Consequences of interactions between the maternal immune system and the preimplantation embryo in cattle

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Summary

Using the cow, three questions related to the importance of the maternal immune system for the developing embryo are addressed: role of semen-induced inflammation for pregnancy establishment, regulation of preimplantation development by molecules that function as soluble mediators of immune cells, and immunological aspects of embryonic signaling by interferon-τ. Unlike rodents, there is no indication that semen modifies the physiology of the mother in a way that promotes embryonic survival. Bulls without seminal vesicles are fertile, artificial insemination results in pregnancy rates similar to natural mating and intrauterine deposition of seminal plasma at the time of insemination does not improve fertility. Regulatory molecules associated with the immune system can be important for development of the embryo. Among the molecules implicated in embryonic growth and survival are colony stimulating factor 2, which can enhance embryo competence to establish and maintain pregnancy, and tumor necrosis factor α, which induces apoptosis and blocks development. Elongation of the trophoblast beginning at Day 14 of development results in large-scale changes in the transcriptome of the embryo, including many genes involved in immune responses. Signaling by interferon-τ and other embryo-derived molecules does not, however, lead to a large-scale change in accumulation of lymphocytes in the endometrium. In contrast, numbers of macrophages and dendritic cells do increase and there is increased expression of genes associated with macrophage activation. Perhaps these antigen-presenting and immunoregulatory cells play an important role in continued survival and development of the conceptus.

Introduction

The preimplantation period in ruminants is bracketed by two events causing local activation of the immune system. The first, deposition of semen in the reproductive tract at estrus, elicits an inflammatory response characterized by migration of neutrophils into the uterine lumen (Howe & Black 1963, Mattner 1968). Near the end of the preimplantation period, enhanced secretion of the embryonic type 1 interferon, IFNT, and possibly other molecules results in expression of a large number of immune-response genes in the endometrium (Klein *et al*. 2006, Walker *et al*. 2010, Cerri *et al*. 2012, Bauersachs *et al*. 2012, Forde *et al*. 2012, Mansouri-Attia *et al*. 2012). Attachment of the trophoblast to the endometrium begins a few days after IFNT secretion is maximal (King *et al*. 1981), marking the end of the preimplantation period.

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The dynamic changes in local immune function exhibited during early pregnancy beg the question as to whether immune cells resident in the reproductive tract exert beneficial or deleterious effects on the preimplantation embryo. Some interactions between the maternal immune system and the embryo could potentially promote embryonic development. If so, disruption of these interactions caused by maternal dysfunction could be a cause of infertility. Supportive of this idea is the observation that endometrium of cows that established pregnancy after embryo transfer had a higher expression of genes involved in T-and B cell signaling and NF-κB signaling at Day 7 after estrus in the estrous cycle preceding embryo transfer than endometrium of cows that did not establish pregnancy (Ponsuksili *et al*. 2012). In addition, endometrial expression of several genes involved in immune function were altered in cows with retarded embryos at Day 7 after estrus as compared to cows with embryos at the correct stage of development (Beltman *et al*. 2013). For example, expression of *IL6, IL10, IFNG* and *IFNA* was higher for endometrium from cows with retarded embryos while expression of *NFKB1* and *IL21* was reduced. It is conceivable that one cause of the reduced developmental competence of embryos produced *in vitro* (Farin *et al*. 2006, Hansen *et al*. 2010) is the lack of exposure of embryos to regulatory signals of immune origin. In other cases, establishment of alternative patterns of immune activation in early pregnancy could potentially harm the embryo. Indeed, endometritis is associated with infertility (Thatcher *et al*. 2010) and with alteration in endometrial expression of immune-modulatory genes (Hoelker *et al*. 2012).

In this paper, the cow will be used as a model to address several questions relevant to understanding the importance of the maternal immune system for the developing embryo. In particular, the possible role of semen-induced inflammation for pregnancy establishment will be assessed, the consequences of exposure of the embryo to molecules that function as soluble mediators of immune cells will be highlighted, and the function of changes in expression of immune-related genes in the endometrium caused by IFNT will be speculated upon.

Inflammatory events induced by semen

The major role of inflammation caused by semen is likely to be promotion of phagocytic removal of microorganisms and spermatozoa deposited in the reproductive tract coincident with mating. Sperm are antigenic in cattle (Lander *et al*. 1990) and cows immunized against sperm have been reported to have lower fertility (Menge 1967, 1969). It has been proposed that inflammatory or immune responses to semen also facilitate the establishment and maintenance of pregnancy by modifying the nature of maternal immune responses (Robertson *et al*. 2013). The best substantiation for this idea comes from studies in the mouse. In this species, seminal plasma has been reported to increase the number of regulatory T cells reactive against paternal antigens (Robertson *et al*. 2013) and to increase expression in the oviduct for genes that stimulate embryonic development (*Lif, Csf2, Il6* and *Egf*) (Bromfield *et al*. 2014). Females mated with males lacking seminal vesicles had reduced conception rates, embryos with poor development to the blastocyst stage and low implantation rates, and offspring which experienced sex-dependent alterations in physiological function that persisted into adulthood (Bromfield *et al*. 2014).

Each year, millions of cattle are bred artificially in a system where the female comes in contact with small volumes of highly-diluted seminal plasma. If semen plays an important role in regulating embryonic development and responsiveness to the maternal immune system, it might be possible to improve fertility and fetal development in cows bred artificially by pharmacological activation of key aspects of the immune response to sperm.

Results in cattle, however, are not supportive of a role for seminal plasma in modulating fertility. Faulkner *et al*. (1968) evaluated consequences of removal of seminal vesicles on bull fertility. When each of five vesiculectomized bulls was used to breed 8-20 heifers each, conception rates were 40, 71, 75, 75 and 100%. Thus, bulls were generally fertile despite the absence of seminal vesicles. Moreover, the percent of ova fertilized after cows and heifers were inseminated artificially with sperm from the testes, epididymis or ejaculate were 0%, 84% and 94%, respectively (n = 106) (Amann & Griel 1974). Fertility was high even when insemination was performed with epididymal sperm that had not been in contact with semen.

It is not known whether there are differences in the nature of immune changes in the reproductive tract following deposition of sperm in cows bred by natural vs artificial insemination. Differences might even depend on semen extender – egg yolk has been reported to block the ability of seminal plasma to promote binding to neutrophils (Alghamdi *et al*. 2009). Given the large dilution of seminal plasma for ejaculates prepared for artificial insemination, it is reasonable to assume that the magnitude of immune deviation in the reproductive tract caused by sperm deposition would be less for cows subjected to artificial insemination than for cows bred naturally. In one study, only 43% of cows had detectable neutrophils in cytobrush samples from the endometrium collected at 4 h after artificial insemination (Kaufmann *et al*. 2009). Despite the differences in volume of seminal plasma deposited in the reproductive tract and the presumed difference in inflammatory response, there were no differences in conception rate between cows bred naturally or via artificial insemination (Landivar *et al*. 1985, Lima *et al*. 2009).

Seminal plasma is a rich source of TGFB and this growth factor may be responsible for semen-induced activation or expansion of regulatory T cells during pregnancy in mice (Ingman & Robertson 2009, Robertson *et al*. 2013). However, Odhiambo *et al*. (2009) found that neither intrauterine deposition of 0.5 ml seminal plasma nor 40 ng TGFB1 at the time of artificial insemination improved pregnancy rate in cattle (Table 1).

Thus, regardless of the model used, there is no evidence that seminal plasma can enhance fertility in cattle.

Table 1. Effect of intrauterine deposition of seminal plasma on fertility of cows bred by artificial insemination.[abc]

			Conception rate (%)		
Trial number	Cow type	No. of animals	Control	Seminal plasma	TGFB
1-3	Beef	763	55.1	58.8	51.0
4	Beef	206	59.5	67.5	–
5	Beef	167	52.4	61.4	–
6	Dairy	800	33.2	37.8	36.3

[a] The control treatment was either intrauterine deposition of bovine serum albumin (trials 1-4) or no additional deposition (trials 5-6).
[b] Differences between treatments were not significant.
[c] Odhiambho *et al*. (2009).

Alterations in embryonic development caused by cytokines associated with the immune system

The microenvironment of the embryo is established by a mucosa populated in part by cells that function in the immune system. The oviduct contains lymphocytes, macrophages and mast

cells (Du Bois *et al*. 1980, Abughrien *et al*. 2000). During early pregnancy (days 5-13), B cells are abundant in the deep stroma and myometrium of the endometrium, T cells are moderately abundant and located in the shallow stroma near the epithelium, and natural killer cells and δγ-T cells are present in low numbers in the shallow stroma (Oliveira *et al*. 2013). During the same time period, macrophages and dendritic cells are present in the stroma, with the former cells abundant and the latter cells present in low numbers (Mansouri-Attia *et al*. 2012).

Epithelial and stromal cells of the endosalpinx and endometrium also participate in innate immunity. Epithelial cells in the endosalpinx of the oviduct express Toll-like receptors *TLR2* and *TLR4* (Kowsar *et al*. 2013). At low concentrations, bacterial lipopolysaccharide (LPS) induced expression of *TLR4, PTGS2, IL1B, NFKB1,* and *TNF* while, at higher concentrations, LPS induced a different set of genes (*TLR2, IL4, IL10* and *PTGES*) (Kowsar *et al*. 2013). Endometrial epithelial and stromal cells also express genes encoding for TLR (Davies *et al*. 2008) and epithelial cells express genes for antimicrobial peptides (Davies *et al*. 2008). Moreover, LPS increased expression of some of these genes and increased secretion of prostaglandin E2, IL6 and IL8 (Davies *et al*. 2008, Turner *et al*. 2014).

There was no effect of pregnancy status at Day 5, 7 or 13 on accumulation of lymphocytes, macrophages or dendritic cells in the endometrium (Mansouri-Attia *et al*. 2012, Oliveira *et al*. 2013). It cannot be ruled out, however, that there are local changes in leukocyte accumulation or activation in the reproductive tract at sites nearby to the embryo. Among the embryonic signals that could activate maternal immune cells include IFNT (expressed as early as the 16-cell or morula stage; Lonergan *et al*. 2003, Yao *et al*. 2009), IL1B and TNF (Muñoz *et al*. 2012). Major histocompatibility antigen class I can be detected on the surface of the blastocyst (Templeton *et al*. 1987, Low *et al*. 1990, Doyle *et al*. 2009) and this molecule could also interact with maternal cells after hatching from the zona. There could also be hormonal regulation of immune cells in the endometrium.

Depending upon the characteristics of immune activation, cytokines produced in the reproductive tract could potentially alter embryonic development in a beneficial or adverse manner. That some cytokines can promote embryonic development is indicated by results of experiments in which embryos were transferred Day 7 after estrus into the uterus of cows that received intrauterine deposition of autologous peripheral-blood mononuclear cells on Day 4. Mononuclear cells were cultured for 24 h in the presence of fetal bovine serum before transfer and appeared to become activated based on an increase in transcript abundance for *IL1B* and several other genes (Ideta *et al*. 2010a). Transfer of mononuclear cells increased pregnancy rate (Ideta *et al*. 2010a). Embryos transferred into cows that received intrauterine deposition of blood mononuclear cells also experienced more extensive development at Day 15 of gestation than embryos transferred to control cows (Ideta *et al*. 2010b). Trophoblast length was greater for embryos recovered from treated cows and a greater percentage of embryos had observable embryonic discs.

There are several cytokines that can improve development of the bovine embryo. The likelihood that an *in vitro* produced embryo developed to the blastocyst stage was improved by culture with IL1B (Paula-Lopes *et al*. 1998), LIF (Neira *et al*. 2010) and CSF2 (de Moraes & Hansen 1997, Loureiro *et al*. 2009, Neira *et al*. 2010, Dobbs *et al*. 2013). Actions of CSF2 to improve development to the blastocyst stage depend upon the overall competence of embryos for development (Dobbs *et al*. 2013). CSF2 increased blastocyst percent when the percent of control embryos developing to the blastocyst stage was low. When it was high, however, CSF2 decreased the percent of embryos becoming a blastocyst.

Treatment with CSF2 also increases ability of the resultant blastocysts to establish and maintain pregnancy. This action has been shown not only in cattle (Loureiro *et al*. 2009) but

also in mice (Sjöblom *et al*. 2005) and humans (Ziebe *et al*. 2013). Exposure of the bovine embryo to CSF2 beginning at Day 5 of development alters several properties of the embryo that might improve competence to establish pregnancy (Fig. 1). These include alterations of expression of several genes related to differentiation (Loureiro *et al*. 2011), increased resistance to apoptosis (Loureiro *et al*. 2011), and an increase in number and pluripotency of cells of the inner cell mass (Loureiro *et al*. 2009, Dobbs *et al*. 2013).

Pro-developmental cytokines could be derived from leukocytes resident in the reproductive tract or from epithelial or stromal cells. Immunoreactive CSF2 is present in both endosalpinx and endometrium throughout the estrous cycle; labeling was greatest in epithelial cells (de Moraes *et al*. 1997, Emond *et al*. 2004). Similarly, immunoreactive IL1B was found in both epithelial and stromal cells of the bovine oviduct and endometrium (Paula-Lopes *et al*. 1999).

While some cytokines enhance embryonic development, other products of immune activation compromise survival of the embryo. In particular, several molecules produced

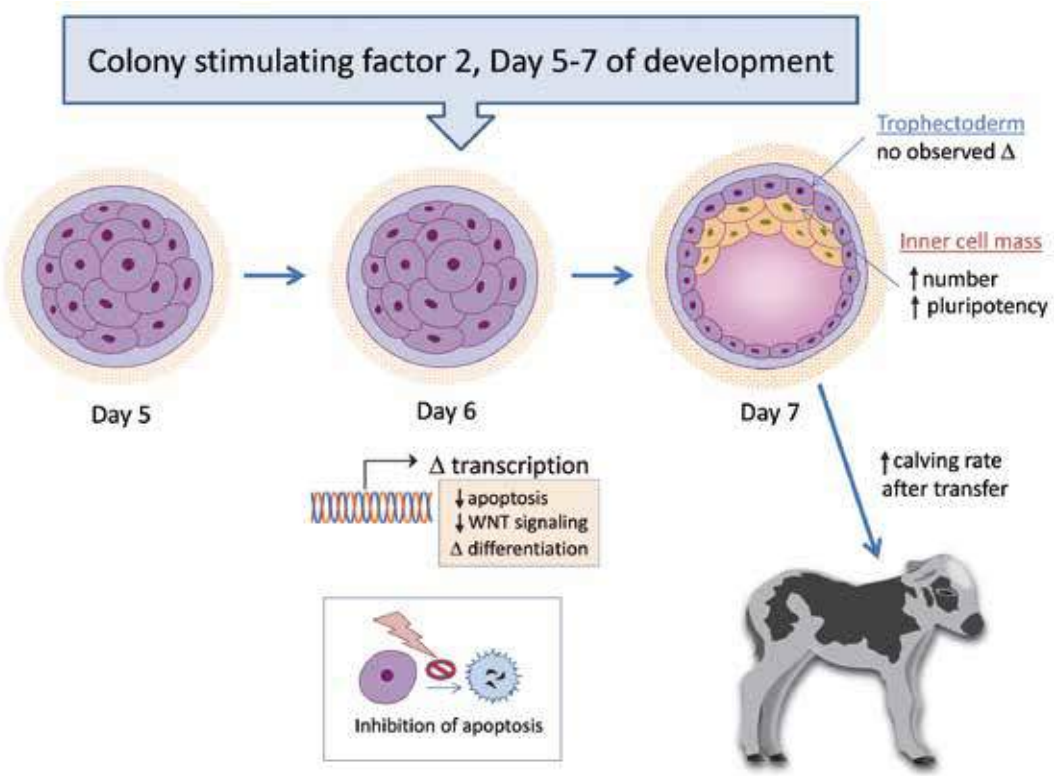

Fig. 1. Actions of CSF2 on the bovine preimplantation embryo. Addition of CSF2 at Day 5 of development changes properties of the resultant morula at Day 6 and blastocyst at Day 7. At Day 6, CSF2 alters gene expression in a way that decreases capacity for apoptosis and canonical WNT signaling as well as alters expression of several other genes involved in differentiation. Moreover, induction of apoptosis is reduced in embryos treated with CSF2. At Day 7, CSF2 increases numbers of cells in the inner cell mass without affecting number of trophectoderm cells. Moreover, isolated clusters of inner cell mass cells are better able to survive passage in a pluripotent state when cultured than cells from control embryos. Additionally, blastocysts treated with CSF2 have increased capacity to continue development to term when transferred to recipients.

as part of the inflammatory response inhibit embryonic development in the preimplantation period, including nitric oxide (Soto *et al*. 2003a), prostaglandin-F2α (PGF2α) (Scenna *et al*. 2004), and TNF (Jackson *et al*. 2012). The action of TNF involves activation of prostaglandin synthesis because it could be blocked by indomethacin (Jackson *et al*. 2012). In addition, TNF can trigger apoptosis in embryonic cells (Soto *et al*. 2003b). Findings from the above-mentioned studies suggest that one of the causes of infertility in cows experiencing endometritis is local production of bioactive molecules inhibitory to the embryo.

The dual nature of immune regulation of embryonic survival, with some molecules enhancing embryo competence for development and some molecules being inhibitory, means that the consequences of immune activation are likely to depend on the nature of the signals activating innate or adaptive immune responses as well as on properties of the embryo. Researchers at the University of Bonn conducted two experiments to identify differences in the endometrial transcriptome between embryo transfer recipients in which pregnancy failure or success occurred. In the one experiment, using embryos produced *in vivo*, among the molecular pathways overrepresented at day 7 in the estrous cycle before transfer for cows that did not establish pregnancy were those for immune response and chemokine activity (Salilew-Wondiom *et al*. 2010). This result could be interpreted to mean that overactivation of immune responses in the endometrial were inimical to pregnancy success. In another study, using embryos produced *in vitro*, endometrium of cows that did not establish pregnancy had lower expression of genes involved in T-and B cell signaling and NF-κB signaling (Ponsuksili *et al*. 2012). Perhaps, for these embryos, where exposure to maternal cytokines did not occur *in vitro*, some activation of immune responses *in utero* was supportive of development. Additional work is needed to understand the characteristics and control of cells producing cytokines in the reproductive tract and the role of those molecules in embryonic development.

Immune consequences of IFNT secretion by the elongated embryo

Prior to rapid elongation of the trophoblast around Day 14-15 of gestation (Betteridge & Fléchon 1988), the presence of the embryo in the reproductive tract causes little if any change in the function of the endometrium. Indeed, through Day 13 of gestation, differences in the transcriptome between endometrium of pregnant and non-pregnant cows were small (Forde et al. 2012) or non-detectable (Forde *et al*. 2011, Bauersachs *et al*. 2012). Similarly, there were few differences in populations of immune cells resident in the endometrium (Mansouri-Attia *et al*. 2012, Oliveira *et al*. 2013). Coincident with elongation, however, the endometrium undergoes large-scale changes in gene expression; transcript abundance changes for hundreds of genes including many involved in immune function (Walker *et al*. 2010, Forde *et al*. 2011, 2012, Bauersachs *et al*. 2012, Cerri *et al*. 2012, Mansouri-Attia *et al*. 2012). For example, Walker et al. (2010) examined differences in gene expression between pregnant and cyclic cows at Day 17 of pregnancy. A total of 1,839 in caruncular tissue and 1,189 genes in intercaruncular tissue were differentially expressed. The four most statistically significant canonical pathways in which these genes were represented were interferon signaling, complement system, role of pattern recognition receptors in the recognition of bacteria and viruses, and antigen presentation.

Changes in endometrial gene expression during Days 15-18 of gestation are caused largely by the increase in *IFNT* secretion by the trophectoderm that occurs coincident with elongation (Robinson *et al*. 2006). Most pregnancy-associated changes in endometrial gene expression at this time can be mimicked by intrauterine infusion of human IFNA2 (Bauersachs *et al*. 2012) or bovine IFNT (Forde *et al*. 2012). The pattern of gene expression induced by type I IFN is

not identical to pregnancy, however, probably because some actions of IFNT are exerted in conjunction with other regulatory molecules such as placental lactogen, cortisol, prostaglandins and progesterone (Dorniak *et al*. 2013). In addition, there might be other regulatory molecules secreted by the embryo at this time in pregnancy including those involved in immune function.

Like other type I interferons, IFNT possesses antiviral activity (Alexenko *et al*. 1997) and the pregnant endometrium around the time of trophoblast elongation exhibits an antiviral state as indicated by upregulation of genes involved in inhibition of viral proliferation (Walker *et al*. 2010, Bauersachs *et al*. 2012, Forde *et al*. 2011, 2012, Cerri *et al*. 2012) as well as increased activity of the antiviral protein 2′5′ oligoadenylate cyclase (Short *et al*. 1991). IFNT can also block lymphocyte proliferation (Skopets *et al*. 1992, Alexenko *et al*. 1997) but it is not known whether T or B cell function is inhibited in the reproductive tract coincident with IFNT secretion. Some endometrial genes that are upregulated at Day 17 of pregnancy are associated with inhibition of lymphocyte function but others are associated with lymphocyte activation (Fig. 2). Examples of the former include *IDO1* (Fallorino *et al*. 2012), molecules of the FASLG signaling cascade (*FAS, FASLG, FADD*), which promote lymphocyte apoptosis (Lettau *et al*. 2011), and the T-cell inhibitory molecule *SERPINA14* (Peltier & Hansen 2001). Examples of the latter include *IL18*, which promotes T cell chemotaxis and maturation of helper T cells (Volin & Koch 2011) and *TNFSF13B*, which stimulates B cell survival and expansion (Goenka *et al*. 2014) and T cell activation (Chen *et al*. 2014). Moreover, despite the upregulation of several chemokine genes in the pregnant endometrium (for example, *CCL2, CCL8, CCL11, CXCL2, CXCL9, CXCL10*, and *CXCL11*; Walker *et al*. 2010, Cerri *et al*. 2012), changes in accumulation of lymphocytes in the endometrium at this time are minimal. At Day 16 of pregnancy, there was no difference between pregnant and non-pregnant cows in the number of B cells or T cells positive for CD4, CD8 or the δγ T cell receptor (Leung *et al*. 2000, Oliveira *et al*. 2013).

The same lack of consistent pattern of regulation is true for NK cells. One function of IFNT

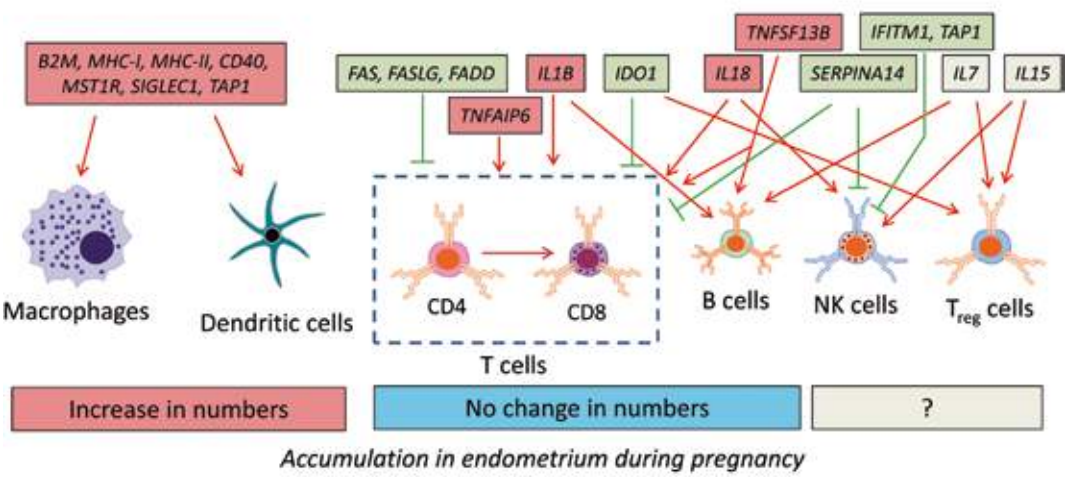

Fig. 2. Changes in gene expression (top) and accumulation of leukocytes (bottom) in the endometrium occurring coincident with trophoblast elongation. Genes in red represent those associated with immune activation, genes in green represent those associated with immune inhibition and genes in gray are unresolved. Lines represent actions of gene products (red, stimulatory; green, inhibitory). The figure is based on results on gene expression from Walker *et al*. (2010) and Cerri *et al*. (2012) and results on leukocyte numbers from Mansouri-Attia *et al*. (2012) and Oliveira *et al*. (2013).

is to activate lytic activity of NK cells (Tuo *et al*. 1993, Tekin & Hansen 2002). Evidence is contradictory, though, as to whether there is increased NK cell activity in the pregnant endometrium during trophoblast elongation. Some genes whose expression is elevated in the endometrium of pregnant cows promote NK cell function, for example, *IL15* and *IL18* (Marçais *et al*. 2013), whereas others are associated with decreased NK cell activity such as *IFITM1* (Yang *et al*. 2005), *SERPINA14* (Tekin & Hansen, 2002), and *TAP1* (Kalkunte *et al*. 2009). Oliveira *et al*. (2013) found that the number of cells in the endometrium at Day 16 that labeled for the NK cell marker NKp46 was lower for pregnant heifers than cyclic heifers. In particular, there was an increase in number of $NKp46^+$ cells from Day 13 to 16 in cyclic animals that did not occur in pregnant animals. In contrast, Vasudevan *et al*. (2013) observed that the proportion of $CD45^+$ leukocytes in Day 17 endometrium that expressed NKp46 was higher for pregnant heifers as compared to cyclic animals.

Three of the genes that were found by Walker *et al*. (2010) to be higher in pregnant cows at Day 17 of pregnancy, *IDO1*, *IL7*, and *IL15*, are involved in generation, proliferation and survival of regulatory T cells (Harnaha *et al*. 2006, Guerin *et al*. 2009, Fallarino *et al*. 2012). This subpopulation of T cells plays an important role to induce anergy against fetal antigens in the mother (Rowe *et al*. 2012). Perhaps IFNT or other embryonic signals released at this time in pregnancy are critical for regulation of maternal regulatory T cells in ruminants. There was, however, no difference between pregnant and cyclic cows in expression of *FOXP3*, a transcription factor associated with regulatory T cells, in the endometrium at Day 16 after estrus (Oliveira *et al*. 2013).

The situation with respect to macrophages and dendritic cells in the endometrium is more clear because there is increased accumulation of cells positive for macrophage and dendritic cell markers at Day 16 of pregnancy (Mansouri-Attia *et al*. 2012). Moreover, many genes associated with activation of these antigen-presenting and immunoregulatory cells are upregulated in the endometrium of pregnant cows (see Fig. 2). Heterogeneity exists in the function of various subsets of macrophages and dendritic cells, with some cell populations promoting inflammation and others limiting immune responsiveness (Butcher & Galkina 2012). During mid- and late-gestation, macrophages resident in the endometrium exhibit a pattern of gene expression consistent with cells being differentiated towards the anti-inflammatory M2 phenotype (Oliveira *et al*. 2010). It will be instructive to determine phenotypes of endometrial macrophages and dendritic cells earlier in pregnancy.

Activation of a massive antiviral response in the pregnant endometrium during the periattachment period distinguishes ruminants from other mammals. *IFNT* arose in evolution about 36 million years ago after ruminants diverged from other mammals so the gene is restricted to ruminant species (Roberts *et al*. 2003). The type I *IFN* locus in cattle has undergone much rearrangement and expansion compared to the mouse and human (Walker & Roberts 2009). One possibility is that *IFNT* was selected in evolution specifically to regulate corpus luteum function. If so, the antiviral and immunoregulatory changes in the endometrium accompanying *IFNT* secretion may not be important for establishment of pregnancy. It has been argued that generation of trophoblast interferons and accompanying changes in the endometrium is conserved more broadly among mammals (Bazer *et al*. 2009) but, if so, the characteristics of trophoblast production of interferons and endometrial response to those molecules is much different in non-ruminants than in ruminants (Roberts *et al*. 2008, Bazer *et al*. 2009). Roberts *et al*. (2008) has speculated that the increased expression of IFN response genes in endometrium at specific stages of pregnancy means that species use a variety of conceptus signals to induce a common set of uterine genes.

Conclusion

There is little evidence for recognition of the developing embryo by the mother until it undergoes trophoblast elongation and IFNT secretion. This is true both with respects to immunological and non-immunological recognition. It is possible that there are local changes in the endosalpinx or endometrium in response to the presence of the embryo but at this point such a conclusion is speculative. The lack of maternal immunological response to the embryo does not necessarily mean that regulatory molecules associated with the immune system are not important for development of the embryo. Embryonic growth and survival can be improved by addition of activated lymphocytes to the endometrium (Ideta *et al*. 2010ab) and competence of the embryo produced *in vitro* to go to term following embryo transfer can be enhanced by exposure to CSF2 during the morula-to-blastocyst transition (Loureiro *et al*. 2009). Embryotrophic cytokines may be derived from leukocytes resident in the endometrium or be products of epithelial cells or stromal fibroblasts. Unlike rodents, there is no indication that semen modifies the physiology of the mother in a way that promotes embryonic survival.

The quiescence of the reproductive tract with respect to presence of the embryo is transformed when trophoblast elongation takes place a few days before formation of the first embryo-maternal attachments accompanying placentation. At this point, the transcriptome of the endometrium undergoes large-scale changes and macrophage and dendritic cell numbers increase. The consequences of these changes for maintenance of the corpus luteum have been recognized and described but implications for the immunological relationship between the embryo and mother are only now being elucidated. The endometrial macrophage may be a key target of regulation by IFNT.

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References

Abughrien BM, Dore MA, McGeady TA & Fitzpatrick E 2000 Intraepithelial leucocytes in the bovine uterine tube. *Cells Tissues and Organs* **166** 20-30.

Alexenko AP, Leaman DW, Li J & Roberts RM 1997 The antiproliferative and antiviral activities of IFN-γ variants in human cells. *Journal of Interferon and Cytokine Research* **17** 769–779.

Alghamdi AS, Lovaas BJ, Bird SL, Lamb GC, Rendahl AK, Taube PC & Foster DN 2009 Species-specific interaction of seminal plasma on sperm-neutrophil binding. *Animal Reproduction Science* **114** 331-344.

Amann RP & Griel LC Jr 1974 Fertility of bovine spermatozoa from rete testis, cauda epididymidis, and ejaculated semen. *Journal of Dairy Science* **57** 212-219.

Bauersachs S, Ulbrich SE, Reichenbach HD, Reichenbach M, Büttner M, Meyer HH, Spencer TE, Minten M, Sax G, Winter G & Wolf E 2012 Comparison of the effects of early pregnancy with human interferon, alpha 2 (IFNA2), on gene expression in bovine endometrium. *Biology of Reproduction* **86** 46.

Bazer FW, Spencer TE & Johnson GA 2009 Interferons and uterine receptivity. *Seminars in Reproductive Medicine* **27** 90-102.

Beltman ME, Forde N, Lonergan P & Crowe MA 2013 Altered endometrial immune gene expression in beef heifers with retarded embryos. *Reproduction Fertility and Development* **25** 966-970.

Betteridge KJ & Fléchon JE 1988 The anatomy and physiology of pre-attachment bovine embryos. *Theriogenology* **29** 155–187.

Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ & Robertson SA 2014 Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *Proceedings of the National Academy of Sciences USA* **111** 2200-2205.

Butcher MJ & Galkina EV 2012 Phenotypic and functional heterogeneity of macrophages and dendritic cell subsets in the healthy and atherosclerosis-prone aorta. *Frontiers in Physiology* **3** 44.

Chen M, Lin X, Liu Y, Li Q, Deng Y, Liu Z, Brand D, Guo

Z, He X, Ryffel B & Zheng SG 2014 The function of BAFF on T helper cells in autoimmunity. *Cytokine and Growth Factor Reviews* http://dx.doi.org/10.1016/j.cytogfr.2013.12.011.

Cerri RL, Thompson IM, Kim IH, Ealy AD, Hansen PJ, Staples CR, Li JL, Santos JE & Thatcher WW 2012 Effects of lactation and pregnancy on gene expression of endometrium of Holstein cows at day 17 of the estrous cycle or pregnancy. *Journal of Dairy Science* **95** 5657-5675.

Davies D, Meade KG, Herath S, Eckersall PD, Gonzalez D, White JO, Conlan RS, O'Farrelly C & Sheldon IM. 2008 Toll-like receptor and antimicrobial peptide expression in the bovine endometrium. *Reproductive Biology and Endocrinology* **6** 53.

de Moraes AAS & Hansen PJ 1997 Granulocyte-macrophage colony-stimulating factor promotes development of *in vitro* produced bovine embryos. *Biology of Reproduction* **57** 1060-1065.

de Moraes AA, Paula-Lopes FF, Chegini N & Hansen PJ 1997 Localization of granulocyte-macrophage colony-stimulating factor in the bovine reproductive tract. *Journal of Reproductive Immunology* **42** 135-145.

Dobbs KB, Khan FA, Sakatani M, Moss JI, Ozawa M, Ealy AD & Hansen PJ 2013 Regulation of pluripotency of inner cell mass and growth and differentiation of trophectoderm of the bovine embryo by colony stimulating factor 2. *Biology of Reproduction* **89** 141.

Dorniak P, Bazer FW & Spencer TE 2013 Biological role of interferon tau in endometrial function and conceptus elongation. *Journal of Animal Science* **91** 1627-1638.

Doyle J, Ellis SA, O'Gorman GM, Aparicio Donoso IM, Lonergan P & Fair T 2009 Classical and non-classical Major Histocompatibility Complex class I gene expression in *in vitro* derived bovine embryos. *Journal of Reproductive Immunology* **82** 48-56.

DuBois JA, Wordinger RJ & Dickey JF 1980 Tissue concentrations of mast cells and lymphocytes of the bovine uterine tube (oviduct) during the estrous cycle. *American Journal of Veterinary Research* **41** 806-808.

Emond V, MacLaren LA, Kimmins S, Arosh JA, Fortier MA & Lambert RD 2004 Expression of cyclooxygenase-2 and granulocyte-macrophage colony-stimulating factor in the endometrial epithelium of the cow is up-regulated during early pregnancy and in response to intrauterine infusions of interferon-tau. *Biology of Reproduction* **70** 54-64.

Fallarino F, Grohmann U & Puccetti P 2012 Indoleamine 2,3-dioxygenase: from catalyst to signaling function. *European Journal of Immunology* **42** 1932-1937.

Farin PW, Piedrahita JA & Farin CE. 2006. Errors in development of fetuses and placentas from *in vitro*-produced bovine embryos. *Theriogenology* **65** 178-191.

Faulkner LC, Hopwood ML & Wiltbank JN 1968 Seminal vesiculectomy in bulls. II. Seminal characteristics and breeding trials. *Journal of Reproduction and Fertility* **16** 179-182.

Forde N, Carter F, Spencer TE, Bazer FW, Sandra O, Mansouri-Attia N, Okumu LA, McGettigan PA, Mehta JP, McBride R, O'Gaora P, Roche JF, Lonergan P. 2011 Conceptus-induced changes in the endometrial transcriptome: how soon does the cow know she is pregnant? *Biology of Reproduction* **85** 144-156.

Forde N, Duffy GB, McGettigan PA, Browne JA, Mehta JP, Kelly AK, Mansouri-Attia N, Sandra O, Loftus BJ, Crowe MA, Fair T, Roche JF, Lonergan P & Evans AC 2012. Evidence for an early endometrial response to pregnancy in cattle: both dependent upon and independent of interferon tau. *Physiological Genomics* **44** 799-810.

Goenka R, Scholz JL, Sindhava VJ & Cancro MP 2014 New roles for the BLyS/BAFF family in antigen-experienced B cell niches. *Cytokine and Growth Factor Reviews* **25** 107-113.

Guerin LR, Prins JR & Robertson SA 2009 Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? *Human Reproduction Update* **15** 517-535**.**

Harnaha J, Machen J, Wright M, Lakomy R, Styche A, Trucco M, Makaroun S & Giannoukakis N 2006 Interleukin-7 is a survival factor for $CD4^+$ $CD25^+$ T-cells and is expressed by diabetes-suppressive dendritic cells. *Diabetes* **55** 158-170.

Hansen PJ, Block J, Loureiro B, Bonilla L & Hendricks KE 2010 Effects of gamete source and culture conditions on the competence of *in vitro*-produced embryos for post-transfer survival in cattle. *Reproduction, Fertility, and Development* **22** 59-66.

Hoelker M, Salilew-Wondim D, Drillich M, Christine GB, Ghanem N, Goetze L, Tesfaye D, Schellander K & Heuwieser W 2012 Transcriptional response of the bovine endometrium and embryo to endometrial polymorphonuclear neutrophil infiltration as an indicator of subclinical inflammation of the uterine environment. *Reproduction, Fertility, and Development* **24** 778-793

Howe GR & Black DL 1963 Spermatozoan transport and leucocytic responses in the reproductive tract of calves. *Journal of Reproduction and Fertility* **6** 305–311.

Ideta A, Sakai S, Nakamura Y, Urakawa M, Hayama K, Tsuchiya K, Fujiwara H & Aoyagi Y 2010a Administration of peripheral blood mononuclear cells into the uterine horn to improve pregnancy rate following bovine embryo transfer. *Animal Reproduction Science* **117** 18-23.

Ideta A, Hayama K, Nakamura Y, Sakurai T, Tsuchiya K, Tanaka S, Yamaguchi T, Fujiwara H, Imakawa K & Aoyagi Y 2010b Intrauterine administration of peripheral blood mononuclear cells enhances early development of the pre-implantation bovine embryo. *Molecular Reproduction and Development* **77** 954-962.

Ingman WV & Robertson SA 2009 The essential roles of TGFB1 in reproduction. *Cytokine and Growth Factor Reviews* **20** 233-239.

Jackson LR, Farin CE & Whisnant S 2012 Tumor necrosis factor alpha inhibits *in vitro* bovine embryo development through a prostaglandin mediated mechanism. *Journal of Animal Science and Biotechnology* **3** 7.

Kalkunte SS, Mselle TF, Norris WE, Wira CR, Sentman CL & Sharma S 2009 Vascular endothelial growth factor C facilitates immune tolerance and endovascular activity of human uterine NK cells at the maternal-fetal interface. *Journal of Immunology* **182** 4085-4092.

Kaufmann TB, Drillich M, Tenhagen BA, Forderung D & Heuwieser W 2009 Prevalence of bovine subclinical endometritis 4h after insemination and its effects on

first service conception rate. *Theriogenology* **71** 385-391. **King GJ, Atkinson BA & Robertson HA** 1981 Development of the intercaruncular areas during early gestation and establishment of the bovine placenta. *Journal of Reproduction and Fertility* **61 4**69-474.

Klein C, Bauersachs S, Ulbrich SE, Einspanier R, Meyer HH, Schmidt SE, Reichenbach HD, Vermehren M, Sinowatz F, Blum H & Wolf E 2006 Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. *Biology of Reproduction* **74** 253-264.

Kowsar R, Hambruch N, Liu J, Shimizu T, Pfarrer C & Miyamoto A 2013 Regulation of innate immune function in bovine oviduct epithelial cells in culture: the homeostatic role of epithelial cells in balancing TH1/TH2 response. *Journal of Reproduction and Development* **59** 470-478.

Lander MF, Hansen PJ & Drost M 1990 Antisperm antibodies in cows after subcutaneous and intrauterine immunisation. *Veterinary Record* **126** 461-462.

Landivar C, Galina CS, Duchateau A & Navarro-Fierro R 1985 Fertility trial in Zebu cattle after a natural or controlled estrus with prostaglandin F2 alpha, comparing natural mating with artificial insemination. *Theriogenology* **23** 421-429.

Lettau M, Paulsen M, Schmidt H & Janssen O 2011 Insights into the molecular regulation of FasL (CD178) biology. *European Journal of Cell Biology* **90** 456-466.

Leung ST, Derecka K, Mann GE, Flint AP & Wathes DC 2000 Uterine lymphocyte distribution and interleukin expression during early pregnancy in cows. *Journal of Reproduction and Fertility* **119** 25-33.

Lima FS, Risco CA, Thatcher MJ, Benzaquen ME, Archbald LF, Santos JE & Thatcher WW 2009 Comparison of reproductive performance in lactating dairy cows bred by natural service or timed artificial insemination. *Journal of Dairy Science* **92** 5456-5466.

Lonergan P, Rizos D, Gutierrez-Adán A, Moreira PM, Pintado B, de la Fuente J & Boland MP 2003 Temporal divergence in the pattern of messenger RNA expression in bovine embryos cultured from the zygote to blastocyst stage *in vitro* or *in vivo*. *Biology of Reproduction* **69** 1424-1431.

Loureiro B, Bonilla L, Block J, Fear JM, Bonilla AQ & Hansen PJ 2009 Colony-stimulating factor 2 (CSF-2) improves development and posttransfer survival of bovine embryos produced *in vitro*. *Endocrinology* **150** 5046-5054.

Loureiro B, Oliveira LJ, Favoreto MG, & Hansen PJ 2011 Colony-stimulating factor 2 inhibits induction of apoptosis in the bovine preimplantation embryo. *American Journal of Reproductive Immunology* **65** 578-588.

Low BG, Hansen PJ, Drost M & Gogolin-Ewens KJ 1990 Expression of major histocompatibility complex antigens on the bovine placenta. *Journal of Reproduction and Fertility* **90** 235-243.

Mansouri-Attia N, Oliveira LJ, Forde N, Fahey AG, Browne JA, Roche JF, Sandra O, Reinaud P, Lonergan P & Fair T 2012 Pivotal role for monocytes/macrophages and dendritic cells in maternal immune response to the developing embryo in cattle. *Biology of Reproduction* **87** 123.

Marçais A, Viel S, Grau M, Henry T, Marvel J & Walzer T 2013 Regulation of Mouse NK Cell Development and Function by Cytokines. *Frontiers in Immunology* **4** 450.

Mattner PE 1968 The distribution of spermatozoa and leucocytes in the female genital tract in goats and cattle *Journal of Reproduction and Fertility* **17** 253–261.

Menge AC 1967 Induced infertility in cattle by iso-immunization with semen and testis. *Journal of Reproduction and Fertility* **13** 445-456.

Menge AC 1969 Early embryo mortality in heifers isoimmunized with semen and conceptus. *Journal of Reproduction and Fertility* **18** 67-74.

Muñoz M, Corrales FJ, Caamaño JN, Díez C, Trigal B, Mora MI, Martín D, Carrocera S & Gómez E 2012 Proteome of the early embryo-maternal dialogue in the cattle uterus. *Journal of Proteome Research* **11** 751-766.

Neira JA, Tainturier D, Peña MA & Martal J 2010 Effect of the association of IGF-I, IGF-II, bFGF, TGF-β1, GM-CSF, and LIF on the development of bovine embryos produced *in vitro*. *Theriogenology* 2010; **73** 595-604.

Odhiambo JF, Poole DH, Hughes L, Dejarnette JM, Inskeep EK & Dailey RA 2009 Pregnancy outcome in dairy and beef cattle after artificial insemination and treatment with seminal plasma or transforming growth factor β-1. *Theriogenology* **72** 566-571.

Oliveira LJ, McClellan S & Hansen PJ 2010 Differentiation of the endometrial macrophage during pregnancy in the cow. *PLoS One* **5** e13213.

Oliveira LJ, Mansourri-Attia N, Fahey AG, Browne J, Forde N, Roche JF, Lonergan P & Fair T 2013 Characterization of the Th profile of the bovine endometrium during the oestrous cycle and early pregnancy. *PLoS One* **8** e75571.

Paula-Lopes FF, de Moraes AA, Edwards JL, Justice JE & Hansen PJ 1998 Regulation of preimplantation development of bovine embryos by interleukin-1β. *Biology of Reproduction* **59** 1406-1412.

Paula-Lopes FF, de Moraes AA & Hansen PJ 1999 The presence of interleukin-1β in the bovine reproductive tract. *Journal of Interferon and Cytokine Research* **19** 279-285.

Peltier MR & Hansen PJ 2001 Immunoregulatory activity, biochemistry, and phylogeny of ovine uterine serpin. *American Journal of Reproductive Immunology* **45** 266-72.

Ponsuksili S, Murani E, Schwerin M, Schellander K, Tesfaye D, & Wimmers K 2012 Gene expression and DNA-methylation of bovine pretransfer endometrium depending on its receptivity after *in vitro*-produced embryo transfer. *PLoS One* **7** e42402.

Roberts RM, Ezashi T, Rosenfeld CS, Ealy AD & Kubisch HM 2003 Evolution of the interferon τ genes and their promoters, and maternal-trophoblast interactions in control of their expression. *Reproduction Supplement* **61** 239-251.

Roberts RM, Chen Y, Ezashi T & Walker AM 2008 Interferons and the maternal-conceptus dialog in mammals. *Seminars in Cell and Developmental Biology* **19** 170-177.

Robertson SA, Prins JR, Sharkey DJ & Moldenhauer LM 2013 Seminal fluid and the generation of regulatory T cells for embryo implantation. *American Journal of Reproductive Immunology* **69** 315-330.

Robinson RS, Fray MD, Wathes DC, Lamming GE & Mann GE 2006 *In vivo* expression of interferon tau mRNA by the embryonic trophoblast and uterine concentrations of interferon tau protein during early pregnancy in the cow. *Molecular Reproduction and Development* **73** 470-474.

Rowe JH, Ertelt JM, Xin L & Way SS 2012 Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. *Nature* **490** 102-106.

Salilew-Wondim D, Hölker M, Rings F, Ghanem N, Ulas-Cinar M, Peippo J, Tholen E, Looft C, Schellander K & Tesfaye D 2010 Bovine pretransfer endometrium and embryo transcriptome fingerprints as predictors of pregnancy success after embryo transfer. *Physiological Genomics* **42** 201-218.

Scenna FN, Edwards JL, Rohrbach NR, Hockett ME, Saxton AM, Schrick FN 2004 Detrimental effects of prostaglandin F2a on preimplantation bovine embryos. *Prostaglandins and Other Lipid Mediators* **73** 215-226.

Short EC Jr, Geisert RD, Helmer SD, Zavy MT & Fulton RW 1991 Expression of antiviral activity and induction of 2′,5′-oligoadenylate synthetase by conceptus secretory proteins enriched in bovine trophoblast protein-1. *Biology of Reproduction* **44** 261-268.

Sjöblom C, Roberts CT, Wikland M, & Robertson SA 2005 Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. *Endocrinology* **146** 2142-2153.

Skopets B, Li J, Thatcher WW, Roberts RM & Hansen PJ 1992 Inhibition of lymphocyte proliferation by bovine trophoblast protein-1 (type I trophoblast interferon) and bovine interferon-α_{11}. *Veterinary Immunology and Immunopathology* **34** 81-96.

Soto P, Natzke RP & Hansen PJ 2003a Identification of possible mediators of embryonic mortality caused by mastitis: actions of lipopolysaccharide, prostaglandin $F_{2\alpha}$, and the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and embryonic development in cattle. *American Journal of Reproductive Immunology* **50**, 263-272.

Soto P, Natzke RP & Hansen PJ 2003b Actions of tumor necrosis factor-alpha on oocyte maturation and embryonic development in cattle. *American Journal of Reproductive Immunology* **50** 380-388.

Tekin S & Hansen PJ 2002 Natural killer-like cells in the sheep: functional characterization and regulation by pregnancy-associated proteins. *Experimental Biology and Medicine* **227** 803-811.

Templeton JW, Tipton RC, Garber T, Bondioli K & Kraemer DC 1987 Expression and genetic segregation of parental BoLA serotypes in bovine embryos. *Animal Genetics* **18** 317-322

Thatcher WW, Santos JE, Silvestre FT, Kim IH & Staples CR 2010 Perspective on physiological/endocrine and nutritional factors influencing fertility in post-partum dairy cows. *Reproduction in Domestic Animals* **45 Suppl 3** 2-14.

Tuo W, Ott TL & Bazer FW 1993 Natural killer cell activity of lymphocytes exposed to ovine, type I, trophoblast interferon. *American Journal of Reproductive Immunology* **29** 26-34.

Turner ML, Cronin JG, Healey GD & Sheldon IM 2014 Epithelial and stromal cells of bovine endometrium have roles in innate immunity and initiate inflammatory responses to bacterial lipopeptides *in vitro* via Toll-like receptors TLR2, TLR1 and TLR6. *Endocrinology* **155** 1453-1465.

Vasudevan, S, Kamat, M. Walusimbi, S, Pate, J. & Ott T 2013 Effects of early pregnancy on endometrial NK cells and CD8+ cells in dairy heifers *American Journal of Reproductive Immunology* **69** 157 (abstr).

Volin MV Koch AE 2011 Interleukin-18: a mediator of inflammation and angiogenesis in rheumatoid arthritis. *Journal of Interferon and Cytokine Research* **31** 745-751.

Walker AM & Roberts RM 2009 Characterization of the bovine type I IFN locus: rearrangements, expansions, and novel subfamilies. *BMC Genomics* **10** 187.

Walker CG, Meier S, Littlejohn MD, Lehnert K, Roche JR & Mitchell MD 2010 Modulation of the maternal immune system by the pre-implantation embryo. *BMC Genomics* **11** 474.

Yang Y, Lee JH, Kim KY, Song HK, Kim JK, Yoon SR, Cho D, Song KS, Lee YH & Choi I 2005 The interferon-inducible 9-27 gene modulates the susceptibility to natural killer cells and the invasiveness of gastric cancer cells. *Cancer Letters* **221** 191-200.

Yao N, Wan PC, Hao ZD, Gao FF, Yang L, Cui MS, Wu Y, Liu JH, Liu S, Chen H & Zeng SM. 2009 Expression of interferon-tau mRNA in bovine embryos derived from different procedures. *Reproduction in Domestic Animals* **44** 132-139.

Ziebe S, Loft A, Povlsen BB, Erb K, Agerholm I, Aasted M, Gabrielsen A, Hnida C, Zobel DP, Munding B, Bendz SH & Robertson SA. 2013 A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for *in vitro* fertilization. *Fertility and Sterility* **99** 1600-1609.