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Transforming growth factor β and the endometrium

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During the oestrous or menstrual cycle and throughout much of pregnancy, the uterine endometrium undergoes rapid, as well as progressive, morphological and functional modification. During the preimplantation stage of pregnancy, the endometrium provides an environment that sustains embryonic development, and then participates in the nidation process. Later, the endometrium contributes the maternal component of the fetomaternal placenta. For a successful pregnancy, the placenta must orchestrate and regulate opposing forces. Trophoblast invasion must be limited to protect the uterus from destruction, while the allogenic fetus must be guarded from maternal immunological attack. Because of their powerful effects on the cellular and molecular processes associated with cellular proliferation and differentiation, angiogenesis and immunomodulation, the transforming growth factor β (TGF-β) polypeptides have been identified as potential modulators of many endometrial functions. Here, we examine the literature concerning cell-specific and temporal patterns of TGF-β expression in the uterine endometrium during the oestrous cycle and pregnancy and evaluate the influence of ovarian steroids on TGF-β expression in a range of species. Studies of the function of TGF-β in the endometrium and at the fetomaternal interface are reviewed and discussed.

The transforming growth factor βs (TGF-β) belong a family of structurally related, dimeric, disulfide-linked polypeptides that includes five TGF-β isoforms, activins, inhibins, Müllerian inhibiting substance, bone morphogenic proteins, and products of the *Xenopus* Vg1 and *Drosophila* decapentaplegic (dpp) genes (reviewed in Rizzino, 1988; Massagué, 1990; Roberts and Sporn, 1990). In mammals, three isoforms of TGF-β (TGF-β1,-β2, and -β3) have been identified. TGF-βs are multifunctional cytokines that influence numerous cellular processes. They can regulate cell proliferation and differentiation positively or negatively depending on cell type and have been implicated in such diverse physiological events as angiogenesis, immune function, steroidogenesis, and tissue remodelling and repair. Since all of these events take place in the uterine endometrium during the oestrous or menstrual cycle and during the establishment of pregnancy, TGF-βs are likely candidates to regulate some of these reproductive functions.

Biological effects of TGF-β are mediated through interactions with cell surface receptors, designated type I, II and III (reviewed in Massagué and Weis-Garcia, 1996). Type I and II receptors are transmembrane proteins containing cytoplasmic serine/threonine kinase domains. TGF-β binds the type II but not type I receptor. The type I receptor recognizes the ligand bound type II receptor with which it forms a heteromeric complex. The type I receptor then undergoes phosphorylation, which is probably catalysed by the type II kinase. Signalling downstream of the type I receptor involves the phosphorylation of one or more cytoplasmic proteins referred to as Smads, complex formation of Smad-2, -3 and -4, and their translocation to the nucleus and association with transcription factors. The type III receptor is a proteoglycan with no cytoplasmic kinase domain and may be involved in presentation of TGF-β to the type II receptor.

TGF-β expression during the oestrous or menstrual cycle: the role of ovarian steroids

The uterine endometrium is a dynamic organ that undergoes remarkable periodic growth, remodelling and breakdown. The cells that compose the endometrium – primarily luminal and glandular epithelium, stroma and endothelium – undergo periods of proliferation, migration, differentiation and death. In the endometrium, these activities are regulated by ovarian steroids while, in other cell systems, many of these processes are influenced by the TGF-β polypeptides (Rizzino, 1988; Massagué, 1990; Roberts and Sporn, 1990).

Expression of TGF-β in endometrium has been characterized in a small number of species during pregnancy and in fewer during oestrous and menstrual cycles. Information is not available on cell-specific and temporal expression of all three mammalian TGF-β isoforms for every species investigated, and there are conflicting reports on both endometrial TGF-β expression and the role of steroids in regulating their expression. An explanation for this may be that the TGF-β isoforms have complex groups of enhancer and silencer regions within their respective promoter regions, but, to our knowledge, none has been reported to have complete steroid response elements. Although this does not preclude their presence further upstream, it indicates that the effects of steroids on TGF-β gene expression may be indirect. In addition, TGF-βs are expressed differentially in various cell types and may be upregulated in one compartment and not another. Furthermore, bioactivity of TGF-βs is regulated at both transcription and at latent protein check points, and transcription does not necessarily indicate protein production (Assoian *et al.*, 1987). Results from the use of *in vitro* cell model systems must be carefully evaluated in light of the demonstration that TGF-β1 is expressed constitutively in cells

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grown on plastic, but downregulated in cells grown on an extracellular matrix (Streuli *et al*., 1993). In addition, TGF-β1 expression is known to be autoinductive and influenced by other growth factors in several cell types, including human endometrial stromal cells (Arici *et al*., 1996). For a clear definition of the role of steroids, additional studies are required that demonstrate consistency of cell-specific expression of TGF-βs in response to both exogenous steroids and the endogenous steroid milieu of the cycle and pregnancy.

In women, expression of TGF-β1, -β2 and -β3 and the type II receptor occurs in all cell types of the endometrium (Chegini *et al.,* 1994a). Within the functionalis region, expression was greatest in luminal and glandular epithelial cells during the late proliferative (days 9–14) to early-to-mid secretory phase (days 16–25) and diminished in the late secretory phase (days 26–28). These results indicate that, after oestrogen priming, TGF-β expression within specific regions of the endometrium is upregulated during times of increasing plasma progesterone concentrations and downregulated during progesterone withdrawal. Mashburn *et al*. (1994) reported that TGF-β1 expression in the stroma was greatest during the secretory phase of the menstrual interval and suggested that it may regulate epithelial cell proliferation and differentiation. Within the stroma, TGF-β2 expression increases during the secretory phase (Gold *et al*., 1994), and addition of progesterone to the culture medium of endometrial explants enhances production of mRNA encoding TGF-β2 (Bruner *et al*., 1995). Arici *et al.* (1996) provided evidence that stromal TGF-β3 expression may be negatively influenced by progesterone and upregulated by oestrogen. Concentrations of mRNA encoding TGF-β3 were observed to be greatest in proliferative phase endometrium compared with those in secretory phase endometrium, decidual tissue or endometrium prepared from women ingesting progestin.

In rodents, oestrogen administration results in at least a transient increase in the expression of TGF-β isoforms and a decrease in the TGF-β type II receptor. Das *et al*. (1992) observed a rapid, temporary increase in TGF-β2, but not in expression of mRNA encoding TGF-β3, in the mouse uterus in response to oestrogen administration. Takahashi *et al.* (1994) reported that diethylstilboestrol (DES) administration to prepubertal mice resulted in increased uterine expression of TGF-β1, -β2 and -β3 mRNA and protein and a decrease in TGF-β binding. Perhaps significant was the observation that increases in steady state concentrations of mRNA occurred within 30 min for TGF-β3 and 3 h for TGF-β1 and -β2. Concentrations of all transcripts declined to baseline values within 6 h, although protein expression was more prolonged and localized primarily to epithelium. Wada *et al*. (1996) observed a decline in TGF-β type II receptors after administration of oestrogen, as well as progesterone and testosterone. In rats, all three TGF-β isoforms were immunolocalized in the endometrium and staining intensities were stronger at dioestrus II and pro-oestrus than at oestrus or dioestrus I (Chegini *et al*., 1994b). Oestrogen administration resulted in increased TGF-β2 in uterine fluids and increased expression of mRNA encoding TGF-β2 in the uterus (Schneider *et al*., 1996).

In ewes, endometrial expression of mRNAs encoding TGF-β1, -β2 and -β3 was greatest during pro-oestrus, coincident with low plasma progesterone concentrations and increasing oestrogen concentrations (Doré *et al*., 1996a,b). TGF-β1 and -β2 immunostaining was prominent in the luminal and glandular epithelium and diffuse in stroma. TGF-β3 immunostaining was intense in the subepithelial stroma of caruncles and diffuse in epithelial cells on the day preceeding oestrus (day 16) but it was barely detectable at other stages of the cycle or in early pregnancy. Oestrogen treatment of ovariectomized ewes resulted in increased expression of mRNAs encoding all three TGF-β isoforms. Progesterone treatment diminished the effects of oestrogen on the expression of TGF-β1 and -β2 but not TGF-β3.

It is apparent that there are differences among species in the regulation of endometrial TGF-β expression. The high expression at late dioestrus and pro-oestrus reported in rats and ewes may indicate a role for these growth factors in the restructuring of the endometrium that occurs during the transition from one cycle to the next. In contrast, the increased production of the TGF-βs in the late proliferative and secretory phase of the menstrual cycle indicates a role in restraining cell proliferation, promoting differentiation (Mashburn *et al*., 1994; Tang *et al*., 1994) and inhibiting extracellular matrix degradation (Bruner *et al*., 1995) (Fig. 1). The differences between endometrial TGF-β expression in women and nonprimate species are difficult to reconcile. Regulation of TGF-β expression is complex and the role of steroids is not well understood. Events unique to the primate reproductive cycle are a prolonged proliferative phase with extensive tissue growth, and menstruation characterized by extensive breakdown. Differences in endometrial physiology among species may require different mechanisms of regulation.

The TGF-βs are recognized to regulate some cellular processes through their stimulatory action on extracellular matrix (ECM) formation, cellular matrix binding receptors and increased synthesis of protease inhibitors and repression of proteases that degrade ECM (Rizzino, 1988). Matrix metalloproteinases (MMP) have been identified as an important class of protease involved in endometrial remodelling (Osteen *et al*., 1994; Salamonsen and Woolley, 1996 and references therein). In women, endometrial MMP expression appears to be negatively regulated by progesterone. Expression of MMPs is suppressed during the secretory phase of the cycle and increased at menstruation, after progesterone withdrawal. In addition, progesterone inhibits MMP production by endometrial stromal cells *in vitro*. Recent studies indicate that TGF-β plays an important role in regulating MMP production in the endometrium during the menstrual cycle. Osteen and colleagues (Bruner *et al*., 1995) examined stromal–epithelial paracrine regulation of matrilysin, an epithelial-specific MMP of the stromelysin family: their results suggest that TGF-β2 is the progesterone-induced, stromal-derived paracrine factor that suppresses epithelial matrilysin production. The suppressive activity may be mediated through interaction with a TGF-β inhibitory element in the gene promoter region similar to that demonstrated for the stromelysin 1 gene in rat fibroblasts (Kerr *et al*., 1990).

The TGF-βs are likely candidates to mediate, in part, the effects of ovarian steroids on the expression of other classes of protease in the endometrium. For example, the plasminogen activators (PA), serine proteases that have been identified in the endometrium, are regulated by ovarian steriods and are believed to play a role in the menstrual process (Casslen *et al*.,

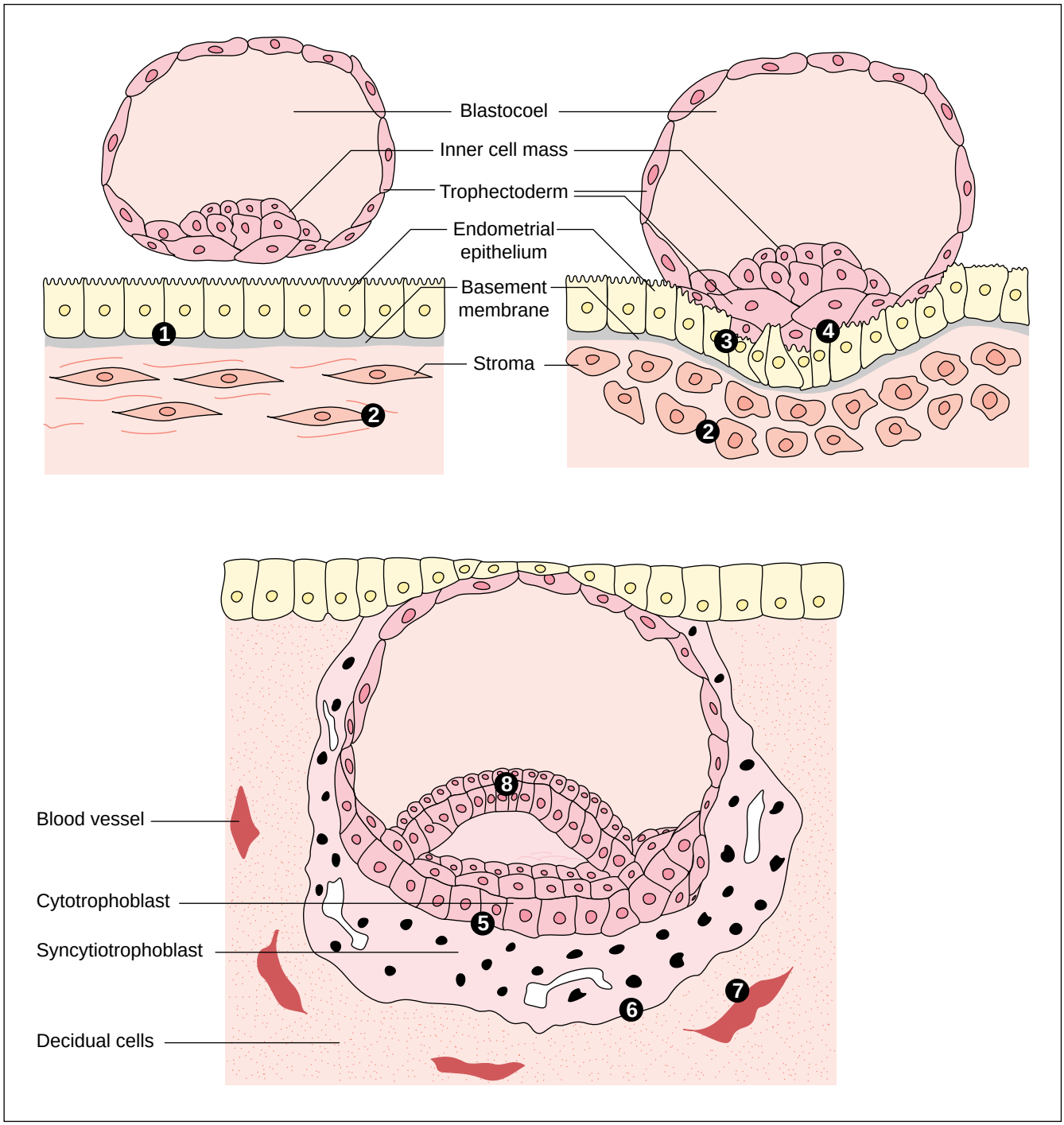

Fig 1. Some proposed sites and actions of transforming growth factor β (TGF-β) in the endometrium and conceptus. (1) Epithelial–stromal interactions; (2) predecidualization and decidualization of stromal cells; (3) trophectoderm cell attachment and (4) migration; (5) trophoblast cell differentiation; (6) regulation of depth of invasion; (7) immunomodulation; and (8) embryonic development.

1986; Schatz and Lockwood, 1993). Production of PAs is suppressed under progesterone dominance and increases with progesterone withdrawal. Progesterone inhibits stromal synthesis of PA and stimulates synthesis of plasminogen activator inhibitor 1 (PAI-1) *in vitro*. TGF-β has been shown to have similar effects on PA and PAI-1 expression in several cell types, including trophoblast (Graham, 1997 and references within) but the role of TGF-β in regulating endometrial PA expression has yet to be determined. Casey and MacDonald (1996) suggested that TGF-β may oppose some actions of progesterone in the endometrium but not others. This view was based on the observation that TGF-β acts in concert with progesterone to

promote stromal decidualization but opposes the stimulatory action of progesterone on expression of stromal enkephalinase.

TGF-β function in endometrium during pregnancy

Proposed regulatory roles of uterine TFG-β during pregnancy include, but are not limited to: decidualization; apoptosis; trophoblast attachment, growth, invasion and differentiation; immunotolerance; cytokine and hormone production; and embryogenesis (some of which are illustrated; Fig. 1). A recurrent observation of studies that characterized the expression of TGF-β in the endometrium during pregnancy was the identification of TGF-β1 or TGF-β2 at the fetal–maternal interface (Kauma *et al*., 1990; Tamada *et al*., 1990; Das *et al*., 1992; Chen *et al*., 1993; Selick *et al*., 1994; Lea *et al*. 1995; Lennard *et al*., 1995). TGF-β in the extracellular matrix of decidual cells (Tamada *et al*., 1990; Graham *et al*., 1992) may provide a reservior of latent TGF-β that is activated by trophoblast-derived proteinases (Graham *et al*., 1992). The identification of TGF-β type I, II and III receptors in the endometrium indicates that these cells are responsive to maternal or embryonic TGF-β (Roelen *et al*., 1994). Slager *et al*. (1993) provided evidence to support the view that TGF-β may be involved in implantation by demonstrating that the injection of neutralizing antibodies, specific for TGF-β2, into the blastocoel of mouse embryos markedly reduced the rate of implantation.

Dungy *et al*. (1991) suggested that TGF-β1 regulates trophoblast invasion and proliferation on the basis of their demonstration that peak expression of mRNA encoding TGF-β1 in human placenta occurred at weeks 17 and 34 of gestation, time points coincident with cessation of trophoblast invasion and cessation of absolute trophoblast growth, respectively. Graham and Lala (1991) demonstrated that exogenous TGF-β1, or conditioned medium from decidual cultures, suppressed trophoblast invasion *in vitro*. Treatment of decidua culture medium with anti-TGF-β antiserum neutralized the anti-invasive activity. Furthermore, the anti-invasive effect of TGF-β may have been mediated by induction of tissue inhibitor of metalloproteinases (TIMP) since neutralizing antibody to TIMP relieved suppressive activity and immunoneutralization of TGF-β resulted in diminished expression of mRNA encoding TIMP.

The invasive activity of human trophoblast cells has been shown to be inhibited by serine proteinase inhibitors as well as inhibitors of MMPs *in vitro* (Yagel, *et al*., 1988). TGF-β1 inhibits human cytotrophoblast production of the serine proteinase urokinanse-type plasminogen activator (uPA) and increases expression of plasminogen activator inhibitor-1 (Graham, 1997). The ability of PAs to generate plasmin is an important function related to cellular invasiveness since plasmin can cleave most proteins of the ECM and activate several MMPs (Vassalli *et al*., 1991). In addition, plasmin may activate latent TGF-β stored in ECM (Lyons *et al*., 1990) which, in turn, may limit invasion by inhibiting MMP and PA production and stimulating their degradation. Regulation of plasmin activity may also promote haemostasis during implantation by inhibition of fibrinolysis and vasoactive protein production (Schatz and Lockwood, 1993).

TGF-β1 stimulates human cytotrophoblast cells to produce oncofetal fibronectin which may be deposited in the ECM at the sites of trophoblast–endometrial attachment (Feinberg *et al*., 1994). These results indicate that fibronectin may serve as a trophoblast–uterine connecting protein that facilitates trophoblast attachment and implantation. It has been suggested that TGF-β regulates trophoblast invasion by stimulating production of adhesive ECM proteins, such as oncofetal fibronectin, and increasing expression of trophoblast cell ECM receptors (Irving and Lala, 1995). This concept is supported by data demonstrating that trophoblast migration is dependent on expression of α_5 and β_1 integrin subunits, the receptor for fibronectin. In addition to its role in regulation of trophoblast attachment, migration and invasion, oncofetal fibronectin localized to the fetal–maternal junction may play an immunomodulatory role in pregnancy maintenance (Feinberg *et al*., 1994). Fibronectin is recognized to influence leukocyte adhesivenesss, chemotaxis and migration. Mice lacking a functional TGF-β1 gene exhibit leukocyte infiltration into multiple organs and a lethal wasting syndrome (Hines *et al*., 1994). Infiltrating leukocytes exhibited increased adhesion to ECM proteins and endothelial cells, indicating alterations in leukocyte adhesive properties. Treatment of the cells with peptides containing cell- and heparin-binding sequences to fibronectin reduced cell adhesion. Treatment of the knockout mice with the same peptides blocked leukocyte infiltration and moderated the wasting syndrome. Results indicate that TGF-induced fibronectin deposition at the fetal–maternal interface may attenuate maternal leukocyte infiltration.

TGF-β at the site of implantation is believed to play a significant role in establishing maternal immunotolerance to the allogenic conceptus. Regulation of immune response by TGF-β varies from chemotaxis to suppression of macrophage and T-cell activity. Leukocytic cells within the mouse decidua express TGF-β2 transcripts (Lea *et al*., 1992) and release a potent immunosuppressive factor that is closely related to TGF-β2 but of a slightly lower mass (Clark *et al*., 1990). In addition, mouse amniotic fluid contains an immunosuppressive factor that has biological properties similar to TGF-β2 (Altman *et al*., 1990). The activities of this factor are neutralized by anti-TGF-β2 specific antibodies but not by anti-TGF-β1-specific antiserum. In mares, high TGF-β1 expression was detected at the interface between the invasive trophoblast cells, which form structures referred to as endometrial cups, and endometrial epithelium (Lennard *et al*., 1995). TGF-β1 expression was associated primarily with leukocytes and endometrial epithelium, and the intensity of the TGF-β signal was proportional to the degree of leukocyte infiltration. Similarly, mRNA encoding TGF-β2 was identified in maternal leukocytes in the region of endometrial cups (Lea *et al*., 1995). Approximately 50% of the TGF-β2 positive cells exhibited biochemical and morphological properties characteristic of eosinophils. It has been suggested for both mice (Clark *et al*., 1990) and mares (Lea *et al*., 1995) that TGF-β production by suppressor cells at the fetomaternal interface serves to protect the conceptus from immunological attack by maternal effector cells.

The TGF-βs are recognized to have a profound influence on proliferation and differentiation of placental cells. TGF-β1 and TGF-β2 inhibit proliferation of first trimester human cytotrophoblast cells *in vitro* (Graham *et al*., 1992). In addition, TGF-β1 stimulates multinuclear cell formation by both first trimester and term trophoblast cells. Since the term cells did not proliferate in culture, multinucleated cell formation was not

necessarily associated with the antiproliferative action of TGF-β. Addition of neutralizing antibody, which recognized both TGF-β1 and TGF-β2, negated the effects of exogenous TGF-β. Antibody alone stimulated proliferation above control concentrations of first term trophoblast cells and inhibited multinuclear cell formation, indicating the presence of endogenous TGF-β activity. However, Morrish *et al*. (1991) demonstrated that TGF-β1 inhibited epidermal growth factor-induced syncytial formation by human cytotrophoblast and dimished production of hCG and placental lactogen.

In contrast to the antiproliferative action of TGF-β on human trophoblast, Munson *et al*. (1996) demonstrated that TGF-β1 and TGF-β2 stimulated proliferation of bovine trophoblast and fetal endometrial epithelial cell lines. Differences in the expression and response to TGF-β among species may be explained, in part, by different placental types. The placentae of ungulates, unlike those of primates and rodents, form neither decidualized endometrium nor invasive or syncytial trophoblast tissue. Instead, placentation involves apposition, adhesion and attachment to both caruncular and intercaruncular regions of the endometrial epithelium. A limited, unique, syncytial formation occurs between migrating trophoblast binucleate cells and endometrial epithelial cells of the caruncles (Wooding, 1984). Munson *et al*. (1996) suggest that the parallel growth of heterologous epithelial cells during epitheliochorial placentation may be regulated, in part, through unique responses to growth factors.

In the endometrium of pregnant ewes, expression of TGF-β1, -β2 and -β3 was lowest on day 16 (Doré *et al*., 1996a), a time of rapid trophoblast growth and the production of trophoblast interferon τ (IFN-τ), which has been shown to diminish endometrial TGF-β expression (Godkin *et al*., 1997). Expression of transcripts for TGF-β1 and -β2 increased markedly at day 23, coincident with the termination of IFN-τ production, and remained high through to day 30. This is the period of initial placentome formation when cotyledons develop on the fetal placenta in areas in contact with maternal caruncles which undergo structural modifications allowing chorionic villi to interdigitate with, but not invade, maternal epithelium and form the crypts of the mature placentome. TGF-βs are likely candidates to play a role in several of the cellular events in this process (Doré *et al*., 1996a).

The critical importance of TGF-β in embryonic development has been demonstrated in mice with a disrupted TGF-β1 gene (Schull *et al*., 1992). Embryos homozygous or heterozygous for the disrupted allelle suffer significant embryonic mortality characterized by defects in extra-embryonic tissues, primarily the yolk sac vasculature and haematopoietic system (Dickson *et al*., 1995). TGF-β1 null fetuses that survive appear as phenotypically normal pups at birth but develop multifocal inflammatory disease resulting in wasting and death (Schull *et al*., 1992). The prenatal survival of TGF-β1 null mice has been attributed, in part, to maternal TGF-β1 which has been shown to cross the placenta and enter the fetus (Letterio *et al*., 1994). A remarkable accomplishment was the survival of a single TGF-β1 null female, rescued from the wasting syndrome by dexamethasone treatment, which gave birth to four live, albeit abnormal, TGF-β1 null pups and three pups heterozygous for the disrupted allelle (Letterio *et al*., 1994). Animal models such as this provide extraordinary opportunities for investigating and identifying the role of TGF-β in reproductive processes, feto–maternal interactions and embryonic development.

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Transforming Growth Factor-Î²1 (TGFÎ²-1), a potent cytokine involved in many diverse cellular responses, is activated from the latent form in the mid-late secretory phase. Previous reports suggest that TGFÎ²-1 might oppose the action of progesterone, although no mechanism has been proposed. We have investigated whether TGFÎ²-1 may have an effect on progesterone receptor (PR) expression or function and inhibit decidualisation.Â These findings highlight the complexity of interactions controlling the hormonal responses of the endometrial stromal cell. This Volume. Transforming growth factor-beta 1 (TGF-Î²1) plays a major role in the etiology of EMs. We aimed to determine whether TGF-Î²1 affects EMs development and progression and its related mechanisms in hypoxic conditions. Methods: Endometrial tissue was obtained from women with or without EMs undergoing surgery from October, 2015 to October, 2016. Endometrial cells were cultured and then exposed to hypoxia and TGF-Î²1 or TGF-Î²1 inhibitors.Â The endometrial tissue samples used for the primary culture were removed and transported immediately to the laboratory. They were chopped to a size of 1 mm3 and washed with PBS three times. Transforming growth factor beta (TGF-Î²) is a multifunctional cytokine belonging to the transforming growth factor superfamily that includes three different mammalian isoforms (TGF-Î² 1 to 3, HGNC symbols TGFB1, TGFB2, TGFB3) and many other signaling proteins. TGFB proteins are produced by all white blood cell lineages. Activated TGF-Î² complexes with other factors to form a serine/threonine kinase complex that binds to TGF-Î² receptors. TGF-Î² receptors are composed of both type 1 and type 2 receptor