Title

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

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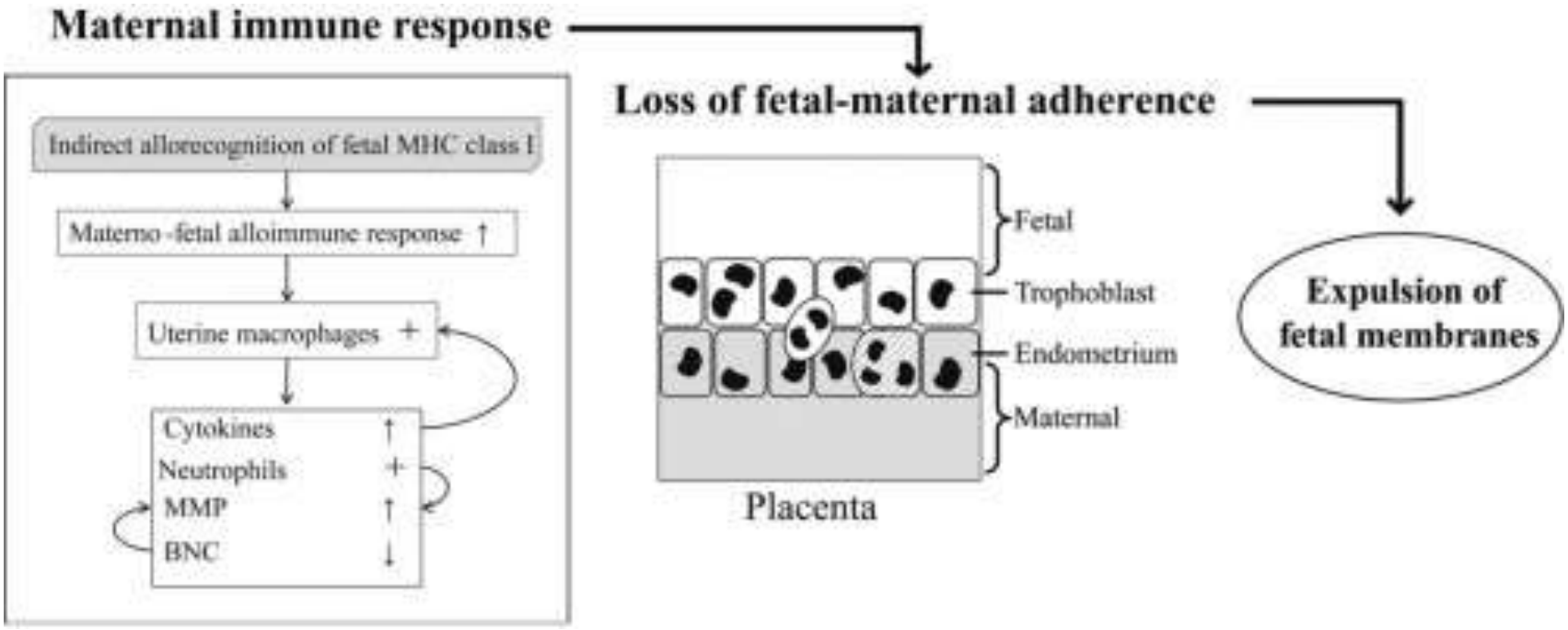
*Highlights

- **MHC class I expression on fetal trophoblasts rises towards the end of gestation**
- **Materno-fetal alloimmunity appears to be important for the timely separation of fetal membranes**
- **Maternal macrophages likely play a crucial role in loss of fetal-maternal adherence**
- **Dam-calf MHC class I compatibility gives a high risk of retained fetal membranes**

Graphical abstract



ABSTRACT

The bovine fetus, like that of other species, is a semi-allograft and 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 MHC class I on the fetal membranes towards 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 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.

Abbreviations: 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 MMP's), CR (coefficient of relationship)

Keywords

Retained fetal membranes; Major Histocompatibility Complex class I; Pregnancy; Alloimmunity, Fetal-maternal adherence; Cattle

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; Benedictus et al., 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 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 mechanism preventing immunological rejection of the fetus (Bainbridge, 2000; Lynge et al., 2014) i) anatomical separation of the fetus from the maternal immune system ii) downregulation of alloantigen expression by the fetus and 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 surface area for 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 extravilluous trophoblasts that invade the uterine tissue and reshape maternal blood vessels (Gude et al., 2004). The anatomy of the bovine placenta assures 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 towards mid gestation expression becomes apparent in interplacentomal regions and rises towards 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 expression of MHC class I with ILA88, a monoclonal antibody that is pan specific for bovine MHC class I, and hypothesized this could reflect

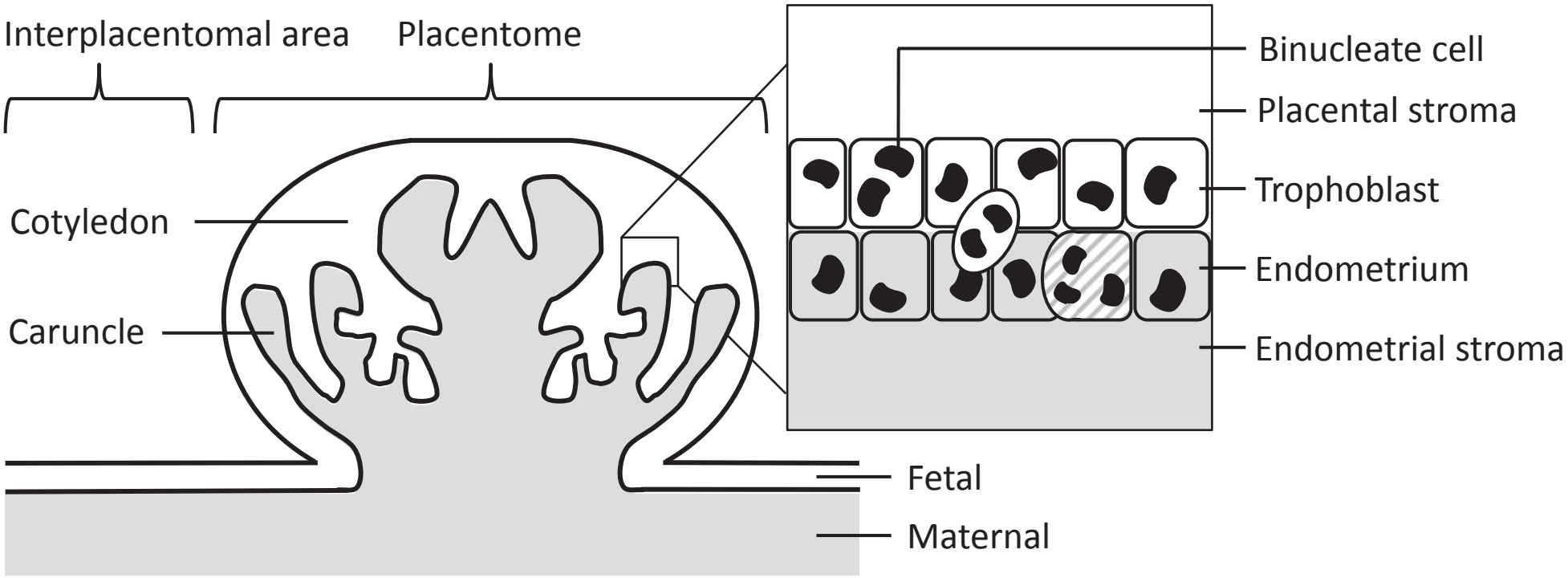


Fig. 1. Bovine placentomes. Placentomes are formed through interdigitation of maternal (caruncle) and fetal (cotyledon) tissue. The apposition of fetal trophoblasts to the maternal endometrium forms a continuous epithelial lining across the placenta. Binucleate cells, specialized trophoblasts, can migrate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

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 soluble bovine NC-MHC class I may be expressed. It is probable that NC-MHC class I expression on bovine trophoblasts has a similar role to HLA-G in humans and that expression of both NC-MHC class I and restricted expression of classical MHC class I by the fetus contributes to the regulation of maternal immunity.

Binucleate cells (**BNC**), specialized cells formed from uni-nucleate trophoblasts and unique to ruminants, can migrate to the endometrium and fuse with maternal cells temporarily forming trinucleate cells (Schlafer et al., 2000; Wooding, 1992, Fig. 1). BNC produce an array of secretory molecules, including placental lactogen, pregnancy associated glycoproteins and many hormones, and likely play a pivotal role in feto-maternal crosstalk (reviewed by Wooding et al. (2005)). BNC have been found to express MHC class I in 'at term' collected placentomes (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 around 230 days of gestation and after dexamethasone induced parturition, respectively. In the study by Bainbridge and colleagues (2001) is was found that not all BNC expressed MHC class I and at present it remains unknown if BNC express MHC class I at the moment of fusion with maternal cells. If this would be the case, this presents 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 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 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 area conflicting, with studies reporting no expression (Davies et al., 2000), downregulation (Low et al., 1990) and normal expression (Chavatte-Palmer et al., 2007).

MHC class I downregulation 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 were 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 depletion of

regulatory T cells in allogeneic pregnancies in mice leads to failure of gestation (Aluvihare et al., 2004), showing the importance of regulatory T cells for successful pregnancy other species. Foxp3+ has been detected in the bovine placenta (Oliveira et al., 2013) and levels of CD4 CD25 T cells rise in 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, γδ T cells were shown to act as regulatory cells (Hoek et al., 2009), of which low numbers have been detected in the endo- and myometrium of pregnant cattle (Oliveira et al., 2013).

Many soluble factors are released at the feto-maternal 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 et al. (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 6 hours after the calf is born (van Werven et al., 1992). Retained fetal membranes (**RFM**), 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 (Gunnink, 1984a; Gunnink, 1984b). Measuring the chemotaxis of maternal leukocytes towards fetal cotyledon extracts revealed that the chemotactic activity of cotyledons obtained from RFM cows was reduced. Also, chemotaxis of leukocytes obtained from RFM cows towards cotyledons from healthy animals was hampered and this could already be observed a week before parturition. Similar results were found by Heuwieser and colleagues (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 hours 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 allorecognition of fetal MHC class I and the ensuing maternal immune response affects fetal-maternal adherence. Next, we

hypothesize what prompts 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 positive 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 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 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 placentomal endometrium are MHC class II positive

(Oliveira and Hansen, 2009) and MHC class I expression is upregulated on placentomal (binucleate) trophoblasts towards 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 indeed occurs 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 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 breakdown and remodeling of the ECM (Chazaud, 2014; Galdiero et al., 2013). Miyoshi et al. (2002) found that a 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 part of these macrophages have a phenotype that supports immune regulation and tissue homeostasis (2010). However, under influence of the materno-fetal alloimmune response uterine macrophages may assume a more inflammatory phenotype towards parturition (Chazaud, 2014; Oliveira et al., 2010) that aids in 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-1β, IL-6, TNF-α) (Chazaud,

2014). In cattle the mRNA expression levels of the pro-inflammatory cytokines IL-1β, IL-6 and IL-8 rise in the cervix towards parturition (van Engelen et al. 2009) and in placental macrophages in humans a shift from an immune regulatory towards an inflammatory phenotype at parturition is believed to aid in 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 intense 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 maternal recognition of fetal MHC class I activates macrophages and induces cytokine production, which, as further explained in the following paragraphs, through 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). MMP's can be activated by many (inflammatory) cytokines (Hirata et al., 2003), including IL-1, IL-6 and TNF-α. Maj and Kankoffer (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 MMP's is inhibited by tissue inhibitors of MMP's (**TIMP**) and both studies found 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 MMP's in the placenta trough 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 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 may 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 break down 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 at 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 towards cotyledons of neutrophils obtained from dams that develop RFM is lower than from dams that release the fetal membranes normally (Kimura et al., 2002). Although neutrophil numbers increase remarkably in the cervix towards 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, 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 for separation of the fetal membranes. Compared to macrophage numbers (Miyoshi et al., 2002; Oliveira and Hansen, 2008), T cell numbers in at term placenta's 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 towards an inflammatory phenotype, characterized by the production of cytokines such as IL-1β, TNF-α and IL-8. The resulting inflammatory milieu can subsequently activatemore 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 regulation of materno-fetal alloimmunity during pregnancy to a maternal immune response that aids in separation of the fetal membranes at

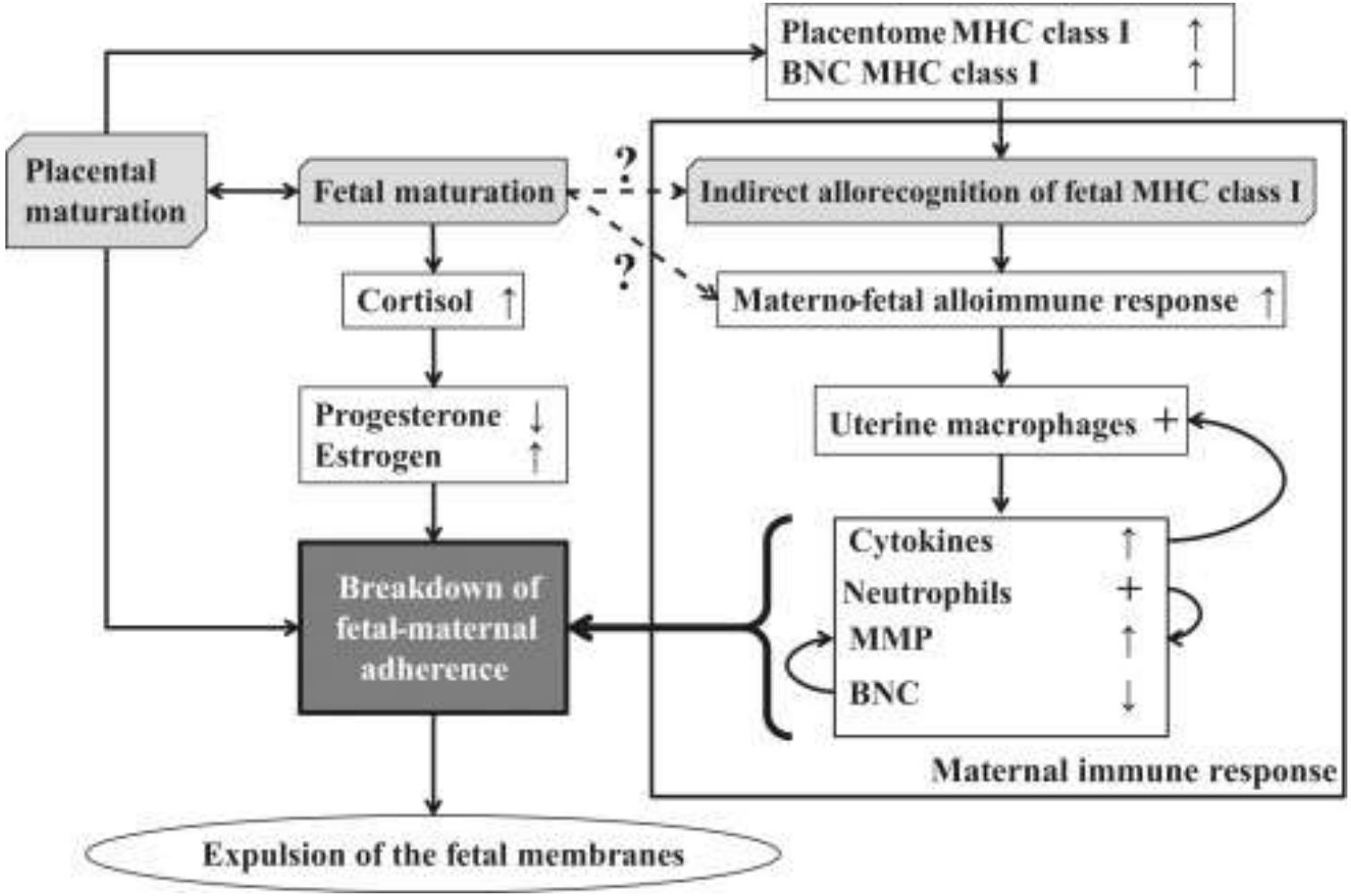


Fig. 2. Processes leading to the breakdown of fetal–maternal adherence. ↑ Upregulation. ↓ Down-regulation. + Activation. MMP matrix metalloproteinases, BNC binucleate cells.

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 as well as 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 mechanism. 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 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 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 for 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 was much higher in two-way compatible than in maternal compatible pregnancies (Fig. 3) (Benedictus et al., 2012). Compatibility of the dam to the calf increased the odds of RFM and suggests that the fetal immune system also plays 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 towards the end of pregnancy (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 towards 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 may contribute to the activation of the maternal innate immune response that leads to the breakdown of fetal-maternal adherence.

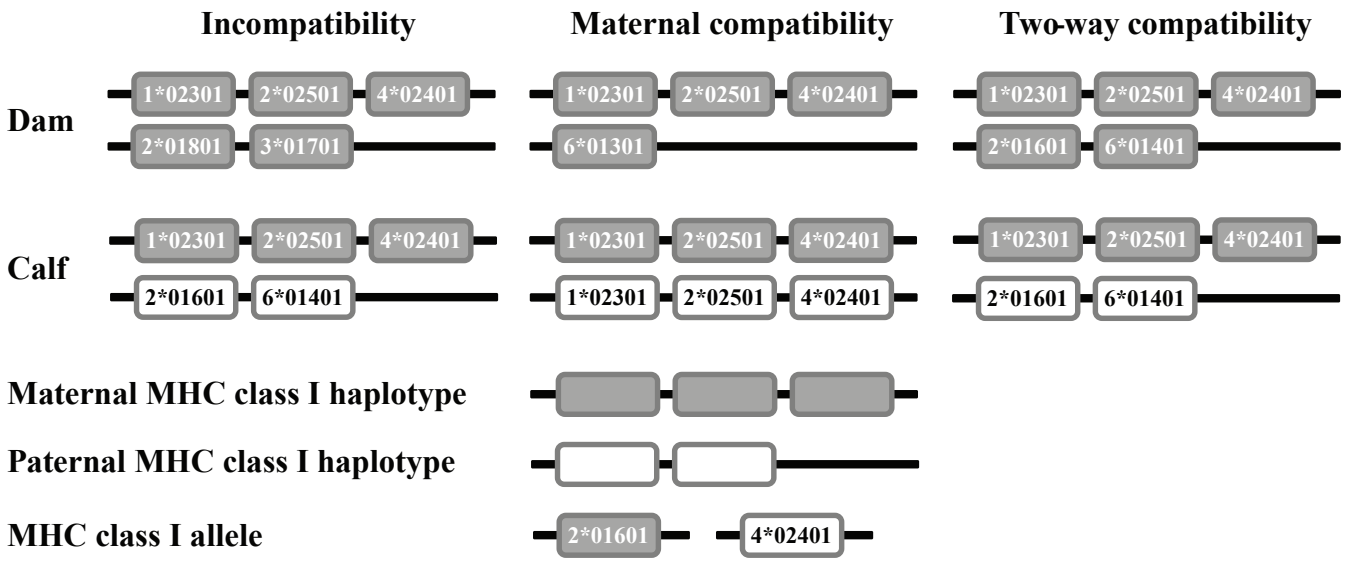


Fig. 3. MHC class I compatibility. In MHC class I incompatible pregnancies the paternally inherited haplotype of the calf is not compatible to the dam, nor is the non-inherited maternal haplotype compatible to the calf. When the paternally inherited haplotype is compatible to the dam, there is maternal compatibility when the non-inherited maternal haplotype is not compatible to 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.)

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 between 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 to type bovine MHC class I (e.g. (Benedictus et al., 2012; Codner et al., 2012; Davies et al., 2006)) are too expensive and labor intense 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 reduce 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 upregulation of MHC class I on fetal (binucleate) trophoblasts at the end of gestation induces a materno-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 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.

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Conflict of interest statement

All three authors declare that they have no conflict of interests