PUBERTAL DEVELOPMENT IN THE MERINO RAM LAMBS AND IMMUNIZATION AGAINST OESTROGENS.

by

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This thesis is dedicated to the memory of my mother,

Edith Pelletier-Auclair

(1925-1990)

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DECLARATION

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

I consent to this thesis being made available for photocopying and loan if accepted for the award of the degree.

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SUMMARY

Several lines of evidence now support the hypothesis that oestrogens regulate many processes of the male reproductive system under physiological conditions.

This thesis presents investigations on pubertal development in South Australian Merino ram lambs and examines the effect of active and passive immunizations against oestradiol-17ß or oestrone on testicular maturation.

All ram lambs were weaned two weeks before the start of the experiment. They were kept indoors, in large pens, under constant lighting (12L:12D) and were fed with commercial sheep pellets and lucerne hay. Each experiment on pubertal development started at 10 or 14 weeks of age and lasted sixteen weeks.

A progressive increase in body weight and testicular volume occurred in all ram lambs studied. Mean LH level tended to decrease as the animals pass through puberty while no distinctive variations in FSH and PRL secretions were noticed. Pituitary responsivess to a GnRH challenge decreased between 14 and 30 weeks of age and probably reflected increasing feedback by gonadal steroids as the steroidogenic activity of the testis increased

Indeed, mean plasma testosterone level as well as testosterone secretion following a hCG challenge increased markedly as the animals matured. Total testicular blood plasma flow (TTBPF) when expressed per unit weight of testis (µl/g/min) was shown to decrease as the testis grows, although total blood flow per testis (ml/min) is increasing. Subcutaneous scrotal temperature seemed to decrease as testicular size increases. Most of the ram lambs had achieved puberty between 22 and 26 weeks of age as confirmed by the presence of spermatozoa in the seminiferous tubules.

Early active immunization against circulating oestradiol-17ß led to an important increase in gonadotropin concentrations (LH and FSH) and tended to improve the rate of testicular growth until 27 weeks of age, however, testicular volume and weight were comparable between control and E_2-immunized ram lambs at 30 weeks of age. Detrimental effects have been observed in some E_2-immunized ram lambs. For instance, we have observed a steep decline in testicular size towards the end of the experiment, presence of large vacuoles within the seminiferous

epithelium and, in one lamb, nearly complete absence of germ cells at 30 weeks of age. TTBPF per testis (ml/min) or per unit weight of testis (µl/g/min)] was more elevated in control than in E_2-immunized lambs. The steroidogenic function of the testis was remarkably enhanced in the E_2-immunized lambs as reflected by the high plasma testosterone concentrations, however, body weights were not improved in these lambs (no anabolic effect).

Testicular biopsies have been taken at 22 and 26 weeks of age in some of these ram lambs. This procedure did not seem to affect significantly subsequent testicular development, however, testosterone secretion measured at the end of the experiment (at 30 weeks of age) was significantly reduced in these ram lambs.

In other experiments, some well-characterized antibodies directed against oestradiol-17ß or oestrone (purified IgG or complete antiserum, showing high specificity and high affinity *in vitro*) have been injected to intact ram lambs during pubertal development (10 or 14 week-duration) or to oestradiol-implanted castrated ram lambs for a short-term period (1 or 2 weeks). Unfortunately, no difference was found between the control and the passively immunized intact ram lambs, and only a slight reduction of the oestradiol effect on gonadotropin secretion have been observed in the wethers passively immunized against oestradiol. This lack of effect may have been due to an insufficient amount of antibodies injected into the animals (low titre) and/or incomplete neutralization of the hormone (e.g. relatively low affinity *in vivo*).

In conjunction with this work on puberty, this thesis includes a one year duration experiment using adult Merino rams actively immunized against testosterone. We have demonstrated that the persistent increment in gonadotropin and testosterone secretions observed in (T-)immunized rams are not sufficient to maintain improved spermatogenic function throughout the year. Furthermore, we have shown that this treatment could lead to a reduction in epididymis weight after twelve months of immunization as well as a reduction in the number of mount culminating in ejaculation performed during a ten-minutes libido trial done on various occasions during the experiment.

PUBLICATIONS

Aspects of the work presented in this thesis have been reported elsewhere:

Abstracts

Auclair, D., Sowerbutts, S.F. and Setchell, B.P. (1993). Active immunization against oestradiol-17ß in developing ram lambs can have a marked effects on the structures and functions of the testis. Proc. Aust. Soc. Reprod. Biol. 25: abstract (submitted), Aug. 23-25 1993, University of Otago, N.Z.

Auclair, D., Sowerbutts, S.F. and Setchell, B.P. (1992). Active immunisation against testosterone in adult Merino rams: A one year-duration study. Proc. Aust. Soc. Reprod. Biol. 24: abstract 83, Sept. 28-30, 1992, University of Adelaide, S.A.

Auclair, D., Sowerbutts, S.F. and Setchell, B.P. (1991). Total testicular blood flow decrease during pubertal development in Merino lambs. Proc. of the 11th North American Testis Workshop, abstract 85, April 24-27, 1991, Montréal, Québec.

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Paper

Setchell, B.P., Auclair, D., Maddocks, S., Mieusset, R. (1991). Perméabilité vasculaire et débit sanguin testiculaire. Contraception, Fertilité, Sexualité, 19, 725-732.