Havemeyer Foundation
Monograph Series No. 17

Proceedings of a Workshop on

COMPARATIVE PLACENTOLOGY

21st – 24th April 2005
Victoria, Canada

Editors: P. D. Sibbons and J. F. Wade

Havemeyer Foundation
Monograph Series No. 17

Proceedings of a Workshop on

COMPARATIVE PLACENTOLOGY

21st – 24th April 2005
Victoria, Canada

Editors: P. Sibbons and J. F. Wade

Suites 3 & 4, 8 Kings Court, Willie Snaith Road, Newmarket, Suffolk CB8 7SG, UK

First published 2005

ISSN 1472-3158

Published by **R & W Communications**

Printed in Great Britain by Quality Print Services (Anglia) Limited

CONTENTS

SESSION 5

EDITORS' FOREWORD

Understanding the complex homeostatic condition of mammalian physiology is the cornerstone of the ability to provide treatments for those physiological defects presenting as disease. Recent advances in epidemiological research have led to the understanding that many of the detrimental conditions seen post-natally and through the whole life span can be associated with events during gestation and the state of the individual at birth. The placenta, together with the maternal and foetal influences on it, is the sole organ responsible for the wellbeing and appropriate development of the attached fetus. It is important, then, that all aspects of the placenta, origin, development, activity, types and modes of failure, are researched and documented so that this knowledge can be used to develop effective management of recognised deficiencies.

The tenuous beginning of a new placenta and the subsequent rapid development of a uniquely decidual organ responsible for the entire wellbeing of the associated foetus or fetuses involves a myriad of structural, chemical and physiological organogenic adjustments to ensure adequate placentogenesis. All of these developmental steps are open to influence from environmental, genetic, single and cumulative physiological insults which, if not adequately defended, can result in one or many organogenic changes. Although not all of these have detrimental sequelae, some can be catastrophic and others can induce subtle alterations in placental function resulting in ranges of morbidity in all ages of progeny from the pregnancy.

These changes are what placentology research strives to identify, model, manipulate and understand by multiple approaches from numerous technological platforms. High on the list of these approaches is the comparison of the many types and functions of placentae from numerous species operating in different modes to achieve the same physiological support systems. Detailed examination of the natural development of placentae across species and the experimental modelling and manipulation of placentogensis in laboratory animals has provided significant data regarding compromising events, their identification, and potential pharmaceutical and other preventive intervention.

The data presented in this Monograph represents the very latest information available with regard to comparative placentology. The range of species currently under study, from mouse to elephant, is a gauge of the interest in, and relevance of, this type of research and the discussion during the workshop from which this monograph is drawn reflects the passion with which placentology researchers approach their subject.

Based on presentations and discussion during the Workshop, assembled formally for this Monograph, several unique and important collaborative research projects were committed to. The data presented here, the discussion at the Workshop and the subsequent collaborative research have, and will continue to, advanced our knowledge of placentogenesis and placental pathogeneses across many species and will take us closer to being able to intervene with appropriate treatments during gestation to prevent subsequent post natal, detrimental conditions.

We are very grateful for the interest and foresight of Gene Pranzo and the support of the Havemeyer Foundation without which this important forum would not be possible.

Paul Sibbons
Jan Wade

HAVEMEYER SCIENTIFIC WORKSHOPS

1981	**First International Workshop on Lymphocyte Alloantigens of the Horse** October - New York City, USA *Organiser: Dr D. F. Antczak*
1982	**Second International Workshop on Lymphocyte Alloantigens of the Horse** October - Cornell University, Ithaca, New York, USA *Organiser: Dr D. F. Antczak*
1983	**Third International Workshop on Lymphocyte Alloantigens of the Horse** April - New Bolton Center, University of Pennsylvania, USA *Organiser: Dr D. F. Antczak*
1984	**First International Symposium on Equine Embryo Transfer** October - Cornell University, Ithaca, New York, USA *Organisers: Drs D. F. Antczak and W. R. Allen*
1985	**Fourth International Workshop on Lymphocyte Alloantigens of the Horse** October - University of Kentucky, USA *Organisers: Drs D. F. Antczak and E. Bailey*
1986	**Workshop on *Corynebacterium equi* Pneumonia of Foals** July - University of Guelph, Canada *Organiser: Dr J. F. Prescott*
1987	**Fifth International Workshop on Lymphocyte Alloantigens of the Horse** October - Louisiana State University, USA *Organisers: Drs D. F. Antczak and J. McClure*
1989	**Second International Symposium on Equine Embryo Transfer** February - Banff, Alberta, Canada *Organisers: Drs D. F. Antczak and W. R. Allen*
1990	**International Workshop on Equine Sarcoids** April - Interlaken, Switzerland *Organisers: Dr D. F. Antczak and Professor S. Lazary*
1992	**Workshop on Equine Neonatal Medicine** January - Naples, Florida *Organisers: Drs D. F. Antczak and P. D. Rossdale*

Third International Symposium on Equine Embryo Transfer
February - Buenos Aires, Argentina
Organisers: Drs D. F. Antczak, W. R. Allen, J. G. Oriol and R. Pashen

1995 **Equine Perinatology**
July - Cambridge, England
Organiser: Dr P. D. Rossdale

Second International Equine Leucocyte Antigen Workshop
July - Lake Tahoe, California, USA
Organisers: Drs D. F. Antczak, P. Lunn and M. Holmes

First International Workshop on Equine Gene Mapping
October - Lexington, Kentucky, USA
Organisers: Drs D. F. Antczak and E. Bailey

Erection and Ejaculation in the Human Male and Stallion: A Comparative Study
October - Mount Joy, Pennsylvania, USA
Organiser: Dr S. M. McDonnell

Bone Remodelling Workshop
October - Corcord, Massachusetts, USA
Organiser: Dr H. Seeherman

1997 **Second International Workshop on Equine Gene Mapping**
October - San Diego, California, USA
Organisers: Drs D. F. Antczak and E. Bailey

Maternal Recognition of Pregnancy in the Mare
January - Dominican Republic
Organisers: Drs W. R. Allen and T. A. E. Stout

Uterine Clearance
March - Gainesville, Florida, USA
Organiser: Dr M. M. LeBlanc

Trophoblast Differentiation
September - Edinburgh, Scotland
Organisers: Drs D. F. Antczak and F. Stewart

1998 **Third International Genome Workshop**
January - San Diego, California, USA
Organisers: Drs D. F. Antczak and E. Bailey

Third International Workshop on Perinatology: Genesis and Post Natal Consequences of Abnormal Intrauterine Developments: Comparative Aspects
February - Sydney, Australia
Organiser: Dr P. D. Rossdale

Horse Genomics and the Genetic Factors Affecting Race Horse Performance
March - Banbury Center, Cold Spring Harbor, New York, USA
Organisers: Drs D. F. Antczak, E. Bailey and J. Witkowski

Allergic Diseases of the Horse
April - Lipica, Slovenia
Organisers: Drs D. F. Antczak, S. Lazary and E. Marti

Equine Placentitis Workshop
October - Lexington, Kentucky, USA
Organisers: Drs D. F. Antczak, W. R. Allen and W. Zent

Septicemia II Workshop
November - Boston, Massachusetts, USA
Organiser: Dr M. R. Paradis

1999

Equine Genome Project
January - San Diego, California, USA
Organisers: Drs D. F. Antczak and E. Bailey

Third International Equine Genome Workshop
June - Uppsala, Sweden
Organisers: Drs D. F. Antczak, E. Bailey and K. Sandberg

Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza
August - Miami, Florida, USA
Organiser: Dr J. Mumford

European Equine Gamete Workshop
September - Lopuszna, Poland
Organisers: Drs W. R. Allen and M. Tischner

Fetomaternal Control of Pregnancy
November - Barbados, West Indies
Organisers: Drs T. Stout and W. R. Allen

2000

Equine Genome Project
January - San Diego, California, USA
Organisers: Drs D. F. Antczak and E. Bailey

Uterine Infections in Mares and Women: A Comparative Study
March - Naples, Florida, USA
Organiser: Dr M. M. LeBlanc

5th International Symposium on Equine Embryo Transfer
July - Saari, Finland
Organiser: Dr T. Katila

2001 **USDA International Plant & Animal Genome Conference**
January - San Diego, California

Equine Immunology in 2001
January - Santa Fe, New Mexico
Organiser: Dr D. P. Lunn

Asthma and Allergies II
April - Hungary
Organisers: S. Lazary and E. Marti

From Elephants to Aids
June - Port Douglas, Australia
Organiser: Professor W. R. Allen

International Equine Gene Mapping
July - Brisbane, Australia
Organiser: K. Bell

Second Meeting of the European Gamete Group (EEGG)
September - Loosdrecht, The Netherlands
Organiser: Dr T. A. E. Stout

Foal Septicemia III
October - Tufts University European Center, Talloires, France
Organiser: M. R. Paradis

Infectious Disease Programme for the Equine Industry and Veterinary Practitioners
October - Marilyn duPont Scott Medical Center, Morvan Park, Virginia, USA
Organisers: Drs J. A. Mumford and F. Fregin

From Epididymis to Embryo
October - Fairmont Hotel, New Orleans, USA
Organiser: Dr L. H-A. Morris

2002 **USDA International Plant & Animal Genome Conference**
January - San Diego, California

Comparative Neonatology/Perinatology
March - Palm Springs, California
Organiser: P. Sibbons

Stallion Behavior IV
June - Reykjavik, Iceland
Organisers: S. McDonell and D. Miller

Rhodococcus Equi II
July - Pullman, Washington
Organiser: J. Prescott

Equine Orthopaedic Infection
August - Dublin, Ireland
Organiser: E. Santschi

Inflammatory Airway Disease
September - Boston, USA
Organiser: Dr E. Robinson

2003 **USDA International Plant and Animal Genome Conference**
January - San Diego, California

Embryonic and Fetal Nutrition
May - Ravello, Italy
Organiser: S. Wilsher

Genomics and the Equine Immunity System
June - Ithaca, New York
Organiser: D. F. Antczak

Fifth International Gene Mapping Workshop
August - Kreuger Park, South Africa
Organiser: E. Baily and E. Vandyke

Equine Recurrent Laryngeal Neuropathy
September - Stratford-upon-Avon, UK
Organisers: P. Dixon and E. Robinson

Transporting Gametes and Embryos
October - Brewster, Massachusetts
Organiser: E. Squires

Third Meeting of the European Gamete Group (EEGG)
October - Pardubice, Czech Republic
Organiser: J. and Z. Müller

Nosocomial Infections and Biosecurity in Equine Hospitals
October - Lexington, USA
Organiser: F. Bain and J. Taub-Dargatz

2004 **USDA International Plant and Animal Genome Conference**
January - San Diego, California

Equine Viral Herpes Virus Workshop
June/July - Tuscany, Italy
Organiser: P. Lunn

Equine Embryo Transfer VI Workshop
August - Rio de Janiero, Brazil
Organiser: M. Alvarenga

Sporting Injuries in Horses and Man: A Comparative Approach
September - Lexington, USA
Organiser: E. J. L. Soulsby

Maternal Recognition of Pregnancy in the Mare III
November - Barbados, West Indies
Organiser: T. A. E. Stout

2005 **USDA International Plant and Animal Genome Conference**
January - San Diego, California
Organiser: J. Mickelson

Comparative Placentology
April - Victoria, Canada
Organiser: P. Sibbons

HAVEMEYER MONOGRAPH SERIES

The following are monographs available to date at a cost of £9.95 each.

Series No 1
PROCEEDINGS OF THE FIRST MEETING OF THE EUROPEAN EQUINE GAMETE GROUP (EEGG)
Editors: W. R. Allen and J. F. Wade
5th–8th September 1999
Lopuszna, Poland

Series No 2
PROCEEDINGS OF A WORKSHOP ON FETOMATERNAL CONTROL OF PREGNANCY
Editors: T. A. E. Stout and J. F. Wade
14th–16th November 1999
Barbados, West Indies

Series No 3
PROCEEDINGS OF THE 5TH INTERNATIONAL SYMPOSIUM ON EQUINE EMBRYO TRANSFER
Editors: T. Katila and J. F. Wade
6th–9th July 2000
Saari, Finland

Series No 4
PROCEEDINGS OF A WORKSHOP ON EQUINE IMMUNOLOGY IN 2001
Editors: D. P. Lunn and J. F. Wade
24th–28th January 2001
Santa Fe, New Mexico

Series No 5
PROCEEDINGS OF THE SECOND MEETING OF THE EUROPEAN GAMETE GROUP (EEGG)
Editors: T. A. E. Stout and J. F. Wade
26th–29th September 2001
Loosdrecht, The Netherlands

Series No 6
PROCEEDINGS OF A WORKSHOP ENTITLED FROM EPIDIDYMIS TO EMBRYO
Editors: L. H-A. Morris, L. Foster and J. F. Wade
18th–21st October 2001
New Orleans, USA

Series No 7
Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza
Editors: J. A. Mumford and J. F. Wade
3rd–5th August 1999
Crowne Plaza Hotel, Miami, Florida, USA
Series No 8

Proceedings of a Workshop on Comparative Neonatology/Perinatology
Editors: Dr P. Sibbons, L. Foster and J. F. Wade
13th–15th March 2002
Palm Springs, California, USA

Series No 9
Proceedings of a Workshop on Inflammatory Airway Disease: Defining the Syndrome
Editors: A. Hoffman, N. E. Robinson and J. F. Wade
30th September–3rd October 2002
Boston, USA

Series No 10
Proceedings of a Workshop on Embryonic and Fetal Nutrition
Editors: S. Wilsher and J. F. Wade
15th–18th May 2003
Ravello, Italy

Series No 11
Proceedings of a Workshop on Equine Recurrent Laryngeal Neuropathy
Editors: P. Dixon, E. Robinson and J. F. Wade
7th–10th September 2003
Stratford-upon-Avon, UK

Series No 12
Proceedings of a Workshop on Transporting Gametes and Embryos
Editors: E. Squires and J. F. Wade
2nd–5th October 2003
Brewster, Massachusetts, USA

Series No 13
Proceedings of the Third Meeting of the European Gamete Group (EEGG)
Editors: J. Müller, Z. Müller and J. F. Wade
12th–15th October 2003
Pardubice, Czech Republic

Series No 14

PROCEEDINGS OF THE 6TH INTERNATIONAL SYMPOSIUM ON EQUINE EMBRYO TRANSFER
Editors: M. Alvarenga and J. F. Wade
4th–6th August 2004
Rio de Janerio, Brazil

Series No 15

PROCEEDINGS OF A WORKSHOP ON SPORTING INJURIES IN HORSES AND MAN: A COMPARATIVE APPROACH
Editors: Lord Soulsby and J. F. Wade
23rd–25th September 2004
Lexington, USA

Series No 16

PROCEEDINGS OF A WORKSHOP ON MATERNAL RECOGNITION OF PREGNANCY IN THE MARE III
Editors: T. A. E. Stout and J. F. Wade
13th–16th November 2004
Barbados, West Indies

If you wish to order copies, please contact R & W Communications, Suites 3 & 4, 8 Kings Court, Willie Snaith Road, Newmarket, Suffolk CB8 7SG, UK, Tel: +44 1638 667600, Fax: +44 1638 667229, e-mail: info@rw-communications.co.uk

SESSION 1:

Chairman:
P. Sibbons

INTERACTIONS AND SUBSTITUTIONS BETWEEN THE YOLK SAC AND CHORIOALLANTOIC PLACENTAE ACROSS EUTHERIAN GROUPS

A. C. Enders

Department of Cell Biology and Human Anatomy, University of California, Davis, California 95616, USA

In Eutherian mammals the vessels of the allantois with or without the endodermal sac are used to form the major respiratory exchange region of the chorioallantoic placenta. As this placenta develops it often displaces the previously developed yolk sac placenta. In the horse for example a choriovitelline placenta is present alone for only a few days; subsequently most of the vascular portion is displaced into the exocelom by growth of the allantois as it forms the allantochorion (Allen 2004). To increase respiratory efficiency the vessels of the allantochorion are closely applied to the uterine epithelium and the trophoblast is thin, decreasing the diffusion distance between vascular systems but also tending to block uterine secretions. This competition for space between the 2 placental types tends to decrease the area available for histotroph uptake. The decrease is compensated in a variety of species by the development within the chorioallantoic placenta of specialised regions of cellular columnar trophoblast. Simple patches of columnar trophoblast cells are found in the placenta of the pangolin and in early stages of development in many artiodactyls and perissodactyls (Mossman 1987). In the pig some of the pouches are more elaborate and have branched villi of columnar trophoblast cells (Dantzer and Leiser 1993). The camelids, another group with a diffuse epitheliochorial placenta, have areolae similar to some of those of the pig (Abd-Elnaeim *et al.* 2003). In the prosimian family *Lorisidae*, pouches with branched villi project from the surface of the placenta and are surrounded by a layer of smooth muscle cells; these structures have been called chorionic vesicles (King 1993).

In a number of species there are areas of columnar trophoblast cells between the bases of the fetal villi. Such regions in the sheep and goat for example are involved in uptake of erythrocytes (Burton 1982). These regions and those in the cetaceans are in a position to ingest secretions and fragments of the tips of the maternal tissue, and consequently have a mixed phagocytic function and could therefore be considered heterophagic.

Typical areolae present as cups overlying the openings of uterine glands are also widespread within carnivores, having been reported in the brown bear, viverids and seals, despite the fact that these species also have specialised haemophagous regions. However there are also regions of columnar trophoblast cells at the tips of the villi, for example in the endotheliochorial placenta of the mink. These regions are in a position to ingest uterine secretions and products of maternal cell breakdown, but do not ordinarily ingest erythrocytes. In the endotheliochorial placenta of the elephant, the regions of erythrocyte ingestion are similar in position to those of the sheep and goat (Allen *et al.* 2003). In the emballonurid bats, yet another group with endotheliochorial placentation, the haemophagous region consists of folds of columnar trophoblast, again in a position to ingest both uterine products and released maternal blood.

Haemophagous regions also occur in a few species with haemochorial placentae. In the hyena these regions occur both at the margins of the haemochorial placenta and at the tips of the villi and ingest materials from the junctional zone, including maternal blood. In the tenrec *Echinops telfairi*, the haemophagous area begins as a region exposed to both glandular secretions and maternal blood, later becoming a solely haemophagous region (Carter *et al.* 2005).

Specialised areas of cellular trophoblast that are available to histotrophic uptake are present in

the chorioallantoic placentae of a wide variety of species. Such regions provide polarised cells for uptake, digestion and export of products from the uterus. This produces a limited region of individual cells that are exposed to maternal products and may have a limited functional life in accumulation of telolysosomes, pigment, etc. Many such regions are potentially heterophagic and relatively few are solely haemophagic.

REFERENCES

Abd-Elnaeim, M.M., Saber, A., Hassan, A., Abou-Elmagd, A., Klisch, K., Jones, C.J. and Leiser, R. (2003) Development of the areola in the early placenta of the one-humped camel (*Camelus dromedarius*): a light, scanning and transmission electron microscopical study. *Anat. Histol. Embryol.* **32,** 326-334.

Allen, W.R. (2004) Development and functions of the equine placenta. In: *Proc. Workshop on the Equine Placenta*, University Kentucky *Ag. Exp. Sta.*, pp 20-33.

Allen, W.R., Mathias, S., Wooding, F.B.P and van Aarde, R.J. (2003) Placentation in the African elephant (*Loxodonta africana*). II. Morphological aspects. *Placenta* **24,** 598-617.

Burton, G.J. (1982) Placental uptake of maternal erythrocytes: a comparative study. *Placenta* **3,** 407-434.

Carter, A.M., Blankenship, T.N., Kunzle, H. and Enders, A.C. (2005) Development of the haemophagous region and labyrinth of the placenta of the tenrec, *Echinops telfairi*. *Placenta* **26,** 251-261.

Dantzer, V. and Leiser, R. (1993) Microvasculature of regular and irregular areolae of the areola-gland subunit of the porcine placenta: structural and functional aspects. *Anat. Embryol.* **188,** 257-267.

King, B.F. (1993) Development and structure of the placenta and fetal membranes of nonhuman primates. *J. Exp. Zool.* **266,** 528-540.

Mossman, H.W. (1987) Vertebrate Fetal Membranes. Rutgers Press, New Brunswick NJ.

UNRAVELLING HUMAN EARLY PREGNANCY: A COMPARATIVE APPROACH

G. J. Burton and E. Jauniaux*

*Department of Anatomy, University of Cambridge and *Academic Department of Obstetrics and Gynaecology, Royal Free and University College London, UK*

The interstitial form of implantation displayed by the human conceptus is almost unique, shared only with the great apes (Mossman 1987). At present the evolutionary advantage this mode of implantation confers is unknown, but it must be considerable because such early and intimate interactions with the maternal endometrium present the conceptus with special challenges. There is mounting evidence that failure of the trophoblastic tissues to meet these challenges underlies the pathogenesis of complications of pregnancy almost unique to the human, such as spontaneous miscarriage and pre-eclampsia.

Recent data suggest that despite their considerable morphological differences the placentae of man and domestic species function in a similar physiological fashion during early pregnancy. During the period of organogenesis, which is characterised by rapid cell proliferation and differentiation, the embryo is particularly susceptible to external agents such as drugs and chemicals. Many teratogenic substances exert their detrimental effects through increased intracellular generation of free radicals (Nicol *et al.* 2000), which may cause direct oxidative damage to the DNA, leading to mutations, or disrupt signalling pathways. Since antioxidant defences cannot provide complete protection against free radical attack, especially against the hydroxyl ion whose reactions are diffusion limited, the best strategy may be to limit the generation of radicals as far as possible. It is notable that in many mammalian species the conceptus remains free within the uterine cavity during the early stages of development, supported by histiotrophic nutrition derived from the uterine glands, the so-called 'uterine milk'. Once this phase is complete, however, haemotrophic exchange becomes dominant in order to meet the increasing metabolic demands of the fetus. In the majority of species this is facilitated by the development of vascularised villous interactions between the maternal and fetal tissues. Often, these 2 forms of nutrition continue in parallel in separate areas of the placental membranes, such as the areolae and non-glandular areas of the pig placenta or the inverted yolk sac and the labyrinth of the mouse (Mossman 1987).

When viewed in these terms the highly invasive form of implantation displayed by the human conceptus appears potentially harmful, for the early onset of haemotrophic exchange usually attributed to it in textbooks of embryology would seem to place the embryo at risk of free radical mediated teratogenesis. However, the recent realisation that the maternal arterial circulation to the placenta is not fully established until towards the end of the first trimester when organogenesis is complete (Hustin and Schaaps 1987; Jauniaux *et al.* 2000), and that prior to this the conceptus is supported by secretions from the uterine glands (Hempstock *et al.* 2004), aligns the human placenta functionally with those of most other mammals.

Despite this apparent equivalence, the placental membranes of the human remain unique is one very important aspect, namely that the same placental structure operates successively for histiotrophic and haemotrophic nutrition. Thus, the villous tree is first bathed by uterine secretions and then by maternal blood. This necessitates that the trophoblast and stromal tissues adapt to a major rise in oxygen concentration at the start of the second trimester (Jauniaux *et al.* 2000). Oxygen can be highly damaging to cells, stimulating both necrosis and apoptosis through oxidative stress. The first trimester syncytiotrophoblast is particularly susceptible due

to low levels of antioxidant defences, and exposure to ambient concentrations of oxygen *in vitro* rapidly leads to disruption of mitochondrial architecture and degeneration (Watson *et al.* 1998). A similar phenomenon occurs during onset of the maternal circulation *in vivo*, for recent data show that onset of the maternal circulation is normally a progressive phenomenon, starting in the periphery of the placenta and extending to the central region (Jauniaux *et al.* 2003). Villi in the periphery display higher levels of oxidative stress than their central counterparts, and this is associated with extensive degeneration of the syncytiotrophoblast. The villi are also avascular, consistent with downregulation of hypoxia-regulated growth factors such as vascular endothelial growth factor (Jauniaux *et al.* 2003). We speculate that ultimately these changes lead to regression of the villi over the superficial pole of the chorionic sac, resulting in the formation of the chorion laeve which is essential for the vaginal birth process at the end of a human pregnancy.

Villous regression appears to be another aspect of human placentation that is unique, perhaps for the great apes. In other species where the villi are not uniformly distributed over the chorionic sac this is the result of differential endometrial receptivity to villous development. Thus, in the ruminant the villous cotyledons only form opposite the maternal caruncles, and in the rhesus monkey the 2 placental discs develop where the blastocyst impinges on the opposite walls of the uterus (Mossman 1987). The regression process has several potential adverse consequences for a pregnancy. Firstly, it will release apoptotic or degenerative products into the maternal circulation that may play a role in priming the maternal inflammatory response that characterises pregnancy (Redman and Sargent 2000). Secondly, it must be carefully co-ordinated because overwhelming trophoblast damage may jeopardise continuance of the pregnancy. Thus, in the majority of cases of missed miscarriage onset of the maternal circulation is both premature and disorganised, occurring throughout the whole placenta. As a result, there is excessive oxidative stress and morphological evidence of extensive degeneration of the syncytiotrophoblast (Hempstock *et al.* 2003; Jauniaux *et al.* 2003). When the placental tissues are retained *in utero* for any length of time under these increased levels of oxygenation regression of the fetal vasculature occurs. There is also a loss of cells from the stromal core due to a combination of increased apoptosis and decreased proliferation. Ultimately, the villi regress in an equivalent fashion to that seen during formation of the chorion laeve.

Oxygen is therefore a powerful agent in remodelling the human villous tree during early pregnancy. Some remodelling is essential, due to the initial formation of villi over the entire chorionic sac. This again may relate to the interstitial mode of implantation bringing the complete surface of the sac into contact with the endometrium, but it is crucial that an extensive part of the sac becomes avillous in order to avoid massive haemorrhage at the time of delivery. Excessive villous regression at this stage of pregnancy may, however, lead to restricted placental development, and to the formation of abnormal placental shapes and cord insertions that are frequently associated with intrauterine growth restriction. A key process in this respect is the regulation of the onset of the maternal circulation. During early pregnancy the terminal portions of the maternal spiral arteries are plugged by aggregations of invading endovascular extravillous trophoblast cells (Hustin and Schaaps 1987), and it is only when these dissipate that free flow of blood into the intervillous space is established. The relative roles of endovascular and interstitial extravillous trophoblast in the physiological conversion of the spiral arteries is uncertain (Kam *et al.* 1999), and it may be that the endovascular population is of principal importance in the initial occlusion of the vessels than in inducing loss of the smooth muscle coat.

Correct physiological conversion of the spiral arteries is an essential pre requisite for successful pregnancy, and failure is associated with a high incidence of pre-eclampsia and intrauterine growth restriction (Brosens 1988). We speculate that increased vasoreactivity associated with incomplete conversion of the arteries exacerbates the intermittent perfusion of the intervillous space that occurs normally, leading to fluctuations in intraplacental oxygen concentration. Manipulations *in vitro* have confirmed that hypoxia-reoxygenation is a powerful inducer of apoptosis and proinflammatory cytokines in the human placenta (Hung *et al.* 2002; Hung *et al.* 2004), both of which may mediate the maternal endothelial cell activation that characterises the syndrome (Roberts and Hubel 1999).

The human placenta therefore appears to be particularly vulnerable to oxidative stress, both at

the transition between the first and second trimesters and in later pregnancy. Oxidative stress may act as a final common pathway for diverse factors acting on pregnancy outcome, for example concomitant metabolic diseases such as diabetes may compound the stress, or dietary lack of micronutrients or genetic polymorphisms may reduce the efficacy of antioxidant defences. In other species the placental tissues develop under a more constant oxygen tension; for example in the mouse the labyrinth is haemochorial from its earliest stages and does not undergo the transition seen in the human. Placental oxidative stress is therefore less of a problem, and this may account for why complications such as spontaneous miscarriage and pre-eclampsia are essentially a human phenomenon.

Acknowledgements

This work was supported by The Wellcome Trust.

References

Brosens, I.A. (1988) The utero-placental vessels at term-the distribution and extent of physiological changes. *Trophoblast Research* **3,** 61-67.

Hempstock, J., Cindrova-Davies, T., Jauniaux, E. and Burton, G.J. (2004) Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy; a morphological and immunohistochemical study. *Reprod. Biol. Endocrinol.* **2,** 58.

Hempstock, J., Jauniaux, E., Greenwold, N. and Burton, G.J. (2003) The contribution of placental oxidative stress to early pregnancy failure. *Human Path.* **34,** 1265-1275.

Hung, T.-H., Charnock-Jones, D.S., Skepper, J.N. and Burton, G.J. (2004) Secretion of tumour necrosis factor-α from human human placental tissues induced by hypoxia-reoxygenation causes endothelial cell activation *in vitro*: a potential mediator of the inflammatory response in pre-eclampsia. *Am. J. Path.* **164,** 1049-1061.

Hung, T.-H., Skepper, J.N., Charnock-Jones, D.S. and Burton, G.J. (2002) Hypoxia/reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in pre-eclampsia. *Circulation Research* **90,** 1274-1281.

Hustin, J. and Schaaps, J.P. (1987) Echographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy. *Am. J. Obstet. Gyn.* **157,** 162-168.

Jauniaux, E., Hempstock, J., Greenwold, N. and Burton, G.J. (2003) Trophoblastic oxidative stress in relation to temporal and regional differences in maternal placental blood flow in normal and abnormal early pregnancies. *Am. J. Path.* **162,** 115-125.

Jauniaux, E., Watson, A.L., Hempstock, J., Bao, Y.-P., Skepper, J.N. and Burton, G.J. (2000) Onset of maternal arterial bloodflow and placental oxidative stress; a possible factor in human early pregnancy failure. *Am. J. Path.* **157,** 2111-2122.

Kam, E.P.Y., Gardner, L., Loke, Y.W. and King, A. (1999) The role of trophoblast in the physiological change in decidual spiral arteries. *Human Reprod.* **14,** 2131-2138.

Mossman, H.W. (1987) Vertebrate fetal membranes:comparative ontogeny and morphology; evolution; phylogenetic significance; basic functions; research opportunities. *London: Macmillan* pp 383.

Nicol, C.J., Zielenski, J., Tsui, L.-C. and Wells, P.G. (2000) An embryoprotective role for glucose-6-phosphate dehydrogenase in developmental oxidative stress and chemical teratogenesis. *FASEB Journal* **14,** 111-127.

Redman, C.W.G. and Sargent, I.L. (2000) Placental debris, oxidative stress and pre-eclampsia. *Placenta* **21,** 597-602.

Roberts, J.M. and Hubel, C.A. (1999) Is oxidative stress the link in the two-stage model of pre-eclampsia? *Lancet* **354,** 788-789.

Watson, A.L., Skepper, J.N., Jauniaux, E. and Burton, G.J. (1998) Susceptibility of human placental syncytiotrophoblastic mitochondria to oxygen-mediated damage in relation to gestational age. *J. Clin. Endocrinol. Metabol.* **83,** 1697-1705.

LOCALISATION OF FIBROBLAST GROWTH FACTORS (FGF) IN PLACENTAE WITH DIFFERING DEGREES OF TROPHOBLAST INVASIVENESS

C. Pfarrer, G. Schuler*, W. R. Allen†, C. Ambrosio**, M. Abd-Elnaeim††, D. Schams*** and R. Leiser‡

*Department of Obstetrics and Gynecology, *Clinic for Obstetrics and Gynaecology of Large and Small Animals, and ‡Department of Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University Giessen, Germany; †Equine Fertility Unit, Mertoun Paddocks, Woodditton Road, Newmarket, Suffolk CB8 9BH, UK; **FMVZ, University of Sao Paulo, Brazil; ††Department of Anatomy and Histology, Assiut University, Egypt; ***Institute of Physiology, Technical University Munich-Weihenstephan, Germany*

Fibroblast growth factors (FGF) are important regulators of angiogenesis and differentiation of cells during embryogenesis and invasive haemochorial placentation (Powers *et al.* 2000). FGF exert their action through selective binding of low molecular weight (MW) isoforms to specific isoforms of high affinity receptors (Ornitz *et al.* 1996) or via nuclear translocation of high MW isoforms (Delrieu 2000). The aim of the present study was to compare the spatiotemporal distribution of specific FGF:receptor pairs in species with differing degrees of trophoblast invasiveness, the non-invasive epitheliochorial horse placenta, the invasive endotheliochorial placenta of dog and cat, and synepitheliochorial cow placentomes representing an intermediate type, due to moderately invasive trophoblast giant cells (TGC). FGF1 and FGF2, together with FGFR (various receptor isoforms) and FGFR2IIIc showing the highest affinity for FGF2 were chosen, due to their action on cells of mesenchymal origin. As stimulator of epithelial cells, FGF7 and its specific receptor FGFR2IIIb were selected.

FGF1, FGF2, FGF7 and FGF receptors (FGFR) were localised in placental tissue sections from horses, cows, cats and dogs in different gestational ages by immunohistochemistry. The presence of the corresponding mRNAs for FGF1, -2, -7, total FGFR, and FGFR2 isoforms IIIb and IIIc was confirmed by in situ hybridisation.

In the horse placenta, FGF1 occurred in the cytoplasm of endothelial cells, and along the feto-maternal contact interface (Fig 1a). FGF2 was expressed in microcotyledonary trophoblast and uterine epithelium as nuclear staining, whereas endothelial cells, and vascular smooth muscle cells all displayed cytoplasmic localisation of FGF2 (Fig 1b). Areolar trophoblast was either negative or showed a light cytoplasmic staining (Fig 1e). FGF7 immunoreactivity was observed in both cytoplasm and nucleus of predominantly trophoblast cells (Fig 1c). FGFR was localised almost exclusively in fetal and maternal endothelium (Fig 1d). Near term the total immunostaining of FGF1, -2, -7 and FGFR declined significantly, and the nuclear signal for FGF2 and FGF7 disappeared.

In bovine placentomes, FGF1 was shown in the cytoplasm of uterine epithelium and trophoblast cells, as well as in nuclei of maternal endothelium and smooth muscle cells (Fig 2a). FGF2 predominantly occurred as cytoplasmic staining in immature TGC, and as nuclear immunoreaction in endothelial cells of large maternal blood vessels (Fig 2b). FGF7 was detected in mononuclear trophoblast, immature TGC and endothelial cells (Fig 2c). A similar localisation was observed for FGFR (Fig 2d). Prior to parturition only few immature TGC remained to express FGFs. In contrast, the connective tissue of the maternal septa, especially blood vessel displayed a distinct staining.

In the placenta of the cat and dog, FGF1 was found in decidual-like cells and fetal endothelium (Fig 3a) as well as in the space between trophoblast cells and endothelial cells of maternal arteries at the fetal side of the placenta. FGF2 in early gestation was expressed in the trophoblast and epithelial cells of the glandular epithelium.

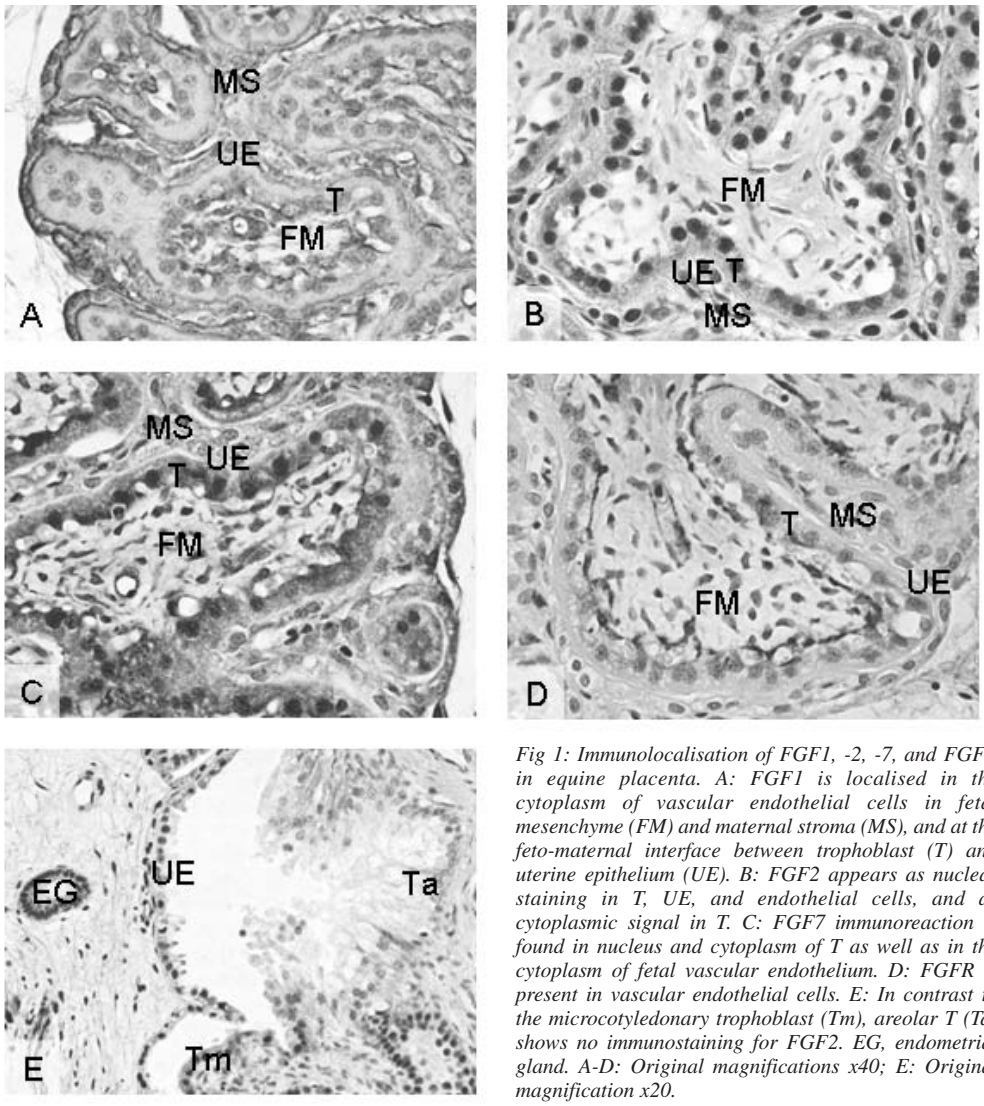

Fig 1: Immunolocalisation of FGF1, -2, -7, and FGFR in equine placenta. A: FGF1 is localised in the cytoplasm of vascular endothelial cells in fetal mesenchyme (FM) and maternal stroma (MS), and at the feto-maternal interface between trophoblast (T) and uterine epithelium (UE). B: FGF2 appears as nuclear staining in T, UE, and endothelial cells, and as cytoplasmic signal in T. C: FGF7 immunoreaction is found in nucleus and cytoplasm of T as well as in the cytoplasm of fetal vascular endothelium. D: FGFR is present in vascular endothelial cells. E: In contrast to the microcotyledonary trophoblast (Tm), areolar T (Ta) shows no immunostaining for FGF2. EG, endometrial gland. A-D: Original magnifications x40; E: Original magnification x20.

Some glandular epithelial cells displayed a distinct nuclear staining. Later in gestation, FGF2 appeared in trophoblast cells in areas of continuing invasion (Fig 3b). FGF7 was found in trophoblast cells, decidual-like cells and a population of circulating white blood cells (Fig 3c). FGFR immunostaining was observed in fetal endothelial cells and decidual-like cells (Fig 3d).

FGF1 and FGF2 are suggested to have different functions in uterine function and conceptus development during the peri-implantation period in pigs, because they are localised in different tissue compartments (Gupta *et al.* 1997). FGF7 stimulates proliferation and differentiation of conceptus trophectoderm in the pig (Ka *et al.* 2001). In the slightly invasive synepitheliochorial sheep placenta at late gestation, increasing FGF2 amounts in the cotyledon imply a role for FGF2 in the regulation of placental angiogenesis, growth and development (Zheng *et al.* 1997). Distinct and non-overlapping roles in ovine uterine functions were proposed for FGF7 and FGF10, since the corresponding mRNAs were localised in different stromal compartments around implantation and in early pregnancy (Chen *et al.* 2000). Thus FGF10 may stimulate epithelial proliferation and differentiation to support the establishment and

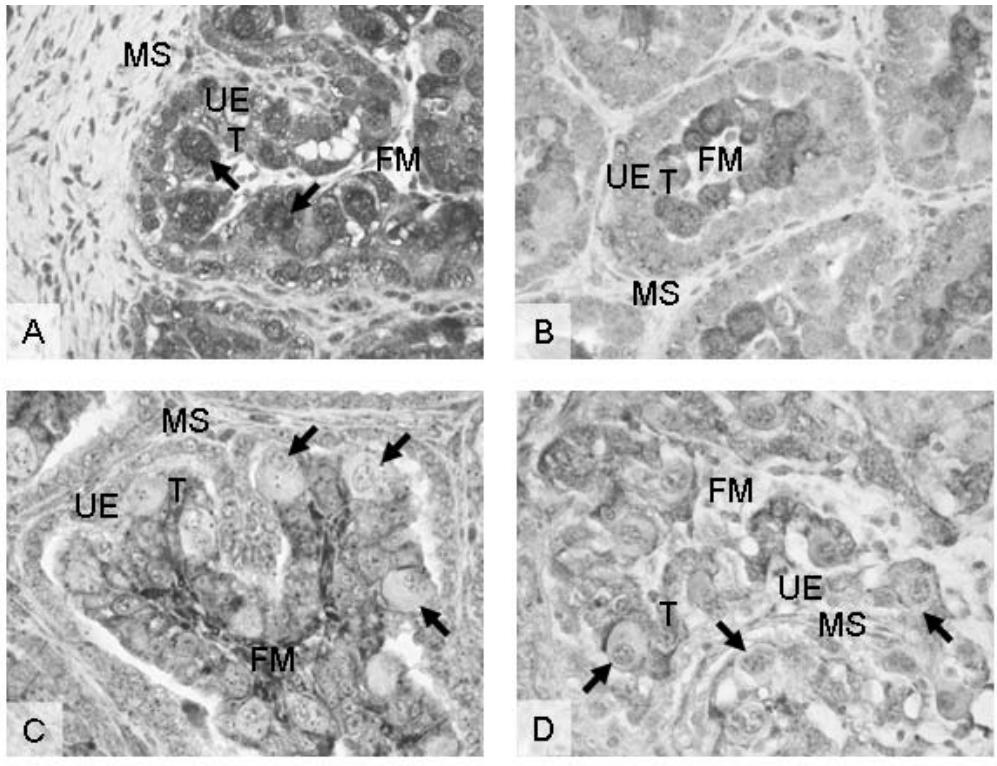

Fig 2: Immunolocalisation of FGF1, -2, -7, and FGFR in bovine placenta. A: FGF1 is expressed almost ubiquitously in the cytoplasm and/or the nuclei of placentomal cells, with the strongest signals found in immature trophoblast giant cells (TGC, arrows). B: FGF2 is localised in trophoblast cells (T), and to much lower extent in endothelial cells and uterine epithelium (UE). C: FGF7 is detected in the blood vessels within the fetal mesenchyme (FM) and in all T, but mature TGC (arrows). D: FGFR is present in endothelial cell of the FM and maternal stroma (MS) as well as in T at the base of the stem villi. Please note that mature TGC are negative (arrows). Original magnifications x40.

the maintenance of pregnancy in sheep (Chen *et al.* 2000). In endotheliochorial placental types FGF have not been described so far. In the human, representing invasive trophoblast placental types, FGF1 and FGF2 are present in the maternal and fetal side of the placenta, suggesting a role for FGF in angiogenesis during early pregnancy, whereas at term, FGF could be associated with more differentiated functions of the trophoblast (Ferriani *et al.* 1994). The nuclear localisation of FGF2 in dividing placental cells supports a role for basic FGF in cytotrophoblast proliferation (Ferriani *et al.* 1994*)*. Human trophoblast cells were to shown to have angiogenic properties *in vitro* (Hamai *et al.* 1998). Also, FGF2 was co-localised to heparan sulphate proteoglycan in specific areas and cytotrophoblast of the human placenta, indicating a functional role of both molecules during early gestation (Mullhauser *et al.* 1996).

High MW isoforms of FGF, and FGFR localised in cell nuclei are supposed to influence gene activities directly (Delrieu 2000). In contrast, low MW isoforms are thought to mediate 'classical' functions as cell proliferation, differentiation and growth, and thus may influence placental angiogenesis (Powers *et al.* 2000).

Due to the subcellular localisation, either in the cytoplasm or in the nucleus of the cells, it may be concluded that low and high MW isoforms are present in the placentae of all species examined and thus other different functions as differentiation and function of trophoblast cells could be supported. The localisation of FGFR in other cell types than vasculature, together with the presence of FGF in the same or neighbouring cells may reflect the correlation of FGF expression and trophoblast invasiveness. The changes near term in the mare or immediately prior to parturition in the cow are suggesting functions associated to

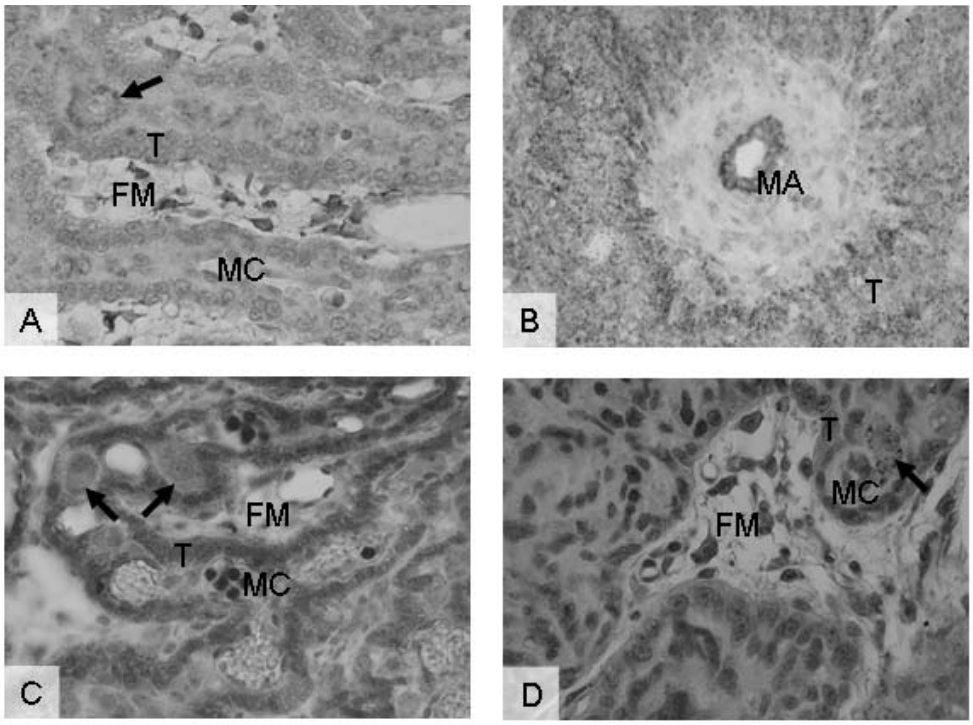

Fig 3: Immunolocalisation of FGF1, -2, -7, and FGFR in feline placenta. A: FGF1 appears in decidual-like cells (arrow), and in endothelial cells in the fetal mesenchyme (FM). In some locations maternal capillaries (MC) are also positive. B: FGF2 is observed in endothelial cell of large maternal arteries (MA). Surrounding invasive trophoblast (T) also show a strong immunoreaction for FGF2. C: FGF7 is found in T, decidual-like cells (arrows), and in white blood cells within maternal capillaries. D: FGFR is localised in decidual-like cells as well as in fetal endothelial cells. Original magnifications x40.

placental growth and function as well as for parturition and post partum tissue remodelling.

References

Chen, C., Spencer, T.E. and Bazer, F.W. (2000) Fibroblast growth factor-10: a stromal mediator of epithelial function in the ovine uterus. *Biol. Reprod.* **63,** 959-966.

Delrieu, I. (2000) The high molecular weight isoforms of basic fibroblast growth factor (FGF-2): an insight into an intracrine mechanism. *FEBS Lett* **468,** 6-10.

Ferriani, R.A., Ahmed, A., Sharkey, A. and Smith, S.K. (1994) Colocalisation of acidic and basic fibroblast growth factor (FGF) in human placenta and the cellular effects of bFGF in trophoblast cell line JEG-3. *Growth Factors* **10,** 259-268.

Gupta, A., Bazer, F.W. and Jaeger, L.A. (1997) Immunolocalisation of acidic and basic fibroblast growth factors in porcine uterine and conceptus tissues. *Biol. Reprod.* **56,** 1527-1536.

Hamai, Y., Fujii, T., Yamashita, T., Kozuma, S., Okai, T. and Taketani, Y. (1998) Evidence for basic fibroblast growth factor as a crucial angiogenic growth factor, released from human trophoblasts during early gestation. *Placenta* **19,** 149-155.

Ka, H., Jaeger, L.A., Johnson, G.A., Spencer, T.E. and Bazer, F.W. (2001) Keratinocyte growth factor is up-regulated by estrogen in the porcine uterine endometrium and functions in trophectoderm cell proliferation and differentiation. *Endocrinology* **142,** 2303-2310.

Mullhauser, J., Marzioni, D., Morroni, M., Vuckovic, M., Crescimanno, C. and Castellucci, M. (1996) Co-distribution of basic fibroblast growth factor and heparan sulfate proteoglycan in the growth zones of the human placenta. *Cell Tissue Res.* **285,** 101-107.

Ornitz, D.M., Xu, J., Colvin, J.S., McEwen, D.G., MacArthur, C.A., Coulier, F., Gao, G. and Goldfarb, M. (1996) Receptor specificity of the fibroblast growth factor family. *J. Biol. Chem.* **271,** 15292-15297.

Powers, C.J., McLeskey, S.W. and Wellstein, A. (2000) Fibroblast growth factors, their receptors and signaling. *Endocr. Relat. Cancer* **7,** 165-197.

Zheng, J., Vagnoni, K.E., Bird, I.M. and Magness, R.R. (1997) Expression of basic fibroblast growth factor, endothelial mitogenic activity, and angiotensin II type-1 receptors in the ovine placenta during the third trimester of pregnancy. *Biol. Reprod.* **56,** 1189-1197.

MODES OF RELEASE OF HUMAN PLACENTAL SYNCYTIOTROPHOBLAST

B. Huppertz

Department of Anatomy II, University Hospital RWTH, Aachen, Germany

During placentation in the horse, the equine trophoblast attaches to the uterine epithelium to generate an epitheliochorial barrier between maternal and fetal circulation within the placenta. The human placenta develops a haemochorial barrier, which is different to the situation in the horse. The uterine epithelium and stroma are invaded by fetal trophoblast finally eroding maternal uterine spiral arteries. This brings the epithelial cover of the placental villi, the villous trophoblast, into direct contact to maternal blood (Benirschke and Kaufmann 2000).

Trophoblast turnover

In the course of a normal turnover of an epithelium, proliferation of basal stem cells is followed by differentiation of post proliferative daughter cells. Turnover finalises in the release of old cells to the outside surface or lumen. In the case of the human placental trophoblast the outside of this epithelium is the maternal blood stream. Hence, any released corpuscle or molecule will be found in the maternal circulation and has to be removed by the mother. Impaired organisation of particle release from the human trophoblast will have adverse consequences for mother and baby.

In an attempt to differentiate histologically the various modes of release from the human villous trophoblast, a morphological investigation has been performed to compare the various structures on the apical surface of the syncytiotrophoblast that may be used to release trophoblast material (Kaufmann and Huppertz In press).

Syncytial sprouts

During early pregnancy until mid-gestation a large number of true syncytial sprouts can be found protruding from the apical surface of the syncytiotrophoblast. They are characterised by the presence of large euchromatic nuclei and represent sites of growth of the villous tree (Fig 1a). Hence these syncytial sprouts are not used for end stages of trophoblast turnover and will not be released into the maternal circulation.

Syncytial knots

Increasing in number during gestation, true syncytial knots are the sites of apoptotic extrusion of aged trophoblast material into the maternal circulation. These structures contain numerous nuclei in more or less advanced stages of late apoptosis displaying chromatin condensation and shrinkage. Syncytial knots are the normal sites of release of syncytial material and the classical endpoint of trophoblast turnover (Fig 1b). Beside the obvious morphology of nuclei, syncytial knots are characterised by the absence of most organelles and the integrity of the surrounding plasma membrane (Huppertz and Kingdom 2004). These corpuscular structures are released into the maternal circulation and are mostly trapped within the capillary network of the maternal lung. Here they are engulfed by alveolar macrophages and hence their number in peripheral blood is extremely low.

Tenney-Parker changes

In some pathological cases including early-onset intrauterine growth retardation and pre-eclampsia, a utero-placental hypoxia is anticipated, although hard data to prove low oxygen inside the placenta are still lacking. In these settings Tenney-Parker changes are observed (Tenney and Parker 1940). These changes are supposed to be syncytial knots

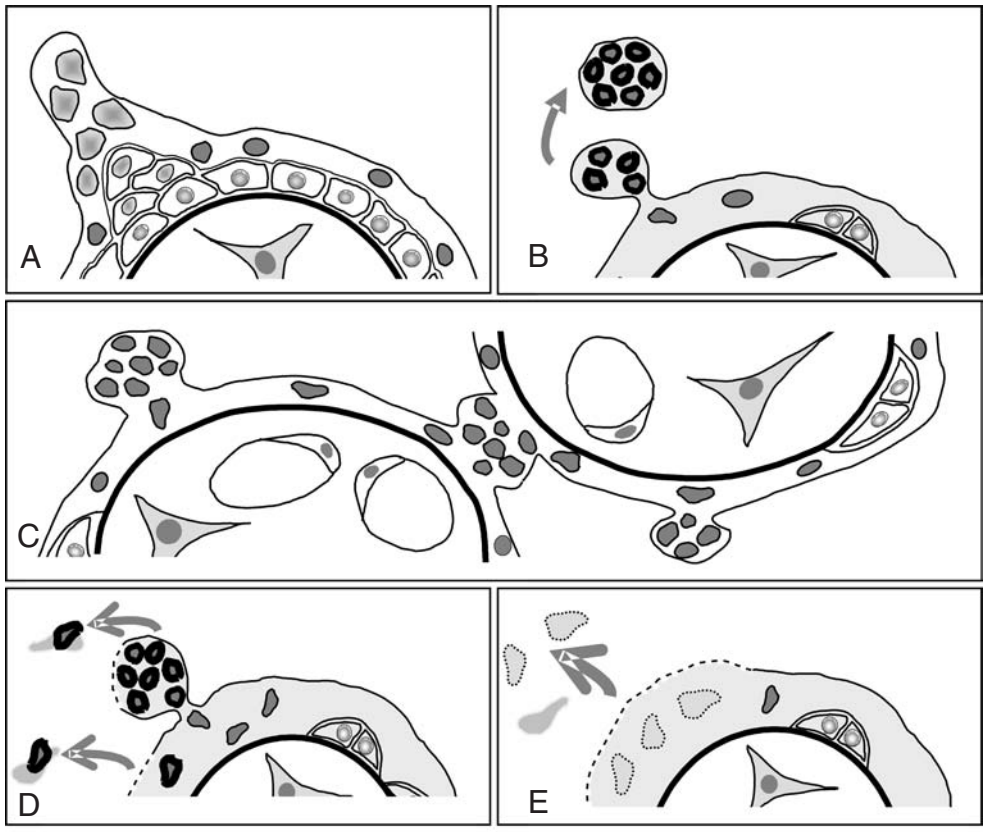

Fig 1: Modes of accumulating nuclei in the syncytiotrophoblast of the human placenta. (A) True syncytial sprouts. The scheme shows multiple layers of cytotrophoblast and the presence of newly fused and large nuclei inside the syncytiotrophoblast only in the sprout while the other nuclei are more condensed. (B) Syncytial knots. The scheme shows the normal end stage of villous trophoblast turnover. The final stage of turnover is the package and release of old material (mostly nuclei) into syncytial knots and extrusion of these structures into the maternal circulation (arrow). (C) Tenney-Parker changes. The scheme shows the seeming bridges between villi and the protrusions at the villous surfaces. They all resemble simply flat sections of a highly branched villous tree and contain morphologically normal syncytial nuclei. (D) Aponecrotic shedding. If the apoptosis cascade fails to develop until its very end and is interrupted and stopped, necrotic release of apoptotic material takes place (arrows). This aponecrotic material could induce inflammation in the mother. (E) Necrotic shedding. If apoptosis does not take place some sites of the syncytium undergo necrosis resulting in the release of necrotic material (arrow) and inducing an inflammatory response of the mother.

but rather they are sectional artifacts caused by flat sectioning of highly branched and distorted villi (Fig 1c). Tenney-Parker changes are characterised by normally structured nuclei inside protrusions of villi or even bridges between villi. Here a 2-dimensional evaluation of 3-dimensional changes generates pictures that mislead the observer. The reason for these changes is the following: In a low oxygen environment (or if the placenta does not correctly sense oxygen) the placental villi try to adapt to this environment by increasing villous surface. This is performed by elongation of placental capillaries and subsequently by increasing the number of branches of villi and this then will lead to the Tenney-Parker changes.

Other modes of release are known that are not associated with clearly defined morphological structures. These modes include aponecrotic and necrotic release of syncytial material.

APONECROSIS

Aponecrosis is defined as follows (Formigli *et al.* 2000): A cell starts the apoptosis cascade but fails

to finalise the cascade. This may be due to energy deprivation or any other cause resulting in downregulation of an energy-depending process such as apoptosis. Finally the already apoptotically cleaved and transformed cellular components are released from the dying cell by breakdown of the plasma membrane, ie necrosis. Inside the syncytiotrophoblast aponecrosis has recently been described and related to pre-eclampsia (Huppertz and Kingdom 2004). The characteristic of aponecrotic release from the syncytiotrophoblast is the presence of apoptotically processed fetal molecules (fragmented DNA, cleaved cytoskeletal proteins) inside the maternal circulation. During aponecrosis the processed molecules are not packed into apoptotic syncytial knots but rather they are released from the syncytiotrophoblast by local breakdown of the apical plasma membrane of the syncytium (Fig 1d). This mode of aponecrotic release seems to prevail in pre-eclampsia and may lead to the activation and damage of the maternal endothelium associated with this syndrome.

Necrosis

Necrotic release of syncytial material is due to any unphysiological damage such as mechanical stress, extreme shear forces or ischaemia. During necrosis the syncytiotrophoblast becomes oedematous; the nuclei increase in size and do not show the classical features of apoptotic nuclei such as condensed chromatin and shrinkage. Syncytial nuclei in necrotic sites may be aggregated or may be distributed evenly without any signs of apoptosis. Finally, breakdown and leakage of the syncytial apical plasma membrane will release cell-free molecules into the maternal circulation (Fig 1e). Now the released molecules do not show features of an organised cleavage, opposite to the findings in aponecrosis. The necrotic release is clearly a pathological phenomenon.

In a specific subset of cases with severe intra uterine growth retardation (IUGR) and absent end-diastolic flow velocities in the umbilical arteries without pre-eclampsia additional patterns of nuclei distribution inside the syncytiotrophoblast were detected (Kaufmann and Huppertz 2005).

Wave-like nuclear pattern

In some of these cases a clear reduction in the number of cytotrophoblasts in combination with a reduced thickness of the syncytiotrophoblast was observed. These cases were characterised by a specific ring-like distribution of nuclei. The seemingly apoptotic nuclei accumulated inside the syncytiotrophoblast in multiple ring-like waves around the vertical axis of the villi, looking like patterns generated by waves on a sandy beach. The underlying pathogenic mechanisms remain obscure linking the pathogenesis of IUGR to this specific form of trophoblast turnover.

Arrested apoptotic shedding

In the subset of IUGR cases mentioned above another rare variation of apoptotic trophoblast turnover was detected. In these cases turnover of trophoblast resulted in the accumulation of clearly apoptotic nuclei within knot-like structures, suggesting that a more or less normal apoptosis cascade triggered the process. But it seems as if the final event, shedding of the knots into the intervillous space was arrested: Hundreds of apoptotic nuclei accumulated in these knots. And moreover, enormous numbers of such giant knots were formed throughout the placenta. In some cases the knots with accumulated nuclei were even larger than the cross section of the producing villus. As in the cases with the wave-like nuclear pattern, also in these cases it can only be speculated whether deficits in the apoptotic cleavage of the syncytial cytoskeleton are present. This would prevent regular delivery of the apoptotic material into the intervillous space.

Conclusions

Accumulations of nuclei within the syncytiotrophoblast of human placental villi only at the first glance appear to be phenotypically rather similar. They are derived from different functional and pathological processes and are normally used for growth of the placenta or turnover of the trophoblast. In pathological cases a large variety of different modes of release can be observed, sometimes missing a clear structure. Finally, the effects of releasing trophoblast

material by different mechanisms should be taken in to account when thinking about fetal and maternal health.

References

Benirschke, K. and Kaufmann, P. (2000) Pathology of the human placenta. Eds: K. Benirshke and P. Kaufmann. *4th Edn. Springer, New York.*

Formigli, L., Papucci, L., Tani, A., Schiavone, N., Tempestini, A., Orlandini, G.E., Capaccioli, S. and Orlandini, S.Z. (2000) Aponecrosis: morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. *J. Cell Physiol.* **182,** 41-49.

Huppertz, B. and Kingdom, J. (2004) Apoptosis in the trophoblast–Role of apoptosis in placental morphogenesis. *J. Soc. Gynecol. Invest.* **11,** 353-362.

Kaufmann, P. and Huppertz, B. (2005) Tenney-Parker changes and apoptotic versus necrotic shedding of trophoblast in normal pregnancy and pre-eclampsia. In: *Pre-eclampsia–Aetiology and Clinical Practice*; Eds: F. Lyall, M. Belfort, *Cambridge University Press* (in press).

Tenney, B. and Parker, F. (1940) The placenta in toxaemia of pregnancy. *Am. J. Obstet. Gynecol.* **39,** 1000-1005.

SESSION 2:

Chairman:
G. Burton

ENDOTHELIAL CELL FUNCTION AND PLACENTAL ANGIOGENESIS

S. Charnock-Jones

Reader in Reproductive Biology, University of Cambridge, Department of Obstetrics and Gynaecology, The Rosie Hospital, Cambridge CB2 2SW, UK

The placenta functions to bring 2 circulatory systems together so that exchange can occur between mother and fetus. As fetal demand increases with gestational age, the capacity for exchange also increases. This is in part accomplished by growth in size of the placenta but there are also refinements of the structure that facilitate exchange. For example, in humans the so-called terminal villi are elaborated in the last third of pregnancy. These initially form as blister-like protrusions on the villous surface that enlarge and develop regions specially adapted for exchange by virtue of the very thin barrier between maternal and fetal blood spaces. In other species, different adaptations occur but the effect is still to bring the 2 circulations into close proximity. In the pig both maternal and fetal capillaries greatly indent the epithelial layers to minimise the diffusion distance between capillaries. Central to these processes are changes in the blood vessels. There is increasing evidence in other systems that the vessels are not merely passive 'plumbing' but that they actively participate in regulating tissue growth and structure. Using a transgenic approach, we have specifically manipulated endothelial apoptosis and this leads to lethal defects in fetal and placental growth. Understanding what processes influence placental blood vessel development is therefore essential for understanding of placental growth, function and pathology.

The key regulators of endothelial cell function have been described and considerable progress made in defining their function in man and mouse. These include the vascular endothelial growth factor and angiopoietin families. VEGF-A is the prototypical member of a family of related growth factors. Additional members described in humans, (with limited but concordant data in other species), are placenta growth factor (PlGF), VEGF-B, VEGF-C and VEGF-D. An additional molecule, (VEGF-E) has been described but this in fact encoded by an orf virus which infects sheep. Such infections result in highly vascularised lesions on the face and mouth of the sheep and this virally encoded protein is able to stimulate host and endothelial cell proliferation – hence the highly vascularised lesions. However, while this protein has both structural and functional similarity to the mammalian VEGFs it is not a mammalian protein (Ferrara 2004).

The function and biological importance of these molecules is less well defined than for VEGF-A but there are clear data indicating that PlGF is able to induce the mobilisation of bone marrow derived endothelial pre-cursors in a similar way to VEGF-A and that, while it is not required for embryonic growth and development, PlGF plays an important role in pathological angiogenesis. PlGF is produced in the placenta (by the trophoblast cells) and is readily detectable in maternal circulation. Perturbations in the level of free PlGF are associated with pre-eclampsia in humans (Levine *et al.* 2004). VEGF-B is not essential for embryonic development but plays a role in cardiac development and VEGF-B-/- mice have impaired response to experimentally induced myocardial ischaemia. VEGF-C is essential for lymphatic development.

Additional complexity results from alternative splicing and the presence of several receptors - VEGFR1 (FLT1), VEGFR2 (KDR in man, flk in mouse) and VEGFR3, (FLT4). There is a soluble splice variant of VEGFR1 (sVEGFR1, sFLT1) which is able to bind and inactivate VEGF-A, PlGF and VEGF-B.

The principal members of the angiopoietin family are ANG1 and ANG2, (there are however

related molecules and there appears to be species specific differences in the distribution of these). Generally, ANG1 acting via the tyrosine kinase TIE2 receptor leads to stable non-leaky vessels and ANG2 antagonises this. Therefore, the action of ANG2 is particularly important during vessel growth and remodelling.

Limited data are available in species other than man and mouse and notable gaps in knowledge remain. However, even in these species, it is clear that the complexity of endothelial regulation that leads to highly differentiated functional capillaries still poses major questions.

One approach to address this complexity is to define the transcripts that are present in the placenta and to determine the conditions which regulate their levels. We have therefore used gene arrays to characterise the transcript populations in human placenta at different times in gestation and under different clinical conditions. We have used cDNA microarrays comprising 15937 probes to determine the transcript profile of human placenta following Caesarean section or long (10 h) labour, the latter causes significant oxidative stress that is detectable in trophoblast and endothelial cells. These array data were processed using the R statistical package, Linear models for Microarray Analysis (Limma) and Cyber-T. They reveal the transcripts that are present and those that are most abundant in the placenta and of particular interest, those that are regulated by labour and oxidative stress. However, because the placenta is obviously composed of several cell types these studies do not reveal clearly the endothelial specific responses. We have therefore used purified endothelial cells *in vitro* to determine the effect on the transcriptome of treatments that alter cell fate (ie growth factor stimulation or withdrawal for example). It is likely that similar regulatory processes activated by these *in vitro* treatments underlie the remodelling that occurs *in vivo*. For these latter studies we have used Affymetrix U95 arrays. The factor withdrawal studies revealed that rather than the level of a few 'master regulator' transcripts changing, there was a co-ordinated increase in pro-apoptotic transcripts (eg TRAIL, TRADD, LARD) and a drop in the pro-survival transcripts (eg TRAF-2, MIHB) and a decrease in those encoding growth factors and tyrosine kinase receptors (eg VEGF-C, IL-8,GP130, FLT1). This could be seen as a feed-forward system where multiple genes are involved in the ultimate cellular response. There were also changes to the glycome which contribute further to this feed-forward process (Johnson *et al.* 2004). It is therefore likely that conditions leading to subtle alterations in the level of multiple transcripts may lead to both physiological and pathological responses. Thus, to understand the processes that regulate the remodelling observed *in vivo* we need to take account, not only of the complexity of the signals received by the cells of interest, but also the integrated response of multiple transcripts and the proteins they encode within the cell.

REFERENCES

Ferrara, N. (2004) Vascular endothelial growth factor: basic science and clinical progress. *Endocr. Rev.* **25,** 581-611.

Levine, R. J., Maynard, S. E., Qian, C., Lim, K. H., England, L. J., Yu, K. F., Schisterman, E. F., Thadhani, R., Sachs, B. P., Epstein, F. H., Sibai, B. M., Sukhatme, V. P. and Karumanchi, S. A. (2004) Circulating angiogenic factors and the risk of pre-eclampsia. *N. Engl. J. Med.* **350,** 672-683.

Johnson, N. A., Sengupta, S., Saidi, S. A., Lessan, K., Charnock-Jones, S. D., Scott, L., Stephens, R., Freeman, T. C., Tom, B. D., Harris, M., Denyer, G., Sundaram, M., Sasisekharan, R., Smith, S. K. and Print, C. G. (2004) Endothelial cells preparing to die by apoptosis initiate a program of transcriptome and

UMBILICAL CORD COMPLICATIONS: PLACENTAL LESIONS AND IMPACT ON ADVERSE PERINATAL OUTCOME

R. N. Baergen

Chief of Perinatal and Pediatric Pathology, Professor of Clinical Pathology and Laboratory Medicine, New York Presbyterian Hospital-Weill Medical College of Cornell University, Department of Pathology, Starr-1002, 520 E. 70th Street, New York, NY 10021, USA

NORMAL UMBILICAL CORD FEATURES

The human umbilical cord is comprised of 2 arteries and one vein, surrounded by Wharton's jelly and covered by a single layer of amnionic epithelium. Wharton's jelly consists primarily of fluid which is sparsely populated with fibroblasts and macrophages, providing a cushion for the umbilical vessels. The average cord length at term is 55 cm but cord lengths up to 300 cm have been described. Long cords, (>70–80 cm) occur in 3.7% of deliveries while short cords (<30–40 cm) occur in 2% (Baergen *et al.* 1994; Benirschke 1994). Cords generally have a twist to the left but right twists are present in about 20% (Lacro *et al.* 1987). A small percentage is untwisted or shows a combination of left and right twists. Normally, there are approximately 0.2 twists or coils per 1 cm of length (umbilical cord coiling index) but hypocoiled and hypercoiled cords exist.

UMBILICAL CORD COMPLICATIONS

Umbilical cord complications in humans are usually the result of mechanical forces and fall into 2 general categories – disruption of the cord with subsequent haemorrhage or direct compression (Spellacy *et al.* 1966; Benirschke 1994). Haemorrhage may result from disruption of a damaged or abnormal umbilical vessel and rarely leads to cord rupture. Velamentous or membranous vessels are also prone to damage. They probably develop secondary to villous atrophy or regression in the area of cord insertion, which leaves the cord behind in the membranes. Thus, these vessels run in the chorion laeve without the benefit of Wharton's jelly. If they rupture, fetal haemorrhage will result and may lead to exsanguination and death.

Direct mechanical compression may also lead to embarrassment of blood flow but this tends to be a chronic rather than acute process (Benirschke 1994). In a cord prolapse, the cord precedes and is compressed by the presenting fetal part (usually the head) during delivery. Likewise, the cord may become entangled around fetal parts, such as the neck (nuchal cord), and significant compression of the umbilical vessels or the large vessels in the neck may occur. Usually cord compression does not become an issue until rupture of membranes when the amniotic fluid allows excessive pressure on the umbilical vessels. If the cord has excessive twisting or coiling, diminished blood flow will develop (Machin *et al.* 2000). Excessive coiling may also result in a stricture at the site of the cord insertion into the umbilicus and fetal demise due to complete loss of umbilical blood flow. By the same mechanism, true knots may cause obstruction of blood flow leading to a similar fate. Excessively short cords are associated with failure to descend through the birth canal during delivery, uterine inversion, abruptio placentae, cord rupture and excessive traction with compression of umbilical vessels (Snider *et al.* 1997). An excessively long cord is associated with cord entanglements and with increased resistance to blood flow through the extended length (Baergen *et al.* 1994).

Cord complications have in common obstruction of blood flow with a decrease in venous return from the placenta, venous stasis and congestion. These may eventually lead to thrombosis in the fetal circulation of the placenta, loss of functional parenchyma and fetal and placental hypoxia due to decreased oxygen and nutrient exchange.

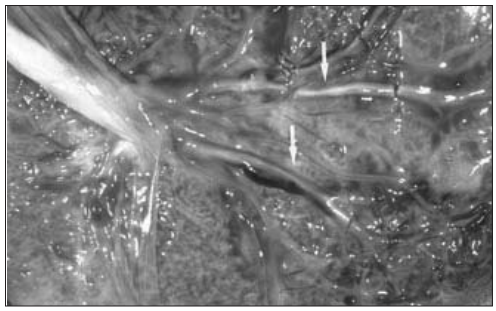

Fig 1: Arrows indicate thrombi in surface chorionic vessels.

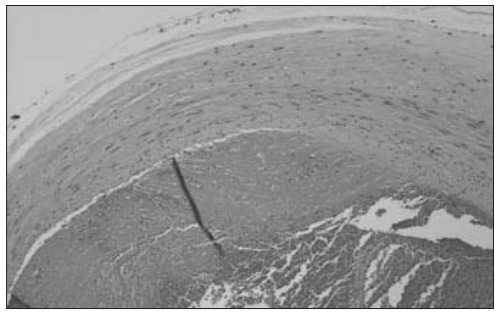

Fig 2: Mural thrombus in chorionic vein.

Placental pathological lesions

The most common placental lesions associated with cord complications are thrombotic lesions, most commonly in chorionic vessels, particularly the veins (Boue *et al.* 1995; Heifetz 1999; Faye-Peterson and Baergen 2001; Redline 2004). Frank thrombosis may be occlusive or non-occlusive. Non-occlusive thrombi are more common in obstructive cord lesions and consist of blood clot and fibrin attached to the endothelial surface of the chorionic or stem vessels (Fig 1 and 2). Some thrombi may present as mural lesions, with fibrin attached to the wall of the vessel or directly incorporated into the wall. Several lesions involving terminal villi have also been described, haemorrhagic endovasculitis (HEV) and more recently villous stromal karyorrhexis. Both terms are somewhat misleading as the origin of this lesion is still in question. Histologically, there is disruption of stem or capillary vessels with extravasation of red blood cells into the villous stroma. Necrosis of villous parenchyma may also occur and in that case there is an associated inflammatory infiltrate. If this process continues the villi may become completely degenerated or avascular with no appreciable capillaries and only hyalinised stroma remaining.

Study of long cords

A study of long umbilical cords aimed to analyse various maternal, fetal and placental factors, microscopic placental lesions and to determine the risk of recurrence of long cords in subsequent pregnancies (Baergen *et al.* 2001; Faye-Peterson and Baergen 2001). To do so, it was necessary to review what is known about the determination of cord length. First, cord length increases throughout pregnancy, although growth slows in the third trimester. Cord length is determined, at least in part, by fetal movement *in utero* (Miller *et al.* 1982). Fetuses who experience intrauterine constraint due to abnormal uterine conformation (uterine anomalies) or to lack of movement, eg skeletal dysplasia, tend to have shortened umbilical cords (Snider 1997). Studies in rats have shown that when animals are given drugs that limit fetal movement or when tethered to structures in the abdominal cavity, they develop short cords while those given free movement develop long cords. A genetic component may be a factor in determination of cord length, but this has not been proven (Moessinger *et al.* 1982).

From a database of 38,000 placentae, we obtained 923 cases of long cords, defined as those >70 cm in length. Placental and autopsy slides were reviewed on all cases where available and chart review was performed on maternal charts in all cases and on paediatric charts in cords greater than 90 cm. Material on 200 matched controls was also reviewed. Results are shown in Table 1 (Baergen *et al.* 2001).

There were 22 neonatal or fetal demises and autopsy was performed on 18. The associated placentae showed features similar to that seen in the entire group, but with a higher percentage of abnormalities. Interestingly, approximately 50% showed cardiac hypertrophy consistent with increased resistance to flow through long cords. Examination of the brain showed ischaemic neuronal injury in 33%, white matter gliosis in 28% and intracerebral haemorrhage, hydrocephalus and other abnormalities in a smaller percentage. The cause of death was a cord complication in 50% of cases, and possibly due to

TABLE 1: Date on long cords

	Long cord	Controls	P value
Delivery complications (%)	3.5	0.5	0.02
Maternal age (yrs)	27.4	25.8	0.05
Parity	2.43	2.17	0.001
Birth weight (gms)	3545	3253	0.001
GA (weeks)	39.6	39.1	0.03
Male sex (%)	57.6	51.3	0.007
Fetal distress (%)	9.1	2.0	0.007
Cord entanglement (%)	12.8	4.0	<0.001
IUGR (%)	2.6	0.5	0.07
IUFD (%)	2.2	0.5	0.06
Right twist (%)	19.0	6.5	<0.001
Marked twist (%)	9.5	0.5	<0.001
True knot (%)	5.2	1.0	0.01
Congestion (%)	7.3	2.5	0.02
Thrombosis (%)	25.0	8.1	<0.001
nRBCs (%)	49.0	11.0	<0.001
Chorangiosis (%)	20.0	3.0	<0.001

a cord complication in an additional 18% (Baergen *et al.* 2001).

Follow up was performed on a subset of cases in which the cord length was over 90 cm and was available in 24 cases up to 4 years of age. Adverse follow up (present in 67% of cases) was defined as either an abnormal neurological examination or abnormal brain imaging. Abnormal brain imaging, seen in 9 cases, consisted of echogenic white matter, changes consistent with anoxic damage, diffuse encephalopathy, intracerebral haemorrhage, or unspecified abnormalities. Abnormal neurological status, seen in 11 cases, consisted of extremity weakness, hypotonia, fine motor deficits, developmental delays, seizures, haemiplegia, cerebral palsy and death. When compared to matched controls, the long cord group had significantly worse follow up (P=0.0001) (Baergen *et al.* 2001).

The risk of recurrence was evaluated by reviewing cord length in pregnancies other than the index pregnancy. The incidence of a long cord in other pregnancies of the control group was 4.0% while the incidence of a second long cord in the long cord group was 6.9% (P=0.02). Furthermore, the mean cord length of "other" pregnancies was 7 cm more in the long cord group than the controls (P=0.04) (Baergen *et al.* 2001).

Summary

In summary, the development of cord length is likely to be multifactorial, including growth during pregnancy, tension on the cord from fetal movement and genetic factors (Miller *et al.* 1982; Baergen *et al.* 2001). Excessive cord length is associated with various maternal, fetal and placental factors as well as adverse outcome including, importantly, growth restriction, fetal demise and neurologic injury (Spellacy *et al.* 1966; Miller *et al.* 1982; Moessinger *et al.* 1982; Beirschke 1994; Boue *et al.* 1995; Snider 1997; Heifetz 1999; Machin *et al.* 2000; Baergen *et al.* 2001; Faye-Peterson and Baergen 2001; Redline 2004). Microscopic placental lesions associated with excessive cord length include those consistent with decreased venous return such as vascular congestion and thrombosis and those associated with fetal and placental hypoxia such as nucleated red blood cells and chorangiosis (Baergen *et al.* 2001; Redline 2004).

This raises several questions. How do cord complications, such as abnormal insertion, length, coiling etc, develop? What are the specific effects of excessive cord length on the flow and resistance through the umbilical cord and the fetal heart? Can diagnostic tests, such as ultrasound, assist in evaluating abnormal cords prenatally and how accurate are they? Finally, are therapeutic interventions possible to prevent adverse outcome?

References

Baergen, R.N., Malicki, D., Behling, C.A. and Benirschke, K. (2001) Morbidity, mortality and placental pathology in excessively long umbilical cords. *Pediatr. Develop. Pathol.* **4,** 144-153.

Benirschke, K. (1994) Obstetrically important lesions of the umbilical cord. *J. Reprod. Med.* **39,** 262-272.

Boue, D.R., Stanley, C. and Baergen, R.N. (1995) Long umbilical cord with torsion and diffuse chorionic vein thrombosis: Multiple associated congenital anomalies including destructive encephalopathy. *J. Perinatol.* **15,** 429-431.

Faye-Peterson, O. and Baergen, R.N. (2001) Long umbilical cords and pre-viable fetal death. *Pediatr. Develop. Pathol.* **4,** 414.

Heifetz, S.A. (1999) Pathology of the umbilical cord. In: *Pathology of the placenta 2nd edn.* Eds: S.H. Lewis and E. Perrin. New York: Churchill Livingstone, pp

107-136.

Lacro, R.V., Jones, K.L. and Benirschke, K. (1987) The umbilical cord twist: origin, direction and relevance. *Am. J. Obstet. Gynecol.* **157,** 833-838.

Machin, G.A., Ackerman, J. and Gilbert-Barnass, E. (2000) Abnormal umbilical cord coiling is associated with adverse perinatal outcomes. *Pediatr. Develop. Pathol.* **3,** 462-471.

Miller, M.E., Jones, M.C. and Smith, D.W. (1982) Tension: the basis of umbilical cord growth. *J. Pediatr.* **101,** 844.

Moessinger, A.C., Blanc, W.A., Marone, P.A. and Polsen, D.C. (1982) Umbilical cord length as an index of fetal activity: experimental study and clinical implications. *Pediatr. Res.* **16,** 109-112.

Snider, W. (1997) Short umbilical cords and lethal skeletal dysplasias. *J. Peritnatol.* **17,** 327-329.

Spellacy, W.N., Gravem, H. and Fisch, R.O. (1966) The umbilical cord complications of true knots, nuchal coils, and cords around the body. *Am. J. Obstet. Gynecol.* **94,** 1136-1142.

Redline, R. (2004) Clinical and pathological umbilical cord abnormalities in fetal thrombotic vasculopathy. *Hum. Pathol.* **35,** 1494-1498.

THE NATURE OF ADVERSE PLACENTAL FEATURES IN EQUINE PREGNANCIES IN THE UK AND THEIR RELATIVE DANGER TO THE FETUS

K. Whitwell

Equine Pathology Consultancy, Moulton, Newmarket, Suffolk CB8 8SG, UK

INTRODUCTION

The threat of losses from communicable diseases in Thoroughbred brood mares, and the need for continuing epidemiological surveillance motivates the UK equine industry to support and encourage detailed diagnostic study of equine fetal and perinatal foal losses. As these investigations are not government funded the routine work is carried out mainly by private diagnostic laboratories, of which there are 2 in Newmarket. An important part of these investigations includes a careful assessment of the placenta, hence a database of information has accrued and highlights the wide range of placental pathologies which have detrimental effects on pregnancy outcome. A familiarity with the normal equine placenta is not the prerogative of pathologists or research workers. As only a tiny minority of mares (those with predicted problems) are hospitalised for parturition 'home births' are the rule and, due to the normally rapid parturition in the mare, stud grooms play the role of nurse and midwife. They are encouraged to examine all placentae prior to disposal. Annual training courses are run in Newmarket for stud personnel in charge of foaling mares. A small part of this includes practical instruction in routine placental surveillance. This helps the stud to identify and draw attention to non-fatal placental defects which may explain postnatal forms of foal compromise. Several veterinary schools also include practical placental assessment for final year students doing equine reproduction electives.

MATERIALS AND METHODS

The materials sourced for this paper are derived from experiences of diagnostic work in Newmarket (examinations of placentae from abortions, stillbirths, neonatal deaths and occasionally from live but compromised foals). Placental information has also been derived from examination of the pregnant or non-pregnant uterus of a small number of mares after their death. Relatively few of the examinations are from non-Thoroughbreds.

Placental examination is carried out by spreading out the chorion in an 'F' shape on a flat well-lit surface, at a convenient (waist) height. The amnion, cord and both sides of the chorion are examined systematically: notes are mapped onto a placenta diagram, including total and amniotic cord lengths and vascular patterns and any unusual dimensions or weights also recorded. Photography is useful. Missing pieces are noted. For diagnostic purposes, sampling for histopathology and for microbiology (bacteriology swab from the chorion 'star' and at least 4 chorion sites sampled into VTM for viral screening) is routinely performed.

RESULTS

Placental pathologies can be restricted to one part (chorion, amnion or umbilical cord) or may involve several sites. Summaries of adverse placental findings are presented in Tables 1 and 2 (and were illustrated at the workshop using a compendium of over 50 colour images).

Allantochorion

Pathologies affecting the villous side of the chorion reflect breeches of the integrity of the utero-chorionic interface. Progress in understanding the nature of the mutual insult has been slow in the past, having to rely on experimental studies and

TABLE 1: Adverse placental features: Summary of utero-chorionic changes

A. Localised separation from the uterus
   i) At site of cervical placentitis
   ii) At site of non-cervical placentitis
   iii) At local pressure/ tear point
B. Sudden generalised separation (premature placental separation (PPS)
   i) Equine Herpes Virus infection
   ii) Undiagnosed
   iii) MRLS (in the USA)
C. Local or general villus hypoplasia
   i) Local areas – overdue undersized foals
   ii) Generalised – premature undersized foal
D. Discontinuity of the uterine lumen
   i) Congenital abnormality eg bipartite uterine body
   ii) Trans-luminal adhesions
E. Altered dimensions of the pregnant uterus
   i) Hydrallantois
   ii) Body pregnancy
   iii) Pointed cervical pole
F. Twin or triplet pregnancy
G. Neoplasia eg papilloma; choriocarcinoma

TABLE 2: Adverse placental features: Summary of amniotic and cord changes

A. Amnionitis
B. Hydrops amnii
C. Perforation or rupture of the amnion
D. Excessive length-associated defects (vascular compromise-based)
E. Funisitis
F. Anomalous umbilical arteries
G. Urachal obstructions and dilatations

serendipitous uterine examinations after pregnant mare demise. Imaging technologies now provide welcome opportunities to assess utero-placental integrity *in vivo*: correlation with subsequent careful placental inspection is likely to yield interpretive data useful to both clinicians and pathologists. Using immunostaining to confirm viral antigen in the vascular endothelial cells of the chorion it has been recently recognised (Smith *et al.* 2004) that an atypical form of EHV-1 abortion exists in which virus-related uterine thrombosis and infarction gives rise to wholesale premature placental separation, PPS, and fetal expulsion before the fetal tissues themselves become infected. This finding has resulted in alteration in the recommended protocol for EHV screening of abortions, chorion samples now being routinely screened. Villus surface pathologies include the utero-placental effects of viral, bacterial and fungal uterine infection, local or generalised separations, villus maldevelopment at sites of endometrial compromise, structural defects acquired and congenital, dimensional disorders associated with fluid dynamics, and twinning. The effects on the progeny in this group are many and diverse.

Pathologies involving non-villous chorionic structures are often related to perturbations within the allantoic cavity and vascular crises. Vascular patterns are likely to reflect events of significance occurring very early in pregnancy: anomalous ones are particularly interesting but interpretation requires accurate early pregnancy data.

Amnion

Pathologies affecting the amnion include amnionitis, fluid dynamics disorders, malformation and perforations by trauma from inside (fetal appendage) or occasionally outside (flank trauma sufficient to cause fetal limb fracture). Their effects include fetal pneumonia, ischaemic loss of appendages and abortion.

Umbilical cord

In the UK, umbilical cord pathology, mainly resulting from tight twisting, is numerically the most important cause of detrimental fetal effects and abortion (Rickets *et al.* 2003; Smith *et al.* 2003). Several conditions are known to be 'associated with' long-cordedness (over 80 cm length) but the actual mechanism of how or why the pathologies arise is not yet clearly defined. Although some of the factors that influence cord length have been determined from statistical studies, it is still not clear whether fetal and uterine activity, or fetal fluid volumes and uterine dimensions play primary or secondary roles in the cord length attained. Detrimental effects on the fetus and placenta reflect sudden or slow reduction in efficiency of vascular perfusion through cord blood vessels, urinary flow down the urachus, and degenerative and reactive change to such shortcomings. Fetal death is a common outcome (Fig 1). It is hard to believe that the fetal heart

Fig 1: Typical appearance of a 6 month abortion associated with excessive cord length. Torsion has led to urachal dilatation, vascular obstruction in cord vessels, poor perfusion within the chorionic villi and fetal death.

would not respond anatomically to a sustained dynamic insult of increasing resistance to flow through the cord vessels. There is currently an absence of objective evidence in the equine species but a recent review of the effects of long cords in the human fetus indicate risks of brain injury are increased, and there is a possibly temporary effect on the heart. On rare occasions cord strangulation results from the wrapping of excessively long allantoic cysts or large yolk sac remnants around the cord.

Discussion

In the UK, by comparison with the past situations in Lexington, USA, the numerically important types of placental pathology are limited. Major losses from placentitis caused by infections such as *Leptospirosis* and *Nocardiform* organisms have not been experienced, nor those from the devastating Mare Reproductive Loss Syndrome or equine amnionitis and fetal loss (EAFL) as seen in Australia It remains important to maintain close surveillance of the causes of equine fetal losses in the UK so that any 'new' and potentially serious placental diseases can be identified early and measures taken to control them. Comparative studies of the human infant and placenta (Baergen *et al.* 2001) provide useful indicators for future equine fetal, foal and placental studies.

References

Baergen, R.N., Malicki, D., Behling, C. and Benirschke, K. (2001) Morbidity, mortality and placental pathology in excessively long umbilical cords: a retrospective study. *Pediatr. Develop. Pathol.* **4,** 144-153.

Ricketts, S.W., Barralet, A. and Whitwell, K.E. (2003) Equine abortion. *Equine vet. Educ. Manual* **6,** 18-21.

Smith, K. C., Blunden, A.S., Whitwell, K. E., Dunn, K.A. and Wales, A.D. (2003) A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. *Equine vet. J.* **35,** 456-501.

Smith, K.C., Whitwell, K.E., Blunden, A.S., Bestbier, M.E., Scase, T.J., Geraghty, R.J., Nugent, J., Davis-Poynter, N.J. and Cardwell, J.M. (2004) Equine herpesvirus-1 abortion: atypical cases with lesions largely or wholly restricted to the placenta. *Equine vet. J.* **36,** 79-82.

SESSION 3:

Chairman:
B. Huppertz

HOW TO MAKE A MYRIAD OF TROPHOBLAST CELL SUBTYPES IN THE MOUSE PLACENTA

J. C. Cross

Department of Biochemistry & Molecular Biology, University of Calgary Faculty of Medicine, Calgary, Alberta, Canada

The trophoblast lineage arises at the blastocyst stage as a simple epithelium (trophectoderm) that, after implantation, differentiates into a variety of cell subtypes in the placenta. Only ~60 trophoblast cells are present in the mouse blastocyst and, therefore, considerable trophoblast proliferation occurs after implantation. However, only the 8–10 trophectodermal cells that overlie and are in direct contact with the inner cell mass (so-called polar trophectoderm) proliferate and subsequently differentiate into the rest of the trophoblast lineage including trophoblast giant cells (TGCs), spongiotrophoblast, glycogen trophoblast cells and syncytiotrophoblast cells of the labyrinth layer (Simmons and Cross 2005). By contrast, the ~50 trophectodermal cells that do not overlie the inner cell mass (mural trophectoderm) are post mitotic and committed to forming 'primary' TGCs after implantation. Polar trophectoderm cells proliferate in response to growth factors produced by the inner cell mass (the fibroblast growth factor FGF4 and the transforming growth factor/Activin related protein Nodal). Trophoblast stem cell lines (TS cells) can be derived from cultured blastocysts or dissected extraembryonic ectoderm/chorion in the presence of FGF4 and Nodal (Tanaka *et al.* 1998; Guzman-Ayala *et al.* 2004). The definitive proof that cells are trophoblast stem cells is that, when they are put back into blastocysts, they contribute to all layers of the placenta (Tanaka *et al.* 1998). When the growth factors are withdrawn from the medium, cultured trophoblast stem cells stop dividing and differentiate into a variety of differentiated cell subtypes. TGC differentiation seems to be the default pathway as >50% of trophoblast stem cells commit to the TGC fate within 48 h after growth factor withdrawal (Hughes *et al.* 2004). By contrast, only ~5% of the cells form multi-nucleated syncytiotrophoblast cells (Hughes *et al.* 2004). Differentiation towards the syncytiotrophoblast fate probably requires signalling input from the allantois (Rossant and Cross 2001).

The molecular signals that control trophoblast cell fates have been defined in mice through transgenic and gene knockout experiments *in vivo* as well as in cultures of trophoblast stem cells. Differentiation and/or maintenance of the various differentiated trophoblast subtypes depend upon key transcription factor genes. Spongiotrophoblast fate is controlled by the bHLH transcription factor Mash2 (Guillemot *et al.* 1994). Another bHLH factor, Hand1, is necessary and sufficient for promoting TGC differentiation (Riley *et al.* 1998; Scott *et al.* 2000). A third factor, Gcm1, is necessary for syncytiotrophoblast differentiation (Anson-Cartwright *et al.* 2000). No specific transcription factors have been identified that determine glycogen trophoblast cell fate. However, glyoogen trophoblast cells express many of the genes and are first evident within the spongiotrophoblast layer, implying that they are simply a specialised subtype of spongiotrophoblast cell (Simmons and Cross 2005). The expression and activity of the Hand1 and Gcm1 transcription factors is normally suppressed in trophoblast stem cells and premature activation of either one is sufficient to over-ride the effects of FGF4/Nodal and cause cell cycle exit and differentiation (Hughes *et al.* 2004).

Despite the molecular insights into factors required for end stage differentiation, the cell lineage origins of these cells remain elusive. To date, all studies of trophoblast lineage development have been indirect. During TGC differentiation, the cultured cells first express genes typical of trophoblast cells from the ectoplacental cone/spongiotrophoblast layer, before expressing TGC-specific genes (Carney *et al.* 1993). The

assumption has been that, during differentiation of TS cells to TGCs, they pass through an intermediate stage. Indeed, isolated ectoplacental cone cells rapidly differentiate into TGCs in culture. In the last few years, several studies have indicated that the picture is more interesting and complicated. In addition to the TGCs that line the implantation site and form the interface with the maternal decidua, a variety of distinct subtypes were discovered, based on morphological and molecular criteria. An endovascular subtype of TGC invades into the maternal spiral arteries to replace endothelial cells (Adamson *et al.* 2002). Other TGC subtypes line the maternal blood canals that bring maternal blood to the base of the labyrinth layer before it enters the sinusoids, and lie within the sinudoidal spaces of the labyrinth (D. Simmons and J. Cross, unpublished data). These various subtypes can be distinguished not only by location but by distinct expression patterns for placental-specific genes of the prolactin/placental lactogen and cathepsin gene families. When cultured TS cells are allowed to differentiate by withdrawal of FGF4/Nodal from the medium, all TGC subtypes appear to form. Retinoic acid has been suggested to promote TGC differentiation (Yan *et al.* 2001), but it specifically induces the mural subtype and suppresses differentiation of the sinusoidal TGC subtype (D. Simmons and J. Cross, unpublished data).

To determine if all TGC subtypes are derived from common precursors, a novel transgenic approach has been used for *in vivo* cell lineage tracing. Fortier *et al. (*2005) developed a transgenic mouse that expresses Cre-recombinase under the control of an ectoplacental cone/spongiotrophoblast-specific promoter from the Tpbpa gene. Cre-recombinase is a sequence specific enzyme that mediates recombination between 2 of its DNA recognition sequences (loxP sites) and DNA that is flanked by loxP sites is deleted as result. We crossed the *Tpbpa-Cre* mice with mice carrying a reporter gene is activated only following Cre-mediated recombination. Because the activation is irreversible and not dependent on ongoing Cre expression, we could irreversibly label all ectoplacental cone/spongiotrophoblast cells and any of their differentiated derivatives including TGCs. Not all TGCs were derived from ectoplacental cone/spongiotrophoblast precursors but that there was significant variation among TGC subtypes. Specifically, essentially all of the spiral artery-TGCs and canal-TGCs, only ~50% of the mural-TGCs and none of the sinusoidal-TGCs are derived from precursors in the ectoplacental cone/spongiotrophoblast layer. These data show that the different TGC subtypes have rather different cell lineage origins.

References

Adamson, S.L., Lu, Y., Whiteley, K. J., Holmyard, D., Hemberger, M., Pfarrer, C. and Cross, J.C. (2002) Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. *Dev. Biol.* **250,** 358-73.

Anson-Cartwright, L., Dawson, K., Holmyard, D., Fisher, S. J., Lazzarini, R. A. and Cross, J. C. (2000) The glial cells missing-1 protein is essential for branching morphogenesis in the chorioallantoic placenta. *Nat. Genet.* **25,** 311-314.

Carney, E.W., Prideaux, V., Lye, S.J. and Rossant, J. (1993) Progressive expression of trophoblast-specific genes during formation of mouse trophoblast giant cells *in vitro*. *Mol. Reprod. Dev.* **34,** 357-368.

Fortier, A.L., Yamamoto, H. and Cross, J. C. (2005) Placental spongiotrophoblast-specific activity of Tpbpa-Cre transgenic mice. *Genesis* In press.

Guillemot, F., Nagy, A., Auerbach, A., Rossant, J. and Joyner, A.L. (1994) Essential role of Mash-2 in extraembryonic development. *Nature* **371,** 333-336.

Guzman-Ayala, M., Ben-Haim, N., Beck, S. and Constam, D.B. (2004) Nodal protein processing and fibroblast growth factor 4 synergize to maintain a trophoblast stem cell microenvironment. *Proc. Natl. Acad. Sci. USA* **101,** 15656-15660.

Hughes, M., Dobric, N., Scott, I.C., Su, L., Starovic, M., St-Pierre, B., Egan, S.E., Kingdom, J.C. and Cross, J.C. (2004) The Hand1, Stra13 and Gcm1 transcription factors override FGF signaling to promote terminal differentiation of trophoblast stem cells. *Dev. Biol. 271,* 26-37.

Riley, P., Anson-Cartwright, L. and Cross, J.C. (1998) The Hand1 bHLH transcription factor is essential for placentation and cardiac morphogenesis. *Nat. Genet.* **18,** 271-275.

Rossant, J. and Cross, J.C. (2001) Placental development: lessons from mouse mutants. *Nat. Rev. Genet.* **2,** 538-548.

Scott, I.C., Anson-Cartwright, L., Riley, P., Reda, D. and Cross, J.C. (2000) The Hand1 basic helix-loop-helix transcription factor regulates trophoblast giant cell differentitation via multiple mechanisms. *Mol. Cell. Biol.* **20,** 530-541.

Simmons, D.G. and Cross, J.C. (2005) Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. *Dev. Biol.* In press.

Tanaka, S., Kunath, T., Hadjantonakis, A.K., Nagy, A. and Rossant, J. (1998) Promotion of trophoblast stem cell proliferation by FGF4. *Science* **282,** 2072-2075.

Yan, J., Tanaka, S., Oda, M., Makino, T., Ohgane, J. and Shiota, K. (2001) Retinoic acid promotes differentiation of trophoblast stem cells to a giant cell fate. *Dev. Biol.* **235,** 422-432.

GESTATIONAL DEPENDENT PET PLACENTAL PATHOGENESIS: DEFINING 2 SPECIFIC SUBSETS

T. I. Ansari, M. Egbor, H. Meyers, C. J. Green and P. D. Sibbons

Department of Surgical Research, NPIMR, Harrow HA1 3UJ, UK

Appropriate placental development is crucial for the growth and survival of the developing fetus. Perturbations either at the implantation stage or later during angiogenesis can severely compromise the overall well-being of the materno-fetal unit. The severity, duration and time of onset of the perturbation can have the potential to result in different pathways and mechanisms being either activated or inactivated.

Pre-eclampsia (PET) and intrauterine growth restriction (IUGR) show evidence of disruption or inhibition in the second wave of trophoblastic invasion which occurs at around week 16 of gestation (Robertson *et al.* 1975). The profound consequence is that physiological vascular changes are restricted to the decidual segments of the utero-placental arteries, leaving the myometrial segments unaltered in their musculoelastic architecture and therefore responsive to vasomotor influences (Brosens *et al.* 1970; Khong *et al.* 1986). Diminished trophoblast invasion leads to utero-placental hypoxia which has a significant influence on the growth and development of the fetal vasculature and ultimately the growth of the placental villi. Fetal development and optimum growth is generally compromised in both IUGR and PET, however, not all fetuses born to mothers with PET are born IUGR resulting in 2 unique subsets within the PET cohort. Both PET and IUGR exhibit signs of initial trophoblast disruption, however, maternal clinical symptoms are only present in PET cases and never in isolated cases of IUGR, perhaps suggesting that the resulting clinical symptoms are not mediated by the placenta but are maternally driven.

Previous quantitative studies investigating placental vascular and villous morphology in cases of isolated PET and IUGR have shown significant discrepancies in the data generated by different studies. Results suggest either morphological similarities (Mayhew *et al.* 2003; Mayhew *et al.* 2004) or differences in villous and capillary surface areas (Clavero-Nunez *et al.* 1971; Mayhew 1996) between control and PET cases. A detailed stereological study was undertaken to investigate placental villous and vascular morphology in cases of isolated PET and IUGR and where PET coexisted with IUGR (ie PET-IUGR) and aged matched controls. Initial results suggest that isolated IUGR shows significant reduction in a number of features estimated eg terminal capillary volumes and surface areas. Isolated PET also showed significant detrimental disruption in terminal villi volume development. However, there were no interactive effects when both PET and IUGR coexisted. The majority of the results obtained were in agreement with the latest published data (Mayhew *et al.* 2003; Mayhew *et al.* 2004).

PET is an aetiologically heterogeneous disorder that occurs in at least 2 subsets, one with normal or enhanced placental function and another involving placental dysfunction and IUGR (Rasmussen and Irgens 2003; Mayhew *et al.* 2004). This observation is based on the findings that early-onset PET (<34 weeks) is associated with placental vascular lesions or reduced utero-placental blood supply (Ghidini *et al.* 1997) leading to reduced birth weight (Long *et al.* 1980). Additionally, recent evidence indicates that in the majority of late-onset PET (>34 weeks) the new born neonate had a normal weight (Odegard *et al.* 2000).

Moore and Redman (1983) reviewed clinical notes from 24 women with severe early-onset PET (<34 weeks) and compared the risk present to 48 randomly selected controls, they concluded that

early-onset PET was significantly different from late-onset PET in terms of risks factors.

By using 34 weeks (the period of greatest morphological change) as a separator between early and late onset PET, it is possible to explore when the observed changes in the isolated PET cases might have occurred. To this effect placentae from all 4 groups spanning the third trimester of pregnancy (ie 25–40 weeks gestation) were stereologically evaluated for features of villous and vascular morphology. Additionally the oxygen diffusive conductance (Dp) (Mayhew *et al.* 1984), a measure of the capacity of the placenta to transfer oxygen, was also determined using a combination of the stereologically estimated variable and physiological constants.

Preliminary analysis of the data suggests that late-onset PET had no demonstrable influence on placental morphology compared with age matched controls. In marked contrast, early-onset PET was associated with a reduction in placental weights, total placental volumes, volume of the intervillous space, terminal villous volumes, volume of terminal capillaries, surface areas of terminal villi and intermediate capillaries and a significant reduction in the terminal villous shape factors.

However, both early- and late-onset IUGR was associated with reductions in the placental weights, placental volumes and surface areas and shape factors. Additionally both early- and late-onset IUGR were associated with reduced linear growth of the placental villi and their vasculature as shown by the significant reduction in the lengths of these features compared with controls. Similar reductions in the volumes, surface areas and lengths were found in both early-and late-onset PET-IUGR and were due to the effects of IUGR when compared with the appropriate control group.

Mean harmonic thickness of the villous membrane did not differ between the early and late onset PET groups. However, villous harmonic thickness was significantly increased in the late onset IUGR groups when compared with the early onset IUGR group. The total Dp showed a significant increase for both early and late onset PET and PET-IUGR.

It has also been suggested that besides utero-placental hypoxia (common to both PET and IUGR) (Mayhew *et al.* 2003), there may be other confounding factor(s) which may have a negligible impact on placental morphology in PET but a substantial impact on placental morphology in IUGR. Oxygen dependent growth factors are crucial in regulating and steering villous development and angiogenesis (Kaufmann *et al.* 2004). The most intensely studied are vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and the Angiopoietin (Ang-1 and Ang-2). Abnormalities in these factors may be responsible for structural placental villous alterations (Maynard *et al.* 2003). During this VEGF-dependent period of angiogenesis, the pattern of capillary growth is one of branching angiogenesis: formation of multiple loops. As oxygen tension rises, an angiogenic switch is triggered such that VEGF is down-regulated and PlGF is up-regulated. Following this switch, capillaries grow by non-branching angiogenesis and this form of vascular growth leads to the formation of terminal villi as well as the inhibition of the global expansion of the feto-placental capillary tree necessary for early placental growth (Mayhew 2003).

In conclusion, isolated early-onset PET is associated with abnormal placental morphology, but placentae from late-onset PET were morphologically similar to placentae from gestational age matched controls, confirming the existence of 2 subsets of this condition and supporting the hypothesis that late-onset PET is a maternal disorder and not a placental disease resulting from disrupted angiogenesis. Although vascular development within the late-onset PET is comparable with age matched controls, the villous membrane requires further investigation in order to explain changes observed in the villous membrane harmonic thickness.

References

Brosens, I.A., Robertson, W.B. and Dixon, H.G. (1970) The role of the spiral arteries in the pathogenesis of pre-eclampsia. *J. Pathol.* **101.**

Clavero-Nunez, J.A., Negueruela, J. and Botella-Llusia, J. (1971) Placental morphometry and placental circulometry. *J. Reprod. Med.* **6,** 209-217.

Ghidini, A., Salafia, C.M. and Pezzullo, J.C. (1997) Placental vascular lesions and likelihood of diagnosis of pre-eclampsia. *Obstet. Gynecol.* **90,** 542-545.

Kaufmann, P., Mayhew, T.M. and Charnock-Jones, D.S. (2004) Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. *Placenta* **25,** 114-126.

Khong, T.Y., De Wolf, F., Robertson, W.B. and Brosens, I. (1986) Inadequate maternal vascular response to placentation in pregnancies complicated by pre-

eclampsia and by small-for-gestational age infants. *Br. J. Obstet. Gynaecol.* **93,** 1049-1059.

Long, P.A., Abell, D.A. and Beischer, N.A. (1980) Fetal growth retardation and pre-eclampsia. *Br. J. Obstet. Gynaecol.* **87,** 13-18.

Mayhew, T.M. (1996) Patterns of villous and intervillous space growth in human placentas from normal and abnormal pregnancies. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **68(1-2),** 75-82.

Mayhew, T.M. (2003) Changes in fetal capillaries during preplacental hypoxia: growth, shape remodelling and villous capillarization in placentae from high-altitude pregnancies. *Placenta* **24,** 191-198.

Mayhew, T.M., Joy, C.F. and Haas, J.D. (1984) Structure-function correlation in the human placenta: the morphometric diffusing capacity for oxygen at full term. *J. Anat.* **139 (Pt 4),** 691-708.

Mayhew T.M., Ohadike, C., Baker, P.N., Crocker, I.P., Mitchell, C. and Ong, S.S. (2003) Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. *Placenta* **24,** 219-226.

Mayhew, T.M., Wijesekara, J., Baker, P.N. and Ong, S.S. (2004) Morphometric evidence that villous development and fetoplacental angiogenesis are compromised by intrauterine growth restriction but not by pre-eclampsia. *Placenta* **25,** 829-833.

Maynard, S.E., Min, J.Y., Merchan, J., Lim, K.H., Li, J., Mondal, S., Libermann, T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E., Epstein, F.H., Sukhatme, V.P. and Karumanchi, S.A. (2003) Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in pre-eclampsia. *J. Clin. Invest.* **111,** 649-658.

Moore, M.P. and Redman, C.W. (1983) Case-control study of severe pre-eclampsia of early onset. *Br. Med. J.* **287,** 580-583.

Odegard, R.A., Vatten, L.J., Nilsen, S.T., Salvesen, K.A. and Austgulen, R. (2000) Pre-eclampsia and fetal growth. *Obstet. Gynecol.* **96,** 950-955.

Rasmussen, S. and Irgens, L.M. (2003) Fetal growth and body proportion in pre-eclampsia. *Obstet. Gynecol.* **101,** 575-583.

Robertson, W.B., Brosens, I. and Dixon, G. (1975) Uteroplacental vascular pathology. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **5,** 47-65.

THE HUMAN PLACENTA AND THE SEARCH FOR STRUCTURAL CORRELATES OF FETAL WELL-BEING

T. M. Mayhew

Centre for Integrated Systems Biology & Medicine, School of Biomedical Sciences, E Floor, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK

INTRODUCTION

In human and other animal pregnancies, integrated growth of different placental compartments helps to maintain fetal well-being. Using stereological sampling and estimation tools to derive 3D spatial information, several groups have quantified placental morphology during gestation (Jackson *et al.* 1992; Mayhew *et al.* 1993a) and in a variety of complicated pregnancies (Burton *et al.* 1989, 1996; Mayhew *et al.* 1990, 1993b, 2003; Bush *et al.* 2000; Mayhew 2002; Ansari *et al.* 2003, 2004). The latter include pre-eclampsia, intrauterine growth restriction (IUGR), sudden infant death, hypobaric hypoxia, maternal cigarette smoking, anaemia, asthma and insulin-dependent (type 1) diabetes mellitus. These databases allow us to examine: 1) growth relationships between different placental tissue compartments and fetal mass (an index of growth and well-being); 2) changes in complicated pregnancies; 3) variables relevant to particular biological processes (eg passive diffusion, vascular and villous morphogenesis and trophoblast turnover).

MATERIALS AND METHODS

Using microscopical sections of placentae generated by hierarchical multistage random sampling schemes, the 3D spatial size and content (volumes, surface areas, lengths, thicknesses) of different tissue compartments (villous, intravillous and intervillous) were quantified with stereological tools. For present purposes, relationships between fetal mass and placental variables in cross-sectional material at different stages of gestation (10 week to term) were examined by correlation and regression analysis. If 2 variables are perfectly positively correlated, their correlation coefficient R = +1. If there is perfect negative correlation, R = -1. For regression analysis, data were log-transformed and used to calculate an allometric relation, $D = AI^B$, where I is the independent variable (fetal mass), D the dependent (placental) variable and A is a constant determined by D and I. When D and I grow commensurately, the exponent B=1. If D grows more slowly than I, B<1; if it grows faster than I, B>1; if it is inversely related to I, B<0. For complicated pregnancies, we identified at term those placental variables which best reflected the changes in fetal mass. All statistical analyses were undertaken using Unistat v5.5 software (Unistat Ltd, London).

RESULTS AND DISCUSSION

Gestation

Almost all placental variables show highly significant (P<0.001) positive correlations with fetal mass. Ranked by Spearman's correlation coefficient, the main variables are placental size (weight or volume), lengths and surface areas of villi and capillaries, volumes of villi and their compartments (fetal capillaries, trophoblast, stroma) and of the intervillous space. Mean thicknesses (arithmetic, harmonic) of the villous membrane (trophoblast and stroma) showed highly significant (P<0.001) negative correlations with fetal mass. When the nature of these relationships was examined, all tissues grew more slowly than fetal mass (B<1) but the lengths, surfaces and volumes of capillaries and villi had the highest exponents and greatest precision of estimation (see Fig 1). Numbers of nuclei (indices of proliferative growth) and thicknesses of tissue

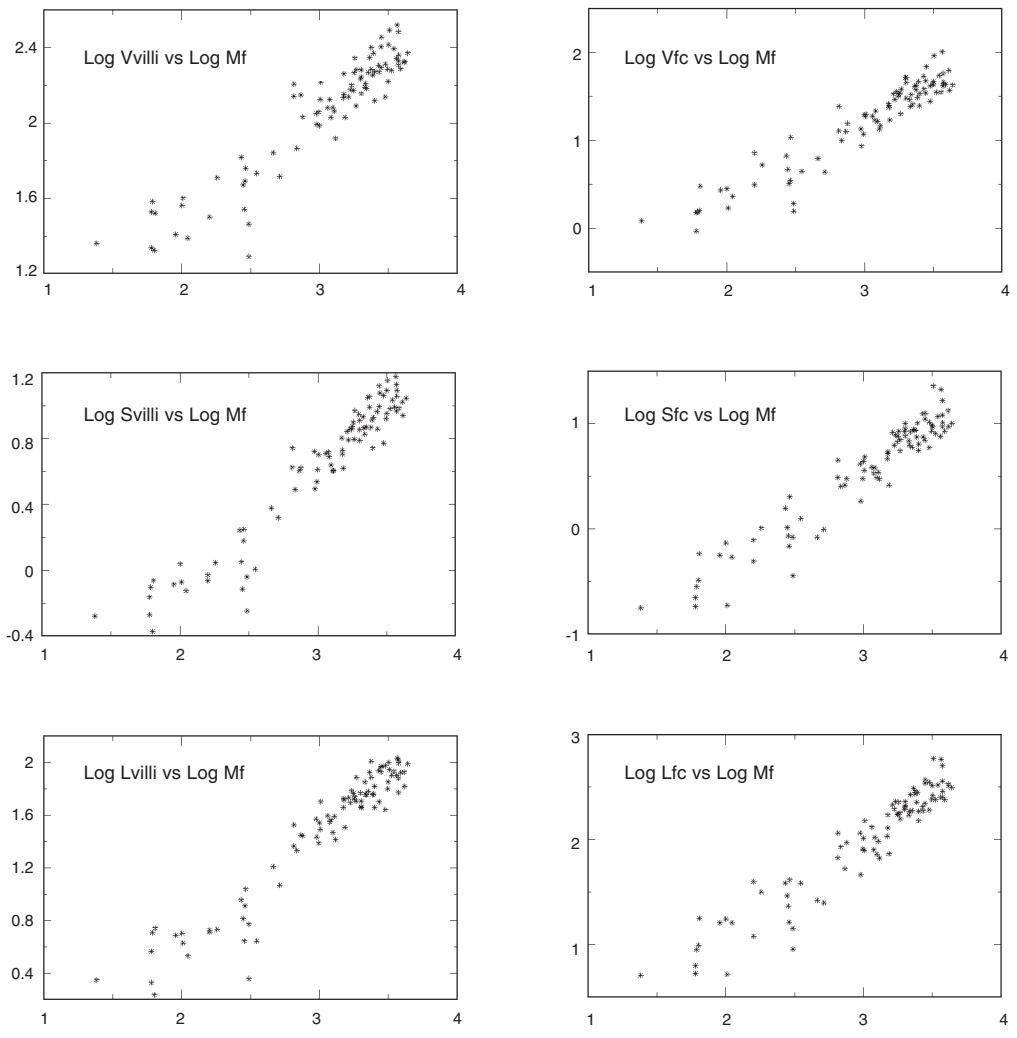

Fig 1: Regression relationships between placental variables and fetal mass (Mf) during uncomplicated pregnancy. All variables were log-transformed and exponents (B) varied from 0.55 (volume of villi) to 0.91 (surface and length of capillaries).

Key: V = volume, S = surface area, L = length, Villi = peripheral villi, fc = fetal capillaries.

layers were less attractive because they were estimated less efficiently (lower precision or greater cost). Interestingly, the only variable which increased in direct proportion to fetal mass (B=1) was functional (total O_2 diffusive conductance) and not structural.

Complicated pregnancies

Similar placental variables were highlighted by studies on term pregnancies associated with compromised fetal growth. More consistent patterns of change were seen for the volumes, surfaces and lengths of villous ingredients and, occasionally, layer thicknesses. In contrast, volumes of non-parenchymal tissues and capillary diameters offered less reliable measures. Similar conclusions were drawn for cases of fetal overgrowth associated with pre-gestational diabetes mellitus (Burton *et al.* 1989, 1996; Mayhew *et al.* 1990, 1993b, 2003; Bush *et al.* 2000; Mayhew, 2002; Ansari *et al.* 2003, 2004).

Biological processes

When considering processes which might be perturbed by particular factors, comparable findings emerge. For instance, hypoxic stress can be monitored effectively by estimating surface areas (villi, capillaries) and mean layer thicknesses (trophoblast, stroma) and then calculating total O_2 diffusive conductances (total Dp). Whilst total Dp increases commensurately with fetal mass during gestation (Mayhew *et al.* 1993a) to maintain specific conductance (= total Dp/fetal mass), responses vary in different complicated pregnancies. Specific Dp increases at high altitude and in pre-gestational diabetes (Mayhew *et al.* 1990, 1993b) but in the former this is due mainly to a decrease in fetal mass and in the latter to an increase in Dp. Specific Dp does not alter in pregnancies associated with maternal cigarette smoking, sudden infant death or small-for-gestational-age (SGA) deliveries (Bush *et al.* 2000; Ansari *et al.* 2003, 2004). In the former 2 pregnancies, neither fetal mass nor total Dp alter but, in SGA pregnancies, both variables decrease proportionately. When assessing these findings, caution must be exercised because changes may not be responses to hypoxia per se but to the presence of confounders (eg toxic ingredients of tobacco smoke in smoking, metabolic disturbances in diabetes).

The events of fetoplacental angiogenesis influence fetal growth as well as villous growth and development. Studies on placentae in uncomplicated pregnancies have shown that angiogenesis can be monitored effectively by estimating the volumes, surfaces and lengths of capillaries, numbers of endothelial cell nuclei, and indices of villous branching. The accompanying villous maturation can be monitored using the volumes, surfaces and lengths of villi, indices of villous capillarisation and trophoblast thickness. In complicated pregnancies which tend to be accompanied by reduced birthweight, angiogenic indices either decrease or do not alter. In contrast, enhanced angiogenesis occurs in pre-gestational diabetes (Burton *et al.* 1996; Mayhew *et al.* 2004). Indices of villous capillarisation based on component densities either do not change or increase, exceptions being pregnancies complicated by maternal smoking or IUGR with absent or reversed end-diastolic flow in umbilical arteries (IUGR+ARED) which have lower volume densities. Capillary: villus length ratios tend to be constant or increase except in pregnancies associated with maternal smoking, pre-gestational diabetes or IUGR+ARED. Increased branching angiogenesis seems to be a feature of several complicated pregnancies but, in IUGR+ARED, there is evidence of increased non-branching angiogenesis (Mayhew *et al.* 2004).

Trophoblast differentiation and turnover influence villous growth and may be perturbed in abnormal pregnancies. Trophoblast comprises a continuously-renewing epithelium with temporal phases of proliferation, recruitment, terminal differentiation and extrusion occurring in distinct spatial compartments. Useful structural measures include the volume and nuclearity of compartments (cytotrophoblast, syncytiotrophoblast, syncytial knots) and their activities and associations (proliferation, apoptosis, necrosis, fibrin deposition). For example, whilst relative numbers of cytotrophoblast and syncytiotrophoblast nuclei do not vary significantly during gestation, the steady state is perturbed in certain circumstances (Mayhew 2001). In high-altitude pregnancies, the steady state favours proliferation over recruitment, or relatively greater extrusion. In pre-eclamptic pregnancies, the incidences of apoptosis (or aponecrosis) and extrusion increase.

ACKNOWLEDGEMENTS

The author expresses thanks to The Medical Research Council (Development Grant Scheme), Wellcome Trust and Anatomical Society of Great Britain and Ireland for research support.

REFERENCES

Ansari, T., Fenlon, S., Pasha, S., O'Neill, B., Gillan, J.E., Green, C.J. and Sibbons, P.D. (2003) Morphometric assessment of the oxygen diffusive conductance in placentae from pregnancies complicated by intra-uterine growth restriction. *Placenta* **24,** 618-626.

Ansari, T., Gillan, J.E., Condell, D., Green, C.J. and Sibbons, P.D. (2004) Analyses of the potential oxygen transfer capability in placentae from infants succumbing to sudden infant death syndrome. *Early Human Devel.* **76,** 127-138.

Burton, G.J., Palmer, M.E. and Dalton, K.J. (1989) Morphometric differences between the placental vasculature of non-smokers, smokers and ex-smokers. *Br. J. Obstet. Gynaecol.* **96,** 907-915.

Burton, G.J., Reshetnikova, O.S., Milovanov, A.P. and Teleshova, O.V. (1996) Stereological evaluation of vascular adaptations in human placental villi to

differing forms of hypoxic stress. *Placenta* **17,** 49-55.

Bush, P.G., Mayhew, T.M., Abramovich, D.R., Aggett, P.G., Burke, M.D. and Page, K.R. (2000) Maternal cigarette smoking and oxygen diffusion across the placenta. *Placenta* **21,** 824-833.

Jackson, M.R., Mayhew, T.M. and Boyd, P.A. (1992) Quantitative description of the elaboration and maturation of villi from 10 weeks of gestation to term. *Placenta* **13,** 357-370.

Mayhew, T.M. (2001) Villous trophoblast of human placenta: a coherent view of its turnover, repair and contributions to villous development and maturation. *Histol. Histopathol.* **16,** 1213-1224.

Mayhew, T.M. (2002) Enhanced fetoplacental angiogenesis in pre-gestational diabetes mellitus: the extra growth is exclusively longitudinal and not accompanied by microvascular remodelling. *Diabetologia* **45,** 1434-1439.

Mayhew, T.M., Charnock-Jones, D.S. and Kaufmann, P. (2004) Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. *Placenta* **25,** 127-139.

Mayhew, T.M., Jackson, M.R. and Boyd, P.A. (1993a) Changes in oxygen diffusive conductances of human placentae during gestation (10–41 weeks) are commensurate with the gain in fetal weight. *Placenta* **14,** 51-61.

Mayhew, T.M., Jackson, M.R. and Haas, J.D. (1990) Oxygen diffusive conductances of human placentae from term pregnancies at low and high altitudes. *Placenta* **11,** 493-503.

Mayhew, T.M., Ohadike, C., Baker, P.N., Crocker, I.P., Mitchell, C. and Ong, S.S. (2003) Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. *Placenta* **24,** 219-226.

Mayhew, T.M., Sørensen, F.B., Klebe, J.G. and Jackson, M.R. (1993b) Oxygen diffusive conductance in placentae from control and diabetic women. *Diabetologia* **36,** 955-960.

GLIAL CELL MISSING IN PLACENTAL DEVELOPMENT: DUAL APPROACHES IN MICE AND HUMANS

J. Kingdom, D. Baczyk, C. Dunk, J. Slevin and S. L. Adamson

Programme in Development and Fetal Health, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada

In mice, trophoblast stem cells (TS cells) produce 2 distinct lineages of trophoblast. Invasive extravillous cytotrophoblast invades the uterine wall to promote blood flow to the implantation site while syncytiotrophoblast lines the labyrinth where maternal-fetal exchange takes place. These cell fate decisions are regulated by specific transcription factors (Cross 2005). The formation of syncytiotrophoblast requires daughter cytotrophoblast cells to exit the cell cycle and undergo syncytial fusion, a process initiated by glial cell missing-1 (Gcm-1). Homozygous null mutants die around e11.5 due to lack of morphogenesis of the chorio-allantoic placenta because the allantoic mesoderm is unable to penetrate the chorionic plate (Anson-Cartwright *et al.* 2000). We recently demonstrated an analogous expression pattern of the human homolog (GCM1) in a subset of villous cytotrophoblast cells in first trimester villous tissue (Baczyk *et al.* 2004). To determine the role of GCM1 in human placental development, the authors first developed an RNAi strategy in the BeWo trophoblast-derived cell line. BeWo cells undergo spontaneous syncytial fusion under control conditions, a process that can be augmented using forskolin (Borges *et al.* 2003). Compared to control conditions, BeWo cells exposed to forskolin demonstrated a 34-fold increase in GCM1 mRNA using real-time PCR (rtPCR) while the transfected combination of 2 RNAi's resulted in 90% inhibition of GCM1 mRNA and undetectable levels of human chorionic gonadotropin in the culture supernatant. These data suggested that GCM1 is critical for syncytiotrophoblast formation in the human placenta.

To explore the role of GCM1 in human placenta, the authors employed a denuded floating villous explant model in first trimester tissues (Baczyk *et al.* 2005). In this model, trypsin exposure can remove 80–90% of syncytiotrophoblast, leaving a 'cobblestone' layer of underlying cytotrophoblasts that regenerate syncytiotrophoblast in the next 40–72 h. The authors have developed anti-sense oligo-nucleotides to GCM1 to test the hypothesis that GCM1 is necessary for syncytiotrophoblast formation in first trimester villi. In initial experiments they added heparin and fibroblast growth factor 4 (FGF4), because these are required for maintenance of TS cells in mice (Hughes *et al.* 2004). Interestingly, the combination of FGF4 and heparin inhibited syncytiotrophoblast formation in favour of proliferation as extravillous cytotrophoblasts while in serum/growth factor – free media the syncytiotrophoblast reformed successfully (Baczyk *et al.* 2005). In denuded explants culture under syncytiotrophoblast-promoting conditions, anti-sense oligo-nucleotides to GCM1 have prevented syncytiotrophoblast formation (manuscript in preparation). These data provide further evidence that GCM1 is required for syncytiotrophoblast formation.

The authors' laboratory (led by Dr S. Lee Adamson) has developed the application of a high-frequency ultrasound bio-microscope (UBM) to observe embryonic and placental development in mice (Foster *et al.* 2002). Using glass micro-pipettes, it has been possible to use the UBM to guide small volume injections into developing fluid-filled cavities beginning at e7.5 (exo-celomic cavity, ectoplacental cleft, amniotic cavity). At e7.75, fluorescent beads were injected into the exo-celomic cavity with >80% success (Slevin *et al.* in press). This method was used to inject a murine RNAi for Gcm1 at e 7.75 before chorio-allantoic fusion. The uterus is exteriorised

by laparotomy at e7.75 to map individual's pregnancies using UBM. Viable pregnancies in the right horn are injected with RNAi while viable pregnancies in the left horn are injected with an equivalent volume (52 nl) of saline or scrambled RNAi. In preliminary experiments thus far (n=3) embryonic survival at e11.5 is greater (75%) in control injected embryos than in RNAi-injected embryos (50%)(unpublished observations). At e11.5 the re-exteriorised uterus is again subjected to UBM to determine fetal viability and measure maximum placental width. The uterus is then removed to a) determine chorio-allantoic fusion in non-viable fetuses, b) determine fetal and placental weight. The placenta is fixed in RNA later for subsequent rtPCR determination of Gcm1 mRNA. In separate experiments whole gestations are fixed and embedded for placental histology. H&E histology has shown an expanded allantois on top of an under-developed chorionic plate, similar to the features observed in Gcm1 -/- mice (Anson-Cartwright *et al.* 2000). The medium-term goal of this project is to develop conditions that mimic human severe IUGR with severe villous maldevelopment and abnormal umbilical artery Doppler flow (Krebs *et al.* 1996). Interestingly, Doppler studies can be obtained from the murine umbilical artery, although end-diastolic frequencies appear rather late (e16.5) in murine pregnancy (14 weeks) (Dr S. Lee Adamson, unpublished observations).

We are presently defining the signalling pathways downstream of Gcm1. The human gene syncytin is known to reside downstream of Gcm1 (Yu *et al.* 2002) and uses the receptor RDR for syncytial fusion. In BeWo cells, arrest of syncytial fusion using RNAi to GCM1 increases RDR mRNA while acceleration of syncytial fusion by forskolin reduces RDR mRNA (Potgens *et al.* 2004). Recently, homologous genes to syncytin in mice have been described (Dupressoir *et al.* 2005) and therefore we are monitoring the effect of RNAi to Gcm1 in mice upon placental expression of candidate downstream genes such as syncytin and its receptor RDR.

Molecular data on these genes regulating syncytiotrophoblast fusion are scant in pathological human pregnancies. GCM1 expression was recently found to be reduced in pre-eclampsia (Chen *et al.* 2004), consistent with 2 papers suggesting reduced expression of the downstream gene syncytin in the same disease (Lee *et al.* 2001; Knerr *et al.* 2002). In BeWo cells, hypoxic (2%) oxygen conditions inhibit syncytiotrophoblast formation and down-regulate syncytin (Kudo *et al.* 2003). Hypoxic, as a result of defective spiral artery invasion, may locally inhibit the GCM1-syncytin-mediated trophoblast turnover pathway, in favour of necrotic shedding and the initiation of pre-eclampsia (Huppertz and Kingdom 2004). To distinguish this possibility from an intrinsic genetic defect in cytotrophoblast cells, we are presently using gene array technology to assess mRNA expression patterns in systematic random-block tissue samples from pregnancies complicated by severe pre-eclampsia, comparing the data with that from BeWo cells in fusing and non-fusing conditions.

In summary, the authors describe human and murine evidence to support a key regulatory role for the transcription factor Gcm1 in villous trophoblast development. Further studies in both species will hopefully unravel the molecular basis of the important placental insufficiency syndromes that result in significant maternal-fetal morbidity.

Acknowledgements

Dr's Kingdom and Adamson are funded by the Canadian Institutes of Health Research, Physician's Services Inc., Ontario and Mount Sinai Hospital Department of Obstetrics & Gynecology, Toronto, Canada

References

Anson-Cartwright, L., Dawson, K., Holmyard, D., Fisher, S.J., Lazzarini, R.A. and Cross, J.C. (2000) The glial cells missing-1 protein is essential for branching morphogenesis in the chorioallantoic placenta. *Nat. Genet.* **25(3),** 311-314.

Baczyk, D., Dunk, C., Huppertz, B., Maxwell, C., Reister, F., Giannoulias, D. and Kingdom, J.C. (2005) Bi-potential behavior of first trimester cytotrophoblasts in first trimester chorionic villi. *Placenta* – In press.

Baczyk, D., Satkunaratnam, A., Nait-Oumesmar, B., Huppertz, B., Cross, J.C. and Kingdom, J.C. (2004) Complex patterns of GCM1 mRNA and protein in villous and extravillous trophoblast cells of the human placenta. *Placenta* **25,** 553-559.

Borges, M., Bose, P., Frank, H.G., Kaufmann, P. and Potgens, A.J. (2003) A 2-colour fluorescence assay for the measurement of syncytial fusion between trophoblast-derived cell lines. *Placenta* **24,** 959-964.

Chen, C.P., Chen, C.Y., Yang, Y.C., Su, T.H. and Chen, H. (2004) Decreased placental GCM1 (glial cells missing) gene expression in pre-eclampsia. *Placenta*

25, 413-421.

Cross, J.C. (2005) How to make a placenta: Mechanisms of trophoblast cell differentiation in mice - A Review. *Placenta* **26,** S3-S9.

Dupressoir, A., Marceau, G., Vernochet, C., Benit, L., Kanellopoulos, C. and Sapin, V. (2005) Heidmann Syncytin-A and Syncytin-B, 2 fusogenic placenta-specific murine envelope genes of retroviral origin conserved in Muridae. *Proc. Natl. Acad. Sci.* **102,** 725-730.

Foster, F.S., Zhang, M.Y., Zhou, Y.Q., Liu, G., Mehi, J., Cherin, E., Harasiewicz, K.A., Starkoski, B.G., Zan, L., Knapik, D.A. and Adamson, S.L. (2002) A new ultrasound instrument for *in vivo* micro-imaging of mice. *Ultrasound Med. Biol.* **28,** 1165-1172.

Hughes, M., Dobric, N., Scott, I.C., Su, L., Starovic, M., St-Pierre, B., Egan, S.E., Kingdom, J.C. and Cross, J.C. (2004) The Hand1, Stra13 and Gcm1 transcription factors override FGF signaling to promote terminal differentiation of trophoblast stem cells. *Dev. Biol.* **271,** 26-37.

Huppertz, B. and Kingdom, J.C. (2004) Apoptosis in the trophoblast--role of apoptosis in placental morphogenesis. *J. Soc. Gynecol. Investig.* **11,** 353-362.

Knerr, I., Beinder, E. and Rascher, W. (2002) Syncytin, a novel human endogenous retroviral gene in human placenta: evidence for its dysregulation in pre-eclampsia and HELLP syndrome. *Am. J. Obstet. Gynecol.* **186,** 210-213.

Krebs, C., Macara, L.M., Leiser, R., Bowman, A.W., Greer, I.A. and Kingdom, J.C. (1996) Intrauterine growth restriction with absent end-diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree. *Am. J. Obstet. Gynecol.* **175,** 1534-1542.

Kudo, Y., Boyd, C.A., Sargent, I.L. and Redman, C.W. (2003) Hypoxia alters expression and function of syncytin and its receptor during trophoblast cell fusion of human placental BeWo cells: implications for impaired trophoblast syncytialisation in pre-eclampsia. *Biochim. Biophys. Acta.* **1638,** 63-71.

Lee, X., Keith, J.C. Jr, Stumm, N., Moutsatsos, I., McCoy, J.M., Crum, C.P., Genest, D., Chin, D., Ehrenfels, C., Pijnenborg, R., van Assche, F.A. and Mi, S. (2001) Downregulation of placental syncytin expression and abnormal protein localisation in pre-eclampsia. *Placenta* **22,** 808-812.

Potgens, A.J., Drewlo, S., Kokozidou, M. and Kaufmann, P. (2004) Syncytin: the major regulator of trophoblast fusion? Recent developments and hypotheses on its action. *Hum. Reprod.* **10,** 487-496.

Yu, C., Shen, K., Lin, M., Chen, P., Lin, C., Chang, G.D. and Chen, H. (2002) GCMa regulates the syncytin-mediated trophoblastic fusion. *J. Biol. Chem.* **277,**

SESSION 4:

Chairman:
P. Sibbons

PLACENTAL AND FETAL MATURATION: MECHANISMS IN ZONARY VERSUS DIFFUSE PLACENTATION

W. R. Allen

University of Cambridge, Equine Fertility Unit, Mertoun Paddocks, Newmarket, Suffolk, CB8 9BH, UK

The domestic horse (*Equus caballus*) and the African elephant (*Loxodonta africana*) are both large, land-based mammals with long gestation periods of, respectively, 11 and 22 months. Yet, curiously, the persistence of functional nephrostomes in the fetal kidney, the existence of the elongated proboscis (trunk) and the absence of a pleural cavity (Short 1962) all indicate a relatively recent evolutionary emergence of the elephant from an aquatic lineage (Gaeth *et al.* 1999).

During early gestation in the horse, the specialised trophoblast cells of the annulate chorionic girdle of the fetal membranes erode and replace the lumenal and glandular epithelia of the endometrium with which they are in close physical contact before they penetrate the basement membranes to enter the stroma. Here they transform morphologically and secrete equine Chorionic Gonadotrophin (eCG) into the maternal circulation during the next 40–60 days. The LH-like biological action of eCG stimulates ovulation and/or luteinisation of secondary follicles in the maternal ovaries and the progesterone secreted by these accessory luteal structures helps to maintain the pregnant state. By Day 100–120 the diffuse, non-invasive, epitheliochorial placenta is secreting sufficient progestagens to support pregnancy without the need for any further contribution from the maternal ovaries.

During early pregnancy in the elephant, an annulate region of the choriovitelline membrane similarly erodes and replaces the lumenal and glandular epithelia. However, the trophoblast cells do not breach the basement membrane and nor do they produce any detectable chorionic gonadotrophin (Allen *et al.* 2002). Instead, they secrete the growth factors, IGF-2 and HGF-SF, which stimulate the endometrial stroma and associated blood vessels to grow upwards at a phenomenal rate to form the vascularised maternal core of the very elongated and folded trophoblast-covered lamellae that constitute the mature zonary placenta. Between 2 and 5 large plum-like corpora lutea of unknown origin are present in the maternal ovaries from the earliest stages of pregnancy and they persist, unsupplemented, throughout gestation (Allen *et al.* 2003).

In the second half of pregnancy the gonads of the horse fetus enlarge dramatically due to hypertrophy and hyperplasia of the interstitial cells and then regress again before term. The interstitial cells secrete large quantities of conventional and 7α-hydroxylated C-19 androgens which are then aromatised to phenolic and Ring β-unsaturated oestrogens by the placenta. These oestrogens play important roles in promoting fetal growth and in stimulating the synthesis and storage of prostaglandin F in the myometrium in readiness for birth. The gonads of the fetal elephant similarly undergo a marked interstitial cell-driven enlargement in the second half of gestation but they secrete only C-21 progestagens which the steroidogenically inert placenta is unable to metabolise further to C-19 androgens or C-18 oestrogens. Whether these fetal gonadal progestagens assist with pregnancy maintenance, or play any role in fetal growth and maturation, remains unknown.

Thus, despite some unusual and interesting similarities between the horse and elephant in early and late placental and fetal development, there remain some striking contrasts in structure and function between these diffuse epitheliochorial versus zonary endotheliochorial styles of placentation.

REFERENCES

Allen, W.R., Mathias, S.S., Wooding, F.B.P., Skidmore, J.A. and van Aarde, R.J. (2002) Placentation in the African elephant (*Loxodonta africana*) I. Endocrinological aspects. *Reprod.* **60,** 105-116.

Allen, W.R., Mathias, S.S., Wooding, F.B.P. and van Aarde, R.J. (2003) Placentation in the African elephant (*Loxodonta africana*) II. Morphological changes in the uterus and placenta throughout gestation. *Placenta* **24,** 598-617.

Gaeth, A.P., Short, R.V. and Renfree, M.B. (1999) The developing renal, reproductive and respiratory systems of the African elephant suggest an aquatic ancestry. *Proc. Nat. Biol. Sci. USA* **96,** 5555-5558.

Short, R.V. (1962) The peculiar lungs of the elephant. *New Scientist* **16,** 570-572.

SESSION 5:

Chairman:
W. R. Allen

AN EARLY GESTATIONAL PORCINE LITTERMATE COMPARISON MODEL FOR DEFINING MECHANISMS CONTROLLING PREGNANCY OUTCOME

C. Tayade, G. P. Black, Y. Fang and B. A. Croy*

*Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada; *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada*

INTRODUCTION

The maternal-fetal interface is a dynamic site in which fetal trophoblast cells interact with maternal tissues including immune cells. The pig is an excellent species for pregnancy studies becauase maternal and fetal tissues from the epithelial-chorial placenta are totally separate and can be cleanly dissected without cross-contamination. In many species, including pigs, endometrium of early normal pregnancy is enriched in innate immune cells, particularly Natural Killer (NK cells) (Croy *et al.* 2003). In species with haemochorial placentation, decidua recruits uterine (u)NK cells that establish conditions for maternal arterial change by producing IFN-γ (Ashkar *et al.* 2000) and synthesis of HIF-1α-regulated VEGF (Li *et al.* 2001). In pigs, conceptuses mediate uNK cell recruitment (Engelhardt *et al.* 2002) but uNK cell functions are unknown.

Pre-natal mortality is common in commercial swine. Of several factors believed responsible, a poor blood supply for the developing embryos is considered a major cause. Commercial pork breeds ovulate 14–16 ova (Anderson *et al.* 1993). Fertilised ova develop as spheres until gestation day (GD) 10, and then rapidly elongate and attach to non-eroded endometrial epithelium between GD 11–13. During elongation, conceptuses secrete oestrogen, which stimulates maternal recognition of pregnancy and alters endometrial secretions (histotroph). Lytic uNK cells are detected from GD 12 (Croy *et al.* 1988); maternal angiogenesis from GD 15 (Winther *et al.* 1999). Approximately 30% of conceptuses die between GD 15–30, the peri- and immediate post attachment intervals with the most rapidly elongating, earliest attaching blastocyts creating a hostile environment for their littermates. Cytokines are very important in maintenance of pregnancy and successful pregnancy is associated with local and systemic shifts from type 1 to type 2 cytokines in many species. Data on pregnancy-induced cytokine shifts are not available in pigs. Porcine conceptus attachment is reported to induce inflammatory cytokines including IL-1β, TGF-β, TNF-α and IFN-γ. In pigs, IL-1β promotes rapid trophoblast elongation (Ross *et al.* 2003).

The Chinese Meishan is a prolific pig whose litter size (14–16) matches its ovulation rate. Reciprocal embryo transfers between Meishan and Yorkshire identified endometrium as the differential regulator of conceptus survival (Wilson *et al.* 1998). The mechanisms accounting for breed differences in endometrium are poorly understood but endometrium associated with Meishans implantation sites is more heavily vascularised than in North American breeds (Biensen *et al.* 1998).

MATERIALS AND METHODS

To address whether endometrial lymphocytes recruited to the early fetal-maternal interface in commercial swine contribute to endometrial vascular development, changes in gene expression between virgin and peri-attachment uteri were studied using Quantitative Realtime PCR. Relative gene expression was examined in different endometrial regions and compared with that of laser-capture, microdissected (LCM) endometrial lymphocytes and trophoblast from the same implantation site. By GD 20, arresting fetuses could be identified (reduced vascularity, length and weight). Samples from arresting sites

were compared with samples from healthy attachment sites.

Results and discussion

The authors found endometrium was not uniform in its expression of VEGF, the gene selected as a marker for angiogenesis. Highest expression occurred in mesometrial endometrium, the side of arterial entry to the uterus. Uterine lymphocytes were major contributors to endometrial transcription of angiogeneic and oxygen sensing genes (VEGF; HIF-1α), greatly exceeding levels of transcription in trophoblasts. HIF-1α binds to the hypoxia response element in the VEGF promoter and is a prime regulator of oxygen homeostasis and angiogenesis. Progressive elevation in HIF-1α expression was observed in healthy conceptus attachment sites suggesting HIF-1α transcription may be promoted by conceptus growth. Maternal endometrium and its lymphocytes cease transcription of angiogenic factors at sites with arresting conceptuses. This change was simultaneous with greatly elevated but highly localised transcription of pro-inflammatory cytokines. Endometrial lymphocytes may sense local (ie individual conceptus) changes in trophoblast as 'danger signals' and respond by destroying their newly promoted endometrial vascular network. Lymphocytes in healthy porcine implantation sites transcribed IFN-γ. Previously, porcine implantation site IFN-γ was only attributed to trophoblast (La Bonnardiere et al. 1991). Our relative quantification studies indicated that lymphocytes, not trophoblasts, are likely to be the major producers of this cytokine during the third week of pig pregnancy. As trophoblasts lack the receptor for IFN-γ, uterus is the most probable target of trophoblastic interferons.

The present study also examined the FASL/FAS system. Trophoblast, endometrium and endometrial lymphocytes dynamically express both molecules and they are differentially in viable and arresting attachment sites. Endometrium and lymphocytes associated with arresting fetuses elevate FAS L and FAS transcription. Elevated FAS L would be expected to protect endometrial lymphocytes and some additional cell types from death. Elevation of FAS in endometrium suggests however that there are maternal targets. FAS expression was elevated in trophoblast of arresting conceptuses as were IFN-γ and IL-15. This appears to be trophoblast's attempt to survive.

The authors have documented dynamic and simultaneous peri-implantation changes in transcription of angiogenic, oxygen sensing, cytokine and cell death pathways in trophoblast, endometrium and maternal lymphocytes in pigs. Changes differ between healthy and apparently normal but arresting conceptus sites. The normal peri-implantation reduction in pig litter size and absence of fetal and maternal cross contamination of collected tissues makes pigs an excellent research model of peri-implantation spontaneous loss of apparently normal conceptuses.

Acknowledgements

This research work was supported by Ontario Pork, Ontario Ministry of Agriculture Food and Rural Affairs, Agriculture Canada and National Science Engineering and Research Council.

References

Anderson, L. H., Christenson, L. K., Christenson, R. K. and Ford, S. P. (1993) Investigations into the control of litter size in swine: II. Comparisons of morphological and functional embryonic diversity between Chinese and American breeds. *J. Anim. Sci.* **71,** 1566-1571.

Ashkar, A. A., Di Santo, J. P. and Croy, B. A. (2000) Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J. Exp. Med.* **192,** 259-720.

Biensen, N. J., Wilson, M. E. and Ford, S. P. (1998) The impact of either a Meishan or Yorkshire uterus on Meishan or Yorkshire fetal and placental development to days 70, 90, and 110 of gestation. *J. Anim. Sci.* **76,** 2169-2176.

Croy, B. A., Waterfield, A., Wood, W. and King, G. J. (1988) Normal murine and porcine embryos recruit NK cells to the uterus. *Cell Immunol.* **115,** 471-480.

Croy, B. A., He, H., Esadeg, S., Wei, Q., McCartney, D., Zhang, J., Borzychowski, A., Ashkar, A. A., Black, G. P., Evans, S. S., Chantakru, S., van den Heuvel, M., Paffaro, V. A. Jr. and Yamada A. T. (2003) Uterine natural killer cells: insights into their cellular and molecular biology from mouse modelling. *Reproduction* **126,** 149-160.

Engelhardt, H., Croy, B. A. and King G. J. (2002) Conceptus influences the distribution of uterine leukocytes during early porcine pregnancy. *Biol. Reprod.* **66,** 1875-1880.

La Bonnardiere, C., Martinat-Botte, F., Terqui, M., Lefevre, F., Zouari, K., Martal, J. and Bazer F. W.

(1991) Production of 2 species of interferon by Large White and Meishan pig conceptuses during the peri-attachment period. *Reprod. Fertil.* **91,** 469-478.

Li, X. F., Charnock-Jones, D. S., Zhang, E, Hiby, S., Malik, S., Day, K., Licence, D., Bowen, J., Gardner, M L., King, A., Loke, Y. W. and Smith, S. K. (2001) Angiogenic growth factor messenger ribonucleic acids in uterine natural killer cells. *J. Clin. Endocrinol. Metab.* **86,** 1823-1834.

Ross, J. W., Malayer, Jr., Ritchey, J. W. and Geisert, R. D. (2003) Characterisation of the interleukin-1beta system during porcine trophoblastic elongation and early placental attachment. *Biol. Reprod.* **69,** 1251-1259.

Wilson, M. E., Biensen, N. J., Youngs, C. R. and Ford, S. P. (1998) Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. *Biol. Reprod.* **58,** 905-910.

Winther, H., Ahmed, A. and Dantzer, V. (1999) Immunohistochemical Localisation of Vascular Endothelial Growth Factor (VEGF) and its Two Specific Receptors, Flt-1 and KDR, in the Porcine Placenta and Non-pregnant Uterus. *Placenta.* **20,** 35-43.

SPECIFIC PLACENTAL LESIONS ARE ASSOCIATED WITH HUMAN MATERNAL THROMBOPHILIA

G. Machin

3931 Cherrilee Crescent, Victoria, British Columbia, V8N 1R7, Canada

Maternal thrombophilia (MT) is associated with adverse obstetric outcomes, including early, severe pregnancy-induced hypertension; intrauterine growth restrictions (IUGR); fetal loss; abruption; maternal venous thombosis/embolism.

Adverse effects on fetal outcome are presumably mediated via the placenta. Previous small published series have shown that MT is associated with 3 placental lesions in which there is heavy fibrin deposition or actual thrombosis. This paper reports on a large series of cases with maternal floor infarction (MFI), massive perivillous fibrin deposition (MPVFD) or fetal stem villous arterial placental thrombosis (SVAT) in which MT testing was done (Table 1).

The material was derived from a group of 714 placentae with MFI, MPVFD and SVAT, alone or in combination. 31% had MT, many being genetic

TABLE 1: Frequency of thrombophilia in placental thrombotic diseases

Lesion	MFI	MPVFD	SVAT	MFI + MPVFD	SVAT + MPVFD	All 3 lesions	Total
Total	105 (100)	498	55	40	12	4	714
Tested for thrombophilia, n, (%)	40 (38)	70 (14)	7 (12)	17 (42)	3 (25)	1 (25)	138 (19)
Thrombophilia positive, n (%)	16 (14)	15 (21)	5 (71)	5 (29)	1 (33)	1 (100)	43 (31)

TABLE 2: Frequencies of specific genetic thrombophilias in placental thrombatic diseases

Lesion	MFI	MPVFD	SVAT	MFI + MPVFD	SVAT + MPVFD	All 3 lesions	Total
Total	16 (100)	15	5	5	1	1	43
S defic	5 (31)	3 (20)	2 (40)	1	0	0	11(26)
C defic	0	1 (5)	0	0	0	0	1 (2)
ATIII defic	0	1 (5)	1 (20)	0	0	0	2 (5)
V-L heteroz	3 (19)	3 (20)	1 (20)	2	0	0	9 (21)
V-L homoz	1 (6)	0	0	0	0	0	1
MTHFR	2 (12)	0	0	0	1	0	3 (7)
LipoA	0	2	0	1	0	0	3 (7)
Multigenic	0	1 (VL/S)	0	0	0	1 (S/C/ATIII)	2
LAC	3 (19)	1 (5)	0	0	0	0	4 (9)
ACL	2 (12)	3 (20)	1 (20)	1	0	0	7 (16)

(Table 2). Thrombophilias tested were: protein S deficiency (S defic), protein C deficiency (C defic), antithrombin III deficiency (ATIII), factor V Leiden mutation, heterozygous and homozygous (V-L), methylene tetrahydrofolate reductase thrombophilic mutations (MTHFR), lipoproteinA abnormalities (LipoA), lupus anticoagulant (LAC) and anticardioplipin antibodies (LAC).

Because the genetic thrombophilias are autosomal dominantly inherited, all cases have 50% possibility that the fetus is also damaging the placenta, quite apart from any paternally-inherited mutations. This may explain, for example, the 50% recurrence risk for MFI. Population gene frequencies are well known for the genetic thrombophilias. Gene frequency is low for deficiencies in ATIII, S and C, with little bio-ethnic variability. However, for V-L and MTHFR, population gene frequencies have bio-ethnic variability and the mutant alleles are quite common in the Caucasian population, reaching levels of 6–10%. In this series, the pick-up rate for protein S deficiency (26%) was striking, at 300 x the population gene frequency of 0.08%.

Genetic thrombophilias are subtle disorders in which the fetus may adversely affect its own environment, but by a genetic predisposition.

Management for MT has been measured in terms of decreased risk for thrombosis/embolism in future pregnancies. No studies have assessed fetal outcomes with low dose heparin therapy, although anecdotal cases appear promising. Because genetic thrombophilia poses a life-long risk in both parents and the child for thrombotic events, thrombophilia testing of all 3 genetic contributors may offer significant benefits for treatable entities, eg MTHFR mutants.

Specific placental lesions offer an efficient method for ascertaining thrombophilic individuals. However, other factors may be operating in these placental lesions, especially syncytiotrophoblastic cell surface abnormalities that predispose to fibrin deposition. These might act synergistically to produce severe degrees of MFI and MPVFD. All of these placental lesions may indicate a tendency for the fetus to lay down fibrin or thrombose vessels in other organs beside placenta, offering a foundation for some cases of brain damage, including cerebral palsy.

PLACENTAL PERTURBATIONS AND PREGNANCY OUTCOME: A COMMON THREAD

C. T. Roberts, A. N. Sferruzzi-Perri, K. L. Kind, J. S. Robinson and J. A. Owens

Research Centre for Reproductive Health, Department Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia 5005

A variety of perturbations of pregnancy are characterised by restricted fetal growth, evident near term. These include maternal undernutrition and cytokine deficiency. Besides the obvious influence of the fetal genome, fetal growth and outcome are regulated by maternal nutrition, which determines the availability of nutrients to the fetus both directly, and indirectly by influencing placental growth and function, which controls their transfer together with oxygen to the fetus.

Recent evidence in animals and humans suggests that factors, which induce poor growth in fetal life, can have persistent effects on the body's structure, physiology and metabolism (Barker 1998). Epidemiological and other evidence in humans shows that low birth weight and/or thinness at birth is associated with a high incidence of adult onset diseases, including type 2 diabetes mellitus, hyperlipidaemia, cardiovascular disease and hypertension in the adult (Barker 1998).

It has been estimated that 70–80% of intrauterine growth restriction is a consequence of placental insufficiency (Regnault *et al.* 2002). Therefore, understanding the cellular and molecular mechanisms involved in placental morphogenesis is critical in identifying potential targets in the prevention or treatment of common pregnancy complications. Studies in non-human species have revealed that not only does maternal undernutrition reduce the abundance of substrates in the maternal circulation required for fetal growth; it also alters placental structural development in a manner that indicates reduced capacity for exchange. These outcomes of nutritional restraint for structural determinants of placental function are similar in both guinea pigs and mice (Roberts *et al.* 2001a and in preparation). The insulin-like growth factors (IGFs) are major peptide mediators of nutritional and endocrine influences on growth and differentiation of many cell types throughout life and IGF-II, in particular, is strongly implicated in placental growth and functional development. Conveniently, the region of the placenta devoted to substrate exchange in these species, the placental labyrinth, is separate to the region of the placenta that synthesises hormones and from which trophoblasts germinate and/or invade the decidua. In mice this is called the junctional zone and in guinea pigs it is the interlobium. We have examined placental size and structure in both mice and guinea pigs after the imposition of mild or moderate maternal food restriction or following maternal treatment with insulin-like growth factor (IGF)–II in early pregnancy.

Mild maternal feed restriction (95% *ad libitum* intake) from before and throughout pregnancy in mice did not alter fetal weight in mid pregnancy (Day 13) but reduced it by 20% near term (Day 18) (Roberts *et al.* 2000). Concomitantly, fetal resorptions were increased by mid gestation, and more than doubled near term, in response to maternal feed restriction. The proportion of the placenta composed of the labyrinth was substantially reduced near term (-16%), while that of the junctional zone was increased (+21%) by mild maternal undernutrition. It has previously been shown that in the mouse, maximum placental volume is reached by Day 16.5, but the proportion of the placenta composed of the labyrinth continues to increase until Day 18.5 at the expense of the junctional zone (Coan *et al.* 2004). We therefore conclude that the changes we have seen may reflect a delay in placental structural and functional maturation.

Similarly, in guinea pigs, moderate maternal feed restriction (70% of *ad libitum* fed/g

bodyweight from 4 weeks before and until Day 35, then 90% of *ad libitum* fed/g bodyweight until Day 60 of pregnancy) prevented this typical increase in the proportion of the placenta that is labyrinthine between mid and late gestation (Roberts *et al.* 2001a). Consequently, the proportion (-70%) and volume (-31%) of the placenta that was devoted to exchange were reduced near term in response to maternal feed restriction. In addition, the weight of both the labyrinth (-20%) and interlobium (-23%) were reduced at mid gestation, while the latter was much less affected near term, in the feed restricted pregnant guinea pig. This shows a clear adverse effect on the exchange component of the placenta and suggests it might be particularly sensitive to nutrient restraint.

Maternal feed restriction in the guinea pig reduced fetal weight in mid (-30%) and late gestation (-31%), but to a lesser extent than placental weight, increasing the ratios of placental to fetal weight (+13–14%) (Roberts *et al.* 2001a). As adults, the offspring of such mothers are hypercholesterolaemic (Kind *et al.* 1999) and have impaired glucose tolerance, hyperinsulinaemia and increased blood pressure (Kind *et al.* 2002; Kind *et al.* 2003). Thus early life programming of syndrome X or the metabolic syndrome occurs in the guinea pig and appears to involve restricted placental functional capacity as well as reductions in the abundance of nutrients in the maternal circulation for transfer to the fetus.

In the guinea pig, as in the mouse, the proportion of the placenta composed of labyrinth increases (+32%) while that accounted for by the interlobium decreases (-42%) between mid and late gestation (Roberts *et al.* 2001a). Consequently, labyrinthine weight increases nearly 5-fold and that of the interlobium about 4-fold over this period in the guinea pig (Roberts *et al.* 2001a). Not only is the former change virtually abolished by maternal feed restriction as noted earlier; any consequences for substrate transfer capacity are exacerbated by additional structural changes within the labyrinth. Thus the surface density of trophoblast, which reflects the exchange surface area per gram of placenta, is reduced by about a third at mid and late gestation in feed restricted mothers (Roberts *et al.* 2001a). Together with the changes in placental and labyrinth size, this reduced total exchange surface area by 36% in mid gestation and 60% in late gestation. In addition, maternal feed restriction increased the arithmetic mean barrier thickness for diffusion in the labyrinthine placenta (+60–70%) at mid and late gestation, which would further reduce substrate transfer capacity.

Consistent with its major role in placental development, IGF-II deletion in mice reduces placental growth and subsequently fetal growth (DeChiara *et al.* 1990). Deletion of placental specific IGF-II transcripts in mice has similar effects which mirror substantially the responses of placental and fetal growth to maternal undernutrition in this species and in the guinea pig (Sibley *et al.* 2004). The placental specific IGF-II deletion also has similar consequences for the abundance and structure of the placental labyrinth to those of maternal undernutrition. This may in part reflect changes in IGF-II abundance induced by maternal undernutrition, which reduces circulating insulin-like growth factor (IGF)–II in the mother in mid pregnancy and the ratio of IGF-II to IGF binding protein-2 (IGFBP-2) in late pregnancy. These endocrine changes correlate with changes in placental structure and suggest that in mid-pregnancy, maternal circulating IGF-II promotes placental structural development, while later in pregnancy, IGFBP-2 inhibits it, and the relative abundance of IGF-II and this BP strongly influences placental structure and function near term (Roberts *et al.* 2001b).

Consistent with this, maternal IGF-II treatment in early to mid pregnancy in the guinea pig increases placental weight at mid gestation (+40%) (Sohlstrom *et al.* 2001) and increases fetal weight near term (+11%) (Sferruzzi-Perri *et al.* 2005). Importantly, maternal IGF-II treatment substantially reduced the number of fetal resorptions (-60%) and increased the number of viable fetuses (+25%). Although placental weight near term was unaffected by the earlier maternal IGF-II treatment, labyrinth volume was increased (+30%) and that of the interlobium unchanged.

In conclusion, the effects of undernutrition of the pregnant mother on fetal and placental development may be mediated, at least in part, by reductions in IGF-II bioavailability, that may be a consequence of reduced placental and/or maternal tissue IGF-II synthesis. Both undernutrition and loss of IGF-II are associated with a delay and apparent reduction in placental structural and functional development. Treating the normal pregnant mother with IGF-II appears to have the opposite effect. We therefore propose that IGF-II deficiency in the mother and/or placenta may

occur in many cases of fetal growth restriction and that treating the pregnant mammal at risk may prevent these cases.

REFERENCES

Barker, D.J.P. (1998) Mothers, babies and health in later life. 2nd ed Churchill Livingstone, London.

Coan, P.M., Ferguson-Smith, A.C. and Burton, G.J. (2004) Developmental dynamics of the definitive mouse placenta assessed by stereology. *Biol. Reprod.* **70,** 1806-1813.

DeChiara, T.M., Efstratiadis, A. and Robertson, E.J. (1990) A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345,** 78-80.

Kind, K.L., Clifton, P.M., Grant, P.A., Owens, P.C., Sohlstrom, A., Roberts, C.T., Robinson, J.S. and Owens, J.A. (2003) Effect of maternal feed restriction during pregnancy on glucose tolerance in the adult guinea pig. *Am. J. Physiol.* **284,** R140-152.

Kind, K.L., Clifton, P.M., Katsman, A.I., Tsiounis, M., Robinson, J.S. and Owens, J.A. (1999) Restricted fetal growth and the response to dietary cholesterol in the guinea pig. *Am. J. Physiol.* **277,** R1675-1682.

Kind, K.L., Simonetta, G., Clifton, P.M., Robinson, J.S. and Owens, J.A. (2002) Effect of maternal feed restriction on blood pressure in the adult guinea pig. *Ex. Physiol.* **87,** 469-477.

Regnault, T.R.H., de Vrijer, B. and Anthony, R.V. (2002) The IGF-II-deficient placenta: aspects of its function. *Trends in Endocrinology and Metabolism* **13,** 410-412.

Roberts, C.T., Ghaoui, R., Fung, D., Khong, T.Y., Robinson, J.S. and Owens, P.C. (2000) The effect of restricted maternal nutrition on fetal weight and decidual leukocyte numbers in mice. In: *14th Rochester Trophoblast Conf.* New York.

Roberts, C.T., Sohlstrom, A., Kind, K.L., Earl, R.A., Khong, T.Y., Robinson, J.S., Owens, P.C. and Owens, J.A. (2001a) Maternal food restriction reduces the exchange surface area and increases the barrier thickness of the placenta in the guinea-pig. *Placenta* **22,** 177-185.

Roberts, C.T., Sohlstrom, A., Kind, K.L., Grant, P.A., Earl, R.A., Robinson, J.S., Khong, T.Y., Owens, P.C. and Owens, J.A. (2001b) Altered placental structure induced by maternal food restriction in guinea pigs: a role for circulating IGF-II and IGFBP-2 in the mother? *Placenta* **22,** S77-S82.

Sferruzzi-Perri, A.N., Owens, J.A., Pringle, K.G., Robinson, J.S. and Roberts, C.T. (2005) Exogenous insulin like growth factors in early pregnancy act via different mechanisms to promote fetal growth. *International Federation of Placenta Associations, Glasgow, Scotland.*

Sibley, C.P., Coan, P.M., Ferguson-Smith, A.C., Dean, W., Hughes, J., Smith, P., Reik, W., Burton, G.J., Fowden, A.L. and Constancia, M. (2004) Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. *PNAS* **101,** 8204-8208.

Sohlstrom, A., Fernberg, P., Owens, J.A. and Owens, P.C. (2001) Maternal nutrition affects the ability of treatment with IGF-I and IGF-II to increase growth of the placenta and fetus, in guinea pigs. *Growth Hormone and IGF Res.* **11,** 392-398.

GROSS AND HISTOLOGICAL OBSERVATIONS ON PLACENTAE FROM ABNORMAL PREGNANCIES

S. Wilsher, J. Ousey and W. R. Allen

University of Cambridge, Department of Veterinary Medicine Equine Fertility Unit, Mertoun Paddocks, Newmarket, Suffolk, CB8 9BH, UK

The development and function of the placenta directly influences the growth and well-being of the fetus in utero. Hence, shortfalls in either the structure of the placenta or its function will be reflected in fetal development. Morphological parameters of the placentae from normal singleton Thoroughbred foalings were described by Whitwell and Jeffcott (1975). More recently, stereological techniques have been used to establish baseline values for the surface density of microcotyledons (Sv), the total microscopic area of feto-maternal contact and placental efficiency for placentae from Thoroughbred mares of varying ages and parities (Wilsher and Allen 2003). In cases of abortion or stillbirth it is common practice for the fetus and placenta to be submitted to a pathology laboratory for investigations. However, in cases of intrauterine growth retardation (IUGR), prolonged gestation, premature placental separation (PPS) and other morphological abnormalities of the placenta or fetus, the placenta is rarely subjected to a full examination, particularly if the foal is viable. In the present study placentae were collected from 20 Thoroughbred mares that had abnormal pregnancies or foals. The cases were grouped on the basis of either a grossly abnormal appearance of the placenta, or an abnormal feature of the pregnancy or foal. Two of the groups are discussed in this abstract. Namely, placentae with abnormal linear dimensions associated with limb deformities in the foal and placentae from mares that showed a prolonged gestation.

Placentae were examined from a number of foals that exhibited flexural limb deformities (n=7), often incorrectly termed contracted tendons. The severity of the deformity ranged from mild (ie responded favourably to treatment) to severe (ie resulting in dystocia and subsequent euthanasia of the foal). In one case the foal exhibited multiple congenital abnormalities. The aetiology of this condition remains unknown, but malpostioning or lack of space *in utero* is popularly blamed. However, large foals or twin foals do not have a greater incidence. Some authors have reported that the incidence of flexural deformities increases with the feeding of a high protein diet given to the mare in late pregnancy.

A number of abnormalities were observed in the placentae of these foals. Linear dimensions were reduced, most notably in the width of both the body and the horns of the placentae. In addition, invagination and folding of the allantochorion was observed in many instances, often at the base of the horns which resulted in curved avillous bands on the chorionic surface. This appeared to have resulted from inelasticity of the blood vessels, that had not expanded to accommodate the growing placenta. Furthermore, a marked degree of oedema was usually associated with these placentae, including accumulation of fluid in the extra-embryonic coelome (EEC). Thus, it appeared that the fetus had been subjected to cramped conditions *in utero* although the underlying reason for the lack of expansion of the allantochorion and the build up of fluid within the EEC remained unclear. Were, for example, the limb deformities actually caused by the cramped conditions *in utero*, or was the lack of expansion of the allantochorion a consequence of reduced fetal movement due to a genetic deformity? A reduction in fetal movements may well lead to a lessening of the mechanical stimuli that determine uterine expansion throughout gestation (Rice 1998). On the other hand, Ginther (1993) noted that a gradual decrease in fetal mobility in the pregnant mare is associated with fetal growth, a

concomitant decrease in the volume of allantoic fluid and closure, by constriction, of the uterine horns. Hence, changes in fluid movement and possibly also hyper-constriction of the uterine body and base of the 2 horns may result in the cramped conditions in late gestation.

Two mares in the survey exhibited prolonged gestations, one a 5-year-old maiden that delivered a healthy foal after 360 days gestation and the other a 15-year-old multiparous mare that produced a non viable foal after 418 days of gestation. The former exhibited a placenta of normal size but this showed a greater than normal density of small, terminal villi on the microcotyledons and a stereologically determined microcotyledon surface density (Sv) of 0.053 μm^{-1}, that was well above the expected value for a mare of that age and parity ($0.034 \pm 0.001 \mu m^{-1}$). Thus, this case of 'hypermaturity' in an equine placenta seemed to mirror the situation in prolonged human pregnancy where hypermaturity of the villous tree results in numerous long, branched and twisted terminal villi (Kaufmann *et al.* 1987). In the second case of prolonged equine gestation, the placenta was exceptionally small, both in terms of its linear measurements and its weight and reflected the small size of the foal (20 kg). Furthermore, the villi showed atrophy and calcification rather than hyperplasia. Thus, the marked gross and histological differences exhibited by these 2 placenta highlight the multifactorial pathogenesis that underlies prolonged gestation in the mare. Although gross examination of the fetal membranes in their entirety affords an opportunity to assess the *in utero* environment, histological examination of the chorionic surface of the diffuse, epitheliochorial equine placenta gives a unique mirror image of the extent and integrity of its attachment to the endometrium via the multitudinous microcotyledons. This, in turn, provides valuable information on the total area of feto-maternal contact and, hence, the growth and well-being of the fetus.

References

Ginther, O.J (1993) Equine fetal kinetics: allantoic-fluid shifts and uterine-horn closures. *Theriogenology* **40,** 241-256.

Kaufmann, P., Luckhardt, M., Schweikhart, G. and Cantle, S.J. (1987) Cross-sectional features and three-dimensional structure of human placental villi. *Placenta* **8,** 235–248.

Rice, G. (1998) Mechano-transcription pathways involved in determining placental size. *Equine vet. J.* **30,** 457.

Wilsher, S. and Allen, W.R. (2003) The effects of maternal age and parity on placental and fetal development in the mare. *Equine vet. J.* **35,** 476-483.

Whitwell, K.E. and Jeffcott, L.B (1975) Morphological

LIST OF PARTICIPANTS

Twink Allen
United Kingdom

Tahera Ansari
United Kingdom

Rebecca Baergen
United States

Graham Burton
United Kingdom

Steve Charnock-Jones
United Kingdom

Jay Cross
Canada

Allen Enders
United States

Berthold Huppertz
Germany

John Kingdom
Canada

Geoff Machin
Canada

Terry Mayhew
United Kingdom

Christiane Pfarrer
Germany

Gene Pranzo
United States

Claire Roberts
Australia

Paul Sibbons
United Kingdom

Chandrakant Tayade
Canada

Jan Wade
United Kingdom

Katherine Whitwell
United Kingdom

Sandra Wilsher
United Kingdom

AUTHOR INDEX