Use of compounds of epididymal and plant origin in maintaining viability of ram spermatozoa

by

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dedication to :

Uma** and the late **Onji** and **Rama

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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Chairussyuhur Arman

Summary

This thesis reports the results of studies aimed at finding better ways of storing ram semen at refrigerator or room temperature, with particular reference to the possibility of using ingredients which are readily available locally in Indonesia. Investigations were extended to examine whether the same techniques for storage were also appropriate for subnormal semen, such as would be produced by rams during periods of heat stress. Motility was assessed visually and using a Hamilton Thorn semen evaluation apparatus.

The first part of the program involved studies to see whether coconut extract had any beneficial effects on the survival of ram spermatozoa and whether quail egg yolk, which is more readily available in Indonesia, could be substituted for hen egg yolk. In citrate glucose based media, quail egg yolk was as effective as hen egg yolk in the storage of ram spermatozoa at 5 or 30°C. Storage at 5°C gave better survival of sperm than storage at 30°C. Diluents containing 15% coconut extract mixed with quail yolk in a citrate buffer preserved motility of ram spermatozoa better at 30°C for 24 or 48 h and at 5°C for up to 240 h, when compared with sperm stored in quail yolk or hen yolk citrate glucose diluents. When the coconut extract was replaced with coconut milk, the motility of the sperm was maintained during storage at 5°C much less well, and in fact no better than in hen yolk citrate glucose diluent.

As the diluents had different pH at the beginning of storage, studies were then conducted to see whether the initial pH of the diluents (7.0 or 7.6) had any influence on the survival of the sperm. The pH of the diluent did not have any significant effect on the overall semen motility characteristics, but diluents containing coconut extract during storage, regardless of starting pH showed consistent advantages with regard to motility, progressive motility, percentage rapid sperm and several other characteristics of sperm motility, as determined by the Hamilton Thorn.

The coconut extract diluent also contained catalase, nystatin and sulfanilamide which were not in the hen yolk citrate glucose diluent. Addition of these compounds, singly or in combination, to egg yolk citrate did not improve the motility of stored ram sperm; indeed, the addition of glucose had a deleterious effect on motility of sperm during storage at either 30 or 5°C. This negative effect was not seen during storage at 30°C when nystatin, catalase and sulfanilamide were present as well as glucose, and at 5°C, the diluent containing nystatin and catalase with no glucose also appeared to have a bad effect. The last result could not be repeated in subsequent experiments, and in the later experiments, the addition of 10% coconut extract had if anything a slightly deleterious effect on the motility of stored sperm. It seems that the lower concentration of coconut extract had a different effect from that seen earlier with 15%, but unfortunately it was not possible to examine systematically the effect of different concentrations of the extract.

Another obvious difference between the coconut extract quail yolk and the hen yolk citrate glucose diluents was in the type and concentration of egg yolk, 5% in the former and 20% in the latter. Therefore a study was undertaken to examine the effects of various concentrations of the same type of egg yolk, in the presence and absence of 15% coconut extract. During storage at 30°C, the addition of 10, 15 or 20% hen egg yolk, in the absence of coconut extract had a very marked deleterious effect on the motility of ram sperm during storage; in the presence of coconut extract, only 20% egg yolk had a bad effect on motility. During storage at 5°C, 5 and 10% egg yolk, in the absence of coconut extract had a beneficial effect on motility, whereas 15 and 20% were deleterious; in the presence of coconut extract, all concentrations of egg yolk had a beneficial effect on motility. Thus the effect of egg yolk on sperm during liquid storage in these diluents appears to be quite different from that on sperm during freezing and thawing.

The deleterious effect of glucose on sperm motility during storage at 30°C was confirmed in the experiments reported in Chapter 8, and fructose had a similar bad effect. Surprisingly, the inclusion of lactose also appeared to reduce motility during storage, whereas inositol, trehalose or sucrose had no consistent effect, when compared with hen yolk citrate diluent. These negative effects of sugars on motility were not seen during

stoage at 5°C. The pH of the diluents fell appreciably during storage at 30°C for 48h to around 5.0 when the diluent contained glucose, fructose or lactose, and to a lesser extent with trehalose, whereas the control diluent without sugar, or with inositol or sucrose showed little change. During storage at 5°C for 288h, the pH fell to around 5.5, and the fall was similar in diluents containing glucose, fructose, trehalose or lactose; again there was little change in the pH of the control diluent or those containing inositol or sucrose. The fall in pH in the diluents containing lactose or trehalose were unexpected, as these sugars have not been shown to be metabolized by sperm. There were significant positive correlations of motility with pH, using either pooled data from before and after storage or just the data at the end of storage for the control diluent or the diluent containing any of the individual sugars.

Restoring the pH to 7.0 with sodium bicarbonate at the end of a period of storage at 30 or 5°C did not have any immediate effect on motility, and had variable effects during 24h of subsequent storage at 30°C. Following the addition of bicarbonate, especially in the absence of glucose, the pH continued to rise, to values above 8.0, whereas there was little subsequent change when an equivalent amount of sodium citrate was added. In this experiment, in contrast to the previous one, the correlations between motility and pH were either non-significant or negative, presumably due to damaging effects of the high pH.

All these studies were with semen from normal rams. In further experiments, the effect of diluents on the storage of semen from rams subjected to intermittent scrotal insulation was examined. The scrotal insulation used, either for 16 or 12 h per day was sufficient to cause a significant fall in the quality of the semen before storage. Between the response of the two insulated rams, there were marked differences, which were repeated in the second experiment on the same animals a year later. The characteristics of motility of sperm showed further deterioration following storage at either 30 or 5°C, and this was significantly less in the diluents containing coconut extract. It would therefore appear that this diluent may have particular advantages for storage of semen, which was not of the highest quality when collected, although the deterioration during storage,

expressed as a percentage of the initial value was similar for the normal and poor quality semen.

In the studies with semen from both the normal and scrotum-insulated rams, there were very high correlations between the values for percent motile sperm, as determined by the Hamilton Thorn machine, and most of the other parameters of motility, with the exception of STR and LIN, which appeared to change less than percent motile. While it is possible that these more sophisticated measurements of motility characteristics may be of some value is assessing semen quality, from the present results, it would seem that there is little advantage in measuring anything other than percent motile sperm.

Publications

The following aspects of the work presented in this thesis have been reported everywhere:

Abstracts

Chairussyuhur, A., Sanchez-Partida, L.G., Maddocks, S. and Setchell, B.P. (1993) Quail yolk and coconut extract in diluents for storage of ram semen at 30 and 5°C.Proc. Aust. Soc. Reprod. Biol. **25**:22

Chairussyuhur, A., Sanchez-Partida, L.G., Zupp, J.L., Maddocks, S. and Setchell, B.P. (1994). Motility characteristics of semen from rams subjected to scrotal insulation, after storage at chilled temperatures for one week. Proc. Aust. Soc. Reprod. Biol. **26**:114

Chairussyuhur, A., Maddocks, S. and Setchell, B.P. (1995). Effect of addition of sugars in the egg yolk citrate-based diluent on the motility of ram semen stored at 30 °C and 5 °C. Proc. Proc. Aust. Soc. Reprod. Biol. **27**:22

Paper

Setchell, B.P., Sanchez-Partida, L.G. and Chairussyuhur, A. (1993). Epididymal constituents and related substances in the storage of spermatozoa: a Review. Reprod. Fertil. Dev. **5**:601-612

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Chapter 1

General Introduction

Although in recent years considerable advances have been made in the development of innovative procedures in the areas of reproductive technology, artificial insemination (AI) which has been used extensively throughout the world, is still the most important single method for the genetic improvement of domestic animals (Foote, 1980; Rasbech, 1993). This technique is a highly successful method for breeding cattle, especially for dairy cattle improvement (Schuh, 1992) and is now accepted as an effective method for breeding certain other species, including sheep.

AI in sheep has been practised for over half a century, originally in the Soviet Union and more recently this technology has also been applied in many other developed countries (Evans, 1988). During the last several decades the numbers of ewes mated by this method increased steadily and it is now estimated that more than 40 million sheep are artificially inseminated every year (Rasbech, 1993).

Most of the developments in the areas of AI, semen storage and synchronization of oestrus in sheep occurred in the developed countries (Rodriguez, 1988), and the technique is now continuously gaining a growing interest especially since the advent of laparoscopic procedures for intra uterine insemination (Evans and Maxwell, 1987) and controlled breeding programs with fixed-time AI (Rasbech, 1993). The potential importance of AI technology and its use in the sheep industry are of considerable and immediate interest to less-developed countries, including Indonesia.

Although AI would share the same advantages and disadvantages in both developed and developing countries, the cost benefit factors are quite different. For example, in developing countries the size of flock is generally small, the use of flock rams is costly and the spread of reproductive diseases by natural service can be a

danger. Besides, there is a chance of increasing the frequency of undesirable recessive genes when using the flock rams. Considering its immense potential in animal breeding, AI in sheep is to be preferred (Schuh, 1992).

In Indonesia the improvement of production from small ruminants (sheep and goats) has been one of the important objectives of the animal industry. There is a desire to adopt AI technology as one of the tools for increasing production and at the same time for improving the genetic performance of these animals under local conditions. One of the main breeds of sheep in Indonesia is the Java Fat-tail sheep. The broad and fat tail of this sheep is known to be a great physical hindrance to effective conception and has been observed to be the major contributing factor to low percent lamb crop realized with this animal (Obst et al, 1980). Improved management practices such as docking should make natural mating feasible. However, social and traditional cultural objections make the practice uncommon. It appears that AI is the only means by which the Fat-tail sheep can be efficiently propagated. However in Indonesia, AI in small ruminants has not been widely practised yet. The desirability of adopting AI in the sheep industry is limited by insufficient experience in the use of techniques and procedures especially in the area of semen processing and preservation. These are regarded as the essential prerequisites for a successful of AI program. Thus, before adopting and implementing AI technology under tropical conditions, it is necessary to establish in preliminary studies, what are the best diluents (extenders) in which the fertilizing capacity of ram semen can be preserved for longer periods of time.

Ram semen can be preserved or stored in liquid form, either at room or cool/chilled temperatures, and in frozen state in liquid nitrogen. Frozen semen made it possible to distribute semen worldwide and to reach areas where insemination with liquid semen from selected males was previously not practical. Nevertheless, the production of frozen semen requires sophisticated techniques and equipment while the necessary machinery and instruments incur heavy maintenance costs. In addition, a substantial portion of sperm are killed during the freezing procedure and sperm that had

been frozen do not survive after thawing as long as those that have been cooled to about 5°C without freezing (Foote, 1993).

In ewes, fertility results with fresh semen are still superior to those with frozen-thawed semen (Salamon, 1972; Langford et al., 1979). This is due to frozen-thawed ram spermatozoa having a reduced capacity to traverse the cervix (Lightfoot and Salamon, 1970), particularly after vaginal insemination (Maxwell and Hewitt, 1986). Therefore, in sheep most AI is performed with diluted liquid semen while only a few ewes are inseminated with frozen semen (Colas, 1983). Fertility of deep-frozen semen is about 20 percent lower than that of fresh semen; the required number of spermatozoa appears to be higher than for fresh semen (Colas, 1975). Intra uterine deposition of frozen-thawed semen gives a higher pregnancy rate than intra vaginal or cervical deposition (Tervit et al, 1984; Maxwell and Hewitt, 1986). However, uterine deposition of semen involves surgery (Killeen and Caffery, 1982; Jabbour and Evans, 1991), which entails some stress and surgical risks for the ewe and may not always be appropriate for routine use. With chilled extended semen, the pregnancy rate may be acceptable even after cervical deposition of semen.

Therefore, studies with the purpose of seeking techniques for dilution and short-term liquid storage of sheep semen using locally available ingredients which are relatively cheap and applicable under tropical developing countries are needed.

Since AI in sheep has become a practical procedure over several decades, different types of semen extenders have been evaluated for their capability to keep ram sperm motile over time at room temperature or chilled. The ingredients in these diluents included citrate, phosphate, tris or glycine buffers, milk (fresh, skimmed or reconstituted), sterilized cream, with or without the addition of egg yolk, sugars, coconut fluid or coconut water, or combinations of the above.

Most of the egg yolk used to dilute sheep semen is derived from hen eggs. A considerable number of quail eggs are produced and consumed in Lombok, Indonesia, where their price is lower than hen eggs. Information regarding the use of quail yolk as

an extender for diluting and preserving ram semen does not appear to be available. Such information is of interest as the proportions of yolk is higher in quail egg than in hen eggs (Fletcher et al, 1983). Since yolk is expensive and under practical conditions a much lower level of yolk can often be used, the use of quail egg yolk as a substitute for hen egg yolk seems to be quite possible.

The use of coconut milk or coconut water (found within the endosperm cavity) as semen diluters has attracted some attention due to its availability in almost all tropical countries. Several studies have been carried out to investigate metabolic activity and/or fertility of bovine (Norman et al, 1962; Islam, 1986), buffalo (Norman et al, 1968), ram (Prasad and Norman, 1968) and buck (Pillai et al, 1978; Pillai and Iyer, 1982) spermatozoa preserved in diluents containing this product. However, there seems to be little or no information on the use of coconut extract (pressed from the coconut endosperm) for diluting and preservation of sheep semen. Such information is of interest, because in comparison with coconut milk, coconut extract is higher in both fat and protein contents (Banzon and Velasco, 1982). Besides, in Lombok coconut trees can be found in every village. Coconut extract is versatile and cheap and can easily be prepared for making semen diluents. It is likely that the use of quail egg yolk and coconut milk or combinations of them could maintain the viability of ram spermatozoa at different temperatures during liquid storage.

It has been known for many years that temperature plays a major role among the various environmental factors affecting reproduction in small ruminants. Elevated body temperatures during periods of high ambient temperatures or pyrexia from disease, lead to testicular degeneration and reduce the percentage of normal and fertile spermatozoa in the ejaculate. In sub-tropical countries, there are seasonal variations in the quality and fertility of ram semen. Rams may retain a satisfactory level of fertility throughout the whole year, but in many instances, fertility is depressed when matings occur during the hot months of the year. Conception failure in ewes mated to heat-stressed rams is related principally to failure of fertilization but embryonic mortality may also be involved (Howarth, 1969; Rathore, 1970). The effects of high temperature

on semen production and quality, observed particularly in tropical areas, seem to exist under Indonesian climatic conditions as well. However, little research in this field has been undertaken (Casu et al, 1991).

Therefore, in order to evaluate the need for new studies under Indonesian husbandry conditions, experiments regarding the effects of elevated temperatures by scrotal insulation, rather than observations on rams running free in the field, on the viability characteristics of spermatozoa are to be pursued. It seemed that the use of quail yolk and coconut extract in semen diluent may support and maintain the viability of low-quality semen from rams subjected to high environmental temperatures.

The epididymis, a highly convoluted duct, through which testicular spermatozoa are transported from the testis, is also known as an organ for sperm storage. The major site of sperm storage is its caudal or tail portion. The tail of the epididymis contains 70 percent of the total number of spermatozoa in the excurrent ducts, whereas the vas deferens contains only 2 percent (Amann, 1981). Under normal conditions sperm are able to survive there for relatively long periods of time. In most mammals they retain their capacity for motility for at least 2 to 3 weeks (Bedford, 1975; Cooper, 1986) and in bats, up to several months (Racey, 1972). The favourable surrounding environment in the epididymis that retains the survival of spermatozoa is thought to be due to the peculiarities in compositions of the luminal fluid; one of which is inositol, a small organic constituent.

Inositol and other polyols have been incorporated in a number of media for the freezing of boar (Salamon et al, 1973) and ram semen (Molinia et al, 1994) and also for thawing frozen ram semen (Salamon and Brandon, 1971). However, there seems to be no information on the use of this sugar alcohol in the semen diluents for short-term liquid preservation of ram semen.

Therefore, it would be worthwhile to investigate the effects of inositol and other related sugars (either mono or disaccharide) which are found in the seminal

plasma and in the luminal fluid of the epididymis, on the viability of ram spermatozoa stored at temperature above freezing.

In the next chapter, techniques for the storage of sheep semen and various diluent components including those plant or epididymal origin that have been tested for preservation of semen from various animals; and also the effects of high temperatures on the quality or fertilizing capacity of spermatozoa will be reviewed.

Chapter 2

Literature Review

2.1. Semen composition and production

2.1.1. Semen composition

Semen consists of the spermatozoa (male gametes) suspended in the liquid or semi gelatinous seminal plasma. Spermatozoa are produced in the testes which lie in the scrotum outside the abdomen in most mammals, while the seminal plasma is formed by the mixed secretions of the testes and the accessory glands (the epididymis, seminal vesicles, prostate) situated along the male genital tract (Garner and Hafez, 1987). Seminal fluid is an isotonic, neutral medium (pH at about 6.5) containing inorganic ions, fructose, inositol, glycerylphosphorylcholine (GPC) and other carbohydrates, citric, sialic and ascorbic acids, steroid hormones and other compounds. The seminal vesicles contribute the largest volume to the seminal fluid (Setchell, 1977; Mann and Lutwak-Mann, 1981; Evans and Maxwell, 1987; Garner and Hafez, 1987).

The composition of the semen produced in each ejaculate differs markedly among species (White, 1976). In rams, the ejaculate volume is lower than in bulls, but sperm concentration is at least three times higher. However, when semen from rams and bulls are compared with that from boars and stallions, semen of these last two is ejaculated in fractions and the volume is much greater, but the sperm density is much lower (Mann and Lutwak-Mann, 1981; Sorensen, 1979).

2.1.2. Semen production.

Spermatozoa are formed in the seminiferous tubules of the testes which make up 90 percent of the testicular mass (Nalbandov, 1964). Each tubule is highly

convoluted and they are about 1500-7000 metres in total length in a 250 gm ram testis (Chemineau et al, 1991) and is about 0.20 mm in diameter (Setchell, 1977). The lumen of the tubule is filled with fluid secreted by the Sertoli cells which collects and transports spermatozoa to the rete testis (Chemineau et al, 1991; Meijer and van Vlissingen, 1993). The tubule is lined by the seminiferous cells which are of two basic types, the spermatogenic cells (spermatogonia, spermatocytes and spermatids) and supporting cells (Sertoli cells) which supply nutrients to the spermatozoa (Chemineau et al, 1991). The spermatogonia are developed from the primordial germ cells which migrate to the germinal crest in the fetus. Once the spermatogonia are located into the seminiferous tubules, they remain quiescent until puberty when they resume dividing and are incorporated in the spermatogenic cycle (Ortavant et al, 1977; Sorensen, 1979). The Sertoli cells have several functions including secretion of fluid, phagocytosis, maturation and release of spermatozoa and synthesis of the intra-tubular androgen binding protein (see Fawcett, 1975).

The lumen of each tubule develops only at puberty (Setchell, 1993) at which time the first release of spermatozoa occurs, but the very early processes of spermatogenesis commence during fetal life. The release of immotile spermatozoa into the lumen of the seminiferous tubule is the final step of spermatogenesis (Chemineau et al, 1991). The lumina of the testis tubules form a loop that opens at both ends into a network of collecting ducts, the rete testis, through the short tubuli recti (Setchell, 1977). The rete in turn, is connected to several tubules constituting the efferent ducts (ductuli efferentes) which lead to the head of the epididymis (near the cranial pole of the testis) where they join to form the single duct of the epididymis (Foster, 1988). In the epididymis, three successive parts can be distinguished, i.e. caput, corpus and cauda where the spermatozoa are stored until ejaculation (Meijer and van Vlissingen, 1993).

2.2. Semen collection

The success of an artificial insemination (AI) program depends upon collection of high-quality semen from genetically superior males that have been kept under good

conditions. Thus the primary objective in semen collection is to obtain maximum output of high quality spermatozoa per ejaculate (Campbell and Lasley, 1975).

Semen from males of the small ruminant species (sheep and goat) that is used for AI can be collected using two main techniques; the first is an artificial vagina and the second is an electro ejaculator. The latter technique has been employed in the past with mixed success: the semen provided has characteristics different from those obtained with an artificial vagina and the volume of the seminal plasma is generally higher; this results in reduced resistance of the sperm to cold shock and less likelihood of surviving of freezing and thawing (Quinn et al, 1968; Entwistle and Martin, 1972). Mattner and Voglmayr (1962) found a higher values of concentration and wave motion in ejaculates collected by artificial vagina than by electro ejaculator. However, a comparison of methods to collect semen in Mexico (Hernandez et al, 1976) showed that the volume of semen and semen quality of Mexican rams were not affected by methods of collection, but the use of an artificial vagina gave higher concentrations than the electro ejaculator.

The method of electro ejaculation is particularly useful to collect semen from rams that are not trained to serve the artificial vagina, or suffer from injury, or when conditions are not conducive to normal mounting and ejaculation (Gomes, 1977; Pineda, 1980). Since this method of collection appears to be painful for the animals (Chemineau et al, 1991) and there is some danger of contamination of the semen sample with urine during collection (Garner, 1991), the artificial vagina method is preferable to electrical stimulation.

In the following section, only collection of ram semen by the aid of an artificial vagina will be discussed.

2.2.1. Preparation of an artificial vagina

The artificial vagina is simple in construction and is designed to simulate natural copulation (Garner, 1991). The type that is used for the ram is essentially similar

to that for the bull (Danish model), except that it is of smaller diameter and shorter (5.5 x20 cm) (Salamon, 1976; Hopkins and Pineda, 1980; Rasbech, 1993). It comprises two layers, an outer casing made from heavy rubber, plastic or other synthetic material with good insulation properties and an inner latex sleeve made of rubber or suitable synthetic layer (Evans and Maxwell, 1987). The space between these two layers forms an enclosed cavity. The casing of the collecting unit is filled via the tap about 2/3 with water maintained at 40-42 °C and inflated with air to the pressure necessary to elicit a complete ejaculation (40-60 mmHg)(Maule, 1962; Hopkins and Pineda, 1980). One of the inner openings of the artificial vagina is lubricated with sterile lubricant to provide a comfortable passage for the penis (Gomes, 1977) and in the other opening a sterile calibrated collecting tube is inserted for 1.5 to 2.0 cm (Evans and Maxwell, 1987) to allow easy semen collection and volume measurement.

2.2.2. Training of males for semen collection

Although the artificial vagina method is painless, quick and simple and results in a better quality of semen, it requires training of rams to ejaculate in the artificial vagina. Training should be done at an early age (7 to 8 months; Hafez, 1987) and begun 2-3 weeks before the artificial insemination program (Evans and Maxwell, 1987). Frequent semen collections should be continued at regular intervals throughout the year. Rams are most easily trained to mount an intact oestrous female or a teaser female that has been ovariectomized and treated with an oestrogen to induce oestrous behaviour (Memon and Ott, 1981). However, some males are readily trained to mount a dummy (phantom), a castrated male, or another male animal (Gomes, 1977). When the ram has mounted the teaser ewe, the sheath (prepuce) should be slightly deflected by the operator's hand and the exposed penis guided into the artificial vagina (Rasbech, 1993). Following ejaculation the semen is collected directly into an insulated sterile collection vessel to prevent temperature shock and placed in an environment where temperature can be maintained at 30-37 °C until the sample can be examined and further processed (Garner, 1991).

Suitable sexual preparation and proper stimulation of the animal by allowing several false mounts before semen is collected may enhance both the quantity and quality of semen (Courot, 1976; Garner, 1991). It has been demonstrated in bulls that sexual preparation prior to collection increased the number of spermatozoa obtained per ejaculate by as much as 100 % (Amann, 1970). In rams, collection of semen between 3 to 5 times per day for 4 to 5 day periods separated by 2 to 3 days rest, does not affect the volume or concentration of spermatozoa (Evans and Maxwell, 1987). Cameron et al (1984) on the other hand reported that only two collections a day are possible without disturbing the volume and sperm concentration, but this varies with the breed and age of the animals.

2.3. Semen evaluation

Semen evaluation is an essential part of artificial insemination programs. Besides being a diagnostic method that may help evaluate the male's reproductive status (Rhodes, 1980) and to ensure that the semen to be used is physiologically normal for the particular animal (Rasbech, 1993), examination of semen quality may also determine the possible degree of dilution rate of semen and thus the number of females which can be inseminated (Evans and Maxwell, 1987; Hafez, 1987).

Several techniques have been used for assessing the quality of ram semen and the conventional assessment is usually limited to the physical parameters: volume, motility, concentration, percentage of live spermatozoa and spermatozoal morphology (Hafez, 1987). Recently, automated, computerized techniques have been developed for evaluation of sperm motion, which will result in more precise, objective measurements of sperm motion than visual (conventional) observations (Amann, 1989). Both semen quantity and quality varies with age, seasonal and temperature effect and the individual within the breed (Memon and Ott, 1981).

2.3.1. Conventional sperm assessment

2.3.1.1. Colour and consistency of semen

The colour of normal ram semen is milky white or pale cream (Evans and Maxwell, 1987). Semen color is affected by its consistency, which according to Gunn et al (1942) may be described as thick creamy, creamy, thin creamy, milky, cloudy, and watery. The consistency of semen gives an idea of the sperm concentration. The highest concentration is obtained with a thick creamy consistency and the lowest concentration is obtained with a thin or watery consistency (Evans and Maxwell, 1987). Simplicio (1982) reported that the score of semen colour and consistency of Brazilian rams (graded from 1 to 5 or turbid to thick creamy), was 3.2 during the rainy season and was 3.6 during the dry season.

The semen in the glass collection tube should be carefully examined for contamination with dust, straw or urine, or blood or pus if the rams are infected. The presence of blood in semen may also be due to injury of the penis during collection and this gives a pink colour. The presence of urine or faeces cause a varying degree of brown, depending on the amount of contamination that will give a typical odor. These contaminants are detrimental to the semen and reduce the life-span and viability of spermatozoa (Herrick and Self, 1962; Hafez, 1987; Evans and Maxwell, 1987).

2.3.1.2. Volume of semen per ejaculate

The measurement of the ejaculate volume is done immediately following collection, usually by reading the graduations marked on the collection tubes (Rasbech, 1993). The ram ejaculate volume ranges from 0.7 to 2 ml (Evans and Maxwell, 1987) but appears very variable from one collection to another. Several factors like age, breed, individual animal, season of the year, the method of collection and the sexual preparation of the animal may also influence the volume of semen ejaculated (Hafez et al, 1955; Garner, 1991).

Wiemer and Ruttle (1987) found a significant effect of age of New Mexican fine wool range rams on the volume of the ejaculate; young rams produced a lower volume than older rams. Studies on the characteristics of semen in Egyptian fat-tailed sheep (Hafez et al, 1955) indicated that the yearly average of semen volume from 9 Ossimi rams was 0.94 ml, while from three rams of the Rahmani breed was 1.17 ml. Semen volumes differed among individual rams observed in this study. The average volume of 151 ejaculates from three 3-year-old Suffolk rams has been reported to be 0.62 ml, with a range of 0.10-1.30 ml by Wiggan and Clark (1967).

Sahni and Roy (1969) reported that the average volume of Bikaneri and Mandia rams reached the highest value during winter (1.08 ml and 0.59 ml, respectively) and the lowest values during summer (1.02 ml and 0.45 ml, respectively). The average ejaculate volume of Brazilian rams recorded by Simplicio (1982) during rainy and dry seasons were 0.67 ml and 0.78 ml , respectively. The volume of the ejaculate correlated positively with the percentage of ewes lambing (Wiggins et al, 1953). Although the predictive value of volume measurement was poor, and the reason of this relationship was not clear.

2.3.1.3. Concentration of the ejaculate (sperm density)

The total count of spermatozoa without considering their shape, size and viability is called sperm concentration or sperm density (Ranjhan and Pathak, 1993). Accurate determination of the number of spermatozoa per millilitre of semen is extremely important because it is a highly variable semen characteristic (Hafez, 1987). The sperm concentration refers to the number of spermatozoa per millilitre of semen, the sperm count is the total number of spermatozoa in the ejaculate. Both are important and should be calculated (Hafez, 1987). The concentration of sperm in the ejaculate is generally estimated by direct visual appreciation of the consistency of the ejaculate, exact counting of spermatozoa in a haemocytometer, turbidimetric measurements or measurement of optical density with a spectrophotometer (Foote, 1969, 1974, 1980; Hafez, 1986). Some more sophisticated electronic machines which are expensive and

not suitable for field practical are also used (Evans and Maxwell, 1987), but these need to be checked against a haemocytometer.

From the concentration of spermatozoa and the volume of ejaculate, the sperm output can be calculated by multiplication (Colenbrander and Kemp, 1990). The dilution rate for semen to be used for insemination is calculated based on both the density and the motility of semen (Rasbech, 1993). Thus the number of females to be inseminated and the optimal number of spermatozoa recommended per insemination dose can theoretically be established (Evans and Maxwell, 1987; Hafez, 1987).

The concentration of spermatozoa in the semen of sheep has been shown to vary from 2 to 6.5 x 10^9 spermatozoa per ml of ejaculated semen (Memon and Ott,1981). A number of factors like age, season of collection, breed, frequency of collection, size of testis, influence sperm density of semen.

Sahni and Roy (1969) reported in Bikaneri sheep that the highest sperm concentration occurred during autumn followed by summer, rain, spring and winter seasons in descending order; whilst in Mandia sheep the highest concentration recorded during spring, rain, summer, winter and autumn in descending sequence. Maqsood (1951) found marked decrease in spermatozoal density in Suffolk rams during non breeding seasons (spring and summer). Amir et al (1986) reported that sperm density of Finn cross rams subjected to frequent ejaculation decreased from the first to the fifth ejaculate from 3.6 to 2.9 x 10^9 . The sperm concentration of different breed of rams in the USA (Rambouillet, Columbia, Targhee and Corriedale) ranged from 0.35 to 7.18 x 10^9 sperm/ml with the mean value of 2.96 x 10^9 sperm/ml (Wiggins et al, 1953). In Poland, Nowakowski and Cwikla (1994) found that adult Polish Merino rams had the larger testis size just before breeding season, which was related to better semen quality. From the results of various studies, it has been established that ram semen of good quality contains 3.5 to 6 x 10^9 spermatozoa/ml (Evans and Maxwell, 1987).

2.3.1.4. Motility of spermatozoa

Although transport of spermatozoa in the female tract from the area of deposition to the area of fertilization is accomplished by factors other than motility of the individual spermatozoa (Moeller and vanDemark, 1955), motility of spermatozoa still appears to be of importance in determining fertilizing capacity. For this reason, degree of motility of spermatozoa is used as one of the criteria for semen evaluation (Herrick and Self, 1962).

Rasbech (1993) categorized three motility examinations namely, motility examination after collection, and before and after freezing. The motility rate found after collection is one of the parameters in deciding dilution rate. The motility rate before freezing is a control of the effectiveness of the dilution, while the motility rate given after freezing is a control of the quality of semen after finalizing the processing.

Accuracy in evaluating motility is achieved only if standard conditions are established for each sample evaluated and the sample maintained at body temperature and examined immediately after collection (Herrick and Self, 1962). The use of time-lapse photography and computer-assisted system analyses that provide more accurate motility assessment, has been developed during the past decade, but the equipment is expensive and rarely used in the field (Wallace, 1992).

Motility or degree of vigor is a combination of progressive movement of the individual spermatozoa and the collective movement of all spermatozoa, the latter often referred to as mass movement or mass motility (Herrick and Self, 1962). Thus, two evaluations should be made on motility, first estimation of wave motion (initial/mass motility) and second estimation of the individual motility of spermatozoa.

2.3.1.4.1. Wave motion (initial/mass motility)

For ram semen evaluation at AI centres, the determination of wave motion (initial or mass motility) of fresh semen is an important criterion as a quick test. This is one of the most easy, rapid and widely used tests of semen quality (Memon and Ott,

1981) which determine whether or not an ejaculate is suitable for further processing (Rasbech, 1993). Initial motility also gives a guide to the percentage of motile spermatozoa (Logue and Isbister, 1992).

Assessment is done as soon as semen collection is made, in order to obtain an appreciation of the wave motion or mass activity of the semen. A drop of pure-non diluted and dense semen is deposited on a glass slide without coverslip and placed on a warmed stage (37-38 °C) under a microscope at a low-power magnification (40x or 100x) (Hopkins and Evans, 1980). Different laboratories use different standards for classifying mass motility (Rasbech, 1993). However, the wave motion is usually scored using a scale ranging from 0 (no wave movements) to 5 (high wave movements) (Wallace, 1991). For the purpose of artificial insemination, high-quality semen should have marked wave motion (scored 4-5) (Evans and Maxwell, 1987).

Factors affecting other semen characteristics (volume, concentration) may also affect sperm motility. For example, Sahni and Roy (1969) reported the influence of season on mass motility in semen from Indian sheep. The highest score of wave motion of Bikaneri rams was 4.81 which occurred during rain season and the lowest score was 4.29 during winter season. In Mandia rams, the highest score (4.65) and the lowest score (3.94) was recorded during summer and autumn seasons. Another study observed that ram semen collected in the autumn had a higher motility score than that collected in the spring (Koger, 1951). However, Hafez et al (1955) found in both Ossimi and Rahmani breeds that semen qualities were better around the spring equinox than at the autumnal equinox under the subtropical climate of Egypt. In the tropical region of Brazil, Simplicio (1982) did not find any differences in mass motility score between rainy and dry seasons. However under tropical conditions in India, the best motility of Corriedale rams was observed during the cooler part of the year (September to February) with motility score ranged from 4.00 to 4.83, and moderate during the hotter months (March to August) with motility score ranged from 0.71 to 3.83 (Sahni and Roy, 1972).

2.3.1.4.2. Individual motility of spermatozoa

For more accurate assessment, instead of evaluation of semen for mass motility the motility of individual sperm can be assessed (Hopkins and Evans, 1980). The motility of individual spermatozoa is assessed by examination of a diluted sample in a droplet preparation under a coverslip on a warm stage (37-38 °C), usually at a magnification of 200-400x (Chemineau, 1991; Rasbech, 1993). Examination at this power (400x) will also allow some initial observation of the morphology of the spermatozoa and the presence of unusual cells in the semen (Logue and Isbister, 1992). Individual motility of spermatozoa is rated as 40, 50, 60, 70% etc. and these values indicate corresponding numbers of progressively motile sperm without including all other types of abnormal motion, e.g. oscillating, circular, backwards etc. (Rasbech, 1993). Samples that do not fulfil the required standard for use in AI are rejected (Hopkins and Evans, 1980). However, due to the time consuming nature of the procedure, this techniques is not always employed.

Wiggan and Clark (1967) reported that the mean percentage of motile sperm from 151 total ejaculates of three 3-year-old Suffolk rams was 76 with the range of 40 to 80 percent. Wiemer and Ruttle (1987) found a reduction in sperm motility as the ram aged. They noted that the percentage of motility of spermatozoa in 1.5, 2 to 5 and 6 years old rams was 80.2, 71.0 and 68.6, respectively. The percentage of individual sperm in samples of Brazilian rams as scored by Simplicio (1982) was slightly higher during rainy season than during dry season.

2.3.1.5. Assessment of percentage of live and dead spermatozoa and morphological abnormalities in sperm

Assessment of percentage of live and dead spermatozoa and morphological abnormalities in sperm is considered an important contribution to prediction of male fertility; and is accepted as a more meaningful technique for differentiating between semen of high and low fertilizing capacity (Peter and Ball, 1986). The visual assessment of live and dead spermatozoa is normally performed at the same time as the

determination of type and number of abnormal cells, i.e. by exact counting the slides prepared by differential staining techniques (Chemineau, 1991).

Several stain mixtures have been used for this physiological and morphological evaluation, e.g. india ink or Wright's and William's stain (Hackett and Macpherson, 1965; Harassymonycz et al, 1976), but the most common stain used today is eosin-nigrosin (Swanson and Bearden, 1951; Mayer et al, 1951; Campbell et al, 1956, Hancock, 1956; Evans and Maxwell, 1987). Basically, live cells are those excluding the stain when it is applied, while dead cells are those stained red with eosin against the dark nigrosin background (Hafez, 1987). These methods are subject to variations between stain batches and are influenced by pH of the stain (Mayer et al, 1951; Entwistle, 1972) and the temperature of the stain and duration of staining (Hancock, 1951; Campbell et al, 1956).

Sperm abnormalities are classified as primary or secondary (Sorensen, 1979) and vary in their importance relative to fertility. It was thought that primary abnormalities originated from the testes and secondary ones in the efferent duct system. Other abnormalities due to damage of spermatozoa that occur during collection, handling and processing of semen for AI are classified as tertiary abnormalities (Hafez, 1987).

Various factors such as age, breed, frequency of collection, season, composition of semen extenders, duration and temperature of storage influence the percentage of live spermatozoa in the semen (Mixner and Saroff, 1954; Dott, 1975; Ranjhan and Pathak, 1993). In the ram, semen of potential sires should contain less than 10 percent dead spermatozoa (Ross, 1989) and less than 25 percent of morphologically abnormal spermatozoa (Hopkins and Evans, 1980).

Wiggins et al (1953) reported that on average there were 80% of live spermatozoa, 79% normal-live spermatozoa, 89% normal spermatozoa and 0.73% abnormal heads in ram semen of European breeds. In Egypt, Hafez et al (1955) found that the annual average of live spermatozoa in fresh and stored samples from nine

Ossimi sheep was 61.32 percent and 43.75 percent, respectively; while the average values from three individual Rahmani sheep were respectively 56.20 percent and 44.72 percent. The percentages of total abnormalities observed in the semen of these breeds were 14.07 in Ossimi rams and 16.11 in Rahmani rams. In the hot semi-arid climate of tropical north east Brazil, the proportions of stained spermatozoa in semen of Brazilian Somali rams were 6.79 percent during the rainy season and 7.11 percent during the dry season (Simplicio, 1982).

Abnormal spermatozoa are produced mainly as a result of disturbances of spermatogenesis. This may be genetic in origin, may be due to disease or adverse environmental conditions. For example, in rams testicular hypoplasia may affect sperm production and infection such as abscesses will cause seminal degeneration (White, 1976). High environmental temperatures have been reported to cause an increase in the proportion of sperm abnormalities (Dutt and Hamm, 1957; Dutt and Simpson, 1957; Moule and Waites, 1963). Furthermore, ram fertility and semen quality are known to be seasonally variable (see review by Lincoln, 1981).

2.3.1.6. pH (hydrogen-ion concentration) and buffering capacity of semen

The amount of secretions from different secretions of accessory sex glands at the time of ejaculation mainly determines the pH and buffering capacity of the semen. Changes in the pH of semen after collection may be regarded as a rough measure of the metabolic activity of spermatozoa that results in production of lactic acid from glycolysis. The accumulation of lactic acid in the semen in turn, lowers pH and decreases sperm motility (Campbell and Lasley, 1975).

Generally, most mammalian spermatozoa are active and survive for the longest period in a medium with optimum pH close to neutrality (White, 1976; Watson, 1990). The natural pH of ram semen after collection has been reported (Emmens, 1959) to vary between 5.9 and 7.3, with a modal value of 6.4 . However, motility of ram spermatozoa reached a maximum at about pH 7.0 (Blackshaw and Emmens, 1951). The pH of semen of the European sheep ranged from 5.5-8.0 with the mean of 6.59 (Wiggins et al, 1953).

Simplicio et al (1982) did not find variations between individuals or between seasons in the pH of semen from Brazilian Somali rams. The average pH during rainy, transitional rainy-dry, dry and transitional dry-rainy seasons was 7.11, 7.01, 6.79 and 6.87, consecutively.

Since ram spermatozoa produce large amounts of lactic acid from seminal plasma fructose (White, 1976) and the buffering ability of normal semen (pH 6.9-7.1) is limited (England, 1993), semen extenders should have additional buffering capacity to extend the shelf-life of spermatozoa. (Ranjhan and Pathak, 1993). For this reason buffers such as phosphate, citrate, Tris, or bicarbonate or combinations of these are included in the diluents (White, 1976). Tiwari et al (1977) found increased production of lactic acid in the diluted of ram semen from 23 mg/ml at 0h to 51 mg/100 ml at 24h of storage at 5°C, which was also reflected in a rapid decrease in the pH of the diluted semen from 6.50 to 5.97 at corresponding times.

2.3.1.7. Other tests for semen quality

A number of other tests for assessing semen quality and predicting male fertility have also been used such as integrity of the acrosome, GOT (glutamic-oxalacetic acid transaminase), fructolysis, lactic acid production, pH change, methylene blue reduction, oxygen uptake, proacrosin content, ability of spermatozoa to travel in different media, including cervical mucus (Chemineau et al, 1991; Memon and Ott, 1981; Wallace, 1991).

2.3.1.8. Relationship between semen quality and fertility

As already shown, a number of parameters were used to assess the quality of semen in the laboratory (*in-vitro* tests). However, the ultimate aim of assessment of semen quality is to relate traits of *in vitro* sperm survival to fertility. This can only be determined by inseminating a large number of females in field trials then evaluating the fertilizing capacity, calculating the actual pregnancy rate, monitoring the ability to maintain pregnancy and finally counting the number(s) of offspring born (*in-vivo* tests).

Many attempts have been made to correlate the results of *in vitro* tests and the fertility of females inseminated with assessed semen, but the results have often been found to be inconsistent. Hafez (1987) suggested that the percentage of motile spermatozoa as evaluated subjectively was positively correlated with fertility. This is supported by Hulet and Ercanbrack (1962), Hulet et al (1965), Linford et al (1976) and Budworth et al (1988) who found a correlation between motility, which is rated as the percentage of motile spermatozoa or mass motility score, and fertility. In contrast, though visual estimation of the percentage of motile sperm cells is the most commonly used laboratory test for evaluating sperm quality, this is not an absolute indicator of sperm fertilizing capacity (Linford et al, 1976). As reported by Eppleston et al (1986), visual motility assessment did not always correlate with fertility in subsequent insemination trials. Similarly, motility has a poor predictive value when correlated with fertility (Watson, 1979). Furthermore, even though assessments of motility done immediately after freezing and thawing or following 1 to 2 hours incubation of samples at 37°C, have been shown significantly correlated with fertility, the predictive value was low (Saacke and White, 1972; Linford et al, 1976).

One of the reasons for inconsistency in fertility results is that most studies have been performed at a defined number of spermatozoa per insemination. Thus alteration of the number of spermatozoa per insemination might reveal a different relation between the characteristics in question and fertility (den Daas, 1992). Another reason might be that the sperm number inseminated was so high that it exceeded the minimal numbers needed for maximal fertility (Amann, 1989) and by the use of semen which has been selected for high quality (Pineda, 1980), so that changes in the number of sperm with a certain characteristics were not further reflected by changes in fertility (den Daas, 1992).

Seminal characteristics are better correlated with fertility when the samples are taken from an unselected population. The coefficient of correlation between seminal characteristics and fertility will depend upon the range of values of the characteristics in the seminal samples used to determine the relationship (Pineda, 1980). However,

fertilizing ability does not depend on a single parameter of the semen but also on the type of oestrus of the female, either natural (Roberts and Houlahan, 1961) or hormonally synchronized (Trounson and Moore, 1974) and on the site of deposition of the semen (Corteel, 1981).

In sheep, the only useful prediction test at the moment is probably the morphological test on the spermatozoa, i.e. percentage of normal or abnormal spermatozoa. The incidence that morphological defects of spermatozoa are associated with impaired fertility has been shown in the earlier studies by Gunn et al (1942), who suggested a reduction of fertility from 80-100 percent to 60 percent when only 1% abnormal spermatozoa were observed; when the number of abnormal spermatozoa exceeded 10%, the rams became severely infertile. Starke (1949) found a greater percentage of abnormal spermatozoa in rams of poor fertility compared with that of normal rams, though the definite level as an indicator of the degree of infertility could not be predicted. He further suggested that it was the type of abnormal spermatozoa that gave a better indication of fertility than the total count of abnormal cells. In his studies, the abnormalities of spermatozoa in semen of poor fertility were specifically characterized by pyriform or narrow heads, enlargement of middle pieces, middle piece beads, or variations in the head size. Chemineau et al (1991) suggested that after natural mating or artificial insemination, the correlations obtained between percentage of normal ram spermatozoa and fertility of the females are close to or higher than 0.50. After deep-freezing of the semen the choice of ejaculates is done by assessing the percentage of abnormal spermatozoa and of living cells after thawing and incubation at 38 °C (Chemineau et al, 1991).

2.3.2. Objective sperm assessment

2.3.2.1. Introduction

Traditional methods of fertility evaluation of a ram by assessing sperm motion such as percentage of all motile cells and progressively motile cells are subjective and highly variable (Sherins, 1991). Although this visual estimation is simple, rapid and

inexpensive, the methods often are not repeatable between examiners and the comparability of results obtained by different research groups seems questionable. Moreover, it is not a reliable assay for predicting fertility (Anzar et al, 1991) and motility characteristics of different ejaculates from the same ram often appear to vary. Inconsistencies associated with this subjective measurements and the need a method for estimating motility accurately, reproducibly and free of bias have prompted the development of objective means for determining sperm motion.

In the last several years there have been a rapid growth of new methods and techniques to measure sperm motility characteristics objectively; they include the use of time-lapse photomicrography (Overstreet et al, 1979), multiple exposure photography (Makler, 1978) and micro cinematography (David et al. 1981). Now the most recent technology employed is computer analysis of digitized video images or computer-assisted sperm analysis (CASA) system (Ellington et al, 1993) e.g. CellSoft, Celltrack/s and HT-2000 (Davis and Katz, 1993). CASA allows objective systematic assessment of sperm motion (Katz and Davis, 1987) and computer algorithms characterize both the pattern and vigor of sperm motion. The CASA methodology increases accuracy and precision of the measurement and allows for standardization among laboratories (Sherins, 1991). It is now being adapted for use either on semen from human (Mack et al, 1988) or domestic livestock (cattle, horse and swine: Jasko et al, 1988; Tuli et al, 1992; ram: Mieusset, 1991; Mieusset, 1992; Sanchez-Partida et al, 1992; Sutiyotin and Thwaites, 1992; dog: Ellington et al, 1993; rabbit: Farrel et al, 1993; turkey: Bakst and Cecil, 1992). Although some information can be obtained on morphological parameters, the predominant feature measured by CASA systems is sperm motility (Katz and Davis, 1987).

2.3.2.2. Potency of CASA system

Since its early development CASA has expanded rapidly and is now utilized widely in both routine and research settings in the fertility laboratory (Katz, 1991). CASA offers great potential and is capable of (a) generating large data set of sperm

characteristics, (b) enabling the identification of several patterns of sperm motility within semen samples in both epididymal (Stephens and Hoskins, 1986) and ejaculated semen (O'Conner et al, 1981; Budworth et al, 1987; Katz and Davis, 1987), (c) providing simultaneous measurement of several motility parameters including the percentage of motile and progressively motile spermatozoa, curvilinear and straight-line velocities, linearity of movement, amplitude of lateral head displacement, and beat cross frequency, in addition to the track motility of individual spermatozoa (Katz and Davis, 1987).

Although there is no doubt that CASA evaluation will become an increasing important method of assessing seminal quality (Amann, 1988), some problems arise in obtaining accurate and precise results (Sherins, 1991). Errors in CASA results can be caused by methods of semen preparation, machine settings, choice of counting chamber and semen sampling bias (Ginsburg et al, 1988; Mack et al, 1988; Mortimer and Mortimer, 1988). Errors may also be due to presence of debris in the sample, specimen concentration and temperature of analysis (Davis, 1992). Among these factors, however, three areas deserve special attention because they are fundamental to CASA systems, namely specimen concentration, percent motility and digitization threshold (Davis and Katz, 1993).

Several studies both in human and animal have reported the inaccuracy in CASA instruments when measuring sperm count at low (below about $20x10^6$ sperm/ml) and high (above about $50x10^6$ sperm/ml) specimen concentration (Budworth et al, 1988; Gill et al, 1988; Mortimer et al, 1988; Neuwinger et al, 1990; Davis and Katz, 1992; Davis et al, 1992). High sperm concentration impacts significantly on estimation of curvilinear velocity (VCL) (Vatman et al, 1988; Mack et al, 1989; Mortimer et al, 1988) due to exclusion of sperm tracks interrupted by cell-cell collisions, which in turn decreased significantly the number of data available for CASA calculations and excluded the faster moving sperm from estimates of VCL and linearity (L) (Vatman et al, 1988). Since the heterogeneity of sperm motion in semen requires relatively long sperm tracking observations to provide accurate measures of VCL and L,

it was recommended that semen more dense than 50 million sperm/ml be diluted to below that value to avoid concentration-induced bias (Sherins, 1991, Davis and Katz, 1993). However, Pedigo et al (1989) reported a negative correlation between sperm density and the coefficient of variation, i.e. the higher the sperm density, the lower the variability of the CASA results. Furthermore, it is questionable whether results obtained at higher sperm densities reflected accurately the real sperm movement characteristics of a semen sample. At higher sperm densities the system might be less discriminating because the numbers of measuring points has increased, and there might be an alteration of the motility pattern of the spermatozoa (Pedigo et al, 1989).

Various studies have reported inaccurate CASA results for sperm motility (Budworth et al, 1988; Gill et al, 1988; Mortimer et al, 1988; Neuwinger et al, 1990; Davis and Katz, 1992). Errors in the computation of percent motility can be caused by errors in the overall sperm count and by biases toward motile or immotile sperm. However, differences between instrument and visual values can also be caused by an inappropriate comparisons of methods (David and Katz, 1993).

A number of studies have shown that the image digitization threshold can significantly affect the accuracy of sperm recognition and tracking of the CASA system (Knuth et al, 1987; Mack et al, 1988; Aanesen and Bendvold, 1989; Blach et al, 1989; Toth et al, 1989, 1991; Benvold and Aanesen, 1990; Davis et al, 1992; Davis and Katz, 1993). Adjusting the quality of the digitized image during CASA, threshold setting, is a critical step which will determine whether or not the sperm head is adequately defined to be included in the computer computations (Mack et al, 1988). Setting-up the digitization threshold must be done subjectively by the observer since up to now there is not an objective method to establish the correct digitization threshold, but CASA instruments should be able to determine the optimum value for each sample or field automatically (Davis and Katz, 1993). Incorrect adjustment to the threshold setting results in loss of data from the sample, variability of data within and between subjects, and differences in data between laboratories (Sherins, 1991).

In view of the lack of consistent CASA results, many attempts have been made to validate the method and to minimize these limitations. CASA systems have been tested extensively to determine the consistency (coefficient of variation) of their results, e.g. the spatial variation within the counting chamber, the variation of the system in measuring a specific sample, the relationship between the computerized and manual analysis, and the relationship between sperm density and the reliability of the measurements (Curry and Watson, 1991; Kolibianakis et al, 1992). Anzar et al (1991) evaluated bull semen using the HTM Analyzer for sperm concentration, motility and other motion parameters and revealed that setting the variables on the machine according to the dimensions and brightness of bull spermatozoa and the background of the extender was better than using the settings for bull semen as recommended in the manufacturer's manual and this significantly affected the accuracy of sperm concentration estimates. Curry and Watson (1991) investigated the effect of increasing chamber depth (10, 20 and 40 um) on ram sperm motility characteristics using the HTM analyzer. They found that Curvilinear velocity, Straight line velocity and Average path velocity were significantly increased with decreasing chamber depth. Linearity and straightness were also significantly increased below 20 um. On the other hand, Amplitude and Lateral head displacement and Beat cross frequency did not appear to be directly correlated with chamber depth.

Although there have been continuous controversies on the estimation of male fertility based upon motility of sperm cells, as determined by visual or objective methods (Ellington et al, 1993), nevertheless, the use of CASA systems will probably be increased with refinements or calibration in instrumentation (Farrell et al, 1993) and standardization of CASA technology (Davis and Katz, 1993). The use of a computerized semen analyzer eliminates the subjective human error and is particularly advantageous in situations where large numbers of ejaculates have to be evaluated or where there are frequent staff changes. It may also be employed to supply more differentiated semen analyses, including the intensity of sperm motility (fast, medium, slow), mean progressive velocity and path velocity, lateral head displacement, and the

like. The only disadvantage is its high price which limits its purchase to laboratories doing a large series of routine assessments such as AI stations or spermatological research (Tuli et al, 1992).

2.3.2.3. Hamilton-Thorn Motility (HTM) Analyzer

2.3.2.3.1. Introduction

There have been many instruments available commercially for over 7 years since computerized sperm analysis was introduced to research laboratories and laboratory medicine (Davis and Katz, 1993). One of the most recent automatic Motility Analyzer is the Hamilton-Thorn Motility (HTM) Analyzer. Several studies have shown that HTM Analyzer appeared to be superior to that of other CASA instruments (Gill et al, 1988; Olds-Clarke et al, 1990; Anzar et al, 1991; Kolibianakis et al, 992; Tuli et al, 1992). For this reasons, the more validated and widely accepted HTM Analyzer will be briefly discussed.

2.3.2.3.2. Principles of the HTM Analyzer

The HTM (Hamilton-Thorn Research, Danvers, MA) is a self-contained instrument for analyzing cell motion, which contains an optical system, memory, microprocessor, screen and printer (Kolibianakis et al, 1992). The system has a variable temperature-controlled stage, and includes a playback system that allows the user to view a particular field and evaluate whether the motile and non-motile cells have been counted (Mahony et al, 1988; Hamilton-Thorn Research, 1989). It uses four different sample chambers: self-filling slide, cannula (flat capillary), Makler and the ABS (Petroff Hauser) slide. The type of chamber preferred depends on the concentration of the sample.

Three illumination options are available with HTM: dark-field, bright-field, and phase contrast. In the dark field-option, the object is illuminated by a near-infra red light source at 882 nm. Light scattered by the sample is collected by a high resolution flat field lens and focused in the image detector surface. The integral solid state CCD

(Charged-Coupled Device) detector produces an electronic image. Once converted into a digital format, the image is then analyzing by processing algorithms which quickly and accurately determine the properties of sperm motion (Gill et al, 1988, HTM Analyzer, 1989). In the dark-field illumination, sperm objects appear as bright cells on a dark background.

The analysis begins with the collection of successive images at equally spaced time intervals. The number of frames (from 5 to 20) and the time intervals (from 7 to 30 frames per second) are specified by the operator in the main gates menu. In most cases 30 frames/sec are assessed. For sperm concentration at less than 50 x 10^6 per ml, 20 frames/sec is recommended, while above 150 x 10^6 per ml the manufacturer recommends 5 frames/sec. Following digitization the instrument removes background artefacts. Objects smaller in size and lower in contrast than operator-specified minimums threshold are eliminated. Assessment of the number of both motile and non-motile objects then provides the basis for motility analysis.

The data generated by HTM and its abbreviations include total concentration of all cells (TOTAL), percentage of motile cells (MOT), progressive cells expressed as a percentage (PROGR), percentage of rapid cells (RAP%), percentage of medium cells (MED%), percentage of slow cells (SLOW%), percentage of static cells (STATIC%), straight line velocity or progressive velocity (VSL), track speed, or curvilinear velocity (VCL), average path velocity (VAP), amplitude of lateral head displacement (ALH), straightness (or linear index) (STR) and linearity (LIN).

2.4. Dilution of semen and use of extenders

2.4.1. Objectives and requirements of semen dilution

As ejaculated spermatozoa are highly dynamic cells and do not survive well, the dilution of semen in appropriate media should provide an environment suitable for their extended storage (Salisbury et al, 1978; Mann and Lutwak-Mann, 1981). The dilution of semen also changes the sperm concentration to a level appropriate for

making multiple inseminations (Picket and Berndston, 1974). Thus, the main objective in diluting semen is to increase the volume of the ejaculate so that a large number of females may be inseminated by a given male. In natural mating, although the male ejaculate deposits several thousand million spermatozoa, only about 100-140 million penetrate the cervix (Evans and Maxwell, 1987), whereas through artificial insemination and the extension of semen, one ejaculate may be used for several hundreds of females (Campbell and Lasley, 1975).

The ideal medium for semen extension will not only increase ejaculate volume but will also be favourable for both survival and longevity of spermatozoa and maintenance of their viability and fertility over extended periods of time (Campbell and Lasley, 1975). Thus, components selected for semen extenders should : (a) provide nutrients as a source of energy (b) contain constituents that provide protection against the harmful effects of cooling and freezing (protect against rapid cooling) (c) provide a buffer to prevent harmful shifts in pH as lactic acid is formed (provide buffering capacity against metabolic acidosis) (d) maintain the proper osmotic pressure and electrolyte balance (e) contain antibiotics that inhibit bacterial (microbial) growth (f) substantially increase the volume of semen so that multiple inseminations can be performed (g) provide an environment in which metabolic activities of the sperm can continue (h) provide some chelating action to protect against toxic ions and (i) contain materials to stabilize enzyme systems and maintain the integrity of membranes (Foote, 1974; Pickett, 1993; Sexton, 1979). In addition, an ideal extender should be simple to prepare, readily reproducible, reasonably inexpensive, and readily available (Campbell and Lasley, 1975).

2.4.2. Components of semen extenders

A variety of extenders have been utilized to dilute ram semen for use in the short (liquid-storage) or long term (frozen storage). Most semen extenders utilize egg yolk, milk, or a combination of the two as a basic ingredient (Salisbury et al, 1978; Foote, 1980). A variety of other biological materials have been tried as extender

components, but none have received wide application (Salisbury et al, 1978; Foote, 1980).

2.4.2.1. Egg-yolk extender

Egg-yolk has been a common ingredient of semen diluents for many years (Lardy and Phillips, 1939; Jones and Martin, 1973) and its effect in preserving the motility, metabolism and fertility of spermatozoa stored at 5 °C is well known (Mayer and Lasley, 1945; Kampschmidt, Mayer and Herman, 1953). In addition to preserving motility, egg yolk protects acrosomal and mitochondrial membranes from cold shock during cooling (Jones and Martin , 1973; Watson, 1991) and freezing (Watson and Martin, 1975). The active elements of the egg yolk that have been claimed to be protective are phospholipids and low-density lipoproteins (LDF) that are non-dialysable and have a molecular weights greater than 1 x 10^5 Da (Vishwanath et al, 1992). Although phospholipids are believed to stabilize the membrane, their mode of interaction is not understood (Amann and Graham, 1993).

2.4.2.2. Milk extender

Milk, either boiled-whole or skim milk has been employed in recent years as a diluter for sheep semen. Cow milk has been successfully used as an extender for bull, ram, boar and stallion semen (Campbell and Lasley, 1975) and is preferable to the milk of other species. Diluents made from whole milk must be heated to 92-95 °C for 8-10 minutes to inactivate the milk protein, lactenin, an anti streptococcal agent found in milk, which is toxic to spermatozoa (Flipse et al, 1954; Melrose, 1962). Use of whole or skim milk in semen extenders protects sperm against cold shock while satisfactorily maintaining fertility, provided the milk is heated prior to use (Salisbury et al, 1961). However, Jones (1965) reported that reconstituted dried skim milk powder did not require heat treatment. The milk protein, casein, has been established as the agent responsible for prevention of cold shock (O'Shea and Wales, 1966).

2.4.2.3. Coconut extender

Coconut milk has been used to extend fresh semen in the tropics where refrigeration is at premium and coconuts are plentiful (Campbell and Lasley, 1975). Bull and buffalo semen may be held at room temperature for one week in coconut milk extender (Norman et al, 1962; 1968). Diluent using coconut milk for dilution of ram and buck semen has also been reported (Prasad and Norman, 1968; Balakrishnan et al, 1978; Balakrishnan and Iyer, 1982; Cagampang et al, 1989).

2.4.2.4. Sugars

The use of sugars in diluents has been shown to be beneficial for preserving bull, ram and cock semen at body and chilled temperatures (Lapwood and Martin, 1966). The inclusion of sugars in semen diluents performs one or more different functions (Watson, 1979). Glycolysable sugars such as glucose, fructose and mannose (Mann and Lutwak-Mann, 1981) and arabinose, which can be oxidized by spermatozoa (White et al, 1954; O'Dell et al, 1959) may provide an energy substrate for promoting sperm motility during incubation. However, the rate at which these sugars may be metabolized, is species-dependent (Mann and Lutwak-Mann, 1981). Sugars may also make a major contribution to the osmolality of the diluent, exerting an osmotic pressure on the sperm cell membrane depending on their permeability coefficients. Finally, sugars may play a cryoprotective role (Nagase et al, 1968).

2.4.2.5. Hydrogen ion concentrations (pH) and buffering agents

Semen extenders should have enough buffer capacity to control shifts of pH which may affect the viability of spermatozoa in fresh-diluted or frozen-thawed semen. Since the optimum pH for mammalian spermatozoa is close to neutral, most diluents are buffered to pH 6.9-7.1 (England, 1993). Phosphate or citrate-buffered semen diluents were initially adjusted around this range (Mann, 1964). Buffer solutions such as phosphate or 2.9-3.2 percent tri sodium citrate dihydrate adjusted to pH 6.9 by the addition of citric acid, have been commonly used in combination with egg yolk. pH

values may vary due to inclusion of other components in the diluent such as sugar or egg yolk, but any combination of these needs to maintain an osmolarity similar to the seminal plasma (Lapwood and Martin, 1972). However, Melrose (1962) commented that the naturally occurring buffers in egg yolk were sufficient to maintain the pH close to neutrality without prior adjustment. A pH 6.5 has been consistently found to give the highest fertility results with bull semen in Tris buffer (Foote, 1972).

2.4.2.6. Antibiotics

For semen preservation it has been customary to include broad-spectrum anti microbial preparations in semen diluents to prevent transmission of disease and to reduce or eliminate the proliferation of micro organisms which produce spermicidal toxins within the media (Melrose, 1962; Coulter, 1992). Generally a combination of penicillin (500-1000 i.u./ml) and streptomycin (500-1000 ug/ml) has been used to provide a broad spectrum of antibacterial activity (Melrose,1962). These antibiotics are generally not toxic to spermatozoa. Salisbury and his co-workers showed that the addition of sulphanilamide (300 mg/ml) to the diluent increased the fertility by some 5 percentage points (Salisbury and Knodt,1947), although later workers could not confirm such a substantial benefit (Melrose, 1962).

2.5. Preservation of semen

2.5.1. Introduction

In an artificial insemination (AI) program, the cardinal concern in extending the service of superior males to large numbers of females is to preserve fertile spermatozoa successfully. Hence, preservation of spermatozoa is a process whereby not only the viability of the cells but also their fertilizing capacity must be maintained during storage (Garner, 1991). Sperm motility, which has been used as a general measure of sperm viability, is important but is not a guarantee of fertility (Salisbury et al, 1978). The development of extenders using egg yolk provided the basis for most of the media used for preservation of spermatozoa (Salisbury et al, 1978). Several factors

must be taken into account when preserving spermatozoa, these include acceptable pH and buffering capacity, temperature, source of metabolizable substrate, osmotic pressure, electrolyte balance, pH and buffering capacity, control of microbial growth and temperature shock, and an appropriate dilution rate (Salisbury et al, 1978; Coulter, 1992).

A large number of artificial inseminations performed throughout the world, especially for sheep in central and eastern Europe, are carried out with undiluted (fresh) semen within a few minutes of semen collection, i.e. without any preservation media (Chemineau et al, 1991). This method gives good fertility levels, but requires the maintenance of the sires in close proximity to the females to be inseminated, thereby severely limiting dissemination of superior germ plasm. In AI centres, dilution and preservation of liquid semen for at least for several days becomes obviously necessary. The development of liquid semen extenders permitted transportation of semen over moderate distances and allowed for wider distribution of superior genes. Moreover, liquid semen has a higher preparation of viable spermatozoa and high fertility (Coulter, 1992). In the next section, the methods of liquid preservation of semen will be discussed.

2.5.2. Preservation of liquid semen (short-term storage)

In some species, and in many conditions, especially when semen can not be frozen, the preservation of liquid semen for artificial insemination is of distinct advantage. For short term storage, liquid semen is generally kept at two different temperatures, first ambient or room temperature and second refrigerator temperature (Gomes, 1977), but potential exist for preservation of semen at body temperature (Jones, 1969; Lapwood and Martin, 1966; Upreti et al, 1991).

2.5.2.1. Preservation at ambient or room temperature

Preservation of liquid semen in places where fluctuations of ambient temperature occur everyday can cause serious problems in maintaining fertile live

spermatozoa (Garner, 1991). Liquid storage of semen requires prevention of bacterial contamination as at room temperature microbes grow rapidly affecting the viability of spermatozoa. The survival of spermatozoa at higher temperatures could be prolonged by methods which reduced or arrested the metabolism of spermatozoa (Maxwell and Salamon, 1993).

Earlier studies on storage of sperm at room temperature have been based on the original Illini Variable Temperature (IVT) extender for preservation of bull sperm which was prepared by bubbling CO_2 gas for 10 minutes through a glucose-citrate-bicarbonate-egg yolk extender (Salisbury and VanDemark, 1961). Bull semen suspended in this extender maintained fertility for 7 days (VanDemark and Sharma, 1957), but subsequent work failed to substantiate this claim (Watson, 1991). Since then other extenders for ambient temperature preservation of semen as a modification of the IVT extender were developed. These have included pH adjustment for self carbonation rather than CO_2 gassing (Cornell University Extender-CUE). These extenders maintained sperm motility at 25°C and preserved fertility for at least 3 days (Foote et al, 1960; Melrose, 1962). In New Zealand, a new ambient temperature extender was developed by incorporating caproic acid and catalase to IVT-CUE extender and gassing with nitrogen. This the so-called Caprogen diluent successfully maintained maximum fertility of bull sperm for 24 hours (Shanon, 1965). Boar semen stored between 15 and 18°C for several days in modified IVT extender (du Mesnil du Buisson and Dauzier, 1958) and other diluents including BL1 and Guelph achieved normal conception rates and litter sizes (Paquignon, 1984). In the Philippines, modified IVT extender was used to dilute buck semen and motility of spermatozoa was maintained up to 50.9 percent for 14 hours of storage at 28°C (Palad and Medina, 1991). This extender was considered to be the best among the extenders used for goat semen preservation in that country. The preservation of ram semen at ambient temperature using IVT-type diluents has not been critically studied, though CO_2-containing diluents were used in Russia and Eastern Europe. This method of storage of ram semen did not give very successful results and its application was limited (Maxwell and Salamon, 1993).

Another ambient temperature extender containing the liquid endosperm of coconut (coconut milk or coconut water) was first developed by Norman (1961). This the so-called coconut milk extender (CME) contained 50% solution of boiled decanted or filtered fresh coconut milk (CM), sodium citrate and anti microbial agents, and was able to maintain the viability of bovine sperm for about a week at room temperature (25°C) (Norman (1961). In following studies, by reducing both the sperm concentration from $2x10^8$ sperm/ml to $1.5x10^7$ sperm/ml, and the CM from 50% to 15%, the buffering capacity of the medium was greatly enhanced and it was possible to maintain the vigorous progressive motility of spermatozoa for 7 days at room temperature (Norman, 1961). When comparative field studies were done, the conception rate of the first service cows inseminated with CME-extended semen stored at room temperature for six days, or milk-glycerol-diluted semen kept at 5°C for 2 days was 61.6% and 76.5%, respectively. In subsequent studies, Norman (1962) suggested that motility of spermatozoa in bull semen extended in modified CME and stored at room temperature (18-30°C) was 65%, 60%, 52% and 46% at day 1, day 3, day 6 and day 10, respectively. The results of fertility trials showed that the conception rate for 2 day old CME-stored sperm was approximately the same as that for 2 day old milk-glycerol stored sperm. Sperm fertilizing capacity decreased with increased time of storage and fell abruptly after the third day of storage.

The use of CME for the storage of bull semen under different climatic conditions and different room temperatures has been reported. For example in Bangladesh, Islam (1986) recorded that at temperature 20-21°C the mean motility of spermatozoa in CME semen on the first day of storage was 68% and dropped below 50% on the sixth day of storage. At temperatures of 10-15°C and 25-28°C the mean motility of spermatozoa in CME semen on the first day of storage was 56% and 55.8%, respectively. On the 6th day of storage it fell to 20.8% and 0% at the above two storage temperatures. CME-stored semen gave significantly better results in inseminations in comparison with that of egg yolk citrate (control) diluted semen. Rao et al (1975) modified CME by adding about 300 mg of glucose and/or fructose into a medium

containing 7% egg yolk and 18% coconut milk to prepare CME-glucose (CME-G), CME-fructose (CME-F) and CME-glucose-fructose (CME-G-F). When bull semen was diluted and preserved at room temperature (20-28°C) for 168 hours, no significant differences were observed among these series of diluents regarding their effects on viability, livability and structural changes of spermatozoa. Thus, addition of glycolysable substrates to CME did not show any beneficial effect.

Coconut milk extender has also been tested for preservation of semen from other species at ambient temperatures. In India coconut milk extender was effective in maintaining sperm motility and the fertilizing capacity of buffalo sperm stored at room temperatures (20-33°C) for 7 days (Norman et al, 1968). Pillai et al (1978) studied the efficiency of CME at three dilution rates (1:100, 1:150 and 1:200) for the preservation of buck semen at 22.6 to 36.4°C. At a dilution rate of 1:100 the average motility was 67%, 48%, 28% and 12% at 0, 24, 48 and 72 hours of storage, respectively, while at dilution rate of 1:150 the average motility rates were 72%, 45%, 30% and 14% at 0, 24, 48 and 72 hours of storage, respectively. When the semen samples were diluted at the rate of 1:200, the average sperm motility was 77%, 57%, 25% and 25% at 0, 24, 48 and 72 hours of storage, respectively. It was noted that the extended semen maintained good motility up to 24 hours at all the three rates of dilution; and dilution at 1:200 was better than the other two dilution rates in terms of maintaining motility. However, the motility of sperm at the three dilution rates was not significantly different. Pillai and Iyer (1982) observed the motility of buck semen diluted in CME and stored at ambient temperature for 96 hours. A dilution rate of 1:200 was preferable to other dilution rates (1:10 and 1:100) at 48 hours.

Various combinations of milk, tris, sodium citrate, yolk alone or their mixtures and other buffers have been used for preservation of ram semen. Kumar and Prasad (1986) diluted semen from Bikaneri rams with 5 different extenders and found that tris and fresh goat milk preserved ram semen with motility above 40% up to 48 hours at room temperature, whereas in reconstituted skim milk, fresh cow milk and fresh skim milk, motility of ram spermatozoa was maintained above 40% only up to 36 hours. In

New Zealand, a technique for storage of ram spermatozoa for up to 3 days at room temperature has been developed (Tervit et al, 1982) which involved inactivation of the sperm by centrifugation through a 7% Ficoll solution. A high proportion of motile sperm (80%) was recovered after 3 days storage. It was shown by Ca^{2+} flux measurements that immotile spermatozoa still had intact plasma membranes. Furthermore, these immotile spermatozoa were shown to be resistant to cold shock (Jansen et al, 1982). However, fertility results obtained following artificial insemination with the reactivated spermatozoa were disappointing and the reasons for this are still unknown.

Ram semen has been successfully stored in the liquid form without serious loss of its fertilizing ability using milk or egg yolk based diluents. The choice between the two types of diluents depends on the temperature selected for storage. Colas (1984) suggested that fertility of ram semen diluted with heated reconstituted skimmed milk and stored for less than 15 hours at 15°C was significantly higher than in egg yolk based diluents whether the oestrus of the ewes was natural or induced. The lambing percentages of the ewes inseminated with semen diluted in egg yolk based diluent and in reconstituted skim milk diluent were 66.4 and 75.4, respectively, in ewes showing natural oestrus. In oestrus synchronized ewes, the lambing percentages were 38.3 and 54.6, respectively, with the above two extenders. However, the fertilizing ability of ram semen diluted with skimmed milk was much lower at 4°C than at 15°C after 5 hours of storage, which was reflected by the lambing percentage of 38.9 versus 75.0, respectively (Colas, 1984). Upreti et al (1991) diluted ram semen in Ram Semen Diluent-1 (RSD-1) containing MOPS (3-(N-morpholino) propane sulphonic acid) and stored at 15°C for 6 hours. Following 6 hours incubation at 38°C, motility of spermatozoa after 6 hours incubation was 70 percent in RSD-1-diluted semen and 21 percent in skim milk-diluted semen. After 24 hours incubation, motility in RSD-1-diluted semen decreased to 45 percent, but no motility was observed in semen diluted in skim milk. Other compounds such as ethylene diamine tetra acetic acid (EDTA), cysteine hydrochloride and glycine singly and/or in combinations with Russian dilutors

(RD) were also added into extenders to assess their effect on the characteristics of ram semen after storage at room temperature for 72 hours (Saxena et al, 1985).

2.5.2.2. Preservation at refrigerator temperature (Chilled semen)

The life span of unprocessed spermatozoa in whole semen is limited to a few hours only. A high temperature is undesirable since the rate of deterioration is rapid due to increased metabolic processes, consequently preservation of semen at room or ambient temperature is limited to only a few days (Tiwari et al, 1977; Upreti et al, 1992). Preservation of fertility of spermatozoa used for artificial insemination for longer periods by freezing would obviously be advantageous. However, liquid nitrogen is not always available everywhere, and the high cost of equipment precludes its application in many developing countries (Foote, 1967). Most semen used for inseminations prior to the availability of frozen semen was kept at refrigerator temperatures (4°-5°C) to reduce metabolic rates and prolong sperm fertility for several days (Gomes, 1977). This method is usually done by cooling down the diluted semen from 30°C, i.e. temperature of dilution, to 15°C or to 5°C and maintaining it at that temperature until it is used (Evans and Maxwell, 1987).

Numerous mixtures have been used to extend and to store ram semen at cool or refrigerator temperature. Most included either egg yolk or milk (and milk by products) or a combination of these two. In addition, mixtures of milk and other chemicals to regulate osmolarity or pH have also been used, e.g. milk-citrate, egg-yolk milk, etc.

Milk (skim or whole milk) as an extender for the preservation of ram semen has been successfully used for many years. The effect is attributed to its protein fraction, which may act as a buffer against changes in pH (Jones, 1969; Watson, 1979) and as a chelating agent against any heavy metals present (Jones, 1969). It may also partially protect spermatozoa during dilution (Blackshaw, 1953) and reduction of temperature for storage (Choong and Wales, 1962). Little research has been done investigating the mechanism of the protective action of milk against cold shock damage

to spermatozoa. However, milk lipoproteins probably act similarly to egg yolk lipoproteins in protecting spermatozoal membranes (Amann and Graham, 1993).

In studies to compare milk from various of sources, Salamon and Robinson (1962) found that heated cow's milk was superior to ewes milk in maintaining fertility of ram spermatozoa. However, Jones (1969) did not find any difference in the protective action of preparations from these two sources. Tiwari et al (1977) examined at 0, 8, and 24 hours' ram semen which had been extended in heated cow's milk and stored at 5 °C. At the three storage intervals, sperm motility was 88.4, 9.0 and 0 % in undiluted semen versus 88.0, 68.4, and 50 % in diluted semen. This suggested the protective action provided by the milk diluent resulted in better viability of the spermatozoa.

Milk diluents also compared favourably with synthetic diluents such as egg-yolk-glucose-citrate as suggested by Emmens and Robinson (1962). Dauzier (1956) diluted ram semen in heated skimmed cow's milk and kept it at 5°C. Ewes inseminated with diluted semen which had been stored for 12 hours gave a conception rate of 51%. Following addition of antibiotics to the milk diluent, a better conception rate of 65% was obtained. Conception rates varied from 6 to 25% when ewes were inseminated with semen stored for 12h in synthetic diluents (citrate, egg yolk-citrate, or egg yolk-phosphate). Other comparative studies (Martin, 1966) suggested that skim milk proved superior to a number of synthetic diluents in maintaining the motility of spermatozoa stored at 5°C for 4 days. However, Blackshaw (1960) could not find any differences between milk and egg yolk-citrate as extenders for incubation at 37°C or subsequent storage at 5°C. However, milk extenders containing antibiotics could be as effective as citrate-buffered diluents for storage ram semen in the chilled state as suggested by Salamon (1962).

In contrast to the above studies, Salamon and Robinson (1962) have reported that citrate-buffered diluents were better than milk diluents for liquid storage of ram semen. In addition, motility of spermatozoa was better in tris diluents than in milk. This

was suggested by Petruzzi (1976), who made a number of comparisons by extending ram semen with 10 % milk powder diluent, pasteurized skimmed cow's milk, powdered milk with 20 % egg yolk, skim milk with 20 % egg yolk, egg yolk-citrate or tris diluent; diluted samples were stored at 5 °C. For the six diluents, respectively, motility averaged 66.85, 64.44, 66.66, 66.00, 69.11, 70.44 % after 4 hours and 30.00, 41.66, 44.44, 41.11, 37.77 and 54.64 % after 6 hours.

Diluents containing milk or egg yolk have also been assessed for their comparative efficacy over different storage periods. For example, Sahni and Roy (1969) reported that milk and milk-containing diluents were significantly superior to all yolk containing diluents, except Cornell University Extender (CUE) at all periods of storage (6, 50 and 106 hours) at 5°C. El-Gaafary et al (1987) found better lambing results with semen diluted in skimmed milk and stored at 5°C for 3 hours than with semen diluted in egg yolk (68% v 60%). However, when storage time at this temperature was extended to 6 hours, egg yolk proved to be a better diluent than skimmed milk as judged by lambing rate (44.0 % v 41.7 %). The deleterious effect of the milk diluent at 5°C was due to rapid accumulation of lactic acid, resulted in a significant drop in pH that caused a high mortality rate of spermatozoa (Tiwari et al, 1977). Lafluf et al (1990) extended ram semen with a sodium citrate-yolk diluent with or without the addition of 10% citric acid, resulting in semen pH of 6.3 and 7.2, respectively. There were no significant differences between the 2 diluents in sperm motility 0, 6, 12, 18 and 24 hours after dilution. The percentages of ewes that did not return to service after insemination with semen with a pH of 6.3 or 7.2 stored at 4 °C for up to 16 hours were 58 and 31%, respectively. Thus the addition of citric acid to semen diluents improves the preservation of ram semen under field conditions.

Egg yolk, particularly its phospholipid component has been shown to protect cell membranes from cold shock and is regularly incorporated in semen diluents (Blackshaw, 1954; Quinn et al, 1980). The level of egg yolk incorporated in the diluent that appear to give good results vary from 5 to 50 percent (Campbell and Lasley, 1975), however, it is commonly used at concentration of 3-25 percent (w/v) (Watson, 1979).

Different amounts of egg yolk are used for protection of sperm of different species (Watson, 1979).

Although egg yolk has been shown to preserve the structure and motility of spermatozoa during chilling and freezing, it has some adverse effects on fertility (Shannon, 1972; Watson and Martin, 1976). Watson and Martin (1976) showed that with fresh diluted ram semen, fertility declined linearly with increasing quantities of egg yolk in the diluent from 0.375 to 6 percent egg yolk. This has been attributed to the action of egg yolk in enhancing the toxicity of dead spermatozoa, perhaps by providing substrates for lipid peroxidation, since the inclusion of catalase, which destroys hydrogen peroxide, reduced the effect (Shannon and Curson, 1972).

Jones and Martin (1973) reported that inclusion of 3% egg yolk in a glucose-phosphate diluent reduced the frequency of changes to the acrosome and mid-piece of ram spermatozoa cooled to 5 °C and stored at this temperature for up to 72 h. Lambing rates were not decreased by cooling the semen extended with egg yolk-glucose phosphate from 35 °C to 5 °C over 2 h, but storage at 5 °C for 24-48 h caused a severe drop in fertility (Watson and Martin, 1976). Fertility was lower when semen was stored in a medium containing 6% rather than 1.5 % egg yolk (Watson and Martin, 1976).

Liquid storage of semen in extenders containing coconut milk at chilled temperatures has been examined in studies on preservation of bull and ram semen. Norman et al (1962) suggested that diluent containing 5% egg yolk and 15% coconut milk was superior to citrate diluent containing 20% egg yolk and skim milk diluent in maintaining motility of bull spermatozoa stored at 5°C for 10 days. The fertilizing capacity of bull semen extended in coconut milk and stored at this temperature compared favourably with that of reported in other more widely used extenders stored at 5°C. However, in comparing bulls semen extended and stored in CME and Russian dilutor (RD) at 4°C for 7 days, Saxena and Tripathi (1977) found that motility of spermatozoa in RD up to 3 days storage was 68.1%, while motility of spermatozoa in CME had fallen to 62.5% after 2 days storage.

Prasad and Norman (1968) tested 4 extenders included Egg Yolk Citrate (EYC), Egg Yolk Tris (EYT), Cornell University Extender (CU-16), and Coconut Milk Extender (CME) to conserve the viability and motility of ram sperm stored at various temperatures (5°-34°C). It was found that the reduction of sperm motility and survival at low temperature (5°C) for 4 weeks in the presence of sulfanilamide could be compensated by the addition of calcium nitrate (0.05%) and glycine (0.9%). The synergistic effect produced by the addition of these compounds to CME made it possible to keep 70% of the initial ram sperm population alive and motile for a week at room temperatures after a continuous storage period of one month at 5°C. The ability of the test extenders to maintain ram sperm at variable temperatures was rated as follows: CME>CU-16>EYT>EYC. Other compounds that may prevent or protect against the effects of accumulation of hydrogen peroxide have also been included in diluents for ram semen. Stojanov et al (1994) reported that the addition of anti oxidants or superoxide dismutase and catalase to tris-glucose-yolk based diluent improved the fertilizing capacity of liquid-stored ram spermatozoa kept over 14 days at 5°C.

Sugars have been included in semen diluents as exogenous energy substrates, as osmotic components and as cryprotective agents (Watson, 1979). Spermatozoa rely primarily (about 90%) on extracellular substrates to meet their energy requirements. This energy is principally derived from carbohydrates. Fructose is the most abundant carbohydrate in the seminal plasma of rams and bulls. In these species the fructose is produced in the seminal vesicles (Mann, 1964). Spermatozoa are capable of glycolysing glucose, fructose and mannose (Mann, 1964) and of oxidizing arabinose (White et al, 1954; O'Dell et al, 1959); these sugars are therefore potential energy sources for promoting sperm motility during incubation.

Several studies have shown an increase in motility or fertility or both when glucose was added to, or partially replaced, diluents containing egg yolk for the storage of semen at temperatures from 46.5 to 5°C (Salisbury and Van Demark, 1946; Ohms and Willet, 1958; Foote and Bratton, 1960). Ohms and Willet (1958) indicated that replacement of yolk-citrate with glucose in semen diluents has led to increased survival

of spermatozoa. Martin (1966) has shown that replacement of part or all of the sodium chloride content (123 mM) by lactose (ranging from 61 to 246mM) in a phosphate-buffered saline (20mM) diluent improved the survival of spermatozoa stored at 37 and 5°C. In further experiments, all diluents obtained from mixtures of diluent containing sodium chloride (31mM) and fructose, glucose, lactose, or sucrose (184mM) were better for the preservation of ram spermatozoa at 5°C than the diluents containing 123mM sodium chloride.

Jones and Martin (1965) have conducted experiments by comparing milk, yolk-citrate, and synthetic diluents containing lactose or fructose for frozen storage of ram spermatozoa. Although lactose was as satisfactory as milk, however, spermatozoa frozen in the lactose diluent had to be resuspended in the fructose synthetic diluent for incubation at 37°C after thawing if survival rate were to remain as high as in those samples frozen and incubated in milk. On the other hand, Lapwood and Martin (1966) in a systematic study of sugars for inclusion in a phosphate-buffered synthetic diluent for ram semen suggested that glucose, mannose, fructose and sucrose were most beneficial at 37 °C, but at 5 °C ribose, xylose, arabinose and galactose were best. This perhaps reflects the differential effect of temperature on membrane permeability of the various sugars.

Another sugar that has been identified as a major chemical constituent in the semen of the boar is inositol (Mann, 1969). This sugar, which is also found in smaller quantities in other species, has been used in diluents to substitute for other sugars. For example, Martin and Richardson (1976) found that ram spermatozoa survived better during incubation at 39°C or chilled to 5°C after dilution if glucose was replaced by an equimolar amount of inositol in the diluent.

In reviewing the use of sugars as diluent components for liquid storage of ram semen, Maxwell and Salamon (1993) concluded that inclusion of some sugars such as glucose and fructose in the diluents for preservation of spermatozoa functioned as energy substrates, while other sugars such as sucrose and lactose may only act to

maintain or increase the osmotic pressure of the diluent. The use of other sugars such as trehalose, which do not provide energy source, but which act extracellularly to maintain osmotic pressure and membrane integrity (Rudolph and Crowe, 1985) may be beneficial in prolonging survival of spermatozoa. However, the addition of trehalose in the whole milk based extender to preserve frozen bull semen did not show significant improvement of fertility after field trials (Foote et al, 1993).

2.6. Environmental factors involved in the reproductive activity

2.6.1. Introduction

In mid and high latitudes and for breeds originating from these areas, photoperiodism is the main factor which controls seasonal breeding of small ruminants (sheep and goats). In both sexes, gonadal activity and sexual behaviour vary according to daylength changes (Lincoln, 1976; Colas, 1983). Other environmental factors, such as temperature, nutrition or social or combinations of any of these factors (Lindsay, 1991) act as modulators of reproductive activity. In contrast, under tropical latitudes, breeds of sheep are not susceptible to these light changes and are able to breed throughout the year (Carles, 1983). Among the various environmental factors, temperature appears to play a more important role influencing reproduction in small ruminants (Colas, 1983). Therefore, the effect of thermal environment on the components of reproductive function, particularly the quality of ram semen and its fertilizing ability will be discussed.

2.6.2. Effect of thermal environment on the quality of ram semen

It has been well known for many years that high ambient temperatures adversely affect the quality of ram semen. Their influence occurs mainly as a result of an increase in testicular temperature or may also occur as a result of pyrexia, failure of one or both testes to descend normally from the abdominal cavity, a condition known as cryptorchidism, or because of lesion or disease that results in inflammation of the testis, scrotum, or epididymis (Freidman et al, 1991; Setchell, 1993). The detrimental effect of

high temperatures on sperm quality can also be experimentally induced by local heating of the scrotum or scrotal insulation (Glover, 1955; Braden and Mattner, 1970; Williamson, 1974; Mieusset et al, 1991; Mieusset et al, 1992) or by keeping animals in hot chambers (Moule and Waites, 1963; Dutt and Hamm, 1957).

An effect of exposure to high environmental temperatures on semen characteristics has been demonstrated by Dutt and Hamm (1957) who transferred sheared or unsheared rams from a control environment to a room where the temperature was 90°F (32°C), with a relative humidity 60 to 65%. A rapid decreased in motility of spermatozoa was found in the unsheared rams from between 80% and 85% in the control period to less than 10% five weeks after the exposure. Sperm concentration also decreased in the unsheared group from between 3.2 and 4.5 x 10^8 cells/ml in the control period to 1.2 x 10^8 cells/ml five weeks after treatment. Other studies (Dutt and Simpson, 1957) also showed lower motility of spermatozoa in the semen of control rams kept in a room without temperature and humidity control (41.8%) than in semen of rams kept in a air-conditioned room (70.3%). Similarly, concentration of spermatozoa in the semen of cooled rams was significantly higher than that of control (3.4 v 2.4 x 10^6 cells/ml) (Dutt and Simpson , 1957).

Although examination of semen characteristics after heat application to the testes revealed an increase in the morphologically abnormal spermatozoa (Moule and Waites, 1963; Dutt and Hamm, 1957; Rathore, 1968; Rathore, 1970; Smith, 1971), the types and relative proportions of abnormal spermatozoa vary considerably between rams (Smith, 1971; Williamson, 1974), ejaculates (Williamson, 1974) and breeds (Lindsay, 1969). The morphological abnormalities may occur to all parts of the spermatic cells, particularly the apical part with increase pyriform heads and acrosome changes (Williamson, 1974; Colas, 1983) and coiled tails or tailless spermatozoa (Colas, 1983). Studies in which the scrota of rams were immersed in a circulating waterbath at approximately 41°C for 2 hr showed a marked increase in the percentage of spermatozoa with abnormal head shapes, tailless heads and tail abnormalities 14 to 15 days after treatment. Other rams treated under the same conditions also showed

similar changes in the morphological abnormalities of spermatozoa, but 16 and 18 days following scrotal heating (Williamson, 1974).

Studies on increasing testicular temperatures either by heating the whole body or by direct heating of the testis by means of scrotal insulation indicated variation in time in the appearance of the affected spermatozoa in the ejaculated semen. Glover (1955) suggested that scrotal insulation which increased testicular temperature by 5°C for 24 hours resulted in the sudden appearance 17-24 days later of tailless spermatozoa in the semen. Glover (1956) also found a high (up to 69%) incidence of decapitated spermatozoa which first appeared in the ejaculate some 20 days after a 24-hour period of scrotal insulation.

Smith (1971) exposed rams in a climatic room under a 10 hour-light and 14 hour-dark regimen and ambient temperature of approximately 41°C for 4, 6, 9 and 13.5 hr. The incidence of abnormal spermatozoa peaked during the third week after all these treatments. When rams scrota were heated within a double-walled polythene chamber by passing hot water (41-44°C) between the walls of the chamber for 1.5 to 2 hr, a sharp increase in the proportion of morphologically abnormal spermatozoa was found 14 to 16 days later (Braden and Mattner, 1970). Mieusset et al (1992) studied characteristics of frozen semen from rams subjected to scrotal insulation which raised subcutaneous scrotal temperature by about 2°C for 16 h/day, for 21 successive days. There was a lower percentage of rapid spermatozoa and a higher number of dead spermatozoa on day 4 and 15 of heating, respectively, in the semen of heated rams compared with control rams. On day 21 of heating, semen from the treated group showed an increase in the number of dead spermatozoa, while the percentages of motile and of rapid spermatozoa decreased. However, the total number of spermatozoa did not differ between the two groups of rams.

The gradual return to normal semen quality after whole body or local heat application on the testis takes 50 to 60 days (Colas, 1983; Casu et al.,1991). The degree of damage depends on the duration and the intensity of heat stress and also on the breed

(Dutt and Hamm, 1957; Lindsay, 1969). The more severe the heat stress, the greater the period of semen damage and of decline in testis weight (Setchell and Waites, 1972).

Hamm (1954) as cited by Dutt and Hamm (1957) had previously shown that high quality semen was not produced in rams transferred in mid-summer to an experimental room maintained at 65°F (18°C) and RH 70 to 80% until after a lapse of six weeks. Semen quality of unsheared heated rams did not return to the level of untreated rams until an interval of eight weeks had elapsed (Dutt and Hamm , 1957). A considerable variation in individual response to the heat stress was noted by Glover (1955), who suggested that ram semen which was most severely damaged following treatment was that which began to degenerate first and regenerate last. Conversely, semen from rams which withstood the treatment best degenerate last and regenerate first.

In rams spermatozoa take about 11-14 days to pass through the epididymis (Ortavant, 1959; Chemineau etal, 1991). Observations on abnormalities of spermatozoa found at day 14 and day 21 after heat treatment by Smith (1971) suggested that cells in the lumen of the seminiferous tubules were the most susceptible to damage by high temperatures. Since all of the semen characteristics studied had returned to normal by the end of collection period, it appeared that the earlier stages of cell division in the spermatogenic cycle had not been adversely affected (Smith, 1971). Similarly in bull, there was a lag time of about 2 wk from the initial exposure to heat until the percentage of motile sperm decreased, suggesting that epididymal sperm may not be affected by heat stress, or that it takes several weeks for any changes to occur in the epididymis that result in a reduction in motile sperm (Meyerhoeffer et al, 1985). This is also supported by Braden and Mattner (1970) who reported that spermatozoa present in the epididymis at the time of heating appeared to be unaffected, but there was considerable damage to spermatozoa developing in the testes.

However these findings were in contrast to other reports in which concentration of the spermatozoa did not reach to pre treatment levels within 60 days

(Moule and Waites, 1963; Waites and Setchell, 1964). It was concluded that the earliest stages of the spermatogenic cycle may have been adversely affected by the experimental treatments. The additive effect of the two consecutive 6 hr treatments and the increased humidity to which the experimental animals were subjected as measured by Moule and Waites (1963) may have been causing the difference in the extent of seminal degeneration.

2.6.3. Effect of thermal environment on the fertilizing ability of ram spermatozoa

The success of spermatozoa in fertilizing eggs is dependent upon the morphological and physiological conditions of the spermatozoa, which is satisfactory if the reproductive organs of males are functioning normally. It is generally accepted that high temperature has a direct harmful effect on the function of the testis (Setchell, 1978; Thatcher and Hansen, 1993) which in turn influences the process of spermatogenesis, depressing semen quality and ultimately fertility.

In a study of ewes bred naturally by Southdown rams early in the breeding season, Dutt (1954) found a low conception rate which was mainly caused by failure of fertilization and early embryonic death; and was partly due to summer temperature (Dutt and Simpson, 1957). Rathore (1968) found a greater number of delayed returns to service in ewes mated to heat-treated rams than in ewes mated to control rams, and suggested that the heat treatment resulted in a higher incidence of embryonic deaths. However, Howarth (1969) did not find significant loss of embryos in ewes mated to heated rams between 7 and 14 days after the beginning of treatment. Braden and Mattner (1970) also suggested that embryonic losses are of minor importance compared with losses due to non-fertilization in ewes mated to heat affected rams.

Recent studies of moderate elevation of intra scrotal temperature of about 2°C by scrotal insulation of conscious rams 16 h/day for as long as 21 consecutive days (Mieusset et al, 1991; Mieusset et al, 1992) did not show an effect on the fertilization capacity of spermatozoa, although there was an increase in the embryonic loss in females inseminated with frozen semen from heated rams as early as day 4 after

treatment. This suggests an effect of heat on the epididymal spermatozoa even though semen characteristics were not altered at all. Thus these studies contradict those of Braden and Mattner (1957) who observed normal viability of eggs fertilized by spermatozoa present in the epididymis at the time of heating.

2.6.4. Heat-sensitive mechanisms contributing to decreased semen quality

The mechanisms by which heat stress depresses fertility have received intense investigation in the male and female using various species for models. Responses of the animal to thermal stress that compromise fertility are multifold, interrelated, and involve direct effects of temperature, altered nervous system regulation, water balance, hormonal alternations, nutritional influences, and biochemical alterations (Thatcher and Collier, 1982). Numerous reports concerning the influence of thermal stress on the reproductive efficiency of males agree that high temperatures can interfere with the process of spermatogenesis in all species of livestock (McDowell, 1972).

The scrota of mammals all have mechanisms which help to protect the testes from overheating (McDowell, 1972). In rams, the testes produce sperm most efficiently when their temperature is 4 to 7°C lower than the body temperature (Moule and Knapp, 1950). Infertility due to disturbances of spermatogenesis can result from the failure of the ram to maintain this optimum temperature (Smith, 1982). Two basic mechanisms are involved to maintain testicular temperature lower than body temperature. Contraction of the tunica dartos, directly sensitive to temperature change, tightens and bunches the scrotum, thereby reducing the exposed surface and also elevating the testes toward the warmer trunk (Meijer and van Vlissinger, 1993). In hot conditions, the tunica dartos relaxes, lowering the testes within the then thin-walled pendulous scrotum (Meijer and van Vlissinger, 1993).

In addition to serving as a protective covering for the testis and its adnexa , the scrotum has an important role in thermoregulation of the testis. It is known that the scrotum is largely responsible for controlling the amount of heat reaching the testes, and scrotal surface area could be an important feature influencing the exchange of heat

between the scrotum and the environment (Fowler, 1968). The spermatic cord of the testes, in which the artery, veins and lymphatic vessels are associated intimately, act as an efficient counter-current heat exchanger (Setchell, 1993). In this region the arterial blood is cooled to scrotal temperature while the venous blood is warmed to body temperature, thereby ensuring that the whole testis is kept at the temperature of the scrotal skin, i.e. several degrees cooler than body cavity, and that the animal is not continually losing body heat through its scrotum (Setchell, 1993).

Heat also may be dissipated through the scrotal wall and body tissues adjacent to the testes (Thatcher and Collier, 1982). Heat loss by radiation is facilitated by the exposed position of the scrotum, the absence of subcutaneous fat within the scrotal fascia and the intra capsular position of large testicular vessels. Additional heat loss by evaporation is allowed by the numerous supply of large sweat glands (Meijer and van Vlissingen, 1993).

As expected, if blood flow decreases then testicular function is compromised. During heat stress, blood flow is redistributed in the body, so that a larger part flows in parts active in heat dissipation, enhancing heat loss from the body. Such redistribution might impair blood flow of the reproductive tract, hence impair its function (Berman, 1991). In the ram though, localized heating of the scrotum at 37 or 40°C (Fowler and Setchell, 1971) caused an increased testicular metabolic rate, which without a compensatory increase in testicular blood flow leads to hypoxia and testicular damage. Dutt et al (1977) suggested that in rams following one week of entire body exposure at 32°C, the wall of the spermatic artery within the pampiniform plexus had thickened and arterial luminal diameter had decreased. Since $PGF_2\alpha$ content of the testis had increased (Dutt et al, 1977), it may be that high levels of $PGF_2\alpha$ may constrict the spermatic artery within the region of the pampiniform plexus region and reduce blood flow to the testis.

High temperatures may decrease levels of gonadotrophin hormone (FSH and LH) produced by the anterior lobe and insufficient amounts of these hormone secretions

could lead to inadequate sex steroid production and consequently to reproductive failure Dutt et al (1977). In fertile men, depression of spermatogenesis was observed after induced increase testicular temperature by 1 to 2°C (Mieusset et al, 1987) and the increase was related to a marked alterations in both exocrine and endocrine functions of the testis (Mieusset et al, 1989). Decreased LH may cause atrophy of the interstitial cells in the testis in males (Habeeb et al, 1992). Gomes et al (1973) concluded that elevated ambient temperature is detrimental to Leydig cell function in the ram. This was based on an evaluation of various endocrine responses in which testosterone decreases were detected in testicular tissue concentrations, spermatic venous concentrations and in vitro biosynthesis of testosterone. There is evidence that heating of the testis alters the functions of all the testicular cells, of which in ram, the most sensitive cell types are pachytene spermatocytes and B-spermatogonia (Waites and Ortavant, 1968). Therefore, it is important to examine metabolic changes in Leydig and Sertoli cells as well as in germ cells. Leydig cells secrete testosterone which regulates spermatogenesis; testosterone is transported to the germ cells and epididymis by androgen-binding protein secreted by Sertoli cells. Reduction of the secretory functions of these cells due to heating of the testes would damage spermatogenesis (Blackshaw, 1970) or sperm maturation.

Therefore, the testicular responses to heat are multifold in which spermatogenesis, semen quality, steroid production, and testicular blood flow are changed in response to heat. As a result a temporary infertility follows after exposure of the testis or the whole animal to heat (Thatcher and Collier, 1982).

2.7. Epididymis and its constituents in the storage of spermatozoa

2.7.1. Introduction

The epididymis is the organ where the spermatozoa pass after they journey through the efferent ducts of the testis (Chemineau et al, 1991). The spermatozoa that leave the testis are neither motile nor capable of fertilization. After passing through epididymis, they become mature spermatozoa, motile and capable of fertilizing ova

(Smith, 1982; Setchell, 1984; Eddy, 1988). The time required for the transport of spermatozoa along the entire length of the epididymal duct varies among species and depends to some extent on frequency of ejaculation (Mann and Lutwak-Mann, 1981). Duration of the epididymal transport is 7 to 9 days in bull (White, 1980), 9 days in rabbit (Mann and Lutwak-Mann, 1981) and 11-14 days in rams (Ortavant, 1959; Chemineau et al, 1991). The transit time may be decreased by 10 to 20 percent in animals which are ejaculating frequently (Garner and Hafez, 1987).

During transit in the epididymis, spermatozoa undergo changes which are recognized as a maturation or ripening. These include structural alterations in both the sperm head and tail, changes in surface properties and constituents, and the acquisition of sustained forward motility (Bellve and O'Brien, 1983). Although the exact site of sperm maturation varies among species, the ability of spermatozoa to fertilize ova is achieved when they have entered the distal region of the epididymis (Waites, 1980). In the ram, the end of the middle part of the corpus epididymidis and the proximal cauda is the place where spermatozoa become motile and fertile (Amann, 1987). The survival of spermatozoa passing through the epididymis depends on androgen production by the testes, which stimulates the epididymal cells (Chemineau et al, 1991).

Once having entered the cauda epididymidis, the sperm cells are stored there until ejaculation. The fluid in the lumen of the epididymis constitutes the milieu in which the spermatozoa survive and remain functional for an extended period. It has been indicated that the storage of spermatozoa in this region occurs in all mammals studied and, in most species, sperm remain viable there for at least two to three weeks (Bedford, 1975; Cooper, 1986). Spermatozoa which are not ejaculated are gradually lost either by excretion in the urine or by phagocytosis and resorption in the epididymis or vas deferens. In the ram, the spermatozoa that are not ejaculated at copulation or voided by masturbation are eliminated periodically during urination (Lino and Braden, 1972).

2.7.2. Epididymal structure

The epididymis, a derivative of the mesonephric duct, is comprised of a single highly coiled tubule that in most mammals, rests on the tunica albuginea of the testes. In artiodactyls, the epididymis is firmly attached to the posterior or superior border of the testis. Based on its shape and position in the testis, the epididymis is divided anatomically into three major regions: caput, corpus and cauda (Hamilton, 1990). The head (caput) region is apposed to the proximal pole of the testis, where the epididymal duct arises from the efferent ducts, the slender body (corpus) terminates in the expanded tail (cauda) region at the distal pole of the testis. The contour of the cauda epididymidis is a visible and palpable feature in the living animal (Meijer and van Vlissingen, 1993).

Based on gross histology and function of the epithelium, Glover and Nicander (1971) defined three segments of the epididymis, namely initial, middle or intermediate and terminal. Although these segments can be distinguished histologically; they do not coincide with the gross anatomic regions (Amann, 1987). Fluid concentration and first stage of sperm maturation occur in the initial segment, then maturation is completed in the middle segment and finally the sperm are stored in the third segment (Setchell, 1991).

The wall of the duct of the epididymis is lined by pseudostratified epithelium columnar cells and has a prominent layer of circular muscle fibers particularly the cauda. The initial segment is characterized by a high epithelium with long straight stereocilia that almost obliterate the lumen which contains very few spermatozoa. In the middle segment, the stereocilia are not so straight and the lumen of the duct is wide and contains many spermatozoa. In the terminal segment, the stereocilia are short; the lumen is very wide and packed with spermatozoa (Glover and Nicander, 1971; Ashdown, 1987). The height of the epithelium decreases progressively from 140 μm in the initial segment to 60 μm in the terminal segment (White, 1976). Conversely, the muscle layer around the duct increases in thickness towards the cauda (Setchell, 1977; Amann,

1987). Peristaltic contractions of this muscle occur during ejaculation (Meijer and van Vlissingen, 1993).

Although the intact epididymis is only a few centimetres long, the total length of the tubule is 2 m in the rat and 6-7 m in the human (Hogarth, 1978; Foster, 1988), 36 m in the bull, 54 m in the boar (Ashdown and Hafez, 1993), about 80 m in the ram (Setchell, 1991) and 86 m in the stallion (Hogarth, 1978).

2.7.3. Epididymal function

In mammals, the epididymis serves a series of principal functions in relation to spermatozoa: maturation of the spermatozoa probably through modification of sperm surface molecules (Amann, 1987; Uhlenbruck, 1993), maintenance of the mature spermatozoa in good condition until ejaculation (Amann, 1987), transport of spermatozoa through the epididymal duct (Mann and Lutwak-Mann, 1981; Amann, 1987), induction and alteration of spermatozoal motility so that ejaculated spermatozoa can penetrate cervical mucus, reach and fertilize the female gamete (Uhlenbruck, 1993), fostering the dissolution of aged or superfluous spermatozoa and resorption of fluid and cellular debris (Burges, 1974). Lastly, the epididymis has the ability to store spermatozoa (Uhlenbruck, 1993).

Maturation of spermatozoa during epididymal transit involves changes in their morphology, composition, and function (Orgebin-Crist et al, 1975; Bedford, 1975; Hamilton, 1975). The morphological changes include distal migration and eventual loss of the cytoplasmic droplet and minor alterations in size and shape of internal structure of the acrosome and midpiece (Branton and Salisbury, 1947; Bedford, 1963). The changes in composition involve the appearance of new surface components and alterations in preexisting surface components; the functional changes include alterations in metabolism, development of the ability to bind the zona pellucida, and modifications in the pattern and effectiveness of flagellar activity (Eddy, 1988).

2.7.4. Composition of luminal fluid

The unique composition of epididymal fluid in which spermatozoa are suspended is a result of absorptive, ion transporting and secretory activities of the cells lining the epididymal duct. The epididymis absorbs almost all of the fluid coming from the testes. In the ram up to 60 ml fluid leave the testis everyday, and most of this is absorbed in the ductuli efferentes of the duct of the epididymis (Crabo, 1965). Ligation of the duct at the junction between testis and epididymis causes fluid accumulation in the testis, which does not occur if the ligature is placed at the junction between caput and corpus (White, 1973).

The fluid in the lumen of the epididymis constitutes the environment not only for the maturation but also for the storage of spermatozoa and it is not unreasonable to suppose that the composition of the fluid may play some part in both processes (Bedford, 1975; Orgebin-Crist et al, 1976). It has been shown, in this connection, that studies in which rabbit spermatozoa were retained in the caput or corpus epididymidis by means of ligature, suggest that the morphological and motility changes may take place without normal migration into the cauda (Glover, 1960; Glover, 1962; Gaddum and Glover, 1965; Bedford, 1967; Orgebin-Crist, 1969). A sojourn in the cauda, however, appears essential for the attainment of full fertility in most animals (Orgebin-Crist, 1967; Paufler and Foote, 1968). Overstreet (1970) suggests that the phenomenon of delayed fertilization resulting from the use of epididymal spermatozoa may be due to the need for an extended period for their capacitation in the female tract.

Fluid reabsorption in the epididymis is associated with a profound modification in ion concentration, such as sodium, potassium and chloride. Rete testis fluid (RTF) has appreciably more potassium and less sodium than blood plasma (Voglmayr et al, 1966). Relative to sodium, the concentration of potassium increases in the epididymis partly as a result of the withdrawal of Na^+, but also partly because of the active secretion of K^+ (Hogarth, 1978). Other features of the ionic composition of RTF are also somewhat different from that of the blood plasma and testicular lymph (Setchell

and Brooks, 1988). The total protein content of the rete testis fluid is less than that of the blood plasma and its nature is different (Waites, 1976).

Fluid resorption and secretion can also cause changes in the concentrations of small organic substances (glycerylphosphorylcholine (GPC), carnitine and inositol) in the epididymal luminal fluid as it passes along the epididymis (Setchell, 1993). In the first part of the epididymis, nearest to the testis, there is substantial reabsorption of fluid leading to an appreciable increase in the concentration of spermatozoa in the proximal caput of epididymis (Setchell and Hinton, 1981). The concentration of inositol also rises at the same time, and appreciable concentrations of glycerophosphocholine (GPC), phosphocholine and inorganic phosphate appear, followed in the distal caput by carnitine. The concentrations of all these substances continue to increase along the epididymis to reach between 50 and 60 mM inositol, carnitine and GPC in the ductus deferens of the rat (Hinton and Setchell, 1980; Hinton et al, 1979; Hinton et al, 1979).

The most striking difference between the RTF and blood plasma is in the glucose and inositol content. RTF normally contain practically no glucose but about 100 times the concentration of inositol that occur in blood plasma (White, 1980). In rodents, there is virtually no glucose in RTF or epididymal fluid, but the inositol concentration, which is already higher in the RTF than blood, rises in the first part of the epididymis; in rodents, more inositol is secreted by the cells lining the epididymal duct, so that in the cauda, concentration of up to 80 mM have been recorded. The situation is quite different in ram, bull and boar, in which the highest inositol levels are reached in the caput, and the concentrations in the ductus deferens are about the same as in RTF (Hinton et al, 1980; Amann, 1987; Setchell and Brooks, 1988).

The possibility that inositol might be utilized by ram and bull testicular sperm as a substrate has been investigated. Athough testicular sperm oxidize inositol only at slow rate when it is added to media in vitro, in vivo inositol may be absorbed into the epididymis and incorporated to phospholipid for subsequent release into the lumen as an energy source for the spermatozoa (Voglmayr, 1975). Inositol is synthesised in the

epididymis from glucose (Robinson and Fritz, 1979) but it is also accumulated from the blood into the cauda epididymidis of the rat (Cooper, 1982; Hinton and Howards, 1982).

The epididymis of domestic species (e.g. bull and boar) is rich in carnitine (Hamilton, 1975). In the rat, and presumably also in other species, carnitine from the blood is concentrated in the epididymis, and high concentrations of carnitine (63 ± 3.3 mM) occur in epididymal plasma collected from the cauda epididymidis. The carnitine level of the fluid is under androgen control and its high concentration must also contribute to the osmotic pressure of the fluid and at least partly redress the loss of sodium chloride in the proximal region of the duct. Epididymal spermatozoa also contain high levels of carnitine along with the enzyme carnitine acetyltransferase, which probably account for the fact that about 5% to 10% of the carnitine in the sperm and epididymal plasma is in the acetyl form (White, 1980).

Carnitine acts as a cofactor in fatty acid oxidation and acetylcarnitine may serve as an energy reservoir and buffer against rapid changes in the concentration of acetyl-CoA in spermatozoa. It has been suggested that the maturation of spermatozoa in the epididymis may be related to accumulation of carnitine in the sperm during epididymal transit (White, 1980). It has also been suggested that carnitine may play an essential role in epididymal spermatozoa metabolism. Since ejaculated spermatozoa are derived only from the ductus deferens and epididymis, the direct correlation between the concentration of carnitine and acetylcarnitine in seminal plasma and the concentration of spermatozoa in ram ejaculates suggests that the ductus deferens and epididymis are also the sole source of seminal carnitine (Brooks , 1979).

The epididymal fluid also contains several enzymes, particularly glycosidases. The alpha-manosidase and beta-N-acetylglucosamidases occur in extraordinarily high concentrations in the epididymal plasma (Mann, 1964). This might also be true of glutamic acid, which occurs in an even higher concentration in ram epididymal plasma

than in testicular fluid, presumably due to selective resorption of fluid in the head of the epididymis (White, 1973).

2.7.5. Effect of epididymal constituents on sperm motility

Most mammalian spermatozoa are relatively quiescent in the epididymis, and rapid flagellar motion begins only after they are released from the epididymis. Spermatozoa released from the efferent ducts or head of the epididymis and suspended in a physiological salt solution swim weakly with a vibratory movement that results in no forward progress. Some spermatozoa from the body of the epididymis are capable of moving more vigorously in a circular path, while the majority of spermatozoa isolated from the tail of the epididymis have a symmetrical flagellar beat, resulting in progressive forward movement (Eddy, 1988).

Several studies tried to identify the epididymal constituents correlated to the initiation and maintenance of sperm motility. Although there appears to be no doubt that rodent sperm are immotile in caudal epididymal plasma (Pholpramool et al, 1985; Turner and Reich, 1987), epididymal sperm from bulls (Pholpramool et al, 1985), rams (White et al, 1959; Jones, 1978) and rabbits (Usselman and Cone, 1983; Turner and Reich, 1985) show movement in droplets of epididymal semen under oil, and become more motile during incubation. However, other studies (Acott and Carr, 1984; Carr and Acott, 1984; Carr et al, 1985) have suggested that in glass capillaries or under coverslips, bull sperm are only weakly motile or are non-progressive before dilution; possibly that the sperm may have adhered to glass or their movement may have been impeded. Clulow et al (1992) observed that caudal sperm from the Australian marsupial *Macropus eugenii* became active during collection or subsequent incubation of undiluted epididymal semen.

Studies on the effects of carnitine on epididymal spermatozoa are complex. Spermatozoa from the rat caput epididymidis, which showed low motility after collection, showed an enhanced motility for at least 20 minutes in the presence of 6 mM L-carnitine, and a transitory stimulation with higher concentrations, in the range found

in the distal caput onwards; sperm with appreciable motility after collection showed a decrease in motility in the presence of L-carnitine at concentrations of 9 mM or higher (Hinton et al, 1981). The motility of caudal rat spermatozoa was inhibited reversibly by 60 mM DL-carnitine, although initiation of motility was not affected (Turner and Giles, 1981). Carnitine can stimulate motility in ejaculated human and bovine spermatozoa (Tanphaichitr, 1977; Deana et al, 1989).

Reports on the effect of GPC on sperm motility are contradictory. One group (Turner et al, 1978; Turner and Giles, 1981) found that GPC in physiological concentrations produced an irreversible inhibition of motility, although part of this effect was due to cadmium contamination of the GPC (see Turner et al, 1978; Turner and Giles, 1981). Another study reported that GPC in concentrations up to 10 mg/ml had no effect on sperm motility (Morton and Chang, 1973). Addition to saline of 5% egg yolk, a rich source of phospholipids other than GPC (Parkinson, 1966), had no stimulatory effect on epididymal sperm motility (Turner and Giles, 1981).

Although inositol, an important constituent of epididymal plasma, and other polyols have been incorporated in a number of media for the freezing of boar semen (Salamon et al, 1973) and for thawing frozen semen (Salamon and Brandon, 1971), there have been no systematic studies on the effects of these substances on epididymal sperm.

The fact that the epididymis can store viable spermatozoa for prolonged periods *in vivo* suggests that a systematic study of the effects of epididymal constituents and related compounds on spermatozoa may help in the formulation of better diluents for preserving spermatozoa during freezing, or storage at cool, room temperature or even body temperature (Setchell et al, 1993).

2.8. The purpose of the study

The benefits of semen preservation are well recognized in the animal industries. However, many of the advances in long-term semen storage involve the use

of special diluents and require refrigeration systems. These are not always available in developing countries. There is therefore a need to develop semen preservation and dilution strategies that can be carried out where refrigeration and commercial diluents are not readily available. In addition, high ambient temperature in the tropics may affect the viability of spermatozoa during preservation. Thus, to sustain the life and fertility of spermatozoa, it is necessary to prolong the lifespan of the spermatozoa by providing in their medium nutrients which are available in those regions, or to slow down their very active metabolism

The aims of the study were to determine the suitability of locally available material such as quail egg yolk for ram semen dilution, and to investigate whether quail egg yolk in combination with compounds which originated from plants, such as coconut extract, can support and maintain viability of ram semen stored at different temperatures.

Temperature is one of the important environmental factors modifying reproduction. Elevated body temperatures during periods of high ambient temperature of the summer or tropical hot season, lead to testicular degeneration and reduce the percentage of normal and fertile spermatozoa in the ejaculate. Therefore, the use of a diluent to preserve semen of sub-standard quality could be useful, as the protective action of a compound may be more important in semen from sub-fertile males.

The second aim of the study was to evaluate ram semen quality following a mild thermal testicular insult accomplished by scrotal insulation and to study the semen production and quality in such heat-stressed rams. The effect of heating the scrotum with a slight increase of temperature but sustained for a long period of time on the semen production and quality was also studied.

The functional significance of the cauda epididymidis as the major site of sperm storage within the male reproductive tract is unquestionable in that the sperm retain their viability there for an appreciable period of time. This appears to be due to

some essential components found in the luminal fluid of the epididymis which may create a favourable environment conditions to enhance survival of the spermatozoa.

The aim of the third study was to elucidate the effect of inositol, one of the small organic compounds in the luminal fluid, on the viability of ram spermatozoa when included in the diluent and stored at different temperatures during varying periods of storage. Since inositol is a sugar alcohol, other sugars which are also found in the luminal fluid or in the seminal plasma such as fructose, glucose, or other disaccharide such as sucrose and lactose were also studied simultaneously in conjunction with inositol.

Chapter 3

Materials and Methods

3.1. Location

All experiments were conducted at the Department of Animal Sciences, Waite Agricultural Research Institute (Waite Campus), University of Adelaide, which is located 7 km south east of Adelaide City.

3.2. Source of sheep

The animals used in the experiments came from different sources, but they were all medium wool Merino sheep. The age of the animals were between 3.5 and 6.5 years.

3.3. General management of the animals

All rams were managed under grazing conditions in the small paddock adjacent to the Department of Animal Sciences building (Waite Campus). The animals were periodically moved into other paddocks to get access to new grass. The teaser ewe was always kept in an individual pen located in the sheep shed. Hay chaff and dry pellets were fed to the animals *ad lib* twice a day and drinking water was supplied automatically to a bowl fitted in the pen.

All the animals were identified by plastic ear tags and stencilled side numbers. The animals were also regularly checked for condition and treated when problems of legs, weakness or poor condition, pink eye, blowfly, etc. were detected. The animals were shorn once a year prior to the summer season.

3.4. Semen collection

In all experiments, the collection of semen was peformed by an artificial vagina using the method described elsewhere (Frank, 1950; Mattner and Voglmayr, 1962; Watson, 1978; Moss et al, 1979 and Evans and Maxwell, 1987). Generally, all rams used in this study were trained in previous experiments, so that they were accustomed to the surrounding environments (the housing condition, the presence of humans) and to mount the teaser (ovariectomized) ewe.

Collection was done in the sheep shed of the Davies Laboratory of the Department of Animal Sciences, University of Adelaide (Waite campus). The collection area was equipped with an oven. During collection the oven was maintained at 48°C for holding the artificial vagina and collecting glasses.

Shortly before the collection, the artificial vagina (AV) was assembled by placing the inner liner inside the casing with approximately equal lengths protruding at each end. The ends of the liner were then folded back over the ends of the casing and secured with rubber bands to hold the liner in place so that leakage of the water was prevented. The assembled-AV was half-filled with warm water until the temperature of the inner lining reached 42°C (checked by inserting a clean thermometer) which was approximate close to body temperature of the female. In order to assist in maintaining the desired internal temperature, the assembled-AV was prewarmed before filling by leaving it in the oven maintained at 48°C. When the internal temperature of the AV was stabilized (42°C), one end of the inner liner was then lubricated with ample vaseline, avoiding excesses that may contaminate the semen. The graduated collection glass was inserted into the other end and while holding it in position, the AV was then inflated by blowing the air via the open tap, which was closed immediately. During the introduction of air, care was exercised to supply the pressure that mimicked the female vaginal orifice of the ewe, but not so great as to prevent or to cause difficult passage of the penis through the entire length of the AV.

An ovariectomized ewe that served as teaser was secured in a collection bail, then the ram released from the pen into the collection area. When the ram mounted the teaser, the operator who knelt down on the right side of the teaser, grasped the sheath gently and deflected the ram's penis into the open end of the AV; ejaculation usually followed immediately. Sometimes when the ram failed to respond or was slow to mount and/or reluctant to ejaculate because the temperature of the AV had fallen below 42°C, the AV was refilled with warmer water. If ejaculation had occured, the ram was returned to the holding pen as soon as it had dismounted. The collecting glass was withdrawn, identified and covered with aluminium foil to avoid direct light, water, dust and other factors that may affect the semen viability (Evans and Maxwell, 1987). It was then placed in an Esky to keep warm and taken immediately to the laboratory, 2-3 minutes walk from the collection site. The collecting glass was then put into the waterbath maintained at 30°C. Within 10 minutes of collection, the semen was processed and assessed.

When collection was finished, all rams were returned to the paddock and the teaser returned to her pen. Afterwards, having drained out the water through the tap, the AV and the rubber liner were thoroughly washed and scrubbed with a mixture of hot water and detergent, then soaked for 30 minutes in water in a bucket. The AV was then rinsed several times with a clean running tap water, followed by reverse osmosis water and finally was allowed to dry by hanging it up.

3.5. Semen processing and dilution

In all experiments, semen was processed and stored at two different temperatures condition: room temperature storage (30°C) to simulate conditions in Indonesia and chilled temperature storage (5°C). The semen was diluted at 30°C with diluents at a ratio of 1:4 (semen:diluent), so the samples (liquid semen) contained reasonable numbers of spermatozoa. The diluent was always added to the semen and never in the opposite way and the addition was done very slowly along the wall of the glass tube to avoid any thermal or osmotic shock.

Hen and Japanese quail eggs were obtained from a retail source in Adelaide. Egg yolk was prepared by cracking the egg shell in half. The egg white was discarded by pouring it from one half cracked-shell to another. When only small amount of egg white was left, the yolk was separated from the egg white by rolling it on a filter paper until dried without causing damage to the yolk. Usually, it was necessary to use several filter papers during each separation. Next, the yolk was punctured using a sterile 10 ml plastic pipette and sucked until all yolk was removed into the pipette. The yolk was then emptied into a clean glass beaker. Other eggs were cracked until the desired volume of the yolk (20 ml) was achieved. All yolks contained in the beaker were then thoroughly mixed. Lastly, according to the amount needed, the yolk was added into the diluents using a 2 or 5 ml plastic syringe and the mixture was made homogeneous by gently shaking the bottle that held the preparation.

3.6. Semen assessment

Traditional manual and the computerised image analysis (Hamilton Thorn Motility Analyzer, Version 6.03-7.2.Q Daintree Ind., Victoria; HTM) methods were used for assessing fresh semen, while for assessing diluted semen, only the computerised image analysis method was used.

3.6.1. Traditional manual semen analysis

The traditional manual semen evaluation (Bishop et al., 1954; Hulet and Ercanbrack, 1962; Evans and Maxwell, 1987) was done shortly after semen was collected. Several semen traits were evaluated as follows:

3.6.1.1. Volume.

Following semen collection, the ejaculate volume was recorded by reading the graduated cylinder without taking into account the frothy part of the ejaculate (Bishop et al., 1954; Evans and Maxwell, 1987). Semen colour was examined simultaneously and categorized as thick creamy, creamy or thin creamy.

3.6.1.2. Motility of spermatozoa

Two types of motility were assessed: 1) mass motility or wave motion and 2) individual motility (percentage of motile spermatozoa).

3.6.1.2.1. Mass motility or wave motion.

To prepare a sample for estimation of mass motility, one drop of fresh semen was placed on a clean, pre-warmed (37°C) microscope slide without a coverslip and observed under the microscope fitted with a warm stage at a magnification of 40x (Bishop et al., 1954; Hulet and Ercanbrack, 1962). The estimation was performed based on the vigour of the wave motion and the collective activity of all spermatozoa. It was scored on a scale of 1 to 4 + (Evans and Maxwell , 1987).

3.6.1.2.2. Individual motility (percentage of motile spermatozoa).

To prepare a sample for estimation of individual motility or percent progressive motility, one drop of fresh semen was placed on a clean, dry, pre-warmed microscope slide (normally the same as for mass motility to save time) and covered with a coverslip (Hulet and Ercanbrack, 1962; Hulet et al., 1965; Linford et al, 1976). This time a greater magnification was used (400x or 1000x) to observe individual spermatozoa. The observations were performed on several fields around the sample and the proportion of the spermatozoa moving forward was assessed as a percentage. Sometimes when the sample was too dense, the examination was repeated at a greater dilution until a good view was achieved. Estimation of the individual motility was done on a scale of 0 to 100.

3.6.1.3. Concentration of spermatozoa

Once motility was evaluated (within 2-5 min), the number of spermatozoa per ml of semen (sperm concentration per ml) was determined with the aid of a haemocytometer (Improved Neubauer cell; Weber, England). An aliquot of 10 µl of fresh semen was drawn into a micropipette and discharged into a 10 ml plastic vial

containing 5 ml of the spermicidal solution {9 g sodium chloride plus 40 ml of 4 % formaldehyde (AnalaR ®, BDH Chemicals, Australia Pty.Ltd., Vict.) in 1L double-distilled water.} to give a dilution rate of 1:500 (semen:spermicidal solution). The vial was then inverted several times to ensure through mixing. Next, the haemocytometer was prepared by firmly pressing the coverslip on to the shoulders of the slide so that the coverslip becomes attached to the haemocytometer. A 10 µl of the sample was drawn using a micropipette and deposited with no air bubble at the edge of the coverslip. This drop spread by capillary action between the counting slide and the coverslip. The same procedure was repeated for the other side of the haemocytometer chamber. If an excess sample was applied and the counting chamber overflowed, the haemocytometer was cleaned and the above procedures were repeated. Finally, the haemocytometer was allowed to stand horizontally for some minutes to enable the spermatozoa to settle evenly on the grid before counting started.

After the sperm had settled, the haemocytometer was placed on the stage of the microscope and the medium-power objective was lowered as far as possible over the coverslip. To make sure that lowering was not too far, it was necessary to look at the distance between the objective and the coverslip from the side of the microscope. Then while looking down the eyepiece, the objective lens was raised slowly until the grid of the haemocytometer was in focus. The sperm cells in 5 large squares, each containing 16 smaller squares were counted. The large squares that were counted lay on the diagonals of the grid in models with 25 large squares. To avoid errors of omission and duplication, those sperm cells that were entirely inside the square and those touching or lying on the top and the right-hand edges of any square were counted. After counting all sperm in the 5 large squares, then the second count was done on the other counting grid of the haemocytometer. The results of first and second counting were averaged. If the difference between the counts on each side of the grid more than 10%, the estimation was repeated. The concentration of spermatozoa per ml of semen was calculated by multiplying number of spermatozoa by a factor determined by the dilution and the volume counted. The area of 5 large squares counted is equivalent to 5/25 = 0.2 mm^2,

and the chamber is 0.1 mm deep, the volume counted = 0.2 mm^2 x 0.1 mm = 0.02 mm^3 or 2 x 10^{-5} ml, i.e. 1/50000 of 1 ml. As the sample had been diluted 10 µl to to 5 ml or 500 times, so the number of sperm in 5 squares is multiplied by 50000 x 5000 i.e. 25 x 10^{-6}. Total sperm count was calculated by multiplying sperm concentration by semen volume. Once counting was finished, immediately the haemocytometer chamber and coverslip were washed in a running clean tap water, followed by RO water and gently blotted dry with a piece of facial tissue. Finally the chamber and coverslip were wiped with a little alcohol and kept for next use.

3.6.1.4. Live and dead ratio and morphology of sperm

For the determination of live and dead sperm and the study of sperm morphology, a special eosin-nigrosin stain was used (Evans and Maxwell, 1987). The stains consisted of:

Eosin (water soluble)	0.835	g
Nigrosin (water soluble)	5.000	g
Sodium citrate.$2H_20$	1.400	g
Distilled reverse osmosis (RO) water to	100	ml

The stains and chemical were weighed and then poured into a 150 ml clean beaker glass containing 40 ml boiling RO water. After adding a magnetic flea, the beaker was put on to a magnetic stirrer and stirred for 20 minutes. The beaker was removed and cooled down for 10 min. The stain solution was then made up to 100 ml by adding RO water to the beaker and finally filtered using Whatman paper No. 4 paper. The stain was pipetted and distributed into several glass tubes, each containing 75 µl of stain which were sealed with plastic caps. All stains were subsequently placed in a refrigerator until use.

Approximately 30 minutes before making the slide, the prepared stains in the tubes were removed from the refrigerator and placed into an oven maintained at 37°C. Similarly, the semen samples for morphological studies were taken from a waterbath

maintained at 30°C and allowed to incubate for 10 minutes in the same oven at 37°C. Once both the stain and the semen reached the same temperature (37°C), two or three drops of semen were mixed with 75 µl the stain thoroughly, but gently using a warm Pasteur pipette and allowed to react for 3 to 5 minutes. A small drop of this mixture was then placed on one end of a warm glass microscope slide and drawn out with the edge of a second slide which served as a spreader so that a thin film was formed. The slide was allowed to air dry and labelled before examination.

The percentage of abnormal spermatozoa was examined under the microscope at 400x magnification (Bishop et al., 1954; Evans and Maxwell, 1987). A total of 200 spermatozoa were counted per slide as either stained or not. The criteria for live or dead sperm was determined as follows: transparent or white heads indicated live sperm cells and pink or coloured heads indicated dead sperm cells (Mayer et el., 1951; Swanson and Bearden, 1951; Sorensen, 1979). For the study of morphology, the same procedure was used and the spermatozoa were recorded as: normal or abnormal, with abnormalities including loose head, coiled tails and bent tails. A total of 200 cells were counted (slightly more than recommended by Salisbury and Mercier, 1945), and the results were expressed as a percentage of normal and abnormal cells.

3.6.2. Objective semen analysis

The procedure for the objective semen analysis depended on whether the semen was fresh or diluted.

3.6.2.1. Fresh semen

Fresh semen collected by artificial vagina was kept in a water bath at 30°C and assessed within 10 minutes after collection. A 100 µl sample from the ejaculate was taken and put into 5 ml polystyrene tube (Disposable products, SA) then mixed slowly with a 900 µl Dulbecco's Phosphate solution (Dulbecco's phosphate buffered saline (PBS) solution, single strength, pH 7.3, CSL, Victoria) at 37°C to avoid thermal shock. The mixture was then shaken thoroughly, but gently in the waterbath maintained at

37°C. A volume of 10 µl of this extended sample was micropipetted into a Makler counting chamber (Sefi-Medical Instruments, Israel) with 10 µm depth, covered with a coverslip, and a few seconds allowed for the sperm to settle. The Makler chamber was placed in the automatic temperature controlled stage of the HTM Analyser machine which was already switched on beforehand, with the stage warm set at 37°C. Normally each sample was analyzed to count not less than 200 cells, which could be counted in 1 to 2 different fields selected among the ones which were clean from debris.

The cell counting depended upon the concentration of the sample. Sometimes when the sample was not concentrated, the number of fields was increased to achieve the required number. Conversely, when the sample was too concentrated (the machine will give warning by 'high density' sign in the monitor), the sample was then further diluted with Dulbecco solution. The machine counts automatically from the first to the last chosen fields and the last one appears on the screen for " playback" function which allows a check to be made to see if the sample was assessed correctly (Mahony et al., 1988; Hamilton Thorn Research, 1989).

The machine marks the motile spermatozoa with a blue dot and the non motile ones with a red dot, thus when the last field is presented all cells should be selected with one of the dots. If all of the cells are dotted, the machine is selecting all cells properly and no more adjustments are needed; when some of the cells were not dotted, then a slight adjustment on the main gates was needed to select all cells in the sample and make the assessment as accurate as possible.

When the sample was assessed and the required number of cells assessed, then results were saved and printed out as a summary at the end of the session. The results were presented on the screen and then printed or recorded on a computer attached to the HTM.

3.6.2.2. Diluted-stored semen

Most of the procedures for assessing diluted-stored semen were the same as for fresh semen, except the dilution rate was different. Semen assessment was done first from samples stored at a temperature of 30°C, afterwards from samples stored at a temperature of 5°C.

The initial assessment of semen to be stored at room temperature was done as soon as semen dilution was completed. Assessment of diluted samples to be stored in the cool room was delayed until the temperature had dropped from 30 to 5°C. A 100 µl aliquot of the diluted sample was pipetted into a clean test tube, then brought from the cool room to the semen laboratory and kept in a waterbath at 30°C. After allowing about 5 minutes for the sperm to equilibrate, the diluted semen was then mixed with 900 µl Dulbecco's solution, then shaken thoroughly in a waterbath maintained at 37°C. A 10 µl aliquot of mixed extended semen was placed on a Makler chamber. The chamber was then loaded into the stage of the HTM Analyzer, warmed to 37°C and all semen parameters were evaluated.

As samples either stored at 30 or 5°C were less concentrated than fresh semen, three to five fields (depending on the density of the sample) were selected for analysis. Each sample was analysed twice from the same fields. Two separate replicates were observed from each semen sample.

For the purpose of this study, only certain semen characteristics assessed by the Hamilton Thorn Motility (HTM) Analyzer were chosen, namely TOTAL, mean ALH, mean LIN, mean VAP, mean VSL, mean VCL, mean STR, MOT%, PROGr%, RAPID%, MEDIUM% and SLOW% (Gill et al., 1988; Hamilton Thorn Research, 1989; Pedigo et al., 1989). All abreviations and characteristics are fully described in Table 3.1.

Table 3.1. The abbreviations used for the semen characteristics measured by the HTM Analyzer system and its definition.

Semen characteristic	Definition
Total sperm (TOTAL)	The sum of all motile and non-motile spermatozoa. The value is given as the total concentration of all cells, the total numbers of cell in an ejaculate and the total number of cells counted.
Mean velocity (mean VAP µm/s)	The five point running average path velocity, averaged over all cells for which VAP>LVV**.
Mean lateral head displacement (mean ALH µm)	Displacement measured in the cell track, averaged over all cells for which the straightness exceeds the threshold straightness (STR>So*), and for which VAP>LVV**.
Mean progressive velocity (mean VSL µm/s)	The velocity measured in a straight line from beginning to end of track, averaged over all cells for which VAP>LVV**.
Mean track speed (VCL µm/s)	Average value of the track speed over all cells for which VAP>LVV**. This is computed by taking the total distance covered by a cell in its track, taking straight lines for each cell between the successive 5 to 20 points acquired, summing the distances and dividing by the total elapsed time.
Mean straightness (mean STR %)	Ratio VCL/VAP. Measures departure of the cell (mean STR %)path from a straight line.
Mean linearity (mean LIN %)	Ratio VSL/VCL. Measures departure of the cell track from a straight line.
Motility (MOT %)	The fraction of total cells for which path velocity VAP>0 (if the slow cells motile:yes option is taken); or for which VAP>a pre-set value for low velocity (LVV**) (If slow cells motile : no option is taken). Therefore the user can choose whether to count all moving cells as motile, or only those moving faster than a chosen velocity. (In the present study all moving cells were counted as motile)
Progressive (PROGR %)	The fraction (percentage) of all cells moving with VAP>MVV*** and STR>So* in a sample.
Rapid (RAPID %)	The fraction of all cells moving with velocity VAP>MVV***.
Medium (MEDIUM %)	The fraction of all cells moving with velocity between LVVand MVV:i.e. MVV***>VAP>LVV**
Slow (SLOW %)	The fraction of all cells moving with velocity (SLOW %) below LVV** i.e. LVV>VAP>0

Modified from Hamilton Thorn Research (1989).

* So=Threshold of STR, the straightness threshold value pre-set by the user.

** LVV=Low VAP velocity, low value of VAP velocity pre-set as a threshold by the user.

*** MVV=Medium VAP velocity, medium value of VAP velocity pre-set as a threshold by the user.

The HTM analyzer system derives this information from video images (either real time or from play-back video recordings), which are captured sequentially (20

frames/s) and digitized by the computer. The digitized (video) frames are then analysed as directed by the software parameters defined in Table 3.2.

Table 3.2. Set-up parameters for the quantification of fresh and diluted ram sperm motility characteristics using the HTM Analyzer system, HTM-2000 (Hamilton Thorn Research, Danvers, MA)

Main gates	Fresh semen	Diluted semen
Analyzer set up		
Temperature	37.0°C	37.0°C
Chamber	Makler (10 µl)	Makler (10 µl)
Image type	Phase contrast	Phase contrast
Field selection	Manual selection	Manual selection
Calculate ALH	yes	yes
Main gates *		
Frames at frame rate	20, at 25/second	20, at 25/second
Minimum contrast	3	14
Minimum size	4	4
Lo/Hi size gates	0.2, 2.2	0.4, 1.9
Lo/Hi intensity	0.2, 2.2	0.4, 1.9
Non motile head size	6	5
Non motile intensity	125	125
Medium VAP value	95	30
Low VAP value	10	10
Slow cells motile	No	No
Threshold STR	60	60

*To calibrate the main gates, some screens were evaluated with the playback function which enables the operator to examine if all non-motile and motile cells have been identified by the machine. With the same function, debris can be avoided (Gill et al., 1988).

3.7. Statistical analysis

Data were subjected to analysis of variance with the split plot design method on arcsin transformed data. Means and standard error of means (SEM) were calculated. When a significant difference was noted among the means, Tukey's test was performed to determine which mean was different from the others. The results was considered significant of $p< 0.05$.

Chapter 4

Coconut extract and quail yolk in diluents for storage of ram semen at room and chilled temperatures

4.1. Viability of ram spermatozoa stored at 30 and 5°C in diluent containing combination of coconut extract and quail yolk

4.1.1. Introduction

Coconut milk (coconut water) as a semen diluter has attracted some attention due to its availability in almost all tropical countries. This product found within the endosperm cavity of the fruit, has been found to be viable "natural" diluent for cattle (Norman et al, 1962;), buffalo (Norman et al, 1968), goat (Pillai et al,1978) and sheep sperm (Prasad and Norman, 1968). However, there seems to be little or no information on the use of coconut extract (fluid pressed from the coconut endosperm) for diluting and preservation of sheep semen. Such information is of interest as the extract has a higher protein content than coconut milk (Banzon and Velasco, 1982), while in tropical country like Indonesia, coconut trees can be found everywhere in the villages. The extract is versatile and cheap and can easily be prepared for making semen diluents.

Egg yolk has been a common constituent of semen diluents since Phillips (1939) discovered its beneficial effects on the survival of bull spermatozoa. Egg yolks that regularly incorporated in semen diluents with citrate, phosphate, sugars and milk, are mainly derived from hen eggs. Information regarding the use of egg yolk of birds other than the hen has been reported by Basu and Berry (1948), who included yolk from turkey eggs in citrate diluent for preservation of bull spermatozoa stored at 40°F (4°C). Recently, quail yolk has been shown to be superior to hen yolk in the freezing of sperm from Poitou jackass (Trimeche et al, 1997). It seem that egg yolk of other species of birds might also be used as a material for diluents.

Information as to the use of quail egg yolk as an extender for dilution and preservation of ram semen is not available. Such information is of interest as the proportions of yolk in quail eggs is higher than in hen eggs (Fletcher et al, 1983; Yannakopoulos and Tserveni-Gousi, 1986). As quail eggs are available in some parts of the world when hen eggs are difficult to obtain, the use of quail egg yolk as a substitute for hen egg yolk could be useful. Thus, the present study was undertaken to investigate the suitability of these local ingredients in semen diluents and examined the survival of ram spermatozoa stored at ambient (30°C) and cool (5°C) temperatures for different periods of time.

4.1.2. Experimental procedure

Single ejaculates were collected with an artificial vagina from each of three Merino rams four times at two weekly intervals. Each ejaculate was split into six portions and each portion diluted 1 + 4 at 30°C no more than 10 minutes after each collection. The diluent used for both storage temperatures was the same as first published by Norman (1962), but slightly modified by the substitution of yolk from quail eggs for the yolk from hen eggs, nystatin was used instead of mycostatin, and 15 ml of coconut extract was used in place of coconut milk or fluid as presented by Norman et al (1962). Salamon's standard diluent as described by Evans and Maxwell (1987) was used for comparison with Norman's diluent. Basically, both diluents consisted of a sodium citrate-egg yolk medium and the components of both diluents are presented in Table 4.1.

The diluted samples were placed in glass culture tubes of 5 ml capacity and sealed with plastic caps. Samples to be stored at 30°C were placed into a plastic rack immersed in a water bath operating at this temperature, while those to be stored at 5°C were removed into a plastic rack immersed in an open plastic box filled with 30°C water and placed in a cool room. Once the temperature of the water in the box reached 5°C and remained there for about 2 hours, the semen samples were then placed into a water bath maintained at 5°C until assessed. In a preliminary experiment, assessment was made after

4 and 8h. In the main experiment, assessment was made up to 48h at 30°C and up to 240h at 5°C.

Table 4.1. Composition of the diluents used for storage of ram semen at room and chilled temperatures.

Component		Salamon's standard diluent *) HYCG or QYCG	Modified Norman's diluent**) CEQY
Sodium citrate ($2H_2O$)	(g)	2.37	2.16
Glucose	(g)	0.80	-
Sodium penicillin	(g)	0.06	0.031
Dihydrostreptomycin sulphate	(mg)	100	68
Sulphanilamide	(mg)	-	300
Catalase	(units)	-	15000
Nystatin	(units)	-	400
Hen or quail egg yolk	(ml)	20	5
Coconut extract	(ml)	-	15
Double distilled water to	(ml)	100	100
Osmolality	(mOsm/kg)	355	294

*) Evans and Maxwell (1987)
**) Norman et al (1962)

4.1.2.1. Preparation of diluent

Preparation of diluent was done one day before semen collection. The chemicals were first dissolved in an appropriate volume of double distilled water (65 ml) and mixed with the antibiotics. Then more water was added to the diluent to make up the final volume/concentrations (80 ml). The prepared diluent was then divided into 4 small bottles, each contained 20 ml diluent, and sealed with parafilm. Finally the bottles were placed in the freezer until use. Shortly before semen collection, the diluents were removed from the freezer and put in a waterbath maintained at 30°C.

4.1.3. Statitical analysis

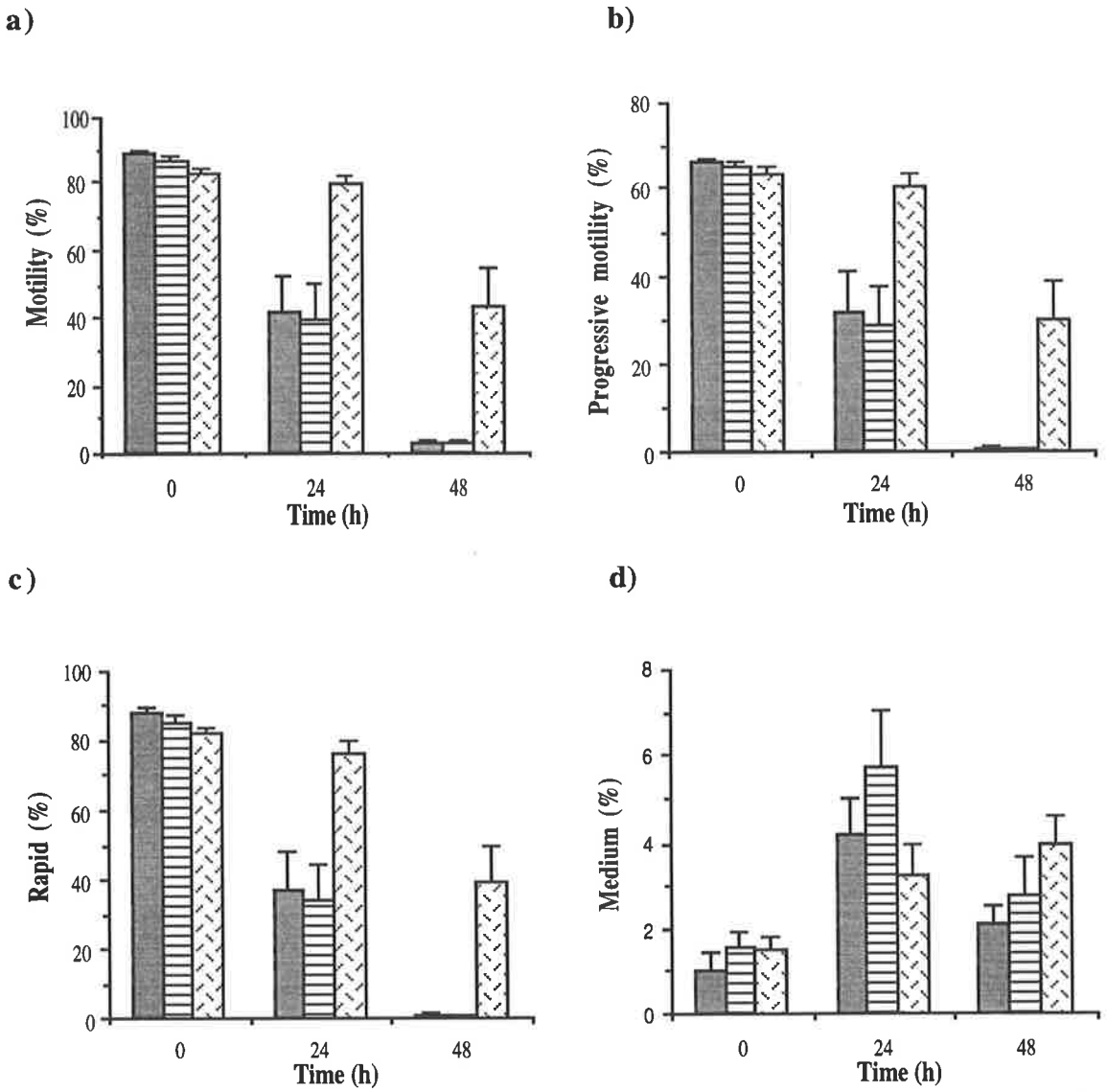
Data were subjected to analysis of variance using split-plot design and arcsin transformation was done for percentage data. Tukey's test was performed to determine percentage significant differences between means.

4.1.4. Results

In the preliminary experiment, hen and quail egg yolk were compared with semen diluted in Salamon standard diluent and stored for 4 or 8h at 30 or 5°C. There were no differences between the yolks from the two species (Figures 4.3 and 4.4.a and b); Table 4.4. (Appendix A).

In the main experiment, at 30°C, there was no significant diluent by time of storage interaction for any sperm motion characteristics, except for mean ALH in which the interaction was significant ($p<0.001$). There was a significant ($p<0.001$) effect of time of storage on the overall sperm motion characteristics. The effect of diluent on these parameters was also significant ($p<0.001$), except for medium, slow, mean LIN and mean STR. In general, an effect of diluents on the sperm motion characteristics was shown after samples were stored for 24h, followed by a marked decrease at 48h storage.

The results presented in Table 4.2 (Appendix A) indicated that at 30°C motility of spermatozoa fell down markedly after 24h of storage in HYCG and QYCG; but in CEQY, motility reduced only slightly. With semen stored for 48h, motility in both HYCG and QYCG diluents droppped abruptly ($p<0.05$) to less than 5%, but in CEQY about 40% of spermatozoa were still motile (Figure 4.1.a). Decreases in mean progressive motility and rapid spermatozoa followed a similar pattern to that of motility of spermatozoa (Figures 4.1.b and c).



Figures 4.1. Motility (a), progressive motility (b), rapid (c) and medium (d) spermatozoa after storage for 0, 24 and 48h at 30°C in hen yolk citrate glucose (■), quail yolk citrate glucose (▤) and coconut extract quail yolk (▣) diluents. Values are Means ± SEM of 12 observations.

A significant increase in mean medium sperm in all diluents was observed after 24h. Although medium sperm in CEQY was lower than that in HYCG or QYCG, the difference was not significant. At 48h of storage, percent medium in HYCG and QYCG decreased again, but in CEQY it continued to increase slightly (Figure 4.1.d).

Mean slow sperm in HYCG and QYCG diluents tended to increase significantly throughout the storage periods, while in CEQY it increased slightly. However, percent slow sperm in CEQY was not significant compared with that in HYCG or QYCG at 24h (Figure 4.1.e).

e)

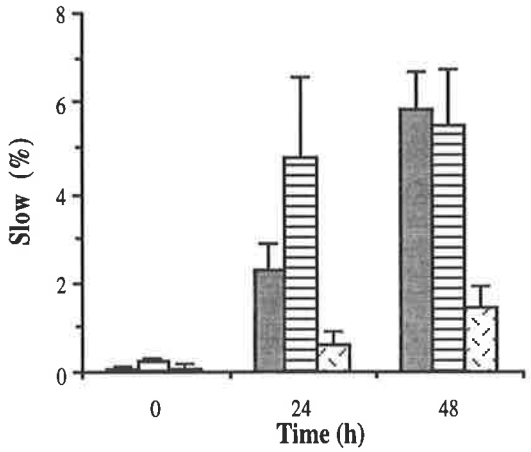
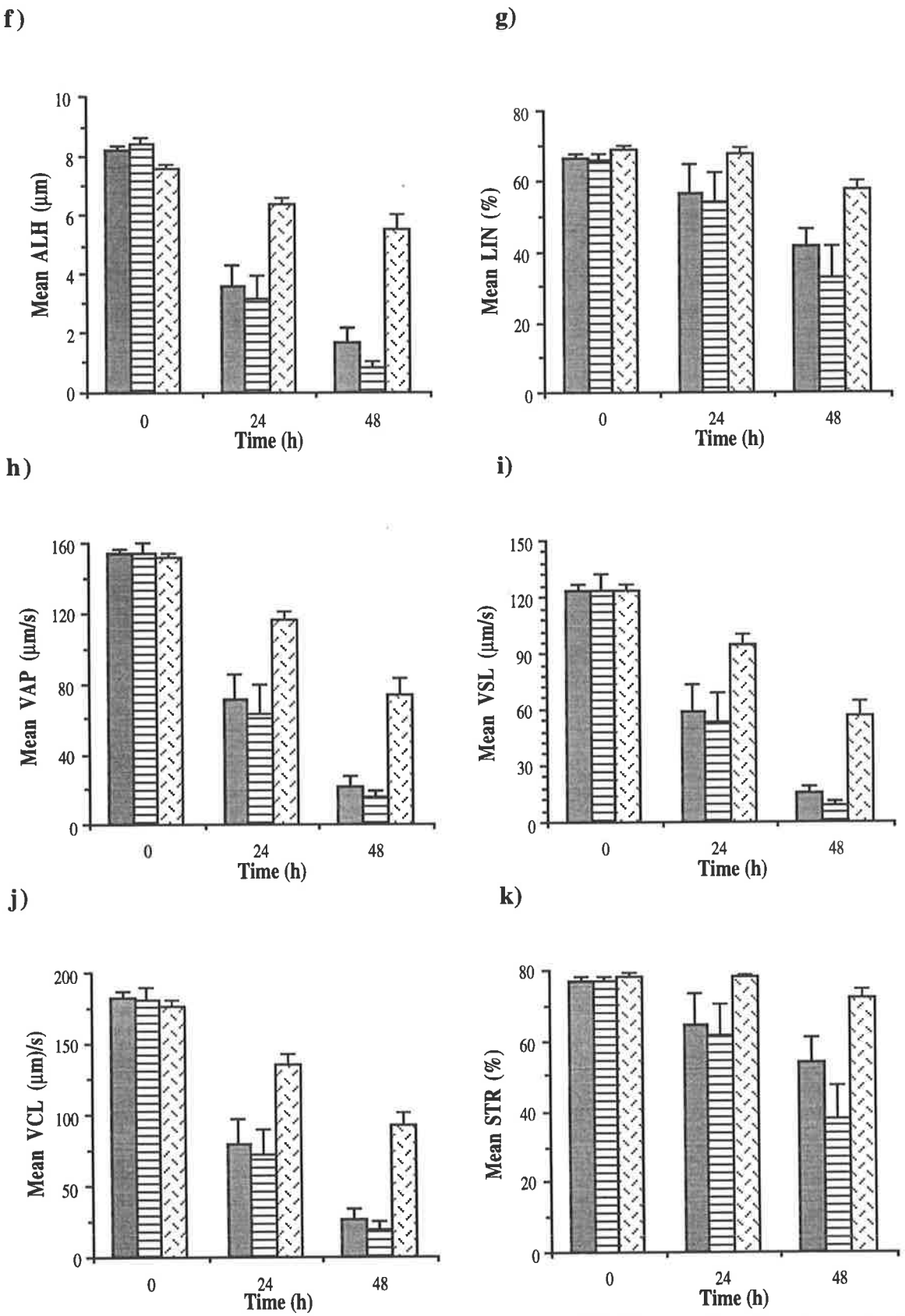


Figure 4.1. Slow (e) spermatozoa after storage for 0, 24 and 48h at 30°C in hen yolk citrate glucose (■), quail yolk citrate glucose (⊟) and coconut extract quail yolk (⊡) diluents. Values are Means ± SEM of 4 observations.

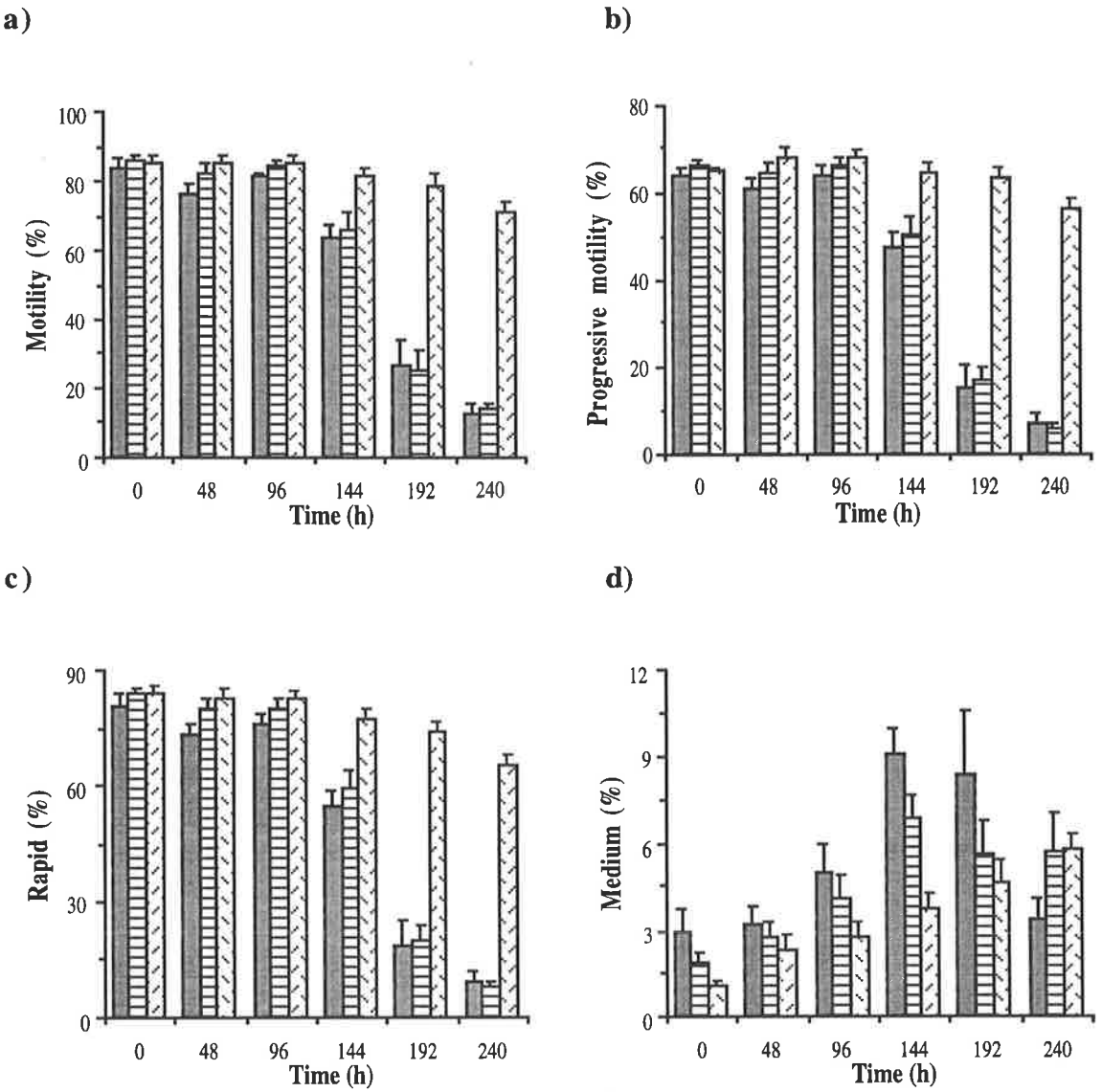
Semen diluted in CEQY had a higher mean ALH, mean VAP, mean VSL and mean VCL than samples diluted in HYCG and QYCG after storage for 24 and 48h, but during these times there was no significant difference between the latter two diluents (Figures 4.1.f,h,i and j). Semen diluted in CEQY had a higher STR than that diluted in QYCG after storage for 48h, however, no significant differences were observed between samples diluted in HYCG and QYCG (Figure 4.1.k). Mean LIN did not alter much after 24h of storage, but a significant ($p<0.05$) decrease in mean LIN in all diluents was observed during storage from 24 to 48h. The differences in mean LIN among the three diluents were not found over time (Figure 4.1.g).



Figures 4.1. Mean ALH (f), mean LIN (g), mean VAP (h), mean VSL (i), mean VCL (j) and mean STR (k) of spermatozoa after storage for 0, 24 and 48h at 30°C in in hen yolk citrate glucose (■), quail yolk citrate glucose (▤) and coconut extract quail yolk (▨) diluents. Values are Means ± SEM of 12 observations.

The percentages of motility and progressive motility in HYCG, QYCG and CEQY diluent stored at 5°C for different periods of time are presented in Table 4.3 (Appendix A). At 5°C, there was a significant ($p<0.001$) diluent by time of storage interaction for all sperm motion characteristics, except for mean LIN.

Percent motility, progressive motility and rapid spermatozoa in all diluents decreased as time of storage increased, however, differences among diluents were not observed up to 144h. However after storage for 192 and 240h, an abrupt decrease ($p<0.05$) in these three parameters was occured in HYCG or QYCG compared with that of CEQY (Figures 4.2.a, b and c).



Figures 4.2. Motility (a), progressive motility (b), rapid (c) and medium (d) spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in in hen yolk citrate glucose (■), quail yolk citrate glucose (▤) and coconut extract quail yolk (▣) diluents. Values are Means ± SEM of 12 observations.

Mean medium sperm in all diluents increased gradually from 0h to 144h of storage, in which the highest values at 144h were recorded in HYCG and QYCG compared to CEQY. After this time, percent medium sperm in these three diluent fluctuated (Figure 4.2.d).

e)

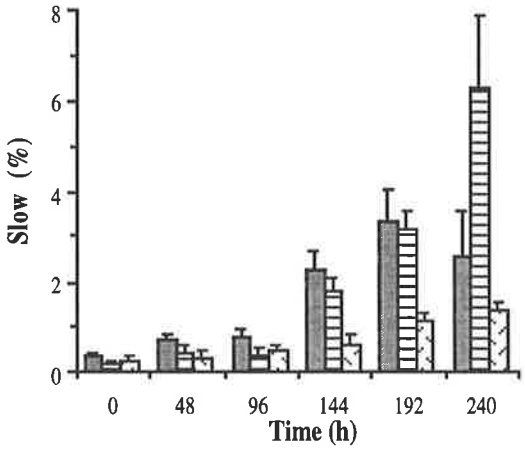
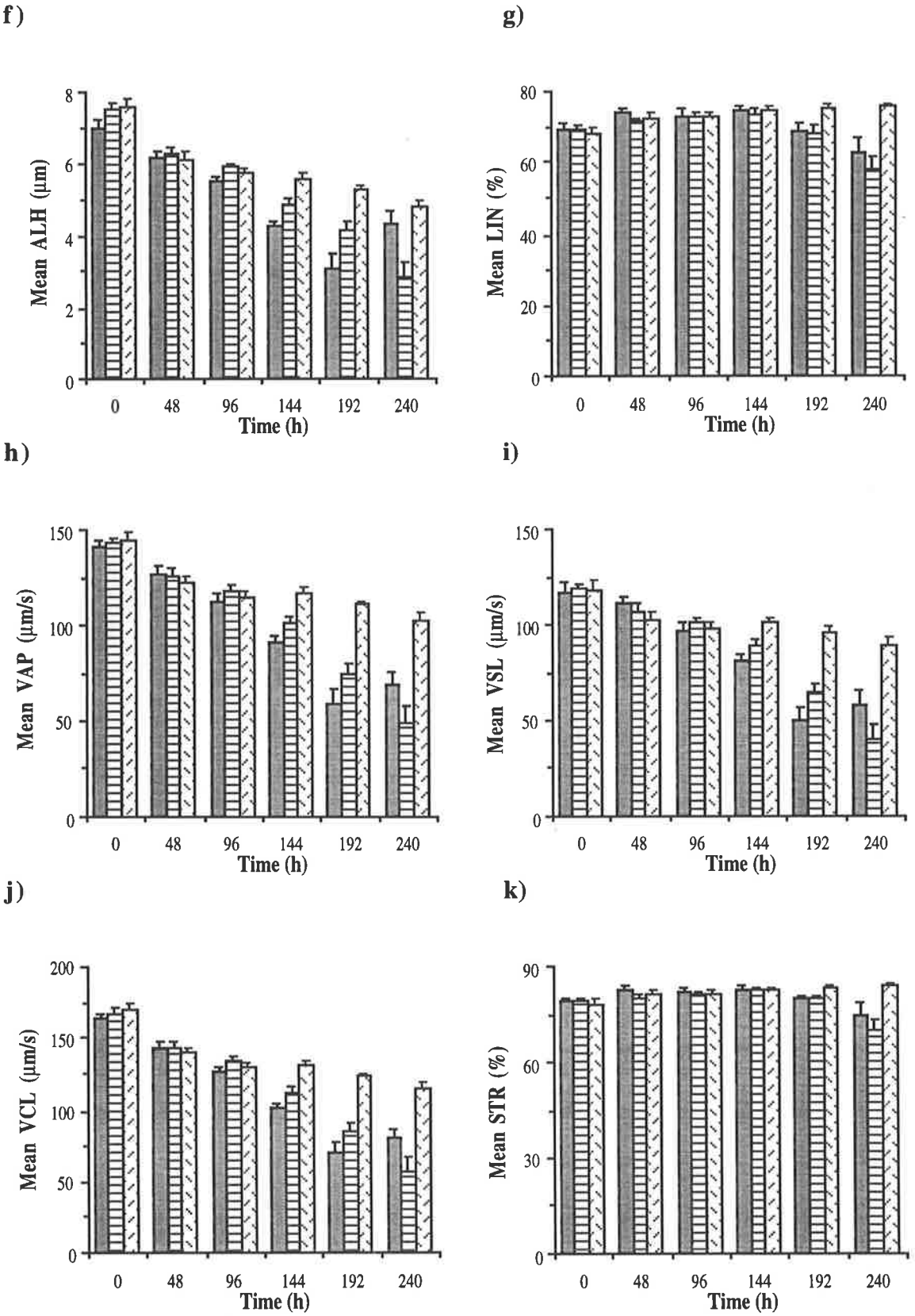


Figure 4.2. Slow (e) spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in in hen yolk citrate glucose (■), quail yolk citrate glucose (▤) and coconut extract quail yolk (▨) diluents. Values are Means ± SEM of 12 observations.

The numbers of slow sperm in all diluents increased irregularly during storage from 0 to 240h. Percent slow was higher ($p<0.05$) in HYCG and QYCG than that in CEQY at 144h. However at 240h, the value was higher in QYCG than in HYCG and CEQY (Figure 4.2.e).

A significant ($p<0.05$) reduction in the mean ALH, mean VAP, mean VSL and mean VCL from samples diluted in HYCG, QYCG and CEQY was noticed after 144h storage, followed by a marked decrease in these parameters at 192 and 240h storage. During this time, semen samples diluted in CEQY were consistently higher in these parameters than those diluted in HYCG and QYCG, but, differences between this two latter diluents were not significant.



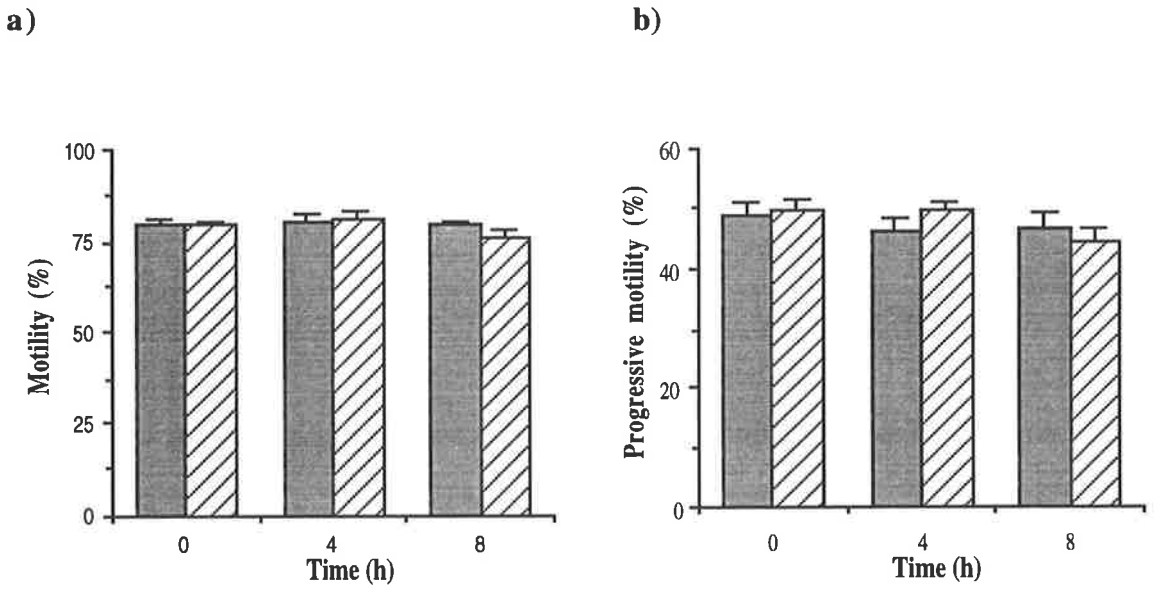
Figures 4.2. Mean ALH (f), mean LIN (g), mean VAP (h), mean VSL (i), mean VCL (j) and mean STR (k) of spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in in hen yolk citrate glucose (■), quail yolk citrate glucose (▣) and coconut extract quail yolk (▣) diluents. Values are Means ± SEM of 12 observations.

There was a gradual decrease in mean ALH in the 3 diluents as time of storage increased. However, mean ALH in HYCG diluent increased again during storage from 192 to 240h. At 192h, mean ALH in CEQY diluent was significantly higher ($p<0.05$) than that in HYCG diluent. At 240h, mean ALH in HYCG or CEQY diluent was significantly ($p<0.05$) higher than that in QYCG diluent (Figure 4.2.f).

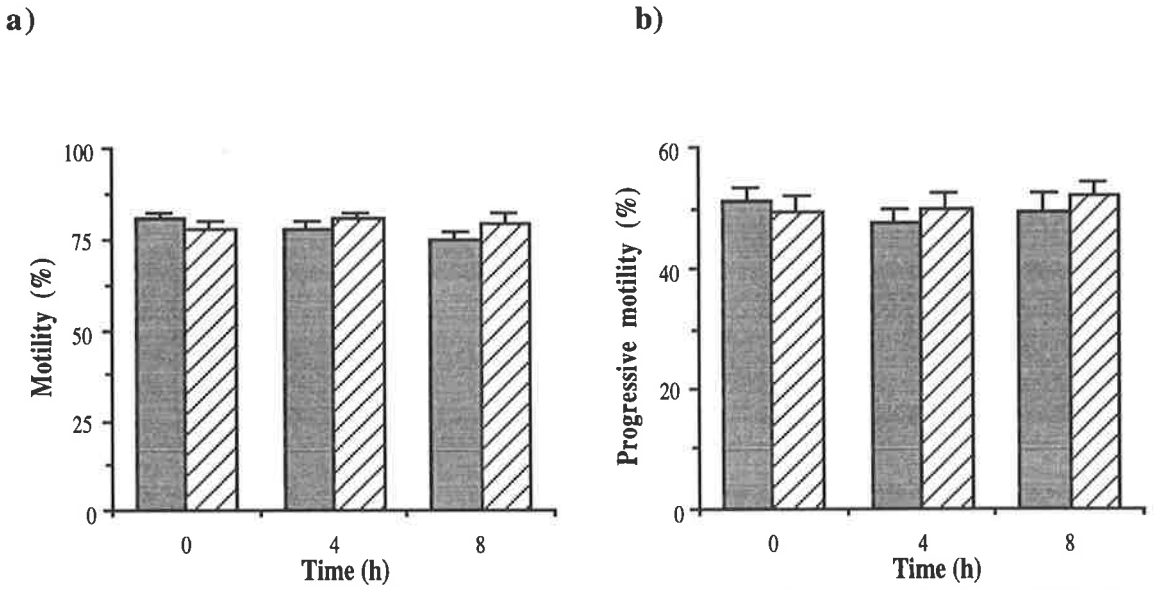
Mean LIN and mean VAP in HYCG and QYCG diluents increased from 0 to 144h and then decreased beyond this time. However, mean LIN and mean VAP in CEQY tended to increase continually up to 240h of storage. At 240, mean LIN in CEQY was higher ($p<0.05$) than that at HYCG or QYCG diluent. For mean VAP, the value at 192h and 240 was higher ($p<0.05$) in samples diluted in CEQY than that in HYCG or QYCG (Figures 4.2.g and h).

After semen had been stored for 192h, there was a significant ($p<0.05$) difference in mean VSL between samples diluted in CEQY and those diluted in HYCG. However, both mean VSL and mean VCL was higher ($p<0.05$) in samples diluted in CEQY than that in HYCG or QYCG after storage for 240h (Figures 4.2.i and j).

Initially mean STR in HYCG and QYCG increased up to 144h of storage, and this decreased in the two consecutive storage periods. However, mean STR in CEQY diluent continually increased up to 240h. At this time, mean STR differed significantly ($p<0.05$) from that of HYCG and QYCG diluents (Figures 4.2.k).



Figures 4.3. Motility (a) and progressive motility (b) of spermatozoa after storage for 8h at 30°C in hen yolk citrate glucose (HYCG)(■) and quail yolk citrate glucose (QYCG)(▨) diluents. Values are Means ± SEM of 3 observations.



Figures 4.4. Motility (a) and progessive motility (b) of spermatozoa after storage for 8h at 5°C in hen yolk citrate glucose (HYCG)(■) and quail yolk citrate glucose (QYCG)(▨) diluents. Values are Means ± SEM of 3 observations.

4.1.5. Discussion

In this study, motility of spermatozoa in CEQY diluent was much better than that in HYCG or QYCG diluent at both temperatures. This suggested that there was a big advantage of coconut extract when added to the diluent. Motility of spermatozoa in HYCG and QYCG diluents were comparable at 30°C up to 48h and 5°C up to 240h. This

might indicate that even at longer times of storage both yolks in citrate-based diluents have the same capabilities in preserving motility of ram spermatozoa. It is still not known whether that advantage was due to inclusion of coconut extract, the other additional components of CEQY, the different amount of yolk in the citrate glucose and coconut extract based diluents, or to different starting pH. In the present studies, pH of citrate glucose diluent was 7.6, while pH of coconut extract diluent was 7.0.

The present results show that ram sperm motility in semen stored at 5°C was maintained better than that stored at 30°C. Bartlett and vanDemark (1962) and Foote and Bratton (1950) who used bull sperm found ambient temperature storage (25°C or above) was inferior to that chilled temperature storage.

There appears no reports on the use of coconut extract in the diluent for preservation of ram semen stored at room or cool temperature. However, the results of this study for samples stored at 30°C could be related to the studies of Pillai and Iyer (1982) with coconut milk. They reported that motility of buck semen extended in coconut milk diluent and stored at room temperature (22.6 to 36.4°C) for 24 and 48h at a ratio of 1:200 (semen:dilution) was 52.34% and 29.22%, respectively. These are lower than that found in the present study for samples stored in CEQY diluent at 30°C, i.e. 79.6% at 24h and 40% at at 48h of storage.

The differences in the percent motile sperm could be due to differences in the species from which the semen was obtained, dilution ratios and a wide range of room temperature applied in those former studies.

It seems that the replacement of glucose by coconut extract (15 ml), in the presence of a lower amount of quail yolk (5 ml), produced a diluent which gave much better preservation of motility than either of the citrate-glucose diluents. This is in accordance with the findings of Norman et al (1962) who found a better motility of bull sperm stored at 5°C in 5% egg yolk and 25% coconut milk diluent than in 20% egg yolk citrate diluent and the fertility of the sperm stored in coconut extract extender with 5% egg yolk "compared favourably with the fertilizing capacity reported for sperm stored at 5°C

in other more widely used diluents". However, the relatively high egg yolk concentration (20 ml) included in HYCG or QYCG diluent may be detrimental to the viability of spermatozoa, though in the present study, the levels of egg yolk were not examined. In contrast, other workers (Foote and Bratton, 1960; Foote et al, 1960) obtained satisfactory results when 20-30% egg yolk was added to diluents meant for storage of bull semen at 4°C. These variations again possibly reflect species differences or an effect of diluent (Watson, 1979; Upretti et al, 1992; Das and Rajkonwar, 1994).

In conclusion, this investigation on ram semen has clearly shown that the substitutition of glucose by coconut extract significantly improved motility during storage at 30 and 5°C; and both yolks are comparable when added in semen diluent for maintaining motility at extended period of times. The similar results obtained with quail yolk and hen yolk contrast with the better recovery of motility by Poitou jackass sperm after freezing and thawing in a medium with quail yolk compared with hen yolk (Trimeche et al, 1997).

4.2. Viability of ram spermatozoa stored at 5°C in diluent containing combination of coconut extract and quail yolk, and of coconut fluid and quail yolk

4.2.1. Introduction

The previous experiment (Section 4.1) indicated that CEQY extender was superior to HYCG or QYCG diluent for preservation of ram semen either stored at room or cool temperatures, but studies by others had used fluid from the endosperm cavity of the fruit, whereas we have had fluid pressed from the coconut endosperm (coconut extract). The experiment presented here compared two diluents containing the two different products directly.

4.2.2. Experimental procedure

Single ejaculates were collected with an artificial vagina from each of three Merino rams, four times at two weekly intervals. Each ejaculate was split into three portions and each portion diluted five fold at 30°C. The diluent used was the same as presented in Table 4.1 (Section 4.1.2). However, for comparison, one quail yolk-citrate based diluent contained coconut extract (CEQY) and the other contained coconut fluid (CFQY). The volume of quail egg yolk was 5 ml and that of coconut extract or coconut fluid was 15 ml. Salamon's standard diluent with hen egg yolk as described by Evans and Maxwell (1987) was used as control diluent. Both coconut materials for this experiment were obtained from commercial coconuts purchased at a local supermarket. The spermatozoa were examined objectively for semen motion characteristics every 48h up to 10 days of storage at 5°C.

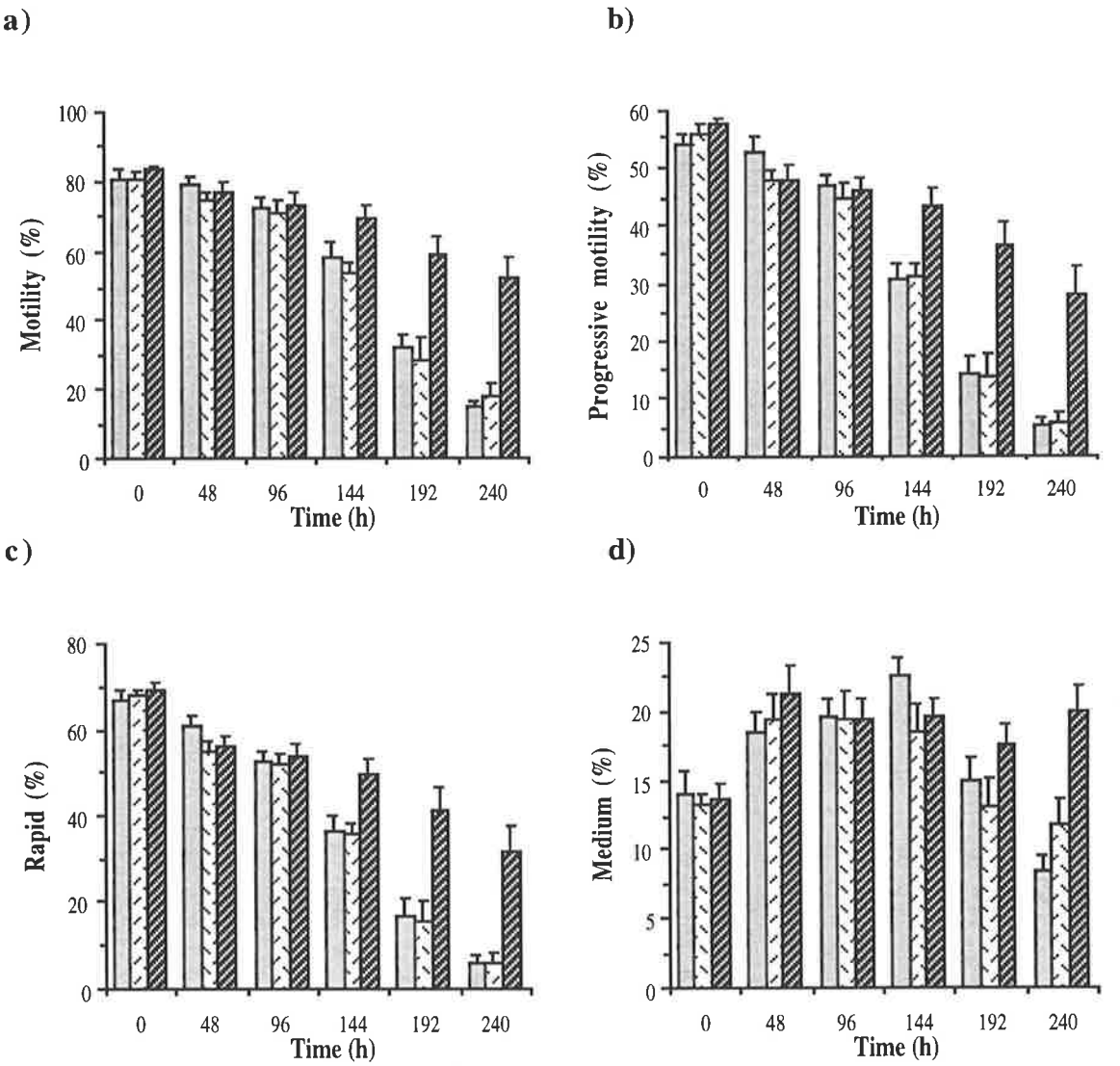
4.2.3. Results

The results of the study indicated that there was no significant differences ($p>0.05$) due to diluents in the numbers of medium sperm, mean ALH, mean LIN, mean VAP, mean VSL, mean VCL and mean STR. However, time of storage had a significant ($p<0.001$) effect on the motility, progressive motility, rapid, medium, slow, static, mean ALH, mean LIN, mean VAP, mean VSL, mean VCL and mean STR. There was an interaction between time of storage and type of diluent on the motility, progressive motility, rapid, medium, slow, mean LIN, mean VAP, mean VSL ($p<0.001$) and mean VCL ($p=0.05$), but not mean STR.

Data on the motion characteristics of ram semen after storage for 240h at 5°C is given in Table 4.5 (Appendix A).

Motility, progressive motility and rapid spermatozoa decreased gradually in the three diluents as time of storage increased from 0 to 96h. However when time of storage extended from 144 to 240h, percent values of these parameters in HYCG and CEQY diluents decreased significantly ($p<0.05$) compared with that of CEQY diluent. During

this times, the first two diluents did not differ ($p>0.05$) between themselves in these parameters (Figures 4.5.a,b and c).



Figures 4.5. Motility (a), progressive motility (b), rapid (c) and medium (d) spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in hen yolk citrate glucose (□), coconut fluid quail yolk (□) and coconut extract quail yolk (▨) diluents. Each value is the mean ± SEM of 12 observations.

The percentage of medium sperm in samples stored from 0 to 144h rose and then fell, whereas samples stored in CEQY showed a rise after 48h storage and then no further change (Figure 4.5.d). There was a gradual increase in the slow from the beginning to the end of storage. The rise was greatest in the samples stored in HYCG, with no difference between the other two diluents (Figure 4.5.e).

e)

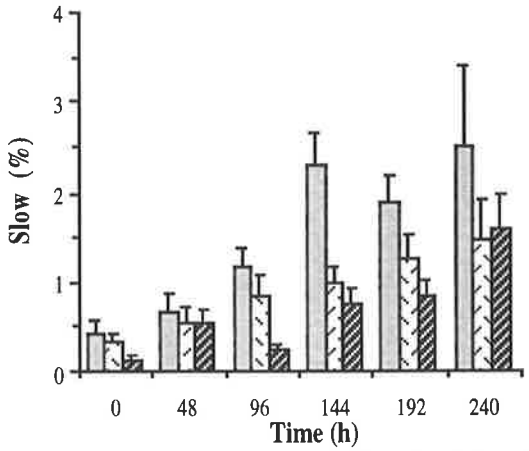


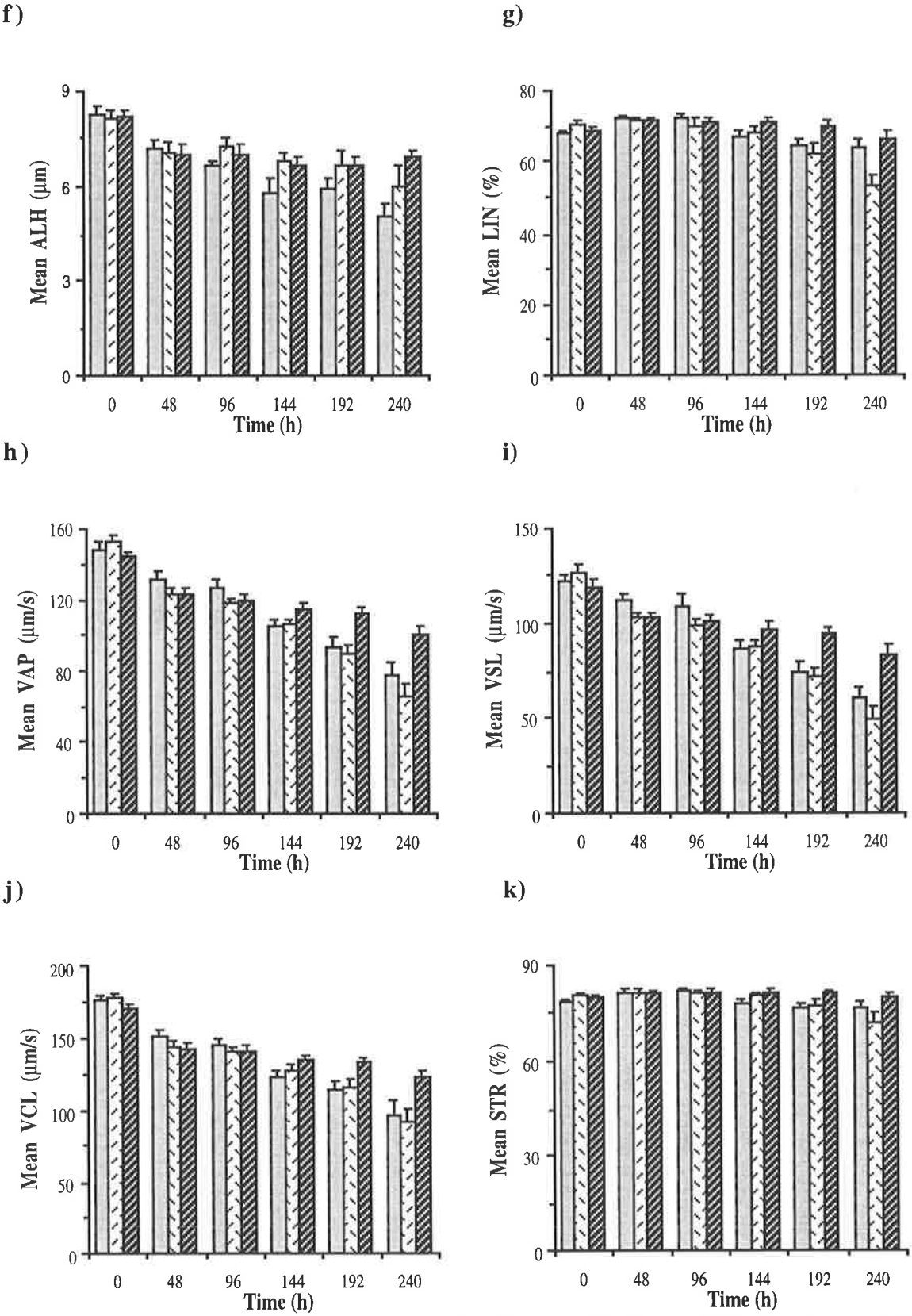
Figure 4.5. Slow (e) spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in hen yolk citrate glucose (□), coconut fluid quail yolk (▨) and coconut extract quail yolk (▩) diluents. Each value is the mean ± SEM of 12 observations.

There was a gradual decrease in mean ALH in all diluents during storage from 0 to 240h. Although samples diluted in CEQY had a higher mean ALH than that diluted in HYCG and CFQY diluent at 240h, the differences in mean ALH were not significant between these three diluents (Figures 4.5.f).

Percent mean LIN did not change much after storage for 192h. At 240h, mean LIN in CFQY was significantly lower ($p<0.05$) than in HYCG and CEQY, and the difference in mean LIN between HYCG and CEQY diluents, however, was not significant (Figures 4.5.g).

A small decrease in mean VAP, mean VSL and mean VCL were seen in all diluents during storage from 0 to 144h. The fall was greater in samples diluted in HYCG and CFQY than that diluted in CEQY, but the difference in these parameters was not significant during this time. However at 192 and 240h, CEQY diluent was found to be superior to HYCG and CFQY diluents in maintaining mean VAP, mean VSL and mean VCL (Figures 4.5.h,i and j).

Mean STR in all diluents remained stable after storage for 144h and then decreased slightly at 192 and 240h. The differences in mean STR between HYCG, QYCG or CEQY were not significant throughout storage period (Figure 4.5.k).



Figures 4.5. Mean ALH (f), mean LIN (g), mean VAP (h), mean VSL (i), mean VCL (j) and mean STR (k) of spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in hen yolk citrate glucose (□), coconut fluid quail yolk (▨) and coconut extract quail yolk (▩) diluents. Each value is the mean ± SEM of 12 observations.

4.2.4. Discussion

In this study, better maintainance of motility of spermatozoa was observed in CEQY diluent than in HYCG or CFQY diluent during storage at 5°C for 10 days. Better viability of ram spermatozoa in CEQY diluent than that in HYCG diluent was consistent with the results of previous studies. In studies in Section 4.1, since no different in motility of spermatozoa between HYCG and QYCG diluent was observed, the former diluent was used as standard as hen yolk is a more commonly used constituent. It is interesting to note that percent motile sperm in CFQY diluent was not better than that in HYCG diluent, even though the basic components of CFQY diluents were similar to that of CEQY diluent. This suggests that the coconut extract is the beneficial constituent of the CEQY medium, not the other factors not present in HYCG.

In the current study, progressive decline in motility of spermatozoa at the longer times of storage in CEQY extender is in agreement with the studies of Norman et al. (1962) who found a linear decrease of the percentage of motile bull spermatozoa over 10 days during storage at 5°C in coconut milk extender (CME). In contrast, this study differs from the findings of Prasad and Norman (1968) who found that CME was able to maintain 70% of the initial ram sperm population alive and motile for a week at room temperatures after a continuous storage period of one month at 5°C. This is probably due to the difference in the composition of the diluent. In their study, the addition of calcium nitrate (0.05%) and glycine (0.9%) in CME extender were able to compensate for the detrimental effect of sulfanilamide in this extender on the viability of spermatozoa kept at low temperature (5°C).

Though the addition of coconut extract in extenders for preservation of ram semen proved significantly beneficial compared with that of coconut fluid and citrate glucose diluents during prolonged incubation at refrigerated temperature, it is important to determine whether the improved motility was due to different starting pH or due to components or compounds in the coconut extract, or the different amount of yolk in the

citrate glucose and coconut extract based diluent. Thus, further study to clarify this finding was needed.

Chapter 5

Effect of initial pH of diluents on the viability of ram spermatozoa stored at room and cool temperatures

5.1. Introduction

During the development of a possible extender for use for storage of semen at room and cool temperatures as reported in the previous studies (Chapter 4, Section 4.1 and 4.2), it was observed that the pH of the two citrate-buffered semen diluents had different starting pH, i.e. citrate glucose diluent started at 7.6 and coconut extract diluent started at pH 7.0.

The pH of semen was found to vary for a given species, although the semen of the majority of mammals is close to neutral (Herman and Madden, 1987). Studies by Swanson and Herman (1944) on 295 samples of dairy bull semen indicate a pH range of 5.8 to 7.4 with an average of 6.3. For the ram, the pH of semen determined after collection varied between 5.9 and 7.3 with a modal value of 6.4 (Emmens, 1959). The pH of semen is also dependent on the ejaculate. Clamohoy and Palad (1967) found that the average pH of buffalo semen in the Phillipine was slightly lower in the first ejaculate than in the second one (6.58 v 6.86).

During storage, the metabolic activity of semen changes due to the effect of the accumulation of metabolic products (Herman and Madden, 1972; England, 1993), i.e. in the form of lactic acid that shifts the pH from alkaline to acidic. If these changes are allowed to persist the viability of spermatozoa may then decrease (Ranjhan and Pathak, 1993). It had also been suggested that the low pH in the cauda was one mechanism of immobilising sperm there (Carr et al, 1985). It was therefore possible that the greater decrease in motility in

citrate-glucose media could be due to a lower pH than in the coconut extract mixture, due either to the metabolism of glucose to lactic acid, or damage to the sperm due to the higher starting pH.

Therefore, as a first step the present study was undertaken to clarify further whether or not the apparent superiority of coconut extract diluent was due to the different starting pH.

5.2. Experimental procedure

Semen samples were obtained from three Merino rams 3.5 to 6.5 years of age. One ejaculate was collected from each ram by means of artificial vagina four times at two weekly intervals. After collection, the individual ejaculate was divided into four portions and each portion diluted five fold (1 semen : 4 diluent) with 4 different diluents at 30°C. Diluents used were sodium citrate-egg yolk based media as presented in Table 4.1 (Chapter 4, Section 4.1.2.). Diluent 1 and 2 were HYCG at pH 7.6 and at pH 7.0, respectively. Diluent 3 and 4 were CEQY at pH 7.6 and at pH 7.0, respectively. pH of diluents was adjusted with citric acid or sodium hydroxide and measurements were done using a digital pH meter (Activon, TPS, Brisbane) after calibration against standard buffer.

The diluted semen samples were placed in 5 ml clean glass tubes, sealed with plastic caps and stored in a water bath maintained at 30°C for room temperature storage. Samples stored at 5°C were cooled to this temperature in the cool room from 30°C over a period of 2 hours. Once the temperature of the water in the container dropped to 5°C, the semen was then assessed.

After various intervals of storage (0, 24 and 48h at 30°C; and 48h intervals up to 10 days at 5°C) the semen in the test tubes were assessed objectively for their motion characteristics.

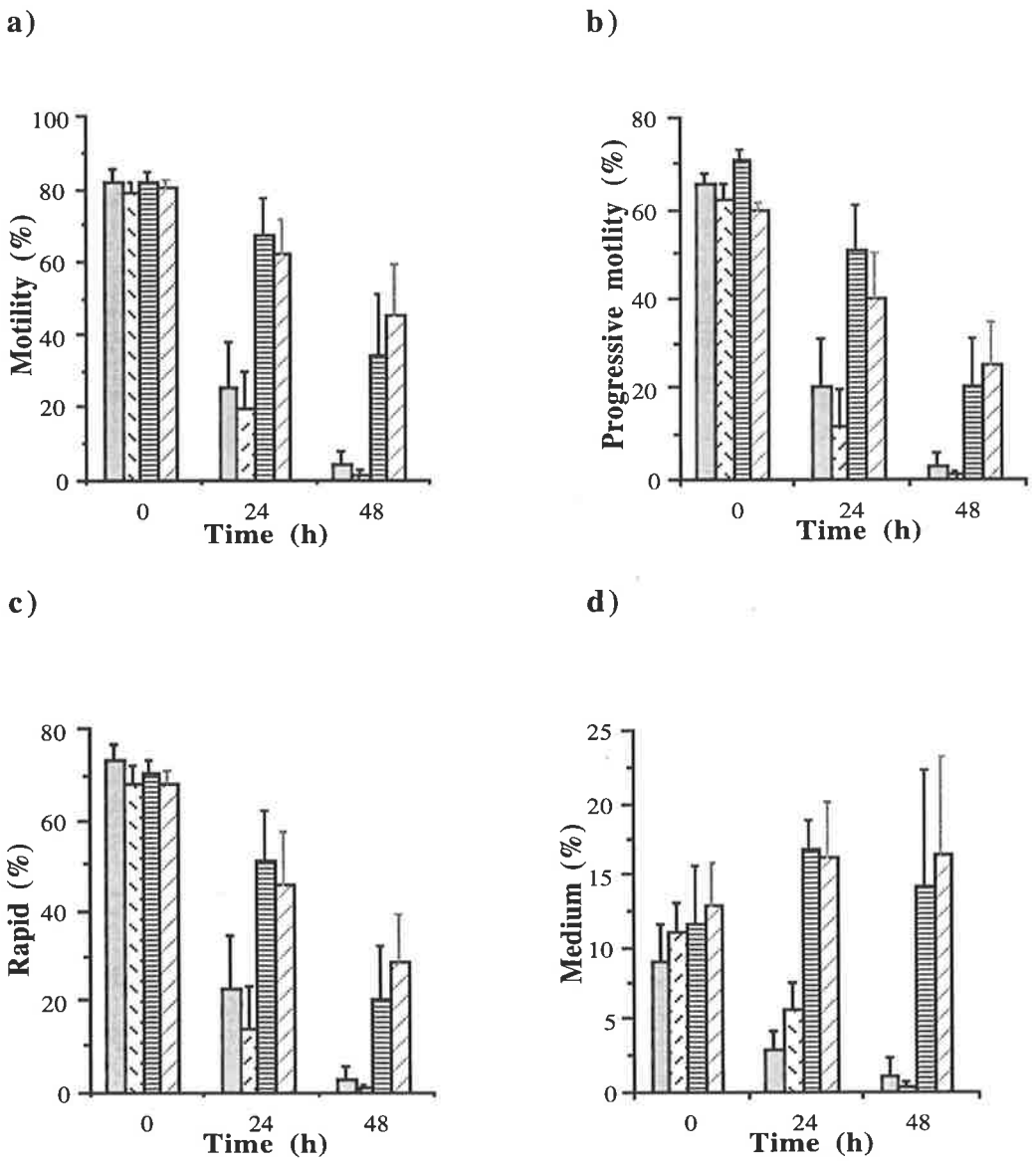
5.3. Results

There were no significant interactions between time of storage, pH and diluent for any of ram semen motion characteristics either stored at 30°C or 5°C. At 30°C, interaction between time and diluent was observed for motility, progressive motility, rapid, mean LIN and mean STR only; at 5°C the interaction between time and diluent was also found for medium, mean ALH and mean VCL. Levels of starting pH did not give any significant effect on the overall semen motion characteristics. Type of diluent produced an effect ($p<0.05$) on medium and mean ALH at 30°C, and on SLOW and mean VAP at 5°C. Time of storage at 30°C affected medium, mean ALH, mean VAP and mean VSL, but at 5°C effects on SLOW and mean VAP were not significant.

The Mean values with SEM for all ram semen motion characteristics in all the 4 diluents at different hours of preservation at 30°C are presented in Table 5.1.(Appendix A).

The percentages of motility, progressive motility and rapid spermatoza from samples stored in HYCG (pH 7.6 or 7.0) and CEQY (pH 7.6 or 7.0) decreased as the time of storage increased. However, this decrease was maximum in both HYCG (pH 7.6 or 7.0) compared with CEQY (pH 7.6 or 7.0). The differences were significant ($p<0.05$) at 24h and 48h storage (Figures 5.1.a,b and c).

There was a gradual decrease in percent medium sperm from 0 to 48h preservation in semen stored in HYCG (pH 7.6 or 7.0) and an increase in medium was observed from 0 to 24h from samples stored in CEQY (pH 7.6 or 7.0)(Figure 5.1.d).



Figures 5.1. Motility (a), progressive motility (b), rapid (c) and medium spermatozoa after storage for 0, 24 and 48h at 30°C in HYCG at pH 7.6 (□) or at pH 7.0 (□); and in CEQY at pH 7.6 (▤) or pH 7.0 (□) diluents. Values are Means ± SEM of 12 observations.

A small difference between the 4 diluents in the percentage of slow spermatozoa was observed. The percentage of slow in HYCG at pH 7.0 increased more rapidly compared with the other three diluents during preservation from 0 to 24h. However, the percentage of slow in CEQY with pH 7.6 was the lowest after storage for 48h. The differences with the other three diluents were not statistically significant (Figure 5.1.e).

e)

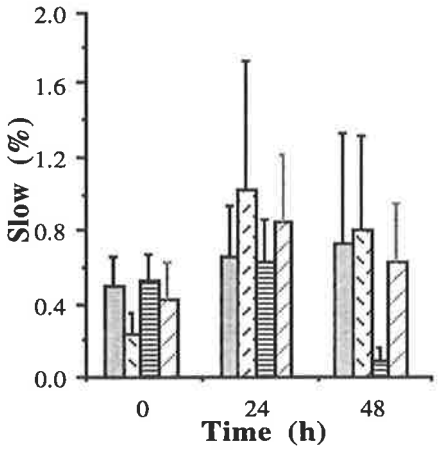
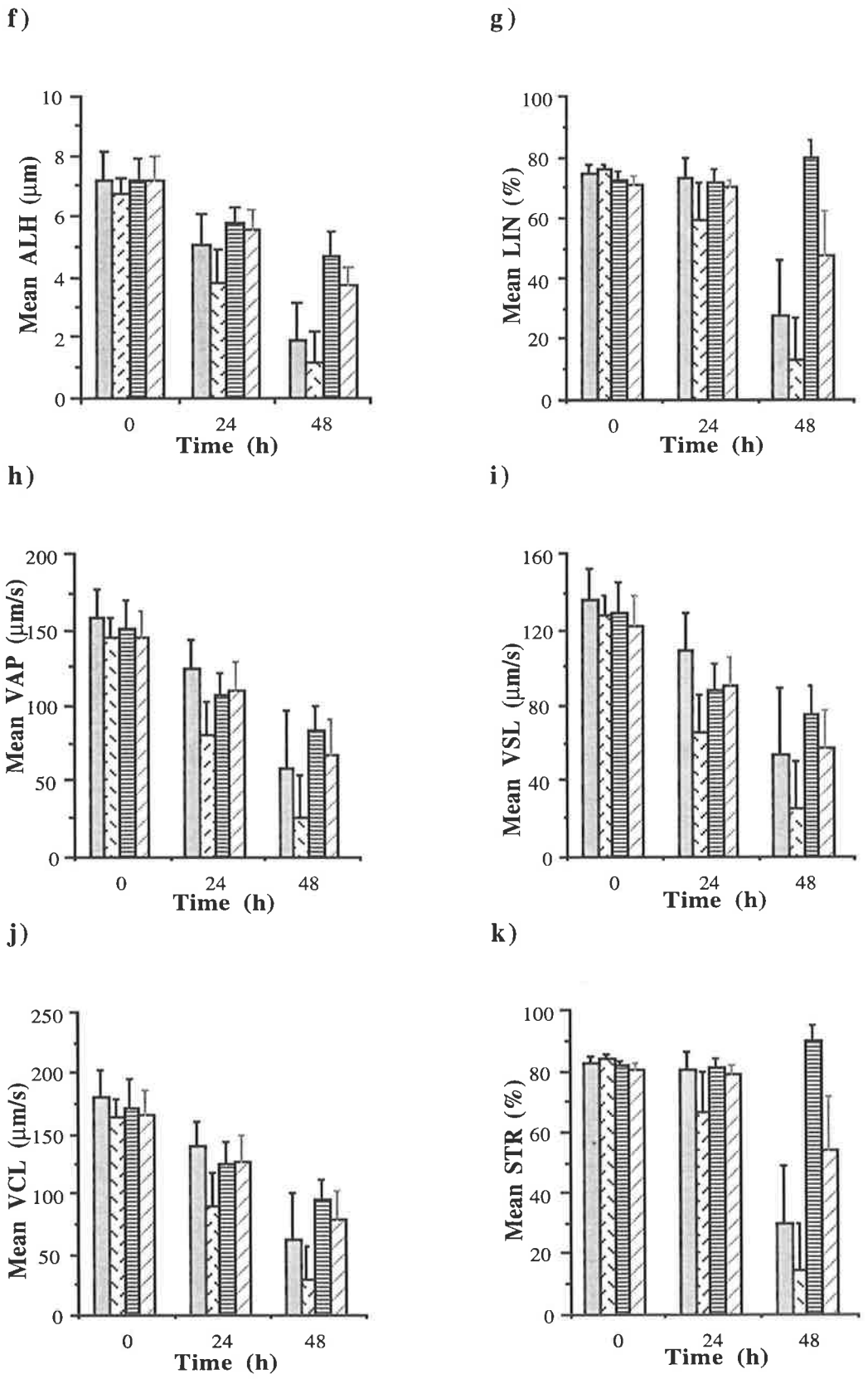


Figure 5.1. Slow (e) spermatozoa after storage for 0, 24 and 48h at 30°C in HYCG at pH 7.6 (▩) or at pH 7.0 (▩); and in CEQY at pH 7.6 (▤) or pH 7.0 (□) diluents. Values are Means ± SEM of 12 observations.

Mean ALH, LIN, VAP, VSL and VCL decreased at the same rate among the 4 diluents from 0 to 24h, but after 48h the value dropped more rapidly in samples stored in HYCG (pH 7.6 or 7.0) than in samples stored in CEQY (pH 7.6 or 7.0)(Figures 5.1.f,g,h,i and j). The differences in these semen motion characteristics between these 2 diluents were significant ($p<0.05$) at 48 h.

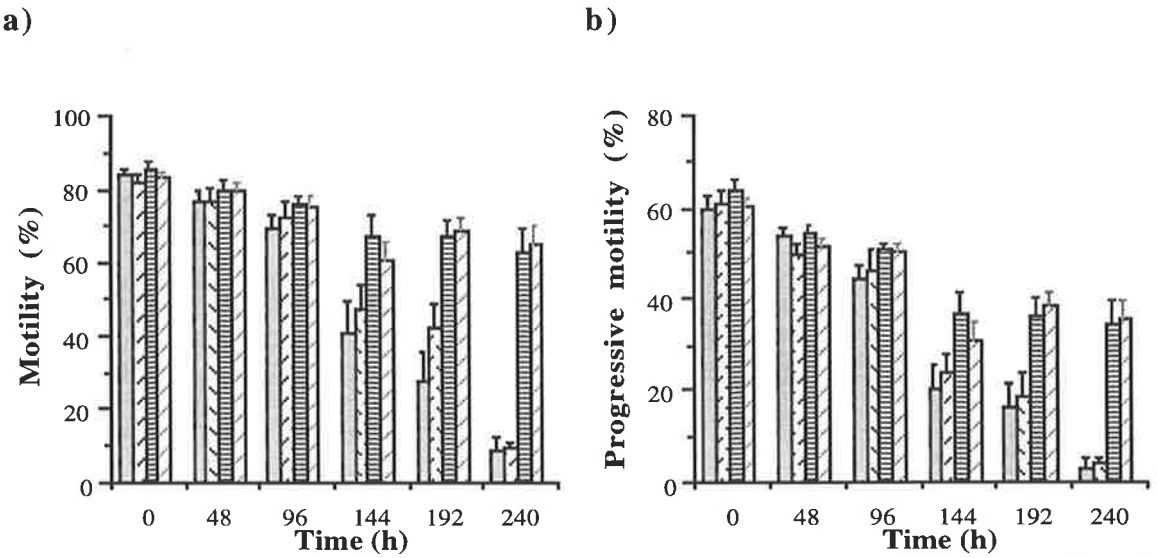
There was not much difference in the percentage of mean STR in the 4 diluents after storage for 24h; but it had decreased abruptly in HYCG (pH 7.6 or 7.0) after 48 h storage. A drop in the mean STR in CEQY at pH 7.0 was observed, but the mean STR at pH 7.6 increased slightly during that time. The differences between these 2 diluents were highly significant ($p<0.05$)(Figure 5.1.k) at 48h of storage.



Figures 5.1. Mean ALH (f), mean LIN (g), mean VAP (h), mean VSL (i), mean VCL (j), and mean STR (k) of spermatozoa after storage for 0, 24 and 48 h at 30°C in HYCG at pH 7.6 (□) or at pH 7.0 (□); and in CEQY at pH 7.6 (▤) or pH 7.0 (▨) diluents. Values are Means ± SEM of 12 observations.

Table 5.2. (Appendix A) shows the Mean values with SEM for all ram semen motion characteristics in all the 4 diluents at different hours of preservation at 5°C.

There was no immediate adverse effect of any diluent on motility, progressive motility and rapid spermatozoa, mean VAP, mean VSL and mean VCL. However, an effect became apparent as the storage period advanced beyond 144h and it was more apparent in HYCG (pH 7.6 or 7.0). Motility, progressive motility, rapid spermatozoa and mean VCL remained at a higher level in CEQY (pH 7.6 or 7.0) than in the HYCG (pH 7.6 or 7.0). The differences between these two sets of diluents were significant ($p<.005$) at 144 to 240h of storage (Figures 5.2.a,b,c,h,i and j).

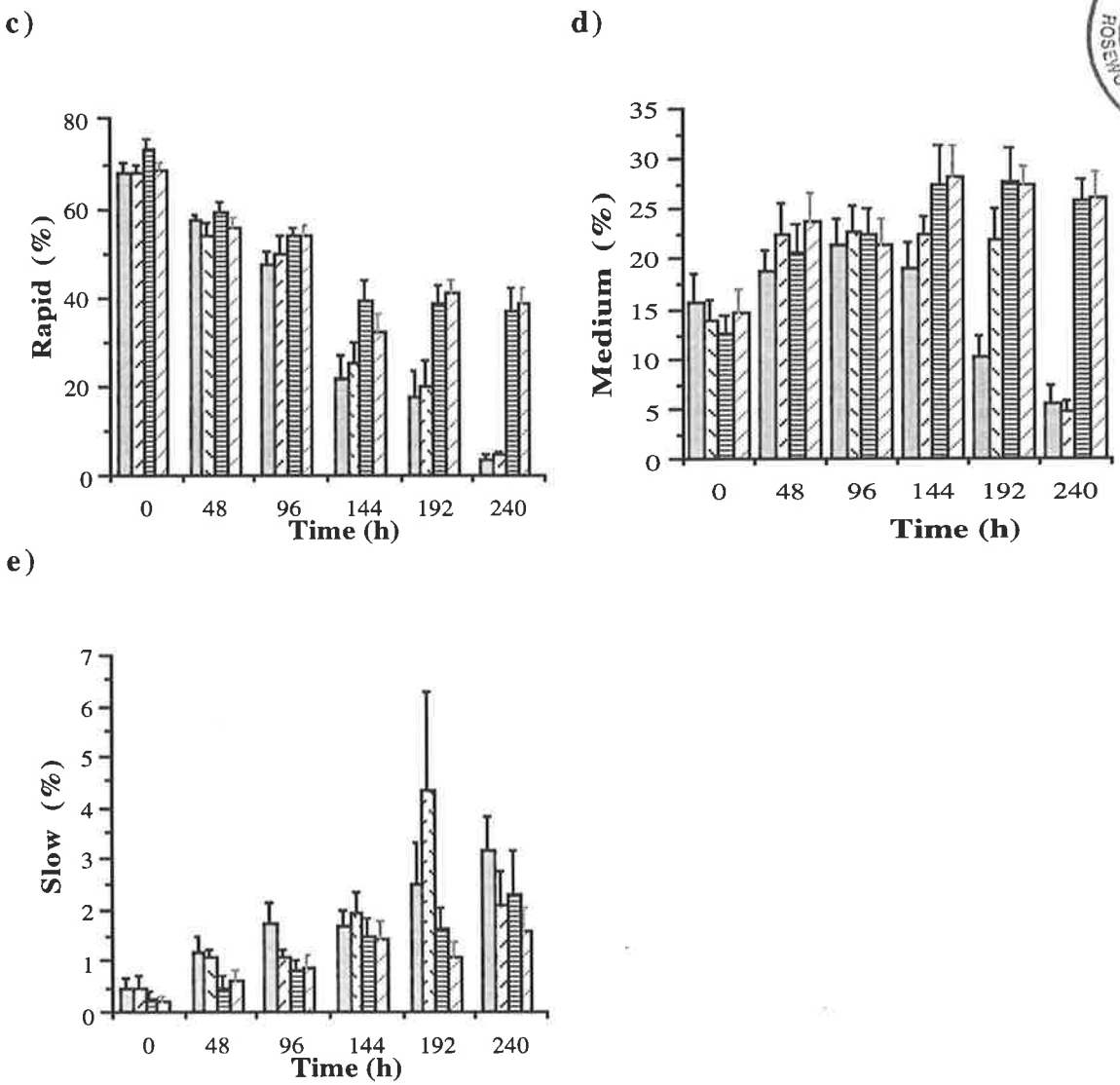


Figures 5.2. Motility (a) and progressive motility of spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in HYCG at pH 7.6 (□) or at pH 7.0 (▨); and in CEQY at pH 7.6 (▤) or pH 7.0 (▨) diluents. Values are Means ± SEM of 12 observations.

The percentage of medium spermatozoa varied between diluents and between the times of storage. The differences between HYCG (pH 7.6 or 7.0) and CEQY (pH 7.6 or 7.0) were significant ($p<0.05$) at 144, 192 and 240h storage (Figure 5.2.d).

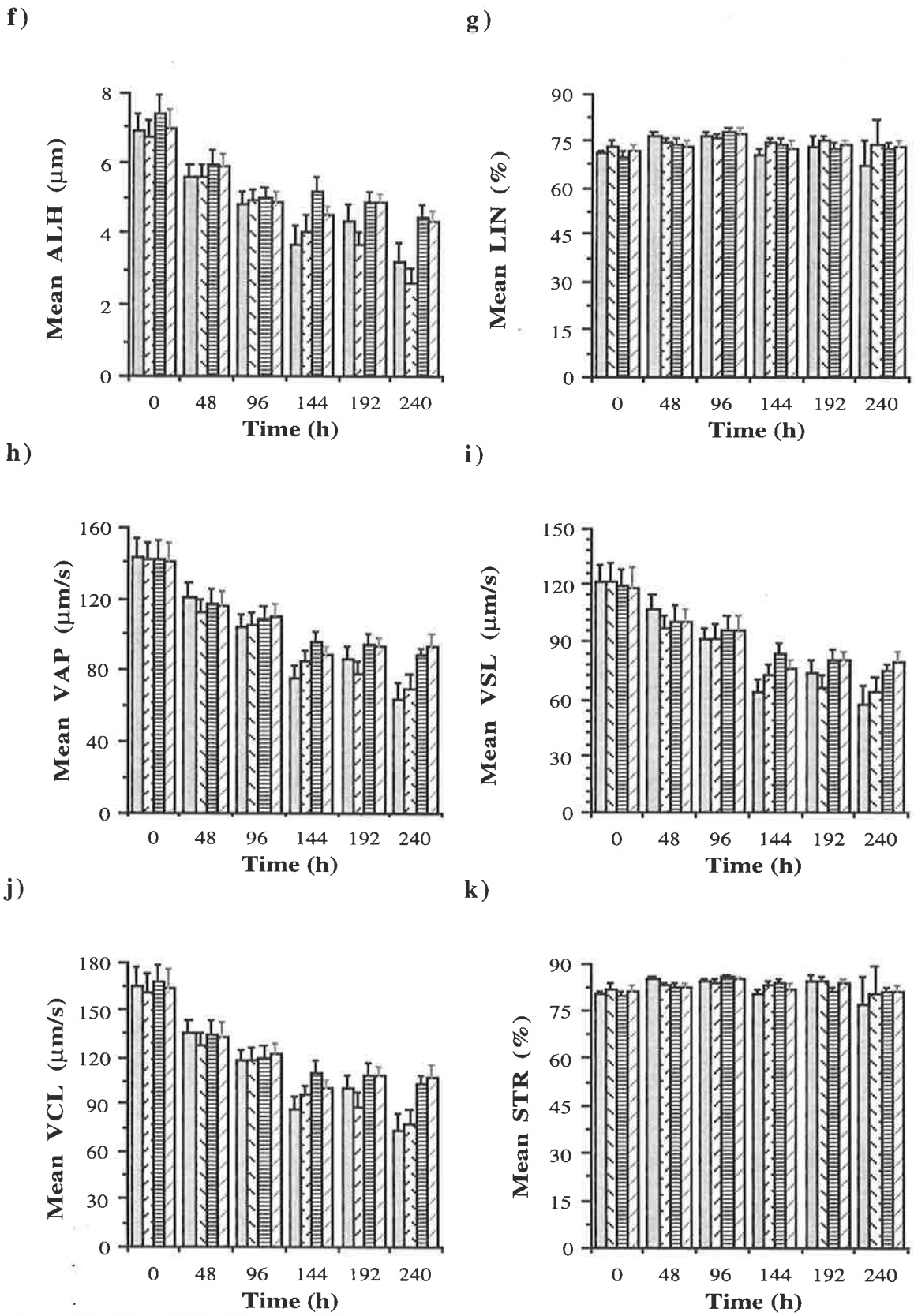
Increases in the percentage of slow spermatozoa observed in the CEQY (pH 7.6 or 7.0) were not obvious at all storage periods. However a rise in the slow became evident in HYCG (pH 7.6 or 7.0) up to 192h storage, then the values fell again at 240h. The

differences in slow between these two sets of diluents were significant ($p<0.05$) (Figure 5.2.e).



Figures 5.2. Rapid (c), medium (d) and slow (e) of spermatozoa after storage for 0, 48, 96, 144, 192 and 240h at 5°C in HYCG at pH 7.6 (□) or at pH 7.0 (▨); and in CEQY at pH 7.6 (▤) or pH 7.0 (▨) diluents. Values are Means ± SEM of 12 observations.

A gradual decrease in mean ALH was observed as the time of storage increased from 0 to 240h. The decrease was less pronounced in semen stored in CEQY (pH 7.6 or 7.0) than in HYCG (pH 7.6 or 7.0) after 240h storage. The difference between these 2 diluents was significant ($p<0.05$) (Figure 5.2.f). No significant differences ($p>0.05$) in mean LIN and mean STR were observed in semen diluted in HYCG (pH 7.6 or 7.0) or CEQY (pH 7.6 or 7.0) during the entire preservation period (Figures 5.2.g and k).



Figures 5.2. Mean ALH (f), mean LIN (g), mean VAP (h), mean VSL (i), mean VCL (j), and mean STR (k) of spermatozoa after storage for 0, 48 96, 144, 192 and 240h at 5°C in HYCG at pH 7.6 (■) or at pH 7.0 (□); and in CEQY at pH 7.6 (▤) or pH 7.0 (▨) diluents. Values are Means ± SEM of 12 observations.

5.4. Discussion

The study shows that the different starting pH did not affect markedly the viability of ram spermatozoa, indicating that they could tolerate a slight degree of alkalinity. Blackshaw and Emmens (1951) suggested that spermatozoa of the ram showed an optimal motility at pH 6.9 - 7.1, but motility was only slightly reduced when the pH was raised to 8.3 to 8.9.

The effectiveness of CEQY diluents in preserving ram spermatozoa stored at room or cool temperatures for various periods of storage was again consistent with the previous study (Chapter 4, Section 4.1 and 4.2). The relatively greater loss of sperm viability on storage at 30°C than at 5°C either in HYCG (pH 7.6 or 7.0) or CEQY (pH 7.6 or 7.0) observed after 24h was probably due to the higher rate of metabolism of the diluted semen at the higher temperature, and consequently greater cumulative adverse effect of metabolites in the diluent that may produce an acidic pH, although, in the present study the pH value during storage was not measured. Upreti et al (1992) observed a decrease in motility of undiluted spermatozoa after incubating at 35°C for up to 60 minutes. A decrease was also observed by Nass-Arden and Breitbart (1990) and attributed to the acidification of semen. Conversely, the ability of ram spermatozoa to survive better during storage at 5°C observed after 144h could be because at the lower temperature there is a lower cumulative adverse effect of metabolites.

In conclusion, it can be stated that no effect of two levels of initial pH on the viability of ram spermatozoa was evident at any storage interval or temperature. Under the conditions of this study, ram semen and diluents used could have had some buffering capacity towards the alkaline side. Therefore, a study to clarify what components or compounds in the coconut extract that gave beneficial effect on the motility characteristics of spermatozoa seemed the next logical step.

Chapter 6

Viability of ram spermatozoa in egg yolk-citrate based diluent, with or without addition of glucose, enzyme additives, sulfanilamide and coconut extract

6.1. Introduction

It has been indicated in the previous experiments that different starting pH levels either 7.6 or 7.0 in HYCG and CEQY diluents had no effect on sperm motility after storage at 30°C for up to 48h or after cooling to 5°C and storage for up to 240h. Thus, it could be assumed that the higher sperm motility observed in semen diluted in CEQY extender was probably due to the effect of coconut extract or due to the combination of the other constituents included in this diluent. It might be that coconut extract contained a fortuitous mixture of nutrients which was beneficial to sperm or contained compounds with special properties which have not been previously considered. This assumption needed to be clarified in a further experiment.

Studies on the preservation of spermatozoa at ambient or room and cool temperatures have involved egg yolk-citrate diluents to which had been added chemically defined salt, sugars and other non electrolytes (Foote et al, 1962). The addition of small amounts of glucose to a yolk buffer diluent has been found to be beneficial for the motility of bovine spermatozoa during storage at cool temperatures (Lardy and Phillips, 1942; Salisbury and Van Demark, 1945; Salisbury, 1946; Milovanov and Sokolovskaja, 1957). Inclusion of catalase in a diluent has been found to be effective in protecting sperm from oxygen damage. Norman et al (1962) included catalase in coconut milk extender for preservation of bull semen and found that fertilizing capacity of spermatozoa was retained up to 7 days at 18 to 30°C. Motility of bull spermatozoa also improved after storage for 12 days at 5°C in diluent containing this enzyme (Foote and Dunn, 1962). In ram, Stojanov et al (1994) showed that the

survival and fertilizing capacity of spermatozoa were enhanced after catalase was included in the diluent. Other enzyme additives such as mycostatin have also been incorporated in the diluent for liquid storage of spermatozoa in order to prevent the growth of fungi, especially when coconut milk extender was used (Norman et al, 1962; Norman et al, 1968; Prasad and Norman, 1968; Pillai et al, 1978; Pillai and Iyer, 1982; Islam, 1986).

Sulfanilamide, penicillin and streptomycin are commonly utilized in diluents. Foote and Bratton (1950) reported that antibacterial agents improved sperm survival at room temperature. The opportunity for bacteria to grow in the diluted semen kept for longer periods even at reduced temperature was considerable unless antibiotics were present in the diluent (Jasko et al, 1993). A combination of penicillin and streptomycin has been shown effectively to control bacterial growth in diluted bull semen (Almquist et al, 1949).

In this chapter, the effect of each of the individual constituents of the coconut extract diluent has been examined.

6.2. Experimental procedure

6.2.1. Semen collection and dilution

Two ejaculates were collected from each of three trained Merino rams by artificial vagina. Semen samples showed an initial individual motility of 80% and mass motility greater than +4 (0 to 5 score) were selected and pooled for this study. Each experiment was done three times in three consecutive weeks. Semen was diluted at ratio 1:4 (semen:diluent). Diluents used were citrate-based medium plus antibiotics (dihydrostreptomycin sulphate and penicillin) containing 10% egg yolk. The concentrations of sodium citrate and egg yolk were chosen to be intermediate between Salamon's standard diluent and Norman's extender as used in the previous experiments.

6.2.2. Semen assessment

Semen was assessed objectively at 0h (initial storage), then at 24 and 48h for samples stored at 30°C. For samples stored at 5°C, an objective assessment was done at 0, 72, 144 and 216 hours of storage.

6.3. Experiment 6.1

This experiment was designed to investigate the viability of ram spermatozoa in citrate-based diluents, when other constituents of coconut extract diluent were included. Two ejaculates from each of three Merino rams were collected and pooled in three consecutive weeks. Each pooled semen samplewas split into 15 aliquots, the first eight aliquots were prepared for 30°C and the second seven aliquots were prepared for 5°C storage. Each of eight and seven aliquots were diluted in eight or seven different diluents, respectively. The composition of the diluents used in Experiment 1 either for storage at 30 or 5°C is presented in Table 6.1.

Table 6.1. Composition of egg yolk citrate (EYC)-based diluents

Ingredient		Diluent							
		EYC	EYC +G	EYC +NC	EYC +NS	EYC +SC	EYC +NSC	EYC +NSCG	EYC +S
Sodium citrate	(g)	2.265	2.265	2.265	2.265	2.265	2.265	2.265	2.265
Streptomycinsulphate	(g)	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Penicillin	(g)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Nystatin	(units)	-	-	400	400	-	400	400	-
Sulfanilamide	(g)	-	-	-	0.3	0.3	0.3	0.3	0.3
Catalase	(units)	-	-	15000	-	15000	15000	15000	-
Glucose	(g)	-	0.8	-	-	-	-	0.8	-
Hen yolk	(ml)	10	10	10	10	10	10	10	10
Double distilled water to	(ml)	100	100	100	100	100	100	100	100
Osmolality	(mOsm/kg)	259	269	257	269	277	267	331	278

The eight diluent treatments were 1) egg yolk citrate (EYC) alone/control, 2) EYC + glucose (+G), 3) EYC + nystatin + catalase (+NC), 4) EYC + nystatin +

sulfanilamide (+NS), 5) EYC + sulfanilamide + catalase (+SC), 6) EYC + nystatin + sulfanilamide + catalase (+NSC), 7) EYC + nystatin + sulfanilamide + catalase + glucose (+NSCG) and 8) EYC + sulfanilamide (+S). These eight diluents were included for storage at 30°C, while for storage at 5°C, only +S diluent was not included.

6.4. Experiment 6.2

Based on Experiment 6.1, Experiment 6.2 was undertaken to confirm changes in sperm motion characteristics at 30 and 5°C as influenced by extension in egg yolk citrate-based diluent alone or in mixtures of egg yolk citrate with combination of enzyme additives, sulfanilamide alone or combination of additives and sulfanilamide. Glucose was excluded in this experiment due to its harmful effect observed in Experiment 6.1.

For three consecutive weeks, two ejaculates from each of three Merino rams were collected and pooled. Each pooled sample was split into 8 aliquots. The first four aliquots were prepared for storage at 30°C, each of which was diluted in four different diluents. The other four were prepared for storage at 5°C and each of them was diluted also in the four diluents. The composition of the diluents used in Experiment 2 either for storage at 30 or 5°C is indicated in Table 6.2. The osmolality of the diluents used in this experiment was not measured.

The 4 diluent treatments were 1) egg yolk citrate (EYC) alone/control, 2) EYC + nystatin + catalase (+NC), 3) EYC + nystatin + sulfanilamide + catalase (+NSC), 4) EYC + sulfanilamide (+S).

Table 6.2. Composition of egg yolk citrate (EYC)-based diluents.

Ingredient		Diluent			
		EYC	EYC +NC	EYC +NSC	EYC +S
Sodium citrate	(g)	2.265	2.265	2.265	2.265
Streptomycin sulphate	(g)	0.100	0.100	0.100	0.100
Penicillin	(g)	0.06	0.06	0.06	0.06
Nystatin	(units)	-	400	400	-
Sulfanilamide	(g)	-	-	0.3	0.3
Catalase	(units)	-	15000	15000	-
Hen yolk	(ml)	10	10	10	10
Double distilled water to	(ml)	100	100	100	100

6.5. Experiment 6.3

As motility and progressive motility of spermatozoa in Experiment 6.2 in the four diluents tested were not significantly different, Experiment 6.3 was designed to further examine the viability of ram semen in these four diluents without coconut extract and with coconut extract. In this experiment, the percentages of live and normal spermatozoa were also recorded to determine whether or not the inclusion of coconut extract had a beneficial effect on these characteristics.

Two ejaculates from each of three Merino rams were collected and pooled in three consecutive weeks. Each pooled semen sample was split into 16 aliquots. Eight of these aliquots were prepared for 30°C storage, each of which was diluted in eight different diluents. The remaining aliquots were prepared for 5°C storage, each of which was also diluted in the eight diluents. The composition of the diluents used in Experiment 6.3 either for storage at 30 or 5°C is presented in Table 6.3. The osmolality of the diluents used in this experiment was not measured.

Table 6.3. Composition of egg yolk citrate (EYC)-based diluents

Ingredient		Diluent							
		EYC	EYC +NC	EYC +NSC	EYC +S	EYC +CE	EYC +NC +CE	EYC +NSC +CE	EYC +S +CE
Sodium citrate	(g)	2.265	2.265	2.265	2.265	2.265	2.265	2.265	2.265
Streptomycin sulphate	(g)	0.100	0.100	0.01	0.100	0.100	0.100	0.100	0.100
Penicillin	(g)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Nystatin	(units)	-	400	400	-	-	400	400	-
Sulfanilamide	(g)	-	-	0.3	0.3	-	-	0.3	0.3
Catalase	(units)	-	15000	15000	-	-	15000	15000	-
Coconut extract	(ml)	-	-	-	-	10	10	10	10
Hen yolk	(ml)	10	10	10	10	10	10	10	10
Double distilled water to	(ml)	100	100	100	100	100	100	100	100

The 8 diluent treatments were 1) egg yolk citrate (EYC) alone/control, 2) EYC + nystatin + catalase (+NC), 3) EYC + nystatin + sulfanilamide + catalase (+NSC), 4) EYC + sulfanilamide (+S), 5) egg yolk citrate + coconut extract (+CE), 6) EYC + nystatin + catalase + coconut extract (+NC+CE), 7) EYC + nystatin + sulfanilamide + catalase + coconut extract (+NSC+CE), 8) EYC + sulfanilamide + coconut extract (+S+CE).

6.6. Results

The results of this study are summarised as follows:

Experiment	Temperature (°C)	Interaction diluent x time	Effect of diluent	Effect of time
6.1	30	Non significant for any of motion characteristics	Non significant for any of motion characteristics	Significantly ($p<0.001$) affected motility characteristics
	5	Significant ($p<0.001$) for all motion characteristics	Significantly ($p<0.001$) affected semen motion characteristics, except slow sperm	Significantly ($p<0.001$) affected motility characteristics
6.2	30	Non significant for any of motion characteristics	Non significant for any of motion characteristics	Significantly ($p<0.001$) affected semen motion characteristics, except for medium sperm and mean STR. For LIN ($p=0.003$)
	5	Non significant for any of motion characteristics	Non significant for any of motion characteristics, except for VAP ($p=0.002$), VCL ($p=0.004$)	Significantly ($p<0.001$) affected semen motion characteristics, except for slow sperm ($p=0.002$)
6.3	30	Non significant for any of motion characteristics	Significantly ($p<0.001$) affected motility, progressive motility, rapid sperm, mean VCL. For mean VAP ($p=0.002$)	Significantly affected motility characteristics, live and dead sperm ($p<0.001$) and normal sperm ($p=0.002$)
	5	Non significant for any of motion characteristics	Non significant for any of motion characteristics	Significantly ($p<0.001$) affected semen motion characteristics, except slow sperm

6.6.1. Experiment 6.1

6.6.1.1. Preservation at 30C

The effect of diluent and time of storage on the motion characteristics of ram semen diluted in a various combinations of egg-yolk citrate-based diluent stored at 30°C is summarized in Table 6.4. (Appendix A).

The mean percentages of motile sperm in all diluents were above 75% at 0h. At 24h, motilities of spermatozoa in +NSC and +NSCG were higher than that in the other 6 diluents. At 48h, motility had fallen to below 25% and the lowest value was recorded in +G. The difference in motile sperm between semen diluted in +G and in EYC diluent was significant ($p<0.05$) at this time. The overall difference in motile sperm between 0 and 24h was not significant, however, the difference between 0 or 24 and 48h was significant ($p<0.05$)(Figure 6.4.a).

a)

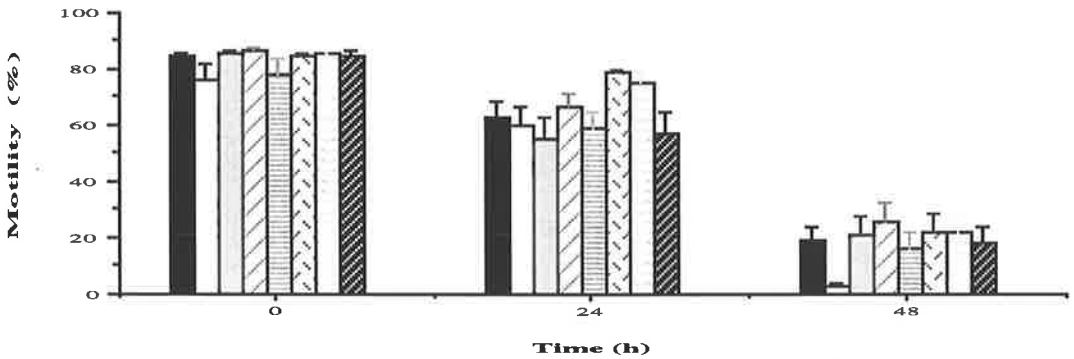
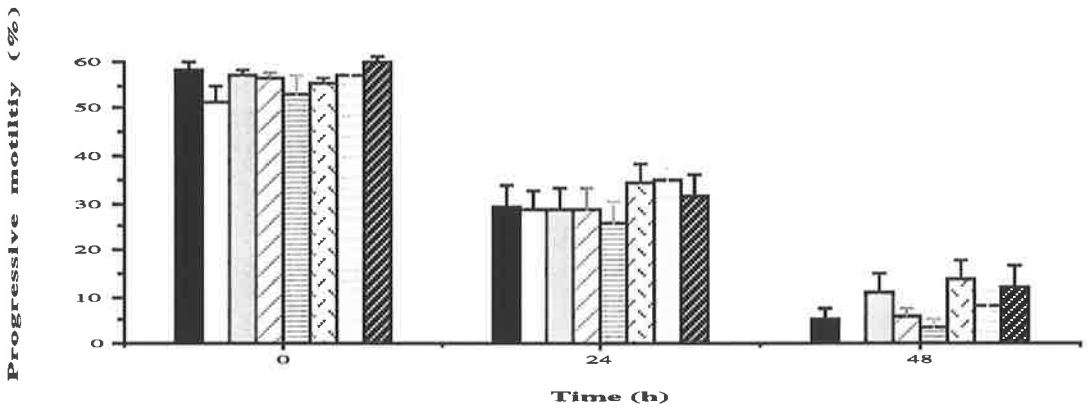


Figure 6.4. Motile (a) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (▨), + sulfanilamide + catalase (▤), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□), + sulfanilamide (▨) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

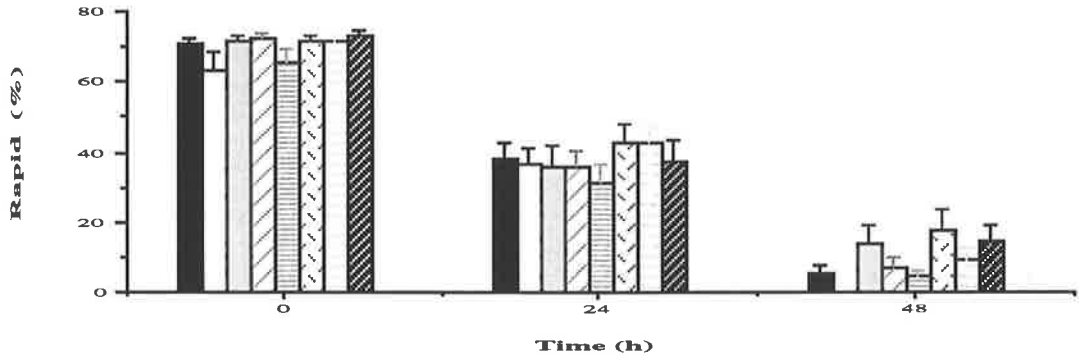
A gradual decrease in the percentages of progressive motile and rapid sperm was observed throughout the incubation period. The means at 0h ranged from 50 to 60% for progressive motile and from 60 to 70% for rapid sperm. Decrease in these parameters became obvious after 48h of storage and reached minimum values of less than 0.50% in +G. During this time, the difference in these parameters between semen diluted in +G and in EYC diluent was significant ($p<0.05$). There were significant

($p<0.05$) differences in this parameters between 0 and 24 or 48h; and between 24 and 48h were significant (Figures 6.4.b and c).

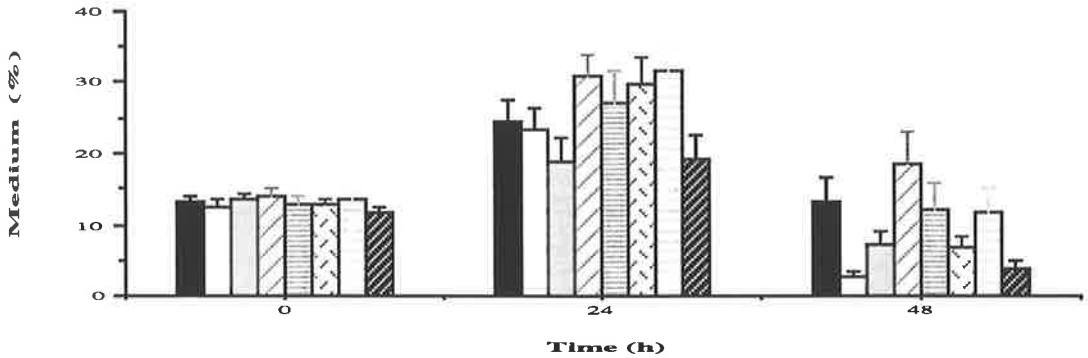
b)



c)



d)



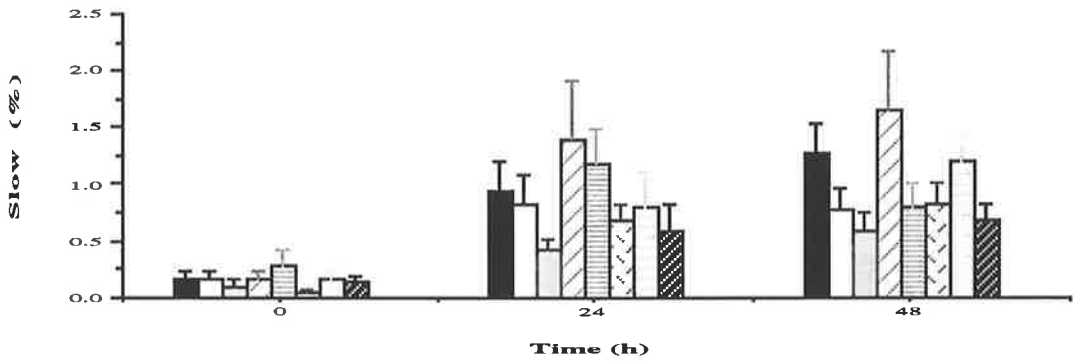
Figures 6.4. Progressive motile (b), rapid (c) and medium (d) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□), + sulfanilamide (▨) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

The mean percentages of medium sperm in all diluents were almost the same during initial storage (between 11 to 14%). At 24h, the values increased to between 18 and 31%, then began to fall to below 20% at 48h with the lowest value again recorded in +G. Although this value numerically lower than values of the other media, the

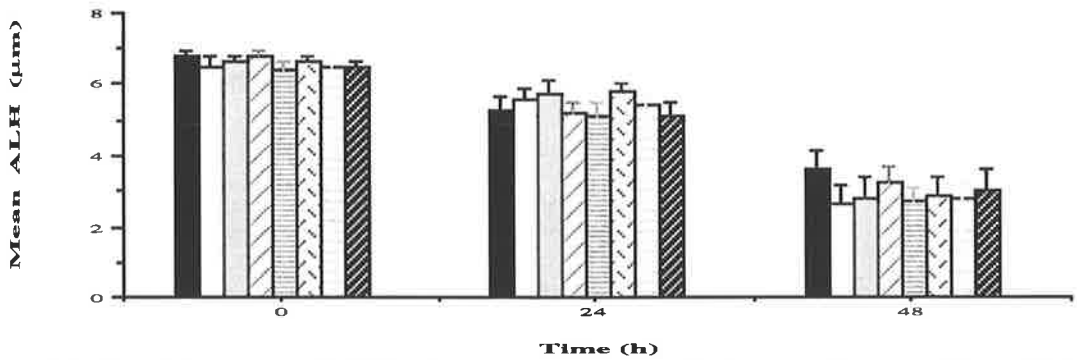
differences were not statistically different. However, there was a significant ($p<0.05$) difference in medium sperm in all diluents between samples stored at 24 and 48h . The difference in medium sperm between samples stored for 24 and 0h was only observed in +NSCG, +NS and +NSC (Figure 6.4.d).

In all diluents, the proportion of slow sperm was below 0.3% at 0h, then increased to between 0.41 and 1.39% at 24h. At 48h, the values rose again to between 0.58 and 1.64%. The differences in slow sperm between samples stored for 0 and 24 or 48h were significant ($p<0.05$)(Figure 6.4.e).

e)



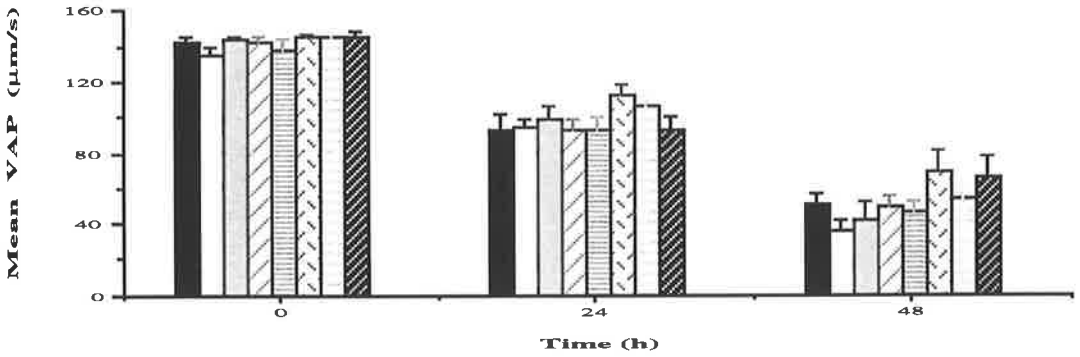
f)



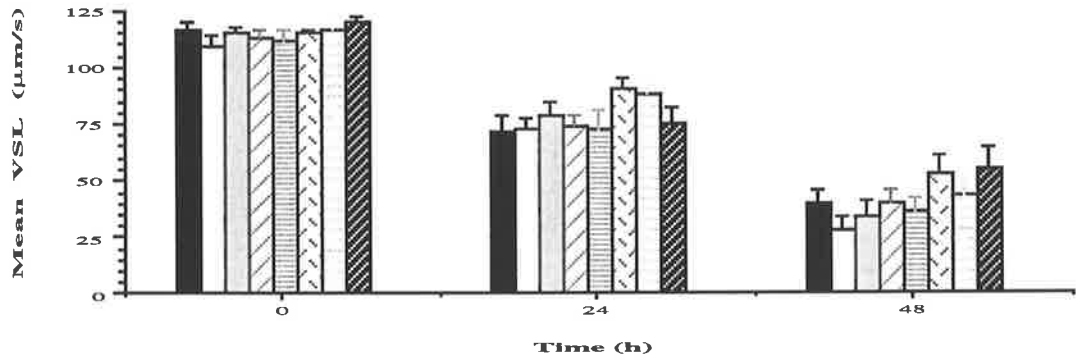
Figures 6.4. Slow (e) and mean ALH (f) of spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (▤), + nystatin + sulfanilamide + catalase (▧), + nystatin + sulfanilamide + catalase + glucose (□), + sulfanilamide (▨) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

Mean ALH from samples diluted in the 8 diluents ranged from 6.41 to 6.80 µm at 0h, then decreased slightly at 24h. Mean ALH continued to decrease until reaching a minimum value of 2.66 µm in +G. The difference in ALH between samples stored at 0 and 24h was not significant, however, it differed significantly ($p<0.05$) between samples incubated for 0 or 24 and for 48h (Figure 6.4.f).

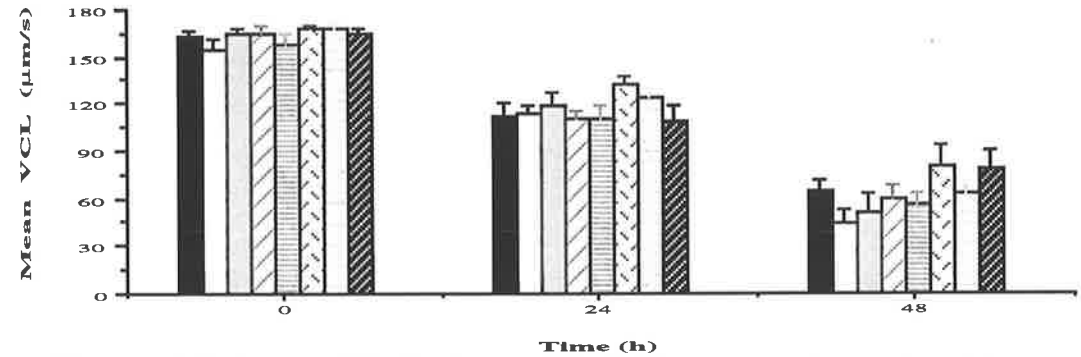
h)



i)



j)



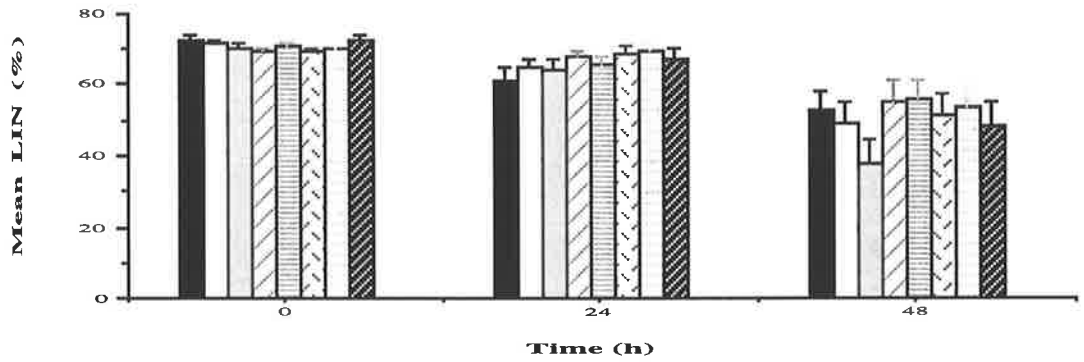
Figures 6.4. Mean VAP (h, mean VSL (i) and mean VCL (j) of spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (▤), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□), + sulfanilamide (▨) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

At 0h, mean VAP, mean VSL and mean VCL in all diluents ranged from 137.20 to 145.73 µm/s, 109.73 to 120.19 µm/s and 153.86 to 167.49 µm/s, respectively. At 24h, +NSCG and +NSC were superior to the other diluents in maintaining these kinematic parameters. At 48h, again +NSC and also +S were better than the other diluents in sustaining these three parameters. However, the differences in mean VAP, mean VSL and mean VCL among all diluents either at 24 or 48h were not significant.

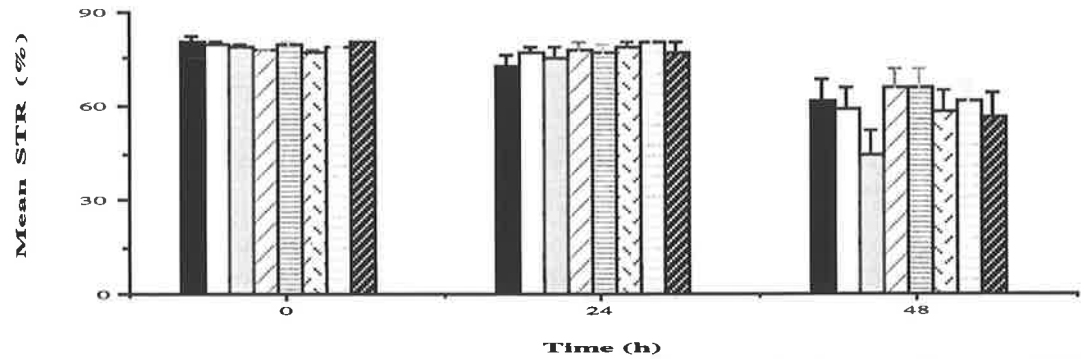
However, they differed ($p<0.05$) between samples stored for 0 and 24 or 48h; and also differed ($p<0.05$) between samples incubated for 24 and 48h (Figures 6.4.h,i and j).

The percentages of LIN and STR in all diluents remained high after 24h storage, and the value for LIN in all diluents ranged from 61 to 70% and for STR ranged from 72 to 80%. However, at 48h mean LIN and STR in +NC had decreased markedly, although the differences in these parameters between +NC and the other 7 diluents at 48h were still not significant. There was, however, a significant ($p<0.05$) difference in mean LIN and STR for all diluents between 0 or 24 and 48h (Figures 6.4.g and k).

g)



k)



Figures 6.4. Mean LIN (g) and mean STR (k) of spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□), + sulfanilamide (□) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

6.6.1.2. Preservation at 5°C

The effect of diluent and time of storage on the motion characteristics of ram spermatozoa diluted in a various combinations of egg-yolk citrate-based diluent and stored at 5°C is presented in Table 6.5 (Appendix A).

The mean percentages of motile spermatozoa in the 7 diluents remained high after storage for 72h. As time of storage advanced to 144h, motility of sperm in +NSCG, +G and +NC fell appreciably. Then by 216h of storage, motility of spermatozoa in these diluents dropped to less than 10%. In contrast, +EYC, +NS, +SC and +NSC were able to maintain the motility of spermatozoa around 70% at 144h, although by 216h the values had decreased to around 60 to 66%. The +NSCG had higher ($p<0.05$) motile sperm than that of +G or +NC at 144h. However, no significant difference among these three diluents was observed at 216h. Motility of spermatozoa in +EYC, +NS, +SC and +NSC differed significantly ($p<0.05$) from that of +NSCG, +G and +NC both at 144 or 216h of storage (Figure 6.5.a).

a)

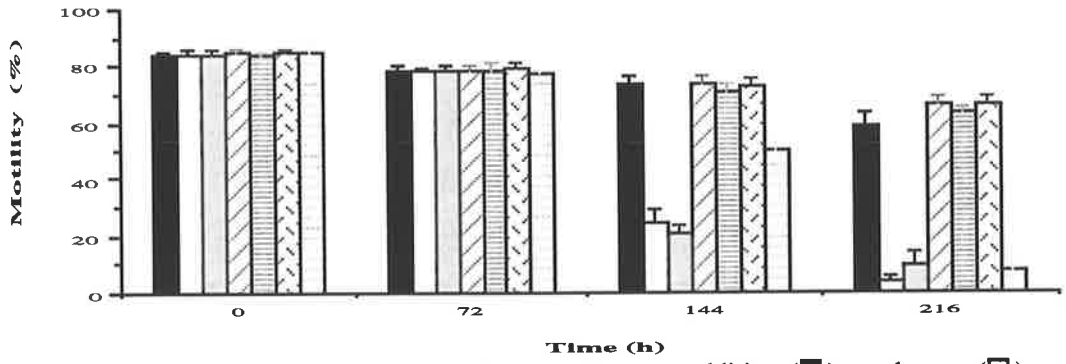
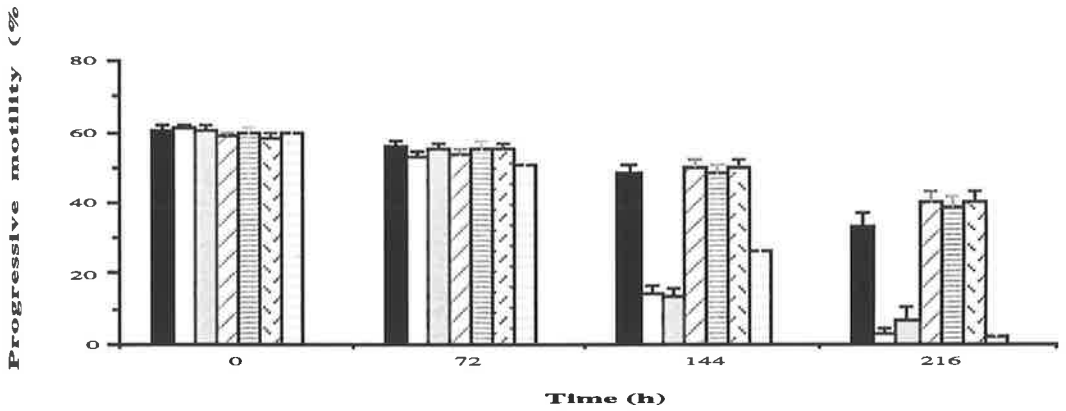


Figure 6.5. Motile (a) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□), and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

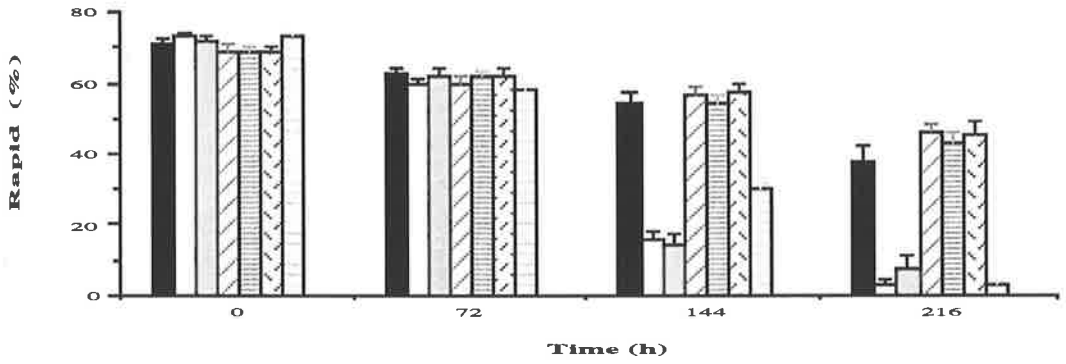
A similar trend that observed in the percentage of motile sperm also occured in the percentages of progressive motile and rapid spermatozoa. At 0h, percent progressive motile and rapid spermatozoa ranged from 58 to 61% and 68 to 72%, respectively. Thereafter, their values decreased slightly at 72h. Subsequently, a sharp reduction in these parameters occured after 216h of storage. The percentages of progressive motile

and rapid sperm from samples extended in +EYC, +NS, +SC and +NSC were greater ($p<0.05$) than those extended in +G, +NC and +NSCG either at 144 or 216h. At 144h, percent progressive motile and rapid spermatozoa were higher ($p<0.05$) in +NSCG than that in +G or +NC; however at 216h, no significance differences ($p>0.05$) were observed in these parameters among the 3 latter diluents (Figures 6.5.b and c).

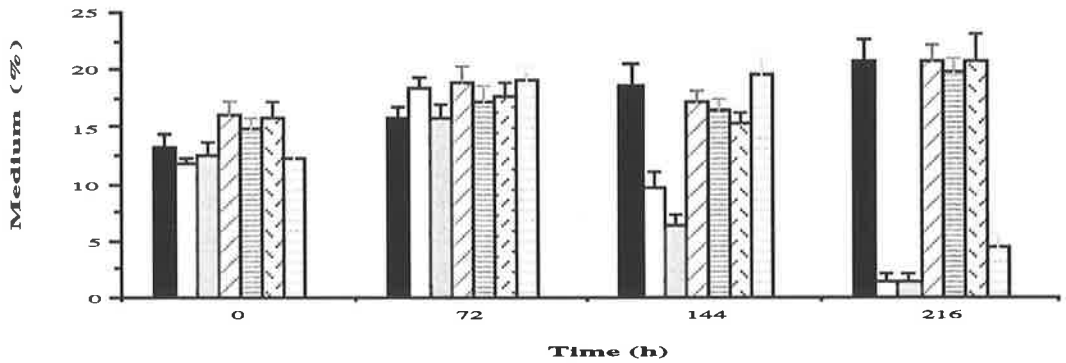
b)



c)



d)



Figures 6.5. Progressive motile (b), rapid (c) and medium (d) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□), and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

The mean percentages of medium sperm in the 7 diluents increased slightly in the same rate from 0 to 72h of storage. As time of storage increased to 144h, a marked decrease in medium sperm was observed in +G and +NC. A sharp decrease in medium sperm was also occured in +NSCG at 216h . The difference in medium sperm for all diluents betwen 0 and 72h was not significant. Percent medium sperm in +EYC, +NSCG and +NS was higher ($p<0.05$) than that in +G or +NC at 144h. In addition, percent medium in +SC or +NSC was significantly ($p<0.05$) higher than that in +NC at this time. At 216h, medium sperm in +EYC, +NS, +SC and +NSC was greater ($p<0.05$) than that in +G, +NC or +NSCG (Figure 6.5.d).

The numbers of slow sperm in +NSCG, +G, EYC and +NC increased steadily from 0 to 144h, then decreased at 216h. However, slow sperm in +NS and +SC continued to rise through 216h. The percentages of slow sperm in +NSCG or +G were significantly ($p<0.05$) greater at 144h than that in +NSCG or +G at 216h. The percentage of slow sperm in +NS was significantly higher ($p<0.05$) at 216h than that of +NS at 0h . At 216h, the percentage of slow sperm in +EYC, +NS, +SC and +NSC was greater ($p<0.05$) than that in +NC. In addition, slow sperm in +NS was significantly ($p<0.05$) different from that in +G (Figure 6.5.e).

e)

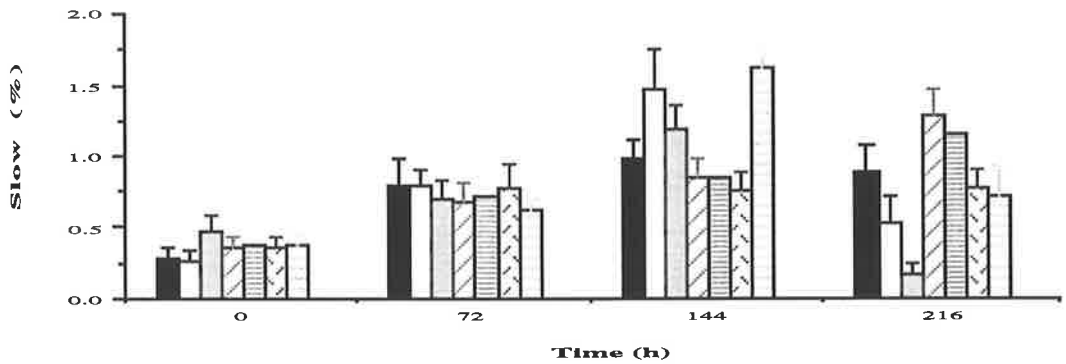


Figure 6.5. Slow (e) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

f)

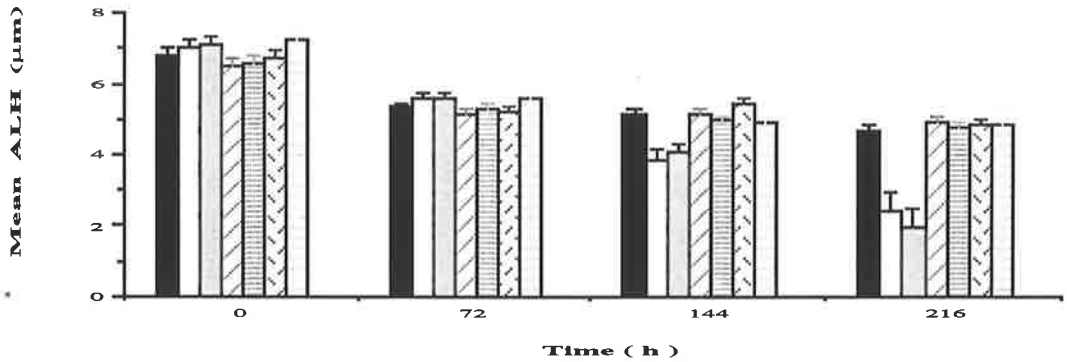


Figure 6.5. Mean ALH (f) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

There was a gradual decline in mean ALH from 0h to 216h. The decrease in +G and +NC diluents became obvious after 144h. At 144h, mean ALH in +NSC was significantly higher ($p<0.05$) than in +G. Although EYC and +NC diluents did not differ in ALH at 216h, they both significantly lower ($p<0.05$) compared with the other 5. No significant difference ($p>0.05$) in ALH was observed between 0 and 72h, however, the difference between 72 and 216h in ALH for samples diluted in +G and +NC was significant ($p<0.05$) (Figure 6.5.f).

g)

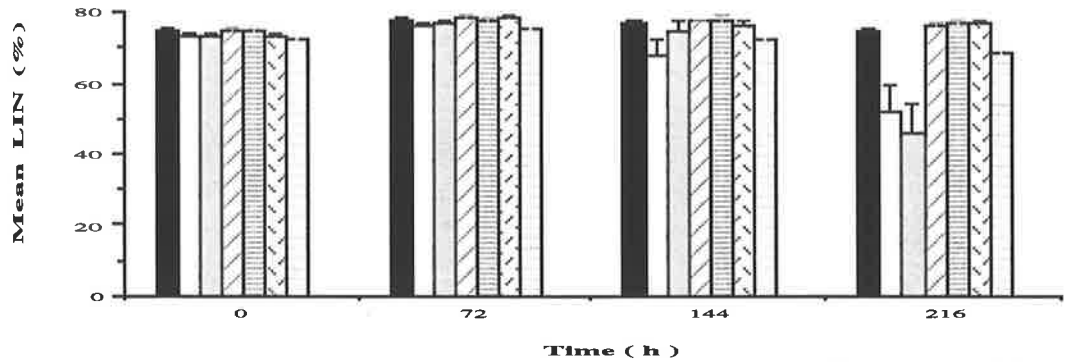
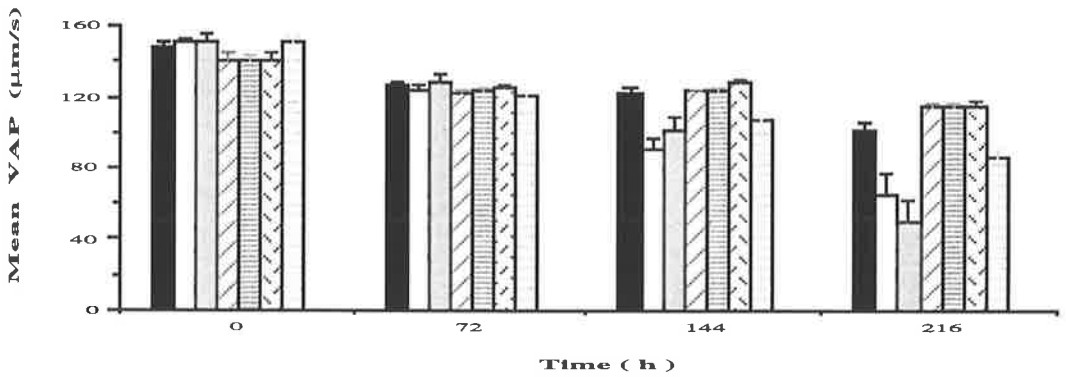


Figure 6.5. Mean LIN (g) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

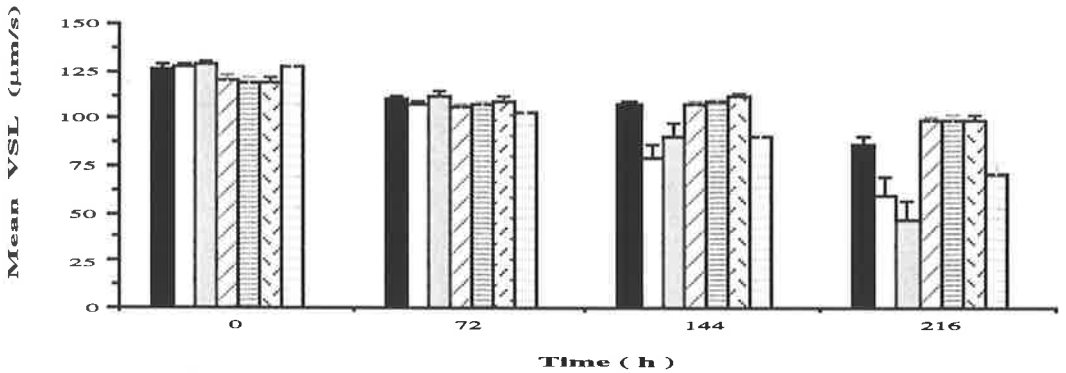
There was a small increase in the percentage of mean LIN from 0h to 72h. At 144h, mean LIN in +NSCG, +G and +NC decreased slightly. After storage for 216h, a decrease in percent LIN became obvious (Figure 6.5.g). No significant difference in

percent LIN between +G and +NC was found at 216h. However, mean LIN in these 2 diluents were significantly ($p<0.05$) lower than that in +EYC, +NSCG, +NS, +SC and +NSC; while differences in LIN among these 5 latter diluents were not significant (Figure 6.5.g).

h)



i)

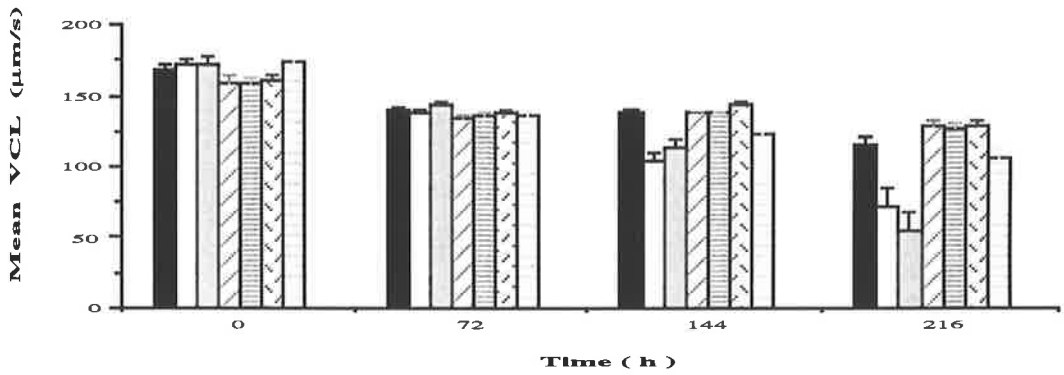


Figures 6.5. Mean VAP (h) and mean VSL (i) spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

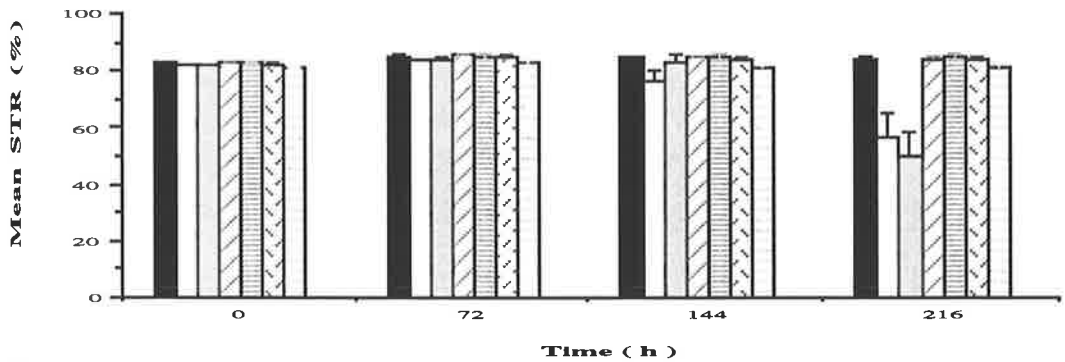
At 0h, mean VAP was recorded between 139 and 151 µm/s, then decreased at 72h. Mean VAP in +G and +NC had decreased sharply from 144h to 216h. At 144h, samples extended in +EYC, +NS, +SC and +NSC had higher ($p<0.05$) mean VAP than those extended in +G. However at 216h, +NS, +SC and +NSC resulted in greater ($p<0.05$) mean VAP than that in +NSCG, +G and +NC. However at 216h, mean VAP in +EYC was higher than that in +G and NC; and +NSCG had higher ($p<0.05$) mean VAP than +NC (Figure 6.5.h).

There was a slight decrease in the mean VSL from about 128 to 103 μm/s at 0h during storage to 72h. Mean VSL in +NSCG, +G and +NC continued to decline at 144h and 216h. Samples extended in +EYC, +NS, +SC or +NSC had a higher ($p<0.05$) mean VSL than those diluted in +NSCG, +G and +NC at 144h. At 216h, mean VSL in +NS, +SC and +NSC were greater than in +NSCG, +G and +NC; and +EYC had also higher ($p<0.05$) mean VSL than that of +G or +NC (Figure 6.5.i).

j)



k)



Figures 6.5. Mean VCL (j) and mean STR (k) of spermatozoa in egg yolk citrate media + no addition (■), + glucose (□), + nystatin + catalase (□), + nystatin + sulfanilamide (□), + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase (□), + nystatin + sulfanilamide + catalase + glucose (□) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

Mean VCL ranged from 158 to 173 μm/s at 0h, and had decreased by 72h. Subsequently, mean VCL in +G and +NC had decreased further between 144h and 216h. Samples extended in +EYC, +NS, +SC or +NSC had a higher ($p<0.05$) mean VCL than those diluted in +NSCG, +G and +NC at 144h. At 216h, mean VCL in +EYC, +NSCG, +NS, +SC and +NSC was higher ($p<0.05$) than that in +G and +NC (Figure 6.5.j).

The percentage of mean STR sperm in all diluents, except in +G and +NC, remained high (above 80%) during storage from 0 to 216h. Mean STR in +G and +NC had decreased slightly at 216h (Figure 6.5.k). The difference in percent STR between +G and +NC was not significant at 216h. However, mean STR in this 2 latter diluents were significantly ($p<0.05$) lower than that in +EYC, +NSCG, +NS, +SC and +NSC; while differences in STR among these 5 latter diluents were not significant (Figure 6.5.k).

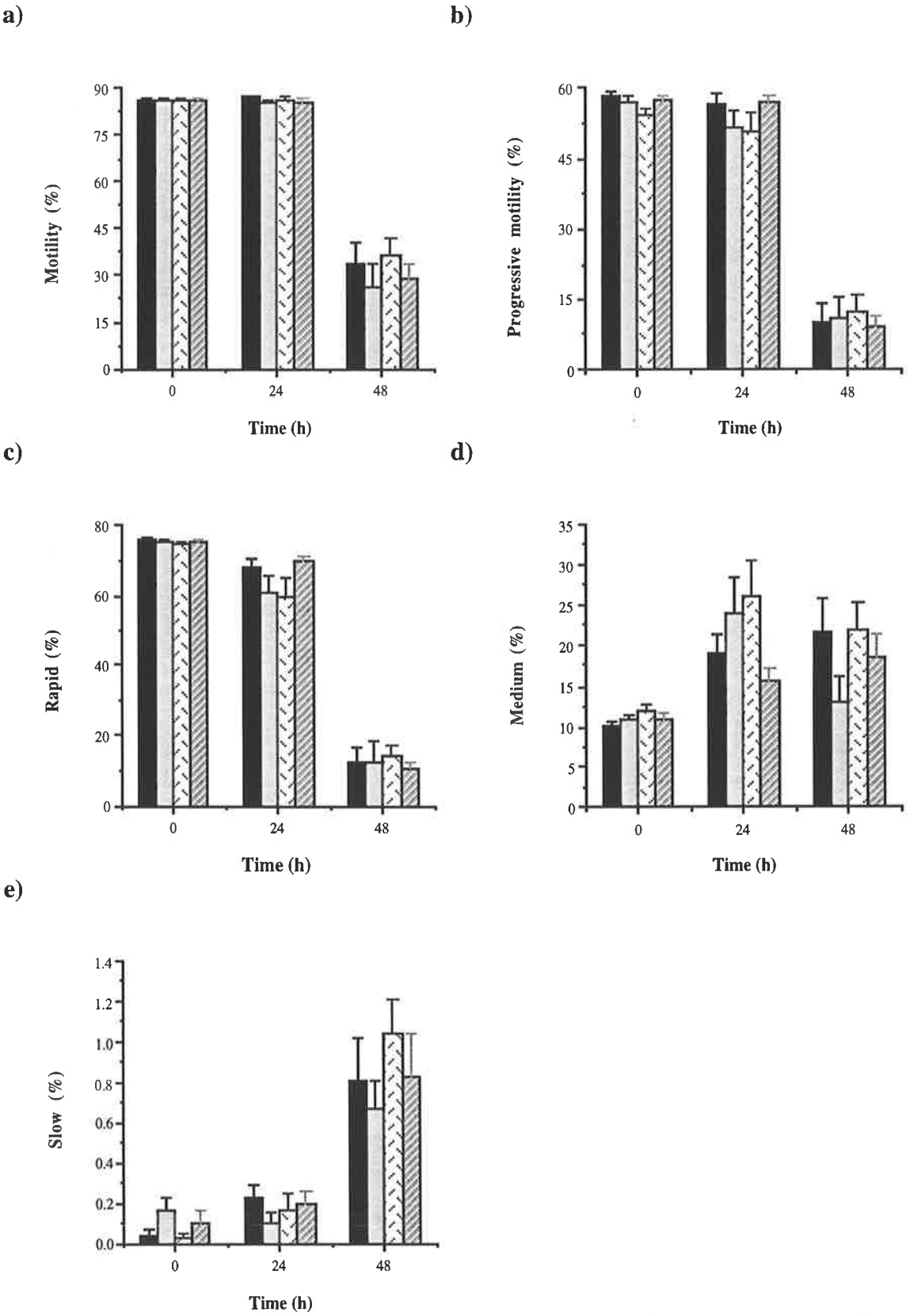
6.6.2. Experiment 6.2

6.6.2.1. Preservation at 30°C

The findings on the effect of different diluents and additives on various motion characteristics of ram semen stored at 30°C are presented in Table 6.6 (Appendix A).

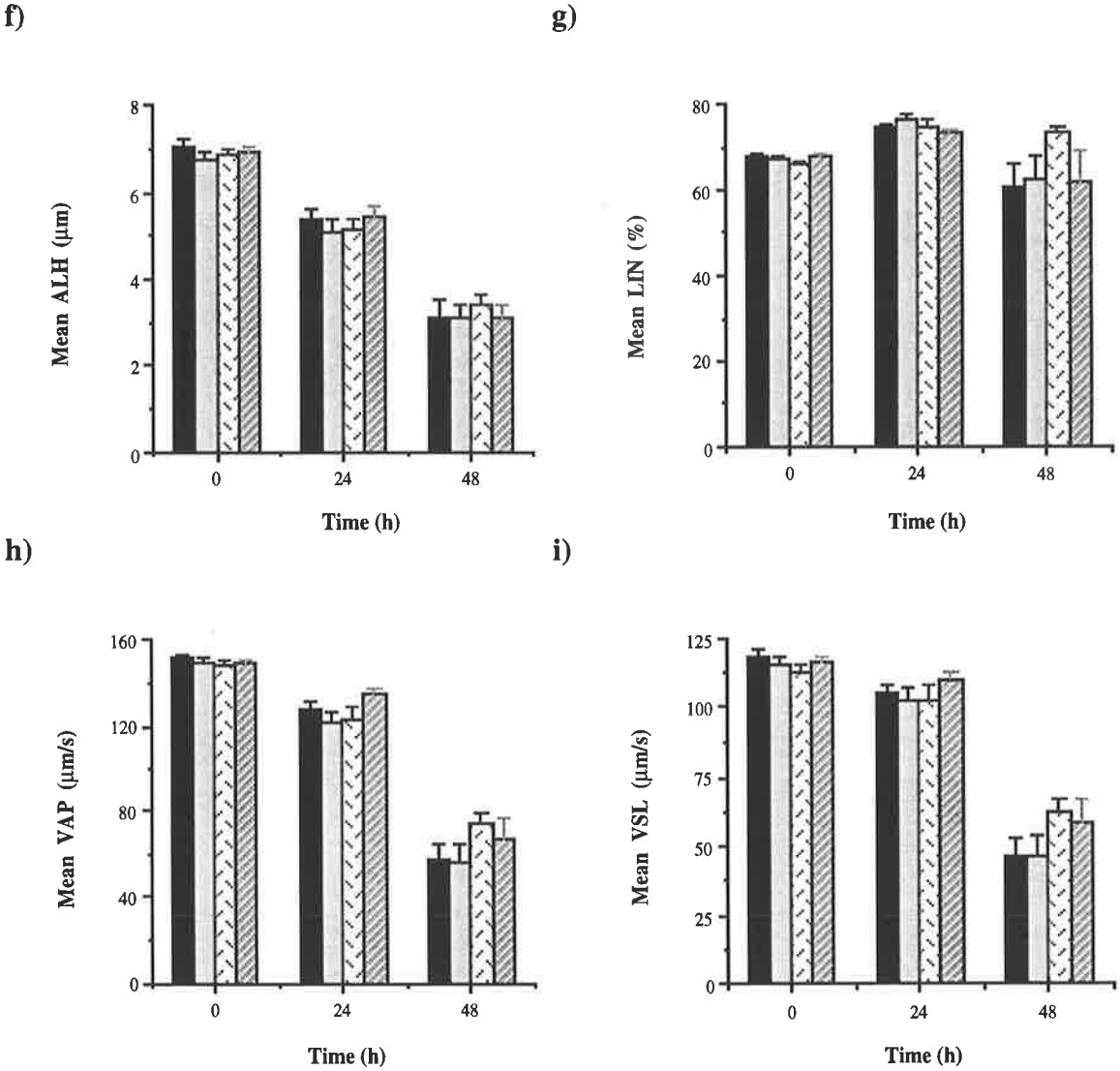
The percentages of motile spermatozoa remained high (around 85%) after storage for 24h, then had decreased markedly by 48h with motile sperm being the lowest in +NC. There was a small reduction in the mean percentages of progressive motile and rapid spermatozoa in the 4 diluents during storage from 0 to 24h. The means at 24h were around 50% for progressive motility and 60% for rapid sperm. After 48h, their motion decreased rapidly to below 10%. The differences in motility, progressive motility and rapid spermatozoa between 0 or 24 and 48h were significant ($p<0.05$) (Figures 6.6.a,b and c).

The percentages of medium sperm in all diluents were almost the same (around 10%) during initial storage (0h). Mean medium sperm in EYC and +S rose to 48h. However, medium sperm in +NC and +NSC increased only up to 24h, and then fell. The differences in medium for each time period were not significant ($p<0.05$)(Figure 6.6.d).



Figures 6.6. Motile (a), progressive motile (b), rapid (c), medium (d) and slow (e) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (▨) and + sulfanilamide (▩) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

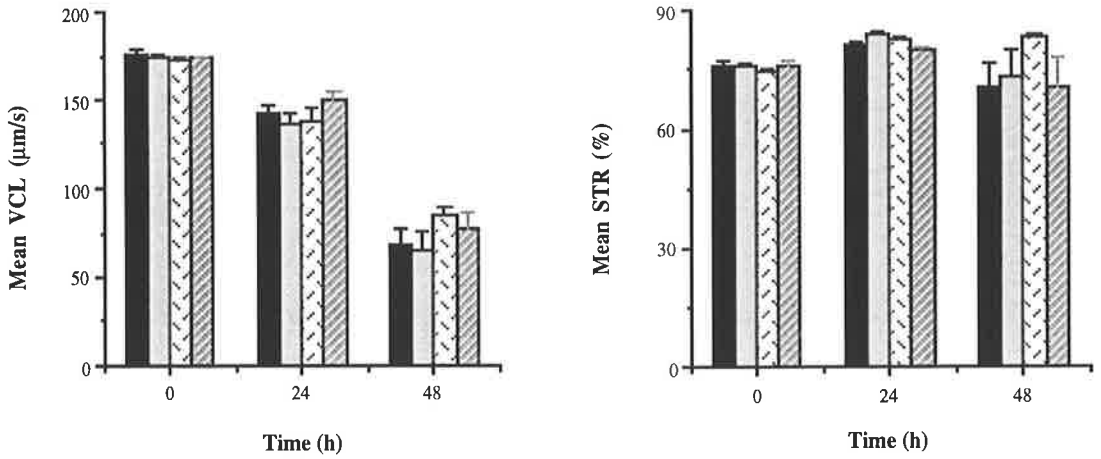
The mean percentage of slow sperm in the 4 diluents were below 0.25% and had increased slightly after storage for 24h. At 48h, slow sperm showed further increases. The differences in slow sperm among diluents between 48 and 0 or 24h were significant ($p<0.05$)(Figures 6.6.e).



Figures 6.6. Mean ALH (f), mean LIN (g), mean VAP (h) and mean VSL (i) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (▣) and + sulfanilamide (▨) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

There was a gradual reduction in the mean ALH, mean VAP, mean VSL and mean VCL for all diluents as the time of storage extended from 0 to 48h. The differences in mean ALH, mean VAP, mean VSL and mean VCL between 0 and 24 or 48h; also between 24 and 48h were significant ($p<0.05$)(Figures 6.6.f,h, i and j).

j) k)



Figures 6.6. Mean VCL (j) and mean STR (k) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□) and + sulfanilamide (▨) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

The percentages of LIN among all diluents increased slightly after 24h of storage. Afterwards there was a slight decrease in mean LIN in all diluents at 48h, to a value lower than that at 0h, except for +NSC remained about the same. The differences in LIN between 0 and 24 or 48h were not significant, however, all diluents showed a higher ($p<0.05$) mean LIN at 24 than that at 48h (Figure 6.6.g).

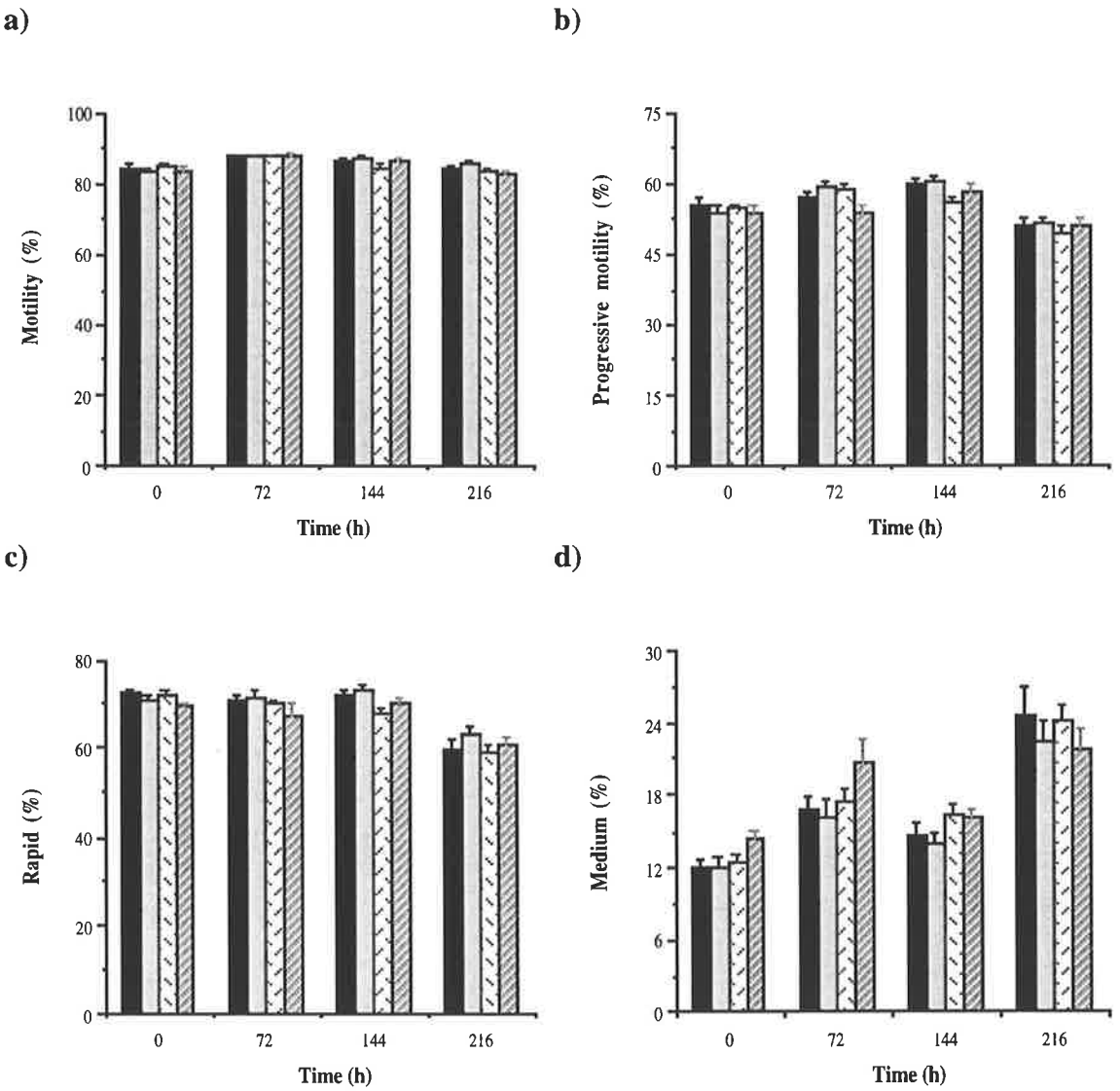
Initially the percentages of mean STR in all diluents increased slightly, except for +NSC, then fell again. The differences in STR for each time period were not significant (Figure 6.6.k).

6.6.2.2. Preservation at 5°C

The effect of diluent and time of storage on the motion characteristics of ram semen diluted in a various combinations of egg-yolk citrate-based diluent stored at 5°C is summarized in Table 6.7. (Appendix A).

The mean percentages of motile, progressive motile and rapid spermatozoa for samples diluted in all diluents were maintained for 144h of storage, then decreased slightly at 216h. The differences in spermatozoal motility between 72 and 0 or 216h were significant ($p<0.05$). For progressive motility, differences were found ($p<0.05$)

between 72 or 144 and 216h. All samples stored at 0 to 144h had greater ($p<0.05$) rapid sperm than those at 216h (Figures 6.7.a,b and c).



Figures 6.7. Motile (a), progressive motile (b), rapid (c) and medium (d) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (◘) and + sulfanilamide (▩) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

Mean medium spermatozoa in all diluents increased between 0h and 72h of storage. Then patterns of irregular fluctuations were observed. Although numerically the percentage of medium sperm was greater at 72h than at 0 or 144h, the differences were not significant. However, there was a significant difference ($p<0.05$) in medium sperm between 216 and 0 to 144h (Figure 6.7.d).

e)

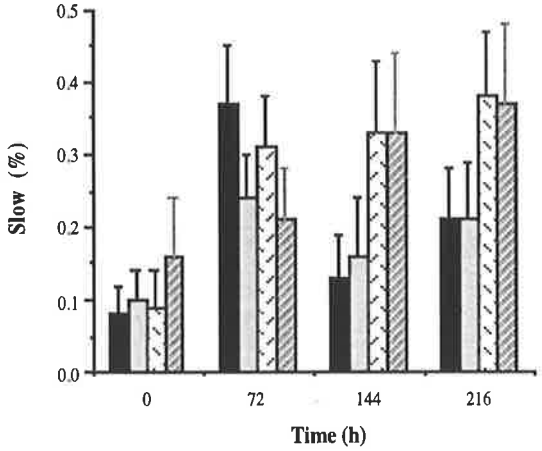
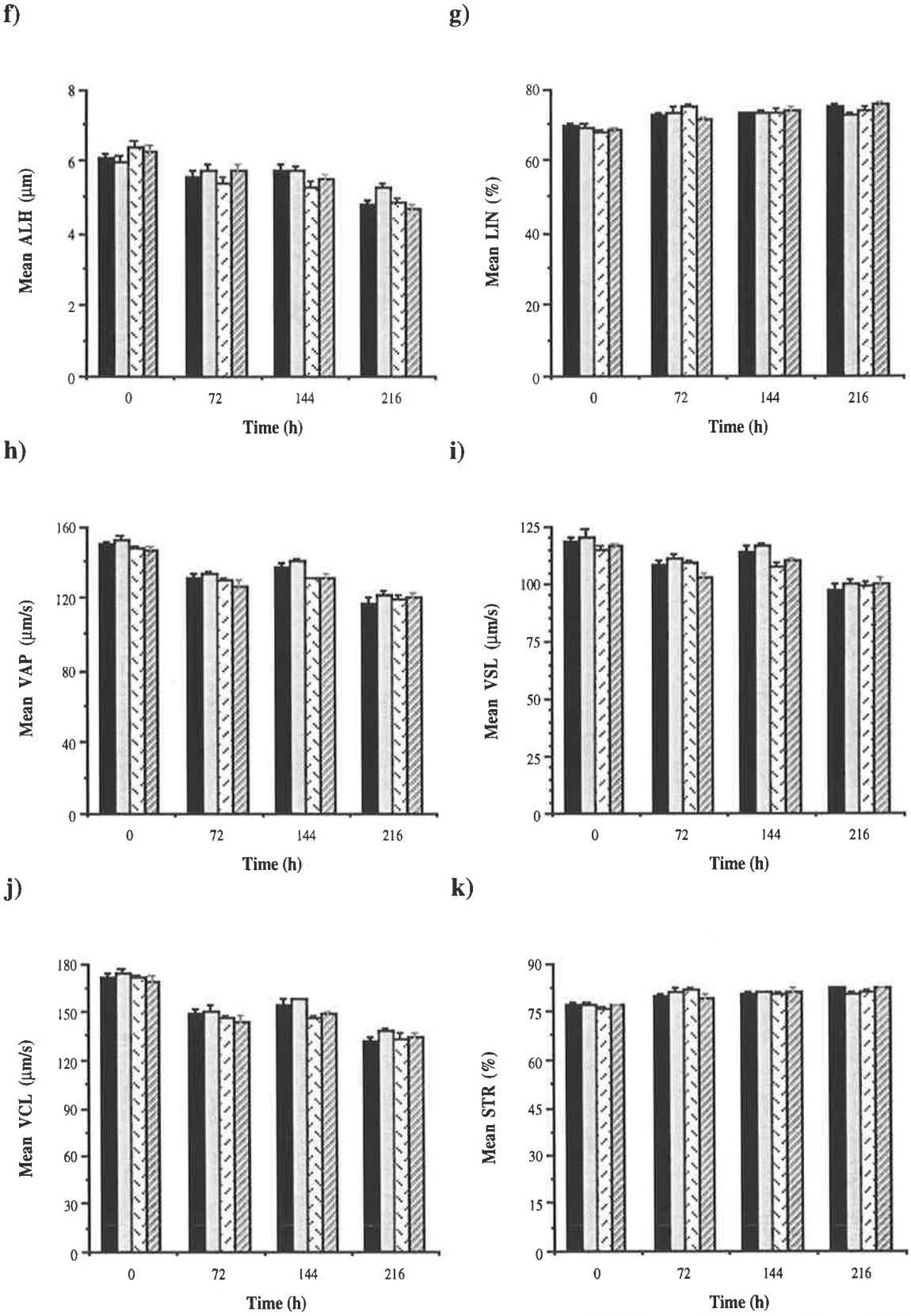


Figure 6.7. Slow (e) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (⊡) and + sulfanilamide (▨) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

The percentages of slow sperm in EYC and +NC increased iregularly from 0 to 216h. However slow sperm in both +NSC and +S tended to increase throughout the storage periods. Slow sperm in EYC was significantly higher ($p<0.05$) at 72h than that at 0h. However, slow sperm in +NC was greater ($p<0.05$) at 216h than that at 0h (Figure 6.7.e).

There was a gradual reduction in the mean of ALH, VAP, VSL and VCL during storage from 0 to 216h. The differences in these parameters between 0 and 72 to 216h, and also between 72 or 144 and 216h were significant ($p<0.05$). However, at 144h, mean VAP for samples extended in +NC was higher ($p<0.05$) than that diluted in +NSC and in EYC. Likewise during this time, VCL for samples diluted in EYC or in +NC was faster ($p<0.05$) than that diluted in +NSC. VCL in EYC was also faster ($p<0.05$) than in +S at 144h of storage (Figures 6.7.f,h,i and j).

The percentages of LIN and STR tended to increase over time. All diluents showed greater ($p<0.05$) values for LIN and STR between 72 and 216h than at 0h (Figures 6.7.g and k).



Figures 6.7. Mean ALH (f), mean LIN (g), mean VAP (h), mean VSL (i), mean VCL (j) and mean STR (k)of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (⧈) and + sulfanilamide (▨) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

6.6.3. Experiment 6.3

6.6.3.1. Preservation at 30°C

The effect of diluent and time of storage on the motion characteristics of ram semen diluted in a various combinations of egg-yolk citrate-based diluent stored at 30°C is summarized in Table 6.8. (Appendix A).

a)

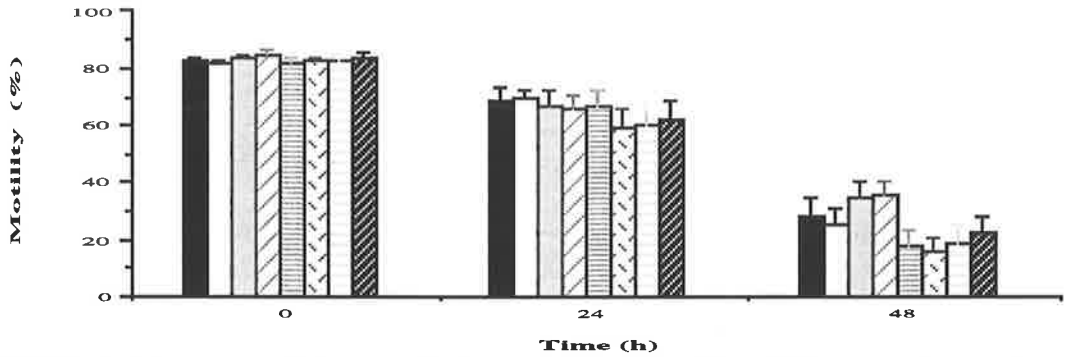


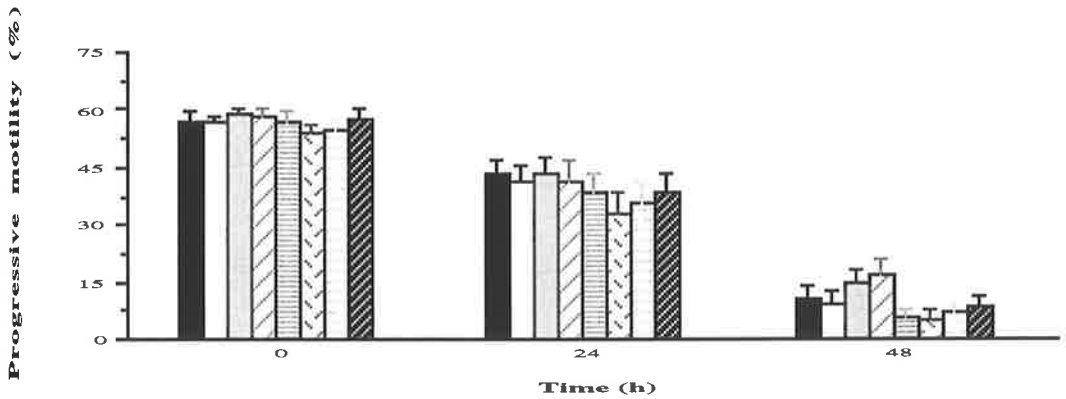
Figure 6.8. Motile (a) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (▩) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

The mean percentages of motility spermatozoa decreased slightly in all diluents during storage from 0 to 24h and the decrease became obvious as the time of storage extended to 48h. The differences in motile sperm between 0 and 24h were not significant ($p>0.05$). However, all diluted samples stored either at 0 or 24h showed a greater ($p<0.05$) motility of spermatozoa than those stored at 48 h. At 48h, motility of spermatozoa was higher ($p<0.05$) for samples diluted in +NSC or +S than those diluted in +CE, +NC+CE and +NSC+CE. The addition of coconut extract, if anything, decreased motility slightly (Figure 6.8.a).

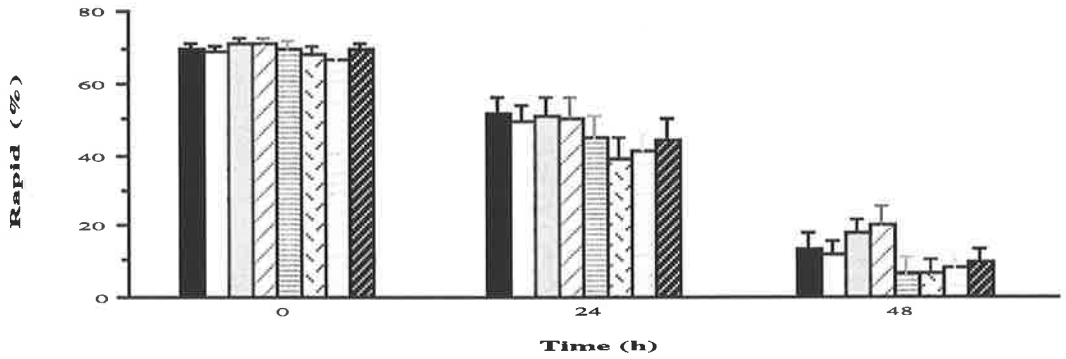
There was a gradual decrease in mean percentages of progressive motility and rapid spermatozoa as the time of storage advanced. The decrease in both parameters followed a pattern similar to that of motility of spermatozoa. The differences in these parameters between 0 and 24 or 48h; and between 24 and 48h were significant ($p<0.05$). At 48h, progressive motile and rapid spermatozoa were higher ($p<0.05$) for

spermatozoa in +NSC and +S than that in in +CE, +NC+CE and +NSC+CE (Figures 6.8.b and c).

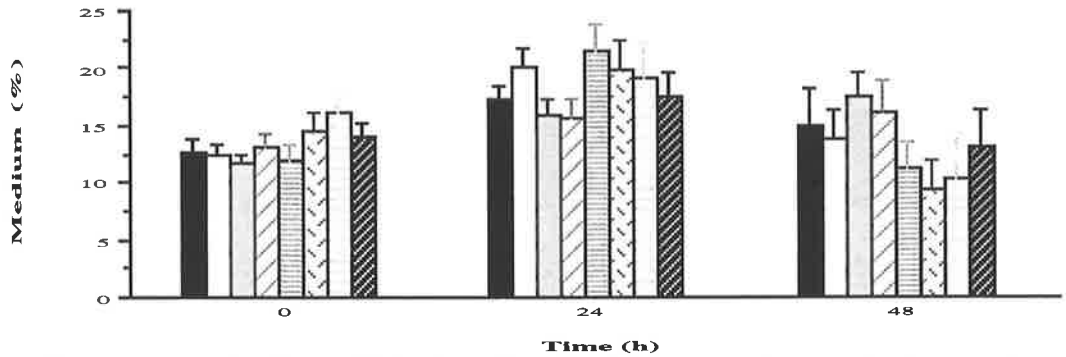
b)



c)



d)



Figures 6.8. Progressive motile (b), rapid (c) and medium (d) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

The percentages of medium sperm in the 8 diluents increased during storage from 0 to 24h, then at 48h medium sperm in +NSC and +S tended to increase. Medium sperm in samples diluted in +CE or +NC+CE and stored for 24h was significantly

($p<0.05$) higher than that stored at 48h. For samples diluted in +NSC+CE, the differences in this parameter were observed between 0 or 24 and 48h. (Figure 6.8.d).

e)

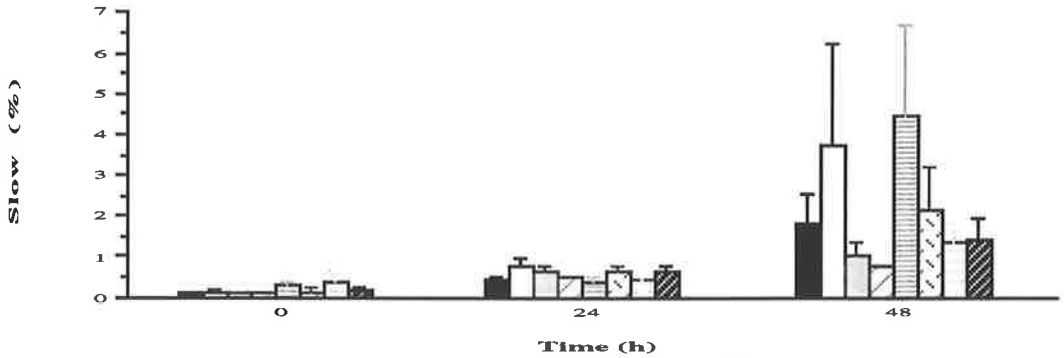
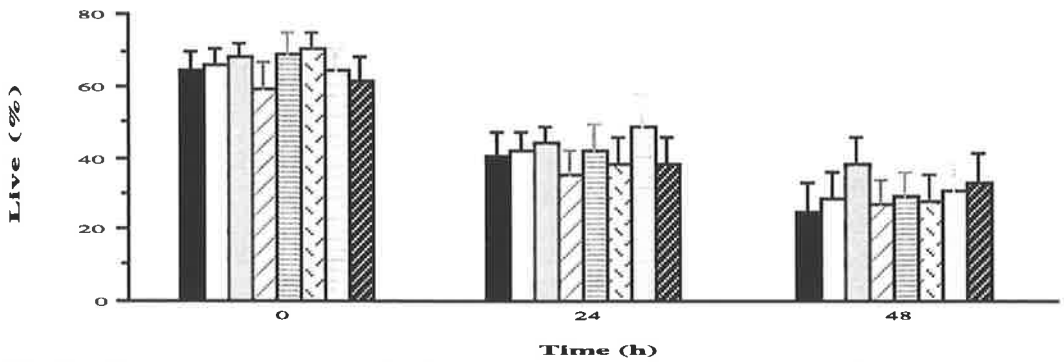


Figure 6.8. Slow (e) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

The mean percentages of slow spermatozoa in all diluents were still less than 1% after storage for 24h. However as time of storage extended to 48h, the increase in slow sperm became obvious especially in +NC and +CE. The difference in slow sperm between 48 and 0h was significant ($p<0.05$) (Figure 6.8.e).

f)



Figures 6.8. Live (f) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

A gradual decrease in the mean percentages of live spermatozoa for sample diluted in all diluents was observed over time. The difference in live sperm between 0 and 24 or 48h was significant ($p<0.05$)(Figures 6.8.f).

g)

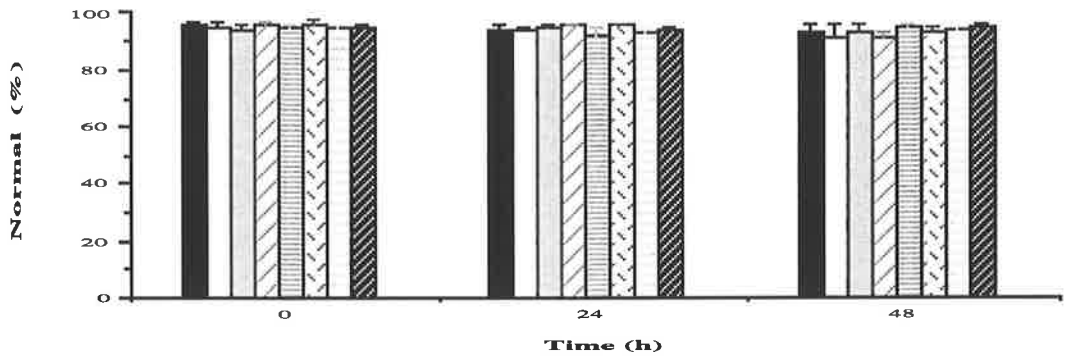


Figure 6.8. Normal (g) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

Mean percentages of normal spermatozoa in all diluents were almost the same throughout the storage periods. Percent normal sperm in +NC and +S was higher ($p<0.05$) at 0 than that at 48h (Figures 6.8.g).

Mean ALH in the 8 diluents decreased during 48h of storage. The differences in mean ALH between 0 or 24 and 48h; and between 24 and 48h were significant ($p<0.05$)(Figure 6.8.h).

h)

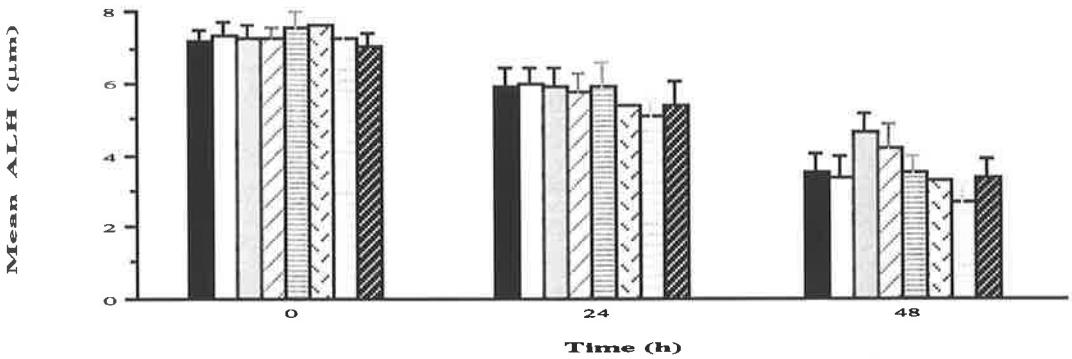


Figure 6.8. Mean ALH (i) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

Mean LIN in all diluents was maintained after storage for 24h, but had decreased to different extent by 48h. Mean LIN at 0 or 24h was higher ($p<0.05$) for

samples diluted in EYC, +NC and +S than that at 48h. For +NSC+CE, a difference in LIN was observed ($p<0.05$) between 24 and 48h (Figure 6.8.i).

i)

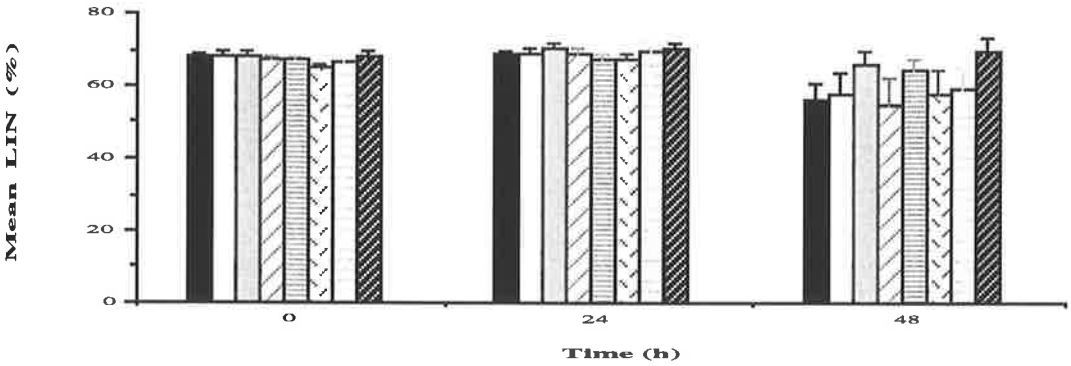


Figure 6.8. Mean LIN (i) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (▤), + nystatin + catalase + coconut extract (▣), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (▩) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

Mean VAP in the various diluents increased between 0h and 48h. Mean VAP in +NSC was significantly higher ($p<0.05$) than that in +NC+CE and +NSC+CE. The differences in mean VAP between 0 or 24 and 48h; and between 24 and 48h were significant ($p<0.05$)(Figure 6.8.j).

j)

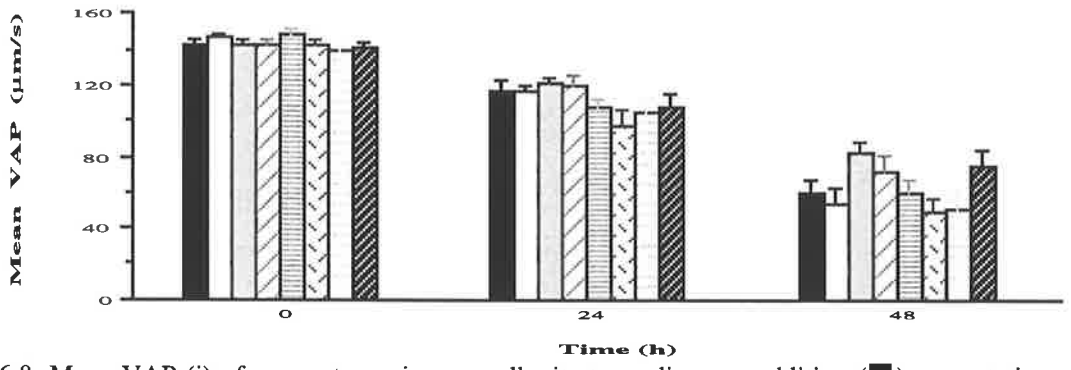


Figure 6.8. Mean VAP (j) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (▤), + nystatin + catalase + coconut extract (▣), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (▩) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

Mean VSL in the 8 diluents decreased between 0h and 48h. The differences in mean VSL between 0 or 24 and 48h; and between 24 and 48h were significant ($p<0.05$) (Figure 6.8.k).

k)

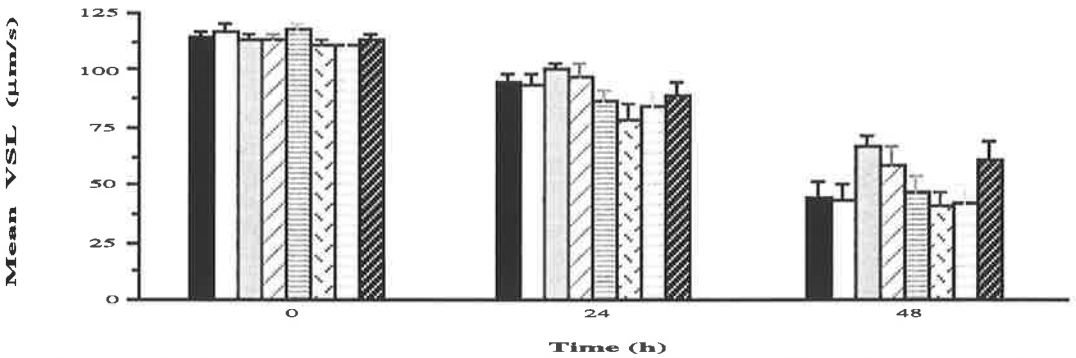


Figure 6.8. Mean VSL (k) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (▣), + sulfanilamide (▨), egg yolk citrate + coconut extract (▤), + nystatin + catalase + coconut extract (▩), + nystatin + sulfanilamide + catalase + coconut extract (▣) + sulfanilamide + coconut extract (▩) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

Mean VCL decreased between 0h and 48h. The differences in mean VCL between 0 or 24 and 48h; and between 24 and 48h were significant ($p<0.05$). During this time mean VCL in +NSC was higher ($p<0.05$) than that in +NC, +CE, +NC+CE and +NSC+CE (Figure 6.8.l).

l)

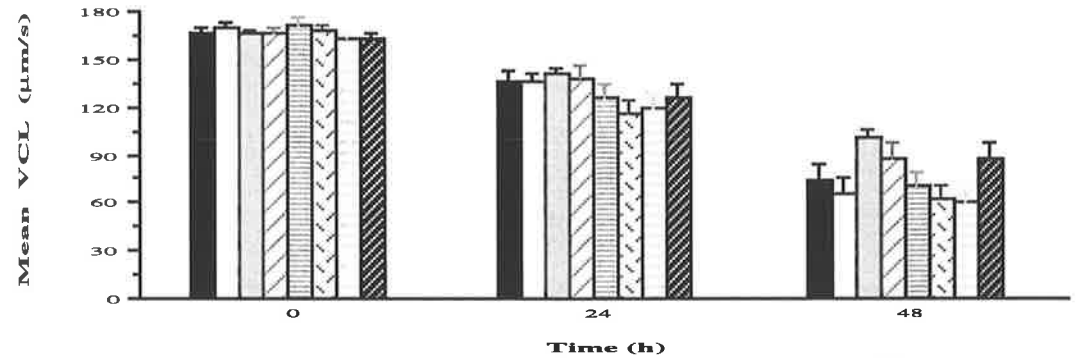


Figure 6.8. Mean VCL (l) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (▣), + sulfanilamide (▨), egg yolk citrate + coconut extract (▤), + nystatin + catalase + coconut extract (▩), + nystatin + sulfanilamide + catalase + coconut extract (▣) + sulfanilamide + coconut extract (▩) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

There were slight enhancements in the percentages of STR in all diluents after 24h of storage. However after 48h of storage, mean STR from samples diluted in EYC, +S or +NC+CE diluent decreased slightly. Those samples diluted in the other 5 diluents remained high (above 70%). The difference in STR among the 8 diluents for each

period of storage was not significant. But, mean STR for samples diluted in +S was higher ($p>0.05$) at 24 than that at 48h (Figure 6.8.m).

m)

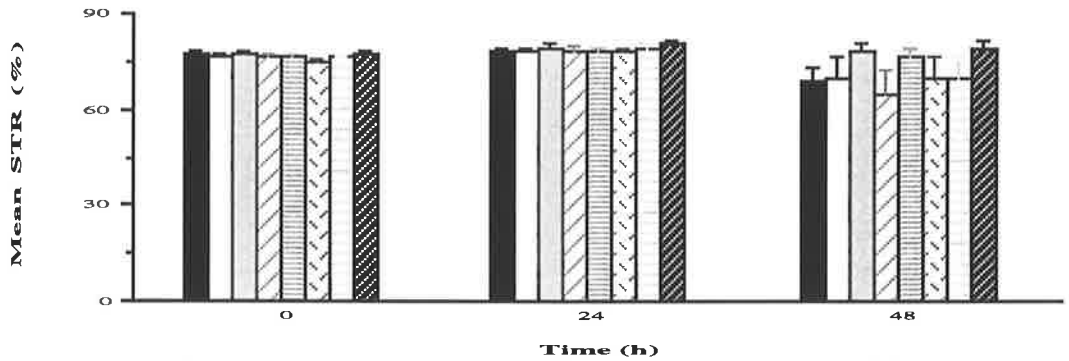


Figure 6.8. Mean STR (m) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 48h at 30°C. Values are Means ± SEM of 3 observations.

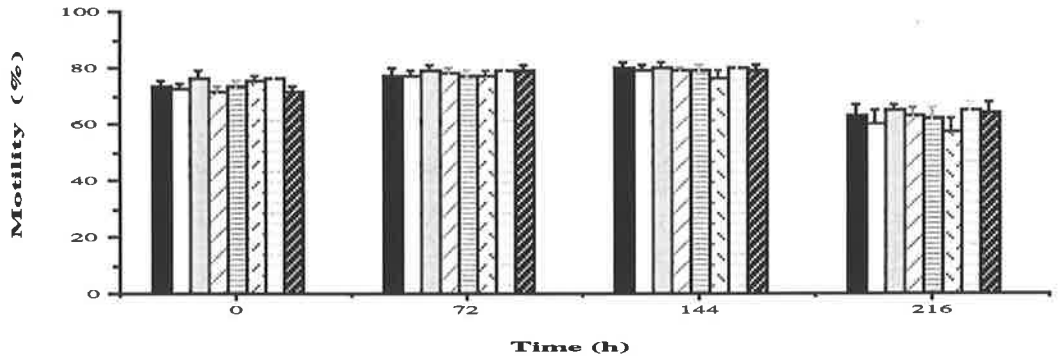
6.6.3.2. Preservation at 5°C

The effect of diluent and time of storage on the motion characteristics of ram semen diluted in a various combinations of egg-yolk citrate-based diluent stored at 5°C is summarized in Table 6.9. (Appendix A).

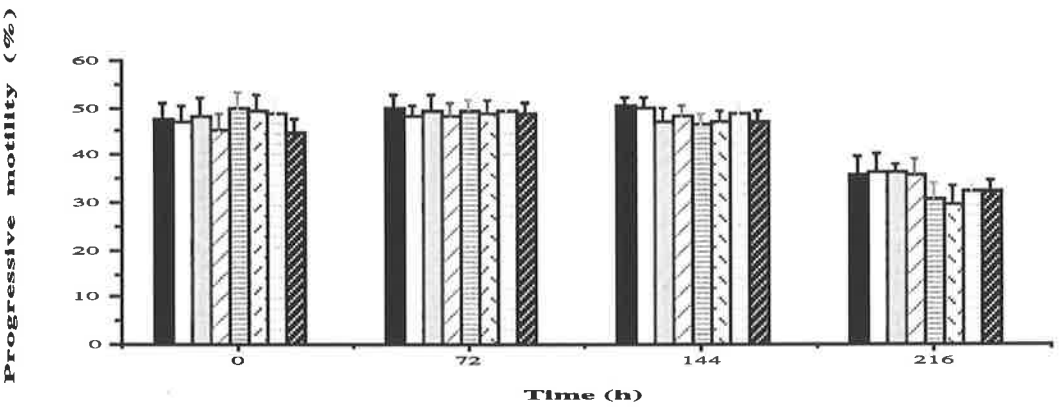
The percentages of motile sperm in all diluents were slightly enhanced after storage for 144h, but had fallen by 216h. For progressive motility, were unchanged at 144h, but then had decreased at 216h. Mean rapid spermatozoa in all diluents decreased from between 56 and 63% at 144h to between 35 and 42% at 216h.

Percent motile, progressive motile and rapid spermatozoa at 0 through 144h was higher ($p<0.05$) than those at 216h. The addition of coconut extract had no effect (Figures 6.9.a,b and c).

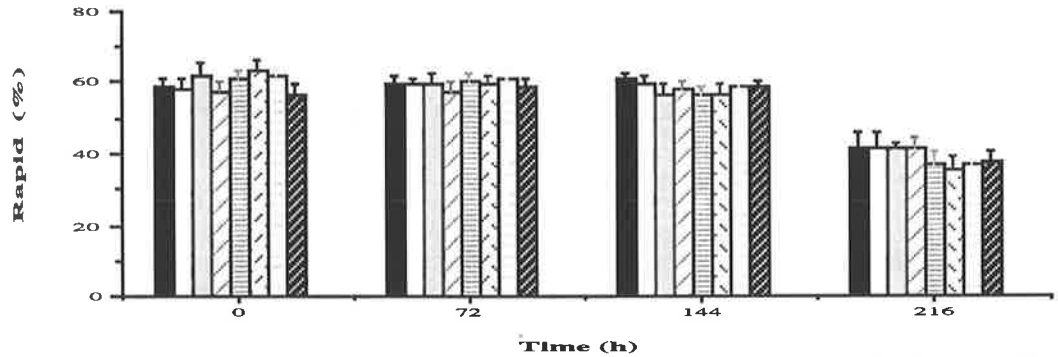
a)



b)

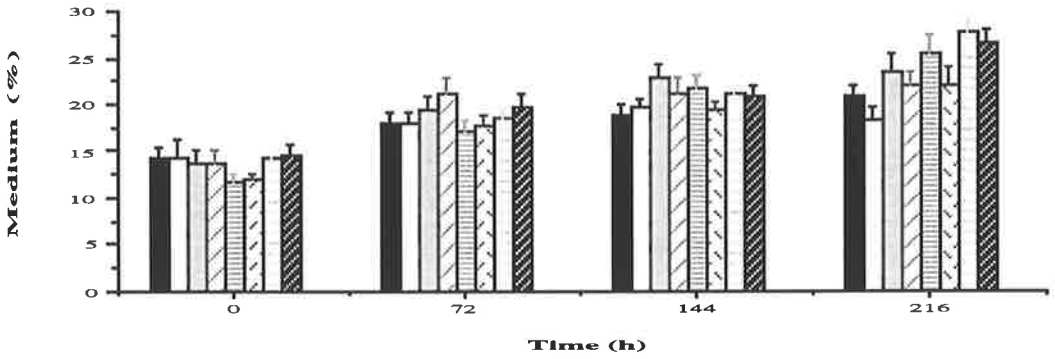


c)

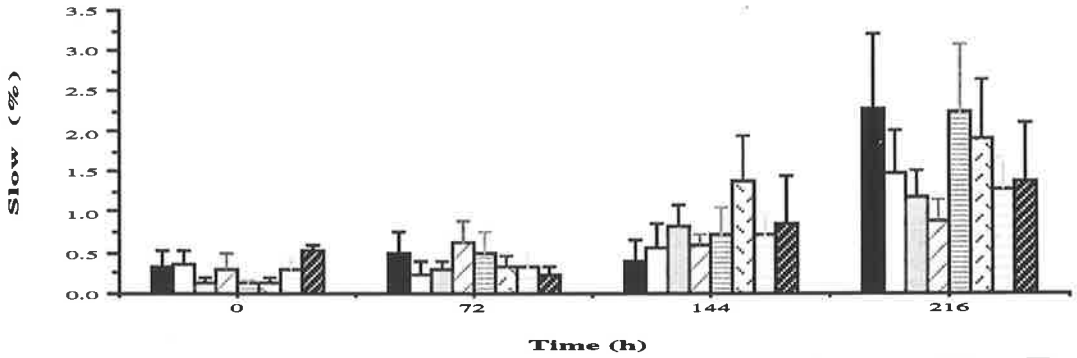


Figures 6.9. Motile (a), progressive motile (b) and rapid (c) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (▤), + nystatin + catalase + coconut extract (▧), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (▨) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

d)



e)



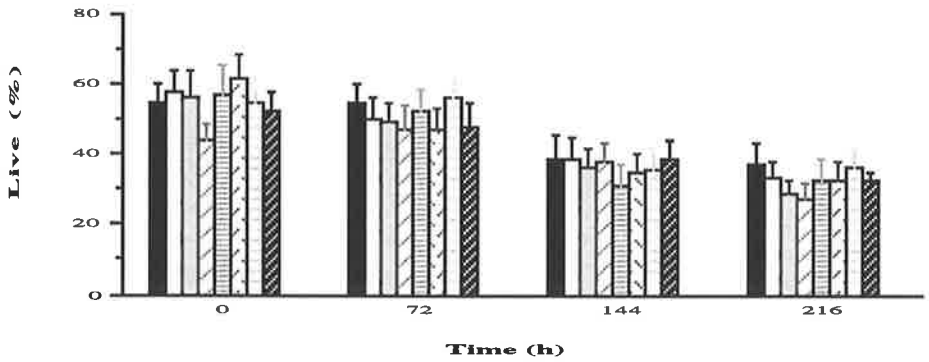
Figures 6.9. Medium (d) and slow (e) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

The percentages of medium sperm in all diluents increased gradually from 0h to 216h. There were significant differences ($p<0.05$) in medium between samples stored at 72 to 216h and at 0h (Figure 6.9.d). There was a continuous increase in the mean percentage of slow spermatozoa in the 8 diluents from 0h to 216h. The difference in slow sperm between samples stored at 216h and those stored at 0 or 72h was significant ($p<0.05$)(Figure 6.9.e).

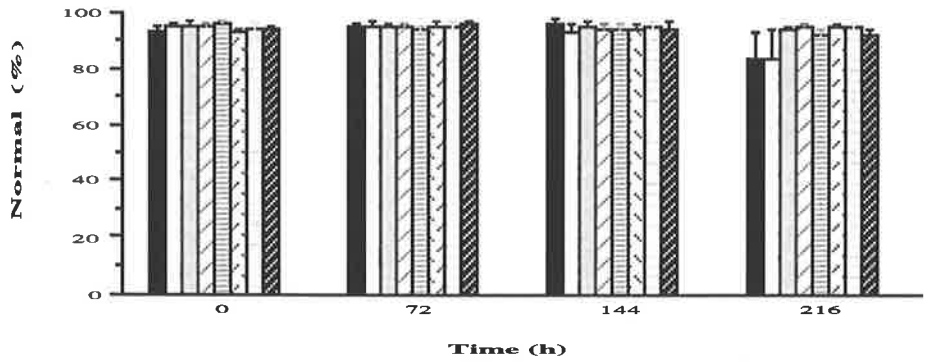
The numbers of live sperm recorded in all diluents decreased during storage. All diluents at 72h showed a higher ($p<0.05$) proportions of live sperm than at 216h (Figure 6.9.f). The percentages of normal sperm in all diluents did not change much after 216h of storage, except for EYC and +NC, in which the values dropped slightly. Percent normal sperm for samples extended in EYC and stored for 72 or 144h was

higher ($p<0.05$) than that stored for 216h. However, +NC had greater ($p<0.05$) normal sperm at 0 to 144h than at 216h (Figure 6.9.g).

f)



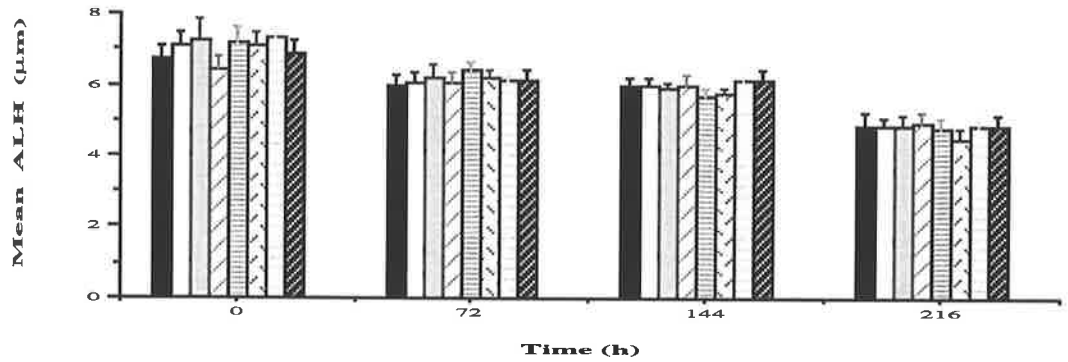
g)



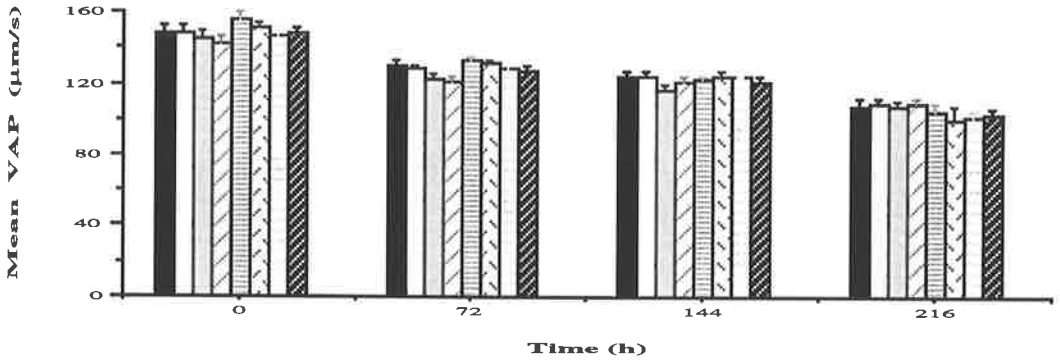
Figures 6.9. Live (f) and normal (g) spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (▣), + sulfanilamide (▨), egg yolk citrate + coconut extract (▤), + nystatin + catalase + coconut extract (▧), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (▩) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

Mean ALH, mean VAP, mean VSL and mean VCL decreased gradually as time of storage increased. The differences in mean ALH, mean VAP, mean VSL and mean VCL between 0 and 72 to 216h were significant ($p<0.05$). There was also significant ($p<0.05$) difference between samples stored at 72 or 144h and 216h. For mean VSL, samples stored at 72h differed significantly ($p<0.05$) with that stored at 216h (Figures 6.9.h, i, j and k).

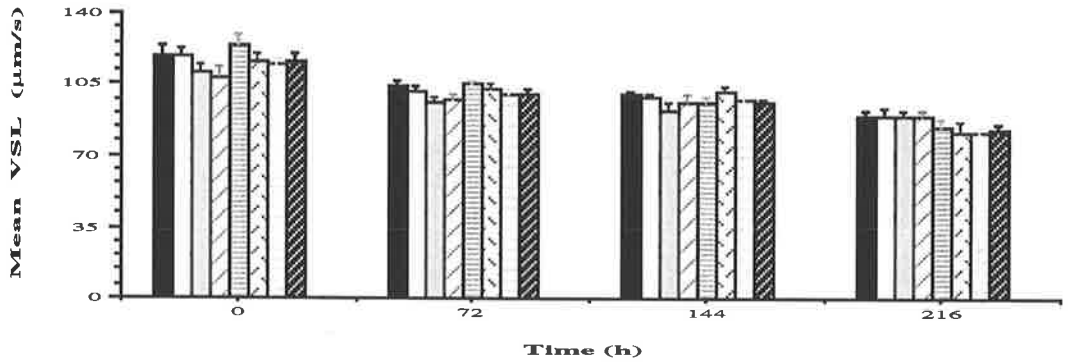
h)



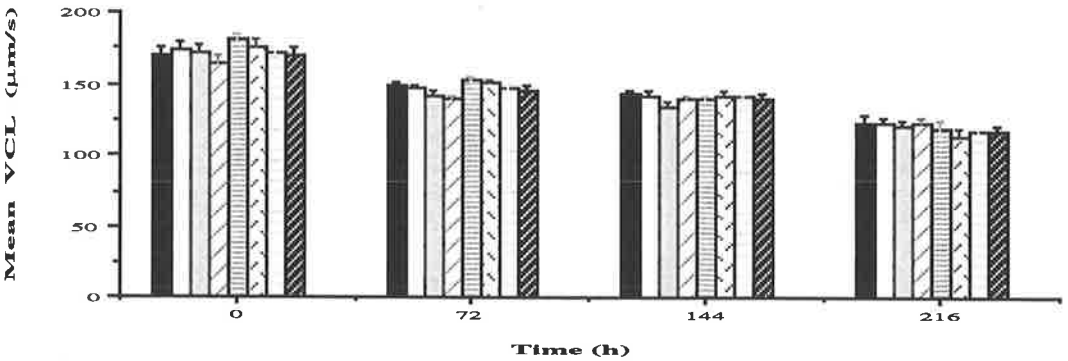
i)



j)



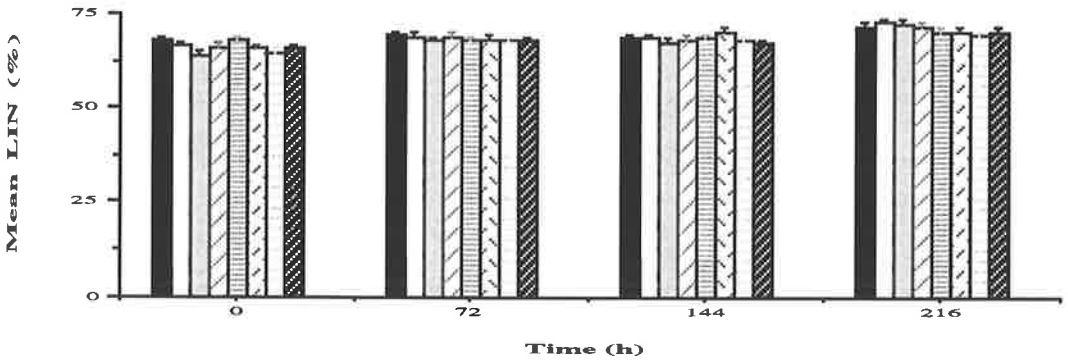
k)



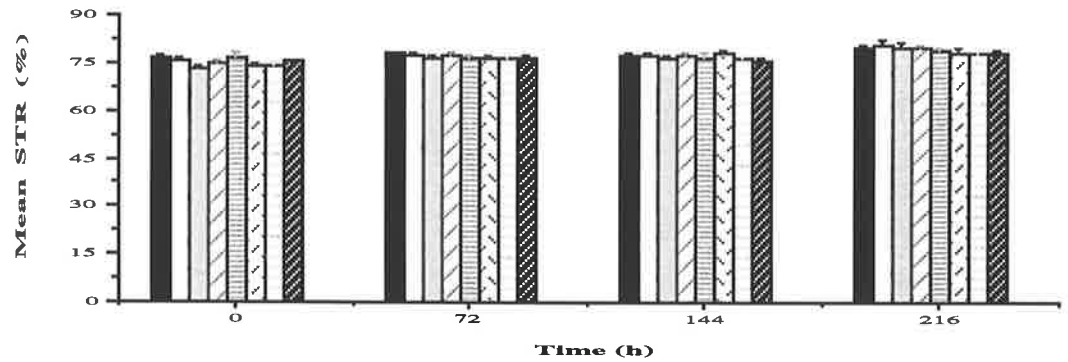
Figures 6.9. Mean ALH (h), mean VAP (i), mean VSL (j) and mean VCL (k) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (▤), + nystatin + catalase + coconut extract (▨), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (▩) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

Both mean LIN and mean STR of spermatozoa in all diluents increased slightly from 0h to 216h. The differences in mean LIN and mean STR between 216 and 0h was significant ($p<0.05$)(Figures 6.9.l and m).

l)



m)



Figures 6.9. Mean LIN (i) and mean STR (m) of spermatozoa in egg yolk citrate media + no addition (■), + nystatin + catalase (□), + nystatin + sulfanilamide + catalase (□), + sulfanilamide (□), egg yolk citrate + coconut extract (□), + nystatin + catalase + coconut extract (□), + nystatin + sulfanilamide + catalase + coconut extract (□) + sulfanilamide + coconut extract (□) and stored for up to 216h at 5°C. Values are Means ± SEM of 3 observations.

6.7. Discussion

The present study shows that most sperm motility and morphological characteristics were as well as or better maintained in diluents without coconut extract as in diluents with coconut extract. This is suprising as the present finding does not confirm the previous findings in Chapters 4 and 5. It is likely that the difference in the levels of egg yolk and coconut extract used might have contributed to the lack of consistent results between the current and the previous study. In the current study (Experiment 6.3), the concentrations of egg yolk and coconut extract used were 10%,

while in the studies in Chapter 4, the concentration of egg yolk used was lower, i.e. 5%, but the concentration of coconut extract was higher, i.e. 15%.

The results obtained in Experiment 6.1 indicate that inclusion of glucose alone in egg yolk citrate diluent (+G), depressed most sperm motion characteristics after 24h and the depression became obvious after 48h of storage at 30°C. Rao et al (1975) also found a marked depression of bull spermatozoal motility when stored at room temperature in citrate-coconut milk diluent containing glucose. However, Sharma and Mahajan (1965), who worked with bull semen found higher motility of spermatozoa when glucose was added to modified IVT diluent with 10% egg yolk after storage for 2 days at room temperatures (27-38°C).

Although glucose is thought to be beneficial to viability of spermatozoa in ambient temperature diluents, because of the increased metabolic activity of sperm held at elevated temperature (Freund et al, 1959), under the conditions of the present study no beneficial effect was apparent. Lapwood and Martin (1966) found that glucose had a slight beneficial effect at 37°C in one experiment when it was used to replace 1/3 or 2/3 of the 185 mM fructose in the diluent, but had no effect when added to a medium containing 123 mM fructose, or when glucose replaced 90% of the fructose.

However, when glucose was included in +NSC diluent (+NSCG) at 30°C, the viability of spermatozoa was maintained as well as in +NSC, which may suggest a beneficial effect of inclusion of enzymes and sulfanilamide in the diluent in overcoming the deleterious effects of glucose,

In Experiment 6.1, +G had a deleterious effect on the viability of spermatozoa during preservation at 5°C. However at this temperature, +G in combination with +NSC diluent (+NSCG) also had a deleterious effect on sperm viability. These results contrast with the finding at 30°C. It was also surprising that in experiment 6.1, +NC at 5°C also appeared to have a bad effect on sperm motility. When nystatin and catalase (+NC) were retested in Experiment 6.2 and 6.3, motility of spermatozoa was not depressed at either temperature.

Inconsistencies of the findings between Experiment 6.1 and Experiment 6.2 could be explained by differences in dilution ratios. In Experiment 1, pooled semen was divided into 15 aliquots, while in Experiment 6.2, it was divided into 8 aliquots. It is likely that the viability rates would be poorer in less concentrated samples than in more concentrated ones when samples were stored at cool temperatures. In contrast, Sengupta and Chaube (1972) reported that extension of buffalo semen at the high dilution rate was more effective in maintaining sperm viability at room temperature than at the lower dilution rate at all storage intervals studied. The depressing effect of +NSC containing glucose (+NSCG) on motility of spermatozoa could also be accounted for by the presence of sulfanilamide in the diluent. Knodt and Salisbury (1946) observed glycolytic activity of bovine spermatozoa during storage at 5°C for 18 days and suggested that the breakdown of glucose to lactic acid was maximally stimulated by sulfanilamide at the level of 300 mg per 100 ml and at dilution rate of 1:9.

The absence of any beneficial effect of sulfanilamide contradicts some earlier observations. Cagampang et al (1989) included sulfanilamide in sodium citrate coconut water-egg yolk diluent for preservation of buck semen and found that sperm motility was comparable to that of standard buck semen diluent (IVT) containing sodium citrate, sodium bicarbonate, potassium chloride, and combination of sulfanilamide plus glucose after storage for up to 3h at room temperatures (25-28°C). Sahni and Roy (1972) suggested that the absence of sulfanilamide in egg yolk citrate diluent caused deterioration of keeping quality of ram semen after 48h of storage due to a rapid multiplication of bacteria at higher temperatures.

The decline in motility of spermatozoa in glucose containing diluents after storage at 5°C indicated the harmful effect of addition of glycolysable substrate in the diluent, possibly because of instant utilization of glucose by spermatozoa which might have released more lactic acid. The present study is in close accordance with the findings of Lapwood and Martin (1966), who found that glucose maintained very poor motility of ram spermatozoa after storage for 3 days at 5°C when compared with ribose, xylose or galactose, with fructose or arabinose giving intermediate results.

Presumably, the deleterious effect of glucose is mediated through conversion to lactate, and consequently reduction of pH to levels which interfere with motility. It is not known whether better buffering capacity of the diluent could maintain pH within a satisfactory range, or whether the low pH might induce a temporary reduction in motility which could be reversed by returning the pH to a neutral range.

However, the most significant finding was that the beneficial effect of coconut extract in semen diluent was not confirmed, but the coconut extract was used at a lower concentration than before. Furthermore, an effect of the other constituents was excluded where the same yolk concentration was used in all media, thereby suggesting that the concentration of egg yolk itself was having a deleterious effect. This warrants further study of the optimal levels of egg yolk that should be incorporated in coconut extract diluent with the higher concentration of coconut extract for obtaining satisfactory numbers of motile sperm for the purpose of AI in sheep.

Chapter 7

Effects of levels of yolk in the presence or absence of coconut extract in citrate-based diluents on the viability of ram spermatozoa stored at 30 and 5°C

7.1. Introduction

Previous work in Chapter 6 designed to screen the various components of coconut extract diluent did not show any significant differences due to the inclusion of the coconut extract or any other constituent. In this experiment, all diluents contained the same concentration of egg yolk. Thus the earlier apparent beneficial effects of coconut extract may have been due instead to a lower concentration of egg yolk, and this possibility was investigated in the present chapter.

There are a number of studies which suggest that high levels of egg yolk are deleterious to spermatozoa, especially ram sperm. First et al (1961) showed that diluents containing 3 or 6% egg yolk maintained motility of ram sperm after freezing and thawing better than those without egg yolk, but also better than those with 12 or 24% egg yolk. However, Salamon and Lightfoot (1969) found that survival of ram spermatozoa after freezing and thawing was very poor without egg yolk, and that 15% was superior to either 5 or 10%. Entwistle & Martin (1972) found that there was a slight but non-significant improvement in motility of ram sperm with 6.5% egg yolk, compared with 13%, with both significantly better than no egg yolk. Watson & Martin (1973, 1975) showed that diluents with 3.75% egg yolk maintained motility of ram sperm after chilling or freezing and thawing better than those with 0.15 or 0.75%, but did not study higher concentrations. However,

these authors subsequently showed that with fresh diluted ram semen, fertility declined with increasing egg yolk concentrations from 0.375 to 6% (Watson & Martin 1976).

With other species, the situation is not so clear-cut. Foote and Bratton (1960) found that motility of bull sperm at 5°C was better with 50% egg yolk than 20% in one experiment increasing storage for 10 days, but 20% was better than 50% in a second experiment with storage for 8 days. In a subsequent study, the same group showed that the fertility of bull bull semen stored for 3 and 4 days at 5°C was only marginally better with 20% egg yolk than with 50% (72.9 and 72.3% non-return rate for 20% egg yolk in two different diluents compared with 66.6% for 50% egg yolk (Foote et al, 1960). Bull sperm frozen in ampoules recover motility after thawing better when the diluent contained between 11 and 24% egg yolk than 6, 35 or 46% (Vandemark et al, 1957), but when frozen in straws, the best results were obtained with diluents containing between 2 and 8% egg yolk, with poorer results with 1, 16 or 32% (Zarazua et al, 1977). Shannon & Curson (1972) found that egg yolk increased the deleterious effect of dead bull sperm on other sperm in the sample. On the other hand, Sahni & Mohan (1990) found that 20% egg yolk preserved the motility of bull sperm following freezing and thawing better than 2.5, 5 or 10%, and while all concentrations tested produced only a less obvious improvement with buffalo sperm, 10% was slightly better than the other concentrations tested. Sperm from a brindled gnu recovered motility after freezing and thawing better in 10% egg yolk than in 20%, and in a second experiment, better in 6.25% than 12.5 or 25% (Watson, 1976). Cat sperm recover motility after freezing and thawing better in diluents containing 5% egg yolk than in those containing 10 or 20% (Glover and Watson, 1987). Trimeche et al (1997) found that 10% quail egg yolk or 5% hen yolk was the optimum concentration for the recovery of motility of Poitou jackas sperm after freezing and thawing, with higher concentrations giving appreciably poorer results.

An attempt had therefore been made to examine the effects of different concentrations of egg yolk on the motility of ram sperm during storage at 30 or 5°C in the presence or absence of coconut extract.

7.2. Experimental procedure

7.2.1. Semen collection and dilution

The first ejaculate from each of three Merino rams was collected once a week with an artificial vagina and then pooled. The experiment was performed 4 times. Pooled semen was then split into 20 parts. The first ten of these aliquots were prepared for 30°C storage and they were diluted with ten different diluents. The second ten of these aliquots were prepared for 5°C storage and were diluted with the ten diluents. The composition of the diluents either for storage at 30°C or 5°C is shown in Tables 7.1 and 7.2.

Table 7.1. Composition of yolk-citrate based diluent (Salamon's Standard Diluent)

Components		Diluent				
		0Y-C	5Y-C	10Y-C	15Y-C	20Y-C
Sodium citrate ($2H_2O$)	(g)	2.37	2.37	2.37	2.37	2.37
Glucose	(g)	0.80	0.80	0.80	0.80	0.80
Sodium penicillin	(g)	0.06	0.06	0.06	0.06	0.06
Dihydrostreptomycin sulphate	(mg)	100	100	100	100	100
Hen yolk	**(ml)**	**0**	**5**	**10**	**15**	**20**
Double distilled water to	(ml)	100	100	100	100	100

Table 7.2. Composition of yolk and coconut extract-citrate based diluent (Norman's Standard Diluent)

Components		Diluent				
		0Y+C	5Y+C	10Y+C	15Y+C	20Y+C
Sodium citrate ($2H_2O$)	(g)	2.37	2.37	2.37	2.37	2.37
Sodium penicillin	(mg)	31	31	31	31	31
Dihydrostreptomycin sulphate	(mg)	68	68	68	68	68
Sulfanilamide	(mg)	300	300	300	300	300
Catalase	(units)	15000	15000	15000	15000	15000
Nystatin	(units)	400	400	400	400	400
Hen yolk	**(ml)**	**0**	**5**	**10**	**15**	**20**
Coconut extract	**(ml)**	**15**	**15**	**15**	**15**	**15**
Double distilled water to	(ml)	100	100	100	100	100

The ten diluent treatments were as follows: 0Y-C (0% egg yolk with no coconut extract), 5Y-C (5% Y), 10Y-C (10% Y), 15Y-C (15% Y), 20Y-C (20% Y), 0Y+C (0% egg yolk +15% coconut extract), 5Y+C (5% Y+15% C), 10Y+C (10% Y+15% C), 15Y+C (15% Y+15% C) and 20Y+C (20% Y+15% C).

7.2.2. Semen assessment

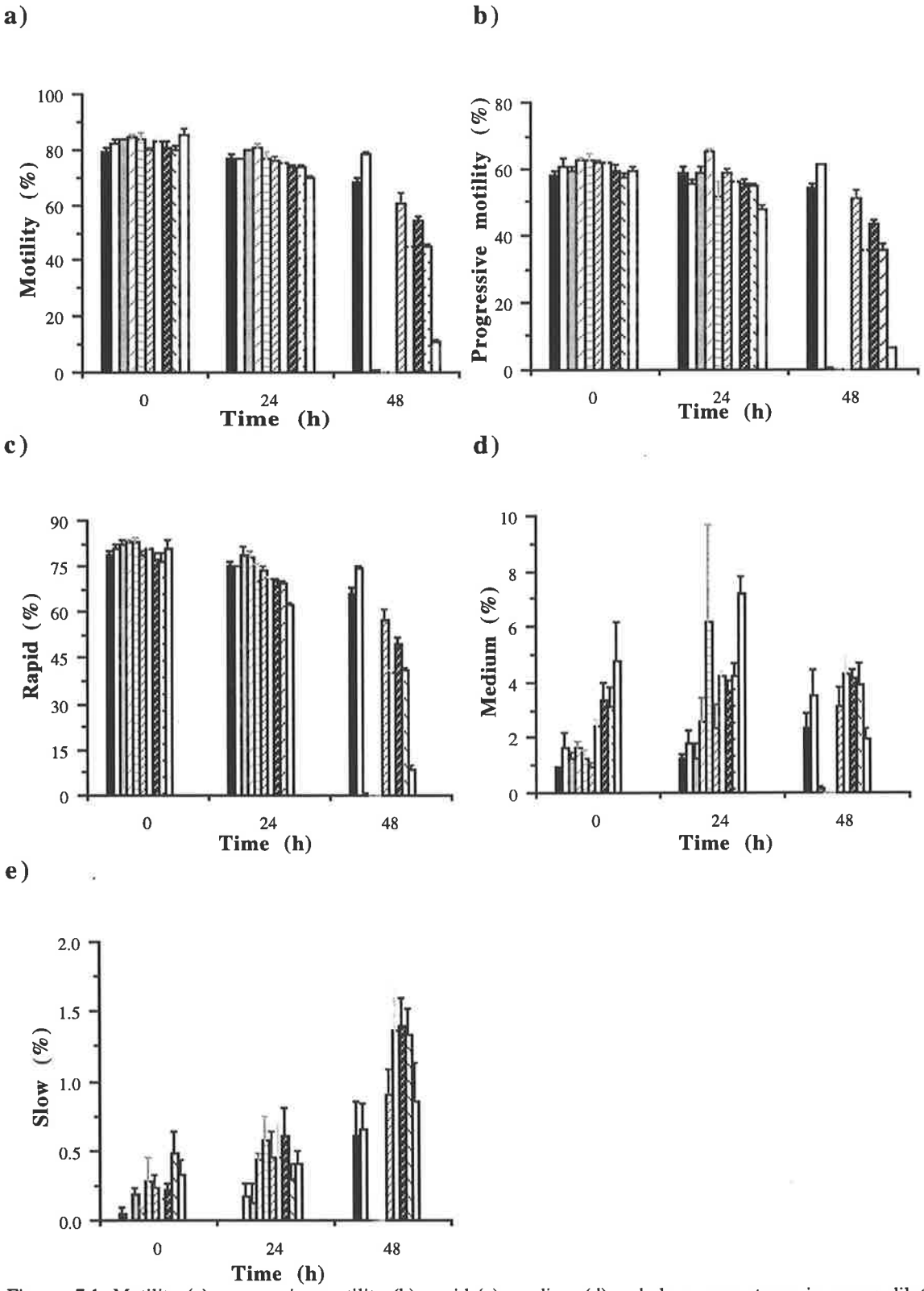
Samples kept at 30°C were assessed objectively at 0, 24 and 48h; while samples kept at 5°C were assessed objectively at 0, 24, 48, 96, 144, 192 and 240h.

7.3. Results

7.3.1. Preservation at 30°C

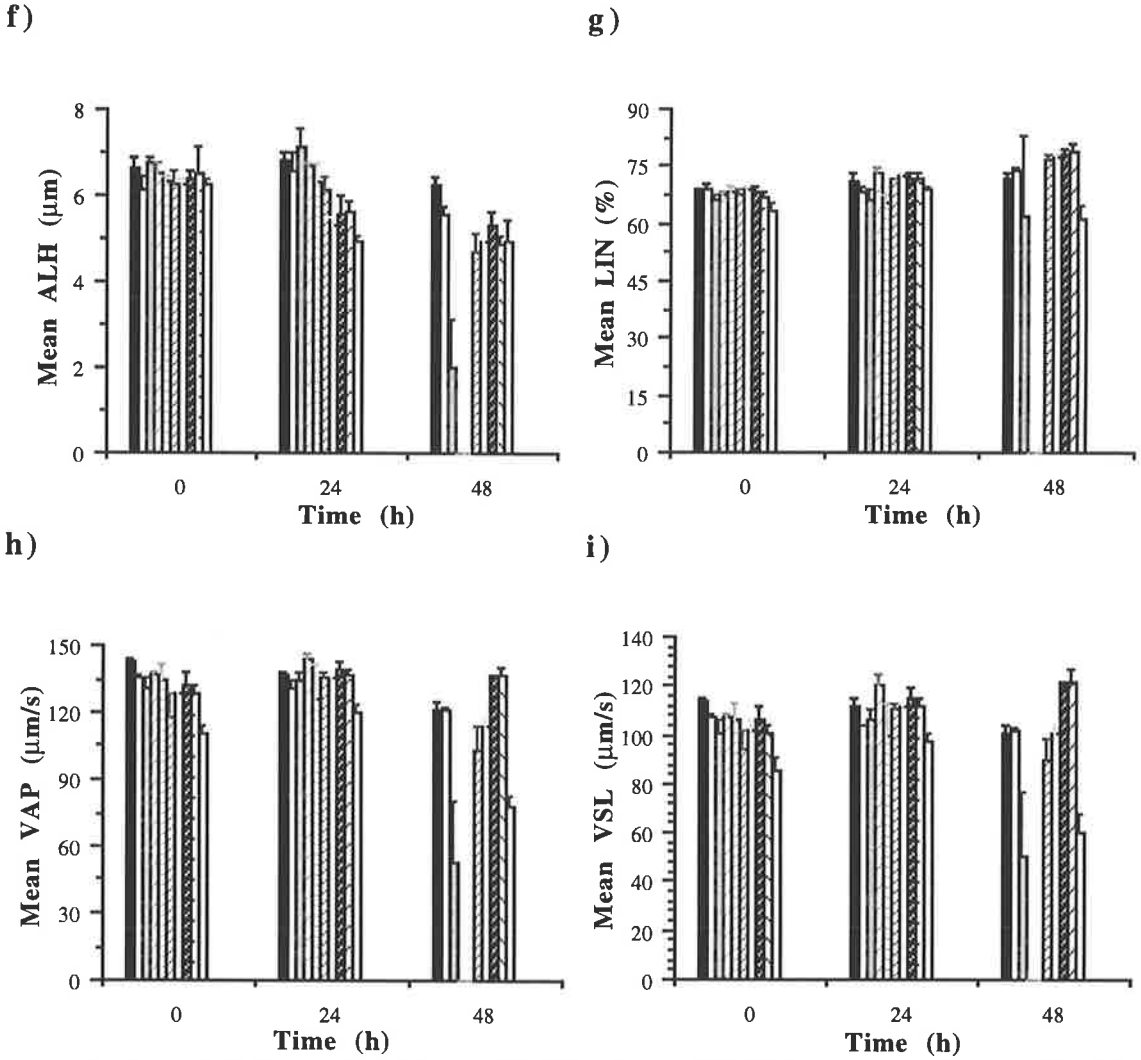
The effect of diluent and time of storage on the motion characteristics of ram semen diluted in ten diluents stored at 30°C is summarized in Table 7.3 (Appendix A). There was a highly significant ($p<.001$) interaction between type of diluents and time of storage in all parameters studied.

No significant difference in motility of spermatozoa among the ten diluents was observed at the start of storage. At 24h, motility of spermatozoa in the diluents with 10 and 15% egg yolk with no coconut extract was significantly higher ($p<0.05$) than that of motility in the diluent without egg yolk. After 48h at 30C, the higher concentrations of egg yolk in the absence of coconut extract produced a severe reduction in sperm motility. This effect of egg yolk was less obvious in the presence of coconut extract, although the 20% egg yolk still caused a marked reduction in motility. However, the motility of the sperm was less when stored with coconut extract, with the lowest concentrations of egg yolk than in the absence of coconut extract (Figure 7.1.a).



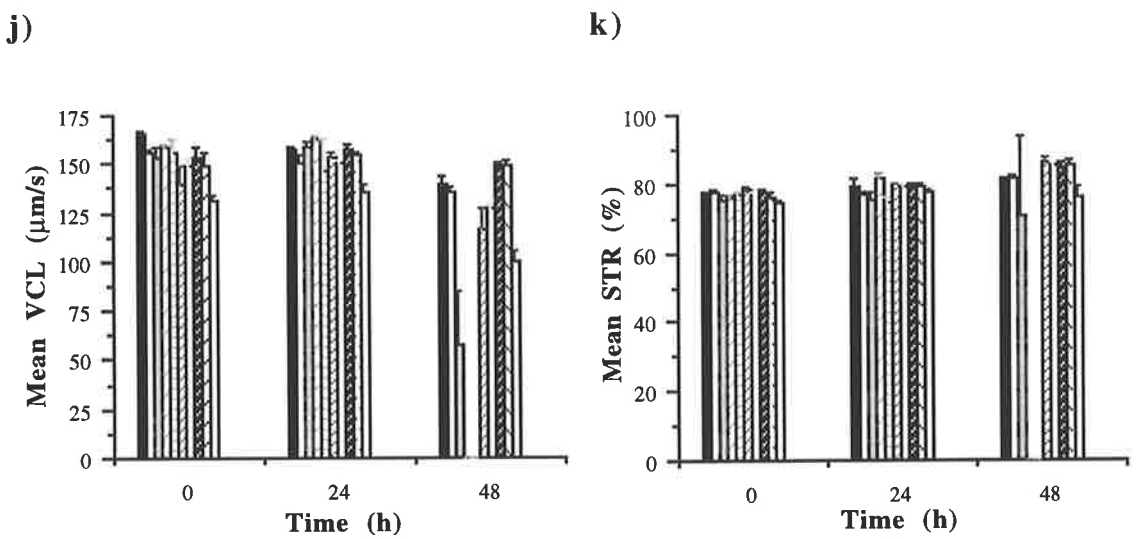
Figures 7.1. Motility (a), progressive motility (b), rapid (c), medium (d) and slow spermatozoa in semen diluted in 0Y-C (■), 5Y-C (□), 10Y-C (▩), 15Y-C (▣), 20Y-C (□), 0Y+C (▨), 5Y+C (□), 10Y+C (▩), 15Y+C (▣) and 20Y+C (□); and stored at 30°C for up to 48h. Values are Means ± SEM of 4 observations.

Similar results were found with percent progressive and percent rapid sperm (Figures 7.1.b and c), while percent medium and percent slow sperm were slightly higher in diluents with coconut extract and the higher concentrations of egg yolk (Figures 7.1.d and e).



Figures 7.1. Mean ALH (f), mean LIN (g), mean VAP (h) and mean VSL (i) of spermatozoa in semen diluted in 0Y-C (■), 5Y-C (□), 10Y-C (▩), 15Y-C (□), 20Y-C (▣), 0Y+C (▨), 5Y+C (□), 10Y+C (▩), 15Y+C (▣) and 20Y+C (□); and stored at 30°C for up to 48h. Values are Means ± SEM of 4 observations.

There were no obvious differences between the mean ALH, mean VAP, mean VSL or mean VCL of sperm stored with the lower concentrations of egg yolk with or without coconut extract (Figures 7.1.f,h,i and j), while percent LIN and percent STR were slightly higher in the presence of coconut extract than in its absence (Figures 7.1.g and k).



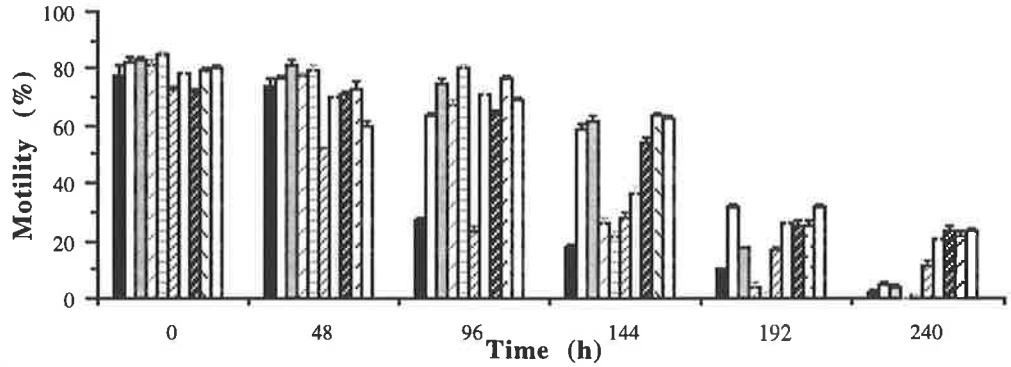
Figures 7.1. Mean VCL (j) and mean STR (k) spermatozoa in semen diluted in 0Y-C (■), 5Y-C (□), 10Y-C (▩), 15Y-C (□), 20Y-C (□), 0Y+C (▨), 5Y+C (□), 10Y+C (▩), 15Y+C (□) and 20Y+C (□); and stored at 30°C for up to 48h. Values are Means ± SEM of 4 observations.

7.3.2. Preservation at 5°C

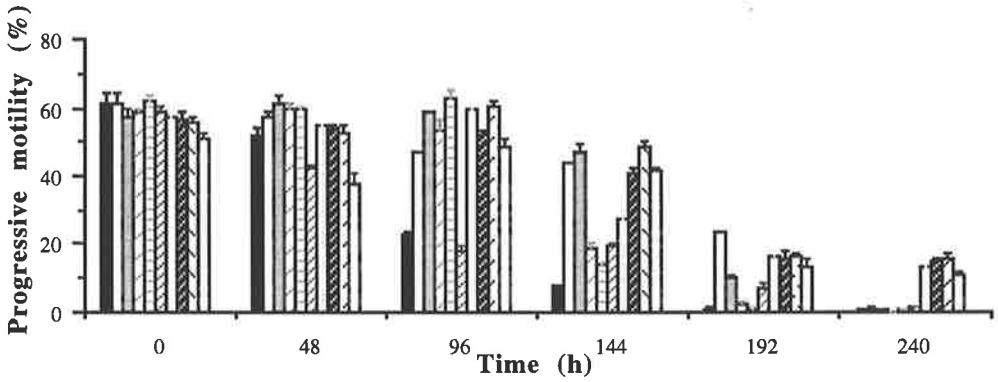
The effect of diluent and time of storage on the motion characteristics of ram spermatozoa diluted in ten diluents stored at 5°C is summarized in Table 7.4. (Appendix A). There was a highly significant interaction ($p<.001$) between type of diluents and time of storage in all parameters studied.

The percentage of motile sperm had fallen by 96h of storage at 5°C in the absence of egg yolk and coconut extract and continued to fall to very low values by 240h. The presence of 5 or 10% egg yolk in the absence of coconut extract maintained motility slightly better in the absence of egg yolk, but higher concentrations (15 and 20%) were less effective than 0% at 192 and 240h. In the presence of 15% coconut extract, motility was maintained at about 20% after 192h of storage at all concentrations of egg yolk, and even in the absence of egg yolk, and in the presence of coconut extract some motility persisted even after 240h storage at 5°C (Figure 7.2.a).

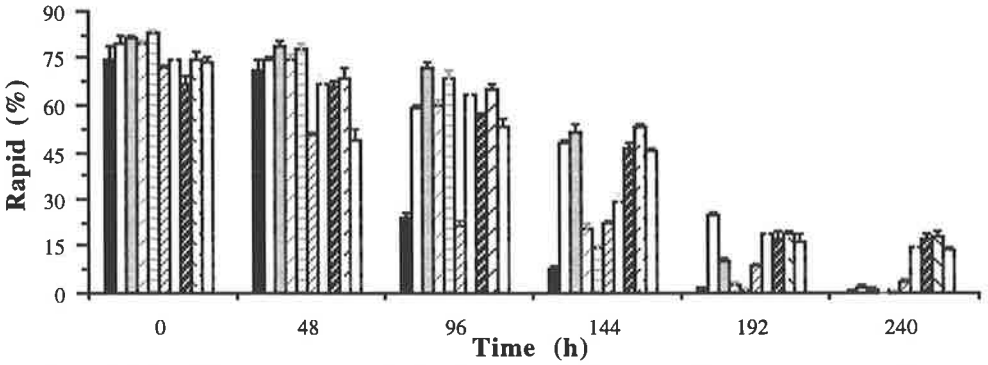
a)



b)



c)

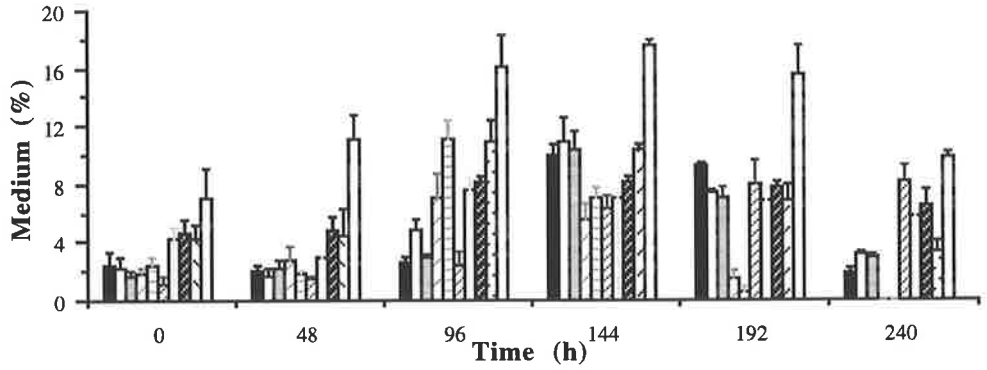


Figures 7.2. Motility (a), progressive motility (b) and rapid (c) spermatozoa in semen diluted in 0Y-C (■), 5Y-C (□), 10Y-C (▩), 15Y-C (▨), 20Y-C (□), 0Y+C (▨), 5Y+C (□), 10Y+C (▩), 15Y+C (▨) and 20Y+C (□); and stored at 5°C for up to 240h. Values are Means ± SEM of 4 observations.

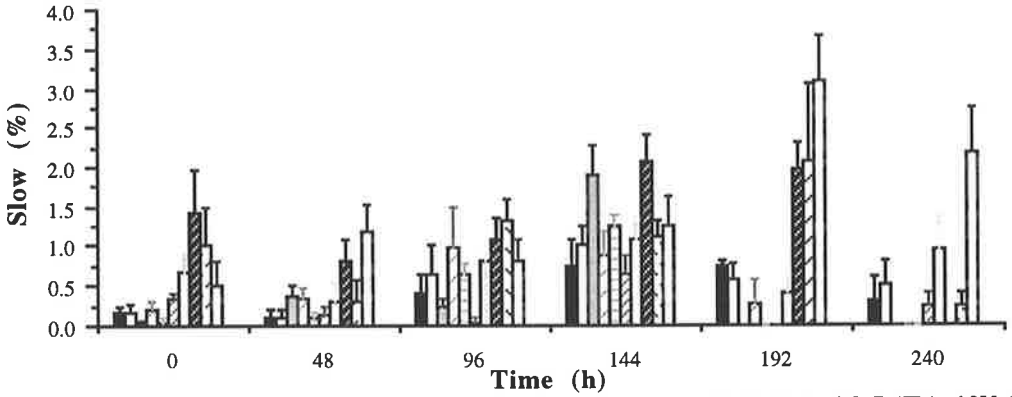
Similar results were obtained with percent progressive and percent rapid sperm (Figures 7.2.b and c). The results with percent medium were less consistent, with high values found with 20% egg yolk in the presence of coconut extract, especially between 96

and 192h. In general, the values in the presence of coconut extract were higher than in its absence (Figure 7.2.d).

d)



e)

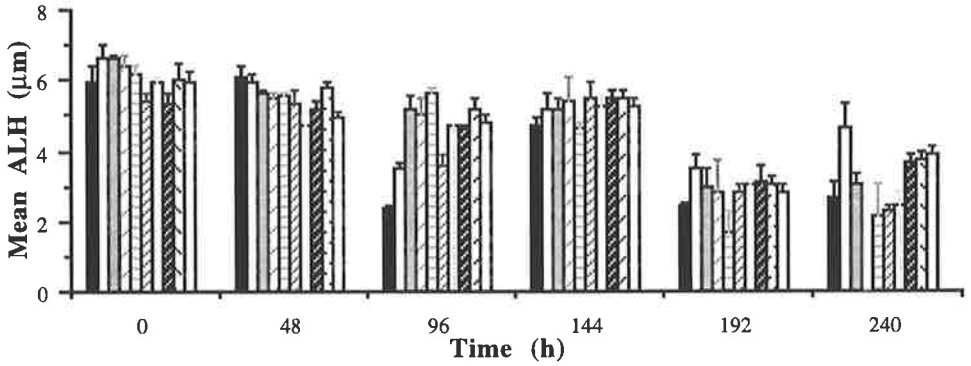


Figures 7.2. Medium (d) and slow (e) spermatozoa in semen diluted in 0Y-C (■), 5Y-C (□), 10Y-C (▩), 15Y-C (□), 20Y-C (□), 0Y+C (▨), 5Y+C (□), 10Y+C (▩), 15Y+C (□) and 20Y+C (□); and stored at 5°C for up to 240h. Values are Means ± SEM of 4 observations.

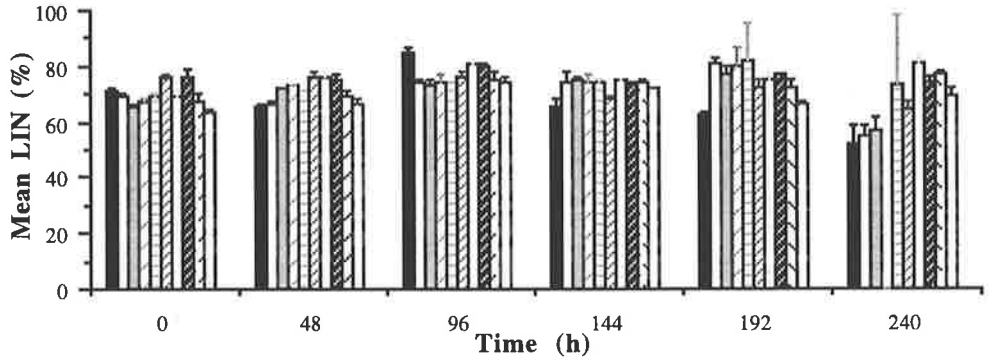
The results for percent slow sperm were also less consistent, but in general higher values were found in the presence of coconut extract than in its absence (Figure 7.2.e). There were no consistent effects of level of egg yolk or the presence of coconut extract in mean ALH, which showed a slight decrease with time of storage (Figure 7.2.f).

Mean VAP, mean VSL and mean VCL, also decreased with time, but to a lesser extent in the presence of coconut extract (Figures 7.2.h,i and j).

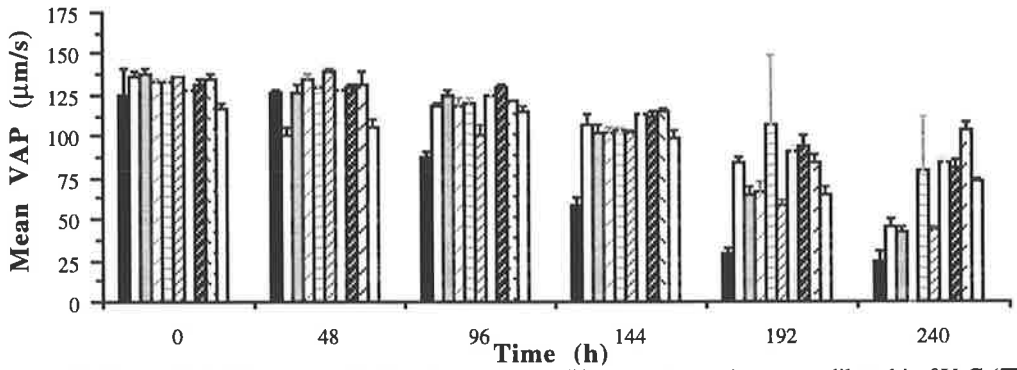
f)



g)



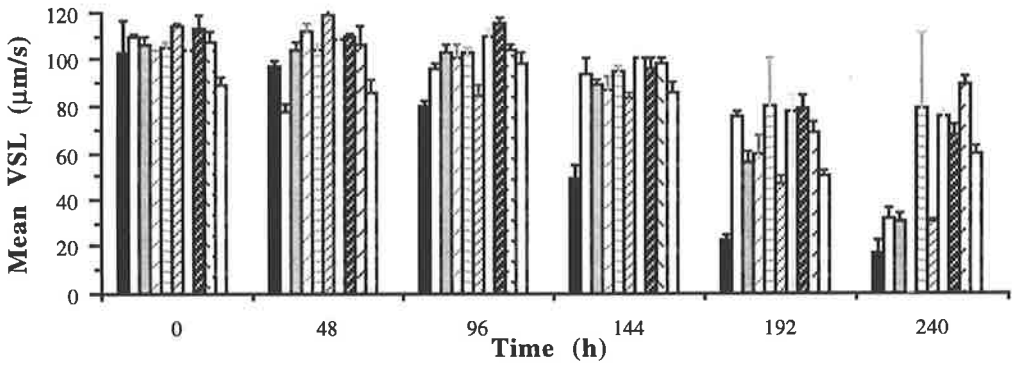
h)



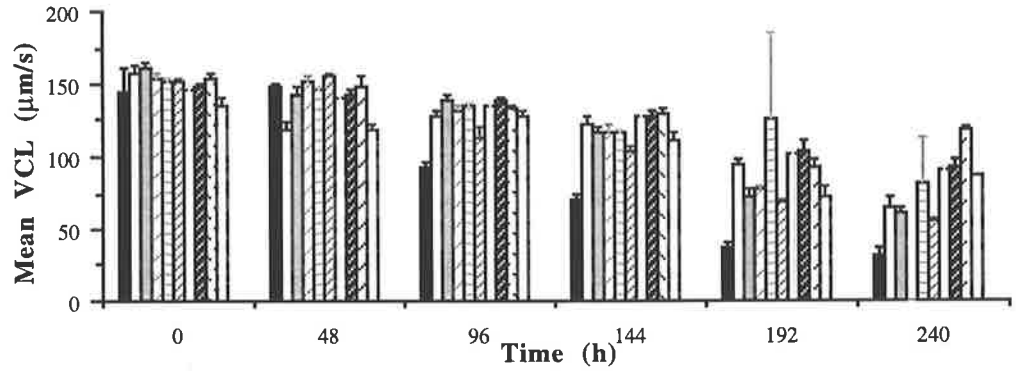
Figures 7.2. Mean ALH (f), mean LIN (g) and mean VAP (h) spermatozoa in semen diluted in 0Y-C (■), 5Y-C (□), 10Y-C (▣), 15Y-C (□), 20Y-C (▣), 0Y+C (▨), 5Y+C (□), 10Y+C (▣), 15Y+C (□) and 20Y+C (□); and stored at 5°C for up to 240h. Values are Means ± SEM of 4 observations.

Mean percentages of LIN and STR had decreased slightly by 240h in the samples stored with no coconut extract, most obviously in the one also containing egg yolk, but otherwise there were few changes (Figures 7.2.g and k).

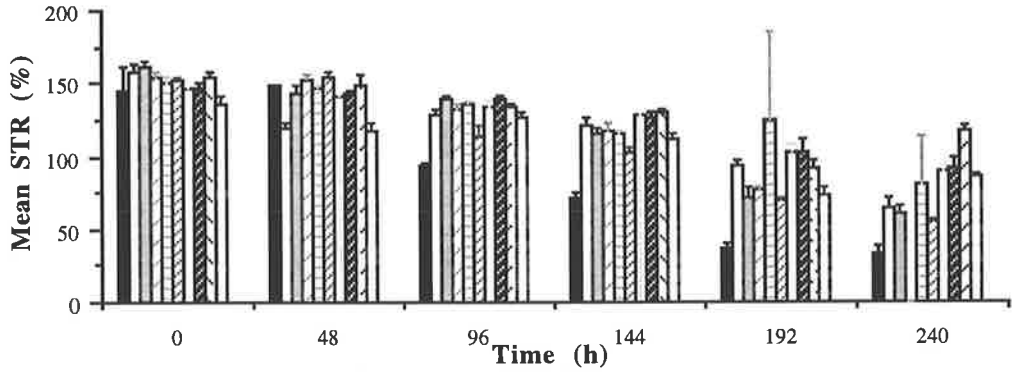
i)



j)



k)



Figures 7.2. Mean VSL (i), mean VCL(j) and mean STR (k) spermatozoa in semen diluted in 0Y-C (■), 5Y-C (□), 10Y-C (▣), 15Y-C (▣), 20Y-C (□), 0Y+C (▨), 5Y+C (□), 10Y+C (▩), 15Y+C (□) and 20Y+C (□); and stored at 5°C for up to 240h. Values are Means ± SEM of 4 observations.

7.4. Discussion

It is clear from the results of the present experiment that concentrations of egg yolk above 5% in the diluents used here are deleterious for the storage of ram sperm, either at 30°C or 5°C. This negative effect of egg yolk thus appears to operate at a lower concentration during liquid storage than during freezing and thawing, where one report (Salamon and Lightfoot, 1969) suggests that 15% gives optimal survival of sperm. However, in view of the present results, and others reported in the literature, it is strange that the widely used text on artificial insemination (Evans and Maxwell, 1987) recommends a level of 20% egg yolk. How egg yolk exerts its damaging effects is not known, possibly reactive oxygen spesies are released from the egg yolk during storage. However, it is clear that the inclusion of coconut extract or one of the other constituents of Norman's medium has a beneficial effect.

The beneficial effect of Norman's diluent in this experiment agree with the findings in chapters 4 and 5, but contrasts with the lack of effect seen in chapter 6. The only explanation which can be offered for this discrepancy is that a slightly lower concentration of coconut extract (10 vs 15%) was inadvertently used in chapter 6. Other constituents of the medium, with the exception of egg yolk which was used at 10% in all samples in chapter 6 were the same in chapters 6 and 7. It is strange that such a small difference in concentration should have such a marked effect, but unfortunately it was not possible to conduct a direct comparison of the effects of different levels of coconut extract.

Chapter 8

Effect of addition of sugars in egg yolk citrate-based diluent on the viability of ram semen stored at 30 and 5°C

8.1. Introduction

It has been indicated in the earlier experiments (chapter 6) that inclusion of glucose in citrate buffered medium had detrimental effects on sperm viability at 30°C, but only in the absence of enzymes. In contrast, it produced negative effects on the viability of ram spermatozoa at 5°C even in the presence of enzymes. Thus a study was conducted to determine in more detail the effect of glucose in comparison with other various sugars such as fructose, inositol, trehalose, lactose or sucrose in citrate-based diluent on the viability of ram semen stored at 30°C for up to 48h; and at 5°C for up to 288h.

Sugars are often included in semen diluents to act as exogenous energy substrates, as osmotic components and as cryoprotective agents (Watson, 1979; Maxwell and Salamon, 1993). Inclusion of sugars such as glucose or fructose which can be metabolized by spermatozoa has been reported to give better storage results (Kampschmidt et al, 1951). Lapwood and Martin (1966) reported that substitution of some sodium chloride by glucose, fructose, ribose, xylose, arabinose or galactose in phosphate-buffered diluent improved the motility of ram spermatozoa at 37°C to approximately the same extent, whereas when storage was for 3 days at 5C, glucose had only a small effect, and arabinose and fructose were less effectrve than ribose, xylose or galactose. Replacement of some sodium chloride by lactose improved the motility of ram sperm stored at 37°C or 5°C and replacement of 92 of 123 mM sodium chloride with 184 mM fructose, glucose, lactose or sucrose improved the survival of ram sperm at 5°C in the presence of 11 mM fructose (Martin, 1966). Observations by Sharma

and Mahajan (1965) revealed an improvement of the survival of bull spermatozoa at room temperature (27-38°C) for 7 days as the level of glucose in the IVT diluent was increased. However, the addition of glycolysable sugars in coconut milk extender did not maintain the percentage of motile or live bull spermatozoa stored at room temperature (20-28°C) for 7 days (Rao et al, 1975).

The use of sugar alcohols such as inositol as cryoprotective agents in ram semen diluents has been reported by Molinia et al (1994); and a non reducing disaccharide, trehalose, proved to be a novel cryoprotectant for chicken spermatozoa as reported by Terada et al (1989). However, there seems to be lack of information regarding the use of inositol or trehalose in the media for maintaning viability of ram semen for short term liquid storage.

This chapter describes the results of a study on the effects of glucose, fructose, inositol, trehalose, lactose or sucrose on the viability of ram semen stored at 30 and at 5°C.

8.2. Experimental procedure

Two ejaculates from each of 3 Merino rams were collected with an artificial vagina and pooled once a week for three consecutive weeks, so each experiment was done 3 times Each pooled sample was divided into 7 aliquots, each of which was diluted five fold in one of 7 different diluents. These were 1) egg yolk citrate (EYC) with no addition/control diluent, 2) EYC+glucose (+G), 3) EYC+fructose (+F), 4) EYC+inositol (+I), 5) EYC+trehalose (+T), 6) EYC+lactose (+L) and 7) EYC+sucrose (+ Su) (Table 8.1).

Table 8.1. Composition of egg yolk-citrate (EYC)-media alone/control, plus glucose (+G), plus fructose (+F), plus inositol (+I), plus trehalose (+T), plus lactose (+L), plus sucrose (+Su).

Ingredient	Dilu	ent					
	EYC	EYC + G	EYC + F	EYC + I	EYC + T	EYC + L	EYC + Su
Sodium citrate (g)	2.265	2.265	2.265	2.265	2.265	2.265	2.265
Streptomycin sulphate (g)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Penicillin (g)	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Glucose (g)	-	0.80	-	-	-	-	-
Fructose (g)	-	-	0.80	-	-	-	-
Inositol (g)	-	-	-	0.80	-	-	-
Trehalose (g)	-	-	-	-	1.52	-	-
Lactose (g)	-	-	-	-	-	1.52	
Sucrose (g)	-	-	-	-	-	-	1.52
Egg yolk (ml)	10	10	10	10	10	10	10
Double distilled water to (ml)	100	100	100	100	100	100	100
Osmolarity (mOsm/kg)	259	269	273	271	282	278	278
pH citrate buffer - yolk (unadjusted)	7.88	7.93	7.86	7.88	7.86	7.76	8.08
pH citrate buffer - yolk (adjusted)*	7.02	7.01	7.01	7.01	7.00	7.01	7.02
pH diluent + yolk	6.98	6.88	6.85	6.88	6.95	7.03	7.05

*pH was adjusted with citric acid

Semen processing was done while both the diluents and the semen were held in a water bath operated at 30°C. Each of those diluted semen was further divided into two equal parts, thus in total there were 14 diluted samples. The first 7 diluted samples were prepared for storage at 30°C and the other 7 diluted samples were used for storage at 5°C. All glass tubes containing diluted semen were sealed with plastic caps and put into a plastic rack, then removed into a plastic box filled with warm water (30°C). Samples to be kept at room temperature were brought to the semen laboratory and put into a water bath operated at this temperature, and assessed objectively at 0, 24 and 48h interval. Samples to be kept at cool temperatures were placed in a cool room, and once the water in the box reached 5°C over 2 hours, the rack then removed to a water bath maintained at 5°C. Semen assessment was done objectively over 4 different storage periods viz 0, 72, 144 and 216 hours. To activate the partially inhibited sperm, a thermostage was used to warm the diluted semen on a Makler glass chamber of the Hamilton Thorn Motility Analyser to 37°C before assessment was done.

The estimations of pH of the diluted semen were also performed at intervals according to time of storage at both temperatures.

8.3. Statistical analysis

Data were subjected to analysis of variance using split-plot design and arcsin transformation was done for percentage data. Tukey's test was performed to determine significant differences between means.

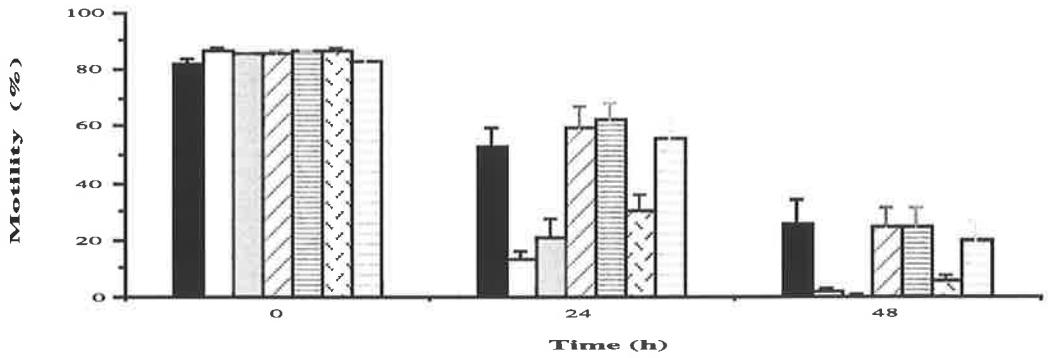
8.4. Results

8.4.1. Preservation at 30°C

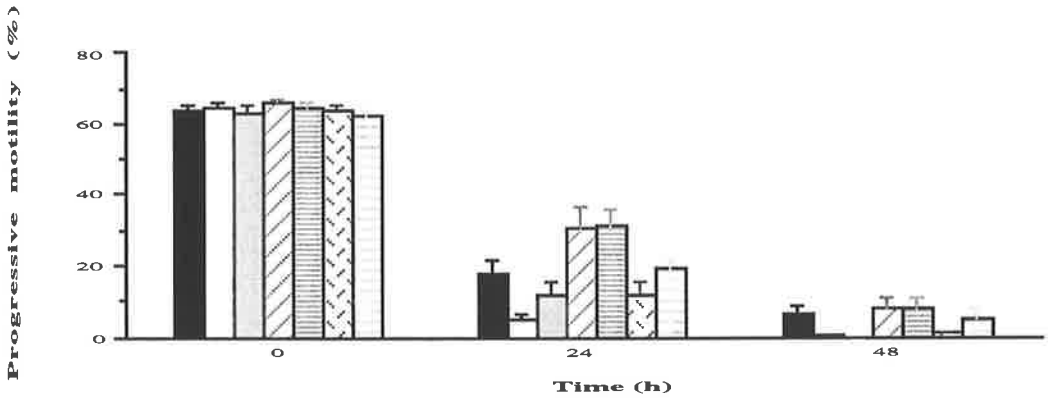
In this experiment, time of storage significantly ($p<0.001$) affected all semen motion characteristics. Diluent had a significant effect on medium, motility, progressive motility, static ($p<0.001$) and mean ALH ($p=0.004$). There was a significant interaction ($p<0.001$) in the percentage of motility and medium sperm between the length of storage and the diluents used. Table 8.2. (Appendix A) shows the mean motion characteristics of ram semen diluted in the seven diluents and stored at 30°C.

The number of motile sperm in all diluents was above 80% at 0h. As time of storage extended, percent motility decreased significantly ($p<0.05$) until it was less than 25% by 48h. When spermatozoa were incubated for 24 or 48h in EYC containing inositol, trehalose or sucrose diluents, there was no significant difference in the ability of the diluents to promote motility compared with that of the control diluent. However, the addition of glucose, fructose or lactose significantly ($p<0.05$) reduced motility at these times (Figure 8.2.a).

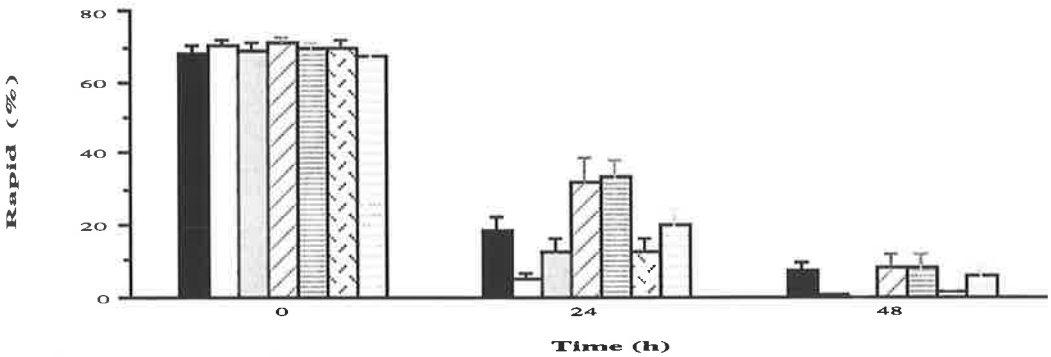
a)



b)



c)



Figures 8.2. Motile (a), progressive motile (b) and rapid (c) spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose(□) and stored at 30°C for up to 48h. Values are Means ± SEM of 3 observations.

At 0h mean progressive motile and rapid spermatozoa were above 60% and around 70%, respectively. With increasing hours of storage, a significant ($p<0.05$) decrease in the numbers of progressive motile and rapid sperm in all diluents was observed. The rate of this forward movement for spermatozoa diluted in inositol- or trehalose-based diluent was greater ($p<0.05$) than those diluted in glucose-, fructose- or lactose-based diluents at 24h. In

addition, the rate of movement for spermatozoa incubated in EYC containing sucrose was also higher ($p<0.05$) than EYC containing glucose diluent. At 48h, all progressive motile and rapid spermatozoa in control or in inositol-, trehalose-, or sucrose-based diluents appeared to be higher (all above 5%) than that in glucose-, fructose- or lactose-based diluents. However, the differences in these parameters among the seven diluents were not significant (Figures 8.2.b and c).

d)

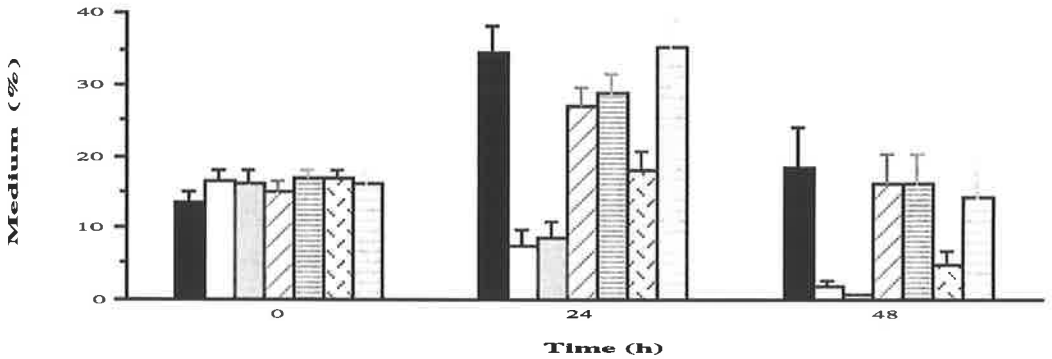


Figure 8.2. Medium (d) spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (▨), + trehalose (▤), + lactose (▨) and + sucrose(□) and stored at 30°C for up to 48h. Values are Means ± SEM of 3 observations.

At 0h, medium sperm in all diluents was around 15%. There was a tendency for medium sperm in most diluents to increase at 24h, after which the values decreased. However for samples diluted in glucose- or fructose-based diluent, the decline in medium sperm occured throughout incubation periods. At 24h, the differences in medium sperm between control or inositol-, trehalose- or sucrose-containing diluents and glucose- or fructose-based diluents were significant ($p<0.05$). At 48h, medium sperm in control or trehalose-containing diluent was significantly ($p<0.05$) higher than that in glucose- or fructose-containing diluent. Besides during this time, the inositol-containing diluent was significantly ($p<0.05$) better in maintaining medium sperm than fructose-containing diluent (Figure 8.2.d).

e)

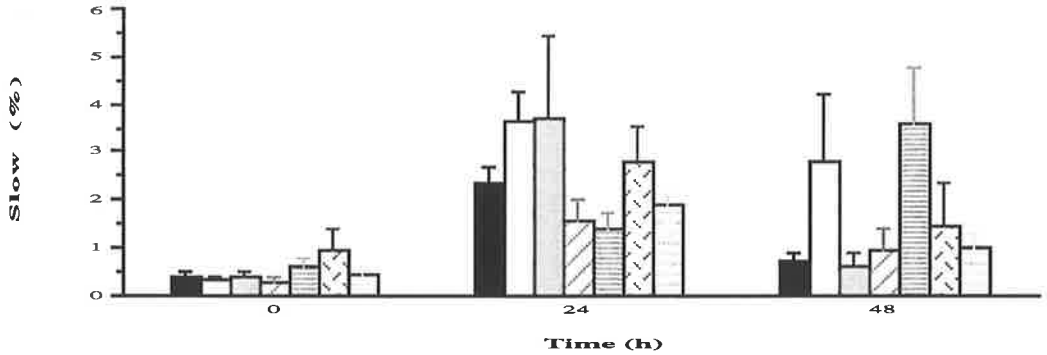


Figure 8.2. Slow (e) spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (▨), + trehalose (▩), + lactose (▨) and + sucrose(□) and stored at 30°C for up to 48h. Values are Means ± SEM of 3 observations.

The percentages of slow sperm in all diluents increased from 0 to 24h and then most decreased at 48h. However, for samples diluted in trehalose-based diluent, the increase in slow sperm continued to 48h of storage. Sperm incubated in control or in glucose-, fructose- or lactose-containing diluents had greater ($p<0.05$) percentages of slow sperm at 24 than at 0 or 48h. The numbers of slow sperm in diluents containing inositol or sucrose was also significantly higher ($p<0.05$) at 24 than at 0h. In addition, slow sperm in trehalose-based diluent was higher ($p<0.05$) at 48 than at 0h (Figure 8.2.e).

f)

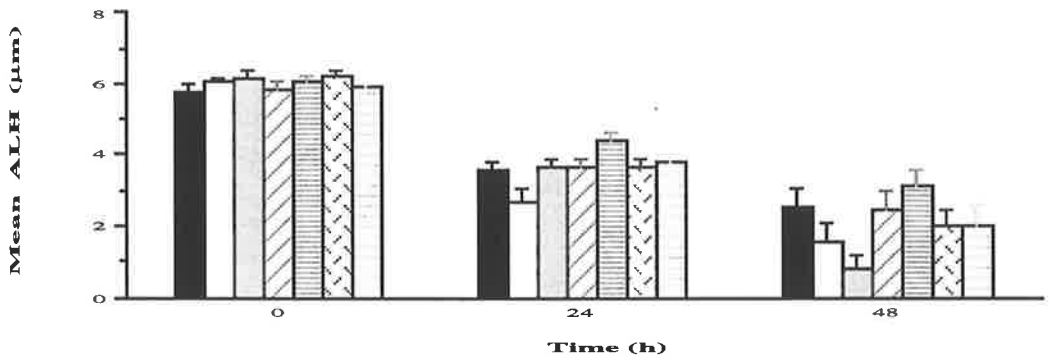
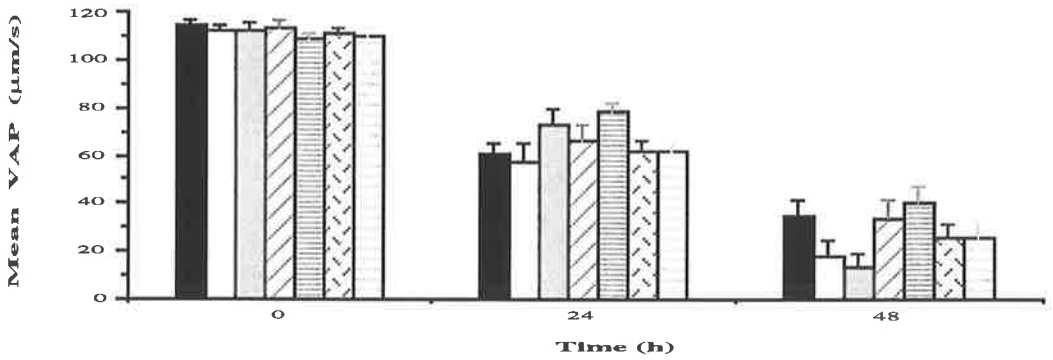


Figure 8.2. Mean ALH (f) of spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (▨), + trehalose (▩), + lactose (▨) and + sucrose(□) and stored at 30°C for up to 48h. Values are Means ± SEM of 3 observations.

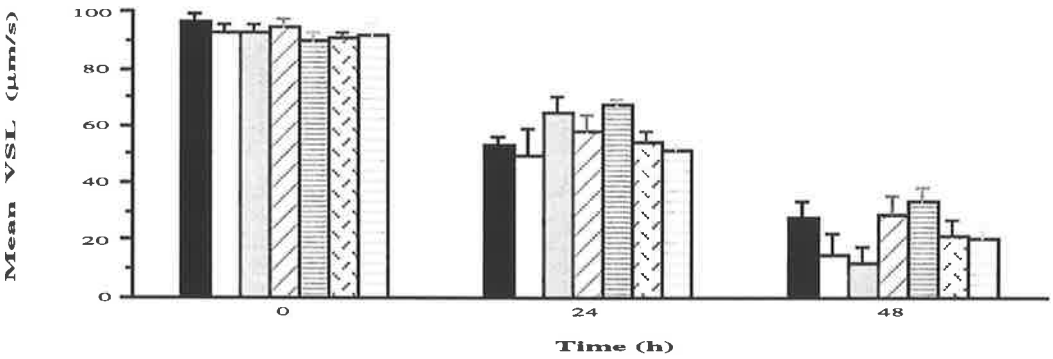
Mean ALH for each diluted semen decreased significantly ($p<0.05$) throughout incubation period. At 48h mean ALH in EYC containing trehalose was greater ($p<0.05$) than

those containing glucose or fructose. Mean ALH in inositol-, trehalose-, lactose- or sucrose-containing diluents did not differ significantly from that of control at this time. The difference between glucose- and fructose-containing diluents was also not significant (Figure 8.2.f).

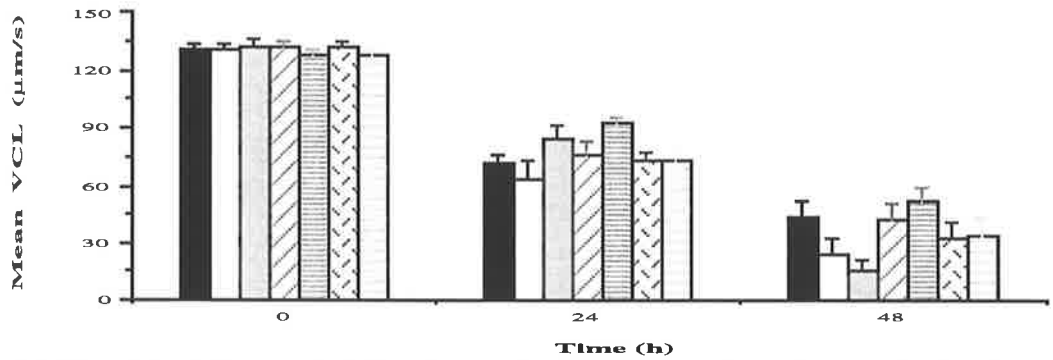
h)



i)



j)



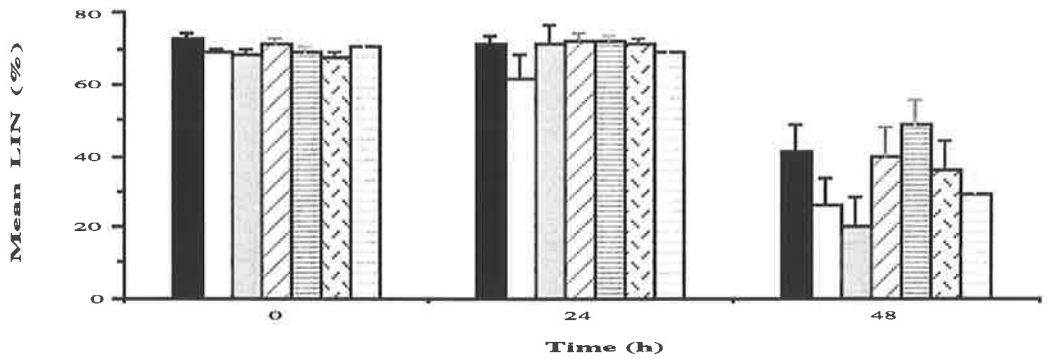
Figures 8.2. Mean VAP (h), mean VSL (i) and mean VCL (j) of spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose (□) and stored at 30°C for up to 48h. Values are Means ± SEM of 3 observations.

At 0h, mean VAP, VSL and VCL in the 7 diluents were almost the same. After 24 and 48h of storage, the values decreased significantly. At 48h, mean VAP, mean VSL and

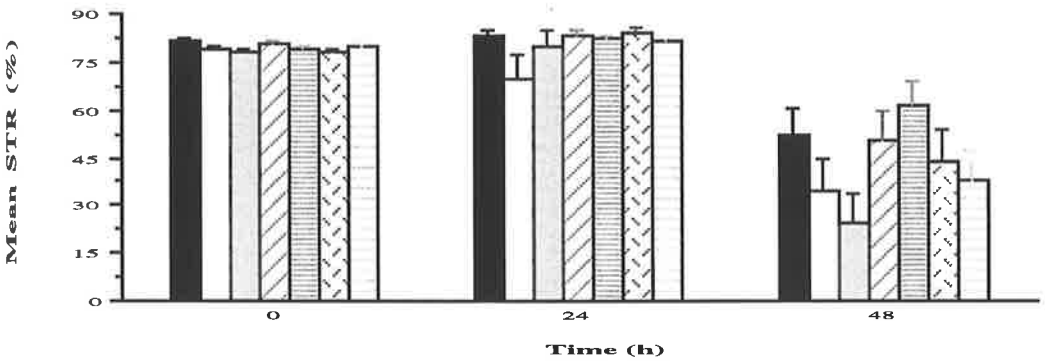
mean VCL in EYC diluent containing glucose or fructose had decreased more compared to the other diluents (Figures 8.2.h,i and j).

The percentages of LIN and STR sperm in all diluents did not change much during storage from 0 to 24h, except for glucose-containing diluent. However after 48h, percent LIN and percent STR in all diluents decreased markedly ($p<0.05$)(Figures 8.2.g and k).

g)



k)

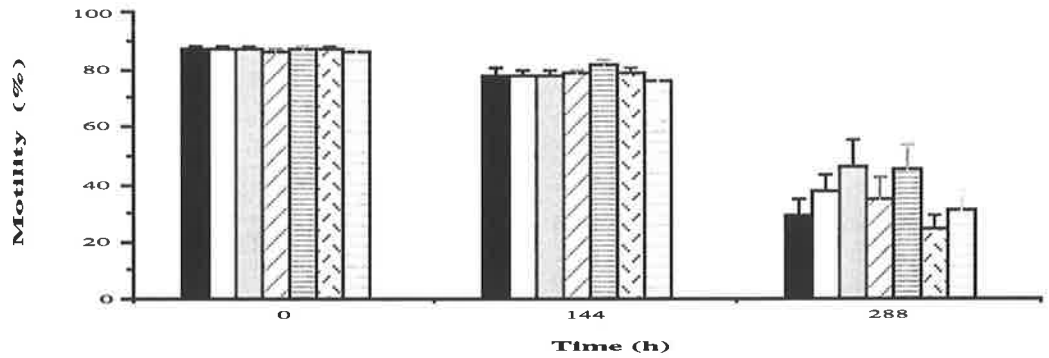


Figures 8.2. Mean LIN (g) and mean STR (k) of spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose (□) and stored at 30°C for up to 48h. Values are Means ± SEM of 3 observations.

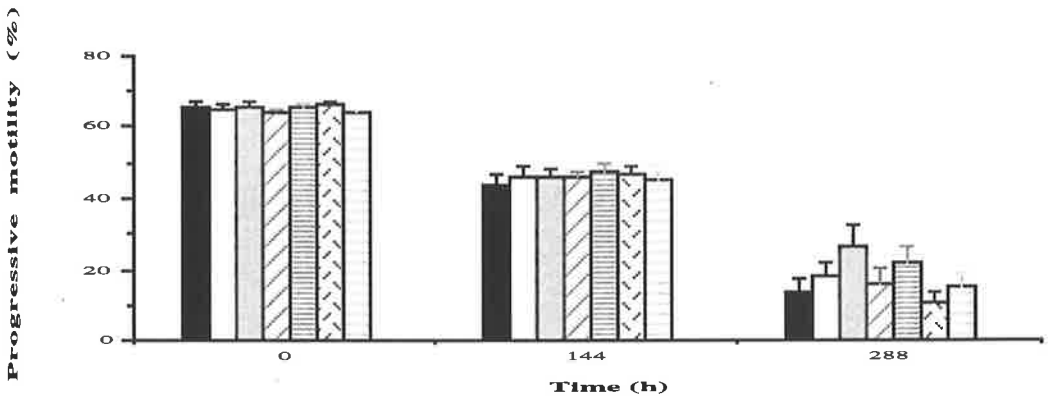
8.4.2. Preservation at 5°C

In this experiment neither diluent nor interaction between diluent by time of storage had any effect on semen motion characteristics at 5°C. However, time of storage significantly ($p<0.001$) affected all semen motion characteristics. The mean motion characteristics of ram spermatozoa surviving in the egg yolk citrate (EYC) diluent alone or its mixtures with a various sugars during 288h of storage at 5°C are given in Table 8.3 (Appendix A).

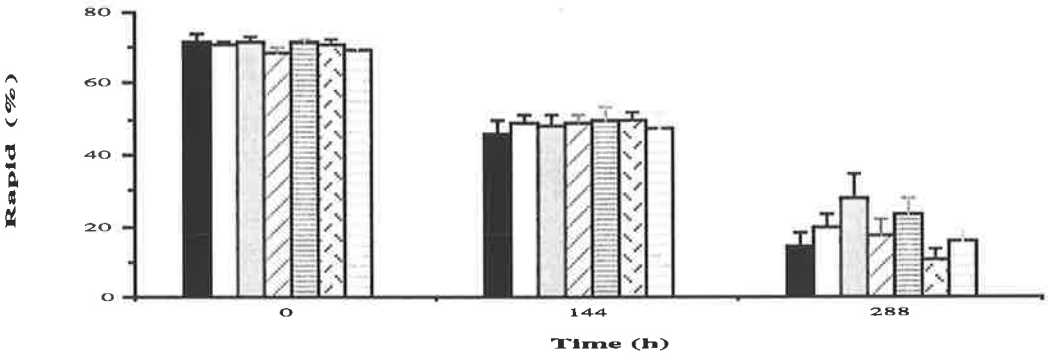
a)



b)



c)



Figures 8.3. Motile (a), progressive motile (b) and rapid (c) spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose(□) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

No significant difference in percent motility, progressive motility and rapid spermatozoa was found between the 7 diluents tested. Small changes in the sperm motion characters in all diluents was observed after 144h of storage. However after 288h, percent motility in all diluents decreased significantly ($p<0.05$), and the lowest motility was obtained in lactose-containing diluent (Figures 8.3.a,b and c).

d)

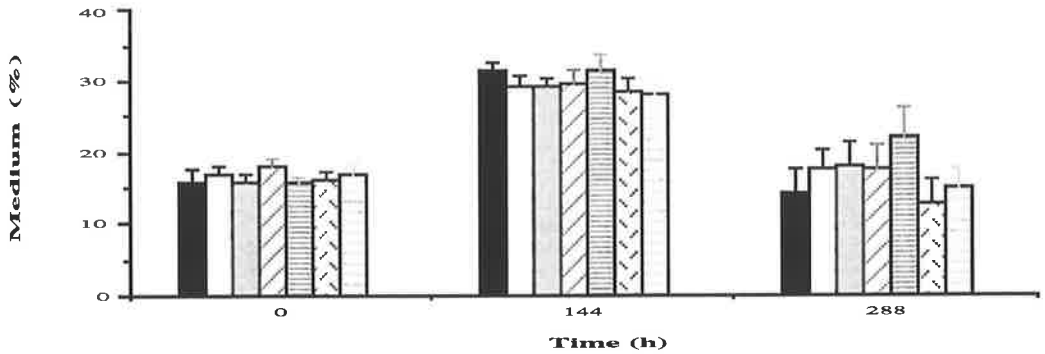
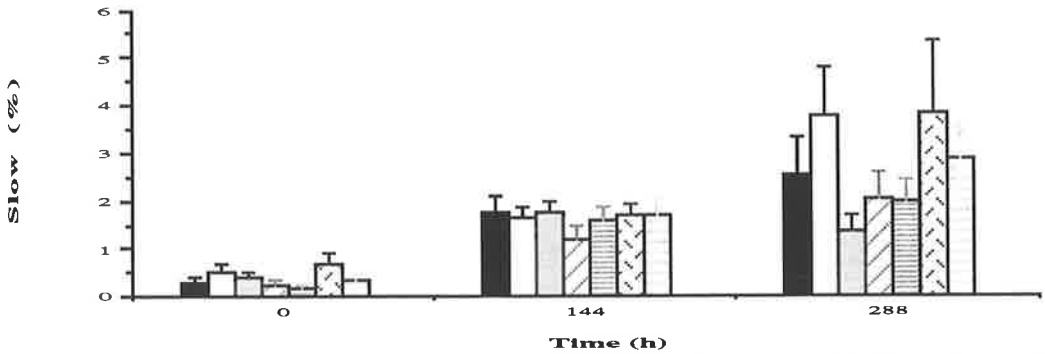


Figure 8.3. Medium (d) spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (▤), + lactose (☑) and + sucrose(□) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

Overall the percentages of medium sperm between the seven diluents were not significant during entire storage period. However, there was a significant ($p<0.05$) increase in medium sperm in all diluents from 0h to 144h; and it had decreased significantly ($p<0.05$) at 288h (Figure 8.3.d). At 0h, mean slow sperm all diluents was found less than 1%, then increased significantly ($p<0.05$) at 144 h. Further storage at 288h tended to increase the percentage of slow sperm, and the values for control, glucose-, lactose-, or sucrose-based diluent was numerically higher than fructose-, inositol- or trehalose-containing diluent (Figure 8.3.e).

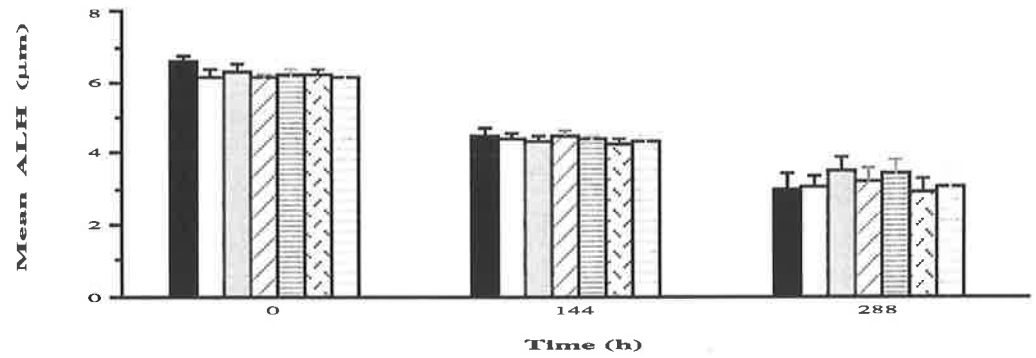
e)



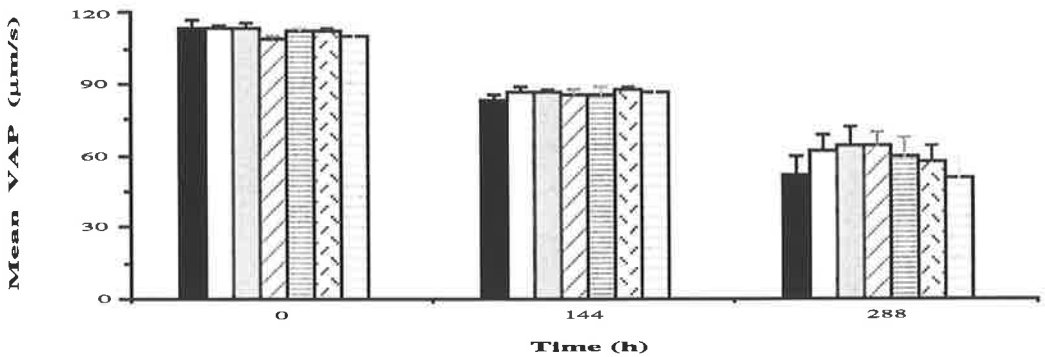
Figures 8.3. Slow (e) of spermatozoa diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (☑), + trehalose (▤), + lactose (☑) and + sucrose(□) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

There was a continuous decrease in mean ALH, mean VAP, mean VSL and mean VCL over time of storage. Mean ALH, mean VAP, mean VSL and mean VCL for samples diluted in the seven diluents and stored at 0h was significantly higher ($p<0.05$) than that stored at 144 or 288h; and the difference in these four kinematics parameters between samples stored at 144 and 288h was also significant ($p<0.05$)(Figures 8.3.f,g,h and i).

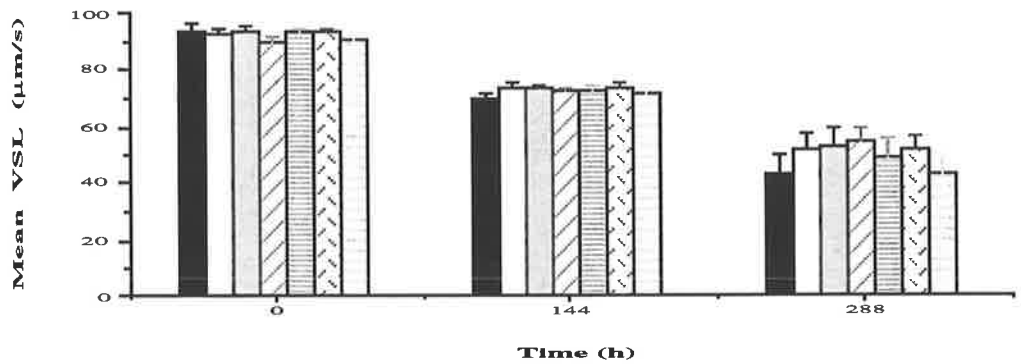
f)



g)

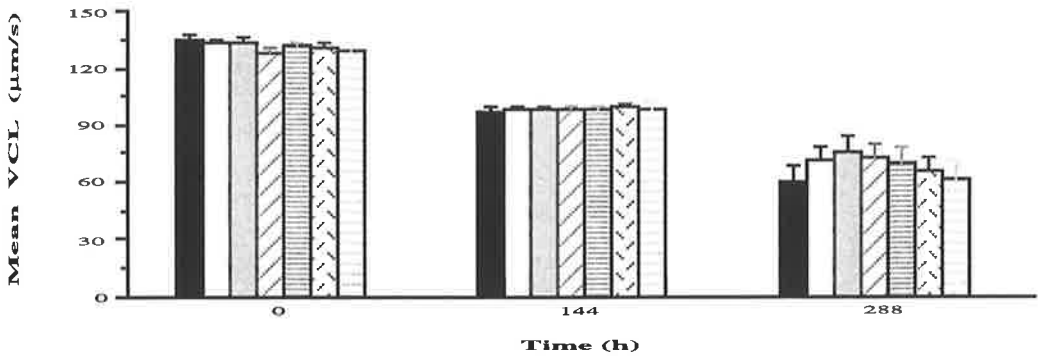


h)



Figures 8.3. Mean ALH (f), mean VAP (g) and mean VSL (h) of spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose (□) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

i)



Figures 8.3. Mean VCL (i) of spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose (□) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

There was a slight increase in the percentage of mean LIN in all diluents at 144h after initiation of storage. After 288h, a small decrease in mean LIN in control, glucose, fructose, inositol or lactose containing diluent was observed. However, in trehalose or sucrose containing diluent, mean LIN fell below 55%. Samples diluted in trehalose or sucrose containing diluent showed a higher ($p<0.05$) mean LIN at 0 or 144h than at 288h (Figure 8.3.j).

j)

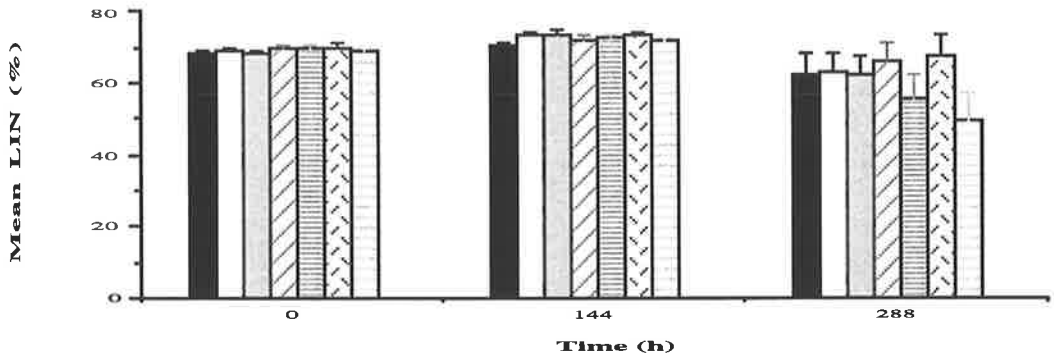


Figure 8.3. Mean LIN (j) of spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose (□) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

Mean STR sperm in all diluents was slightly enhanced after 144h of storage. When time of storage was extended to 288h, mean STR in trehalose or sucrose containing diluents had decreased more than the other remaining diluents. For samples diluted in trehalose

containing diluent, mean STR was higher ($p<0.05$) at 144h than that at 288h. For samples diluted in sucrose containing diluent, mean STR was also higher ($p<0.05$) at 0 or 144 than that at 288h (Figure 8.3.k).

k)

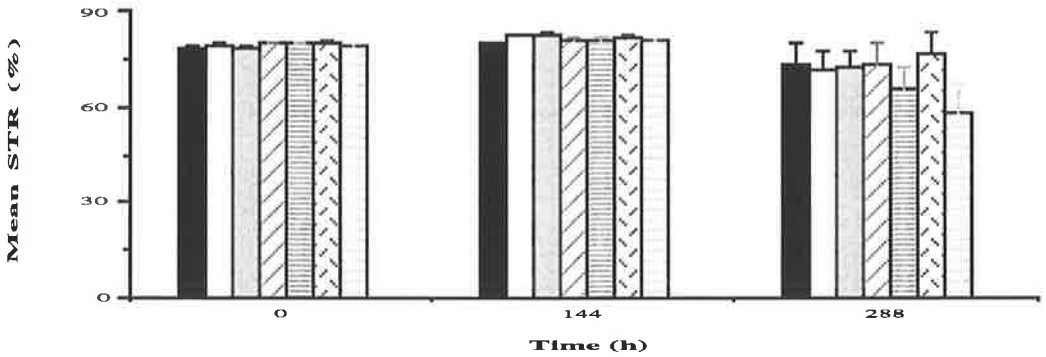


Figure 8.3. Mean STR (k) of spermatozoa in semen diluted in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (□), + inositol (□), + trehalose (□), + lactose (□) and + sucrose (□) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

8.4.3. pH of stored diluted semen

In this study, interactions of pH of diluent by time of storage ($p<0.001$) was observed both at 30 and 5°C. Table 8.4 and Table 8.5 (Appendix A) shows the mean pH reading of diluted ram semen stored up to 48h at 30°C and up to 288h at 5°C, respectively, in seven different diluents.

Observations at 30°C suggested that the difference in pH between inositol, trehalose, lactose or sucrose containing diluent and the control was not significant at 0h. However, pH of these diluents, except lactose, was significantly ($p<0.05$) higher than the pH of glucose or fructose diluent. At 24h, no significant difference in pH between inositol or sucrose containing diluent and the control was observed. However, pH of inositol, sucrose and control diluents were significantly ($p<0.05$) higher than the pH of glucose, fructose, trehalose and lactose containing diluent. The differences in pH between trehalose or lactose and glucose or fructose diluents were also significant ($p<0.05$). At 24h, pH of trehalose diluent was significantly ($p<0.05$) higher than pH of lactose diluent. At 48h, pH of inositol

and sucrose diluents did not differ from pH of control. However, they were significantly ($p<0.05$) higher than pH of glucose, fructose, trehalose and lactose diluents. Trehalose containing diluent had a higher ($p<0.05$) pH than that of pH of glucose diluent at 48h of storage (Figure 8.4.a).

a)

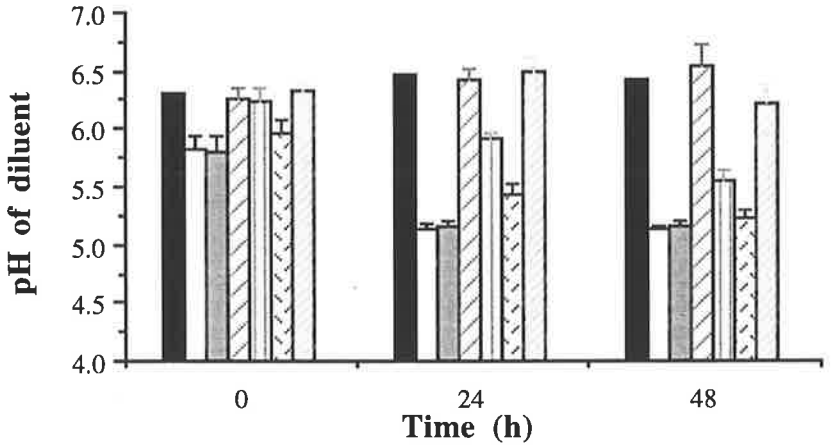


Figure 8.4. Mean pH (a) of diluted semen in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (▩), + inositol (▨), + trehalose (▦), + lactose (▤) and + sucrose (▥) and stored at 30°C for up to 48h. Values are Means ± SEM of 3 observations.

Observations at 5°C suggested that the changes in pH were less than at 30°C. No significant difference was observed between pH of inositol, trehalose or sucrose containing diluent and the control at 0h. However, pH of inositol containing diluent was significantly ($p<0.05$) higher than that of glucose, fructose and lactose containing diluents, while pH of trehalose, sucrose and control diluents were significantly higher ($p<0.05$) than pH of fructose diluent. Although there were no significant differences in pH between inositol or sucrose and control diluents, they were all significantly ($p<0.05$) higher than pH of glucose, fructose, trehalose and lactose diluents either at 144 or 288h of storage (Figure 8.4.b).

b)

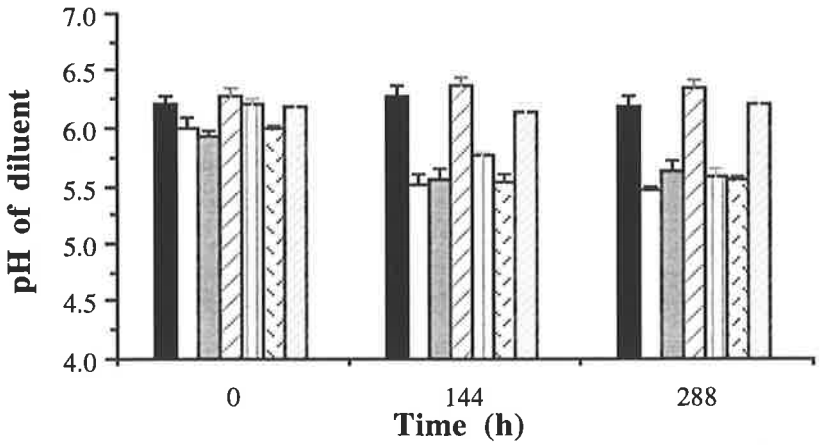


Figure 8.4. Mean pH (b) of diluted semen in egg yolk citrate media alone (control) (■), + glucose (□), + fructose (▩), + inositol (▨), + trehalose (▦), + lactose (▧) and + sucrose (▤) and stored at 5°C for up to 288h. Values are Means ± SEM of 3 observations.

During the present investigation, efforts were also made to establish relationship between various of pH of diluents and motility of spermatozoa. In this case, to make an appropriate comparisons among diluents, those diluted semen with pH values of less than 5 were excluded in the analysis of correlation. This is because below this pH all motility had ceased and the pH of the control diluent remained above 6 after storage for 48h at 30°C or 288h at 5°C. The correlation was based on pH of storage at 24h at 30°C or at 144h of storage at 5°C or pH of pooled data at 0 and 24h of storage at 30°C; and at 0 and 144h of storage at 5°C. The coefficients of correlation (r) of the relationship between pH of diluents and motility of spermatozoa are presented in Table 8.6 and Table 8.7. (Appendix A).

Sperm motility was positively correlated with pH of control or any other sugar-containing diluents at 30°C whether data were analyzed for a single time of storage or pooled. The motility had also significant and positive relationship with pH of all diluted semen at 5°C, except for inositol containing diluent with pooled data and sucrose containing diluent for 0h and 144h of storage.

8.5. Discussion

In this study inclusion of glucose, fructose or lactose in EYC diluents had detrimental effects on the viability of ram spermatozoa preserved at 30°C up to 48h. The results with glucose confirmed the findings of the previous studies (chapter 6) and similar results were obtained by other workers in bulls (Rao et al, 1975) and buffaloes (Kumar et al, 1993). Glucose and fructose serve as energy substrates for spermatozoa. In this study, that these readily metabolizable sugars did not support motility of ram spermatozoa could be due to either exhaution of available energy substrates, a drop in pH due to build up of lactic acid, or a combination of these factors (Mann, 1964; Bearden and Fuquay, 1997). Foote (1964) suggested that at higher temperatures, pH decreased to a greater extent as a consequence of the higher rate of metabolism. Measurements of pH of EYC diluents containing glucose, fructose or lactose after 48h of storage indicated that the pH had already fallen to around 5 (see Figure 8.4). Thus an inverse relationship was evident between metabolism and longevity of motility, as suggested by significant positive correlations between pH of diluents and motility of spermatozoa. Higher correlation coefficients observed in glucose (r=0.970), fructose (r=0.827) and lactose (r=0.899) diluents suggests that sharp decreases in pH occurred as hours of preservation increase, which in turn reduced the numbers of motile sperm in diluents containing these sugars (see Table 8.6, Appendix A). Blackshaw and Emmens (1951) showed that ram semen diluted in a buffer with a pH between 5.4 and 5.8 had slightly lower motility than a sample diluted at 6.7 to 7.1, but importantly they did not examine intermediate or lower pH buffers. These authors also showed that bull sperm diluted in buffer at pH 5.3 to 5.9 and 6.4 to 7.0 showed similar motility, while motility was markedly reduced with buffer at pH 4.2 - 4.6. Salisbury and Kinney (1957) studied the effect of diluents at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 on the motility of bull sperm and found that motility was reduced in the more acidic environments, from maximal values seen at 7.0 and above.

In the study, negative effects of glucose and fructose on the motility of spermatozoa shown during preservation at 30°C did not occur when diluted semen was preserved at 5°C. The observations recorded here confirmed the results obtained by other workers (Salisbury and VanDemark, 1945; Adler and Rasbech, 1956; Ohm and Willett, 1958; and Foote and Bratton, 1960 with bull semen), (Wilcox, 1960 with cock semen) and (Sengupta and Chaube, 1972 with buffalo semen). However, the present results contradict the findings in the previous investigation at 5°C as described in chapter 6, but as pH was not measured in the previous experiment, no explanation can be offered for this discrepancy. The present study shows that though citrate-fructose was slightly better than citrate-glucose in maintaining motility of ram spermatozoa, statistically the difference was not significant. This is in close agreement with that of Maxwell (1978) who observed slightly better results with Tris-fructose than with Tris-glucose diluent in protecting ram spermatozoa during cooled storage for 4 days. The beneficial effect of fructose might due to its preferential utilisation by spermatozoa to any other naturally occurring oxidizable substrate in semen (Yaseen and El-Kamesh, 1970).

Lapwood and Martin (1966) showed that either glucose or fructose, as well as ribose, xylose, arabinose or galactose maintained motility better than control during storage for 6 hours in a phosphate buffered medium at 37°C, but during storage for 3 days at 5°C glucose had only a small beneficial effect and, and fructose or arabinose was less effective than ribose, xylose or galactose. They and Martin (1966) also studied the effects of other sugars including lactose in the presence of fructose, but the results are not relevant to the present studies, except that during storage for 3 to 8 days at 5°C, glucose had a consistently deleterious effect, while xylose was the only sugar that had a consistently beneficial effect.

It was observed in the present study that the pH of the diluted semen stored at 5°C in control, inositol and sucrose diluents showed only a slight decline, and while there were falls in pH with glucose, fructose, trehalose and lactose, there were much less than at 30°C (Table 8.5) . This may be because of the limitation of metabolic activity of cooled sperm at lower

temperatures or might reflect their use of endogenous energy substrates during the initial storage time. Observations in bull semen by Kampschmidt et al (1951) showed a decrease in the motility of spermatozoa in medium containing glucose after day 5 of storage at 5°C, which was in parallel with the decrease in pH, while the pH of the diluted semen in citrate-containing no sugar was maintained. Tomar and Desai (1961) preserved buffalo semen at 5-7°C and found that pH of diluted semen in yolk glycine with glucose and fructose fell to about 5.9 after 72h of storage, while in sodium citrate containing medium it decreased to about 6.1.

The fall in pH during incubation with lactose or trehalose was surprising as there appears to be no information on this metabolism by sperm, except for an unsubstantiated statement by Martin (1966) that "lactose can not be utilized by spermatozoa". We can not say whether the fall in pH was due to the metabolism of the trehalose or lactose or to the presence of metabolisable contaminants in the samples used.

With regard to the osmolarity of all diluents which ranged from 247 to 290 milliosmoles, it seems that within this range ram spermatozoa could survive well without any detrimental effect. Maxwell (1978) stored ram spermatozoa in iso or hypertonic diluents (>200 milliosmoles), which are less harmful than hypotonic diluents (Blackshaw and Emmens, 1951). Similarly, Johnson et al (1955) reported that diluents with tonicity varied from 230 to 420 milliosmoles did not affect the viability of bull spermatozoa when stored in the liquid state.

From the findings of the present studies, it therefore could be concluded that none of sugars, except glucose, fructose and lactose had any effect on the viability of ram spermatozoa in sodium citrate diluent, and glucose, fructose or lactose had deleterious effects. However, at 5°C none of the sugars had any beneficial or deleterious effect on the viability of spermatozoa up to 144h. Beyond this time of storage the viability of spermatozoa deteriorated quickly. Hence, it is advisable to preserve ram spermatozoa in citrate diluent only

up to these times. Finally, it is also clear from this study that even a simple EYC-buffer without sugar seems to be an adequate extender for ram semen for short-term storage at room or chilled temperatures.

Chapter 9

Attempted reactivation of ram spermatozoa with sodium bicarbonate after storage at 30 and 5°C in egg yolk citrate diluents with or without glucose

9.1. Introduction

In the previous experiment (Chapter 8), it was demonstrated that inclusion of sugars such as glucose, fructose or lactose in egg yolk citrate-buffer, reduced motility characteristics of ram spermatozoa after storage for 24h at 30°C. However at 5°C, the motility characteristics were maintained by all sugars up to 144h, and beyond this time then deteriorated. In addition, the capabilities of these EYC-sugar containng diluents in maintaining motility characteristics were no better than that of the EYC without sugar under storage at either temperatures. It has been indicated that decreases in motility characteristics in some diluents coincided with decreases in pH. A pH value of 5 was observed at 48h in those egg yolk citrate-sugar containing diluents when stored at 30°C. However in EYC without sugar, the pH remained high (above 6) at this time.

Attempts to reactivate the immobilized spermatozoa, although for short periods of time, have been made by addition of carbon dioxide (Shettles, 1940) and increase in temperature or alkalinization (Milovanov and Khabibulin, 1933, Bernstein and Beskhlebnov, 1939; Emmens, 1947). Dubincik (1936) indicated that motility of spermatozoa ceased at a pH of 4.2, but after a shift to alkalinity, sperm motility was resumed. At a pH of 3.4 an irreversible reaction occured which prevented the sperm from regaining motility even when the pH was raised. Kampschmidt et al (1951) suggested that motility in the glucose- and fructose- containing diluents could be maintained at a higher

level during prolonged storage if the pH drop was averted by using sodium bicarbonate buffer.

Kaeser and Ludwick (1952) cited by Rickard et al (1957) observed that motility of spermatozoa increased after adding various amounts of sodium carbonate to diluted semen, though the optimum motility was somewhat dependent on storage temperature. Rickard et al (1957) activated bovine spermatozoa diluted in egg yolk-citrate diluent using sodium carbonate and found that samples incubated at 37 to 39°C showed somewhat better livability at pH 7.1 to 7.4 than at pH 6.5 to 6.8. Conversely, in samples stored at 4 to 6°C, better livability was observed at pH 6.5 to 6.8 than at pH 7.1 to 7.4.

Willett and Ohms (1958) observed complete inactivation of bull spermatozoa after storage in egg yolk-glucose in combination with lactic acid and sodium hydroxide solutions at pH 6.4, but not in yolk-citrate diluents at a similar pH. Reactivation of spermatozoa after storage in the lactate medium could be accomplished by increasing pH to levels above 7.00 and motility was maintained for at least 1h at room temperature or for 24h at 5°C. Reversible acid inhibition can serve as a means of extending the life-span of sperm for several days at room temperature (Norman et al, 1958)

An *in vitro* study to clarify the role of bicarbonate on the sperm activation process at ejaculation was done by Tajima et al (1987) in pigs. They found that bicarbonate quickly increased the motility, respiration rate and cAMP content of the porcine epididymal sperm. In more recent studies, Si and Okuno (1993, 1995) confirmed that bicarbonate was essential for activating mouse sperm motility. A sluggish motility of caudal epididymal spermatozoa was observed after dilution in isotonic sucrose solution. However, by adding bicarbonate to this solution spermatozoa were reactivated and became vigorously motile. It has also been suggested (Carr et al, 1985) that a low pH in the cauda epididymidis may be one factor in rendering the sperm there temporarily immotile.

In view of the previous findings, it seemed possible that *in vitro* immobilized ram spermatozoa could be reactivated by addition of sodium bicarbonate. The aim of the study was, therefore, to determine whether raising the pH with sodium bicarbonate had any effect on the motility characteristics of ram spermatozoa during storage at 30 and 5°C in egg yolk-buffer with or without sugar.

9.2. Experimental procedure

Three Merino rams were used for semen collection. Semen from each ram was collected by artifical vagina once a week for 3 consecutive weeks, so each experiment was done 3 times and the ejaculates from three rams were pooled on each occasion. The pooled semen was then split into 8 similar aliquots, each of which was put into a small glass tube. The first four tubes (tube 1, 2, 3, 4) were prepared for room temperature storage and the second four ones (tube 5, 6, 7, 8) were used for chilled temperature storage. Semen samples were diluted with egg yolk citrate-glucose diluent and egg yolk citrate diluent as shown in Table 9.1 at the ratio 1:4 (semen:diluent) i.e. 5 fold.

Table 9.1. Composition of egg yolk citrate (EYC) diluent with or without glucose.

Ingredients	Diluent	
	Egg yolk citrate-glucose (EYCG)	Egg yolk citrate (EYC)
Sodium citrate $2H_2O$ (g)	2.265	2.265
Dihydrostreptomycin sulphate (g)	0.10	0.10
Penicillin (g)	0.06	0.06
Glucose (g)	0.8	-
Egg yolk (ml)	10	10
Double distilled water to (ml)	100	100
Osmolality (mOsm/kg)	259	269
pH diluent before addition of egg yolk (unadjusted)	7.84	7.80
pH diluent before addition of egg yolk (adjusted)*	7.02	7.02
pH diluent after addition of egg yolk	6.93	6.93

* pH of each diluent was adjusted to around 7.0 using citric acid.

Each semen sample in tubes 1, 2, 5 and 6 was diluted with egg yolk citrate-glucose (EYCG) diluent; while each semen sample in tubes 3, 4, 7 and 8 was diluted with egg yolk-

citrate (EYC) diluent. To those diluted samples in tubes 1 and 5 (labelled as +G+B) and tubes 2 and 6 (labelled as +B) sodium bicarbonate was added (see below) after a preliminary incubation. of 96h at 5°C or 12h at 30°C. At the same time, a similar volume of sodium citrate was added to tubes 2 and 6 (labelled as +G+C) and to tubes 4 and 8 (see Summary below).

Room temperature storage (30°C) for 12h.					Cool temperature storage (5°C) for 96h.				
Semen sample in tube	Semen diluted with	Diluted semen added with	Labelled as	Used as	Semen sample in tube	Semen diluted with	Diluted semen added with	Labelled as	Used as
1	EYCG	Sodium bicarbonate	+G+B	Treatment	5	EYCG	Sodium bicarbonate	+G+B	Treatment
2	EYCG	Sodium citrate	+G+C	Control	6	EYCG	Sodium citrate	+G+C	Control
3	EYC	Sodium bicarbonate	+B	Treatment	7	EYC	Sodium bicarbonate	+B	Treatment
4	EYC	Sodium citrate	+C	Control	8	EYC	Sodium citrate	+C	Control

After the semen sample in each tube was diluted, its pH was measured. This was regarded as the initial pH for samples that were immediately stored at 30°C or for samples that were kept in the cool room until the temperature dropped from 30 to 5°C.

9.2.1. Semen assessment and alkalinization

For samples stored at 30°C objective semen assessment was done using Hamilton Thorn Motility Analyzer at -12h and 0h from addition of bicarbonate. Measurements of pH and glucose concentration of diluted semen in tube 1 (+G+B) and tube 3 (+B) were also done at these times. Alkalinization was performed by adding a solution of sodium bicarbonate (1M NaHCO3), about 10 µl at a time so that the pH in these tubes was raised to as close as possible to 7.00. It was observed in the preliminary experiments that to raise the pH of the diluted semen to this pH, about 250 µl of this buffer solution was needed.

Measurements of pH and glucose concentration of diluted semen in tubes 2 (+G+C) and tube 4 (+C) were also done and then each of these received the same volume of 0.1N sodium citrate-buffer, pH = 7.0 as that of sodium bicarbonate that had been added to tube 1 and 3 (250 µl). Having received this solution, the pH in tube 2 and 4 was again measured. Semen assessment was done within an hour after alkalinization then observations were made at given time intervals (12 and 24h of storage) where both pH and glucose concentration were also measured.

Initial objective assessment of cool-stored semen (at time -96h before addition of bicarbonate or citrate buffer) was done as soon as the temperature reached 5°C. Semen samples were then removed from cool room to the Semen Laboratory and put in a waterbath (30°C) and allowed to warm for several minutes. Assessment was also made for samples that had been stored at 30°C before addition of bicarbonate or citrate buffer. During assessment of -96h and 0h-stored diluted semen, pH and glucose concentration of samples in each tube were also measured.

Following addition of bicarbonate or citrate, all semen samples were then incubated at 30°C in a waterbath and assessment was done within an hour. Subsequent observations were made after 12 and 24h of incubation, at which times pH and glucose concentration were also measured.

Objective assessment of each semen sample stored either at 30 or 5°C was done twice and the duplicates were averaged.

9.2.2. Glucose concentration measurement

Measurement of glucose concentration in the semen samples was done using a blood glucose meter (Ames Glucometer®3), which is for *in vitro* diagnostic use. The machine is accompanied with the test strip, a film plastic strip with a reagent test pad for determining the concentration of glucose in the samples.

The test pad reaction is based on the action of enzymes; glucose oxidase and peroxidase. The glucose oxidase catalyzes the oxidation of glucose in samples in the presence of oxygen in the atmosphere, producing gluconic acid and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide oxidizes the tetramethylbenzidine (reduced form) turning the test pad blue with an intensity proportional to the glucose concentration.

The colour on the reacted test pad of the strip was compared to the color chart on the Glucofilm bottle label. The comparison was done within one minute after the instrument reading was completed using the same strip. The test provides a quantitative measurement of glucose in whole samples from 1.1 to 27.7 mM/L.

9.3. Statistical analysis

The percentage data was angular transformed and analyses of variance were performed. Significance differences between means were determined using Tukey'test. Simple correlation analysis was also conducted to correlate pH of diluted semen and motility of sperm.

9.4. Results

9.4.1. pH diluent and glucose concentration at 30 and 5°C

The data with respect to the changes in pH and glucose concentration have been presented in Tables 9.2 and 9.3. There was a significant ($p<0.001$) interaction between diluent and time of storage on pH. However, glucose concentration was significantly ($p<0.001$) affected by time of storage from addition of bicarbonate at 30°C.

The initial pH of the four diluents at 30°C, -12h before addition of bicarbonate ranged from 6.15 to 6.62. After storage but before addition of bicarbonate or citrate buffer, pH of all diluents had decreased significantly ($p<0.05$) and there was a significantly

($p<0.05$) smaller fall in pH in diluted semen without addition of glucose than in those with added glucose. After 12h of subsequent incubation, pH increased significantly ($p<0.05$) more in diluents to which bicarbonate was added than those to which sodium citrate was added. After 24h of storage, pH of diluents to which bicarbonate was added was still significantly ($p<0.05$) higher than those to which sodium citrate was added (Table 9.2).

Glucose concentration in the diluents was 44.4 mM/L at -12h before addition of bicarbonate at 30°C and fell to 21.83 and 20.97 mM/L in +G+B and +G+C, respectively at 0h. However at 12 and 24h of following alkalinization, glucose contents had decreased further ($p<0.05$), so that by the end of incubation, glucose was undetectable (Table 9.2).

The data with regard to the changes in pH and glucose concentration at 5°C have been presented in Table 9.3. There was a significant ($p<0.001$) interaction between diluent and time of storage on pH. The glucose concentration continued to fall after the addition of bicarbonate, but the extent of the fall in glucose concentration at 5°C was surprising.

At the time of addition of bicarbonate, pH of glucose containing diluents had decreased significantly ($p<0.05$) to approximately 5.65, but in diluents containing no glucose, pH showed a smaller decrease to approximately 6.42. The differences in pH between the two diluents with or without glucose at 0h were significant ($p<0.05$). pH of diluents to which bicarbonate was added was higher than that of diluents to which citrate was added. At 12 and 24h of subsequent incubation, pH of +G+B, +B and +C was significantly ($p<0.05$) higher than that of +G+C. The difference in pH between +G+B or +B and +C was significant ($p<0.05$) at 12h. pH in +B was also higher ($p<0.05$) than that in +G+B or +C at 24h (Table 9.3).

Table 9.2. pH and glucose concentration in egg yolk citrate with glucose plus sodium bicarbonate (+G+B) or plus sodium citrate (+G+C); egg yolk citrate without glucose plus sodium bicarbonate (+B) or plus sodium citrate (+C) and storage at 30°C (Mean ± SEM, n = 3).

Variable	Diluent	Time before addition of bicarbonate or citrate buffer		Time after addition of bicarbonate or citrate buffer	Time after addition of bicarbonate or citrate buffer	
		-12h	0h	0h	12h	24h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
pH	+G+B	6.15 ± 0.04	5.72 ± 0.14	7.02 ± 0.01	7.73 ± 0.40	6.77 ± 0.36
	+G+C	6.15 ± 0.04	5.65 ± 0.19	6.22 ± 0.04	6.00 ± 0.07	5.79 ± 0.06
	+B	6.61 ± 0.03	6.47 ± 0.01	7.04 ± 0.01	8.43 ± 0.03	7.80 ± 0.23
	+C	6.62 ± 0.03	6.48 ± 0.04	6.63 ± 0.02	6.92 ± 0.06	6.89 ± 0.09
Glucose concentration (mM/L)	+G+B	44.4 *	21.83 ± 4.26	- -	6.97 ± 0.50	<1.10
	+G+C	44.4 *	20.97 ± 3.21	- -	7.50 ± 1.80	<1.10

* Calculated from amount of glucose added

Table 9.3. pH and glucose concentration in egg yolk citrate with glucose plus sodium bicarbonate (+G+B) or plus sodium citrate (+G+C); egg yolk citrate without glucose plus sodium bicarbonate (+B) or plus sodium citrate (+C) and storage at 5°C for 96h before and for 24h after alkalinization and incubation at 30 °C (Mean ± SEM, n = 3).

Variable	Diluent	Time before addition of bicarbonate or citrate buffer		Time after addition of bicarbonate or citrate buffer	Time after addition of bicarbonate or citrate buffer	
		-96h	0h	0h	12h	24h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
pH	+G+B	6.55 ± 0.19	5.65 ± 0.09	7.00 ± 0.00	8.09 ± 0.38	8.09 ± 0.38
	+G+C	6.36 ± 0.00	5.65 ± 0.06	5.98 ± 0.03	5.94 ± 0.04	5.94 ± 0.04
	+B	6.70 ± 0.07	6.42 ± 0.00	7.00 ± 0.00	8.43 ± 0.33	8.46 ± 0.35
	+C	6.71 ± 0.07	6.43 ± 0.01	6.53 ± 0.01	7.00 ± 0.21	7.00 ± 0.21
Glucose concentration (mM/L)	+G+B	44.4 *	16.67 ± 1.78	- -	<1.10	<1.10
	+G+C	44.4 *	17.90 ± 3.71	- -	<1.10	<1.10

* Calculated from amount of glucose added

Initially at -96h before addition of bicarbonate, glucose concentration in the two diluents was 44.4 mM/L, then decreased significantly ($p<0.05$) to 16.67 and 17.90 mmol/L in +G+B and +G+C, respectively. Up to 12h after the time of alkalinization, glucose concentrations continued to decrease significantly ($p<0.05$) until they became undetectable. The concentrations remained low when incubation time was extended to 24h (Table 9.3).

9.4.2. Motility of sperm stored at 30°C

Despite the appreciable changes in pH in some media, at the time of addition of bicarbonate, the percentage of motile sperm in the four diluents had not changed greatly and remained high (above 80%). There was no immediate effect of adding bicarbonate and changing pH to 7.0. At 12h of the second incubation, motility decreased slightly in +G+B and in +C, however in +G+C and +B, motility decreased to a much greater extent. At 24h of the second incubation, motility of spermatozoa in +G+C and in +B had decreased more ($p<0.05$) than that in +G+B and +C (Figure 9.1.a).

a)

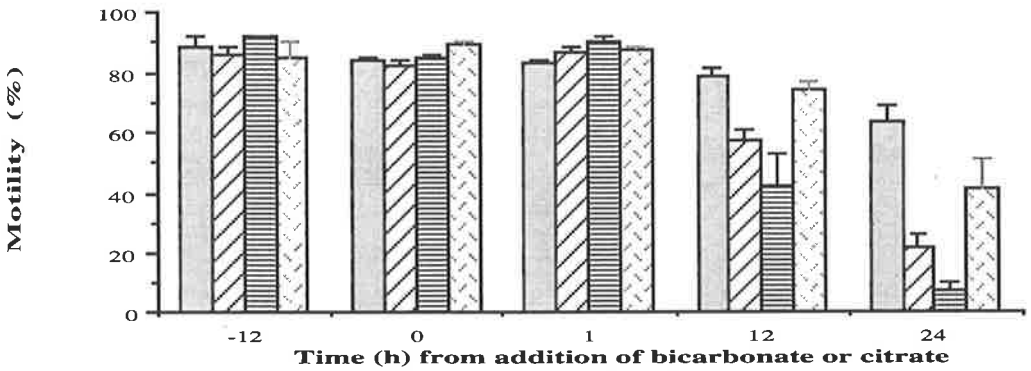
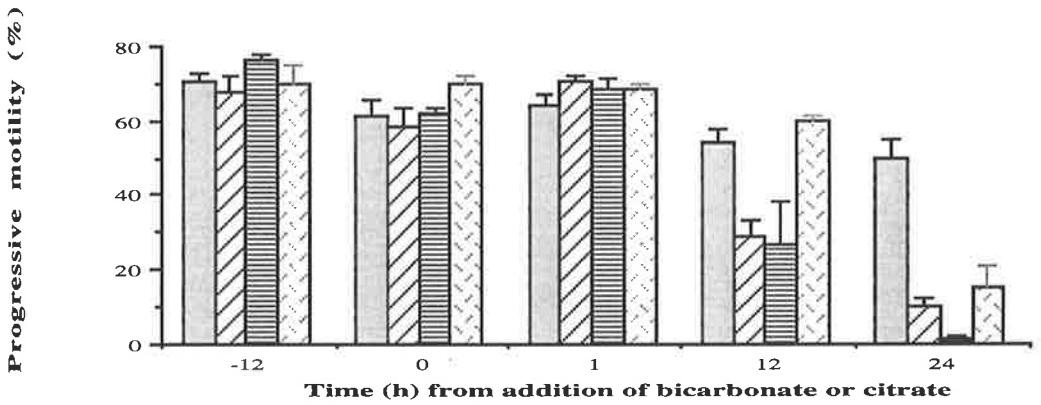


Figure 9.1. Motility (a) of spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▣) diluent storage at 30°C. Values are Means ± SEM of 3 observations.

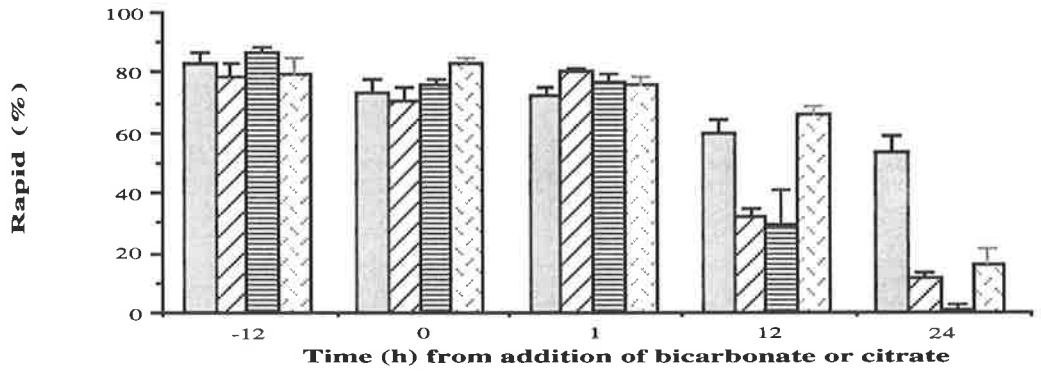
A small reducion in progressive motile and rapid spermatozoa was observed during the first incubation in all diluents, except for +C. At 1h of the second incubation, the numbers of progressive motile and rapid sperm had increased significantly ($p<0.05$).

However after 12h of the second incubation, both parameters either in +G+C or +B decreased significantly ($p<0.05$) compared with that of +G+B or +C. At 24h of the second incubation, progressive motile and rapid spermatozoa in +G+C, +B and +C were much lower ($p<0.05$) than that of +G+B (Figures 9.1.b and c).

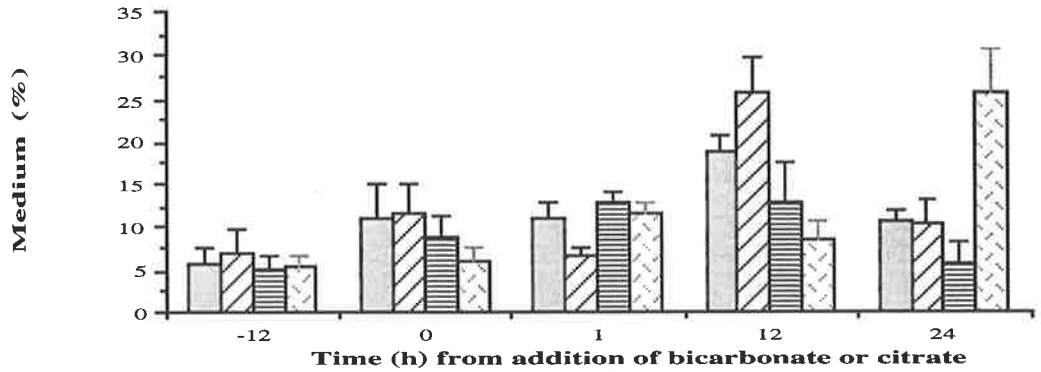
b)



c)



d)



Figures 9.1. Progressive motility (b), rapid (c) and medium (d) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (□) diluent storage at 30°C. Values are Means ± SEM of 3 observations.

The proportion of medium sperm was below 15% at both 0h of the first and 1h of the second incubation. At 12h of the second incubation, medium sperm in +G+B or +G+C was significantly higher ($p<0.05$) than that in +C. At 24h, +C diluent had the highest ($p<0.05$) medium sperm compared with the other diluents; and medium sperm in +G+B differed significantly ($p<0.05$) to that in +B (Figure 9.1.d).

e)

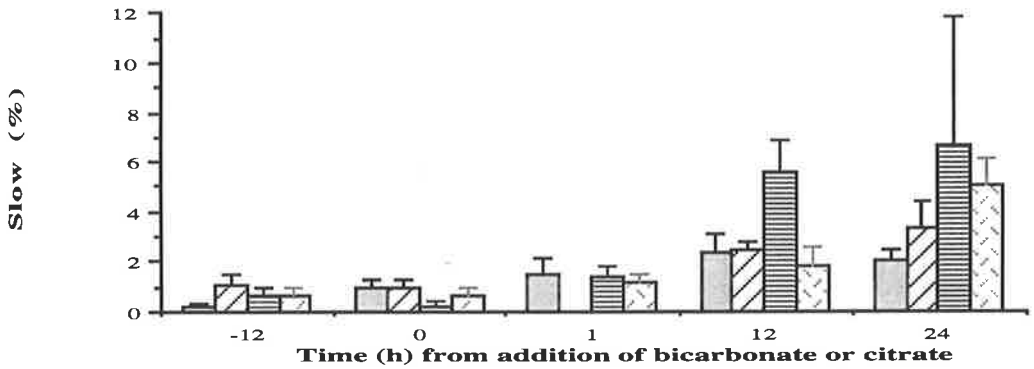
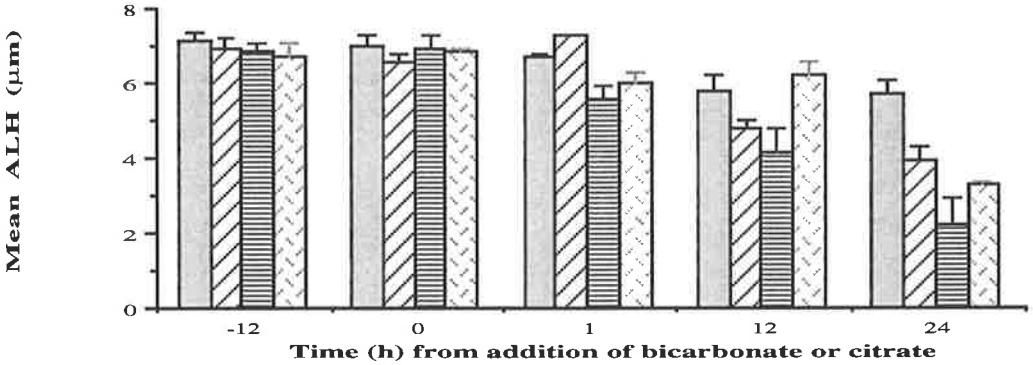


Figure 9.1. Slow (e) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (□), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (□) diluent storage at 30°C. Values are Means ± SEM of 3 observations.

At the end of the first incubation, the numbers of slow sperm in all diluents was below 2%. A gradual increase in slow sperm was observed in all diluents, except for +B, as time advanced from 12 to 24h of the second incubation. The numbers of slow sperm in +B diluent was higher ($p<0.05$) than that of the others at 12h of the second incubation. However at 24h, percent slow sperm in +B only differed ($p<0.05$) from that of +G+B and +G+C; and +C differed ($p<0.05$) from that of +G+B (Figure 9.1.e).

Mean ALH in the four diluents had not changed much during the first incubation and the value was approximately 7 μm. After 1h of the second incubation, mean ALH in +G+B and +G+C was significantly ($p<0.05$) higher than that in +B and +C. After 12h of the second incubation, mean ALH in +C was significantly higher ($p<0.05$) than that of +G+C and +B. At 24h mean ALH in +G+B or +G+C was higher ($p<0.05$) than that of +B (Figure 9.1.f).

f)



g)

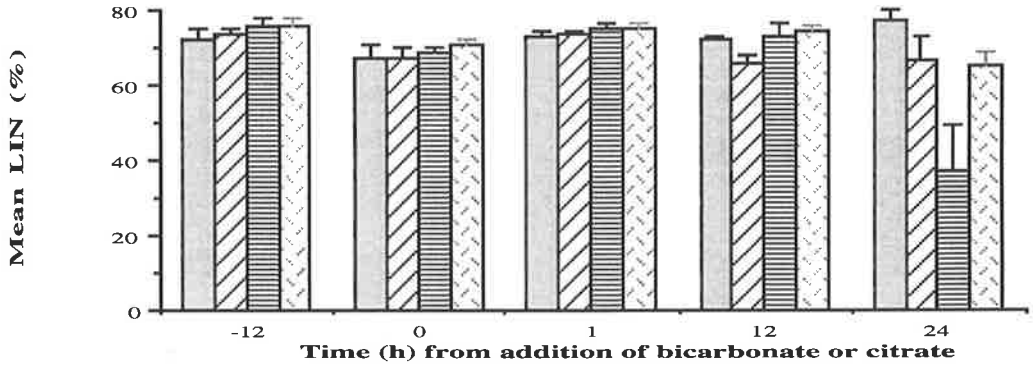


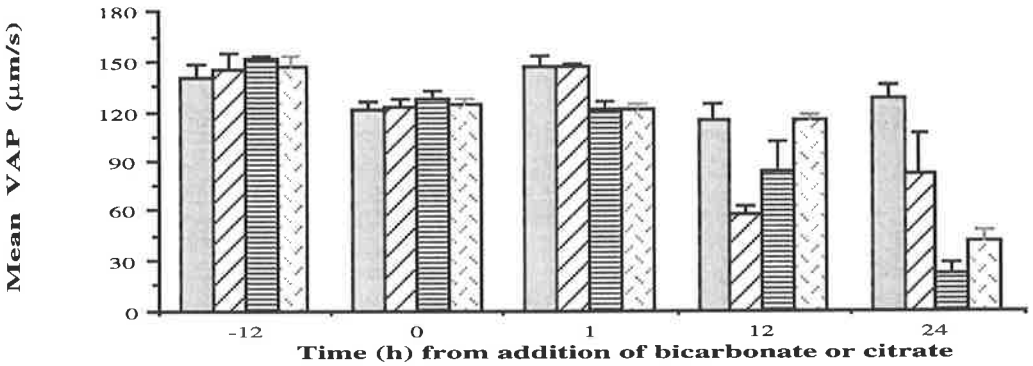
Figure 9.1. Mean ALH (f) and mean LIN (g) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▧) diluent storage at 30°C. Values are Means ± SEM of 3 observations.

No significant difference in mean LIN was observed between the four diluents either at 0h of the first or 12h of the second incubation. However at 24h of the second incubation, mean LIN in +G+B was higher ($p<0.05$) than that of +B and +C. Mean LIN in +G+C or +C was also higher ($p<0.05$) than that of +B (Figure 9.1.g).

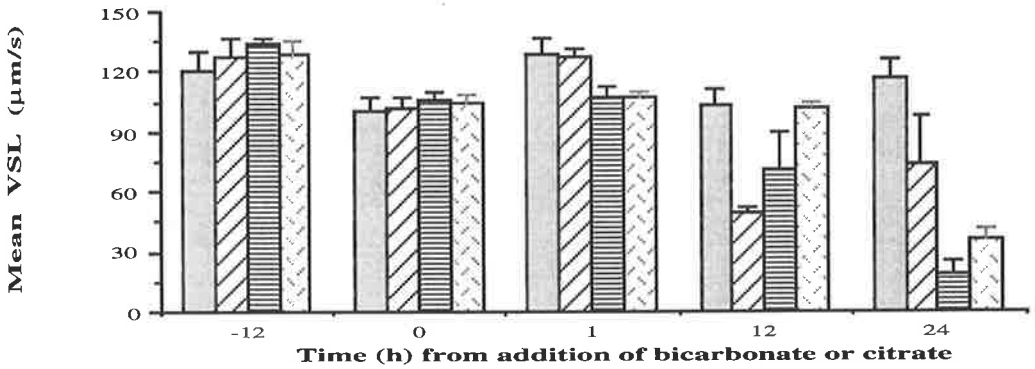
Mean VAP, mean VSL and mean VCL in glucose containing diluents were higher ($p<0.05$) compared with those with no glucose at 1h of the second incubation. Mean VAP, mean VSL and mean VCL in +G+B or +C was higher ($p<0.05$) than that in +G+C or +B at 12h of the second incubation; and also higher ($p<0.05$) in +B than that in +G+B. At 24h of the second incubation, mean VAP, mean VSL and mean VCL in glucose containing

diluents were significantly ($p<0.05$) higher compared with those of diluents containing no glucose (Figures 9.1.h,i and j).

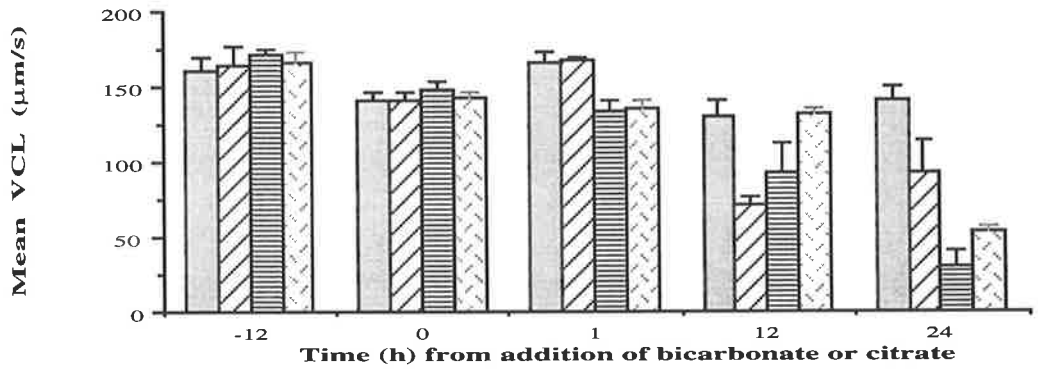
h)



i)



j)



Figures 9.1. Mean VAP (h), mean VSL (i) and mean VCL (j) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▢) diluent storage at 30°C. Values are Means ± SEM of 3 observations.

The percentage of STR in all diluents remained constant (around 80%) at 1h of the second incubation. After 24h of the second incubation, percent STR in +B decreased

markedly to around 50%, but the difference in this parameter among the four diluents was not significant (Figure 9.1.k).

k)

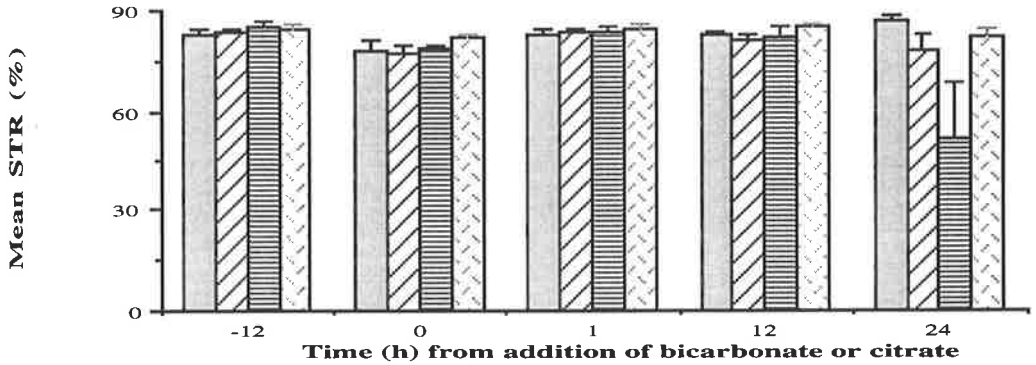


Figure 9.1. Mean STR (k) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (□), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (□) diluent storage at 30°C. Values are Means ± SEM of 3 observations.

As can be seen (Table 9.5, Appendix A), there was a highly significant ($p<0.001$) interaction between diluent and time of storage at 30°C on motility, progressive motility, rapid, slow, mean ALH, mean VAP, mean VSL and mean VCL of spermatozoa, except for mean STR. A significant interaction between diluent and time of storage on mean LIN ($p=0.027$) and medium spermatozoa ($p=0.024$) was also observed.

9.4.3. Motility of sperm stored for 96h at 5°C then at 30°C for 24h

Despite the appreciable changes in pH in some media, motility of spermatozoa in the 4 diluents remained high (above 80%) after 96h of storage at 5°C. At 1h of the second incubation, motility was unchanged. However after 12 and 24h of the second incubation, percent motility in +G+B, +G+C and +B decreased ($p<0.05$) more than that of +C. When bicarbonate was added to glucose containing diluents, percent motile sperm at 12h of the second incubation was higher ($p<0.05$) than when citrate was added to this diluents, but at 24h of the second incubation, the case was reversed (Figure 9.2.a).

a)

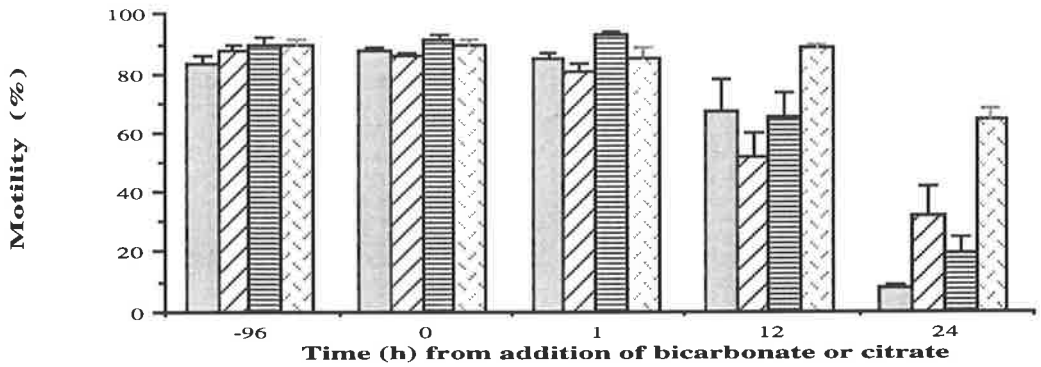


Figure 9.2. Motile (a) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▧) diluent storage at 5°C between -96h and 0h and at 30°C thereafter. Values are Means ± SEM of 3 observations.

The percentage of progressive motile sperm in all diluents, except +G+C, did not change significantly during the first period of incubation. Then at 1h of the second incubation at 30°C, progressive motile sperm in all diluents decreased slightly. After 12h of the second incubation, percent progressive motile in +G+B, +G+C and +B had decreased more ($p<0.05$) than that in +C. At 24h, progressive motility in all diluents had fallen further ($p<0.05$); and +G+C or +C had higher ($p<0.05$) percent progressive motile than did +G+B and +B (Figure 9.2.b).

b)

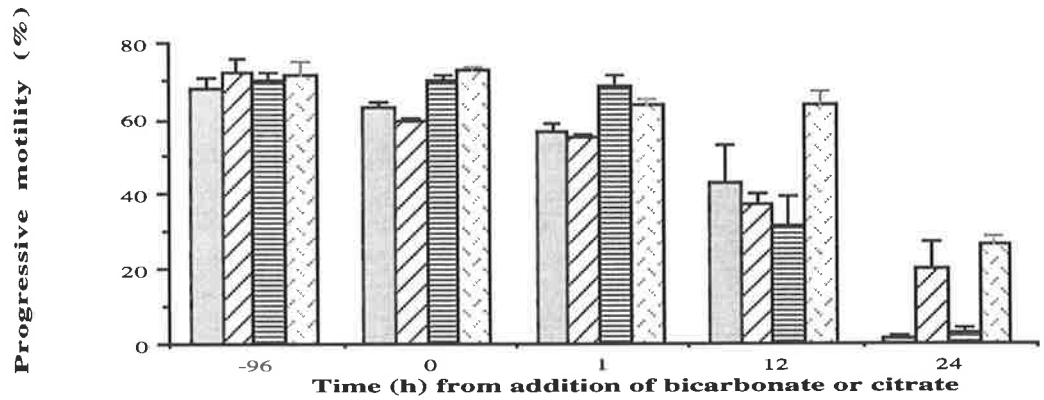


Figure 9.2. Progressive motile (b) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▧) diluent storage at 5°C between -96h and 0h and at 30°C thereafter. Values are Means ± SEM of 3 observations.

The numbers of rapid sperm among the four diluents remained high (80%) at the end of the first incubation and only decreased slightly after 1h of the second incubation. Although at 12h of the second incubation rapid sperm in +G+B, +G+C and +B had decreased more than in +C, the differences were not significant ($p<0.05$). After 24h, rapid sperm in +G+C and +C decreased more than that in +G+B and +B (Figure 9.2.c).

c)

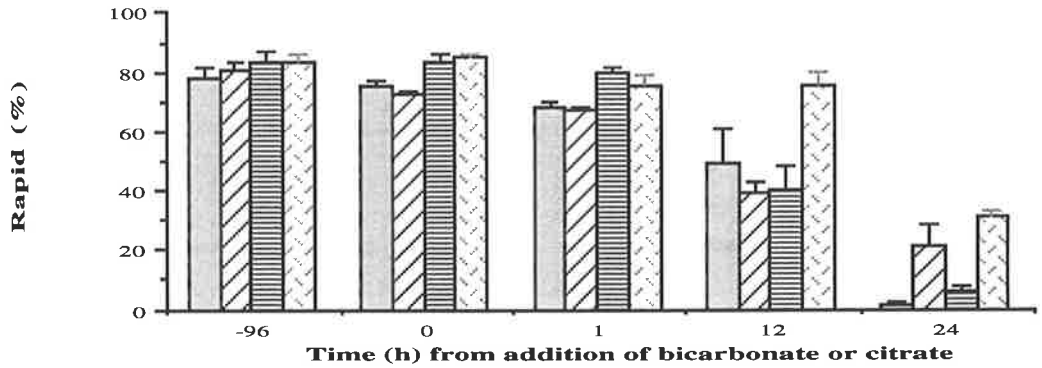


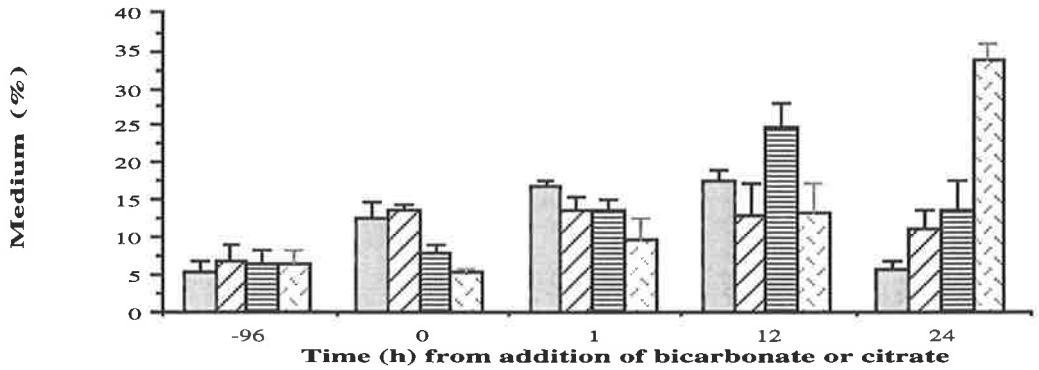
Figure 9.2. Rapid (c) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▧) diluent storage at 5°C between -96h and 0h and at 30°C thereafter. Values are Means ± SEM of 3 observations.

The percentage of medium sperm in semen samples diluted in diluent with glucose was higher ($p<0.05$) than that of samples without glucose at the time of addition of bicarbonate or citrate. At 1h of the second incubation, medium sperm in all diluents had increased, a difference in this parameter was observed only between +G+B and +C. After 12h of the second incubation, medium sperm in +B was higher ($p<0.05$) than that in +G+C and +C . However at 24h, medium sperm in +C diluent was higher ($p<0.05$) than in any of the others; and the difference in medium sperm between +B and +G+B was also significant ($p<0.05$)(Figure 9.2.d).

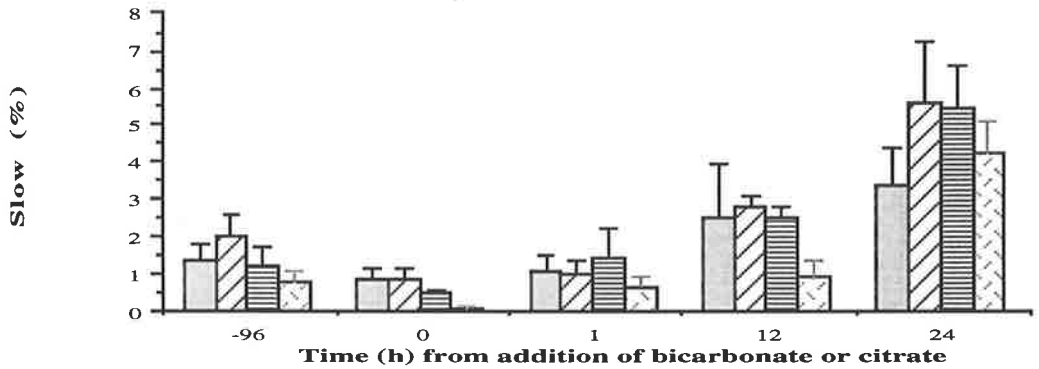
At the time of addition of bicarbonate or citrate, the numbers of slow sperm in all diluents was below 2% and maintained this level at 1 or 12h of the second incubation. However at 24h, percent slow sperm in all diluents had increased to above 3%. The difference in slow sperm in all diluents between samples incubated at 24h and at -96h or

0h of the first incubation, and at 1 to 12h of the second incubation was significant ($p<0.05$)(Figure 9.2.e).

d)



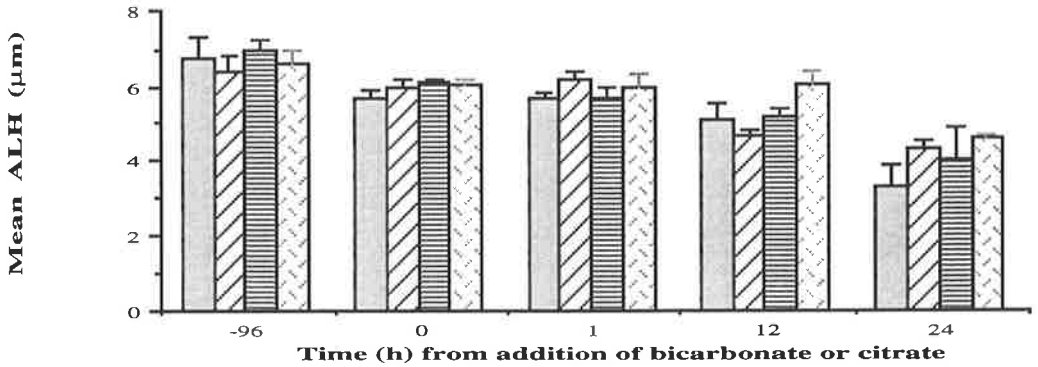
e)



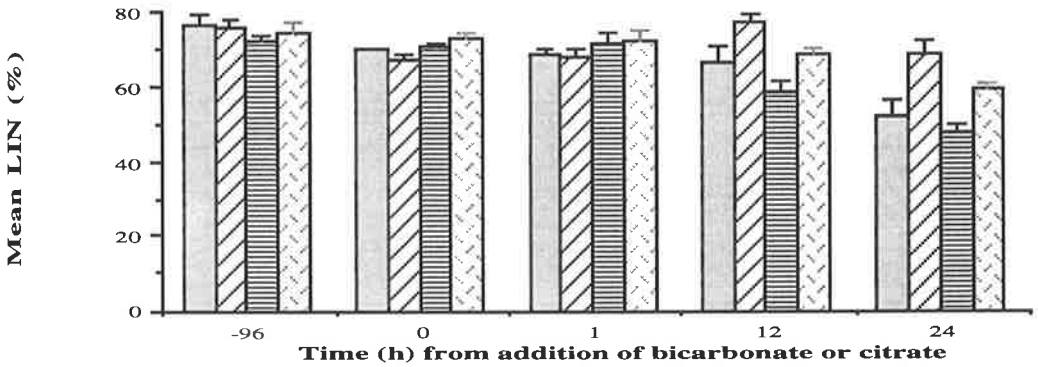
Figures 9.2. Medium (d) and slow (e) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (□), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (□) diluent storage at 5°C between -96h and 0h and at 30°C thereafter. Values are Means ± SEM of 3 observations.

Mean ALH in +G+B and +B had decreased significantly ($p<0.05$) at the time of addition of bicarbonate or citrate. However in +G+C and +C, the decrease was not significant. No significant changes in mean ALH was also observed at 1h of the second incubation. However at 12hof the second incubation, a significant decrease ($p<0.05$) in mean ALH had occured in +G+B and +G+C, but not in +B. At 24h, mean ALH in all diluents, except +G+C had decreased further significantly ($p<0.05$)(Figure 9.2.f).

f)



g)



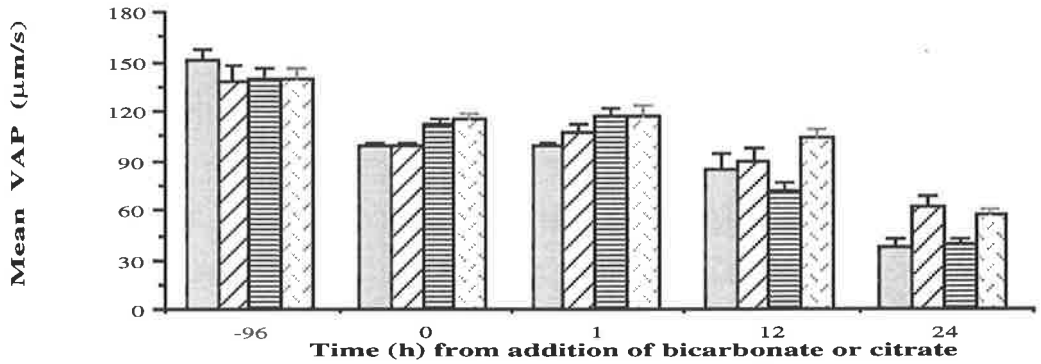
Figures 9.2. Mean ALH (f) and mean LIN (g) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▣) diluent storage at 5°C between -96h and 0h and at 30°C thereafter. Values are Means ± SEM of 3 observations.

The percentage of mean LIN in all diluents tended to be lower at the end of the first incubation. At 1h after alkalinization, mean LIN did not change greatly, however at 12h of the second incubation, mean LIN in +B was significantly lower ($p<0.05$) compared with that of +G+B, +G+C and +C. Then at 24h, a significant ($p<0.05$) further decrease in mean LIN was observed in all diluents, and percent LIN in +G+C or +C was higher than that in +G+B or +B (Figure 9.2.g).

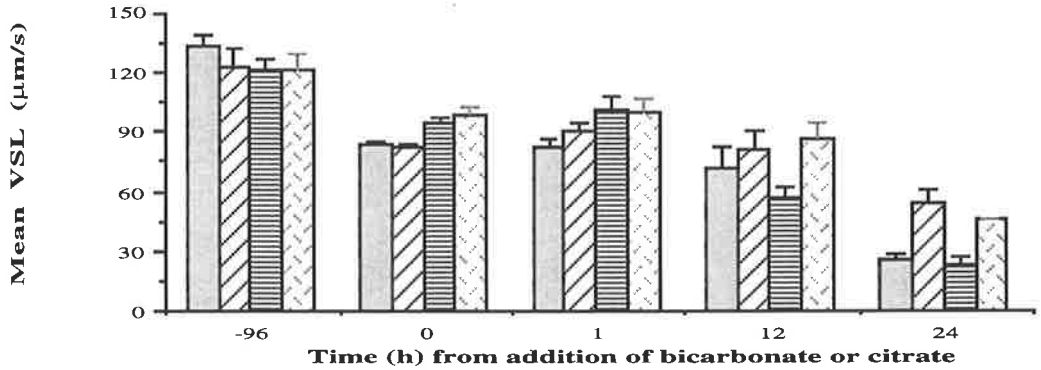
At the start of the first incubation, mean VAP in all diluents ranged from 138 to 150 µm/s, then during the first incubation it decreased significantly ($p<0.05$) to between 99 to 114 µm/s. At 1h of the second incubation, mean VAP in most diluents tended to be

higher. However, by 12h of the second incubation mean VAP had decreased significantly ($p<0.05$) and by 24h the values ranged from 38 to 61 μm/s (Figure 9.2.h).

h)



i)



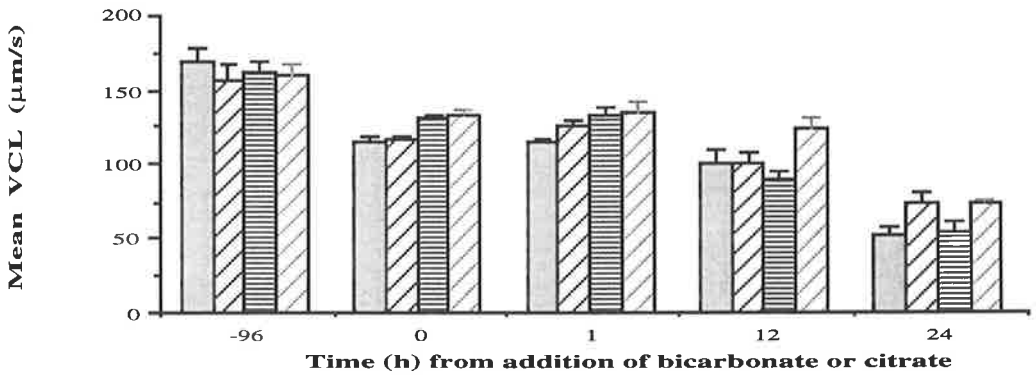
FigureS 9.2. Mean VAP (h), mean VSL (i) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▨) diluent storage at 5°C between -96h and 0h and at 30°C thereafter. Values are Means ± SEM of 3 observations.

Mean VSL in all diluents was between 121 and 132 μm/s at -96h of the second incubation. This decreased significantly ($p<0.05$) to between 82 and 98 μm/s at the second incubation . A slight increase in mean VSL was then observed in most diluents at 1h of the second incubation. However at 12 or 24h of the second incubation, mean VSL in all diluents had fallen again and appeared to be faster ($p<0.05$) in +G+B and +B than the other two diluents (Figure 9.2.i).

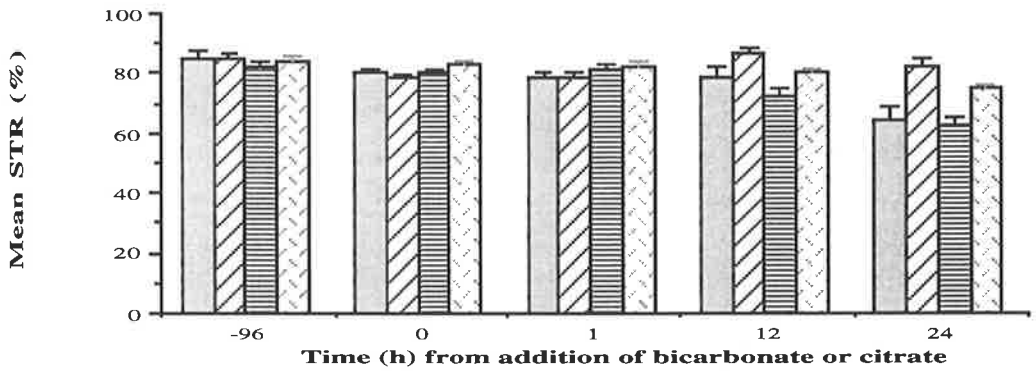
A significant ($p<0.05$) decrease in mean VCL in all diluents was observed at 0h from addition of bicarbonate or citrate. After 1h of the second incubation, mean VCL in all

diluents was slightly higher. However after 12 and 24h of the second incubation, mean VCL in all diluents tended to be significantly lower ($p<0.05$)(Figure 9.2.j).

j)



k)



Figures 9.2. Mean VCL (j) and STR (k) spermatozoa in egg yolk citrate glucose with bicarbonate (+G+B) (□), egg yolk citrate glucose with citrate (+G+C) (▨), egg yolk citrate with bicarbonate (+B) (▤) and egg yolk citrate with citrate (+C) (▥) diluent storage at 5°C between -96h and 0h and at 30°C thereafter. Values are Means ± SEM of 3 observations.

A small fall in the percentage of STR in all diluents was observed at the time of addition of bicarbonate or citrate, but this then remained constant at 12h of the second incubation. At this time, the difference in percent STR between +G+C or +C and +B was significant ($p<0.05$); percent STR in +G+C and +C was significantly ($p<0.05$) less compared with that in +G+B and +B (Figure 9.2.k).

The results of the study (Table 9.6., Appendix A) indicated that time of storage had a significant ($p<0.001$) effects on the percentage of slow, mean ALH, mean VAP, mean VSL and mean VCL of spermatozoa. There was significant interaction between

diluent and time of storage on the percentage of motility (p=0.004), progressive motility (p=0.017), rapid (p=0.043), medium, mean STR, (p=0.002) and percentage of mean LIN (p=0.013) of spermatozoa.

9.4.4. Correlation of pH and motility

The correlations between motility and pH of diluted semen are presented in Table 9.4. No significant correlation between motility and pH was observed in any diluent when data time from -12 and 0h at 30°C or at -96 and 0h at 5°C were pooled. When data from 12 and 24h of the second incubation at 30°C after preliminary storage at 30 or 5°C were pooled, a significant (p<0.001) positive correlation between motility and pH was observed in +G+C diluents, but negative correlation in +G+B and +B diluent, and there was no significant correlation between motility and pH in +C diluent when data from the two incubations (before and after addition of citrate) were pooled. There were significant negative correlations with +G+B, +B and +C treatments and no significant correlation with +G+C treatment.

Table 9.4. Correlation coefficients (r) between motility of spermatozoa and pH of diluted semen in egg yolk citrate glucose with addition of bicarbonate (+G+B), egg yolk citrate glucose with addition of citrate (+G+C), egg yolk citrate with additionn of bicarbonate (+B) and egg yolk citrate with addition of citrate (+C) diluents and their estimated equations.

	EYCG	EYCGC	EYC	EYCC
Before alkalinization (pooled temperature 30 and 5°C)	r = - 0.104 y = 6.91 - 0.0105 x	r = 0.385 y = 2.70 + 0.0380 x	r = 0.384 y = 5.544 + 0.01126 x	r = 0.103 y = 6.630 + 0.00225 x
After alkalinization (pooled temperature 30 and 5°C)	r = - 0.453*** y = 8.159 - 0.01101 x	r = 0.599*** y = 5.7902 + 0.00339 x	r = - 0.626*** y = 8.519 - 0.012 x	r = - 0.310 y = 7.137 - 0.00417 x
Pooled temperature and before and after alkalinization	r = - 0.568*** y = 8.424 - 0.02121 x	r = 0.229 y = 5.819 + 0.00222 x	r = - 0.748*** y = 8.634 - 0.01924 x	r = - 0.433*** y = 7.236 - 0.00646 x

*** p<0.001

9.5. Discussion

It was observed in the present study that at a pH between 5.65 and 5.72 at 30°C, motility of some samples was markedly reduced, while in others there was little effect. This range of pH was close to that reported by Norman et al (1958), who observed an inhibition of sperm motility at pH 5.58-5.80. They further suggested that the inhibitory effect of excessive amounts of lactic acid could be reversed provided that pH did not fall below 5.5, however, if pH dropped below this level irreversible enzyme denaturation occurs and the excess hydrogen ion becomes lethal to the sperm. In the present study, pH of diluted semen at the end of the first incubation had fallen only to 5.65, so it seemed possible that neutralization of the acidity could restore motility of the spermatozoa. Sodium bicarbonate has been known to be essential for activating motility and metabolism of bull (Murdoch and White, 1968; Kampschmidt et al, 1951; Rickard et al, 1957; Willett and Ohms, 1958; Norman et al, 1958); pig (Tajima et al, 1987) and mouse spermatozoa (Si and Okuno, 1993; 1995). In the present study, there was virtually no change in motility of ram spermatozoa after bicarbonate or citrate was added. It seems that the activation of immobilized sperm motility by sodium bicarbonate may be species dependent (Si and Okuno, 1993).

It was observed that in +G+C diluent, decreased motility characteristics during incubation at 30°C after the addition of citrate buffer were accompanied by declines in glucose content and pH, indicating that this sugar was probably metabolized by spermatozoa to lactate for their energy substrate as it was during the first incubation in both diluents containing glucose. Sperm respiration and glycolysis during *in vitro* preservation are directly correlated with pH (Norman et al, 1958). In contrast, pH of +C diluent was maintained above 6 which provides evidence of a lowered sperm metabolic rate in this diluent.

In the first part of the present study, with both incubations at 30°C, the better maintenance of motility in the +G+B samples than in +G+C or +B samples was

presumably due to better control of the pH in the range between 6.5 and 7.7. Conditions either more acidic (as in +G+C) or more alkaline (as in +B) than this may have had deleterious effects on motility of the sperm.

In the second part of this study in which preliminary storage was at 5°C, the glucose concentrations in the diluents to which the sugar had been added, also decreased appreciably. This might suggest that this exogenous sugar has been utilized by spermatozoa for their motility activities, even at the lower temperature, or alternatively, reduced glucose concentrations could also be attributed to metabolic activity of spermatozoa during the course of cooling from 30 to 5°C (Tiwari et al, 1977) followed by minimum activity during storage from the time the temperature had reached 5°C. Unfortunately glucose concentrations were not measured at the end of the cooling period. Furthermore, decrease in pH of diluted semen containing glucose at the end of the first incubation might also suggest that lactic acid was formed during glycolysis which could have changed the pH from alkaline towards acidic. Salisbury and Van Demark (1945) suggested that bull spermatozoa diluted with yolk-citrate utilized glucose at a relatively uniform rate, regardless of the quality of semen, during storage at 5°C and the amount of lactic acid produced under these conditions was directly correlated with the livability of bull spermatozoa. Glucose uptake by ram spermatozoa has been shown to be 0.42µmole/h/10^8 sperm at 37°C (Scott et al, 1962). Assuming a halving of this rate for each fall of 10°C, this would correspond to a rate of 0.27 µmole/h/10^8 sperm at 30°C and 0.050 µmole/h/10^8 sperm at 5°C. In the present experiments, semen containing 2.62 x 10^9 spermatozoa per ml was diluted 1:4,so the concentrations in the incubations was 5.24 x 10^8. Thus one would predict consumption of 17.0µmoles/ml over 12h at 30°C and 25.2 µmoles/ml over 96h at 5 °C. These values correspond reasonably well with the changes observed (22.6, 14.1 and 16.2 µmoles/ml over 12 h at 30 and 27.2 over 96 h at 5°C (see Table 9.2). Glucose metabolism by the spermatozoa could therefore have been responsible for the changes in

glucose concentrations observed, and in any case, the addition of antibiotics should have minimized the effect of bacterial contamination.

The study shows that after 1h of the second incubation, no immediate increase in motility due to alkalinization was observed and comparison between diluents indicated poorer maintenance of motility in +G+B than in +C after 24h of the second incubation at 30°C. Thus, alkalinization of glucose containing diluent with sodium bicarbonate or sodium citrate was not effective in maintaining motility characteristics in samples after storage for 4 days at 5°C followed by incubation at 30°C for 24h when comparison is made with egg yolk citrate-containing diluent to which sodium citrate was added. The present evidence does not agree with those of Willett and Ohms (1951), who stored bull semen at 5°C for 7 days and then within 5 minutes after addition of sodium bicarbonate, observed a marked increase in motility of the inhibited-spermatozoa at 37°C in yolk-glucose-lactate either at pH 6.8 (normal pH) or 6.45 (low pH). This difference could be attributed to the difference in pH between the present study and the experiment of Willett and Ohms (1951) in which, alkalinization of EYC containing sugar was done when pH of the diluted semen had fallen below 6, although semen had been stored for only 4 days.

The failure to maintain of motility when bicarbonate was added after incubation in glucose-containing medium at 5°C may have been due to the continuing increase in pH during the second incubation period (24h) after the pH adjustments by addition of sodium bicarbonate; this rise was greater in +B than in +G+B diluents but both showed substantial falls in motility, presumably because of the continuing high pH. Foote and Bratton (1960) observed that the pH of the self-carbonating CUE buffer rose during storage of bull sperm at 5 and 25°C. Foote (1964) also suggested that the level of citric acid included usually prevented major shifts in pH, but occasionally unexplained rises in pH above the neutral point happened, accompanied by a reduction in sperm motility.

This study shows that low pH itself has little effect on motility as indicated by good motility after 12h at 30°C or 96h at 5°C with pH in the range 5.65 to 5.72. Furthermore, the positive correlations found between motility and pH in the previous chapter were not found in the present study. The time of storage (12h or 24h at 30°C and 96 vs 144h at 5°C) were different, but presumably this discrepancy indicated that factors other than pH are involved in the determination of motility.

This becomes even clearer when one considers the negative correlations found with the data obtained after addition of bicarbonate or citrate, or the pooled data. Under these conditions, the pH was presumably rising to sufficiently high levels in some samples to exert a deleterious effect on motility.

It is therefore apparent that simple addition of bicarbonate is not sufficient to maintain motility during storage, even though this was sufficient to restore pH to the neutral range initially; the subsequent inexplicable rise of pH to alkaline values may in itself had a negative effect on motility.

Chapter 10

The effects of elevated testicular temperature on the viability of ram spermatozoa

10.1. Introduction

It was seen in previous experiments (Chapters 4, 5 and 7) that normal ram sperm stored at 30°C for 48h and at 5°C for 10 days have better viability characteristics when stored in a novel coconut extract-quail egg yolk (CEQY) diluent, than when a conventional hen yolk-citrate glucose (HYCG) diluent is used. However, summer temperature extremes in the field may compromise normal sperm quality. In rams, scrotal temperatures can become elevated during summer by several degrees (Setchell et al, 1994) and it has been shown experimentally by whole body heating (Dutt and Hamm, 1957; Dutt and Simpson, 1957) or scrotal insulation (Braden and Mattner, 1970; Smith, 1971, Mieusset et al, 1991; Mieusset et al, 1992) that rises of this magnitude are sufficient to reduce sperm quality and produce reductions in mass motility and the percentage of live spermatozoa and increases in the numbers of abnormal cells (Chemineau et al, 1991). There may also be a change in the ability of these sperm to survive at room or cool temperatures.

In the light of previous work, it seems that information regarding the effects of increased testicular temperature on the viability characteristics of fresh diluted semen is not readily available. Therefore, two experiments which were designed to evaluate motility and morphological characteristics of fresh diluted ram spermatozoa following increased testicular temperatures accomplished by scrotal insulation for 16h/day for 21 days (first experiment) and for 12h/day for 28 days (second experiment) were conducted; and will be discussed in the sections below of this chapter.

10.2. Viability of semen from rams subjected to scrotal insulation for 16h/day, after storage at room and chilled temperatures for six hours

The objective of this study was to evaluate the effects of elevated testicular temperature for 16h/day for 21 consecutive days on the viability characteristics of ram spermatozoa diluted in CEQY and HYCG diluents stored at 30 or 5°C for 6 hours.

10.2.1. Experimental procedure

10.2.1.1. Animals

Four 3-5 year-old fully fleeced mature Merino rams weighing 65-75 kg were used in the study. The animals were kept in individual pens in a room with controlled temperature and light, at 21°C under a regimen of 16h light and 8h dark. Two animals chosen at random, were used as controls and the other two submitted to a regimen of scrotal insulation for 16h/d (17:00 to 09:00) for 21 consecutive days. All animals were fed a standard ration (one scoop of pellets and one handful of hay) twice a day with free access of water.

10.2.1.2. Thermal insult to the testes

The purpose of the scrotal insulation was to create a thermal insult to the testes, mimicking a mild, naturally occuring environmental interference with testicular thermoregulation.

Thermal insult to the testes was induced by enclosing the scrotum of each treated ram with a bag contained insulated material (aluminium foil) sandwiched between two layers of cotton cloth (Mieusset et al, 1992). These layers were machine quilted together and then sewn into a supplementary cover of waterproof cotton cloth on the outside. The bag was held around the scrotum by four tapes tied over the back of the rams to obtain complete coverage of the scrotum and scrotal neck up to the body wall. The bag was fitted to be loose enough not to interfere with the circulation but sufficiently secure to avoid removal or slippage. As the bag on removal was always wet because of scrotal sweating, it was necessary to turn the bag inside out before drying it in a hot room.

10.2.1.3. Temperature measurements.

Scrotal surface temperatures were measured using thermal probes (YSI 409A, Yellow Springs Instrument Co, Ohio, USA) glued to the median raphe of the scrotum about halfway down the testis. Recordings were made every 10 minutes for about 60 minutes before and 60 minutes after the bags were applied and removed on rams 12 and 16 on 3 occasions in consecutive weeks.

10.2.1.4. Semen collection, processing and assessing

In this experiment, testicular insulation commenced each day immediately before the lights were switched off at 1700 pm and ended when the lights were switched on at 0900 am on the following day. Semen samples were collected once a week from all rams by artificial vagina. Semen evaluation was done during scrotal insulation, after scrotal insulation and continued until full recovery had occurred. However, pre-insulation semen was collected from all rams and subjective assesment of motility and progressive motility of spermatozoa suggested that the four rams had high quality semen (G. Ekpe, personal communications).

Single ejaculates from each ram were split into four aliquots, each of which was diluted five fold (one part semen : four parts diluents) at 30°C using diluents as shown in Table 4.1 (Chapter 4). Samples to be stored at 30°C were maintained at this temperature in a water bath and semen assessment was done following incubation at 0 and 6h at 30°C. Those to be stored at 5°C were removed into a cool room, allowed to cool down from 30 to 5°C over 2h and then maintained at 5°C in a water bath for 0 or 6h until assessed. When making the assessment, these samples were first rewarmed at 30°C in a water bath and immediately assessed.

The volume of semen per ejaculate from each group of rams was measured immediately after collection. Total sperm count per ejaculate in fresh semen from each group of rams was calculated using a haemocytometer and motility was assessed subjectively as described in Chapter 3, Section 3.6.1.3. and 3.6.1.2. The viability of

diluted spermatozoa was assessed objectively using Hamilton Thorn Motility Analyser (HTM Analyser, Daintree Ind., Australia) as described in Chapter 3, Sections 3.6.2.2.

For sperm morphology examination, the diluted semen from each ejaculate, after storage at 30 or 5°C for 0 and 6h, respectively, was stained by mixing 2-3 drops of diluted semen with 75 µl eosin-nigrosin stain (Evans and Maxwell, 1978) at 37°C. A drop of the mixtures was then placed on a clean sterile glass slide and a smear was made as described in Chapter 3, Section 3.6.1.4. Sperm morphology was quantified by observation of 200 sperm/stained smear using light microscopy at 1250 x magnification. Individual spermatozoa were assigned to only one morphological category even if they exhibited several abnormalities. The categories included normal sperm morphology and sperm abnormalities such as tailess heads, coiled tails and bent tails. The same slides were also used in assessing the percentage of live and dead sperm by examining 200 sperm.

10.2.2. Statistical analysis

Actual percentages were tabulated, but statistical inferences were made using the angular transformed data. Data were subjected to analysis of variance and differences between groups were determined by Tukey's test.

10.2.3. Results

Semen characteristics, assessed by computerised image analysis and manual assessment of morphology of spermatozoa of diluted semen are presented as averaged data of individual rams within treatment groups at different collection days in Tables 10.a to 10.k. in Appendix B of the thesis. The volume of semen per ejaculate and manual assessments of the total sperm count (TSC) and percent motile sperm per ejaculate of each ram throughout experimental periods are presented in Table 10.1 in Appendix B.

Mean scrotal surface temperature recorded as series of measurements between 70 and 10 minutes before the bags were applied was 31.69 $\pm$ 0.17 (30 observations in 2 series on rams 12 and 16) and ranged from 30.85 $\pm$ 0.08 to 32.74 $\pm$ 0.39 for individual

series, when ambient temperature was between 19.0 and 21.2°C. On another occasion, when ambient temperature had dropped to between 17.9 and 18.6°C, scrotal surface temperature was 27.22 ± 0.26 (14 observations in single series on the same two rams). When the bags were applied, scrotal temperature rose by a mean of 2.14 ± 0.33°C (mean of 4 differences from the above individual pre-insulation means and those obtained from 26 observations in 2 series each on the two rams between 10 and 70 minutes after application of the bags) at the higher ambient temperature, and by 3.88 ± 0.08°C (14 observations in single series on the two rams) at the lower ambient temperature. At the end of the 16h insulation period, mean scrotal temperature was 34.22 ± 0.14°C (48 observations in 3 series on each of the two rams), ranging from 33.37 ± 0.37 to 35.22 ± 0.11 for individual series, and was not affected by the ambient temperature. When the bags were removed, scrotal surface temperature dropped by 2.98 ± 0.11°C (mean of 4 differences from the above individual means and those obtained at the higher ambient temperature between 10 and 70 minutes after removal of the bags) or by 5.07 ± 1.13 (mean of 2 differences at the lower ambient temperature)(Figure 10.1).

The effect on semen volume, total sperm count and motility of fresh semen manually assessed from individual rams of both groups is presented in Figures 10.2, 10.3 and 10.4. As the number of animals in each group was small (only two rams), comparison between individual rams within and between groups could not be performed as with a single degree of freedom, differences may sometimes fail to be detected due to numerical coincidences in the dummy variate during statistical analysis. However, statistical analysis of pooled data from both rams within each group indicated that no significant effect of thermal stress on the volume of semen per ejaculate; however, the effect on total sperm count and motility of spermatozoa was significant ($p<0.001$).

For the purpose of discussion, the results of semen volume, total sperm count and motility of undiluted fresh semen manually assessed are described on an individual ram basis.

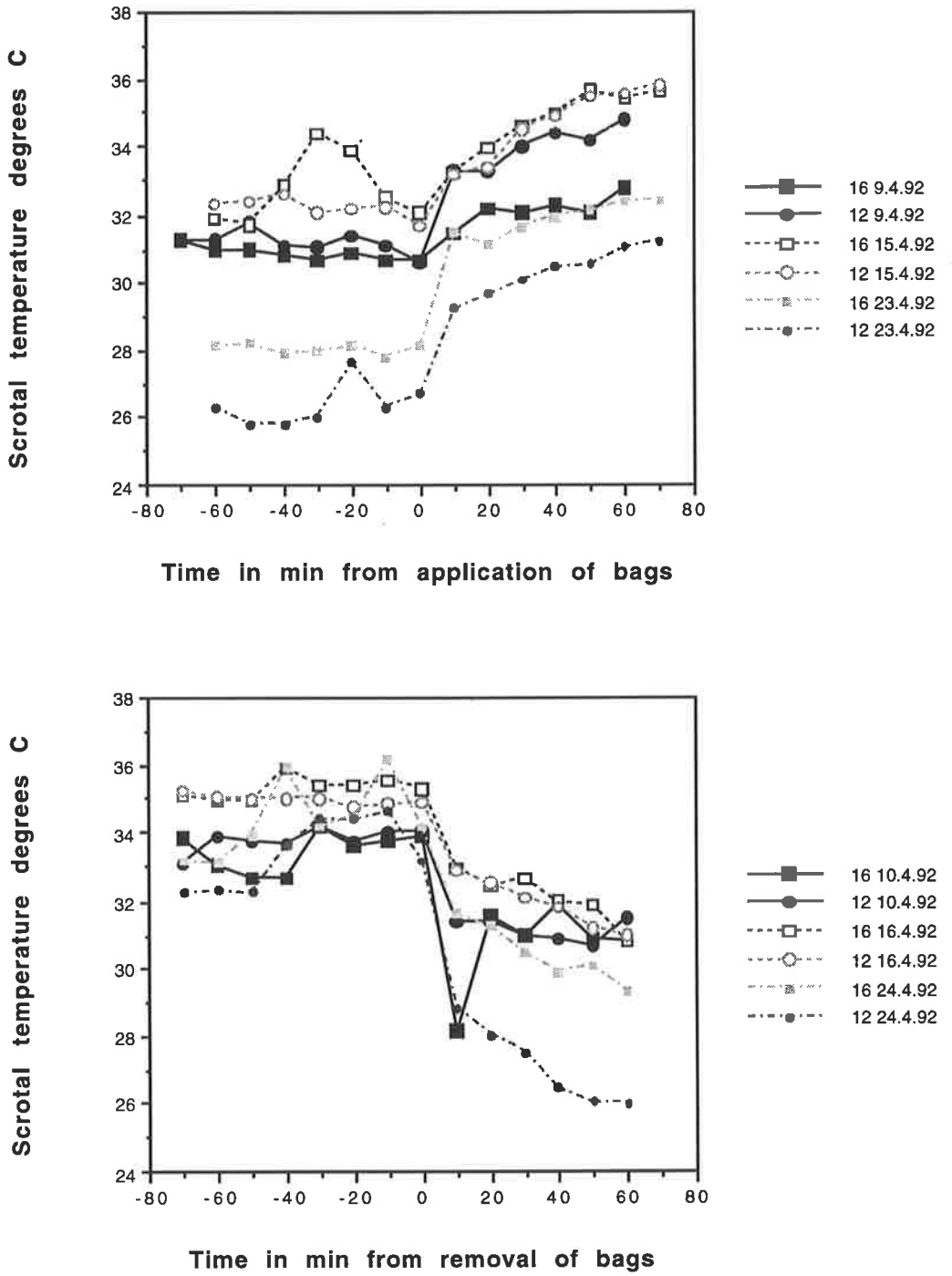


Figure 10.1. Scrotal suface temperatures in two rams (12 and 16) before and after application and removal of insulating bags. On the first two occasions, the environmental temperature was around 20°C, on the third occasion it was around 18°C.

The volume of semen produced by the two groups of rams did not differ throughout the observation periods. The volume ranged from 0.60 to 1.80 ml, although the control group did produce a higher semen volume on day 23, 30 and 51 compared with the insulated group (Figure 10.2).

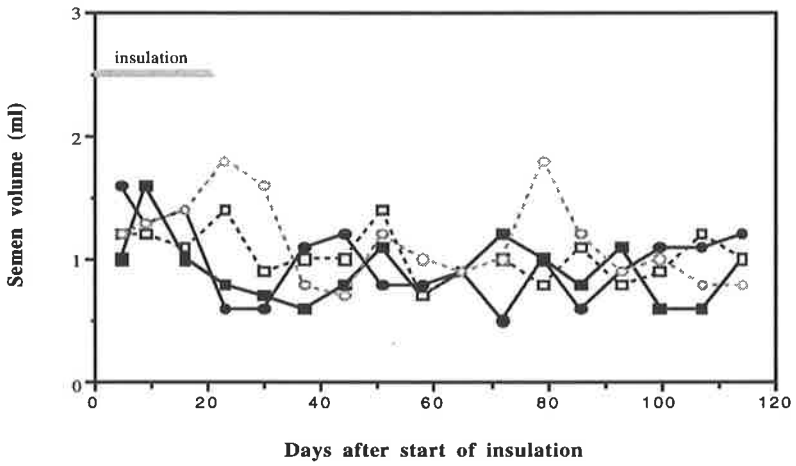


Figure 10.2. Semen volume in fresh ejaculate assessed manually from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

Nine days after scrotal insulation was started, total sperm count of the insulated rams was not affected. However, an effect became apparent at day 16 of scrotal insulation. At this time, total sperm count in the two rams from the treated group decreased gradually up to day 37. An increase in total sperm count was then observed in Ram No. 16 at day 44 and 51. Then at day 58, total sperm count in this ram decreased again. However, it then rose steadily up to day 79, after which a pattern of irregular variation in total sperm count in this ram occurred. In contrast, total sperm count in Ram No.12 remained below 0.5 x 10^9 sperm/ejaculate from day 44 through day 86. After this time, a sharp increase in total sperm count from this ram occurred. Rams of the control group showed a higher total sperm count from day 16 through day 72 compared with rams of the insulated group (Figure 10.3). Very low sperm counts were obtained for all rams on day 86 and from control ram 3 on day 30.

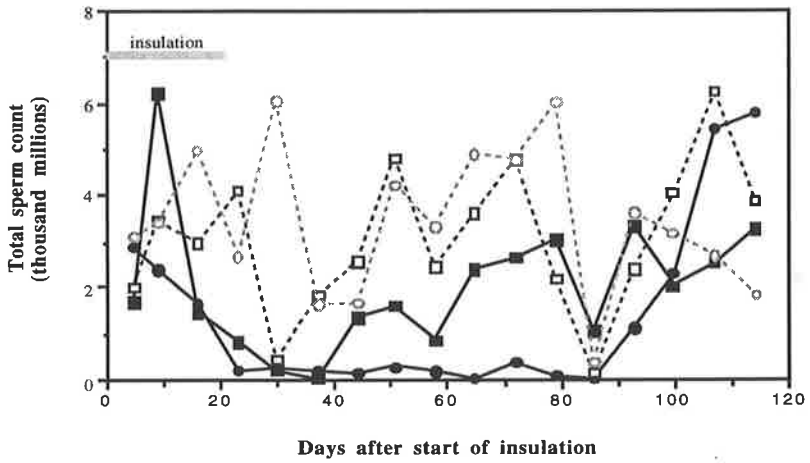


Figure 10.3.Total sperm count in fresh ejaculate assessed manually from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

When sperm motility of fresh undiluted semen from the two groups of rams was compared 4 or 9 days after scrotal insulation was started, no difference was detected. After 16 days of scrotal insulation, a sharp decrease in motility of spermatozoa from the insulated rams was observed compared with that of control rams. The decrease in percent motile in the insulated group continued until it reached zero percent by day 23 (Figure 10.4). Motility of spermatozoa in this group began to increase at day 30, i.e. 9 days after scrotal insulation was terminated. Both rams showed decreases in their sperm motility again at day 37; in Ram No. 16 it decreased to 11%, but in Ram No. 12, it decreased to zero. Motility of spermatozoa from Ram No. 16 began to increase again at day 44 and this increase continued to day 77. In Ram No. 12, however, it increased only to day 44 (22%), after that it tended to decrease again to 4% at day 51 and almost reached zero percent at day 58. Sperm motility of ram No.12 started to increase again from day 65 to day 79, then a slight decrease in motility occured at day 86. At day 93, a rapid increase in motility of spermatozoa from Ram No.12 was observed and then by day 114 sperm motility returned to the normal value (Figure 10.4).

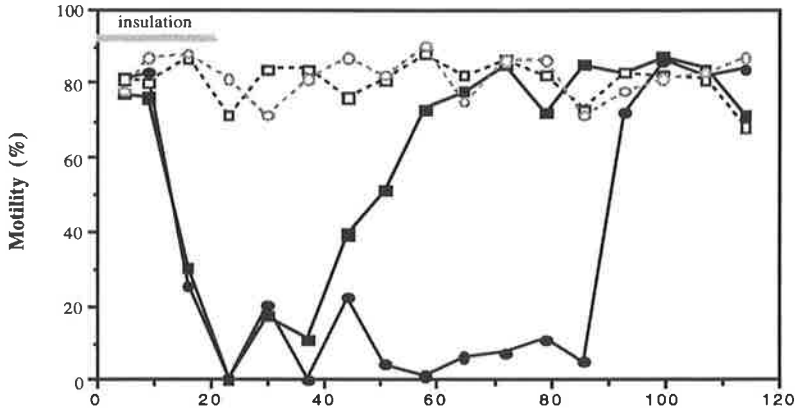


Figure 10.4. Motility of fresh semen manually assessed from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

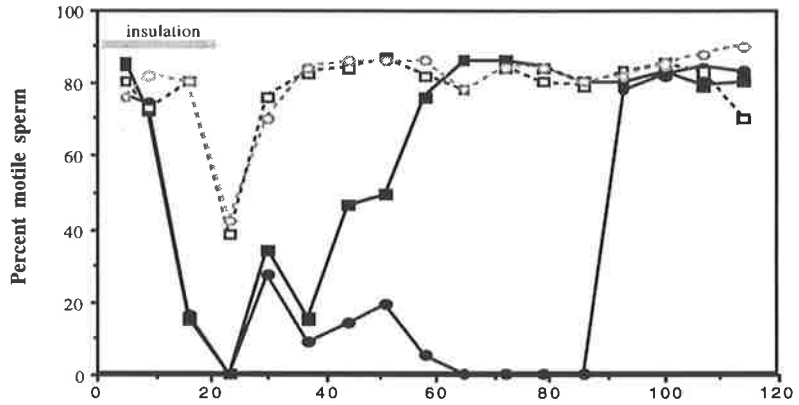


Figure 10.5. Percent motile sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

Considering the actual mean individual data of fresh diluted semen prior to storage, the results showed that mean motility of spermatozoa determined objectively in the 4 individual rams ranged from 72 to 85% on days 5 and day 9 after start of insulation. A sharp decrease in motility in both insulated rams No.16 and No.12 was observed at day 16. At day 23 after start of insulation, motility of the sperm from both the insulated rams dropped to zero percent. At day 30, i.e. 9 days after the end of insulation, motility in both rams had begun to recover, and in ram 16, it continued to improve to reach control values around day 60. However, the recovery in ram 12 was only partial and

temporary, and motility fell to 0% again between days 64 and 86; thereafter there was a rapid return to control levels (Figure 10.5).

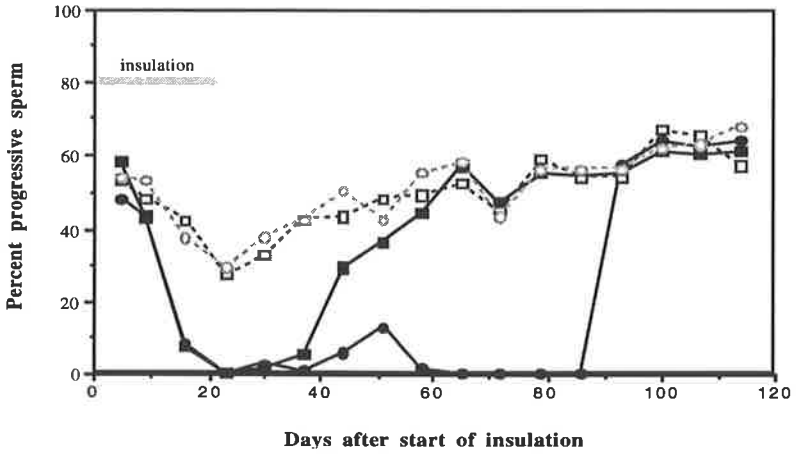


Figure 10.6. Percent progressive motile sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

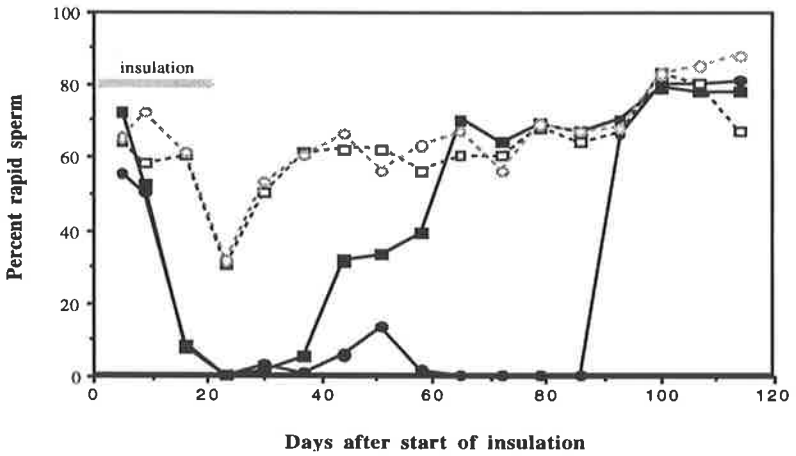


Figure 10.7. Percent rapid sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

Progressive motile and rapid sperm in the insulated rams No.16 or No.12 also began to decrease at day 16 after start of insulation and by day 23 both parameters decreased to zero percent, and remained low until day 37. A gradual increase in percent progressive and rapid sperm in insulated ram No.16 was observed from day 44 and control values were reached around day 60. However in ram No.12, a small increase in these two motility characteristics occured only at day 44 and 51, after which at day 58 the values decreased again to below 2%. Percent progressive and rapid sperm remained at

zero percent up to day 86. Both characters reached the same values as controls from day 93 (Figures 10.6 and 10.7).

A rapid decrease in the numbers of medium sperm in both insulated rams occurred at day 16 and on day 23, no motility was observed in the insulated rams. By day 30 medium sperm in insulated ram No.16 increased again, and thereafter was not consistently different from the controls. Medium sperm in insulated ram No.12 also recovered on day 30, this thereafter decreased again `from day 37 and from days 65 through 86 medium sperm in this insulated ram remained at zero percent. Medium sperm began to rise again at day 93, but dropped in the remaining days of insulation, as did the controls and the other insulated ram (Figure 10.8).

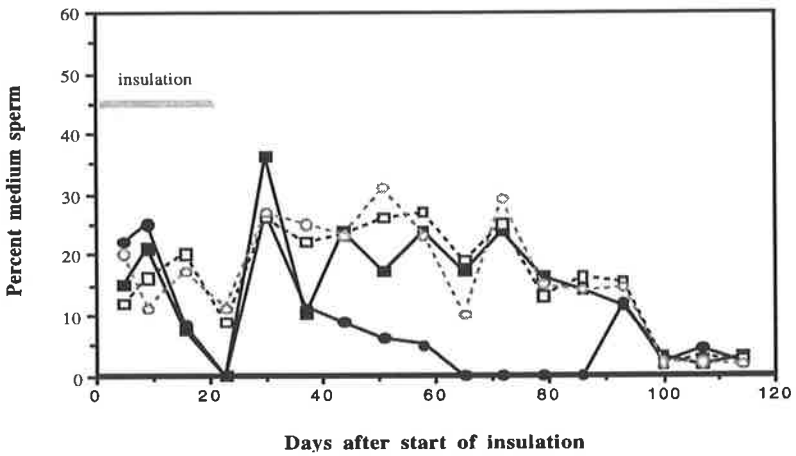


Figure 10.8. Percent medium sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

The percentage of slow sperm in the 4 individual rams remained low until after 9 days of scrotal insulation. In day 16 until about day 60, percent slow sperm increased irregularly in the insulated rams. Thereafter, percent slow sperm in all rams was low throughout the rest of the experiment (Figure 10.9).

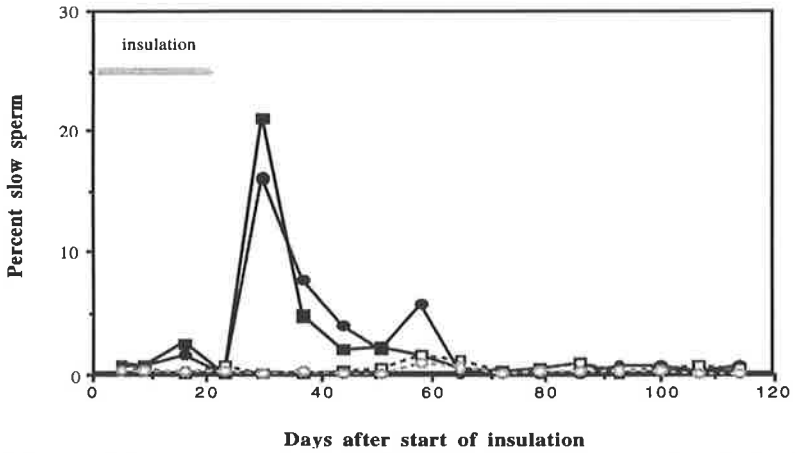


Figure 10.9 Percent slow sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

The numbers of dead sperm were already greater than control in the insulated rams by day 9 when this measurement was first made, and then increased to almost 100% in both insulated rams on days 23 and 30. Then the percentage fell in ram 16, but remained high for ram 12 until after day 60, when for the next three collections, there were too few sperm for a meaningful estimate to be made. From day 93 onwards, there were no differences between insulated and control rams. The percentage dead sperm in the semen from the control rams showed a slight increase over the period of observation, for unknown reasons (Figure 10.10.a).

The numbers of abnormal sperm was also greater than control in the insulated rams from day 9, the first observations, and remained higher than control in both insulated rams until about day 60, when the controls showed a slight increase, and the difference between the insulated and control rams disappeared. Too few sperm were available from ram 12 between days 65 and 80 for meaningful estimates to be made (Figure 10.10.b).

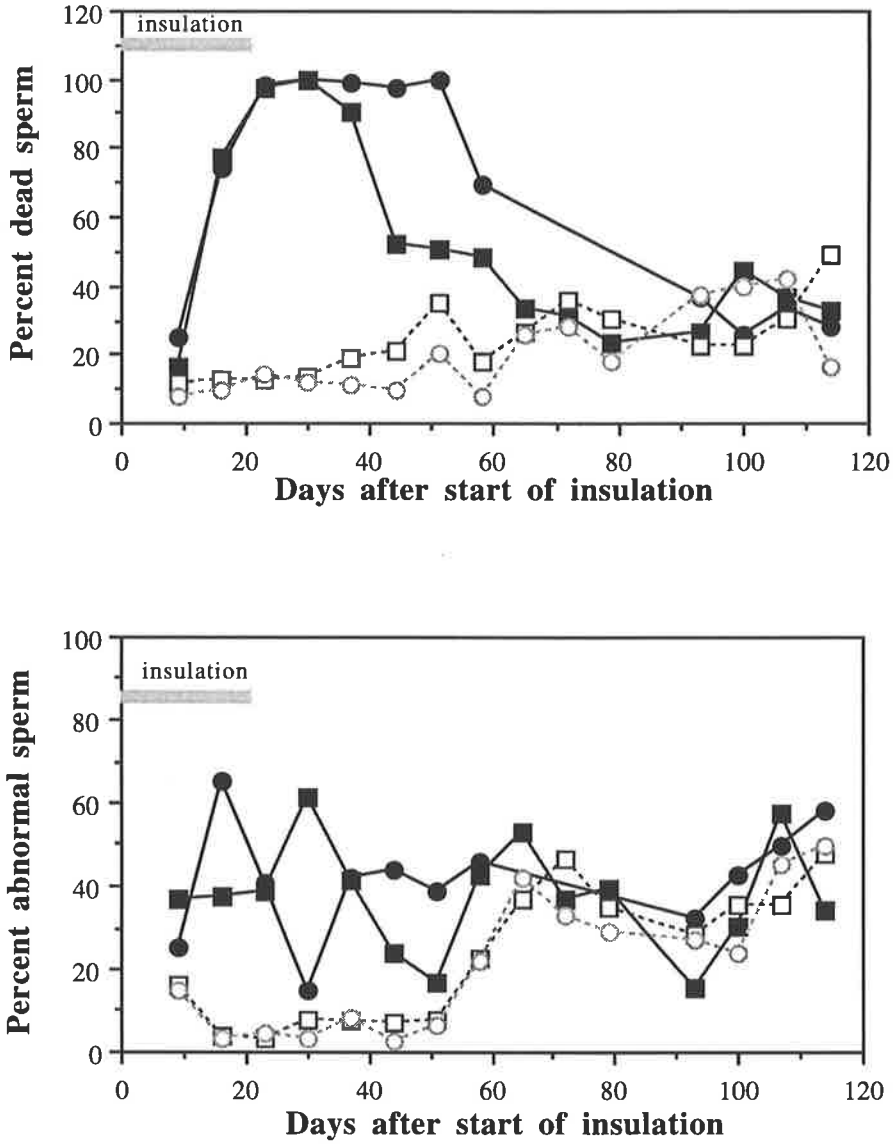
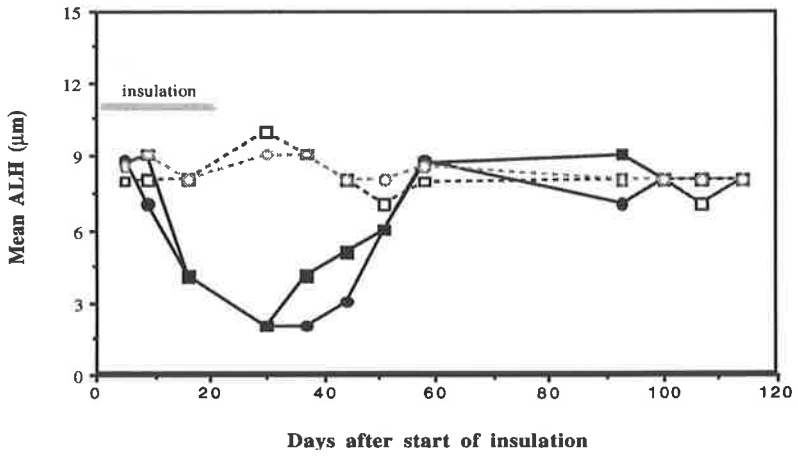


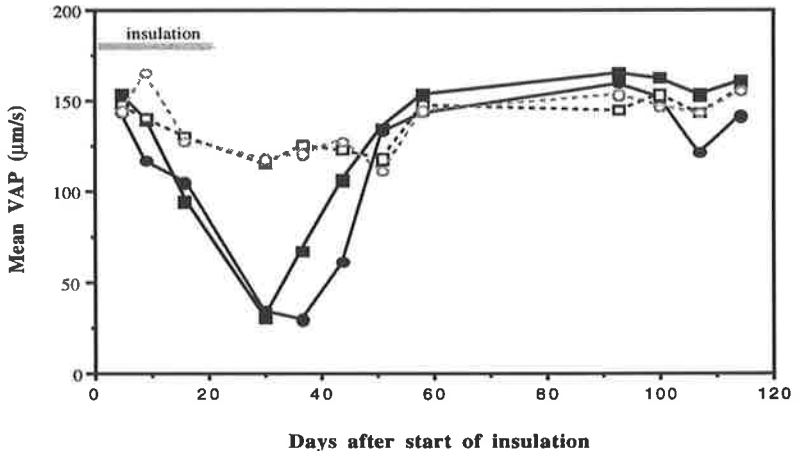
Figure 10.10. Percent dead sperm (a) and percent abnormal sperm (b) in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

Mean ALH, mean VAP, mean VSL and mean VCL of spermatozoa in the four rams did not differ greatly on days 5 and 9 of the scrotal insulation. Decrease in these kinematic parameters in the insulated rams was started after day 9 and continued to day 30. An increase in these four kinematic parameters in the insulated rams occured from day 37 to day 44, and this increase was faster in ram No.16 than in ram No.12. By day 51, mean ALH, mean VAP, mean VSL and mean VCL of spermatozoa in the two groups of rams returned to control values, which were then maintained to the end of the period of observation. (Figures 10.11.a,b,c,d).

a)



b)



c)

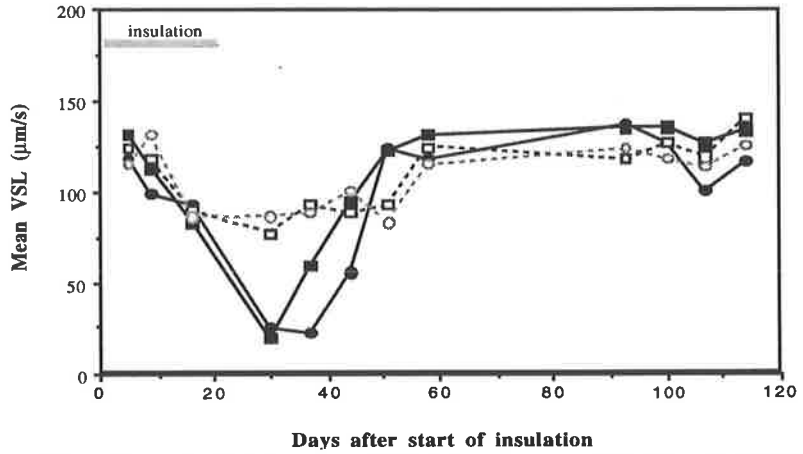
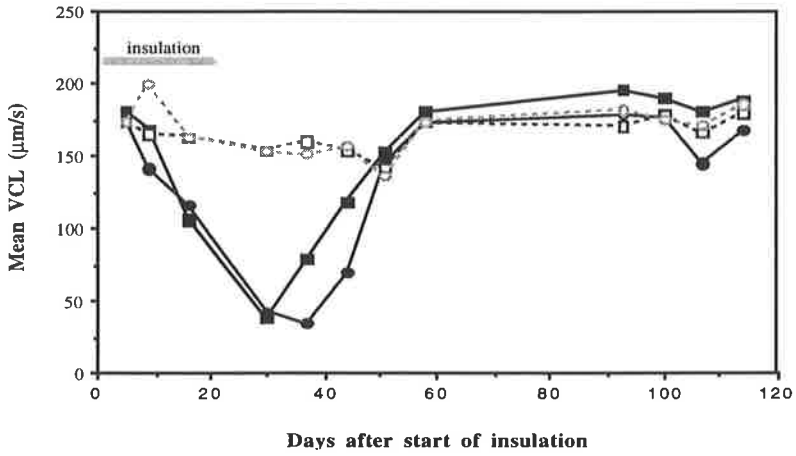
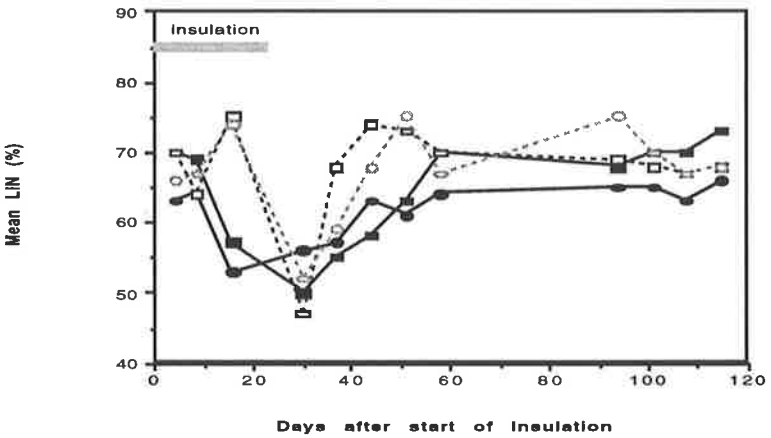


Figure 10.11. Mean ALH (a), mean VAP (b) and mean VSL (c) of spermatozoa measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

d)



e)



f)

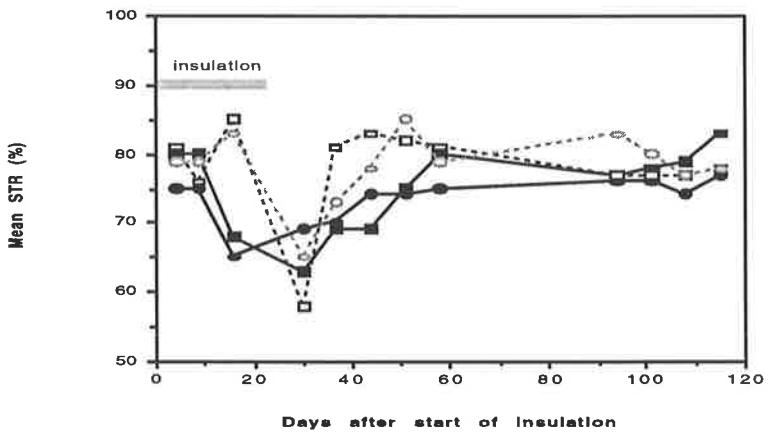


Figure 10.11. Mean VCL (d), mean LIN (e) and mean STR(f) of spermatozoa measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 16 h/day for 21 consecutive days.

Interaction between day of collection and whether insulated or not was significant for percent motility, medium, dead, mean LIN, mean VAP, mean VSL, mean VCL and mean STR ($p<0.001$), slow ($p=0.007$), live ($p=0.009$) and normal spermatozoa ($p=0.002$), but not for progressive motility, rapid, loose head, bent tail, abnormal sperm and mean ALH of spermatozoa.

Table 10.1.2. (Appendix B) shows the effect of storage at 30 and 5°C on changes in sperm motility and morphological characteristics of diluted semen from controls and 16h/day of scrotally insulated rams.

Statistical analysis of the data from each day of collection indicated a highly significant ($p<0.001$) effect of 16 h/day for 21 days of testicular heating on the viability characteristics of stored diluted ram semen. There were no significant interactions between storage temperature, day of collection and whether insulated or not for percent motility or any morphological characteristic of spermatozoa, except percent coiled tail sperm ($p=0.005$).There was a significant interaction between day of collection and insulation on changes in sperm motility and slow ($p<0.05$); changes in live, dead, mean LIN and mean STR ($p<0.001$) of spermatozoa.

With four variables, persentation of all data was very difficult. Therefore, data were simplified to facilitate interpretation. First, in order to evaluate response of spermatozoa from individual rams to scrotal insulation based on their motility and morphology characteristics, the actual data of diluted semen from individual rams after storage for 6h at 30 or 5°C in CEQY or HYCG diluent were pooled and averaged.

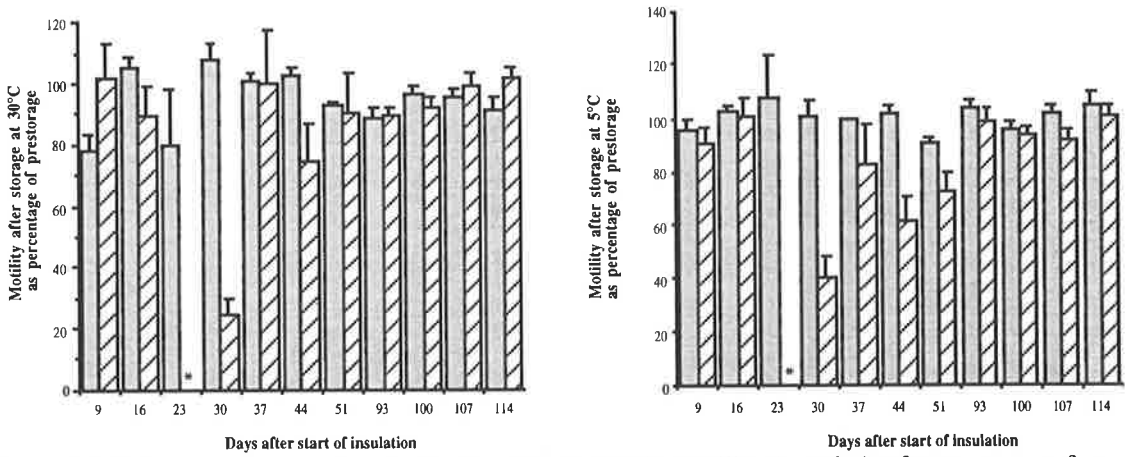
Second, to examine the effect of incubation or storage at 2 different temperatures (30 and 5°C) on motility and morphological characteristics, data from each collection were expressed as the percentage of samples after storage for 6h as the percentage of prestorage samples at 0h.

Third, to observe the diluent effect during storage at 2 different temperatures (30 or 5°C) on sperm motility and morphological characteristics, data were divided into three

periods. Period 1 (during insulation) represent pooled data from three collections (day 13, 20 and 27). Period 2 (after insulation) represent pooled data from four collections (day 34, 41, 48 and 55). Period 3 (full recovery) represent pooled data from four-day collections (day 97, 104, 111 and 118). The data to be analyzed were also expressed as the percentage of samples after storage at 6h as the percentage of prestorage samples at 0h.

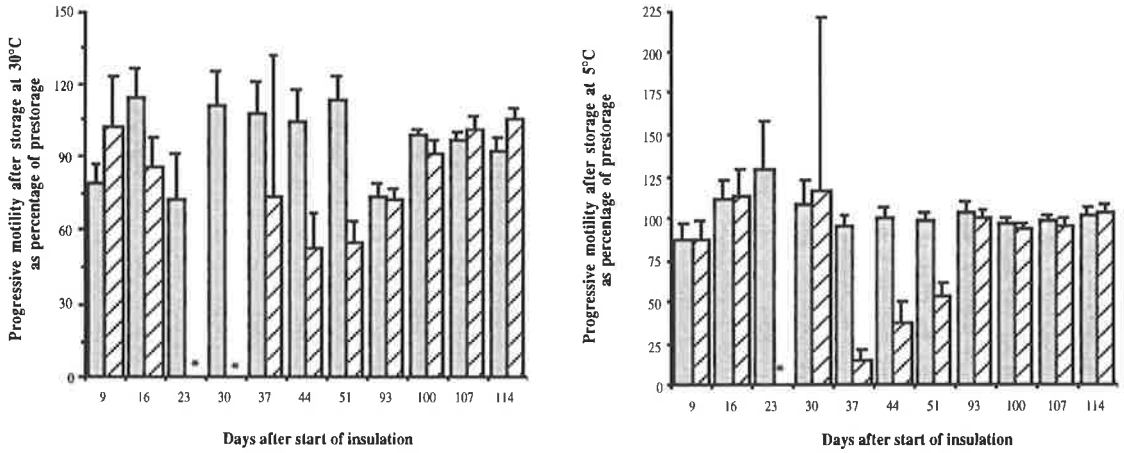
No marked differences between the two groups of rams up to 16 days was observed with regard to change during storage in motility of spermatozoa at either temperature. From day 30, storage for 6h caused a much greater reduction in motility in sperm from the insulated rams at both 30 and 5°C. This effect was less obvious at day 37, but became clear again at day 44. On day 51, there was no greater decrease during storage at 30 in motility of sperm from the insulated rams, although there was still a small difference during storage at 5°C, and thereafter there was no difference of the effect of storage on the motility of sperm from the two groups of rams (Figures 10.12.a and a').

a) **30°C** **a')** **5°C**



Figures 10.12. Changes during storage for 6h at 30 or 5°C in motility (a and a') of spermatozoa from control (□) and 16h/d scrotally insulated rams (▨).*indicate that no figure could be calculated because the value was zero before storage

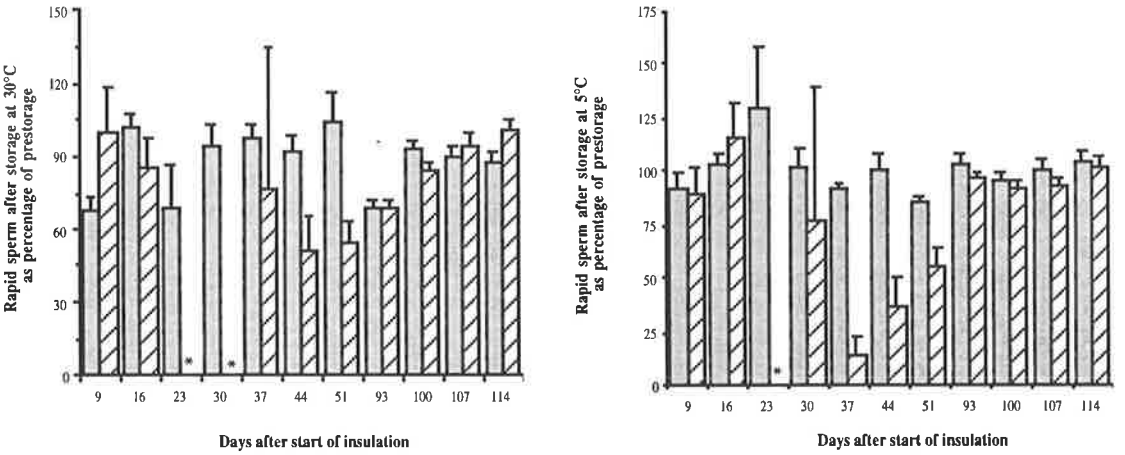
b) 30°C b') 5°C



Figures 10.12. Changes during storage for 6h at 30 or 5°C in progressive motility (b and b') spermatozoa from control (□) and 16h/d scrotally insulated rams (▨).*indicate that no figure could be calculated because the value was zero before storage

Changes during storage at 30 or 5°C in progressive motile and rapid spermatozoa in the insulated and control groups were not different up to 16 days of insulation at 30 and 5°C. However from 23 days from the start of insulation, storage caused a much greater fall in the semen from insulated rams than controls. This effect persisted until 51 days after the start, and thereafter there were no differences in the response of sperm from the two groups of rams (Figures 10.12.b, b' and c, c').

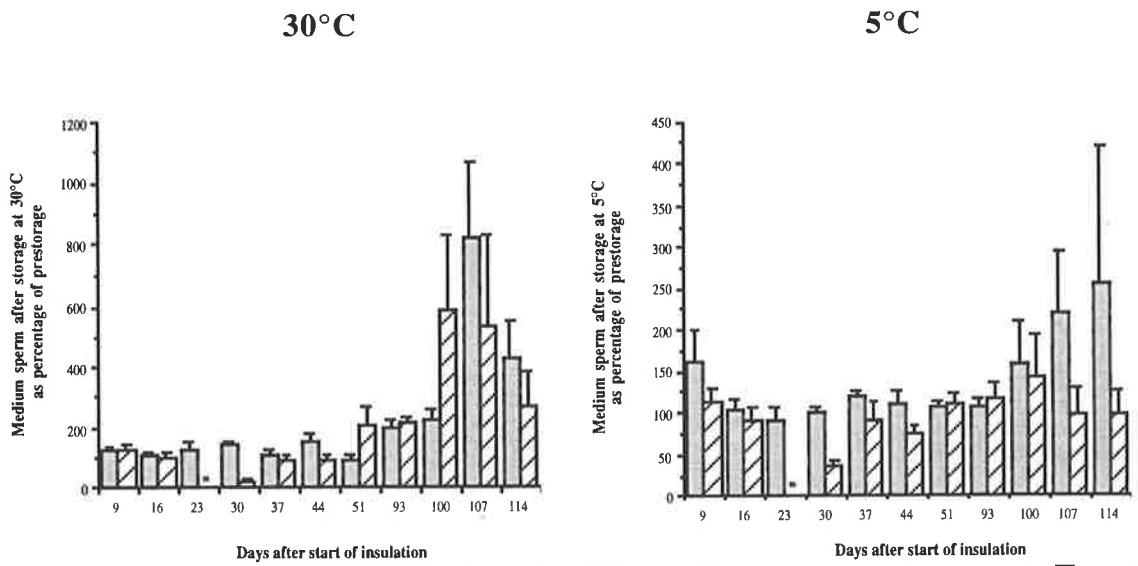
c) c')



Figures 10.12. Changes during storage for 6h at 30 or 5°C in rapid (c and c') spermatozoa from control (□) and 16h/d scrotally insulated rams (▨).*indicate that no figure could be calculated because the value was zero before storage

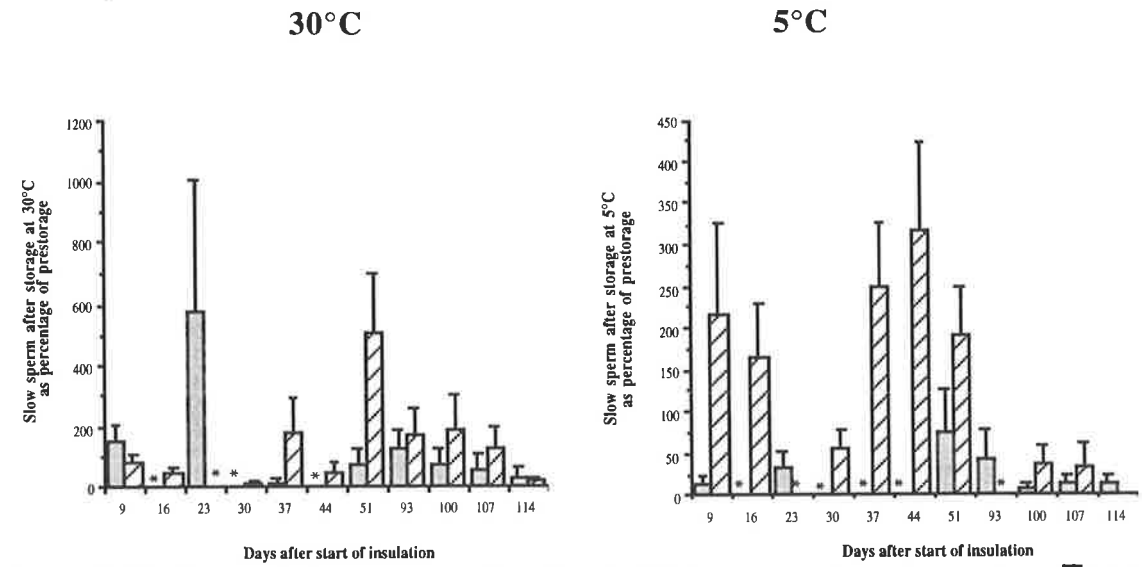
Change during storage in medium sperm in the two groups was similar up to 16 days of scrotal insulation at both temperatures. Thereafter, there were small increases in the

percentage of medium sperm during storage in both groups,with no consistent differences between semen from insulated and control rams (Figure 10.13).



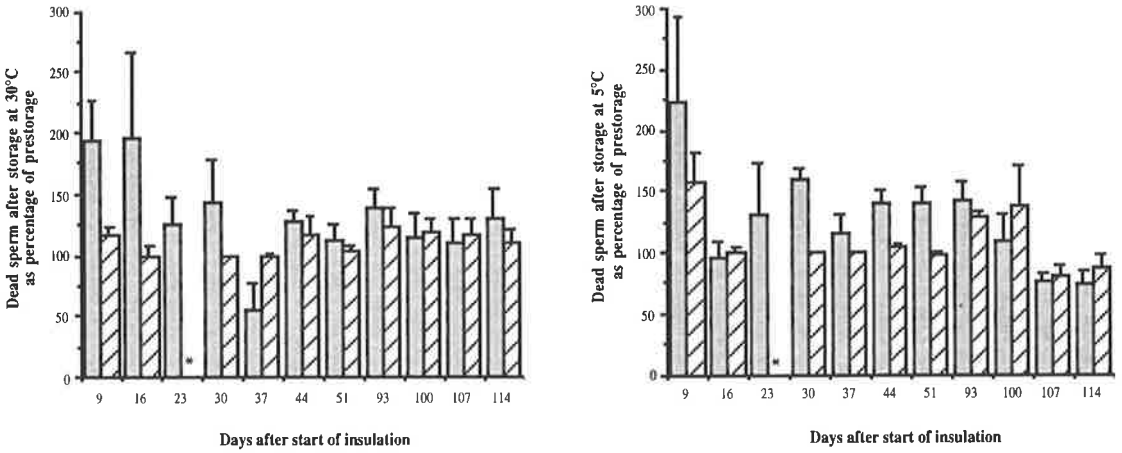
Figures 10.13. Changes during storage for 6h at 30 or 5°C in medium spermatozoa from control (□) and 16h/d scrotally insulated rams (■).* indicate that no figure could be calculated because the value was zero before storage

The percentage of slow sperm rose during storage to a variable extent. After 9 days of insulation, the increase was similar at 30°C, but much greater at 5°C for semen frm the insulated rams. This difference persisted until 51 days, and semen from insulated rams showed a greater increase in slow sperm than control during storage at 30°C on days 37 and 51 (Figure 10.14).

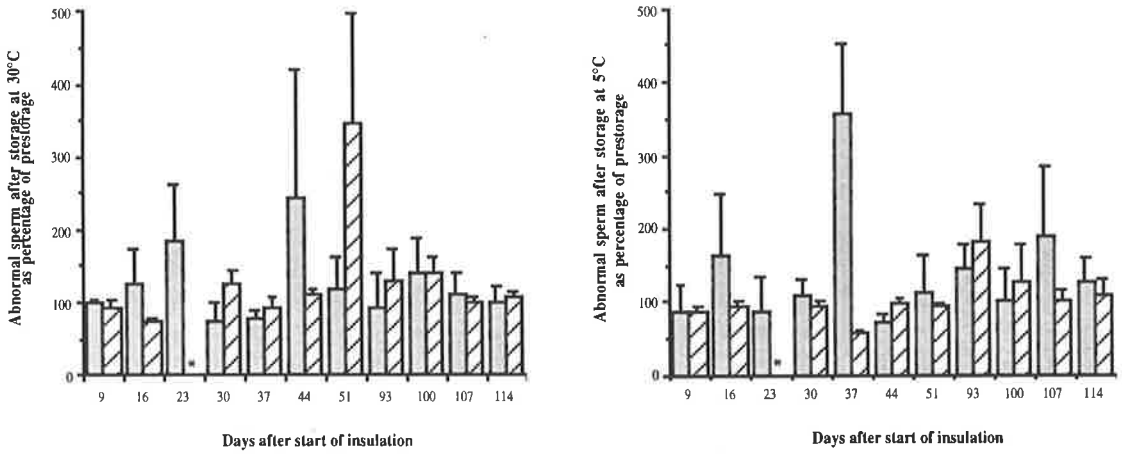


Figures 10.14. Changes during storage for 6h at 30 or 5°C in slow spermatozoa from control (□) and 16h/d scrotally insulated rams (■).* indicate that no figure could be calculated because the value was zero before storage

a) 30°C 5°C



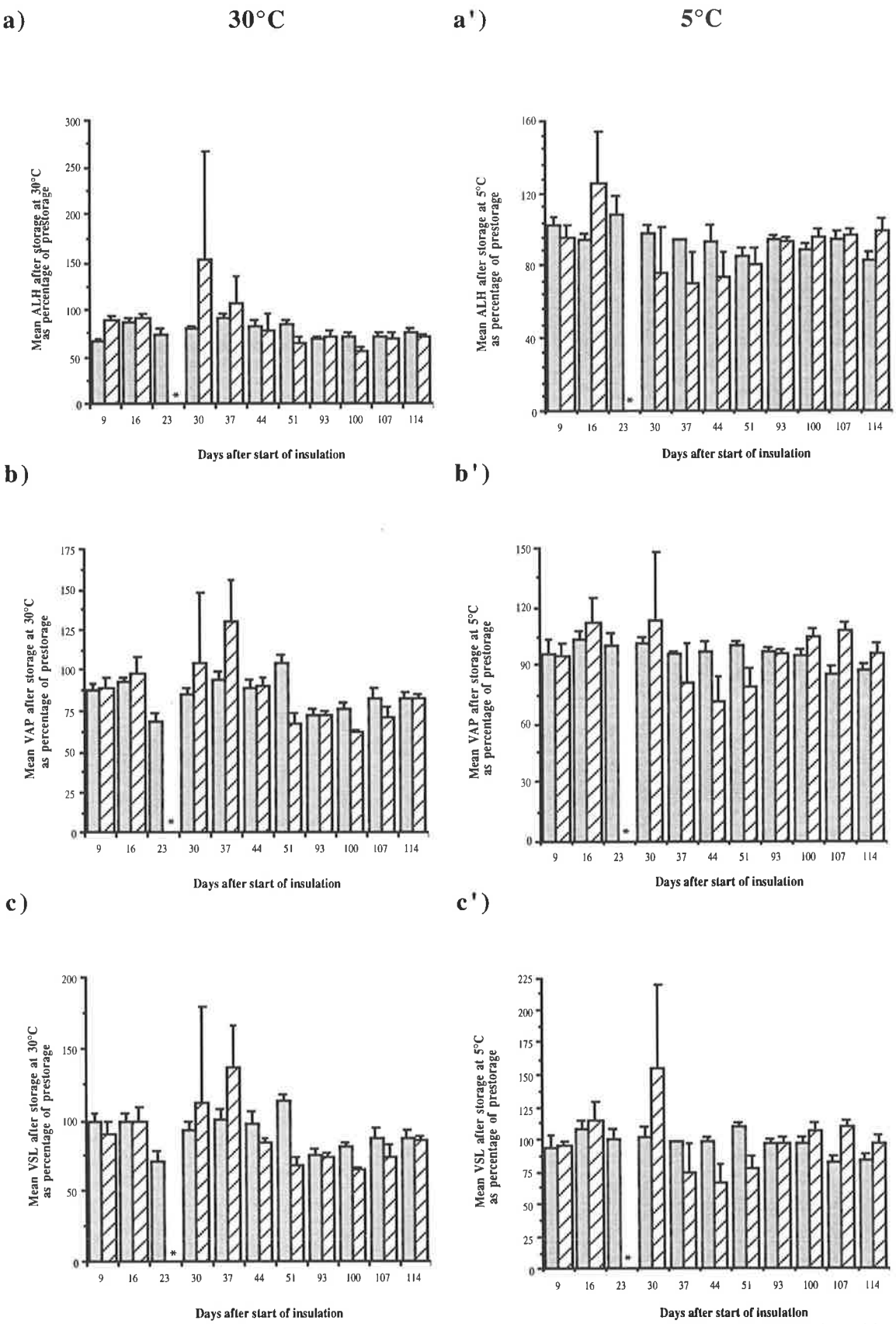
b)



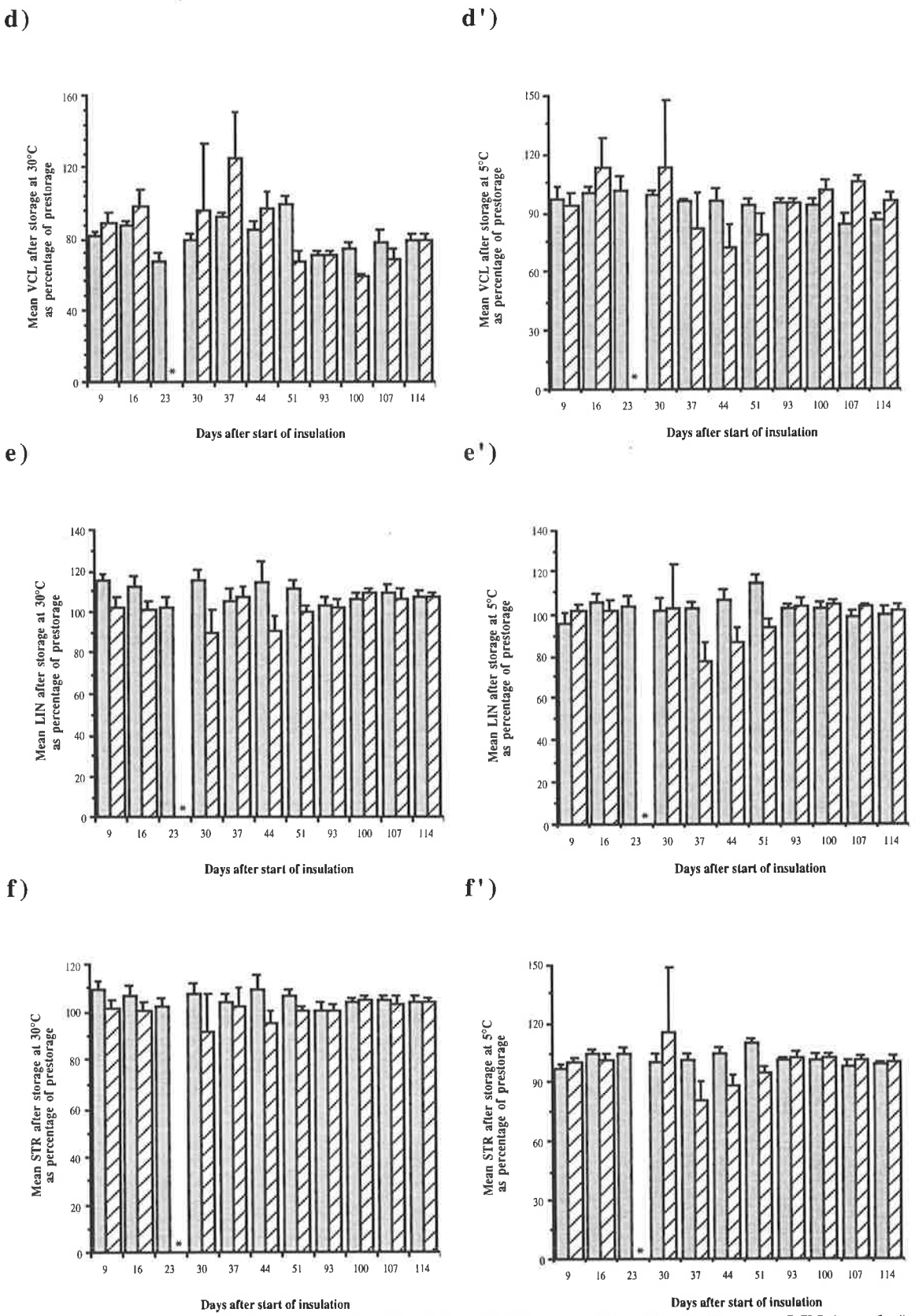
Figures 10.15. Changes during storage for 6h at 30 or 5°C in dead (a) and abnormal (b) spermatozoa from control (□) and 16h/d scrotally insulated rams (■).* indicate that no figure could be calculated because the value was zero before storage

No significant difference was observed in changes during storage in dead or abnormal sperm between the insulated and control groups (Figures 10.15.a and b).

Changes during storage in mean ALH, mean VAP, mean VSL and mean VCL of the two groups of rams were not significant up to day 16 after start of scrotal insulation. From day 30 to day 51 after the start of insulation, semen from the insulated rams showed slightly greater falls during storage at 5°C ($p<0.05$) in mean ALH, mean VAP, mean VSL and mean VCL compared with controls . During storage at 30°C, the changes in mean ALH,VAP, VSL and VCL were similar in semen collected from the insulated and control groups (Figures 10.16.a,a' to f,f').



Figures 10.16. Changes during storage for 6h at 30 or 5°C in mean ALH (a and a'), mean VAP (b and b') and mean VSL (c and c') of spermatozoa from control (□) and 16h/d scrotally insulated rams (◘).* indicate that no figure could be calculated because the value was zero before storage



Figures 10.16. Changes during storage for 6h at 30 or 5°C in mean VCL (d and d'), mean LIN (e and e') and mean STR (f and f') of spermatozoa from control (□) and 16h/d scrotally insulated rams (▣).* indicate that no figure could be calculated because the value was zero before storage

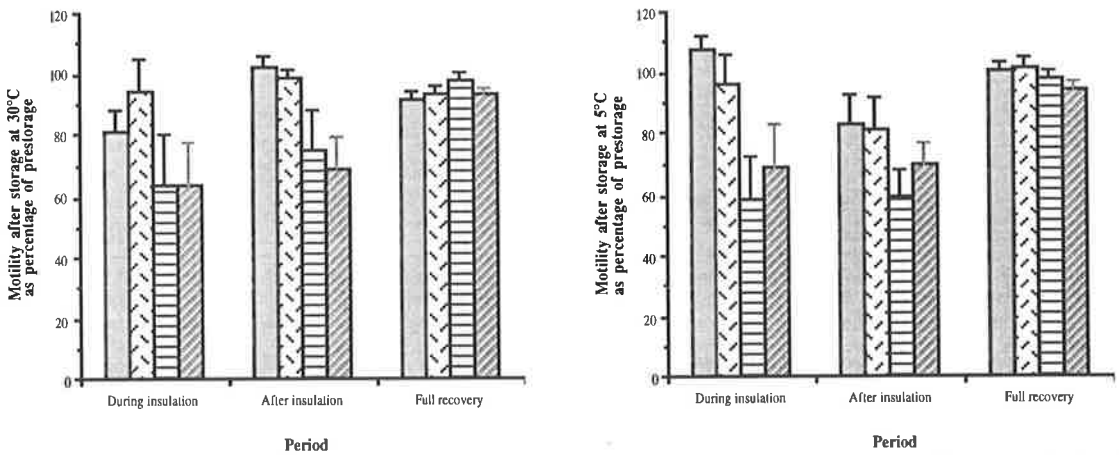
Semen from rams in the insulated group showed a significantly ($p<0.05$) greater decrease in mean LIN and STR during storage compared with controls either at 30 or 5°C between days 30 and 44 after the start of insulation (Figures 10.16.e,e' and f,f').

The effect of diluents on changes during storage in motility and morphological characteristics of spermatozoa in semen from control and 16h/d scrotally insulated rams at two different temperatures is summarized in Table 10.1.3 (Appendix B).

There were no significant interactions of diluent x insulation x temperature x day of collection on motility and morphological characteristics of spermatozoa. A significant interaction of diluent x temperature x day of collection was observed in the changes in medium ($p<0.001$) and coiled tail ($p=0.002$) spermatozoa (Tables 10.1.4 a to k, Appendix B).

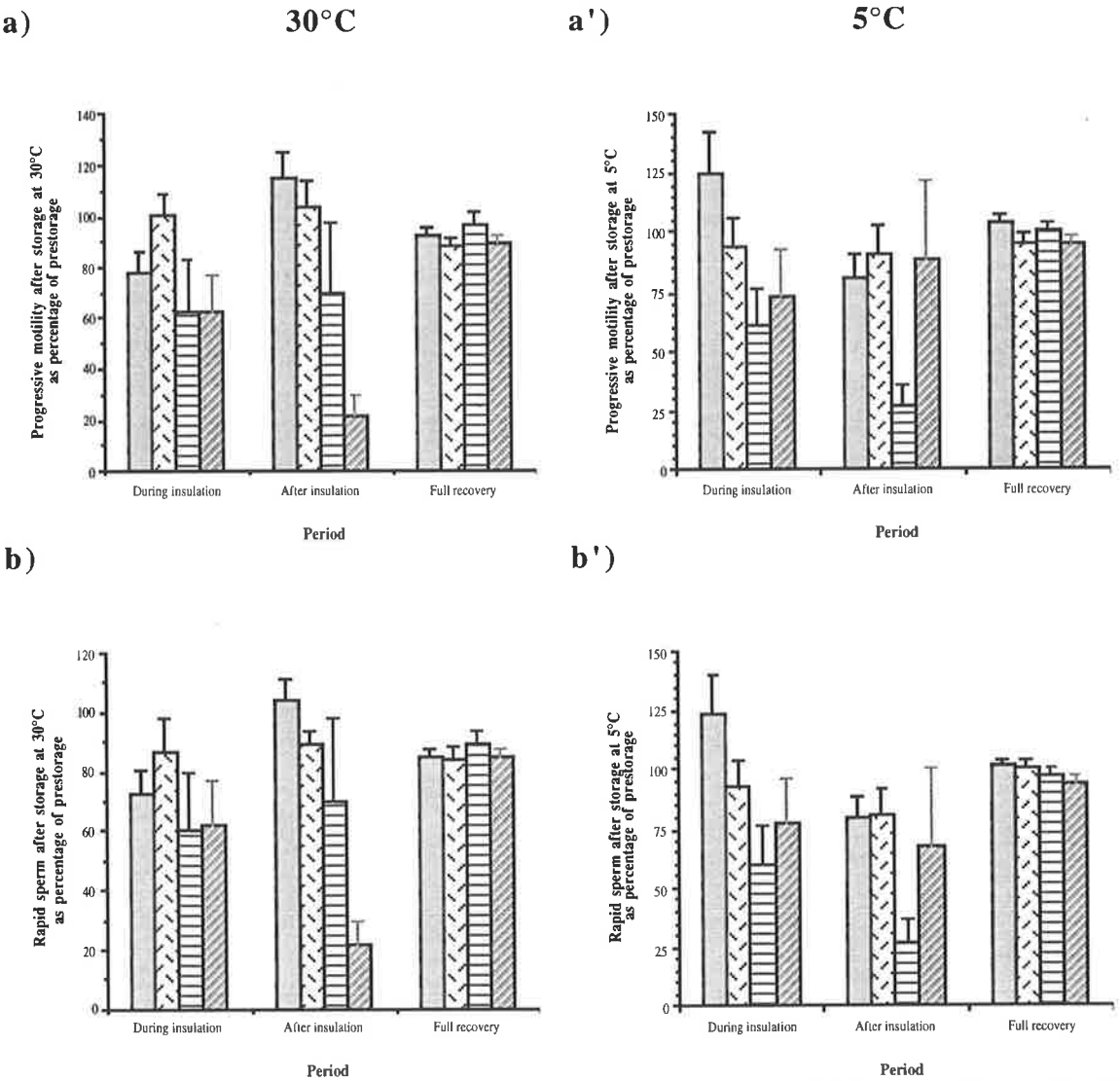
The percentage motile spermatozoa and the percentage progessive and rapid sperm in semen from control rams decreased in both diluents and at both temperatures to a significantly ($p<0.05$) lower extent than sperm from the insulated rams during the insulation period and immediately afterwards. However, the changes were similar in the two groups when the other characteristics of the motility of the sperm from the insulated rams had returned to normal (Figures 10.17.a and a').

a) **30°C** **a')** **5°C**



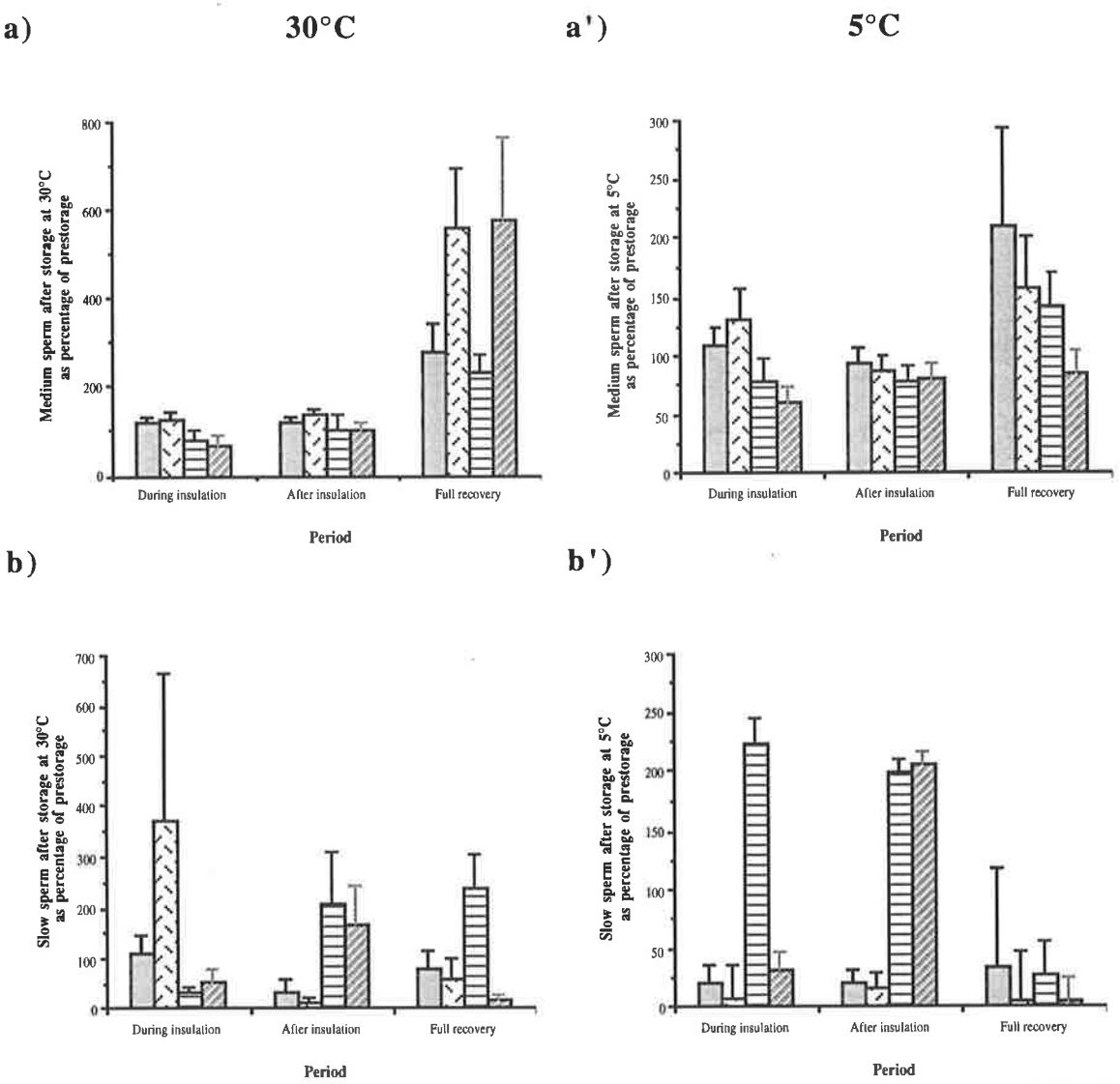
Figures 10.17. Effect of diluent on changes during storage for 6h at 30 or 5°C in motility (a and a') of spermatozoa in semen from control diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (▨) and in semen from 16h/d scrotally insulated rams diluted in hen yolk citrate glucose (▤) or in coconut extract quail yolk (▩) diluents.

There were no effects of the diluent on the changes in percent progressive motile or rapid sperm during storage of sperm collected during the insulation period, but in the time after insulation before recovery of the sperm characteristics had occurred, sperm from the insulated rams stored less well in CEQY at 30°C, but better at 5°C (Figures 10.18. a,a' and b, b').



Figures 10.18. Effect of diluent on changes during storage for 6h at 30 or 5°C in progressive motility (a and a') and rapid (b and b') spermatozoa in semen from control diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (▨) and in semen from 16h/d scrotally insulated rams diluted in hen yolk citrate glucose (⊟) or in coconut extract quail yolk (▨) diluents.

There were no consistent effects of diluent on the changes during storage in medium sperm in semen from the two groups of rams, while the percent slow sperm increased more during storage in semen from insulated rams during the post-insulation period, with no consistent effect of diluent (Figure 10.19.a,a' and b,b').



Figures 10.19. Effect of diluent on changes during storage for 6h at 30 or 5°C in medium (a and a') and slow (b and b') spermatozoa in semen from control diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (□) and in semen from 16h/d scrotally insulated rams diluted in hen yolk citrate glucose (⊟) or in coconut extract quail yolk (⊟) diluents.

During and after insulation, the change during storage in live sperm in semen from control rams was less ($p<0.05$) than in semen from insulated rams in samples diluted in HYCG or CEQY and kept at 30 or 5°C. The diluent had no consistent effect. (Figures 10.20.a and a').

Scrotal insulation did not affect change during storage in percent normal sperm in either diluent or at either temperature (Figures 10.20.b and b'). However, scrotal insulation increased ($p<0.05$) the rise in coiled tail sperm in samples diluted in HYCG and stored at 30 or 5°C (data not shown).

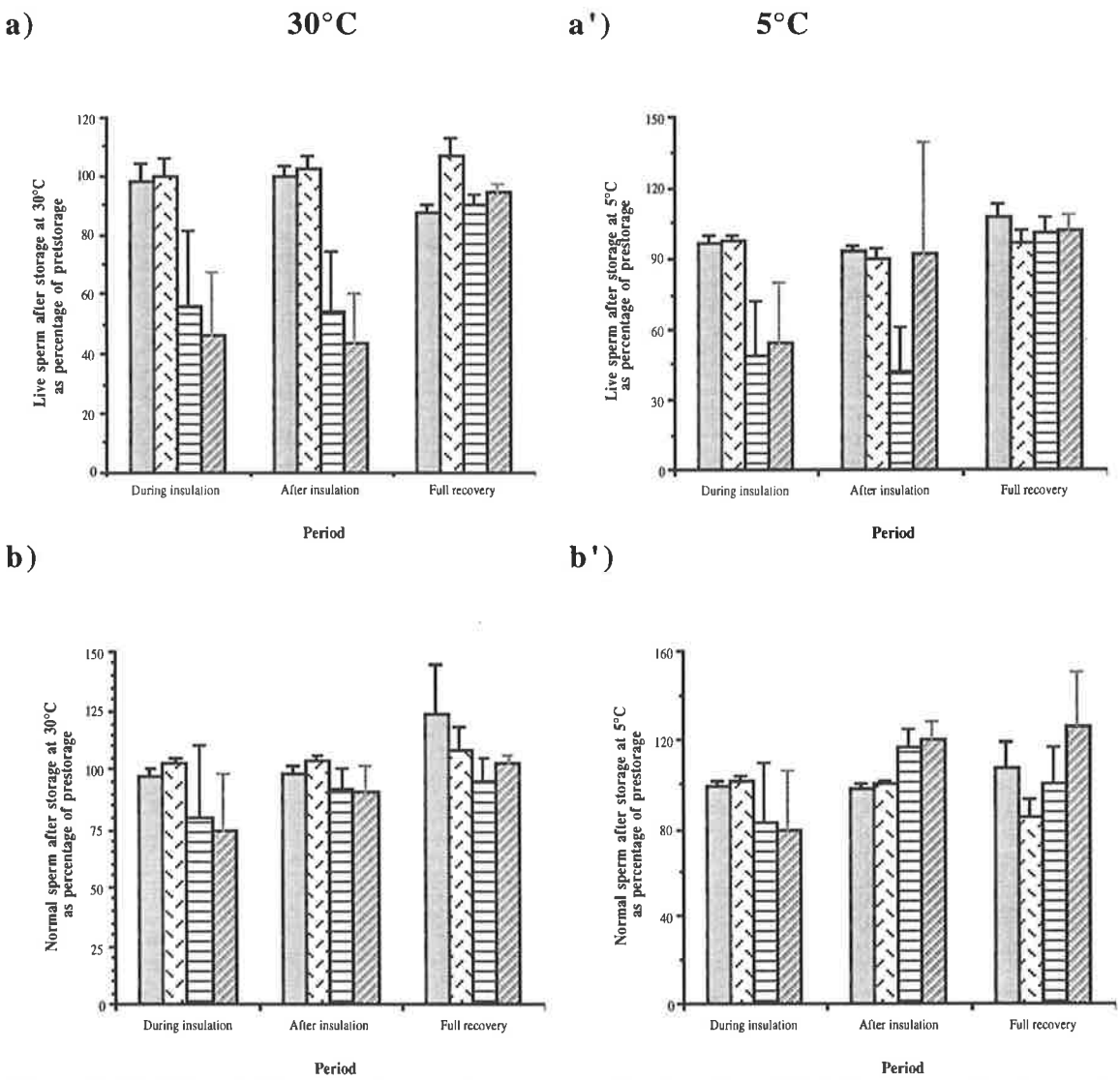
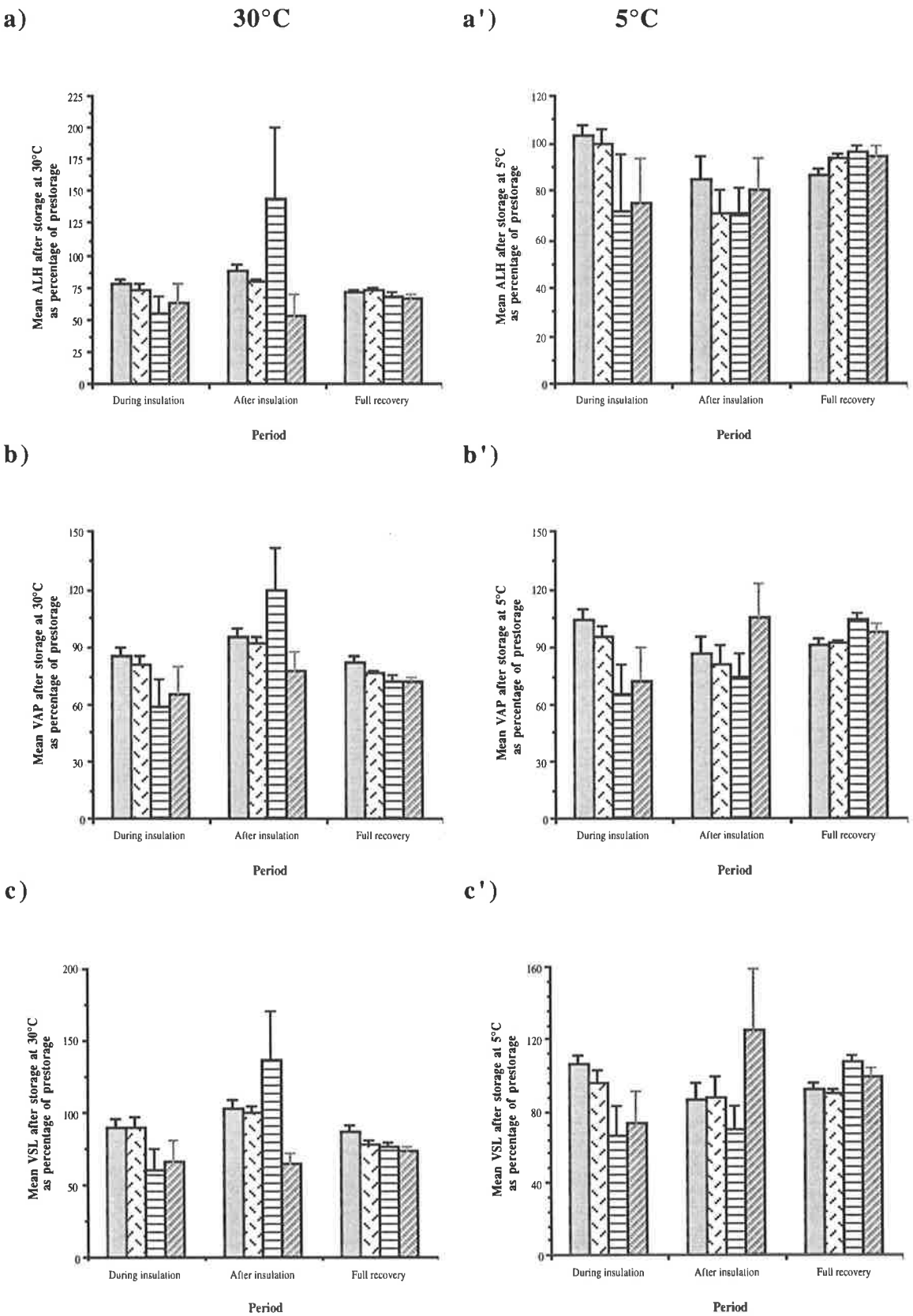
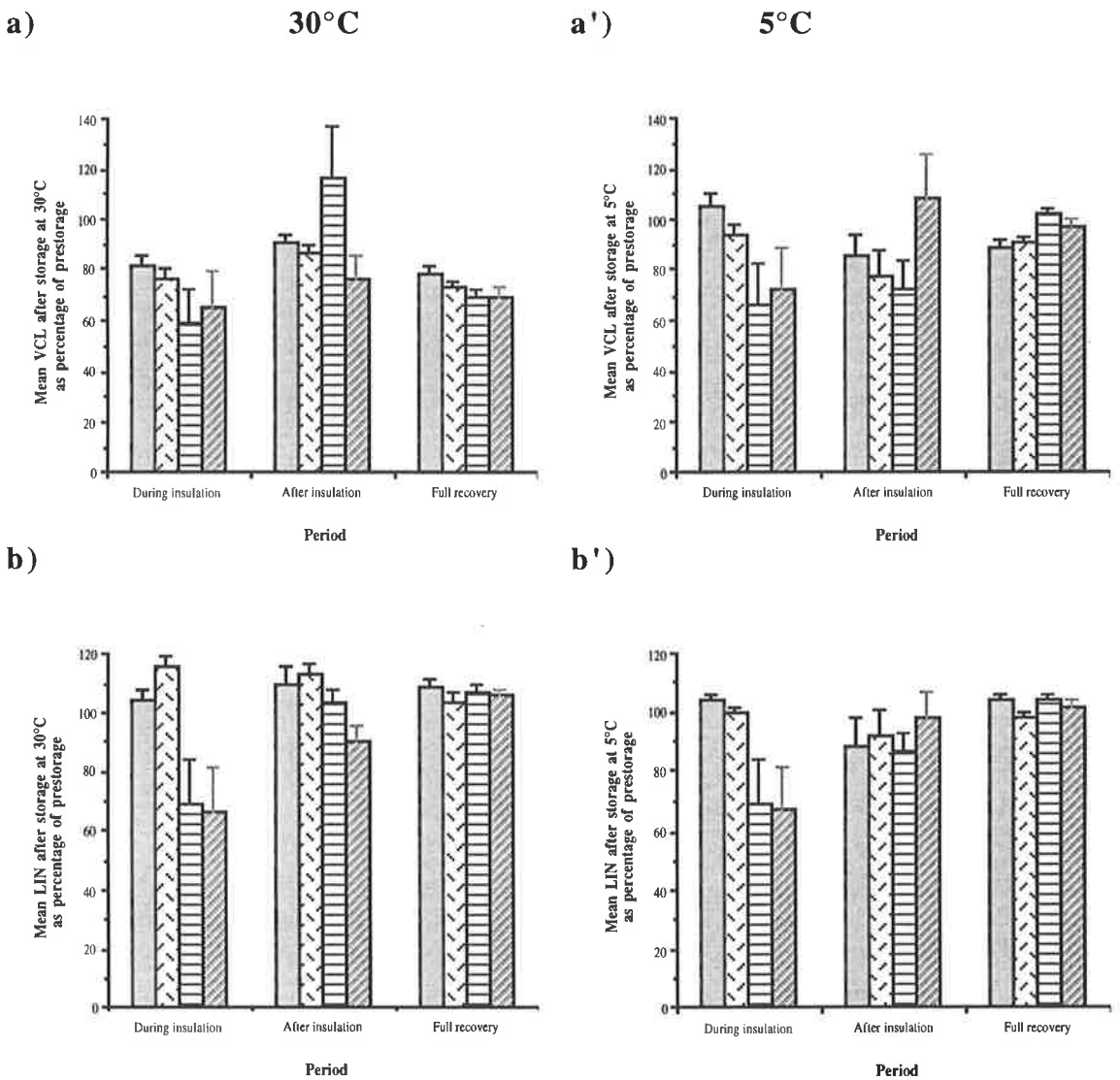


Figure 10.20. Effect of diluent on changes during storage for 6h at 30 or 5°C in live (a and a') and normal (b and b') spermatozoa in semen from control diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (▨) and in semen from 16h/d scrotally insulated rams diluted in hen yolk citrate glucose (▭) or in coconut extract quail yolk (▨) diluents.

Although changes during storage in mean ALH, mean VAP, mean VSL and mean VCL of spermatozoa in semen from control rams and diluted in HYCG or CEQY stored at 30 or 5°C was slightly less than that of sperm from insulated rams, the differences in these kinematic parameters were not significant (at either temperature. There were no consistent differences between the two diluents (Figures 10.21.a,a', b,b',c,c' and 10.22.a, a').

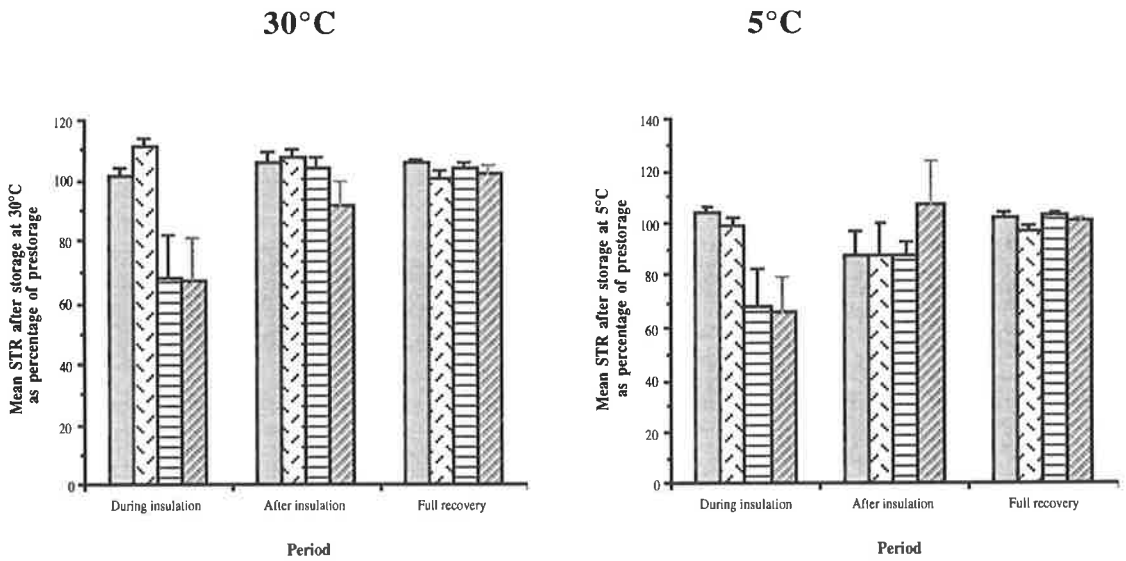


Figures 10.21. Effect of diluent on changes during storage for 6h at 30 or 5°C in mean ALH (a and a'), mean VAP (b and b') and mean VSL (c and c') of spermatozoa in semen from control diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (▨) and in semen from 16h/d scrotally insulated rams diluted in hen yolk citrate glucose (■) or in coconut extract quail yolk (▩) diluents.



Figures 10.22. Effect of diluent on changes during storage for 6h at 30 or 5°C in mean VCL (a and a') and mean LIN (b and b') of spermatozoa in semen from control diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (▨) and in semen from 16h/d scrotally insulated rams diluted in hen yolk citrate glucose (▤) or in coconut extract quail yolk (▩) diluents.

Changes during storage in mean LIN and mean STR in samples from control rams diluted in HYCG or CEQY and stored at room and cool temperatures were significantly ($p<0.05$) less than in semen from insulated rams during insulation. However, changes in these parameters were similar in the semen of the treated and control rams after insulation and following full recovery periods and there was no consistent effect of diluent (Figures 10.22.b,b' and 10.23).



Figures 10.23. Effect of diluent on changes during storage for 6h at 30 or 5°C in mean STR of spermatozoa in semen from control diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (□) and in semen from 16h/d scrotally insulated rams diluted in hen yolk citrate glucose (■) or in coconut extract quail yolk (▨) diluents.

10.2.4. Discussion

In this study, volume of semen collected from experimental rams showed little change during scrotal insulation. This agrees with the observation that heat stress did not influence semen volume from other species subjected to increased ambient temperatures in dairy bulls (Johnson and Branton, 1953) and in boars (Wettemann et al, 1976). The lower total sperm count observed in the heated compared with the control rams was in agreement with the findings of Mieusset et al (1991). In contrast, Quintana Casares (1993) did not find any significant effect on total sperm count with up to 21 days of scrotal insulation for 16 h/d. In addition, decrease due to increased testicular temperature by scrotal insulation in motility of individual undiluted spermatozoa manually assessed supports the findings of previous studies in rams (Dutt and Hamm, 1957; Dutt and Simpson, 1957; Moule and Waites, 1963; Howarth, 1969) and in bulls (Meyerhoeffer et al, 1985).

It is seen that following 16 h/d of elevated testicular temperature, motility of undiluted semen began to fall at day 16 after start of insulation and then by day 23 no motility was observed (zero motility) in the semen of insulated rams (Figure 10.4.). This is in agreement with those reported in the previous studies (Dutt and Hamm, 1957; Moule

and Waites, 1963, Rathore, 1969; Smith, 1971; Mieusset et al, 1992). As sperm spend up to 14 days in the epididymis, this suggests that sperm about to leave the testis were affected. However, a second decrease in motility of undiluted semen occurred on day 37 in insulated ram No.12 suggesting that developing germ cells in the seminiferous tubules were also damaged by the elevated scrotal temperature.

At day 44 (23 days after termination of scrotal insulation), motility of undiluted spermatozoa in the insulated rams began to increase again. However, there were important differences in the response of individual rams, with the increase in motility from ram No. 12 not occuring as soon as in ram No.16. At day 51 (30 days after termination of scrotal insulation), motility of spermatozoa remained higher in ram No.16 than that in ram No.12. Even at day 58, while motility of ram No.16 continued to increase gradually, motility of ram No.12 tended to decrease further and though it increased again from day 65, the value up to day 86 remained below 11%. The reason for the decrease in sperm motility in ram No.12 during these later times is unknown. The timing of the fall in motility might reflect different sensitivities of the various stages of developing spermatozoa to increased testicular temperature (Friedman et al, 1991). In this study, although increases in scrotal temperatures due to insulation were uniform as the bags were applied at random each day to the two animals, and the temperature reached were similar, the severity of the response between ram No 12 and ram No 16 was quite different. This agreed with the studies of Dun (1956), who also observed considerable variation between individual rams in the extent of the change of semen characteristics following exposure to heat.

In relation to the recovery time, it was observed that motility of spermatozoa in semen from ram No.16 returned to control values within 37 days following the end of insulation, i.e. at day 58 after start of insulation. However, in ram No.12, it took 57 days after insulation was stopped or 93 days after the start of insulation. This difference in the recovery time was mainly due to a further drop in this parameter which occurred in ram No.12 between days 51 and 86. The recovery time observed in ram No.16 was very similar to that observed by Mieusset et al (1991). In their study, they found that motility

of spermatozoa from rams whose testes were insulated for 16 h/d for 144 consecutive days after the end of treatment returned to normal values by about 40 days after the end of insulation. For ram No.12, the delay was slightly longer than that seen by Mieusset et al (1991). However, it was close to those of Moule and Waites (1963), who observed that the complete recovery was not apparent until 60 days after heat treatment had been terminated.

In studies on the effect of 16h/day scrotal insulation on motility and morphological characteristics of diluted semen from individual rams, it was found that the effect began on day 16 of scrotal insulation and it became obvious by day 23. This effect seemed to be consistent and reflected the pattern of changes in degeneration of semen characteristics observed in undiluted semen. However, decreases in sperm motility in diluted semen at day 23 in both control rams was unexpected, as observations on fresh undiluted semen did not indicate lower values in this character. It could be due to unrecognised technical errors during preparation of diluent or dilution of semen or the semen samples may have been inadvertently subjected to cold schock.

Decreased viability of diluted sperm (motility, progressive motility and rapid spermatozoa) due to testicular insulation was coincident with increases in the percentages of dead and morphologically abnormal sperm in diluted semen seen at day 16, which became more apparent at day 23. Considering that epididymal transport takes approximately 14 days in rams (Ortavant, 1959), it is likely that in this study heat stress interfered less with sperm maturation in the epididymis rather than the spermatogenic process. These results are similar to the findings of Dutt and Hamm, (1957), Moule and Waites (1963), Fowler (1968) and Mieussett, et al (1991; 1992), who could not find abnormal spermatozoa in the semen of the heated rams until about 14 days after treatment; this showed that spermatozoa present in the epididymis during treatment were resistent to heat. Glover (1955) reported that in rams the numbers of abnormal spermatozoa increased between days 17 and 24 after a short heat treatment. Austin et al (1961) suggested that morphological abnormalities were probably due to heat damage to

spermatids and mature sperm; dead sperm were due to injury to spermatocytes in addition to more mature cells.

In this study when the two rams within the insulated group were compared, variation between ram No.16 and No.12 occurred with regard to the numbers of dead and abnormal sperm during scrotal insulation. Return of these morphological characteristics to pre-treatment levels was achieved in ram No 16 within 44 days and in ram No.12 72 days from the termination of scrotal insulation. In addition, variation between the two individual rams within the insulated group also occurred in mean ALH, mean LIN, mean VAP, mean VSL, mean VCL and mean STR of spermatozoa. In general, all these kinematic characteristics reached the same values as that of the controls within 30 days after termination of insulation or at day 51 after the start of insulation.

In the present study, the most common specific abnormality of diluted spermatozoa collected during the insulation period was coiled tails, followed by bent tails and detached heads. This is in close agreement with the findings of Glover (1956) and Smith (1971) who observed in fresh semen that the first type of abnormality of ram spermatozoa subjected to heat stress was coiled tails, which was followed by the appearance of tailess heads. In bulls, the first type of morphological abnormality to appear was also coiled tails (Johnston et al, 1963). Dott (1975) postulated that tailess heads was due to sperm degeneration occurring in the epididymis (secondary abnormality) or because semen was not handled properly (tertiary abnormality).

It was observed that during the time when motility of spermatozoa in fresh undiluted semen from insulated rams had dropped to zero percent, there was also a large drop in sperm concentration in their ejaculates, and as a result, assessment of viability characteristics, live, dead, morphologically normal and abnormal spermatozoa of diluted semen from the insulated group could not be performed. Damage to spermatocytes may have been responsible for decreased sperm concentration following the longer period of scrotal insulation in the insulated group. This may indicate that the further development of some spermatids, and of diplotene and pachytene spermatocytes (Ortavant, 1959) is

adversely affected by elevated testicular temperature due to scrotal insulation. The results agree with the earlier findings reviewed by Van Demark and Free (1970) that spermatocytes and spermatids are more sensitive to increased temperature than are spermatogonia within the testis and spermatozoa in the epididymis.

At the same time as the characteristics of the spermatozoa from the insulated rams were showing differences, these sperm were also less able to withstand storage for 6h at either 30 or 5°C. As there is no simple explanantion for the changes seen during storage of semen from control animals, it is probably premature to suggest reasons for the greater susceptibility of the sperm from the insulated rams. However, it may be relevant that Shannon and Curson (1972) noticed that dead sperm in a sample reduced the survival of the live sperm, an effect they attributed to peroxide generated by amino acid oxidase. Nevertheles, this observation emphasises the need to optimize storage conditons for sperm collected from animals which may be of poor fertility.

In this study, regardless of the type of diluent, the effect of insulation on the response to storage for 6h at 30 or 5°C in motility and morphological characteristics of diluted spermatooa started on day 30 of the insulation. This again is consistent with the previous observations on undiluted semen from the insulated rams. It is also quite similar to those reported by Vogler et al (1991), who, working with bulls, observed a depression in scrotally insulated animals of viability of spermatozoa in semen that had been frozen-thawed and incubated for 3h at 37°C. Mieusset et al (1991) also found lowered motility in frozen-thawed spermatozoa from insulated rams.

Observations on pooled data to evaluate the effect of type of diluent indicated that changes during storage in motility characteristics (motile, progressive motile and rapid spermatozoa) in semen from control and insulated rams were similar in HYCG or CEQY diluent at 30°C or at 5°C. Thus in this study, CEQY was comparable to HYCG and did not show its beneficial effect when used to preserve semen from fertile or sub-fertile rams during insulation. The storage period chosen (6h) may not have been sufficient for differences due to diluent to have become apparent. In the earlier

experiment, the difference between CEQY and HYCG diluents on the motility of sperm stored at 5°C did not become apparent before 144h, and while there were differences after after 24h of storage at 30°C, shorter times were not examined.

It could be concluded from the results of this experiment that increased testicular temperature by scrotal insulation for 16 h/day for 21 consecutive days causes dramatic changes in the viability characteristics and morphological profile of ram spermatozoa. The adverse effect of scrotal insulation was more pronounced in one insulated ram than in the other. Beneficial effects of CEQY diluent in maintaining the viability of spermatozoa were not apparent in this study while sperm from insulated rams tolerated storage less well than sperm from control rams. Moreover, the harmful effects of testicular heating on the viability of ram spermatozoa are temporary and recovery occurs within 40 to 65 days after the end of heat treatment.

10.3. Viability of semen from rams subjected to scrotal insulation, after storage at chilled temperatures for one week

10.3.1. Introduction

It has been indicated from the results of the above experiment (Section 10.1) that raised testicular temperature by insulating ram scrota for 16h/d for 21 consecutive days depressed the viability characteristics and increased the number of morphological abnormalities of ram spermatozoa. The magnitude of response and the type of abnormal spermatozoa produced between the two rams in the insulated group varied.

It is seen that the depression in these characteristics was especially more severe in one insulated animal (Ram No 12) in that semen quality of this ram did not return to the normal level until an interval of 65 days had elapsed after the end of insulation. In addition, it was observed that while sperm from the insulated rams responded less well than controls in a short period of storage (6h) at 30 or 5°C, no clearcut beneficial effect of CEQY diluent was apparent in maintaining the viability of the spermatozoa. It seemed that by reducing the intensity of the heat stress, and by prolonging the period of time of

storage at chilled temperatures, the novel diluent may be more effective in maintaining the viability of spermatozoa from nonfertile or subfertile rams.

The second study was therefore undertaken to investigate the effect of CEQY and HYCG diluents on the survival for 7 days at 5°C, of sperm from 2 normal rams and from 2 rams whose testes were subjected to elevated temperatures (about 2°C) by scrotal insulation for 12h/d for 28 consecutive days.

10.3.2. Experimental procedure

The same animals as in the first experiment were used about 1 year later and methods of scrotal insulation, semen collection, semen processing and semen diluent were as described in Section 10.2.1., except that the time during which the scrota were insulated, was 12h/d (09:00 to 21:00) for a duration of 28 days.

Semen samples were collected once a week for all rams by artificial vagina for 2 weeks before, 4 weeks during and 5 weeks after the insulation period. Single ejaculates from each ram was split into four aliquots, each of which was diluted five fold (one part semen : four parts diluent) at 30°C using diluents as shown in Table 4.1 (Chapter 4). Diluted samples were then removed into a cool room, allowed to cool down from 30 to 5°C over 2 hours and then maintained at 5°C in a water bath until assessed. When making assessment, these samples were first rewarmed at 30°C in a waterbath. Semen assessment was done following incubation for 0, 96 and 168 hours.

The volume of semen per ejaculate from each group of rams was measured immediately after collection. Total sperm count per ejaculate in fresh semen from each group of rams was assessed subjectively as described in Chapter 3, Section 3.9.13. The viability of the spermatozoa in the diluted semen was assessed objectively using a Hamilton Thorn Motility Analyser (HTM Analyser, Daintree Ind., Australia) as described in Chapter 3, Section 3.6.2.2.

For sperm morphology examination, the diluted semen from each ejaculate, after storage at 5°C for 0, 96 and 168 hours, was stained by mixing 2-3 drops of diluted

semen with 75 µl eosin-nigrosin stain (Evans and Maxwell, 1978) at 37°C. A drop of mixture was then placed in a clean sterile glass slide and a smear was made as described in Chapter 3, Section 3.6.1.4. Sperm morphology was quantified by observation of 200 sperm/stained smear using light microscopy at 1250 x magnification. Individual spermatozoa were assigned to only one morphological category even if they exhibited several abnormalities. The categories included normal sperm morphology and sperm abnormalities such as tailess heads (head loose), coiled tails and bent tails. The same slides were also used in assessing the percentage of live and dead sperm by examining 200 sperm.

10.3.3. Statistical analysis

Actual percentages were tabulated, but statistical inferences of the percentage data were made using the angular transformed analyses. Data were subjected to analysis of variance and differences between groups were determined by Tukey's test.

10.3.4. Results

Sperm motility characteristics assessed by computerised image analysis and morphology of spermatozoa assessed manually in diluted semen from individual rams within treatment groups at different insulation periods are given in Tables 10.2.4.a to k in Appendix C of the thesis. Table 10.1.1. in Appendix B shows the volume of semen per ejaculate and the total sperm count and percent motile sperm per ejaculate assessed manually for each ram throughout the experiment.

To facilitate interpretation, data were simplified as in the previous study.

Firstly, in order to evaluate response of individual rams due to scrotal insulation based on their motility and morphology characteristics, the actual data of diluted semen from individual rams prior to storage at 5°C in CEQY or HYCG diluent were graphed.

Secondly, to examine the effect of incubation or storage at 5°C on motility characteristics, data of each collection were expressed as the values for samples after storage for 168h as a percentage of pre-storage values.

Finally, to observe the effect of diluent at 5°C on sperm motility and morphological characteristics, data throughout observations were divided into three periods. Period 1 (before insulation) representated of pooled data from two days collection (day -11 and day -3). Period 2 (during insulation) representated of pooled data from four days collection (day 4, 10, 13 and 25). Period 3 (after insulation) representated of pooled data from five days collection (day 32, 50, 77, 84 and 105). The data to be analyzed were also expressed as the values for samples after storage for 168h as a percentage of pre-storage values.

In the present study, scrotal insulation had no significant effect on the volume of semen per ejaculate and total sperm count during the treatment. The insulated rams had higher semen volume compared with the controls during heat stress; conversely, total sperm count in controls was higher than that of the heated groups. However, the differences in these two semen characteristics were not statistically significant.

Between day -11 before and day 25 after the start of scrotal insulation, semen volume of the 4 individual rams did not differ markedly, and ranged from 0.5 to 1.0 ml. Variation in semen volume from the 4 individual rams was observed between day 32 and 105 after start of insulation, but there appeared to be no consistent effect of insulation (Figure 10.24).

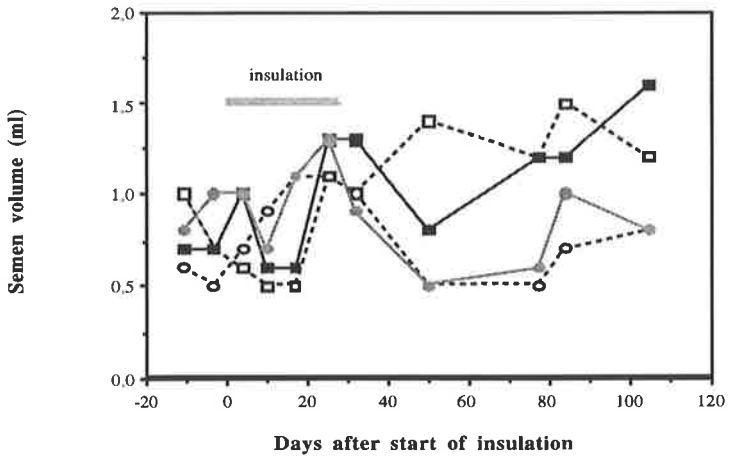


Figure 10.24. Semen volume in fresh ejaculate assessed manually from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

All rams within control and insulated groups showed similar total sperm counts up to day 10 of scrotal insulation. On day 17, decreases in total sperm count in the insulated rams was observed, being 0.17 $x10^9$ in ram No.16 and 0.10 $x10^9$ in ram No.12. Thereafter, variation in total sperm count in the 4 rams occurred although again there was no consistent effect of insulation (Figure 10.25).

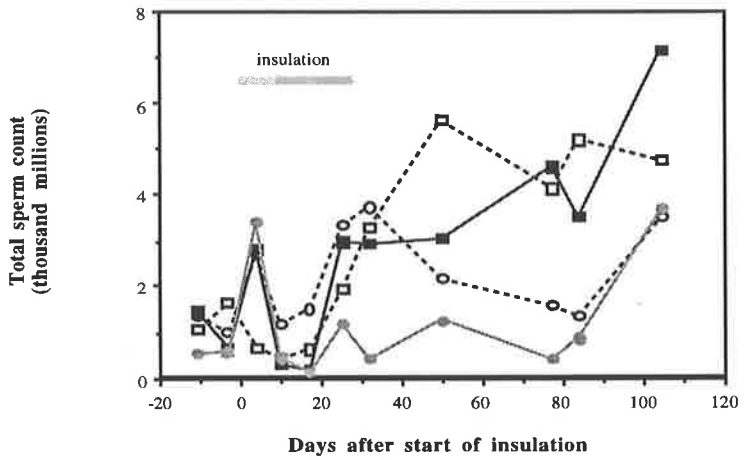


Figure 10.25.Total sperm count in fresh ejaculate assessed manually from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12h/day for 28 consecutive days.

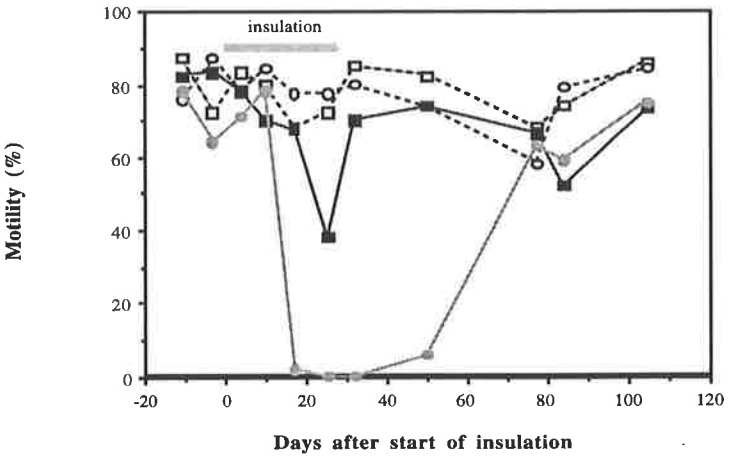


Figure 10.26. Motility of fresh semen manually assessed from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

Statistical analysis of data from fresh diluted semen did not show any significant interaction between day of collection and heat treatment for percent motility, progressive motility, medium, rapid, slow, and all morphological characteristics of spermatozoa. However, interaction between day of collection and heat treatment was significant for mean ALH (p=0.002), mean VAP (p=0.005), mean VCL (p=0.001) and mean STR (p=0.005) of spermatozoa.

On day -11 and -3 before heat treatment, motility of spermatozoa as assessed subjectively from individual rams in the insulated and control groups was similar. Up to day 10 after the start of scrotal insulation, motility of spermatozoa in the insulated rams remained high (above 71%). However, at day 17, motility of spermatozoa in insulated ram No.16 decreased slightly, while in ram No.12 it decreased rapidly to 2%. At day 25, the decreases in motility continued, reaching 38% in ram No.16, but in ram No.12 it decreased to zero percent and remained at this level until day 32. At day 32, motility in ram No.16 increased again to 70%. In ram No.12, an increase in motility of spermatozoa occured at day 50, and by day 80, it had reached almost the same value of that of control rams (Figure 10.26).

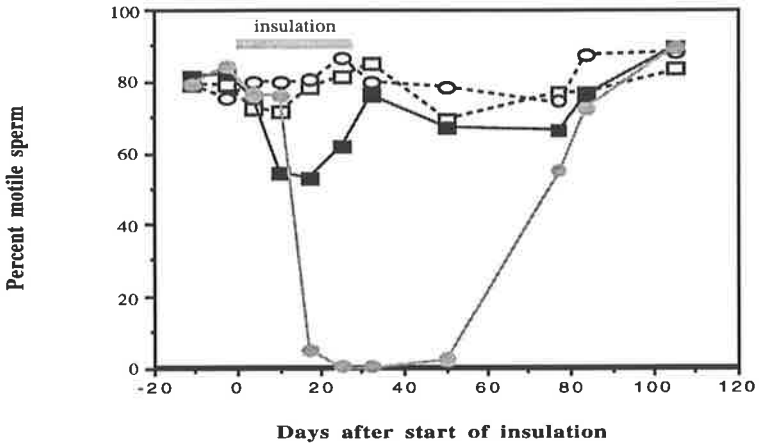


Figure 10.27. Percent motile sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

Motility of spermatozoa assessed objectively in the 4 rams were almost the same between day -11 and day -3 before and at day 4 after scrotal insulation. At day 10 after start of insulation, motility in Ram No.16 dropped slightly, while in Ram No.12 it remained high. At day 17, however, sperm motility in Ram No.12 decreased abruptly and by day 25 motility became less than 1%. In Ram No.16, motility only decreased to 52% at day 17 and increased again on day 25 and 32. A slight decrease in the percent motile sperm was observed in Ram No.16 in the next two collections, i.e. day 50 and 77 and then motility increased again to the same levels as that of controls by day 83. In Ram No.12 percent motile sperm remained low between day 32 and day 50, however, it had increased by day 77 and the increase continued to day 84. Thereafter it remained at almost the same value as Ram No.16 and the controls. Percent motile sperm in the control rams No.3 and No. 31 did not alter markedly throughout experimental periods (Figure 10.27).

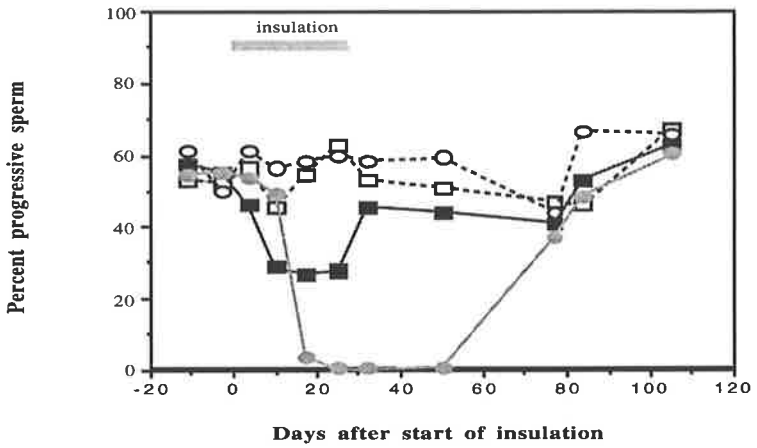


Figure 10.28. Percent progressive motile sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

Progressive motile and rapid sperm in the insulated rams No.16 or No.12 did not differ to that of the controls rams No.3 or No.31 at days -11 or -3 before insulation or on day 4 after insulation. A slight decrease in percent progresive and rapid sperm in Ram No.16 from day 4 to day 10 was observed with no change in Ram No.12. Then a sharp decrease in progressive and rapid sperm in Ram No.12 occured from day 10 to day 17, and by day 25 the value of these two characteristics became zero. Percent progressive and rapid sperm in the insulated ram No.12 remained low from day 32 to day 50, while in insulated ram No.16 the values were lower than controls in days 10, 19 and 23 and increased again at day 25 (Figures 10.28 and 10.29).

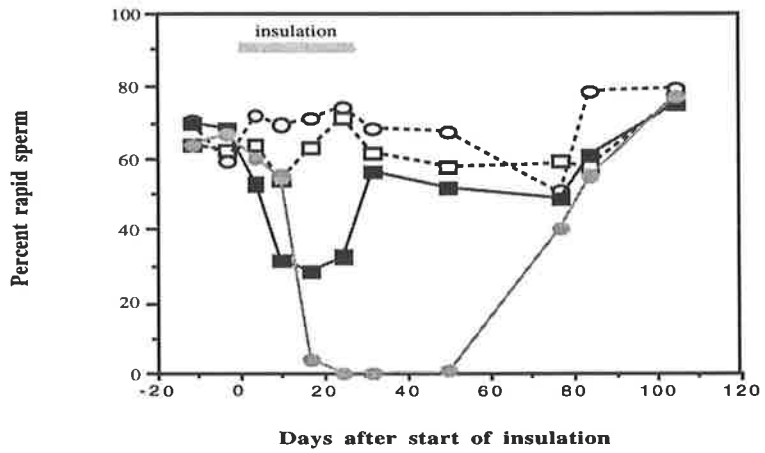


Figure 10.29. Percent rapid sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

The numbers of medium sperm in the insulated rams was higher than that of control on day 4 and day 10. On day 17 medium sperm in ram No.16 increased and peaked on day 25, conversely in ram No.12 it fell markedly to a low level on day 32 which was maintained until day 50. Medium sperm in insulated rams reached similar values to that of controls on day 77 (Figure 10.30).

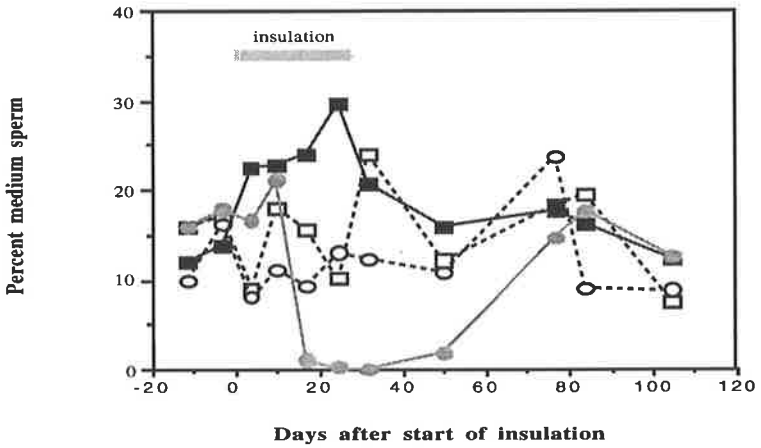


Figure 10.30. Percent medium sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

A small rise in slow sperm in ram No.16 occurred on days 10, 17 and 25 after start of insulation. In ram No.12, however, slow sperm decreased from day 10 to day 17 and reached zero on days 25 and 32 (Figure 10.31).

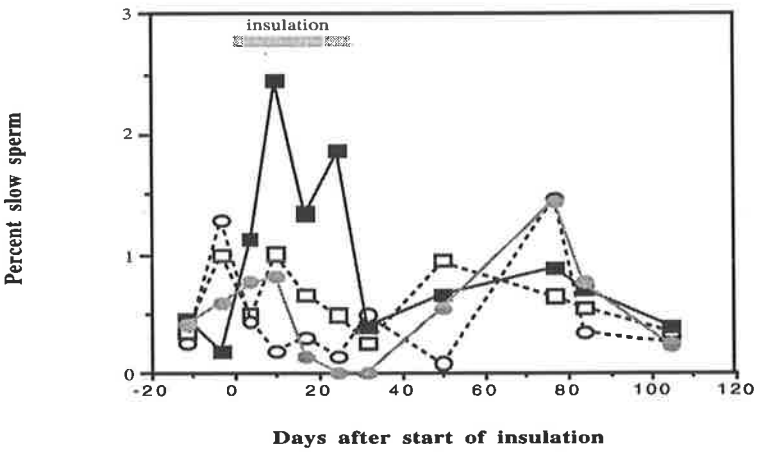
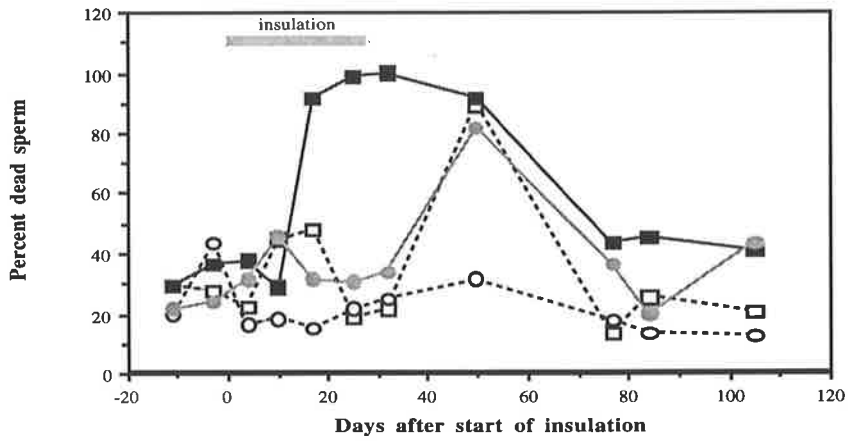


Figure 10.31. Percent slow sperm measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

During scrotal insulation on day 4, percent dead sperm in both insulated rams were higher than control; in ram 12 there was then a sharp rise on day 17, so that from then until day 50, all the sperm from this ram appeared dead.The percentage dead then fell again but remained higher than control to the end of the period of observation. The percentage dead sperm in semen from ram 16 was slightly higher than control on days 25, 32, 77 and 104, but was similar to control on day 83. On day 50, ram 16 and one of the controls showed a sudden increase in percentage dead sperm, but this was probably an artefact of staining (Figure 10.32a).

a)



b)

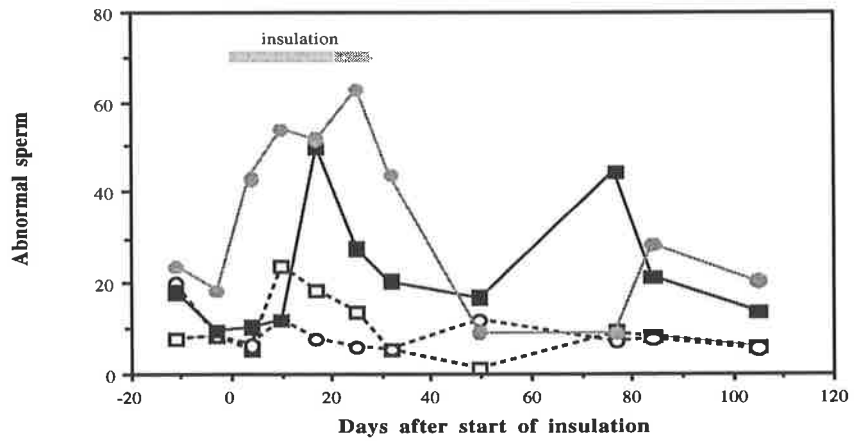
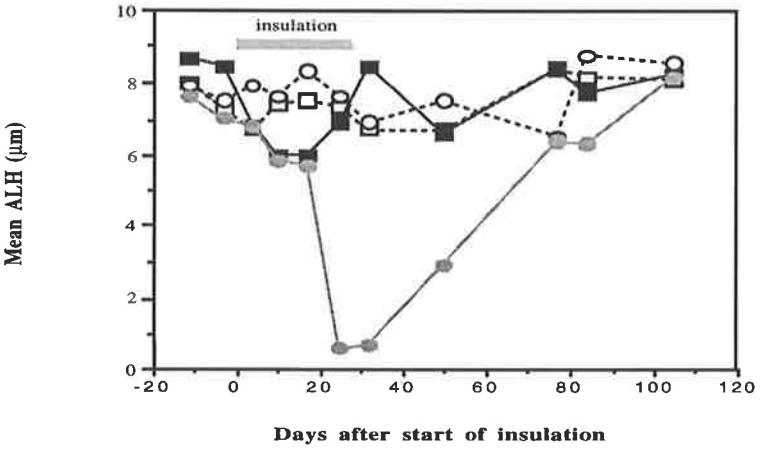


Figure 10.32. (a) Percent dead and (b) percent abnormal spermatozoa in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

The numbers of abnormal sperm in the control rams remained at less than 25% throughout the experiment. During insulation, abnormal sperm in ram No.12 showed an

increase between days 4 and 50, and again in the last two samples, while in ram No.16, there was an increase in abnormal sperm which began on day 17 and continued to the end of the period of observation (Figure 10.32.b).

a)



b)

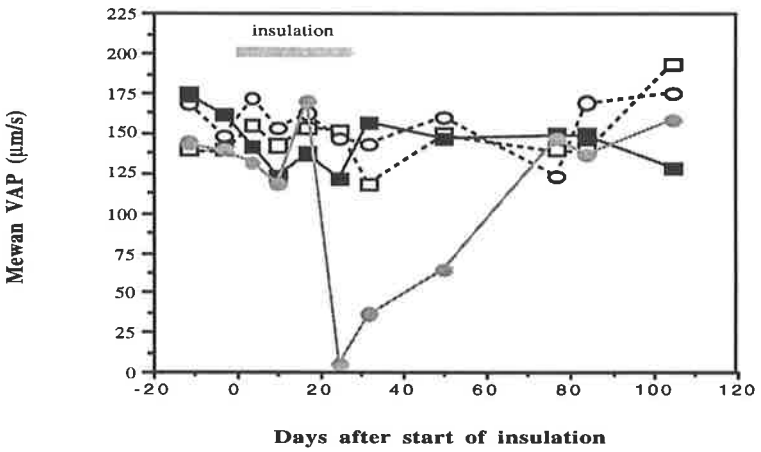
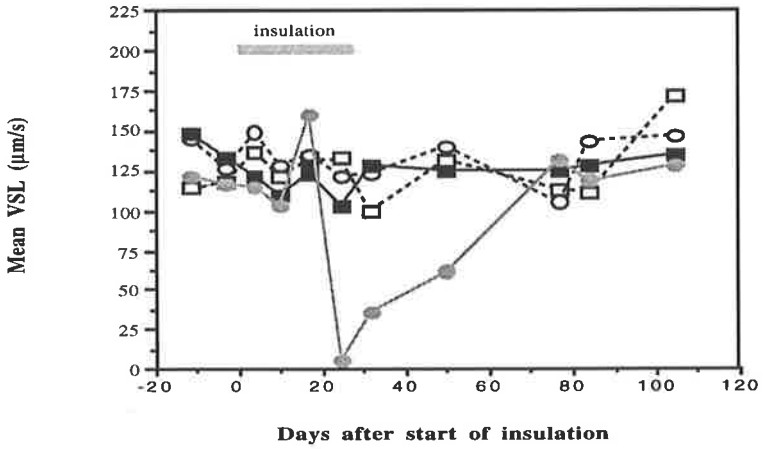


Figure 10.33. Mean ALH (a) and mean VAP (b) of spermatozoa measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

Mean ALH in the 4 rams on days -11 and day -3 was above 6 µm. During scrotal insulation from day 4 through day 25, mean ALH in the insulated rams was slightly lower than that of control rams. However, the decrease in mean ALH in insulated ram No.12 became more obvious at day 25 and values for this ram remained lower than control until day 50; for ram 16 ALH had returned to control levels from day 25 on. (Figure 10.33.a).

Mean VAP, mean VSL and mean VCL in individual rams of the two groups did not change markedly either before insulation or up to day 17 after start of insulation. However, a decrease in these three kinematics parameters occured in insulated ram No.12 on day 25 followed by a return towards control on day 32 and 50, with no differences from control apparent from day 77 onwards; the values for ram 16 did not differ from control at any time. (Figure 10.33.b, c and d).

c)



d)

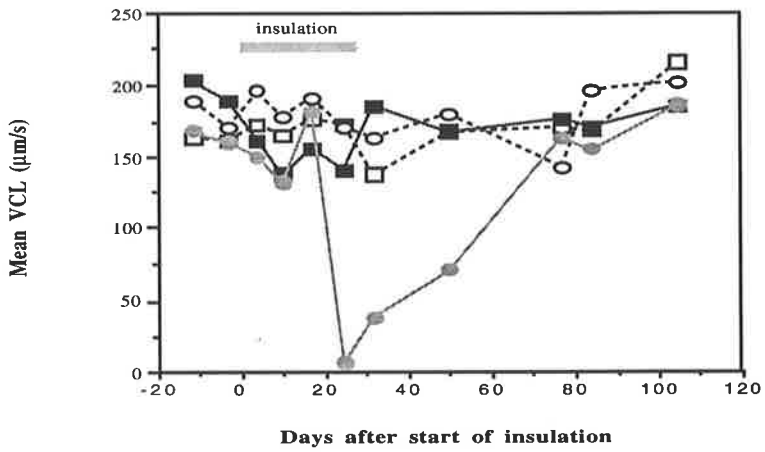
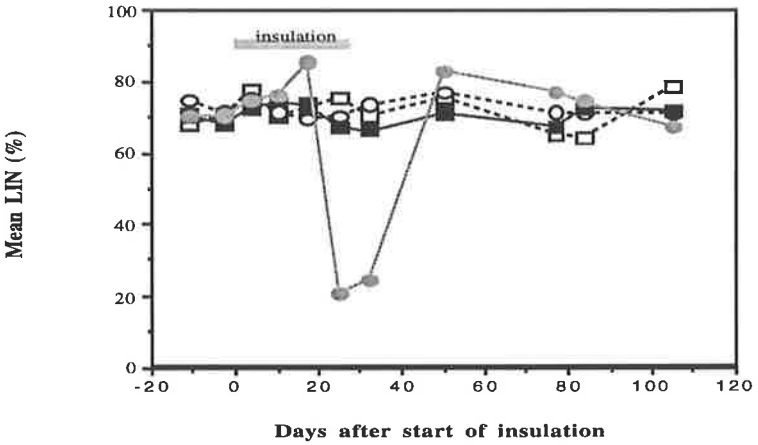


Figure 10.33. Mean VSL (c) and mean VCL (d) of spermatozoa measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

Mean LIN and STR showed little change, except in insulated ram No.12, in which these characters were lower than control on days 25 and 32 after the start of insulation, but then returned to normal (Figure 10.34.a and b).

a)



b)

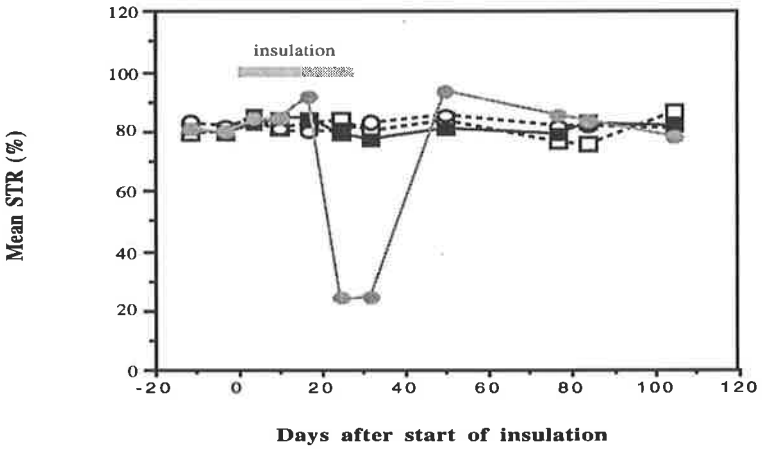


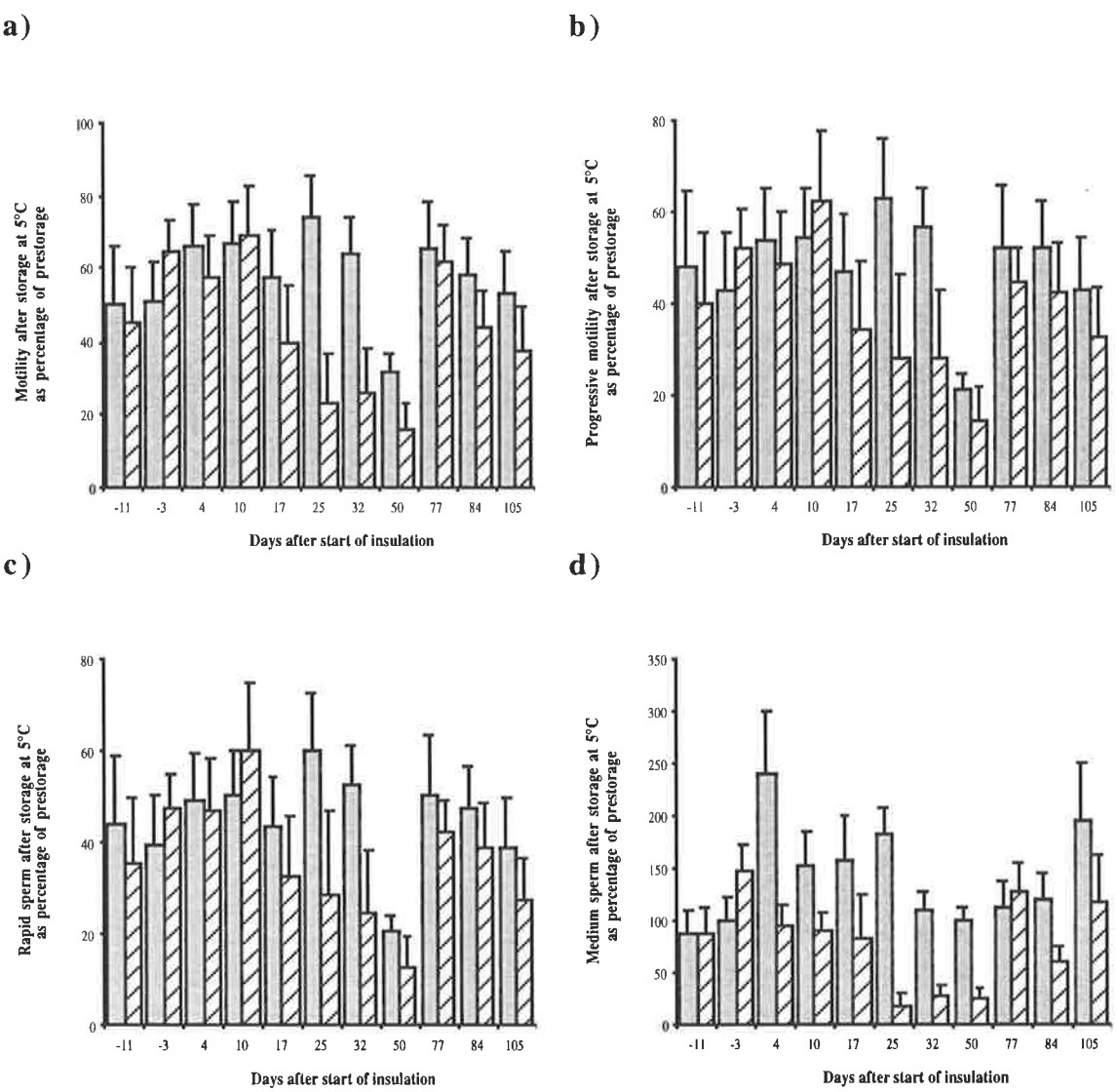
Figure 10.34. Mean LIN (a) and mean STR (b) of spermatozoa measured with the Hamilton Thorn system in diluted semen from control ram No. 3 (□), control ram No. 31 (○) and scrotally insulated ram No. 16 (■), scrotally insulated ram No. 12 (●) for 12 h/day for 28 consecutive days.

Table 10.2.2. (Appendix C) demonstrates the effect of storage at 5°C on changes in motility and morphological characteristics of diluted semen from controls and 12h/d scrotally insulated rams.

Statistical analysis of the data from each day of collection did not indicate a significant interaction between diluent x time of storage x heating on sperm motility and morphological characteristics throughout the experimental periods (Tables 10.a. to 10.k.).

No significant differences were observed in the changes during storage in motility, progressive motility and rapid spermatozoa between the two groups of rams at

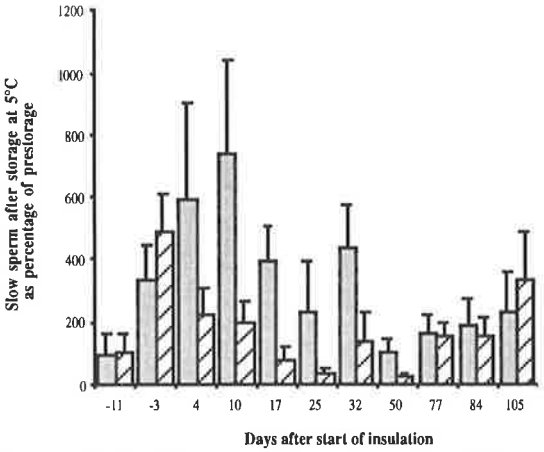
day -11 and -3. Semen from insulated rams showed greater changes during storage than controls in these three motility characters from day 17 to day 77. However, the overall differences were not significant (Figures 10.35.a,b and c).



Figures 10.35. Changes during storage for 168h at 5°C in motility (a), progressive motility (b), rapid (c) and medium (d) spermatozoa from control (■) and 12h/d scrotally insulated (▨) rams

Percentages of medium sperm tended to remain constant or increase during storage of control semen, whereas between days 25 and 50, this value fell in semen from insulated rams.(Figure 10.35.d).

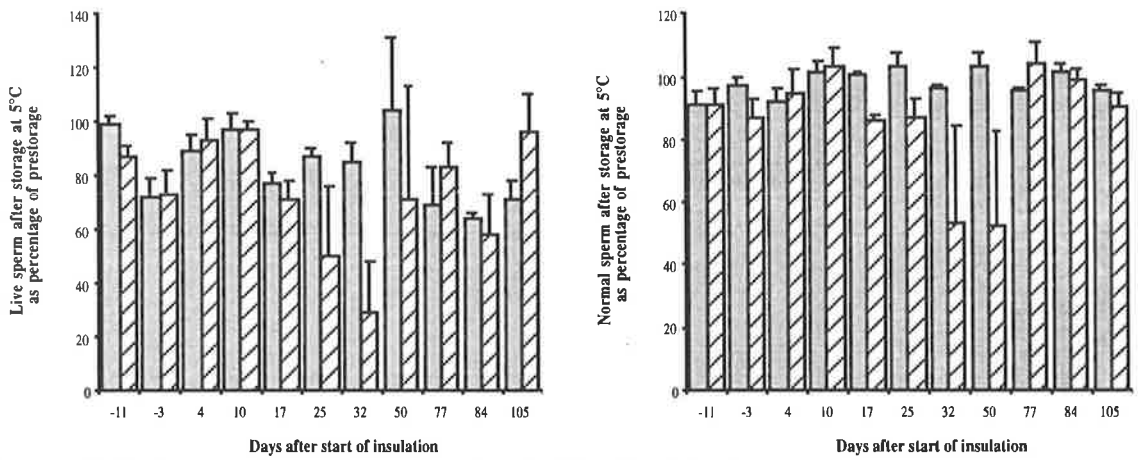
The percentage of slow sperm also tended to increase during storge in semen from the control rams, and to fall in semen form the insulated rams between days 17 and 50 after the start of insulation (Figure 10.36).



Figures 10.36. Changes during storage for 168h at 5°C in slow spermatozoa from control (■) and 12h/d scrotally insulated (☐) rams

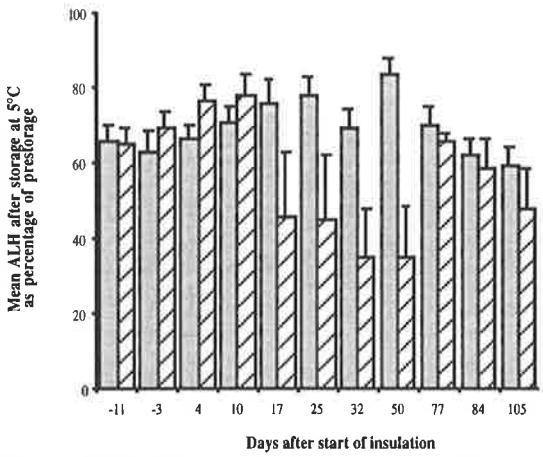
No marked difference in changes during storage in the percentage live or morphologically normal spermatozoa was observed between the two groups of rams up to day 10. On day 17 from the start of scrotal insulation, percent normal sperm fell more during storage in semen from the insulated rams than controls, and percent live and percent normal both fell more during storage in semen from insulated rams from day 25 to day 50. Thereafter, semen from control and insulated rams responded similarly to storage (Figures 10.37 a and b).

a) **b)**

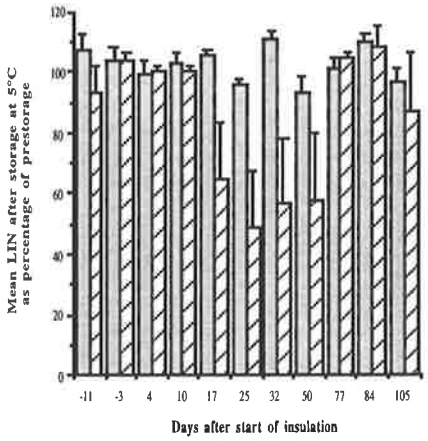


Figures 10.37. Changes during storage for 168h at 5°C in live (a) and normal (b) spermatozoa from control (■) and 12h/d scrotally insulated (☐) rams.

a)



b)

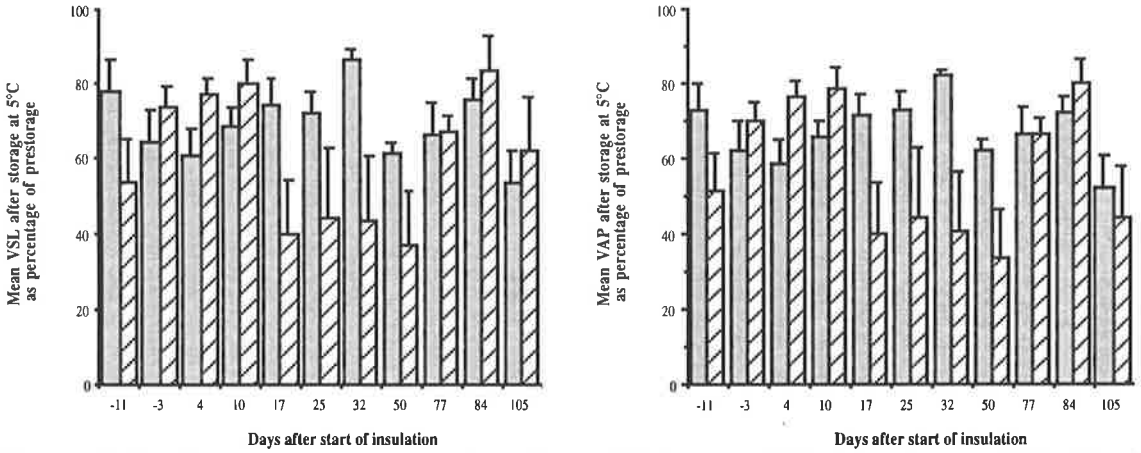


Figures 10.38. Changes during storage for 168h at 5°C in mean ALH (a) and mean LIN (b) of spermatozoa from control (■) and 12h/d scrotally insulated (▨) rams

Mean ALH of diluted semen fell only slightly during storage and there was no difference between the two groups of rams up to day 10 after the start of insulation. From day 17 to 50, mean ALH fell more during storage of semen frominsulated rams than controls, but there were no differences from day 77 onwards (Figure 10.38.a).

Mean LIN of diluted semen changed very little during storage of semen from control rams, or from the insulated rams up to day 10 after the start of insulation.From day 17 to day 50, mean LIN fell more during storage of semen from insulated rams. However, the difference in the change during storage in this parameter between the insulated and control groups was not significant. (Figures 10.38.b).

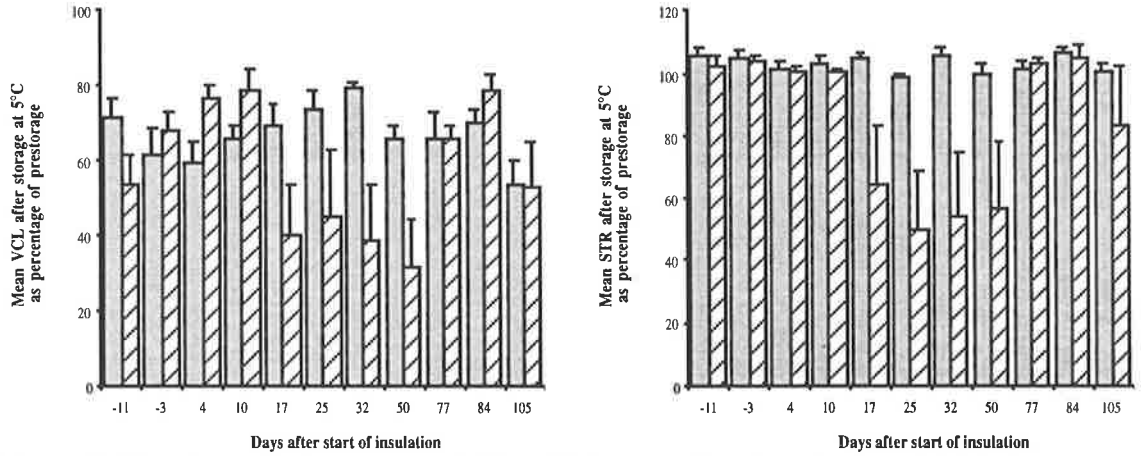
a) b)



Figures 10.39. Changes during storage for 168h at 5°C in mean VSL (a) and mean VAP (b) of spermatozoa from control (■) and 12h/d scrotally insulated (▨) rams

Mean VSL, mean VAP and mean VCL fell slightly during storage of semen from control rams or from insulated rams up to day 10 after the start of insulation. There was a greater fall during storage between days 17 and 50 in semen from the insulated rams, but thereafter, the response of semen from the control and insulated rams was similar (Figures 10.39.a,b, 10.40.a).

a) b)



Figures 10.40. Changes during storage for 168h at 5°C in mean VCL (a) and mean STR (b) of spermatozoa from control (■) and 12h/d scrotally insulated (□) rams

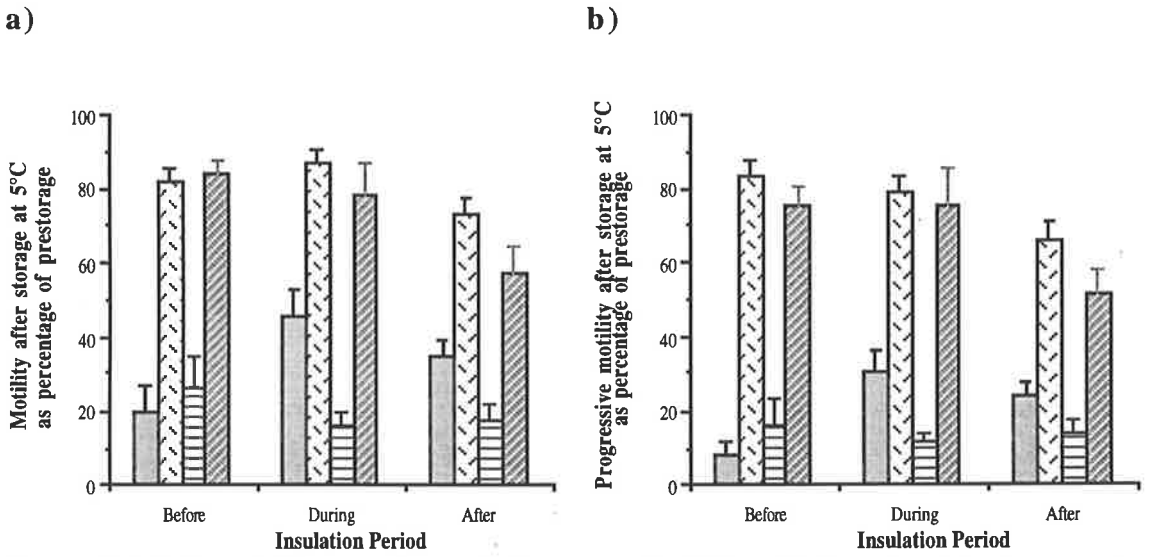
Mean STR of diluted semen from control rams did not fall significantly during storage, but in semen from insulated rams, this value fell significanlty during storage

between days 17 and 50 after the startof insulation. The difference between the insulated rams and controls was significant ($p<0.05$) only at day 25 and 32 (Figure 10.40.b).

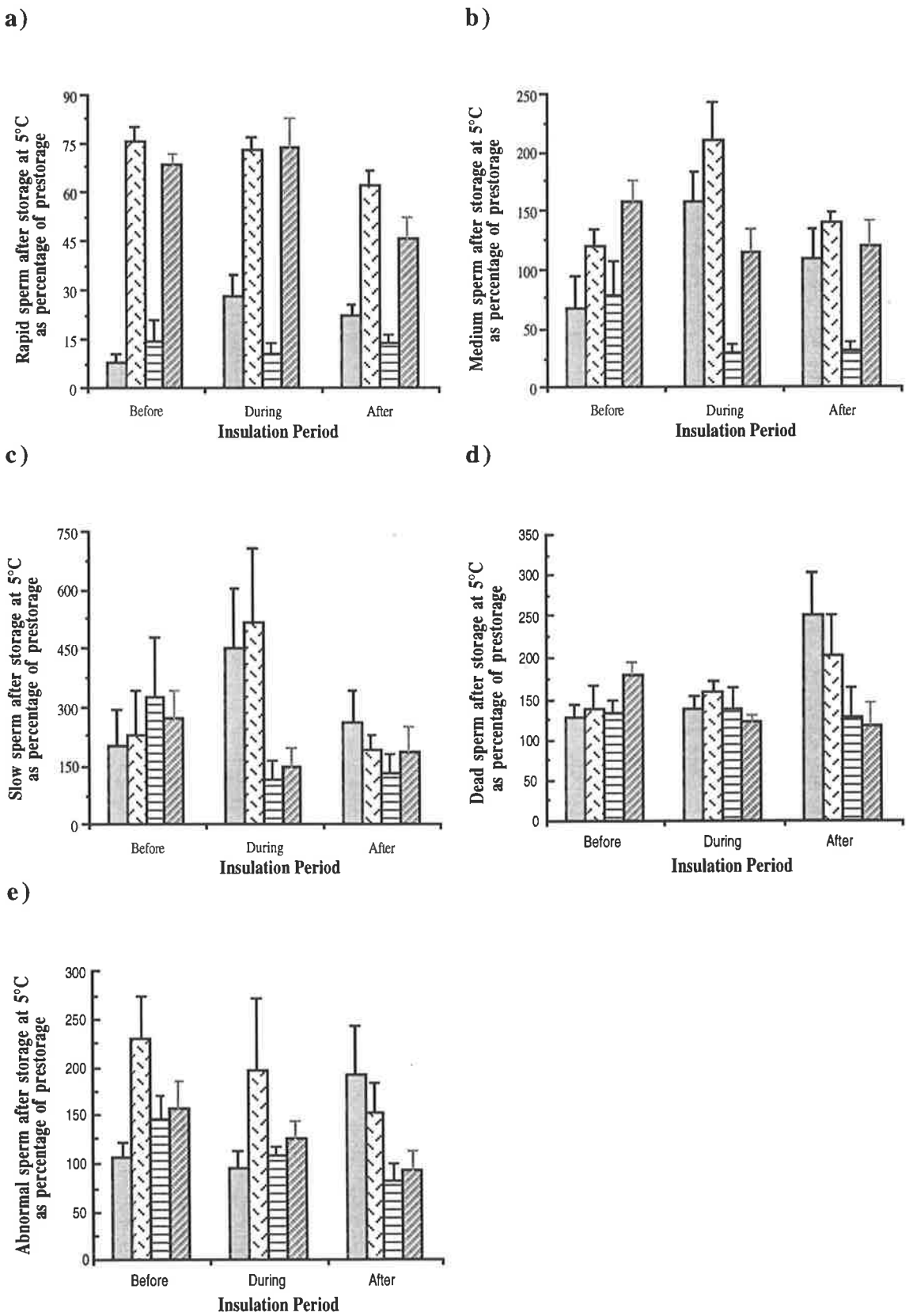
The effect of diluents on changes during storage in motility and morphological characteristics of spermatozoa in semen from control and 12h/d scrotally insulated rams at two different temperatures is summarized in Table 10.2.3. (Appendix C). Overall, no interaction of diluent x heating was observed on any changes in sperm motility and morphological characteristics.

At all time periods and for both treatment groups, falls during storage in motility of spermatozoa were significantly ($p<0.05$) less when CEQY was used as the diluent than with HYCG (Figure 10.41.a). CEQY also reduced the change during storage in % progressive, % rapid and % medium sperm (Figure 10.41.b, 10.42.a,b), mean VAP, VSL and VCL (Figure 10.43 c,d,e) when compared with HYCG for semen from both control and insulated rams. There was no consistent effect of diluent on the change during storage in % slow sperm (Figure 10.42.c), nor in % dead sperm (Figure 10.42.d) or abnormal sperm (Figure 10.42.e). There was also a tendency with some characteristics of motility (% motile, % progressive, % rapid and % medium, Figures 10.41.a,b, 10.42. a,b) for the semen from the insulated rams during the period of insulation to a show a greater effect of the medium (CEQY vs HYCG) on the change during storage, compared with semen from the control rams examined at the same time. However, this difference was largely due to differences in the response of semen from the control ram collected either in the pre-insulation or insulation periods, when these rams did not receive any different treatment, rather than changes in the semen from the insulated rams. The true significance of this difference must therefore remain uncertain. All other characteristics of motility showed similar effects of the two media during storage, except that mean ALH, LIN and STR all fell during storage in semen collected during and after insulation only if it was stored in HYCG storage; if the semen was stored in CEQY, there were no changes in these parameters during storage (Figure 10.43.a,b,f). These observations, although not conclusive, suggest that the advantage of CEQY compared with HYCG for storage of

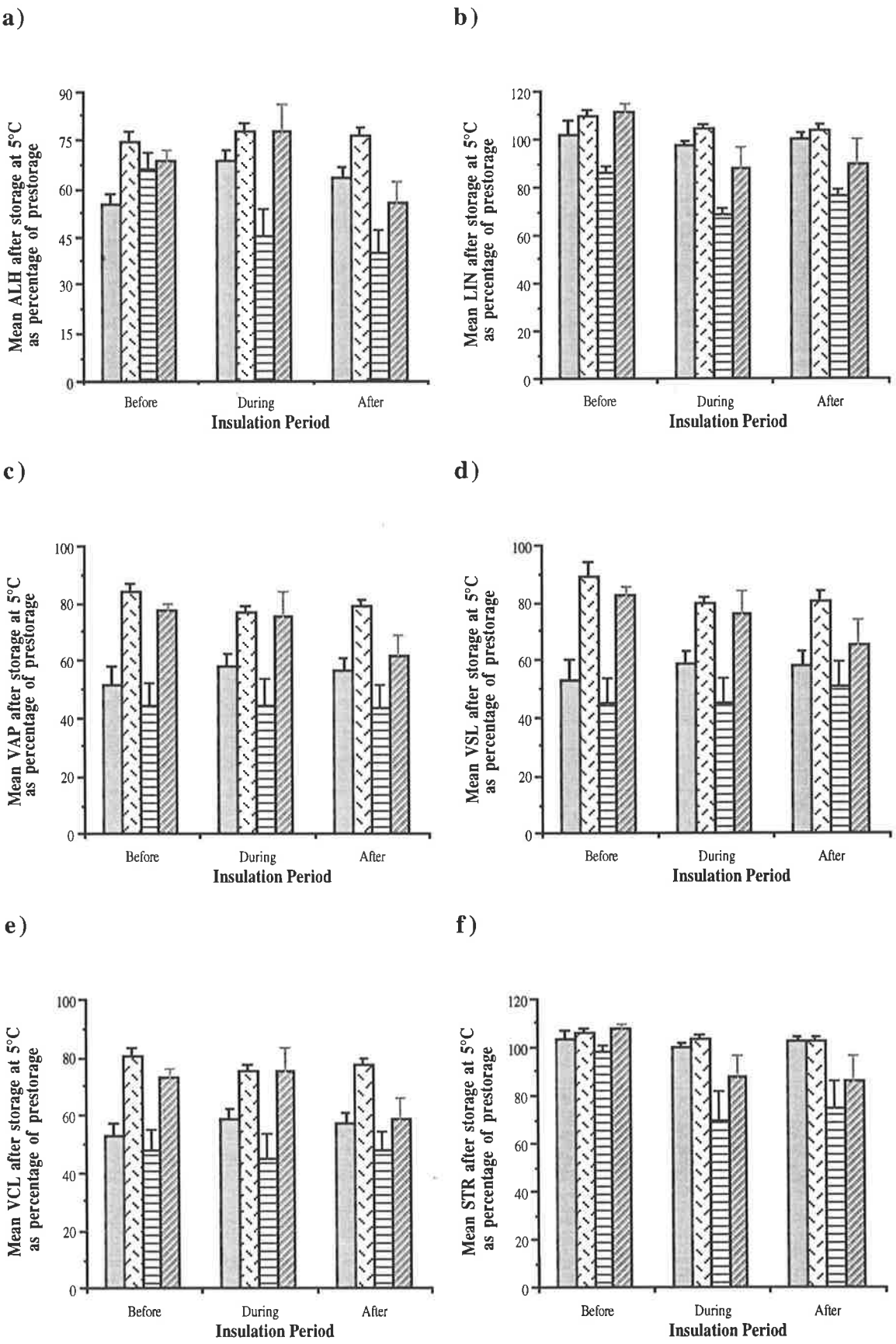
semen may be particularly valuable for semen which was already subnormal in ALH, LIN or STR.



Figures 10.41. Effect of diluent on changes during storage for 168h at 5°C in motility (a) and progressive motility (b) spermatozoa in control semen diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (□) and in semen from 12h/d scrotally insulated rams diluted in hen yolk citrate glucose (⊟) or in coconut extract quail yolk (▨) diluents.



Figures 10.42. Effect of diluent on changes during storage for 168h at 5°Cin rapid (a), medium (b), slow (c), dead (d) and abnormal (e) spermatozoa at 5°C in control semen diluted in hen yolk citrate glucose (▣) or in coconut extract quail yolk (□) and in semen from 12h/d scrotally insulated rams diluted in hen yolk citrate glucose (⊟) or in coconut extract quail yolk (▨) diluents.



Figures 10.43. Effect of diluent on changes during storage for 168h at 5°Cin mean ALH (a), LIN (b), VAP (c), VSL(d), VCL (e) and STR (f) of spermatozoa at 5°C in control semen diluted in hen yolk citrate glucose (□) or in coconut extract quail yolk (▨) and in semen from 12h/d scrotally insulated rams diluted in hen yolk citrate glucose (▤) or in coconut extract quail yolk (▩) diluents.

10.3.5. Discussion

In the present experiments, the observation that semen volume per ejaculate and total sperm count in the heated rams remained unchanged during scrotal insulation was quite similar to the findings reported by Mieusset et al (1991), who could not see any significant effect of heat stress on the total sperm count in rams scrotally insulated for 8 h/day for 162 consecutive days. However, when scrotal insulation was 16 h/d for 144 consecutive days, total sperm count was depressed slightly from day 100 of testicular heating (Mieusset et al, 1992). In the earlier experiment in the chapter, insulation for 16h/day for 21 days also had no consistent effect on sperm numbers.

In the present study, after 12h/d of scrotal insulation, motility characteristics of undiluted semen began to drop at day 17 after start of insulation. The rate of decrease in motility between the two insulated rams was again faster in ram No.12 than that of ram No.16. In ram 12, it reached a base level on day 17 which then continued until day 32, while in ram No.16, it decreased only at day 25, afterwards returned to control values. Thus as observed in the first experiment, variation between individual rams occurred in this second experiment suggesting that ram No.12 was consistently more sensitive to the effects of heat than ram No.16 and showed effects sooner. Similar results were also reported by Glover (1955), Moule and Waites (1963) and Williamson (1974) in rams and it was suggested that the variation in time of response between rams after scrotal heating was probably due to differences in epididymal passage time between individual rams (Amir and Ortavant, 1968). Differences between rams in response to a standard amount of scrotal heating have been recorded by other workers (Gunn et al, 1942, Moule and Waites, 1963, Dun 1956), and it was reported that two strains of Merino rams selected for different degrees of skin wrinkle (Folds plus and minus strains) also showed marked differences in their susceptibility to heat (Fowler and Dun, 1966).

It appeared that reducing the intensity of the heat treatment from 16h/d to 12h/d but increasing the duration of insulation from 21 to 28 days resulted in different

susceptibility of insulated rams No.16 and No.12 to seminal degeneration as suggested by time of recovery.

Considering the effect of storage on changes in motility (motile, progressive motile, rapid, medium, slow and static spermatozoa), morphological (live and normal spermatozoa) and kinematics (mean ALH, mean LIN, mean VAP, mean VSL, mean VCL and mean STR) characteristics of diluted spermatozoa during weekly collection, all values fell more during storage of semen collected from day 17 up to day 50 after the start of insulation. This greater effect of storage on motility characteristics of semen from the insulated rams was reflected by the changes in motility of undiluted semen from insulated rams. The results were consistent with the results found in the first experiment in this chapter. In general, most changes in motility, morphological and kinetic characteistics of diluted spermatozoa began to recover after day 50, i.e. 22 days after the end of scrotal insulation.

In this experiment changes during storage in dead and morphologically abnormal diluted spermatozoa observed during testicular insulation were not markedly different from the control. However, the greater falls during storage in motile, progressive motile and rapid spermatozoa in the insulated rams compared with the controls occurred at the same time as depression of changes in live and morphological abnormalities of diluted spermatozoa. The changes in the numbers of live and abnormal spermatozoa are similar to the findings of Austin et al (1961). The fact that abnormal sperm are not usually noted in the ejaculates until about 14 days after heating (Dutt and Hamm, 1957; Moule and Waites, 1963; Fowler, 1968; Mieusset et al, 1992) indicates that spermatozoa present in the epididymis at the time of heating are relatively unaffected, in contrast to the developing germ cells in the testis.

Considering the effect of diluents, the present study shows that some motility characteristics were better maintained in semen from control or insulated rams diluted and stored for 168h with CEQY than with HYCG, and this effect could be observed before, during and after insulation period. The time of storage (168h) was greater than that

(144h) at which the first differences between CEQY and HYCG diluents became apparent in Chapter 4, but less than the time (192h) at which the effects became maximal. For the results after storage for 96h, i.e. before any differences were seen in Chapter 4, there was only a small difference between CEQY and HYCG (90 vs 74% after 96h storage as % of prestorage, respectively) and no consistent difference between insulated and control rams (see Appendix for data). Furthermore there appeared to be only subtle differences in the responses of sperm from the insulated and control rams to the different diluents, and the variability of the responses makes it difficult to evaluate these results.

As in the previous experiment, it is probably too early to speculate on the reason why the sperm for the insulated rams are more susceptible to the effects of storage, although as discussed earlier, the liberation of peroxide by dead or dying sperm seems a possible factor.

In conclusion, a small increase in scrotal temperatures for short periods of time has a rather severe effect on the viability characteristics of ram semen and on the proportion of live and normal spermatozoa. However, this effect was again more severe in one insulated ram No.12 than the other, which is consistent with the results of the previous experiment. Motility characteristics deteriorated more during storage in HYCG than in CEQY, and the effect was more marked for a few characteristics in sperm from the insulated rams.

Chapter 11

General Discussion

Studies were undertaken to examine the potential efficacy of coconut extract as a semen diluent in combination with quail yolk in citrate buffer. During *in vitro* storage, viability characteristics of spermatozoa were better maintained in coconut extract quail yolk diluent than in hen yolk or quail yolk citrate without coconut extract in semen samples stored 2 or 10 days at 30°C or 5°C, respectively. In addition, it appears that of these two storage temperatures, liquid chilled storage provided better conditions for spermatozoa to survive than room temperatures did. Our study supports the findings of Bartlett and VanDemark (1962) and Foote and Bratton, (1960); but did not agree with that of Shannon and Curson (1984), who found that bull spermatozoa retained motility and fertility better when stored at 16°C than at 5°C.

Trimeche et al (1997) recently found that quail yolk was better than hen yolk in preserving the motility of Poitou jackass sperm during freezing and thawing. Comparison between hen and quail yolk in "Salamon's standard diluent" for preservation of ram semen did not show any significant difference in motility of spermatozoa between the yolks during short term storage (8h) at 30 or 5°C, and Basu and Berry (1948) had found that hen and turkey yolk gave comparable results in short term storage of bull spermatozoa. The results of experiment 4.1 also showed that even during long term storage (48h at 30°C or 240h at 5°C) both yolks in citrate-based diluents remained comparable in preserving motility of ram spermatozoa.

In order to determine whether there were a consistent beneficial results from using compounds of plant origin, comparisons were made between coconut extract quail yolk and

coconut water quail yolk and hen yolk citrate diluents, as described in experiment 4.2 (chapter 4). The study shows that the improved viability of spermatozoa stored at room or refrigerated tmperatures was more pronounced in diluent containing coconut extract than those containing coconut water or the conventional hen yolk citrate diluent over prolonged incubation periods, and suggested that it was the coconut extract, not the other additivis in the diluent which was having the beneficial effect. Even when compared with studies on the motility of buck spermatozoa diluted in coconut water and stored for 48h at room temperatures as reported by Pillai and Iyer (1982), our study still showed a higher motility of ram spermatozoa in diluent containing coconut extract at that time. It seems that our observations represent the first report to date of the use of coconut extract and quail yolk in a diluent. Unfortunately, the beneficial effect of coconut extract in quail yolk-citrate diluent as observed in these studies, could not be tested in fertility trials because of time and project fund limitations.

No information was available on the role of coconut extract in the preservation of viability characteristics of ram spermatozoa, as the study did not elucidate its chemical composition, but there seem to be several factors that would be responsible for this maintenance of viability. It has been reported that coconut extract contains protein, carbohydrate and fats and that the percentage of protein in coconut extract was higher than that in coconut water (Banzon and Velasco, 1982).

It is possible that better motility of spermatozoa in coconut extract and quail yolk diluents compared with coconut fluid and citrate glucose diluents was because of the different starting pH or of the components in the coconut extract, other constituents of the diluent, or the level of yolks in citrate glucose and coconut extract based diluents. However, experiments in chapter 5 to examine the first three possibilities did not indicate any marked effects of the different starting pH in coconut extract quail yolk (at pH 7.6 or pH 7.0) and in citrate-glucose (at pH 7.6 or pH 7.0) diluents on the viability of ram semen, indicating that ram spermatozoa can tolerate some degree of alkalinity, as had been reported by Blackshaw

and Emmens (1951). However, the effectiveness of coconut extract and quail yolk diluent in maintaining motility found in this experiment was again consistent with the previous studies in chapter 4.

Further work (chapter 6) to clarify the main findings of the previous investigations, by screening each of the components of the coconut extract diluent, showed that none of the other additives in the coconut extract medium had any beneficial effect, indeed glucose appeared to have deleterious effects. Furthermore, in these experiments, there was no significant difference between diluents without coconut extract and those with coconut extract with regard to the maintenance of sperm viability during storage at 30 or 5°C. This was unexpected in view of the results of the studies in chapters 4 and 5. This inconsistentcy may have been due to the different levels or ratios of egg yolk and coconut extract incorporated in the diluent.

As the beneficial effect of coconut extract in semen diluents was not confirmed in studies described in chapter 6, probably due to a lower concentration of this extract used than in the earlier experiments, and the effect of the other constituents was excluded where the same yolk concentration was used in all media, it seemed possible that the concentration of egg yolk itself was having a deleterious effect. Thus, the study described in chapter 7 attempted to determine the optimum level of egg yolk for incorporation in ram semen diluents with or without addition of coconut extract. The results indicated the deleterious effect of yolk on sperm motility during storage at room and cool temperatures when added at levels higher than 5%. However, this effect of egg yolk is likely to operate at lower concentration during liquid storage than during freezing and thawing, as studied by Salamon and Lightfoot (1969), where the survival of ram spermatozoa reached optimal levels when 15% of egg yolk was added to the diluent.

In addition, it has been indicated in experiment in chapter 6 that when glucose was present in citrate diluent, ram semen deteriorated during preservation at 30 or 5°C. The

present study supports that of Rao et al (1975) in terms of preservation at 30°C, but contrasts with that of Mahajan (1965) who worked with bull semen. However, combination of glucose and enzymes and sulfanilamide in the diluent at 30°C resulted in a maintenance of motility, while at 5°C this was not the case. Thus, further study to determine in more detail the effect of glucose and the comparative effects of other sugars in citrate buffer on the viability characteristics of spermatozoa was conducted, and the results were presented in chapter 8. It was found that none of the sugars tested maintained motlility any better than a diluent without sugar, and furthermore, glucose, fructose or lactose had deleterious effect on motility characteristics during storage at 30°C. The results with glucose confirmed the observations in the experiment of chapter 6. Decreases in sperm viability in these metabolizable sugar containing diluents may indicate that under *in vitro* storage conditions, ram spermatozoa were capable of deriving energy from the substrates present in these sugars, but that the end-products of this metabolism may have been harmful. These findings supported well the observations of Rao et al (1975) and Kumar et al (1993).

In contrast with the finding of the experiment at 30°C, glucose and fructose maintained motility of spermatozoa at 5°C, but again no better than sugar-free medium. This confirmed the findings of other workers (Salisbury and VanDemark, 1945; Adler and Rasbech, 1956; Ohm and Wilett, 1958; and Foote and Bratton, 1960 with bull semen; Wilcox 1960 with cock semen; Sengupta and Chaube, 1972 with buffalo semen). However, experiments in chapter 8 did not confirm the observations at 5°C as explained in chapter 6.The decrease in sperm motility was correlated with the decrease in pH. The changes in pH suggested utilization of lactose and trehalose, and this was an unexpected finding, as metabolism of these sugars by sperm has not been reported.

In relation to the use of other sugars, this study shows similarities with those reported by previous workers (Martin and Richardson, 1976; Terada et al, 1989; Lapwood and Martin, 1966 and Kumar et al, 1993), who observed the beneficial effect of inclusion of inositol, trehalose or sucrose in maintaining motility of spermatozoa. However, addition of

lactose in citrate buffer had a deleterious effect at 30°C, possibly due to breakdown of the disaccharide into its constituents monosaccharides, glucose, and galactose. Our findings support the results of Martin (1966) and Lapwood and Martin (1966), but differed with that of Arns et al (1987).

Following observations in the previous experiment that inclusion of glucose caused reduction in pH and deleterious effects on the motility characteristics of ram spermatozoa, and because of temporarily immobilization of sperm in the cauda epididymidis may be at least partly due to the low pH found in luminal fluid there (see Setchell et al, 1993), attempts were made to restore the motility of immobilized spermatozoa by returning pH to neutral. It was hoped to simulate the changes seen in semen during ejaculation when acid epididymal semen is mixed with alkaline accessory fluid produced in the accessory reproductive organs.

Therefore studies on the effect of alkalinization of semen with sodium bicarbonate on the motility characteristics of spermatozoa during storage at 30 and 5°C in egg yolk citrate diluent with or without glucose were carried out in chapter 9. A marked decrease in motility of spermatozoa in some samples occurred at pH between 5.65 and 5.72 at 30°C, while in others the effect of pH was little. The effect was more variable than that reported by Norman et al (1958) who observed that motility of all sperm was inhibited at pH 5.58 - 5.80.

During preliminary storage at 30°C in diluents containing glucose, concomitant decreases in glucose content and pH occurred, suggesting that glucose was being metabolized by spermatozoa as their energy substrate. However, when the pH of the diluted semen was raised from 5.65 to close to 7.00 with sodium bicarbonate, little improvement was observed in most motility characteristics of spermatozoa at 30°C. However, during the second incubation for 24h, motility of spermatozoa was better preserved in diluents containing glucose with the pH returned to 7, than when glucose was omitted or pH was not raised to 7. This is probably due to better control of the pH in the range between 6.5 and 7.7, while conditions more acidic or more alkaline than this may have had deleterious effects on

motility of the sperm. Our study at 30°C supports the finding of Emmens (1947) and Si and Okuno (1993, 1995).

The lack of maintenance of motility when bicarbonate was added after incubation in glucose-containing diluent stored at 5°C probably reflects on the continuing increase in pH during the second incubation period (24h). The results of this study are consistent with the observations reported by Foote and Bratton (1960) and Foote (1964) and those of Tiwari et al (1977) and Salisbury and Van Demark (1945).

All previous studies were mainly concerned with *in vitro* preservation of semen from normal rams. It is obvious that heating of the scrotum can have a direct effect upon the testis and epididymis, and as one of the main functions of the scrotum is to control scrotal temperature, any disruption of this can interfere with spermatogenesis. As high temperatures are maintained throughout the year in the tropical regions, it was considered important to study the effect of elevated testicular temperature by scrotal insulation on the survival of spermatozoa during storage. The information gained from this study would be useful from a reproductive management point of view, as mating strategies could be arranged at certain times, when semen production and quality reach their peak.

Therefore, *in vitro* preservation using coconut extract-quail yolk and hen yolk-citrate diluents was studied using semen from heat stressed rams in comparison with semen from normal rams in two experiments. In experiment 1, an increase in subcutaneous scrotal temperature of about 2°C for 16h/day for 21 consecutive days decreased motility of undiluted semen from insulated rams within 16 days after start of insulation onwards. Our study supports the findings of previous studies in rams (Dutt and Hamm, 1957; Dutt and Simpson, 1957; Moule and Waites, 1963; Howarth, 1969; Rathore, 1969; Smith, 1971; Mieusset et al, 1991, 1992).

In this study, the effect was much more severe in one ram (No.12) than the other (No.16). Also, the time of recovery was different between these two rams, being much

longer in ram No.12 than in No 16. Variation like this between individual rams was also reported by Dun (1955), Glover (1955), Moule and Waites, (1963) and Williamson (1974). Observations on diluted semen from these insulated rams also indicated that a decrease during storage in motility and morphological characteristics occurred at the same time as the fall in motility.

Storage at 30 and 5°C produced greater changes in motility and morphological characteristics of spermatozoa in semen from insulated rams than in semen from control rams. The present study also showed that these characteristics were maintained to a similar extent in semen diluted in coconut extract-quail yolk diluent and in citrate glucose-hen yolk diluent, but storage in these experiments was for a comparatively short time.

In a second experiment, done with the same rams one year later, 12h/day of scrotal insulation led to a reduction in motility of undiluted spermatozoa from individual insulated rams from day 17 after start of scrotal insulation. Again, the effect was much more obvious in ram No.12 than in ram No.16. Although in both experiments, the extent of exposure to heat was different, the time of occurrence of the effect on the semen of the two treated rams was almost the same, i.e. about 2 weeks after start of insulation. However, time of recovery after 12h/day insulation was achieved in a shorter time than after 16h/day, suggesting cells earlier in the spermatogenesis process has affected by the more severe heating, even though it was for a shorter period.

Consistent with previous observations, decrease in motility characteristics of spermatozoa in fresh diluted semen was also reflected in a decrease of motility during storage of spermatozoa in semen from insulated rams. Similarly, an effect of storage was also reflected in changes in morphological characteristics of spermatozoa from the two insulated rams. Furthermore, this second study showed that diluent containing coconut extract was better than that based on citrate and glucose in maintaining the viability characteristics of

spermatozoa over longer periods and that this beneficial effect was more marked in some regards in semen from rams subjected heat stress.

It is interesting to note from the findings in the two scrotal insulation experiments, that the greater severity of the effect experienced by ram No.12 as observed in the first experiment (16 h/d) was repeatable in the second experiment (12h/d) one year later. While this observation was only on two animals, the difference could be due to genetic differences beween the rams. It would be interesting if semen from these rams could be used in artificial insemination trials, with examination of the sensitivity to heat of their male offspring. If this is so, then only males that give good semen samples during insulation and are thus more resistant to heat stress should be choosen as potential sires.

The final relevant point arising from these studies relates to the relative value of the various measurement of semen quality made by the Hamilton-Thorn apparatus, and those done manually. It is clear that under some circumstances, the objective evaluation of semen can pick up changes in motility that were not apparent from the manual evaluation (compare Figures 10.4 and 10.5). However, of the characteristics determined by the Hamilton-Thorn, percent medium and slow sperm seem to show only minor changes and percent motile is highly correlated with percent progressive and percent rapid (Table 11.1). The more detailed measurements of the motility characteristics such as mean ALH, mean VSL, etc. also seem to correlate well with percent motile sperm, suggesting that not only does the proportion of motile sperm fall after storage or heating the testis, but that the ability of the motile sperm to swim normally is also compromised to about the same extent. This may be relevant in explaining the apparent reduced ability of sperm from these rams to fertilize ova *in vitro*, even when comparable numbers are selected by a swim-up procedure (Ekpe et al, 1993).

While percent motile sperm was highly correlated with most other motion and kinematics parameters, the correlation was less for mean LIN and mean STR, but, these two parameters appear to change less than percent motile sperm.

From studies presented in this thesis, there is good evidence that use of coconut extract and quail yolk in the diluent supports and maintains the viability characteristics of ram spermatozoa during storage at room or refrigerated temperatures, whether the semen is collected from normal-fertile rams or from abnormal or subfertile rams. Future work needs to be conducted to determine what would be the best levels of coconut extract to be included in ram semen diluent in order to improve motility of ram spermatozoa during liquid storage either at 30 or 5°C. Finally, the beneficial effect of this compound in semen diluents for stored *in vitro* has to be proved through a fertility trials, especially under tropical conditions in Indonesia.

Artificial insemination in sheep is now becoming a recognised practice in some developed countries and techniques of processing and preservation of semen has been continuously developed. Results obtained during this study demonstrated succesful evidence on the use of techniques of processing and short term liquid storage of ram semen at room and cool temperatures. These techniques are particularly useful and could be of relevance in the tropical regions, such as Indonesia. In this country, sheep remained to be considered as an important source of protein in the human diets. With increasing human population and the concern for sustainable food production, an application of this reproductive technology is regarded as a potential tool for increasing livestock production in general, and sheep production in particular.

Table 11.1. Correlations of percent motile sperm with other characterisitics of sperm motility.

Exp No.	Treatment	Motility range	Prog	Rap	Med	Slo	ALH	VAP	VSL	VCL	LIN	STR
4.1	CG vs CE	3 - 90	.998	.998	-.32	-.92	.88	.94	.95	.92	.75	.69
4.2	CE vs CF	15 - 80	.992	.987	.54	-.79	.78	.94	.95	.91	.84	.79
5.1 & 5.2	Difft pH	2 - 85	.965	.956	.59	-.40	.87	.82	.80	.82	.58	.58
6.4 & 6.5	G,C,N &S	2 - 85	.964	.967	.53	-.42	.91	.92	.90	.92	.80	.77
6.6 & 6.7	C,N & S	28 - 85	.987	.973	-.13	-.88	.83	.91	.94	.89	.62	.53
6.8 & 6.9	CE & CNS	15 - 83	.981	.975	.24	-.73	.88	.91	.92	.90	.55	.39
7.3.1	Yolk %30	1 - 85	.985	.997	.08	-.42	.84	.79	.65	.84	.08	-.02
7.3.2	Yolk %5	1 - 85	.988	.990	-.01	-.01	.84	.85	.80	.86	.06	-.18
8	Sugars	0 - 85	.966	.962	.49	-.63	.93	.94	.93	.94	.75	.74
9	HCO3	5 - 90	.965	.971	-.18	-.88	.90	.84	.81	.85	.71	.66
10.1	Insul 16	4 - 89	.918	.943	.25	-.46	.83	.75	.65	.79	.41	.43
10.2	Insul 12	0 - 89	.977	.973	.56	-.18	.83	.80	.77	.81	.51	.49

Abbreviations: CG: citrate-glucose diluent; CE: coconut extract diluent; CF: coconut fluid diluent; G: glucose; C: catalase; N: nystatin; S: sulfamilamide; HCO3: sodium bicarbonate; Insul 16: scrotal insulation for 16 h/day; Insul 12: scrotal insulation for 12 h/day.

Chapter 11

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Appendix A.

Table 4.2. Characteristics of ram semen stored at 30°C in hen yolk citrate glucose (HYCG), quail yolk citrate glucose (QYCG) and (coconut extract quail yolk) CEQY diluent for 24h (Mean ± SEM, n = 12).

Semen characteristic	Diluent	Time of storage at 30°C		
		0h	24h	48h
		Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	HYCG	88.88 ± 1.15	41.48 ± 10.94	2.93 ± 0.68
	QYCG	87.06 ± 1.61	39.67 ± 10.83	3.17 ± 0.98
	CEQY	83.53 ± 1.27	79.46 ± 2.92	43.35 ± 11.30
PROG (%)	HYCG	66.44 ± 0.68	31.78 ± 9.07	0.65 ± 0.34
	QYCG	64.85 ± 1.27	28.78 ± 9.01	0.30 ± 0.17
	CEQY	63.38 ± 1.44	60.03 ± 3.14	30.26 ± 8.51
RAPID (%)	HYCG	87.85 ± 1.44	37.28 ± 10.77	0.85 ± 0.41
	QYCG	85.47 ± 1.80	33.91 ± 10.73	0.41 ± 0.21
	CEQY	82.00 ± 1.40	76.21 ± 3.51	39.36 ± 10.94
MEDIUM (%)	HYCG	1.04 ± 0.41	4.19 ± 0.88	2.09 ± 0.44
	QYCG	1.57 ± 0.36	5.75 ± 1.29	2.75 ± 0.92
	CEQY	1.51 ± 0.31	3.24 ± 0.67	3.95 ± 0.66
SLOW (%)	HYCG	0.07 ± 0.07	2.25 ± 0.57	5.86 ± 0.85
	QYCG	0.26 ± 0.08	4.77 ± 1.78	5.46 ± 1.31
	CEQY	0.13 ± 0.07	0.61 ± 0.29	1.44 ± 0.50
ALH (μm)	HYCG	8.17 ± 0.17	3.59 ± 0.72	1.70 ± 0.42
	QYCG	8.41 ± 0.19	3.13 ± 0.80	0.79 ± 0.27
	CEQY	7.56 ± 0.15	6.36 ± 0.25	5.47 ± 0.47
LIN (%)	HYCG	66.55 ± 1.15	56.46 ± 8.20	41.26 ± 4.96
	QYCG	65.93 ± 1.89	54.12 ± 7.98	32.91 ± 8.32
	CEQY	68.54 ± 1.05	67.81 ± 1.53	57.53 ± 2.61
VAP (μm/s)	HYCG	153.16 ± 4.10	70.34 ± 15.74	21.30 ± 5.39
	QYCG	153.08 ± 6.83	63.53 ± 15.89	15.12 ± 4.28
	CEQY	150.90 ± 3.57	115.74 ± 6.10	73.09 ± 9.11
VSL (μm/s)	HYCG	123.09 ± 3.93	59.77 ± 14.06	14.80 ± 4.79
	QYCG	124.16 ± 8.14	53.85 ± 14.12	9.06 ± 2.49
	CEQY	123.00 ± 3.42	94.58 ± 5.16	56.37 ± 7.81
VCL (μm/s)	HYCG	182.35 ± 4.58	79.65 ± 17.51	27.03 ± 6.27
	QYCG	181.76 ± 6.89	72.70 ± 17.87	19.10 ± 5.91
	CEQY	176.79 ± 4.14	135.60 ± 6.82	91.97 ± 10.18
STR (%)	HYCG	77.02 ± 1.09	64.37 ± 9.27	54.06 ± 6.72
	QYCG	76.76 ± 1.68	61.56 ± 9.09	37.78 ± 9.62
	CEQY	78.25 ± 0.99	78.00 ± 1.12	72.58 ± 1.86

Table 4.3. Characteristics of ram semen stored at 5°C in hen yolk citrate glucose (HYCG), quail yol k citrate glucose (QYCG) and coconut extract quail yolk (CEQY) diluent for 240h (Mean ± SEM, n = 12).

Semen charac-teristic	Diluent	Time of storage at 5°C											
		0h		48h		96h		144h		192h		240h	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
MOT	HYCG	83.84	2.43	76.31	2.98	81.06	1.76	63.62	3.70	27.09	7.09	12.70	3.02
(%)	QYCG	85.64	1.36	82.50	2.92	84.33	1.84	65.81	5.43	25.40	5.19	13.83	1.83
	CEQY	85.20	1.79	85.07	2.15	85.21	1.81	80.90	2.97	78.77	2.86	71.04	2.94
PROG	HYCG	63.83	2.10	60.94	2.41	63.75	2.72	47.49	3.40	15.34	5.31	7.02	2.30
(%)	QYCG	66.65	1.10	64.40	2.42	66.42	1.59	50.32	4.31	16.84	3.41	5.70	1.27
	CEQY	64.84	1.37	68.12	2.05	68.03	1.63	64.55	2.29	63.28	2.73	56.06	2.38
RAPID	HYCG	80.92	3.09	73.11	3.26	76.03	2.45	54.55	3.93	18.75	6.37	9.30	2.81
(%)	QYCG	83.81	1.55	79.78	3.26	80.23	2.35	58.98	5.29	19.77	4.23	8.09	1.84
	CEQY	84.16	1.90	82.73	2.30	82.49	2.01	77.23	3.31	74.17	2.81	65.24	2.98
MEDIUM	HYCG	2.95	0.78	3.21	0.58	5.03	0.95	9.08	0.93	8.34	2.24	3.40	0.67
(%)	QYCG	1.85	0.40	2.73	0.58	4.11	0.79	6.83	0.86	5.63	1.10	5.73	1.24
	CEQY	1.05	0.27	2.33	0.51	2.70	0.61	3.68	0.55	4.60	0.80	5.81	0.45
SLOW	HYCG	0.35	0.10	0.68	0.17	0.73	0.23	2.26	0.42	3.35	0.68	2.57	0.96
(%)	QYCG	0.18	0.11	0.43	0.16	0.37	0.18	1.79	0.31	3.15	0.37	6.28	1.62
	CEQY	0.26	0.13	0.31	0.15	0.51	0.10	0.59	0.19	1.12	0.19	1.42	0.16
ALH	HYCG	7.83	0.99	7.80	1.12	7.26	1.86	4.29	0.09	3.10	0.41	4.34	0.34
(µm)	QYCG	7.49	0.23	6.27	0.18	5.90	0.12	4.90	0.14	4.12	0.26	2.87	0.39
	CEQY	7.58	0.27	6.13	0.22	5.74	0.13	5.56	0.19	5.25	0.13	4.80	0.21
LIN	HYCG	69.27	1.64	74.00	1.34	73.24	1.73	74.61	1.33	68.81	1.86	62.94	3.86
(%)	QYCG	69.20	1.23	71.55	1.15	72.82	1.17	73.76	1.44	68.08	2.27	58.17	3.50
	CEQY	67.97	1.99	72.64	1.53	72.99	1.31	74.81	1.12	75.27	1.06	75.49	1.01
VAP	HYCG	140.46	3.99	127.26	4.05	112.93	3.66	91.57	3.03	59.05	6.98	68.80	7.09
(µm/s)	QYCG	143.18	2.54	125.97	3.85	118.20	2.23	100.93	3.80	74.80	5.72	49.69	8.50
	CEQY	144.93	3.63	121.94	3.53	115.11	3.08	117.09	2.63	110.64	2.59	102.27	4.53
VSL	HYCG	116.95	4.43	110.31	4.51	96.96	3.82	81.17	3.01	49.95	6.23	58.37	7.44
(µm/s)	QYCG	118.56	2.31	106.43	4.16	100.78	2.73	88.85	3.77	64.39	5.46	39.93	8.18
	CEQY	117.74	4.76	102.90	3.88	97.59	3.35	100.91	2.34	95.52	2.70	88.98	4.18
VCL	HYCG	163.22	4.45	143.46	4.26	127.16	3.48	101.48	2.99	69.36	8.06	80.62	6.53
(µm/s)	QYCG	167.75	3.58	143.57	3.92	133.99	2.36	112.63	3.90	85.93	5.62	58.37	8.66
	CEQY	170.57	3.93	139.56	3.75	130.58	2.97	131.38	3.21	123.45	2.65	115.26	4.78
STR	HYCG	79.07	1.37	82.62	1.07	81.86	1.30	82.98	1.12	79.90	1.14	75.10	3.20
(%)	QYCG	79.37	1.00	80.39	0.94	81.41	0.96	82.58	1.01	79.76	1.29	70.10	3.28
	CEQY	77.97	1.59	81.60	1.15	81.56	1.01	82.82	0.76	83.07	0.87	83.76	0.87

Table 4.4. Characteristics of ram semen stored at 30 and 5°C in hen yolk citrate glucose (HYCG) and quail yolk citrate glucose (QYCG) for 8h (Mean ± SEM, n = 12).

Semen characteristic	Time of storage at 30°C in HYCG diluent			Time of storage at 5°C in HYCG diluent		
	0h	4h	8h	0h	4h	8h
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	79.37 ± 1.95	80.47 ± 2.29	79.09 ± 1.62	80.42 ± 2.10	77.67 ± 2.25	74.70 ± 2.47
PROG (%)	48.77 ± 2.24	45.94 ± 2.44	46.90 ± 2.29	50.95 ± 1.84	47.41 ± 2.50	49.65 ± 2.42
RAPID (%)	61.07 ± 2.50	57.78 ± 3.24	56.37 ± 2.78	62.96 ± 2.89	58.30 ± 2.67	57.25 ± 2.81
MEDIUM (%)	18.32 ± 1.79	22.68 ± 1.36	22.70 ± 2.22	17.47 ± 1.23	19.37 ± 1.07	17.45 ± 1.64
SLOW (%)	0.30 ± 0.11	0.45 ± 0.20	0.40 ± 0.21	0.29 ± 0.13	0.24 ± 0.14	0.27 ± 0.15
ALH (µm)	8.83 ± 0.21	8.22 ± 0.38	7.27 ± 0.35	8.19 ± 0.29	7.87 ± 0.31	7.68 ± 0.25
LIN (%)	63.20 ± 1.39	61.58 ± 1.78	66.95 ± 1.47	65.77 ± 1.02	65.71 ± 1.65	70.91 ± 1.62
VAP (µm/s)	146.25 ± 3.99	127.20 ± 3.33	125.32 ± 5.24	144.98 ± 3.49	138.03 ± 3.16	140.05 ± 2.87
VSL (µm/s)	117.91 ± 4.37	100.84 ± 3.05	103.30 ± 4.57	118.52 ± 3.13	113.81 ± 3.81	119.89 ± 3.23
VCL (µm/s)	176.92 ± 3.86	155.62 ± 4.68	147.73 ± 6.10	172.46 ± 4.27	164.26 ± 3.57	163.06 ± 3.04
STR (%)	75.40 ± 1.14	74.14 ± 1.53	78.05 ± 1.12	77.15 ± 0.82	77.10 ± 1.36	81.52 ± 1.26

Semen characteristic	Time of storage at 30°C in QYCG diluent			Time of storage at 5°C in QYCG diluent		
	0h	4h	8h	0h	4h	8h
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	79.38 ± 1.51	81.44 ± 2.16	76.10 ± 2.21	77.58 ± 2.42	80.52 ± 1.87	79.18 ± 2.91
PROG (%)	49.59 ± 1.88	49.39 ± 1.90	44.13 ± 2.73	49.63 ± 2.39	49.84 ± 2.41	51.81 ± 2.56
RAPID (%)	63.61 ± 2.00	62.01 ± 2.59	52.42 ± 3.17	62.08 ± 2.91	61.70 ± 2.65	62.65 ± 3.20
MEDIUM (%)	15.75 ± 1.25	19.43 ± 1.44	23.68 ± 2.09	15.50 ± 1.46	18.80 ± 1.58	16.54 ± 1.87
SLOW (%)	0.23 ± 0.14	0.17 ± 0.10	0.46 ± 0.20	0.12 ± 0.06	0.35 ± 0.25	0.33 ± 0.17
ALH (µm)	9.10 ± 0.27	8.33 ± 0.29	7.42 ± 0.28	8.64 ± 0.29	8.56 ± 0.28	8.12 ± 0.31
LIN (%)	63.20 ± 1.86	64.38 ± 1.40	65.78 ± 1.60	64.94 ± 1.69	64.79 ± 1.94	67.54 ± 1.82
VAP (µm/s)	149.36 ± 3.74	136.24 ± 4.36	123.84 ± 4.90	146.79 ± 3.42	140.74 ± 3.74	140.94 ± 3.65
VSL (µm/s)	119.17 ± 4.68	110.11 ± 4.24	102.62 ± 4.52	118.87 ± 3.82	114.53 ± 4.14	116.97 ± 4.02
VCL (µm/s)	180.67 ± 3.78	164.32 ± 5.29	146.83 ± 5.74	176.68 ± 4.23	169.41 ± 3.55	166.30 ± 4.14
STR (%)	75.08 ± 1.46	76.25 ± 1.22	77.56 ± 1.35	76.66 ± 1.35	76.75 ± 1.55	78.60 ± 1.44

Table 4.5. Characteristics of ram semen stored at 5°C in hen yolk citrate glucose (HYCG), coconut fluid quail yolk (CFQY) and coconut extract quail yolk (CEQY) diluent for 240h (Mean ± SEM, n = 12)

Semen charac-teristic	Diluent	Time of storage at 5°C					
		0h	48h	96h	144h	192h	240h
		Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
MOT (%)	HYCG	80.6 ±2.54	79.0 ±2.25	72.5 ±2.41	58.7 ±4.16	31.9 ±4.02	14.9 ±1.97
	CFQY	80.8 ±1.7	74.2 ±3.0	71.4 ±2.9	54.0 ±3.0	29.0 ±5.7	18.2 ±3.4
	CEQY	83.0 ±1.3	77.2 ±2.9	73.3 ±2.9	69.2 ±3.8	59.0 ±5.8	52.1 ±6.0
PROG (%)	HYCG	66.5 ±2.8	60.6 ±2.7	52.9 ±2.2	36.1 ±3.7	17.0 ±3.7	6.5 ±1.4
	CFQY	67.6 ±1.6	54.9 ±2.4	51.9 ±2.7	35.6 ±2.8	15.9 ±4.1	6.5 ±2.2
	CEQY	69.5 ±1.3	55.9 ±2.9	53.8 ±2.5	49.5 ±3.7	41.3 ±5.0	32.1 ±5.8
RAPID (%)	HYCG	53.8 ±2.3	52.8 ±2.3	46.9 ±2.1	30.6 ±3.0	14.2 ±2.9	5.2 ±1.1
	CFQY	55.8 ±1.73	47.5 ±2.02	44.7 ±2.60	31.1 ±2.55	13.7 ±3.77	5.5 ±2.02
	CEQY	57.7 ±0.9	47.8 ±2.7	46.1 ±2.2	43.3 ±3.2	36.4 ±4.3	28.1 ±5.3
MEDIUM (%)	HYCG	14.2 ±1.5	18.4 ±1.6	19.7 ±1.4	22.6 ±1.3	14.9 ±1.8	8.4 ±1.1
	CFQY	13.2 ±0.9	19.4 ±2.0	19.5 ±2.0	18.4 ±2.1	13.0 ±2.0	11.7 ±1.9
	CEQY	13.6 ±1.2	21.3 ±2.0	19.5 ±1.4	19.7 ±1.2	17.7 ±1.4	20.0 ±1.9
SLOW (%)	HYCG	0.4 ±0.2	0.7 ±0.2	1.2 ±0.2	2.3 ±0.4	1.9 ±0.3	2.5 ±0.9
	CFQY	0.3 ±0.1	0.5 ±0.2	0.8 ±0.3	1.0 ±0.2	1.3 ±0.3	1.5 ±0.4
	CEQY	0.1 ±0.1	0.5 ±0.2	0.2 ±0.1	0.7 ±0.2	0.8 ±0.2	1.6 ±0.4
ALH (µm)	HYCG	8.3 ±0.22	7.2 ±0.27	6.6 ±0.15	5.8 ±0.41	5.9 ±0.29	5.0 ±0.47
	CFQY	8.2 ±0.2	7.1 ±0.3	7.2 ±0.3	6.8 ±0.3	6.7 ±0.4	6.0 ±0.7
	CEQY	8.2 ±0.2	7.0 ±0.3	7.0 ±0.3	6.6 ±0.3	6.6 ±0.3	6.9 ±0.3
LIN (%)	HYCG	68.0 ±0.9	71.9 ±1.2	72.3 ±1.2	67.2 ±1.6	64.3 ±1.9	63.9 ±2.2
	CFQY	70.2 ±1.2	71.3 ±1.4	69.7 ±2.1	68.1 ±1.8	62.2 ±2.5	53.3 ±2.8
	CEQY	69.0 ±0.9	71.4 ±1.1	70.9 ±1.8	70.9 ±1.9	69.6 ±2.1	66.3 ±2.3
VAP (µm/s)	HYCG	148.6 ±3.4	131.1 ±4.2	126.7 ±4.9	104.5 ±4.2	93.3 ±5.7	77.9 ±7.6
	CFQY	152.1 ±3.3	122.6 ±3.1	118.6 ±2.3	106.2 ±2.9	89.4 ±5.7	66.3 ±6.7
	CEQY	144.3 ±3.4	122.5 ±3.5	119.7 ±2.7	114.6 ±3.5	111.5 ±3.5	100.1 ±4.9
VSL (µm/s)	HYCG	122.2 ±3.5	111.5 ±3.8	109.1 ±5.2	86.5 ±3.7	74.9 ±4.9	60.8 ±5.7
	CFQY	127.0 ±3.4	103.0 ±3.1	99.0 ±3.1	87.9 ±3.0	71.4 ±5.6	49.7 ±6.5
	CEQY	119.2 ±3.2	102.7 ±3.3	100.5 ±2.7	96.9 ±3.6	93.7 ±4.0	82.9 ±5.3
VCL (µm/s)	HYCG	175.9 ±4.0	151.3 ±4.7	144.7 ±4.5	122.8 ±4.8	113.7 ±6.2	96.5 ±9.6
	CFQY	176.9 ±3.7	143.6 ±3.9	141.1 ±2.6	127.9 ±3.2	116.0 ±6.8	92.3 ±8.6
	CEQY	169.9 ±3.8	142.9 ±4.1	140.9 ±3.4	134.7 ±3.7	133.1 ±3.1	123.6 ±4.3
STR (%)	HYCG	78.6 ±0.8	81.8 ±0.8	82.2 ±0.9	78.1 ±1.3	76.6 ±1.5	76.6 ±1.9
	CFQY	80.2 ±0.9	81.5 ±1.0	81.0 ±1.3	80.3 ±1.1	77.5 ±1.7	71.7 ±3.2
	CEQY	79.5 ±0.7	81.4 ±0.8	81.5 ±1.3	81.6 ±1.2	81.1 ±1.3	80.0 ±1.3

Table 5.1. Means values with SEM for ram semen motion characteristics in different diluents at different hours preservations at temperture 30°C. (Mean ± SEM, n = 12).

Semen charac-teristic	Diluent-pH	Time of storage at 30°C		
		0h	24h	48h
		Mean ± Sem	Mean ± Sem	Mean ± Sem
MOT (%)	HYCG-7.6	82.37 ± 3.18	25.40 ± 12.68	4.13 ± 3.75
	HYCG-7.0	78.87 ± 2.86	19.73 ± 9.99	1.15 ± 1.15
	CEQY-7.6	81.93 ± 3.04	67.23 ± 10.93	34.24 ± 17.23
	CEQY-7.0	80.42 ± 2.57	61.85 ± 9.96	44.68 ± 15.02
PROG (%)	HYCG-7.6	65.15 ± 2.67	20.23 ± 10.63	2.97 ± 2.59
	HYCG-7.0	61.80 ± 3.47	11.73 ± 7.80	0.82 ± 0.82
	CEQY-7.6	62.40 ± 2.43	44.45 ± 10.37	18.42 ± 10.85
	CEQY-7.0	59.63 ± 1.59	39.97 ± 10.39	25.33 ± 9.61
RAPID (%)	HYCG-7.6	73.32 ± 3.68	22.57 ± 12.05	2.97 ± 2.59
	HYCG-7.0	67.87 ± 3.98	14.08 ± 9.09	0.82 ± 0.82
	CEQY-7.6	70.32 ± 2.88	50.47 ± 11.43	20.14 ± 11.64
	CEQY-7.0	67.65 ± 3.15	45.78 ± 11.46	28.28 ± 10.91
MEDIUM (%)	HYCG-7.6	9.07 ± 2.41	2.83 ± 1.43	1.17 ± 1.17
	HYCG-7.0	11.00 ± 2.11	5.68 ± 1.91	0.33 ± 0.33
	CEQY-7.6	11.60 ± 4.08	16.75 ± 2.09	14.08 ± 8.27
	CEQY-7.0	12.77 ± 3.05	16.07 ± 3.96	16.42 ± 6.71
SLOW (%)	HYCG-7.6	0.50 ± 0.16	0.65 ± 0.28	0.72 ± 0.62
	HYCG-7.0	0.23 ± 0.12	1.02 ± 0.71	0.80 ± 0.51
	CEQY-7.6	0.52 ± 0.15	0.63 ± 0.24	0.08 ± 0.07
	CEQY-7.0	0.42 ± 0.20	0.85 ± 0.36	0.63 ± 0.32
ALH (μm)	HYCG-7.6	7.22 ± 0.91	5.05 ± 1.01	1.93 ± 1.22
	HYCG-7.0	6.72 ± 0.58	3.82 ± 1.13	1.12 ± 1.12
	CEQY-7.6	7.18 ± 0.85	5.78 ± 1.00	4.68 ± 0.97
	CEQY-7.0	7.13 ± 0.78	5.58 ± 0.91	3.73 ± 1.24
LIN (%)	HYCG-7.6	74.48 ± 2.51	73.58 ± 6.25	28.15 ± 17.86
	HYCG-7.0	75.78 ± 1.15	59.23 ± 12.25	13.40 ± 13.40
	CEQY-7.6	73.08 ± 1.96	71.68 ± 4.06	79.82 ± 5.65
	CEQY-7.0	71.05 ± 2.71	70.37 ± 2.53	47.20 ± 15.07
VAP (μm/s)	HYCG-7.6	158.58 ± 18.33	124.10 ± 19.49	58.37 ± 38.44
	HYCG-7.0	146.00 ± 12.19	79.20 ± 23.12	26.40 ± 26.39
	CEQY-7.6	150.17 ± 19.79	106.28 ± 15.03	83.04 ± 15.09
	CEQY-7.0	144.53 ± 17.73	110.30 ± 18.45	66.77 ± 23.59
VSL (μm/s)	HYCG-7.6	135.95 ± 16.08	108.08 ± 20.15	53.68 ± 35.68
	HYCG-7.0	127.82 ± 11.11	66.03 ± 19.41	24.87 ± 24.86
	CEQY-7.6	128.13 ± 17.90	88.10 ± 12.71	75.32 ± 14.79
	CEQY-7.0	121.13 ± 16.37	90.00 ± 15.80	56.27 ± 20.52
VCL (μm/s)	HYCG-7.6	180.15 ± 21.32	140.58 ± 20.42	61.62 ± 40.40
	HYCG-7.0	164.52 ± 13.86	90.60 ± 26.76	28.63 ± 28.63
	CEQY-7.6	171.87 ± 22.58	124.32 ± 18.84	94.90 ± 16.99
	CEQY-7.0	166.13 ± 20.17	127.45 ± 20.63	77.40 ± 26.63
STR (%)	HYCG-7.6	83.05 ± 2.01	80.55 ± 5.41	29.97 ± 18.97
	HYCG-7.0	84.25 ± 0.95	66.18 ± 13.42	14.83 ± 14.83
	CEQY-7.6	82.08 ± 1.41	80.98 ± 2.93	89.84 ± 5.08
	CEQY-7.0	80.38 ± 2.27	79.35 ± 2.54	54.28 ± 17.27

Table 5.2. Semen motion characteristics in different diluents at different hours preservations at temperture 5°C. (Mean ± SEM, n = 12).

Semen characteristics	Time (h)	Diluents with 2 pH levels stored at 5°C				Semen characteristics	Time (h)	Diluents with 2 pH levels stored at 5°C			
		HYCG		CEQY				HYCG		CEQY	
		7.6	7.0	7.6	7.0			7.6	7.0	7.6	7.0
		Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem			Mean ± Sem	Mean Sem	Mean ± Sem	Mean ± Sem
MOT (%)	0	83.67 ± 2.07	81.53 ± 2.11	85.70 ± 1.39	82.98 ± 1.84	ALH (μm)	0	6.93 ± 0.46	6.77 ± 0.46	7.40 ± 0.55	6.95 ± 0.54
	48	76.29 ± 2.79	76.69 ± 3.75	79.98 ± 2.08	79.59 ± 1.89		48	5.55 ± 0.39	5.55 ± 0.38	5.94 ± 0.40	5.88 ± 0.34
	96	69.09 ± 3.48	72.45 ± 4.42	76.03 ± 2.11	75.53 ± 2.14		96	4.80 ± 0.36	4.93 ± 0.35	5.00 ± 0.33	4.92 ± 0.24
	144	40.76 ± 8.31	47.66 ± 6.18	67.14 ± 5.53	60.80 ± 4.98		144	3.74 ± 0.47	4.07 ± 0.45	5.15 ± 0.41	4.52 ± 0.24
	192	27.93 ± 7.94	42.39 ± 6.42	66.97 ± 4.76	68.41 ± 3.87		192	4.32 ± 0.51	3.73 ± 0.34	4.88 ± 0.28	4.88 ± 0.24
	240	9.00 ± 3.42	9.30 ± 2.08	62.78 ± 5.91	65.13 ± 4.75		240	3.22 ± 0.55	2.62 ± 0.40	4.47 ± 0.32	4.32 ± 0.31
PROG (%)	0	60.00 ± 2.36	61.00 ± 2.10	63.93 ± 1.91	60.07 ± 1.90	LIN (%)	0	70.66 ± 1.25	72.93 ± 2.00	69.82 ± 1.44	71.36 ± 2.46
	48	53.74 ± 1.62	49.58 ± 2.61	54.25 ± 1.78	51.00 ± 2.24		48	76.91 ± 1.13	74.28 ± 1.28	73.92 ± 1.41	73.40 ± 1.50
	96	44.08 ± 2.86	46.22 ± 4.40	50.48 ± 1.77	50.23 ± 1.98		96	76.38 ± 1.23	75.43 ± 1.64	77.96 ± 0.95	77.33 ± 1.37
	144	20.38 ± 5.32	23.71 ± 4.08	37.20 ± 4.28	30.66 ± 4.06		144	70.49 ± 2.24	74.59 ± 1.09	73.53 ± 1.85	72.85 ± 2.05
	192	16.31 ± 5.55	18.71 ± 5.16	36.17 ± 4.00	38.35 ± 3.14		192	73.44 ± 3.36	74.87 ± 1.57	72.44 ± 1.63	73.70 ± 1.48
	240	3.14 ± 1.68	4.16 ± 1.29	34.18 ± 5.08	35.43 ± 4.11		240	66.53 ± 8.60	73.82 ± 8.30	72.58 ± 1.46	73.33 ± 1.46
RAPID (%)	0	67.76 ± 2.46	67.64 ± 2.29	73.17 ± 2.41	68.38 ± 2.17	VAP (μm)/s)	0	143.10 ± 10.72	141.09 ± 10.87	142.87 ± 10.33	140.68 ± 11.08
	48	57.45 ± 1.49	54.12 ± 2.75	59.10 ± 2.08	55.91 ± 2.48		48	121.08 ± 7.82	112.76 ± 6.59	117.17 ± 9.11	115.86 ± 8.49
	96	47.58 ± 3.10	49.66 ± 4.65	53.51 ± 1.96	54.01 ± 2.27		96	104.76 ± 6.26	104.88 ± 7.16	108.09 ± 7.37	109.39 ± 7.09
	144	21.82 ± 5.75	25.44 ± 4.29	39.63 ± 4.42	32.47 ± 4.23		144	75.58 ± 7.23	84.93 ± 5.44	95.96 ± 6.58	88.72 ± 3.98
	192	17.84 ± 5.74	20.45 ± 5.34	39.10 ± 4.07	41.09 ± 3.05		192	85.27 ± 7.41	76.96 ± 7.74	94.77 ± 5.76	93.81 ± 4.43
	240	3.54 ± 1.73	4.52 ± 1.28	36.83 ± 5.19	38.84 ± 3.93		240	63.59 ± 10.02	69.81 ± 7.81	89.01 ± 3.30	93.52 ± 6.52
MEDIUM (%)	0	15.90 ± 2.64	13.89 ± 2.22	12.54 ± 1.89	14.58 ± 2.35	VSL (μm)/s)	0	120.78 ± 10.09	120.99 ± 10.94	118.95 ± 9.31	118.72 ± 11.26
	48	18.83 ± 2.16	22.57 ± 2.99	20.88 ± 2.65	23.70 ± 3.05		48	107.06 ± 7.57	97.38 ± 6.36	100.53 ± 8.97	99.64 ± 8.20
	96	21.51 ± 2.42	22.78 ± 2.42	22.54 ± 2.43	21.51 ± 2.61		96	91.62 ± 5.89	91.56 ± 7.05	96.12 ± 7.36	96.23 ± 7.30
	144	18.95 ± 2.82	22.25 ± 2.09	27.55 ± 3.91	28.32 ± 3.05		144	63.09 ± 6.67	73.27 ± 4.66	83.22 ± 6.49	76.05 ± 4.68
	192	10.07 ± 2.30	21.94 ± 3.21	27.85 ± 3.42	27.32 ± 2.06		192	74.20 ± 6.65	65.98 ± 6.74	80.33 ± 5.65	80.64 ± 4.04
	240	5.45 ± 2.04	4.77 ± 1.10	25.94 ± 1.99	26.26 ± 2.37		240	56.73 ± 10.32	63.48 ± 7.84	74.90 ± 3.07	78.82 ± 5.97
SLOW (%)	0	0.48 ± 0.19	0.50 ± 0.19	0.28 ± 0.12	0.21 ± 0.10	VCL (μm)/s)	0	165.15 ± 11.60	161.33 ± 11.74	166.75 ± 11.90	163.18 ± 12.20
	48	1.19 ± 0.25	1.05 ± 0.20	0.49 ± 0.22	0.63 ± 0.20		48	135.21 ± 8.81	127.32 ± 7.33	133.05 ± 10.05	132.47 ± 9.17
	96	1.72 ± 0.42	1.07 ± 0.18	0.83 ± 0.18	0.88 ± 0.27		96	116.72 ± 7.11	117.73 ± 7.55	119.73 ± 8.19	121.44 ± 7.61
	144	1.67 ± 0.31	1.95 ± 0.38	1.49 ± 0.37	1.43 ± 0.33		144	86.95 ± 8.26	95.98 ± 6.75	110.83 ± 7.24	101.48 ± 3.88
	192	2.48 ± 0.84	4.33 ± 1.91	1.63 ± 0.40	1.08 ± 0.31		192	99.58 ± 9.01	88.60 ± 8.97	109.09 ± 6.50	108.78 ± 5.07
	240	3.13 ± 0.68	2.07 ± 0.66	2.31 ± 0.84	1.61 ± 0.43		240	74.59 ± 10.12	78.34 ± 8.82	103.59 ± 4.49	107.26 ± 7.45
						STR (%)	0	80.37 ± 1.03	82.02 ± 1.77	79.78 ± 1.16	80.84 ± 1.99
							48	85.05 ± 0.91	82.84 ± 1.03	82.57 ± 1.26	82.67 ± 1.23
							96	84.09 ± 0.89	83.68 ± 1.28	85.68 ± 0.83	84.69 ± 1.19
							144	80.23 ± 1.88	82.92 ± 1.11	83.54 ± 1.00	82.18 ± 1.62
							192	84.48 ± 2.21	84.10 ± 1.31	81.41 ± 1.34	83.37 ± 1.25
							240	76.87 ± 8.52	80.21 ± 8.51	81.36 ± 1.21	81.53 ± 1.26

Table 6.4. Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with glucose (+G), with nystatin and catalase (+NC), with nystatin and sulfanilamide (+NS), with sulfanilamide and catalase (+SC), with nystatin, sulfanilamide and catalase, (+NSC), with nystatin, sulfanilamide, catalase and glucose (+NSCG), with sulfanilamide (+S); and stored at 30°C for up to 48 h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	Time (h) 24 Mean ± SEM	Time (h) 48 Mean ± SEM	Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	Time (h) 24 Mean ± SEM	Time (h) 48 Mean ± SEM
MOT (%)	EYC	83.81 ± 1.24	62.06 ± 6.19	18.74 ± 5.42	MEDIUM (%)	EYC	13.21 ± 0.89	24.27 ± 3.06	13.23 ± 3.35
	+G	76.27 ± 5.10	59.89 ± 6.29	2.82 ± 0.76		+G	12.49 ± 1.04	23.38 ± 2.89	2.53 ± 0.70
	+NC	85.39 ± 0.74	55.13 ± 6.95	20.94 ± 6.80		+NC	13.46 ± 0.80	18.85 ± 3.19	7.03 ± 2.05
	+NS	86.30 ± 0.70	66.73 ± 4.58	25.90 ± 6.41		+NS	13.94 ± 0.91	31.03 ± 2.78	18.40 ± 4.44
	+SC	77.91 ± 5.16	58.65 ± 6.15	16.44 ± 5.52		+SC	12.67 ± 1.23	27.19 ± 4.30	11.84 ± 3.93
	+NSC	84.24 ± 1.01	78.39 ± 1.63	22.25 ± 6.45		+NSC	12.66 ± 0.80	29.87 ± 3.53	6.72 ± 1.52
	+NSCG	85.08 ± 0.92	74.56 ± 3.84	21.23 ± 6.03		+NSCG	13.36 ± 0.82	31.64 ± 3.62	11.59 ± 3.59
	+S	84.88 ± 0.97	57.03 ± 7.55	18.24 ± 5.88		+S	11.49 ± 0.85	19.31 ± 3.37	3.87 ± 1.01
PROG (%)	EYC	58.50 ± 1.30	29.16 ± 4.47	5.11 ± 2.24	SLOW (%)	EYC	0.17 ± 0.05	0.93 ± 0.27	1.26 ± 0.27
	+G	51.27 ± 3.64	28.79 ± 4.04	0.23 ± 0.08		+G	0.16 ± 0.06	0.83 ± 0.25	0.77 ± 0.19
	+NC	56.93 ± 1.11	28.79 ± 4.49	11.08 ± 4.20		+NC	0.10 ± 0.05	0.41 ± 0.10	0.58 ± 0.16
	+NS	56.36 ± 1.23	29.01 ± 3.95	5.84 ± 1.82		+NS	0.16 ± 0.06	1.39 ± 0.53	1.64 ± 0.53
	+SC	53.06 ± 3.79	25.72 ± 4.76	3.60 ± 1.34		+SC	0.27 ± 0.16	1.18 ± 0.31	0.80 ± 0.21
	+NSC	55.35 ± 1.08	34.42 ± 4.12	13.63 ± 4.52		+NSC	0.05 ± 0.02	0.69 ± 0.13	0.81 ± 0.19
	+NSCG	56.82 ± 0.89	35.04 ± 4.04	8.42 ± 2.65		+NSCG	0.17 ± 0.05	0.80 ± 0.30	1.20 ± 0.24
	+S	59.84 ± 1.25	31.29 ± 4.84	12.22 ± 4.39		+S	0.14 ± 0.05	0.59 ± 0.22	0.68 ± 0.15
RAPID (%)	EYC	70.60 ± 1.65	37.81 ± 5.38	5.52 ± 2.32					
	+G	63.77 ± 4.52	36.49 ± 4.78	0.30 ± 0.10					
	+NC	71.90 ± 1.10	36.29 ± 5.59	13.86 ± 5.20					
	+NS	72.34 ± 1.15	35.71 ± 4.53	7.51 ± 2.31					
	+SC	65.24 ± 4.56	31.46 ± 5.52	4.60 ± 1.63					
	+NSC	71.57 ± 1.35	43.12 ± 4.99	17.48 ± 5.90					
	+NSCG	71.73 ± 1.07	42.92 ± 4.76	9.65 ± 3.03					
	+S	73.38 ± 1.51	37.71 ± 5.79	14.37 ± 5.14					

Table 6.4. (continued). Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with glucose (+G), with nystatin and catalase (+NC), with nystatin and sulfanilamide (+NS), with sulfanilamide and catalase (+SC), with nystatin, sulfanilamide and catalase, (+NSC), with nystatin, sulfanilamide, catalase and glucose (+NSCG), with sulfanilamide (+S); and stored at 30°C for up to 48 h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	Time (h) 24 Mean ± SEM	Time (h) 48 Mean ± SEM	Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	Time (h) 24 Mean ± SEM	Time (h) 48 Mean ± SEM
ALH (µm)	EYC	6.74 ± 0.19	5.24 ± 0.38	3.64 ± 0.53	VSL (µm/s)	EYC	117.16 ± 2.7	71.43 ± 7.59	40.06 ± 5.33
	+G	6.46 ± 0.29	5.61 ± 0.29	2.66 ± 0.52		+G	109.73 ± 4.68	73.12 ± 4.66	27.55 ± 5.87
	+NC	6.64 ± 0.15	5.69 ± 0.4	2.78 ± 0.6		+NC	115.41 ± 2.72	79.23 ± 6.11	33.16 ± 8.01
	+NS	6.8 ± 0.18	5.2 ± 0.29	3.25 ± 0.45		+NS	113.52 ± 2.48	74.45 ± 4.6	39.15 ± 6.07
	+SC	6.41 ± 0.26	5.1 ± 0.36	2.69 ± 0.39		+SC	112.17 ± 4.95	73.07 ± 7.42	36.5 ± 5.7
	+NSC	6.62 ± 0.16	5.82 ± 0.24	2.89 ± 0.47		+NSC	115.1 ± 1.94	90.28 ± 5.3	52.54 ± 8.3
	+NSCG	6.51 ± 0.17	5.44 ± 0.23	2.79 ± 0.43		+NSCG	117.09 ± 2.34	87.61 ± 5.5	43.07 ± 7.24
	+S	6.45 ± 0.2	5.1 ± 0.44	3.05 ± 0.54		+S	120.19 ± 2.28	74.9 ± 7.37	54.56 ± 10.26
LIN (%)	EYC	72.51 ± 1.26	61.17 ± 3.33	52.37 ± 5.71	VCL (µm/s)	EYC	162.38 ± 3.87	111.14 ± 9.71	64.39 ± 6.88
	+G	71.21 ± 1.17	64.87 ± 2.3	49.17 ± 6.27		+G	153.86 ± 6.81	114.04 ± 5.43	44.38 ± 7.74
	+NC	70.22 ± 1.01	63.9 ± 3	37.75 ± 7.04		+NC	164.94 ± 2.99	119.35 ± 7.94	50.67 ± 11.58
	+NS	69.04 ± 1.04	67.78 ± 2.14	55.45 ± 5.68		+NS	165.27 ± 3.68	110.3 ± 5.74	59.7 ± 8.45
	+SC	70.84 ± 0.81	65.47 ± 2.49	55.91 ± 5.3		+SC	157.65 ± 6.6	110.03 ± 8.4	54.77 ± 7.42
	+NSC	69.11 ± 0.88	68.72 ± 1.95	50.92 ± 6.14		+NSC	167.49 ± 2.22	131.64 ± 6.41	80.33 ± 12.45
	+NSCG	70.31 ± 0.53	69.9 ± 1.69	53.4 ± 5.82		+NSCG	167.39 ± 3.28	124.59 ± 6.3	62.5 ± 9.11
	+S	72.64 ± 0.89	66.76 ± 3.28	48.23 ± 6.71		+S	165.29 ± 3.06	108.05 ± 9.71	77.85 ± 12.36
VAP (µm/s)	EYC	141.51 ± 3.11	92.56 ± 8.65	51.49 ± 5.86	STR (%)	EYC	80.96 ± 1.03	72.5 ± 3.35	62.34 ± 6.24
	+G	134 ± 5.87	93.53 ± 5.19	35.16 ± 6.6		+G	79.76 ± 1.09	77.25 ± 1.85	59.39 ± 6.89
	+NC	142.93 ± 2.63	99.13 ± 7.23	41.93 ± 9.95		+NC	78.62 ± 0.86	75.57 ± 3.24	44.37 ± 8.1
	+NS	142.36 ± 2.93	92.84 ± 5.1	48.64 ± 7.16		+NS	77.71 ± 0.83	78.61 ± 1.79	66.01 ± 6.44
	+SC	137.2 ± 5.76	92.06 ± 7.98	45.95 ± 6.48		+SC	79.54 ± 0.75	77.46 ± 2.21	66.09 ± 6.1
	+NSC	144.57 ± 1.88	112.06 ± 5.87	69.12 ± 11		+NSC	77.48 ± 0.74	78.72 ± 1.42	58.6 ± 6.78
	+NSCG	145.04 ± 2.64	106.73 ± 6.04	54.28 ± 8.29		+NSCG	78.66 ± 0.41	80.16 ± 1.32	61.31 ± 6.59
	+S	145.73 ± 2.5	92.05 ± 8.78	66.61 ± 11.42		+S	80.34 ± 0.71	77.5 ± 3.36	56.53 ± 7.36

Table 6.5. Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with glucose (+G), with nystatin and catalase (+NC), with nystatin and sulfanilamide (+NS), with sulfanilamide and catalase (+SC), with nystatin, sulfanilamide and catalase, (+NSC), with nystatin, sulfanilamide, catalase and glucose (+NSCG), with sulfanilamide (+S); and stored at 5°C for up to 216h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	72 Mean ± SEM	144 Mean ± SEM	216 Mean ± SEM
MOT (%)	EYC	84.06 ± 1.74	78.26 ± 3.39	72.92 ± 5.10	58.33 ± 8.79
	+G	84.53 ± 1.92	77.73 ± 2.73	25.02 ± 6.74	4.05 ± 2.67
	+NC	84.43 ± 1.87	78.19 ± 3.20	20.79 ± 5.88	9.21 ± 8.10
	+NS	84.72 ± 1.99	78.88 ± 2.75	73.39 ± 4.96	66.30 ± 4.96
	+SC	83.78 ± 1.64	78.72 ± 3.28	70.71 ± 3.84	62.87 ± 4.31
	+NSC	84.69 ± 1.61	79.37 ± 3.16	72.57 ± 5.00	66.25 ± 4.73
	+NSCG	85.23 ± 1.91	77.43 ± 2.57	49.95 ± 4.87	7.31 ± 3.27
PROG (%)	EYC	60.47 ± 2.60	55.67 ± 3.24	48.17 ± 4.88	33.44 ± 6.33
	+G	61.31 ± 1.69	52.80 ± 2.54	14.14 ± 4.33	2.91 ± 2.22
	+NC	60.52 ± 2.04	54.75 ± 3.21	13.22 ± 4.37	7.03 ± 6.46
	+NS	58.61 ± 2.24	53.34 ± 2.55	49.59 ± 3.69	40.30 ± 4.49
	+SC	59.50 ± 2.13	54.97 ± 3.41	48.49 ± 3.12	38.77 ± 3.87
	+NSC	58.31 ± 2.22	55.02 ± 3.02	49.89 ± 3.56	40.16 ± 5.05
	+NSCG	60.07 ± 1.83	50.88 ± 2.12	26.41 ± 2.28	2.27 ± 0.96
RAPID (%)	EYC	70.85 ± 2.77	62.35 ± 3.76	54.25 ± 5.58	38.05 ± 7.25
	+G	72.80 ± 2.30	59.41 ± 2.94	15.44 ± 4.77	3.31 ± 2.52
	+NC	71.84 ± 2.34	62.29 ± 3.88	14.33 ± 4.77	7.70 ± 7.08
	+NS	68.78 ± 3.08	59.94 ± 3.14	56.25 ± 4.41	45.54 ± 5.12
	+SC	68.92 ± 2.31	61.43 ± 3.74	54.24 ± 3.71	43.04 ± 4.42
	+NSC	68.86 ± 2.50	61.80 ± 3.44	57.28 ± 4.30	45.42 ± 5.78
	+NSCG	72.87 ± 2.14	58.29 ± 2.58	30.30 ± 2.57	2.90 ± 1.23

Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	72 Mean ± SEM	144 Mean ± SEM	216 Mean ± SEM
MEDIUM (%)	EYC	13.23 ± 2.00	15.91 ± 1.36	18.68 ± 3.01	20.83 ± 3.17
	+G	11.73 ± 1.07	18.31 ± 1.74	9.58 ± 2.54	1.49 ± 0.91
	+NC	12.58 ± 1.84	15.89 ± 1.83	6.45 ± 1.47	1.51 ± 1.08
	+NS	15.96 ± 2.35	18.93 ± 2.24	17.13 ± 1.64	20.73 ± 2.71
	+SC	14.84 ± 1.80	17.29 ± 2.22	16.47 ± 1.87	19.84 ± 2.15
	+NSC	15.81 ± 2.30	17.58 ± 2.13	15.29 ± 1.78	20.83 ± 3.76
	+NSCG	12.35 ± 0.91	19.13 ± 2.00	19.66 ± 2.94	4.43 ± 2.21
SLOW (%)	EYC	0.29 ± 0.11	0.80 ± 0.31	0.98 ± 0.25	0.89 ± 0.31
	+G	0.26 ± 0.13	0.79 ± 0.19	1.47 ± 0.49	0.53 ± 0.31
	+NC	0.48 ± 0.20	0.70 ± 0.23	1.18 ± 0.32	0.17 ± 0.14
	+NS	0.36 ± 0.14	0.69 ± 0.22	0.86 ± 0.20	1.28 ± 0.26
	+SC	0.38 ± 0.13	0.72 ± 0.21	0.84 ± 0.21	1.16 ± 0.33
	+NSC	0.36 ± 0.14	0.78 ± 0.27	0.76 ± 0.23	0.77 ± 0.23
	+NSCG	0.38 ± 0.14	0.62 ± 0.14	1.62 ± 0.32	0.71 ± 0.36

Table 6.5.(continued). Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with glucose (+G), with nystatin and catalase (+NC), with nystatin and sulfanilamide (+NS), with sulfanilamide and catalase (+SC), with nystatin, sulfanilamide and catalase, (+NSC), with nystatin, sulfanilamide, catalase and glucose (+NSCG), with sulfanilamide (+S); and stored at 5°C for up to 216h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	72 Mean ± SEM	144 Mean ± SEM	216 Mean ± SEM	Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	72 Mean ± SEM	144 Mean ± SEM	216 Mean ± SEM
ALH (µm)	EYC	6.79 ± 0.22	5.33 ± 0.12	5.15 ± 0.13	4.68 ± 0.18	VSL (µm/s)	EYC	126.39 ± 3.2	110.54 ± 1.88	106.67 ± 2.92	86.9 ± 3.95
	+G	7.03 ± 0.19	5.58 ± 0.12	3.87 ± 0.28	2.44 ± 0.46		+G	127.17 ± 1.83	107.41 ± 2.01	78.94 ± 6.55	59.23 ± 10.23
	+NC	7.11 ± 0.21	5.58 ± 0.14	4.06 ± 0.26	1.93 ± 0.53		+NC	128.14 ± 2.67	111.56 ± 2.45	91.37 ± 5.68	45.91 ± 10.84
	+NS	6.52 ± 0.21	5.14 ± 0.14	5.16 ± 0.13	4.89 ± 0.14		+NS	119.36 ± 3.04	106.26 ± 1.48	106.73 ± 1.68	98.64 ± 2.45
	+SC	6.58 ± 0.2	5.29 ± 0.13	4.95 ± 0.12	4.77 ± 0.15		+SC	119.19 ± 2.6	107.75 ± 1.8	108.61 ± 1.51	99.12 ± 2.2
	+NSC	6.73 ± 0.22	5.21 ± 0.12	5.44 ± 0.17	4.86 ± 0.13		+NSC	118.84 ± 3.06	109.25 ± 1.96	111.01 ± 2.14	98.52 ± 2.97
	+NSCG	7.25 ± 0.22	5.6 ± 0.11	4.92 ± 0.12	4.81 ± 0.35		+NSCG	126.74 ± 2.14	103.61 ± 1.72	89.85 ± 1.95	70.66 ± 6.9
LIN (%)	EYC	74.7 ± 0.78	77.96 ± 0.58	77.06 ± 0.84	74.76 ± 1.14	VCL (µm/s)	EYC	167.97 ± 4.78	139.84 ± 2.18	136.81 ± 3.21	114.48 ± 5.12
	+G	73.44 ± 0.58	76.39 ± 0.59	68.08 ± 4.2	52.03 ± 7.42		+G	171.43 ± 3.12	137.69 ± 2.33	102.52 ± 6.87	71.81 ± 12.1
	+NC	73.07 ± 0.83	77.2 ± 0.54	74.68 ± 3.23	45.78 ± 8.26		+NC	172.77 ± 3.94	142.7 ± 3.02	113.14 ± 6.06	55.14 ± 12.47
	+NS	74.49 ± 0.61	78.72 ± 0.64	77.29 ± 0.72	76.29 ± 0.93		+NS	159.07 ± 4.71	134.14 ± 1.91	136.58 ± 1.93	127.91 ± 2.87
	+SC	74.85 ± 0.87	77.92 ± 0.74	78.1 ± 0.79	77.14 ± 0.8		+SC	158.43 ± 3.85	136.33 ± 1.89	137.06 ± 1.7	126.91 ± 3.02
	+NSC	73.52 ± 0.75	78.38 ± 0.53	76.33 ± 1.12	76.71 ± 0.79		+NSC	160.19 ± 4.42	137.86 ± 2.01	143.69 ± 1.75	127.57 ± 3.65
	+NSCG	72.13 ± 0.69	75.53 ± 0.74	72.37 ± 0.8	68.77 ± 2.62		+NSCG	173.48 ± 3.41	135.31 ± 1.66	122.12 ± 2.05	106.09 ± 6.58
VAP (µm/s)	EYC	148.1 ± 3.8	126.79 ± 1.99	122.68 ± 3.12	101.23 ± 4.84	STR (%)	EYC	82.94 ± 0.57	84.89 ± 0.44	84.54 ± 0.62	83.95 ± 0.76
	+G	150.39 ± 2.37	123.97 ± 2.19	90.55 ± 6.79	65.37 ± 11.02		+G	81.89 ± 0.42	83.81 ± 0.45	76.2 ± 4.42	56.67 ± 8.01
	+NC	151.59 ± 3.24	129.1 ± 2.74	101.99 ± 5.95	50.45 ± 11.71		+NC	81.66 ± 0.62	84.26 ± 0.43	82.52 ± 3.31	49.71 ± 8.78
	+NS	140.44 ± 3.84	121.57 ± 1.69	122.96 ± 1.69	114.15 ± 2.65		+NS	82.7 ± 0.49	85.48 ± 0.46	84.5 ± 0.6	84.02 ± 0.68
	+SC	139.86 ± 3.08	123.62 ± 1.79	124.37 ± 1.45	113.74 ± 2.58		+SC	82.96 ± 0.64	84.87 ± 0.5	84.79 ± 0.57	84.75 ± 0.56
	+NSC	140.66 ± 3.61	125.16 ± 1.96	128.69 ± 1.69	114.16 ± 3.42		+NSC	81.95 ± 0.55	85.03 ± 0.4	83.61 ± 0.86	84.33 ± 0.63
	+NSCG	151.41 ± 2.51	121.26 ± 1.65	106.89 ± 1.97	85.79 ± 6.41		+NSCG	80.83 ± 0.51	83.15 ± 0.55	81.06 ± 0.65	80.91 ± 2.01

Table 6.6. Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S); and stored at 30°C for up to 48h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	Time (h) 24 Mean ±SEM	Time (h) 48 Mean ±SEM	Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	Time (h) 24 Mean ±SEM	Time (h) 48 Mean ±SEM	Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	Time (h) 24 Mean ±SEM	Time (h) 48 Mean ±SEM	Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	Time (h) 24 Mean ±SEM	Time (h) 48 Mean ±SEM
MOT (%)	EYC	85.82 ±0.71	86.8 ±0.9	33.82 ±6.86	MEDIUM (%)	EYC	10.1 ±0.53	18.94 ±2.26	21.62 ±4.11	ALH (µm)	EYC	7.07 ±0.16	5.39 ±0.23	3.11 ±0.39	VSL (µm/s)	EYC	119 ±1.75	105.9 ±2.54	46.34 ±6.39
	+NC	85.93 ±0.77	85 ±1.15	26.03 ±7.51		+NC	10.9 ±0.64	24.14 ±4.32	13.08 ±2.91		+NC	6.75 ±0.19	5.11 ±0.24	3.07 ±0.36		+NC	116.2 ±1.88	103.2 ±4.19	46.64 ±6.67
	+NSC	86.01 ±0.53	85.9 ±1.00	36.19 ±5.76		+NSC	11.9 ±0.90	26.05 ±4.51	22.01 ±3.16		+NSC	6.88 ±0.13	5.16 ±0.26	3.43 ±0.21		+NSC	113.5 ±2.20	102.9 ±5.36	62.52 ±4.13
	+S	85.92 ±0.51	85.6 ±0.92	29.03 ±4.41		+S	10.9 ±0.76	15.75 ±1.43	18.62 ±2.62		+S	6.94 ±0.14	5.45 ±0.21	3.06 ±0.35		+S	117 ±1.60	109.9 ±2.89	58.66 ±7.69
PROG (%)	EYC	58.11 ±1.02	56.5 ±2.06	10.32 ±3.7	SLOW (%)	EYC	0.04 ±0.03	0.23 ±0.06	0.81 ±0.21	LIN (%)	EYC	67.57 ±0.94	74.23 ±1.03	60.63 ±5.26	VCL (µm/s)	EYC	176.3 ±1.69	143.3 ±3.73	67.73 ±9.08
	+NC	56.71 ±1.15	51.3 ±3.80	10.74 ±4.67		+NC	0.16 ±0.07	0.11 ±0.04	0.67 ±0.14		+NC	67.41 ±0.84	76.56 ±0.95	62.27 ±5.47		+NC	173.9 ±2.16	136.2 ±6.19	66.18 ±9.30
	+NSC	54.56 ±0.92	50.6 ±4.02	12.63 ±3.05		+NSC	0.03 ±0.02	0.17 ±0.08	1.04 ±0.17		+NSC	66.01 ±0.6	74.73 ±1.11	73.27 ±0.95		+NSC	172.4 ±2.62	137.8 ±7.43	84.93 ±4.88
	+S	57.46 ±0.86	56.9 ±1.06	9.24 ±2.02		+S	0.1 ±0.06	0.2 ±0.06	0.83 ±0.22		+S	67.75 ±0.69	73.25 ±0.81	62.13 ±6.86		+S	173.1 ±2.00	150.7 ±4.18	76.91 ±9.26
RAPID (%)	EYC	75.67 ±0.95	67.9 ±2.64	12.22 ±4.40						VAP (µm/s)	EYC	151.3 ±1.36	127.7 ±3.04	57.44 ±7.84	STR (%)	EYC	76.32 ±0.74	81.32 ±0.81	70.54 ±6.14
	+NC	75.05 ±1.00	60.8 ±4.73	12.95 ±5.68							+NC	149.3 ±1.61	121.6 ±5.38	56.27 ±8.24		+NC	75.89 ±0.68	83.91 ±0.89	73.32 ±6.41
	+NSC	74.09 ±0.99	59.8 ±5.00	14.18 ±3.47							+NSC	147.8 ±2.46	122.3 ±6.58	74.44 ±4.62		+NSC	74.66 ±0.58	82.64 ±0.77	83.36 ±0.81
	+S	74.99 ±0.92	69.8 ±1.44	10.41 ±2.27							+S	149 ±1.57	134.1 ±3.41	67.81 ±8.37		+S	76.38 ±0.57	80.16 ±0.56	70.52 ±7.73

Table 6.7. Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S); and stored at 5°C for up to 216h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	72 Mean ±SEM	144 Mean ±SEM	216 Mean ±SEM
MOT (%)	EYC	84.43 ±1.18	87.69 ±0.53	86.62 ±0.63	84.28 ±0.80
	+NC	83.02 ±1.14	87.41 ±0.74	87.25 ±0.64	85.65 ±0.69
	+NSC	84.73 ±0.65	87.32 ±0.48	84.27 ±1.11	83.03 ±1.12
	+S	83.98 ±1.12	88.05 ±0.67	86.64 ±0.61	82.67 ±1.01
PROG (%)	EYC	55.34 ±1.26	57.07 ±1.08	59.4 ±1.57	50.65 ±2.05
	+NC	54.07 ±1.21	59.07 ±1.49	60.26 ±1.06	51.65 ±1.20
	+NSC	54.77 ±0.76	58.62 ±0.87	56.11 ±0.99	49.32 ±1.48
	+S	53.78 ±1.30	53.93 ±1.69	58.41 ±1.00	50.82 ±1.85
RAPID (%)	EYC	72.44 ±1.09	70.85 ±1.39	71.97 ±1.36	59.71 ±2.40
	+NC	71.1 ±0.90	71.29 ±1.78	73.38 ±0.90	63.18 ±1.61
	+NSC	72.28 ±0.93	69.86 ±1.17	67.82 ±1.21	58.87 ±1.75
	+S	69.47 ±1.20	67.46 ±2.50	70.57 ±0.98	60.87 ±1.94

Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	72 Mean ±SEM	144 Mean ±SEM	216 Mean ±SEM
MEDIUM (%)	EYC	11.99 ±0.63	16.84 ±1.07	14.63 ±1.13	24.58 ±2.54
	+NC	11.92 ±0.86	16.12 ±1.51	13.87 ±0.92	22.46 ±1.69
	+NSC	12.46 ±0.62	17.47 ±1.07	16.42 ±0.76	24.18 ±1.29
	+S	14.5 ±0.64	20.57 ±2.20	16.06 ±0.77	21.79 ±1.74
SLOW (%)	EYC	0.08 ±0.04	0.37 ±0.08	0.13 ±0.06	0.21 ±0.07
	+NC	0.1 ±0.04	0.24 ±0.06	0.16 ±0.08	0.21 ±0.08
	+NSC	0.09 ±0.05	0.31 ±0.07	0.33 ±0.10	0.38 ±0.09
	+S	0.16 ±0.08	0.21 ±0.07	0.33 ±0.11	0.37 ±0.11

Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	72 Mean ±Sem	144 Mean ±SEM	216 Mean ±SEM
ALH (µm)	EYC	6.05 ±0.16	5.55 ±0.19	5.76 ±0.10	4.82 ±0.10
	+NC	5.96 ±0.17	5.7 ±0.20	5.71 ±0.13	5.26 ±0.11
	+NSC	6.37 ±0.16	5.36 ±0.22	5.28 ±0.14	4.84 ±0.13
	+S	6.28 ±0.18	5.74 ±0.17	5.5 ±0.13	4.65 ±0.14
LIN (%)	EYC	69.41 ±0.97	72.49 ±0.71	72.96 ±0.68	75.4 ±0.49
	+NC	69.14 ±1.17	73.72 ±1.13	73.29 ±0.77	72.79 ±0.68
	+NSC	67.71 ±0.60	74.79 ±0.97	73.43 ±0.99	74.07 ±0.92
	+S	68.42 ±0.86	71.42 ±0.79	74.09 ±1.09	75.48 ±0.60
VAP (µm/s)	EYC	149.36 ±2.07	131.66 ±2.15	137.4 ±2.24	116.8 ±3.08
	+NC	151.88 ±2.83	133.12 ±2.22	140.2 ±1.42	121.5 ±2.50
	+NSC	147.54 ±1.42	129.96 ±1.58	130.3 ±1.45	119.1 ±2.25
	+S	146.99 ±1.70	126.47 ±2.92	131.9 ±1.26	119.9 ±2.65

Semen characteristic	Diluent	Time (h) 0 Mean ±SEM	72 Mean ±SEM	144 Mean ±SEM	216 Mean ±SEM
VSL (µm/s)	EYC	118.36 ±1.80	107.81 ±1.69	113.66 ±2.32	97.53 ±2.66
	+NC	120.24 ±3.24	110.66 ±1.65	116.06 ±1.68	99.52 ±1.98
	+NSC	114.83 ±1.45	108.78 ±1.26	107.3 ±1.62	98.93 ±2.05
	+S	115.91 ±1.73	102.43 ±2.10	109.84 ±1.22	100.32 ±2.31
VCL (µm/s)	EYC	171.24 ±2.50	149.21 ±2.84	155.03 ±2.50	130.93 ±3.44
	+NC	173.91 ±3.20	150.38 ±3.00	157.38 ±1.55	137.7 ±2.82
	+NSC	171.02 ±1.86	145.56 ±2.32	146.12 ±1.84	133.42 ±2.53
	+S	169.74 ±2.03	143.75 ±3.43	148.62 ±1.99	133.94 ±3.16
STR (%)	EYC	77.08 ±0.82	79.94 ±0.57	80.39 ±0.55	82.17 ±0.44
	+NC	76.78 ±1.03	81.33 ±0.90	80.52 ±0.64	80.36 ±0.56
	+NSC	75.76 ±0.47	81.87 ±0.76	80.28 ±0.80	81 ±0.76
	+S	76.64 ±0.64	79.38 ±0.72	81.39 ±0.86	82.08 ±0.48

Table 6.8. Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S), EYC media with coconut extract (+CE), with nystatin, catalase and coconut extract (NC+CE), with nystatin, sulfanilamide, catalase and coconut extract (NSC+CE), with sulfanilamide and coconut extract (S+CE); and stored at 30°C for up to 48h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h)			Semen characteristic	Diluent	Time (h)		
		0	24	48			0	24	48
		Mean ± SEM	Mean ± SEM	Mean ± SEM			Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	EYC	82.05 ± 1.64	68.79 ± 4.59	27.97 ± 7.00	MEDIUM (%)	EYC	12.61 ± 1.08	17.33 ± 1.14	14.96 ± 3.31
	+NC	81.24 ± 1.31	69.43 ± 3.27	25.21 ± 6.30		+NC	12.31 ± 0.97	20.19 ± 1.66	13.67 ± 2.60
	+NSC	83.14 ± 1.06	67.13 ± 5.54	34.87 ± 5.79		+NSC	11.62 ± 0.90	15.93 ± 1.34	17.52 ± 2.27
	+S	84.25 ± 1.12	65.29 ± 6.36	36.24 ± 7.08		+S	13.17 ± 1.08	15.57 ± 1.72	16.21 ± 2.73
	+CE	81.86 ± 1.79	66.51 ± 6.12	17.78 ± 5.21		+CE	12.04 ± 1.21	21.60 ± 2.29	11.12 ± 2.51
	+NC+CE	82.93 ± 0.87	58.64 ± 6.93	15.79 ± 5.44		+NC+CE	14.50 ± 1.69	19.93 ± 2.62	9.32 ± 2.54
	+NSC+CE	82.81 ± 0.74	60.18 ± 7.59	18.63 ± 7.25		+NSC+CE	16.09 ± 1.54	19.25 ± 3.11	10.32 ± 3.89
	+S+CE	83.69 ± 1.16	61.39 ± 7.29	22.60 ± 5.90		+S+CE	14.12 ± 1.12	17.67 ± 2.07	13.09 ± 3.17
PROG (%)	EYC	57.22 ± 2.42	43.07 ± 4.31	10.08 ± 3.76	SLOW (%)	EYC	0.09 ± 0.05	0.42 ± 0.11	1.83 ± 0.71
	+NC	57.06 ± 1.59	41.66 ± 3.89	9.18 ± 3.46		+NC	0.11 ± 0.06	0.74 ± 0.24	3.72 ± 2.51
	+NSC	59.10 ± 1.75	43.54 ± 4.16	14.57 ± 3.44		+NSC	0.07 ± 0.04	0.63 ± 0.13	1.04 ± 0.34
	+S	58.10 ± 2.34	41.64 ± 5.29	16.92 ± 4.31		+S	0.07 ± 0.05	0.48 ± 0.22	0.77 ± 0.25
	+CE	56.89 ± 2.69	38.27 ± 5.02	5.03 ± 2.82		+CE	0.27 ± 0.10	0.38 ± 0.10	4.45 ± 2.28
	+NC+CE	54.07 ± 2.36	32.77 ± 5.71	4.69 ± 2.93		+NC+CE	0.14 ± 0.09	0.64 ± 0.15	2.17 ± 1.06
	+NSC+CE	55.03 ± 2.82	36.06 ± 5.30	6.57 ± 3.31		+NSC+CE	0.38 ± 0.22	0.43 ± 0.11	1.33 ± 0.48
	+S+CE	57.80 ± 2.33	38.32 ± 5.31	8.04 ± 3.37		+S+CE	0.16 ± 0.08	0.60 ± 0.17	1.44 ± 0.50
RAPID (%)	EYC	69.46 ± 2.01	51.46 ± 5.20	12.98 ± 4.80					
	+NC	68.91 ± 1.28	49.26 ± 4.35	11.53 ± 4.46					
	+NSC	71.52 ± 1.05	51.22 ± 4.96	17.36 ± 4.37					
	+S	71.08 ± 1.39	49.73 ± 6.14	20.04 ± 5.17					
	+CE	69.81 ± 2.29	44.92 ± 5.75	6.68 ± 4.05					
	+NC+CE	68.43 ± 1.80	38.71 ± 6.48	6.48 ± 4.14					
	+NSC+CE	66.71 ± 2.00	40.92 ± 6.01	8.31 ± 4.43					
	+S+CE	69.56 ± 1.63	43.72 ± 6.01	9.53 ± 4.02					

Table 6.8 (continued). Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S), EYC media with coconut extract (+CE), with nystatin, catalase and coconut extract (NC+CE), with nystatin, sulfanilamide, catalase and coconut extract (NSC+CE), with sulfanilamide and coconut extract (S+CE); and stored at 30°C for up to 48h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0	24	48	Semen characteristic	Diluent	Time (h) 0	24	48
		Mean ± SEM	Mean ± SEM	Mean ± SEM			Mean ± SEM	Mean ± SEM	Mean ± SEM
LIVE (%)	EYC	64.06 ± 5.85	40.72 ± 6.42	24.61 ± 8.47	LOOSE HEAD (%)	EYC	0.17 ± 0.12	0.50 ± 0.32	1.72 ± 1.42
	+NC	65.94 ± 4.12	41.95 ± 4.94	28.11 ± 7.46		+NC	0.44 ± 0.20	0.30 ± 0.11	0.22 ± 0.12
	+NSC	68.17 ± 3.90	43.72 ± 4.76	37.83 ± 7.81		+NSC	0.72 ± 0.31	0.33 ± 0.12	0.28 ± 0.12
	+S	59.00 ± 7.72	35.00 ± 6.46	27.00 ± 6.44		+S	0.11 ± 0.07	0.39 ± 0.14	0.72 ± 0.49
	+CE	69.17 ± 5.91	41.89 ± 7.34	28.94 ± 7.33		+CE	0.28 ± 0.09	1.00 ± 0.55	0.56 ± 0.39
	+NC+CE	70.56 ± 4.62	38.06 ± 7.30	27.22 ± 7.58		+NC+CE	0.39 ± 0.16	0.44 ± 0.15	0.44 ± 0.18
	+NSC+CE	64.83 ± 6.08	49.00 ± 8.44	30.50 ± 7.42		+NSC+CE	0.67 ± 0.19	0.28 ± 0.12	0.22 ± 0.12
	+S+CE	61.56 ± 6.79	38.56 ± 7.31	32.78 ± 8.64		+S+CE	0.50 ± 0.19	0.33 ± 0.12	0.22 ± 0.15
DEAD (%)	EYC	35.94 ± 5.85	59.28 ± 6.42	75.39 ± 8.47	COILED TAIL (%)	EYC	3.33 ± 0.84	5.11 ± 1.52	4.39 ± 1.63
	+NC	34.06 ± 4.12	45.85 ± 5.28	71.89 ± 7.46		+NC	3.75 ± 2.14	4.90 ± 0.81	7.56 ± 3.56
	+NSC	31.83 ± 3.90	56.28 ± 4.76	62.17 ± 7.81		+NSC	3.78 ± 1.24	3.89 ± 0.84	5.39 ± 1.96
	+S	28.00 ± 5.07	65.00 ± 6.46	66.00 ± 7.45		+S	3.56 ± 0.98	3.39 ± 0.62	6.28 ± 1.48
	+CE	30.83 ± 5.91	58.11 ± 7.34	71.06 ± 7.33		+CE	4.06 ± 1.34	6.50 ± 2.32	3.83 ± 0.95
	+NC+CE	29.44 ± 4.62	61.94 ± 7.30	72.78 ± 7.58		+NC+CE	3.28 ± 0.98	3.61 ± 0.90	4.33 ± 1.06
	+NSC+CE	35.17 ± 6.08	51.00 ± 8.44	68.50 ± 6.98		+NSC+CE	3.61 ± 0.71	5.94 ± 1.95	5.00 ± 1.36
	+S+CE	38.44 ± 6.79	61.44 ± 7.31	67.22 ± 8.64		+S+CE	3.61 ± 0.65	4.61 ± 0.78	4.11 ± 0.90
NORMAL (%)	EYC	95.78 ± 1.01	93.50 ± 1.69	92.78 ± 3.04	BENT TAIL (%)	EYC	0.72 ± 0.25	0.89 ± 0.34	1.11 ± 0.35
	+NC	94.62 ± 2.40	93.75 ± 1.11	91.17 ± 3.89		+NC	1.19 ± 0.62	1.05 ± 0.34	1.06 ± 0.38
	+NSC	94.22 ± 1.62	94.94 ± 0.92	93.22 ± 2.02		+NSC	1.28 ± 0.53	0.83 ± 0.26	1.11 ± 0.22
	+S	95.06 ± 1.33	95.22 ± 0.78	91.28 ± 1.50		+S	1.50 ± 0.52	1.00 ± 0.24	1.67 ± 0.30
	+CE	94.28 ± 1.79	92.00 ± 2.40	94.39 ± 0.80		+CE	2.50 ± 1.63	1.06 ± 0.27	1.22 ± 0.34
	+NC+CE	95.78 ± 1.35	95.11 ± 1.02	93.44 ± 1.38		+NC+CE	0.56 ± 0.33	0.83 ± 0.29	1.78 ± 0.49
	+NSC+CE	94.83 ± 1.10	92.28 ± 2.17	93.78 ± 1.37		+NSC+CE	0.89 ± 0.37	1.50 ± 0.33	1.00 ± 0.24
	+S+CE	94.94 ± 0.81	94.11 ± 0.90	94.83 ± 0.98		+S+CE	0.94 ± 0.26	0.94 ± 0.28	0.83 ± 0.17

Table 6.8 (continued). Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S), EYC media with coconut extract (+CE), with nystatin, catalase and coconut extract (NC+CE), with nystatin, sulfanilamide, catalase and coconut extract (NSC+CE), with sulfanilamide and coconut extract (S+CE); and stored at 30°C for up to 48h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	24 Mean ± SEM	48 Mean ± SEM	Semen characteristic	Diluent	Time (h) 0 Mean ± SEM	24 Mean ± SEM	48 Mean ± SEM
ALH (µm)	EYC	7.18 ± 0.34	5.94 ± 0.50	3.48 ± 0.55	VSL (µm/s)	EYC	114.56 ± 2.96	94.59 ± 3.66	44.44 ± 6.92
	+NC	7.34 ± 0.39	5.96 ± 0.46	3.35 ± 0.61		+NC	117.79 ± 3.04	94.43 ± 3.57	43.10 ± 6.45
	+NSC	7.28 ± 0.40	5.92 ± 0.54	4.64 ± 0.50		+NSC	113.57 ± 2.54	100.18 ± 2.63	66.37 ± 4.34
	+S	7.28 ± 0.32	5.78 ± 0.55	4.19 ± 0.66		+S	112.90 ± 3.67	97.07 ± 5.50	58.28 ± 8.09
	+CE	7.60 ± 0.32	5.95 ± 0.54	3.52 ± 0.41		+CE	118.01 ± 3.20	86.87 ± 4.78	46.98 ± 6.59
	+NC+CE	7.62 ± 0.40	5.37 ± 0.63	3.29 ± 0.46		+NC+CE	110.99 ± 3.21	78.62 ± 6.82	39.95 ± 6.61
	+NSC+CE	7.29 ± 0.39	5.09 ± 0.52	2.71 ± 0.46		+NSC+CE	110.88 ± 3.41	84.97 ± 6.69	41.59 ± 8.73
	+S+CE	7.08 ± 0.33	5.41 ± 0.65	3.34 ± 0.57		+S+CE	113.33 ± 3.09	89.05 ± 5.84	61.12 ± 8.48
LIN (%)	EYC	68.21 ± 0.70	68.51 ± 1.41	56.19 ± 4.53	VCL (µm/s)	EYC	166.67 ± 3.70	137.16 ± 6.28	73.71 ± 10.13
	+NC	67.92 ± 1.16	68.94 ± 1.22	57.93 ± 5.65		+NC	170.80 ± 3.03	136.58 ± 5.11	66.07 ± 10.39
	+NSC	68.11 ± 1.02	70.46 ± 1.37	65.82 ± 3.31		+NSC	166.52 ± 2.59	141.30 ± 4.75	100.53 ± 5.03
	+S	67.27 ± 0.79	69.03 ± 1.37	54.97 ± 6.81		+S	166.42 ± 3.50	138.81 ± 7.62	87.04 ± 11.16
	+CE	67.26 ± 0.66	67.27 ± 1.20	64.24 ± 3.22		+CE	172.88 ± 3.65	127.44 ± 7.45	69.85 ± 8.70
	+NC+CE	64.97 ± 0.89	67.42 ± 1.50	57.82 ± 6.64		+NC+CE	169.20 ± 3.42	115.36 ± 9.97	61.13 ± 8.92
	+NSC+CE	66.42 ± 1.01	69.99 ± 1.83	59.46 ± 6.03		+NSC+CE	164.23 ± 3.68	120.09 ± 9.09	59.81 ± 10.72
	+S+CE	68.13 ± 0.93	70.32 ± 1.43	69.71 ± 3.20		+S+CE	163.65 ± 2.75	125.46 ± 8.91	87.01 ± 10.49
VAP (µm/s)	EYC	142.36 ± 3.23	117.54 ± 5.25	58.92 ± 8.73	STR (%)	EYC	77.76 ± 0.67	78.22 ± 1.03	69.22 ± 4.46
	+NC	146.43 ± 2.80	116.61 ± 4.02	53.56 ± 8.47		+NC	77.22 ± 0.79	78.63 ± 0.95	70.41 ± 6.25
	+NSC	142.47 ± 2.47	121.86 ± 3.48	82.62 ± 4.91		+NSC	77.60 ± 0.73	79.82 ± 0.89	78.49 ± 2.14
	+S	141.92 ± 3.32	119.64 ± 6.28	71.85 ± 9.68		+S	76.80 ± 0.76	78.64 ± 1.19	64.92 ± 7.35
	+CE	147.76 ± 3.21	107.50 ± 5.80	58.98 ± 7.92		+CE	76.93 ± 0.75	78.21 ± 1.05	76.94 ± 2.34
	+NC+CE	142.18 ± 2.94	97.60 ± 8.18	49.09 ± 8.03		+NC+CE	75.09 ± 0.79	78.22 ± 1.20	69.93 ± 6.83
	+NSC+CE	139.53 ± 3.26	104.05 ± 7.69	51.03 ± 9.94		+NSC+CE	76.46 ± 0.81	79.36 ± 1.28	70.53 ± 6.47
	+S+CE	140.99 ± 2.73	107.48 ± 7.21	74.83 ± 9.86		+S+CE	77.34 ± 0.80	80.58 ± 0.97	79.37 ± 2.07

Table 6.9. Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S), EYC media with coconut extract (+CE), with nystatin, catalase and coconut extract (NC+CE), with nystatin, sulfanilamide, catalase and coconut extract (NSC+CE), with sulfanilamide and coconut extract (S+CE); and stored at 5°C for up to 216h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0	72	144	216	Semen characteristic	Diluent	Time (h) 0	72	144	216
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	EYC	73.15 ± 1.83	77.67 ± 2.1	80.28 ± 1.19	62.76 ± 4.19	MEDIUM (%)	EYC	14.21 ± 1.25	17.83 ± 1.4	18.95 ± 1.04	20.91 ± 1.2
	+NC	72.64 ± 2.19	77.29 ± 1.94	79.39 ± 1.9	60.4 ± 4.15		+NC	14.39 ± 1.84	17.94 ± 1.19	19.62 ± 0.88	18.38 ± 1.17
	+NSC	75.91 ± 2.95	78.99 ± 1.91	80.01 ± 1.47	64.91 ± 2.12		+NSC	13.77 ± 1.32	19.47 ± 1.36	22.89 ± 1.47	23.55 ± 1.91
	+S	70.98 ± 2.14	78.51 ± 1.6	78.93 ± 1.28	63.08 ± 3.32		+S	13.64 ± 1.32	21.13 ± 1.63	21.07 ± 1.74	21.96 ± 1.57
	+CE	73.1 ± 1.97	77.57 ± 1.87	78.65 ± 1.96	62.27 ± 3.25		+CE	11.68 ± 0.98	17.01 ± 1.23	21.56 ± 1.45	25.3 ± 2.18
	+NC+CE	75.39 ± 2.14	77.59 ± 1.97	76.15 ± 2.75	57.49 ± 4.55		+NC+CE	11.92 ± 0.63	17.71 ± 1.18	19.34 ± 1.12	22.03 ± 1.84
	+NSC+CE	76 ± 1.76	79.68 ± 1.53	79.94 ± 1.42	65.06 ± 2.59		+NSC+CE	14.36 ± 1.17	18.43 ± 1.23	21 ± 0.97	27.77 ± 1.71
	+S+CE	70.91 ± 2.45	78.83 ± 1.87	79.64 ± 1.64	64.38 ± 3.26		+S+CE	14.57 ± 1.25	19.69 ± 1.29	20.94 ± 0.91	26.36 ± 1.5
PROG (%)	EYC	47.76 ± 3.02	49.92 ± 2.75	50.2 ± 1.76	35.71 ± 3.84	SLOW (%)	EYC	0.34 ± 0.17	0.5 ± 0.26	0.4 ± 0.24	2.29 ± 0.92
	+NC	47.38 ± 3.21	48.08 ± 2.67	49.71 ± 2.09	36.44 ± 3.61		+NC	0.35 ± 0.16	0.23 ± 0.17	0.56 ± 0.3	1.49 ± 0.51
	+NSC	48.14 ± 3.71	49.19 ± 3.28	47.12 ± 2.78	36.01 ± 2.03		+NSC	0.12 ± 0.07	0.3 ± 0.09	0.82 ± 0.27	1.19 ± 0.33
	+S	45.28 ± 3.69	48.09 ± 3.14	48.09 ± 2.58	35.56 ± 3.36		+S	0.29 ± 0.18	0.63 ± 0.25	0.58 ± 0.15	0.89 ± 0.26
	+CE	49.57 ± 3.38	49.2 ± 2.05	46.23 ± 2.44	30.81 ± 3.32		+CE	0.11 ± 0.06	0.5 ± 0.26	0.71 ± 0.34	2.23 ± 0.83
	+NC+CE	49.41 ± 3.42	48.51 ± 2.75	46.88 ± 2.49	29.82 ± 3.49		+NC+CE	0.12 ± 0.07	0.33 ± 0.12	1.38 ± 0.57	1.91 ± 0.74
	+NSC+CE	48.57 ± 3.02	49.43 ± 2.43	48.81 ± 2.09	32.22 ± 2.32		+NSC+CE	0.29 ± 0.15	0.34 ± 0.13	0.72 ± 0.26	1.29 ± 0.39
	+S+CE	44.89 ± 2.82	48.44 ± 2.36	47.28 ± 2.09	32.26 ± 2.24		+S+CE	0.52 ± 0.19	0.22 ± 0.12	0.86 ± 0.38	1.37 ± 0.54
RAPID (%)	EYC	58.95 ± 2.55	59.84 ± 2.37	61.32 ± 1.77	41.88 ± 4.47						
	+NC	58.26 ± 2.78	59.36 ± 2.18	59.78 ± 2.28	42.04 ± 3.97						
	+NSC	62.13 ± 3.68	59.53 ± 2.92	57.11 ± 2.58	41.36 ± 1.54						
	+S	57.35 ± 3.12	57.39 ± 2.88	57.85 ± 2.37	41.14 ± 3.21						
	+CE	61.42 ± 2.41	60.58 ± 1.92	57.07 ± 2.21	36.98 ± 3.88						
	+NC+CE	63.46 ± 2.68	59.88 ± 2.28	56.81 ± 2.93	35.47 ± 3.87						
	+NSC+CE	61.65 ± 2.55	61.27 ± 1.89	58.94 ± 1.77	37.26 ± 2.11						
	+S+CE	56.32 ± 3.11	59.14 ± 2.3	58.69 ± 2.12	38.02 ± 2.52						

Table 6.9 (continued). Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S), EYC media with coconut extract (+CE), with nystatin, catalase and coconut extract (NC+CE), with nystatin, sulfanilamide, catalase and coconut extract (NSC+CE), with sulfanilamide and coconut extract (S+CE); and stored at 5°C for up to 216h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0	72	144	216	Semen characteristic	Diluent	Time (h) 0	72	144	216
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LIVE (%)	EYC	54.50 ± 5.30	54.67 ± 5.50	38.61 ± 6.28	37.28 ± 5.92	LOOSE HEAD (%)	EYC	1.06 ± 0.52	1.78 ± 0.63	1.11 ± 0.51	1.33 ± 0.55
	+NC	57.94 ± 5.27	50.06 ± 5.88	38.83 ± 5.60	33.44 ± 4.06		+NC	0.72 ± 0.38	2.17 ± 0.98	3.00 ± 1.57	1.28 ± 0.63
	+NSC	56.12 ± 7.23	48.89 ± 5.73	36.39 ± 5.24	28.33 ± 4.28		+NSC	0.63 ± 0.28	0.67 ± 0.30	1.06 ± 0.50	2.17 ± 0.77
	+S	43.75 ± 4.64	47.22 ± 6.47	37.44 ± 5.71	27.22 ± 4.13		+S	1.12 ± 0.91	1.78 ± 0.72	1.78 ± 0.76	1.56 ± 0.73
	+CE	56.81 ± 8.07	51.89 ± 6.41	30.89 ± 5.99	32.22 ± 6.03		+CE	0.50 ± 0.30	1.67 ± 0.81	1.22 ± 0.54	1.33 ± 0.69
	+NC+CE	61.81 ± 6.41	47.06 ± 5.63	34.83 ± 5.21	32.28 ± 5.53		+NC+CE	1.62 ± 1.07	1.00 ± 0.30	1.33 ± 0.43	0.94 ± 0.66
	+NSC+CE	54.33 ± 7.49	55.78 ± 6.87	35.61 ± 5.92	36.44 ± 5.08		+NSC+CE	1.89 ± 0.84	1.39 ± 0.75	1.33 ± 0.60	1.61 ± 0.85
	+S+CE	52.00 ± 5.98	47.89 ± 6.86	38.61 ± 5.46	32.00 ± 2.40		+S+CE	0.78 ± 0.51	1.17 ± 0.38	2.78 ± 2.05	3.11 ± 1.28
DEAD (%)	EYC	45.50 ± 5.30	45.33 ± 5.50	61.39 ± 6.28	62.78 ± 5.92	COILED TAIL (%)	EYC	4.94 ± 0.93	3.11 ± 1.18	2.28 ± 0.77	3.72 ± 0.80
	+NC	42.06 ± 5.27	49.94 ± 5.88	61.17 ± 5.60	66.56 ± 4.06		+NC	4.00 ± 0.76	2.11 ± 0.84	2.50 ± 0.92	3.44 ± 0.62
	+NSC	43.88 ± 7.23	51.11 ± 5.73	63.61 ± 5.24	71.67 ± 4.28		+NSC	3.06 ± 0.69	4.11 ± 0.73	2.89 ± 0.44	3.22 ± 0.76
	+S	56.25 ± 4.64	52.78 ± 6.47	62.56 ± 5.71	72.22 ± 4.33		+S	3.44 ± 1.29	3.33 ± 1.02	3.39 ± 0.97	2.83 ± 0.51
	+CE	43.19 ± 8.07	48.11 ± 6.41	69.11 ± 5.99	67.78 ± 6.03		+CE	2.50 ± 0.38	3.39 ± 0.88	3.94 ± 0.91	5.56 ± 1.32
	+NC+CE	38.19 ± 6.41	52.94 ± 5.63	65.17 ± 5.21	67.72 ± 5.53		+NC+CE	4.50 ± 0.66	2.94 ± 1.02	4.06 ± 1.25	3.78 ± 0.63
	+NSC+CE	45.67 ± 7.49	44.22 ± 6.87	64.39 ± 5.92	63.56 ± 5.08		+NSC+CE	3.22 ± 0.89	2.94 ± 0.73	3.00 ± 0.99	3.00 ± 1.10
	+S+CE	48.00 ± 5.98	52.11 ± 6.86	61.37 ± 5.47	68.00 ± 2.40		+S+CE	3.83 ± 1.07	2.72 ± 0.68	2.72 ± 0.64	4.22 ± 1.27
NORMAL (%)	EYC	92.67 ± 1.57	94.39 ± 1.77	96.00 ± 1.33	83.22 ± 9.79	BENT TAIL (%)	EYC	1.33 ± 0.47	0.72 ± 0.26	0.61 ± 0.22	1.44 ± 0.39
	+NC	94.61 ± 0.88	94.44 ± 1.85	93.06 ± 2.69	83.72 ± 9.88		+NC	0.67 ± 0.19	0.72 ± 0.38	1.44 ± 0.58	1.28 ± 0.47
	+NSC	95.25 ± 1.03	94.50 ± 1.09	95.28 ± 0.92	93.67 ± 1.46		+NSC	1.06 ± 0.32	0.72 ± 0.32	0.78 ± 0.25	0.94 ± 0.36
	+S	94.25 ± 1.64	94.22 ± 1.43	93.94 ± 1.65	94.67 ± 1.00		+S	1.19 ± 0.35	0.67 ± 0.22	0.89 ± 0.32	0.94 ± 0.26
	+CE	96.06 ± 0.49	93.89 ± 1.42	93.89 ± 1.56	91.89 ± 1.89		+CE	0.94 ± 0.20	0.94 ± 0.36	0.94 ± 0.42	1.22 ± 0.38
	+NC+CE	92.75 ± 1.09	95.06 ± 1.33	93.61 ± 2.03	94.67 ± 1.27		+NC+CE	1.12 ± 0.18	1.00 ± 0.33	1.00 ± 0.51	0.61 ± 0.16
	+NSC+CE	93.78 ± 1.66	94.83 ± 1.50	94.94 ± 1.79	94.39 ± 1.64		+NSC+CE	1.11 ± 0.26	0.83 ± 0.30	0.72 ± 0.32	1.00 ± 0.24
	+S+CE	93.78 ± 1.37	95.44 ± 1.19	94.00 ± 2.44	91.83 ± 2.04		+S+CE	1.61 ± 0.25	0.67 ± 0.34	0.56 ± 0.18	0.83 ± 0.26

Table 6.9 (continued). Motion characteristics of ram spermatozoa in egg yolk citrate (EYC) media without addition, with nystatin and catalase (+NC), with nystatin, sulfanilamide and catalase (+NSC), with sulfanilamide (+S), EYC media with coconut extract (+CE), with nystatin, catalase and coconut extract (NC+CE), with nystatin, sulfanilamide, catalase and coconut extract (NSC+CE), with sulfanilamide and coconut extract (S+CE); and stored at 5°C for up to 216h (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time (h) 0	72	144	216	Semen characteristic	Diluent	Time (h) 0	72	144	216
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
ALH (µm)	EYC	6.7 ± 0.36	5.95 ± 0.34	5.99 ± 0.18	4.86 ± 0.3	VSL (µm/s)	EYC	118.82 ± 5.4	104.71 ± 2.2	99.49 ± 1.76	89.25 ± 3.19
	+NC	7.12 ± 0.34	6.02 ± 0.3	5.97 ± 0.23	4.84 ± 0.24		+NC	117.89 ± 4.76	101.98 ± 1.85	99.22 ± 1.52	90.28 ± 3.02
	+NSC	7.26 ± 0.57	6.19 ± 0.36	5.86 ± 0.21	4.86 ± 0.28		+NSC	110.44 ± 3.88	96.49 ± 2.72	92.59 ± 2.98	89.3 ± 2.53
	+S	6.36 ± 0.43	6.02 ± 0.29	6 ± 0.25	4.91 ± 0.3		+S	108.91 ± 4.56	97.33 ± 2.92	96.93 ± 2.84	89.98 ± 2.35
	+CE	7.18 ± 0.39	6.39 ± 0.28	5.67 ± 0.23	4.74 ± 0.29		+CE	123.89 ± 4.99	105.05 ± 2.12	96.25 ± 2.24	84.16 ± 4.24
	+NC+CE	7.06 ± 0.41	6.14 ± 0.26	5.73 ± 0.16	4.47 ± 0.26		+NC+CE	116.4 ± 3.45	103.31 ± 2.32	101.34 ± 2.17	81.23 ± 5.34
	+NSC+CE	7.29 ± 0.37	6.13 ± 0.28	6.13 ± 0.2	4.83 ± 0.28		+NSC+CE	114.04 ± 3.18	101.01 ± 2.29	97.98 ± 1.27	82.31 ± 1.75
	+S+CE	6.89 ± 0.37	6.09 ± 0.34	6.09 ± 0.27	4.84 ± 0.3		+S+CE	116.24 ± 3.95	100.24 ± 2.49	95.59 ± 2.07	83.49 ± 1.84
LIN (%)	EYC	67.63 ± 1.03	69.5 ± 0.82	68.57 ± 1	71.59 ± 1.19	VCL (µm/s)	EYC	171.02 ± 5.2	148.17 ± 3.03	143.06 ± 2.65	122.78 ± 4.64
	+NC	66.4 ± 0.87	68.85 ± 1.04	68.64 ± 0.7	72.54 ± 1.27		+NC	173.54 ± 5.33	146.81 ± 2.41	141.84 ± 3.05	121.92 ± 4.12
	+NSC	63.65 ± 1.16	67.66 ± 1.06	67.28 ± 1.3	71.92 ± 1.33		+NSC	171.75 ± 5.16	141.2 ± 2.94	135.02 ± 2.92	121.41 ± 2.93
	+S	66.01 ± 1.17	68.54 ± 1.44	68.04 ± 1.36	71.19 ± 1.45		+S	164.38 ± 5.7	139.35 ± 2.17	139.57 ± 2.31	123.33 ± 2.57
	+CE	67.73 ± 0.96	67.91 ± 0.9	68.54 ± 1.18	70.07 ± 1.22		+CE	180.78 ± 4.5	152.77 ± 3.01	139.09 ± 2.82	118.57 ± 5.96
	+NC+CE	65.5 ± 0.77	67.88 ± 1.02	70.01 ± 1.11	69.91 ± 1.35		+NC+CE	176.44 ± 3.54	150.65 ± 2.78	141.9 ± 2.85	113.08 ± 6.93
	+NSC+CE	64.66 ± 0.75	67.91 ± 0.96	67.53 ± 0.83	69.1 ± 1.05		+NSC+CE	172.61 ± 3.86	147.61 ± 2.66	141.83 ± 2.14	117.07 ± 2.36
	+S+CE	66.02 ± 0.69	67.94 ± 0.8	66.86 ± 1.12	69.91 ± 1.16		+S+CE	170.84 ± 4.45	145.58 ± 3.92	140.59 ± 3.35	117.64 ± 2.99
VAP (µm/s)	EYC	148.16 ± 4.87	129.99 ± 2.35	124.6 ± 2.16	107.9 ± 3.93	STR (%)	EYC	76.62 ± 1.02	77.74 ± 0.8	77.14 ± 0.95	80.19 ± 1.04
	+NC	148.52 ± 4.74	128.17 ± 1.88	123.5 ± 2.44	108.3 ± 3.81		+NC	76.04 ± 0.85	77.4 ± 1.13	77.35 ± 0.82	81.04 ± 0.96
	+NSC	145.06 ± 3.94	121.91 ± 2.79	116.9 ± 3.07	107.39 ± 2.61		+NSC	73.21 ± 0.91	76.73 ± 0.86	76.47 ± 1.13	80.22 ± 1.13
	+S	141.34 ± 4.62	120.7 ± 2.15	120.9 ± 2.15	109.06 ± 2.16		+S	74.64 ± 1.25	77.44 ± 1.27	77.03 ± 1.24	79.51 ± 1.18
	+CE	155.43 ± 3.93	131.91 ± 2.27	121.6 ± 2.15	103.23 ± 5.31		+CE	76.82 ± 1.09	76.75 ± 0.86	76.7 ± 1.15	79.05 ± 0.97
	+NC+CE	150.84 ± 2.74	130.83 ± 2.31	124.6 ± 2.35	99.78 ± 6.41		+NC+CE	74.36 ± 0.73	76.47 ± 1.08	78.28 ± 1.09	78.53 ± 0.95
	+NSC+CE	146.86 ± 3.3	127.96 ± 2.48	122.8 ± 1.61	101.29 ± 2.25		+NSC+CE	74.11 ± 0.82	76.71 ± 0.86	76.53 ± 0.79	78.13 ± 0.79
	+S+CE	147.02 ± 3.91	126.52 ± 3.34	121.4 ± 2.76	102.83 ± 2.7		+S+CE	75.3 ± 0.8	76.59 ± 0.68	75.93 ± 0.96	78.59 ± 0.85

Table 7.3. Characteristics of ram semen stored at 30°C in 0Y-C (0% egg yolk/Y with no coconut extract), 5Y-C (5%Y), 10Y-C (10% Y), 15Y-C (15% Y), 20Y-C (20% Y), 0Y+C (0% egg yolk/Y+15% coconut extract/C), 5Y+C (5% Y+15% C), 10Y+C (10% Y+15% C),15Y+C (15% Y+15% C) and 20Y+C (20% Y+15% C) diluent for 24h. (Mean ± SEM, n=4).

Semen charac-teristic	Diluent	Time of storage at 30°C		
		0h	24h	48h
		Mean ± Sem	Mean ± Sem	Mean ± Sem
MOT (%)	0Y-C	79.15 ± 1.65	76.45 ± 1.46	68.53 ± 1.39
	5Y-C	82.20 ± 1.23	76.57 ± 0.41	78.12 ± 1.18
	10Y-C	83.68 ± 1.03	80.10 ± 2.31	0.40 ± 0.24
	15Y-C	84.65 ± 0.79	80.68 ± 1.82	0.00 ± 0.00
	20Y-C	84.03 ± 1.72	76.53 ± 2.19	0.00 ± 0.00
	0Y+C	79.87 ± 1.11	75.75 ± 1.61	60.25 ± 4.19
	5Y+C	83.00 ± 1.13	75.10 ± 1.29	44.95 ± 1.15
	10Y+C	80.48 ± 2.24	73.57 ± 0.55	53.97 ± 1.60
	15Y+C	80.02 ± 1.46	73.98 ± 0.54	44.65 ± 0.82
	20Y+C	85.45 ± 1.86	69.60 ± 0.88	10.65 ± 1.35
PROG (%)	0Y-C	58.27 ± 1.19	58.70 ± 2.08	54.03 ± 1.35
	5Y-C	60.92 ± 2.01	55.63 ± 1.06	61.08 ± 0.37
	10Y-C	59.45 ± 1.35	58.42 ± 2.38	0.28 ± 0.19
	15Y-C	62.35 ± 0.73	65.00 ± 1.08	0.00 ± 0.00
	20Y-C	62.83 ± 1.95	51.47 ± 4.51	0.00 ± 0.00
	0Y+C	61.80 ± 0.79	58.65 ± 1.60	50.88 ± 2.96
	5Y+C	62.10 ± 0.57	56.00 ± 1.13	35.78 ± 1.11
	10Y+C	59.67 ± 1.64	55.75 ± 0.90	43.42 ± 1.27
	15Y+C	57.45 ± 1.28	54.82 ± 0.88	35.8 ± 1.78
	20Y+C	59.22 ± 1.35	47.72 ± 1.14	6.32 ± 0.39
RAPID (%)	0Y-C	78.33 ± 1.66	75.20 ± 1.64	66.18 ± 1.56
	5Y-C	80.60 ± 1.33	74.80 ± 0.50	74.62 ± 0.43
	10Y-C	82.40 ± 1.23	78.82 ± 2.38	0.28 ± 0.19
	15Y-C	83.05 ± 0.63	78.10 ± 2.28	0.00 ± 0.00
	20Y-C	82.72 ± 1.92	70.37 ± 5.18	0.00 ± 0.00
	0Y+C	78.93 ± 1.04	73.43 ± 1.64	57.13 ± 3.52
	5Y+C	80.55 ± 1.17	70.95 ± 0.84	40.63 ± 0.76
	10Y+C	77.07 ± 2.16	69.90 ± 0.72	49.80 ± 1.92
	15Y+C	76.87 ± 2.15	69.78 ± 0.52	40.78 ± 1.34
	20Y+C	80.73 ± 2.92	62.40 ± 0.64	8.63 ± 1.09
MEDIUM (%)	0Y-C	0.85 ± 0.10	1.23 ± 0.21	2.37 ± 0.49
	5Y-C	1.62 ± 0.53	1.80 ± 0.45	3.53 ± 0.91
	10Y-C	1.27 ± 0.22	1.25 ± 0.55	0.13 ± 0.08
	15Y-C	1.62 ± 0.25	2.58 ± 0.86	0.00 ± 0.00
	20Y-C	1.27 ± 0.30	6.15 ± 3.54	0.00 ± 0.00
	0Y+C	0.95 ± 0.18	2.33 ± 0.85	3.10 ± 0.74
	5Y+C	2.45 ± 0.17	4.18 ± 0.46	4.28 ± 0.62
	10Y+C	3.37 ± 0.61	3.67 ± 0.43	4.15 ± 0.33
	15Y+C	3.15 ± 0.71	4.18 ± 0.52	3.92 ± 0.73
	20Y+C	4.75 ± 1.43	7.20 ± 0.60	1.98 ± 0.34

Table 7.3.(continued). Characteristics of ram semen stored at 30°C in 0Y-C (0% egg yolk/Y with no coconut extract), 5Y-C (5%Y), 10Y-C (10% Y), 15Y-C (15% Y), 20Y-C (20% Y), 0Y+C (0% egg yolk/Y+15% coconut extract/C), 5Y+C (5% Y+15% C), 10Y+C (10% Y+15% C),15Y+C (15% Y+15% C) and 20Y+C (20% Y+15% C) diluent for 24h. (Mean ± SEM, n=4).

Semen charac-teristic	Diluent	Time of storage at 30°C		
		0h	24h	48h
		Mean ± Sem	Mean ± Sem	Mean ± Sem
SLOW (%)	0Y-C	0.05 ± 0.05	0.00 ± 0.00	0.60 ± 0.25
	5Y-C	0.00 ± 0.00	0.17 ± 0.10	0.65 ± 0.19
	10Y-C	0.18 ± 0.06	0.13 ± 0.13	0.00 ± 0.00
	15Y-C	0.05 ± 0.05	0.43 ± 0.05	0.00 ± 0.00
	20Y-C	0.28 ± 0.17	0.58 ± 0.17	0.00 ± 0.00
	0Y+C	0.23 ± 0.09	0.45 ± 0.18	0.90 ± 0.18
	5Y+C	0.15 ± 0.10	0.45 ± 0.26	1.37 ± 0.31
	10Y+C	0.22 ± 0.05	0.60 ± 0.20	1.40 ± 0.20
	15Y+C	0.48 ± 0.16	0.30 ± 0.11	1.33 ± 0.19
	20Y+C	0.32 ± 0.12	0.40 ± 0.09	0.85 ± 0.28
ALH (μm)	0Y-C	6.65 ± 0.23	6.82 ± 0.17	6.25 ± 0.21
	5Y-C	6.10 ± 0.31	6.58 ± 0.39	5.55 ± 0.22
	10Y-C	6.72 ± 0.16	7.12 ± 0.42	2.00 ± 1.15
	15Y-C	6.50 ± 0.23	6.70 ± 0.06	0.00 ± 0.00
	20Y-C	6.30 ± 0.16	6.32 ± 0.43	0.00 ± 0.00
	0Y+C	6.28 ± 0.29	6.12 ± 0.31	4.70 ± 0.43
	5Y+C	6.27 ± 0.12	5.32 ± 0.18	4.93 ± 0.15
	10Y+C	6.37 ± 0.19	5.57 ± 0.41	5.30 ± 0.30
	15Y+C	6.53 ± 0.62	5.60 ± 0.25	4.87 ± 0.18
	20Y+C	6.23 ± 0.15	4.95 ± 0.13	4.95 ± 0.47
LIN (%)	0Y-C	68.28 ± 0.91	70.80 ± 2.43	71.53 ± 1.60
	5Y-C	69.03 ± 1.28	68.22 ± 1.50	73.72 ± 0.93
	10Y-C	66.20 ± 1.62	66.22 ± 2.60	61.72 ± 21.39
	15Y-C	67.53 ± 0.98	72.85 ± 1.34	0.00 ± 0.00
	20Y-C	68.25 ± 1.39	65.25 ± 2.59	0.00 ± 0.00
	0Y+C	67.75 ± 1.48	71.55 ± 0.20	76.68 ± 1.21
	5Y+C	69.10 ± 0.43	72.20 ± 0.79	77.22 ± 1.01
	10Y+C	68.00 ± 1.71	71.62 ± 1.73	77.95 ± 1.18
	15Y+C	66.90 ± 1.63	71.65 ± 1.19	78.85 ± 1.95
	20Y+C	63.32 ± 2.04	68.85 ± 0.97	61.40 ± 3.32
VAP (μm/s)	0Y-C	142.60 ± 1.77	136.65 ± 1.77	121.18 ± 3.85
	5Y-C	135.35 ± 1.28	131.15 ± 2.83	121.12 ± 1.37
	10Y-C	130.60 ± 4.48	134.80 ± 2.6	53.30 ± 26.56
	15Y-C	136.75 ± 0.78	143.48 ± 2.36	0.00 ± 0.00
	20Y-C	134.98 ± 6.09	126.50 ± 15.6	0.00 ± 0.00
	0Y+C	118.15 ± 9.96	135.40 ± 2.35	102.93 ± 10.82
	5Y+C	128.42 ± 4.59	135.30 ± 0.92	113.98 ± 4.74
	10Y+C	131.68 ± 5.88	139.55 ± 3.78	136.50 ± 0.55
	15Y+C	127.82 ± 4.63	136.75 ± 1.92	136.55 ± 4.24
	20Y+C	110.03 ± 4.24	120.02 ± 3.55	77.70 ± 4.72

Table 7.3.(continued). Characteristics of ram semen stored at 30°C in 0Y-C (0% egg yolk/Y with no coconut extract), 5Y-C (5%Y), 10Y-C (10% Y), 15Y-C (15% Y), 20Y-C (20% Y), 0Y+C (0% egg yolk/Y+15% coconut extract/C), 5Y+C (5% Y+15% C), 10Y+C (10% Y+15% C),15Y+C (15% Y+15% C) and 20Y+C (20% Y+15% C) diluent for 24h. (Mean ± SEM, n=4).

Semen charac-teristic	Diluent	Time of storage at 30°C		
		0h	24h	48h
		Mean ± Sem	Mean ± Sem	Mean ± Sem
VSL (µm/s)	0Y-C	113.30 ± 1.80	111.35 ± 3.58	100.12 ± 3.85
	5Y-C	107.42 ± 0.70	103.40 ± 0.77	102.12 ± 1.22
	10Y-C	100.52 ± 5.82	105.55 ± 4.77	50.53 ± 25.95
	15Y-C	107.07 ± 1.15	120.07 ± 4.19	0.00 ± 0.00
	20Y-C	106.35 ± 5.89	99.15 ± 13.85	0.00 ± 0.00
	0Y+C	94.12 ± 7.84	110.72 ± 2.38	89.95 ± 9.01
	5Y+C	102.22 ± 3.93	111.28 ± 1.29	100.68 ± 4.31
	10Y+C	105.70 ± 5.93	114.47 ± 4.84	120.70 ± 0.79
	15Y+C	100.83 ± 2.90	111.85 ± 2.72	121.92 ± 5.23
	20Y+C	85.40 ± 4.86	97.45 ± 3.37	60.27 ± 7.57
VCL (µm/s)	0Y-C	164.77 ± 2.28	156.80 ± 1.81	139.27 ± 3.74
	5Y-C	155.52 ± 2.05	150.42 ± 4.40	135.62 ± 2.23
	10Y-C	153.20 ± 4.91	158.73 ± 2.72	57.95 ± 26.98
	15Y-C	159.17 ± 1.32	162.35 ± 1.82	0.00 ± 0.00
	20Y-C	155.40 ± 6.86	146.37 ± 16.72	0.00 ± 0.00
	0Y+C	139.48 ± 9.24	153.32 ± 2.99	116.20 ± 11.37
	5Y+C	148.65 ± 5.02	150.77 ± 0.44	126.70 ± 4.25
	10Y+C	153.15 ± 4.93	157.20 ± 3.31	149.3 ± 1.58
	15Y+C	149.28 ± 6.09	154.30 ± 1.94	148.60 ± 3.32
	20Y+C	131.60 ± 2.23	135.83 ± 3.94	99.68 ± 5.53
STR (%)	0Y-C	76.50 ± 0.68	79.25 ± 2.16	80.57 ± 1.03
	5Y-C	77.22 ± 1.04	76.55 ± 1.08	81.43 ± 0.60
	10Y-C	75.00 ± 1.56	75.55 ± 1.95	70.28 ± 23.51
	15Y-C	76.22 ± 0.59	81.37 ± 1.25	0.00 ± 0.00
	20Y-C	76.60 ± 1.22	74.43 ± 1.80	0.00 ± 0.00
	0Y+C	78.50 ± 0.85	79.57 ± 0.41	85.82 ± 1.56
	5Y+C	77.57 ± 0.30	78.87 ± 0.77	85.15 ± 1.18
	10Y+C	77.57 ± 0.79	78.93 ± 1.28	84.75 ± 0.91
	15Y+C	76.03 ± 1.35	78.92 ± 0.92	85.42 ± 1.42
	20Y+C	74.47 ± 0.90	77.48 ± 0.66	75.70 ± 3.68

Table 7.4.(continued). Characteristics of ram semen stored at 5°C in 0Y-C (0% egg yolk/Y with no coconut extract), 5Y-C (5%Y), 10Y-C (10% Y), 15Y-C (15% Y), 20Y-C (20% Y), 0Y+C (0% egg yolk/Y+15% coconut extract/C), 5Y+C (5% Y+15% C), 10Y+C (10% Y+15% C),15Y+C (15% Y+15% C) and 20Y+C (20% Y+15% C) diluent for 240h. (Mean ± SEM, n=4).

Semen charac-teristic	Diluent	Time of storage at 5°C					
		0h	48h	96h	144h	192h	240h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
VSL	0Y-C	102.95 ± 13.61	97.35 ± 1.91	80.18 ± 2.48	48.82 ± 5.80	22.90 ± 2.17	17.67 ± 5.00
(µm/s)	5Y-C	109.20 ± 1.71	78.05 ± 3.58	95.95 ± 2.48	93.20 ± 7.34	75.60 ± 2.04	32.30 ± 4.47
	10Y-C	105.90 ± 3.68	103.62 ± 3.71	102.90 ± 2.83	89.02 ± 2.86	56.08 ± 3.99	31.25 ± 2.54
	15Y-C	103.67 ± 0.88	112.17 ± 2.82	100.18 ± 6.24	87.03 ± 5.14	59.97 ± 7.75	0.00 ± 0.00
	20Y-C	105.57 ± 1.90	104.20 ± 1.68	102.60 ± 1.99	94.35 ± 2.57	79.72 ± 20.74	79.40 ± 31.97
	0Y+C	113.97 ± 1.25	119.05 ± 3.23	84.98 ± 4.70	83.12 ± 2.94	47.38 ± 2.45	30.83 ± 1.68
	5Y+C	103.82 ± 3.93	108.57 ± 3.76	109.95 ± 3.60	100.50 ± 2.48	78.25 ± 6.33	75.45 ± 4.71
	10Y+C	113.03 ± 5.32	109.18 ± 2.22	115.70 ± 1.51	96.05 ± 4.28	78.70 ± 5.64	66.87 ± 4.95
	15Y+C	107.28 ± 5.14	106.68 ± 7.32	104.48 ± 1.55	98.75 ± 1.37	68.03 ± 5.19	89.22 ± 2.80
	20Y+C	89.27 ± 2.81	85.48 ± 5.67	98.68 ± 4.64	85.15 ± 4.57	49.92 ± 2.72	59.40 ± 3.14
VCL	0Y-C	144.57 ± 17.25	147.80 ± 1.40	93.10 ± 2.33	70.97 ± 3.88	36.83 ± 3.30	32.18 ± 5.58
(µm/s)	5Y-C	157.80 ± 4.93	119.23 ± 4.47	128.70 ± 2.52	121.92 ± 5.08	93.55 ± 4.21	64.87 ± 6.97
	10Y-C	161.85 ± 2.93	143.27 ± 4.63	139.27 ± 2.88	115.75 ± 4.10	72.07 ± 6.14	60.58 ± 5.11
	15Y-C	154.28 ± 3.63	152.22 ± 3.61	131.50 ± 4.16	116.93 ± 5.65	77.22 ± 1.79	0.00 ± 0.00
	20Y-C	151.30 ± 3.18	146.60 ± 2.32	135.10 ± 2.78	116.33 ± 1.31	125.50 ± 59.83	81.12 ± 32.73
	0Y+C	152.25 ± 1.27	154.80 ± 2.74	113.05 ± 7.73	102.95 ± 4.06	68.95 ± 2.34	54.70 ± 2.62
	5Y+C	146.60 ± 1.85	140.72 ± 2.40	134.52 ± 2.83	127.65 ± 3.94	102.10 ± 5.29	90.20 ± 2.94
	10Y+C	147.30 ± 3.21	142.82 ± 2.79	139.60 ± 1.34	127.43 ± 3.70	103.05 ± 8.28	92.53 ± 6.02
	15Y+C	154.50 ± 2.74	148.02 ± 7.77	134.12 ± 1.92	129.43 ± 3.01	92.57 ± 5.07	117.68 ± 3.25
	20Y+C	136.10 ± 5.51	118.27 ± 4.32	126.97 ± 4.20	111.10 ± 5.31	72.68 ± 6.13	86.35 ± 1.16
STR	0Y-C	80.65 ± 0.44	74.25 ± 0.70	90.03 ± 1.15	78.50 ± 2.08	76.20 ± 1.47	71.32 ± 2.59
(%)	5Y-C	77.70 ± 1.23	76.17 ± 0.84	79.80 ± 0.90	83.15 ± 2.02	88.12 ± 1.20	75.85 ± 1.94
	10Y-C	74.18 ± 1.46	79.18 ± 0.77	80.75 ± 1.14	83.78 ± 0.68	85.15 ± 1.81	77.87 ± 3.83
	15Y-C	75.87 ± 1.12	80.12 ± 0.83	81.18 ± 1.98	83.07 ± 2.29	91.33 ± 4.24	0.00 ± 0.00
	20Y-C	77.35 ± 1.17	78.15 ± 0.71	81.90 ± 0.55	83.05 ± 1.21	85.85 ± 10.53	74.25 ± 24.75
	0Y+C	82.60 ± 0.98	83.32 ± 1.14	82.35 ± 1.97	78.40 ± 1.39	81.80 ± 1.05	76.32 ± 3.44
	5Y+C	77.95 ± 0.99	82.27 ± 0.92	86.43 ± 1.26	83.10 ± 0.63	82.27 ± 4.13	87.17 ± 3.22
	10Y+C	83.30 ± 2.66	81.90 ± 0.75	85.37 ± 0.30	81.60 ± 1.11	82.00 ± 0.54	82.97 ± 1.25
	15Y+C	75.67 ± 1.93	77.32 ± 1.33	82.70 ± 1.50	82.10 ± 1.29	78.93 ± 2.83	83.87 ± 1.14
	20Y+C	72.30 ± 1.02	75.17 ± 1.69	81.65 ± 1.51	81.18 ± 0.94	74.68 ± 2.17	79.95 ± 1.99

Table 7.4.(continued). Characteristics of ram semen stored at 5°C in 0Y-C (0% egg yolk/Y with no coconut extract), 5Y-C (5%Y), 10Y-C (10% Y), 15Y-C (15% Y), 20Y-C (20% Y), 0Y+C (0% egg yolk/Y+15% coconut extract/C), 5Y+C (5% Y+15% C), 10Y+C (10% Y+15% C),15Y+C (15% Y+15% C) and 20Y+C (20% Y+15% C) diluent for 240h. (Mean ± SEM, n=4).

Semen charac-teristic	Diluent	Time of storage at 5°C					
		0h	48h	96h	144h	192h	240h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
SLOW	0Y-C	0.18 ± 0.06	0.10 ± 0.10	0.40 ± 0.23	0.75 ± 0.33	0.73 ± 0.10	0.30 ± 0.30
(%)	5Y-C	0.17 ± 0.10	0.10 ± 0.10	0.65 ± 0.38	1.02 ± 0.25	0.57 ± 0.21	0.50 ± 0.33
	10Y-C	0.03 ± 0.03	0.38 ± 0.13	0.25 ± 0.09	1.90 ± 0.37	0.00 ± 0.00	0.00 ± 0.00
	15Y-C	0.20 ± 0.12	0.33 ± 0.13	0.97 ± 0.52	0.88 ± 0.32	0.28 ± 0.28	0.00 ± 0.00
	20Y-C	0.05 ± 0.05	0.10 ± 0.06	0.65 ± 0.14	1.25 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
	0Y+C	0.33 ± 0.09	0.15 ± 0.10	0.05 ± 0.05	0.65 ± 0.22	0.00 ± 0.00	0.25 ± 0.14
	5Y+C	0.68 ± 0.25	0.32 ± 0.21	0.80 ± 0.11	1.08 ± 0.27	0.40 ± 0.24	0.95 ± 0.39
	10Y+C	1.43 ± 0.52	0.83 ± 0.24	1.08 ± 0.28	2.07 ± 0.33	1.98 ± 0.32	0.00 ± 0.00
	15Y+C	1.00 ± 0.49	0.32 ± 0.24	1.33 ± 0.25	1.12 ± 0.19	2.08 ± 0.96	0.25 ± 0.15
	20Y+C	0.50 ± 0.30	1.20 ± 0.32	0.83 ± 0.27	1.27 ± 0.37	3.08 ± 0.59	2.18 ± 0.55
ALH	0Y-C	5.97 ± 0.43	6.12 ± 0.25	2.35 ± 0.05	4.72 ± 0.26	2.42 ± 0.09	2.65 ± 0.47
(μm)	5Y-C	6.62 ± 0.39	5.93 ± 0.24	3.47 ± 0.22	5.18 ± 0.45	3.52 ± 0.40	4.65 ± 0.65
	10Y-C	6.60 ± 0.07	5.62 ± 0.13	5.17 ± 0.40	5.20 ± 0.28	2.97 ± 0.51	3.08 ± 0.24
	15Y-C	6.43 ± 0.26	5.45 ± 0.20	5.05 ± 0.45	5.43 ± 0.65	2.82 ± 0.91	0.00 ± 0.00
	20Y-C	6.18 ± 0.24	5.53 ± 0.10	5.65 ± 0.13	4.65 ± 0.13	1.65 ± 0.62	2.17 ± 0.91
	0Y+C	5.43 ± 0.17	5.32 ± 0.40	3.58 ± 0.31	5.52 ± 0.42	2.85 ± 0.18	2.25 ± 0.19
	5Y+C	5.93 ± 0.20	4.75 ± 0.31	4.72 ± 0.15	5.27 ± 0.38	3.03 ± 0.17	2.47 ± 0.32
	10Y+C	5.37 ± 0.28	5.15 ± 0.24	4.65 ± 0.05	5.45 ± 0.25	3.10 ± 0.47	3.65 ± 0.25
	15Y+C	6.02 ± 0.49	5.78 ± 0.19	5.20 ± 0.26	5.47 ± 0.28	3.02 ± 0.28	3.70 ± 0.29
	20Y+C	5.93 ± 0.34	4.95 ± 0.12	4.83 ± 0.21	5.22 ± 0.23	2.80 ± 0.26	3.92 ± 0.23
LIN	0Y-C	70.95 ± 1.40	65.37 ± 0.72	84.87 ± 1.22	65.25 ± 2.73	62.33 ± 0.99	52.15 ± 6.93
(%)	5Y-C	68.77 ± 1.66	66.30 ± 1.06	73.87 ± 1.01	74.37 ± 3.06	80.70 ± 1.78	55.15 ± 3.22
	10Y-C	65.15 ± 1.54	71.65 ± 0.74	73.32 ± 1.41	75.15 ± 0.51	76.50 ± 3.07	57.03 ± 4.37
	15Y-C	67.03 ± 1.30	72.60 ± 0.92	74.32 ± 2.18	73.93 ± 3.09	79.65 ± 7.27	0.00 ± 0.00
	20Y-C	69.15 ± 1.22	70.73 ± 0.70	73.83 ± 0.53	73.77 ± 1.18	81.73 ± 13.50	73.45 ± 24.49
	0Y+C	75.68 ± 1.14	76.05 ± 1.40	75.93 ± 2.03	67.95 ± 1.54	72.52 ± 2.50	64.25 ± 2.86
	5Y+C	69.52 ± 1.39	76.40 ± 1.14	80.48 ± 1.65	74.85 ± 0.75	75.43 ± 4.18	81.12 ± 3.69
	10Y+C	75.80 ± 2.89	75.25 ± 1.25	80.08 ± 0.36	73.25 ± 1.16	76.50 ± 0.90	74.15 ± 1.49
	15Y+C	67.47 ± 2.58	69.62 ± 2.00	75.47 ± 2.03	73.87 ± 1.51	72.35 ± 3.11	76.68 ± 1.05
	20Y+C	63.10 ± 1.02	66.37 ± 2.26	74.12 ± 1.88	71.80 ± 0.72	65.92 ± 1.51	69.05 ± 2.84
VAP	0Y-C	124.65 ± 16.20	126.25 ± 1.25	88.15 ± 2.48	58.53 ± 4.99	29.82 ± 2.75	24.30 ± 6.13
(μm/s)	5Y-C	136.05 ± 3.39	101.20 ± 4.18	117.70 ± 2.48	107.18 ± 6.22	84.65 ± 3.02	44.88 ± 4.69
	10Y-C	138.25 ± 2.94	127.15 ± 4.54	124.27 ± 3.20	102.55 ± 4.02	64.57 ± 4.93	41.58 ± 3.18
	15Y-C	132.20 ± 2.41	135.00 ± 3.10	118.50 ± 4.54	101.40 ± 4.60	66.30 ± 7.20	0.00 ± 0.00
	20Y-C	132.40 ± 2.54	129.65 ± 1.38	120.35 ± 2.60	104.17 ± 1.71	106.68 ± 42.57	80.20 ± 32.30
	0Y+C	136.05 ± 0.65	139.18 ± 2.18	100.87 ± 6.64	101.47 ± 2.85	58.95 ± 2.60	44.33 ± 1.80
	5Y+C	127.98 ± 3.11	128.73 ± 3.21	124.25 ± 3.44	113.95 ± 2.82	91.27 ± 5.86	84.12 ± 2.98
	10Y+C	131.75 ± 2.91	128.85 ± 2.08	130.12 ± 1.62	112.50 ± 3.27	93.40 ± 6.90	80.25 ± 5.01
	15Y+C	134.93 ± 3.33	130.95 ± 8.00	120.97 ± 0.62	114.87 ± 1.66	84.50 ± 5.31	104.18 ± 4.36
	20Y+C	117.03 ± 3.47	104.78 ± 4.86	114.43 ± 4.47	98.97 ± 4.99	64.05 ± 5.07	72.30 ± 2.01

Table 7.4.(continued). Characteristics of ram semen stored at 5°C in 0Y-C (0% egg yolk/Y with no coconut extract), 5Y-C (5%Y), 10Y-C (10% Y), 15Y-C (15% Y), 20Y-C (20% Y), 0Y+C (0% egg yolk/Y+15% coconut extract/C), 5Y+C (5% Y+15% C), 10Y+C (10% Y+15% C),15Y+C (15% Y+15% C) and 20Y+C (20% Y+15% C) diluent for 240h. (Mean ± SEM, n=4).

Semen charac-teristic	Diluent	Time of storage at 5°C					
		0h	48h	96h	144h	192h	240h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
VSL (μm/s)	0Y-C	102.95 ± 13.61	97.35 ± 1.91	80.18 ± 2.48	48.82 ± 5.80	22.90 ± 2.17	17.67 ± 5.00
	5Y-C	109.20 ± 1.71	78.05 ± 3.58	95.95 ± 2.48	93.20 ± 7.34	75.60 ± 2.04	32.30 ± 4.47
	10Y-C	105.90 ± 3.68	103.62 ± 3.71	102.90 ± 2.83	89.02 ± 2.86	56.08 ± 3.99	31.25 ± 2.54
	15Y-C	103.67 ± 0.88	112.17 ± 2.82	100.18 ± 6.24	87.03 ± 5.14	59.97 ± 7.75	0.00 ± 0.00
	20Y-C	105.57 ± 1.90	104.20 ± 1.68	102.60 ± 1.99	94.35 ± 2.57	79.72 ± 20.74	79.40 ± 31.97
	0Y+C	113.97 ± 1.25	119.05 ± 3.23	84.98 ± 4.70	83.12 ± 2.94	47.38 ± 2.45	30.83 ± 1.68
	5Y+C	103.82 ± 3.93	108.57 ± 3.76	109.95 ± 3.60	100.50 ± 2.48	78.25 ± 6.33	75.45 ± 4.71
	10Y+C	113.03 ± 5.32	109.18 ± 2.22	115.70 ± 1.51	96.05 ± 4.28	78.70 ± 5.64	66.87 ± 4.95
	15Y+C	107.28 ± 5.14	106.68 ± 7.32	104.48 ± 1.55	98.75 ± 1.37	68.03 ± 5.19	89.22 ± 2.80
	20Y+C	89.27 ± 2.81	85.48 ± 5.67	98.68 ± 4.64	85.15 ± 4.57	49.92 ± 2.72	59.40 ± 3.14
VCL (μm/s)	0Y-C	144.57 ± 17.25	147.80 ± 1.40	93.10 ± 2.33	70.97 ± 3.88	36.83 ± 3.30	32.18 ± 5.58
	5Y-C	157.80 ± 4.93	119.23 ± 4.47	128.70 ± 2.52	121.92 ± 5.08	93.55 ± 4.21	64.87 ± 6.97
	10Y-C	161.85 ± 2.93	143.27 ± 4.63	139.27 ± 2.88	115.75 ± 4.10	72.07 ± 6.14	60.58 ± 5.11
	15Y-C	154.28 ± 3.63	152.22 ± 3.61	131.50 ± 4.16	116.93 ± 5.65	77.22 ± 1.79	0.00 ± 0.00
	20Y-C	151.30 ± 3.18	146.60 ± 2.32	135.10 ± 2.78	116.33 ± 1.31	125.50 ± 59.83	81.12 ± 32.73
	0Y+C	152.25 ± 1.27	154.80 ± 2.74	113.05 ± 7.73	102.95 ± 4.06	68.95 ± 2.34	54.70 ± 2.62
	5Y+C	146.60 ± 1.85	140.72 ± 2.40	134.52 ± 2.83	127.65 ± 3.94	102.10 ± 5.29	90.20 ± 2.94
	10Y+C	147.30 ± 3.21	142.82 ± 2.79	139.60 ± 1.34	127.43 ± 3.70	103.05 ± 8.28	92.53 ± 6.02
	15Y+C	154.50 ± 2.74	148.02 ± 7.77	134.12 ± 1.92	129.43 ± 3.01	92.57 ± 5.07	117.68 ± 3.25
	20Y+C	136.10 ± 5.51	118.27 ± 4.32	126.97 ± 4.20	111.10 ± 5.31	72.68 ± 6.13	86.35 ± 1.16
STR (%)	0Y-C	80.65 ± 0.44	74.25 ± 0.70	90.03 ± 1.15	78.50 ± 2.08	76.20 ± 1.47	71.32 ± 2.59
	5Y-C	77.70 ± 1.23	76.17 ± 0.84	79.80 ± 0.90	83.15 ± 2.02	88.12 ± 1.20	75.85 ± 1.94
	10Y-C	74.18 ± 1.46	79.18 ± 0.77	80.75 ± 1.14	83.78 ± 0.68	85.15 ± 1.81	77.87 ± 3.83
	15Y-C	75.87 ± 1.12	80.12 ± 0.83	81.18 ± 1.98	83.07 ± 2.29	91.33 ± 4.24	0.00 ± 0.00
	20Y-C	77.35 ± 1.17	78.15 ± 0.71	81.90 ± 0.55	83.05 ± 1.21	85.85 ± 10.53	74.25 ± 24.75
	0Y+C	82.60 ± 0.98	83.32 ± 1.14	82.35 ± 1.97	78.40 ± 1.39	81.80 ± 1.05	76.32 ± 3.44
	5Y+C	77.95 ± 0.99	82.27 ± 0.92	86.43 ± 1.26	83.10 ± 0.63	82.27 ± 4.13	87.17 ± 3.22
	10Y+C	83.30 ± 2.66	81.90 ± 0.75	85.37 ± 0.30	81.60 ± 1.11	82.00 ± 0.54	82.97 ± 1.25
	15Y+C	75.67 ± 1.93	77.32 ± 1.33	82.70 ± 1.50	82.10 ± 1.29	78.93 ± 2.83	83.87 ± 1.14
	20Y+C	72.30 ± 1.02	75.17 ± 1.69	81.65 ± 1.51	81.18 ± 0.94	74.68 ± 2.17	79.95 ± 1.99

Table 8.2. Semen characteristics incubated at 30°C in egg yolk citrate (EYC) media-alone (control), plus glucose, plus fructose, plus inositol, plus trehalose plus lactose and plus sucrose (Mean ± SEM, n= 3).

Semen characteristic	Diluent	Time (h)			Semen characteristic	Diluent	Time (h)		
		0	24	48			0	24	48
		Mean ±SEM	Mean ±SEM	Mean ±SEM			Mean ±SEM	Mean ±SEM	Mean ±SEM
MOT (%)	Control	81.94 ±2.17	53.08 ±6.36	25.55 ±7.78	MEDIUM (%)	Control	13.46 ±1.44	34.48 ±3.94	18.47 ±5.56
	Glucose	86.84 ±0.93	12.58 ±3.35	2.14 ±0.73		Glucose	16.48 ±1.32	7.34 ±2.26	1.79 ±0.59
	Fructose	85.41 ±0.71	20.86 ±6.17	0.59 ±0.25		Fructose	16.17 ±1.85	8.59 ±2.15	0.54 ±0.22
	Inositol	85.94 ±0.86	59.25 ±7.12	24.44 ±6.92		Inositol	14.71 ±1.61	27.14 ±2.61	16.07 ±4.30
	Trehalose	86.45 ±0.57	62.25 ±5.41	24.39 ±6.45		Trehalose	16.73 ±1.31	28.71 ±2.76	16.21 ±3.99
	Lactose	86.78 ±0.88	30.34 ±5.71	5.94 ±1.92		Lactose	16.61 ±1.44	17.86 ±2.71	4.99 ±1.68
	Sucrose	83.46 ±1.04	55.97 ±5.89	19.87 ±6.36		Sucrose	16.20 ±1.38	35.44 ±3.39	14.24 ±4.82
PROG (%)	Control	63.39 ±1.70	17.97 ±3.51	6.68 ±2.38	SLOW (%)	Control	0.38 ±0.10	2.36 ±0.33	0.72 ±0.20
	Glucose	64.41 ±1.30	5.03 ±1.45	0.36 ±0.15		Glucose	0.30 ±0.08	3.64 ±0.64	2.79 ±1.44
	Fructose	63.18 ±1.77	11.56 ±4.03	0.05 ±0.05		Fructose	0.36 ±0.14	3.71 ±1.76	0.58 ±0.27
	Inositol	65.72 ±1.27	30.47 ±5.95	8.25 ±3.03		Inositol	0.28 ±0.09	1.57 ±0.44	0.96 ±0.41
	Trehalose	64.36 ±1.51	31.63 ±4.38	7.51 ±3.19		Trehalose	0.62 ±0.16	1.41 ±0.32	3.61 ±1.14
	Lactose	63.63 ±1.47	11.82 ±3.55	0.89 ±0.37		Lactose	0.93 ±0.49	2.82 ±0.71	1.46 ±0.90
	Sucrose	62.17 ±1.43	19.48 ±3.74	5.23 ±2.78		Sucrose	0.44 ±0.15	1.92 ±0.26	1.01 ±0.29
RAPID (%)	Control	68.47 ±2.11	18.60 ±3.63	7.08 ±2.49					
	Glucose	70.37 ±1.26	5.24 ±1.48	0.36 ±0.15					
	Fructose	69.24 ±1.87	12.28 ±4.35	0.05 ±0.05					
	Inositol	71.24 ±1.71	32.12 ±6.37	8.38 ±3.09					
	Trehalose	69.73 ±1.44	33.56 ±4.67	8.18 ±3.53					
	Lactose	70.16 ±1.63	12.48 ±3.72	0.96 ±0.37					
	Sucrose	67.25 ±1.51	20.51 ±3.94	5.63 ±2.94					

Table 8.2. (continued). Semen characteristics incubated at 30°C in egg yolk citrate (EYC) media-alone (control), plus glucose, plus fructose, plus inositol, plus trehalose plus lactose and plus sucrose (Mean ± SEM, n= 3).

Semen characteristic	Diluent	Time (h)			Semen characteristic	Diluent	Time (h)		
		0	24	48			0	24	48
		Mean ±SEM	Mean ±SEM	Mean ±SEM			Mean ±SEM	Mean ±SEM	Mean ±SEM
ALH (μm)	Control	5.79 ±0.17	3.61 ±0.20	2.53 ±0.49	VSL (μm/s)	Control	96.39 ±2.79	52.89 ±3.53	27.96 ±5.66
	Glucose	6.04 ±0.15	2.67 ±0.38	1.57 ±0.52		Glucose	92.47 ±3.15	49.87 ±7.43	14.58 ±4.61
	Fructose	6.19 ±0.18	3.62 ±0.29	0.79 ±0.35		Fructose	92.50 ±3.23	64.61 ±5.95	12.11 ±5.35
	Inositol	5.86 ±0.22	3.67 ±0.23	2.47 ±0.50		Inositol	94.74 ±2.50	58.11 ±5.54	29.17 ±6.50
	Trehalose	6.09 ±0.14	4.44 ±0.17	3.16 ±0.45		Trehalose	89.55 ±2.69	67.30 ±2.44	33.12 ±5.51
	Lactose	6.26 ±0.14	3.70 ±0.21	1.99 ±0.49		Lactose	90.79 ±2.59	53.96 ±4.13	21.33 ±5.46
	Sucrose	5.92 ±0.11	3.79 ±0.25	2.02 ±0.55		Sucrose	92.21 ±2.94	51.65 ±3.76	20.29 ±5.89
LIN (%)	Control	72.70 ±1.54	71.39 ±2.03	41.44 ±7.48	VCL (μm/s)	Control	131.44 ±3.19	71.30 ±4.32	43.93 ±8.55
	Glucose	69.06 ±1.23	61.35 ±7.12	26.25 ±7.78		Glucose	131.56 ±2.66	63.98 ±8.83	24.26 ±7.65
	Fructose	68.43 ±1.22	71.26 ±5.14	20.31 ±8.25		Fructose	132.61 ±3.62	84.49 ±6.30	15.29 ±6.37
	Inositol	71.44 ±1.40	71.90 ±2.12	39.89 ±7.93		Inositol	131.89 ±3.32	76.79 ±6.67	42.27 ±8.78
	Trehalose	69.26 ±1.24	72.39 ±1.05	48.95 ±6.62		Trehalose	128.11 ±2.91	92.41 ±3.86	51.53 ±7.70
	Lactose	67.57 ±1.24	70.99 ±1.97	36.01 ±8.50		Lactose	132.22 ±2.75	73.56 ±4.42	32.98 ±7.58
	Sucrose	70.59 ±1.07	69.27 ±2.14	29.39 ±7.30		Sucrose	128.78 ±2.63	73.49 ±4.57	33.98 ±9.25
VAP (μm/s)	Control	114.54 ±2.71	60.98 ±4.06	34.73 ±6.90	STR (%)	Control	81.63 ±1.24	83.89 ±1.34	51.87 ±9.10
	Glucose	112.67 ±2.69	57.14 ±8.15	18.34 ±5.83		Glucose	78.92 ±1.05	69.82 ±7.85	34.14 ±10.12
	Fructose	112.98 ±3.08	73.67 ±6.11	13.06 ±5.61		Fructose	78.56 ±1.10	80.26 ±5.10	24.06 ±9.56
	Inositol	113.83 ±2.62	66.97 ±6.42	33.91 ±7.39		Inositol	80.74 ±1.14	83.63 ±1.23	50.37 ±9.79
	Trehalose	109.26 ±2.56	79.57 ±3.23	40.67 ±6.54		Trehalose	79.09 ±1.03	82.51 ±0.95	61.27 ±8.14
	Lactose	111.66 ±2.46	62.11 ±4.49	26.02 ±6.22		Lactose	78.11 ±0.97	84.38 ±1.42	44.03 ±9.97
	Sucrose	110.92 ±2.57	61.83 ±4.22	26.07 ±7.31		Sucrose	80.37 ±0.94	81.46 ±1.64	37.92 ±9.31

Table 8.3. Semen characteristics incubated at 5°C in egg yolk citrate (EYC) media-alone (control), plus glucose, plus fructose, plus inositol, plus trehalose plus lactose and plus sucrose (Mean ± SEM, n= 3).

Semen characteristic	Diluent	Time (h)			Semen characteristic	Diluent	Time (h)		
		0	144	288			0	144	288
		Mean ±SEM	Mean ±SEM	Mean ±SEM			Mean ±SEM	Mean ±SEM	Mean ±SEM
MOT (%)	Control	87.35 ±0.91	77.96 ±2.93	28.58 ±6.53	MEDIUM (%)	Control	15.93 ±1.74	31.63 ±0.93	14.27 ±3.41
	Glucose	87.38 ±0.63	77.99 ±1.92	37.11 ±6.36		Glucose	16.87 ±0.93	29.28 ±1.49	17.73 ±2.64
	Fructose	87.34 ±1.15	77.78 ±2.47	45.94 ±9.05		Fructose	15.72 ±1.34	29.22 ±1.07	17.81 ±3.64
	Inositol	86.57 ±1.11	78.80 ±1.34	34.64 ±7.83		Inositol	18.06 ±1.21	29.87 ±1.58	17.47 ±3.49
	Trehalose	87.26 ±0.81	81.57 ±1.89	45.30 ±8.16		Trehalose	15.79 ±0.85	31.65 ±2.06	22.16 ±4.00
	Lactose	86.91 ±1.03	78.54 ±1.76	23.86 ±5.82		Lactose	16.18 ±0.99	28.58 ±1.75	12.96 ±3.08
	Sucrose	86.11 ±0.92	75.91 ±3.19	30.63 ±6.42		Sucrose	16.92 ±1.4 0	28.19 ±1.67	14.78 ±2.86
PROG (%)	Control	65.51 ±1.65	43.49 ±2.98	13.55 ±3.59	SLOW (%)	Control	0.28 ±0.12	1.75 ±0.35	2.53 ±0.80
	Glucose	64.87 ±1.18	46.37 ±2.29	18.57 ±3.83		Glucose	0.51 ±0.19	1.62 ±0.28	3.78 ±1.00
	Fructose	65.78 ±1.19	46.31 ±2.21	26.12 ±6.04		Fructose	0.42 ±0.12	1.74 ±0.27	1.33 ±0.38
	Inositol	63.61 ±1.22	45.86 ±1.82	16.03 ±4.28		Inositol	0.25 ±0.12	1.22 ±0.26	2.04 ±0.58
	Trehalose	65.49 ±0.90	47.04 ±2.98	21.63 ±4.44		Trehalose	0.18 ±0.06	1.61 ±0.28	1.96 ±0.47
	Lactose	65.98 ±1.25	46.98 ±1.75	10.68 ±2.78		Lactose	0.71 ±0.20	1.69 ±0.23	3.86 ±1.51
	Sucrose	63.75 ±1.23	45.01 ±3.74	15.18 ±3.54		Sucrose	0.33 ±0.12	1.71 ±0.32	2.86 ±0.69
RAPID (%)	Control	71.41 ±1.74	46.31 ±3.23	14.31 ±3.81					
	Glucose	70.53 ±1.20	48.71 ±2.50	19.38 ±4.03					
	Fructose	71.64 ±1.34	48.57 ±2.43	28.13 ±6.61					
	Inositol	68.52 ±1.54	48.93 ±1.92	17.19 ±4.63					
	Trehalose	71.47 ±0.97	49.91 ±3.26	23.15 ±4.87					
	Lactose	70.72 ±1.27	49.98 ±1.97	10.90 ±2.84					
	Sucrose	69.20 ±1.49	47.72 ±4.05	15.85 ±3.75					

Table 8.3. (continued). Semen characteristics incubated at 5°C in egg yolk citrate (EYC) media-alone (control), plus glucose, plus fructose, plus inositol, plus trehalose plus lactose and plus sucrose (Mean ± SEM, n= 3).

Semen characteristic	Diluent	Time (h)			Semen characteristic	Diluent	Time (h)		
		0	144	288			0	144	288
		Mean ±SEM	Mean ±SEM	Mean ±SEM			Mean ±SEM	Mean ±SEM	Mean ±SEM
ALH (µm)	Control	6.58 ±0.14	4.52 ±0.19	2.97 ±0.43	VSL (µm/s)	Control	93.73 ±2.58	69.80 ±1.83	43.58 ±6.25
	Glucose	6.17 ±0.19	4.39 ±0.16	3.08 ±0.30		Glucose	93.11 ±1.59	73.61 ±1.88	52.09 ±5.59
	Fructose	6.31 ±0.18	4.37 ±0.13	3.49 ±0.41		Fructose	93.37 ±2.45	73.71 ±1.34	52.96 ±6.41
	Inositol	6.13 ±0.12	4.53 ±0.13	3.23 ±0.33		Inositol	90.48 ±1.42	72.30 ±1.84	54.66 ±5.45
	Trehalose	6.20 ±0.14	4.39 ±0.15	3.42 ±0.42		Trehalose	93.33 ±1.52	72.59 ±2.06	49.12 ±6.47
	Lactose	6.24 ±0.16	4.27 ±0.12	2.91 ±0.38		Lactose	93.42 ±1.80	74.03 ±1.93	51.37 ±5.76
	Sucrose	6.16 ±0.18	4.37 ±0.14	3.09 ±0.48		Sucrose	90.68 ±2.44	71.94 ±2.57	43.34 ±6.97
LIN (%)	Control	68.23 ±0.95	70.70 ±0.76	62.31 ±5.83	VCL (µm/s)	Control	135.11 ±3.04	96.77 ±2.37	60.34 ±8.13
	Glucose	68.90 ±1.04	73.54 ±1.20	62.85 ±5.76		Glucose	133.12 ±1.75	98.39 ±1.93	71.62 ±6.84
	Fructose	68.38 ±0.79	74.01 ±0.86	62.53 ±4.83		Fructose	133.83 ±3.04	97.97 ±1.68	75.01 ±8.64
	Inositol	69.49 ±0.88	72.36 ±1.03	65.94 ±5.88		Inositol	128.35 ±1.74	97.99 ±1.79	72.91 ±7.04
	Trehalose	69.70 ±1.14	72.69 ±1.07	55.83 ±6.62		Trehalose	132.28 ±1.41	97.87 ±2.71	70.31 ±8.67
	Lactose	70.19 ±1.05	73.21 ±1.01	67.51 ±6.22		Lactose	131.77 ±2.20	99.05 ±2.03	66.41 ±7.15
	Sucrose	69.17 ±1.08	72.18 ±0.92	49.31 ±7.75		Sucrose	129.96 ±3.03	98.77 ±3.14	61.78 ±9.58
VAP (µm/s)	Control	114.24 ±2.74	83.78 ±2.16	51.66 ±7.47	STR (%)	Control	78.95 ±0.78	80.21 ±0.53	73.33 ±6.62
	Glucose	113.16 ±1.38	86.36 ±1.89	62.23 ±6.25		Glucose	79.13 ±0.78	82.47 ±0.89	71.57 ±6.29
	Fructose	113.79 ±2.48	86.32 ±1.47	63.97 ±7.58		Fructose	78.66 ±0.65	82.68 ±0.70	72.76 ±5.20
	Inositol	109.25 ±1.46	85.91 ±1.83	64.14 ±6.32		Inositol	79.81 ±0.71	81.41 ±0.78	73.94 ±6.45
	Trehalose	112.68 ±1.15	86.04 ±2.30	59.72 ±7.75		Trehalose	79.89 ±0.92	81.26 ±0.89	65.63 ±7.25
	Lactose	112.18 ±1.70	87.57 ±1.78	57.79 ±6.33		Lactose	80.66 ±0.80	81.58 ±0.92	76.98 ±6.78
	Sucrose	110.77 ±2.56	86.31 ±2.94	51.34 ±8.24		Sucrose	79.10 ±0.83	81.02 ±0.77	58.53 ±8.86

Table 8.4. pH values for ram semen in egg yolk citrate media alone (control) or containing a various sugars at different hours of storage at 30°C. (Mean ± SEM, n = 3).

Diluent	Time of storage at 30°C						
	0h		24h		48h		pH
	Mean	± SEM	Mean	± SEM	Mean	± SEM	shift*
Control	6.31	± 0.04	6.47	± 0.11	6.43	± 0.19	-0.12
Glucose	5.83	± 0.11	5.13	± 0.04	5.12	± 0.04	0.71
Fructose	5.80	± 0.13	5.16	± 0.03	5.16	± 0.04	0.64
Inositol	6.26	± 0.10	6.42	± 0.09	6.54	± 0.18	-0.28
Trehalose	6.23	± 0.12	5.91	± 0.06	5.55	± 0.08	0.68
Lactose	5.96	± 0.12	5.43	± 0.09	5.23	± 0.06	0.73
Sucrose	6.34	± 0.06	6.49	± 0.12	6.22	± 0.16	0.12

* decrease of pH from 0 to 48h

Table 8.5. pH values for ram semen in egg yolk citrate media-alone (control) or -containing a various sugars at different hours of storage at 5°C. (Mean ± SEM, n = 3).

Diluent	Time of storage at 5°C						
	0h		144h		288h		pH
	Mean	± SEM	Mean	± SEM	Mean	± SEM	shift*
Control	6.21	± 0.06	6.28	± 0.10	6.18	± 0.09	0.03
Glucose	5.99	± 0.10	5.51	± 0.09	5.46	± 0.04	0.53
Fructose	5.92	± 0.05	5.55	± 0.11	5.63	± 0.09	0.29
Inositol	6.28	± 0.07	6.38	± 0.07	6.36	± 0.05	-0.08
Trehalose	6.20	± 0.06	5.76	± 0.04	5.58	± 0.07	0.62
Lactose	5.99	± 0.04	5.53	± 0.07	5.55	± 0.04	0.44
Sucrose	6.19	± 0.04	6.13	± 0.07	6.20	± 0.05	-0.01

* decrease of pH from 0 to 288h

Table 8.6. Correlation coefficients (r) between sperm motility and pH of diluted semen

Diluent	Pooled time (0 and 24h) at 30°	Pooled time (0 and 144h) at 5°C
Control	r = 0.398*	r = 0.953*
Glucose	r = 0.970 **	r = 0.865**
Fructose	r = 0.827**	r =0.805**
Inositol	r = 0.472*	r =0.388 (NS)
Trehalose	r = 0.582**	r = 0.437*
Lactose	r = 0.899**	r = 0.860**
Sucrose	r = 0.547*	r = 0.390*

** p<0.001
* p<0.05

Table 8.7. Correlation coefficients (r) between sperm motility and pH of diluted semen

Diluent	Time (24h) at 30°	Time (144h) at 5°C
Control	r = 0.716**	r = 0.989*
Glucose	r = 0.546 *	r = 0.824*
Fructose	r = 0.539*	r =0.757*
Inositol	r = 0.555*	r =0.999**
Trehalose	r = 0.553**	r = 0.950**
Lactose	r = 0.795**	r = 0.657*
Sucrose	r = 0.825*	r = 0.554 (NS)

** p<0.001
* p<0.05

Table 9.5. Semen characteristics in egg yolk citrate with glucose plus sodium bicarbonate (+G+B) or plus sodium citrate (+G+C); egg yolk citrate without glucose plus sodium bicarbonate (+B) or plus sodium citrate (+C) and storage at 30°C (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time before addition of bicarbonate or citrate buffer at 30°C		Time after addition of bicarbonate or citrate buffer at 30°C		
		-12h	0h	1h	-12h	24h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	+G+B	88.88 ± 3.06	84.25 ± 1.00	83.42 ± 1.05	78.45 ± 3.19	64.05 ± 4.92
	+G+C	86.32 ± 2.44	82.32 ± 2.14	86.68 ± 2.43	57.38 ± 3.37	21.55 ± 3.92
	+B	91.90 ± 0.53	84.62 ± 1.95	89.98 ± 2.33	42.12 ± 10.63	6.98 ± 3.03
	+C	85.15 ± 4.94	89.37 ± 1.12	87.90 ± 1.31	74.58 ± 2.65	41.45 ± 9.08
PROG (%)	+G+B	70.82 ± 1.90	60.57 ± 4.68	64.05 ± 2.90	53.82 ± 3.91	49.68 ± 5.15
	+G+C	68.03 ± 3.97	58.37 ± 4.76	70.40 ± 1.49	29.10 ± 3.39	9.43 ± 2.19
	+B	76.22 ± 1.71	61.43 ± 1.96	68.38 ± 2.71	26.37 ± 11.38	1.30 ± 0.66
	+C	69.62 ± 4.77	70.12 ± 1.79	68.07 ± 1.50	59.52 ± 1.56	15.02 ± 5.40
RAPID (%)	+G+B	83.20 ± 3.14	73.33 ± 4.15	72.33 ± 2.70	59.57 ± 4.63	53.22 ± 5.67
	+G+C	79.10 ± 4.60	70.85 ± 4.79	79.85 ± 1.57	31.70 ± 3.09	11.15 ± 2.36
	+B	86.82 ± 1.76	75.67 ± 3.17	76.97 ± 2.92	29.28 ± 12.12	1.30 ± 0.66
	+C	79.83 ± 5.46	83.28 ± 1.81	76.23 ± 2.28	66.17 ± 2.34	15.90 ± 5.46
MEDIUM	+G+B	5.67 ± 1.90	10.95 ± 4.32	11.08 ± 1.77	18.90 ± 1.86	10.83 ± 1.03
	+G+C	7.20 ± 2.60	11.47 ± 3.51	6.83 ± 0.90	25.70 ± 3.90	10.40 ± 2.83
	+B	5.08 ± 1.55	8.97 ± 2.41	13.00 ± 1.36	12.83 ± 4.94	5.68 ± 2.57
	+C	5.30 ± 1.39	6.07 ± 1.61	11.68 ± 1.38	8.43 ± 2.12	25.57 ± 5.29
SLOW (%)	+G+B	0.18 ± 0.12	0.93 ± 0.34	1.52 ± 0.71	2.38 ± 0.78	2.03 ± 0.45
	+G+C	1.15 ± 0.36	0.97 ± 0.36	0.00 ± 0.00	2.53 ± 0.30	3.38 ± 1.11
	+B	0.65 ± 0.31	0.28 ± 0.19	1.37 ± 0.53	5.58 ± 1.30	6.63 ± 5.17
	+C	0.68 ± 0.25	0.68 ± 0.31	1.18 ± 0.31	1.85 ± 0.74	5.03 ± 1.12

Table 9.5. (continued). Semen characteristics in egg yolk citrate with glucose plus sodium bicarbonate (+G+B) or plus sodium citrate (+G+C); egg yolk citrate without glucose plus sodium bicarbonate (+B) or plus sodium citrate (+C) and storage at 30°C (Mean ± SEM, n = 3)

Semen characteristic	Diluent	Time before addition of bicarbonate or citrate buffer at 30°C		Time after addition of bicarbonate or citrate buffer at 30°C		
		-12h	0h	1h	12h	24h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Mean ALH (µm)	+G+B	7.15 ± 0.24	7.02 ± 0.27	6.75 ± 0.08	5.82 ± 0.37	5.70 ± 0.37
	+G+C	6.90 ± 0.32	6.63 ± 0.20	7.27 ± 0.08	4.83 ± 0.18	3.92 ± 0.39
	+B	6.87 ± 0.27	6.97 ± 0.36	5.62 ± 0.27	4.15 ± 0.62	2.17 ± 0.76
	+C	6.75 ± 0.35	6.87 ± 0.08	6.03 ± 0.24	6.23 ± 0.36	3.25 ± 0.11
Mean LIN (%)	+G+B	72.58 ± 2.30	67.62 ± 3.50	72.88 ± 1.80	72.53 ± 0.72	77.47 ± 2.33
	+G+C	74.20 ± 1.25	67.37 ± 3.03	73.87 ± 0.84	66.25 ± 1.45	66.78 ± 6.35
	+B	75.93 ± 2.00	69.02 ± 1.55	75.12 ± 1.72	72.68 ± 4.16	37.38 ± 11.93
	+C	76.08 ± 1.73	71.33 ± 1.31	75.05 ± 1.68	74.62 ± 1.70	65.07 ± 4.17
Mean VAP (µm/s)	+G+B	139.08 ± 8.64	120.38 ± 6.13	145.90 ± 8.07	115.40 ± 8.63	126.90 ± 8.74
	+G+C	145.57 ± 9.96	122.08 ± 4.99	145.85 ± 3.38	58.30 ± 3.60	82.25 ± 23.70
	+B	150.45 ± 2.26	127.50 ± 4.47	120.23 ± 5.65	82.70 ± 18.81	21.72 ± 7.90
	+C	146.47 ± 6.95	123.62 ± 3.44	120.32 ± 3.75	114.03 ± 3.81	41.98 ± 5.90
Mean VSL (µm/s)	+G+B	120.48 ± 9.26	100.53 ± 6.47	129.18 ± 8.64	103.00 ± 7.59	116.58 ± 9.31
	+G+C	128.12 ± 9.12	101.45 ± 6.01	128.30 ± 3.26	49.60 ± 3.24	73.10 ± 24.99
	+B	133.92 ± 3.23	106.37 ± 3.45	106.58 ± 5.63	71.82 ± 17.58	18.00 ± 6.79
	+C	129.13 ± 6.84	105.28 ± 3.53	107.05 ± 3.09	101.03 ± 3.93	35.78 ± 6.13
Mean VCL (µm/s)	+G+B	160.87 ± 9.32	140.87 ± 6.00	166.38 ± 7.68	131.32 ± 9.88	141.45 ± 8.42
	+G+C	165.85 ± 11.00	141.48 ± 4.97	167.58 ± 3.28	72.13 ± 4.11	93.32 ± 22.23
	+B	171.67 ± 2.72	148.25 ± 5.87	135.32 ± 5.82	92.23 ± 19.36	30.10 ± 10.55
	+C	166.03 ± 7.97	143.73 ± 3.20	136.58 ± 4.38	131.63 ± 4.69	53.08 ± 4.83
Mean STR (%)	+G+B	82.77 ± 2.06	78.42 ± 2.64	83.13 ± 1.23	83.15 ± 0.65	86.80 ± 1.69
	+G+C	83.67 ± 1.05	77.42 ± 2.58	84.05 ± 0.50	81.70 ± 1.24	78.08 ± 5.04
	+B	85.38 ± 1.53	79.25 ± 0.92	84.08 ± 1.25	81.98 ± 3.41	51.63 ± 16.66
	+C	85.03 ± 1.21	81.92 ± 1.03	84.93 ± 1.52	85.15 ± 1.07	82.02 ± 2.55

Table 9.6. Semen characteristics in egg yolk citrate with glucose plus sodium bicarbonate (+G+B) or plus sodium citrate (+G+C); egg yolk citrate without glucose plus sodium bicarbonate (+B) or plus sodium citrate (+C) (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time before addition of bicarbonate or citrate buffer at 5°C		Time after addition of bicarbonate or citrate buffer at 30°C		
		-96h	0h	1h	12h	24h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	+G+B	83.07 ± 2.82	87.47 ± 0.79	84.75 ± 1.84	67.17 ± 10.74	7.78 ± 1.07
	+G+C	87.35 ± 2.03	85.92 ± 1.06	81.05 ± 2.21	52.20 ± 7.88	32.20 ± 9.40
	+B	89.87 ± 2.44	91.33 ± 1.77	93.18 ± 0.75	65.13 ± 8.55	19.23 ± 5.59
	+C	89.73 ± 1.57	90.03 ± 1.47	84.70 ± 3.81	88.38 ± 1.52	64.95 ± 2.79
PROG (%)	+G+B	68.38 ± 2.15	63.23 ± 1.78	56.85 ± 2.23	42.93 ± 10.29	1.13 ± 0.35
	+G+C	72.43 ± 3.19	59.50 ± 0.70	55.03 ± 0.85	37.18 ± 3.28	20.10 ± 6.76
	+B	70.27 ± 2.08	70.20 ± 1.87	68.75 ± 2.63	31.73 ± 7.47	3.07 ± 0.86
	+C	71.88 ± 2.98	72.67 ± 1.02	63.62 ± 1.54	63.85 ± 3.50	26.07 ± 2.35
RAPID (%)	+G+B	77.80 ± 4.04	74.88 ± 2.02	67.90 ± 2.26	49.45 ± 11.43	1.78 ± 0.43
	+G+C	80.52 ± 3.37	72.25 ± 1.46	67.35 ± 1.17	39.23 ± 3.74	21.18 ± 7.15
	+B	83.35 ± 3.61	83.45 ± 2.38	79.73 ± 2.03	40.42 ± 7.96	5.83 ± 1.82
	+C	83.15 ± 2.51	84.88 ± 1.15	75.08 ± 3.55	75.20 ± 4.97	31.22 ± 2.03
MEDIUM (%)	+G+B	5.25 ± 1.58	12.58 ± 1.94	16.88 ± 0.61	17.68 ± 1.57	6.00 ± 0.87
	+G+C	6.87 ± 2.21	13.65 ± 0.62	13.68 ± 1.77	12.98 ± 4.37	11.00 ± 2.63
	+B	6.48 ± 1.76	7.87 ± 1.10	13.43 ± 1.46	24.73 ± 3.11	13.40 ± 4.32
	+C	6.57 ± 1.65	5.17 ± 0.75	9.60 ± 2.86	13.18 ± 3.87	33.75 ± 2.02
SLOW (%)	+G+B	1.33 ± 0.47	0.87 ± 0.26	1.05 ± 0.46	2.53 ± 1.38	3.38 ± 1.00
	+G+C	1.97 ± 0.61	0.90 ± 0.22	1.03 ± 0.30	2.77 ± 0.35	5.60 ± 1.66
	+B	1.22 ± 0.52	0.45 ± 0.10	1.42 ± 0.77	2.47 ± 0.29	5.47 ± 1.12
	+C	0.77 ± 0.29	0.08 ± 0.08	0.62 ± 0.29	0.93 ± 0.45	4.27 ± 0.81

Table 9.6. (continued). Semen characteristics in egg yolk citrate with glucose plus sodium bicarbonate (+G+B) or plus sodium citrate (+G+C); egg yolk citrate without glucose plus sodium bicarbonate (+B) or plus sodium citrate (+C) (Mean ± SEM, n = 3).

Semen characteristic	Diluent	Time before addition of bicarbonate or citrate buffer at 5°C		Time after addition of bicarbonate or citrate buffer at 30°C		
		-96h	0h	1h	12h	24h
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Mean ALH (µm)	+G+B	6.75 ± 0.55	5.70 ± 0.20	5.70 ± 0.09	5.05 ± 0.47	3.27 ± 0.60
	+G+C	6.40 ± 0.39	5.98 ± 0.17	6.15 ± 0.20	4.65 ± 0.15	4.28 ± 0.21
	+B	6.95 ± 0.30	6.07 ± 0.13	5.68 ± 0.24	5.20 ± 0.21	4.02 ± 0.87
	+C	6.57 ± 0.43	6.03 ± 0.15	5.98 ± 0.34	6.03 ± 0.32	4.60 ± 0.06
Mean LIN (%)	+G+B	76.77 ± 2.74	69.83 ± 0.63	68.45 ± 1.48	67.02 ± 3.65	52.03 ± 4.54
	+G+C	76.42 ± 1.61	67.30 ± 1.13	68.15 ± 2.11	76.85 ± 2.52	68.65 ± 3.61
	+B	72.37 ± 1.85	70.83 ± 1.11	71.83 ± 2.62	59.07 ± 3.02	47.37 ± 2.67
	+C	74.33 ± 2.67	72.77 ± 1.63	72.38 ± 2.69	68.90 ± 1.46	59.32 ± 1.66
Mean VAP (µm/s)	+G+B	150.63 ± 6.43	99.52 ± 2.08	98.45 ± 3.09	84.60 ± 9.98	38.07 ± 4.11
	+G+C	138.60 ± 9.19	99.07 ± 1.51	108.08 ± 3.46	89.17 ± 8.37	61.17 ± 7.26
	+B	140.62 ± 5.99	112.12 ± 2.82	116.72 ± 5.77	72.28 ± 4.69	38.95 ± 3.83
	+C	139.65 ± 6.80	114.97 ± 3.21	116.83 ± 6.10	103.17 ± 6.09	57.83 ± 1.45
Mean VSL (µm/s)	+G+B	132.80 ± 5.41	83.48 ± 1.75	83.13 ± 3.73	72.05 ± 10.27	25.12 ± 3.95
	+G+C	123.32 ± 8.59	82.18 ± 1.37	90.28 ± 3.71	81.57 ± 8.69	53.88 ± 7.10
	+B	121.32 ± 5.67	94.30 ± 2.70	101.32 ± 6.29	57.23 ± 5.48	23.82 ± 2.92
	+C	121.77 ± 6.61	98.92 ± 3.99	100.23 ± 5.80	87.18 ± 4.74	45.53 ± 1.67
Mean VCL (µm/s)	+G+B	169.77 ± 8.47	115.83 ± 2.49	114.77 ± 2.48	100.10 ± 10.75	52.13 ± 6.03
	+G+C	156.33 ± 10.46	116.72 ± 1.66	125.72 ± 3.43	100.87 ± 7.98	73.67 ± 7.19
	+B	162.53 ± 7.15	130.00 ± 2.96	133.20 ± 5.06	90.23 ± 4.03	54.75 ± 6.06
	+C	159.17 ± 7.73	133.50 ± 3.15	134.95 ± 6.72	122.72 ± 7.30	74.45 ± 0.90
Mean STR (%)	+G+B	85.22 ± 2.05	80.35 ± 0.89	79.23 ± 1.20	79.00 ± 3.11	64.22 ± 4.18
	+G+C	85.32 ± 1.46	78.50 ± 0.89	78.83 ± 1.48	87.05 ± 1.43	82.90 ± 2.28
	+B	82.63 ± 1.33	80.88 ± 0.80	81.50 ± 1.91	72.95 ± 2.37	62.58 ± 2.78
	+C	83.72 ± 1.99	83.10 ± 1.39	82.35 ± 2.08	80.20 ± 1.23	75.02 ± 1.48

Appendix B.

Table 10.1.1. Manual assessment of semen volume, total sperm count and motility of undiluted semen collected from control and 16h/day scrotally insulated rams.

Days after start of insulation	Semen volume (ml)				Total sperm count ($x10^9$)				Motility (%)			
	Control rams		Insulated rams		Control rams		Insulated rams		Control rams		Insulated rams	
	No 03	No 31	No 16	No 12	No 03	No 31	No 16	No 12	No 03	No 31	No 16	No 12
5	**1.20**	**1.20**	**1.00**	**1.60**	**2.03**	**3.11**	**1.69**	**2.90**	**81.00**	**78.00**	**77.00**	**81.00**
9	**1.20**	**1.30**	**1.60**	**1.30**	**3.45**	**3.38**	**6.20**	**2.37**	**80.00**	**87.00**	**76.00**	**83.00**
16	**1.10**	**1.40**	**1.00**	**1.40**	**2.97**	**4.95**	**1.45**	**1.63**	**87.00**	**88.00**	**30.00**	**25.00**
23	1.40	1.80	0.80	0.60	4.08	2.68	0.82	0.21	71.00	81.00	0.00	0.00
30	0.90	1.60	0.70	0.60	0.44	6.02	0.21	0.26	84.00	71.00	17.00	20.00
37	1.00	0.80	0.60	1.10	1.85	1.63	0.04	0.18	84.00	81.00	11.00	0.00
44	1.00	0.70	0.80	1.20	2.56	1.66	1.35	0.15	76.00	87.00	39.00	22.00
51	1.40	1.20	1.10	0.80	4.80	4.20	1.57	0.30	81.00	82.00	51.00	4.00
58	0.70	1.00	0.70	0.80	2.46	3.30	0.88	0.19	88.00	90.00	73.00	1.00
65	0.90	0.90	0.90	0.90	3.60	4.88	2.37	0.05	82.00	75.00	78.00	6.00
72	1.00	1.00	1.20	0.50	4.74	4.74	2.64	0.41	86.00	86.00	85.00	7.00
79	0.80	1.80	1.00	1.00	2.17	5.99	3.03	0.10	82.00	86.00	72.00	11.00
86	1.10	1.20	0.80	0.60	0.16	0.41	1.05	0.04	73.00	71.00	85.00	5.00
93	0.80	0.90	1.10	0.90	2.37	3.62	3.33	1.11	83.00	78.00	83.00	72.00
100	0.90	1.00	0.60	1.10	4.04	3.17	2.02	2.32	82.00	81.00	87.00	86.00
107	1.20	0.80	0.60	1.10	6.22	2.68	2.53	5.39	81.00	83.00	84.00	82.00
114	1.00	0.80	1.00	1.20	3.84	1.83	3.24	5.76	68.00	87.00	71.00	84.00

Table 10.1.2. Effect of storage at two different temperatures (30 and 5°C) on changes in motility and morphological characteristics of semen from control and 16h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (6h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Days after start of insulation	30°C		5°C	
		Control rams	Insulated rams	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT	**9**	**78.08 ± 4.75**	**101.39 ± 11.44**	**95.98 ± 3.53**	**91.07 ± 5.90**
(%)	**16**	**105.47 ± 3.11**	**89.40 ± 10.33**	**102.73 ± 1.81**	**100.13 ± 6.96**
	23	**80.35 ± 18.05**	**0.00 ± 0.00**	**107.35 ± 16.26**	**0.00 ± 0.00**
	30	108.05 ± 5.03	24.09 ± 5.82	101.05 ± 5.36	40.45 ± 7.75
	37	101.15 ± 2.60	99.88 ± 17.67	37.13 ± 18.12	74.87 ± 17.33
	44	102.90 ± 2.49	74.64 ± 12.28	101.79 ± 2.17	61.53 ± 8.89
	51	92.89 ± 1.53	90.04 ± 13.63	91.03 ± 1.72	73.03 ± 6.27
	93	88.65 ± 3.25	89.48 ± 2.94	103.28 ± 3.63	98.79 ± 4.69
	100	96.47 ± 2.91	92.16 ± 3.38	95.88 ± 2.76	92.76 ± 3.89
	107	95.30 ± 3.09	99.58 ± 4.05	101.89 ± 3.10	91.48 ± 3.79
	114	91.71 ± 3.58	101.43 ± 4.39	104.90 ± 4.37	101.18 ± 3.61
PROG	**9**	**79.63 ± 6.91**	**102.39 ± 21.27**	**87.80 ± 8.88**	**88.07 ± 10.56**
(%)	**16**	**114.15 ± 12.06**	**85.43 ± 11.93**	**111.83 ± 9.98**	**113.70 ± 15.40**
	23	**72.93 ± 17.79**	**0.00 ± 0.00**	**129.09 ± 29.28**	**0.00 ± 0.00**
	30	111.05 ± 14.40	0.00 ± 0.00	107.88 ± 14.19	117.34 ± 103.41
	37	107.89 ± 12.87	73.85 ± 58.37	35.91 ± 17.74	12.61 ± 8.26
	44	104.75 ± 13.58	52.78 ± 14.21	100.45 ± 6.34	37.02 ± 13.49
	51	112.84 ± 9.84	54.85 ± 8.95	98.71 ± 4.92	52.98 ± 9.25
	93	73.82 ± 5.47	72.54 ± 4.03	103.50 ± 5.97	100.45 ± 4.05
	100	98.82 ± 2.33	91.80 ± 4.56	96.14 ± 4.44	93.47 ± 4.24
	107	95.97 ± 3.77	100.81 ± 6.27	97.99 ± 4.37	94.83 ± 5.07
	114	92.78 ± 4.97	105.02 ± 5.19	102.55 ± 3.46	103.61 ± 4.13
RAPID	**9**	**67.52 ± 5.84**	**98.88 ± 18.89**	**90.97 ± 8.76**	**89.77 ± 12.73**
(%)	**16**	**101.99 ± 5.32**	**85.48 ± 11.82**	**103.50 ± 4.19**	**115.87 ± 16.59**
	23	**68.89 ± 17.48**	**0.00 ± 0.00**	**129.74 ± 28.07**	**0.00 ± 0.00**
	30	94.34 ± 9.11	0.00 ± 0.00	101.83 ± 8.45	76.60 ± 63.06
	37	97.22 ± 5.98	76.73 ± 58.56	34.41 ± 16.83	12.61 ± 8.26
	44	91.10 ± 7.13	51.26 ± 14.00	100.19 ± 7.95	37.16 ± 13.27
	51	103.94 ± 12.53	54.52 ± 8.46	84.77 ± 3.43	54.95 ± 9.58
	93	68.02 ± 4.13	68.87 ± 3.00	103.01 ± 5.73	96.70 ± 2.60
	100	92.90 ± 3.30	83.77 ± 3.77	95.37 ± 3.26	91.62 ± 4.64
	107	89.71 ± 3.82	94.43 ± 4.63	100.72 ± 4.45	92.44 ± 4.40
	114	87.15 ± 4.23	100.05 ± 4.63	104.45 ± 5.24	102.52 ± 4.43
MEDIUM (%)	**9**	**124.50 ± 12.36**	**123.86 ± 17.22**	**162.78 ± 38.75**	**114.89 ± 15.77**
(%)	**16**	**114.71 ± 5.92**	**101.45 ± 19.01**	**104.51 ± 12.73**	**91.03 ± 15.16**
	23	**127.69 ± 22.48**	**0.00 ± 0.00**	**89.50 ± 18.79**	**0.00 ± 0.00**
	30	142.18 ± 13.23	24.92 ± 6.12	100.47 ± 6.29	36.36 ± 7.35
	37	113.82 ± 9.15	95.42 ± 15.45	45.69 ± 22.44	83.87 ± 23.17
	44	153.38 ± 25.01	90.80 ± 18.49	110.69 ± 13.79	73.88 ± 11.01
	51	96.25 ± 13.84	204.05 ± 63.32	106.07 ± 5.93	111.37 ± 12.60
	93	196.70 ± 25.13	216.34 ± 17.76	106.77 ± 10.49	117.81 ± 18.83
	100	224.88 ± 34.08	588.87 ± 243.08	159.28 ± 52.82	141.99 ± 54.28
	107	818.86 ± 246.60	538.30 ± 289.51	219.76 ± 74.45	96.53 ± 33.49
	114	424.79 ± 126.74	272.37 ± 113.32	253.49 ± 165.11	98.70 ± 27.86

Table 10..1.2. (continued) Effect of storage at two different temperatures (30 and 5°C) on changes in motility and morphological characteristics of semen from control and 16h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (6h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Days after start of insulation	30°C		5°C	
		Control rams	Insulated rams	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
SLOW	**9**	**153.90 ± 50.19**	**80.37 ± 30.13**	**12.50 ± 12.50**	**218.75 ± 108.54**
(%)	**16**	**0.00 ± 0.00**	**49.91 ± 15.59**	**0.00 ± 0.00**	**164.58 ± 63.53**
	23	**574.34 ± 431.51**	**0.00 ± 0.00**	**32.14 ± 21.72**	**0.00 ± 0.00**
	30	0.00 ± 0.00	16.28 ± 8.03	0.00 ± 0.00	54.95 ± 25.08
	37	15.28 ± 15.28	180.93 ± 112.74	0.00 ± 0.00	221.34 ± 85.52
	44	0.00 ± 0.00	53.47 ± 24.91	0.00 ± 0.00	316.60 ± 104.95
	51	71.43 ± 51.51	504.75 ± 191.75	76.87 ± 50.70	190.82 ± 59.05
	93	125.00 ± 58.33	171.95 ± 84.72	45.00 ± 33.11	0.00 ± 0.00
	100	72.02 ± 53.14	188.12 ± 116.03	7.92 ± 5.19	35.26 ± 24.06
	107	57.50 ± 57.50	130.21 ± 65.31	11.72 ± 11.72	31.25 ± 31.25
	114	32.14 ± 32.14	17.50 ± 17.50	12.85 ± 9.52	0.00 ± 0.00
LIVE	**9**	**89.12 ± 4.68**	**94.52 ± 2.53**	**92.11 ± 2.38**	**91.07 ± 2.83**
(%)	**16**	**92.39 ± 4.12**	**101.14 ± 26.81**	**100.88 ± 2.03**	**109.29 ± 16.73**
	23	**95.62 ± 3.86**	**0.00 ± 0.00**	**99.90 ± 2.06**	**0.00 ± 0.00**
	30	95.41 ± 3.76	0.00 ± 0.00	87.42 ± 1.52	0.00 ± 0.00
	37	112.95 ± 6.09	58.53 ± 34.87	99.10 ± 1.48	64.00 ± 37.37
	44	95.83 ± 0.67	90.15 ± 11.29	95.06 ± 0.40	151.88 ± 83.68
	51	100.33 ± 3.94	46.13 ± 26.63	82.77 ± 6.85	51.60 ± 29.80
	93	88.22 ± 4.21	92.75 ± 4.07	84.01 ± 2.42	84.41 ± 3.70
	100	101.33 ± 9.30	89.97 ± 5.01	94.18 ± 9.36	100.78 ± 14.46
	107	98.87 ± 8.36	92.72 ± 6.58	115.42 ± 4.48	113.72 ± 5.21
	114	99.71 ± 12.39	93.81 ± 5.76	112.78 ± 3.99	105.15 ± 4.05
DEAD	**9**	**194.17 ± 33.55**	**117.08 ± 6.30**	**223.36 ± 70.30**	**157.18 ± 24.42**
(%)	**16**	**196.75 ± 68.55**	**99.88 ± 8.47**	**95.10 ± 15.15**	**100.63 ± 3.61**
	23	**125.97 ± 22.71**	**0.00 ± 0.00**	**131.68 ± 42.09**	**0.00 ± 0.00**
	30	142.53 ± 35.89	100.00 ± 0.00	159.65 ± 7.63	100.00 ± 0.00
	37	55.07 ± 21.87	100.18 ± 1.63	115.40 ± 15.68	98.92 ± 0.66
	44	126.40 ± 10.22	117.31 ± 14.21	138.92 ± 11.55	104.93 ± 2.86
	51	111.19 ± 14.77	104.66 ± 2.19	138.94 ± 13.45	98.59 ± 0.89
	93	137.28 ± 16.24	123.32 ± 13.77	141.18 ± 17.66	127.33 ± 6.20
	100	115.02 ± 20.49	119.66 ± 9.65	109.04 ± 20.44	137.29 ± 34.54
	107	110.46 ± 18.53	116.14 ± 13.58	75.85 ± 6.92	80.78 ± 7.45
	114	130.94 ± 23.71	110.36 ± 11.09	73.77 ± 11.21	87.74 ± 10.38
NORMAL	**9**	**100.46 ± 1.35**	**106.67 ± 9.41**	**104.43 ± 4.17**	**106.05 ± 3.41**
(%)	**16**	**99.62 ± 0.84**	**128.80 ± 6.07**	**102.24 ± 3.32**	**112.30 ± 8.66**
	23	**98.08 ± 1.58**	**0.00 ± 0.00**	**101.90 ± 1.66**	**0.00 ± 0.00**
	30	104.22 ± 3.45	98.28 ± 3.96	99.87 ± 1.11	125.86 ± 16.08
	37	105.57 ± 3.17	104.25 ± 6.73	95.55 ± 1.27	138.00 ± 6.38
	44	97.74 ± 3.87	97.35 ± 5.14	101.33 ± 0.64	100.16 ± 2.80
	51	100.87 ± 5.09	87.81 ± 7.27	100.74 ± 3.17	109.63 ± 8.63
	93	120.08 ± 11.45	110.79 ± 18.70	92.21 ± 7.64	90.14 ± 6.96
	100	112.54 ± 25.75	87.43 ± 6.47	107.41 ± 20.47	127.09 ± 50.36
	107	98.33 ± 19.56	98.93 ± 10.12	86.75 ± 18.96	107.81 ± 16.45
	114	132.82 ± 33.74	97.32 ± 3.65	99.93 ± 11.79	129.05 ± 33.39

Table 10.1.2. (continued). Effect of storage at two different temperatures (30 and 5°C) on changes in motility and morphological characteristics of semen from control and 16h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (6h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Days after start of insulation	30°C		5°C	
		Control rams	Insulated rams	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD	**9**	**106.23 ± 14.89**	**176.04 ± 105.91**	**118.44 ± 32.78**	**170.46 ± 62.48**
(%)	**16**	**150.00 ± 86.60**	**81.96 ± 27.24**	**126.92 ± 124.37**	**98.42 ± 11.87**
	23	**100.00 ± 57.74**	**0.00 ± 0.00**	**16.67 ± 16.67**	**0.00 ± 0.00**
	30	100.00 ± 67.70	194.34 ± 44.17	0.00 ± 0.00	105.35 ± 17.76
	37	229.17 ± 207.21	97.17 ± 20.79	75.00 ± 47.87	75.60 ± 12.68
	44	50.00 ± 28.87	112.69 ± 17.09	100.00 ± 57.74	109.27 ± 20.53
	51	113.89 ± 95.62	465.35 ± 241.47	112.50 ± 112.50	124.35 ± 63.09
	93	35.00 ± 23.63	91.67 ± 71.20	62.50 ± 47.32	39.58 ± 17.80
	100	50.00 ± 28.87	58.33 ± 47.87	79.17 ± 73.72	62.50 ± 37.50
	107	0.00 ± 0.00	125.00 ± 75.00	0.00 ± 0.00	100.00 ± 70.71
	114	0.00 ± 0.00	52.50 ± 49.22	25.00 ± 25.00	51.19 ± 28.20
COILED TAIL	**9**	**93.75 ± 6.25**	**197.27 ± 62.13**	**64.58 ± 36.38**	**48.49 ± 9.92**
(%)	**16**	**58.33 ± 25.00**	**78.21 ± 8.58**	**125.00 ± 47.87**	**98.83 ± 4.91**
	23	**81.25 ± 73.15**	**0.00 ± 0.00**	**37.50 ± 37.50**	**0.00 ± 0.00**
	30	157.50 ± 63.03	97.92 ± 36.70	183.33 ± 108.65	106.87 ± 35.87
	37	89.93 ± 35.81	118.25 ± 47.41	483.25 ± 202.11	54.85 ± 26.36
	44	37.50 ± 21.65	102.67 ± 34.76	48.96 ± 31.75	49.00 ± 28.86
	51	325.00 ± 158.77	201.87 ± 89.00	61.46 ± 46.69	138.82 ± 54.32
	93	102.65 ± 10.53	193.43 ± 83.86	155.54 ± 71.56	123.19 ± 34.88
	100	104.61 ± 57.07	224.30 ± 84.74	88.38 ± 28.15	93.04 ± 22.87
	107	114.03 ± 39.14	115.60 ± 12.58	91.92 ± 21.57	89.71 ± 17.38
	114	91.00 ± 13.58	216.25 ± 62.45	191.75 ± 93.73	98.00 ± 14.74
BENT TAIL	**9**	**104.08 ± 15.43**	**76.66 ± 19.72**	**88.21 ± 38.37**	**97.99 ± 21.91**
(%)	**16**	**112.50 ± 51.54**	**84.42 ± 15.85**	**33.33 ± 23.57**	**88.66 ± 8.48**
	23	**308.34 ± 230.69**	**0.00 ± 0.00**	**86.31 ± 54.58**	**0.00 ± 0.00**
	30	34.88 ± 14.72	76.95 ± 19.33	92.50 ± 48.88	77.53 ± 4.72
	37	66.67 ± 11.77	100.84 ± 16.94	250.00 ± 86.60	36.65 ± 6.55
	44	295.83 ± 216.71	115.91 ± 28.36	17.50 ± 11.81	67.08 ± 9.73
	51	212.44 ± 162.89	376.04 ± 160.14	58.75 ± 13.29	81.25 ± 18.84
	93	155.12 ± 115.02	166.73 ± 88.40	156.18 ± 36.56	238.92 ± 75.27
	100	156.80 ± 49.29	141.24 ± 19.94	118.55 ± 63.88	130.68 ± 58.12
	107	110.15 ± 28.23	95.89 ± 6.86	237.13 ± 140.44	103.11 ± 14.21
	114	101.29 ± 21.98	103.68 ± 8.59	120.95 ± 27.67	109.33 ± 26.89
ABNORMAL	**9**	**97.92 ± 6.15**	**92.32 ± 12.27**	**85.23 ± 36.33**	**85.72 ± 7.05**
(%)	**16**	**125.00 ± 47.99**	**75.58 ± 1.68**	**163.86 ± 81.22**	**95.36 ± 7.14**
	23	**185.00 ± 76.21**	**0.00 ± 0.00**	**87.45 ± 45.87**	**0.00 ± 0.00**
	30	74.95 ± 26.20	124.90 ± 18.48	106.04 ± 26.45	91.57 ± 9.57
	37	79.39 ± 12.08	94.78 ± 12.72	356.67 ± 96.17	54.55 ± 6.70
	44	244.36 ± 177.09	112.42 ± 8.84	70.32 ± 10.19	97.48 ± 6.03
	51	117.89 ± 45.93	345.91 ± 151.49	110.33 ± 53.99	94.00 ± 3.48
	93	94.60 ± 46.33	129.78 ± 44.05	144.00 ± 35.87	180.62 ± 51.03
	100	142.48 ± 46.33	139.43 ± 21.79	100.33 ± 43.38	125.75 ± 51.45
	107	110.58 ± 29.88	100.97 ± 5.95	187.75 ± 98.48	99.47 ± 13.98
	114	98.74 ± 23.59	108.21 ± 6.63	126.83 ± 32.63	106.03 ± 24.99

Table 10.1.2. (continued) Effect of storage at two different temperatures (30 and 5°C) on changes in motility and morphological characteristics of semen from control and 16h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (6h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Days after start of insulation	30°C		5°C	
		Control rams	Insulated rams	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
ALH	**9**	**67.76 ± 2.02**	**88.68 ± 5.02**	**102.27 ± 4.73**	**95.29 ± 6.80**
(µm)	**16**	**86.84 ± 3.43**	**90.32 ± 6.19**	**94.66 ± 3.67**	**125.45 ± 29.35**
	23	**73.58 ± 6.03**	**0.00 ± 0.00**	**107.66 ± 10.03**	**0.00 ± 0.00**
	30	78.90 ± 4.22	151.43 ± 116.87	98.45 ± 4.39	75.63 ± 24.96
	37	89.91 ± 4.97	106.68 ± 29.01	35.35 ± 17.25	62.76 ± 18.98
	44	81.98 ± 6.50	76.63 ± 18.65	93.00 ± 8.99	73.54 ± 13.43
	51	84.13 ± 5.39	64.32 ± 6.41	85.36 ± 3.94	80.14 ± 9.87
	93	70.00 ± 1.76	72.44 ± 4.94	93.65 ± 2.62	92.32 ± 3.65
	100	70.76 ± 4.89	57.12 ± 3.09	88.47 ± 3.62	94.85 ± 4.91
	107	71.33 ± 4.21	69.80 ± 4.62	93.62 ± 5.46	96.51 ± 3.15
	114	76.00 ± 4.19	70.93 ± 2.58	83.34 ± 3.31	98.97 ± 6.21
LIN	**9**	**114.97 ± 4.27**	**102.24 ± 4.55**	**95.39 ± 4.96**	**101.37 ± 2.80**
(%)	**16**	**112.44 ± 5.10**	**101.52 ± 3.87**	**105.87 ± 4.06**	**102.01 ± 4.95**
	23	**102.36 ± 4.85**	**0.00 ± 0.00**	**103.40 ± 4.99**	**0.00 ± 0.00**
	30	115.04 ± 5.86	89.78 ± 10.77	101.34 ± 5.85	102.59 ± 20.63
	37	105.58 ± 6.40	107.07 ± 4.99	38.54 ± 18.84	75.45 ± 9.04
	44	114.53 ± 9.76	91.12 ± 7.52	106.30 ± 5.59	86.44 ± 6.61
	51	111.15 ± 3.88	100.20 ± 2.75	114.68 ± 4.22	93.34 ± 3.93
	93	102.81 ± 3.96	102.39 ± 3.89	102.32 ± 1.79	103.66 ± 4.08
	100	106.29 ± 3.15	108.90 ± 2.56	102.69 ± 3.12	104.24 ± 1.98
	107	109.56 ± 4.10	106.04 ± 4.99	98.16 ± 3.58	103.02 ± 1.82
	114	106.90 ± 4.01	107.45 ± 2.09	100.00 ± 2.79	101.60 ± 3.33
VAP	**9**	**87.77 ± 4.22**	**88.96 ± 6.59**	**95.86 ± 7.81**	**94.84 ± 6.10**
(µm/s)	**16**	**92.74 ± 3.05**	**97.52 ± 10.28**	**103.01 ± 4.20**	**112.25 ± 12.97**
	23	**68.52 ± 5.60**	**0.00 ± 0.00**	**99.69 ± 7.03**	**0.00 ± 0.00**
	30	85.41 ± 4.03	104.99 ± 42.69	100.68 ± 3.86	113.30 ± 34.71
	37	94.62 ± 4.05	130.51 ± 24.88	36.05 ± 17.60	82.78 ± 20.19
	44	88.76 ± 5.23	90.25 ± 6.10	97.43 ± 5.02	71.40 ± 12.51
	51	104.50 ± 4.56	67.88 ± 5.99	99.43 ± 2.60	78.48 ± 10.28
	93	72.95 ± 3.27	72.59 ± 1.89	96.62 ± 2.62	95.52 ± 3.13
	100	77.12 ± 2.94	61.59 ± 1.14	94.88 ± 3.59	104.16 ± 5.04
	107	82.58 ± 6.35	70.73 ± 6.91	84.63 ± 4.88	107.54 ± 4.04
	114	82.01 ± 4.33	83.00 ± 2.38	87.12 ± 3.48	96.16 ± 5.37

Table 10.1.2. (continued) Effect of storage at two different temperatures (30 and 5°C) on changes in and morphological characteristics of semen from control and 16h/day scrotally insulated rams (Mean ± SEM, n = 8 for motility characteristics and n = 4 for morphological characteristics). Mean expressed as the percentage of samples after storage (6h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Days after start of insulation	30°C		5°C	
		Control rams	Insulated rams	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
VSL	**13**	**98.87 ± 5.61**	**90.41 ± 8.14**	**94.10 ± 9.72**	**94.23 ± 5.00**
(µm/s)	**20**	**98.87 ± 6.20**	**99.37 ± 9.89**	**108.13 ± 7.24**	**115.25 ± 13.94**
	27	**71.29 ± 6.74**	**0.00 ± 0.00**	**100.05 ± 8.60**	**0.00 ± 0.00**
	34	93.05 ± 6.84	111.55 ± 68.86	102.32 ± 7.74	155.51 ± 64.09
	41	100.23 ± 8.09	137.57 ± 29.04	36.84 ± 17.99	74.00 ± 21.39
	48	97.12 ± 9.52	83.59 ± 4.00	99.47 ± 3.63	67.68 ± 14.36
	55	113.55 ± 4.00	67.85 ± 6.75	110.06 ± 2.29	76.67 ± 10.72
	97	75.01 ± 5.12	74.62 ± 2.72	97.58 ± 3.64	97.67 ± 5.56
	104	80.38 ± 3.24	64.82 ± 1.92	96.75 ± 5.12	107.40 ± 6.03
	111	87.47 ± 6.64	73.43 ± 8.88	82.91 ± 5.15	110.35 ± 4.91
	118	86.58 ± 5.65	85.95 ± 2.87	85.67 ± 3.60	97.20 ± 6.47
VCL	**13**	**81.02 ± 3.37**	**88.53 ± 6.11**	**96.92 ± 6.37**	**93.62 ± 6.36**
(µm/s)	**20**	**88.02 ± 1.89**	**97.51 ± 9.64**	**100.53 ± 2.91**	**113.46 ± 14.88**
	27	**67.98 ± 4.22**	**0.00 ± 0.00**	**101.43 ± 7.30**	**0.00 ± 0.00**
	34	79.64 ± 2.74	96.31 ± 37.44	99.23 ± 2.37	113.26 ± 34.66
	41	92.26 ± 2.32	124.65 ± 25.67	35.84 ± 17.50	83.68 ± 18.83
	48	84.62 ± 4.32	96.97 ± 9.01	96.00 ± 6.51	71.75 ± 12.23
	55	98.57 ± 4.99	67.32 ± 5.87	94.28 ± 3.42	79.35 ± 10.10
	97	70.84 ± 2.58	70.88 ± 2.05	95.39 ± 2.14	94.70 ± 1.89
	104	74.82 ± 3.19	58.80 ± 1.27	93.92 ± 3.25	102.06 ± 4.69
	111	78.35 ± 6.14	68.59 ± 6.22	84.41 ± 4.59	106.02 ± 3.47
	118	79.22 ± 3.63	79.59 ± 2.45	86.22 ± 3.28	95.52 ± 4.71
STR	**13**	**109.80 ± 3.05**	**101.68 ± 3.03**	**96.29 ± 3.11**	**99.86 ± 2.21**
(%)	**20**	**107.43 ± 3.68**	**100.92 ± 3.50**	**103.96 ± 3.09**	**101.16 ± 3.27**
	27	**102.80 ± 3.03**	**0.00 ± 0.00**	**108.63 ± 8.00**	**0.00 ± 0.00**
	34	108.32 ± 3.60	92.01 ± 16.11	100.06 ± 4.22	115.19 ± 33.01
	41	103.82 ± 4.40	102.97 ± 7.39	38.22 ± 18.68	79.02 ± 9.14
	48	108.98 ± 6.37	95.53 ± 4.88	104.21 ± 3.84	88.28 ± 5.55
	55	106.90 ± 2.30	101.08 ± 2.00	109.53 ± 2.60	95.19 ± 2.81
	97	101.02 ± 3.04	100.93 ± 2.41	101.09 ± 1.33	102.74 ± 2.96
	104	103.87 ± 2.27	105.15 ± 1.94	101.73 ± 2.39	102.55 ± 1.43
	111	105.30 ± 2.02	103.60 ± 3.29	98.33 ± 2.78	101.47 ± 1.59
	118	104.06 ± 2.58	104.11 ± 1.53	98.61 ± 2.05	100.80 ± 1.83

Table 10.1.3. Effect of diluent at two different temperaturers (30 and 5°C) on changes in motility and morphological characteristics of semen from control and 16h/day scrotally insulated rams (Mean ± SEM, n = 12 for period during insulation and 16 for after insulation and full recovery). Mean represented pooled data within each period and expressed as the percentage of samples after storage (6h) as the percentage of samples prestorage (0h)

Semen characteristic	Period	Diluent	30°C		5°C	
			Control rams	Insulated rams	Controlrams	Insulated rams
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	**During Insulation**	**HYCG**	**81.66 ± 7.2**	**63.68 ± 16.37**	**107.88 ± 4.4**	**58.85 ± 13.58**
		CEQY	**94.27 ± 10.99**	**63.51 ± 14.32**	**96.16 ± 9.85**	**68.62 ± 14.78**
	After Insulation	HYCG	103.14 ± 3.03	75.45 ± 12.65	83.69 ± 8.6	59.38 ± 8.47
		CEQY	99.36 ± 1.95	68.87 ± 10.56	81.81 ± 10.3	69.93 ± 7.14
	Full recovery	HYCG	92.13 ± 2.39	97.63 ± 3.37	101.21 ± 2.15	97.74 ± 2.87
		CEQY	93.93 ± 2.26	93.70 ± 2.11	101.77 ± 2.91	94.38 ± 2.94
PROG (%)	**During Insulation**	**HYCG**	**77.71 ± 8.95**	**62.12 ± 20.44**	**125.15 ± 17.14**	**60.70 ± 15.32**
		CEQY	**100.10 ± 13.14**	**63.09 ± 14.13**	**93.99 ± 12.40**	**73.81 ± 18.34**
	After Insulation	HYCG	115.17 ± 10.21	69.23 ± 28.55	80.56 ± 10.01	26.80 ± 9.21
		CEQY	103.10 ± 6.69	21.50 ± 8.38	90.91 ± 11.99	88.72 ± 54.01
	Full recovery	HYCG	92.65 ± 2.73	95.85 ± 5.67	104.43 ± 2.63	100.71 ± 3.19
		CEQY	88.05 ± 4.68	89.24 ± 3.36	95.66 ± 3.46	95.47 ± 3.03
RAPID (%)	**During Insulation**	**HYCG**	**72.32 ± 8.15**	**60.36 ± 19.07**	**123.53 ± 16.14**	**59.95 ± 16.16**
		CEQY	**86.62 ± 11.25**	**62.55 ± 13.99**	**92.61 ± 10.85**	**77.14 ± 18.94**
	After Insulation	HYCG	104.23 ± 7.20	69.75 ± 28.69	79.81 ± 9.30	27.53 ± 9.30
		CEQY	89.07 ± 4.44	21.50 ± 8.22	80.79 ± 10.64	67.34 ± 32.66
	Full recovery	HYCG	84.73 ± 3.27	88.78 ± 4.83	101.50 ± 3.10	97.15 ± 2.99
		CEQY	84.16 ± 3.99	84.78 ± 3.21	100.28 ± 3.62	94.49 ± 3.01
MEDIUM (%)	**During Insulation**	**HYCG**	**118.47 ± 9.73**	**80.79 ± 21.36**	**107.81 ± 15.70**	**78.51 ± 19.99**
		CEQY	**126.14 ± 14.22**	**69.41 ± 18.58**	**130.05 ± 27.72**	**58.77 ± 15.21**
	After Insulation	HYCG	118.70 ± 12.32	105.33 ± 34.61	94.01 ± 11.24	78.32 ± 11.70
		CEQY	134.12 ± 12.62	102.26 ± 20.78	87.45 ± 11.98	79.38 ± 12.53
	Full recovery	HYCG	276.70 ± 67.93	230.28 ± 40.73	211.49 ± 83.03	142.40 ± 27.74
		CEQY	555.92 ± 139.19	577.66 ± 185	158.15 ± 42.00	85.11 ± 19.47
SLOW (%)	**During Insulation**	**HYCG**	**111.81 ± 36.13**	**32.22 ± 12.18**	**21.43 ± 14.87**	**223.82 ± 79.37**
		CEQY	**373.69 ± 293.86**	**54.63 ± 22.73**	**8.33 ± 8.33**	**31.74 ± 17.49**
	After Insulation	HYCG	32.64 ± 25.65	208.07 ± 105.14	21.25 ± 21.25	199.63 ± 43.97
		CEQY	10.71 ± 10.71	169.64 ± 74.39	17.19 ± 17.19	205.04 ± 70.17
	Full recovery	HYCG	81.25 ± 34.03	236.67 ± 69.05	34.05 ± 17.15	27.50 ± 18.98
		CEQY	62.08 ± 38.06	17.22 ± 10.07	4.69 ± 4.69	5.75 ± 4.88
LIVE (%)	**During Insulation**	**HYCG**	**98.15 ± 5.73**	**55.69 ± 25.61**	**96.23 ± 2.51**	**49.45 ± 22.41**
		CEQY	**100.31 ± 6.07**	**46.34 ± 20.76**	**97.83 ± 1.78**	**53.92 ± 25.31**
	After Insulation	HYCG	99.59 ± 3.54	53.83 ± 21.03	92.21 ± 2.67	42.70 ± 18.18
		CEQY	102.68 ± 3.94	43.57 ± 17.02	89.95 ± 3.89	91.03 ± 48.43
	Full recovery	HYCG	87.17 ± 3.16	89.83 ± 4.32	106.54 ± 6.33	100.81 ± 6.79
		CEQY	106.90 ± 6.33	94.79 ± 2.34	96.65 ± 5.41	101.22 ± 6.59
DEAD (%)	**During Insulation**	**HYCG**	**118.64 ± 34.22**	**68.53 ± 21.73**	**196.72 ± 53.77**	**83.37 ± 28.6**
		CEQY	**131.49 ± 30.83**	**76.31 ± 24.63**	**116.90 ± 12.63**	**87.36 ± 33.21**
	After Insulation	HYCG	117.08 ± 21.38	102.47 ± 1.83	135.91 ± 11.48	100.58 ± 1.21
		CEQY	100.52 ± 16.77	108.60 ± 7.28	140.54 ± 8.33	100.64 ± 1.56
	Full recovery	HYCG	143.32 ± 11.95	119.18 ± 8.32	88.46 ± 13.05	107.35 ± 10.73
		CEQY	103.52 ± 10.93	115.56 ± 7.93	111.46 ± 14.26	109.23 ± 18.80
NORMAL (%)	**During Insulation**	**HYCG**	**97.88 ± 3.04**	**80.17 ± 30.32**	**99.41 ± 2.67**	**82.93 ± 27.09**
		CEQY	**102.60 ± 2.20**	**74.16 ± 24.06**	**101.84 ± 2.56**	**79.75 ± 26.00**
	After Insulation	HYCG	98.14 ± 3.66	91.91 ± 8.04	98.30 ± 1.54	116.56 ± 8.40
		CEQY	104.50 ± 1.88	90.53 ± 10.69	100.45 ± 1.27	120.25 ± 8.39
	Full recovery	HYCG	124.16 ± 20.02	94.80 ± 10.44	107.60 ± 11.47	100.44 ± 16.58
		CEQY	107.64 ± 10.90	102.45 ± 3.89	85.50 ± 7.72	126.70 ± 24.49
LOOSE HEAD (%)	**During Insulation**	**HYCG**	**375.82 ± 296.26**	**43.57 ± 23.72**	**97.57 ± 30.71**	**55.57 ± 32.23**
		CEQY	**81.11 ± 33.18**	**143.79 ± 71.43**	**42.50 ± 30.10**	**108.47 ± 47.46**
	After Insulation	HYCG	336.11 ± 222.79	107.98 ± 23.87	118.75 ± 56.65	102.57 ± 31.86
		CEQY	35.42 ± 12.38	2068.46 ± 1947.59	25.00 ± 25.00	104.72 ± 10.90
	Full recovery	HYCG	25.00 ± 16.37	105.42 ± 48.61	37.50 ± 26.31	90.62 ± 35.34
		CEQY	17.50 ± 12.78	58.33 ± 31.97	45.83 ± 36.83	48.51 ± 18.00

Table 10.1.3. (continued) Effect of diluent at two different temperaturers (30 and 5°C) on changes in motility and morphological characteristics of semen from control and 16h/day scrotally insulated rams (Mean ± SEM, n = 12 for period during insulation and 16 for after insulation and full recovery). Mean represented pooled data within each period and expressed as the percentage of samples after storage (6h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Period	Diluent	30°C		5°C	
			Control rams	Insulated rams	Controlrams	Insulated rams
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL (%)	**During Insulation**	**HYCG**	**83.61 ± 21.39**	**90.14 ± 47.77**	**316.67 ± 163.64**	**29.73 ± 10.71**
		CEQY	**59.72 ± 23.81**	**111.28 ± 54.64**	**79.67 ± 35.47**	**39.22 ± 20.88**
	After Insulation	HYCG	147.71 ± 88.69	91.63 ± 21.29	243.75 ± 128.67	65.93 ± 15.52
		CEQY	157.29 ± 45.90	123.86 ± 37.58	144.79 ± 58.75	108.88 ± 35.50
	Full recovery	HYCG	98.24 ± 21.89	237.50 ± 58.01	92.29 ± 20.41	121.23 ± 17.93
		CEQY	107.86 ± 24.97	137.30 ± 18.99	171.41 ± 54.10	80.68 ± 9.71
BENT TAIL (%)	**During Insulation**	**HYCG**	**331.79 ± 253.95**	**67.41 ± 25.43**	**134.23 ± 64.12**	**50.71 ± 20.11**
		CEQY	**87.60 ± 10.28**	**43.95 ± 15.41**	**148.78 ± 54.79**	**39.04 ± 21.09**
	After Insulation	HYCG	241.09 ± 127.67	185.86 ± 75.74	93.75 ± 47.05	71.79 ± 10.38
		CEQY	63.82 ± 22.76	149.01 ± 64.62	115.62 ± 46.38	59.47 ± 8.92
	Full recovery	HYCG	153.26 ± 55.02	157.35 ± 41.63	153.59 ± 73.10	146.36 ± 27.30
		CEQY	108.42 ± 25.34	96.42 ± 4.74	163.03 ± 29.26	144.67 ± 47.45
ABNORMAL (%)	**During Insulation**	**HYCG**	**173.71 ± 57.65**	**63.06 ± 23.27**	**238.55 ± 92.65**	**46.70 ± 16.80**
		CEQY	**87.67 ± 16.32**	**56.82 ± 19.21**	**114.33 ± 37.91**	**46.81 ± 16.05**
	After Insulation	HYCG	191.41 ± 87.38	243.79 ± 136.81	211.02 ± 70.60	84.54 ± 8.73
		CEQY	75.50 ± 11.50	442.30 ± 329.83	110.66 ± 28.22	84.26 ± 7.04
	Full recovery	HYCG	116.33 ± 27.25	138.18 ± 21.81	128.75 ± 51.20	131.04 ± 20.58
		CEQY	106.87 ± 24.13	101.01 ± 5.48	150.87 ± 24.82	124.89 ± 34.13
ALH (µm)	**During Insulation**	**HYCG**	**78.41 ± 3.25**	**55.10 ± 12.21**	**103.35 ± 4.57**	**72.30 ± 23.46**
		CEQY	**73.71 ± 4.70**	**64.23 ± 14.07**	**99.71 ± 6.44**	**74.86 ± 18.82**
	After Insulation	HYCG	88.38 ± 4.03	145.08 ± 55.83	85.07 ± 9.40	70.52 ± 11.19
		CEQY	79.09 ± 3.09	54.45 ± 15.28	71.01 ± 9.24	80.55 ± 13.02
	Full recovery	HYCG	71.55 ± 2.55	68.78 ± 2.85	86.24 ± 2.89	96.25 ± 3.05
		CEQY	72.50 ± 2.96	66.37 ± 3.34	93.30 ± 2.57	95.07 ± 3.39
LIN (%)	**During Insulation**	**HYCG**	**104.27 ± 3.58**	**69.03 ± 15.08**	**103.69 ± 2.55**	**68.91 ± 14.95**
		CEQY	**115.58 ± 3.94**	**66.81 ± 14.38**	**99.43 ± 4.93**	**66.68 ± 14.42**
	After Insulation	HYCG	109.99 ± 5.81	103.99 ± 3.95	88.58 ± 9.28	86.86 ± 5.81
		CEQY	113.15 ± 3.35	90.09 ± 5.69	91.84 ± 11.73	98.19 ± 9.13
	Full recovery	HYCG	109.12 ± 1.82	106.80 ± 2.50	104.01 ± 1.84	104.42 ± 1.66
		CEQY	103.66 ± 3.18	105.59 ± 2.52	97.57 ± 1.87	101.83 ± 2.31
VAP (µm)	**During Insulation**	**HYCG**	**84.94 ± 4.92**	**58.97 ± 14.57**	**104.20 ± 4.77**	**65.48 ± 15.77**
		CEQY	**81.08 ± 4.45**	**65.36 ± 14.24**	**94.83 ± 5.40**	**72.58 ± 16.69**
	After Insulation	HYCG	95.02 ± 3.98	118.99 ± 22.59	86.08 ± 8.81	74.22 ± 12.30
		CEQY	91.62 ± 3.10	77.83 ± 9.44	80.72 ± 10.22	105.35 ± 17.13
	Full recovery	HYCG	81.59 ± 3.84	72.32 ± 3.24	90.58 ± 3.38	103.57 ± 3.13
		CEQY	75.73 ± 2.11	71.63 ± 3.29	91.05 ± 2.22	98.12 ± 3.39
VSL (µm)	**During Insulation**	**HYCG**	**89.49 ± 6.24**	**60.27 ± 14.97**	**106.29 ± 5.33**	**66.59 ± 15.51**
		CEQY	**89.87 ± 6.38**	**66.25 ± 14.50**	**95.22 ± 8.11**	**73.06 ± 17.53**
	After Insulation	HYCG	102.04 ± 6.56	136.49 ± 34.42	86.61 ± 9.18	69.73 ± 12.74
		CEQY	99.93 ± 3.97	63.79 ± 7.17	87.73 ± 11.13	125.01 ± 34.04
	Full recovery	HYCG	86.83 ± 4.24	75.66 ± 4.41	92.55 ± 3.72	106.77 ± 3.64
		CEQY	77.88 ± 3.01	73.75 ± 3.30	88.90 ± 3.13	99.54 ± 4.54
VCL (µm)	**During Insulation**	**HYCG**	**81.10 ± 3.95**	**58.53 ± 14.19**	**105.43 ± 4.76**	**65.86 ± 16.85**
		CEQY	**76.92 ± 3.16**	**65.49 ± 14.27**	**93.82 ± 3.92**	**72.20 ± 16.45**
	After Insulation	HYCG	90.76 ± 3.22	116.27 ± 20.69	85.37 ± 8.84	72.19 ± 11.39
		CEQY	86.78 ± 3.04	76.35 ± 9.02	77.30 ± 9.87	108.63 ± 16.89
	Full recovery	HYCG	78.27 ± 3.55	69.58 ± 2.66	88.92 ± 3.20	101.70 ± 2.85
		CEQY	73.34 ± 1.96	69.35 ± 3.47	91.05 ± 1.90	97.45 ± 2.86
STR (%)	**During Insulation**	**HYCG**	**101.70 ± 2.41**	**67.81 ± 14.69**	**104.07 ± 2.21**	**68.34 ± 14.68**
		CEQY	**111.66 ± 2.19**	**67.26 ± 14.42**	**98.96 ± 3.21**	**65.67 ± 14.11**
	After Insulation	HYCG	105.74 ± 3.76	104.06 ± 3.61	87.75 ± 8.89	87.94 ± 5.10
		CEQY	108.27 ± 2.11	91.74 ± 8.01	88.26 ± 11.10	107.62 ± 16.19
	Full recovery	HYCG	105.91 ± 1.22	104.01 ± 1.56	102.43 ± 1.39	102.71 ± 1.19
		CEQY	101.22 ± 1.99	102.88 ± 1.78	97.45 ± 1.43	101.07 ± 1.57

Table 10.1.4.a. Characteristics of semen collected at day 9 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	67.47 ± 9.92	71.62 ± 2.97	79.18 ± 2.84	62.03 ± 5.51
		CEQY	74.07 ± 4.73	64.23 ± 2.79	68.95 ± 1.32	71.75 ± 3.97
	Control	HYCG	75.05 ± 2.39	64.87 ± 2.92	77.10 ± 3.11	73.90 ± 2.09
		CEQY	76.80 ± 4.59	54.15 ± 7.81	75.28 ± 4.56	71.78 ± 5.19
PROG (%)	Insulated	HYCG	38.40 ± 8.68	33.03 ± 5.50	49.40 ± 2.10	33.73 ± 6.19
		CEQY	40.92 ± 4.20	35.80 ± 2.35	40.20 ± 1.94	43.05 ± 3.43
	Control	HYCG	46.93 ± 3.33	39.28 ± 3.00	49.35 ± 4.03	48.55 ± 4.18
		CEQY	45.97 ± 3.01	34.70 ± 6.41	53.10 ± 1.02	39.72 ± 4.87
RAPID (%)	Insulated	HYCG	46.10 ± 10.45	38.13 ± 6.36	63.40 ± 4.17	39.40 ± 7.06
		CEQY	46.77 ± 4.91	40.67 ± 2.24	45.20 ± 2.57	52.65 ± 4.39
	Control	HYCG	57.65 ± 5.23	43.13 ± 3.04	62.47 ± 6.74	60.88 ± 3.90
		CEQY	61.45 ± 5.48	37.45 ± 7.91	65.97 ± 4.46	53.80 ± 8.48
MEDIUM (%)	Insulated	HYCG	21.35 ± 1.51	33.53 ± 5.27	15.77 ± 1.50	22.65 ± 1.66
		CEQY	27.27 ± 4.23	23.58 ± 2.41	23.72 ± 1.93	19.12 ± 2.95
	Control	HYCG	17.40 ± 3.30	21.77 ± 1.91	14.65 ± 4.26	13.02 ± 1.86
		CEQY	15.33 ± 1.78	16.70 ± 1.07	9.30 ± 0.85	17.98 ± 4.45
SLOW (%)	Insulated	HYCG	1.00 ± 0.34	1.12 ± 0.22	0.20 ± 0.08	1.45 ± 0.19
		CEQY	0.78 ± 0.28	0.55 ± 0.19	0.83 ± 0.32	0.20 ± 0.20
	Control	HYCG	0.47 ± 0.17	1.35 ± 0.32	0.08 ± 0.08	0.00 ± 0.00
		CEQY	0.28 ± 0.17	0.80 ± 0.29	0.40 ± 0.14	0.13 ± 0.13
LIVE SPERM (%)	Insulated	HYCG	76.00 ± 1.50	74.50 ± 1.50	85.50 ± 0.50	78.00 ± 4.00
		CEQY	76.00 ± 9.00	69.50 ± 11.00	80.00 ± 9.00	72.25 ± 3.75
	Control	HYCG	87.00 ± 0.00	74.50 ± 9.00	93.75 ± 2.75	84.25 ± 0.75
		CEQY	91.00 ± 3.50	84.25 ± 2.75	88.50 ± 1.00	83.50 ± 5.00
DEAD SPERM (%)	Insulated	HYCG	24.00 ± 1.50	25.50 ± 1.50	14.50 ± 0.50	22.00 ± 4.00
		CEQY	24.00 ± 9.00	30.50 ± 11.00	20.00 ± 9.00	27.75 ± 3.75
	Control	HYCG	13.00 ± 0.00	25.50 ± 9.00	6.25 ± 2.75	15.75 ± 0.75
		CEQY	9.00 ± 3.50	15.75 ± 2.75	11.50 ± 1.00	16.50 ± 5.00
NORMAL SPERM (%)	Insulated	HYCG	65.50 ± 4.00	64.25 ± 3.25	69.25 ± 6.25	73.50 ± 1.00
		CEQY	56.00 ± 0.00	64.50 ± 11.00	75.75 ± 4.25	79.75 ± 5.25
	Control	HYCG	81.50 ± 1.00	82.25 ± 2.75	84.75 ± 2.25	88.75 ± 7.75
		CEQY	81.75 ± 0.75	81.75 ± 1.25	90.50 ± 5.00	94.00 ± 2.00
LOOSE HEAD SPERM (%)	Insulated	HYCG	5.00 ± 3.00	0.75 ± 0.25	1.50 ± 1.50	3.25 ± 2.75
		CEQY	1.50 ± 0.00	5.00 ± 2.00	3.00 ± 2.50	5.75 ± 4.25
	Control	HYCG	4.75 ± 3.75	4.25 ± 3.25	4.50 ± 3.50	7.00 ± 6.50
		CEQY	5.25 ± 2.25	5.50 ± 1.00	2.25 ± 0.25	3.00 ± 1.50

Table 10.1.4.a. (continued) Characteristics of semen collected at day 9 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	6.25 ± 0.25	14.25 ± 4.75	15.25 ± 5.25	9.00 ± 4.00
		CEQY	6.50 ± 0.00	11.00 ± 8.50	12.00 ± 7.00	6.25 ± 5.25
	Control	HYCG	2.25 ± 0.25	2.00 ± 0.50	4.50 ± 3.50	0.75 ± 0.75
		CEQY	2.00 ± 1.00	2.00 ± 1.00	3.25 ± 2.75	0.50 ± 0.00
BENT TAIL SPERM (%)	Insulated	HYCG	23.25 ± 6.75	20.75 ± 1.25	14.00 ± 2.50	14.25 ± 0.25
		CEQY	36.00 ± 0.00	19.50 ± 4.50	9.25 ± 0.25	8.25 ± 4.25
	Control	HYCG	11.50 ± 2.50	11.50 ± 0.00	6.25 ± 2.25	3.50 ± 0.50
		CEQY	11.00 ± 2.00	10.75 ± 1.25	4.00 ± 2.50	2.50 ± 0.50
ABNOR-MAL SPERM (%)	Insulated	HYCG	34.50 ± 4.00	35.75 ± 3.25	30.75 ± 6.25	26.50 ± 1.00
		CEQY	44.00 ± 0.00	35.50 ± 11.00	24.25 ± 4.25	20.25 ± 5.25
	Control	HYCG	18.50 ± 1.00	17.75 ± 2.75	15.25 ± 2.25	11.25 ± 7.75
		CEQY	18.25 ± 0.75	18.25 ± 1.25	9.50 ± 5.00	6.00 ± 2.00
ALH (µm)	Insulated	HYCG	7.83 ± 0.49	6.35 ± 0.33	9.32 ± 0.33	7.53 ± 0.66
		CEQY	7.67 ± 0.57	7.20 ± 0.13	7.65 ± 0.52	8.33 ± 0.27
	Control	HYCG	8.15 ± 0.10	5.65 ± 0.21	8.50 ± 0.56	8.00 ± 0.43
		CEQY	8.90 ± 0.41	5.87 ± 0.31	8.40 ± 0.56	9.18 ± 0.53
LIN (%)	Insulated	HYCG	62.53 ± 2.38	65.43 ± 2.44	62.75 ± 1.21	66.05 ± 3.35
		CEQY	68.35 ± 3.04	67.20 ± 1.19	67.45 ± 2.43	65.78 ± 2.84
	Control	HYCG	67.12 ± 0.50	71.80 ± 1.80	65.73 ± 0.72	67.85 ± 2.49
		CEQY	62.33 ± 3.19	76.10 ± 1.36	69.85 ± 4.17	60.30 ± 2.48
VAP (µm/s)	Insulated	HYCG	122.82 ± 14.84	99.22 ± 8.54	144.40 ± 4.50	119.10 ± 7.90
		CEQY	120.60 ± 7.49	111.42 ± 2.88	124.07 ± 5.68	132.07 ± 4.63
	Control	HYCG	139.23 ± 12.14	131.08 ± 7.16	152.68 ± 14.35	161.87 ± 3.82
		CEQY	153.52 ± 5.57	123.07 ± 7.00	166.50 ± 5.72	138.35 ± 16.69
VSl (µm/s)	Insulated	HYCG	99.95 ± 13.48	82.78 ± 8.16	113.87 ± 2.16	99.25 ± 6.48
		CEQY	101.50 ± 4.94	93.15 ± 3.68	106.53 ± 4.60	106.97 ± 4.00
	Control	HYCG	113.62 ± 9.78	116.93 ± 5.95	123.30 ± 10.68	134.20 ± 3.95
		CEQY	120.73 ± 7.99	110.90 ± 5.25	139.50 ± 6.43	105.55 ± 13.09
VCL (µm/s)	Insulated	HYCG	147.57 ± 16.30	117.73 ± 9.40	176.37 ± 5.40	141.67 ± 11.09
		CEQY	144.02 ± 9.65	134.20 ± 2.90	148.75 ± 7.65	158.10 ± 5.55
	Control	HYCG	165.87 ± 12.45	146.03 ± 8.04	181.02 ± 16.78	188.85 ± 4.36
		CEQY	186.30 ± 5.81	137.10 ± 7.27	194.73 ± 8.36	170.30 ± 18.31
STR (%)	Insulated	HYCG	74.78 ± 1.83	77.40 ± 2.08	75.12 ± 1.50	77.77 ± 2.89
		CEQY	80.10 ± 2.37	79.45 ± 0.98	80.55 ± 2.06	77.45 ± 2.22
	Control	HYCG	78.53 ± 0.31	81.37 ± 1.42	76.87 ± 0.90	77.90 ± 1.68
		CEQY	73.97 ± 2.73	85.53 ± 1.71	80.30 ± 3.47	72.82 ± 1.85

Table 10.1.4.b. Characteristics of semen collected at day 16 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	18.20 ± 2.62	13.88 ± 3.72	15.50 ± 3.19	14.45 ± 2.10
		CEQY	12.20 ± 1.88	12.30 ± 2.15	16.80 ± 1.09	17.10 ± 1.30
	Control	HYCG	79.05 ± 2.39	81.15 ± 2.52	81.55 ± 2.39	83.25 ± 1.48
		CEQY	78.97 ± 3.20	84.87 ± 1.16	82.65 ± 1.97	85.12 ± 0.41
PROG (%)	Insulated	HYCG	7.55 ± 2.73	4.05 ± 0.37	5.58 ± 0.62	6.53 ± 1.48
		CEQY	7.55 ± 1.20	7.25 ± 0.92	8.03 ± 0.89	8.48 ± 1.73
	Control	HYCG	35.65 ± 3.31	36.55 ± 6.54	39.90 ± 1.23	40.17 ± 1.01
		CEQY	37.28 ± 4.37	44.47 ± 2.63	40.17 ± 5.86	46.15 ± 3.31
RAPID (%)	Insulated	HYCG	7.75 ± 2.72	4.22 ± 0.34	6.05 ± 0.66	7.37 ± 1.98
		CEQY	7.83 ± 1.37	7.37 ± 0.96	8.85 ± 0.96	9.42 ± 1.88
	Control	HYCG	56.22 ± 2.36	55.18 ± 4.73	65.00 ± 1.99	62.88 ± 1.26
		CEQY	59.38 ± 3.59	61.97 ± 1.84	63.60 ± 3.23	69.57 ± 3.50
MEDIUM (%)	Insulated	HYCG	10.45 ± 2.18	9.63 ± 4.01	9.45 ± 2.61	7.07 ± 1.01
		CEQY	4.37 ± 0.57	4.93 ± 1.38	8.03 ± 0.69	7.68 ± 2.42
	Control	HYCG	22.83 ± 1.00	25.98 ± 3.18	16.58 ± 1.33	20.40 ± 0.74
		CEQY	19.60 ± 1.23	22.90 ± 2.46	19.05 ± 1.52	15.55 ± 3.13
SLOW (%)	Insulated	HYCG	4.32 ± 0.85	1.85 ± 1.21	1.10 ± 0.34	1.52 ± 0.64
		CEQY	0.95 ± 0.16	0.55 ± 0.18	1.37 ± 0.25	1.12 ± 0.40
	Control	HYCG	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10
		CEQY	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LIVE SPERM (%)	Insulated	HYCG	18.25 ± 5.25	26.25 ± 16.25	18.00 ± 5.50	18.75 ± 3.25
		CEQY	28.75 ± 1.75	21.25 ± 3.75	19.00 ± 7.50	18.00 ± 1.00
	Control	HYCG	90.75 ± 3.25	81.00 ± 5.00	88.00 ± 1.50	88.00 ± 1.50
		CEQY	89.50 ± 1.50	85.25 ± 4.25	87.50 ± 0.50	89.00 ± 3.50
DEAD SPERM (%)	Insulated	HYCG	81.75 ± 5.25	73.75 ± 16.25	82.00 ± 5.50	81.25 ± 3.25
		CEQY	71.25 ± 1.75	78.75 ± 3.75	81.00 ± 7.50	82.00 ± 1.00
	Control	HYCG	9.25 ± 3.25	19.00 ± 5.00	12.00 ± 1.50	12.00 ± 1.50
		CEQY	10.50 ± 1.50	14.75 ± 4.25	12.50 ± 0.50	11.00 ± 3.50
NORMAL SPERM (%)	Insulated	HYCG	48.75 ± 11.25	60.50 ± 9.50	50.75 ± 17.75	51.75 ± 11.75
		CEQY	46.50 ± 12.00	59.75 ± 10.75	49.75 ± 14.75	56.50 ± 10.50
	Control	HYCG	98.50 ± 0.00	97.75 ± 1.25	90.25 ± 1.75	97.25 ± 0.75
		CEQY	98.00 ± 0.00	98.00 ± 1.50	98.50 ± 0.50	95.25 ± 2.25
LOOSE HEAD SPERM (%)	Insulated	HYCG	13.75 ± 2.75	11.75 ± 5.75	17.25 ± 8.75	15.75 ± 4.75
		CEQY	16.75 ± 4.25	10.50 ± 0.50	17.25 ± 6.25	15.25 ± 3.75
	Control	HYCG	0.25 ± 0.25	0.75 ± 0.75	3.25 ± 3.25	1.25 ± 0.75
		CEQY	0.25 ± 0.25	0.75 ± 0.75	0.50 ± 0.50	3.00 ± 2.00

Table 10.1.4.b. (continued) Characteristics of semen collected at day 16 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	19.50 ± 8.50	16.25 ± 5.25	18.25 ± 6.25	19.50 ± 6.50
		CEQY	22.50 ± 7.00	16.00 ± 7.00	19.75 ± 7.25	17.75 ± 6.25
	Control	HYCG	0.25 ± 0.25	0.25 ± 0.25	3.25 ± 1.25	1.00 ± 1.00
		CEQY	1.25 ± 0.25	0.75 ± 0.25	0.75 ± 0.25	1.50 ± 0.50
BENT TAIL SPERM (%)	Insulated	HYCG	18.00 ± 5.50	11.50 ± 1.50	13.75 ± 2.75	13.00 ± 0.50
		CEQY	14.25 ± 0.75	13.75 ± 3.25	13.25 ± 1.25	10.50 ± 0.50
	Control	HYCG	1.00 ± 0.00	1.25 ± 0.75	3.25 ± 0.25	0.50 ± 0.50
		CEQY	0.50 ± 0.00	0.50 ± 0.50	0.25 ± 0.25	0.25 ± 0.25
ABNOR-MAL SPERM (%)	Insulated	HYCG	51.25 ± 11.25	39.50 ± 9.50	49.25 ± 17.75	48.25 ± 11.75
		CEQY	53.50 ± 12.00	40.25 ± 10.75	50.25 ± 14.75	43.50 ± 10.50
	Control	HYCG	1.50 ± 0.00	2.25 ± 1.25	9.75 ± 1.75	2.75 ± 0.75
		CEQY	2.00 ± 0.00	2.00 ± 1.50	1.50 ± 0.50	4.75 ± 2.25
ALH (µm)	Insulated	HYCG	4.12 ± 0.62	3.40 ± 0.52	3.65 ± 0.62	4.18 ± 1.12
		CEQY	4.37 ± 0.22	4.22 ± 0.24	4.25 ± 0.65	4.37 ± 0.63
	Control	HYCG	7.90 ± 0.57	7.20 ± 0.37	8.33 ± 0.47	8.15 ± 0.41
		CEQY	7.93 ± 0.39	6.45 ± 0.22	7.53 ± 0.46	6.80 ± 0.45
LIN (%)	Insulated	HYCG	70.67 ± 3.28	71.15 ± 3.08	74.00 ± 1.43	74.90 ± 5.06
		CEQY	80.23 ± 1.55	81.22 ± 1.72	71.78 ± 3.30	72.83 ± 2.00
	Control	HYCG	53.83 ± 2.67	58.10 ± 5.24	54.42 ± 1.85	55.05 ± 1.15
		CEQY	53.95 ± 2.25	62.63 ± 1.52	55.28 ± 3.87	60.28 ± 2.45
VAP (µm/s)	Insulated	HYCG	88.35 ± 16.55	76.15 ± 12.79	89.05 ± 9.86	95.77 ± 5.64
		CEQY	119.90 ± 2.41	123.23 ± 8.66	98.10 ± 6.37	106.10 ± 15.56
	Control	HYCG	125.05 ± 3.42	113.75 ± 5.28	133.55 ± 4.89	128.60 ± 1.52
		CEQY	126.42 ± 4.42	118.90 ± 4.18	131.15 ± 5.72	142.80 ± 7.58
VSl (µm/s)	Insulated	HYCG	79.05 ± 15.70	68.28 ± 11.07	78.70 ± 9.07	84.10 ± 3.55
		CEQY	110.35 ± 3.51	117.37 ± 8.74	82.20 ± 7.37	92.55 ± 14.84
	Control	HYCG	86.12 ± 5.48	80.98 ± 9.42	90.30 ± 6.07	89.10 ± 2.04
		CEQY	85.97 ± 5.55	87.82 ± 4.23	90.22 ± 8.39	102.60 ± 8.38
VCL (µm/s)	Insulated	HYCG	98.67 ± 18.87	86.25 ± 14.52	100.62 ± 11.66	109.33 ± 9.96
		CEQY	131.43 ± 2.38	134.10 ± 8.56	112.17 ± 6.05	121.07 ± 17.46
	Control	HYCG	159.55 ± 1.01	139.18 ± 3.04	167.30 ± 6.05	162.35 ± 2.03
		CEQY	160.25 ± 3.48	142.05 ± 4.03	164.58 ± 5.04	170.28 ± 7.10
STR (%)	Insulated	HYCG	82.98 ± 3.80	81.92 ± 2.89	83.57 ± 1.50	84.67 ± 3.21
		CEQY	87.85 ± 1.23	89.75 ± 1.64	81.53 ± 2.30	81.82 ± 2.00
	Control	HYCG	65.95 ± 2.13	68.60 ± 4.53	65.65 ± 1.81	67.15 ± 1.24
		CEQY	65.47 ± 1.83	72.32 ± 1.23	66.43 ± 3.65	69.45 ± 1.53

Table 10.1.4..c. Characteristics of semen collected at day 23 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	47.08 ± 4.83	24.75 ± 4.57	32.90 ± 4.40	40.50 ± 4.02
		CEQY	35.42 ± 5.86	32.20 ± 6.66	42.93 ± 7.93	31.20 ± 2.15
PROG (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	33.17 ± 4.35	14.13 ± 3.29	18.33 ± 3.91	27.25 ± 2.74
		CEQY	26.10 ± 6.11	20.45 ± 2.86	30.42 ± 5.75	21.12 ± 2.74
RAPID (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	37.15 ± 4.85	14.55 ± 3.34	20.45 ± 4.36	30.83 ± 3.36
		CEQY	27.83 ± 6.65	20.62 ± 2.92	33.68 ± 6.20	23.75 ± 2.38
MEDIUM (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	9.93 ± 2.62	10.18 ± 3.17	12.48 ± 2.78	9.70 ± 3.70
		CEQY	7.57 ± 1.42	11.58 ± 4.86	9.25 ± 2.09	7.43 ± 0.73
SLOW (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	0.38 ± 0.13	1.15 ± 0.27	0.75 ± 0.38	0.92 ± 0.45
		CEQY	0.30 ± 0.18	6.95 ± 6.03	0.00 ± 0.00	0.40 ± 0.40
LIVE SPERM (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	87.75 ± 1.25	87.75 ± 0.25	90.25 ± 6.25	89.00 ± 2.00
		CEQY	84.50 ± 1.00	77.00 ± 5.00	85.25 ± 1.25	86.00 ± 2.50
DEAD SPERM (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	12.25 ± 1.25	12.25 ± 0.25	9.75 ± 6.25	11.00 ± 2.00
		CEQY	15.50 ± 1.00	23.00 ± 5.00	14.75 ± 1.25	14.00 ± 2.50
NORMAL SPERM (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	97.50 ± 0.50	93.75 ± 1.75	97.00 ± 1.00	97.00 ± 1.50
		CEQY	97.25 ± 0.25	97.25 ± 1.75	93.75 ± 2.75	97.25 ± 1.25
LOOSE HEAD SPERM (%)	Insulated	HYCG	no sperm	no sperm	no sperm	no sperm
		CEQY	no sperm	no sperm	no sperm	no sperm
	Control	HYCG	1.00 ± 0.50	1.50 ± 1.50	0.75 ± 0.75	0.75 ± 0.25
		CEQY	0.75 ± 0.75	1.50 ± 1.50	0.00 ± 0.00	1.00 ± 0.00

Table 10.1.4..c. (continued) Characteristics of semen collected at day 23 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	0.50 ± 0.50	1.75 ± 1.25	1.00 ± 0.00	0.75 ± 0.75
		CEQY	1.00 ± 1.00	0.50 ± 0.00	3.75 ± 1.75	1.00 ± 1.00
BENT TAIL SPERM (%)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	1.00 ± 0.50	3.00 ± 2.00	1.25 ± 0.25	1.50 ± 1.00
		CEQY	1.00 ± 0.50	0.75 ± 0.25	2.50 ± 1.00	0.75 ± 0.25
ABNORMAL SPERM (%)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	2.50 ± 0.50	6.25 ± 1.75	3.00 ± 1.00	3.00 ± 1.50
		CEQY	2.75 ± 0.25	2.75 ± 1.75	6.25 ± 2.75	2.75 ± 1.25
ALH (µm)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	8.75 ± 0.19	6.50 ± 0.23	7.18 ± 0.59	8.27 ± 0.37
		CEQY	8.00 ± 0.51	5.82 ± 1.03	7.70 ± 0.67	7.28 ± 0.97
LIN (%)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	70.82 ± 2.35	68.80 ± 3.03	66.78 ± 2.64	70.82 ± 4.01
		CEQY	71.22 ± 2.90	75.90 ± 4.26	71.55 ± 3.24	71.22 ± 4.22
VAP (µm/s)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	168.95 ± 9.47	113.68 ± 12.24	146.52 ± 13.30	154.85 ± 13.59
		CEQY	154.02 ± 9.77	105.50 ± 13.06	156.15 ± 7.69	141.78 ± 6.49
VSl (µm/s)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	147.75 ± 9.82	101.23 ± 12.50	129.07 ± 12.44	136.80 ± 14.59
		CEQY	137.05 ± 9.37	98.95 ± 13.64	134.57 ± 10.04	120.70 ± 9.03
VCL (µm/s)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	198.32 ± 8.58	132.25 ± 12.71	166.87 ± 12.65	184.40 ± 12.65
		CEQY	180.73 ± 11.51	124.07 ± 12.03	180.60 ± 5.07	162.45 ± 5.10
STR (%)	Insulated	HYCG	**no sperm**	**no sperm**	**no sperm**	**no sperm**
		CEQY	**no sperm**	**no sperm**	**no sperm**	**no sperm**
	Control	HYCG	82.82 ± 1.54	80.52 ± 2.17	77.12 ± 1.83	83.32 ± 2.86
		CEQY	82.55 ± 2.16	89.20 ± 2.07	81.20 ± 1.98	81.15 ± 2.73

Table 10.1.4..d. Characteristics of semen collected at day 30 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	17.33 ± 5.30	4.18 ± 1.13	38.72 ± 5.40	15.25 ± 0.84
		CEQY	25.98 ± 4.93	4.02 ± 1.30	37.33 ± 5.11	13.47 ± 4.62
	Control	HYCG	74.43 ± 4.42	81.72 ± 1.47	79.92 ± 2.86	74.43 ± 5.20
		CEQY	77.20 ± 2.01	80.62 ± 3.18	78.10 ± 1.14	84.30 ± 1.77
PROG (%)	Insulated	HYCG	0.90 ± 0.46	0.23 ± 0.22	1.40 ± 0.69	0.85 ± 0.85
		CEQY	0.75 ± 0.50	0.00 ± 0.00	2.63 ± 2.18	2.92 ± 2.92
	Control	HYCG	33.83 ± 5.21	32.55 ± 2.53	35.57 ± 3.12	30.35 ± 6.47
		CEQY	34.90 ± 4.11	38.70 ± 4.35	33.00 ± 1.19	41.03 ± 2.36
RAPID (%)	Insulated	HYCG	0.90 ± 0.46	0.23 ± 0.22	2.40 ± 1.00	1.28 ± 1.27
		CEQY	0.75 ± 0.50	0.00 ± 0.00	2.85 ± 2.15	2.92 ± 2.92
	Control	HYCG	49.08 ± 4.48	44.67 ± 3.11	52.15 ± 3.50	47.60 ± 7.69
		CEQY	52.55 ± 2.96	48.42 ± 5.01	51.13 ± 1.62	56.93 ± 2.70
MEDIUM (%)	Insulated	HYCG	16.43 ± 4.97	3.93 ± 1.04	36.33 ± 6.22	14.00 ± 0.94
		CEQY	25.20 ± 5.39	4.02 ± 1.30	34.47 ± 5.65	10.55 ± 3.98
	Control	HYCG	25.32 ± 2.18	37.00 ± 2.78	27.78 ± 4.29	26.85 ± 3.71
		CEQY	24.65 ± 1.13	32.23 ± 2.77	26.95 ± 1.62	27.37 ± 1.35
SLOW (%)	Insulated	HYCG	19.00 ± 6.07	4.55 ± 0.98	17.00 ± 6.87	12.18 ± 4.32
		CEQY	26.37 ± 11.97	6.52 ± 5.81	13.95 ± 7.10	3.95 ± 2.98
	Control	HYCG	0.10 ± 0.10	0.15 ± 0.15	0.10 ± 0.10	0.00 ± 0.00
		CEQY	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.08	0.00 ± 0.00
LIVE SPERM (%)	Insulated	HYCG	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		CEQY	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Control	HYCG	92.75 ± 3.25	85.50 ± 9.00	82.75 ± 0.25	71.25 ± 2.25
		CEQY	92.75 ± 1.25	91.75 ± 5.25	82.25 ± 2.25	73.00 ± 3.00
DEAD SPERM (%)	Insulated	HYCG	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
		CEQY	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Control	HYCG	7.25 ± 3.25	14.50 ± 9.00	17.25 ± 0.25	28.75 ± 2.25
		CEQY	7.25 ± 1.25	8.25 ± 5.25	17.75 ± 2.25	27.00 ± 3.00
NORMAL SPERM (%)	Insulated	HYCG	65.50 ± 20.50	66.00 ± 16.50	57.50 ± 22.50	65.00 ± 12.50
		CEQY	72.00 ± 19.50	67.25 ± 17.75	55.50 ± 27.50	63.25 ± 19.25
	Control	HYCG	86.75 ± 9.75	93.75 ± 5.75	96.50 ± 1.00	97.00 ± 0.00
		CEQY	97.75 ± 0.75	97.50 ± 0.50	96.00 ± 0.00	95.25 ± 2.25
LOOSE HEAD SPERM (%)	Insulated	HYCG	18.00 ± 12.50	27.00 ± 16.00	27.75 ± 19.25	23.50 ± 12.50
		CEQY	18.00 ± 15.00	24.50 ± 15.00	33.75 ± 24.25	28.00 ± 14.50
	Control	HYCG	0.25 ± 0.25	0.75 ± 0.75	0.50 ± 0.50	1.00 ± 0.00
		CEQY	1.00 ± 0.00	0.50 ± 0.00	0.25 ± 0.25	0.00 ± 0.00

Table 10.1.4..d. (continued) Characteristics of semen collected at day 30 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	2.25 ± 0.75	2.00 ± 1.00	4.00 ± 0.00	3.75 ± 1.25
		CEQY	3.75 ± 2.25	2.50 ± 1.00	3.50 ± 1.50	3.00 ± 1.00
	Control	HYCG	3.75 ± 1.25	3.25 ± 3.25	1.25 ± 0.25	1.00 ± 0.50
		CEQY	0.50 ± 0.00	1.25 ± 0.25	2.00 ± 1.00	3.25 ± 1.75
BENT TAIL SPERM (%)	Insulated	HYCG	14.25 ± 8.75	5.00 ± 0.50	10.75 ± 3.25	7.75 ± 1.25
		CEQY	6.25 ± 2.25	5.75 ± 1.75	7.25 ± 4.75	5.75 ± 3.75
	Control	HYCG	9.25 ± 8.25	2.25 ± 1.75	1.75 ± 0.75	1.00 ± 0.50
		CEQY	0.75 ± 0.75	0.75 ± 0.25	1.75 ± 1.25	1.50 ± 0.50
ABNOR-MAL SPERM (%)	Insulated	HYCG	34.50 ± 20.50	34.00 ± 16.50	42.50 ± 22.50	35.00 ± 12.50
		CEQY	28.00 ± 19.50	32.75 ± 17.75	44.50 ± 27.50	36.75 ± 19.25
	Control	HYCG	13.25 ± 9.75	6.25 ± 5.75	3.50 ± 1.00	3.00 ± 0.00
		CEQY	2.25 ± 0.75	2.50 ± 0.50	4.00 ± 0.00	4.75 ± 2.25
ALH (µm)	Insulated	HYCG	1.90 ± 0.64	1.30 ± 0.90	3.00 ± 0.77	1.63 ± 0.43
		CEQY	1.92 ± 0.58	0.63 ± 0.50	1.50 ± 0.69	2.70 ± 1.24
	Control	HYCG	8.75 ± 0.51	7.37 ± 0.29	9.02 ± 0.59	9.42 ± 0.33
		CEQY	9.48 ± 0.50	6.85 ± 0.45	9.42 ± 0.21	8.60 ± 0.33
LIN (%)	Insulated	HYCG	55.15 ± 5.66	51.70 ± 2.47	51.78 ± 4.72	53.23 ± 3.84
		CEQY	41.13 ± 4.26	34.82 ± 8.59	46.40 ± 9.85	37.17 ± 14.97
	Control	HYCG	54.13 ± 3.87	58.15 ± 2.55	54.95 ± 2.64	49.70 ± 3.16
		CEQY	51.25 ± 2.90	61.45 ± 1.61	50.40 ± 0.59	55.95 ± 1.57
VAP (µm/s)	Insulated	HYCG	25.33 ± 3.64	27.72 ± 10.42	36.75 ± 9.79	30.28 ± 14.65
		CEQY	34.75 ± 7.03	18.35 ± 6.20	31.80 ± 11.78	40.22 ± 29.14
	Control	HYCG	116.53 ± 5.28	96.23 ± 2.62	116.85 ± 5.39	112.30 ± 7.74
		CEQY	118.60 ± 5.47	102.93 ± 5.04	113.70 ± 1.66	119.20 ± 3.63
VSl (µm/s)	Insulated	HYCG	18.35 ± 3.94	19.40 ± 8.98	24.25 ± 7.69	18.90 ± 9.96
		CEQY	20.68 ± 5.38	7.18 ± 2.21	22.97 ± 10.46	34.15 ± 28.65
	Control	HYCG	83.97 ± 7.60	71.03 ± 3.50	83.07 ± 4.87	74.90 ± 7.75
		CEQY	82.35 ± 7.36	79.80 ± 4.57	77.40 ± 2.03	86.90 ± 3.21
VCL (µm/s)	Insulated	HYCG	30.62 ± 3.57	33.88 ± 12.55	47.55 ± 11.37	34.90 ± 15.26
		CEQY	43.00 ± 7.99	21.25 ± 5.82	37.83 ± 13.10	47.85 ± 31.58
	Control	HYCG	150.42 ± 4.82	119.20 ± 2.04	151.23 ± 7.50	147.98 ± 7.66
		CEQY	157.23 ± 4.67	124.93 ± 6.25	150.12 ± 2.67	150.60 ± 4.68
STR (%)	Insulated	HYCG	66.15 ± 5.70	63.13 ± 1.67	65.07 ± 5.04	64.83 ± 6.29
		CEQY	55.10 ± 5.31	44.38 ± 13.42	59.47 ± 14.58	51.60 ± 18.09
	Control	HYCG	68.10 ± 3.22	70.85 ± 1.96	68.80 ± 2.48	63.55 ± 2.76
		CEQY	65.68 ± 2.58	73.20 ± 1.42	64.55 ± 0.79	69.12 ± 1.41

Table 10.1.4..e. Characteristics of semen collected at day 37 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	9.82 ± 2.59	10.40 ± 4.25	15.82 ± 1.15	10.72 ± 3.39
		CEQY	10.90 ± 2.77	8.85 ± 1.50	17.75 ± 3.17	11.43 ± 3.12
	Control	HYCG	80.97 ± 1.72	82.32 ± 2.66	83.37 ± 1.40	81.30 ± 0.50
		CEQY	85.95 ± 1.20	86.23 ± 1.91	84.48 ± 1.45	82.30 ± 0.00
PROG (%)	Insulated	HYCG	1.83 ± 1.09	4.78 ± 3.32	2.15 ± 1.25	2.50 ± 1.57
		CEQY	2.87 ± 2.10	0.10 ± 0.10	2.53 ± 1.48	1.28 ± 0.75
	Control	HYCG	39.13 ± 5.77	40.03 ± 6.40	42.70 ± 3.34	39.20 ± 5.10
		CEQY	45.00 ± 3.99	46.17 ± 3.16	40.13 ± 2.55	36.20 ± 0.00
RAPID (%)	Insulated	HYCG	1.83 ± 1.09	4.98 ± 3.33	2.62 ± 1.53	2.50 ± 1.57
		CEQY	2.87 ± 2.10	0.20 ± 0.20	2.53 ± 1.48	1.28 ± 0.75
	Control	HYCG	53.13 ± 3.69	52.78 ± 5.96	63.78 ± 1.53	56.20 ± 2.60
		CEQY	62.63 ± 3.48	58.72 ± 3.80	61.15 ± 1.23	58.50 ± 0.00
MEDIUM (%)	Insulated	HYCG	8.05 ± 1.54	5.43 ± 0.93	13.20 ± 0.93	8.23 ± 2.55
		CEQY	8.00 ± 1.37	8.65 ± 1.33	15.25 ± 4.62	10.15 ± 2.76
	Control	HYCG	27.80 ± 3.48	29.55 ± 4.06	19.60 ± 0.63	25.15 ± 2.15
		CEQY	23.28 ± 2.66	27.52 ± 3.59	23.27 ± 1.79	23.80 ± 0.00
SLOW (%)	Insulated	HYCG	3.85 ± 0.84	4.55 ± 2.00	5.90 ± 2.34	7.22 ± 2.28
		CEQY	8.65 ± 2.90	3.67 ± 2.34	7.25 ± 4.24	6.35 ± 3.53
	Control	HYCG	0.22 ± 0.22	0.28 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
		CEQY	0.00 ± 0.00	0.15 ± 0.15	0.00 ± 0.00	0.00 ± 0.00
LIVE SPERM (%)	Insulated	HYCG	6.75 ± 3.75	7.25 ± 7.25	5.25 ± 5.25	6.00 ± 6.00
		CEQY	7.00 ± 5.50	6.00 ± 6.00	3.00 ± 3.00	4.25 ± 4.25
	Control	HYCG	85.25 ± 2.25	92.50 ± 6.50	90.75 ± 3.25	90.50 ± 0.50
		CEQY	75.75 ± 8.25	88.00 ± 3.00	89.50 ± 1.00	88.00 ± 0.50
DEAD SPERM (%)	Insulated	HYCG	93.25 ± 3.75	92.75 ± 7.25	94.75 ± 5.25	94.00 ± 6.00
		CEQY	93.00 ± 5.50	94.00 ± 6.00	97.00 ± 3.00	95.75 ± 4.25
	Control	HYCG	14.75 ± 2.25	7.50 ± 6.50	9.25 ± 3.25	9.50 ± 0.50
		CEQY	24.25 ± 8.25	12.00 ± 3.00	10.50 ± 1.00	12.00 ± 0.50
NORMAL SPERM (%)	Insulated	HYCG	65.75 ± 2.25	66.50 ± 8.00	54.00 ± 3.50	76.50 ± 3.00
		CEQY	59.25 ± 1.75	63.50 ± 0.50	55.50 ± 1.00	74.25 ± 6.25
	Control	HYCG	82.00 ± 3.50	84.25 ± 0.25	98.50 ± 0.00	92.25 ± 0.25
		CEQY	82.25 ± 3.25	88.50 ± 0.50	97.25 ± 0.25	94.75 ± 1.25
LOOSE HEAD SPERM (%)	Insulated	HYCG	16.75 ± 1.75	15.25 ± 6.75	21.50 ± 3.00	13.75 ± 2.25
		CEQY	17.25 ± 1.25	17.00 ± 2.50	20.00 ± 0.50	17.00 ± 4.50
	Control	HYCG	2.00 ± 1.00	4.50 ± 4.00	0.50 ± 0.00	0.75 ± 0.25
		CEQY	2.25 ± 1.25	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00

Table 10.1.4..e. (continued) Characteristics of semen collected at day 37 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	6.00 ± 2.00	4.50 ± 2.00	8.50 ± 3.50	2.50 ± 0.50
		CEQY	4.50 ± 2.00	5.50 ± 1.00	5.75 ± 1.25	3.75 ± 2.25
	Control	HYCG	2.00 ± 0.50	2.50 ± 1.50	0.75 ± 0.25	5.50 ± 0.50
		CEQY	2.00 ± 0.50	1.00 ± 1.00	2.00 ± 0.50	3.00 ± 0.50
BENT TAIL SPERM (%)	Insulated	HYCG	11.50 ± 2.50	13.75 ± 0.75	16.00 ± 3.00	7.25 ± 0.25
		CEQY	19.00 ± 5.00	14.00 ± 1.00	18.75 ± 0.25	5.00 ± 0.50
	Control	HYCG	14.00 ± 3.00	8.75 ± 5.25	0.25 ± 0.25	1.50 ± 0.50
		CEQY	13.50 ± 1.50	10.25 ± 0.75	0.75 ± 0.25	2.25 ± 0.75
ABNOR-MAL SPERM (%)	Insulated	HYCG	34.25 ± 2.25	33.50 ± 8.00	46.00 ± 3.50	23.50 ± 3.00
		CEQY	40.75 ± 1.75	36.50 ± 0.50	44.50 ± 1.00	25.75 ± 6.25
	Control	HYCG	18.00 ± 3.50	15.75 ± 0.25	1.50 ± 0.00	7.75 ± 0.25
		CEQY	17.75 ± 3.25	11.50 ± 0.50	2.75 ± 0.25	5.25 ± 1.25
ALH (µm)	Insulated	HYCG	2.20 ± 0.70	3.85 ± 1.26	4.53 ± 0.82	2.97 ± 1.86
		CEQY	3.47 ± 0.77	1.02 ± 0.40	2.87 ± 1.07	2.37 ± 0.93
	Control	HYCG	8.32 ± 0.55	7.57 ± 0.48	9.42 ± 0.31	9.35 ± 0.25
		CEQY	8.07 ± 0.50	7.07 ± 0.64	9.33 ± 0.56	9.20 ± 0.00
LIN (%)	Insulated	HYCG	59.07 ± 6.82	65.08 ± 3.70	63.50 ± 1.66	44.22 ± 10.91
		CEQY	60.13 ± 3.58	60.72 ± 3.95	72.78 ± 3.44	59.25 ± 7.13
	Control	HYCG	57.77 ± 4.32	58.85 ± 4.38	54.97 ± 3.39	53.30 ± 5.70
		CEQY	59.42 ± 2.95	63.25 ± 2.79	53.32 ± 2.31	52.70 ± 0.00
VAP (µm/s)	Insulated	HYCG	39.22 ± 14.11	82.72 ± 36.76	50.80 ± 8.87	37.95 ± 15.94
		CEQY	44.70 ± 17.78	25.72 ± 2.53	55.70 ± 23.50	37.25 ± 14.57
	Control	HYCG	117.37 ± 9.48	110.42 ± 6.26	130.35 ± 2.50	119.30 ± 3.00
		CEQY	123.43 ± 7.74	114.78 ± 3.62	120.05 ± 0.93	120.40 ± 0.00
VSl (µm/s)	Insulated	HYCG	31.95 ± 13.81	74.95 ± 35.68	41.65 ± 7.97	28.50 ± 15.36
		CEQY	39.15 ± 17.43	18.68 ± 3.60	49.75 ± 22.55	30.98 ± 13.62
	Control	HYCG	88.15 ± 11.94	83.60 ± 8.60	93.03 ± 5.50	84.50 ± 9.00
		CEQY	92.43 ± 7.68	91.23 ± 4.77	84.65 ± 2.76	83.40 ± 0.00
VCL (µm/s)	Insulated	HYCG	44.07 ± 15.26	92.28 ± 40.08	62.47 ± 8.74	50.08 ± 21.42
		CEQY	53.63 ± 19.40	28.93 ± 3.08	63.17 ± 25.61	44.58 ± 16.73
	Control	HYCG	146.90 ± 9.52	136.27 ± 5.19	166.55 ± 1.33	155.20 ± 0.60
		CEQY	151.93 ± 10.09	137.62 ± 5.70	154.80 ± 2.79	156.10 ± 0.00
STR (%)	Insulated	HYCG	68.08 ± 6.90	76.85 ± 4.63	77.25 ± 2.11	55.68 ± 13.70
		CEQY	77.30 ± 2.50	69.87 ± 5.55	83.37 ± 2.96	72.35 ± 8.20
	Control	HYCG	70.57 ± 3.73	71.55 ± 3.95	68.10 ± 2.93	67.25 ± 5.45
		CEQY	71.30 ± 2.35	74.85 ± 2.51	67.02 ± 2.04	66.10 ± 0.00

Table 10.1.4..f. Characteristics of semen collected at day 44 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	31.50 ± 7.08	23.23 ± 10.33	39.72 ± 7.14	23.60 ± 10.03
		CEQY	26.52 ± 12.85	28.45 ± 15.44	35.15 ± 18.25	25.17 ± 13.02
	Control	HYCG	82.60 ± 2.86	86.00 ± 1.64	88.35 ± 1.12	89.37 ± 2.47
		CEQY	83.45 ± 1.74	84.38 ± 0.94	86.03 ± 1.84	87.87 ± 1.31
PROG (%)	Insulated	HYCG	14.80 ± 5.17	10.30 ± 4.91	20.45 ± 3.19	9.90 ± 5.55
		CEQY	13.90 ± 8.08	12.97 ± 7.49	17.47 ± 10.24	12.15 ± 7.08
	Control	HYCG	49.68 ± 7.09	51.68 ± 2.97	47.53 ± 2.52	42.40 ± 4.59
		CEQY	42.63 ± 5.07	38.67 ± 4.65	44.77 ± 1.35	49.83 ± 1.23
RAPID (%)	Insulated	HYCG	15.45 ± 5.54	10.30 ± 4.91	21.97 ± 3.65	10.73 ± 5.90
		CEQY	15.27 ± 8.93	13.72 ± 7.97	18.83 ± 11.05	13.08 ± 7.59
	Control	HYCG	64.28 ± 2.88	63.03 ± 1.46	63.48 ± 4.12	61.55 ± 9.25
		CEQY	63.33 ± 2.93	51.73 ± 5.86	60.95 ± 4.40	62.13 ± 4.00
MEDIUM (%)	Insulated	HYCG	16.05 ± 1.96	12.90 ± 5.45	17.72 ± 4.67	12.90 ± 4.25
		CEQY	11.18 ± 4.57	14.70 ± 7.57	16.30 ± 7.29	12.10 ± 5.55
	Control	HYCG	18.33 ± 2.38	22.97 ± 2.73	24.90 ± 3.54	27.83 ± 6.83
		CEQY	20.18 ± 1.35	32.67 ± 5.91	25.05 ± 3.41	25.70 ± 2.91
SLOW (%)	Insulated	HYCG	10.35 ± 3.45	3.07 ± 0.84	2.62 ± 0.37	3.50 ± 1.02
		CEQY	1.08 ± 0.66	1.75 ± 0.47	0.82 ± 0.19	3.30 ± 0.81
	Control	HYCG	0.13 ± 0.13	0.43 ± 0.43	0.00 ± 0.00	0.10 ± 0.10
		CEQY	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.25	0.00 ± 0.00
LIVE SPERM (%)	Insulated	HYCG	25.75 ± 21.75	23.25 ± 18.75	23.25 ± 20.25	20.00 ± 19.00
		CEQY	30.75 ± 28.75	18.75 ± 16.75	21.25 ± 20.75	18.75 ± 16.75
	Control	HYCG	83.25 ± 5.75	80.50 ± 5.50	84.50 ± 3.50	80.50 ± 3.50
		CEQY	82.75 ± 7.25	78.50 ± 6.00	88.50 ± 6.00	84.00 ± 6.50
DEAD SPERM (%)	Insulated	HYCG	74.25 ± 21.75	76.75 ± 18.75	76.75 ± 20.25	80.00 ± 19.00
		CEQY	69.25 ± 28.75	81.25 ± 16.75	78.75 ± 20.75	81.25 ± 16.75
	Control	HYCG	16.75 ± 5.75	19.50 ± 5.50	15.50 ± 3.50	19.50 ± 3.50
		CEQY	17.25 ± 7.25	21.50 ± 6.00	11.50 ± 6.00	16.00 ± 6.50
NORMAL SPERM (%)	Insulated	HYCG	70.75 ± 4.75	64.25 ± 5.25	65.75 ± 6.25	66.75 ± 10.75
		CEQY	67.00 ± 14.00	68.50 ± 9.00	60.50 ± 15.50	60.25 ± 15.75
	Control	HYCG	95.75 ± 2.25	90.25 ± 5.75	94.50 ± 3.00	95.00 ± 3.00
		CEQY	95.75 ± 1.25	96.75 ± 1.75	95.50 ± 2.50	97.50 ± 1.50
LOOSE HEAD SPERM (%)	Insulated	HYCG	17.75 ± 11.25	21.25 ± 10.75	21.00 ± 9.50	22.25 ± 15.75
		CEQY	24.50 ± 17.00	21.25 ± 14.25	26.75 ± 20.25	29.00 ± 19.00
	Control	HYCG	0.50 ± 0.00	0.25 ± 0.25	0.50 ± 0.50	1.00 ± 1.00
		CEQY	1.00 ± 0.50	0.75 ± 0.75	0.25 ± 0.25	1.00 ± 0.00

Table 10.1.4..f. (continued) Characteristics of semen collected at day 44 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	7.00 ± 5.50	8.75 ± 6.75	6.25 ± 6.25	7.25 ± 6.75
		CEQY	3.75 ± 3.75	6.00 ± 5.50	6.50 ± 6.00	5.50 ± 5.00
	Control	HYCG	2.00 ± 2.00	2.00 ± 1.00	2.25 ± 2.25	3.00 ± 3.00
		CEQY	2.25 ± 1.75	1.50 ± 1.50	2.00 ± 2.00	1.25 ± 1.25
BENT TAIL SPERM (%)	Insulated	HYCG	4.50 ± 1.00	5.75 ± 1.25	7.00 ± 3.00	3.75 ± 1.75
		CEQY	4.75 ± 0.75	4.25 ± 0.25	6.25 ± 1.25	5.25 ± 1.75
	Control	HYCG	1.75 ± 0.25	7.50 ± 6.50	2.75 ± 1.25	1.00 ± 1.00
		CEQY	1.00 ± 0.00	1.00 ± 1.00	2.25 ± 0.25	0.25 ± 0.25
ABNOR-MAL SPERM (%)	Insulated	HYCG	29.25 ± 4.75	35.75 ± 5.25	34.25 ± 6.25	33.25 ± 10.75
		CEQY	33.00 ± 14.00	31.50 ± 9.00	39.50 ± 15.50	39.75 ± 15.75
	Control	HYCG	4.25 ± 2.25	9.75 ± 5.75	5.50 ± 3.00	5.00 ± 3.00
		CEQY	4.25 ± 1.25	3.25 ± 1.75	4.50 ± 2.50	2.50 ± 1.50
ALH (µm)	Insulated	HYCG	4.83 ± 0.42	4.20 ± 0.15	4.93 ± 0.20	3.95 ± 0.85
		CEQY	2.58 ± 1.06	3.15 ± 1.33	4.12 ± 0.89	3.45 ± 1.08
	Control	HYCG	8.55 ± 0.91	6.93 ± 0.34	7.45 ± 0.87	7.28 ± 1.24
		CEQY	8.95 ± 0.39	7.07 ± 0.68	7.93 ± 1.08	6.72 ± 0.92
LIN (%)	Insulated	HYCG	68.08 ± 3.04	71.60 ± 0.74	75.32 ± 2.87	64.50 ± 6.67
		CEQY	70.73 ± 6.45	55.23 ± 10.98	70.65 ± 4.26	61.10 ± 7.30
	Control	HYCG	62.33 ± 7.62	68.10 ± 3.31	62.23 ± 4.10	62.00 ± 5.03
		CEQY	54.95 ± 3.48	61.60 ± 4.90	60.85 ± 5.61	67.43 ± 4.37
VAP (µm/s)	Insulated	HYCG	90.12 ± 9.98	72.25 ± 6.46	112.47 ± 3.29	71.45 ± 21.76
		CEQY	61.28 ± 27.46	54.67 ± 22.33	69.97 ± 17.60	62.80 ± 24.76
	Control	HYCG	136.90 ± 12.48	121.50 ± 2.37	120.33 ± 8.40	118.30 ± 11.41
		CEQY	125.62 ± 4.25	107.90 ± 6.56	117.87 ± 7.60	112.53 ± 7.01
VSl (µm/s)	Insulated	HYCG	81.37 ± 8.96	66.57 ± 5.89	102.50 ± 3.70	61.10 ± 21.00
		CEQY	53.25 ± 25.04	45.92 ± 22.05	61.60 ± 16.07	54.17 ± 23.89
	Control	HYCG	106.65 ± 17.63	99.15 ± 4.85	91.23 ± 4.85	87.47 ± 5.75
		CEQY	89.40 ± 6.54	81.32 ± 5.39	88.17 ± 3.25	90.25 ± 1.81
VCL (µm/s)	Insulated	HYCG	101.62 ± 10.38	80.98 ± 6.52	125.25 ± 2.51	80.53 ± 23.65
		CEQY	67.60 ± 29.53	64.87 ± 24.10	79.18 ± 20.14	71.00 ± 27.71
	Control	HYCG	166.30 ± 10.89	141.57 ± 2.32	146.67 ± 14.12	143.22 ± 17.35
		CEQY	160.25 ± 4.08	132.55 ± 10.08	146.12 ± 13.84	134.78 ± 12.78
STR (%)	Insulated	HYCG	79.95 ± 2.07	82.53 ± 0.24	84.28 ± 2.40	74.97 ± 5.34
		CEQY	78.37 ± 4.14	69.55 ± 9.54	80.85 ± 4.21	70.32 ± 7.83
	Control	HYCG	73.40 ± 6.12	78.22 ± 2.57	72.85 ± 2.78	72.35 ± 3.98
		CEQY	67.70 ± 3.02	73.10 ± 3.98	72.10 ± 4.39	78.03 ± 3.30

Table 10.1.4..g. Characteristics of semen collected at day 51 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	34.75 ± 7.87	35.92 ± 6.96	37.13 ± 7.12	27.73 ± 8.16
		CEQY	34.40 ± 8.69	24.65 ± 7.16	30.53 ± 11.70	25.40 ± 10.85
	Control	HYCG	86.40 ± 0.65	81.78 ± 2.11	86.70 ± 0.86	77.68 ± 2.27
		CEQY	86.45 ± 0.88	78.80 ± 1.94	86.68 ± 0.92	80.10 ± 1.66
PROG (%)	Insulated	HYCG	20.67 ± 3.36	15.02 ± 3.13	26.27 ± 6.24	13.20 ± 5.65
		CEQY	20.62 ± 3.66	8.35 ± 2.48	17.67 ± 7.76	13.60 ± 6.70
	Control	HYCG	38.25 ± 3.86	47.77 ± 0.49	50.97 ± 1.09	46.13 ± 2.84
		CEQY	48.40 ± 1.95	46.28 ± 2.66	42.48 ± 6.02	44.40 ± 4.40
RAPID (%)	Insulated	HYCG	22.62 ± 3.85	16.50 ± 3.70	27.10 ± 6.41	14.25 ± 6.05
		CEQY	21.80 ± 4.19	8.70 ± 2.41	18.75 ± 8.21	14.75 ± 7.30
	Control	HYCG	46.93 ± 7.32	52.90 ± 0.93	62.60 ± 0.72	52.70 ± 3.40
		CEQY	62.13 ± 2.44	52.90 ± 3.55	62.70 ± 4.97	53.75 ± 6.12
MEDIUM (%)	Insulated	HYCG	12.13 ± 4.30	19.42 ± 3.34	10.02 ± 0.83	13.48 ± 2.21
		CEQY	12.63 ± 4.63	15.92 ± 5.01	11.75 ± 3.80	10.60 ± 3.67
	Control	HYCG	39.47 ± 6.88	28.87 ± 2.88	24.10 ± 0.33	24.90 ± 3.25
		CEQY	24.32 ± 2.68	25.92 ± 2.27	23.95 ± 4.42	26.35 ± 5.19
SLOW (%)	Insulated	HYCG	1.67 ± 0.85	4.23 ± 1.12	2.00 ± 0.07	5.55 ± 1.76
		CEQY	2.33 ± 0.63	7.68 ± 2.88	2.08 ± 0.78	3.83 ± 0.46
	Control	HYCG	0.28 ± 0.16	1.33 ± 0.58	0.12 ± 0.12	1.18 ± 0.27
		CEQY	0.17 ± 0.17	0.65 ± 0.38	0.10 ± 0.10	0.55 ± 0.23
LIVE SPERM (%)	Insulated	HYCG	27.50 ± 26.00	24.50 ± 24.50	23.00 ± 23.00	24.00 ± 24.00
		CEQY	25.00 ± 24.50	23.00 ± 23.00	24.25 ± 24.25	24.75 ± 24.75
	Control	HYCG	75.00 ± 10.00	75.00 ± 5.00	68.75 ± 6.75	59.75 ± 0.75
		CEQY	76.75 ± 8.25	76.00 ± 3.00	68.75 ± 5.25	54.25 ± 13.25
DEAD SPERM (%)	Insulated	HYCG	72.50 ± 26.00	75.50 ± 24.50	77.00 ± 23.00	76.00 ± 24.00
		CEQY	75.00 ± 24.50	77.00 ± 23.00	75.75 ± 24.25	75.25 ± 24.75
	Control	HYCG	25.00 ± 10.00	25.00 ± 5.00	31.25 ± 6.75	40.25 ± 0.75
		CEQY	23.25 ± 8.25	24.00 ± 3.00	31.25 ± 5.25	45.75 ± 13.25
NORMAL SPERM (%)	Insulated	HYCG	88.50 ± 6.50	81.00 ± 3.00	55.25 ± 25.25	55.25 ± 25.25
		CEQY	90.75 ± 4.75	75.00 ± 8.00	52.25 ± 28.25	57.75 ± 25.25
	Control	HYCG	90.25 ± 3.25	83.25 ± 1.25	94.50 ± 1.00	93.00 ± 4.50
		CEQY	84.75 ± 0.25	92.75 ± 2.25	94.25 ± 2.25	97.00 ± 1.50
LOOSE HEAD SPERM (%)	Insulated	HYCG	4.00 ± 2.00	10.75 ± 7.25	32.25 ± 30.25	34.25 ± 28.25
		CEQY	4.25 ± 1.25	14.00 ± 12.00	38.75 ± 31.25	35.75 ± 27.75
	Control	HYCG	2.75 ± 1.75	2.50 ± 1.50	0.75 ± 0.25	2.25 ± 2.25
		CEQY	5.75 ± 4.25	0.25 ± 0.25	0.00 ± 0.00	0.25 ± 0.25

Table 10.1.4..g. (continued) Characteristics of semen collected at day 51 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	5.25 ± 2.75	3.75 ± 3.25	7.75 ± 5.75	5.25 ± 3.25
		CEQY	2.50 ± 1.50	8.00 ± 4.00	3.50 ± 3.00	3.75 ± 2.25
	Control	HYCG	0.50 ± 0.50	7.25 ± 0.25	2.00 ± 1.00	3.00 ± 2.00
		CEQY	1.50 ± 0.50	4.50 ± 2.50	2.50 ± 1.50	1.25 ± 0.75
BENT TAIL SPERM (%)	Insulated	HYCG	2.25 ± 1.75	4.50 ± 1.00	4.75 ± 0.75	5.25 ± 0.25
		CEQY	2.50 ± 2.00	3.00 ± 0.00	5.50 ± 0.00	2.75 ± 0.25
	Control	HYCG	6.50 ± 5.50	7.00 ± 0.00	2.75 ± 0.25	1.75 ± 0.25
		CEQY	8.00 ± 4.50	2.50 ± 0.00	3.25 ± 0.75	1.50 ± 0.50
ABNOR-MAL SPERM (%)	Insulated	HYCG	11.50 ± 6.50	19.00 ± 3.00	44.75 ± 25.25	44.75 ± 25.25
		CEQY	9.25 ± 4.75	25.00 ± 8.00	47.75 ± 28.25	42.25 ± 25.25
	Control	HYCG	9.75 ± 3.25	16.75 ± 1.25	5.50 ± 1.00	7.00 ± 4.50
		CEQY	15.25 ± 0.25	7.25 ± 2.25	5.75 ± 2.25	3.00 ± 1.50
ALH (µm)	Insulated	HYCG	6.40 ± 0.37	4.10 ± 0.30	5.95 ± 0.51	4.60 ± 1.27
		CEQY	6.52 ± 0.40	4.03 ± 0.60	5.53 ± 0.94	4.80 ± 1.05
	Control	HYCG	6.55 ± 0.76	5.82 ± 0.11	7.37 ± 0.18	6.55 ± 0.54
		CEQY	7.47 ± 0.83	5.60 ± 0.32	8.58 ± 0.19	7.02 ± 0.41
LIN (%)	Insulated	HYCG	75.45 ± 2.01	75.10 ± 1.08	77.82 ± 1.53	68.48 ± 6.51
		CEQY	73.82 ± 1.89	74.07 ± 2.57	70.50 ± 3.09	69.93 ± 3.82
	Control	HYCG	65.95 ± 3.77	72.05 ± 0.81	64.35 ± 1.05	70.23 ± 3.21
		CEQY	65.05 ± 4.60	72.07 ± 1.36	55.65 ± 2.95	66.40 ± 1.37
VAP (µm/s)	Insulated	HYCG	137.27 ± 6.82	96.37 ± 6.12	143.83 ± 6.31	91.25 ± 24.93
		CEQY	137.23 ± 11.51	87.00 ± 14.76	117.87 ± 13.24	114.22 ± 18.06
	Control	HYCG	99.90 ± 6.23	109.32 ± 3.98	119.30 ± 1.74	121.85 ± 6.26
		CEQY	114.45 ± 6.99	111.75 ± 3.72	121.42 ± 7.65	118.05 ± 10.16
VSl (µm/s)	Insulated	HYCG	124.82 ± 7.38	86.20 ± 5.13	132.57 ± 7.05	81.90 ± 23.80
		CEQY	124.55 ± 11.96	79.57 ± 15.98	107.87 ± 12.47	103.68 ± 17.85
	Control	HYCG	79.15 ± 2.43	94.28 ± 2.56	95.53 ± 2.26	104.05 ± 5.41
		CEQY	89.55 ± 1.93	96.12 ± 3.30	87.37 ± 8.55	96.72 ± 7.81
VCL (µm/s)	Insulated	HYCG	152.90 ± 7.74	105.80 ± 7.22	158.68 ± 6.93	103.35 ± 27.41
		CEQY	151.77 ± 11.20	96.40 ± 15.81	132.35 ± 16.15	128.30 ± 21.54
	Control	HYCG	120.18 ± 9.84	123.25 ± 4.62	143.37 ± 2.46	140.65 ± 7.95
		CEQY	138.50 ± 11.72	126.60 ± 4.25	154.07 ± 6.23	140.23 ± 12.28
STR (%)	Insulated	HYCG	85.10 ± 1.88	84.82 ± 0.73	86.65 ± 1.32	79.50 ± 5.35
		CEQY	83.43 ± 1.49	85.25 ± 1.92	82.42 ± 2.95	81.65 ± 4.48
	Control	HYCG	77.02 ± 3.07	81.40 ± 0.90	76.18 ± 1.04	80.65 ± 2.28
		CEQY	76.18 ± 3.67	81.68 ± 1.33	68.70 ± 2.09	77.55 ± 1.42

Table 10.1.4..h. Characteristics of semen collected at day 93 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	78.45 ± 2.20	69.55 ± 4.13	75.60 ± 3.06	78.97 ± 5.09
		CEQY	75.82 ± 1.86	68.25 ± 1.77	85.20 ± 1.34	79.03 ± 4.77
	Control	HYCG	79.53 ± 1.74	69.60 ± 4.02	82.03 ± 3.28	86.62 ± 0.74
		CEQY	85.35 ± 1.69	76.40 ± 3.07	83.03 ± 1.84	83.10 ± 3.55
PROG (%)	Insulated	HYCG	57.25 ± 2.51	38.60 ± 2.60	55.07 ± 1.90	58.13 ± 2.56
		CEQY	54.47 ± 2.48	41.90 ± 2.24	57.13 ± 2.76	53.90 ± 1.08
	Control	HYCG	47.27 ± 1.06	39.60 ± 3.26	52.50 ± 1.39	59.45 ± 3.30
		CEQY	59.63 ± 2.47	37.75 ± 2.39	53.65 ± 3.42	49.70 ± 3.56
RAPID (%)	Insulated	HYCG	66.18 ± 2.73	43.55 ± 4.06	64.85 ± 2.85	65.47 ± 3.53
		CEQY	65.95 ± 2.74	47.40 ± 1.78	72.35 ± 1.86	66.87 ± 2.88
	Control	HYCG	63.83 ± 2.01	45.28 ± 3.54	66.00 ± 1.33	72.77 ± 2.52
		CEQY	72.30 ± 1.66	46.65 ± 3.43	67.20 ± 2.90	63.45 ± 3.80
MEDIUM (%)	Insulated	HYCG	12.27 ± 1.05	25.98 ± 2.91	10.72 ± 0.93	13.48 ± 2.06
		CEQY	9.90 ± 0.92	20.85 ± 0.24	12.90 ± 1.62	12.15 ± 2.08
	Control	HYCG	15.68 ± 1.09	24.35 ± 1.83	16.03 ± 2.37	13.85 ± 2.26
		CEQY	13.05 ± 1.36	29.75 ± 3.10	15.82 ± 1.58	19.65 ± 1.24
SLOW (%)	Insulated	HYCG	0.38 ± 0.13	1.67 ± 0.48	0.10 ± 0.10	0.20 ± 0.20
		CEQY	0.27 ± 0.21	0.45 ± 0.18	0.20 ± 0.14	0.00 ± 0.00
	Control	HYCG	0.40 ± 0.10	0.95 ± 0.26	0.38 ± 0.15	0.40 ± 0.31
		CEQY	0.08 ± 0.08	0.88 ± 0.59	0.00 ± 0.00	0.08 ± 0.07
LIVE SPERM (%)	Insulated	HYCG	69.75 ± 1.75	64.00 ± 7.00	62.50 ± 3.50	54.75 ± 7.75
		CEQY	74.75 ± 7.75	69.75 ± 2.75	65.25 ± 7.25	53.50 ± 8.00
	Control	HYCG	76.25 ± 2.25	67.25 ± 5.75	64.75 ± 9.25	56.00 ± 6.50
		CEQY	72.00 ± 4.50	63.25 ± 2.75	66.50 ± 13.50	53.50 ± 8.50
DEAD SPERM (%)	Insulated	HYCG	30.25 ± 1.75	36.00 ± 7.00	37.50 ± 3.50	45.25 ± 7.75
		CEQY	25.25 ± 7.75	30.25 ± 2.75	34.75 ± 7.25	46.50 ± 8.00
	Control	HYCG	23.75 ± 2.25	32.75 ± 5.75	35.25 ± 9.25	44.00 ± 6.50
		CEQY	28.00 ± 4.50	36.75 ± 2.75	33.50 ± 13.50	46.50 ± 8.50
NORMAL SPERM (%)	Insulated	HYCG	66.00 ± 23.00	71.50 ± 0.50	80.25 ± 3.25	66.50 ± 8.00
		CEQY	73.00 ± 4.00	71.75 ± 0.25	85.00 ± 9.00	82.00 ± 1.00
	Control	HYCG	85.25 ± 10.25	87.75 ± 1.75	61.50 ± 0.00	63.75 ± 4.75
		CEQY	62.00 ± 8.00	83.25 ± 2.75	80.50 ± 5.00	64.75 ± 0.25
LOOSE HEAD SPERM (%)	Insulated	HYCG	2.75 ± 1.75	1.50 ± 1.50	3.00 ± 1.00	1.25 ± 0.75
		CEQY	1.75 ± 1.25	1.00 ± 1.00	1.50 ± 1.50	1.25 ± 1.25
	Control	HYCG	0.50 ± 0.00	0.25 ± 0.25	0.25 ± 0.25	0.50 ± 0.50
		CEQY	1.75 ± 0.75	0.50 ± 0.50	0.50 ± 0.50	0.25 ± 0.25

Table 10.1.4..h. (continued) Characteristics of semen collected at day 93 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	10.25 ± 7.25	11.25 ± 1.75	6.00 ± 1.50	10.25 ± 0.75
		CEQY	8.25 ± 3.75	10.50 ± 2.50	6.00 ± 4.00	3.75 ± 2.25
	Control	HYCG	2.75 ± 0.25	3.00 ± 0.00	9.00 ± 3.50	6.50 ± 3.00
		CEQY	7.25 ± 4.25	6.00 ± 2.50	4.50 ± 2.50	6.00 ± 1.00
BENT TAIL SPERM (%)	Insulated	HYCG	21.00 ± 17.50	15.75 ± 0.75	10.75 ± 5.75	22.00 ± 9.50
		CEQY	17.00 ± 1.00	16.75 ± 3.25	7.50 ± 3.50	13.00 ± 4.50
	Control	HYCG	11.50 ± 10.00	9.00 ± 1.50	29.25 ± 3.75	29.25 ± 7.25
		CEQY	29.00 ± 3.00	10.25 ± 0.25	14.50 ± 3.00	29.00 ± 1.00
ABNOR-MAL SPERM (%)	Insulated	HYCG	34.00 ± 23.00	28.50 ± 0.50	19.75 ± 3.25	33.50 ± 8.00
		CEQY	27.00 ± 4.00	28.25 ± 0.25	15.00 ± 9.00	18.00 ± 1.00
	Control	HYCG	14.75 ± 10.25	12.25 ± 1.75	38.50 ± 0.00	36.25 ± 4.75
		CEQY	38.00 ± 8.00	16.75 ± 2.75	19.50 ± 5.00	35.25 ± 0.25
ALH (µm)	Insulated	HYCG	7.92 ± 0.59	5.65 ± 0.25	7.40 ± 0.58	7.30 ± 0.74
		CEQY	7.80 ± 0.52	5.52 ± 0.33	7.48 ± 0.35	6.47 ± 0.54
	Control	HYCG	7.97 ± 0.16	5.62 ± 0.13	7.87 ± 0.25	7.20 ± 0.47
		CEQY	8.05 ± 0.26	5.58 ± 0.23	7.95 ± 0.38	7.60 ± 0.15
LIN (%)	Insulated	HYCG	72.00 ± 1.98	71.58 ± 1.27	73.30 ± 3.03	74.42 ± 2.46
		CEQY	71.57 ± 3.65	74.47 ± 1.74	67.82 ± 2.94	71.20 ± 4.91
	Control	HYCG	63.05 ± 0.97	70.65 ± 1.60	66.28 ± 2.19	69.60 ± 2.25
		CEQY	69.90 ± 2.15	65.20 ± 1.03	65.87 ± 2.15	65.50 ± 1.83
VAP (µm/s)	Insulated	HYCG	168.28 ± 2.27	114.90 ± 3.87	164.52 ± 3.20	151.48 ± 1.66
		CEQY	164.18 ± 2.56	126.22 ± 1.10	150.55 ± 6.45	147.85 ± 4.20
	Control	HYCG	144.35 ± 1.79	113.62 ± 2.88	148.70 ± 7.41	146.52 ± 4.24
		CEQY	159.65 ± 3.67	106.70 ± 5.13	143.15 ± 4.37	134.45 ± 1.45
VSl (µm/s)	Insulated	HYCG	143.73 ± 3.98	99.28 ± 3.13	141.72 ± 5.56	132.05 ± 1.43
		CEQY	138.07 ± 4.32	110.17 ± 1.07	121.87 ± 7.32	122.12 ± 8.93
	Control	HYCG	112.93 ± 1.96	96.75 ± 3.46	120.95 ± 8.91	121.70 ± 5.47
		CEQY	133.68 ± 5.36	85.12 ± 5.23	114.32 ± 4.16	106.55 ± 2.62
VCL (µm/s)	Insulated	HYCG	193.07 ± 4.32	129.87 ± 3.88	187.87 ± 5.18	173.60 ± 4.95
		CEQY	189.48 ± 6.20	140.62 ± 2.34	176.70 ± 7.91	170.67 ± 4.13
	Control	HYCG	172.40 ± 2.64	129.35 ± 2.58	175.60 ± 6.55	169.23 ± 4.05
		CEQY	186.30 ± 3.12	123.77 ± 5.64	171.12 ± 5.92	160.62 ± 3.05
STR (%)	Insulated	HYCG	81.25 ± 1.75	80.80 ± 0.80	82.07 ± 2.40	83.78 ± 1.50
		CEQY	81.20 ± 2.68	82.70 ± 1.27	77.48 ± 2.44	79.75 ± 3.97
	Control	HYCG	73.75 ± 1.07	80.03 ± 1.26	76.82 ± 1.70	79.00 ± 1.77
		CEQY	80.00 ± 1.58	74.75 ± 1.08	76.75 ± 1.89	76.18 ± 1.80

Table 10.1.4..i. Characteristics of semen collected at day 100 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	82.10 ± 1.83	79.60 ± 2.01	80.08 ± 4.19	76.78 ± 1.74
		CEQY	83.62 ± 1.63	72.97 ± 4.77	81.45 ± 1.48	72.25 ± 4.11
	Control	HYCG	81.98 ± 2.91	76.98 ± 3.18	85.05 ± 1.88	82.32 ± 2.25
		CEQY	86.85 ± 1.88	85.55 ± 1.69	85.45 ± 1.31	80.90 ± 3.45
PROG (%)	Insulated	HYCG	59.13 ± 2.59	59.10 ± 3.01	62.30 ± 3.51	60.38 ± 1.55
		CEQY	66.48 ± 3.38	55.02 ± 2.57	60.58 ± 3.47	53.65 ± 3.56
	Control	HYCG	63.27 ± 2.81	60.50 ± 2.63	64.87 ± 2.05	63.00 ± 2.03
		CEQY	61.60 ± 2.03	62.88 ± 3.63	66.55 ± 3.25	62.83 ± 4.85
RAPID (%)	Insulated	HYCG	78.82 ± 2.58	70.05 ± 3.13	77.83 ± 3.97	73.75 ± 2.81
		CEQY	82.85 ± 1.36	65.12 ± 5.20	78.15 ± 2.52	68.20 ± 4.60
	Control	HYCG	79.18 ± 2.87	70.18 ± 3.14	83.20 ± 2.45	80.37 ± 2.20
		CEQY	85.32 ± 1.74	82.45 ± 2.26	84.03 ± 1.21	78.75 ± 4.11
MEDIUM (%)	Insulated	HYCG	3.27 ± 0.98	9.52 ± 1.33	2.28 ± 0.92	3.03 ± 1.12
		CEQY	0.77 ± 0.33	7.85 ± 1.31	3.33 ± 1.24	4.05 ± 1.51
	Control	HYCG	2.83 ± 0.58	6.80 ± 0.36	1.87 ± 0.61	1.95 ± 0.38
		CEQY	1.52 ± 0.30	3.05 ± 1.20	1.42 ± 0.14	2.15 ± 1.15
SLOW (%)	Insulated	HYCG	0.18 ± 0.10	2.33 ± 0.14	0.42 ± 0.25	0.82 ± 0.39
		CEQY	0.13 ± 0.13	0.80 ± 0.23	1.25 ± 0.53	0.27 ± 0.17
	Control	HYCG	0.30 ± 0.18	0.73 ± 0.36	0.48 ± 0.27	0.33 ± 0.03
		CEQY	0.23 ± 0.08	0.40 ± 0.31	0.00 ± 0.00	0.28 ± 0.17
LIVE SPERM (%)	Insulated	HYCG	65.25 ± 0.75	55.00 ± 5.50	61.00 ± 16.00	62.25 ± 0.75
		CEQY	67.50 ± 4.50	64.50 ± 4.50	66.50 ± 18.00	58.50 ± 7.50
	Control	HYCG	67.75 ± 10.75	61.25 ± 2.75	67.25 ± 3.25	67.50 ± 14.00
		CEQY	67.75 ± 15.75	72.50 ± 7.00	72.00 ± 4.00	64.50 ± 14.00
DEAD SPERM (%)	Insulated	HYCG	34.75 ± 0.75	45.00 ± 5.50	39.00 ± 16.00	37.75 ± 0.75
		CEQY	32.50 ± 4.50	35.50 ± 4.50	33.50 ± 18.00	41.50 ± 7.50
	Control	HYCG	32.25 ± 10.75	38.75 ± 2.75	32.75 ± 3.25	32.50 ± 14.00
		CEQY	32.25 ± 15.75	27.50 ± 7.00	28.00 ± 4.00	35.50 ± 14.00
NORMAL SPERM (%)	Insulated	HYCG	76.75 ± 1.75	59.50 ± 2.50	53.00 ± 11.50	51.00 ± 24.50
		CEQY	74.50 ± 3.00	72.75 ± 8.25	50.50 ± 18.00	54.25 ± 24.25
	Control	HYCG	68.00 ± 21.00	84.50 ± 4.50	62.50 ± 2.50	79.25 ± 11.25
		CEQY	82.25 ± 4.75	70.00 ± 1.00	68.25 ± 4.25	58.00 ± 19.50
LOOSE HEAD SPERM (%)	Insulated	HYCG	1.50 ± 1.50	0.75 ± 0.25	1.00 ± 0.00	1.00 ± 0.50
		CEQY	0.25 ± 0.25	1.00 ± 0.00	0.25 ± 0.25	0.50 ± 0.00
	Control	HYCG	0.25 ± 0.25	0.50 ± 0.00	0.00 ± 0.00	0.50 ± 0.50
		CEQY	1.00 ± 0.50	0.25 ± 0.25	3.25 ± 2.75	1.25 ± 0.25

Table 10.1.4..i. (continued) Characteristics of semen collected at day 100 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	4.00 ± 2.00	10.75 ± 1.75	8.00 ± 3.50	6.25 ± 0.75
		CEQY	6.00 ± 3.00	4.75 ± 1.25	6.00 ± 2.00	5.25 ± 2.25
	Control	HYCG	5.50 ± 3.00	1.00 ± 0.00	6.75 ± 0.25	2.75 ± 0.25
		CEQY	2.75 ± 1.25	4.00 ± 0.00	3.75 ± 0.75	5.00 ± 0.50
BENT TAIL SPERM (%)	Insulated	HYCG	17.75 ± 5.25	29.00 ± 4.50	38.00 ± 8.00	41.75 ± 24.25
		CEQY	19.25 ± 6.25	21.50 ± 7.00	43.25 ± 15.75	40.00 ± 26.50
	Control	HYCG	26.25 ± 17.75	14.00 ± 4.50	30.75 ± 2.75	17.50 ± 11.50
		CEQY	14.00 ± 3.00	25.75 ± 0.75	24.75 ± 6.25	35.75 ± 20.25
ABNOR-MAL SPERM (%)	Insulated	HYCG	23.25 ± 1.75	40.50 ± 2.50	47.00 ± 11.50	49.00 ± 24.50
		CEQY	25.50 ± 3.00	27.25 ± 8.25	49.50 ± 18.00	45.75 ± 24.25
	Control	HYCG	32.00 ± 21.00	15.50 ± 4.50	37.50 ± 2.50	20.75 ± 11.25
		CEQY	17.75 ± 4.75	30.00 ± 1.00	31.75 ± 4.25	42.00 ± 19.50
ALH (µm)	Insulated	HYCG	7.60 ± 0.11	4.30 ± 0.15	7.07 ± 0.40	6.43 ± 0.19
		CEQY	8.35 ± 0.23	4.80 ± 0.51	7.52 ± 0.18	7.33 ± 0.48
	Control	HYCG	7.93 ± 0.33	5.00 ± 0.35	7.80 ± 0.31	6.80 ± 0.32
		CEQY	7.95 ± 0.16	6.22 ± 0.61	7.85 ± 0.14	7.00 ± 0.36
LIN (%)	Insulated	HYCG	64.22 ± 0.39	71.52 ± 1.01	70.00 ± 2.07	73.02 ± 1.42
		CEQY	69.80 ± 2.27	74.05 ± 2.33	66.70 ± 2.07	69.18 ± 1.18
	Control	HYCG	70.30 ± 3.99	74.98 ± 2.37	69.18 ± 2.66	70.45 ± 1.85
		CEQY	63.35 ± 0.62	66.87 ± 4.37	69.10 ± 1.68	71.03 ± 1.88
VAP (µm/s)	Insulated	HYCG	144.95 ± 5.50	88.77 ± 3.12	146.35 ± 9.32	149.42 ± 5.04
		CEQY	177.57 ± 5.54	109.87 ± 4.90	144.75 ± 8.70	150.23 ± 6.22
	Control	HYCG	165.28 ± 15.02	119.53 ± 3.28	149.68 ± 1.50	143.55 ± 4.47
		CEQY	141.45 ± 2.06	114.15 ± 5.48	153.85 ± 3.54	143.62 ± 7.85
VSl (µm/s)	Insulated	HYCG	116.75 ± 6.27	75.90 ± 2.94	121.35 ± 8.36	128.03 ± 5.12
		CEQY	150.02 ± 7.91	96.28 ± 5.79	119.72 ± 8.53	126.95 ± 5.17
	Control	HYCG	140.50 ± 17.09	106.00 ± 4.58	123.78 ± 3.98	120.15 ± 4.82
		CEQY	109.18 ± 1.48	90.53 ± 1.20	127.92 ± 4.72	121.35 ± 6.49
VCL (µm/s)	Insulated	HYCG	171.80 ± 5.65	99.98 ± 3.40	168.87 ± 10.20	169.32 ± 5.69
		CEQY	207.40 ± 5.67	123.05 ± 5.34	170.75 ± 9.57	173.58 ± 6.62
	Control	HYCG	190.37 ± 14.57	132.87 ± 2.23	174.43 ± 2.17	165.65 ± 5.85
		CEQY	170.35 ± 3.43	134.57 ± 9.13	179.73 ± 2.65	166.57 ± 9.72
STR (%)	Insulated	HYCG	74.93 ± 0.43	80.45 ± 1.13	79.37 ± 1.54	81.45 ± 1.17
		CEQY	80.32 ± 2.26	82.47 ± 1.61	77.53 ± 2.32	79.22 ± 1.04
	Control	HYCG	79.85 ± 3.39	83.28 ± 1.63	79.03 ± 2.29	79.85 ± 1.41
		CEQY	74.28 ± 0.47	76.65 ± 3.48	79.22 ± 1.34	80.80 ± 1.85

Table 10.1.4.. j. Characteristics of semen collected at day 107 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen characteristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	79.65 ± 3.13	79.07 ± 4.16	82.15 ± 0.74	72.73 ± 3.29
		CEQY	83.50 ± 2.11	82.62 ± 3.06	80.27 ± 3.01	75.68 ± 5.67
	Control	HYCG	85.90 ± 1.17	84.62 ± 1.02	81.40 ± 2.22	83.30 ± 4.14
		CEQY	85.85 ± 2.67	78.52 ± 2.27	85.18 ± 2.78	86.07 ± 2.57
PROG (%)	Insulated	HYCG	58.45 ± 4.17	60.88 ± 3.62	61.25 ± 3.14	54.88 ± 1.88
		CEQY	63.40 ± 3.82	59.75 ± 1.04	60.63 ± 2.18	59.63 ± 3.27
	Control	HYCG	64.87 ± 0.72	64.42 ± 0.53	58.88 ± 1.42	62.50 ± 3.54
		CEQY	64.18 ± 4.00	58.83 ± 3.99	66.72 ± 2.12	59.72 ± 1.25
RAPID (%)	Insulated	HYCG	76.72 ± 2.69	73.32 ± 5.00	76.97 ± 2.32	68.77 ± 3.92
		CEQY	82.12 ± 1.94	76.08 ± 4.70	77.18 ± 3.13	73.33 ± 5.70
	Control	HYCG	83.95 ± 1.53	79.87 ± 1.44	76.82 ± 3.65	77.73 ± 5.94
		CEQY	85.35 ± 2.63	71.35 ± 3.16	82.78 ± 3.43	82.30 ± 2.80
MEDIUM (%)	Insulated	HYCG	2.93 ± 0.81	5.75 ± 1.36	5.20 ± 1.66	3.95 ± 1.09
		QYCG	1.40 ± 0.37	6.57 ± 2.29	3.10 ± 0.67	2.35 ± 0.63
	Control	HYCG	1.92 ± 0.44	4.75 ± 2.17	4.60 ± 1.70	5.57 ± 1.87
		QYCG	0.52 ± 0.07	7.17 ± 1.05	2.37 ± 0.95	3.83 ± 1.07
SLOW (%)	Insulated	HYCG	0.25 ± 0.09	1.00 ± 0.15	0.85 ± 0.21	0.25 ± 0.25
		CEQY	0.05 ± 0.05	0.85 ± 0.85	0.30 ± 0.19	0.20 ± 0.20
	Control	HYCG	0.25 ± 0.13	0.10 ± 0.10	0.50 ± 0.38	1.45 ± 0.51
		CEQY	0.23 ± 0.13	1.27 ± 0.48	0.25 ± 0.17	0.00 ± 0.00
LIVE SPERM (%)	Insulated	HYCG	66.50 ± 0.00	60.50 ± 9.50	65.50 ± 8.00	68.75 ± 4.75
		CEQY	68.25 ± 3.25	64.25 ± 1.75	58.50 ± 1.00	71.25 ± 1.75
	Control	HYCG	72.50 ± 7.00	61.50 ± 8.00	59.00 ± 2.00	71.25 ± 7.25
		CEQY	59.50 ± 10.00	67.25 ± 10.75	63.25 ± 3.75	69.75 ± 3.25
DEAD SPERM (%)	Insulated	HYCG	33.50 ± 0.00	39.50 ± 9.50	34.50 ± 8.00	31.25 ± 4.75
		CEQY	31.75 ± 3.25	35.75 ± 1.75	41.50 ± 1.00	28.75 ± 1.75
	Control	HYCG	27.50 ± 7.00	38.50 ± 8.00	41.00 ± 2.00	28.75 ± 7.25
		CEQY	40.50 ± 10.00	32.75 ± 10.75	36.75 ± 3.75	30.25 ± 3.25
NORMAL SPERM (%)	Insulated	HYCG	44.25 ± 11.75	39.50 ± 14.50	40.50 ± 9.00	41.50 ± 3.50
		CEQY	47.00 ± 8.50	51.00 ± 2.50	54.50 ± 4.00	56.75 ± 8.25
	Control	HYCG	57.50 ± 8.50	37.00 ± 1.50	72.25 ± 14.75	56.00 ± 18.50
		CEQY	51.50 ± 3.50	67.50 ± 9.00	57.75 ± 15.75	53.00 ± 23.50
LOOSE HEAD SPERM (%)	Insulated	HYCG	0.50 ± 0.00	1.25 ± 0.25	0.50 ± 0.00	1.00 ± 0.50
		CEQY	0.00 ± 0.00	1.25 ± 0.75	0.25 ± 0.25	0.25 ± 0.25
	Control	HYCG	0.00 ± 0.00	0.25 ± 0.25	0.75 ± 0.75	0.50 ± 0.50
		CEQY	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.50	0.00 ± 0.00

Table 10.1.4.. j. (continued) Characteristics of semen collected at day 107 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	10.00 ± 1.00	11.25 ± 3.25	12.75 ± 1.75	11.25 ± 1.25
		CEQY	10.50 ± 4.50	11.75 ± 3.25	10.75 ± 4.75	7.75 ± 0.25
	Control	HYCG	5.25 ± 0.25	9.25 ± 1.25	2.75 ± 0.25	3.50 ± 0.00
		CEQY	8.50 ± 0.50	4.25 ± 0.25	6.75 ± 0.25	3.75 ± 0.25
BENT TAIL SPERM (%)	Insulated	HYCG	45.25 ± 10.75	48.00 ± 11.00	46.25 ± 7.25	46.25 ± 5.25
		CEQY	42.50 ± 4.00	36.00 ± 0.00	34.50 ± 0.50	35.25 ± 7.75
	Control	HYCG	37.25 ± 8.25	53.50 ± 0.00	24.25 ± 15.25	40.00 ± 19.00
		CEQY	40.00 ± 3.00	28.25 ± 9.25	34.50 ± 16.00	43.25 ± 23.25
ABNOR-MAL SPERM (%)	Insulated	HYCG	55.75 ± 11.75	60.50 ± 14.50	59.50 ± 9.00	58.50 ± 3.50
		CEQY	53.00 ± 8.50	49.00 ± 2.50	45.50 ± 4.00	43.25 ± 8.25
	Control	HYCG	42.50 ± 8.50	63.00 ± 1.50	27.75 ± 14.75	44.00 ± 18.50
		CEQY	48.50 ± 3.50	32.50 ± 9.00	42.25 ± 15.75	47.00 ± 23.50
ALH (µm)	Insulated	HYCG	8.02 ± 0.48	5.97 ± 0.26	6.72 ± 0.41	6.37 ± 0.08
		CEQY	7.90 ± 0.41	5.03 ± 0.46	7.12 ± 0.20	6.95 ± 0.47
	Control	HYCG	7.95 ± 0.10	6.15 ± 0.55	7.07 ± 0.43	6.08 ± 0.25
		CEQY	8.25 ± 0.63	5.33 ± 0.19	6.82 ± 0.32	6.82 ± 0.57
LIN (%)	Insulated	HYCG	65.70 ± 3.82	70.08 ± 1.50	67.10 ± 1.41	69.48 ± 1.10
		CEQY	66.47 ± 2.76	68.60 ± 1.86	69.25 ± 3.20	70.65 ± 1.81
	Control	HYCG	66.60 ± 1.19	71.23 ± 1.63	67.45 ± 2.94	70.45 ± 0.70
		CEQY	65.15 ± 4.73	71.95 ± 1.78	69.78 ± 1.47	63.78 ± 2.90
VAP (µm/s)	Insulated	HYCG	146.32 ± 8.74	112.87 ± 13.21	115.85 ± 10.13	124.93 ± 5.46
		CEQY	144.60 ± 7.85	90.05 ± 8.52	141.70 ± 19.72	147.77 ± 16.62
	Control	HYCG	150.40 ± 6.37	130.07 ± 13.69	136.55 ± 4.87	113.78 ± 10.49
		CEQY	141.87 ± 5.04	109.25 ± 4.38	142.45 ± 6.30	120.70 ± 3.03
VSl (µm/s)	Insulated	HYCG	119.17 ± 11.79	95.48 ± 11.70	95.38 ± 9.10	105.57 ± 3.23
		CEQY	116.80 ± 5.54	73.40 ± 5.86	119.35 ± 19.44	125.95 ± 15.70
	Control	HYCG	121.87 ± 6.07	109.05 ± 10.20	111.32 ± 3.43	95.85 ± 7.92
		CEQY	112.80 ± 8.58	93.22 ± 5.11	118.78 ± 4.18	93.43 ± 3.35
VCL (µm/s)	Insulated	HYCG	174.40 ± 7.69	130.50 ± 13.54	137.18 ± 11.11	145.60 ± 6.04
		CEQY	173.47 ± 10.35	105.20 ± 11.33	164.60 ± 19.24	170.92 ± 17.19
	Control	HYCG	177.93 ± 5.73	149.25 ± 16.64	158.98 ± 7.06	129.40 ± 11.14
		CEQY	171.75 ± 2.02	123.22 ± 4.40	166.20 ± 8.06	143.18 ± 4.19
STR (%)	Insulated	HYCG	76.85 ± 3.29	80.23 ± 0.77	78.62 ± 1.20	79.85 ± 1.59
		CEQY	77.57 ± 2.42	78.93 ± 1.78	79.68 ± 2.03	80.62 ± 1.62
	Control	HYCG	77.20 ± 0.74	80.25 ± 1.07	77.25 ± 2.47	79.75 ± 0.49
		CEQY	76.22 ± 3.63	80.88 ± 1.54	79.45 ± 1.06	73.97 ± 2.11

Table 10.1.4.. k. Characteristics of semen collected at day 114 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Insulated	HYCG	78.67 ± 4.74	81.10 ± 3.34	81.72 ± 3.55	82.20 ± 2.98
		CEQY	81.78 ± 1.55	80.42 ± 2.33	83.35 ± 3.99	83.82 ± 2.25
	Control	HYCG	81.55 ± 5.37	71.37 ± 4.13	78.68 ± 5.63	77.78 ± 4.75
		CEQY	80.32 ± 5.12	76.80 ± 7.08	77.97 ± 7.43	84.70 ± 3.62
PROG (%)	Insulated	HYCG	60.45 ± 3.36	65.25 ± 3.20	60.20 ± 2.75	65.10 ± 1.02
		CEQY	63.80 ± 1.98	64.03 ± 1.20	64.57 ± 3.30	63.10 ± 1.92
	Control	HYCG	63.83 ± 2.82	58.80 ± 5.68	62.00 ± 4.23	62.50 ± 4.08
		CEQY	63.50 ± 1.84	59.40 ± 5.07	60.35 ± 3.68	62.18 ± 1.48
RAPID (%)	Insulated	HYCG	76.25 ± 5.11	78.40 ± 3.49	78.60 ± 4.92	79.60 ± 2.98
		CEQY	80.48 ± 1.82	76.95 ± 2.08	80.57 ± 4.15	82.03 ± 2.14
	Control	HYCG	79.65 ± 5.75	66.27 ± 5.62	76.65 ± 6.18	73.77 ± 5.15
		CEQY	78.82 ± 5.37	71.85 ± 7.93	75.40 ± 7.62	82.90 ± 4.42
MEDIUM (%)	Insulated	HYCG	2.45 ± 0.47	2.70 ± 0.67	3.12 ± 1.69	2.58 ± 0.59
		QYCG	1.27 ± 0.35	3.48 ± 1.12	2.80 ± 0.19	1.78 ± 0.91
	Control	HYCG	1.92 ± 0.50	5.10 ± 1.62	2.00 ± 0.61	4.07 ± 1.70
		QYCG	1.48 ± 0.42	4.97 ± 1.01	2.57 ± 0.85	1.83 ± 0.86
SLOW (%)	Insulated	HYCG	0.20 ± 0.20	0.35 ± 0.22	0.70 ± 0.39	0.17 ± 0.17
		CEQY	0.20 ± 0.12	0.30 ± 0.18	0.10 ± 0.10	0.20 ± 0.20
	Control	HYCG	0.23 ± 0.17	0.75 ± 0.38	0.65 ± 0.43	0.53 ± 0.38
		CEQY	0.00 ± 0.00	0.40 ± 0.28	0.20 ± 0.12	0.18 ± 0.10
LIVE SPERM (%)	Insulated	HYCG	63.50 ± 3.00	59.00 ± 10.00	71.25 ± 3.75	71.50 ± 2.00
		CEQY	72.75 ± 2.75	69.50 ± 8.50	71.00 ± 0.50	78.00 ± 5.50
	Control	HYCG	72.75 ± 14.25	61.25 ± 14.25	64.50 ± 10.00	77.00 ± 12.50
		CEQY	66.75 ± 23.25	72.75 ± 13.75	65.50 ± 18.50	69.00 ± 17.50
DEAD SPERM (%)	Insulated	HYCG	36.50 ± 3.00	41.00 ± 10.00	28.75 ± 3.75	28.50 ± 2.00
		CEQY	27.25 ± 2.75	30.50 ± 8.50	29.00 ± 0.50	22.00 ± 5.50
	Control	HYCG	27.25 ± 14.25	38.75 ± 14.25	35.50 ± 10.00	23.00 ± 12.50
		CEQY	33.25 ± 23.25	27.25 ± 13.75	34.50 ± 18.50	31.00 ± 17.50
NORMAL SPERM (%)	Insulated	HYCG	68.75 ± 0.75	63.25 ± 1.75	51.75 ± 17.25	45.00 ± 0.00
		CEQY	53.25 ± 12.25	54.25 ± 10.75	45.75 ± 22.25	60.00 ± 8.00
	Control	HYCG	30.50 ± 7.00	55.25 ± 5.75	50.00 ± 7.50	55.00 ± 0.50
		CEQY	60.00 ± 1.00	47.00 ± 11.00	65.00 ± 17.50	54.25 ± 6.75
LOOSE HEAD SPERM (%)	Insulated	HYCG	2.75 ± 2.25	0.25 ± 0.25	0.75 ± 0.75	1.75 ± 0.25
		CEQY	0.50 ± 0.00	0.50 ± 0.50	7.75 ± 2.75	2.75 ± 2.25
	Control	HYCG	5.50 ± 5.00	0.00 ± 0.00	0.50 ± 0.50	0.50 ± 0.50
		CEQY	0.00 ± 0.00	2.25 ± 1.75	0.00 ± 0.00	0.25 ± 0.25

Table 10.1.4.. k. (continued) Characteristics of semen collected at day 114 from 16h/d scrotally insulated and control rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 30 or 5°C for up to 6h (Mean ± SEM, n = 4).

Semen charac-teristic	Treated ram	Diluent	Time of storage at 30°C		Time of storage at 5°C	
			0h	6h	0h	6h
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
COILED TAIL SPERM (%)	Insulated	HYCG	4.00 ± 2.00	8.00 ± 0.00	7.75 ± 1.75	9.25 ± 3.25
		CEQY	4.00 ± 0.50	6.75 ± 1.75	6.00 ± 4.00	4.00 ± 2.00
	Control	HYCG	9.75 ± 0.25	7.75 ± 0.75	8.00 ± 0.50	7.75 ± 3.25
		CEQY	8.75 ± 0.75	8.75 ± 1.75	2.50 ± 1.00	5.25 ± 1.75
BENT TAIL SPERM (%)	Insulated	HYCG	24.50 ± 0.50	28.50 ± 2.00	39.75 ± 14.75	44.00 ± 3.00
		CEQY	42.25 ± 12.75	38.50 ± 12.00	40.50 ± 15.50	33.25 ± 8.25
	Control	HYCG	54.25 ± 1.75	37.00 ± 5.00	41.50 ± 7.50	36.75 ± 4.25
		CEQY	31.25 ± 0.25	42.00 ± 7.50	32.50 ± 16.50	40.25 ± 8.75
ABNOR-MAL SPERM (%)	Insulated	HYCG	31.25 ± 0.75	36.75 ± 1.75	48.25 ± 17.25	55.00 ± 0.00
		CEQY	46.75 ± 12.25	45.75 ± 10.75	54.25 ± 22.25	40.00 ± 8.00
	Control	HYCG	69.50 ± 7.00	44.75 ± 5.75	50.00 ± 7.50	45.00 ± 0.50
		CEQY	40.00 ± 1.00	53.00 ± 11.00	35.00 ± 17.50	45.75 ± 6.75
ALH (µm)	Insulated	HYCG	8.53 ± 0.34	6.03 ± 0.37	8.15 ± 0.19	8.00 ± 0.47
		CEQY	8.00 ± 0.20	5.68 ± 0.28	7.50 ± 0.25	7.40 ± 0.70
	Control	HYCG	8.00 ± 0.32	5.95 ± 0.18	7.70 ± 0.21	6.05 ± 0.30
		CEQY	8.02 ± 0.45	6.10 ± 0.35	8.20 ± 0.32	7.20 ± 0.36
LIN (%)	Insulated	HYCG	67.80 ± 1.09	73.20 ± 2.12	65.22 ± 1.69	70.07 ± 2.16
		CEQY	68.62 ± 1.47	73.28 ± 1.84	69.30 ± 0.84	66.23 ± 2.48
	Control	HYCG	70.75 ± 2.83	77.50 ± 1.94	71.37 ± 1.10	73.78 ± 0.72
		CEQY	69.45 ± 3.60	71.35 ± 0.86	68.80 ± 3.32	66.03 ± 2.10
VAP (µm/s)	Insulated	HYCG	159.98 ± 9.67	130.95 ± 9.17	143.65 ± 2.84	156.65 ± 4.43
		CEQY	153.20 ± 8.18	127.90 ± 1.11	146.07 ± 5.75	121.45 ± 6.21
	Control	HYCG	159.23 ± 7.01	135.55 ± 9.65	162.18 ± 2.64	134.45 ± 7.86
		CEQY	151.10 ± 5.57	117.45 ± 3.58	151.80 ± 4.34	137.87 ± 1.51
VSl (µm/s)	Insulated	HYCG	132.80 ± 9.47	112.33 ± 9.16	117.32 ± 2.39	133.07 ± 4.69
		CEQY	126.37 ± 7.36	109.28 ± 2.19	121.82 ± 5.37	98.57 ± 5.60
	Control	HYCG	135.10 ± 9.43	122.65 ± 9.59	139.40 ± 1.95	116.97 ± 6.48
		CEQY	125.87 ± 7.35	100.57 ± 3.85	127.72 ± 6.96	110.43 ± 2.82
VCL (µm/s)	Insulated	HYCG	189.07 ± 10.86	147.65 ± 10.65	173.00 ± 5.10	181.85 ± 5.73
		CEQY	180.50 ± 9.68	145.27 ± 2.12	170.40 ± 6.39	145.95 ± 8.73
	Control	HYCG	184.12 ± 4.26	150.68 ± 10.27	186.98 ± 4.07	152.27 ± 8.65
		CEQY	177.90 ± 6.15	135.15 ± 3.28	179.62 ± 5.04	162.75 ± 3.25
STR (%)	Insulated	HYCG	78.75 ± 0.99	81.93 ± 1.78	77.47 ± 1.60	80.68 ± 1.81
		CEQY	78.85 ± 1.36	82.07 ± 1.50	79.43 ± 0.54	77.40 ± 1.97
	Control	HYCG	80.78 ± 2.05	85.82 ± 1.56	81.30 ± 1.11	82.78 ± 0.63
		CEQY	80.32 ± 3.44	81.25 ± 0.73	80.25 ± 2.98	76.27 ± 1.67

Appendix C.

Table 10.2.1. Manual assessment of semen volume, total sperm count and motility of undiluted semen collected from control and 12h/day scrotally insulated rams.

Days after start of insulation	Semen volume (ml)				Total sperm count ($x10^9$)				Motility (%)			
	Control rams		Insulated rams		Control rams		Insulated rams		Control rams		Insulated rams	
	No 03	No 31	No 12	No 16	No 03	No 31	No 12	No 16	No 03	No 31	No 12	No 16
-11	1.00	0.60	0.80	0.70	1.05	1.35	0.52	1.43	87.00	76.00	78.00	82.00
-3	0.70	0.50	1.00	0.70	1.64	0.97	0.55	0.64	72.00	87.00	64.00	83.00
4	**0.60**	**0.70**	**1.00**	**1.00**	**0.64**	**2.69**	**3.40**	**2.83**	**83.00**	**78.00**	**71.00**	**78.00**
10	**0.50**	**0.90**	**0.70**	**0.60**	**0.42**	**1.16**	**0.43**	**0.28**	**80.00**	**84.00**	**78.00**	**70.00**
17	**0.50**	**1.10**	**1.10**	**0.60**	**0.61**	**1.50**	**0.10**	**0.17**	**68.00**	**77.00**	**2.00**	**67.00**
25	**1.10**	**1.10**	**1.30**	**1.30**	**1.95**	**3.31**	**1.15**	**2.97**	**72.00**	**77.00**	**0.00**	**38.00**
32	1.00	1.00	0.90	1.30	3.29	3.72	0.39	2.91	85.00	80.00	0.00	70.00
50	1.40	0.50	0.50	0.80	5.63	2.16	1.25	3.04	82.00	74.00	6.00	74.00
77	1.20	0.50	0.60	1.20	4.11	1.58	0.41	4.62	68.00	58.00	63.00	66.00
84	1.50	0.70	1.00	1.20	5.19	1.35	0.84	3.53	74.00	79.00	59.00	52.00
105	1.20	0.80	0.80	1.60	4.74	3.51	3.67	7.17	86.00	84.00	75.00	73.00

Table 10.2.2. Effect of storage at 5°C on changes in motility and morphological characteristics of semen from control and 12h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (168h) as the percentage of samples prestorage (0h)

Semen characteristic	Days after start of insulation	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM
MOT	-11	50.56 ± 15.47	44.99 ± 15.60
(%)	-3	50.90 ± 10.63	64.88 ± 8.05
	4	**66.45 ± 11.03**	**57.37 ± 11.94**
	10	**66.73 ± 11.37**	**68.74 ± 13.93**
	17	**57.30 ± 12.91**	**39.37 ± 15.87**
	25	**74.08 ± 11.54**	**22.79 ± 14.06**
	32	64.12 ± 9.74	25.51 ± 12.55
	50	32.18 ± 4.36	15.96 ± 7.20
	77	65.58 ± 13.07	61.98 ± 9.45
	84	58.24 ± 9.60	43.75 ± 10.50
	105	52.82 ± 11.66	37.61 ± 11.96
PROG	-11	47.99 ± 16.88	40.05 ± 15.47
(%)	-3	43.18 ± 12.35	52.26 ± 8.44
	4	**53.78 ± 11.34**	**48.71 ± 11.27**
	10	**54.76 ± 10.60**	**62.10 ± 15.89**
	17	**47.06 ± 12.20**	**34.58 ± 14.71**
	25	**62.80 ± 13.43**	**28.27 ± 18.17**
	32	56.79 ± 8.70	27.80 ± 14.98
	50	21.18 ± 3.61	14.71 ± 7.22
	77	52.32 ± 13.59	44.62 ± 7.41
	84	52.42 ± 10.19	42.27 ± 10.73
	105	42.93 ± 11.61	32.62 ± 11.16
RAPID	-11	43.84 ± 15.17	35.83 ± 13.75
(%)	-3	39.54 ± 11.28	47.37 ± 7.84
	4	**49.23 ± 10.39**	**47.14 ± 11.19**
	10	**50.22 ± 9.92**	**59.80 ± 15.51**
	17	**43.31 ± 11.01**	**32.56 ± 13.41**
	25	**60.01 ± 12.82**	**28.63 ± 18.60**
	32	52.94 ± 8.38	24.89 ± 13.45
	50	20.59 ± 3.57	12.85 ± 6.45
	77	50.44 ± 12.91	42.45 ± 6.64
	84	47.58 ± 9.22	39.03 ± 9.73
	105	39.29 ± 10.47	27.81 ± 9.21
MEDIUM (%)	-11	86.83 ± 24.91	88.20 ± 26.21
(%)	-3	99.38 ± 22.48	148.01 ± 25.07
	4	**240.52 ± 59.99**	**95.29 ± 19.46**
	10	**153.78 ± 32.89**	**90.90 ± 15.93**
	17	**158.18 ± 42.72**	**83.34 ± 42.02**
	25	**183.34 ± 23.56**	**18.03 ± 10.66**
	32	110.13 ± 19.27	27.40 ± 11.39
	50	99.47 ± 13.98	25.78 ± 10.48
	77	113.20 ± 24.26	127.27 ± 27.63
	84	121.19 ± 22.59	60.14 ± 15.58
	105	194.22 ± 55.45	117.43 ± 44.43

Table 10.2.2. (continued) Effect of storage at 5°C on changes in motility and morphological characteristics of semen from control and 12h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (168h) as the percentage of samples prestorage (0h)

Semen characteristic	Days after start of insulation	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM
SLOW	-11	96.56 ± 71.23	105.77 ± 56.22
(%)	-3	333.44 ± 110.84	493.48 ± 120.11
	4	**590.86 ± 313.14**	**223.53 ± 86.55**
	10	**737.08 ± 300.30**	**202.81 ± 64.64**
	17	**392.40 ± 116.03**	**75.62 ± 42.74**
	25	**230.17 ± 164.74**	**35.61 ± 18.68**
	32	432.69 ± 142.11	135.83 ± 98.67
	50	107.50 ± 41.74	23.21 ± 15.26
	77	161.77 ± 57.28	151.73 ± 42.64
	84	187.86 ± 86.91	151.90 ± 62.26
	105	231.00 ± 127.75	332.74 ± 153.24
LIVE	-11	99.34 ± 2.71	87.26 ± 3.72
(%)	-3	72.00 ± 6.93	73.83 ± 8.10
	4	**89.21 ± 6.31**	**93.76 ± 6.97**
	10	**97.28 ± 5.83**	**96.86 ± 2.88**
	17	**77.01 ± 4.23**	**71.15 ± 7.34**
	25	**87.24 ± 2.42**	**50.43 ± 25.42**
	32	85.40 ± 6.47	29.55 ± 18.50
	50	104.33 ± 26.97	71.62 ± 41.48
	77	69.56 ± 14.25	83.79 ± 8.42
	84	63.95 ± 2.49	58.66 ± 14.82
	105	71.49 ± 7.08	95.82 ± 14.64
DEAD	-11	107.00 ± 9.74	137.08 ± 10.76
(%)	-3	159.12 ± 21.02	173.63 ± 23.48
	4	**154.37 ± 30.08**	**120.06 ± 12.02**
	10	**123.31 ± 19.67**	**106.12 ± 4.10**
	17	**164.93 ± 20.12**	**141.75 ± 24.19**
	25	**149.59 ± 7.20**	**155.83 ± 45.84**
	32	145.83 ± 16.82	88.31 ± 51.68
	50	115.46 ± 15.03	45.09 ± 26.04
	77	305.99 ± 125.56	128.60 ± 13.78
	84	272.77 ± 37.69	238.49 ± 66.87
	105	295.15 ± 94.43	109.21 ± 21.21
NORMAL	-11	90.69 ± 4.47	91.44 ± 5.33
(%)	-3	97.32 ± 2.22	86.78 ± 6.04
	4	**91.91 ± 4.85**	**94.28 ± 8.05**
	10	**101.49 ± 3.03**	**103.10 ± 6.18**
	17	**100.60 ± 1.22**	**85.96 ± 1.79**
	25	**102.97 ± 4.07**	**86.87 ± 5.84**
	32	96.58 ± 0.75	53.67 ± 31.08
	50	102.99 ± 4.80	52.37 ± 30.25
	77	95.09 ± 1.21	104.06 ± 6.64
	84	101.52 ± 1.99	99.14 ± 2.97
	105	95.41 ± 1.95	90.13 ± 4.62

Table 10.2.2. (continued) Effect of storage at 5°C on changes in motility and morphological characteristics of semen from control and 12h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (168h) as the percentage of samples prestorage (0h)

Semen characteristic	Days after start of insulation	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM
LOOSE HEAD	-11	41.07 ± 36.47	35.47 ± 16.57
(%)	-3	0.00 ± 0.00	50.38 ± 17.77
	4	**25.00 ± 25.00**	**0.00 ± 0.00**
	10	**195.00 ± 108.13**	**0.00 ± 0.00**
	17	**283.33 ± 140.44**	**110.90 ± 16.63**
	25	**12.50 ± 12.50**	**223.61 ± 83.90**
	32	225.00 ± 193.11	25.60 ± 14.84
	50	12.50 ± 12.50	50.00 ± 50.00
	77	25.00 ± 25.00	123.96 ± 73.49
	84	8.33 ± 8.33	121.76 ± 32.81
	105	33.33 ± 23.57	115.62 ± 39.98
COILED TAIL	-11	255.07 ± 86.65	164.96 ± 27.74
(%)	-3	148.91 ± 26.90	299.37 ± 77.07
	4	**312.09 ± 166.29**	**102.43 ± 20.70**
	10	**92.16 ± 16.07**	**124.29 ± 21.50**
	17	**76.11 ± 16.27**	**113.57 ± 5.81**
	25	**77.14 ± 19.18**	**117.07 ± 13.02**
	32	156.67 ± 34.48	48.89 ± 28.34
	50	181.80 ± 172.80	29.69 ± 18.29
	77	115.59 ± 10.89	113.32 ± 28.92
	84	89.39 ± 21.39	101.22 ± 12.85
	105	181.15 ± 38.41	156.69 ± 22.00
BENT TAIL	-11	191.67 ± 64.31	112.38 ± 18.80
(%)	-3	154.17 ± 71.16	118.39 ± 31.98
	4	**256.25 ± 60.22**	**90.99 ± 21.86**
	10	**102.39 ± 27.49**	**163.84 ± 79.53**
	17	**122.03 ± 54.64**	**107.23 ± 9.71**
	25	**77.22 ± 45.14**	**114.95 ± 44.23**
	32	256.25 ± 215.63	69.94 ± 42.38
	50	212.50 ± 100.78	16.67 ± 11.79
	77	396.67 ± 204.69	200.49 ± 102.87
	84	76.25 ± 10.28	115.28 ± 63.07
	105	306.25 ± 199.05	271.67 ± 123.93
ABNORMAL	-11	199.74 ± 59.66	132.78 ± 16.14
(%)	-3	135.71 ± 24.73	172.08 ± 27.41
	4	**295.87 ± 140.82**	**98.19 ± 19.60**
	10	**98.26 ± 16.99**	**127.31 ± 30.76**
	17	**99.79 ± 14.13**	**114.10 ± 2.68**
	25	**92.43 ± 21.33**	**130.73 ± 18.94**
	32	167.21 ± 20.32	46.10 ± 26.69
	50	249.62 ± 139.20	28.56 ± 17.77
	77	159.43 ± 12.29	119.69 ± 20.31
	84	86.91 ± 25.40	96.51 ± 10.32
	105	198.16 ± 48.60	145.97 ± 23.57

Table 10.2.2. (continued) Effect of storage at 5°C on changes in motility and morphological characteristics of semen from control and 12h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (168h) as the percentage of samples prestorage (0h)

Semen characteristic	Days after start of insulation	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM
ALH	-11	65.91 ± 4.54	65.05 ± 4.57
(μm)	-3	63.30 ± 5.39	69.61 ± 3.92
	4	**67.02 ± 3.64**	**76.73 ± 4.30**
	10	**70.92 ± 4.33**	**78.10 ± 6.13**
	17	**76.29 ± 6.08**	**46.15 ± 17.06**
	25	**78.21 ± 4.53**	**45.02 ± 17.17**
	32	69.82 ± 4.56	34.88 ± 13.52
	50	83.76 ± 4.44	35.41 ± 13.69
	77	70.59 ± 4.44	65.79 ± 2.48
	84	62.54 ± 4.41	58.95 ± 8.01
	105	59.44 ± 4.72	48.22 ± 10.62
LIN	-11	107.61 ± 5.11	93.44 ± 8.90
(%)	-3	104.04 ± 3.90	104.02 ± 2.47
	4	**99.52 ± 3.67**	**100.50 ± 2.07**
	10	**102.62 ± 4.25**	**100.31 ± 2.09**
	17	**105.02 ± 2.51**	**64.53 ± 19.41**
	25	**96.37 ± 1.36**	**48.91 ± 18.64**
	32	110.40 ± 2.96	56.50 ± 21.37
	50	93.25 ± 4.66	57.80 ± 22.05
	77	101.21 ± 3.42	104.37 ± 2.23
	84	109.36 ± 3.21	107.98 ± 6.77
	105	96.79 ± 4.69	87.18 ± 19.32
VAP	-11	73.48 ± 6.69	51.76 ± 10.02
(μm/s)	-3	62.14 ± 8.01	70.02 ± 5.19
	4	**59.16 ± 6.36**	**76.64 ± 4.28**
	10	**66.31 ± 4.35**	**78.58 ± 6.14**
	17	**71.22 ± 6.33**	**39.88 ± 14.03**
	25	**73.07 ± 4.86**	**44.79 ± 18.15**
	32	82.27 ± 1.99	40.64 ± 16.13
	50	62.04 ± 2.94	33.48 ± 12.95
	77	66.50 ± 7.79	66.47 ± 4.40
	84	72.34 ± 4.44	80.41 ± 6.31
	105	52.38 ± 8.22	44.43 ± 13.20

Table 10.2.2. (continued) Effect of storage at 5°C on changes in motility and morphological characteristics of semen from control and 12h/day scrotally insulated rams (Mean ± SEM, n = 4). Mean expressed as the percentage of samples after storage (168h) as the percentage of samples prestorage (0h)

Semen characteristic	Days after start of insulation	Control rams	Insulated rams
		Mean ± SEM	Mean ± SEM
VSL	-11	77.67 ± 8.95	53.68 ± 11.52
(µm/s)	-3	64.69 ± 8.72	73.91 ± 5.75
	4	**61.01 ± 7.04**	**77.43 ± 4.29**
	10	**68.69 ± 5.28**	**80.08 ± 6.52**
	17	**74.21 ± 7.12**	**40.03 ± 14.23**
	25	**72.73 ± 5.24**	**44.62 ± 18.30**
	32	86.89 ± 2.60	43.81 ± 17.28
	50	61.40 ± 2.92	37.13 ± 14.44
	77	66.58 ± 8.45	67.29 ± 4.69
	84	76.03 ± 5.57	83.91 ± 8.75
	105	53.54 ± 8.74	62.34 ± 14.52
VCL	-11	71.76 ± 5.00	53.33 ± 8.11
(µm/s)	-3	61.51 ± 7.01	68.31 ± 4.84
	4	**59.52 ± 5.51**	**76.36 ± 4.17**
	10	**65.81 ± 3.60**	**78.61 ± 6.06**
	17	**69.51 ± 5.63**	**39.92 ± 13.91**
	25	**73.84 ± 4.60**	**45.50 ± 18.00**
	32	79.10 ± 1.90	38.47 ± 15.25
	50	66.22 ± 3.35	31.98 ± 12.24
	77	66.13 ± 7.25	65.54 ± 4.10
	84	70.27 ± 3.46	79.08 ± 4.35
	105	53.22 ± 6.94	53.14 ± 11.89
STR	-11	105.59 ± 2.84	102.53 ± 3.58
(%)	-3	104.59 ± 2.27	103.61 ± 1.90
	4	**101.65 ± 2.11**	**100.60 ± 1.49**
	10	**103.12 ± 2.45**	**100.80 ± 1.10**
	17	**104.50 ± 2.00**	**64.24 ± 19.05**
	25	**98.25 ± 1.18**	**49.57 ± 18.76**
	32	106.37 ± 1.92	54.61 ± 20.65
	50	99.41 ± 4.01	56.48 ± 21.39
	77	101.65 ± 2.27	103.32 ± 1.90
	84	106.48 ± 1.93	105.20 ± 4.52
	105	100.97 ± 1.83	83.80 ± 18.39

Table 10.2.3. Effect of diluent at 5°C on changes in motility and morphological characteristics of semen from control and 12h/day scrotally insulated rams (Mean ± SEM, n = 8 for Before, n = 16 for During, and n = 20 for After Insulation). Mean represented pooled data within each period and expressed as the percentage of samples after storage (168h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Insulation Period	Control rams		Heated rams	
		HYCG	CEQY	HYCG	CEQY
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	Before	19.63 ± 7.57	81.83 ± 4.33	25.76 ± 8.68	84.12 ± 4.25
"	**During**	**45.24 ± 7.68**	**87.04 ± 4.00**	**15.97 ± 4.21**	**78.17 ± 8.81**
"	After	34.50 ± 5.19	73.40 ± 4.64	17.97 ± 4.07	57.23 ± 7.44
PROG (%)	Before	8.06 ± 3.41	83.10 ± 5.01	16.42 ± 7.02	75.89 ± 4.42
"	**During**	**30.07 ± 6.40**	**79.13 ± 3.99**	**11.17 ± 2.97**	**75.66 ± 9.56**
"	After	23.94 ± 3.80	65.83 ± 5.50	14.20 ± 3.34	51.11 ± 7.17
RAPID (%)	Before	7.69 ± 3.09	75.69 ± 4.34	14.73 ± 6.30	68.47 ± 3.74
"	**During**	**28.35 ± 5.98**	**73.04 ± 4.15**	**10.61 ± 2.84**	**73.46 ± 9.42**
"	After	22.19 ± 3.50	61.76 ± 5.02	13.47 ± 3.30	45.88 ± 6.43
MEDIUM (%)	Before	67.62 ± 26.84	118.59 ± 15.21	78.37 ± 28.63	157.83 ± 17.34
"	**During**	**158.39 ± 24.44**	**209.52 ± 33.50**	**28.95 ± 8.21**	**114.82 ± 20.10**
"	After	107.79 ± 25.99	139.02 ± 9.98	31.60 ± 7.30	120.08 ± 21.87
SLOW (%)	Before	204.69 ± 90.30	225.31 ± 114.82	327.58 ± 150.63	271.67 ± 73.58
"	**During**	**455.26 ± 147.81**	**520.01 ± 190.67**	**117.85 ± 42.57**	**150.92 ± 47.36**
"	After	261.88 ± 81.79	190.21 ± 40.61	127.94 ± 49.87	186.47 ± 65.40
LIVE (%)	Before	41.75 ± 16.44	43.92 ± 17.09	43.90 ± 16.73	36.65 ± 14.26
"	**During**	**44.10 ± 11.59**	**43.58 ± 11.41**	**33.91 ± 11.36**	**44.05 ± 11.46**
"	After	39.39 ± 10.90	39.56 ± 9.43	31.21 ± 10.08	36.68 ± 11.28
DEAD (%)	Before	64.41 ± 25.28	68.65 ± 28.92	66.50 ± 26.15	88.85 ± 34.57
"	**During**	**69.46 ± 19.13**	**78.60 ± 21.56**	**69.39 ± 21.69**	**61.55 ± 16.31**
"	After	125.55 ± 38.22	101.49 ± 32.52	64.35 ± 22.17	57.59 ± 19.62
NORMAL (%)	Before	49.46 ± 18.71	44.54 ± 16.92	45.69 ± 17.43	43.43 ± 16.66
"	**During**	**51.47 ± 13.34**	**47.78 ± 12.40**	**45.54 ± 12.09**	**47.01 ± 12.23**
"	After	48.94 ± 11.26	49.37 ± 11.36	41.74 ± 11.83	38.13 ± 10.76
LOOSE HEAD (%)	Before	18.75 ± 18.75	1.79 ± 1.79	26.49 ± 12.55	16.44 ± 10.07
"	**During**	**85.21 ± 44.56**	**43.75 ± 31.58**	**50.85 ± 29.26**	**32.78 ± 17.44**
"	After	11.67 ± 6.96	49.17 ± 39.91	38.49 ± 15.35	48.89 ± 19.83
COILED TAIL (%)	Before	53.55 ± 20.81	148.44 ± 63.28	120.70 ± 53.32	113.56 ± 55.95
"	**During**	**41.66 ± 15.75**	**97.72 ± 48.86**	**55.80 ± 15.39**	**58.54 ± 16.05**
"	After	93.85 ± 37.07	51.07 ± 15.70	43.21 ± 13.85	46.75 ± 14.61
BENT TAIL (%)	Before	71.25 ± 41.23	101.67 ± 48.90	40.16 ± 17.73	75.22 ± 29.07
"	**During**	**58.02 ± 20.18**	**81.45 ± 31.83**	**42.99 ± 13.12**	**76.26 ± 27.51**
"	After	150.83 ± 62.96	98.75 ± 50.62	40.37 ± 16.90	94.44 ± 39.49
ABNORMAL (%)	Before	53.08 ± 21.36	114.64 ± 48.19	73.61 ± 29.60	79.59 ± 32.68
"	**During**	**47.73 ± 15.10**	**98.86 ± 43.99**	**54.44 ± 14.88**	**63.14 ± 18.24**
"	After	96.61 ± 33.15	75.66 ± 23.32	40.17 ± 12.77	47.20 ± 14.33

Table 10.2.3. (continued) Effect of diluent at 5°C on changes in motility and morphological characteristics of semen from control and 12h/day scrotally insulated rams (Mean ± SEM, n = 8 for Before, n = 16 for During, and n = 20 for After Insulation). Mean represented pooled data within each period and expressed as the percentage of samples after storage (168h) as the percentage of samples prestorage (0h)

Semen charac-teristic	Insulation Period	Control rams		Heated rams	
		HYCG	CEQY	HYCG	CEQY
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
ALH (µm)	Before	54.79 ± 3.51	74.42 ± 3.21	66.30 ± 4.88	68.36 ± 3.70
"	**During**	**68.56 ± 3.60**	**77.66 ± 2.79**	**45.02 ± 8.99**	**77.98 ± 8.16**
"	After	63.25 ± 3.37	76.47 ± 2.68	40.37 ± 6.67	55.68 ± 6.60
LIN (%)	Before	101.91 ± 5.54	109.74 ± 2.68	85.77 ± 6.12	111.69 ± 2.80
"	**During**	**97.17 ± 2.18**	**104.59 ± 2.02**	**69.36 ± 12.24**	**87.76 ± 8.81**
"	After	100.45 ± 2.85	103.89 ± 2.70	76.23 ± 11.80	89.37 ± 10.44
VAP (µm/s)	Before	51.83 ± 5.98	83.79 ± 3.05	44.38 ± 8.13	77.41 ± 2.65
"	**During**	**58.22 ± 3.94**	**76.66 ± 2.38**	**44.59 ± 8.85**	**75.36 ± 8.25**
"	After	56.73 ± 4.23	78.70 ± 2.55	43.54 ± 7.68	61.42 ± 7.60
VSL (µm/s)	Before	52.94 ± 7.30	89.43 ± 4.46	44.78 ± 9.13	82.80 ± 2.90
"	**During**	**58.74 ± 4.28**	**79.58 ± 2.65**	**45.11 ± 9.01**	**75.97 ± 8.45**
"	After	58.42 ± 4.77	80.76 ± 3.29	50.76 ± 8.45	65.64 ± 8.20
VCL (µm/s)	Before	52.72 ± 4.46	80.55 ± 2.52	47.99 ± 7.08	73.65 ± 2.83
"	**During**	**58.97 ± 3.44**	**75.38 ± 2.30**	**45.04 ± 8.88**	**75.15 ± 8.08**
"	After	57.42 ± 3.56	77.82 ± 2.29	47.33 ± 7.36	58.69 ± 7.19
STR (%)	Before	103.71 ± 3.14	106.47 ± 1.69	98.65 ± 2.75	107.50 ± 1.84
"	**During**	**100.09 ± 1.48**	**103.69 ± 1.34**	**69.74 ± 12.20**	**87.86 ± 8.68**
"	After	103.24 ± 1.45	102.76 ± 1.88	74.93 ± 11.43	86.37 ± 9.98

Table 10.2.4.a. Characteristics of semen collected on day -11 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± Sem, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem
MOT (%)	HYCG	79.03 ± 3.11	79.05 ± 1.54	54.38 ± 9.81	22.73 ± 7.32	8.37 ± 4.56	3.87 ± 2.01
	CEQY	79.47 ± 2.95	81.35 ± 2.93	78.10 ± 1.25	73.25 ± 5.97	71.47 ± 2.72	68.57 ± 4.83
PROG (%)	HYCG	55.83 ± 5.26	55.05 ± 3.45	24.85 ± 10.27	8.10 ± 3.51	1.83 ± 1.07	0.28 ± 0.16
	CEQY	57.58 ± 1.65	56.33 ± 1.35	52.60 ± 2.96	47.10 ± 5.86	53.05 ± 1.37	44.58 ± 4.44
RAPID (%)	HYCG	64.40 ± 4.17	64.95 ± 4.23	27.02 ± 11.55	8.38 ± 3.58	2.40 ± 1.30	0.35 ± 0.21
	CEQY	68.40 ± 1.42	67.73 ± 2.71	57.65 ± 2.46	52.00 ± 7.10	57.08 ± 1.81	47.78 ± 4.24
MEDIUM (%)	HYCG	14.63 ± 2.46	14.08 ± 2.77	27.33 ± 1.85	14.30 ± 3.79	5.97 ± 3.45	3.52 ± 1.80
	CEQY	11.07 ± 2.34	13.63 ± 0.64	20.45 ± 1.69	21.25 ± 2.02	14.43 ± 1.27	20.75 ± 1.85
SLOW (%)	HYCG	0.38 ± 0.24	0.42 ± 0.25	1.80 ± 0.65	1.70 ± 0.65	1.25 ± 0.73	0.50 ± 0.25
	CEQY	0.20 ± 0.20	0.43 ± 0.14	1.12 ± 0.26	1.35 ± 0.67	1.10 ± 0.27	1.48 ± 0.42
LIVE (%)	HYCG	75.25 ± 0.75	73.75 ± 2.75	75.75 ± 5.25	70.75 ± 0.25	74.00 ± 2.00	66.50 ± 8.50
	CEQY	76.25 ± 8.25	75.50 ± 5.00	76.50 ± 2.50	73.50 ± 12.50	76.00 ± 3.50	64.00 ± 5.50
DEAD (%)	HYCG	24.75 ± 0.75	26.25 ± 2.75	24.25 ± 5.25	29.25 ± 0.25	26.00 ± 2.00	33.50 ± 8.50
	CEQY	23.75 ± 8.25	24.50 ± 5.00	23.50 ± 2.50	26.50 ± 12.50	24.00 ± 3.50	36.00 ± 5.50
NORMAL (%)	HYCG	82.25 ± 7.25	78.00 ± 1.50	88.50 ± 4.00	79.75 ± 9.25	80.75 ± 7.75	74.75 ± 5.25
	CEQY	90.50 ± 4.50	80.50 ± 7.50	83.75 ± 5.75	76.00 ± 11.50	75.50 ± 6.50	70.50 ± 12.50

Table 10.2.4..a. (continued). Characteristics of semen collected on day -11 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± Sem, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem
LOOSE HEAD (%)	HYCG	2.00 ± 2.00	3.25 ± 0.25	3.25 ± 1.75	1.00 ± 1.00	3.00 ± 3.00	1.00 ± 0.00
	CEQY	2.00 ± 1.50	2.00 ± 0.50	2.75 ± 2.75	1.75 ± 1.25	0.25 ± 0.25	1.00 ± 1.00
COILED TAIL (%)	HYCG	10.75 ± 5.25	13.00 ± 1.50	5.25 ± 2.25	17.25 ± 9.75	10.75 ± 4.25	19.25 ± 6.25
	CEQY	4.00 ± 2.00	13.75 ± 7.75	7.75 ± 2.75	17.50 ± 11.50	15.75 ± 7.25	23.25 ± 12.25
BENT TAIL (%)	HYCG	5.00 ± 0.00	5.75 ± 0.25	3.00 ± 0.00	2.00 ± 0.50	5.50 ± 0.50	5.00 ± 1.00
	CEQY	3.50 ± 1.00	3.75 ± 0.25	5.75 ± 0.25	4.75 ± 1.25	8.50 ± 1.00	5.25 ± 1.25
ALH (µm)	HYCG	7.73 ± 0.38	7.43 ± 0.38	4.35 ± 0.51	3.85 ± 0.39	4.37 ± 0.26	5.10 ± 0.40
	CEQY	8.13 ± 0.21	8.88 ± 0.39	5.55 ± 0.33	5.70 ± 0.64	6.05 ± 0.36	5.32 ± 0.25
LIN (%)	HYCG	71.40 ± 3.71	71.75 ± 1.65	78.35 ± 1.49	76.90 ± 3.22	74.93 ± 8.74	50.50 ± 2.35
	CEQY	70.68 ± 3.01	68.43 ± 0.92	77.55 ± 2.38	76.50 ± 0.91	77.93 ± 1.75	79.57 ± 0.82
VAP (µm/s)	HYCG	150.60 ± 9.79	159.30 ± 10.96	84.40 ± 14.50	69.55 ± 9.61	93.80 ± 17.86	41.13 ± 9.30
	CEQY	155.50 ± 9.73	157.20 ± 7.49	118.43 ± 3.23	117.02 ± 9.82	132.20 ± 3.51	119.60 ± 4.51
VSL (µm/s)	HYCG	127.80 ± 10.38	136.60 ± 10.19	76.50 ± 11.87	63.30 ± 9.19	81.93 ± 19.69	31.68 ± 7.16
	CEQY	129.50 ± 11.48	131.20 ± 6.05	104.43 ± 4.33	104.15 ± 7.60	117.80 ± 2.71	108.30 ± 4.72
VCL (µm/s)	HYCG	172.80 ± 9.19	183.30 ± 11.75	93.65 ± 16.13	78.87 ± 9.69	106.90 ± 13.30	61.97 ± 12.80
	CEQY	179.20 ± 7.60	186.20 ± 9.68	130.55 ± 2.74	130.05 ± 11.70	146.60 ± 4.23	131.80 ± 4.53
STR (%)	HYCG	81.33 ± 2.81	81.48 ± 1.21	87.85 ± 2.28	88.53 ± 2.08	84.48 ± 4.92	77.70 ± 3.87
	CEQY	80.22 ± 2.04	79.70 ± 0.42	85.10 ± 1.62	85.35 ± 0.84	85.90 ± 1.16	87.37 ± 0.80

Table 10.2.4..b. Characteristics of semen collected on day -3 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± Sem, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem
MOT	HYCG	77.45 ± 3.02	81.35 ± 2.23	64.00 ± 5.30	73.53 ± 5.30	21.52 ± 9.60	37.40 ± 5.47
(%)	CEQY	76.80 ± 3.21	84.62 ± 2.14	82.98 ± 1.10	72.75 ± 5.68	56.07 ± 2.11	70.37 ± 4.02
PROG	HYCG	53.80 ± 1.47	55.97 ± 0.71	39.77 ± 2.80	45.08 ± 4.60	6.62 ± 3.14	17.95 ± 4.20
(%)	CEQY	48.30 ± 3.89	54.07 ± 0.96	54.15 ± 2.44	46.60 ± 3.74	35.17 ± 2.43	39.08 ± 1.64
RAPID	HYCG	62.85 ± 3.11	66.62 ± 1.46	43.33 ± 3.50	49.43 ± 5.60	6.98 ± 3.34	19.03 ± 4.50
(%)	CEQY	58.30 ± 4.44	67.73 ± 1.81	61.90 ± 2.96	53.08 ± 4.35	39.03 ± 2.38	44.53 ± 1.60
MEDIUM	HYCG	14.55 ± 1.72	14.75 ± 1.10	20.62 ± 3.10	24.12 ± 3.50	14.57 ± 6.38	18.40 ± 3.95
(%)	CEQY	18.55 ± 2.27	16.90 ± 2.13	21.10 ± 2.10	19.70 ± 1.35	17.05 ± 2.00	25.85 ± 3.79
SLOW	HYCG	1.23 ± 0.38	0.50 ± 0.16	2.12 ± 0.30	1.82 ± 0.40	2.25 ± 1.01	2.43 ± 0.25
(%)	CEQY	1.03 ± 0.43	0.25 ± 0.10	0.70 ± 0.22	1.12 ± 0.45	1.70 ± 0.19	1.45 ± 0.25
LIVE	HYCG	64.00 ± 4.50	66.50 ± 10.50	46.00 ± 0.50	62.00 ± 10.50	44.50 ± 11.00	56.25 ± 4.25
(%)	CEQY	65.00 ± 11.50	74.00 ± 2.00	44.50 ± 11.50	55.50 ± 6.00	47.75 ± 1.75	46.00 ± 7.00
DEAD	HYCG	36.00 ± 4.50	33.50 ± 10.50	54.00 ± 0.50	38.00 ± 10.50	55.50 ± 11.00	43.75 ± 4.25
(%)	CEQY	35.00 ± 11.50	26.00 ± 2.00	55.50 ± 11.50	44.50 ± 6.00	52.25 ± 1.75	54.00 ± 7.00
NORMAL	HYCG	90.25 ± 0.25	86.00 ± 6.00	90.75 ± 0.75	77.50 ± 13.50	90.00 ± 3.50	75.00 ± 11.00
(%)	CEQY	93.00 ± 0.00	86.25 ± 2.75	93.25 ± 0.25	82.25 ± 8.25	88.25 ± 0.75	75.25 ± 13.75

Table 10.2.4..b. (continued). Characteristics of semen collected on day -3 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± Sem, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem
LOOSE HEAD	HYCG	0.75 ± 0.75	2.25 ± 1.25	1.00 ± 0.50	1.75 ± 0.25	0.00 ± 0.00	2.00 ± 1.50
(%)	CEQY	0.00 ± 0.00	3.50 ± 2.00	0.75 ± 0.75	1.25 ± 0.75	0.25 ± 0.25	0.75 ± 0.25
COILED TAIL	HYCG	6.75 ± 0.25	7.00 ± 5.00	4.25 ± 2.25	18.00 ± 12.50	7.25 ± 1.25	20.50 ± 13.00
(%)	CEQY	4.25 ± 0.25	7.25 ± 0.25	2.50 ± 0.50	13.50 ± 8.50	8.00 ± 0.50	19.25 ± 12.25
BENT TAIL	HYCG	2.25 ± 0.75	4.75 ± 2.25	4.00 ± 1.00	2.75 ± 1.25	2.75 ± 2.25	2.50 ± 0.50
(%)	CEQY	2.75 ± 0.25	3.00 ± 1.00	3.50 ± 1.50	3.00 ± 0.50	3.50 ± 1.50	4.75 ± 1.25
ALH	HYCG	7.25 ± 0.33	7.43 ± 0.36	5.25 ± 0.40	5.85 ± 0.30	3.75 ± 0.25	4.60 ± 0.18
(µm)	CEQY	7.47 ± 0.28	7.97 ± 0.57	6.48 ± 0.28	6.42 ± 0.35	5.53 ± 0.31	6.05 ± 0.19
LIN	HYCG	72.52 ± 1.70	71.15 ± 0.57	76.00 ± 2.60	75.10 ± 0.80	71.70 ± 2.56	71.87 ± 1.76
(%)	CEQY	69.05 ± 1.57	67.45 ± 2.28	72.18 ± 1.56	74.35 ± 1.81	74.97 ± 2.30	71.97 ± 1.90
VAP	HYCG	147.00 ± 5.78	150.80 ± 6.40	110.90 ± 2.10	118.50 ± 6.30	61.83 ± 7.60	91.70 ± 7.83
(µm/s)	CEQY	140.00 ± 3.21	148.40 ± 7.20	121.50 ± 3.87	126.30 ± 5.78	114.00 ± 1.51	115.60 ± 4.66
VSL	HYCG	126.50 ± 4.21	127.30 ± 6.08	98.57 ± 3.70	105.40 ± 5.00	54.45 ± 7.21	81.35 ± 7.38
(µm/s)	CEQY	116.70 ± 3.11	120.90 ± 5.80	103.20 ± 2.22	109.40 ± 4.02	99.70 ± 1.87	99.60 ± 5.50
VCL	HYCG	168.00 ± 7.33	173.70 ± 7.56	123.20 ± 0.90	133.20 ± 7.10	73.40 ± 5.97	103.90 ± 7.98
(µm/s)	CEQY	162.10 ± 3.78	174.50 ± 10.30	138.50 ± 4.98	142.80 ± 8.01	127.50 ± 1.76	131.60 ± 4.66
STR	HYCG	82.07 ± 1.39	80.80 ± 0.56	84.72 ± 1.60	84.60 ± 0.40	84.75 ± 1.56	82.28 ± 1.19
(%)	CEQY	79.25 ± 1.19	77.75 ± 1.88	81.22 ± 1.22	83.40 ± 1.42	83.70 ± 1.37	81.78 ± 1.67

Table 10.2.4..c. Characteristics of semen collected on day 4 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± Sem, n = 4).

Semen charac-teristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem	Mean ± Sem
MOT	HYCG	73.80 ± 3.31	77.60 ± 1.93	65.00 ± 2.30	69.60 ± 5.23	34.92 ± 12.56	20.62 ± 3.71
(%)	CEQY	78.10 ± 1.61	73.57 ± 1.80	72.12 ± 3.88	73.37 ± 4.23	64.83 ± 2.11	64.93 ± 0.47
PROG	HYCG	57.38 ± 2.88	52.47 ± 3.90	45.40 ± 5.21	39.90 ± 3.27	19.95 ± 10.44	11.00 ± 2.45
(%)	CEQY	59.98 ± 1.40	46.83 ± 3.14	49.88 ± 4.99	43.03 ± 5.94	42.17 ± 2.65	35.45 ± 0.93
RAPID	HYCG	64.65 ± 3.47	58.35 ± 3.89	48.40 ± 5.70	42.60 ± 3.87	20.45 ± 10.73	11.63 ± 2.63
(%)	CEQY	70.43 ± 2.45	53.93 ± 4.13	54.35 ± 5.51	47.20 ± 5.89	45.22 ± 2.38	39.45 ± 1.01
MEDIUM	HYCG	9.17 ± 0.94	19.27 ± 3.07	16.60 ± 4.79	27.00 ± 2.90	14.55 ± 2.02	9.00 ± 1.57
(%)	CEQY	7.65 ± 1.40	19.67 ± 2.60	17.83 ± 4.85	26.25 ± 2.13	19.65 ± 1.39	25.53 ± 0.72
SLOW	HYCG	0.60 ± 0.17	0.95 ± 0.39	1.50 ± 0.33	2.33 ± 0.48	1.75 ± 0.67	1.80 ± 0.37
(%)	CEQY	0.30 ± 0.16	0.93 ± 0.28	1.12 ± 0.35	0.78 ± 0.30	2.00 ± 0.39	1.57 ± 0.14
LIVE	HYCG	78.50 ± 1.50	55.25 ± 4.25	56.75 ± 13.75	69.25 ± 5.25	78.00 ± 1.00	53.25 ± 4.75
(%)	CEQY	83.50 ± 4.50	76.50 ± 2.00	56.00 ± 2.50	75.00 ± 7.50	66.25 ± 8.25	68.75 ± 0.75
DEAD	HYCG	21.50 ± 1.50	44.75 ± 4.25	43.25 ± 13.75	30.75 ± 5.25	22.00 ± 1.00	46.75 ± 4.75
(%)	CEQY	16.50 ± 4.50	23.50 ± 2.00	44.00 ± 2.50	25.00 ± 7.50	33.75 ± 8.25	31.25 ± 0.75
NORMAL	HYCG	92.75 ± 1.75	75.75 ± 13.25	89.00 ± 2.50	71.75 ± 21.25	89.75 ± 0.75	69.00 ± 25.00
(%)	CEQY	95.50 ± 1.00	71.50 ± 19.50	89.25 ± 0.25	70.50 ± 20.00	83.00 ± 8.00	72.00 ± 20.00

Table 10.2.4..c. (continued). Characteristics of semen collected on day 4 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen charac-teristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD	HYCG	0.50 ± 0.00	1.00 ± 0.50	0.00 ± 0.00	0.50 ± 0.50	0.25 ± 0.25	0.00 ± 0.00
(%)	CEQY	0.00 ± 0.00	0.00 ± 0.00	0.75 ± 0.25	0.50 ± 0.50	1.25 ± 1.25	0.50 ± 0.50
COILED TAIL	HYCG	5.00 ± 1.50	20.25 ± 12.25	8.25 ± 2.75	23.50 ± 20.00	6.75 ± 1.75	27.75 ± 23.25
(%)	CEQY	2.75 ± 0.75	25.25 ± 18.25	8.25 ± 0.25	26.00 ± 21.00	10.25 ± 5.75	24.50 ± 17.50
BENT TAIL	HYCG	1.75 ± 0.25	3.00 ± 0.50	2.75 ± 0.25	4.25 ± 1.75	3.25 ± 0.75	3.25 ± 1.75
(%)	CEQY	1.75 ± 0.25	3.25 ± 1.25	1.75 ± 0.25	3.00 ± 0.50	5.50 ± 1.00	3.00 ± 2.00
ALH	HYCG	6.72 ± 0.47	6.62 ± 0.27	5.07 ± 0.50	5.45 ± 0.17	4.07 ± 0.21	4.57 ± 0.10
(µm)	CEQY	7.93 ± 0.46	6.95 ± 0.50	5.53 ± 0.26	5.92 ± 0.32	5.72 ± 0.34	5.75 ± 0.06
LIN	HYCG	78.50 ± 1.96	73.78 ± 1.16	81.20 ± 0.89	76.10 ± 0.98	75.10 ± 5.73	76.10 ± 1.91
(%)	CEQY	73.85 ± 1.05	73.03 ± 1.14	79.17 ± 1.78	72.83 ± 2.19	76.45 ± 3.16	71.33 ± 0.92
VAP	HYCG	161.10 ± 5.64	135.75 ± 2.46	118.00 ± 10.60	111.00 ± 7.20	79.00 ± 15.67	98.25 ± 7.62
(µm/s)	CEQY	164.67 ± 7.04	136.20 ± 11.03	120.60 ± 7.29	115.40 ± 8.75	111.80 ± 2.52	108.08 ± 2.26
VSL	HYCG	142.35 ± 5.00	118.28 ± 1.66	107.00 ± 9.43	99.30 ± 6.67	71.62 ± 15.93	87.40 ± 7.27
(µm/s)	CEQY	141.00 ± 5.01	117.28 ± 9.06	108.10 ± 6.82	101.20 ± 9.21	99.05 ± 1.79	93.35 ± 2.57
VCL	HYCG	177.83 ± 7.65	154.33 ± 3.48	128.00 ± 11.70	124.00 ± 7.62	89.37 ± 13.65	110.22 ± 6.63
(µm/s)	CEQY	188.27 ± 9.38	155.30 ± 12.85	132.90 ± 7.23	130.80 ± 8.93	126.90 ± 4.54	123.58 ± 2.32
STR	HYCG	86.07 ± 1.52	83.40 ± 0.96	88.40 ± 0.66	85.10 ± 0.95	85.67 ± 3.24	85.30 ± 1.25
(%)	CEQY	82.87 ± 0.73	82.75 ± 0.91	86.97 ± 1.19	82.65 ± 1.79	86.00 ± 2.30	81.75 ± 0.79

Table 10.2.4..d. Characteristics of semen collected collected on day 10 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT	HYCG	71.62 ± 5.41	68.90 ± 4.50	56.00 ± 9.86	51.83 ± 10.63	41.17 ± 16.36	24.70 ± 5.18
(%)	CEQY	79.80 ± 0.76	61.20 ± 8.68	73.57 ± 4.88	64.37 ± 6.74	63.30 ± 8.44	60.67 ± 6.30
PROG	HYCG	47.55 ± 5.53	40.73 ± 3.68	33.20 ± 6.53	26.58 ± 7.29	19.40 ± 8.99	9.70 ± 2.39
(%)	CEQY	53.65 ± 1.80	37.25 ± 8.67	46.80 ± 5.61	30.40 ± 2.84	39.10 ± 5.40	34.83 ± 4.63
RAPID	HYCG	56.38 ± 7.63	44.50 ± 4.22	38.65 ± 9.10	28.42 ± 8.23	22.15 ± 10.32	10.05 ± 2.57
(%)	CEQY	66.18 ± 2.00	41.70 ± 9.84	52.45 ± 6.74	33.25 ± 3.52	43.63 ± 7.41	37.67 ± 5.33
MEDIUM	HYCG	15.25 ± 2.23	24.40 ± 0.85	17.35 ± 0.97	23.37 ± 3.01	19.00 ± 6.19	14.65 ± 3.42
(%)	CEQY	13.65 ± 2.14	19.52 ± 1.78	21.12 ± 1.97	31.10 ± 3.30	19.65 ± 1.28	23.02 ± 2.30
SLOW	HYCG	1.03 ± 0.48	1.58 ± 0.54	1.93 ± 0.42	1.83 ± 0.43	1.55 ± 0.43	2.53 ± 0.71
(%)	CEQY	0.15 ± 0.06	1.65 ± 0.47	0.88 ± 0.10	1.55 ± 0.57	1.70 ± 0.77	2.05 ± 0.58
LIVE	HYCG	69.00 ± 12.50	63.50 ± 7.50	67.00 ± 7.00	66.50 ± 4.00	66.50 ± 8.00	63.75 ± 5.25
(%)	CEQY	68.50 ± 13.50	62.75 ± 9.25	60.25 ± 4.25	64.50 ± 0.50	64.75 ± 4.25	58.50 ± 10.00
DEAD	HYCG	31.00 ± 12.50	36.50 ± 7.50	33.00 ± 7.00	33.50 ± 4.00	33.50 ± 8.00	36.25 ± 5.25
(%)	CEQY	31.50 ± 13.50	37.25 ± 9.25	39.75 ± 4.25	35.50 ± 0.50	35.25 ± 4.25	41.50 ± 10.00
NORMAL	HYCG	78.75 ± 5.25	63.50 ± 18.50	85.00 ± 5.00	66.00 ± 19.50	84.00 ± 7.00	67.50 ± 13.00
(%)	CEQY	85.75 ± 6.75	70.75 ± 23.75	85.25 ± 5.25	66.00 ± 20.00	82.75 ± 7.25	67.50 ± 20.50

Table 10.2.4..d. (continued). Characteristics of semen collected on day 4 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD (%)	HYCG	1.75 ± 0.75	0.75 ± 0.75	1.00 ± 1.00	0.25 ± 0.25	2.25 ± 2.25	0.00 ± 0.00
	CEQY	0.50 ± 0.00	0.25 ± 0.25	1.25 ± 1.25	0.50 ± 0.50	1.50 ± 1.00	0.50 ± 0.50
COILED TAIL (%)	HYCG	16.75 ± 3.75	32.25 ± 19.25	10.75 ± 3.25	29.25 ± 18.75	11.25 ± 3.75	30.00 ± 12.50
	CEQY	9.75 ± 5.25	26.75 ± 21.75	9.75 ± 3.75	31.00 ± 18.50	12.00 ± 7.00	28.75 ± 19.75
BENT TAIL (%)	HYCG	2.75 ± 0.75	3.50 ± 0.00	3.25 ± 0.75	4.50 ± 1.00	2.50 ± 1.00	2.50 ± 0.50
	CEQY	4.00 ± 1.50	2.25 ± 1.75	3.75 ± 0.25	2.50 ± 2.00	3.75 ± 0.75	3.25 ± 1.25
ALH (µm)	HYCG	6.80 ± 0.29	5.87 ± 0.41	5.68 ± 0.52	4.87 ± 0.41	4.85 ± 0.42	3.78 ± 0.18
	CEQY	8.20 ± 0.15	5.93 ± 0.18	6.20 ± 0.20	5.02 ± 0.27	5.78 ± 0.60	5.37 ± 0.39
LIN (%)	HYCG	72.78 ± 1.21	75.30 ± 0.66	74.47 ± 3.09	74.05 ± 1.26	69.03 ± 0.34	74.68 ± 2.06
	CEQY	68.62 ± 1.89	74.87 ± 0.76	72.80 ± 1.62	75.25 ± 1.63	75.43 ± 3.13	75.90 ± 2.27
VAP (µm/s)	HYCG	142.35 ± 5.91	116.90 ± 5.32	114.67 ± 6.37	97.30 ± 4.54	83.10 ± 10.05	79.60 ± 10.45
	CEQY	152.85 ± 3.32	122.20 ± 1.01	120.30 ± 2.02	100.03 ± 1.74	113.50 ± 3.53	108.12 ± 3.73
VSL (µm/s)	HYCG	121.62 ± 4.03	103.62 ± 4.50	99.55 ± 2.85	87.03 ± 2.95	70.70 ± 8.59	72.02 ± 9.92
	CEQY	126.03 ± 4.11	107.57 ± 0.54	103.43 ± 2.20	88.70 ± 3.15	99.32 ± 0.72	96.72 ± 3.52
VCL (µm/s)	HYCG	161.90 ± 7.38	131.50 ± 6.63	129.05 ± 8.74	109.28 ± 5.21	97.65 ± 10.37	88.95 ± 10.52
	CEQY	178.42 ± 2.05	136.48 ± 1.34	136.12 ± 2.16	112.33 ± 0.94	127.47 ± 6.25	121.25 ± 5.03
STR (%)	HYCG	81.85 ± 1.07	84.70 ± 0.12	83.55 ± 3.05	84.30 ± 1.28	81.12 ± 1.01	84.72 ± 0.68
	CEQY	78.78 ± 1.32	84.30 ± 0.31	81.78 ± 1.14	84.78 ± 1.40	84.28 ± 2.54	85.62 ± 1.76

Table 10.2.4..e. Characteristics of semen collected on day 17 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT	HYCG	79.23 ± 2.60	28.62 ± 13.98	39.93 ± 15.45	26.12 ± 9.36	26.02 ± 14.35	0.13 ± 0.13
(%)	CEQY	79.02 ± 1.19	28.60 ± 13.87	70.90 ± 3.79	27.50 ± 13.56	64.85 ± 5.50	18.95 ± 8.54
PROG	HYCG	57.75 ± 1.48	14.32 ± 6.64	26.62 ± 12.23	11.27 ± 2.75	11.05 ± 6.06	0.00 ± 0.00
(%)	CEQY	54.03 ± 1.33	15.40 ± 6.84	47.90 ± 2.55	16.70 ± 7.95	40.65 ± 3.32	10.60 ± 4.86
RAPID	HYCG	66.48 ± 2.65	15.83 ± 7.58	27.98 ± 13.02	11.60 ± 2.99	12.38 ± 6.84	0.00 ± 0.00
(%)	CEQY	66.82 ± 2.96	16.62 ± 7.48	52.20 ± 2.99	17.50 ± 8.38	45.97 ± 4.12	10.85 ± 4.94
MEDIUM	HYCG	12.73 ± 2.08	12.77 ± 6.60	11.93 ± 2.48	14.53 ± 6.93	13.68 ± 7.55	0.13 ± 0.13
(%)	CEQY	12.17 ± 1.99	11.98 ± 6.51	18.70 ± 1.90	9.93 ± 5.20	18.87 ± 1.66	8.10 ± 3.66
SLOW	HYCG	0.67 ± 0.23	0.67 ± 0.31	0.88 ± 0.27	1.30 ± 0.65	2.68 ± 0.80	0.68 ± 0.68
(%)	CEQY	0.25 ± 0.10	0.77 ± 0.65	1.43 ± 0.43	0.92 ± 0.36	1.08 ± 0.31	1.30 ± 0.50
LIVE	HYCG	69.00 ± 16.00	37.50 ± 29.00	70.75 ± 9.75	34.75 ± 25.75	51.50 ± 17.00	20.00 ± 13.00
(%)	CEQY	68.25 ± 16.75	40.25 ± 31.25	69.75 ± 12.25	38.50 ± 27.50	56.00 ± 16.00	30.25 ± 23.25
DEAD	HYCG	31.00 ± 16.00	62.50 ± 29.00	29.25 ± 9.75	65.25 ± 25.75	48.50 ± 17.00	80.00 ± 13.00
(%)	CEQY	31.75 ± 16.75	59.75 ± 31.25	30.25 ± 12.25	61.50 ± 27.50	44.00 ± 16.00	69.75 ± 23.25
NORMAL	HYCG	86.00 ± 4.50	52.75 ± 0.25	87.75 ± 5.25	50.00 ± 2.50	87.25 ± 6.25	44.00 ± 0.50
(%)	CEQY	88.25 ± 6.25	46.00 ± 2.00	88.25 ± 2.75	48.75 ± 1.75	88.00 ± 4.50	40.75 ± 2.75

Table 10.2.4..e. (continued). Characteristics of semen collected on day 17 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen charac-teristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD (%)	HYCG	1.00 ± 0.50	11.00 ± 8.50	3.25 ± 2.75	11.50 ± 8.00	4.75 ± 1.75	12.25 ± 9.25
	CEQY	1.00 ± 1.00	8.25 ± 6.75	1.50 ± 1.00	10.25 ± 7.25	1.00 ± 1.00	11.50 ± 10.50
COILED TAIL (%)	HYCG	11.00 ± 3.00	27.75 ± 10.25	7.25 ± 1.25	32.75 ± 9.25	5.50 ± 2.00	33.25 ± 11.25
	CEQY	8.50 ± 4.00	36.00 ± 12.00	7.00 ± 1.00	33.25 ± 13.75	8.50 ± 3.50	39.00 ± 15.50
BENT TAIL (%)	HYCG	2.00 ± 1.00	8.50 ± 1.50	1.75 ± 1.25	5.75 ± 3.75	2.50 ± 2.50	10.50 ± 2.50
	CEQY	2.25 ± 1.25	9.75 ± 3.25	3.25 ± 0.75	7.75 ± 4.75	2.50 ± 0.00	8.75 ± 2.25
ALH (µm)	HYCG	7.40 ± 0.14	5.58 ± 0.42	4.50 ± 0.55	4.10 ± 0.31	5.45 ± 0.89	0.13 ± 0.13
	CEQY	8.43 ± 0.42	6.10 ± 0.38	5.95 ± 0.03	6.80 ± 0.45	6.60 ± 0.21	5.37 ± 0.22
LIN (%)	HYCG	73.62 ± 0.90	78.57 ± 3.88	81.10 ± 1.42	82.18 ± 4.16	76.78 ± 3.87	23.23 ± 23.23
	CEQY	68.12 ± 1.42	80.07 ± 4.75	76.70 ± 1.02	78.50 ± 2.88	72.10 ± 2.42	77.87 ± 1.90
VAP (µm/s)	HYCG	158.02 ± 2.55	148.25 ± 8.60	102.20 ± 11.51	105.95 ± 12.26	99.57 ± 16.20	5.50 ± 5.50
	CEQY	156.50 ± 4.18	157.90 ± 10.89	125.00 ± 2.68	153.00 ± 9.67	123.70 ± 6.12	117.30 ± 5.11
VSL (µm/s)	HYCG	136.65 ± 2.15	137.37 ± 10.56	93.82 ± 10.08	98.45 ± 13.10	88.75 ± 15.69	5.45 ± 5.45
	CEQY	128.40 ± 2.73	146.30 ± 12.34	111.00 ± 2.82	141.00 ± 10.56	107.10 ± 8.09	107.60 ± 6.61
VCL (µm/s)	HYCG	180.37 ± 2.77	163.03 ± 7.46	111.30 ± 12.61	115.45 ± 10.85	111.35 ± 16.74	5.87 ± 5.87
	CEQY	185.00 ± 6.09	172.30 ± 8.66	140.00 ± 2.41	169.00 ± 9.33	142.40 ± 4.35	129.40 ± 4.58
STR (%)	HYCG	82.95 ± 0.89	87.45 ± 2.45	89.08 ± 0.78	90.57 ± 2.16	86.68 ± 3.55	24.80 ± 24.80
	CEQY	78.85 ± 1.27	88.47 ± 3.12	85.10 ± 0.82	87.10 ± 1.80	82.43 ± 1.70	87.78 ± 2.07

Table 10.2.4..f. Characteristics of semen collected on day 25 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	HYCG	88.62 ± 0.67	31.25 ± 18.20	81.80 ± 1.45	14.77 ± 8.70	39.58 ± 3.93	1.33 ± 0.82
	CEQY	78.85 ± 3.65	30.48 ± 17.47	78.20 ± 2.27	21.55 ± 13.39	81.12 ± 0.25	26.35 ± 15.22
PROG (%)	HYCG	65.70 ± 1.45	14.65 ± 8.63	54.80 ± 1.95	5.97 ± 3.47	18.20 ± 2.07	0.43 ± 0.25
	CEQY	56.48 ± 1.75	13.15 ± 7.71	54.97 ± 2.55	8.90 ± 6.18	55.22 ± 0.52	14.20 ± 8.21
RAPID (%)	HYCG	77.93 ± 1.14	17.23 ± 10.30	61.78 ± 2.04	6.22 ± 3.60	20.67 ± 2.34	0.50 ± 0.30
	CEQY	66.62 ± 1.81	14.95 ± 8.83	61.72 ± 2.65	10.35 ± 7.28	62.17 ± 1.35	16.17 ± 9.36
MEDIUM (%)	HYCG	10.73 ± 1.09	14.07 ± 8.13	20.00 ± 2.19	8.52 ± 5.09	18.90 ± 1.94	0.80 ± 0.50
	CEQY	12.20 ± 2.90	15.52 ± 8.87	16.48 ± 1.61	11.23 ± 6.53	18.92 ± 1.37	10.15 ± 5.86
SLOW (%)	HYCG	0.08 ± 0.08	0.75 ± 0.46	0.70 ± 0.16	1.12 ± 0.85	2.35 ± 0.70	0.20 ± 0.12
	CEQY	0.52 ± 0.19	1.10 ± 0.73	0.30 ± 0.09	1.10 ± 0.75	0.23 ± 0.09	1.08 ± 0.63
LIVE (%)	HYCG	73.75 ± 0.25	31.25 ± 31.25	83.25 ± 0.75	27.50 ± 27.50	57.75 ± 8.75	13.00 ± 13.00
	CEQY	80.50 ± 3.50	35.25 ± 35.25	78.25 ± 8.25	32.25 ± 32.25	74.50 ± 5.00	27.00 ± 27.00
DEAD (%)	HYCG	26.25 ± 0.25	68.75 ± 31.25	16.75 ± 0.75	22.50 ± 22.50	42.25 ± 8.75	37.00 ± 37.00
	CEQY	19.50 ± 3.50	64.75 ± 35.25	21.75 ± 8.25	17.75 ± 17.75	25.50 ± 5.00	23.00 ± 23.00
NORMAL (%)	HYCG	95.50 ± 0.50	62.50 ± 13.00	79.25 ± 4.75	28.00 ± 28.00	92.25 ± 1.25	28.00 ± 28.00
	CEQY	94.25 ± 0.75	73.50 ± 10.00	92.50 ± 1.00	34.00 ± 34.00	91.00 ± 1.00	32.25 ± 32.25

Table 10.2.4..f. (continued). Characteristics of semen collected on day 25 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control Mean ± SEM	Heated Mean ± SEM	Control Mean ± SEM	Heated Mean ± SEM	Control Mean ± SEM	Heated Mean ± SEM
LOOSE HEAD (%)	HYCG	0.50 ± 0.50	11.00 ± 0.50	7.25 ± 0.75	5.75 ± 5.75	0.50 ± 0.50	2.75 ± 2.75
	CEQY	0.25 ± 0.25	5.50 ± 0.00	0.00 ± 0.00	2.00 ± 2.00	2.00 ± 2.00	1.50 ± 1.50
COILED TAIL (%)	HYCG	2.75 ± 0.25	22.50 ± 13.00	10.50 ± 4.00	15.00 ± 15.00	4.75 ± 1.25	16.25 ± 16.25
	CEQY	4.25 ± 1.75	16.25 ± 8.75	6.00 ± 1.50	12.75 ± 12.75	4.75 ± 0.75	13.00 ± 13.00
BENT TAIL (%)	HYCG	1.25 ± 0.75	4.00 ± 0.50	3.00 ± 1.50	1.25 ± 1.25	2.50 ± 2.00	3.00 ± 3.00
	CEQY	1.25 ± 1.25	4.75 ± 1.25	1.50 ± 0.50	1.25 ± 1.25	2.25 ± 0.25	3.25 ± 3.25
ALH (µm)	HYCG	7.48 ± 0.23	3.45 ± 2.00	6.22 ± 0.18	3.62 ± 1.29	5.08 ± 0.46	2.92 ± 1.69
	CEQY	7.47 ± 0.15	4.02 ± 1.75	6.23 ± 0.22	2.75 ± 1.63	6.65 ± 0.26	3.25 ± 1.88
LIN (%)	HYCG	72.37 ± 1.39	33.90 ± 19.60	73.95 ± 1.60	58.38 ± 20.10	67.92 ± 0.97	30.20 ± 17.50
	CEQY	72.92 ± 2.09	53.75 ± 18.34	75.57 ± 0.79	33.88 ± 19.56	72.00 ± 1.34	35.22 ± 20.35
VAP (µm/s)	HYCG	149.40 ± 3.63	62.42 ± 36.20	119.80 ± 3.78	77.85 ± 26.00	91.70 ± 2.99	41.08 ± 23.70
	CEQY	148.70 ± 5.38	63.45 ± 31.07	122.40 ± 2.16	50.92 ± 30.01	125.40 ± 2.49	65.40 ± 37.76
VSL (µm/s)	HYCG	125.90 ± 2.76	53.18 ± 30.70	102.90 ± 4.15	71.85 ± 24.30	76.10 ± 2.49	33.65 ± 19.50
	CEQY	126.30 ± 6.36	54.35 ± 26.10	105.45 ± 1.91	43.40 ± 25.45	106.50 ± 2.01	56.43 ± 32.58
VCL (µm/s)	HYCG	171.20 ± 4.33	72.52 ± 42.00	135.60 ± 3.48	87.05 ± 29.00	107.20 ± 4.17	52.20 ± 30.10
	CEQY	170.40 ± 3.79	73.93 ± 36.37	138.23 ± 2.91	58.85 ± 34.70	144.70 ± 3.34	73.97 ± 42.72
STR (%)	HYCG	81.37 ± 1.22	39.90 ± 23.00	82.82 ± 1.15	65.85 ± 22.30	79.00 ± 0.70	38.20 ± 22.10
	CEQY	82.45 ± 1.39	62.83 ± 21.30	84.15 ± 0.28	39.45 ± 22.78	81.87 ± 0.96	40.15 ± 23.18

Table 10.2.4..g. Characteristics of semen collected on day 32 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	HYCG	83.35 ± 1.73	38.97 ± 22.27	70.50 ± 6.15	30.20 ± 17.93	32.43 ± 3.25	8.43 ± 5.09
	CEQY	81.58 ± 2.68	37.68 ± 21.76	76.72 ± 2.60	34.45 ± 19.95	72.82 ± 1.64	30.23 ± 17.60
PROG (%)	HYCG	55.50 ± 1.92	23.15 ± 13.20	50.25 ± 6.60	18.98 ± 11.20	19.17 ± 0.92	3.85 ± 2.26
	CEQY	55.50 ± 2.55	22.27 ± 12.89	54.40 ± 3.07	23.48 ± 13.65	44.08 ± 4.48	20.90 ± 12.16
RAPID (%)	HYCG	64.35 ± 1.10	28.30 ± 16.16	55.35 ± 7.15	21.75 ± 12.91	20.25 ± 0.99	4.02 ± 2.39
	CEQY	64.28 ± 4.11	27.77 ± 16.04	60.30 ± 3.63	26.30 ± 15.34	48.15 ± 5.34	23.67 ± 13.76
MEDIUM (%)	HYCG	18.95 ± 2.68	10.65 ± 6.25	15.15 ± 1.07	8.45 ± 5.02	12.18 ± 2.43	4.40 ± 2.71
	CEQY	17.30 ± 4.72	9.92 ± 5.73	16.43 ± 2.58	8.13 ± 4.73	24.62 ± 5.09	6.55 ± 3.84
SLOW (%)	HYCG	0.15 ± 0.05	0.18 ± 0.10	0.83 ± 0.36	0.88 ± 0.53	2.10 ± 0.63	0.60 ± 0.60
	CEQY	0.57 ± 0.26	0.20 ± 0.12	0.65 ± 0.36	0.68 ± 0.39	1.02 ± 0.30	0.33 ± 0.26
LIVE (%)	HYCG	73.75 ± 0.25	31.25 ± 31.25	83.25 ± 0.75	27.50 ± 27.50	57.75 ± 8.75	13.00 ± 13.00
	CEQY	80.50 ± 3.50	35.25 ± 35.25	78.25 ± 8.25	32.25 ± 32.25	74.50 ± 5.00	27.00 ± 27.00
DEAD (%)	HYCG	26.25 ± 0.25	68.75 ± 31.25	16.75 ± 0.75	22.50 ± 22.50	42.25 ± 8.75	37.00 ± 37.00
	CEQY	19.50 ± 3.50	64.75 ± 35.25	21.75 ± 8.25	17.75 ± 17.75	25.50 ± 5.00	23.00 ± 23.00
NORMAL (%)	HYCG	95.50 ± 0.50	62.50 ± 13.00	79.25 ± 4.75	28.00 ± 28.00	92.25 ± 1.25	28.00 ± 28.00
	CEQY	94.25 ± 0.75	73.50 ± 10.00	92.50 ± 1.00	34.00 ± 34.00	91.00 ± 1.00	32.25 ± 32.25

Table 10.2.4..g. (continued). Characteristics of semen collected on day 32 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen charac-teristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD	HYCG	0.50 ± 0.50	11.00 ± 0.50	7.25 ± 0.75	5.75 ± 5.75	0.50 ± 0.50	2.75 ± 2.75
(%)	CEQY	0.25 ± 0.25	5.50 ± 0.00	0.00 ± 0.00	2.00 ± 2.00	2.00 ± 2.00	1.50 ± 1.50
COILED TAIL	HYCG	2.75 ± 0.25	22.50 ± 13.00	10.50 ± 4.00	15.00 ± 15.00	4.75 ± 1.25	16.25 ± 16.25
(%)	CEQY	4.25 ± 1.75	16.25 ± 8.75	6.00 ± 1.50	12.75 ± 12.75	4.75 ± 0.75	13.00 ± 13.00
BENT TAIL	HYCG	1.25 ± 0.75	4.00 ± 0.50	3.00 ± 1.50	1.25 ± 1.25	2.50 ± 2.00	3.00 ± 3.00
(%)	CEQY	1.25 ± 1.25	4.75 ± 1.25	1.50 ± 0.50	1.25 ± 1.25	2.25 ± 0.25	3.25 ± 3.25
ALH	HYCG	6.93 ± 0.23	4.65 ± 2.00	5.85 ± 0.34	3.62 ± 2.10	4.15 ± 0.37	2.33 ± 1.35
(µm)	CEQY	6.67 ± 0.40	4.45 ± 2.57	5.87 ± 0.29	3.73 ± 2.16	5.30 ± 0.27	3.60 ± 2.08
LIN	HYCG	70.72 ± 2.44	57.75 ± 20.56	77.85 ± 0.52	36.08 ± 20.85	80.82 ± 1.41	37.53 ± 21.67
(%)	CEQY	73.07 ± 2.51	32.33 ± 18.68	76.67 ± 0.25	36.13 ± 20.86	77.32 ± 0.79	36.75 ± 21.22
VAP	HYCG	130.70 ± 7.07	115.67 ± 38.85	122.70 ± 6.49	75.20 ± 43.43	103.07 ± 4.79	50.25 ± 29.04
(µm/s)	CEQY	129.57 ± 9.45	76.10 ± 43.94	120.50 ± 3.67	75.62 ± 43.79	110.53 ± 7.51	75.57 ± 43.69
VSL	HYCG	110.40 ± 7.97	101.45 ± 34.01	108.50 ± 5.95	65.22 ± 37.66	93.42 ± 4.73	46.08 ± 26.65
(µm/s)	CEQY	110.10 ± 8.60	62.10 ± 35.87	105.20 ± 3.09	65.80 ± 38.06	97.15 ± 6.89	65.48 ± 37.84
VCL	HYCG	150.70 ± 6.72	128.85 ± 44.06	136.00 ± 7.16	85.62 ± 49.47	112.20 ± 4.05	55.63 ± 32.14
(µm/s)	CEQY	148.60 ± 10.67	91.30 ± 52.71	134.90 ± 4.08	86.57 ± 50.17	124.15 ± 8.42	85.68 ± 49.53
STR	HYCG	80.80 ± 1.90	63.00 ± 21.55	85.43 ± 0.44	40.97 ± 23.67	87.70 ± 0.80	42.47 ± 24.52
(%)	CEQY	82.23 ± 1.97	38.40 ± 22.18	84.75 ± 0.33	41.15 ± 23.76	85.40 ± 0.67	41.58 ± 24.00

Table 10.2.4..h. Characteristics of semen collected on day 50 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	HYCG	72.52 ± 5.05	34.45 ± 18.80	48.92 ± 7.11	17.83 ± 10.41	19.85 ± 5.70	7.85 ± 4.97
	CEQY	74.62 ± 2.75	34.80 ± 18.97	44.38 ± 9.39	18.75 ± 11.42	28.03 ± 2.96	13.25 ± 8.30
PROG (%)	HYCG	54.10 ± 4.89	22.50 ± 12.70	28.40 ± 5.06	9.95 ± 5.88	11.22 ± 4.14	3.97 ± 3.00
	CEQY	55.30 ± 1.80	21.37 ± 12.20	27.22 ± 8.46	14.98 ± 9.21	12.43 ± 1.69	8.80 ± 5.59
RAPID (%)	HYCG	62.15 ± 5.69	25.15 ± 14.30	31.37 ± 5.99	10.23 ± 6.06	11.65 ± 4.32	3.97 ± 3.00
	CEQY	62.33 ± 1.70	26.65 ± 15.36	29.85 ± 9.59	15.60 ± 9.68	14.63 ± 2.44	9.25 ± 5.91
MEDIUM (%)	HYCG	10.38 ± 1.17	9.32 ± 4.60	17.55 ± 2.65	7.60 ± 4.39	8.17 ± 1.66	3.87 ± 2.24
	CEQY	12.33 ± 1.33	8.15 ± 3.62	14.52 ± 1.22	3.15 ± 1.82	13.38 ± 0.57	4.03 ± 2.42
SLOW (%)	HYCG	0.60 ± 0.32	0.58 ± 0.33	1.33 ± 0.10	0.98 ± 0.57	0.83 ± 0.33	0.63 ± 0.37
	CEQY	0.40 ± 0.26	0.60 ± 0.20	1.37 ± 0.35	0.30 ± 0.17	0.65 ± 0.27	0.45 ± 0.29
LIVE (%)	HYCG	38.75 ± 30.25	14.25 ± 4.25	39.25 ± 27.75	20.50 ± 19.00	33.00 ± 17.50	12.50 ± 12.50
	CEQY	40.25 ± 27.75	12.75 ± 5.75	39.00 ± 24.00	14.25 ± 11.75	37.25 ± 29.25	14.00 ± 14.00
DEAD (%)	HYCG	61.25 ± 30.25	85.75 ± 4.25	60.75 ± 27.75	79.50 ± 19.00	67.00 ± 17.50	37.50 ± 37.50
	CEQY	59.75 ± 27.75	87.25 ± 5.75	61.00 ± 24.00	85.75 ± 11.75	62.75 ± 29.25	36.00 ± 36.00
NORMAL (%)	HYCG	94.00 ± 4.50	82.50 ± 6.50	87.75 ± 1.25	86.25 ± 5.25	94.50 ± 3.00	47.75 ± 47.75
	CEQY	93.25 ± 5.75	91.50 ± 1.00	95.25 ± 0.25	90.25 ± 5.25	97.50 ± 1.50	47.25 ± 47.25

Table 10.2.4..h. (continued). Characteristics of semen collected on day 50 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD (%)	HYCG	0.50 ± 0.50	2.75 ± 2.75	1.75 ± 1.75	2.50 ± 1.50	0.25 ± 0.25	0.25 ± 0.25
	CEQY	0.75 ± 0.25	0.50 ± 0.00	0.50 ± 0.00	2.50 ± 2.50	0.25 ± 0.25	0.50 ± 0.50
COILED TAIL (%)	HYCG	4.75 ± 4.25	11.25 ± 3.25	9.00 ± 1.00	9.50 ± 3.00	2.75 ± 0.75	1.75 ± 1.75
	CEQY	5.00 ± 5.00	5.50 ± 1.50	2.50 ± 0.00	4.50 ± 2.50	1.50 ± 1.00	1.50 ± 1.50
BENT TAIL (%)	HYCG	0.75 ± 0.25	3.50 ± 0.50	1.50 ± 0.50	1.75 ± 0.75	2.50 ± 2.00	0.25 ± 0.25
	CEQY	1.00 ± 0.50	2.50 ± 0.50	1.75 ± 0.25	2.75 ± 0.25	0.75 ± 0.75	0.75 ± 0.75
ALH (µm)	HYCG	7.07 ± 0.50	4.50 ± 0.78	5.52 ± 0.43	2.12 ± 1.23	5.87 ± 0.57	2.15 ± 1.26
	CEQY	7.12 ± 0.20	4.95 ± 1.43	5.87 ± 0.80	2.45 ± 1.42	6.00 ± 0.53	2.45 ± 1.43
LIN (%)	HYCG	75.22 ± 0.81	80.35 ± 3.52	76.07 ± 3.32	41.75 ± 24.11	73.07 ± 0.60	40.17 ± 23.30
	CEQY	76.87 ± 3.02	73.55 ± 4.19	75.03 ± 2.61	44.03 ± 25.42	68.05 ± 5.34	41.83 ± 24.15
VAP (µm/s)	HYCG	162.50 ± 5.80	106.50 ± 19.90	111.40 ± 6.78	49.03 ± 28.31	93.35 ± 9.13	43.03 ± 25.70
	CEQY	145.40 ± 3.69	103.50 ± 27.37	106.20 ± 8.10	68.32 ± 39.52	97.07 ± 2.97	54.58 ± 31.52
VSL (µm/s)	HYCG	141.80 ± 3.89	96.32 ± 15.80	98.30 ± 6.67	46.40 ± 26.79	84.07 ± 7.04	41.38 ± 24.90
	CEQY	128.10 ± 6.16	88.58 ± 21.41	93.30 ± 4.79	64.75 ± 37.41	80.80 ± 2.62	50.72 ± 29.29
VCL (µm/s)	HYCG	182.20 ± 7.60	116.70 ± 22.40	125.90 ± 8.32	52.85 ± 30.52	111.50 ± 10.30	47.47 ± 27.90
	CEQY	163.90 ± 2.62	119.10 ± 33.22	123.00 ± 11.97	72.12 ± 41.74	117.10 ± 6.83	58.75 ± 33.93
STR (%)	HYCG	83.57 ± 0.72	88.37 ± 3.50	84.87 ± 2.47	45.92 ± 26.52	87.72 ± 1.66	45.93 ± 26.50
	CEQY	85.55 ± 2.56	85.42 ± 4.22	85.22 ± 2.27	46.63 ± 26.92	79.90 ± 4.56	45.18 ± 26.08

Table 10.2.4..i. Characteristics of semen collected on day 77 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	HYCG	73.43 ± 1.06	60.27 ± 2.22	64.25 ± 3.11	59.95 ± 3.62	30.37 ± 14.60	24.95 ± 5.48
	CEQY	77.40 ± 1.84	60.80 ± 5.14	72.05 ± 4.07	61.72 ± 4.42	69.50 ± 2.05	49.70 ± 1.77
PROG (%)	HYCG	44.03 ± 0.65	35.53 ± 2.94	36.17 ± 4.44	36.20 ± 3.69	9.20 ± 5.03	10.15 ± 2.54
	CEQY	46.90 ± 2.30	42.40 ± 2.86	42.28 ± 1.73	39.00 ± 1.34	38.60 ± 2.71	26.20 ± 3.57
RAPID (%)	HYCG	52.22 ± 1.43	40.00 ± 2.84	40.65 ± 3.95	40.28 ± 4.41	11.25 ± 6.28	11.80 ± 3.36
	CEQY	56.70 ± 4.14	48.60 ± 4.84	49.17 ± 3.26	43.30 ± 2.40	44.20 ± 2.17	27.70 ± 4.12
MEDIUM (%)	HYCG	21.17 ± 0.70	20.30 ± 2.57	23.60 ± 2.40	19.65 ± 1.34	19.12 ± 8.33	13.15 ± 2.35
	CEQY	20.70 ± 2.97	12.20 ± 0.49	22.87 ± 2.50	18.45 ± 2.37	25.20 ± 1.76	22.00 ± 3.47
SLOW (%)	HYCG	1.23 ± 0.37	1.20 ± 0.16	2.80 ± 0.42	2.55 ± 0.85	1.48 ± 0.69	0.98 ± 0.34
	CEQY	0.85 ± 0.50	1.10 ± 0.51	1.30 ± 0.34	1.30 ± 0.62	1.62 ± 0.53	1.60 ± 0.38
LIVE (%)	HYCG	86.00 ± 3.50	58.00 ± 5.00	59.00 ± 4.50	48.75 ± 2.25	41.00 ± 11.00	49.50 ± 7.00
	CEQY	83.00 ± 1.00	62.50 ± 2.00	61.25 ± 11.25	47.50 ± 9.00	75.50 ± 9.00	50.25 ± 1.25
DEAD (%)	HYCG	14.00 ± 3.50	42.00 ± 5.00	41.00 ± 4.50	51.25 ± 2.25	59.00 ± 11.00	50.50 ± 7.00
	CEQY	17.00 ± 1.00	37.50 ± 2.00	38.75 ± 11.25	52.50 ± 9.00	24.50 ± 9.00	49.75 ± 1.25
NORMAL (%)	HYCG	92.25 ± 3.25	68.50 ± 23.00	92.25 ± 3.25	71.75 ± 5.75	88.25 ± 3.75	70.75 ± 14.75
	CEQY	92.00 ± 1.00	78.50 ± 12.50	94.25 ± 0.25	73.50 ± 13.00	87.00 ± 3.50	78.00 ± 10.00

Table 10.2.4..i. (continued). Characteristics of semen collected on day 77 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD (%)	HYCG	0.25 ± 0.25	2.50 ± 1.50	0.50 ± 0.50	1.25 ± 0.25	1.00 ± 0.50	2.50 ± 2.00
	CEQY	0.00 ± 0.00	0.75 ± 0.75	0.75 ± 0.75	2.25 ± 1.75	1.00 ± 1.00	2.50 ± 2.50
COILED TAIL (%)	HYCG	5.50 ± 2.50	23.25 ± 18.75	5.50 ± 3.00	21.75 ± 5.75	5.75 ± 1.75	22.25 ± 13.25
	CEQY	6.50 ± 2.00	16.00 ± 8.00	3.00 ± 0.50	20.00 ± 11.00	8.00 ± 3.50	13.25 ± 6.25
BENT TAIL (%)	HYCG	2.00 ± 0.50	5.75 ± 2.75	1.75 ± 0.25	5.25 ± 0.25	5.00 ± 2.50	4.50 ± 0.50
	CEQY	1.50 ± 1.00	4.75 ± 3.75	2.00 ± 1.50	4.25 ± 0.25	4.00 ± 1.00	6.25 ± 1.25
ALH (µm)	HYCG	7.40 ± 0.37	7.28 ± 0.57	5.98 ± 0.58	6.05 ± 0.62	4.87 ± 0.72	4.50 ± 0.54
	CEQY	7.45 ± 0.85	7.52 ± 0.64	6.02 ± 0.40	6.35 ± 0.60	5.58 ± 0.51	5.32 ± 0.62
LIN (%)	HYCG	68.00 ± 2.45	69.65 ± 3.47	71.00 ± 4.36	73.03 ± 2.90	65.45 ± 4.05	72.53 ± 4.71
	CEQY	67.40 ± 1.81	73.70 ± 3.17	71.35 ± 3.17	74.42 ± 5.08	71.80 ± 4.61	77.00 ± 2.74
VAP (µm/s)	HYCG	132.43 ± 1.26	136.57 ± 6.07	110.30 ± 2.52	120.50 ± 6.51	65.70 ± 9.21	88.40 ± 6.02
	CEQY	129.00 ± 8.68	157.00 ± 4.27	117.90 ± 3.10	132.10 ± 4.10	106.00 ± 2.01	107.00 ± 13.17
VSL (µm/s)	HYCG	110.60 ± 1.23	117.62 ± 6.77	94.07 ± 4.44	104.80 ± 5.62	53.07 ± 5.65	75.28 ± 1.60
	CEQY	106.00 ± 5.38	137.00 ± 4.58	99.80 ± 4.34	117.40 ± 7.17	89.10 ± 4.80	95.50 ± 10.64
VCL (µm/s)	HYCG	157.10 ± 3.97	157.70 ± 5.62	127.90 ± 2.81	136.40 ± 8.76	80.03 ± 12.66	101.42 ± 9.08
	CEQY	152.00 ± 12.81	179.00 ± 7.29	135.80 ± 3.44	148.90 ± 3.42	122.00 ± 2.26	121.00 ± 16.30
STR (%)	HYCG	79.42 ± 1.88	80.02 ± 2.30	81.03 ± 2.90	82.55 ± 2.12	79.03 ± 3.82	82.50 ± 4.17
	CEQY	78.30 ± 1.36	83.40 ± 2.35	81.12 ± 2.35	83.60 ± 3.59	81.40 ± 3.35	86.40 ± 1.96

Table 10.2.4..j. Characteristics of semen collected on day 84 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT	HYCG	85.15 ± 3.89	74.45 ± 1.34	62.40 ± 7.47	52.50 ± 4.79	30.88 ± 8.21	14.45 ± 7.29
(%)	CEQY	78.68 ± 3.44	73.93 ± 1.90	73.80 ± 3.31	60.55 ± 7.07	59.15 ± 6.00	54.85 ± 6.13
PROG	HYCG	57.78 ± 6.31	53.13 ± 2.28	37.50 ± 9.87	34.50 ± 3.59	18.75 ± 7.81	9.50 ± 5.20
(%)	CEQY	54.05 ± 6.26	47.80 ± 0.39	46.75 ± 5.27	38.75 ± 3.17	37.75 ± 5.72	34.75 ± 5.57
RAPID	HYCG	69.57 ± 6.71	59.17 ± 2.70	43.30 ± 10.37	37.00 ± 4.02	20.00 ± 7.78	10.00 ± 5.20
(%)	CEQY	65.87 ± 6.17	55.67 ± 0.76	52.75 ± 5.74	43.00 ± 4.14	42.25 ± 6.90	37.50 ± 6.60
MEDIUM	HYCG	15.63 ± 3.44	15.27 ± 1.51	19.20 ± 3.01	15.50 ± 0.70	11.07 ± 1.42	4.40 ± 2.08
(%)	CEQY	12.80 ± 2.99	18.23 ± 1.53	20.85 ± 2.65	17.60 ± 3.01	17.15 ± 2.27	17.50 ± 1.15
SLOW	HYCG	0.35 ± 0.16	0.85 ± 0.21	1.00 ± 0.32	1.62 ± 0.37	1.37 ± 0.83	0.52 ± 0.22
(%)	CEQY	0.50 ± 0.26	0.60 ± 0.15	0.77 ± 0.14	0.95 ± 0.27	2.02 ± 1.72	1.00 ± 0.36
LIVE	HYCG	81.50 ± 6.00	65.50 ± 12.50	71.50 ± 8.50	55.50 ± 2.50	51.75 ± 7.75	33.00 ± 15.50
(%)	CEQY	80.25 ± 6.25	69.75 ± 12.25	62.00 ± 8.50	58.00 ± 2.00	51.75 ± 1.25	40.75 ± 0.25
DEAD	HYCG	18.50 ± 6.00	34.50 ± 12.50	28.50 ± 8.50	44.50 ± 2.50	48.25 ± 7.75	67.00 ± 15.50
(%)	CEQY	19.75 ± 6.25	30.25 ± 12.25	38.00 ± 8.50	42.00 ± 2.00	48.25 ± 1.25	59.25 ± 0.25
NORMAL	HYCG	91.50 ± 0.50	73.75 ± 2.25	87.00 ± 5.00	73.50 ± 2.50	95.25 ± 0.75	76.00 ± 1.00
(%)	CEQY	92.75 ± 0.75	81.75 ± 0.25	92.00 ± 1.00	74.50 ± 0.00	91.75 ± 2.25	77.75 ± 0.25

Table 10.2.4..j. (continued). Characteristics of semen collected on day 84 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD (%)	HYCG	1.00 ± 0.50	4.50 ± 4.00	0.25 ± 0.25	5.75 ± 5.25	0.25 ± 0.25	6.75 ± 5.75
	CEQY	0.50 ± 0.50	3.00 ± 2.00	1.25 ± 1.25	5.25 ± 4.75	0.50 ± 0.50	2.50 ± 2.00
COILED TAIL (%)	HYCG	5.00 ± 1.50	18.00 ± 5.50	11.00 ± 4.50	18.25 ± 2.25	3.00 ± 1.00	14.50 ± 3.50
	CEQY	5.25 ± 0.75	12.50 ± 1.50	4.00 ± 1.50	17.75 ± 6.25	6.50 ± 2.50	15.25 ± 3.75
BENT TAIL (%)	HYCG	2.50 ± 0.50	3.75 ± 0.75	1.75 ± 0.25	2.50 ± 0.50	1.50 ± 0.00	2.75 ± 1.25
	CEQY	1.50 ± 1.00	2.75 ± 0.75	2.75 ± 1.25	2.50 ± 1.50	1.25 ± 0.75	4.50 ± 1.50
ALH (µm)	HYCG	8.33 ± 0.21	6.93 ± 0.30	6.32 ± 0.29	5.60 ± 0.67	4.50 ± 0.35	2.87 ± 0.46
	CEQY	8.52 ± 0.19	7.05 ± 0.59	5.85 ± 0.33	6.55 ± 0.51	6.37 ± 0.36	5.10 ± 0.80
LIN (%)	HYCG	67.72 ± 2.04	75.60 ± 1.31	69.80 ± 4.94	78.00 ± 2.12	73.72 ± 6.21	80.12 ± 10.60
	CEQY	67.43 ± 2.20	71.23 ± 1.21	75.12 ± 2.38	74.72 ± 2.64	73.35 ± 0.15	79.18 ± 2.84
VAP (µm/s)	HYCG	150.80 ± 7.80	146.70 ± 5.65	116.00 ± 10.93	119.00 ± 4.59	98.57 ± 11.60	104.80 ± 11.90
	CEQY	156.40 ± 10.32	137.12 ± 5.34	118.90 ± 7.11	130.90 ± 4.46	125.70 ± 7.71	119.32 ± 8.95
VSL (µm/s)	HYCG	124.70 ± 8.07	129.70 ± 5.35	97.40 ± 12.91	107.00 ± 2.74	85.00 ± 13.50	94.12 ± 18.50
	CEQY	128.48 ± 10.83	116.50 ± 4.49	103.10 ± 7.56	114.10 ± 4.99	109.55 ± 6.92	107.18 ± 6.12
VCL (µm/s)	HYCG	177.40 ± 7.83	165.90 ± 5.73	137.00 ± 6.55	133.00 ± 7.46	113.70 ± 8.59	120.30 ± 1.35
	CEQY	184.92 ± 9.74	158.37 ± 6.10	134.70 ± 5.99	147.60 ± 6.42	144.05 ± 8.94	132.12 ± 11.81
STR (%)	HYCG	78.47 ± 1.67	84.45 ± 0.96	80.30 ± 3.25	86.60 ± 1.18	83.03 ± 4.14	87.00 ± 7.70
	CEQY	78.15 ± 1.90	81.18 ± 0.77	83.55 ± 1.50	83.90 ± 2.03	83.45 ± 0.45	87.35 ± 1.89

Table 10.2.4..k. Characteristics of semen collected on day 105 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
MOT (%)	HYCG	86.10 ± 2.41	89.55 ± 1.06	79.15 ± 2.67	38.68 ± 15.18	25.55 ± 13.93	6.22 ± 3.80
	CEQY	85.07 ± 1.53	89.28 ± 0.70	79.20 ± 1.50	68.95 ± 8.56	63.67 ± 2.37	61.03 ± 4.12
PROG (%)	HYCG	67.47 ± 2.06	59.10 ± 4.92	56.50 ± 1.62	20.68 ± 9.94	10.00 ± 5.70	3.92 ± 2.42
	CEQY	65.28 ± 1.17	63.55 ± 2.59	60.33 ± 2.55	41.47 ± 7.58	46.55 ± 2.74	36.33 ± 5.29
RAPID (%)	HYCG	77.83 ± 1.82	72.85 ± 5.59	61.60 ± 2.41	23.27 ± 11.31	10.72 ± 6.11	4.00 ± 2.48
	CEQY	77.18 ± 1.51	78.43 ± 0.85	66.25 ± 1.88	46.60 ± 9.12	49.73 ± 2.86	39.00 ± 5.58
MEDIUM (%)	HYCG	8.23 ± 0.99	14.10 ± 2.37	17.53 ± 0.47	15.40 ± 4.01	14.80 ± 7.86	2.20 ± 1.32
	CEQY	7.90 ± 0.42	10.85 ± 1.14	13.00 ± 1.36	22.35 ± 1.29	13.95 ± 1.40	22.05 ± 2.63
SLOW (%)	HYCG	0.13 ± 0.08	0.30 ± 0.15	1.35 ± 0.32	1.05 ± 0.50	1.62 ± 0.75	0.90 ± 0.56
	CEQY	0.45 ± 0.21	0.30 ± 0.13	0.70 ± 0.34	1.12 ± 0.56	1.50 ± 0.27	1.35 ± 0.39
LIVE (%)	HYCG	83.00 ± 1.50	58.50 ± 3.50	80.75 ± 1.25	62.00 ± 4.00	63.25 ± 6.75	46.00 ± 7.50
	CEQY	84.25 ± 6.75	57.00 ± 1.50	53.75 ± 9.75	56.75 ± 5.25	55.25 ± 6.25	63.50 ± 10.50
DEAD (%)	HYCG	17.00 ± 1.50	41.50 ± 3.50	19.25 ± 1.25	38.00 ± 4.00	36.75 ± 6.75	54.00 ± 7.50
	CEQY	15.75 ± 6.75	43.00 ± 1.50	46.25 ± 9.75	43.25 ± 5.25	44.75 ± 6.25	36.50 ± 10.50
NORMAL (%)	HYCG	95.75 ± 0.25	84.75 ± 1.25	89.00 ± 5.00	82.25 ± 0.75	88.25 ± 0.75	81.25 ± 7.75
	CEQY	93.00 ± 0.00	81.50 ± 5.50	92.25 ± 2.75	85.75 ± 2.75	91.75 ± 0.75	69.00 ± 6.50

Table 10.2.4..k. (continued). Characteristics of semen collected on day 105 from control and 12h/day scrotally insulated rams and diluted in hen yolk citrate glucose (HYCG) or coconut extract quail yolk (CEQY) diluents and stored at 5°C for up to 168h (Mean ± SEM, n = 4).

Semen characteristic	Diluent	Time of storage (h) at 5°C					
		0		96		168	
		Control	Heated	Control	Heated	Control	Heated
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
LOOSE HEAD (%)	HYCG	0.25 ± 0.25	4.50 ± 3.50	0.50 ± 0.00	1.50 ± 0.50	1.75 ± 1.75	1.50 ± 0.50
	CEQY	1.00 ± 0.50	2.25 ± 1.25	0.25 ± 0.25	2.75 ± 0.25	0.50 ± 0.00	2.50 ± 1.00
COILED TAIL (%)	HYCG	3.25 ± 0.25	10.00 ± 4.00	8.00 ± 4.00	13.75 ± 1.25	7.50 ± 1.00	14.25 ± 6.25
	CEQY	4.50 ± 0.00	14.25 ± 7.25	5.00 ± 2.00	9.25 ± 1.75	5.75 ± 1.25	21.25 ± 5.75
BENT TAIL (%)	HYCG	0.75 ± 0.25	0.75 ± 0.75	2.50 ± 1.00	2.50 ± 0.00	2.50 ± 2.00	3.00 ± 1.00
	CEQY	1.50 ± 0.50	2.00 ± 0.50	2.50 ± 0.50	2.25 ± 1.25	2.00 ± 0.50	7.25 ± 1.75
ALH (µm)	HYCG	8.30 ± 0.15	7.65 ± 0.31	5.40 ± 0.19	5.75 ± 0.59	4.40 ± 0.27	2.37 ± 1.37
	CEQY	8.28 ± 0.30	8.75 ± 0.12	5.82 ± 0.38	6.12 ± 0.29	5.37 ± 0.44	5.70 ± 0.20
LIN (%)	HYCG	74.68 ± 2.28	70.47 ± 0.93	79.70 ± 1.42	70.22 ± 1.00	64.32 ± 4.33	43.85 ± 25.34
	CEQY	74.28 ± 2.07	68.40 ± 1.64	80.95 ± 1.76	74.67 ± 1.29	79.82 ± 1.45	77.18 ± 2.67
VAP (µm/s)	HYCG	181.50 ± 7.43	117.10 ± 39.06	132.60 ± 2.27	99.03 ± 16.23	60.58 ± 14.67	59.43 ± 34.36
	CEQY	186.40 ± 4.46	167.40 ± 4.54	143.50 ± 3.80	125.20 ± 2.07	134.50 ± 5.77	118.00 ± 5.15
VSL (µm/s)	HYCG	157.40 ± 8.83	124.80 ± 4.91	118.50 ± 3.02	85.75 ± 13.97	52.25 ± 13.36	56.92 ± 32.96
	CEQY	160.30 ± 6.35	138.10 ± 5.92	128.40 ± 3.50	110.30 ± 0.91	120.10 ± 4.72	105.90 ± 6.21
VCL (µm/s)	HYCG	205.70 ± 6.67	173.50 ± 7.71	143.80 ± 1.53	116.30 ± 16.83	76.45 ± 14.01	64.12 ± 37.03
	CEQY	211.80 ± 3.07	196.30 ± 3.96	156.10 ± 4.33	142.70 ± 2.24	147.70 ± 6.24	132.30 ± 3.26
STR (%)	HYCG	83.45 ± 1.83	80.12 ± 1.16	86.37 ± 1.07	82.30 ± 1.63	81.32 ± 1.76	47.33 ± 27.33
	CEQY	83.15 ± 1.58	78.87 ± 1.49	87.50 ± 1.14	84.10 ± 0.79	86.75 ± 1.23	86.43 ± 1.51