

# Progesterone During Pregnancy: Endocrine–Immune Cross Talk in Mammalian Species and the Role of Stress

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## Keywords

NK cells, pregnancy, progesterone

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Submitted June 16, 2007;  
accepted June 21, 2007.

## Citation

Arck P, Hansen PJ, Mulac Jericevic B, Piccinni M-P, Szekeres-Bartho J. Progesterone during pregnancy: endocrine-immune cross talk in mammalian species and the role of stress. *Am J Reprod Immunol* 2007; 58:268–279

doi:10.1111/j.1600-0897.2007.00512.x

## Introduction

Pregnancy is characterized by overall hormonal changes. Progesterone (P) is critical for not only the establishment but also for the maintenance of pregnancy, as its functions support ovulation and uterine as well as mammary gland development. Compared to the low levels (1–2 nmol/L) during the follicular phase of the menstrual cycle P concentrations increase to 15–20, 35–50, and 20–40 nmol/L in the early-, mid-, and late-luteal phases respectively.

The major source of P during pregnancy is the corpus luteum of the ovary and, if pregnancy occurs, in

Progesterone is critical for the establishment and the maintenance of pregnancy, both by its endocrine and immunological effects. The genomic actions of progesterone are mediated by the intracellular progesterone receptors; A and B. A protein called P-induced blocking factor (PIBF), by inducing a T<sub>H2</sub> dominant cytokine production, mediates the immunological effects of progesterone. Progesterone plays a role in uterine homing of NK cells and up-regulates HLA-G gene expression, the ligand for various NK inhibitory receptors. At high concentrations progesterone is a potent inducer of Th2-type cytokines as well as of LIF and M-CSF production by T cells. Though a key role for progesterone in creating local immunosuppression has been conserved during the evolution of an epitheliochorial placenta, there has been some divergence in the pattern of endocrine-immunological cross talk in Bovidae. In sheep, uterine serpin, a progesterone-induced endometrial protein, mediates the immunosuppressive effects of progesterone. Epidemiological studies suggest the role of stress in premature pregnancy termination and exposure to stress induces abortion in mice via a significant reduction in progesterone levels, accompanied by reduced serum levels of PIBF. These effects are corrected by progesterone supplementation. These findings indicate the significance of a progesterone-dependent immuno-modulation in maternal tolerance of the fetus, which is discussed in this review.

many species, including humans and rodents, P production is eventually sustained by the placenta.<sup>1</sup> In humans, P production gradually rises during gestation to reach a level of 3 µg/g of placental tissue (1–10 µM), whereas the serum concentrations of P range from about 100 to 500 nM during pregnancy.<sup>2</sup> The need for P in maintaining pregnancy is shown by the fact that blocking of P binding sites causes abortion in human and also in various animal species. Besides its endocrine effects P acts as an ‘immunosteroid’. Successful pregnancy depends on maternal tolerance of the fetal ‘semi-allograft’ (Clark et al., current issue). Here, P blocks very early T-cell

lymphopoiesis during pregnancy<sup>3</sup> and controls the bias towards a pregnancy protective immune milieu,<sup>4</sup> which involves an immunomodulatory protein, known as P-induced blocking factor (PIBF).<sup>5</sup>

Such observations might be just the 'tip of the iceberg' of insights into the cross talk between the endocrine and the immune systems,<sup>6–8</sup> the underlying mechanisms that operate throughout gestation are not completely understood.

### Progesterone, its receptors and mechanisms of actions

Progesterone (P), one of the key players in the interaction between the endocrine and immune systems, regulates menstrual bleeding, tissue repair and regeneration, inflammation, angiogenesis, blastocyst implantation, and maintenance of pregnancy. These events result from precisely coordinated activity of molecular pathways that ensure endometrial cell proliferation, differentiation, cell survival, leukocyte trafficking, apoptosis, and angiogenesis.

Genomic and non-genomic pathways mediate the biological activities of P. The P regulated genomic pathway depends on two progesterone receptor (nPR) isoforms, PR-A and PR-B, both members of the nuclear receptor superfamily of transcription factors.<sup>9,10</sup> PR-A and PR-B are the products of the same gene, transcribed under control of two distinct promoters. The PR-A and PR-B isoforms differ, in that PR-B contains an additional N-terminal stretch of approximately 165 amino acids.

In addition to regulation by P, the transcriptional activity of nPR isoforms can be regulated through alternation of expression level, interaction with co-activators and post-translational modification of nPR.<sup>9,10</sup> Spatial and temporal expression of the PR-A and PR-B vary in reproductive tissues as a consequence of the developmental and the hormonal status as well as of carcinogenesis.

In mice, null mutation of the nPR gene revealed that transcriptional activity of nPR controls the uterine immune environment as well as endometrial receptivity and decidualization.<sup>11,12</sup> Also functionally active nPRs in the thymus are required for thymic involution during pregnancy and for a normal fertility.<sup>13</sup> Studies with mice in which PR-A and PR-B expression were selectively ablated demonstrate that PR-A and PR-B isoforms are functionally distinct transcription factors.<sup>13,14</sup> Briefly, P-induced activation of PR-A is both necessary and sufficient for the

establishment and the maintenance of pregnancy, but elicits reduced pregnancy-stimulated mammary gland morphogenesis. In contrast, P-induced transcriptional activity of the PR-B isoform is insufficient for implantation and the maintenance of pregnancy, and mice lacking PR-A are infertile. These findings imply that the relative expression of the two isoforms is critical for the appropriate reproductive tissue responses to P.<sup>15</sup>

Although the genomic pathway of P action has been extensively studied, so far only a few P-regulated genes have been identified in the peri-implantation uterus. These genes include amphiregulin (a member of EGF superfamily), histidine decarboxylase (an enzyme that converts histidine to histamine), Hox-A10 and Hox-A11 (both members of homeobox gene family), calcitonin (a peptide hormone), proenkephalin (neuropeptide), immune response gene 1 (Irg-1), MUC1 (a glycoprotein component of apical glycocalyx), indian hedgehog (mophogen), and galectin-1.<sup>16</sup>

Hox-A10 deficiency in mice leads to severe local immunological disturbances, characterized by a polyclonal proliferation of T cells that occurs in absence of the normal P-mediated immunosuppression in the peri-implantation uterus.<sup>17</sup> Natural killer (NK) cell constitute the predominant leukocyte population present in endometrium at the time of implantation and early pregnancy (Santoni et al.<sup>18</sup>, current issue). Hox-A10 deficiency in mice alters region specific gene expression and compromises NK cell differentiation, but not trafficking of NK precursors cells during decidualization.<sup>19</sup>

Estrogen-induced MUC1 is expressed by human villous syncytio- and cytotrophoblast as well as by invasive extravillous cytotrophoblast.<sup>20</sup> It was proposed that blastocyst implantation is regulated by a uterine barrier, whereby a high density of MUC1 at the epithelial cell surface can inhibit blastocyst adhesion.<sup>21</sup> In mice MUC1 expression in the uterine epithelium is down-regulated during the window of implantation, and studies on genetically modified mice suggest that PR-A antagonizes Muc1 expression, which may subsequently allow blastocyst adhesion.<sup>22</sup>

Indian hedgehog (Ihh) is another gene, regulated by P. Ihh plays a critical role in communication (required for embryo implantation) between the uterine epithelium and stroma.<sup>23</sup> Ihh belongs to hedgehog family of morphogens that regulate cell proliferation, differentiation and cell-cell communication, all of which may be involved in successful

decidualization and maintenance of fetal tolerance. Finally, galectin-1, which is also under the regulation of P, appears to be of importance, since its expression is down regulated on placental villous tissues from patients with spontaneous miscarriages.<sup>24</sup>

Non-genomic actions of P include (a) P-induced acrosomal reaction, (b) P-induced resumption of meiosis, and (c) P induced decrease in neuronal excitability and anesthesia.<sup>25</sup> The non-genomic actions are characterized by a fast response to P (latency of minutes rather than hours) and no requirement for *de novo* protein or RNA synthesis. Non-genomic actions of P appear to operate through membrane specific G protein-coupled receptors.

Proper functional communication between the genomic and non-genomic P-regulated signaling pathways could be critical for the establishment of a correct endocrine-immune interaction in human endometrium during the establishment and maintenance of pregnancy.<sup>25</sup>

Clearly, a profound knowledge of the key molecular signals that are essential for the establishment of the receptive uterus will open therapeutic approaches in the development of new strategies for the treatment of implantation failures. Although technological advancement in functional genomics and proteomics allows identification of the differentially regulated genes during the implantation window<sup>26,27</sup> ethical considerations preclude an in-depth investigation of early molecular events in humans. Therefore, animal models, such as mouse knockout models will continue to be invaluable tools for studying the molecular events involved in the establishment and maintenance of pregnancy.<sup>28</sup>

### The effect of P on the immune system

With the exception of human leukocyte (HLA)-C, polymorphic major histocompatibility complex (MHC) is not expressed on human trophoblast, and this creates a unique immunological situation. Though decidual macrophages and dendritic cells can present fetal antigens to both decidual CD4<sup>+</sup> and CD8<sup>+</sup> cells, trophoblast-presented antigens are unlikely to be recognized in an MHC-restricted fashion. In the decidua, there is a significantly increased number of activated  $\gamma/\delta$  TCR-positive cells.<sup>29,30</sup> As most  $\gamma/\delta$  T cells are capable to recognize unprocessed foreign antigens without MHC restriction, they could be candidates for 'seeing' trophoblast presented antigens. In peripheral blood of healthy pregnant

women the number of  $\gamma/\delta$  T cells is a significantly increased, and almost all of them express nPRs,<sup>31</sup> suggesting a prior activation. These cells could be of decidual origin, which, after activation by trophoblast presented antigens, appear in peripheral circulation.

Uterine dendritic cells (DC) have been proposed to serve as a switchboard between fetal rejection and tolerance.<sup>32-34</sup> DCs are the most potent antigen presenting cells (APCs) involved in the innate immune response and in the maintenance of tolerance.<sup>35</sup> The regulation of their maturation, migration, and expression of stimulatory and costimulatory molecules has major consequences on the immune response, whereby endogenous factors regulating DC function are poorly understood. Immature DCs exhibit a tolerogenic phenotype, characterized by low expression of costimulatory molecules (CD40, CD80, and CD86), low production of proinflammatory cytokines, increased production of IL-10, and capacity to induce regulatory T cells with suppressive actions, all of which will promote pregnancy maintenance. Immature DC reside in early pregnancy decidua in humans<sup>32,36</sup> and mice<sup>34,37</sup> and possibly serve as sentinel cells of the tissue environment for potential danger signals. However, in murine pregnancies with high abortion rates, an increase of mature APC can be observed.<sup>34</sup> By blocking crucial ligands required on APCs to induce T-cell activation, mechanisms of fetal tolerance are restored in abortion-prone pregnancies.<sup>38</sup> P has been shown to inhibit mature dendritic cells as well as DC-stimulated proliferation of T cells in a receptor-mediated fashion.<sup>39</sup>

The effect of P on the immune system of pregnant women could be partly receptor-mediated.<sup>40-43</sup> Recent finding suggest that P might act directly through membrane specific P receptors to suppress T-cell activation during pregnancy.<sup>44</sup> Following recognition of fetal antigens, activated maternal  $\gamma/\delta$  T cells express nPRs,<sup>31</sup> and upon P binding, they produce a mediator namely PIBF.<sup>5,45</sup> In urine samples of healthy pregnant women, PIBF concentration continuously increases until the 37th week of gestation, followed by a slow decrease until term. In pregnancies that end up in miscarriage or pre-term delivery, urinary PIBF levels fail to increase during pregnancy.<sup>46</sup>

By signaling via the Jak/STAT pathway,<sup>47</sup> PIBF induces a T<sub>H2</sub> dominant cytokine production<sup>48</sup> and in a cytokine-mediated way blocks NK activity.<sup>49</sup>

Neutralization of endogenous PIBF activity in pregnant mice by specific anti-PIBF antibody causes

a significant reduction in the number of viable fetuses, and this is associated with an increased splenic NK activity, together reduced IL-10 and increased interferon- $\gamma$  (IFN- $\gamma$ ) production of the spleen cells.<sup>50</sup> These are corrected by treatment of the pregnant animals with anti-NK antibodies,<sup>50</sup> suggesting that in mice PIBF contributes to the success of pregnancy and that the major part of its pregnancy-protective effect lies in controlling NK activity.

In rodents and also in human hormonally controlled uterine NK cells play an important role in creating a suitable environment for the establishment of pregnancy.<sup>51</sup> The temporal and spatial distribution of these cells suggests that one of the functions of these cells might be the control of placentation. Decidual NK cells secrete an array of angiogenic factors and induce vascular growth in the decidua. By producing interleukin-8 and interferon-inducible protein-10 chemokines decidual NK cells regulate trophoblast invasion.<sup>52</sup>

Henderson et al.<sup>53</sup> demonstrated the absence of PR in purified decidual NK cells, and thus a genomic action of P on these cells is unlikely. Nevertheless, P affects decidual NK cells in several ways.

Van den Heuvel et al.<sup>42</sup> reported that P plays a role in uterine homing of NK cells by promoting NK cell interactions with the endothelium. NK cell migration to the endometrium is also supported by sex-hormone-induced specific endometrial production of chemokines.<sup>54</sup>

Decidual NK cells show a low spontaneous cytotoxic activity in spite of their high perforin content.<sup>55</sup> Many of them express inhibitory receptors that recognize non-polymorphic MHC molecules. P has been shown to up-regulate HLA-G gene expression,<sup>56</sup> and the increased availability of P protects the trophoblast from NK-mediated killing.

Based on their cytokine secretion profile human CD4<sup>+</sup> T helper cells, can be subdivided into at least three distinct functional subsets.<sup>57,58</sup>

Human type 1 (Th1) CD4<sup>+</sup> T cells that produce interleukin (IL-2), tumor necrosis factor (TNF- $\alpha$ ), and IFN- $\gamma$  are the main effectors of host defense against infections by intracellular parasites. On the other hand, human type 2 (Th2) CD4<sup>+</sup> T cells produce IL-4, IL-5, IL-13 and IL-10, which together with IL-4 inhibit several macrophage functions. A third type (Th0) produces both Th1- and Th2-type cytokines. Recently additional CD4<sup>+</sup> cell subsets have been identified: Th3 cells, which produce TGF- $\beta$  and IL-10,<sup>59</sup> Th17

cells, which produce IL-17A and IL-22,<sup>60</sup> and regulatory T cells (T reg) secreting IL-10 and TGF- $\beta$ .<sup>61</sup>

Cytokines produced by APCs and lymphocytes affect the development of Th1 and Th2 responses both *in vitro* and *in vivo*. IFN- $\gamma$ , IL-12, and IFN- $\alpha$  exert critical effects on CD4<sup>+</sup> subset maturation by inducing Th1 expansion,<sup>62,63</sup> while IL-4 is needed for Th2 cell maturation.<sup>62</sup> Steroid hormones control the cytokine profile of T cells. Glucocorticoids and 1,25-dihydroxy-vitamin D3 increase IL-4,<sup>64,65</sup> whereas dihydrotestosterone decreases IL-4 and IL-5 production.<sup>66</sup> The polypeptide hormone relaxin predominantly produced by the corpus luteum and decidua during pregnancy favors the development of IFN- $\gamma$ -producing T cells.<sup>67</sup> In two murine T-cell lines (NIMP-TH1 and EL4) dexamethasone negatively regulates IL-5 gene expression, whereas testosterone and P induce the expression of IL-5 gene.<sup>68</sup>

Progesterone also affects the differentiation of resting peripheral blood T cells into Th1-, Th0-, or Th2-like clones and the production of IL-4 by human T-cell clones<sup>4</sup> via the development of antigen-specific CD4<sup>+</sup> T cell lines with an enhanced ability to produce IL-4 and IL-5.<sup>4</sup> In addition, P induces IL-4 mRNA expression and the production of detectable amounts of IL-4 in Th1-type T-cell clones (able to produce IFN- $\gamma$  only without P).<sup>4</sup> IL-4 production by Th1 T-cell clones in response to P was associated with the expression of CD30 (a molecule preferentially expressed by IL4-producing T cells).<sup>4</sup>

These results indicate that P at concentrations that are higher than those found in serum during pregnancy, but comparable to those present at the fetomaternal interface,<sup>1</sup> functions as a potent inducer of Th2-type cytokine production, which could be independently confirmed by others.<sup>2</sup> Further, P promotes the development of LIF (leukemia inhibitory factor),<sup>69</sup> as well as macrophage colony-stimulating factor (M-CSF)-producing T cells.<sup>70</sup> Progesterone-induced LIF (essential for the embryo implantation) and M-CSF (important for pregnancy development) production was mediated by IL-4, produced by T cells in response to P. The effects of P were hormone-specific, as P receptors are found on activated T cells and P analogues (4 pregnen 20  $\beta$ -ol 3-one, 4 pregnen 20- $\alpha$  ol 3-one, and 5 pregnen 3  $\beta$ -ol 20-one) have no effect on cytokine production by either T-cell lines and clones.<sup>4,40</sup> The mechanisms by which P acts on T-cell differentiation are still unknown.

It has been suggested that a Th1 to Th2 switch at the fetomaternal interface plays a role in the

maintenance of successful pregnancy.<sup>71</sup> In line with this, placental IL-4 mRNA expression in mice was found to be 5- to 10-fold higher than in peripheral blood.<sup>72</sup> Furthermore, defective IL-4 production by decidual CD4<sup>+</sup> as well as CD8<sup>+</sup> T cells, and defective of IL-10, LIF, and M-CSF production by decidual CD4<sup>+</sup> T cells were detectable in women with unexplained recurrent abortion at the time of miscarriage.<sup>69</sup> These data suggest that in human the success of pregnancy is associated with the production of Th2-type cytokines, LIF and M-CSF by T cells at materno-fetal interface. P, which, at concentrations comparable with those present at the materno-fetal interface during pregnancy, is not only a potent inducer of Th2-type cytokines (i.e. IL-4 and IL-5),<sup>4</sup> but also of LIF and M-CSF production by T cells<sup>69,70</sup> may be at least in part responsible for a Th2 switch at maternofetal interface.<sup>73</sup> IL-4 produced by decidual Th2 cells can in turn promote the development of T cells producing LIF and M-CSF,<sup>69,70</sup> which seem to be important for embryo implantation and development. Both IL-4 and IL-10 can inhibit the development and function of Th1 cells and macrophages, thus preventing the allograft rejection.

These findings indicate that the immunological effects of P contribute to the complex network of regulatory pathways in the cause of fetal allograft survival.

#### The effect of stress on the immune response and the outcome of pregnancy

The hypothalamic-pituitary-adrenal (HPA) axis exerts an inhibitory effect on the female reproductive system when activated by stress. Corticotrophin releasing hormone (CRH) inhibits hypothalamic gonadotropin releasing hormone (GnRH) secretion, and glucocorticoids inhibit pituitary luteinizing hormone and ovarian steroid hormones, estrogen and P.

Psychosocial stress has been shown to alter cytokine production by peripheral lymphocytes of pregnant women during the first trimester of pregnancy.

Some of the pregnancy complications cannot be explained by either maternal or fetal pathologies.<sup>74–77</sup> Several epidemiological studies support the notion that the onset of miscarriages may be attributable to high levels of perceived stress. Repeated miscarriages can induce anxiety and even depression. Emotional stress, due to repeated pregnancy losses might also contribute to further miscarriages. However, to date, the unconfined acceptance of stress as a cause for

pregnancy loss in everyday clinical practice is limited by contradictory observations.<sup>78,79</sup> Such contradictory results may be explained by the diverse experimental design, such as correlating the temporal coincidence of stressful life events or self-reported stress perception to the onset of spontaneous abortion. Further, the lack of appropriate tools to evaluate stress perception is clearly a limitation in cohort studies. For humans, little physiological evidence exists in support of this hypothesis and thus, identification of risk factors for stress-triggered miscarriages requires further research.<sup>80–82</sup>

Emerging evidence indicates that mediators of the HPA axis, such as CRH and the glucocorticoid cortisol may serve as such stress indicators.<sup>83</sup> High levels of glucocorticoids exert adverse effects on the uterus and fetus, and inhibit pituitary luteinizing hormone, and ovarian estrogen, and P secretion.<sup>84</sup> Such inhibitory effects of stress hormones on the female reproductive system are responsible for the 'hypothalamic' amenorrhea of stress, and – as shown in mice – may also account for inadequate levels of P during pregnancy, subsequently resulting in spontaneous abortion.<sup>84</sup> The concept of stress-triggered inhibition of P is supported by experimental evidence from animal studies. Exposure to stress in the form of restraint<sup>85</sup> or sound<sup>86</sup> induces abortion in pregnant mice via a significant reduction in P levels, accompanied by reduced serum levels of P-induced blocking factor (PIBF) and diminished expression of PRs at the feto-maternal interface.<sup>87</sup> Administration of the P derivative dydrogesterone increases levels of PIBF and restores the pregnancy-protective immune milieu in the mouse model of stress-triggered abortions, as well as in humans with threatened abortion.<sup>87–89</sup> Such endocrine-immune cross talk is exceedingly dependent on a specific CD8<sup>+</sup> T-cell population, since depletion of CD8 led to a termination of the pregnancy protective effect of P substitution in mice, whereby the precise phenotype of this specific, pregnancy-protective CD8 cell population, e.g. the co-expression of the  $\alpha\beta$  or  $\gamma\delta$  T-cell receptor, remains to be elucidated.<sup>88</sup> The notion of decreased levels of P in response to stress could also be confirmed in other mammalian species, such as in elks.<sup>90</sup>

In addition to the 'classical' stress mediators, such as CRH, adrenocorticotropin (ACTH), cortisol or catecholamines,<sup>91</sup> the neurotrophin nerve growth factor (NGF) or the neuropeptide Substance P are progressively recognized as a pivotal regulator of the

stress response cascade.<sup>92–94</sup> Thus, future research addressing the potential threat of such stress mediators on progesterone production and pregnancy maintenance is needed.

**Endocrine-immune cross talk in Bovidae: insights into the immunological consequences of evolution of the epitheliochorial placenta.**

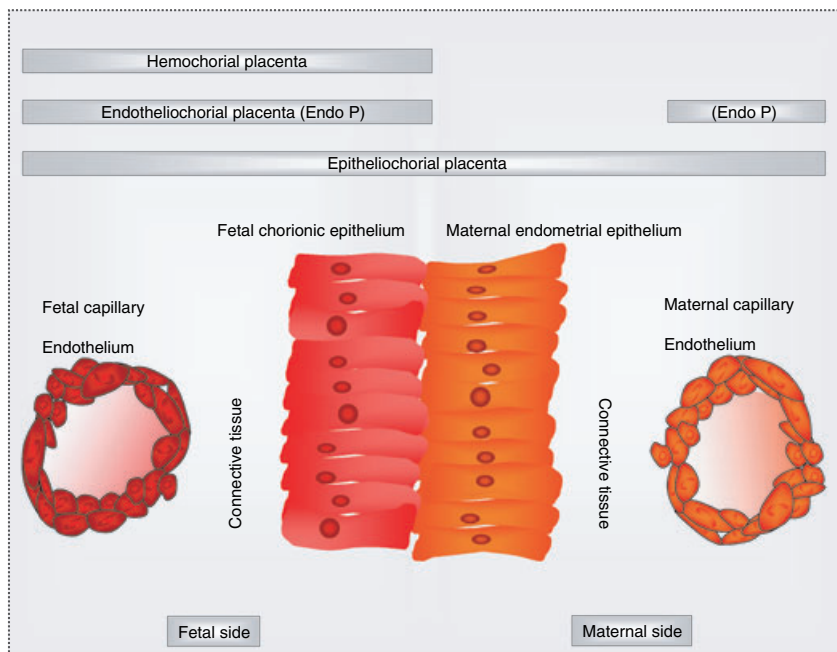
Besides mice and humans the Bovidae have been one of the most extensively studied clades in mammalian reproduction; whereby examination of the specializations acquired by these animals during evolution provides insights into immunological adjustments to pregnancy. Such features are essential in reproduction in eutherian mammals and represent clade-specific solutions to the immunological problem of viviparity.

The Bovidae diverged as a separate family of pecoran ruminants about 24–29 million years ago during the Late Oligocene epoch.<sup>95</sup> While the basic pattern of reproduction in ruminants is similar to other mammals, there are distinct features including placental anatomy. Ruminants possess an epitheliochorial placenta characterized by apposition of fetal and maternal tissues. Invasion of the maternal system either does not occur or is limited to migration of trophoblast cells into the maternal endometrial epithelium to form a syncytium (Fig. 1).<sup>96,97</sup>

Evolutionary advantages conferred by an epitheliochorial placenta include more efficient transport of nutrients.<sup>96</sup> In addition, fetal-maternal competition may be reduced in species with epitheliochorial species because the mother has greater control over maternal blood flow to the placenta.<sup>96</sup> It is also possible that there are immunological consequences of epitheliochorial placentation. As compared to species with invasive placenta, access of maternal leukocytes to fetal placental tissue is restricted physically by several cell layers and there may be reduced opportunity for pieces of trophoblast tissue to enter draining lymph nodes and peripheral circulation of the mother.

Whether these differences actually confer increased immunological fitness for the placenta with respect to maternal immunological recognition is not known. In any case, the immunological relationship between the conceptus and mother in Bovidae is similar in many ways to that for other mammals. Expression of MHC antigens on the trophoblast is largely down-regulated<sup>98,99</sup> although, at least in the cow, there is limited expression of class I MHC molecules by trophoblast in later pregnancy.<sup>99</sup> Down-regulation of MHC antigen expression may be an important requirement for successful pregnancies: cloned bovine conceptuses, which experience high rates of fetal loss, can express aberrantly high levels of MHC class I protein associated with

**Fig. 1** Comparative understanding of the placenta in different species: Placentas are variously classified, i.e. by their macroscopic appearance, or according to its intimacy of fetal-maternal contact. Here, the main placental types have been described as epitheliochorial (three maternal layers and three fetal layers), endotheliochorial (one maternal layer, three fetal layers), hemochorial (no maternal layers; three fetal layers). (Note: placentas can also express a mosaic type). Hence, six cellular layers that can potentially be between the fetal and maternal blood cells. Epitheliochorial (6-layer) placentas are common in pigs, cows, horses, and sheep. Endotheliochorial (4-layer) placentas are frequently found in dogs, cats, seals, and ferrets. Hemochorial placentas (where the maternal blood cells are in direct contact with the fetal chorion) are seen in humans, rats, and mice.<sup>114</sup>



increased accumulation of maternal lymphocytes in endometrial stroma.<sup>100</sup>

Experiments from the sheep indicate that extensive remodeling of the leukocyte population in the uterus takes place during pregnancy. Macrophages accumulate in large numbers in the endometrial stroma during pregnancy<sup>101</sup> and granulated  $\gamma\delta$ -T cells become abundant in luminal epithelium of the interplacentomal regions during mid- and late-pregnancy.<sup>102,103</sup> Non-granulated T cells in the glandular epithelium decline during pregnancy while numbers of these cells in luminal epithelium first decline and then return to levels seen in non-pregnant ewes.<sup>103</sup> It is not known whether there are changes in NK-cell populations in the endometrium during pregnancy because of the paucity of immunological and functional assays for these cells in ruminants. Limited evidence in sheep suggests that there is no large increase in endometrial NK-cell numbers in late pregnancy when compared with cyclic ewes.<sup>104</sup> It may be that these cells, which play a crucial role in vascular remodeling in mice,<sup>105</sup> are not important players in at least some species with epitheliochorial placentation because of differences in endometrial vascular architecture.

Use of a unilaterally pregnant model in sheep (where pregnancy is surgically confined to one uterine horn) has revealed that accumulation of macrophages is due to both systemic signals (numbers of cells in the non-pregnant uterine horn of the unilaterally pregnant ewe higher than amounts in uteri of non-pregnant ewes) and locally produced signals (number of cells in the uterus of unilaterally ligated ewes higher in the pregnant horn than in the non-pregnant horn).<sup>102</sup> Accumulation of  $\gamma\delta$  T cells is a result of unidentified systemic signals.<sup>103</sup> However, local placentally derived signals may be involved in activation of  $\gamma\delta$  T cells. There was increased expression of CD25 from  $\gamma\delta$  T cells isolated from the pregnant uterine horn of unilaterally pregnant ewes when compared with the non-pregnant horn.<sup>29</sup>

As previously outlined, one of the key regulators of uterine immune function is P. In cattle, the placental contribution to P synthesis is low throughout most of pregnancy and also low in sheep until day 50 of pregnancy (gestation length = 147 days). Thus, for all or part of pregnancy, lymphocytes at the fetal-maternal interface are probably not exposed to the high concentrations required to inhibit lymphocyte function.<sup>106</sup> Administration of P to sheep can block tissue graft rejection *in utero* when injected to

achieve concentrations in blood too low to directly inhibit lymphocyte proliferation.<sup>107,108</sup> Thus, P regulates tissue rejection responses indirectly by inducing secretory molecules from the uterine endometrium that can regulate immune function.

Interestingly, the major P-induced immunoregulatory molecule in sheep is a member of the serine proteinase inhibitor family called uterine serpin. Ovine uterine serpin can block lymphocyte proliferation *in vitro* in sheep<sup>109</sup> and NK-cell-mediated abortion *in vivo* in mice.<sup>110</sup> Cattle, goats, and pigs also secrete uterine serpin from the endometrium but its role in immune function in these species has not been documented. Strikingly, the gene for uterine serpin is not present in human or mouse as determined by queries of genomic databases. The gene for uterine serpin arose early in mammalian evolution<sup>111</sup> and it may be that the gene has been retained only in species with epitheliochorial placentation.

Local signals controlling macrophage accumulation and activation status of  $\gamma\delta$  T cells have not been identified. The bovine placenta expresses non-classical MHC antigens,<sup>112</sup> and it may be that, as has been postulated for species with invasive placenta,<sup>113</sup> these molecules act to regulate macrophages and dendritic cells in the uterus to direct immune responses to favor conceptus survival.

## Conclusions

Progesterone is critical for the establishment and the maintenance of pregnancy, both by its endocrine and immunological effects, as shown by a progesterone-dependent immunomodulation in maternal tolerance of the fetus, e.g. via PIBF and uterine homing of NK cells and up-regulation of HLA-G gene expression in mice and/or humans. Adequate levels of progesterone may be inhibited upon high stress perception, followed by reduced serum levels of PIBF and diminished expression of progesterone receptors at the feto-maternal interface. These effects are corrected by progesterone supplementation.

Some divergence in the pattern of endocrine-immunological cross talk is present in Bovidae, such as the lack of placental progesterone synthesis or the presence of uterine serpin, a progesterone-induced endometrial protein, which may mediate the immunosuppressive effects of progesterone in sheep. By reason of their evolutionary conservation, these may be essential features of viviparity in eutherian mammals.

## Acknowledgements

P Arck, B Mulac Jericevic, M-P Piccinni and J Szekeres-Bartho are members of the European Network of Excellence on Embryo Implantation Control, supported by EU Contract No. 512040.

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