CSIRO PUBLISHING

www.publish.csiro.au/journals/rfd *Reproduction, Fertility and Development*, 2006, **18**, 71–76

Placental function in development and disease

James C. Cross

Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta T2N 4N1, Canada. Email: jcross@ucalgary.ca

Abstract. The placenta is an organ that clinicians and embryologists would all agree is important for pregnancy success. Unfortunately, however, they too often ignore it when they are exploring causes for embryonic, fetal and perinatal complications. The core function of the placenta is to mediate the transport of nutrients between the maternal and fetal circulation, but it also has critical endocrine functions that alter different maternal physiological systems in order to sustain pregnancy. Both its development and ongoing functions can be dynamically regulated by environmental factors, including nutrient status and tissue oxygenation. In recent years, mainstream attention has begun to shift onto the placenta and it is now becoming clear that placental pathology is associated with several complications in human and animal pregnancies, including embryonic lethality, fetal growth restriction, pre-eclampsia and the high rates of fetal deaths observed after nuclear transfer (cloning).

Introduction

The placenta is the first of the fetal organs to develop and has several fascinating and critical functions. It mediates implantation and establishes the interface for nutrient and gas exchange between the maternal and fetal circulation, as well as initiating maternal recognition of pregnancy, altering the local immune environment and altering maternal cardiovascular and metabolic functions through the production of paracrine and endocrine hormones. Abnormalities in any one of these functions can be associated with poor pregnancy outcome, ranging from the mild (intrauterine growth restriction; IUGR) to the severe (implantation failure and embryonic, fetal or perinatal death). The present review describes recent insights into how placental development and function are regulated and the potential role for primary placental pathologies in explaining a variety of complications in human and animal pregnancies.

General anatomy of the placenta

The gross anatomy of the placenta varies considerably among mammalian species (Perry 1981; Leiser and Kaufmann 1994; Wooding and Flint 1994). Given these differences, people often hesitate to extrapolate information from one species to another. This is unfortunate because, when one looks at the core functional cell types, several features are conserved across species as diverse as humans, rodents, equids and ruminants (Cross *et al*. 2003). At the most basic level, the fetal placenta is composed of an outer epithelial layer derived from the trophectoderm (trophoblast) and an inner vascular and stromal layer derived from the allantois (extra-embryonic mesoderm). The trophoblast layer goes on to subspecialise and, in general, fulfils two functions in every mammalian species. First, it generates the extensive surface area for nutrient exchange and provides the barrier between the maternal and fetal circulation. Second, trophoblast cells interact closely with the uterus and produce hormones that target maternal physiological systems and promote maternal blood flow to the implantation site.

In most mammals, the trophoblast layer becomes organised into highly branched villous structures, although how the villi are organised varies considerably (Wooding and Flint 1994). In pigs, the villi are spread diffusely across the placenta. In other species, they are clustered either into a band (horse) or into cotyledons. Rodents have a single cotyledon, ruminants (sheep, cow) have multiple cotyledons scattered across the placental surface and humans have multiple cotyledons that are grouped together resembling a single disc. In rodents and primates, the uterine epithelium is eroded such that maternal blood comes into direct contact with the trophoblast surface of the villi (haemochorial). Rodents have three trophoblast cell layers, including two syncytial layers and a single mononuclear cell type, whereas primates have a single syncytial layer plus an underlying mononuclear trophoblast cell layer. In contrast with these invasive species, the endometrium remains intact (epitheliochorial) in pigs, ruminants and horses. The invasive/endocrine trophoblast cell subtype varies considerably in the extent of uterine invasion. Pig trophoblast cells do not invade at all and ruminant binucleate cells migrate essentially only the distance of one cell diameter to enter the endometrium (Wooding and Flint 1994). In contrast, rodent trophoblast giant cells and glycogen trophoblast cells migrate several hundred microns into the uterus in association with spiral arteries and diffusely

 10.1071/RD05121 1031-3613/06/020071

into the interstitium of the decidua, respectively (Adamson *et al*. 2002). Extravillous cytrophoblast cells in primates also invade in these two patterns, but much more deeply, and, indeed, normally as far as the myometrial cell layer of the uterus (Pijnenborg 1996).

Nutritional and endocrine functions of the placenta

The function of the placenta during early gestation is primarily to mediate implantation of the embryo into the uterus and, secondarily, to produce hormones that induce maternal recognition of pregnancy, the term used to describe the means by which the lifespan of the corpus luteum is extended, preventing the end of the ovarian cycle. The specific hormones used to induce maternal recognition of pregnancy vary among species. Pig embryos secrete oestrogen (Flint *et al*. 1978), whereas ruminant embryos produce an interferon (Spencer *et al*. 2004) to suppress uterine production of the luteolytic factor prostaglandin $F_{2\alpha}$. Primate (Hearn *et al*. 1991) and horse (Allen 1978) embryos produce a chorionic gonadotrophin that directly stimulates luteal progesterone production. In rodents, mating alone produces a prolonged luteal phase (pseudopregnancy) and then placental lactogens, which are produced in the second half of gestation, have luteotrophic effects (Forsyth 1994).

After implantation, the major function of the placenta is to mediate, as well as to regulate, nutrient uptake from the mother to the fetus. It does so by forming the highly branched villous structure and establishing its own circulation connected to the umbilical cord (Watson and Cross 2005). Different nutrients transfer either passively or by active transporters (Hay 1994; Reik *et al*. 2003; Watson and Cross 2005). The placenta is also able to accumulate glycogen and this is most marked in rodents, in cells called glycogen trophoblast cells (Simmons and Cross 2005). In mice, glycogen accumulation is limited to the latter half of gestation and specifically occurs in a subset of cells in the spongiotrophoblast layer after embryonic day 12.5 that later invade the wall of the uterus (Adamson *et al*. 2002). Glycogen accumulation is increased in diabetic pregnancies (Barash *et al*. 1985; Padmanabhan and Shafiullah 2001), but is stimulated by insulin (Goltzsch *et al*. 1987). In contrast, glycogen content is reduced in insulin-like growth factor (IGF)-II mutant placentas (Lopez *et al*. 1996), suggesting that IGF-II may act in a paracrine manner within the placenta to regulate glucose uptake and the synthesis of glycogen, perhaps by acting through the insulin receptor. Glycogen deposits are a common pathological finding in different tissues, but because placental accumulation occurs in all pregnancies and it appears to be highly regulated, it may have a normal physiological function.

In addition to having these functions in nutrient uptake, the placenta also plays an active role in regulating maternal physiology to the nutritional benefit of the fetus. First, the invasive trophoblast population produces angiogenic factors and vasodilators and, in some species, actually invades the arteries also to increase blood flow to the implantation site (Cross *et al*. 2002). Second, the placenta has been shown, in several species, to produce hormones that stimulate the pregnancy associated increase in maternal blood cell production and blood volume (Conrad *et al*. 1996; Kim *et al*. 2001; Klemcke *et al*. 2001; Zhou *et al*. 2002, 2005). Third, the placenta produces several metabolic hormones, such as placental lactogens and placental growth hormone, that can alter insulin production and promote insulin resistance in maternal tissues, resulting in increased glucose availability to the fetus (Soares *et al*. 1998; Linzer and Fisher 1999). The placenta also produces leptin (Pelleymounter *et al*. 1995) and ghrelin (Tschop *et al*. 2000), hormones that suppress and stimulate appetite, respectively.

Cellular and molecular aspects of placental development

The placenta is derived from two major cell types. The trophoblast cell lineage originates from the trophectoderm at the blastocyst stage. The stromal and vascular components of the placenta are derived from the allantois, which grows out from the embryo proper to make contact with the overlying trophoblast layer. Villous development initiates only at points of chorioallantoic attachment and this process is particularly vulnerable to perturbation by genetic (Watson and Cross 2005), epigenetic (Spindle *et al*. 1996) or nutritional (Wallace *et al*. 1999) influences. The cellular and molecular mechanisms underlying the development of the placenta are best understood in the mouse as a result of studies involving experimental embryology, culture trophoblast stem cells and analysis of mouse mutants that have defects in placental development. We now know of more than 100 genes that are critical for placental development and their functions have been reviewed in detail elsewhere (Hemberger and Cross 2001; Rossant and Cross 2001; Simmons and Cross 2005; Watson and Cross 2005). In addition to cell intrinsic mechanisms, placental development is also highly regulated by oxygen levels (Genbacev *et al*. 1997; Adelman *et al*. 2000; Caniggia *et al*. 2000) and the availability of nutrients in the maternal circulation (Cross and Mickelson 2005). This means that the basic pattern of placental development can be altered by environmental influences and, moreover, that placental development can attempt to compensate for other defects. A good example is the placental phenotype in *Rb* mouse mutants. The mutant placenta shows poor villous development, but the villi that do form are hypervascularised (Wu *et al*. 2003).

Placental pathologies associated with pregnancy complications

Placental mal-development in cloned animals

Attempts to clone most animal species using nuclear transfer technology have met with very high rates of embryonic,

fetal and perinatal mortality (Sakai *et al*. 2005). Although the specific pathologies in the fetus vary, changes in the placenta are the most common finding. Initial studies had described only the placenta in those rare pregnancies that made it to term. In cattle and sheep, the term placenta in clones is characterised by a reduced number of cotyledons, hydroallantois and hypertrophy of remaining placentomes (Hill *et al*. 1999; De Sousa *et al*. 2001). Interestingly, the maternal caruncles are also underdeveloped, implying that primary fetoplacental defects indirectly affect the mother also (Hashizume *et al*. 2002). In more recent studies, examination of first trimester, cloned conceptuses showed that failure to establish cotyledons is common and likely accounts for the large number of conceptuses that fail during the first trimester (Hill *et al*. 2000; Hashizume *et al*. 2002). Presumably only those conceptuses that have a threshold minimum number of cotyledons are able to survive and make it to term. Because cotyledonary size can undergo a compensatory increase if the number of placentomes is reduced by carunclectomy (Anthony *et al*. 2003), the hypertrophy observed in placentas from clones is likely to be simply a secondary change. In mice, the term placenta in clones is enlarged owing to expansion of the spongiotrophoblast layer, but whether this is a primary or secondary change is unclear (Tanaka *et al*. 2001; Ogura *et al*. 2002; Singh *et al*. 2004).

The common finding of placental defects in cloned mice, cows and sheep suggests placental pathology as a major cause of pregnancy complications. This hypothesis has been supported by chimera studies in which tetraploid blastomeres are mixed with preimplantation cloned embryos. In these chimeras, the tetraploid cells can contribute to trophoblast derivatives, but not the embryos/fetus proper (Cross 2001). Tetraploid chimeras in both mice (Eggan *et al*. 2001) and cattle (Iwasaki *et al*. 2000) do better than regular clones, implying that trophoblast lineage defects in clones account for at least some of the pregnancy complications.

Placental dysfunction in human intrauterine growth restriction and pre-eclampsia

Placental pathology has long been associated with IUGR and pre-eclampia in humans. However, it is important to note that the specific changes that are observed can be rather variable. The IUGR placenta most frequently has lesions within the villi, including inflammation and infarcts (Salafia *et al*. 1992, 1995). In severe, early onset disease, there is often a significant reduction in villous surface area (Macara *et al*. 1996), perhaps suggesting primary defects in villous formation. Pre-eclampsia is fundamentally a maternal disease characterised by hypertension, renal glomerulosclerosis leading to proteinuria, uricaemia and, in severe cases, seizures. The placenta in pre-eclampsia usually has a normal weight and surface area (Teasdale 1985). However, there is marked proliferation of villous cytotrophoblast cells (Fox 1964; Jones and Fox 1980; Arnholdt *et al*. 1991; Redline and Patterson 1995) and the syncytiotrophoblast shows focal necrosis (Jones and Fox 1980). In addition, trophoblast invasion of the uterine spiral arteries and their remodelling of the spiral arteries is frequently reduced (Brosens *et al*. 1972; Gerretsen *et al*. 1981; Khong *et al*. 1986). Intrauterine growth restriction is sometimes, although not always, observed in pre-eclampsia. When it is, the surface area of placental villi is reduced and is associated with a decrease in umbilical blood flow (Jackson *et al*. 1995). The underlying cause of pre-eclampsia is a matter of considerable debate, but recent studies in mice suggest that primary fetoplacental lesions are sufficient to initiate the disease (Cross 2003).

Altered placental development in under- and overnourished animal models

Studies in rats, sheep and guinea-pigs have shown that both feed restriction and overfeeding affect development of the placental villi. In rats, protein restriction (8% *v.* 20% dietary protein) from the time of mating leads to an increase in villous surface area without affecting overall placental volume, implying more extensive branching of the villi (Doherty *et al*. 2003). This implies that trophoblast cells can alter their development most likely in an attempt to compensate for the protein restriction, although the underlying mechanism is not clear. Importantly, however, the underlying vasculature volume does not undergo a proportional increase. The pups are mildly growth restricted (approximately 10% smaller by term), most likely because, in the absence of a proportional increase in vascularisation, the uptake efficiency of the placenta may not have improved despite the larger surface area available. Another model involves maternal iron restriction before and during pregnancy, which leads to anaemia. This perturbation also produces placentas with larger villous surface areas, indicating that trophoblast differentiation can be regulated by tissue oxygen levels (Lewis *et al*. 2001). This finding is consistent with observations in knockout mice lacking hypoxia-inducible factor (HIF; Adelman *et al*. 2000). Curiously, despite the fact that angiogenesis in most vascular beds is thought to be regulated by oxygen levels, maternal iron restriction in the rat does not result in a proportional increase in fetoplacental vascular volume (Lewis *et al*. 2001).

In sheep, both feed restriction and overfeeding can lead to IUGR (Wallace *et al*. 1999, 2004; Redmer *et al*. 2004; Reynolds *et al*. 2005). Cotyledon number is most affected by overnourishment during the first trimester when the cotyledons are being formed, whereas cotyledon size is most affected by nutritional status in the second and third trimesters (Wallace *et al*. 1999). Overfeeding during the first and second trimesters results in reduced trophoblast proliferation (Wallace *et al*. 2004) and expression of angiogenic factors (Redmer *et al*. 2004; Reynolds *et al*. 2005) and is associated with smaller cotyledons that are poorly vascularised. Any one of these manipulations is associated with IUGR. Collectively,

these data indicate that, in rats and sheep, at least, nutrient status has major effects on the development of the villous structures in the placenta. The placenta can alter its development in an attempt to compensate but, beyond a certain point, the placenta can no longer compensate for altered nutritional status and IUGR results.

The effect of nutritional status on placental development has also been examined in guinea-pigs, an animal whose placenta has considerable structural similarity to that of humans. The most common guinea-pig model involves severe total feed intake, in which the pregnant dams are fed only 50–70% of the normal *ad libidum* intake, resulting in birthweights that are reduced by approximately 30%. By the end of gestation, the villous layer of the placenta (the labyrinth) is reduced in overall volume and surface area (Dwyer *et al*. 1992; Roberts *et al*. 2001*a*). Therefore, there is no obvious attempt of the guinea-pig placenta to try and compensate for undernutrition. At a mechanistic level, expression of IGF-I and -II, and the ratio of the IGFs to IGF binding proteins, is reduced in response to feed restriction (Sohlstrom *et al*. 1998; Roberts *et al*. 2001*b*, 2002; Olausson and Sohlstrom 2003). Insulin-like growth factor levels are correlated with the extent of villous branching and are inversely correlated with thickness of the trophoblast barrier (Roberts *et al*. 2001*b*, 2002), similar to changes seen in IGF-II-deficient mice (Constancia *et al*. 2002; Sibley *et al*. 2004). The response of the guinea-pig placenta to overall nutrient restriction is obviously rather different than that of the rat and sheep in response to protein and energy restriction, as discussed above. It is difficult to explain the differences at present. They could reflect a species difference or a difference in the effects of protein compared with total diet restriction.

Conclusions and future directions

We have learned, in the past few years, that the development of the placenta is highly regulated and is therefore susceptible to perturbation. Several genetic, epigenetic, nutritional and environmental factors have been identified and are associated with complications in pregnancy outcome ranging from embryonic lethality to fetal growth restriction. However, this work is rather underdeveloped and much more work needs to be performed to bridge insights from different animal models and humans. In addition, because the placenta is involved in mediating nutrient uptake as well as regulating maternal metabolic and cardiovascular function, much more work needs to be performed to understand how an abnormal placenta contributes to pregnancy complications common to humans and animals. Finally, it is clear that clinically useful markers of placental pathology are required in order to provide early diagnosis of pregnancy complications. The challenge to the field is that although many, many markers have been advocated over the years, there are still no clear-cut specific tests of placental function.

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**, 358–373. doi:10.1016/S0012-1606(02)90773-6

Adelman, D. M., Gertsenstein, M., Nagy, A., Simon, M. C., and Maltepe, E. (2000). Placental cell fates are regulated *in vivo* by HIF-mediated hypoxia responses. *Genes Dev.* **14**, 3191–3203. doi:10.1101/GAD.853700

Allen, W. R. (1978). Maternal recognition of pregnancy and immunological implications of trophoblast–endometrium interactions in equids. *Ciba Found. Symp.* **64**, 323–352.

Anthony, R. V., Scheaffer, A. N., Wright, C. D., and Regnault, T. R. (2003). Ruminant models of prenatal growth restriction. *Reprod. Suppl.* **61**, 183–194.

Arnholdt, H., Meisel, F., Fandrey, K., and Lohrs, U. (1991). Proliferation of villous trophoblast of the human placenta in normal and abnormal pregnancies. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **60**, 365–372.

Barash, V., Gutman, A., and Shafrir, E. (1985). Fetal diabetes in rats and its effect on placental glycogen. *Diabetologia* **28**, 244–249. doi:10.1007/BF00282241

Brosens, I. A., Robertson, W. B., and Dixon, H. G. (1972). The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstet. Gynecol. Ann.* **1**, 177–191.

Caniggia, I., Mostachfi, H., Winter, J., Gassmann, M., Lye, S. J., Kuliszewski, M., and Post, M. (2000). Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). *J. Clin. Invest.* **105**, 577–587.

Conrad, K. P., Benyo, D. F., Westerhausen-Larsen, A., and Miles, T. M. (1996). Expression of erythropoietin by the human placenta. *FASEB J.* **10**, 760–768.

Constancia, M., Hemberger, M., Hughes, J., Dean, W., Ferguson-Smith, A., *et al*. (2002). Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* **417**, 945–948. doi:10.1038/NATURE00819

Cross, J. C. (2001). Factors affecting the developmental potential of cloned mammalian embryos. *Proc. Natl Acad. Sci. USA* **98**, 5949–5951. doi:10.1073/PNAS.111182398

Cross, J. C. (2003). The genetics of pre-eclampsia: a feto-placental or maternal problem? *Clin. Genet.* **64**, 96–103. doi:10.1034/J.1399-0004.2003.00127.X

Cross, J. C., and Mickelson, L. (2005). Nutritional influences on implantation and placental development. *Nutr. Rev.*, in press.

Cross, J. C., Hemberger, M., Lu, Y., Nozaki, T., Whiteley, K., Masutani, M., and Adamson, S. L. (2002). Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. *Mol. Cell. Endocrinol.* **187**, 207–212. doi:10.1016/S0303-7207(01)00703-1

Cross, J. C., Baczyk, D., Dobric, N., Hemberger, M., Hughes, M., Simmons, D. G., Yamamoto, H., and Kingdom, J. C. (2003). Genes, development and evolution of the placenta. *Placenta* **24**, 123–130. doi:10.1053/PLAC.2002.0887

De Sousa, P. A., King, T., Harkness, L., Young, L. E., Walker, S. K., and Wilmut, I. (2001). Evaluation of gestational deficiencies in cloned sheep fetuses and placentae. *Biol. Reprod.* **65**, 23–30.

Doherty, C. B., Lewis, R. M., Sharkey, A., and Burton, G. J. (2003). Placental composition and surface area but not vascularization are altered by maternal protein restriction in the rat. *Placenta* **24**, 34–38. doi:10.1053/PLAC.2002.0858

Dwyer, C. M., Madgwick, A. J., Crook, A. R., and Stickland, N. C. (1992). The effect of maternal undernutrition on the growth and development of the guinea pig placenta. *J. Dev. Physiol.* **18**, 295–302.

Eggan, K., Akutsu, H., Loring, J., Jackson-Grusby, L., Klemm, M., Rideout, W. M., 3rd, Yanagimachi, R., and Jaenisch, R. (2001). Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation. *Proc. Natl Acad. Sci. USA* **98**, 6209–6214. doi:10.1073/PNAS.101118898

Flint, A. P., Burton, R. D., Gadsby, J. E., Saunders, P. T., and Heap, R. B. (1978). Blastocyst oestrogen synthesis and the maternal recognition of pregnancy. *Ciba Found. Symp.* **64**, 209–238.

Forsyth, I. A. (1994). Comparative aspects of placental lactogens: structure and function. *Exp. Clin. Endocrinol.* **102**, 244–251.

Fox, H. (1964). The villous cytotrophoblast as an index of placental ischaemia. *J. Obstet. Gynaecol. Br. Commonw.* **71**, 885–893.

Genbacev, O., Zhou, Y., Ludlow, J. W., and Fisher, S. J. (1997). Regulation of human placental development by oxygen tension. *Science* **277**, 1669–1672. doi:10.1126/SCIENCE.277.5332.1669

Gerretsen, G., Huisjes, H. J., and Elema, J. D. (1981). Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation. *Br. J. Obstet. Gynaecol.* **88**, 876–881.

Goltzsch, W., Bittner, R., Bohme, H. J., and Hofmann, E. (1987). Effect of prenatal insulin and glucagon injection on the glycogen content of rat placenta and fetal liver. *Biomed. Biochim. Acta* **46**, 619–622.

Hashizume, K., Ishiwata, H., Kizaki, K., Yamada, O., Takahashi, T., *et al*. (2002). Implantation and placental development in somatic cell clone recipient cows. *Cloning Stem Cells* **4**, 197–209. doi:10.1089/15362300260339485

Hay, W. W., Jr (1994). Placental transport of nutrients to the fetus. *Horm. Res.* **42**, 215–222.

Hearn, J. P., Webley, G. E., and Gidley-Baird, A. A. (1991). Chorionic gonadotrophin and embryo-maternal recognition during the peri-implantation period in primates. *J. Reprod. Fertil.* **92**, 497–509.

Hemberger, M., and Cross, J. C. (2001). Genes governing placental development. *Trends Endocrinol. Metab.* **12**, 162–168. doi:10.1016/S1043-2760(01)00375-7

Hill, J. R., Roussel, A. J., Cibelli, J. B., Edwards, J. F., and Hooper, N. L. (1999). Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology* **51**, 1451–1465. doi:10.1016/S0093-691X(99)00089-8

Hill, J. R., Burghardt, R. C., Jones, K., Long, C. R., Looney, C. R., *et al*. (2000). Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol. Reprod.* **63**, 1787–1794.

Iwasaki, S., Campbell, K. H., Galli, C., and Akiyama, K. (2000). Production of live calves derived from embryonic stem-like cells aggregated with tetraploid embryos. *Biol. Reprod.* **62**, 470–475.

Jackson, M. R., Walsh, A. J., Morrow, R. J., Mullen, J. B., Lye, S. J., and Ritchie, J. W. (1995). Reduced placental villous tree elaboration in small-for-gestational-age pregnancies: relationship with umbilical artery Doppler waveforms. *Am. J. Obstet. Gynecol.* **172**, 518–525. doi:10.1016/0002-9378(95)90566-9

Jones, C. J. P., and Fox, H. (1980). An ultrastructural and ultrahistochemical study of the human placenta in maternal pre-clampsia. *Placenta* **1**, 67–76.

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.

Kim, M. J., Bogic, L., Cheung, C. Y., and Brace, R. A. (2001). Placental expression of erythropoietin mRNA, protein and receptor in sheep. *Placenta* **22**, 484–489. doi:10.1053/PLAC.2001.0681

Klemcke, H. G., Vallet, J. L., Christenson, R. K., and Pearson, P. L. (2001). Erythropoietin mRNA expression in pig embryos. *Anim. Reprod. Sci.* **66**, 93–108. doi:10.1016/S0378-4320(01)00084-7

Leiser, R., and Kaufmann, P. (1994). Placental structure: in a comparative aspect. *Exp. Clin. Endocrinol.* **102**, 122–134.

Lewis, R. M., Doherty, C. B., James, L. A., Burton, G. J., and Hales, C. N. (2001). Effects of maternal iron restriction on placental vascularization in the rat. *Placenta* **22**, 534–539. doi:10.1053/PLAC.2001.0679

Linzer, D. I., and Fisher, S. J. (1999). The placenta and the prolactin family of hormones: regulation of the physiology of pregnancy. *Mol. Endocrinol.* **13**, 837–840. doi:10.1210/ME.13.6.837

Lopez, M. F., Dikkes, P., Zurakowski, D., and Villa-Komaroff, L. (1996). Insulin-like growth factor II affects the appearance and glycogen content of glycogen cells in the murine placenta. *Endocrinology* **137**, 2100–2108. doi:10.1210/EN.137.5.2100

Macara, L., Kingdom, J. C., Kaufmann, P., Kohnen, G., Hair, J., More, I. A., Lyall, F., and Greer, I. A. (1996). Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. *Placenta* **17**, 37–48.

Ogura, A., Inoue, K., Ogonuki, N., Lee, J., Kohda, T., and Ishino, F. (2002). Phenotypic effects of somatic cell cloning in the mouse. *Cloning Stem Cells* **4**, 397–405. doi:10.1089/153623002321025078

Olausson, H., and Sohlstrom, A. (2003). Effects of food restriction and pregnancy on the expression of insulin-like growth factors-I and -II in tissues from guinea pigs. *J. Endocrinol.* **179**, 437–445. doi:10.1677/JOE.0.1790437

Padmanabhan, R., and Shafiullah, M. (2001). Intrauterine growth retardation in experimental diabetes: possible role of the placenta. *Arch. Physiol. Biochem.* **109**, 260–271. doi:10.1076/APAB.109.3.260.11596

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., and Collins, F. (1995). Effects of the obese gene product on body weight regulation in *ob*/*ob* mice. *Science* **269**, 540–543.

Perry, J. S. (1981). The mammalian fetal membranes. *J. Reprod. Fertil.* **62**, 321–335.

Pijnenborg, R. (1996). The placental bed. *Hypertens. Pregnancy* **15**, 7–23.

Redline, R. W., and Patterson, P. (1995). Pre-eclampsia is associated with an excess of proliferative immature intermediate trophoblast. *Hum. Pathol.* **26**, 594–600. doi:10.1016/0046-8177(95)90162-0

Redmer, D. A., Wallace, J. M., and Reynolds, L. P. (2004). Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. *Domest. Anim. Endocrinol.* **27**, 199–217. doi:10.1016/J.DOMANIEND.2004.06.006

Reik, W., Constancia, M., Fowden, A., Anderson, N., Dean, W., Ferguson-Smith, A., Tycko, B., and Sibley, C. (2003). Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *J. Physiol.* **547**, 35–44. doi:10.1113/JPHYSIOL.2002.033274

Reynolds, L. P., Borowicz, P. P., Vonnahme, K. A., Johnson, M. L., Grazul-Bilska, A. T., Redmer, D. A., and Caton, J. S. (2005). Placental angiogenesis in sheep models of compromised pregnancy. *J. Physiol.* **565**, 43–58. doi:10.1113/JPHYSIOL.2004.081745

Roberts, C. T., Sohlstrom, A., Kind, K. L., Earl, R. A., Khong, T. Y., Robinson, J. S., Owens, P. C., and Owens, J. A. (2001*a*). Maternal food restriction reduces the exchange surface area and increases the barrier thickness of the placenta in the guinea-pig. *Placenta* **22**, 177–185. doi:10.1053/PLAC.2000.0602

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. (2001*b*). 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**(Suppl. A), S77–82. doi:10.1053/PLAC.2001.0643

Roberts, C. T., Kind, K. L., Earl, R. A., Grant, P. A., Robinson, J. S., Sohlstrom, A., Owens, P. C., and Owens, J. A. (2002). Circulating insulin-like growth factor (IGF)-I and IGF binding proteins-1 and -3 and placental development in the guinea-pig. *Placenta* **23**, 763–770.

Rossant, J., and Cross, J. C. (2001). Placental development: lessons from mouse mutants. *Nat. Rev. Genet.* **2**, 538–548. doi:10.1038/35080570

Sakai, R. R., Tamashiro, K. L., Yamazaki, Y., and Yanagimachi, R. (2005). Cloning and assisted reproductive techniques: influence on early development and adult phenotype. *Birth Defects Res. C Embryo Today* **75**, 151–162. doi:10.1002/BDRC.20042

Salafia, C. M., Vintzileos, A. M., Silberman, L., Bantham, K. F., and Vogel, C. A. (1992). Placental pathology of idiopathic intrauterine growth retardation at term. *Am. J. Perinatol.* **9**, 179–184.

Salafia, C. M., Ernst, L. M., Pezzullo, J. C., Wolf, E. J., Rosenkrantz, T. S., and Vintzileos, A. M. (1995). The very low birthweight infant: maternal complications leading to preterm birth, placental lesions, and intrauterine growth. *Am. J. Perinatol.* **12**, 106–110.

Sibley, C. P., Coan, P. M., Ferguson-Smith, A. C., Dean, W., Hughes, J., *et al*. (2004). Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. *Proc. Natl Acad. Sci. USA* **101**, 8204–8208. doi:10.1073/PNAS.0402508101

Simmons, D. G., and Cross, J. C. (2005). Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. *Dev. Biol.* **284**, 12–24. doi:10.1016/J.YDBIO.2005.05.010

Singh, U., Fohn, L. E., Wakayama, T., Ohgane, J., Steinhoff, C., *et al*. (2004). Different molecular mechanisms underlie placental overgrowth phenotypes caused by interspecies hybridization, cloning, and Esx1 mutation. *Dev. Dyn.* **230**, 149–164. doi:10.1002/DVDY.20024

Soares, M. J., Muller, H., Orwig, K. E., Peters, T. J., and Dai, G. (1998). The uteroplacental prolactin family and pregnancy. *Biol. Reprod.* **58**, 273–284.

Sohlstrom, A., Katsman, A., Kind, K. L., Roberts, C. T., Owens, P. C., Robinson, J. S., and Owens, J. A. (1998). Food restriction alters pregnancy-associated changes in IGF and IGFBP in the guinea pig. *Am. J. Physiol.* **274**, E410–E416.

Spencer, T. E., Johnson, G. A., Burghardt, R. C., and Bazer, F. W. (2004). Progesterone and placental hormone actions on the uterus: insights from domestic animals. *Biol. Reprod.* **71**, 2–10. doi:10.1095/BIOLREPROD.103.024133

Spindle, A., Sturm, K. S., Flannery, M., Meneses, J. J., Wu, K., and Pedersen, R. A. (1996). Defective chorioallantoic fusion in mid-gestation lethality of parthenogenone-tetraploid chimeras. *Dev. Biol.* **173**, 447–458. doi:10.1006/DBIO.1996.0039

Tanaka, S., Oda, M., Toyoshima, Y., Wakayama, T., Tanaka, M., *et al*. (2001). Placentomegaly in cloned mouse concepti caused by expansion of the spongiotrophoblast layer. *Biol. Reprod.* **65**, 1813–1821.

Teasdale, F. (1985). Histomorphometry of the human placenta in maternal preeclampsia. *Am. J. Obstet. Gynecol.* **152**, 25–31.

Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* **407**, 908–913. doi:10.1038/35038090

Wallace, J. M., Bourke, D. A., Aitken, R. P., and Cruickshank, M. A. (1999). Switching maternal dietary intake at the end of the first trimester has profound effects on placental development and fetal growth in adolescent ewes carrying singleton fetuses. *Biol. Reprod.* **61**, 101–110.

Wallace, J. M., Aitken, R. P., Milne, J. S., and Hay, W. W., Jr (2004). Nutritionally mediated placental growth restriction in the growing adolescent: consequences for the fetus. *Biol. Reprod.* **71**, 1055–1062. doi:10.1095/BIOLREPROD.104.030965

Watson, E. D., and Cross, J. C. (2005). Development of structures and transport functions in the mouse placenta. *Physiology* **20**, 180–193.

Wooding, F. B. P., and Flint, A. P. F. (1994). Placentation. In 'Marshall's Physiology of Reproduction', 4th edn. (Ed. G. E. Lamming.) pp. 233–460. (Chapman and Hall: New York, USA.)

Wu, L., de Bruin, A., Saavedra, H. I., Starovic, M., Trimboli, A., *et al*. (2003). Extra-embryonic function of Rb is essential for embryonic development and viability. *Nature* **421**, 942–947. doi:10.1038/NATURE01417

Zhou, B., Lum, H. E., Lin, J., and Linzer, D. I. (2002). Two placental hormones are agonists in stimulating megakaryocyte growth and differentiation. *Endocrinology* **143**, 4281–4286. doi:10.1210/EN.2002-220447

Zhou, B., Kong, X., and Linzer, D. I. (2005). Enhanced recovery from thrombocytopenia and neutropenia in mice constitutively expressing a placental hematopoietic cytokine. *Endocrinology* **146**, 64–70. doi:10.1210/EN.2004-1011