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),
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 epitheliocorial 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.
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.

Fig. 1. Gross anatomy of different developmental stages of the bovine placenta (A-G) and histology of the feto-maternal interface in cattle (H).
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 INF (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 &
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 vessels 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 Neuregulin (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 | time frame | Ref. |
|---------|----------------------|--------------------|------------|------|
|         | ECM | MS | ME | MBM | MV | FS | UTC | FBM | TGC | FV |          |          |
| Coll-I  | x  | x | nm | nm | nm | nm | x | nm | nm | nm | nm | gd 0-30 | Yamada et al. 2002 |
| Coll-I  | x  | nm | nm | x | x | x | x | nm | nm | nm | x | 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 | x | nm | nm | nm | x | x | gm 1-9 | Boos et al. 2003b |
| Coll-IV | nm | nm | nm | x | nm | nm | nm | nm | nm | nm | nm | gd 0-30 | Yamada et al. 2002 |
| Coll-IV | x  | nm | x | x | x | nm | x | nm | x | nm | x | gd 18-30 | Macintyre et al. 2002 |
| Coll-IV | x  | - | x | x | - | - | - | - | - | - | - | gd 80-270 | Pfarrer et al. 2003 |
| FN      | x  | x | nm | nm | nm | nm | x | nm | nm | nm | nm | gd 0-30 | Yamada et al. 2002 |
| FN      | x  | - | x | x | x | x | x | x | - | - | - | gd 14-21 | Maclaren & Wildeman 1995 |
| LAMA    | nm | x | x | x | x | x | nm | x | nm | x | gd 80-270 | Pfarrer et al. 2003 |
| LAMA    | x  | nm | x | x | nm | nm | nm | nm | nm | nm | x | gd 18-30 | Macintyre et al. 2002 |
| LAMA    | x  | - | x | x | x | - | - | - | - | - | - | gd 80-270 | Pfarrer et al. 2003 |
| Integrins | MS | ME | MBM | IV | FS | UTC | FBM | TGC | FV | time frame | Ref. |
| alpha1  | x  | x | nm | nm | nm | nm | x | nm | nm | nm | nm | gd 18-30 | Macintyre et al. 2002 |
| alpha1  | x  | - | - | x | x | - | - | - | - | - | - | gd 80-270 | Pfarrer et al. 2003 |
| alpha2  | x  | - | x | x | - | x | x | x | x | x | x | gd 80-270 | Pfarrer et al. 2003 |
| alpha3  | x  | - | nm | nm | nm | nm | x | nm | nm | nm | x | gd 80-270 | Macintyre et al. 2002 |
| alpha4  | x  | - | x | nm | nm | nm | x | nm | nm | nm | - | gd 80-270 | Pfarrer et al. 2003 |
| alpha5  | nm | nm | nm | nm | nm | nm | x | nm | nm | nm | nm | gd 14-21 | Maclaren & Wildeman 1995 |
| alpha5  | x  | - | x | x | x | x | x | x | x | x | x | gd 80-270 | Pfarrer et al. 2003 |
| alpha6  | x  | x | nm | nm | nm | nm | x | nm | nm | nm | x | gd 80-270 | Pfarrer et al. 2003 |
| alpha6  | x  | x | - | x | x | x | x | x | x | x | x | gd 80-270 | Pfarrer et al. 2003 |
| alphaV  | x  | - | x | x | - | x | - | - | - | - | - | gd 80-270 | Pfarrer et al. 2003 |
| beta1   | nm | nm | nm | nm | nm | nm | x | x | x | x | nm | gd 14-21 | Maclaren & Wildeman 1995 |
| beta1   | x  | x | nm | nm | nm | nm | x | nm | 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 | nm | x | x | x | 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 | time frame | Ref.          |
|--------------|-----------------------|--------------------|------------|--------------|
|              | MS | ME | MV | FS | UTC | TGC | FV |                |
| MMP2         | x  | x  | x  | x  | x⁴ | -  | -  | x              | gm 1-9     | Walter & Boos 2001 |
| MMP2         | x  | -  | x  | x⁵ | -  | -  | x  | gm 3-9        | Dilly et al. 2011 |
| MMP2         | x² | -  | nm | nm | x² | x⁶ | nm | gm 50-275     | Mishra et al. 2012 |
| MMP9         | x  | x  | x  | -  | -  | -  | -  | x              | gm 1-9      | Walter & Boos 2001 |
| MMP14        | x² | x² | nm | nm | x² | x² | nm | gm 3-9        | Dilly et al. 2011 |
| MMP14        | x² | x² | nm | nm | x² | x² | nm | gm 50-275     | Mishra et al. 2012 |
| TIMP1        | 12 days before calving | Streyle et al. 2012 |
| TIMP2        | -  | -  | x  | x  | x  | -  | -  | x              | gm 1-9      | Walter & Boos 2001 |
| TIMP2        | -  | -  | -  | -  | -  | -  | -  | x              | gm 3-9      | Dilly et al. 2011 |
| ENMMPRIN     | -  | x² | nm | nm | x² | x² | nm | gm 50-275     | Mishra et al. 2012 |
| GF           |        |    |    |    |    |    |    |                |
| FGFR1        | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| FGFR2        | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| FGFR7        | -  | x⁶ | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| FGFR         | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| FGFR1        | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| FGFR2        | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGF         | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGFR1       | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGFR2       | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGF         | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGFR1       | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGFR2       | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGF         | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGFR1       | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| VEGFR2       | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| PAF-R        | x⁴ | x⁸ | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| PAF-AH       | x⁴ | x⁸ | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| EGFR         | -  | x² | -  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| ErbB-2       | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| ErbB-3       | x¹²|x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| ErbB-4       | x¹³|x¹³| x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| EGF          | -  | x¹²|x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| AREG         | x¹³ | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |
| NRG-1        | x  | x  | x  | x  | x  | x  | x  | -  | x              | gd 150-270  | Pfarrer et al. 2006a |

MS: maternal stroma; ME: maternal epithelium; MV: maternal vessels; FS: fetal stroma; UTC: uninuclear trophoblast cell; TGC: trophoblast giant cell; FV: fetal vesel; gd: gestational day; gm: gestational month; nm: not mentioned; ²expression increase over the course of gestation; ³expression decrease shortly before term; ⁴expression only at the end of gestation; ⁵expression vanished after normal calving; ⁶very weak immunoreaction; ⁷expression vanished with ongoing gestation; ⁸expression increase shortly before term; ⁹variable expression; ¹⁰expression only at the begin of gestation; ¹¹only migrastational (80-120day) expression; ¹2expression decrease over the course of gestation; ¹³expression decrease during the last trimester of gestation; ¹⁴immature TGC; ¹⁵mature 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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