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**TROPHOBLAST CELLS OF RUMINANT PLACENTAS –
A MINIREVIEW**

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Abstract

Understanding of ruminant placental structure and function is essential for veterinarians and researchers. The ruminant placenta is classified as cotyledonary and synepitheliochorial on the bases of its gross anatomical features and histological characteristics respectively. The richly vascularized embryonic chorioallantois is lined on its outer surface by cells of the trophodermal epithelium. These cells which assume specialized functions are referred to as trophoblast cells. Two morphologically and functionally distinct cell types have been recognized in the trophoderm of the placenta of ruminant animals. These are the mononucleate trophoblast cells and the binucleate trophoblast cells. The occurrence, morphological characteristics, and specialized functions of these trophoblast cells, in relation to conceptus nutrition and survival *in utero* are discussed in this review.

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INTRODUCTION

The word 'placenta' is taken from Latin meaning "flat cake" from the apparent gross similarity of the human discoid placenta to round flat loaves of unleavened bread commonly made in ancient times (Schlafer *et al*, 2000). Although the placentas of all eutherian species provide common structural and functional features, there are species variations in the gross appearance and microscopic structure of the placenta. In ruminant ungulates, the placenta is classified as cotyledonary on the basis of its gross anatomical features. It exhibits discrete areas of attachment, the placentomes, formed by interaction of patches of the chorioallantois with the endometrium. The foetal portion of the placentome is the cotyledon, while the maternal contact sites are the caruncles.

During the blastocyst stage of development, the embryo develops a fluid-filled central cavity, the blastocoel, which is surrounded by a single layer of cells known as the trophoctoderm. These cells, which remain as the outermost layer of the conceptus, form a single layer that covers the outside of the placenta. Together with the somatic (parietal) mesoderm, they constitute the chorion. The allantois, a sac-like structure that arises as an out-pocketing of the foetal hindgut ultimately expands to fuse with the chorion, forming the chorioallantois (Schlafer *et al*, 2000). The richly vascularized chorioallantois is lined on the outside by cells of the trophoctodermal epithelium. These cells assume specialized functions and are referred to as trophoblast cells. Two morphologically and functionally distinct cell types have been recognized in the trophoctoderm of the placenta of ruminant animals. These are the mononucleate trophoblast cells and the binucleate trophoblast cells (Wimsatt, 1951; Greenstein *et al*, 1958). The mononucleate cells form the majority of the interface and are primarily involved in nutrient exchange. In contrast, the binucleate cells are synthetic cells that produce hormones such as placental lactogen and progesterone (Duello *et al*, 1986; Myers and Reimers, 1988).

MONONUCLEATE TROPHOBLAST CELLS:

Mononucleate trophoblast cells are mononuclear cuboidal to columnar cells that show typical features of epithelial cells. They are located on a basal lamina and are connected to each other by junctional complexes including tight junctions (Dent, 1973; Wooding *et al*, 1994). These cells constitute about four-fifths of the trophoblast population (Boshier and Holloway, 1977). Each mononucleate trophoblast cell possesses a single irregularly-shaped nucleus with a single large nucleolus and finely dispersed chromatin. The cytoplasm contains small Golgi bodies, polysomes, cisternae of rough endoplasmic reticulum and ovoid to elongated mitochondrial profiles that are located towards the apex of the cell (Boshier and Holloway, 1977). Also present in the cytoplasm are accumulations of lipid droplets, and vesicles of different sizes, some of which contain degenerating material (King *et al*, 1980). The apical surface membranes of mononucleate trophoblast cells are modified to form microvillar processes that interdigitate with similar processes arising from the maternal uterine epithelial cells thus, forming the foeto-maternal contact zone (Bjorkman, 1969; Dent, 1973).

Role in maternal recognition of pregnancy

‘Maternal recognition of pregnancy’ is the term used to describe how a mother responds (physiologically) to the presence of a conceptus in her reproductive tract (Demmers *et al*, 2001). Since the corpus luteum regresses, unless ‘rescued’ by the implanting conceptus, the evolution of the placenta has probably been a major determinant in governing the way in which the early conceptus signals its presence to the mother (Roberts *et al*, 1997). Failure of the conceptus to signal its presence at the appropriate time leads to pregnancy loss. The ruminant artiodactyls (sheep, cattle, goats, and deer) have evolved a means of rescuing the corpus luteum during early pregnancy. This means of rescuing the corpus luteum appears to be unique to this group of mammals in which implantation is both late in onset and superficial in extent (Wooding, 1992). According to a review by Roberts *et al* (1999), the ovine corpus luteum regresses at the end of a normal estrous cycle (16 days post-estrus) in response to the pulsatile release of prostaglandin F_{2α} (PGF_{2α}), which is produced by the uterine endometrium (Flint, 1995; Lamming *et al*, 1995; Thatcher *et al*, 1995; Spencer *et al*, 1996; Bazer *et al*, 1997; Martal *et al*, 1997). Generation of luteolytic episodes of PGF_{2α} secretion requires luteal secretion of oxytocin and the interaction of circulating oxytocin with its receptor, located principally on endometrial epithelial cells (Demmers *et al*, 2001). Thus, this pulsatility which is under the control of oxytocin is essential for luteolysis to occur. However, the presence of a viable conceptus diminishes, and in most cases, totally abolishes the pulsatile release of PGF_{2α} (Roberts *et al*, 1999).

The maintenance of ovine corpus luteum during early pregnancy was shown to result from the production of a proteinaceous factor present in the pre-implantation conceptus (Martal *et al*, 1979). This factor, initially called trophoblastin, and later, ovine trophoblast protein 1 (oTP-1) (Godkin *et al*, 1982) is now known as ovine Interferon-tau (oIFN-τ) (Roberts *et al*, 1999). It was found to be the major secretory product of the ovine conceptus trophoblast cells between day 13 and day 16 of pregnancy, the time of maternal recognition of pregnancy in sheep (Moor and Rowson, 1964; 1966a; 1966b). A bovine interferon-tau (bIFN-τ) has been characterized as a secretory product of the trophoblast cells of the bovine conceptus (Bartol *et al*, 1985; Leaman and Roberts, 1992). The bIFN-τ is produced between day 10 and day 24 of gestation, and was shown to have anti-luteolytic properties. Similarly, a caprine interferon-tau (cIFN-τ) was identified in goats (Guillomot *et al*, 1998; Ealy *et al*, 2004), being secreted between day 16 and day 21 of pregnancy. Northern blotting of blastocyst mRNA (Stewart *et al*, 1989) and *in situ* hybridization (Farin *et al*, 1990) demonstrated that the expression of interferon-tau is localized to the extra-embryonic trophoblast of ruminant conceptuses. Thus, trophoblast cells of ruminant placentas play a very important role in maternal recognition of pregnancy and survival of the conceptus through the secretion of interferon-tau. Peak production of interferon-tau occurs at day 16 of pregnancy in sheep (Godkin *et al*, 1982) and day 17 of pregnancy in cattle (Bartol *et al*, 1985).

Since interferon-tau was not detected in peripheral blood of pregnant ewes, it is thought to act directly on the uterine endometrium. The expression of type-1 interferon receptor subunits, IFNAR-1 and IFNAR-2, by endometrial cells have been reported (Kaluz *et*

al, 1996). The primary mechanism by which interferon-tau inhibits corpus luteum regression is by suppressing the pulsatile release of endometrial prostaglandin F_{2α} (PGF_{2α}) (Vallet *et al*, 1988; Ott *et al*, 1992). IFN-τ prevents oxytocin receptor expression in the endometrial epithelium, thereby preventing pituitary- and luteal-derived oxytocin from stimulating endometrial PGF_{2α} synthesis and release (Flint *et al*, 1992). Northern blotting (Stewart *et al*, 1989) showed that this effect is exerted at the level of gene transcription. In addition to controlling oxytocin receptor gene expression in the endometrium, IFN-τ affects the synthesis of other cytokines that contribute to the immuno-modulation required to prevent rejection of the conceptus. IFN-τ increases the expression of Interferon-gamma (IFN-γ) and interleukin 4 (IL-4) by bovine lymphocytes *in vitro* (Tuo *et al*, 1999) and reduces the proliferative responses of lymphocytes to interleukin 2 (IL-2) (Niwano *et al*, 1989). It also increases endometrial cyclo-oxygenase 2 (COX-2) concentrations and the production of prostaglandin E₂ (PGE₂) which is a putative leutotrophic agent (Dannet-Desnoyers *et al*, 1994; Asselin *et al*, 1997a; 1997b; Xiao *et al*, 1998; Parent *et al*, 2003). Furthermore, IFN-τ induces the ubiquitin cross-reactive protein, also known as interferon-stimulated gene-17 product (ISG-17) (Hansen *et al*, 1999), which controls cytosolic protein processing through the proteasome, osteopontin (Johnson *et al*, 1999a; 1999b) that promotes cell – cell attachment. This may be involved in attachment of the blastocyst to the endometrial surface during implantation.

Role in foetal nutrition

The placenta is the only channel for transport of nutrients to the conceptus, and the demands of this structure increases exponentially to term, together with a wide range of structural modifications (Wooding and Flint, 1994). The ruminant placenta has two major regions namely, the interplacentomal region involved in histotrophic transfer, and the placentomal region that functions in haemotrophic exchange of nutrients and metabolites between the dam and the foetus (Schlafer *et al*, 2000). In the interplacentomal areas, there is simple apposition between the foetal membranes and the uterine epithelium, but in the placentomes, the endometrial surface exhibits deep caruncular crypts, which are penetrated by long, profusely branched cotyledonary villi of foetal chorioallantois (Davis *et al*, 2000). This arrangement results in a greatly enhanced surface area between foetal and maternal compartments in the placentomes. It is estimated to be about 130 square metres in cow (Russe and Sinowatz, 1991). There are regional variations in the patterns of gene expression and specialized functions of trophoblast cells in the placentomal and interplacentomal regions of the ruminant placenta (Muson *et al*, 1989; Wooding *et al*, 1996a). However, morphological features common to trophoblast cells in these two regions include the presence of microvilli on the apical surface of the cells (King *et al*, 1980; Guillomot, 1995), as well as, the presence of junctional complexes including tight junctions and desmosomes between adjacent trophoblast cells (Dent, 1973). Whereas the microvilli provide an increased surface area for absorption of materials, the tight junctions serve to maintain separate foetal and maternal compartments. Challier and Bintein, (1989) suggested that the main resistance to trans-placental transfer lies in the trophoblastic layer. Comparison of feto-maternal fluxes of different metabolically inert lipid-insoluble molecules (urea, erythritol, mannitol, and Cr-

EDTA) revealed a very sharp decrease in the permeability of the sheep placenta as molecular size increased (Stacey *et al*, 1977). The sheep placenta exhibited a size selectivity that excludes molecules of 0.45nm radius, and so, permits but a poor passive diffusion. Thus, the placenta behaves like a low-permeability barrier containing specific mechanisms of trans-cellular transport for nutrients and other substances needed for foetal growth and metabolism. Macromolecules are transported across the placenta by facilitated diffusion or endocytosis.

During implantation, trophoblast papillae develop on the areas of the conceptus facing uterine gland openings (Guillomot and Guay, 1982; Wooding *et al*, 1982). These were found to be temporary structures that disappeared by day 20 of pregnancy in sheep (Wooding *et al*, 1982). These trophoblast papillae penetrated well down into the lumina of the uterine glands. They were interpreted to be a system by which the conceptus is immobilized on the uterine epithelium and/or a histotrophic mechanism for absorbing secretory products (Guillomot and Guay, 1982). Endometrial glands synthesize and secrete a complex array of proteins and related substances termed histotroph (Wimsatt, 1950; Amoroso, 1952; Bazer, 1975). These glands are located in the interplacentomal regions of the ruminant placenta (Gray *et al*, 2001a; Spencer and Bazer, 2004). The histotroph which is a complex mixture of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins, and other substances is necessary for the nourishment of the conceptus, and acts as a primary regulator of conceptus survival, development, production of pregnancy recognition signals, implantation and placentation (Bazer *et al*, 1979; Roberts and Bazer, 1988; Bartol *et al*, 1999; Carson *et al*, 2000; Gray *et al*, 2001b). In addition to haemotrophic nutrition, the conceptus requirement for histotroph remains critical throughout pregnancy in domestic animals with synepitheliochorial placenta such as the ruminants. Uterine gland-secreted histotroph bathe the conceptus and is absorbed by the trophoblast cells especially at the placental areolae. Areolae are unique placental structures that develop over the mouth of each uterine gland as specialized areas for absorption and transport of uterine histotroph in ruminants and pigs (Spencer and Bazer, 2004). There are different mechanisms for trans-placental transport of different nutrients. Glucose transport is by facilitated diffusion using Glucose transporters 1 and 3 (GT 1 and GT 3). GT-1 and 3 gene expressions were identified in the trophoblast cells of ruminant placenta (Currie *et al*, 1997; Ehrhardt and Bell, 1997).

Transport of calcium ions across the placental trophoblast cells have been studied (Mellor and Matheson, 1977; Care *et al*, 1990; 1996; Care, 1991; Abbas *et al*, 1993; Jones *et al*, 1997; Morgan *et al*, 1997; Nikitenko *et al*, 1998). Maternal calcium transport across the ovine placenta is against a concentration gradient (Mellor and Matheson, 1977) and so, must be an active process (Care *et al*, 1990; Abbas *et al*, 1993). Foetal calcium need increases exponentially during the second half of gestation, when bone formation is proceeding rapidly (Grace *et al*, 1986). Interplacentomal trophoblast cells are capable of active up-take (Jones *et al*, 1997) and transport (Abbas *et al*, 1993) of calcium ions. The 9-KDa calcium binding protein (Calbindin-D_{9k}; 9CBP) which is characteristic of epithelia actively transporting calcium is present in ruminant trophoblast cells during the second half of gestation (Wooding *et al*, 1996a; Morgan *et al*, 1997). Immunocytochemical and *in situ* hybridization studies (Morgan *et al*, 1997; Nikitenko *et al*, 1998) demonstrated that significant increase in the

amount of 9CBP protein and messenger RNA during pregnancy in sheep and cattle is localized to the mononucleate trophoblast cells in the interplacentomal regions of the placenta. Parathyroid hormone-related protein (Care *et al*, 1990; 1996; Care, 1991) and calcitriol (1, 25(OH)₂ vitamin D₃) (Jones *et al*, 1997) play important roles in the control of the overall placental calcium transport.

Another important mechanism for trans-placental transport of macromolecules involves phagocytic activities of mononucleate trophoblast cells. An area of specialized phagocytic trophoblast cells is found in the areolae of the interplacentomal chorioallantois overlying the openings of endometrial glands. Microscopic accumulations of uterine milk secreted by endometrial glands in this region are phagocytized by the trophoblast cells (Schlafer *et al*, 2000). Similarly, mononucleate trophoblast cells lining the arcade zones (located towards the fetal side, between the bases of cotyledonary villi) of the placentomes are involved in phagocytosis of macromolecules especially erythrocytes (Burton *et al*, 1976; Myagkaya and Vreeling-Sindelarova, 1976; Santos *et al*, 1996; Pereira *et al*, 2001). Extravasated maternal blood which escapes from the capillaries and other larger vessels (Burton *et al*, 1976) accumulates in the maternal-foetal interface at the arcade zone resulting in the formation of haematomas, from which the maternal blood cells are engulfed by the trophoblasts. This is thought to be an important mechanism for the trans-placental transport of iron needed by the developing foetus for erythropoiesis (Myagkaya and Daems, 1979; Myagkaya *et al*, 1979). The presence of haemoglobin-derived pigments (Myagkaya and Schellens, 1981) and ferrocyanide reaction products (Myagkaya *et al*, 1984) were demonstrated in lysosomal structures involved in the breakdown of maternal erythrocytes ingested by chorionic epithelial cells, suggesting that trivalent iron is liberated from digested haemoglobin. In addition to erythrocytes, the trophoblastic cells in the erythrophagic regions also engulf maternal leukocytes and neighbouring epithelial cells and giant cells (Myagkaya and Schellens, 1981).

BINUCLEATE TROPHOBLAST CELLS:

Mature ruminant binucleate cells are large cells that have a characteristic structure quite different from the surrounding trophoblastic mononucleate epithelial cells. These binucleate cells represent about 15 – 20 percent of trophoblastic cells at the beginning of implantation and throughout pregnancy in ruminants (Wooding and Wathes, 1980; Wooding, 1982). It is generally accepted that binucleate trophoblast cells originate from mononucleate trophoblast cells by acytokinetic mitoses (Wimsatt, 1951; Bjorkman, 1968). They were thought to arise directly from 'trophoblast stem cells' (Greenstein *et al*, 1958), but there is no evidence for a stem cell population (Wooding, 1992). It has been proposed that any normal mononucleate trophoblast cell can give rise to a binucleate cell by consecutive nuclear divisions, the second without subsequent cytokinesis (Wooding, 1992; Wooding and Flint, 1994).

During the early stages of their development, the binucleate cells are randomly scattered and located deep within the trophoblastic layer in an intraepithelial position, such that they make no contact with either the basement membrane or the apical microvillar border of the trophoblast (Wooding, 1984; Wango *et al*, 1990). These young cells appear as small

round (or spherical) cells whose cytoplasm stain darker than the surrounding mononucleate trophoblast cells. The dark staining is due to the presence of numerous cytoplasmic ribosomes in the binucleate cells (Wango *et al*, 1990). Ultrastructural features of the mature binucleate cell include the presence of extensive array of rough endoplasmic reticulum, numerous small mitochondria, and well-developed large Golgi bodies, from which arise considerable numbers of characteristic large membrane-bound granules which occupy more than 50% of the cell volume (Wooding *et al*, 1980; Wooding, 1992). The granules are positive to Periodic Acid Schiff (PAS) staining and can also be demonstrated by silver impregnation (Klisch *et al*, 1999). In addition, the granules are remarkably visible in electron-micrographs following staining with phosphotungstic acid (Wooding, 1980; 1984). The features of trophoblast binucleate cells are similar in cow, sheep, goats and deer (Wooding, 1992). The vast majority of ruminant binucleate cells contain two nuclei, but the presence of mononuclear and trinuclear forms have been reported (Klisch *et al*, 1999). The mononuclear and trinuclear cells of the trophoblast observed by Klisch and his co-workers resemble the binuclear cells in all respects, except for the number of nuclei. Therefore, these authors proposed the term 'Trophoblast Giant Cells' (TGC) for these three forms of giant cells found in the trophoblast of ruminant placentas. The mononuclear TGC often exhibit lobulation of the nucleus, and this was regarded as a sign that these cells originate from abortive mitoses that were blocked before the completion of karyokinesis (Klisch *et al*, 1999). The occurrence of trinuclear cells was attributed to tripolar acytokinetic mitoses of trophoblast cells, during which the arrangement of the chromosomes at metaphase is typically Y-shaped (Klisch *et al*, 1999).

Binucleate cells are not uniformly distributed in the trophoblast, but tend to occur in small clusters (Wooding, 1984). Although the binucleate cells present in the placental and inter-placental regions of the ruminant placenta are anatomically similar, there is regional variation in the SBU3 antigen expression of binucleate cells in these two regions. During development, binucleate cells in both regions possess two nuclei, many rough endoplasmic reticulum cisternae and extensive Golgi bodies that produce the characteristic large granules typical of the fully mature binucleate cell. However, immunocytochemical studies using the monoclonal antibody, SBU3, demonstrated that SBU3 antigen expression is restricted to the binucleate cell granules in the placentomes, but there were no similar antigen expressions in interplacental binucleate cells (Lee *et al*, 1985; 1986a; 1986b). Furthermore, these subpopulations of binucleate cells exhibit different selective synthetic capacity and functional specialization (Wooding *et al*, 1996b).

Role in Conceptus implantation

Implantation is the process that results in attachment of the conceptus to the maternal endometrium, leading to the establishment of the placental structures. Guillomot *et al* (1981) described three stages of the implantation process in ruminants namely; (i) a long pre-attachment period during which the conceptus elongates considerably, (ii) an apposition stage when cellular contacts are established between foetal trophoblast layer and maternal uterine epithelium, and (iii) an adhesion stage that gives rise to the cellular structure of an

epitheliochorial placenta. The role of binucleate trophoblast cells in implantation have been extensively studied in many ruminant species (Wooding *et al*, 1980; Wooding, 1984; Lee *et al*, 1986c; Wango *et al*, 1990). Young binucleate cells reside in an intra-epithelial position between the mononucleate trophoblast cells. As these cells mature and accumulate the characteristic large membrane-bound granules in their cytoplasm, they begin to migrate towards the chorionic microvillar junction with the maternal endometrium. During this migration, binucleate cells are capable of passing through the tight junctions between adjacent mononucleate cells, without disrupting the junctions. This ability of binucleate cells to migrate through tight junctions without interrupting its continuity was characterized by freeze fracture techniques in later stages of pregnancy in sheep (Wooding and Morgan, 1983). Passage through tight junctions is accomplished when the binucleate cell extends an apical cytoplasmic process to insert in, and form part of the tight junction (Wango *et al*, 1990), such that the compartmentalization of foetal and maternal components of the placenta is not compromised in the process. Morphometric quantitation indicates that about 15 – 20 percent of the binucleate cells are migrating through tight junctions at any one time and this rate continues right up to parturition (Wooding, 1983; Wooding *et al*, 1986).

The binucleate cells migrate across the foeto-maternal interface to fuse with columnar cells of the maternal uterine epithelium forming trinucleate hybrid cells (Wooding, 1984; Wango *et al*, 1990; Wooding, 1992). The enlargement of the trinucleate cells to form multinuclear syncytial plaques occurs by continued binucleate cell migration and fusion with trinucleate cells (Wooding, 1984; Wooding, 1992). This capacity of binucleate cells to migrate and fuse with maternal uterine epithelial cells resulting in the formation of syncytial plaques gave rise to the classification of ruminant placenta as synepitheliochorial (Wooding, 1992). The fate of the syncytial plaques varies among the different species of ruminant animals. In cow and deer, the syncytial plaques are present at implantation, but become displaced by overgrowth of mononuclear uterine epithelial cells (Wathes and Wooding, 1980). Subsequent migration of binucleate cells in the placentomes of these species produces only transient trinucleate cells with no barrier or structural role (Wooding and Wathes, 1980). In sheep and goats, the caruncular syncytial plaques continue as a consistent feature in the placentomes throughout pregnancy (Wooding, 1984; Wango *et al*, 1990; Wooding, 1992). These plaques increase in size by continued binucleate cell migration and fusion with existing syncytial plaques until each syncytial plaque contains about 25 nuclei (Wooding *et al*, 1981; Wango *et al*, 1990; Wooding, 1992). Binucleate cell migration and syncytial plaque formation also takes place in the interplacentomal region of the placenta. However, the syncytial plaques formed in this region are rapidly displaced by uterine epithelial cells especially around the mouths of uterine glands (Wooding, 1984). Although no specific structural function has been ascribed to the syncytial plaques, it has been suggested that they may be important in reducing the diffusion distance between maternal and foetal blood capillaries, since the syncytial plaques are shorter than the uterine epithelium they replace (Wooding, 1984).

Endocrine functions of binucleate cells

Migration and fusion of binucleate cells with maternal endometrial epithelial cells are important mechanisms for the delivery of the binucleate cell granules into the maternal compartment. Using selective phosphotungstic acid (PTA) staining for the binucleate cell granules, Wooding, (1984) demonstrated that the characteristic binucleate cell granules are present in maternal trinucleate cells and syncytial plaques. He showed that soon after cell fusion, the PTA-staining granules stream down to the maternal face of the trinucleate or syncytial cell, where images suggesting exocytosis of the granules are evident. Thus, trinucleate cells and syncytial plaques are thought to serve in delivering the contents of the binucleate cell granules close to the maternal blood circulation. These granules have been shown to contain the hormone, placental lactogen (Duello *et al*, 1986; Wooding and Beckers, 1987; Anthony *et al*, 1995). Ovine placental lactogen is a nonglycosylated, single-chain, 23-kDa protein (Gertler and Djiane, 2002). The onset of ovine placental lactogen production on Day 16 of gestation is concomitant with increases in osteopontin and the initiation of expression of uterine milk protein by the endometrial glandular epithelium (Johnson *et al*, 1999a; 1999b; Stewart *et al*, 2000). In the ovine uterus, placental lactogen binding sites are specific to the endometrial glandular epithelium expressing prolactin receptors (Stewart *et al*, 2000; Noel *et al*, 2003), and temporal changes in conceptus production of this hormone are correlated with endometrial gland morphogenesis and increased production of uterine milk protein and osteopontin by the glandular epithelium during pregnancy (Johnson *et al*, 2003; Spencer *et al*, 2004). This implies that placental lactogen may play an important role in stimulating endometrial gland morphogenesis and differentiated function during pregnancy in ruminants, and so facilitate foetal growth and development. In addition to placental lactogen, binucleate cells are thought to produce other hormones such as prolactin-related protein-1 (Zieler *et al*, 1990; Kessler *et al*, 1991; Anthony *et al*, 1995), pregnancy associated glycoproteins (Zoli *et al*, 1991; 1992; Green *et al*, 2000), oestradiol (Matamoros *et al*, 1994) and progesterone (Reimers *et al*, 1985; Wango *et al*, 1991). These hormones are important factors controlling caruncular and uterine gland morphogenesis, differentiation and functions, necessary for foetal nutrition and survival *in utero*.

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