Implantation in Ruminants: Changes in Pre-Implantation, Maternal Recognition of Pregnancy, Control of Attachment and Invasion - Review -

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ABSTRACT : As high as 50% of pregnancies are known to fail and the majority of such losses occur during the peri-implantation period. For the establishment of pregnancy in mammalian species, therefore, implantation of the conceptus to the maternal endometrium must be completed successfully. Physiological events associated with implantation differ among mammals. In ruminant ungulates, an elongation of the trophohlast in early conceptus development is required before the attachment of the conceptus to the uterine endometrium. Moreover, implantation sites are restricted to each uterine caruncula where tissue remodeling, feto-maternal cell fusion and placentation take place in a coordinated manner. These unique events occur under strict conditions and are regulated by numerous factors from the uterine endometrium and trophoblast in a spatial manner. Interferon-tau (IFN-τ), a conceptus-derived anti-luteolytic factor, which rescues corpus luteum from its regression in ruminants, is particularly apt to play an important role as a local regulator in coordination with other factors, such as TGF-β, Cox-2 and MMPs at the attachment and placentation sites. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 6 : 845-855*)

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INTRODUCTION

Implantation is characterized by the first intimate relationship between the maternal tissues and developing conceptus. For implantation and the subsequent placental formation, many physiological events need to be completed sequentially. After conception in the oviduct, a fertilized ovum begins its cleavage and forms the morula. During a period from the compaction of the morula to the formation of the blastocyst, cells on the outside of the morula differentiate into the trophectoderm and the remaining cells become inner cell mass (ICM). The formation of the blastocyst occurs after the conceptus moves into the uterus on day 4 (day 0 = first day of estrus) in the ruminant ungulates. The hatching, when the blastocyst comes out of the zona pellucida, occurs on day 8 in the ewe and cow. In rodents and primates, the blastocyst begins its attachment to the uterine epithelium soon after the hatching is completed; but in ruminants, an additional morphological change, elongation of the blastocyst, is required before the attachment of the conceptus to the uterine endometrium. Maternal recognition of pregnancy, the prolongation of corpus luteum function and continuous production of progesterone, must occur while the blastocyst elongation takes place. When trophoblast elongation is completed on day 16 to 18, the attachment between the blastocyst and the uterine epithelium begins, and placentation begins on day 20 and 22 of gestation in the ewe and cow, respectively. In ruminant ungulates, implantation occurs at carunculas, predetermined sites, on the endometrium. Only those that proceed beyond the stages of attachment and placentation go through the gestational period of approximately 149 and 270 days in the ewe and cow, respectively. Up to 50% of pregnancies are known to fail, and 22% and 80% of the embryonic losses from human and farm animal species, respectively, result from spontaneous abortion during the peri-implantation period (Wilcox et al., 1988; Roberts et al., 1990). Although technical aspects of *in vitro* fertilization (IVF), embryo transfer (ET) and cloning have been improved, the pregnancy rate still remains at only 11% (Gregory, 1998). It has been suggested that the low rate of pregnancy results from implantation failure and, therefore, the process and mechanism of normal implantation must be elucidated in order to improve the rate of successful pregnancy. However, studies of implantation are in the beginning stages, particularly in the studies on farm animals. The focus of this review is on the processes of implantation in ruminant ungulates, which can be divided into four sequential events: 1) estrous cycle

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and luteal regression, 2) physiological changes in pre-implantation, 3) maternal recognition of pregnancy, and 4) regulation of attachment and invasion processes.

ESTROUS CYCLE AND LUTEAL REGRESSION IN RUMINANTS

Estrous cycle

The average length of each estrous cycle is 16-17 days in the ewe and 21 days in the cow. The estrous cycle is divided into two phases, follicular and luteal. During the follicular phase, follicular development and selection of dominant follicles for ovulation are regulated accurately via the hypothalamus-pituitary-ovary axis. Although follicle stimulating hormone (FSH) from the anterior pituitary stimulates numerous follicles to develop, most follicles go through apoptosis and become atretic, and only one or two preovulatory follicles are ovulated at each estrus. The preovulatory follicles secrete large amounts of inhibin and estrogen. Inhibin suppresses FSH secretion from the pituitary in a negative feedback manner. This suppression reduces the development of subordinate follicles while estrogen suppresses the gonadotropin releasing hormone (GnRH) pulse generator, resulting in the reduction of luteinizing hormone (LH) secretion from the pituitary. However, progressively elevating estrogen secreted from the "selected" preovulatory follicles accelerates the GnRH pulse generator in a positive feedback manner. Although a preovulatory LH surge may be independent of the GnRH pulse generator (Nishihara et al., 1999), estrogen at the threshold level induces an LH surge that triggers ovulation. After ovulation, the remaining cells from follicular tissues form corpus luteum (CL) that secretes progesterone.

Mechanism of luteal regression

In the ruminant ungulates, luteal regression is caused by pulsatile secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus. Processes of luteal regression are characterized by loss of progesterone production (functional regression) and tissue destruction such as physiological cell death and apoptosis (structural regression) (Hoyer, 1998). Hoyer also indicated that functional regression of CL is stimulated by $PGF_{2\alpha}$ via an activation of its membrane receptor located on large luteal cells of the ovine species. However, whether structural regression is also initiated by $PGF_{2\alpha}$ is still unknown.

In the luteal phase, dominant secretion of progesterone by CL blocks the expression of both estrogen and oxytocin receptors on the uterine endometrium (McCracken et al., 1984). In ruminants, two or three waves of follicular development occur during the estrous cycle (Ginther et al., 1989; Kaneko et al., 1995). However, follicles of the first or second wave do not result in ovulation because dominant secretion of progesterone suppresses the GnRH pulse generator, which does not allow the developing follicles to receive sufficient amounts of gonadotropins. A reduction of progesterone secretion, rather than the next follicular development, is suspected to be an essential event that triggers luteal regression. In most mammals except canine and murine species, CL life-span is approximately 14 days during estrous or menstrual cycles, and this also holds true for pseudopregnant rats and mice. Regardless of species, therefore, there exists a common, as well as unique mechanism which maintains CL life-span and initiates luteal regression after about 2 weeks. 20α-hydroxy-steroid dehydrogenase (20α-HSD) in rat luteal tissues, which catalyzes conversion of progesterone to a biologically inactive steroid, is expressed at the end of the estrous cycle and pseudopregnancy (Matsuda et al., 1990). Inhibition of 20α-HSD activity partially suppresses the reduction in peripheral progesterone levels at the end of pseudopregnancy (Yoshida et al., 1997). Recent studies suggest that along with 20α-HSD, 26-cholesterol hydroxylase (P450c26) which negatively regulates cholesterol utility in the luteal tissue, is also involved in the reduction of progesterone production in the rat luteal tissue at the end of the functional luteal phase (Yoshida et al., 1999). However, further investigations are required to elucidate molecular mechanisms by which the expression of 20α-HSD and P450c26 are regulated at the end of luteal phases.

After the reduction of progesterone secretion, estrogen from growing follicles up-regulates the expression of estrogen and oxytocin receptors located at the uterine endometrium. Oxytocin, secreted from the posterior pituitary and CL in a pulsatile manner, induces an episodic release of $PGF_{2\alpha}$ from the uterine endometrium, which in turn regulates a release of oxytocin. This positive feedback loop accelerates the functional regression of CL (Fuchs, 1987), however, a factor(s) which initiates this positive feedback loop is unclear. Recently, it was reported that platelet-activating factor (PAF) may act as an endogenous pulse generator for $PGF_{2\alpha}$ release (Chami et al., 1999). Chami et al. suggested that the milieu of steroid hormone by which the expression of oxytocin receptor is up-regulated could also induce the expression of PAF from the uterine endometrium in ewes. PAF acts on the uterus in an autocrine manner and induces low-amplitude $PGF_{2\alpha}$ pulses, which in turn regulate oxytocin release from CL. Establishment of a positive feedback loop between $PGF_{2\alpha}$ and oxytocin results in the induction of high-amplitude $PGF_{2\alpha}$ pulses that cause CL regression, leading to the

subsequent follicular phase.

PHYSIOLOGICAL CHANGES IN PRE-IMPLANTATION

In order for the maternal recognition of pregnancy to occur successfully, early conceptus development and uterine preparation for its receptive state must occur simultaneously during the pre-implantation period. The extent of conceptus and uterine development must be synchronized; the conceptus must reach the blastocyst stage and be elongated, and the endometrium must undergo certain developmental changes that allow the conceptus to attach (implantation window).

Early conceptus development and elongation

In mice, the presence of the pre-implantation conceptus is not an absolute requirement for priming maternal environments for the process of implantation. *In vitro* fertilized ova can easily be grown to the blastocyst stage in a simple medium, and embryos up to the blastocyst stage can be successfully transferred to the uterus of the recipient. However, conceptuses derived from the *in vitro* culture system can be distinguished from their *in vivo* counterparts by their morphology, impaired developmental rates or lower pregnancy rates after embryo transfer. Therefore, an additional factor(s) from epithelial cells of the oviduct and/or uterus are required for proper conceptus development beyond the blastocyst stage. The presence of growth factors and cytokines, such as TGF-α and -β, IGF, and PDGF, is found in the uterus of ruminant ungulates (Kane et al., 1997). However, roles of these factors on the conceptus development are not known and those processes may also be regulated by uterine and/or conceptus factors yet unidentified.

After trophectoderm formation, the conceptus in ruminant ungulates begins a rapid and extensive elongation and reaches approximately 20 cm in length. Although the regulation of conceptus elongation is not well understood, the extent of interferon-tau (IFN-τ) expression by the trophectoderm may be associated with the degree of its elongation since amounts of IFN-τ production seem to parallel trophoblast elongation. The factors that enhance IFN-τ expression will be discussed in a later section.

During the period of conceptus elongation, unique intra-epithelial binucleate cells appear within the trophectoderm and increase in frequency in the areas of the trophectoderm apposed to caruncular areas of the uterine epithelium (Wooding, 1984). These binucleate cells have large Golgi bodies and play important roles in subsequent implantation events, but a factor(s) which regulates the formation of binucleate cells is not known at present. Since the number of binucleate cells facing the uterine carunculas is greater than that in the inter-carunculas, formation of binucleate cells is somehow regulated through interactions between the trophectoderm and uterine carunculas.

Uterine preparation and receptivity

An attainment of uterine receptivity, which is controlled by steroid hormones, several growth factors and cytokines, is essential for the conceptus implantation to the uterine endometrium. Experiments that demonstrate these processes have been carried out in primates, human and rodents, but few have been done with farm animals. In general, a milieu of estrogen dominance within the uterus is inadequate for the conceptus implantation, while progesterone antagonizes the action of estrogen and accelerates the development and maturation of the endometrium in preparation for the conceptus attachment and placentation. However, some evidences suggest that along with progesterone, estrogen is also required for the process of conceptus implantation to the receptive uterus. Changes in estrogen and progesterone production induce the expression of several growth factors and cytokines from the endometrium (Tabibzach, 1994; Ace and Okulicz, 1995; Giudice, 1995), which in turn affect the uterine receptivity, but the regulation of these factors in a spatial and temporal manner is unclear at present. At least two events are required for the attainment of uterine receptivity: reduction or loss of an anti-adhesive molecule from the uterine endometrium and an appearance of cell adhesion molecules on the uterine endometrial surface.

Muc-1, one of high molecular weight mucin glycoproteins, is present on the surface of the female reproductive tract and functions as a molecule for the protection of the uterus from inflammation or infection. Previous studies indicated that Muc-1 may function as an anti-adhesive molecule during the pre-implantation period in several species (Pemberton et al., 1992; Carson et al., 1998). In pigs and mice, the expression of Muc-1 mRNA and protein is downregulated during the peri-implantation period. Estrogen stimulates Muc-1 expression while progesterone antagonizes the stimulatory actions of estrogen (Surveyor et al., 1995; Bowen et al., 1996). Treatment of mice with the anti-progestin could maintain the expression of uterine Muc-1 during the peri-implantation period and prevent the implantation (Vinisanum and Martin, 1990). These results suggest that in pigs and mice, the increase in progesterone levels from mature CL correlates with reduced Muc-1 expression during the luteal phase or pregnancy. On the other hand, in rabbits, the expression of Muc-1 during the peri-implantation period is elevated in the uterine epithelium, except at implantation sites where

its expression is reduced, suggesting that some embryonic signals are required for Muc-1 reduction in this species (Hoffman et al., 1998). Although it is unclear whether or not embryonic signals are required for the reduction of Muc-1 in ruminants, loss of Muc-1 may be essential for the establishment of uterine receptivity during the peri-implantation period.

Integrins are a large family of cell surface receptors that mediate the attachment of extracellular matrix (ECM) in almost all cell types. It is known that the expression of integrins on uterine epithelial and trophoblast cells is modulated during the estrous cycle and pregnancy, and the elevation of integrin expression during the peri-implantation period is required for the subsequent attachment and invasion. The number of functional integrin molecules and patterns of these integrin expressions are different among species, suggesting that although several common integrins have been identified, species-specific differences may reflect the major differences in the invasive or non-invasive modes of implantation (Lessey et al., 1994; Burghardt et al., 1997). Integrins have an important role in not only the attachment of the conceptus to the maternal endometrium, but also the transcriptional regulation of several genes. The attachment of ECM to integrins in tumor cells generates cytoplasmic signaling, especially via the mitogen activated protein kinase (MAPK), resulting in activation of a family of target genes crucial to the invasion, matrix metalloproteinases (MMP) (Crowe and Shuler, 1999). However, signaling pathways of MMPs in the conceptus and roles of integrins ECM in the establishment of uterine receptivity are only beginning and numerous questions remain unanswered.

In ruminants, the elongated conceptuses are able to attach and implant to the carunculas located throughout the uterine horns. It seems likely that the attainment of this uterine receptivity is restricted to the caruncular regions. However, this restriction cannot be explained by expression patterns of steroid hormones or several cytokines. In the caprine endometrium, integrin expression is not regulated by ovarian steroids (Guillomot, 1999), while in non-ruminant species, integrin expression is modulated by steroid hormones during the estrous cycle and in early pregnancy. An unique mechanism may be involved in the attainment of uterine receptivity in the ruminant ungulates; for example, binucleate cells of the trophoblast may be conditioned locally by a factor(s) released from uterine carunculas.

MATERNAL RECOGNITION OF PREGNANCY

Although, in some species, progesterone is secreted by extragonadal tissues during the latter half of gestation (Porter et al., 1982), continuous secretion of progesterone from CL is essential for the establishment and maintenance of pregnancy. This is one of the earliest maternal responses that distinguish a normal ovarian cycle from pregnancy, and it is collectively called maternal recognition of pregnancy (Short, 1969). Two patterns of maternal pregnancy recognition or the maintenance of the CL life span exist among species: luteotrophic and antiluteolytic. In primates and horses, trophoblasts secrete chorionic gonadotropins (CG) while in rodents, the act of mating induces a prolactin surge from the posterior pituitary, both produce luteotrophic effects that stimulate CL and maintain production of progesterone (Porter et al., 1982; Hearn et al., 1991; Soares et al., 1991). In ruminant animal species, an antiluteolytic rather than luteotrophic factor sustains the production of progesterone. Such an antiluteolytic factor affects the secretory pattern of $PGF_{2\alpha}$, which is identified as a physiological initiator of luteal regression in many species including ruminant and porcine species. In pigs, an antiluteolytic factor is estrogen produced by trophoblasts (Bazer and Thatcher, 1989). In ruminants, IFN-τ has been considered as a trophectodermal factor implicated in the initiation of maternal recognition of pregnancy (Martal et al., 1979; Godkin et al., 1982; Imakawa et al., 1987).

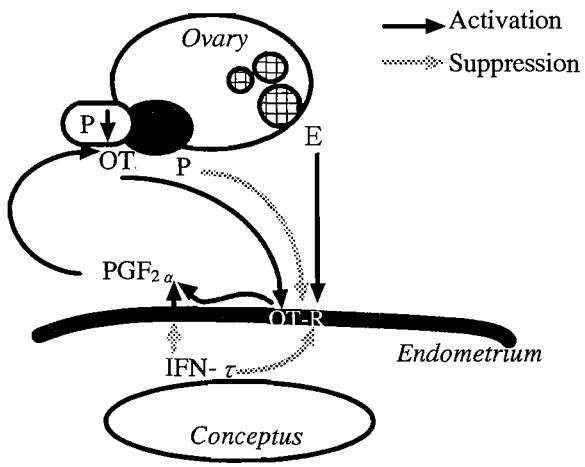

Figure 1. Maintenance of CL function in the ruminant ungulates. When a steroid hormone milieu changes from progesterone (P) to estrogen (E) dominance due to luteal regression, oxytocin receptor (OT-R) expression is enhanced and oxytocin (OT)-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) positive feedback loop is then established. In pregnant animals, IFN-τ produced from the conceptus suppresses the expression of OT-R and, thus, modulates $PGF_{2\alpha}$ production, resulting in the maintenance of progesterone secretion. Solid arrow indicates "activation" and dotted arrow for "suppression" or "inactivation".

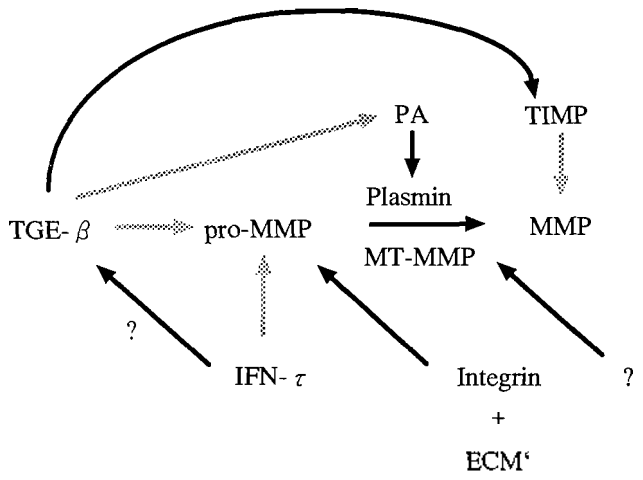

Figure 2. Working hypothesis on the regulation of matrix metalloproteinase (MMP) expression during the peri-implantation period. At the endometrium, integrin signaling by ECM induces expression of pro-MMP, which is converted to an active form MMP by plasmin and(or) membrane type MMP (MT-MMP). Plasminogen is converted to an active form by plasminogen activator (PA), but a regulatory factor(s) for MT-MMP expression is not known. TGF-β and IFN-τ, produced from the conceptus during the peri-implantation period, control an over-expression of MMP at implantation sites, resulting from the suppression of pro-MMP and PA production, and activation of tissue inhibitor of metalloproteinase (TIMP) which blocks MMP activity. However, molecular mechanisms by which TGF-β and IFN-τ are regulated are not well understood at present. Solid arrow indicates "activation" and dotted arrow for "suppression" or "inactivation". "?" suggests a potential role, which has not been proved/demonstrated yet.

Mechanism of pregnancy recognition

In ewes, conceptuses produce large quantities of IFN-τ (up to 100 μg/conceptus/day) on days 13-16 of pregnancy (Godkin et al., 1982; Imakawa et al., 1995). Much evidence from *in vivo* and *in vitro* experiments show that intrauterine injections of recombinant IFN-τ attenuate the secretory pattern of $PGF_{2\alpha}$ from endometrial cells (Vallet et al., 1988; Godkin et al., 1997), and extend inter-estrous intervals in ruminants (Martal et al., 1990; Ott et al., 1993). These observations suggest that large amounts of IFN-τ from conceptuses on days 13-16 of pregnancy inhibit pulsatile release of $PGF_{2\alpha}$ from the uterus, resulting in the prevention of CL regression. However, mechanisms of IFN-τ that inhibit pulsatile release of $PGF_{2\alpha}$ are complicated and not well understood at present.

When the luteal regression begins, estrogen induces the expression of endometrial estrogen and oxytocin receptors. In contrast, when IFN-τ is administered before the expected increase in oxytocin and estrogen receptors, the expression of these endometrial receptors is blocked (Flint et al., 1991; Spencer and Bazer, 1996). It is likely that oxytocin receptor expression is prevented by a preliminary inhibition of the estrogen receptor (Spencer et al., 1995; Spencer and Bazer, 1996). However, whether IFN-τ directly suppresses oxytocin receptor expression has not been demonstrated. Moreover, IFN-τ also suppresses the production of PAF, which affects the pulsatile secretion of $PGF_{2\alpha}$ (Chami et al., 1999). These evidences suggest that IFN-τ prevents the promotion of the positive feedback loop between $PGF_{2\alpha}$ and oxytocin, resulting in the maintenance of luteal function.

Several reports suggest that IFN-τ could act directly on prostaglandin metabolism. Through the production of prostaglandin synthesis inhibitor, IFN-τ is able to inhibit the synthesis of prostaglandins by endometrial cells (Gross et al., 1988; Tamby et al., 1993; Thatcher et al., 1995). In other model systems, however, IFN-τ increases the synthesis of prostaglandins and the expression of cyclooxygenase-2, PGE2 and $PGF_{2\alpha}$ synthase enzymes at the uterine endometrium (Asselin et al., 1997a; Asselin et al., 1997b; Charpigny et al., 1997). It is likely that IFN-τ changes the pattern of prostaglandin synthesis by increasing luteotrophic PGE2 production rather than $PGF_{2\alpha}$. This would then be a primary reason for IFN-τ eliciting the maternal recognition of pregnancy because PGE2 overcomes the luteolytic effect of $PGF_{2\alpha}$ for luteal maintenance (Henderson et al., 1977). A recent study suggests that the balance of steroid hormones, estrogen and progesterone, modulate the prostaglandin synthesis (Xiao et al., 1998). To define the direct effect of IFN-τ on prostaglandin synthesis, further experiments are required to determine the cooperative effect between IFN-τ and steroid hormones on prostaglandin synthesis, and elucidate the mechanism by which endometrial PGE2 and $PGF_{2\alpha}$ syntheses are regulated differently by cyclooxygenase.

Regulation of IFN-τ expression

Based on the origin and serological characteristics, an IFN family is divided; type I IFN including IFN-α, -β and -ω, and type II IFN including IFN-γ. Because of structural resemblance, IFN-τ is considered to be a member of the type I IFN family (Imakawa et al., 1987; Stewert et al., 1987; Roberts et al., 1992). Although type I IFN consists of many ligands, only one receptor has been realized. Both IFN-α and IFN-τ are known to bind to the same receptor, type I IFN receptor, which consists of at least two subunits at the uterine endometrium (Han et al., 1997). In addition, the presence of a third subunit

for the receptor complex has been suspected (Cleary et al., 1994). It has been proposed that differential effects exhibited by various type I IFN are due to a combination of two or three type I IFN receptor subunits.

Regulatory mechanisms by which IFN-τ production by the trophoblast is controlled during early pregnancy are not completely understood. The expression of other type I IFNs such as IFN-α and -β is induced by virus or double-stranded RNA, but IFN-τ expression is not induced by any of those factors. Ovine IFN-τ mRNA begins to increase from day 11, reaches a peak on day 14-16, begins to decrease thereafter, and no expression is detected on day 23 (Hansen et al., 1988; Farin et al., 1989). The induction of IFN-τ expression appears to be genetically pre-determined without influences of maternal uterine environments because IFN-τ mRNA and protein are found right after hatching from the blastocysts cultured following *in vitro* oocyte maturation and fertilization in cows (Hernandez-Ledezma et al., 1992; Stojkovic et al., 1995). Levels of IFN-τ production by the *in vitro* derived blastocysts are considerably lower than those found in the conceptuses *in vivo*, however, IFN-τ expression is enhanced by the exposure to maternal uterine environments (Hernandez-Ledezma et al., 1992). Furthermore, a few investigations are available for the regulation of IFN-τ gene expression (Leaman et al., 1994; Yamaguchi et al., 1999a; Yamaguchi et al., 1999b), but a lack of ruminant trophoblast cell lines has delayed progress in these investigations.

Although cytokines, GM-CSF and IL-3, have been determined as endometrial factors that enhance IFN-τ expression (Imakawa et al., 1993; Imakawa et al., 1995), the mechanisms by which endometrial GM-CSF and IL-3 are regulated are unknown. The expression of GM-CSF mRNA from the endometrial cells is induced by estrogen but not progesterone in mice and ewes (Robertson et al., 1996; McGuire et al., in preparation), however, both estrogen and progesterone are required for the production of these proteins in the ewe (McGuire et al., in preparation). It is suspected that the transition of steroid milieu from progesterone to estrogen, resulting from the initiation of luteal regression, may determine the initial expression of endometrial GM-CSF. However, the level of GM-CSF production in pregnant ewes is higher than that in cyclic ewes, indicating that a pregnancy factor(s), such as IFN-τ or physical existence of the conceptus may be required. Recently, it was reported that the expression of Cox-2 is seen before the enhancement of IFN-τ expression in pregnant ewes (Charpigny et al., 1997). Since various growth factors are known to be induced by Cox-2 expression (O'Bannion et al., 1992; Hamasaki et al., 1993), involvement of Cox-2 in the production of GM-CSF or other IFN-τ inducible factors is suggested. As mentioned above, while the conceptus elongates rapidly and extensively, large amounts of IFN-τ are produced. Conversely, the extensive elongation of the trophectoderm may result in high levels of IFN-τ production. It is unknown whether the enhancement of IFN-τ expression results from GM-CSF and IL-3 or from the conceptus elongation stimulated by GM-CSF and IL-3.

The expression of IFN-τ begins to decrease from day 16 and disappears on day 23. It has been reported that the termination of IFN-τ expression results from the attachment of the trophectoderm to the maternal carunculas (Guillomot et al., 1990), however, a biochemical factor(s) which regulates the cessation of IFN-τ has not been determined. TGF-β may directly suppress IFN-τ expression: a) trophoblast cells express TGF-β receptor, b) the production of TGF-β from the conceptus increases from day 16 when the expression of IFN-τ begins to decrease (Imakawa et al., 1998), and c) IFN-τ gene contains a nucleotide sequence identical to TGF-β inhibitory element (TIE) in its promoter/enhancer region (Nephew et al., 1993). On the other hand, since TGF-β has a role in controlling cell proliferation, TGF-β may terminate the trophoblast elongation, which in turn result in the reduction of IFN-τ expression. Recent study suggests that a type I IFN receptor appears in day 16-18 conceptuses, suggesting that in addition to a paracrine factor to the endometrium, IFN-τ may have a direct effect on the conceptus development in an autocrine manner (Imakawa et al., in preparation). It is also possible that IFN-τ may regulate the expression of some genes from the conceptus, for example, TGF-β may be regulated by IFN-τ, but details are unknown at present.

As stated above, IFN-τ expression is restricted to the period of peri-implantation in ruminants. In addition to a well-documented anti-luteolytic effect of IFN-τ *in vivo*, anti-proliferative and anti-viral effects of IFN-τ are found *in vitro* and the importance of those effects has not been elucidated. Furthermore, the relationship between the production of IFN-τ and the formation of binucleate cells is still unknown.

CONTROL OF ATTACHMENT AND INVASION

After the elongation of the conceptus, the establishment of uterine receptivity and the rescue of luteal regression are completed, the attachment of the conceptus to the maternal endometrium is initiated on day 16 of gestation in the ewe. Although of the type of placentation in ruminants is non-invasive (synepitheliochorial placenta), endometrial tissue remodeling is a prerequisite for the formation of placental cotyledons

and the development of angiogenesis in the uterine caruncula. There is fusion of fetal chorionic binucleate cells with those of the uterine epithelium forming the feto-maternal trinucleate cells, partial invasion of the trophoblast cells to maternal endometrium and reorganization of the vessels (Wooding, 1984). These tissue remodelings are required for degradation and penetration of ECM components, particularly basement membranes that separate the epithelial cells from the underlying or surrounding connective tissue stroma and prevent the cell invasion. When the attachment occurs, cell contacts are established between the tips of the uterine microvilli and the smooth trophoblastic cell membranes. Integrins, which appear on the cell surface of the trophoblast and uterine epithelium, bond ECMs, followed by the production of tissue remodeling factors, matrix metalloproteinases (MMPs). These MMPs degrade ECMs and help the conceptus to penetrate the uterine stroma.

MMPs are a group of zinc-requiring enzymes that play a major role in tissue remodeling in both normal and pathological processes. Along with tissue inhibitors of MMPs (TIMPs) that inhibit effects of MMPs by the formation of 1:1 complexes, these enzymes are synthesized and secreted locally by the resident cells. Many MMP members are known to be involved in tumor cell invasion (Birkedal-Hansen, 1995; Jones and Walker, 1997). Those factors that have been important during implantation processes are MMP-1 (interstitial collagenase), MMP-2 (gelatinase A; 72kDa gelatinase), MMP-3 (stromelysin-1) and MMP-9 (gelatinase B; 92kDa gelatinase). MMP-2 and MMP-9 are particularly important because these enzymes can degrade basement membranes of epithelial cell layers. Most MMPs (except MMP-3) are secreted as inactive precursors (proMMPs), which are converted to the active form by the proteolytic removal of a proMMP-domain. The serine-protenases, such as trypsin, plasmin, and kallikrein, are candidates for the activation of most proMMPs excluding proMMP-2 (Graham and Lala, 1992). Recently, membrane-type matrix metalloproteinase-1 (MT-MMP-1), which can activate proMMP-2 in tumor cells, was discovered (Sato et al., 1994). Human MMP-3 and MT-MMPs are produced as active forms. Since MMP-3 and MT-MMPs have furin, the Golgi-associated proteinase recognition motif which regulates intracellular activation, these enzymes are activated within the constitutive secretory pathway (Pei and Weiss, 1995). It is suspected that binucleate cells with large Golgi bodies may express MMP-3 and MT-MMPs during the implantation period in ruminants. Three related genes encoding MT-MMP-2, MT-MMP-3 and MT-MMP-4 have been described (Takino et al., 1995; Will and Hinzmann, 1995; Puente et al., 1996), but their roles are unknown at present. It is suspected that during tissue remodeling at implantation sites, as well as cancer invasion, the activation of proMMPs is important and is controlled by the serine-proteinase and MT-MMP (at least MT-MMP-1), resulting in the prevention of over-expression and sequential autodigestion of MMPs, and thus over-invasion of the trophoblast. However, it is still unclear whether the expression of these activators is restricted to the binucleate cells within implantation sites (uterine caruncle).

Control of MMPs production at attachment and invasion sites

In order for the trophoblast to proceed to the process beyond the attachment stage, the local production and activation of MMPs are required and are probably regulated spatially and temporally. However, little is known about the regulation of MMPs in all species, especially in ruminants. Both trophoblast and endometrial cells in ruminants are able to produce proMMPs. However, MMP-2 (gelatinase) activity of uterine flushing media from day 16 pregnant ewes is higher than that of day 16 non-pregnant ones, suggesting that the trophoblast promotes the activation of proMMP-2 (Salamonsen et al., 1986). These observations suggest that the trophoblast (probably binucleate cells) may express MT-MMP-1 abundantly on the surface which accelerates the production of active MMP-2 during the implantation period. Furthermore, the trophoblast is able to produce the plasminogen activator (PA), which modulates the production of plasmin and accelerates the production of active forms of other MMPs. Factors that activate MT-MMP-1 and PA expression are not known at present, however, the attachment between fetal-integrins on the trophoblast (binucleate cells) to endometrial ECMs, which causes generation of cytoplasmic signalling and induction of some other genes, may be a potential factor for such activation (Zhang et al., 1996). In the maternal endometrium, the binding of maternal-integrins on the uterine epithelial cells to ECMs also induces the expression of some other genes. The production of proMMPs may be enhanced, however, the stromal cells rather than the epithelial cells are major sources of proMMPs (Salamonsen et al., 1993). It is possible that proMMP expression at the stromal cells is induced by a factor(s) produced at the epithelial cells due to the integrin signaling.

During the implantation period, temporal expression of IFN-τ, TGF-β and Cox-2 genes by trophoblast cells has so far been characterized (Imakawa et al., 1998; Chami et al., 1999). IFN-τ is able to suppress the production of MMPs from the endometrial cells in culture, and this suppression is not mediated by the attenuation of endometrial prostaglandins (Salamonsen et al., 1994). However, it is unknown whether IFN-τ

can directly act on MMP production at the stromal cells or whether other factors may affect endometrial MMP expression, because IFN-τ receptor is present on the epithelial cells but not on the stromal cells. The production of TGF-β from the ovine trophoblast begins to increase when IFN-τ production reaches its highest level on day 16 of gestation (Imakawa et al., 1998). TGF-β, produced from decidual tissues in the human, has been reported to inhibit the activity of MMPs by suppressing PA production and by increasing TIMP-1 production (Graham, 1997). TGF-β from the trophoblast may control the production and activation of MMPs. Cox-2 expression by trophoblast cells and the associated prostaglandin production at the endometrium may be involved in the control of trophoblast invasion. Factors induced by Cox have been shown to suppress the production of MMPs and enhance the expression of TIMPs (Salvatori et al., 1992; Mertz et al., 1994). In contrast, prostaglandins are known to promote the synthesis of MMPs in other model systems (Mauviel et al., 1994), suggesting that the balance between types of prostaglandins is the most important determinant in the regulation of MMPs production. At present, few data are available explaining the possible mechanism by which these factors are regulated spatially and temporally during the implantation period. However, these factors undoubtedly play an important role even in non-invasive trophoblast cells that remain in close apposition with the intact uterine epithelium in the ruminant ungulates.

In conclusion, the conceptus and maternal uterus could develop independently, however, biochemical and physical interactions between two cell types are a prerequisite for the successful attachment and placentation. In ruminants, a trophoblast factor, IFN-τ, plays a role in the maternal recognition of pregnancy. Either directly or indirectly, IFN-τ may also function as a local regulator at implantation sites: the suppression of cell proliferation and over-invasion of the conceptus, and control of immune response potentially elicited by the maternal immune system. In order to understand the processes of successful pregnancy in the ruminant ungulates, further experiments are required to elucidate functions of IFN-τ and other genes as local regulators and to determine mechanisms of their expression during the attachment and placentation periods.

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