



Review article

The role of placental MHC class I expression in immune-assisted separation of the fetal membranes in cattle

Lindert Benedictus^{a,*}, Ad P. Koets^{a,b}, Victor P.M.G. Rutten^{a,c}^a Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.165, 3508 TD Utrecht, The Netherlands^b Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.151, 3508 TD Utrecht, The Netherlands^c Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Private Bag X04, Onderstepoort 0110, South Africa

ARTICLE INFO

Article history:

Received 27 January 2015

Received in revised form 27 April 2015

Accepted 20 May 2015

Keywords:

Retained fetal membranes

Major histocompatibility complex class I

Pregnancy

Alloimmunity

Fetal–maternal adherence

Cattle

ABSTRACT

The bovine fetus, like that of other species, is a semi-allograft and the regulation of materno-fetal alloimmunity is critical to prevent its immunological rejection. In cattle, a materno-fetal alloimmune response may be beneficial at parturition. It is hypothesized that upregulation of major histocompatibility complex (MHC) class I on the fetal membranes toward the end of gestation induces a maternal alloimmune response that activates innate immune effector mechanisms, aiding in the loss of the adherence between the fetal membranes and the uterus. Loss of fetal–maternal adherence is pivotal for the timely expulsion of the fetal membranes and the absence (or reduction) of the maternal immune response may lead to retained fetal membranes, a common reproductive disorder of cattle. Currently, there is no effective treatment for retained fetal membranes and a better understanding of materno-fetal alloimmune-assisted separation of the fetal membranes may lead to novel targets for the treatment of retained fetal membranes. In this review, the regulation of materno-fetal alloimmunity during pregnancy in cattle, with a focus on placental MHC class I expression, and the importance of maternal alloimmunity for the timely separation of the fetal membranes, are discussed.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The bovine fetus inherits and expresses paternal antigens and is thus semi-allogeneic to the maternal immune system. Preventing immunological rejection of the fetus is,

therefore, critical for a successful pregnancy. Maternal antibodies against paternal alloantigens are induced in up to 64% of multiparous cattle and can be detected as early as the second trimester of gestation (Hines and Newman, 1981), showing that the materno-fetal immune response is regulated, rather than suppressed, and is normally not harmful to the fetus. In cattle, delayed expulsion of the fetal membranes, a common reproductive disorder, is associated with a reduced maternal (allo)immune response (Benedictus et al., 2011, 2012; Gunnink, 1984b; Joosten et al., 1991), suggesting that at parturition a materno-fetal alloimmune response may be beneficial for the separation of the fetal membranes. The regulation of materno-fetal alloimmunity

Abbreviations: MHC, major histocompatibility complex; NC-MHC class I, non-classical MHC class I; BNC, binucleate cells; RFM, retained fetal membranes; ECM, extracellular matrix; MMP, matrix metalloproteinases; TIMP, tissue inhibitors of MMPs; CR, coefficient of relationship.

* Corresponding author. Tel.: +31 30 2534608; fax: +31 30 2533555.

E-mail addresses: LindertBenedictus@gmail.com (L. Benedictus), a.p.koets@uu.nl (A.P. Koets), v.rutten@uu.nl (V.P.M.G. Rutten).

<http://dx.doi.org/10.1016/j.jri.2015.05.003>

0165-0378/© 2015 Elsevier Ireland Ltd. All rights reserved.

during pregnancy in cattle, with a focus on placental MHC class I expression, and the importance of maternal alloimmunity for the timely separation of the fetal membranes, are discussed.

2. Placental MHC class I expression and regulation of materno-fetal alloimmunity during pregnancy

There are three basic mechanisms preventing immunological rejection of the fetus (Bainbridge, 2000; Lynge et al., 2014):

- (i) Anatomical separation of the fetus from the maternal immune system;
- (ii) Down-regulation of alloantigen expression by the fetus;
- (iii) Regulation of the maternal immune response in the uterus.

Care should be taken in extrapolating findings on the regulation of materno-fetal immunity during pregnancy from other species to cattle. There are large differences in placental morphology between species and the common ancestor of species with long gestation periods (e.g., horse, cattle, and humans) had a short gestation period. Therefore, mechanisms to regulate materno-fetal immunity during pregnancy (for a prolonged time) have evolved separately in these lineages and are likely to be species-specific (Bainbridge, 2000).

In the bovine placenta fetal trophoblasts and maternal endometrium form a continuous epithelial lining across the whole placenta (Fig. 1) (Schlafer et al., 2000). Specialized structures called placentomes form through interdigitation of maternal (caruncle) and fetal (cotyledon) epithelium, thereby increasing the surface area for the exchange of waste and nutrients (Schlafer et al., 2000). Bovine placental histology is in strong contrast to the human placenta, where fetal trophoblasts are directly in contact with maternal blood and extravillous trophoblasts that invade the uterine tissue and reshape maternal blood vessels (Gude et al., 2004). The anatomy of the bovine placenta ensures that there is minimal contact between the maternal immune system and fetal cells.

Allogeneic MHC class I is highly immunogenic and in several species it has been shown that MHC class I is down-regulated on fetal trophoblasts, e.g., humans, horse and pig (Bainbridge, 2000). In cattle MHC class I expression on fetal trophoblasts is down-regulated in early pregnancy, but toward mid-gestation expression becomes apparent in interplacentomal regions and rises toward the end of gestation (Davies et al., 2000; Low et al., 1990). In the placentomes, at the area of most intimate contact, there is no MHC class I expression on the trophoblasts (Chavatte-Palmer et al., 2007; Davies et al., 2000; Low et al., 1990). In an elegant study by Davies et al. (2006) it was shown that interplacentomal trophoblasts transcribe very high levels of non-classical MHC class I, indicating that a proportion of the MHC class I proteins expressed by bovine trophoblasts is non-classical. Ellis et al. (1998) detected transcription of MHC class I in late gestation placentome-derived trophoblasts, but could not detect the expression of MHC class I with ILA88, a monoclonal antibody that is pan-specific for bovine MHC class I, and hypothesized that this could reflect the expression of non-classical MHC class I (NC-MHC class I). Since there are no bovine NC-MHC class I-specific antibodies, it is currently impossible to differentiate classical and non-classical MHC class I protein expression in the bovine placenta. In human pregnancies HLA-G, a NC-MHC class I, is highly expressed on trophoblasts, both on the cell membrane and in soluble form, and is believed to be of importance for immune regulation, suppression, and tolerance induction at the fetal–maternal interface (Lynge et al., 2014). Davies et al. (2006) found multiple splice variants of one non-classical allele, including a variant with a deletion of the transmembrane domain, indicating that soluble bovine NC-MHC class I may be expressed. It is probable that NC-MHC class I expression on bovine trophoblasts plays a similar role to HLA-G in humans and that expression of NC-MHC class I and restricted expression of classical MHC class I by the fetus contribute to the regulation of maternal immunity.

Binucleate cells (BNC), specialized cells formed from uninucleate trophoblasts and unique to ruminants, can migrate to the endometrium and fuse with maternal cells, temporarily forming trinucleate cells (Fig. 1) (Schlafer et al., 2000; Wooding, 1992). BNC produce an array of secretory molecules, including placental lactogen,

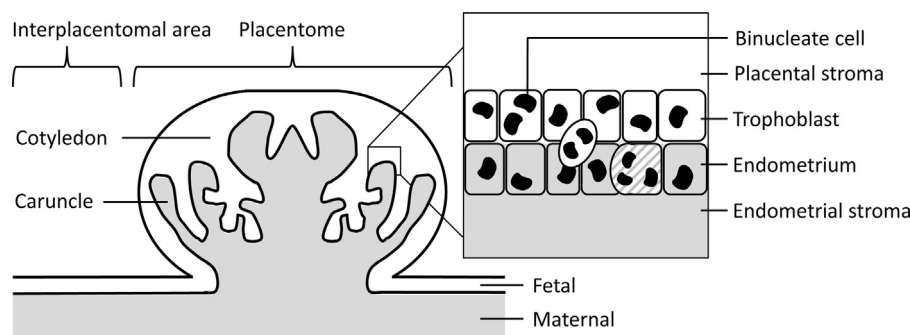


Fig. 1. Bovine placentomes. Placentomes are formed through interdigitation of maternal (caruncle) and fetal (cotyledon) tissue. The apposition of fetal trophoblasts to the maternal endometrium forms a continuous epithelial lining across the placenta. Binucleate cells, specialized trophoblasts, can migrate to the maternal side and may fuse with endometrial cells, forming trinucleate hybrid cells (shaded).

pregnancy-associated glycoproteins and many hormones, and likely play a pivotal role in fetomaternal crosstalk, as reviewed by Wooding et al. (2005). BNC have been found to express MHC class I in placentomes collected 'at term' collected (Bainbridge et al., 2001; Ellis et al., 1998) and transcribed both classical and non-classical MHC class I (Bainbridge et al., 2001). On the other hand, Davies et al. (2000) and Chavatte-Palmer et al. (2007) could not detect MHC class I expression on BNC. However, these studies looked at BNC at around 230 days' gestation and after dexamethasone-induced parturition respectively. In the study by Bainbridge and colleagues (2001) it was found that not all BNC expressed MHC class I and at present it remains unknown whether or not BNC express MHC class I at the moment of fusion with maternal cells. If this were the case, it would present an interesting situation for allorecognition, as this enables the presentation of fetal antigens on both fetal and maternal MHC class I and the expression of maternal antigens on fetal MHC class I, thereby increasing the chance of the allorecognition of fetal MHC class I. Although the results regarding the MHC class I expression of BNC are not conclusive, invasive trophoblasts in horse also upregulate MHC class I and Bainbridge (2000) hypothesized that the upregulation of MHC class I on invasive trophoblasts possibly contributes to the induction of tolerance to paternal MHC class I. Indeed, for the induction of antigen-specific regulatory T cells the cognate antigen of the T cell has to be present (Sela et al., 2011). Expression of classical MHC class I on invasive (binucleate) trophoblast cells, in combination with the expression of NC-MHC class I and the immunosuppressive and tolerogenic environment of the placenta, could, although this has not been studied in cattle, lead to the induction of paternal MHC class I-specific regulatory T cells in the dam.

Maternal endometrium expresses MHC class I throughout pregnancy in the interplacentomal area (Davies et al., 2000; Low et al., 1990). Findings regarding maternal MHC class I expression in the placentomes are conflicting, with studies reporting no expression (Davies et al., 2000), down-regulation (Low et al., 1990), and normal expression (Chavatte-Palmer et al., 2007).

MHC class I down-regulation is a common immune evasion method of infectious organisms and NK cells can detect and kill cells with low or no MHC class I expression. The human NC-MHC class I gene HLA-G is known to inhibit NK cells and cytotoxic T cells and can induce regulatory T cells (Lynge et al., 2014). Although numbers are low, NK cells have been detected in the endometrium of pregnant cattle (Oliveira et al., 2013). Based on the analogy between bovine and human NC-MHC class I, expression of NC-MHC class I on bovine trophoblasts may inhibit NK cells and contribute to the induction of regulatory T cells. In humans reduced levels of regulatory T cells in the placenta and peripheral blood are associated with pre-eclampsia and preterm labor (Quinn and Parast, 2013) and the depletion of regulatory T cells in allogeneic pregnancies in mice leads to the failure of gestation (Aluvihare et al., 2004), showing the importance of regulatory T cells for successful pregnancy in other species. Foxp3 has been detected in the bovine placenta (Oliveira et al., 2013) and levels of CD4⁺CD25⁺ T cells rise in the peripheral blood of pregnant cows (Oliveira and

Hansen, 2008). However, there is evidence that CD4⁺CD25⁺ Foxp3⁺ T cells do not have regulatory functions in cattle (Hoek et al., 2009). Instead, $\gamma\delta$ T cells were shown to act as regulatory cells (Hoek et al., 2009), low numbers of which have been detected in the endo- and myometrium of pregnant cattle (Oliveira et al., 2013).

Many soluble factors are released at the fetomaternal interface and systemically during pregnancy (e.g., uterine serpins, pregnancy hormones) and contribute further to the modulation of the maternal immune system. However, these are outside the scope of this review and are discussed in Oliveira et al. (2012) and Hansen (2013).

3. Materno-fetal alloimmune-assisted separation of the fetal membranes

3.1. Physiology of the separation of the fetal membranes and retained fetal membranes

In cattle the fetal membranes are normally expelled within six hours after the calf is born (van Werven et al., 1992). Retained fetal membranes (RFM), the persistence of the adherence between the fetal membranes and the maternal placenta after parturition, is a frequently occurring postpartum disorder in cattle (Laven and Peters, 1996; van Werven et al., 1992) and is associated with reduced reproductive performance (Joosten et al., 1988; van Werven et al., 1992) and economic losses (Joosten et al., 1988).

Loss of adherence between the fetal and the maternal epithelium together with contractions of the uterus lead to the expulsion of the fetal membranes. The loss of fetal-maternal adherence in cattle is believed to involve several processes:

- (i) Collapse of the fetal-placental circulation, leading to shrinking of the placentomal villi (Laven and Peters, 1996).
- (ii) Placental maturation, characterized by a decrease in the number and the height of maternal epithelial cells (Boos et al., 2003; Laven and Peters, 1996) and a drop in BNC numbers (Gross et al., 1991; Williams et al., 1987).
- (iii) Breakdown of the extracellular matrix linking the fetal and maternal epithelium (Beagley et al., 2010).

The first indications for the involvement of the maternal immune system in the loss of fetal-maternal adherence and the occurrence of RFM were provided by Gunnink (1984a, 1984b). Measuring the chemotaxis of maternal leukocytes toward fetal cotyledon extracts revealed that the chemotactic activity of cotyledons obtained from RFM cows was reduced. Also, chemotaxis of leukocytes obtained from RFM cows toward cotyledons from healthy animals was hampered and this could already be observed a week before parturition. Similar results were found by Heuwieser et al. (1985). Kimura et al. (2002) found that the functioning of neutrophils from RFM cows was impaired and that this was also apparent before parturition. However, the best indication for the direct involvement of the maternal immune system in placental separation was the finding that MHC class I compatibility between

fetus and dam gives a high risk of RFM in the dam (Benedictus et al., 2012; Joosten et al., 1991). Comparison of cytokine levels and leukocyte subsets in placental tissue between MHC class I-compatible and -incompatible pregnancies approximately 24 h before parturition showed that MHC class I compatibility had a direct influence on the maternal immune response at the placenta (Davies et al., 2004). These results indicate that around parturition allogeneic MHC class I expressed on fetal trophoblasts elicits a materno-fetal alloimmune response that aids in the “loss” of fetal–maternal adherence. Conversely, the absence (or reduction) of materno-fetal alloimmunity in MHC class I-compatible pregnancies leads to RFM. In the following section we discuss how the allorecognition of fetal MHC class I and the ensuing maternal immune response affects fetal–maternal adherence. Next, we hypothesize what might prompt the alloimmune-assisted separation of the fetal membranes at the end of gestation.

3.2. Immune-assisted loss of fetal–maternal adherence

First, we questioned which mechanism of allorecognition is (most) important for the fetal MHC class I-driven materno-fetal alloimmune response. The maternal and fetal epithelia are largely intact following separation of the fetal membranes (Laven and Peters, 1996; Williams et al., 1987), indicating that the loss of fetal adherence is not a destructive process. Therefore, direct allorecognition of fetal MHC class I on trophoblasts by cytotoxic CD8⁺ T cells and subsequent killing is not a likely route of materno-fetal alloimmune-assisted separation of the fetal membranes. CD8⁺ T cells are present in the placenta during pregnancy (Davies et al., 2004; Oliveira et al., 2013), but not in great numbers. Davies et al. (2004) detected a drop in CD8⁺ T cells around parturition in MHC class I-incompatible pregnancies, but not in MHC class I-compatible pregnancies. The mechanism causing the drop in CD8 T cell numbers is not known, but appears to be related to the recognition of fetal MHC class I and could potentially be caused by non-classical MHC class I-induced, FAS receptor-mediated apoptosis of activated CD8⁺ T cells in incompatible pregnancies (Fournel et al., 2000). Expression of MHC class I on trophoblasts of first-trimester, somatic-cell, nuclear transfer cloned bovine fetuses can lead to immune-mediated abortion (Hill et al., 2002). Characterization of lymphocyte populations in the placenta of somatic cell nuclear transfer pregnancies revealed that CD4⁺ T cells were the dominant population (Davies et al., 2004), indicating the indirect presentation of alloantigens via self-MHC class II and activation of CD4⁺ T cells is the most likely route of immune-mediated abortion in these cloned pregnancies. Similarly, recognition of fetal MHC class I around parturition most likely involves the indirect pathway of allorecognition. Maternal macrophages residing in the placental endometrium are MHC class II-positive (Oliveira and Hansen, 2009) and MHC class I expression is up-regulated on placental (binucleate) trophoblasts toward parturition (Bainbridge et al., 2001; Davies et al., 2000). Apoptosis of trophoblasts and subsequent phagocytosis by macrophages allows the presentation of fetal MHC class I via maternal MHC class II. The production of fetal

alloantigen-specific IgG antibodies, which can be detected during pregnancy (Hines and Newman, 1981), depends on self-MHC II-restricted CD4⁺ T cell help and shows that the indirect pathway of allorecognition does indeed occur during pregnancy.

The next question we addressed was how does the maternal alloimmune response facilitate the loss of fetal–maternal adherence? Breakdown of the extracellular matrix (ECM) linking the fetal and maternal epithelium is thought to be very important in the separation of the fetal membranes (Beagley et al., 2010). Indeed, many genes associated with the degradation of the ECM are upregulated around parturition (Streyl et al., 2012) and disruption of the ECM by the infusion of collagenase into the placenta led to a marked reduction in the retention time of fetal membranes in experimentally induced RFM (Eiler and Hopkins, 1993). Macrophages are potent producers of many cytokines and play an important role in the breakdown and remodeling of the ECM (Chazaud, 2014; Galdiero et al., 2013). Miyoshi et al. (2002) found that the reduced function of uterine macrophages was associated with the occurrence of RFM. Oliveira and Hansen showed that during pregnancy large numbers of maternal macrophages accumulate in the uterus (2008, 2009) and that at least some of these macrophages have a phenotype that supports immune regulation and tissue homeostasis (2010). However, under the influence of the materno-fetal alloimmune response uterine macrophages may assume a more inflammatory phenotype toward parturition (Chazaud, 2014; Oliveira et al., 2010) that aids in the breakdown of the ECM. Macrophages with an inflammatory phenotype are characterized by the release of reactive oxygen and nitrogen intermediates, chemokines and inflammatory cytokines (e.g., IL-1 β , IL-6, TNF- α) (Chazaud, 2014). In cattle, the mRNA expression levels of the pro-inflammatory cytokines IL-1 β , IL-6, and IL-8 rise in the cervix toward parturition (Van Engelen et al., 2009) and in placental macrophages in humans a shift from an immune regulatory toward an inflammatory phenotype at parturition is believed to aid in the degradation of the ECM (Nagamatsu and Schust, 2010). Comparing MHC class I-incompatible and -compatible pregnancies, Davies et al. (2004) found higher numbers of maternal macrophages in incompatible than in compatible pregnancies. Furthermore, in incompatible pregnancies higher amounts of IL-2 were detected and macrophages stained less intensely for TNF- α (Davies et al., 2004). CD4⁺ T cells are the major source of IL-2 and in this context increased IL-2 likely results from the activation of CD4⁺ T cells by uterine macrophages presenting fetal allogeneic MHC class I. Release of TNF- α from activated macrophages may be an explanation for the decreased TNF- α staining in MHC class I-incompatible pregnancies. These results imply that the maternal recognition of fetal MHC class I might activate macrophages and induce cytokine production, which, as further explained in the following paragraphs, through the direct and indirect effects of macrophages, can lead to breakdown of the ECM and to loss of fetal–maternal adherence.

Placental maturation is characterized by increased apoptosis of trophoblasts and maternal endothelium (Boos et al., 2003) and is one of the processes believed to be

involved in the loss of fetal–maternal adherence (Boos et al., 2003; Laven and Peters, 1996). Uterine macrophages produce TNF- α (Davies et al., 2004), which can induce apoptosis in cells and as such may influence placental maturation. Matrix metalloproteinases (MMP) are enzymes capable of breaking down the ECM. MMP-2, MMP-9, and MMP-14 have been detected in the bovine placenta (Dilly et al., 2011; Maj and Kankofer, 1997; Walter and Boos, 2001) and are upregulated before parturition (Streyl et al., 2012). MMPs can be activated by many (inflammatory) cytokines (Hirata et al., 2003), including IL-1, IL-6, and TNF- α . Maj and Kankofer (1997) found lower MMP-2 and MMP-9 enzyme activity in animals with spontaneous RFM, but after induced parturition Walter and Boos (2001) and Dilly and colleagues (2011) found no differences in MMP-2, MMP-9, and MMP-14 between non-RFM and RFM cows. However, the activity of MMPs is inhibited by tissue inhibitors of MMPs (TIMP) and both studies found that the presence of TIMP-2 in the bovine placenta is restricted to BNC (Dilly et al., 2011; Walter and Boos, 2001). In normal pregnancies there is a steep drop in BNC numbers and degranulation of BNC before parturition, while in RFM, BNC numbers remain high (Gross et al., 1991; Schlafer et al., 2000; Williams et al., 1987). The drop in BNC numbers before parturition may increase the activity of MMPs in the placenta through the withdrawal of TIMP-2. Interestingly, around parturition, BNC numbers were lower in MHC class I-incompatible than in -compatible pregnancies (Davies et al., 2004), indicating that allorecognition of fetal MHC class I is directly related to the drop in BNC normally seen before parturition. We hypothesize that cytokines resulting from the materno-fetal alloimmune response around parturition might influence the life cycle of BNC, inducing degranulation or apoptosis (e.g., TNF- α), leading to the drop in BNC numbers. Neutrophils also have the ability to remodel or breakdown the ECM (Galdiero et al., 2013). In humans, IL-8 stimulates the release of MMP-9 from neutrophils. IL-8 is an important chemotactic factor for neutrophils in term cotyledons (Kimura et al., 2002) and normally, the expression of IL-8 in placentomes is upregulated around parturition (Streyl et al., 2012). IL-8 serum levels around parturition were lower in RFM than in non-RFM dams. Furthermore, the chemotaxis toward cotyledons of neutrophils obtained from dams that develop RFM is lower than obtained from dams that release the fetal membranes normally (Kimura et al., 2002). Although neutrophil numbers increase remarkably in the cervix toward parturition (Van Engelen et al., 2009), Miyoshi et al. (2002) report that immediately post-partum neutrophil numbers in the placenta are low. Nevertheless, the data from Kimura et al. (2002) indicate that neutrophils may play a role in the loss of fetal–maternal adherence.

Together these data indicate that innate immune effector mechanisms, rather than adaptive ones, lead to the actual breakdown of fetal–maternal adherence. Indeed, in cattle numerous genes associated with innate immunity are upregulated around parturition (Streyl et al., 2012) and in humans inflammation and innate immune cells are believed to play a pivotal role in parturition as well (Christiaens et al., 2008). In spite of this, the high risk of RFM in MHC class I-compatible pregnancies (Benedictus

et al., 2012; Joosten et al., 1991) and the direct effect of MHC class I compatibility on the maternal immune response in the uterus (Davies et al., 2004) shows that an adaptive immune response to fetal MHC class I is also critical to the separation of the fetal membranes. Compared with macrophage numbers (Miyoshi et al., 2002; Oliveira and Hansen, 2008), T cell numbers in term placentas are low (Miyoshi et al., 2002). Therefore, we hypothesize that around parturition CD4 T cells, activated through indirect allorecognition of fetal MHC class I, activate uterine macrophages and induce a switch toward an inflammatory phenotype, characterized by the production of cytokines such as IL-1 β , TNF- α , and IL-8. The resulting inflammatory milieu can subsequently activate more macrophages. As detailed above, these cytokines and activated macrophages (and possibly neutrophils) aid in the loss of fetal–maternal adherence. We conclude that the alloimmune response against fetal MHC class I serves as a trigger that activates innate immune effector mechanisms leading to the breakdown of fetal–maternal adherence (Fig. 2).

3.3. What prompts the alloimmune-assisted separation of the fetal membranes?

Fetal MHC class I is expressed on interplacentomal trophoblasts throughout the third trimester of pregnancy, while fetal–maternal adherence is unaffected. Currently, it is not known what prompts the shift from the regulation of materno-fetal alloimmunity during pregnancy to a maternal immune response that aids in the separation of the fetal membranes at parturition. Fetal BNC are the cells in most intimate contact with maternal tissue, since they migrate to the endometrium and fuse with maternal endometrial cells (Schlafer et al., 2000; Wooding, 1992). MHC class I expression on BNC is detected only around parturition (Bainbridge et al., 2001; Ellis et al., 1998) and BNC numbers are affected by MHC class I compatibility between dam and calf (Davies et al., 2004). BNC are present in the placentomal and in the interplacentomal regions. However, since trophoblasts from placentomal villi do not express MHC class I during pregnancy (Chavatte-Palmer et al., 2007; Davies et al., 2000; Low et al., 1990), expression of MHC class I on BNC around parturition presents a striking change in fetal MHC class I expression in placentomes especially. We hypothesize that allorecognition of MHC class I expressed at the end of pregnancy on BNC in placentomes could be the trigger of the maternal alloimmune-assisted separation of the fetal membranes. Since placentomes are most important for the attachment between the maternal and fetal parts of the placenta, breakdown of fetal–maternal adherence in these areas may be of particular importance for the timely release of the fetal membranes.

Of course many other changes occur at the end of pregnancy that could also influence the regulation of materno-fetal alloimmunity, including placental maturation (Boos et al., 2003; Laven and Peters, 1996), remodeling of the maternal and fetal epithelium, and the hormonal changes that occur around parturition (Senger, 2003). In most RFM cases the calf is born normally and, therefore, separation of the fetal membranes and birth of the calf appear to be governed by (partially) separate mechanisms.

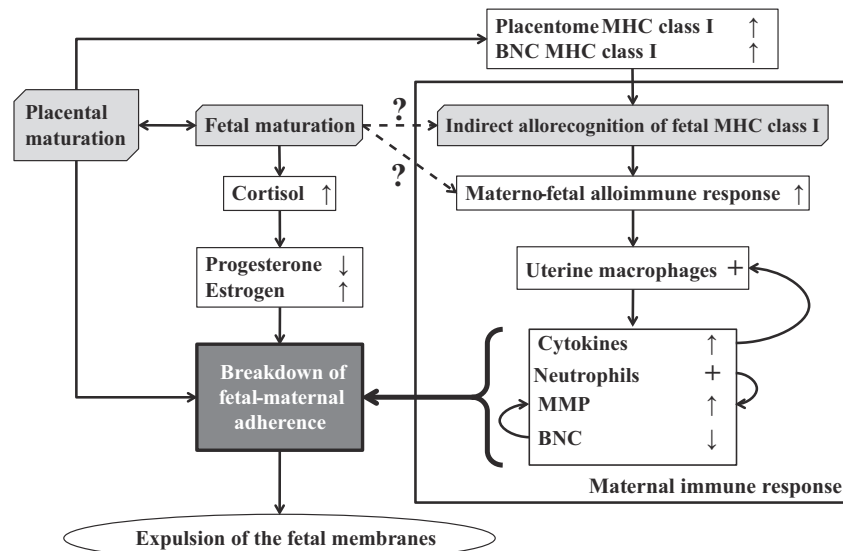


Fig. 2. Processes leading to the breakdown of fetal–maternal adherence. ↑ Upregulation. ↓ Down-regulation. + Activation. MMP matrix metalloproteinases, BNC binucleate cells.

In cattle the mature fetus produces cortisol, which triggers a cascade of hormonal changes that eventually initiates parturition (Senger, 2003). Emulating these hormonal changes with corticosteroids, prostaglandins or progesterone receptor blockers to induce parturition leads to the successful birth of the calf, but is associated with a high rate of RFM (25–100%) (Benedictus et al., 2011; Dilly et al., 2011; Shenavai et al., 2012). This shows that the hormonal changes seen around parturition alone are not sufficient for successful separation of the fetal membranes. Shenavai and colleagues (2012) found that placental maturation, i.e., changes in maternal endometrium and a drop in the number of BNC, did not occur after induction of parturition with corticosteroids, prostaglandins or progesterone receptor blockers. Above, we reasoned that the materno-fetal alloimmune response likely contributes to placental maturation and results from our group (Benedictus et al., 2011) showed that following the induction of parturition

with corticosteroids the occurrence of RFM is associated with reduced chemotactic activity of the fetal cotyledons and, therefore, with impaired alloimmune-assisted separation of the fetal membranes. We postulate that fetal maturation and the hormonal changes occurring around parturition are pivotal to the birth of the calf, whereas placental maturation and the materno-fetal alloimmune response are most important for separation of the fetal membranes (Fig. 2).

We found that the odds of RFM were much higher in two-way compatible than in maternal compatible pregnancies (Fig. 3) (Benedictus et al., 2012). Compatibility of the dam with the calf increased the odds of RFM and suggests that the fetal immune system might also play a role in the separation of the fetal membranes. The immune system of the calf is fully functional at the end of gestation (Cortese, 2009) and the number of fetal macrophages in the fetal membranes rises toward the end of pregnancy

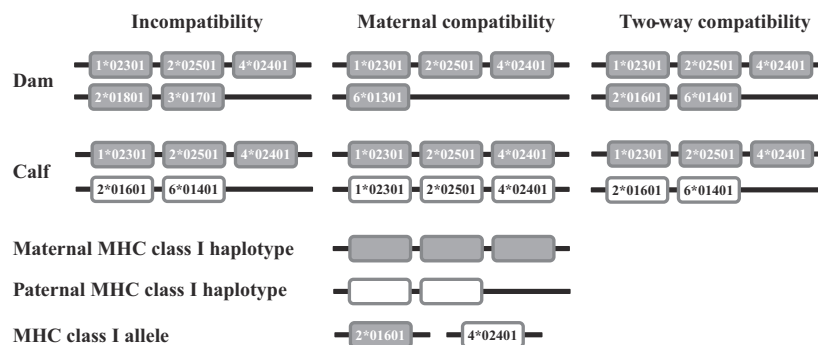


Fig. 3. Major histocompatibility complex (MHC) class I compatibility. In MHC class I-incompatible pregnancies the paternally inherited haplotype of the calf is not compatible with the dam, nor is the non-inherited maternal haplotype compatible with the calf. When the paternally inherited haplotype is compatible with the dam, there is maternal compatibility when the non-inherited maternal haplotype is not compatible with the calf and two-way compatibility when the non-inherited maternal haplotype is compatible. (Bovine MHC class I haplotypes contain 1 to 3 functional loci.).

(Schlafer et al., 2000). Spontaneous parturition in humans is associated with fetal monocyte activation (Steinborn et al., 1999) and in mice surfactant protein-A production in the lungs of the fetus at the end of pregnancy is believed to activate macrophages, which migrate toward the maternal side of the placenta, where they produce inflammatory cytokines that initiate parturition (Condon et al., 2004). Therefore, we hypothesize that cytokines produced as a result of a fetal immune response against maternal alloantigens might contribute to the activation of the maternal innate immune response that leads to the breakdown of fetal–maternal adherence.

4. Reducing MHC class I compatibility as a preventive measure for RFM

Currently, there is no effective treatment for RFM (Beagley et al., 2010) and many of the identified risk factors for the occurrence of RFM are difficult to prevent. However, reducing MHC class I compatibility between dam and calf through controlled breeding may be a feasible measure to prevent RFM. The chance of MHC class I compatibility between dam and calf is related to their coefficient of relationship (CR; i.e., their degree of kinship). Indeed, results from our group (Benedictus et al., 2013) indicate that there might be a positive association between the CR of the dam and calf and the occurrence of RFM. A similar association has been found in Frisian horses (Sevinga et al., 2004). Theoretically, a 5% increase in CR between dam and calf gives a 10% higher change of MHC class I compatibility. However, the effect of CR on the occurrence of RFM was small and current breeding practice already minimizes the CR between dam and calf. Hence, reducing the CR between dam and calf will only have a minimal effect on the incidence of RFM. Although MHC class I haplotypes in a population are diverse, there are usually a handful of common haplotypes occurring at a high frequency (Codner et al., 2012). Therefore, MHC class I compatibility occurring through chance is relatively high and higher than compatibility occurring through common ancestry. Selective breeding of MHC class I typed dams and sires would avert the occurrence of MHC class I-compatible pregnancies. Since following normal parturition approximately half of the RFM cases are associated with MHC class I compatibility (Benedictus et al., 2012; Joosten et al., 1991), such an approach would be expected to substantially reduce the incidence of RFM. Current methods of typing bovine MHC class I, e.g. (Benedictus et al., 2012; Codner et al., 2012; Davies et al., 2006), are too expensive and labor intensive for use in general breeding practice. However, considering that sequencing costs are dropping rapidly, averting MHC class I compatibility between dam and calf through selective breeding of sequence-based MHC class I typed animals may be a cost-effective innovative approach to reducing the incidence of RFM in the near future.

5. Conclusion

Loss of adherence between the fetal membranes and the uterus is pivotal for the timely expulsion of the fetal membranes. We conclude that the upregulation of MHC

class I on fetal (binucleate) trophoblasts at the end of gestation induces a maternal–fetal alloimmune response that is crucial for the required loss of fetal–maternal adherence. We hypothesize that CD4 T cells, activated through indirect allorecognition of fetal MHC class I, stimulate uterine macrophages and that activated macrophages play a central role in the breakdown of the extracellular matrix linking the fetal and maternal epithelium both directly and indirectly through the secretion of cytokines (most notably IL-8 and TNF- α) and the activation of downstream mechanisms. Absence (or reduction) of the maternal alloimmune response may lead to the persistence of fetal–maternal adherence and thereby cause retention of the fetal membranes, an important reproductive disorder in cattle. Currently, there is no effective treatment for retained fetal membranes and a better understanding of the immune-assisted loss of fetal–maternal adherence may lead to novel targets for the treatment of retained fetal membranes. MHC class I compatibility between dam and calf is an important risk factor for RFM and since the costs of sequencing are dropping rapidly, selective breeding of sequence-based MHC class I typed animals may be a cost-effective preventive measure for RFM in the near future.

Conflict of interest

All three authors declare that they have no conflict of interests.

Acknowledgements

The authors wish to thank Mirjam Nielen for proof-reading the manuscript.

References

- Aluvihare, V.R., Kallikourdis, M., Betz, A.G., 2004. Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* 5, 266–271.
- Bainbridge, D.R., 2000. Evolution of mammalian pregnancy in the presence of the maternal immune system. *Rev. Reprod.* 5, 67–74.
- Bainbridge, D.R., Sargent, I.L., Ellis, S.A., 2001. Increased expression of major histocompatibility complex (MHC) class I transplantation antigens in bovine trophoblast cells before fusion with maternal cells. *Reproduction* 122, 907–913.
- Beagley, J.C., Whitman, K.J., Baptiste, K.E., Scherzer, J., 2010. Physiology and treatment of retained fetal membranes in cattle. *J. Vet. Intern. Med.* 24, 261–268.
- Benedictus, L., Jorritsma, R., Knijn, H.M., Vos, P.L., Koets, A.P., 2011. Chemotactic activity of cotyledons for mononuclear leukocytes related to occurrence of retained placenta in dexamethasone induced parturition in cattle. *Theriogenology* 76, 802–809.
- Benedictus, L., Thomas, A.J., Jorritsma, R., Davies, C.J., Koets, A.P., 2012. Two-way calf to dam major histocompatibility class I compatibility increases risk for retained placenta in cattle. *Am. J. Reprod. Immunol.* 67, 224–230.
- Benedictus, L., Koets, A.P., Kuijpers, F.H., Joosten, I., van, E.P., Heuven, H.C., 2013. Heritable and non-heritable genetic effects on retained placenta in Meuse-Rhine-Yssel cattle. *Anim. Reprod. Sci.* 137, 1–7.
- Boos, A., Janssen, V., Mulling, C., 2003. Proliferation and apoptosis in bovine placentomes during pregnancy and around induced and spontaneous parturition as well as in cows retaining the fetal membranes. *Reproduction* 126, 469–480.
- Chavatte-Palmer, P., Guillomot, M., Roiz, J., Heyman, Y., Laigre, P., Servely, J.L., Constant, F., Hue, I., Ellis, S.A., 2007. Placental expression of major histocompatibility complex class I in bovine somatic clones. *Cloning Stem Cells* 9, 346–356.

- Chazaud, B., 2014. Macrophages: supportive cells for tissue repair and regeneration. *Immunobiology* 219, 172–178.
- Christiaens, I., Zaragoza, D.B., Guilbert, L., Robertson, S.A., Mitchell, B.F., Olson, D.M., 2008. Inflammatory processes in preterm and term parturition. *J. Reprod. Immunol.* 79, 50–57.
- Codner, G.F., Stear, M.J., Reeve, R., Matthews, L., Ellis, S.A., 2012. Selective forces shaping diversity in the class I region of the major histocompatibility complex in dairy cattle. *Anim. Genet.* 43, 239–249.
- Condon, J.C., Jeyasuria, P., Faust, J.M., Mendelson, C.R., 2004. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4978–4983.
- Cortese, V.S., 2009. Neonatal immunology. *Vet. Clin. North Am. Food Anim. Pract.* 25, 221–227.
- Davies, C.J., Fisher, P.J., Schlafer, D.H., 2000. Temporal and regional regulation of major histocompatibility complex class I expression at the bovine uterine/placental interface. *Placenta* 21, 194–202.
- Davies, C.J., Hill, J.R., Edwards, J.L., Schrick, F.N., Fisher, P.J., Eldridge, J.A., Schlafer, D.H., 2004. Major histocompatibility antigen expression on the bovine placenta: its relationship to abnormal pregnancies and retained placenta. *Anim. Reprod. Sci.* 82–83, 267–280.
- Davies, C.J., Eldridge, J.A., Fisher, P.J., Schlafer, D.H., 2006. Evidence for expression of both classical and non-classical major histocompatibility complex class I genes in bovine trophoblast cells. *Am. J. Reprod. Immunol.* 55, 188–200.
- Dilly, M., Hambruch, N., Shenavai, S., Schuler, G., Froehlich, R., Haeger, J.D., Ozalp, G.R., Pfarrer, C., 2011. Expression of matrix metalloproteinase (MMP)-2, MMP-14 and tissue inhibitor of matrix metalloproteinase (TIMP)-2 during bovine placentation and at term with or without placental retention. *Theriogenology* 75, 1104–1114.
- Eiler, H., Hopkins, F.M., 1993. Successful treatment of retained placenta with umbilical cord injections of collagenase in cows. *J. Am. Vet. Med. Assoc.* 203, 436–443.
- Ellis, S.A., Sargent, I.L., Charleston, B., Bainbridge, D.R., 1998. Regulation of MHC class I gene expression is at transcriptional and post-transcriptional level in bovine placenta. *J. Reprod. Immunol.* 37, 103–115.
- Fournel, S., Aguerre-Girr, M., Huc, X., Lenfant, F., Alam, A., Toubert, A., Bensussan, A., Le, B.P., 2000. Cutting edge: soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8⁺ cells by interacting with CD8. *J. Immunol.* 164, 6100–6104.
- Galdiero, M.R., Bonavita, E., Barajon, I., Garlanda, C., Mantovani, A., Jaillon, S., 2013. Tumor associated macrophages and neutrophils in cancer. *Immunobiology* 218, 1402–1410.
- Gross, T.S., Williams, W.F., Russek-Cohen, E., 1991. Cellular changes in the peripartum bovine fetal placenta related to placental separation. *Placenta* 12, 27–35.
- Gude, N.M., Roberts, C.T., Kalonis, B., King, R.G., 2004. Growth and function of the normal human placenta. *Thromb. Res.* 114, 397–407.
- Gunnink, J.W., 1984a. Pre-partum leucocytic activity and retained placenta. *Vet. Q.* 6, 52–54.
- Gunnink, J.W., 1984b. Retained placenta and leucocytic activity. *Vet. Q.* 6, 49–51.
- Hansen, P.J., 2013. Physiology and Endocrinology Symposium: maternal immunological adjustments to pregnancy and parturition in ruminants and possible implications for postpartum uterine health: is there a prepartum-postpartum nexus? *J. Anim. Sci.* 91, 1639–1649.
- Heuwieler, W., Grunert, E., Ehlert, R., 1985. Quantitative determination of the chemotactic activity of extirpated bovine placentalomas with special reference to postpartal discharge. *Berl. Munch. Tierarztl. Wochenschr.* 98, 401–409.
- Hill, J.R., Schlafer, D.H., Fisher, P.J., Davies, C.J., 2002. Abnormal expression of trophoblast major histocompatibility complex class I antigens in cloned bovine pregnancies is associated with a pronounced endometrial lymphocytic response. *Biol. Reprod.* 67, 55–63.
- Hines, H.C., Newman, M.J., 1981. Production of foetally stimulated lymphocytotoxic antibodies by multiparous cows. *Anim. Blood Groups Biochem. Genet.* 12, 201–206.
- Hirata, M., Sato, T., Tsumagari, M., Shimada, A., Nakano, H., Hashizume, K., Ito, A., 2003. Differential regulation of the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases by cytokines and growth factors in bovine endometrial stromal cells and trophoblast cell line BT-1 in vitro. *Biol. Reprod.* 68, 1276–1281.
- Hoek, A., Rutten, V.P., Kool, J., Arkesteijn, G.J., Bouwstra, R.J., Van, R., Koets, I.A.P., 2009. Subpopulations of bovine WC1(+) gammadelta T cells rather than CD4(+)CD25(high) Foxp3(+) T cells act as immune regulatory cells ex vivo. *Vet. Res.* 40, 6.
- Joosten, I., Stelwagen, J., Dijkhuizen, A.A., 1988. Economic and reproductive consequences of retained placenta in dairy cattle. *Vet. Rec.* 123, 53–57.
- Joosten, I., Sanders, M.F., Hensen, E.J., 1991. Involvement of major histocompatibility complex class I compatibility between dam and calf in the aetiology of bovine retained placenta. *Anim. Genet.* 22, 455–463.
- Kimura, K., Goff, J.P., Kehrli Jr., M.E., Reinhardt, T.A., 2002. Decreased neutrophil function as a cause of retained placenta in dairy cattle. *J. Dairy Sci.* 85, 544–550.
- Laven, R.A., Peters, A.R., 1996. Bovine retained placenta: aetiology, pathogenesis and economic loss. *Vet. Rec.* 139, 465–471.
- Low, B.G., Hansen, P.J., Drost, M., Gogolnewens, K.J., 1990. Expression of major histocompatibility complex antigens on the bovine placenta. *J. Reprod. Fertil.* 90, 235–243.
- Lynge, N.L., Djurisic, S., Hviid, T.V., 2014. Controlling the immunological crosstalk during conception and pregnancy: HLA-G in reproduction. *Front. Immunol.* 5, 198.
- Maj, J.G., Kankofer, M., 1997. Activity of 72-kDa and 92-kDa matrix metalloproteinases in placental tissues of cows with and without retained fetal membranes. *Placenta* 18, 683–687.
- Miyoshi, M., Sawamukai, Y., Iwanaga, T., 2002. Reduced phagocytotic activity of macrophages in the bovine retained placenta. *Reprod. Domest. Anim.* 37, 53–56.
- Nagamatsu, T., Schust, D.J., 2010. The immunomodulatory roles of macrophages at the maternal-fetal interface. *Reprod. Sci.* 17, 209–218.
- Oliveira, L.J., Hansen, P.J., 2008. Deviations in populations of peripheral blood mononuclear cells and endometrial macrophages in the cow during pregnancy. *Reproduction* 136, 481–490.
- Oliveira, L.J., Hansen, P.J., 2009. Phenotypic characterization of macrophages in the endometrium of the pregnant cow. *Am. J. Reprod. Immunol.* 62, 418–426.
- Oliveira, L.J., McClellan, S., Hansen, P.J., 2010. Differentiation of the endometrial macrophage during pregnancy in the cow. *PLoS One* 5, e13213.
- Oliveira, L.J., Barreto, R.S., Perecin, F., Mansouri-Attia, N., Pereira, F.T., Meirelles, F.V., 2012. Modulation of maternal immune system during pregnancy in the cow. *Reprod. Domest. Anim.* 47 (Suppl. 4), 384–393.
- Oliveira, L.J., Mansourri-Attia, N., Fahey, A.G., Browne, J., Forde, N., Roche, J.F., Loneragan, P., Fair, T., 2013. Characterization of the Th profile of the bovine endometrium during the oestrous cycle and early pregnancy. *PLoS One* 8, e75571.
- Quinn, K.H., Parast, M.M., 2013. Decidual regulatory T cells in placental pathology and pregnancy complications. *Am. J. Reprod. Immunol.* 69, 533–538.
- Schlafer, D.H., Fisher, P.J., Davies, C.J., 2000. The bovine placenta before and after birth: placental development and function in health and disease. *Anim. Reprod. Sci.* 60–61, 145–160.
- Sela, U., Olds, P., Park, A., Schlesinger, S.J., Steinman, R.M., 2011. Dendritic cells induce antigen-specific regulatory T cells that prevent graft versus host disease and persist in mice. *J. Exp. Med.* 208, 2489–2496.
- Senger, P.L., 2003. Placentation, the endocrinology of gestation and parturition. In: *Pathways to Pregnancy and Parturition. Current Conceptions*, Richmond, pp. 304–346.
- Sevinga, M., Binns, M., Barkema, H.W., Hesselink, J.W., Weijden, G.C., Taverne, M.A., Lenstra, J.A., 2004. Retained Placenta in Friesian Mares and Major Histocompatibility Complex Compatibility of Mare and Foal. *Utrecht University, Utrecht*, pp. 59–67, PhD Thesis.
- Shenavai, S., Preissing, S., Hoffmann, B., Dilly, M., Pfarrer, C., Ozalp, G.R., Caliskan, C., Seyrek-Intas, K., Schuler, G., 2012. Investigations into the mechanisms controlling parturition in cattle. *Reproduction* 144, 279–292.
- Steinborn, A., Sohn, C., Sayehli, C., Baudendistel, A., Huwelmeier, D., Solbach, C., Schmitt, E., Kaufmann, M., 1999. Spontaneous labour at term is associated with fetal monocyte activation. *Clin. Exp. Immunol.* 117, 147–152.
- Streyl, D., Kennngott, R., Herbach, N., Wanke, R., Blum, H., Sinowatz, F., Wolf, E., Zerbe, H., Bauersachs, S., 2012. Gene expression profiling of bovine periparturient placentomes: detection of molecular pathways potentially involved in the release of foetal membranes. *Reproduction* 143, 85–105.
- Van Engelen, E., de Groot, M.W., Breeveld-Dwarkasing, V.N.A., Everts, M.E., Weyden, G.C., Taverne, M.A.M., Rutten, V.P.M.G., 2009. Cervical ripening and parturition in cows are driven by a cascade of pro-inflammatory cytokines. *Reprod. Dom. Anim.* 44, 834–841.
- van Werven, T., Schukken, Y.H., Lloyd, J., Brand, A., Heeringa, H.T., Shea, M., 1992. The effects of duration of retained placenta on reproduction, milk production, postpartum disease and culling rate. *Theriogenology* 37, 1191–1203.

- Walter, I., Boos, A., 2001. Matrix metalloproteinases (MMP-2 and MMP-9) and tissue inhibitor-2 of matrix metalloproteinases (TIMP-2) in the placenta and interplacental uterine wall in normal cows and in cattle with retention of fetal membranes. *Placenta* 22, 473–483.
- Williams, W.F., Margolis, M.J., Manspeaker, J., Douglass, L.W., Davidson, J.P., 1987. Peripartum changes in the bovine placenta related to fetal membrane retention. *Theriogenology* 28, 213–223.
- Wooding, F.B., Roberts, R.M., Green, J.A., 2005. Light and electron microscope immunocytochemical studies of the distribution of pregnancy associated glycoproteins (PAGs) throughout pregnancy in the cow: possible functional implications. *Placenta* 26, 807–827.
- Wooding, F.B.P., 1992. The synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. *Placenta* 13, 101–113.