Placental development and its control in cattle

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Summary

This review aims to provide an overview of the current knowledge regarding the development and function of the bovine placenta, starting with the gross anatomical morphology and histology. In addition, detailed information on trophoblast giant cell formation (TGC) and the complex interplay of the extracellular matrix (ECM) proteins, integrins and growth factor systems is given. Drugs and pathological conditions (e.g. cloned placenta, retained placenta) are also reviewed. Knowledge derived from cell culture studies is embedded and set in proportion to the data gained from in vivo experiments. Finally, new discoveries and the major challenges for future work on the bovine placenta close the circle, in order to provide a complete picture of what is known about this fascinating, synepitheliochorial organ.

Introduction

In the bovine placenta fetal cotyledons and maternal caruncles form placentomes, which are the main areas of exchange between fetus and mother. In the past the fetal-maternal interhemal barrier was characterized as syndesmochorial, meaning that invading trophoblast is directly opposed to endometrial stroma, due to an assumed destruction of the uterine epithelium (Grosser 1927). However, subsequent studies revealed that the uterine epithelium persists throughout gestation; therefore, the term epitheliochorial placenta was proposed (Bjorkman 1969). To further specify the nomenclature, which is based on layers between fetal and maternal circulation as well as on the physiology, Wooding *et al.* (1992) introduced the more specific term synepitheliochorial, thus emphasizing alterations in uterine epithelium by hybrid cell formation (Wooding 1992). To date, this specification is generally accepted.

Like other mammalian species, diseases compromising the placenta and its function are also present in bovine. One of the most prominent is the retention of fetal membranes (RFM), which can affect the reproductive performance and lead to considerable economic loss at the herd level (Laven & Peters 1996). Even though in the past numerous attempts were made to tackle this pathology by using a variety of sophisticated methodologies (e.g. gene expression analysis),

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Dedicated to Prof. Dr. Rudolf Leiser for sharing with me his contagious excitement for bovine placentation and mentoring my scientific career without restricting my creativity.

RFM still remains a major economic problem. This review attempts to give a comprehensive overview of the development of the bovine placenta including placental morphology, trophoblast development, cell-cell and cell-matrix interactions, new discoveries and major future challenges. In addition to in vivo data that is available, we chose to incorporate a range of findings from in vitro studies since it has become more and more popular to use cell-culture based systems to investigate distinct, isolated aspects of placental development or functionality in a controlled environment.

General morphology

In the mature placenta of cattle, groups of (fetal) chorioallantoic villi, named cotyledons, are anchored in maternal endometrial crypts, which are part of the caruncle. Each fetal villus contains an outer trophoblast layer, underlined by mesenchyme containing multiple blood vessels. The outermost uterine cell layer, neighboring the trophoblast, is the uterine epithelium, which is also underlined by stromal tissue and multiple blood vessels (Buse *et al.* 2013). Fetal villi themselves can be divided into stem, intermediate and terminal villi, whilst the villous blood vessels can be accordingly classified as stem arteries and veins, intermediate arterioles and venules and terminal capillaries. This cotyledonary architecture of the fetal placenta is like the one found in the human hemochorial placenta (Leiser *et al.* 1997). Interestingly, the villous placental blood vessels of ruminants show adaptational changes to hypoxic environmental stress, analogous to changes occurring in placentas of e.g. heavy smokers (Krebs *et al.* 1997, Pfarrer *et al.* 1999). During bovine implantation the chorioallantoic sac enlarges from gestational day (gd) 16 to 27 (for review (Assis Neto *et al.* 2010). Within this period the development of cotyledons starts near to the embryo and later spreads over the chorioallantoic sac. Exclusively the allantochorion of the cotyledons attaches to the uterine caruncles forming so-called placentomes. Due to this sequential formation the most developed placentomes can be found close to the embryo (Leiser 1975). The caruncles themselves number up to 100 to 140 and are devoid of endometrial glands. In the nonpregnant animal the caruncular precursors are mushroom-shaped nodules that have already been observed in fetal endometrium and undergo histological remodeling up to 12 months after birth in order to become caruncles (Atkinson *et al.* 1984). During implantation and placentation in cattle major structural and ultrastructural changes occur (Wathes & Wooding 1980, Leiser 1975), which finally lead to the mature bovine placenta. Actual growth is observed until gd 170; after that enlargement of the feto-maternal interface is accomplished by branching of the villous trees and corresponding crypts (Leiser *et al.* 1997). Histologically, the synepitheliochorial ruminant placenta contains 80% polarized uninucleate trophoblast cells (UTC) and 20% non-polarized trophoblast giant cells (TGC). Like the UTC the uterine epithelium (UE) is also polarized. Both cell layers possess apical microvilli, which lie in close association with each other. The remaining gap is termed intervillous space. In epitheliochorial and synepitheliochorial placentae this space is 15-20nm wide and contains ancient pregnancy-associated glycoproteins (Wooding *et al.* 2005b). In the synepitheliochorial placenta of sheep extracellular matrix (ECM) proteins like osteopontin are distinctly located in the intervillous space and have been suggested to mediate trophoblast attachment to the endometrium (Johnson *et al.* 2003). The gross anatomy and histology of the bovine placenta at different developmental stages are shown in Fig. 1.

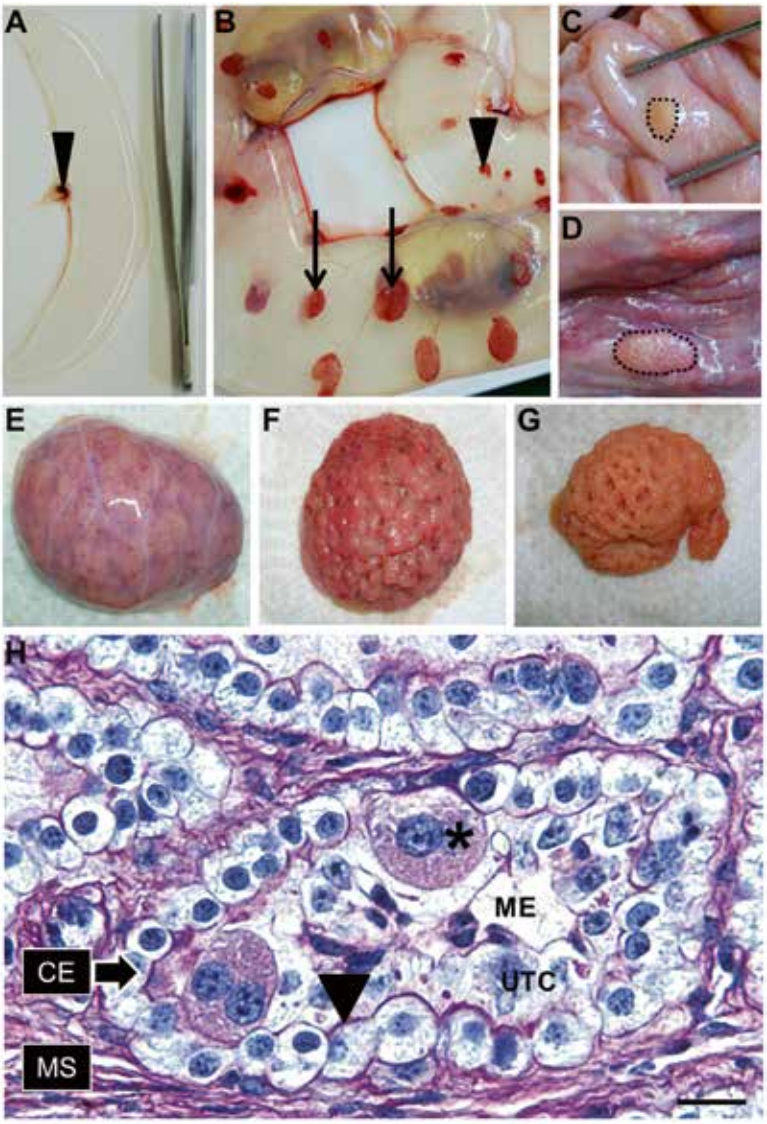

Fig. 1. Gross anatomy of different developmental stages of the bovine placenta (A-G) and histology of the feto-maternal interface in cattle (H).

All samples were obtained from the local abattoir and the gestational day (GD) was determined according to (Assis Neto *et al.* 2010). (A) Early stage of bovine placental development: no cotyledons on the chorioallantoic sac (CAS) (length: 14cm, width: 0.7-1.4cm) are visible. The size of the embryo (GD 16-17) (thin arrowhead) is 0.8cm. (B) More advanced stages of bovine placenta: multiple cotyledons (caliber: 2-2,2cm) (*pars fetalis* of placenta) can be seen on the CAS. The most developed are close to the embryo (GD 28) (arrows), whilst the least developed are located at the end of the CAS (arrowheads). The size of the embryo is 8cm. The images (C) and (D) display endometrial caruncles (*pars maternalis* of placenta) close to the embryo, which have been indicated by dotted lines. (C) Caruncle (calibre: 0.5cm) in early pregnancy belonging to the embryo in image (A). No crypts are observed. (D) Caruncle (calibre: 1-1.3cm) belonging to the embryos in image (B): shallow caruncular crypts can be seen. (E-G) Bovine mid-gestational placentome (5.-6. month of gestation; size 4-4.4cm). (E) Intact placentome (*partes fetalis and maternalis*). On the fetal side chorioallantoic blood vessels supplying the cotyledon are visible. (F-G) The same placentome separated: a cotyledon (F) containing multiple fetal villi that were formerly anchored in the crypts of the maternal caruncle (G). (H) Histological section stained with PAS (Periodic Acid Schiff): The core of the fetal villus is made up of mesenchyme (ME) lying beyond the uninucleate trophoblast (UTC), located on a basement membrane. Among the UTC two trophoblast giant cells (asterisks) can be seen, one of which is binucleate. The caruncular epithelium (CE) itself is located on a basement membrane and is underlined by maternal stroma (MS). The feto-maternal interface (between UTC and CE) is partially strongly stained (arrowhead). Scale bar = 20μm.

Trophoblast Giant Cells (TGC)

TGC (Klisch *et al.* 1999a) are considered to be moderately invasive (Pfarrer *et al.* 2003) because they migrate through chorionic tight junctions towards the UE and fuse with singular UE to form hybrid cells, which exocytose TGC products into the maternal compartment of the placenta. Due to the fact that the majority of TGC have two nuclei they are also termed binucleate cells (BNC) or in older literature diplokaryocytes. They arise from uninucleate trophoblast cells (UTC) (Wimsatt 1951) by tripolar acytokinetic mitosis and are polyploid (Klisch *et al.* 1999ab). TGC begin to form during bovine implantation from gd 16-33 (Wathes & Wooding 1980, Leiser 1975, Greenstein *et al.* 1958) and make up 20% of all trophoblast cells throughout gestation (Wooding 1992). Their number decreases dramatically at the end of gestation, when TGC can only be sporadically detected in individual villi (Klisch *et al.* 2006). In cattle hybrid cells generally form through fusion of one TGC with one uterine epithelial cell, and therefore mostly contain three nuclei. In contrast, ovine TGC continuously fuse with existing hybrid cells, thereby forming syncytia with up to 25 nuclei (Wooding 1992). The fact that the ovine feto-maternal interface almost exclusively consists of these large hybrid syncytia may be one reason that RFM is not common in sheep. In past studies it was discussed whether or not TGC could arise from all UTC or exclusively from a stem cell population (Greenstein *et al.* 1958, Wimsatt 1951). The mature placenta in rodents is known to have multiple TGC subtypes that have distinct cell lineage origins (Hu & Cross 2010). Apart from that, murine TGC that differentiate from trophoblast stem cell lines showed invasive capacity thereby resembling normal trophoblast cells in vivo (Hemberger *et al.* 2004). In bovine, little is known about the stages of differentiation from a uninucleated trophoblast cell (UTC) to an invading TGC or the underlying reasons for the drastic change in biological activity between these two cell populations. Trophoblast cell lines provide valuable data for investigating this process, which is one of the most intriguing characteristics of trophoblast cells. A common feature of blastocyst (Shimada *et al.* 2001) and placenta-derived bovine trophoblast cells in culture (Hambruch *et al.* 2009) is the loss of TGC, most likely because no more UTC undergo TGC differentiation in vitro (Shimada *et al.* 2001, Hambruch *et al.* 2009). Yet, singular TGC, which have either attached or spontaneously differentiated, can be seen in early passages of bovine primary (Hambruch *et al.* 2009) or permanent trophoblast cell lines (Hambruch *et al.* 2009, Shimada *et al.* 2001). The loss of TGC and the continuous presence of proliferating UTC, from which TGC in vivo originate, makes such cell lines an ideal tool to study TGC differentiation and possibly identify its regulators. Different conclusions have been drawn from such in vitro experiments. Nakano *et al.* (2002b) have demonstrated that extracellular matrix, namely type I collagen (COL1) could induce up to 3% TGC from blastocystal UTC. The resulting TGC showed expression of bovine placental lactogen (bPL, CSH1), no cytokeratin expression, and polyploid nuclei (Nakano *et al.* 2002b). Yet, the expression of pregnancy-associated glycoproteins and the total in vivo TGC percentage (20% of all trophoblast cells) has not been observed (Nakano *et al.* 2002b, Nakano *et al.* 2002a), indicating differentially regulated steps in TGC differentiation. UTC isolated from bovine placentomes so far have not been shown to differentiate into TGC (Hambruch *et al.* 2009). One reason could be the fact that the UTC placental environment (polarized cells with apical contact to uterine epithelial cells after epithelial-mesenchymal transformation) is completely different from the preimplantative blastocystal one. In addition, the co-localization of transcription factors in bovine trophoblast cells in vivo has pointed towards the importance of proteins like DLX3 (distal-less homeobox 3), SP1 (specificity protein 1) and PPARG (peroxisome proliferator-activated receptor gamma; Degrelle *et al.* 2011) in the regulation of TGC formation. Other transcription factors like OCT4 (octamer-binding transcription factor 4), NANOG and CDX2 (caudal type homeobox 2) have only been analyzed during the development of blastocyst stage embryos (Madeja *et al.* 2013)

in vitro but not in placental tissue. Yet, others like GATA2 and GATA3 (GATA binding protein 2/3) have been found to affect endogenous CDX2 and INFT (interferon tau) expression (Bai *et al.* 2009) and might also play a role in TGC development. Multiple growth factors like Fibroblast Growth Factor (FGF) (Pfarrer *et al.* 2006a), Vascular Endothelial Growth Factor (VEGF) (Pfarrer *et al.* 2006b) and Platelet-Activating Factor (PAF) (Bucher *et al.* 2006) are co-localized in TGC and could act upstream of the previously mentioned transcription factors. In addition, others have suggested that steroid synthesis could be the most striking cause for TGC differentiation (Schuler *et al.* 2008, Khatri *et al.* 2013). Furthermore, another hypothesis on the development of TGC has been proposed, namely endogenous retroviruses, which are latently present in the mammalian genome (Black *et al.* 2010, Koshi *et al.* 2012). Such retroviral genes have been detected in the bovine placenta in vivo (Baba *et al.* 2011) and in vitro where they are involved in the expression of TGC-specific genes during the differentiation process (Koshi *et al.* 2011, Koshi *et al.* 2012).

Cell-cell and cell-matrix interactions

Caruncular and placentomal growth, elaboration of villous trees as well as regression after birth requires extensive tissue remodeling during all stages of gestation and postpartum. Any remodeling is based on loosening/dissociation of cell-matrix junctions within the connective tissue of the maternal crypts and the fetal mesenchyme of the villous trees. Here fibroblasts are the major source for ECM molecules like fibronectin (FN1), type I collagen and type IV collagen (COL4) and laminin. They also produce a variety of matrix metalloproteinases (MMPs) for the remodeling of those components (Kalluri & Zeisberg 2006). In bovine, ECM molecules have been detected in the placenta from gestational day (gd) 80 to 270 in addition to the subunits of their respective integrin receptors (Pfarrer *et al.* 2003). These integrins are known to serve multiple functions at the feto-maternal interface in domestic animals during implantation (Burghardt *et al.* 2002). MMPs are a family of zinc-dependent endopeptidases that collectively are capable of degrading all extracellular matrix molecules. The MMP family can be subdivided into four different groups, which are the archetypal MMPs like collagenases, the gelatinases (e.g. MMP9), the matrilysins and the convertase-activatable MMPs (e.g. MMP14) that can be secreted or are membrane-associated (Fanjul-Fernandez *et al.* 2010). MMPs are regulated by four specific tissue inhibitors of matrix metalloproteinases (TIMPs) and by a variety of growth factors, cytokines and chemokines (Clark *et al.* 2008). Furthermore, MMPs cleave intracellular substrates like cytoskeletal proteins and are located in multiple cellular compartments, demonstrating the importance and wide range of MMP mediated processes (Cauwe & Opdenakker 2010). Placental cells share many molecular circuits with cancer cells (Ferretti *et al.* 2007). Specifically, the biological activities of MMPs (e.g. for cell proliferation, invasion and angiogenesis) have been confirmed for cancer cells (Egeblad & Werb 2002, Murray & Lessey 1999). Therefore, in the placenta similar ways of action are likely during implantation, gestation and around parturition.

Several studies have detected MMPs and tissue inhibitors of metalloproteinases (TIMPs) in bovine placental tissue throughout gestation (Kizaki *et al.* 2008, Walter & Boos 2001) or at term in comparison to animals suffering from RFM (Dilly *et al.* 2011, Streyl *et al.* 2012, Walter & Boos 2001). The gelatinases MMP2 and 9 are located in multiple placental compartments and appear to be the most likely candidates to play roles in ECM remodeling throughout gestation (Walter & Boos 2001, Kizaki *et al.* 2008). In term placental tissue membrane-bound MMP14 was also expressed on a protein level (Dilly *et al.* 2011). The main TIMP expressed in the placenta on a protein level is TIMP2, which is distinctly located only in TGC (Walter &

Boos 2001, Dilly *et al.* 2011). Furthermore, the mRNA of extracellular matrix metalloproteinase inducer (EMMPRIN), another factor that is able to regulate MMP14 and 2 expression, was located in bovine placental cell populations in increasing quantities throughout gestation (Mishra *et al.* 2012). Other members of the MMP (MMP1, 3, 9, 13, and 16) and TIMP family (TIMP1, 3 and 4) have so far only been detected on an mRNA level (Streyl *et al.* 2012) in term placental tissue, apparently due to the lack of proper antibodies.

Regarding the localization of ECM molecules and MMP substrates, considerable data are available. Throughout gestation multiple ECM molecules have been detected in the bovine placenta (Pfarrer *et al.* 2003, Boos *et al.* 2000). In placentomes, type I collagen and type III collagen are localized in the mesenchyme and the endometrial maternal stroma as well as blood vessel walls, while type IV collagen is observed beneath all epithelia, myometrium and also in the walls of vessels. Interestingly, type I collagen cannot be observed in the mesenchyme during early pregnancy, but later on increases strongly in midpregnancy (Boos *et al.* 2003b). The walls of large fetal and maternal blood vessel continuously express fibronectin and laminin throughout gestation. At gd 270 a local loss of type IV collagen and laminin has been observed in the maternal basement membrane. During early pregnancy TGC express high amounts of laminin and the corresponding ITGA6B1 integrin receptor homogeneously in the cytoplasm, suggesting TGC invasion along a self-produced matrix. Later on (gd 220-270) this localization shifts to a membrane-associated one. The expression of various integrin receptors throughout gestation has also been examined within the bovine placenta (Pfarrer *et al.* 2003). Molecules of the ECM and its corresponding integrin receptors have also been localized in bovine endometrium during the estrous cycle (Boos, 2000) and during bovine implantation in endometrium and trophoblast (MacIntyre *et al.* 2002, MacLaren & Wildeman 1995). In cattle TGC fusion with uterine epithelium (UE) during implantation has been proposed as being capable of changing the integrin/ECM composition of the subepithelial, endometrial stroma (MacIntyre *et al.* 2002). A distinct local change of stromal ECM (type I collagen content) upon implantation has been observed in goats (Guillomot 1999). In cattle, conflicting data on type I collagen downregulation in the subepithelial endometrial stroma in early pregnancy and/or the end of the cycles exist (Yamada *et al.* 2002, Boos, 2000), Due to the known extensive crosstalk of cell associated integrins with ECM proteins and many other growth factors and cytokine receptors such alterations are to be expected. For example, growth factor signaling affects the expression of a variety of molecules like MMP, TIMP, integrins and extracellular matrix molecules (ECM) during the establishment of human pregnancy (McEwan *et al.* 2009). In the bovine placenta multiple growth factor systems have been detected in the bovine placenta like FGF (Pfarrer *et al.* 2006a), VEGF (Pfarrer *et al.* 2006b), PAF (Bucher *et al.* 2006), EGF, Amphiregulin and Neuroregulin (Akbalik & Ketani 2013), Insulin-like growth factor (Ravelich *et al.* 2004) and Transforming growth factor β (TGFB) (Ravelich *et al.* 2006, Sugawara *et al.* 2010). Others, like bone morphogenetic protein (BMP) were found only in the bovine trophectoderm prior to uterine attachment (Pennington & Ealy 2012). In this context the relevance of the ECM should be highlighted again, since it plays a crucial and complex role during growth factor signaling. The ECM proteins are able to bind a great variety of soluble growth factors thereby regulating their bioavailability and integrating multivalent signals to the cell in a timely and spatially organized manner (Taipale & Keski-Oja 1997). To gain insights into the mechanistic aspects of how such growth factor systems could potentially influence MMP, TIMP, ECM molecules or integrin expression in bovine placental cell, in vitro studies were undertaken in the past. Such studies used bovine endometrial stroma cells and bovine blastocyst (Hirata *et al.* 2003b) or placental trophoblast cells (Dilly *et al.* 2010) and observed that growth factors like EGF, tumor necrosis factor (TNF) or ECM molecules like type I collagen, affect the expression of components of the MMP/TIMP system (Dilly *et al.* 2010, Hirata *et al.* 2003a, Hirata *et al.* 2003b). The maternal and fetal localization of proteins in the bovine placenta is shown in Table 1.

Table 1. Distribution pattern of proteins in bovine placentomal tissue over the course of implantation and gestation (analyzed by immunohistochemistry)

Protein	Maternal Localisation				Fetal Localisation						
ECM	MS	ME	MBM	MV	FS	UTC	FBM	TGC	FV	time frame	Ref.
Coll-I	x	x	nm	nm	nm	x	nm	nm	nm	gd 0-30	Yamada et al. 2002
Coll-I	x	nm	nm	x	x[1,2]	nm	nm	nm	x[1,2]	gm 1-9	Boos et al. 2003b
Coll-I	x	-	x	x	-	-	-	-	-	gd 80-270	Pfarrer et al. 2003
Coll-III	x	nm	nm	x	x[2]	nm	nm	nm	x[2]	gm 1-9	Boos et al. 2003b
Coll-IV	nm	nm	x	nm	nm	nm	x	nm	nm	gd 0-30	Yamada et al. 2002
Coll-IV	x	nm	x	x	x	nm	x	nm	x	gd 18-30	MacIntyre et al. 2002
Coll-IV	x	nm	x	x	x	nm	x	nm	x	gm 1-9	Boos et al. 2003b
Coll-IV	x	-	x[3]	x	x	x	x	-	x	gd 80-270	Pfarrer et al. 2003
FN	x	x	nm	nm	nm	x	nm	nm	nm	gd 0-30	Yamada et al. 2002
FN	x	nm	nm	nm	nm	-	-	-	nm	gd 14-21	MacLaren & Wildeman 1995
FN	x	-	x	x	x	x	x	-	x	gd 80-270	Pfarrer et al. 2003
LAMA	nm	x	x	x	nm	x	x	nm	x	gd 0-30	Yamada et al. 2002
LAMA	x	nm	x	x	x	nm	x	nm	x	gd 18-30	MacIntyre et al. 2002
LAMA	x	-	x[3]	x	-	-	-	x	-	gd 80-270	Pfarrer et al. 2003
Integrins	MS	ME	MBM	MV	FS	UTC	FBM	TGC	FV	time frame	Ref.
alpha1	x	x	nm	nm	nm	x	nm	nm	nm	gd 18-30	MacIntyre et al. 2002
alpha1	x	-	-	x	x	-	-	-	x	gd 80-270	Pfarrer et al. 2003
alpha2	x	-	-	x	x	-	-	x	x	gd 80-270	Pfarrer et al. 2003
alpha3	x	x	nm	nm	nm	x	nm	nm	x	gd 18-30	MacIntyre et al. 2002
alpha3	x	-	-	x	x	-	-	-	-	gd 80-270	Pfarrer et al. 2003
alpha4	x	-	-	x	x	-	-	-	x	gd 80-270	Pfarrer et al. 2003
alpha5	nm	nm	nm	nm	x	-	nm	nm	nm	gd 14-21	MacLaren & Wildeman 1995
alpha5	x	-	-	x	x	-	-	-	x	gd 80-270	Pfarrer et al. 2003
alpha6	x	x	nm	nm	nm	x	nm	nm	x	gd 18-30	MacIntyre et al. 2002
alpha6	x	x	-	x	x	x	-	x	x	gd 80-270	Pfarrer et al. 2003
alphaV	x	-	-	x	-	-	-	-	-	gd 80-270	Pfarrer et al. 2003
beta1	nm	nm	nm	nm	x	x	x	x	nm	gd 14-21	MacLaren & Wildeman 1995
beta1	x	x	nm	nm	nm	x	nm	x	x	gd 18-30	MacIntyre et al. 2002
beta1	x	x	-	x	x	x	-	x	x	gd 80-270	Pfarrer et al. 2003
beta1	x	x	x	nm	x	x	x	x	nm	gd 80-270	Bridger et al. 2008
beta3	x	-	-	x	-	-	-	-	-	gd 80-270	Pfarrer et al. 2003
beta4	x	-	-	x	-	-	-	-	-	gd 80-270	Pfarrer et al. 2003

MS: maternal stroma; ME: maternal epithelium; MBM: maternal basement membrane; MV: maternal vessels; FS: fetal stroma; UTC: uninuclear trophoblast cell; FBM: fetal basement membrane; TGC: trophoblast giant cell; FV: fetal vessel; gd: gestational day; gm: gestational month; nm: not mentioned

[1] expression only after gd 60; [2] expression increase over the course of gestation; [3] expression decrease shortly before term.

Table 1. (continued)

Protein	Maternal Localisation			Fetal Localisation					
MMPs	MS	ME	MV	FS	UTC	TGC	FV	time frame	Ref.
MMP2	x	x	x	x	x[4]	-	x	gm 1-9	Walter & Boos 2001
MMP2	x	-	x	x[5]	-	-	x	gm 3-9	Dilly et al. 2011
MMP2	x[2]	-	nm	nm	x[2]	x[6]	nm	gd 50-275	Mishra et al. 2012
MMP9	x	x	x	-	-	-	x	gm 1-9	Walter & Boos 2001
MMP14	x[7]	x[7]	-	x[2,5]	x[4]	-	x	gm 3-9	Dilly et al. 2011
MMP14	x[2]	x[2]	nm	nm	x[2]	x[2]	nm	gd 50-275	Mishra et al. 2012
MMP14	-	x[3]	-	-	-	-	x	12 days bevor calving	Streyl et al. 2012
TIMP1	-	x	-	x	x	x	-	12 days bevor calving	Streyl et al. 2012
TIMP2	-	-	-	-	-	x	-	gm 1-9	Walter & Boos 2001
TIMP2	-	-	-	-	-	x	-	gm 3-9	Dilly et al. 2011
EMMPRIN	-	x[2]	nm	nm	x[2]	x[2]	nm	gd 50-275	Mishra et al. 2012
GF	MS	ME	MV	FS	UTC	TGC	FV	time frame	Ref.
FGF1	x	x	-	x	x	x[im, m]	-	gd 150-270	Pfarrer et al. 2006a
FGF2	x	x	-	x	x[3]	x[im,3]	-		
FGF7	-	x[6]	x		x	x[im]	x		
FGFR	x	-	x	x	x	x[im]	x		
FGF1	-	x[7]	x[4]	-	-	x[im]	x[4]		
FGF2	-	x[3]	x[4]	-	-	x[im,3]	x[4]		
FGF7	x	x[6,8]	x[6]	-	-	x[im,3]	x[6]		
FGFR	x[6,8]	x[6,8]	-	-	-	x[im,3]	-		
FGF2IIIb	x[6,8]	x[6,8]	-	-	-	x[im,3]	-		
FGF2IIIc	x[6,8]	x[6,8]	-	-	-	x[im,3]	-		
VEGF	x	-	-	nm	x	x[9]	x	gd 18-48	Pfarrer et al. 2006b
VEGFR1	x	x	x	-	x	x[9]	x		
VEGFR2	x	x	x	-	x	x[9]	x		
VEGF	x	x	x[4,11]	-	x	x	x	gd 80 to term	Pfarrer et al. 2006b
VEGFR1	x	x[10]	x	-	x[10]	x	-		
VEGFR2	x[10]	x	x	x[6]	x	-	x[6,4]		
PAF-R	x[4]	x[8]	x	-	-	x[im,3]	x	gd 30 to term	Bucher et al. 2006
PAF-AH	-	x[8]	x[8]	-	-	x[im,3]	x[8]		
EGFR	-	x[12]	-	-	x[12]	x	-	gd 59-271	Akbalik & Ketani 2013
ErbB-2	x	x	-	x[12]	x[12]	x[12]	-		
ErbB-3	x[12]	x	-	x[13]	x	x	-		
ErbB-4	x[13]	x[13]	-	x[13]	x[12]	x	-		
EGF	-	x[12]	-	x[12]	x[12]	x[12]	-		
AREG	-	x[13]	-	x	x	x[12]	-		
NRG-1	-	x[13]	-	x	x	x[13]	-		

MS: maternal stroma; ME: maternal epithelium; MV: maternal vessels; FS: fetal stroma; UTC: uninuclear trophoblast cell; TGC: trophoblast giant cell; FV: fetal vessel; gd: gestational day; gm: gestational month; nm: not mentioned; [2] expression increase over the course of gestation; [3] expression decrease shortly before term; [4] expression only at the end of gestation; [5] expression vanished after normal calving; [6] very weak immunoreaction; [7] expression vanished with ongoing gestation; [8] expression increase shortly before term; [9] variable expression; [10] expression only at the begin of gestation; [11] only migestational (80-120day) expression; [12] expression decrease over the course of gestation; [13] expression decrease during the last trimester of gestation; [im] immature TGC; [m] mature TGC.

Drugs and pathology

During pregnancy/gestation, drugs or infectious agents can cross the placental barrier and harm placental integrity and the fetus (Giaginis *et al.* 2012, Johnson *et al.* 1994). At the same time nutrients have to selectively cross the placental barrier in a controlled manner to ensure optimal embryo growth. Nutrient and drug transport across the human placenta is either mediated by a variety of active transporters or by passive diffusion (Lager & Powell 2012, Giaginis *et al.* 2012). In the bovine placenta, proteins responsible for maternal-fetal Ca^{2+} transport (Sprekeler *et al.* 2012) and glucose transport have been located on different placental membrane layers (Wooding *et al.* 2005a). In addition, the prominent drug efflux carrier P-glycoprotein 1 (ABCB1), known for its role in the human placenta (Neumanova *et al.* 2014), is also functionally expressed in the bovine placenta and in bovine caruncular epithelial cells in vitro, mediating a basal-to-apical flux (Waterkotte *et al.* 2011). Therefore, such endometrial cells might be a proper in vitro model to study drug transport across the placental barrier of cattle. During gestation the bovine placenta is targeted by multiple infectious agents like parasites (Maley *et al.* 2003), viruses (Swasdipan *et al.* 2002) or bacteria (Hansen *et al.* 2011). Such agents have been either suggested as the cause for abortions (de Oliveira *et al.* 2010) or are also viewed critically in regard to pathogenicity (Agerholm 2013). In rodents and non-human primates, which are commonly employed for risk toxicity assessment, extensive data concerning placental lesions are available (Cline *et al.* 2013). Similar lesions (e.g. placental necrosis) are also observed in cattle (Gibney *et al.* 2008). Yet, placental morphological anomalies can also be caused due to non-infectious reasons like twinning (Benirschke 1995) or somatic cell nuclear transfer (SCNT) (Chavatte-Palmer *et al.* 2012). Furthermore, SCNT pregnancies also differ in their endometrial reaction to the fetus (Bauersachs *et al.* 2009).

One of the most common conditions occurring in cattle following parturition is RFM, which is associated with increased postpartum disease, decreased milk production and reduced reproductive performance and therefore is economically important (Laven & Peters 1996). Past studies analyzed a variety of factors in RFM such as apoptosis, and proliferation (Boos *et al.* 2003a), the expression of singular MMP/TIMP proteins (Dilly *et al.* 2011) and the change in expression of multiple genes by microarray analysis (Streyl *et al.* 2012). Very different causes for RFM have been proposed ranging from breed to stress and oxidative damage (Kankofer 2002). In addition, it is known that induction of parturition by different protocols also represents a major cause for RFM (Dilly *et al.* 2011). Normally at term a massive destruction of collagen and other ECM components accompanies uterine involution and expulsion of fetal membranes (McNaughton & Murray 2009). Failure of this process appears to be the underlying main cause of RFM, since it was demonstrated that collagenase injection via the umbilical arteries released the retained membranes (Eiler & Hopkins 1993). Therefore, it stands to reason that the regulation of the activity of endogenous collagenase (MMPs) in the bovine placenta plays a key role in the timely release of fetal membranes. One candidate for such a signal might be 12-oxo-eicosatetraenoic acid (12-oxoETE), which shows a peak concentration in maternal blood prior to release of fetal membranes and induces detachment of cells from culture vessels. In animal experiments injection with 12-oxoETE resulted in rapid release of fetal membranes (Kamada *et al.* 2012).

New discoveries and major challenges

In the past years multiple discoveries have been made, providing new information on the development and physiology of the bovine placenta. One such discovery is surely the use of cell lines for mechanistic studies on growth factor action (Hambruch *et al.* 2009, Dilly *et al.*

2010, Hirata *et al.* 2003b), TGC differentiation (Nakano *et al.* 2002b, Haeger *et al.* 2011) and cell-matrix interactions (Bridger *et al.* 2008). However, one of the main pitfalls is the isolation and characterization of such cell lines, particularly trophoblast lines (Haeger *et al.* 2011, Shimada *et al.* 2001, Talbot *et al.* 2010) due to cross contamination with other placental cell types (Bridger *et al.* 2007, Feng *et al.* 2000). Another drawback is the loss of TGC differentiation from such cell lines and the limited options to induce formation in vitro (Hambruch *et al.* 2009, Haeger *et al.* 2011, Shimada *et al.* 2001, Nakano *et al.* 2002a). More recently 3-dimensional cell culture models (spheroids) have been employed to study TGC formation (Haeger *et al.* 2011), trophoblast attachment (Sakurai *et al.* 2012) and endometrial $PGF_{2\alpha}$ (Prostaglandin $F_{2\alpha}$) secretion (Yamauchi *et al.* 2003). Yet, the use of bovine placental cell lines might still prove to be a valuable tool in the future, as important observations in human trophoblast physiology (e.g. syncytialization) have also been made in cell culture systems (Kliman *et al.* 1986). In addition, studies have been published, which provide clues for further research on placental development including the following: (1) the fact that bovine trophoblast expresses mesenchymal-genes after attachment to the endometrium (Yamakoshi *et al.* 2012), which represents a dramatic change to mid-gestation as far as vimentin is concerned (Haeger *et al.* 2011), underlining the plasticity of bovine trophoblast cells during different stages of gestation; and (2) the hypothesis that endogenous retroviruses are evolutionary driving forces for placental structure, TGC differentiation and cell fusion in the bovine placenta (Koshi *et al.* 2012, Spencer & Palmarini 2012, Black *et al.* 2010, Nakaya *et al.* 2013). Furthermore, future work in the field of placental development should also be directed at the cellular effects of trophoblastic glycoproteins like pregnancy-associated glycoproteins (PAG), and their functional implications at the feto-maternal interface (Wooding *et al.* 2005b).

Conclusions

Due to the economic importance of bovine reproduction extensive data are available on the development of the placenta in cattle. Nevertheless, even though new intriguing facts have been discovered, the understanding of economically important diseases like RFM still poses a major challenge. A wide variety of important questions are available for future research studies to be undertaken by bovine and/or ruminant placentologists, since it has become clear that placental physiology and pathology are important. Despite new technological opportunities (e.g. microarray analysis), techniques such as electron microscopy have yielded an incredible amount of valuable data (Wathes & Wooding 1980, Wooding *et al.* 1994, Leiser 1975, Leiser *et al.* 1997) and could still be employed in future research. Interestingly, adaptive angiogenesis in the ruminant placenta (Krebs *et al.* 1997) shows remarkable similarities to adaptive angiogenesis in fetal villi in placentas of heavy smokers (Pfarrer *et al.* 1999). Therefore, researchers working predominantly on human placentation might also consider mechanisms associated with bovine placentation since the villous feto-maternal interdigitation and the branching of villi is similar to human placental villi.

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