PROGESTERONE AND INTERFERON TAU REGULATED GENES IN THE ENDOMETRIUM OF THE OVINE UTERUS AND EXPRESSION OF INTERFERON STIMULATED GENES IN THE CORPUS LUTEUM DURING EARLY PREGNANCY IN SHEEP

A Thesis

by

HYO WON AHN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2008

Major Subject: Physiology of Reproduction

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Approved by:

Co-Chairs of Committee:	Fuller W. Bazer
	Thomas E. Spencer
Committee Members:	Robert C. Burghardt
	Gregory A. Johnson
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ABSTRACT

Progesterone and Interferon Tau Regulated Genes in the Endometrium of the Ovine Uterus and Expression of Interferon Stimulated Genes in the Corpus Luteum During Early Pregnancy in Sheep. (May 2008)

Hyo Won Ahn, B.S., Konkuk University

Co-Chairs of Advisory Committee: Dr. Fuller W. Bazer
Dr. Thomas E. Spencer

During early pregnancy in ruminants, progesterone (P4) from the corpus luteum (CL) and interferon tau (IFNT) from the conceptus act on the endometrium to regulate genes including interferon stimulated genes (ISGs) that are hypothesized to be important for uterine receptivity and conceptus growth. Previous custom ovine cDNA array analyses identified candidate genes that were regulated by pregnancy, P4 and/or IFNT in the ovine uterus.

The first study validated pregnancy, P4 and/or IFNT regulated genes identified by previous custom ovine cDNA microarray analyses. *ACTA2, COL3A1, POSTN, SPARC, S100A2, STAT5A* and *TAGLN* were examined. *POSTN* was upregulated by P4 and *S100A2* was downregulated by IFNT. Moreover, functional studies showed that *POSTN* stimulated attachment of ovine trophectoderm cells. However, neither *COL3A1, SPARC, ACTA2* nor *TAGLN* was regulated by pregnancy, P4 or IFNT in the ovine endometrium. Collectively, these results confirmed that *POSTN* and *S100A2* are P4 and IFNT regulated, respectively, and likely involved in uterine receptivity to conceptus implantation during early pregnancy.

The second study determined expression of ISGs in the CL of pregnant ewes. *MX1, MX2, ISG15, OAS1,* and *RSAD2* mRNAs were increased on Day 14 of pregnancy and maintained to Day 18, indicating that IFNT induced expression of ISGs in the CL. These results confirmed that locally produced

IFNT has paracrine effects and also endocrine effects on reproductive organs other than the uterine endometrium and maternal immune system.

DEDICATION

To my parents
YOUNG-CHE AHN & GIL-BOK CHOI

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I would like to thank my mentors, Drs. Fuller Bazer and Thomas Spencer, for their patience, guidance and extraordinary support throughout the course of this research. I also would like to thank my committee members, Drs. Robert Burghardt and Gregory Johnson, for their tremendous influence throughout my research program.

I also would like to thank all past and present members of the Bazer/Spencer laboratory. In particular, I would like to express appreciation for the tremendous assistance from Dr. Kanako Hayashi and Dr. JoAnn Fleming. Thanks also go to my colleagues in the Physiology of Reproduction program, the Genetics Program, and the College of Veterinary Medicine and Biomedical Research for their advice and encouragement.

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CHAPTER I
INTRODUCTION

Maternal recognition of pregnancy and implantation of the conceptus in early pregnancy are crucial for successful establishment of pregnancy. The ruminant conceptus secretes the pregnancy recognition signal, interferon tau (IFNT), which prevents luteolysis of the corpus luteum (CL). The CL produces progesterone (P4) which is required for initiation and maintenance of pregnancy by making the uterus a permissive environment for conceptus development, implantation, placentation and successful fetal and placental development to term (Spencer & Bazer, 2004). Progesterone receptors (PGR) are expressed in the endometrial epithelia and stroma during the early to midluteal phase, allowing direct regulation of a number of genes by P4 via activation of the PGR (Spencer *et al.*, 2004a). IFNT acts in a paracrine manner to block development of the uterine luteolytic mechanism to suppress release of uterine prostaglandin F2α (PGF) pulses which lead to luteolysis (Flint *et al.*, 1992). P4 and IFNT play a central role in establishment of pregnancy in ruminants, and changes of gene expression in this period of early pregnancy in the uterine endometrium are regulated by the action of P4 from the CL and then IFNT from the conceptus. To elucidate genes involved in conceptus-endometrial interaction in ruminants, gene expression profiling has been used to identify candidate genes regulated by P4 and/or IFNT (Gray *et al.*, 2006). During diestrus, progesterone levels increase and act via PGR to block expression of *estrogen receptor alpha* (*ESR1*) and then *oxytocin receptor* (*OXTR*) in endometrial luminal epithelium (LE)/superficial glandular epithelium (sGE) after Days 11 to 12 (McCracken *et al.*, 1984). Continuous exposure of the uterus to progesterone for 8 to 10 days down- regulates expression of *PGR* in endometrial

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This thesis follows the style of *Reproduction.*

epithelia after Days 11 to 12 (Spencer *et al.*, 1995), allowing increases in epithelia after Days 11 to 12 (Spencer *et al.*, 1995), allowing increases in expression of *ESR1* and *OXTR* in LE/sGE. However, during early pregnancy, IFNT acts in a paracrine manner to bind to interferon receptors on the endometrial LE and inhibit transcription of the *ESR1* gene through a signaling pathway involving interferon regulatory factor 2 (IRF2) (Choi *et al.*, 2001b, Fleming *et al.*, 2001). These actions of IFNT on the *ESR1* gene prevent OXTR formation, thereby preventing oxytocin-induced release of luteolytic pulses of PGF which allows for maintenance of the CL and its production of progesterone required for establishment and maintenance of pregnancy.

Also, IFNT stimulates transcription of IFN-stimulated genes (ISGs) that appear to play roles in endometrial differentiation and conceptus implantation during early pregnancy (Spencer & Bazer, 2004). IFNT utilizes the classical Janus kinase-signal transducer and activator of transcription (JAK-STAT) cell signaling pathway used by all Type I IFNs. All cell types in the endometrium have IFNA receptor 1 (IFNAR1) and IFNA receptor 2 (IFNAR2) but ISGs are not expressed by all endometrial cell types because the JAK-STAT signaling pathway is inhibited by IRF2, a potent repressor of transcription, that is expressed in the LE/sGE (Choi *et al.*, 2001b). However, there are some genes induced or stimulated by IFNT in LE/sGE such as *WNT7A*, *LGALS15*, *CTSL*, and *CST3* of the ovine uterus (Gray *et al.*, 2006, Kim *et al.*, 2003b, Song *et al.*, 2005, 2006). These novel ISGs must be transactivated via an alternative cell signaling pathway(s), and they are proposed to play important roles during early pregnancy in ruminants.

Expression of the *IFNT* gene is unique compared to other interferons. *IFNT* is not induced by virus (Guesdon *et al.*, 1996). *IFNT* expression is limited to the period of early pregnancy and to the conceptus trophectoderm while other IFNs are expressed by many types of cells in most tissues (Roberts *et al.*, 1992). IFNT is synthesized and secreted between Days 10 and 20 with maximal production between Days 14 to16 in sheep (Bazer *et al.*, 1997, Roberts *et al.*, 1999). It has

been thought that IFNT has only paracrine actions in the endometrium. However, when recombinant ovine IFNT (roIFNT) was administered to pregnant ewes by either intrauterine (i.u.) or subcutaneous (s.c.) routes, both treatments were equally effective in induction of ISGs (*MX1* and *ISG15*) in the CL (Spencer *et al.*, 1999b). Moreover, there was an increase of ISG expression in peripheral blood leukocytes (PBLs) and peripheral blood mononuclear cells (PBMCs) in pregnant ewes and cows (Gifford *et al.*, 2007, Yankey *et al.*, 2001). Thus, IFNT may exert paracrine effects on the endometrium as well as endocrine effects on the CL.

CHAPTER II
LITERATURE REVIEW

Early pregnancy and conceptus development

Conceptus development is initiated with fertilization, which is fusion of gametes that occurs in the oviduct. Fusion of male and female pronuclei results in a zygote that is surrounded by the zona pellucida, a glycoprotein membrane surrounding the vitelline membrane. Then, the zygote undergoes cleavage or cell division without an increase in cytoplasm. After the first cleavage, cells called blastomeres undergo subsequent cleavages to 4, 8, 16 cells and so on to form a small ball of blastomeres called the morula. After the morula stage, cells of the embryo undergo: (1) compaction which is combination of flattening and positioning of surface microvilli asymmetrically; (2) formation of tight junctions and polarization; (3) cavitation, or the formation of a fluid-filled cavity called the blastocoel; and (4) differentiation of cells into the inner cell mass or embryonic disc and trophectoderm which contribute to formation of the blastocyst (Hafez, 1993, Senger, 2005).

Between cells in the inner and outer portions of morulae, gap junctions and tight junctions, respectively, are formed. Gap junctions allow intercellular communication which may enable the inner cells to remain in a defined cluster. Tight junctions alter permeability and polarization of the outer cells to allow formation of the blastocoel. Different junctional complexes between cells are believed to effect differentiation of cells into two populations of cells in the blastocyst stage, inner cell mass and trophectoderm. The inner cell mass gives rise to the embryo proper and primary germ layers of the embryo (endoderm, mesoderm, ectoderm) in later stages (Hafez, 1993). The trophectoderm takes up nutrients for conceptus development (Senger, 2005).

The fertilized ovum moves toward the uterus and embryos enter the uterus at various stages of development in different species, but most enter at the morula stage and some enter the uterus as early as the 4-cell stage. In the uterus, embryos develop to the blastocyst stage. Enzymatic dissolution of the zona pellucida and expansion of the blastocoel enables shedding of the zona pellucida in the uterus (Hafez, 1993). Zona hatching is followed by a rapid phase of blastocyst development and growth. In domestic ruminants and pigs, the blastocyst elongates during the latter stages of implantation, but this unique developmental event does not occur in laboratory rodents, horses, primates or humans (Allen & Stewart, 2001, Guillomot *et al.*, 1993, Spencer *et al.*, 2004b). The period of conceptus elongation coincides with secretion of maternal recognition of pregnancy signals e.g., IFNT from conceptuses in ruminants (Ashworth & Bazer, 1989). At this time, the trophectoderm acquires the ability to attach or adhere to uterine luminal epithelium (LE), which appears to be the primary site of hormonally regulated uterine receptivity in sheep and other species with central type implantation.

Before or during elongation of the blastocyst, the endoderm is derived from the inner cell mass. Mesoderm is also derived from the inner cell mass and migrates between the endoderm and trophectoderm. After cavitation, the inner layer of endoderm of foregut and mesoderm become yolk sac, endoderm of hindgut and mesoderm becomes the allantois and trophectoderm and mesoderm becomes chorion. Extraembryonic membranes are formed before implantation in ruminants and pigs and after implantation in human and rodents (Carson *et al.*, 2000, Guillomot *et al.*, 1993, Renfree, 1982, Spencer *et al.*, 2004b).

As illustrated in Fig. 2.1, the morula enters the uterus on Day 4 of pregnancy in sheep. The blastocyst is formed on Day 6, and then the zona pellucida is shed between Days 8 and 9. Between Days 8 and 10, endodermal cells migrate from

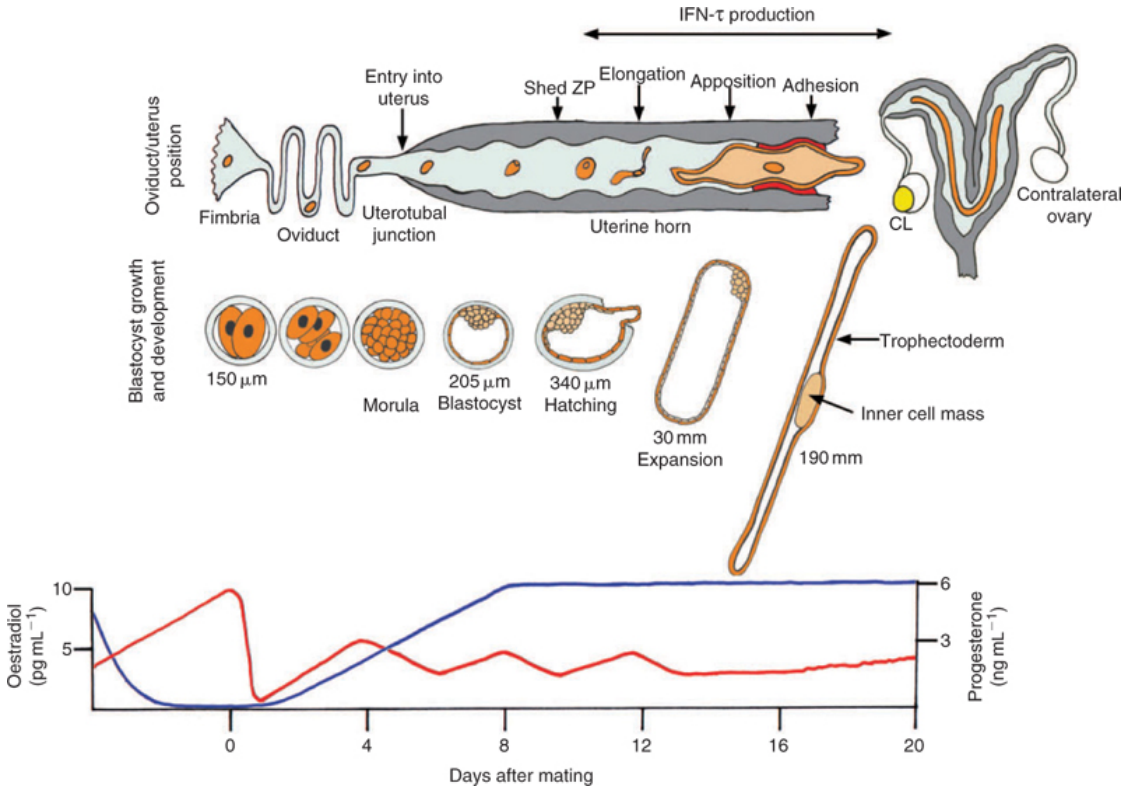

Figure 2.1. Events of early pregnancy in sheep. The embryo enters the uterus on Day 4 and the blastocyst is formed on Day 6. The zona pellucida is shed between Days 8 and 9. The blastocyst starts to elongate on Day 13 and undergoes extreme morphological changes from spherical to tubular and filamentous forms, reaching 10 cm long on Day 14. After Day 14, there is progressive apposition of the conceptus trophectoderm as it becomes closely associated with the endometrial LE followed by unstable adhesion. By Day 16, the trophoblast adheres firmly to endometrial LE in both intercaruncular and caruncular areas. From Spencer et al., 2007 and originally drawn by Dr. Greg A. Johnson.

the inner cell mass and line up against the trophectoderm cells and a basement membrane is formed between them. Beginning on Day 12, the blastocyst starts to elongate, reaching a final length of 10-22cm (Rowson & Moor, 1966, Wintenberger-Torres & Flechon, 1974). On Day 13, the embryonic disc appears (Guillomot *et al.*, 1981). On Day 14, the conceptus has a filamentous form and is about 10cm long. Conceptus elongation continues until Day 17 when it reaches a length of 25cm or more (Wintenberger-Torres & Flechon, 1974). Conceptus elongation and production of IFNT occur coincidentally (Farin *et al.*, 1989, Gray *et al.*, 2002).

Implantation and placentation

Implantation in sheep

As illustrated in Fig. 2.2, implantation, which can be defined as attachment of placental membranes to uterine endometrium, takes place beginning at the blastocyst stage in domestic ruminants in five phases: (1) shedding of zona pellucida; (2) precontact and blastocyst orientation; (3) apposition; (4) adhesion; and (5) endometrial fusion (Guillomot, 1995, Guillomot *et al.*, 1981, Guillomot *et al.*, 1993). After shedding of the zona pellucida on Days 8 to 9, the blastocyst undergoes a morphological change from spherical, to tubular (by Day 10) and then to a filamentous form (Days 13-14). Apposition, a close association but unstable adhesion of trophectoderm and endometrial LE, begins with a reduction in microvilli covering the trophectoderm between Days 13 and 15. A major event of apposition is interdigitation of cytoplasmic projections of trophectoderm cells and uterine epithelial microvilli. Also, finger-like villi or papillae from the trophoblast not only absorb histotroph from uterine glands but may also facilitate apposition through an anchoring effect (Wooding *et al.*, 1982). MUC1, the glycocalyx covering the apical surface of endometrial LE is reduced coincident with loss of

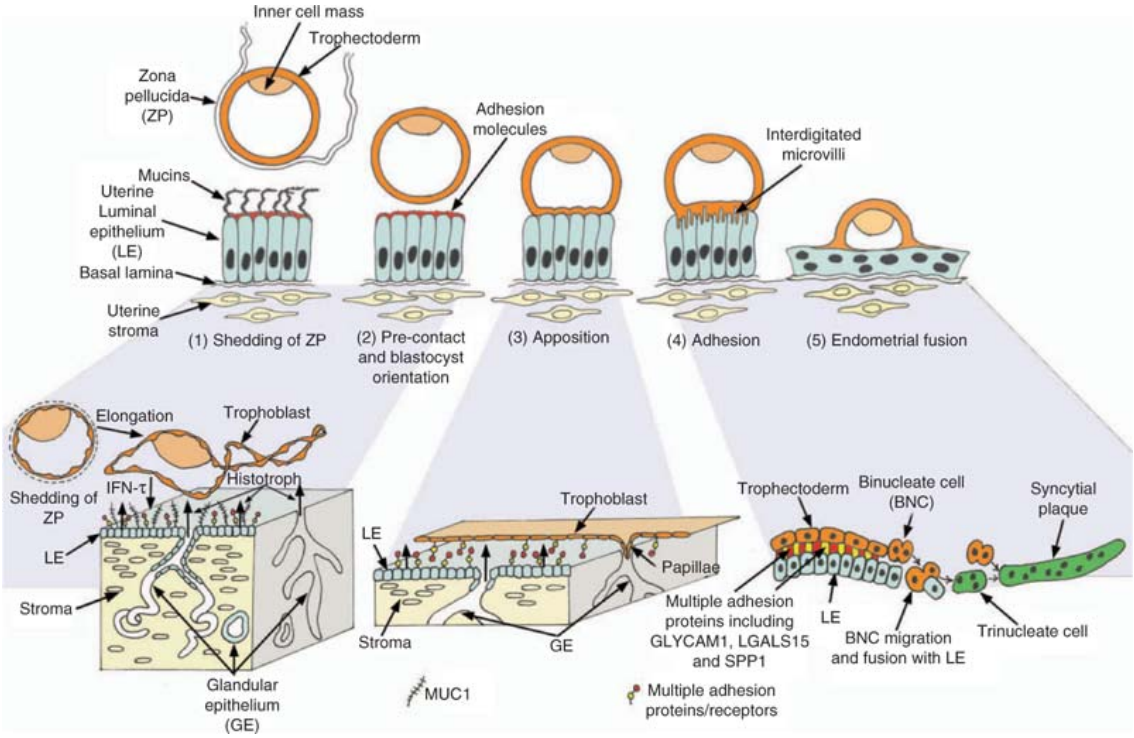

Figure 2.2. Phases of conceptus implantation in sheep. The zona pellucida is shed between Days 8 and 9 (phase 1). As the blastocyst approaches the endometrial LE, the loss of MUC1 occurs locally at implantation sites from Day 9 to 17, which is coincident with loss of PGR from the uterine LE. Loss of MUC1 allows close contact between trophectoderm and endometrial LE and exposure to adhesive molecules (phase 2). Apposition of the conceptus starts around Day 14, with finger-like villi or papillae developed from the trophoblast, penetrating into the mouths of uterine glands on Days 15 to 18 (phase 3). These papillae absorb histotroph from the uterine glands and also act as an anchor. After Day 16, the blastocyst is firmly attached by interdigitation of uterine epithelial microvilli and cytoplasmic projections of trophoblast cells (phase 4). The trophoblast giant binucleate cells (BNCs) are differentiated from the mononuclear trophoblast cells by Day 16 and the BNC fuses with the endometrial LE, forming syncytia (phase 5). From Spencer et al., 2007 and originally drawn by Dr. Greg A. Johnson.

PGR from LE and it is no longer present by Day 17 (Johnson *et al.*, 2001a). GLYCAM1 (Spencer *et al.*, 1999a), LGALS15 (Farmer *et al.*, 2007, Gray *et al.*, 2004), secreted phoshoprotein 1 or osteopontin (SPP1; (Johnson *et al.*, 2003a) and integrins (Johnson *et al.*, 2001a) are well known adhesive molecules or receptors during implantation in sheep. The loss of MUC1 and actions of adhesion molecules via related receptors are required for successful conceptus attachment. From Day 16, the trophoblast begins to adhere firmly to endometrial LE. Interdigitation of the trophectoderm and endometrial LE occurs in both caruncular and intercaruncular areas of the endometrium until about Day 22. The trophoblast giant binucleated cells (BNCs), arose from mononuclear trophectoderm cells, are formed, migrated and fused with endometrial LE thus syncytial plaques are formed.

Placentation in sheep

Placentation is formation and growth of the placenta, a transient organ for metabolic exchange between the conceptus and the mother. The placenta is formed when fetal tissues acquire contact or fusion with maternal tissues for exchange of nutrients, gases and metabolites. The fetal membranes involved in formation of the placenta include the yolk sac, allantois, amnion, and chorion (Perry, 1981). The chorion is attached to the uterus. The amnion surrounds the fetus as a close-fitting, tough membrane filled with amnionic fluid under slight pressure which provides the fetus with a liquid environment and buoyancy to allow it to develop independent of the forces of gravity. The allantois is a fluid-filled membrane in which there is accumulation of fluid from the embryo/fetus, as well as the accumulation and storage of nutrients such as proteins, sugars, amino acids and water (Bazer & First, 1983). Thus, the placenta has nutritive, respiratory, endocrine and immunosuppressive roles crucial for development and growth of fetus (King *et al.*, 1982). The placenta is the regulator of exchange of nutrients

and gases between the fetus and the mother. Also, as a transient endocrine organ, the placenta produces progesterone (P4), estrogen (E2), chorionic somatomammotropin hormone 1 (CSH1; also known as placental lactogen) and placental growth hormone.

The ovine placenta has similar characteristics to those of other domestic ruminants. First, domestic ruminants have a synepitheliochorial placenta, with the epithelium modified by migration of BNCs and their fusion with endometrial LE to form fetomaternal syncytia throughout pregnancy (Wooding., 1992). Second, ruminants have cotyledonary placentae. Cotyledons represent a placental unit of highly branched villous, tree-like folds with abundant blood vessels and connective tissue. The placentome of the cotyledonary placenta consists of a fetal cotyledon (part of chorioallantois), and a maternal uterine caruncle. Placentomes provide specialized structures for efficient hematotrophic nutrition and gas exchange between the fetal and maternal circulations because of their very close proximity for exchanging oxygen and micronutrients via countercurrent mechanisms (Reynolds *et al.*, 2005). In sheep, about 90 to 100 placentomes are evenly distributed across the placenta (Hafez, 1993).

During early placentation, the trophoblast binucleate giant cells (BNCs) are differentiated from mononuclear trophectoderm cells around Day 14 of pregnancy (Wooding., 1982). These binucleate cells fuse with endometrial LE to form syncytia. The roles of BNCs are: (1) to form syncytia essential for successful implantation and growth of placenta; and (2) to produce protein and steroid hormones, e.g, CSH1 and P4, that regulate maternal physiology. At about Day 16 of pregnancy in sheep, the chorion initiates attachment to the endometrium. During formation of the placentomes, chorionic villi protrude into crypts in the caruncle, forming a concave structure between Days 20 and 60 of gestation in sheep (Dunlap *et al.*, 2006). Furthermore, in the intercotyledonary areas, fetal and maternal epithelia are simply apposed except for modifications of the trophoblast

over uterine gland openings to form areolae which absorb secretions from uterine glands and have been best described in pigs (Perry, 1981).

Interferon tau (IFNT)

IFNT is a member of the Type I interferon (IFN) family along with interferon alpha (IFNA), beta (IFNB), delta (IFND), and omega (IFNW). IFNT was first identified as protein X or Trophoblastin (Bazer *et al.*, 1996, Imakawa *et al.*, 1987) and also termed ovine trophoblast protein-1 (oTP-1) as the first primary protein secreted by the ovine conceptus (Godkin *et al.*, 1982). After cDNA cloning and amino acid sequencing, it was designated ovine IFNT (Bazer *et al.*, 1997). IFNT arose from IFNW in the Ruminantia about 36 million years ago (Demmers *et al.*, 2001), sharing 70% homology. Bovine IFNT and ovine IFNT cDNAs share a higher degree of homology than ovine IFNT and ovine IFNW (Bazer *et al.*, 1996). IFNT has high amino acid sequence homology across ruminant species. However, there are differences among IFNT proteins among species. Bovine IFNT is glycosylated with N-linked oligosaccharides while ovine IFNT is not glycosylated and caprine IFNT proteins exist as both glycosylated and nonglycosylated forms (Demmers *et al.*, 2001).

Expression of the *IFNT* gene is different from that of other interferons. First, *IFNT* is not induced by virus (Guesdon *et al.*, 1996) although IFNT has potent antiviral activity. Second, *IFNT* gene expression is limited to the conceptus trophectoderm while other IFNs are expressed by many types of cells in most tissues. IFNT is synthesized and secreted from mononuclear cells of the conceptus trophectoderm between Days 10 and 20 with maximal production between Days 14 to16 (Bazer *et al.*, 1997, Roberts *et al.*, 1999). In cattle, IFNT is produced between Days 16 and 24 of gestation (Demmers *et al.*, 2001) with maximal production on Day 17 of pregnancy (Bartol *et al.*, 1985). Caprine IFNT is

secreted between Days 16 and 21 of pregnancy. Increases in IFNT on Day 13 in sheep and Day 15 in cattle correspond with elongation of conceptuses from spherical to filamentous forms (Ashworth & Bazer, 1989).

IFNT from conceptuses plays a central role in establishment of pregnancy in ruminants. Homogenates of the ovine conceptus from Days 14 and 15 extended CL lifespan and interestrus intervals when infused into uteri of cyclic ewes. However, intrauterine infusion of conceptus homogenates from Days 21 to 25 or intravenous injection of conceptus homogenates did not extend CL lifespan (Rowson & Moor, 1967). Introduction of either ovine trophoblastic vesicles or recombinant ovine IFNT into the uterine lumen of cows blocked luteolysis and extended the interestrus interval (Helmer *et al.*, 1989, Heyman *et al.*, 1984, Meyer *et al.*, 1992). These results suggest that IFNT acts in a paracrine manner as the pregnancy recognition signal to prevent development of the luteolytic mechanism. IFNT alone is sufficient for maternal recognition of pregnancy (Bazer, 1992). Similar to other Type I IFNs, IFNT has antiviral (Pontzer *et al.*, 1990), antiproliferative (Bazer & Johnson, 1991, Roberts, 1989), antitumor (Pontzer *et al.*, 1990), immunomodulatory (Bazer & Johnson, 1991, Roberts, 1989), and therapeutic (Bazer & Johnson, 1991, Bekisz *et al.*, 2004, Martal *et al.*, 1998) properties.

As a member of the Type I IFN family, IFNT stimulates transcription of IFN-stimulated genes (ISGs) that appear to play roles in endometrial differentiation and conceptus implantation during early pregnancy (Spencer & Bazer, 2004). As illustrated in Fig. 2.3.a, IFNT utilizes the classical JAK-STAT cell signaling pathway used by all Type I IFNs. All cell types in the endometrium have IFNA receptor 1 (IFNAR1) and IFNA receptor 2 (IFNAR2) with highest expression in the uterine LE (Rosenfeld *et al.*, 2002). Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) constitutively associate with cytoplasmic domains of IFNAR1 and IFNAR2 (Colamonici *et al.*, 1994, Novick *et al.*, 1994). After phosphorylation of signal transducer and activator of transcription (STAT) 1 and 2, they form homodimers of

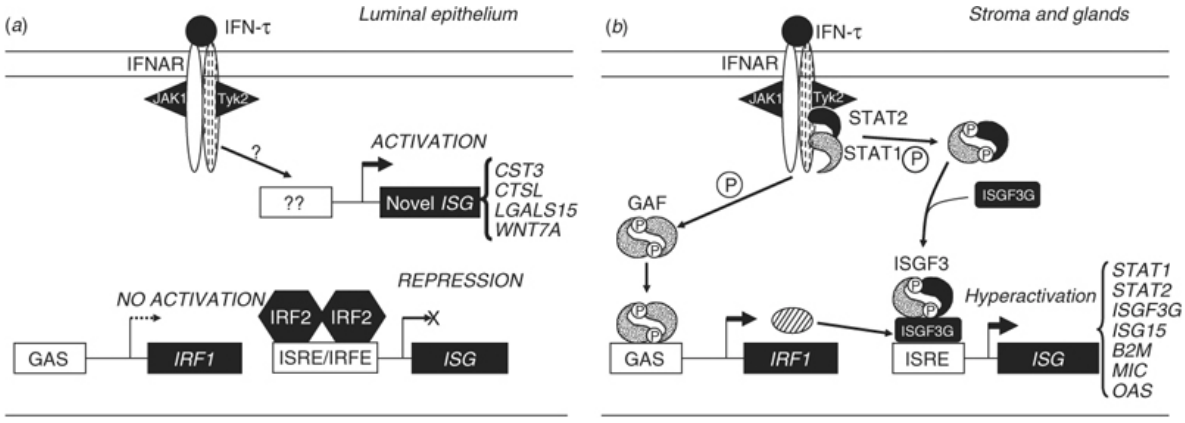

Figure 2.3. Schematic illustrating current working hypothesis on interferon tau (IFNT) signalling in the ovine uterine endometrium. IFNT binds to the Type I IFN receptor (IFNAR) present on cells of the ovine endometrium. In cells of the stroma and middle to deep GE (Fig. 2.3.b), IFNT-mediated association of IFNAR subunits facilitates cross-phosphorylation and activation of janus kinase (JAK), which, phosphorylates the receptor and signal transducers and activators of transcription (STAT) 1 and 2. Both STAT1 and STAT2 are then released from the receptor and form a heterodimer. Interferon stimulated transcription factor 3γ (ISGF3G) is formed by association of a STAT1–STAT2 heterodimer and ISGF3G in the cytoplasm, translocates to the nucleus and activate genes containing an IFN-stimulated response element (ISRE), such as STAT1, STAT2, ISGF3G, B2M, ISG15, MIC and OAS. Gamma-activated factor (GAF) is formed by STAT1 homodimers, which translocate to the nucleus and transactivate genes containing a gamma-activation sequence (GAS) element, such as interferon regulatory factor 1 (IRF1). IRF1 can also bind and transactivate ISRE. Therefore, hyperactivation of ISG expression occurs. In LE and sGE (Fig.2.3a), this signaling pathway is inactive due to the presence of IRF2, a potent repressor of transcription. IRF2 binds ISRE and inhibit expression of classic ISGs (*STAT1, STAT2, ISGF3G, B2M, ISG15, MIC, OAS*). However, some novel ISGs such as *WNT7A, CST3, CTSL,* and *LGALS15* are expressed in response to IFNT activation of an alternate cell signalling pathway. Adapted from Spencer et al., 2007.

STAT1 (GAF) and heterodimers of STAT1 and STAT2. These dimers translocate to the nucleus. GAF binds to the gamma activation sequence (GAS) to transactivate genes containing GAS elements such as IFN regulatory factor 1 (IRF1). Heterodimers of STAT1 and STAT2 bind to IRF9 to form ISGF3 and transactivate genes containing IFN stimulated response elements (ISRE) including *STAT1, STAT2, IRF9, B2M, ISG15, MHC* and *OAS* (Choi *et al.*, 2003, Choi *et al.*, 2001a, Johnson *et al.*, 2000, Johnson *et al.*, 2002, Johnson *et al.*, 1999, Johnson *et al.*, 2001b).

ISGs are not expressed by all endometrial cell types. Many classical ISGs (*B2M*, *GBP2, IFI27, IFIT1, ISG15, IRF9, MIC, OAS*, *RSAD2*, *STAT1,* and *STAT2*) are induced or upregulated by IFNT in glandular epithelium (GE) and stroma (Fig. 2.3.a., but not in LE and superficial GE (sGE) of the ovine uterus as shown in Fig.2.3.b. (Choi *et al.*, 2003, Choi *et al.*, 2001b, Johnson *et al.*, 1999, Johnson *et al.*, 2001b, Kim *et al.*, 2003b, Song *et al.*, 2007b) because the JAK-STAT cell signaling pathway is inhibited by IRF2, a potent repressor of transcription, that is expressed in LE/sGE (Choi *et al.*, 2001b). However, there are some genes induced or stimulated by IFNT in LE/sGE such as *WNT7A*, *LGALS15*, *CTSL*, and *CST3* of the ovine uterus (Gray *et al.*, 2006, Kim *et al.*, 2003b, Song *et al.*, 2005, 2006). These novel ISGs must be transactivated via an alternative cell signaling pathway(s), and they are proposed to play important roles during early pregnancy in ruminants. To date, ras-related C3 botulinum substrate 1 (RAC1), p38/mitogen activated protein kinase (p38 MAPK), insulin receptor substrate (IRS), the phosphotidylinositol-3 kinase (PI3K) cascade, and v-crk sarcoma virus CT10 oncogene homolog (avian)-like (CRKL), a member of the CDC2 related kinase family, are candidate signaling mechanisms known to be activated by Type I IFNs (Bekisz *et al.*, 2004). Further, suppressor of cytokine signaling (SOCS1-3) is also upregulated in endometria by pregnancy and IFNT (Sandra *et al.*, 2005).

Model for antiluteolytic effects of IFNT on the ovine endometrium

As an antiluteolysin, IFNT blocks development of the uterine luteolytic mechanism to suppress release of uterine pulses of luteolytic PGF2α pulses (Flint *et al.*, 1992, Vallet *et al.*, 1987). Hormonal regulation via their respective receptors illustrated in Fig. 2.4. In cyclic ewes, estrogen from Graafian follicles at estrus stimulates *estrogen receptor alpha* (*ESR1*), *progesterone receptor* (*PGR*), and *oxytocin receptor* (*OXTR*) expression (Spencer & Bazer, 1995). During diestrus, progesterone levels increase and act via PGR to block expression of ESR1 and then OXTR in endometrial LE/sGE after Days 11 to 12 which is referred to as the period of 'progesterone block' (McCracken *et al.*, 1984). Therefore, ESR1 and OXTR are not detected in endometrial LE/sGE between Days 5 and 11 of the estrous cycle, i.e., during most of diestrus. Effects of progesterone on *OXTR* gene expression are via suppression of ESR1. In the ovine *OXTR* gene, there are several Sp1 elements in the promoter region that appear to mediate responsiveness to ligand activated ESR1 (Fleming *et al.*, 2006). However, continuous exposure of the uterus to progesterone for 8 to 10 days down-regulates expression of PGR in endometrial epithelia after Days 11 to 12 (Spencer *et al.*, 1995), allowing for rapid increases in expression of ESR1 on Day 13 and OXTR on Day 14 in LE/sGE. Down-regulation of *PGR* expression by progesterone may involve PGR-mediated decreases in *PGR* gene transcription. The pulsatile release of oxytocin from the CL and posterior pituitary induces release of luteolytic pulses of PGF. During early pregnancy, IFNT acts in a paracrine manner to bind IFNARs on the endometrial LE and inhibit transcription of the ESR1 gene through a signaling pathway involving IRF2 (Choi *et al.*, 2001b, Fleming *et al.*, 2001). These actions of IFNT on the *ESR1* gene prevent OXTR formation, thereby preventing oxytocin-induced release of luteolytic pulses of PGF2α which allows for maintenance of the CL and its production of progesterone required for establishment and maintenance of pregnancy.

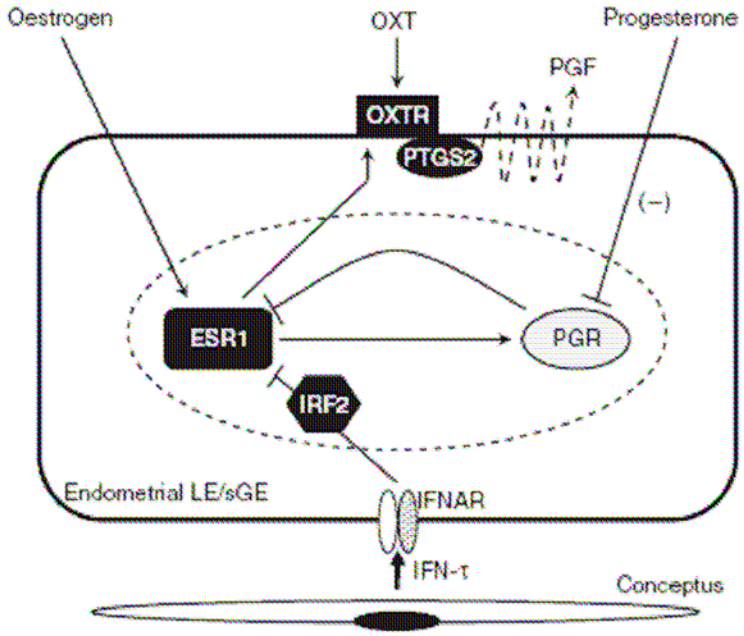

Figure 2.4. Illustration of hormonal regulation of the endometrial antiluteolytic mechanisms and antiluteolytic effects of IFNT, the conceptus signal for pregnancy recognition on the maternal endometrium in the ovine uterus. During the periimplantation period, continuous exposure to P4 downregulates PGR expression in the endometrial epithelia. IFNT from the conceptus suppress transcription of ESR1 and E2-induced OXTR genes, thereby preventing the ability of oxytocin to induce luteolytic pulses of PGF. However, during the estrous cycle, ESR1 expression increases as PGR expression decreases after Days 11 to 13 the, E2, then induces OXTR expression on Days 13 to 14, which allows oxytocin to induce release of luteolytic pulse of PGF on Days 15 to 16. Adapted from Spencer *et al*., 2007.

Interferon stimulated genes (ISGs) and implantation/placentation

During early pregnancy, IFNT from ruminant conceptuses induces expression of ISGs (Hansen *et al.*, 1999, Spencer *et al.*, 2007a, Spencer *et al.*, 2007b). Many ISGs are hypothesized to play roles in implantation and/or placentation, including endometrial differentiation, implantation and development of the conceptus. Selected ISGs expressed in the ovine uterus are described here with their differential cellular localization and functional implications in implantation and placentation.

Galectin 15 (LGALS15)

LGALS15 (also known as OVGAL11) was first shown to be induced in gastrointestinal tissue and secreted into the intestinal lumen after infection of sheep with the helminth parasite *Haemonchus contortus* (Dunphy *et al.*, 2000). *LGALS15* mRNA was detected in endometrial LE and sGE both in cyclic and pregnant ewes. In cyclic ewes, *LGALS15* mRNA was low on Day 10, increased to Day14, and decreased on Day 16. In pregnant ewes, *LGALS15* mRNA was detected after Day 10 and increased by Day 20. *LGALS15* mRNA was induced by progesterone and stimulated by IFNT (Gray *et al.*, 2004, Satterfield *et al.*, 2006). LGALS15 protein is concentrated near and on the apical surface of LE and sGE and abundant in the histotroph recovered from the uterine lumen. LGALS15 is associated with crystalline inclusion bodies in endometrial LE and conceptus trophectoderm (Gray *et al.*, 2005, Gray *et al.*, 2004). Functional *in vitro* studies suggested that LGALS15 is involved in blastocyst elongation and implantation in sheep as it stimulates trophectoderm cell proliferation and migration via the JNK signaling pathway, attachment via its RGD sequence, and focal adhesion formation *in vitro* (Farmer *et al.*, 2007).

Wingless-type MMTV integration site family, member 7A (WNT7A)

WNT genes are regulators of cell fate, death, and differentiation, and interaction between cells (Polakis, 2000). Signaling pathways activated by WNTs are divided into canonical and noncanonical pathways (Logan & Nusse, 2004). WNTs are important regulators of uterine morphogenesis, uterine receptivity to the embryo, and blastocyst implantation in the mouse (Dey *et al.*, 2004, Mohamed *et al.*, 2005). In sheep, *WNT7A* is a novel gene induced in the endometrial LE and superficial GE of the uterus by IFNT from Days 11 to 20 via a STAT1-independent pathway. Intrauterine infusion of IFNT increased *WNT7A* mRNA, demonstrating a paracrine action of IFNT on expression of *WNT7A* (Kim *et al.*, 2003b). WNT signaling via beta catenin (CTNNB1) has been implicated in bovine trophoblast giant BNC differentiation (Kim *et al.*, 2003b). Recent results showed that WNT7A stimulated trophectoderm cell proliferation and transcription of genes involved in cell proliferation and differentiation such as msh homeobox 1 (*MSX1*) and *MSX2* via the canonical signaling pathway (Hayashi *et al.*, 2007).

Interferon stimulated gene 15 ubiquitin-like modifier (ISG15)

ISG15 (also known as ISG17/UCRP) was first identified in mouse Ehrlich ascites tumor cells as being highly induced by IFN treatment (Farrell *et al.*, 1979). ISG15, also called ubiquitin cross-reactive protein (UCRP) due to its cross-reactivity with ubiquitin-specific antibodies (Haas *et al.*, 1987), is synthesized as a 17 kDa precursor termed ISG17 and processed to a 15 kDa protein by a specific protease which is critical for conjugation to target proteins (Austin *et al.*, 1996, Johnson *et al.*, 1998, Johnson *et al.*, 1999, Kim & Zhang, 2003). Upon interferon treatment, ISG15 can be detected in cells in both free and conjugated forms. In the ovine uterus, *ISG15* mRNA is localized to LE, sGE, and stromal cells. *ISG15* mRNA is detected from the LE on Day 11, then from sGE and stratum compactum stroma between Day 11 to Day 13 of pregnancy and is abundant between Days 15

and 19, extending to the deep GE (dGE), stratum spongiosum stroma and myometrium which is coincident with a loss of *ISG15* mRNA in the LE (Johnson *et al.*, 1999). *ISG15* mRNA is maintained through Day 30 of pregnancy in sheep, but is confined to stratum compactum stroma. Also, *ISG15* mRNA is localized to maternal caruncular stroma surrounding the interdigitating placental chorionic villi. Interestingly, *ISG15* is expressed throughout pregnancy although it may decrease in late pregnancy of sheep (Joyce *et al.*, 2005). Similar results have been found in mouse that pregnancy-specific *ISG15* expression increased during implantation (Austin *et al.*, 2003). Moreover, *ISG15* mRNA in peripheral blood leukocytes (PBLs) was increased in pregnant diary cows compared to nonpregnant cows (Gifford *et al*., 2007). This evidence suggest that ISG15 is not only part of an antiviral response, but also critical factor at the uterine-placental interface during conceptus development, implantation and placentation.

Myxovirus resistance (MX)

MX is induced by Type I IFNs and it is a potent inhibitor of viral replication (Horisberger & Gunst, 1991). MX is also a functional monomeric GTPase with roles in intracellular protein and vesicle trafficking (Horisberger., 1992). Expression of *MX* is induced in all cells that possess Type I IFN receptors and has been used as an indicator of viral infection (Haller *et al.*, 1998). Among domestic animals, expression of *MX* is found in uteri of cows, pigs, sheep, and mares (Hicks *et al.*, 2003, Ott *et al.*, 1998). In sheep, *MX1* mRNA and protein were localized in LE, sGE, stroma and myometrium. *MX1* mRNA increased from Day 11 to maximum levels on Day 15, then decreased to moderate levels that were maintained throughout pregnancy in the ovine uterus. However, low expression of *MX1* mRNA is detected in cyclic or bred but nonpregnant ewes (Ott *et al.*, 1998). *MX1* mRNA is also detected in peripheral blood mononuclear cells of pregnant ewes until Day 30. Similar results have been reported for dairy cows in which

MX1 and *MX2* gene expression increases in peripheral blood leukocytes (PBLs) of pregnant, but not bred but nonpregnant cows (Gifford *et al.*, 2007).

2',5'-oligoadenylate synthetase (OAS)

Activated by viral response, OAS polymerizes ATP into 2'-5' linked oligomers to activate the endonuclease RNase L that cleaves viral and cellular RNA (Chebath *et al.*, 1987, Kumar *et al.*, 2000, Marié & Hovanessian, 1992). OAS is induced by Type I IFNs and has a role in inhibition of viral replication, as well as cell growth, differentiation and apoptosis (Benech *et al.*, 1985, Chebath *et al.*, 1987, Marié & Hovanessian, 1992). In cyclic ewes, OAS protein is low in stroma and GE. In pregnant ewes, OAS protein is increased in the stroma and deep GE, from Day 15, maintained on Day 17, and decreased on Day 19 of pregnancy. Further, *OAS* mRNA is regulated by IFNT which requires presence of P4 (Johnson *et al.*, 2001b, Mirando *et al.*, 1991). In pregnant cows, OAS is stimulated higher in uterine epithelium than stroma. IFNT treatment increases OAS in all endometrial cell types (Schmitt *et al.*, 1993).

Radical S-adenosyl methionine domain containing 2 (RSAD2) and Interferon-induced with helicase C domain 1 (IFIH1)

RSAD2 (alias viperin) is a cytoplasmic antiviral protein induced by Type I IFNs that can inhibit infection of cells with human cytomegalovirus (Chin & Cresswell, 2001). IFIH1 (alias MDA5) is a RNA helicase that, through its ATP-dependent unwinding of RNA, promotes mRNA degradation and is involved in innate immune defense against viruses. IFIH1 is an IFNT induced gene involved in induction of apoptosis (Kang *et al.*, 2002 , Kang *et al.*, 2004). *RSAD2* and *IFIH1* mRNAs are both found in ovine uterus during early pregnancy and in response to intrauterine IFNT (Song *et al.*, 2007b). RSAD2 and IFIH2 are suggested to have a

role(s) in induction of the antiviral state and modulation of local immune cells that may be beneficial to conceptus implantation (Song *et al.*, 2007b).

Cathepsin L (CTSL) and Cystatin C (CST3)

Cathepsins are a family of lysosomal proteases that can degrade extracellular matrix (ECM) molecules like collagens, laminin, fibronectin, and proteoglycans (Kirschke *et al.*, 1998), while CTS3, a low-molecular weight secretory protein, inhibits lysosomal cysteine proteinases, including cathepsins (Abrahamson *et al.*, 1986, Hall *et al.*, 1995). In the ovine uterus, CTSL is up-regulated by IFNT and CST3 is regulated by IFNT and P4, with both showing same cell-specific localization during early pregnancy (Song *et al.*, 2005, Song *et al.*, 2006). In mice, CTSB, CTSL and CST3 are necessary for embryo development and decidualization during the periimplantation period (Afonso *et al.*, 1997). Therefore, regulation of both the protease and its inhibitor by IFNT may affect endometrial remodeling and regulation of invasive actions of ovine conceptuses.

Gene expression profiling in the ovine and bovine endometria during early pregnancy

Gene expression profiling has identified important factors for implantation, placentation and conceptus growth during early pregnancy in cows and ewes. Using cDNA microarray and/or suppression subtraction hybridization (SSH), new genes were reported and validated by RT-PCR, real-time PCR, slot blot hybridization, and *in situ* hybridization (Bauersachs *et al.*, 2006, Cammas *et al.*, 2005, Chen *et al.*, 2007, Gray *et al.*, 2006, Ishiwata *et al.*, 2003, Klein *et al.*, 2006). Genes involved in uterine receptivity for conceptus attachment were studied by

comparing transcriptome changes between endometria of nonpregnant and pregnant cows or ewes (Bauersachs *et al.*, 2006, Gray *et al.*, 2006, Klein *et al.*, 2006). Genes regulated by the pregnancy recognition signal IFNT in human cells and ovine LE cells were identified (Chen *et al.*, 2007, Kim *et al.*, 2003b). In sheep, genes regulated by pregnancy, P4 and/or IFNT in the endometrium were identified (Gray *et al.*, 2006). Many genes involved in cell attachment, cell adhesion, modulation of the maternal immune system, remodeling of the endometrium, regulation of transcription, and signal transduction, interferon stimulated genes (ISGs) were found in these studies (Bauersachs *et al.*, 2006, Klein *et al.*, 2006). Finding genes regulated by P4 and/or IFNT by transcriptional profiling and validating them with a proteomic/metabolic approach will offer better insight into conceptus-endometrial interaction during early pregnancy in cows and sheep (Spencer *et al.*, 2007a). The genes described in the following sections and in Chapter III of this thesis are briefly discussed below. They were originally identified by Gray and coworkers (2006).

Actin, alpha 2, smooth muscle, aorta (ACTA2)

Actins constitute a family of highly conserved and homologous cytoskeletal proteins that are requisite for cellular function. Vertebrates have six tissue-specific actin isoforms: two in striated muscle cells (skeletal (ACTA1) and cardiac (ACTC)), two in smooth muscle cells (SMCs) (vascular (ACTA2) and visceral (ACTG2)) and two in nonmuscle cells (ACTB and ACTG1) (McHugh *et al.*, 1991 , Zhu *et al.*, 2006). ACTA2 null mice were fertile and were used to demonstrate that ACTA2 has a role in regulating vascular contractility and blood pressure homeostasis, but not formation of the cardiovascular system. ACTA2 is the most abundant protein in SMCs, occupying about 40% of total cellular protein and 70% of total actin (Schildmeyer *et al.*, 2000). Tissue-specific expression of actin isoforms, regulated expression of isoforms during muscle differentiation, and coexpression of multiple

isoforms in various cell types were reported and ACTA2 is a component of contractile sarcomeres and smooth muscle myofibrils of warm-blooded vertebrate muscle tissues (Barja *et al.*, 1986, Carroll *et al.*, 1986, Chang *et al.*, 1984, McHugh & Lessard, 1988, Owens., 1995, Owens & Thompson, 1986). ACTA2 has been used as a marker of cells undergoing decidualization in sheep and primates (Johnson *et al.*, 2003b, Kim *et al.*, 1998).

Collagen, type III, alpha 1 (COL3A1)

Collagens are major components of all extracellular matrix (ECM). COL3A1 is a type of fibrillar collagens which includes type I, II, III, V and XI. COL3A1 is a homotrimer consisting of three identical α-1 chains. COL3A1 is found in stretchable connective tissues such as the blood vessel walls, hollow organs like the uterus and the skin, giving tensile strength (Aumailley & Gayraud, 1998, Burgeson & Nimni, 1992, van der Rest & Garrone, 1991). Mutation in COL3A1 causes Ehlers-Danlos Syndrome (EDS), especially Type III (vascular type), with symptoms of bruising, bleeding, rupture of hollow organs, and fragile uteri (De Paepe & Malfait, 2004). Uterine collagens (COL1A1, COL3A1, and COL4A1) show type and location-specific patterns. Aggregation of uterine collagens including COL3A1 constitutes thick and thin collagen fibrils in pregnant murine endometrium during decidualization, with COL3A1 localized in both thin and thick fibrils (Spiess & Zorn, 2007). In cows, COL3A1 is the dominant collagen in the uterine wall of non-pregnant females (Boos, 2000), and is present throughout gestation in all fetal membranes and the uterine wall. It is localized with COL1A1 but COL3A1 dominates in quantity. Within the bovine placentome, COL3A1 was prevalent on the maternal side (Boos *et al.*, 2003).

Periostin (POSTN)

POSTN is a secreted cell adhesion protein of relatively unknown function that has homology with the insect growth cone guidance protein fasciclin I (Kruzynska-Frejtag *et al.*, 2004, Takeshita *et al.*, 1993). POSTN was also identified as an osteoblast-specific factor and, as a homophilic adhesion molecule during bone formation, it can support osteoblastic cell line attachment and spreading (Horiuchi *et al.*, 1999). POSTN is also induced by transforming growth factor beta 1 (TGFB1) which promotes the attachment and spreading of fibroblasts (Skonier *et al.*, 1992). POSTN stimulates cell adhesion, motility, and migration via integrin αvβ3 and αvβ5 subunits (Gillan *et al.*, 2002). POSTN is overexpressed in various types of human cancer such as lung, rain, ovary, breast cancers and thyroid, and non small cell lung carcinoma (Gillan *et al.*, 2002, Sasaki *et al.*, 2002, Sasaki *et al.*, 2001a, Sasaki *et al.*, 2003 , Sasaki *et al.*, 2001b, Sasaki *et al.*, 2001, Shao *et al.*, 2004). Female *Postn* null mice are infertile due to failure to cycle although male null mice is fertile (Rios *et al.*, 2005). POSTN is highly expressed in many tissues including placenta, uterus, mammary gland, and fetal tissues (Gillan *et al.*, 2002). Expression of POSTN in rat carotid arteries and in cultured vascular smooth muscle cells (VSMCs) is stimulated by growth factors acting via the phosphoinositide-3-kinase (PIK3) dependent signaling pathway (Li *et al.*, 2006). Mesenchyme-specific POSTN induces cell invasive activity and metastasis through epithelial-mesenchymal transition via coactivation of epidermal growth factor (EGF) receptor and integrins (Yan & Shao, 2006).

S100 calcium binding protein A2 (S100A2)

The S100 family of proteins share calcium-binding EF-hand motifs (Donato, 2001). The S100 proteins are small, acidic proteins of 10-12kDA, found exclusively in vertebrates. About 23 proteins reported to be in this family have broad intracellular and extracellular functions. Function of many S100 proteins are

related to tumor, acting as either promoter or suppressor proteins (Salama *et al.*, 2007). Among them, S100A2 is a tumor suppressor in many cancers including breast, lung, and prostate cancers by site-specific hypermethylation (Salama *et al.*, 2007, Wicki *et al.*, 1997). However, S100A2 is also increased in some cancers such as esophageal squamous cell carcinoma and non-small cell lung carcinomas. How S100A2 acts as either a tumor promoter or tumor suppressor is unclear, but its tumor suppressive function may be due to suppression of PTGS2 (or COX2) expression (Tsai *et al.*, 2006). Also, S100A2 is involved in enhancing p53 transcriptional activity while other members inhibit p53 activity (Marenholz *et al.*, 2004 , Semov *et al.*, 2005). S100A2 is localized to both nuclear and cytoplasmic fractions of all squamous and transitional epithelia (Cross *et al.*, 2005). S100A2 was preferentially expressed in normal mammary epithelial cells but not breast tumor cells (Lee *et al.*, 1992).

Secreted protein, acidic, cysteine-rich (SPARC)

SPARC (also known as osteonectin, BMN-40, 43K protein) is a collagen-binding glycoprotein that appears to regulate cell growth through interactions with ECM and cytokines (Lane & Sage, 1994). SPARC null mice showed decreased bone formation due to fewer oetsoblasts and osteoclasts, as the previous name osteonectin implies. SPARC is classified as matricellular protein, which is not an ECM component itself but modulates cell-matrix interactions and cell function. SPARC mediates adhesion, characterized by disruption of focal adhesions and reorganization of actin stress fibers (Bornstein & Sage, 2002). SPARC inhibits cell proliferation *in vitro* (Brekken & Sage, 2001), induces apoptosis (Yiu *et al.*, 2001), binds to cytokine/growth factors like vascular endothelial growth factor (VEGF) and fibroblast growth factor two (FGF2) and regulates cell growth, growth factor production, angiogenesis, matrix deposition or assembly, cell attachment and migration (Bornstein & Sage, 2002). SPARC is an upstream regulator of several

collagens (Zhou *et al.*, 2006). SPARC is coordinately down-regulated with COL3A1 in IL11 receptor α null mice which fail to undergo normal uterine decidualization (White *et al.*, 2004).

Signal transducer and activator of transcription 5A (STAT5A)

STAT5A, a member of signal transducer and activator of transcription in JAK-STAT signaling pathway (Darnell, 1997), was initially identified in mammary gland tissue as a prolactin-induced transcription factor named mammary gland factor (MGF) (Wakao *et al.*, 1994). STAT5 consists of two highly related genes encoding STAT5a and STAT5b proteins, with high similarity (Liu *et al.*, 1995). These proteins differ mainly at the carboxyl terminus and present distinct DNA-binding specificities due to a single amino acid difference in the DNA binding domain (Boucheron *et al.*, 1998). These two proteins probably regulate expression of common as well as distinct genes. *Stat5a* knockout mice develop normally and are indistinguishable from wild-type litter mates in size, weight, and fertility. However, mammary lobuloalveolar outgrowth during pregnancy was reduced, and the females failed to lactate after parturition due to a failure of terminal differentiation of the mammary glands (Liu *et al.*, 1997) . These results demonstrate that STAT5A is important for mammogenesis and lactogenesis. In addition, *Stat5a/b* knockout mice have defects in development of functional ovarian CL that result in female infertility that is not characteristic of either *Stat5a* or *Stat5b* knockout mice which indicates redundancy of the STAT5A and STAT5B protein functions. IFNT activates STATs in cells of the ovine endometrium. Phosphorylated STAT5A/B (pSTAT5A/B) was transiently translocated to the nucleus after ovine LE cells were stimulated by IFNT *in vitro* (Stewart *et al.*, 2001). Immunoreactive pSTAT5A/B protein was affected transiently by IFNT, suggesting dynamic changes in STAT5A action during early pregnancy.

Transgelin (TAGLN)

TAGLN (also known as SM22) is an abundant-smooth muscle specific 22 kDa protein, the function of which is unknown. TAGLN has been found in both visceral and vascular smooth muscle, and its expression is very high in aorta, lung, uterus, and intestine. In chicken gizzard, three types of isoforms are reported (α, β, γ). Transgelin serves as an actin-binding and organizing polypeptide (Kobayashi *et al.*, 1994, Shapland *et al.*, 1993). TAGLN is commonly used as an early marker of smooth muscle tissues. TAGLN deficient mouse showed changes in the actin filament distribution although no obvious abnormalities in vascular smooth muscle were detected (Zhang *et al.*, 2001). TAGLN is found in smooth-muscle containing organs including uterus, intestine, gizzard, esophagus and aorta (Lees-Miller *et al.*, 1987).

CHAPTER III
PROGESTERONE AND INTERFERON TAU REGULATED GENES IN THE OVINE UTERINE ENDOMETRIUM

Introduction

Maternal recognition of pregnancy and implantation of the conceptus in early pregnancy are crucial for establishment of pregnancy. The ruminant conceptus secretes the pregnancy recognition signal, interferon tau (IFNT), which prevents luteolysis of the corpus luteum (CL). The CL produces progesterone (P4) which is required for successful pregnancy by making uterus a permissive environment for embryo development, implantation, placentation and successful fetal and placental development to term (Spencer & Bazer, 2004). In a number of mammalian uteri, progesterone receptors (PGR) are expressed in the endometrial epithelia and stroma during the early to midluteal phase, allowing direct regulation of a number of genes by progesterone via activation of the PGR. The loss of PGR in the endometrial luminal epithelium (LE) and then glandular epithelium (GE) is associated with reprogramming of gene expression in the endometrium (Spencer *et al.*, 2004a). IFNT acts on the endometrium in a cell-specific manner to regulate expression of IFN-stimulated genes (ISGs) that are hypothesized to play roles in uterine receptivity and conceptus implantation (Spencer & Bazer, 2004). Gene expression in this period of pregnancy in the uterine endometrium is regulated by the action of P4 from CL and then IFNT from the conceptus. Previously, a custom endometrial cDNA array from Day 14 pregnant ewes was constructed and used to identify pregnancy, P4 and/or IFNT-regulated genes identified in uteri of sheep (Gray *et al.*, 2006). A subset of genes listed in Table 3.1 was found by this microarray to be increased or decreased in response to pregnancy, P4 and/or IFNT.

Table 3.1. Candidate pregnancy, progesterone and IFNT regulated genes [a]

Symbol	Name	Day 14 Pregnant/Cyclic [b]	Effect of P4 [b]	Effect of IFNT [b]
ACTA2	actin, alpha 2, smooth muscle, aorta	↓	-	-
COL3A1	collagen, type III, alpha 1	↓	-	↓
SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	↓	-	↓
POSTN	periostin, osteoblast specific factor	↑	↑	↓
S100A2	S100 calcium binding protein A2	-	-	↓
STAT5A	signal transducer and activator of transcription 5A	↑	↑	↑
TAGLN	transgelin	-	-	↑

[a] From Gray et al., 2006

[b] ↑= increase; ↓= decrease; - = no effect

Periostin (POSTN) is upregulated in epithelial ovarian tumors and supports adhesion and migration of ovarian epithelial cells (Gillan *et al.*, 2002). S100 calcium binding protein A2 (S100A2) is highly expressed in kidney, lung, breast epithelia and down-regulated in cells which acquire tumorigenic phenotype (Wicki *et al.*, 1997). Collagen, type III, alpha 1 (COL3A1) and secreted protein, acidic, cysteine-rich (SPARC), are extracellular matrix (ECM) components, that are regulated by interleukin 11 (IL11) during decidualization in mice (White *et al.*, 2004). Actin, alpha 2, smooth muscle, aorta (ACTA2) is expressed in myometrium and its expression is altered during pregnancy to prepare the myometrium for development of optimal contraction during labor (Shynlova *et al.*, 2005). Transgelin (TAGLN) is an actin-binding protein involved in actin organization by gelling actin (Shapland *et al.*, 1993). Signal transducer and activator of transcription 5A (STAT5A) is one member of the signal transducer and activator of transcription in JAK-STAT signaling pathway that mediates the action of lactogenic hormones (Darnell, 1997).

Although genes in Table 3.1 have been identified as candidate of pregnancy, P4 and/or IFNT-regulated genes in the ovine uterine endometrium, confirmation of the spatio-temporal changes in their expression in the endometrium during early pregnancy and in response to P4 and IFNT have not been investigated. Our working hypothesis is that the genes identified by custom microarray analysis of ovine endometria have physiological roles in establishing uterine receptivity for successful implantation during pregnancy. Therefore, these studies were conducted to confirm regulation of those candidate genes by pregnancy, P4 and/or IFNT.

Materials and methods

Animals

Mature crossbred Suffolk ewes (*Ovis aries*) were observed daily for estrus in the presence of vasectomized rams and were used in experiments after they had exhibited at least two estrous cycles of normal duration (16~18 days). At estrus, ewes were assigned randomly to cyclic or pregnant status. All experimental and surgical procedures were in agreement with the Guide for the Care and Use of Agriculture Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas A&M University.

Study One

At estrus (Day 0), ewes were mated to an intact ram and then hysterectomized (n = 5 ewes/Day) on either Day 10, 12, 14, or 16 of the estrous cycle or Day 10, 12, 14, 16, 18, or 20 of pregnancy. On Days 10 to 16 of pregnancy, the uterine lumen was flushed with 20 ml of sterile 10 mm Tris-HCl (pH 8.0) to confirm pregnancy by observing the presence of one or more morphologically normal conceptus(es). Uterine flushes were not obtained from either Day 18 or 20 of pregnancy because the conceptus is firmly adhered to the endometrial LE and basal lamina (Guillomot *et al.*, 1981). Uterine flushes were clarified by centrifugation (3000 x g for 30 min at 4ºC) and frozen at -80ºC for Western blot analysis. At hysterectomy, several sections (~0.5cm) from the midportion of each uterine horn ipsilateral to the corpus luteum were fixed in fresh 4% paraformaldehyde in PBS (pH 7.2). After 24 h, fixed tissues were changed to 70% ethanol for 24 h and then embedded in Paraplast-Plus (Oxford Labware, St. Louis, MO) for analyses using *in situ* hybridization and immunohistochemistry. The remaining endometrium was physically dissected from myometrium, frozen in

liquid nitrogen, and stored at -80°C for subsequent RNA extraction. In monovulatory pregnant ewes, uterine tissue samples were marked as either contralateral or ipsilateral to the ovary bearing the corpus luteum. No tissues from the contralateral uterine horn were used for study.

Study Two

Cyclic crossbred ewes (n = 20) were checked daily for estrus and then ovariectomized and fitted with indwelling uterine catheters on Day 5 as described previously (Gray *et al.*, 2006). Ewes were then randomly assigned (n = 5 per treatment) and treated daily as follows: (1) 50 mg P4 from days 5-16 and i.u. with 200µg control proteins from days 11-16 (P4+CX); (2) P4 and 75 mg ZK 136,317 (P4 receptor antagonist ZK 136,317; Schering AG, Berlin, Germany) from days 11-16 and i.u. control proteins (P4+ZK+CX); (3) P4 and i.u. IFNT ($2x10^7$ antiviral units) from days 11-16 (P4+IFN); or (4) P4 and ZK and IFNT (P4+ZK+IFN). The P4 and ZK were administered daily in corn oil vehicle. All ewes were hysterectomized on Day 17. Each uterine horn of each ewe received twice-daily injections of either CX proteins (50µg/horn/injection) or recombinant ovine IFNT ($5x10^6$ antiviral units/horn/injection). Recombinant ovine IFNT was produced in *Pichia pastoris* and purified as described previously (Van Heeke *et al.*, 1996). Proteins were prepared for i.u. injection as described previously (Spencer *et al.*, 1999b). This regimen of P4 and IFNT mimics the effects of P4 and the conceptus on endometrial expression of hormone receptors and IFNT-stimulated genes during early pregnancy in ewes (Song *et al.*, 2006). Uteri were processed for histology and the endometria obtained for RNA extraction as described in Study 1.

RNA isolation

Total cellular RNA was isolated from frozen samples of endometrium using

Trizol reagent (Life Technologies, Inc., Bethesda, MD) according to manufacturer's recommendations. The quantity and quality of total RNA was determined by spectrometry and denaturing agarose gel electrophoresis, respectively.

Cloning of partial cDNA

Partial cDNAs for ovine *ACTA2, COL3A1, POSTN, S100A2, SPARC, STAT5A* and *TAGLN* were generated by RT-PCR using total RNA from either Day 16 or Day 18 pregnant ovine endometrium specific primers and annealing temperatures summarized in Table 3.2 and methods described previously (Hayashi *et al.*, 2007). PCR products of the predicted size were cloned into pCRII using T/A cloning kit (Invitrogen) and their sequences were verified by sequencing using an ABI PRISM Dye Terminator Cycle Sequencing Kit and ABI PRISM automated DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA).

Slot blot hybridization analyses

Steady-state levels of mRNA in ovine endometria were assessed by slot blot hybridization as described previously (Song *et al.*, 2007a). Radiolabeled antisense and cRNA probes were generated by *in vitro* transcription using [α-^{32}P] UTP. Denatured total endometrial RNA (20 µg) from each ewe was hybridized with radiolabeled cRNA probes. To correct for variation in total RNA loading, a duplicate membrane was hybridized with radiolabeled antisense 18S cRNA (pT718S; Ambion, Austin, TX). After washing, blots were digested with RNase A and radioactivity associated with slots quantified using a Typhoon 8600 MultiImager (Molecular Dynamics, Piscataway, NJ). Data are expressed as relative units.

Table 3.2. Sequences of primers used for RT-PCR and cloning

Gene	Sequence (5'-3'): forward and reverse	GenBank	Annealing Temperature (°C)	Product Size (bp)
ACTA2	GTGTGTGACAATGGCTCTGG TCTCAAAGTCCAGGGCTACG	BC102699	55.1	649
COL3A1	GAAAGCCTTGAAGCTGATGG TGCTCTGAAAATGGGCTAGG	L47641	54.9	552
POSTN	CCATCTGTGGACAGAAAACG CACCATTTGTTGCAATCTGG	D13666	55	465
S100A2	AGAGGGCGACAAGTTCAAGC ATGAGGGCCAGGAAGACG	BC102570	56.1	192
SPARC	GGCCTGGATCTTCTTTCTCC AGTCCAGGTGGAGTTTGTGG	J03233	55	428
STAT5A	TGACCTGCTCATCAACAAGC AATAGGACCCTGCACACTGG	NM_001012673	54.9	634
TAGLN	GTCCTTCCTATGGCATGAGC CTGTAGGCCGATGACATGC	NM_001046149	54.6	521

In situ *hybridization analyses*

Cell-specific localization of mRNA expression in sections (5 μm) of the ovine uterus was determined by radioactive *in situ* hybridization analysis using methods described previously (Choi *et al.*, 2001b, Spencer *et al.*, 1999b). Radiolabeled antisense and sense cRNA probes were generated by in vitro transcription using linearized plasmid cDNA templates, RNA polymerases, and [α-^{35}S] UTP. Deparaffinized, rehydrated, and deproteinated uterine tissue sections were hybridized with radiolabeled antisense or sense cRNA probes. After hybridization, washing, and ribonuclease A digestion, slides were dipped in NTB-2 liquid photographic emulsion (Kodak, Rochester, NY) and exposed at 4ºC for 3 days to 4 weeks. Slides were developed in Kodak D-19 developer, counterstained with Gill's hematoxylin (Fisher Scientific), and then dehydrated through a graded series of alcohol to xylene. Coverslips were then affixed with Permount (Fisher Scientific).

Immunohistochemistry

Immunolocalization of POSTN protein was performed in crosssections (5 μm) of paraffin-embedded uteri (n=5 ewes/day/status) using specific antibodies and a Vectastain Elite ABC rabbit IgG Kit (PK-6101; Vector Laboratories, Burlingame, CA) according to manufacturer's recommendations. Rabbit polyclonal antibody specific to POSTN protein (ab14041, Abcam Inc., Cambridge, MA) was used for immunohistochemistry at 0.5 μg/ml of final concentration. Negative controls were performed in which the primary antibody was substituted with the same concentration of normal rabbit IgG (Sigma, St. Louis, MO). Multiple tissue sections from each ewe were processed as sets within an experiment. Immunoreactive proteins were visualized using diaminobenzidine tetrahydrochloride (Sigma Chemical Co.). Sections were subsequently dehydrated and coverslips were affixed with Permount.

Western blot analyses

Protein concentrations of uterine flushes were determined using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA) with BSA as the standard. Proteins were denatured and separated by 10% SDS PAGE and Western blot analysis conducted as described previously (Spencer *et al.*, 1999a). Rabbit polyclonal POSTN antibody (cat no. ab14041, Abcam Inc., Cambridge, MA) at 1.0 µg/ml was used in analyses. Blots were washed, and placed in Affinity purified antibody Peroxidase labeled Goat anti-Rabbit IgG (H+L) (1:10,000 dilution of 1 mg/ml stock; cat no. 474-1506, KPL Inc., Gaithersburg, MD). Immunoreactive POSTN protein was detected using enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL) and X-OMAT AR x-ray film (Kodak) according to the manufacturer's recommendations.

Attachment assay

Ovine trophectoderm cells (oTr) were isolated and cultured from Day 15 ovine conceptuses and cultured as described previously (Farmer *et al.*, 2007). Attachment assays were conducted using oTrF cells and methods as described previously (Farmer *et al.*, 2007). Information about Cell suspension plates with 24 wells (Greiner Multiwell Tissue Culture Plates, PGC Scientific Co, Monroe, NC) were coated with either BSA (Bovine Serum Albumin Fraction V, Pierce, Rockford, IL) as a negative control, bovine FN (fibronectin 0.1% [w/v] solution from bovine plasma, Sigma, St. Louis, MO) as a positive control, or recombinant human POSTN protein (R&D Systems, Minneapolis, MN) in triplicate and allowed to dry overnight in a sterile hood at room temperature. Wells were then blocked with 1 ml BSA (10 mg/ml) in PBS for 1 h and rinsed three times with 1 ml per well serum and insulin-free DMEM. Equal numbers of freshly trypsinized oTrF cells were seeded into each well, and plates were incubated for 1.5 h. Wells were washed

three times with 1 ml serum- and insulin-free medium to remove unattached cells. Cell numbers were determined using a Janus Green assay as described previously (Farmer et al., 2007). The entire experiment was repeated at least three times with different passages of oTrF cells.

Migration assay

Migration assays were conducted with oTrF cells as described previously (Farmer *et al.*, 2007). Briefly, oTrF cells (50,000 cells per 100 µl serum and insulin-free DMEM) were seeded on 8 µm pore Transwell inserts (Corning Costar #3422, Corning, NY). Treatments were then added to each well (n=3 wells per treatment) that included combinations of: (a) serum and insulin-free DMEM medium (600 µl); (b) recombinant human POSTN protein (R&D Systems, Minneapolis, MN) at 10 ng or 100 ng; (c) BSA (Bovine Serum Albumin Fraction V, Pierce, Rockford, IL) at 10 ng or 100 ng (d) trophoblast growth medium including serum and insulin as a positive control. After 12 h, cells on the upper side of the inserts were removed with a cotton swab. For evaluation of cells that migrated onto the lower surface, inserts were fixed in 50% ethanol for 5 min. The transwell membranes were then removed, placed on a glass slide with the side containing cells facing up, overlaid with Prolong antifade mounting reagent with DAPI, and overlaid with a cover slip (Invitrogen-Molecular Probes, Eugene, OR, USA). The migrated cells were systematically counted using a Zeiss Axioplan 2 fluorescence microscope with Axiocam HR digital camera and Axiovision 4.3 software (Carl Zeiss Microimaging, Thornwood, NY). The entire experiment was repeated at least three times with different passages of oTrF cells.

Statistical analyses

Data from all quantitative studies were subjected to least squares ANOVA

using the General Linear Models procedures of the Statistical Analysis System (SAS Institute, Cary, NC). Slot blot hybridization data were corrected for differences in sample loading by using the 18S rRNA data as a covariate. Data are presented as the least-squares means with overall standard error (SE). A P value < 0.05 was considered as significant.

Results

In situ hybridization analysis of uteri from cyclic and pregnant ewes (Study One)

In situ hybridization detected *S100A2* mRNA predominantly in the LE and GE of the ovine endometrium as well as the conceptus (Fig. 3.1). In cyclic ewes, *S100A2* mRNA appeared to increase between Days 10 and 16. In pregnant ewes, *S100A2* mRNA was lowest on Days 10 and 12, increased on Days 14 and 16, and decreased on Days 18 and 20. *S100A2* mRNA was present in both conceptus trophectoderm and endoderm.

POSTN mRNA was observed predominantly in the stratum compactum stroma, and caruncle but not in LE, GE or conceptus on Days 18 and 20 (Fig. 3.1). In cyclic ewes, *POSTN* mRNA was lowest on Day 10, increased on Day 12 and was then undetectable between Days 12 and 14. In pregnant ewes, *POSTN* mRNA was constitutively expressed in the stroma and particular abundant in the caruncular stroma.

COL3A1 mRNA was detected in intercaruncular stroma and caruncle of the ovine endometrium and endoderm of the conceptus (Fig. 3.2). In cyclic ewes, *COL3A1* mRNA decreased from Days 10 to 14 and then increased substantially on Day 16. In pregnant ewes, *COL3A1* mRNA was low in the stroma between Days 10 and 20. Abundant *COL3A1* mRNA was detected in the caruncular stroma and the endoderm of conceptuses on Days 18 and 20 of pregnancy.

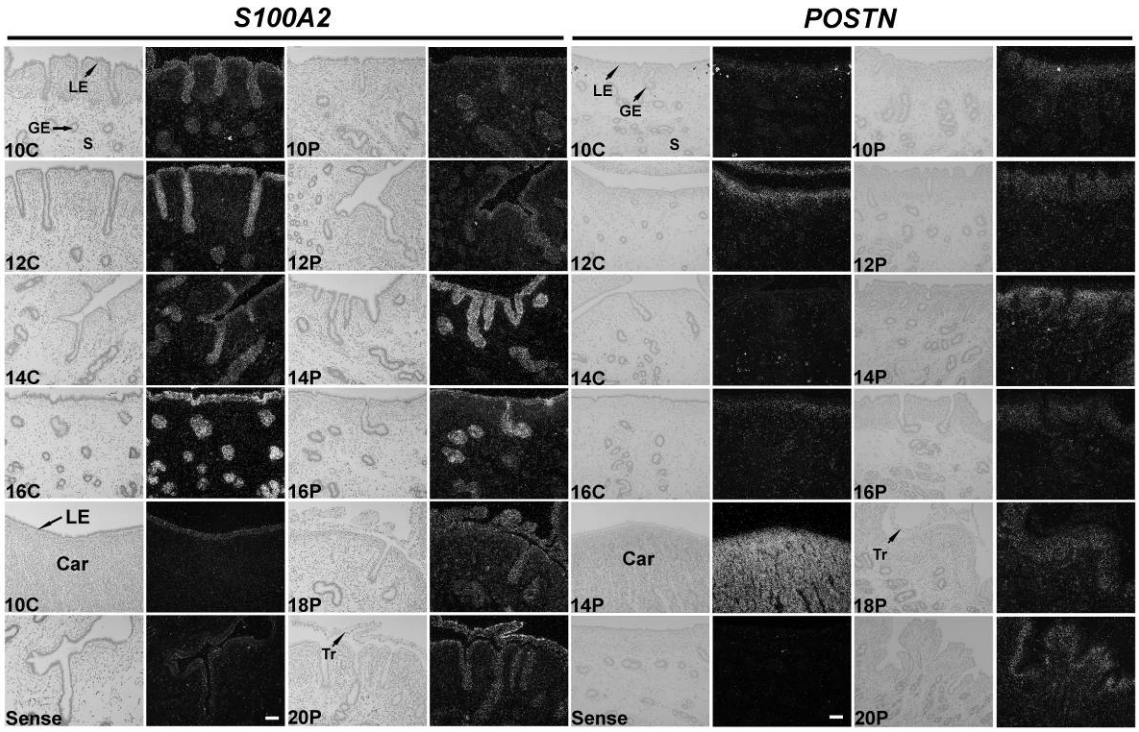

Figure 3.1. *In situ* localization of *S100A2* and *POSTN* mRNAs in cyclic (C) and pregnant (P) ovine endometria. Cross-sections of uteri were hybridized with radiolabeled antisense or sense ovine *S100A2* and *POSTN* cRNA probes. *S100A2* mRNA is localized in LE, GE, and conceptus on Days 18 and 20. *POSTN* mRNA is localized in stroma and caruncle. Bright-field photomicrographs (left) and dark-field photomicrographs (right) were taken for each gene. Legend: LE, luminal epithelium; GE, glandular epithelium; S, stroma; Car, caruncle; Tr, trophectoderm; Scale bar represents 10 μm.

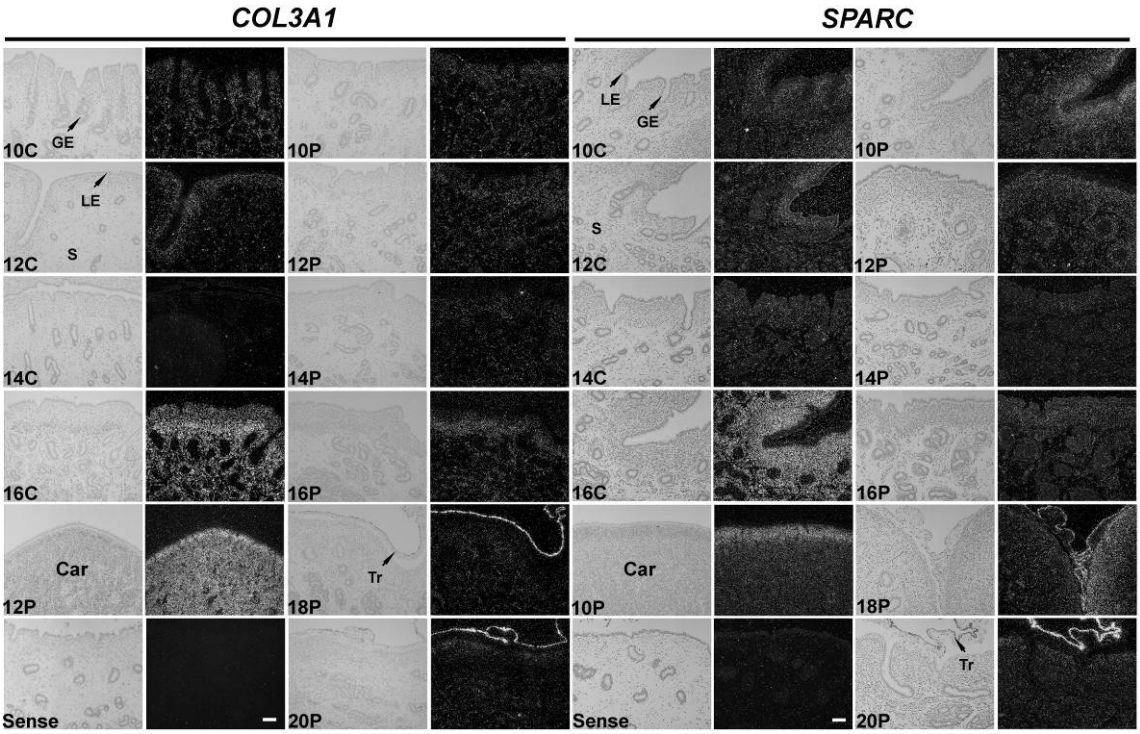

Figure 3.2. *In situ* localization of *COL3A1* and *SPARC* mRNAs in cyclic (C) and pregnant (P) ovine endometria. Cross-sections of uteri were hybridized with radiolabeled antisense or sense ovine *COL3A1* and *SPARC* cRNA probes. *COL3A1* and *SPARC* mRNAs are localized in stroma, caruncle, and conceptus on Days 18 and 20. Bright-field photomicrographs (left) and dark-field photomicrographs (right) were taken for each gene. Legend: LE, luminal epithelium; GE, glandular epithelium; S, stroma; Car, caruncle; Tr, trophectoderm; Scale bar represents 10 μm.

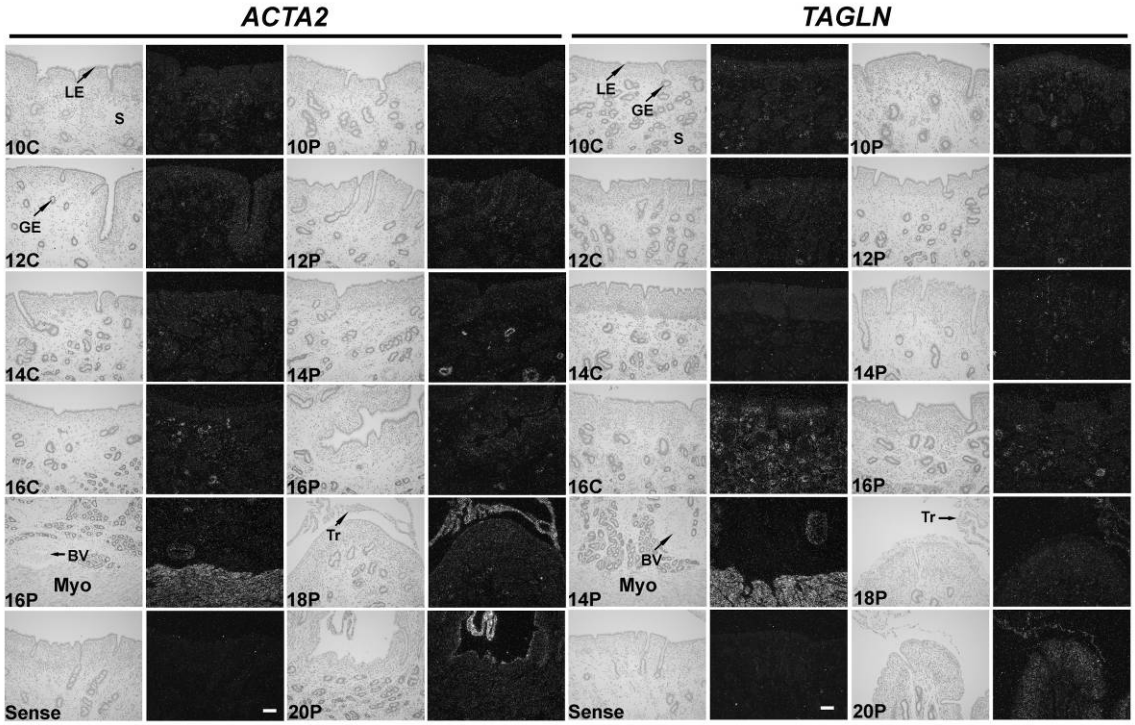

Figure 3.3. *In situ* localization of *ACTA2* and *TAGLN* mRNAs in cyclic (C) and pregnant (P) ovine endometria. Cross-sections of uteri were hybridized with radiolabeled antisense or sense ovine *ACTA2* and *TAGLN* cRNA probes. *ACTA2* mRNA was localized in stroma, myometrium, and conceptus on Days 18 and 20. Bright-field photomicrographs (left) and dark-field photomicrographs (right) were taken for each gene. Legend: LE, luminal epithelium; GE, glandular epithelium; S, stroma; M, myometrium; BV, blood vessel; Tr, trophectoderm; Scale bar represents 10 μm.

Similar temporal and spatial alteration in *SPARC* mRNAs were observed during the cycle and early pregnancy in the ovine uteri.

ACTA2 and *TAGLN* mRNAs were low to undetectable in most cells of the endometrium, except for the blood vessels in both cyclic and pregnant ewes (Fig. 3.3). However, *TAGLN* mRNA was increased in the stroma of Day 16 cyclic ewes. Abundant *ACTA2* and *TAGLN* mRNA was observed in the myometrium both in cyclic and pregnant ovine uteri. Abundant *ACTA2,* but not *TAGLN*, mRNA was expressed in the conceptus trophectoderm on Days 18 and 20 of pregnancy.

STAT5A mRNA was below the limits of detection in both cyclic and pregnant ewes (data not shown).

Steady-state levels on S100A2 and POSTN mRNAs in the cyclic and pregnant endometria (Study One)

The abundance and spatio-temporal pattern of mRNA localization in cyclic and pregnant uteri were considered for *S100A2* and *POSTN* for further study with respect to possible regulation by P4 and/or IFNT. Steady-state levels of *S100A2* and *POSTN* mRNAs in endometria from cyclic and pregnant ewes were determined by slot blot hybridization analyses (Fig. 3.4). *S100A2* mRNA was affected by day and status ($P<0.05$). In cyclic ewes, *S100A2* mRNA increased nearly five-fold between Days 12 and 16 (linear, $P<0.01$). In pregnant ewes, *S100A2* mRNA was increased from Days 12 to 14 and then decreased to Day 20 (quadratic, $P<0.05$). *POSTN* mRNA was also affected by day and status ($P<0.05$). In cyclic ewes, *POSTN* mRNA was high on Day 12 and then decreased till Day 16 (linear, $P<0.05$). In pregnant ewes, *POSTN* mRNA increased from Day 10 to Day 12, and remained elevated thereafter.

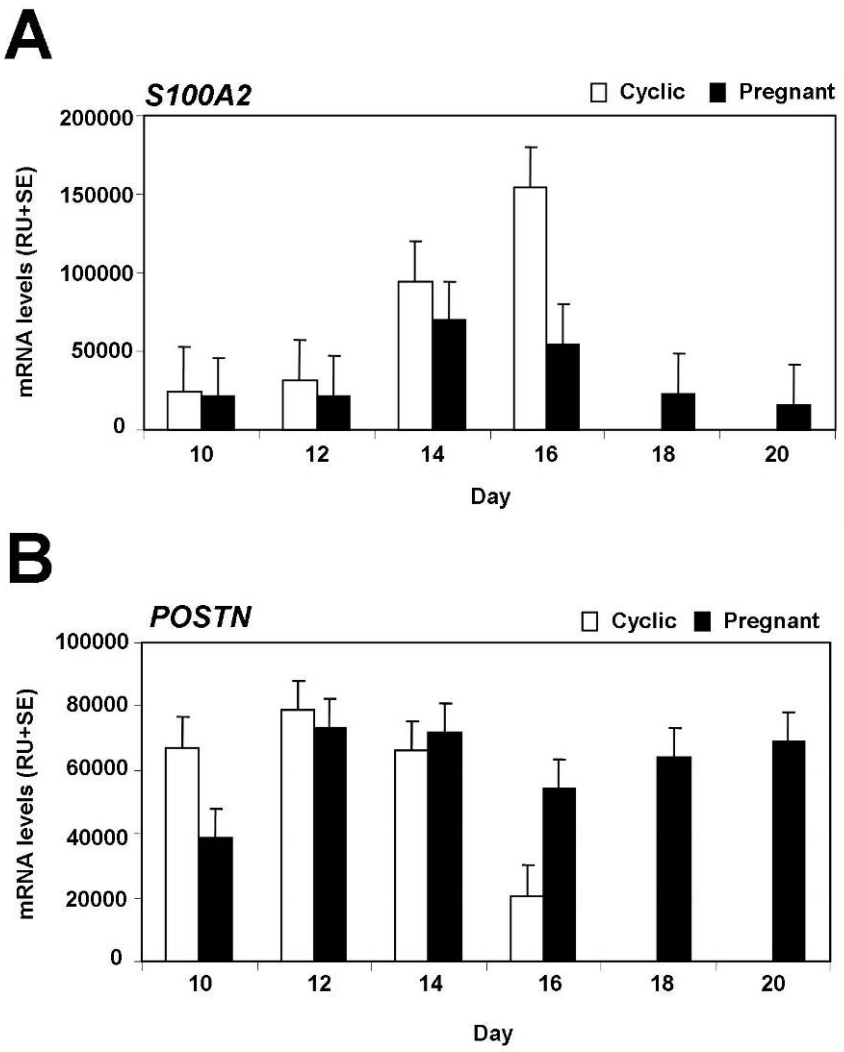

Figure 3.4. Steady-state levels of *S100A2* (A) and *POSTN* (B) mRNAs in endometria from cyclic and early pregnant ewes as determined by slot blot hybridization analysis. (A) *S100A2* mRNA was increased from Day 12 to Day 16 ($P<0.01$) in cyclic ewes. In pregnant ewes, *S100A2* mRNA was increased from Day 12 to Day 14 ($P<0.01$) and decreased to Day 20 ($P<0.05$). (B) In cyclic ewes, *POSTN* mRNA was high in Day 12, then decreased till Day 16 ($P<0.05$). In pregnant ewes, *POSTN* mRNA was increased on Day 12, decreased till Day 16 ($P<0.05$) and maintained thereafter. Data are presented as least-square mean relative units (RU) with standard error (SE).

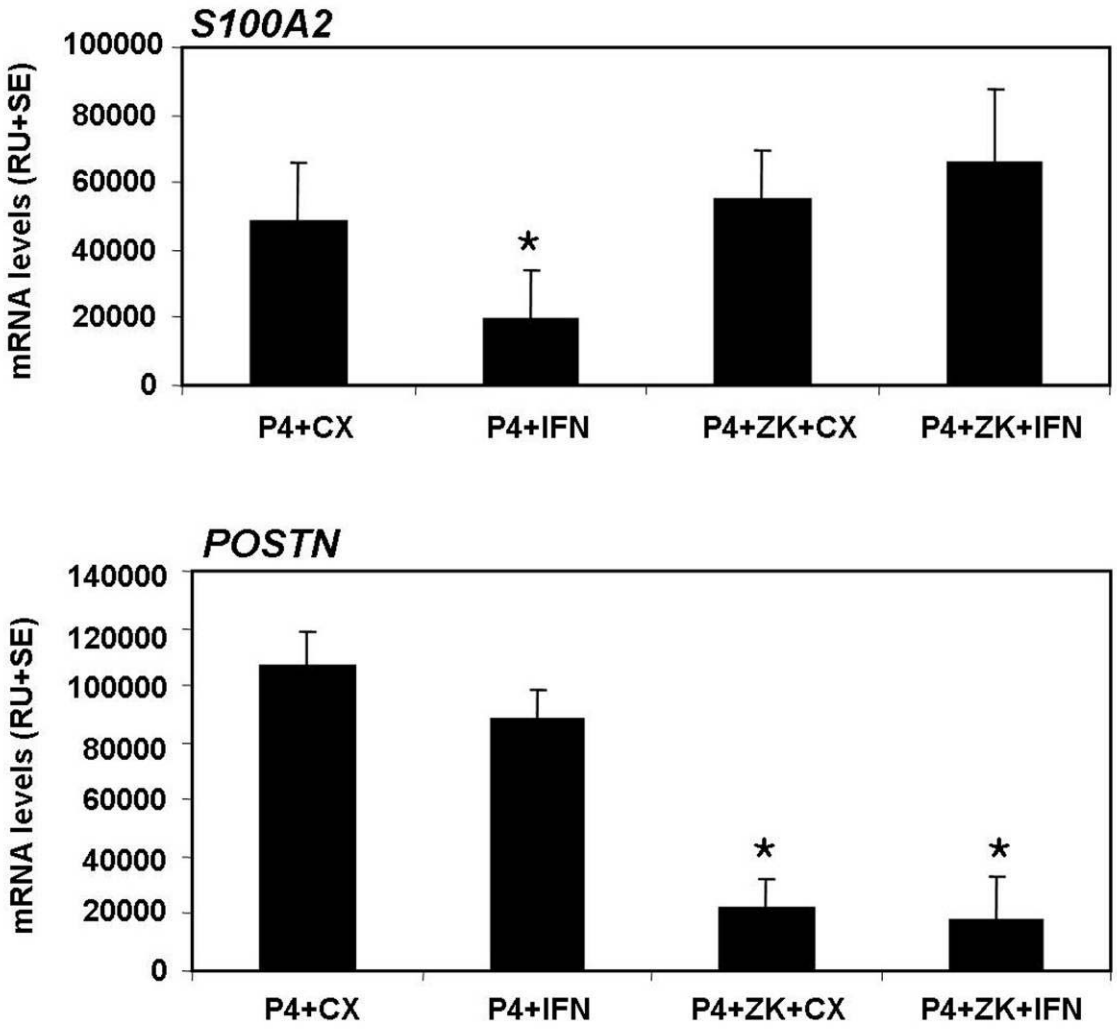

Figure 3.5. Steady-state levels of *S100A2* and *POSTN* mRNAs in endometria from Study Two. Intrauterine infusion of IFNT decreased *S100A2* mRNA in the presence of P4 alone (P4+CX vs P4+IFN, $P<0.05$) but not in P4+ZK treated ewes (P4+ZK+CX vs P4+ZK+IFN, $P>0.10$). The PGR antagonist ZK treatment tended to reduce *POSTN* mRNA (P4+CX vs P4+ZK+CX, $P>0.05$) but roIFNT did not affect to *POSTN* mRNA levels (P4+CX vs P4+IFN, $P>0.10$). The asterisk (*) denotes an effect of treatment. Data are presented as least-square mean relative units (RU) with standard error (SE).

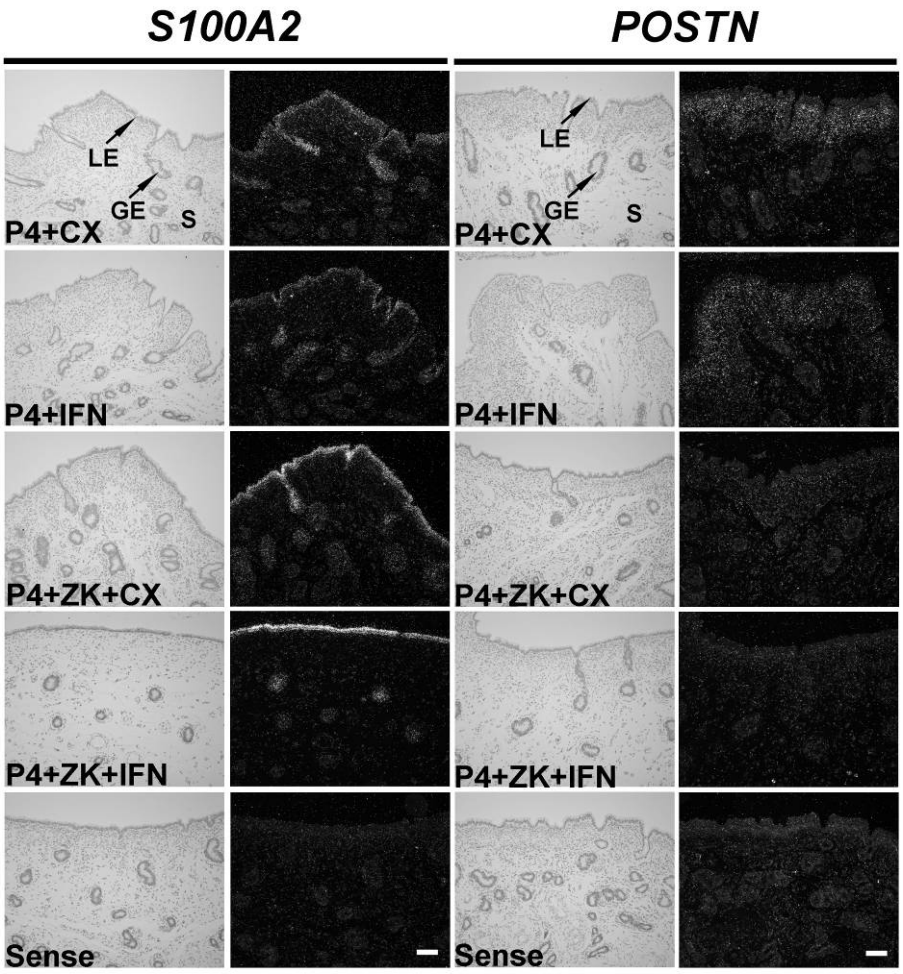

Figure 3.6. *In situ* localization of *S100A2* and *POSTN* mRNAs in endometria from pregnant ewes in Study Two. *S100A2* mRNA tended to be less abundant in P4+IFN treated ewes compared to ewes from other treatment groups. P4 increased *POSTN* mRNA in the stroma (primarily stratum compactum stroma) of P4-treated ewes (P4+CX and P4+IFN). Legend: LE, luminal epithelium; GE, glandular epithelium; S, stroma; Scale bar represents 10 μm.

Effects of P4 and IFNT on S100A2 and POSTN mRNA expression (Study Two)

To determine effects of P4 and IFNT on *S100A2* and *POSTN*, Study 2 was conducted as described in Materials and Methods. Briefly, cyclic ewes were ovariectomized on Day 5, treated with P4 or P4 and the PR antagonist ZK136,317, and intrauterine infusion of control protein (CX) or recombinant ovine IFNT (roIFNT). As shown in Fig. 3.5, intrauterine roIFNT decreased steady-state levels of *S100A2* mRNA about 2.5-fold (P4+CX vs P4+IFN, $P<0.05$). Treatment of ewes with the P4 did not affect *S100A2* mRNA (P4+CX vs P4+ZK+CX, $P>0.10$). Intrauterine roIFNT did not affect *S100A2* mRNA levels in endometria from ewes receiving P4+ZK (P4+ZK+CX vs P4+ZK+IFN, $P>0.10$).

For ewes receiving only P4, roIFNT did not affect steady-state levels of *POSTN* mRNAs in the endometria (P4+CX vs P4+IFN, $P>0.10$). However, treatment of ewes with the ZK 136,317 PGR antagonist decreased *POSTN* mRNA about 4.8-fold (P4+CX vs P4+ZK+CX, $P=0.001$). *In situ* hybridization results (Fig. 3.6.) confirm that *S100A2* mRNA was reduced in P4+IFNT treated ewes in the LE and superficial GE (sGE). Increased *POSTN* mRNA was localized in the stratum compactum stroma of P4-treated ewes (P4+CX and P4+IFNT) but not P4+ZK treated ewes (P4+ZK+CX and P4+ZK+IFN).

Immunoreactive POSTN protein

POSTN protein was observed in the stratum compactum stroma, area adjacent to GE, and caruncles in the ovine endometrium (Fig. 3.7). In the stratum compactum stroma, POSTN protein was localized either in the subepithelial area adjacent to LE or in areas in close proximity to stratum spongiosum stroma. Abundance of POSTN protein during the estrous cycle and early pregnancy is summarized in Table 3.3. In cyclic ewes, POSTN protein increased on Day 12 and was maintained. In pregnant ewes, POSTN protein increased on Day 12,

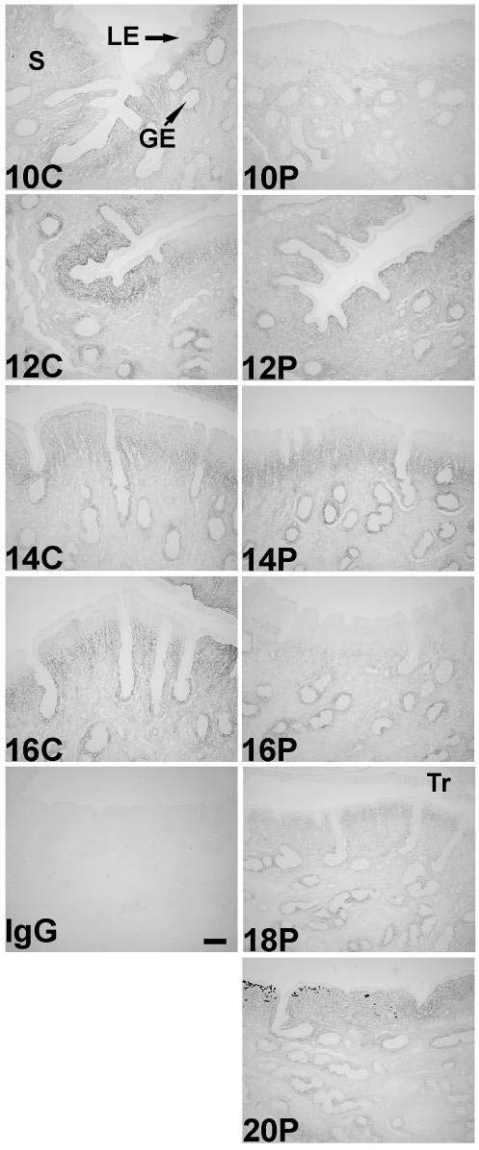

Figure 3.7. Immunolocalization of POSTN protein in cyclic (C) and pregnant (P) ovine endometria. Immunoreactive proteins were detected using specific rabbit polyclonal antibody against POSTN protein. For the IgG control, normal rabbit IgG was substituted for the primary antibody. POSTN protein was localized in the stroma, area adjacent to GE, and caruncle. POSTN protein increased on Day 12 in both cyclic and pregnant ewes and was maintained in cyclic ewes, but decreased in pregnant ewes. Legend: LE, luminal epithelium; GE, glandular epithelium; S, stroma; Tr, trophectoderm. Scale bar represents 10 μm.

Table 3.3. Distribution and relative abundance of immunoreactive POSTN protein in endometria of cyclic and pregnant ewes in Study One.[a]

		Endometrium							
		Intercaruncular					Caruncular		
PND	status	LE	GE, shallow	GE, deep	Stroma, compactum	Stroma, spongiosum	LE	Stroma	Myometrium
10	C	-	-	-	++/+++	+	-	+/++	-
12	C	-	-	-	+++	+	-	+	-
14	C	-	-	-	++	+	-	++	-
16	C	-	-	-	++/+++	+	-	N.D. [b]	-
10	P	-	-	-	-/+	+	-	+	-
12	P	-	-	-	++/+++	+	-	++	-
14	P	-	-	-	++/+++	+	-	-	-
16	P	-	-	-	-/+	+	-	++	-
18	P	-	-	-	++	+	-	++/+++	-
20	P	-	-	-	++/+++	+	-	++/+++	-

[a] Protein staining intensity was evaluated visually as absent (-), weak (+), moderate (++), or strong (+++).

[b] N.D. Not determined

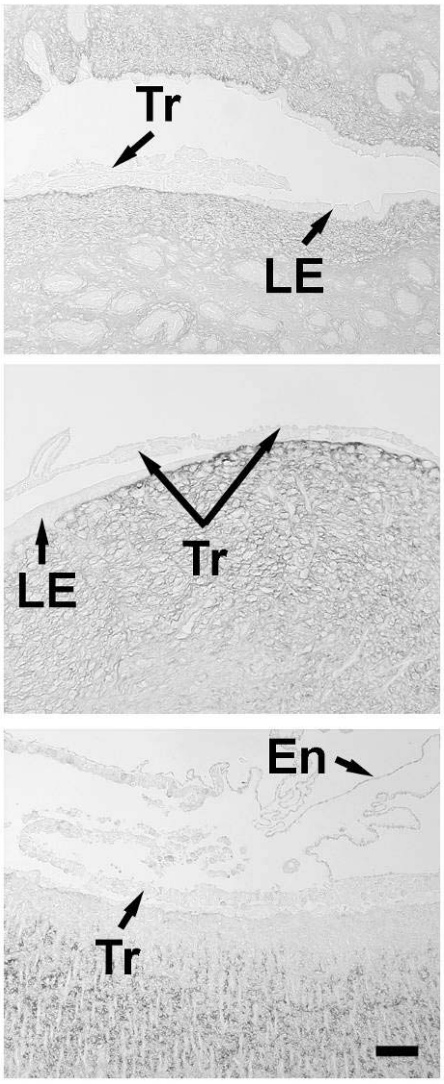

Figure 3.8. Representative photomicrographs of immunoreactive POSTN protein in endometria from pregnant ewes between Days 16 to 20. Abundant POSTN protein was in the stratum compactum stroma proximal to the LE. In the caruncle, crystallization of POSTN protein was noted but this was not detected in either cyclic or pregnant ewes from earlier days. Legend: LE, luminal epithelium; Tr, trophectoderm; En, endoderm; Car, caruncle; Cr, crystals; Scale bar represents 20 µm.

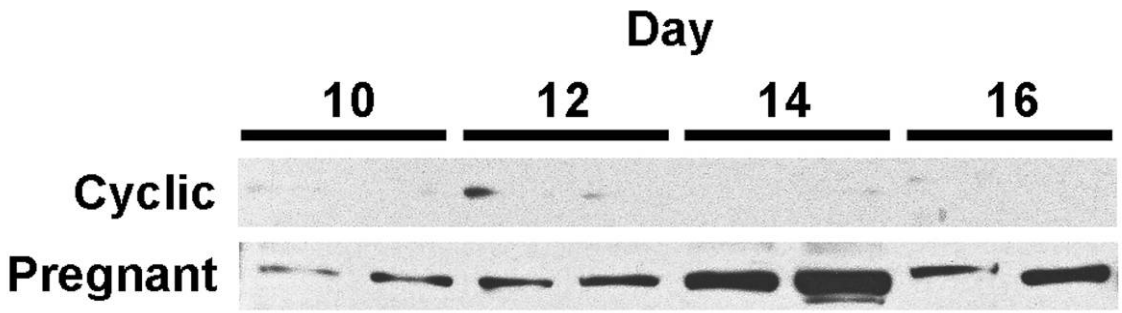

Figure 3.9. Representative Western blot analysis of POSTN protein in uterine flushes from cyclic and pregnant ewes. Proteins in uterine flushes were separated by 10% SDS-PAGE (40µg/lane), and immunoreactive POSTN protein was detected using specific rabbit polyclonal antibody. POSTN protein of about 90 kDa was detected in pregnant, but not cyclic ewes on Days 14 and 16.

decreased to Day 16, and then increased again. Less POSTN protein was detected in pregnant than cyclic ewes on Day 16. On Days 16 to 20 of pregnancy, interesting features were found (Fig. 3.8). In intercaruncular areas and on the sides of concave caruncle, abundant POSTN protein was accumulated at the apical area of the endometrium just beneath the LE. In the caruncles, focal accumulation of POSTN protein on Days 18 and 20 was different from cyclic ewes and pregnant ewes on earlier days. A 90 kDa immunoreactive POSTN protein was also detected in uterine flushes (Fig. 3.9). POSTN protein was detected primarily in the uterine flushes from pregnant ewes and was most abundant on Days 14 and 16 of pregnancy.

Effect of POSTN on attachment and migration of trophectoderm cells

POSTN protein promotes cell adhesion and motility via integrin αvβ3 and αvβ5 subunits (Gillan *et al.*, 2002). Integrins, a family of heterodimeric transmembrane glycoprotein receptors, bind extracellular matrix (ECM) proteins to mediate cellular differentiation, motility and adhesion through numerous cell signaling pathways (Giancotti & Ruoslahti, 1999). During the periimplantation period of pregnancy in sheep, integrin subunits α (v,4,5) and β (1,3,5) are constitutively expressed on apical surfaces of both conceptus trophectoderm and endometrial LE (Johnson *et al.*, 2001a). Therefore, attachment and migration analyses were conducted to test the hypothesis that POSTN affects attachment and migration function of ovine trophectoderm cells (Fig. 3.10). For the attachment assay, wells of suspension culture plates were precoated with either 10 ng and 100 ng of recombinant human POSTN (rhPOSTN), bovine albumin serum (BSA) as the negative control, or bovine fibronectin (bFN) as the positive control. For migration assay, cells were treated in serum and insulin-free DMEM with 10 ng and 100 ng of rhPOSTN protein or BSA and cultured in a Transwell plate. Relative to wells coated with BSA, higher concentration of rhPOSTN

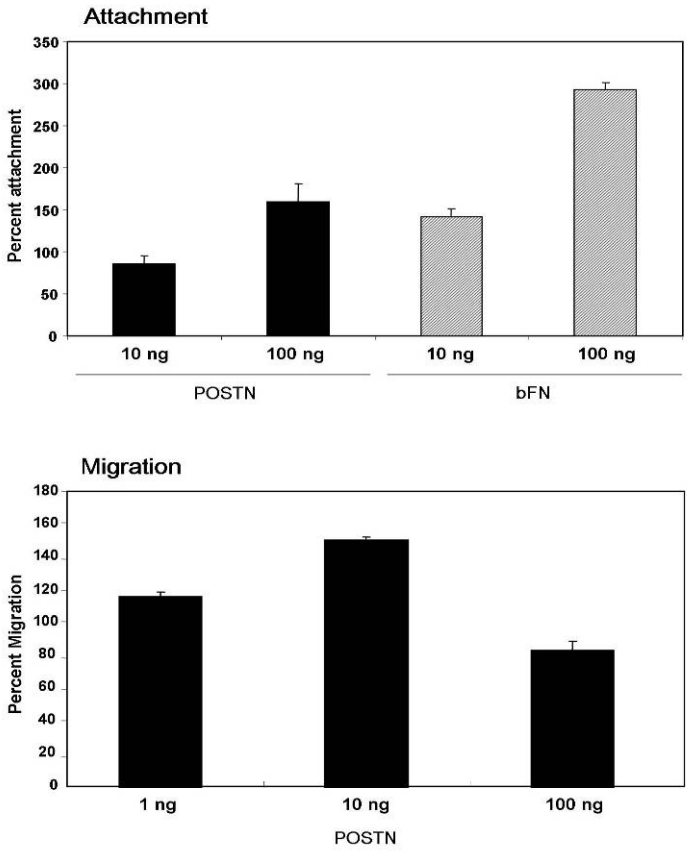

Figure 3.10. POSTN stimulates attachment and migration of ovine trophectoderm cells. For attachment, wells of suspension culture plates were precoated overnight with either BSA (negative control), bovine fibronectin (positive control), or recombinant human POSTN. Equal numbers of cells were added to each well, and the number of attached cells was determined after 1.5hr of incubation. For migration, cells were cultured in a Transwell plate in serum- and insulin-free medium and treated with recombinant human POSTN. Data are presented as percent increase of attachment or migration on POSTN treatment groups above corresponding BSA treatment groups. Higher concentration of recombinant POSTN protein stimulated attachment of oTrF cells. 10ng of recombinant POSTN protein was the most effective treatment in stimulating migration of oTrF cells.

(100ng) stimulated greater cell attachment. However, 10ng of rhPOSTN was most effective in stimulating oTrF cell migration.

Discussion

There was discordance between results from the microarray analysis (Gray *et al.*, 2006) and corroboration analysis of several genes examined in this study. The endometrium is an organ of dynamic change, with autocrine and paracrine factors acting in a spatio-temporal manner, under complex regulation by hormones and their relevant receptors, and with various cell signaling pathways being activated during the estrous cycle and early pregnancy. Thus, comparing differential gene regulation between one specific day of the estrous cycle and pregnancy or among treatment groups using microarray data may not provide the entire frame of regulation of expression of a artcitular gene during the peri-implantation period of pregnancy. These factors highlight the necessity of corroborating microarray data in studies of gene expression by cells of the endometrium. In complex tissues like uterine endometria, localization of mRNA using *in situ* hybridization provides important temporal and spatial information about gene expression that allows increased insight into the mechanistic functions of gene products within complex epitheliomesenchymal tissues (White & Salamonsen, 2005). In addition, a modern technique laser-capture microdissection (LCM) enables isolation of LE and GE from the endometria to identify genes uniquely expressed in the LE or GE during implantation in mice (Niklaus & Pollard, 2006). Furthermore, gene profiling using microarray analyses must extend to proteomic and functional genomic research (Horcajadas *et al.*, 2007) to understand the ultimate roles of genes in organ systems.

ACTA2 was expected to be downregulated by pregnancy and *TAGLN* upregulated by IFNT based on microarray data (Gray *et al.*, 2006). *In situ*

hybridization revealed that abundant *ACTA2* mRNA primarily in the myometrium as studies in human and monkey and indicated that *ACTA2* is the primary type of actin in the myometrium (Cavaille, 1985, Cavaille *et al.*, 1986). However, *ACTA2* and *TAGLN* mRNAs were not detected in LE, GE or stroma in the present study although these cell types have major roles in early pregnancy, and no changes in these mRNAs were found in either myometrium or blood vessels. Therefore, the results of the present study do not confirm P4 and/or IFNT regulation of ACTA2 and TAGLN in ovine endometria during pregnancy. Abundant *ACTA2* mRNA was maintained throughout pregnancy and there was an alteration in gamma-actin mRNA expression in myometrium of rat uteri (Shynlova *et al.*, 2005). During early pregnancy in sheep, low levels of ESR1 and PGR in the myometrium allows P4 to maintain a quiescent myometrium (Spencer & Bazer, 1995). Also, α-smooth muscle actin is a marker of decidualization-like changes of stromal cells, along with secreted phosphoprotein 1 or osteopontin (SPP1) and desmin. Alpha-smooth muscle actin mRNA appears on intercaruncular and caruncular areas of stromal cells from Day 25 through Day 120 of pregnancy but not during the estrous cycle or earlier stages of pregnancy which is cinsistent with results of the present study (Johnson *et al.*, 2003b). *TAGLN*, also called as SM22α, is a marker for smooth muscle cells and smooth muscle-containing tissues including the uterus (Duband *et al.*, 1993, Gimona *et al.*, 1992, Lees-Miller *et al.*, 1987, Shanahan *et al.*, 1993). In the present study, *TAGLN* mRNA was localized in similar pattern to that for *ACTA2* mRNA in myometrium, stroma and blood vessels, but not conceptus. Interestingly, *TAGLN* mRNA was highly expressed on Day 16 of pregnancy, which is different from *ACTA2* mRNA in uteri on the same day. *TAGLN* is a repressor for *MMP9* (Nair *et al.*, 2006), a member of metalloproteinases (MMPs) which plays a role in uterine endometrial tissue remodeling, and *MMP9* regulated by estrogen in mouse uterus (Zhang *et al.*, 2007). *TAGLN* mRNA expression might be effected by estrogen in cyclic ewes as shown in Fig. 3.3. Even though MMPs in ovine uteri during early pregnancy are not well characterized, their inhibitors are regulated by

P4 or IFNT, e.g., cathepsin L (CTSL) and cystatin C (CST3) (Song *et al.*, 2006, Song *et al.*, 2007a) implicate potential actions during early pregnancy. Collectively, results indicate that *TAGLN* may have effects in the ovine uterus other than actin-organization.

COL3A1 and *SPARC* were expected to be down-regulated by IFNT and pregnancy (Gray *et al.*, 2006). Pregnancy was associated with down-regulation of *COL3A1* mRNA, but gene expression was not regulated by either P4 or IFNT (see Fig. 3.2). In this study, *COL3A1* mRNA was quite abundant during proestrus of ewes (Day 16 cycle; Fig. 3.2), suggesting that estrogen upregulates *COL3A1* in the ovine uterine stroma. Estrogen regulates expression of collagen types I, III, and V in rat uteri (Frankel *et al.*, 1988) and uterine endometria of postmenopausal women treated with estrogen had increased abundance of collagen types I, III and VI in endometrial stroma. Interestingly, administration of estrogen not only increased collagen types I and III, which are commonly colocalized, but also changed the ratio between two (Iwahashi *et al.*, 1997). *COL3A1* is the collagen type increases tensile strength (Aumailley & Gayraud, 1998, Burgeson & Nimni, 1992, van der Rest & Garrone, 1991). Less collagen fibrils surrounding endometrial stromal cells were found during early pregnancy in mice (Finn, 1971). Reduced *COL3A1* in pregnant compared to cyclic uteri might be related to the decrease in junctional complexes in the uterus for easier paracellular transport of molecules to and from the conceptus. Junctional proteins in the LE decrease from Day 12 of both cyclic and pregnant ewes, making a potentially “leaky” paracellular space that would allow transport of molecules from the uterine stroma and/or selective movement of serum transudate directly to the conceptus (Satterfield *et al.*, 2007). SPARC, a matricellular protein, is not an ECM component itself, but it modulates cell-matrix interactions and cell function (Bornstein & Sage, 2002). SPARC is an upstream regulator of several collagens including COL3A1 (Zhou *et al.*, 2006). *SPARC* and *COL3A1* had simimar mRNA localization patterns (see Fig. 3.2.), suggesting possible regulation of both *COL3A1* and *SPARC* by estrogen in

cyclic ewes. It should also be noted that COL3A1 and SPARC are particularly abundant in conceptus trophectoderm. *COL3A1* and *SPARC* are coordinately down-regulated in IL11 receptor α-null mice which are unable to undergo normal uterine decidualization (White *et al.*, 2004). Collectively, *SPARC* and *COL3A1* may have a cooperative role during early pregnancy in ovine uterine endometrium and/or conceptus trophectoderm. In this study, *COL3A1* and *SPARC* mRNAs were observed in endoderm of the ovine conceptus. During extramebryonic membrane formation, endoderm splits into two layers and give rise to yolk sac and allantois. In bovine conceptuses, *COL3A1* mRNA was detected low on Day 20 and increased through Day 36 in the chorioallantois. COL3A1 is the major secretory protein from bovine chorioallantoic membranes and associated with development of placenta (Shang *et al.*, 1997). Also, *Sparc* is detected in extraembryonic membranes and placenta (Howe *et al.*, 1988). Thus, it is speculated that COL3A1 and SPARC secreted from endoderm together play a role in extraembryonic development in the ovine conceptus as well. Moreover, Sparc secreted from mesenchymal parietal endoderm cells enhances early cardiomyogenesis *in vitro* (Stary *et al.*, 2006), suggesting similar role of SPARC in ovine embryogenesis.

S100A2 was expected to be downregulated by IFNT (Gray *et al.*, 2006) and the present study confirmed that *S100A2* is an IFNT downregulated gene. As illustrated in Fig. 3.5, IFNT down-regulated *S100A2* mRNA only in ewes treated with P4 (P4+CX vs P4+IFN). However, in the absence of P4, IFNT did not down-regulate *S100A2* mRNA (P4+ZK+CX vs P4+ZK+IFN). This result suggests that IFNT regulation on *S100A2* is P4-dependent. Previous reports are contradictory in that *S100A2* does not have IFN response elements (IREs), but IFN-α (IFNA) which is a member of Type I IFN induced *S100A2* expression in human melanoma cells with co-stimulation of the transforming growth factor beta 1 (TGFB1) signaling pathway (Foser *et al.*, 2006). The roles of *S100A2,* other than tumor suppressor and in the uterus, have not been reported. One explanation might be that *S100A2*

regulates prostaglandin-endoperoxide synthase 2 or COX2 (*PTGS2*). *S100A2* suppresses *PTGS* mRNA in squamous cell carcinoma (Salama *et al.*, 2007, Tsai *et al.*, 2006). In ovine endometrium, *PTGS2* mRNA levels are higher in pregnant than cyclic ewes, which is opposite to the expression pattern for *S100A2* mRNA. In addition, *PTGS2* and *S100A2* mRNAs are both localized to LE and sGE of uteri from cyclic and pregnant ewes (Kim *et al.*, 2003a). Expression of *S100A2* on conceptus or trophectoderm cells has not been reported. S100A8 is a member of S100 alpha family and it regulates fetal-maternal rejection that embryo is resorbed by the mother in *S100A8* null mice (Passey *et al.*, 1999). Therefore, it will be interesting to examine S100A2 at the time of implantation to find out its role in fetal-maternal interaction in sheep.

This is the first study on *POSTN* in early pregnancy and implantation. POSTN, a secreted ECM protein originally isolated from osteoblasts (Takeshita *et al.*, 1993), shares homology with fasciclin I, which is a cell adhesion protein guiding growth cone in insects (Elkins *et al.*, 1990). *POSTN* increased cell migration, invasion and adhesion in tumorigenic 293T cells (Yan & Shao, 2006) and *POSTN* has been reported in human uterus, placenta and mammary gland. Recombinant POSTN protein stimulated ovarian epithelial cell adhesion and motility via αvβ3 and αvβ5 integrins (Gillan *et al.*, 2002). Female *POSTN* null mice are infertile due to failure to cycle, but male null mice are fertile (Rios *et al.*, 2005). *POSTN* was expected to be up-regulated by P4, down-regulated by IFNT and up-regulated by pregnancy (Gray *et al.*, 2006). Result from this study indicates that *POSTN* is regulated by P4 but neither IFNT or pregnancy regulated its expression. As an ECM component, *POSTN* mRNA was localized in the intercaruncular stratum compactum stroma and caruncle of ovine uteri. As illustrated in Fig 3.8, abundant POSTN protein was localized along at the apical side of stratum compactum stroma and the site of conceptus attachment on Days 18 and 20 of pregnancy, while it was absent in the conceptus. Functional studies also demonstrated that rhPOSTN protein stimulated attachment of ovine

trophectoderm cells *in vitro*. *POSTN* contains FAS-1 binding domain which enables POSTN to interact with integrin (Kim *et al.*, 2000). With stimulation of growth factors like FGF2, *POSTN* stimulates vascular smooth muscle cells *in vitro* and effects of *POSTN* were inhibited by silencing PI3K-AKT cell signaling pathway (Li *et al.*, 2006) which is present in the ovine trophectoderm (Jin-Young Kim, unpublished results). POSTN may act as a ligand to induce signaling cascade like PI3-kinase signaling pathway to stimulate attachment and/or migration of trophectoderm cells. These results suggest that POSTN secreted either into the uterine lumen or transported between paracellular spaces and the stroma may serves as an ECM ligand for integrin subunits present on conceptus trophectoderm. It may then activate unknown or known signaling pathways (e.g., PI3-kinase signaling pathway (Yan & Shao, 2006)) to support attachment during via interactions with integrin subunits in a dose dependent manner. Abundant POSTN was found in caruncles on Days 18 and 20 of pregnancy, and *POSTN* is expressed in the human and mouse placentae (Gillan *et al.*, 2002, Rios *et al.*, 2005). Thus, POSTN in the caruncle may have an unknown role in placentation and proper function of the placenta during mid and late pregnancy.

Collectively, results from the present study confirm that *S100A2* and *POSTN* are IFNT and P4 regulated genes, respectively. Above all, *POSTN* was not only one of the stromal ECM proteins, but it also stimulated attachment of ovine trophectoderm cells similar to LGALS15 and SPP1 (Farmer *et al.*, 2007, Johnson *et al.*, 2003a). Future studies will be directed toward identification of signaling pathways involved in conceptus implantation following activation by POSTN. As an IFNT downregulated gene, *S100A2* might have affect in either luteotrophic/luteolytic mechanism involving prostaglandins. Functional studies for *S100A2* related to action of *PTGS2* on synthesis of prostaglandins during early pregnancy will be essential.

CHAPTER IV
EXPRESSION OF INTERFERON STIMULATED GENES IN THE CORPUS LUTEUM DURING EARLY PREGNANCY IN SHEEP

Introduction

Interferon tau (IFNT), a member of the type I interferon (IFN) family, is the pregnancy recognition signal from conceptuses of ruminants. Ovine IFNT is secreted from mononuclear cells of the conceptus trophectoderm between Days 10 and 20 with maximal production between Days 14 to 16 (Bazer *et al.*, 1997, Roberts *et al.*, 1999). During early pregnancy, IFNT acts in a paracrine manner on the uterine endometrium to block production of luteolytic $PGF_{2\alpha}$ pulses by suppressing expression of estrogen receptor alpha (ESR1) and thus oxytocin receptor (OXTR) in the luminal epithelium (LE) and superficial glandular epithelium (sGE), therefore preventing regression of CL (Bazer, 1992). Also, IFNT stimulates expression of IFN-stimulated genes (ISGs) that appear to play roles in endometrial differentiation and conceptus implantation during early pregnancy (Spencer & Bazer, 2004).

Administration of recombinant ovine IFNT (roIFNT) by intrauterine (i.u.) or subcutaneous (s.c.) injections was both effective in induction of ISGs (*MX1* and *ISG15*) in the CL whereas only i.u. roIFNT was effective in induction of ISGs and suppression of ESR1 and OXTR in the uterine endometrium (Spencer *et al.*, 1999b). This result generated a hypothesis that both i.u. (paracrine) and s.c. (endocrine) IFNT acts on CL to induce expression of ISGs and three potential mechanisms were suggested. First, IFNT may exit the uterus and act in an endocrine manner to induce ISGs in the CL. Second, IFNT might induce substances in the endometrium that have paracrine/endocrine effects on the ovary and CL. Third, IFNT may exert effects on uterine blood cells, uterine mucosa or

circulating immune cells which could then exert effects on the uterus and the CL (Spencer *et al.*, 1999b, Yankey *et al.*, 2001).

Supporting the hypothesis that IFNt exerts effects on uterine blood cells, uterine mucosa or circulating immune cellsm uterus and CL, several reports indicated effects of IFNT on the circulating immune cells during early pregnancy. In pregnant ewes, *MX1* expression in peripheral blood mononuclear cells (PBMCs) is increased (Yankey *et al.*, 2001), and *MX1*, *MX2*, and *ISG15* mRNAs are more abundant in peripheral blood leukocytes (PBLs) of pregnant dairy cows (Gifford *et al.*, 2007), whereas *ISG15* mRNA was low in blood cells from non-pregnant dairy cows (Han *et al.*, 2006). Moreover, *RSAD2* expression in immune cells in the uterine stroma is induced by IFNT (Song *et al.*, 2007b).

Therefore, it was hypothesized that IFNT stimulates immune cells in the endometrium that travel to the CL to modify its function. Expression of ISGs by the ovine endometrium during the estrous cycle and early pregnancy has been investigated, but a similar analysis has not been performed in CL. Therefore, this study was conducted to determine expression of ISGs in CL during the period of IFNT secretion in ewes. ISGs known to be expressed by the ovine endometrium, i.e., *MX1, MX2, ISG15, OAS1,* and *RSAD2* were evaluated in ovine CL at the mRNA level.

Materials and methods

Animals

Mature crossbred Suffolk ewes (Ovis aries) were observed daily for estrus in the presence of vasectomized rams and were used in experiments after they had exhibited at least two estrous cycles of normal duration (16 to 18 days). At estrus, ewes were assigned randomly to cyclic or pregnant status. All experimental and surgical procedures were in agreement with the Guide for the Care and Use of Agriculture Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas A&M University.

Experimental design

At estrus (Day 0), ewes were mated to an intact ram (n=5 ewes/day). The uterine lumen was flushed with 20 ml sterile 10 mM Tris-HCl (pH 8.0) to confirm pregnancy by observing the presence of one or more morphologically normal conceptuses. Corpora lutea were collected from ovaries of pregnant ewes on Days 13, 14, 15, 16 and 18. Corpora lutea and endometria were frozen in liquid nitrogen, and stored at - 80 °C for subsequent RNA extraction.

RNA isolation

Total cellular RNA was isolated from frozen samples of endometrium using Trizol reagent (Life Technologies, Inc., Bethesda, MD) according to manufacturer's recommendations. The quantity and quality of total RNA was determined by spectrometry and denaturing agarose gel electrophoresis, respectively.

Cloning of partial cDNA

Partial cDNAs for ovine *MX1, MX2, ISG15, OAS1, and RSAD2* were generated by RT-PCR using total RNA from Day 16 or Day 18 pregnant ovine endometria using specific primers based on ovine *MX1* (Genbank EF028200; forward, 5'-ACA TGA AAC GGA GTC CAA GG-3', reverse, 5'- TGC CAG GAA GGT CTA TCA GG -3'), ovine *MX2* (Genbank AY859475; forward, 5'- AGG TCA TGC AGA ACC TCA CC -3', reverse, 5'- TAA TTT CCA TGG CCT TCT GG -3'), and bovine *OAS1* (Genbank NM178108; forward, 5'- ATC AGG AGA CAG CTG GAA GC -3', reverse, '- TCC AAA ACA GTC CGA AAT CC - 3') using methods described previously (Hayashi *et al.*, 2007). Primers for ovine *ISG15* and the plasmid for ovine *RSAD2* (Song *et al.*, 2007b) were kindly provided by Dr. Gwonhwa Song (Texas A&M University, College Station, TX, USA). PCR amplification was conducted as follows: 1) 95ºC for 2 min; 2) 95ºC for 30 sec; 3) 54.6ºC (for *OAS1*), 54.9ºC (for *MX2*) and 55ºC (for *MX1* and *ISG15*) for 1min; 4) 72ºC for 1 min for 33 cycles; and 5) 72ºC for 7 min. Partial cDNAs for ovine *MX1, MX2, ISG15* and *OAS1* PCR products of the correct size were cloned into pCRII using a T/A cloning kit (Invitrogen) and their sequences verified using an ABI PRISM Dye Terminator Cycle Sequencing Kit and ABI PRISM automated DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA).

Slot blot hybridization analyses

Steady-state levels of mRNA in ovine endometria were assessed by slot blot hybridization using methods described previously (Song *et al.*, 2007a). Radiolabeled antisense and cRNA probes were generated by *in vitro* transcription using linearized plasmid cDNA templates, RNA polymerases and [α-^{32}P] UTP. Denatured total endometrial RNA (20 µg) from each ewe was hybridized with radiolabeled cRNA probes. To correct for variation in total RNA loading, a duplicate membrane was hybridized with radiolabeled antisense 18S cRNA

(pT718S; Ambion, Austin, TX). After washing, blots were digested with RNase A and radioactivity associated with slots quantified using a Typhoon 8600 MultiImager (Molecular Dynamics, Piscataway, NJ). Data are expressed as relative units.

Statistical analyses

Data from all quantitative studies were subjected to least squares ANOVA using the General Linear Models procedures of the Statistical Analysis System (SAS Institute, Cary, NC). Slot blot hybridization data were corrected for differences in sample loading by using the 18S rRNA data as a covariate. Data are presented as the least-squares means with overall standard errors (SE). A P value equal to or less than 0.05 was considered to be significant.

Results

Steady-state levels of *MX1, ISG15, OAS1, RSAD2* (linear, $P<0.05$) and *MX2* (quadratic, $P<0.05$) mRNAs in CL during the periimplantation period were affected by day of pregnancy. Abundance of *MX1, MX2, ISG15, OAS1* and *RSAD2* mRNAs was low on Day 13, increased rapidly on Day 14 and maintained to Day 18 (effect of day, $P<0.05$). Between Days 13 and 14, *MX1* and *MX2* mRNAs increased 8.8-fold and 4.2-fold, respectively (Fig. 4.1), whereas *ISG15, OAS1* and *RSAD2* mRNAs increased 9.6-fold, 25.3-fold, and 15.4-fold, respectively (Fig. 4.2). There were no significant changes in mRNA level for any of the ISGs studied between Days 14 and 18 of pregnancy.

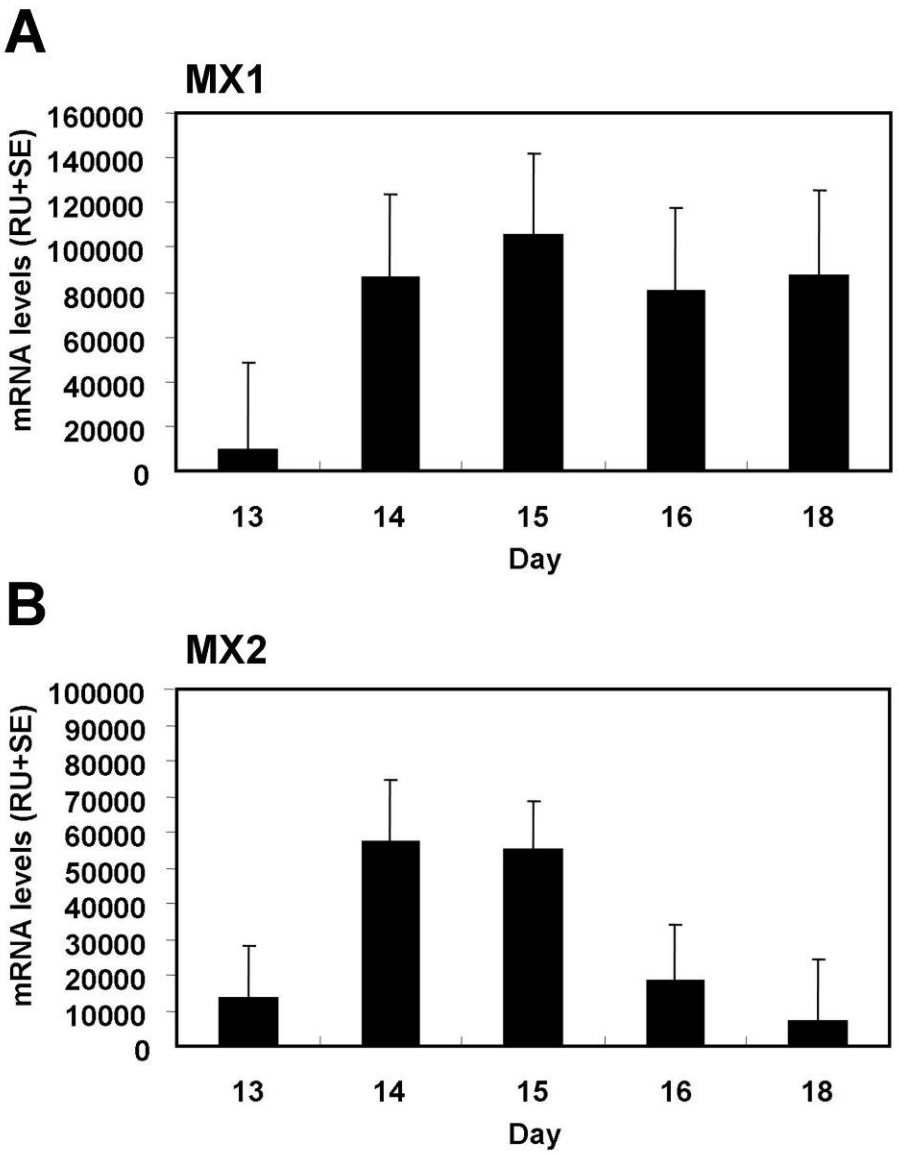

Figure 4.1. Steady-state levels of *MX1* (A) and *MX2* (B) mRNAs in CL on Days 13, 14, 15, 16 and 18 of pregnant ewes. (A) *MX1* mRNA increased from Day 13 to Day 14 (linear, $P<0.05$) and was maintained thereafter. (B) *MX2* mRNA increased from Days 13 to 14 and 15 (quadratic, $P<0.05$), and then decreased. Data are presented as least-square mean relative units (RU) with standard error (SE).

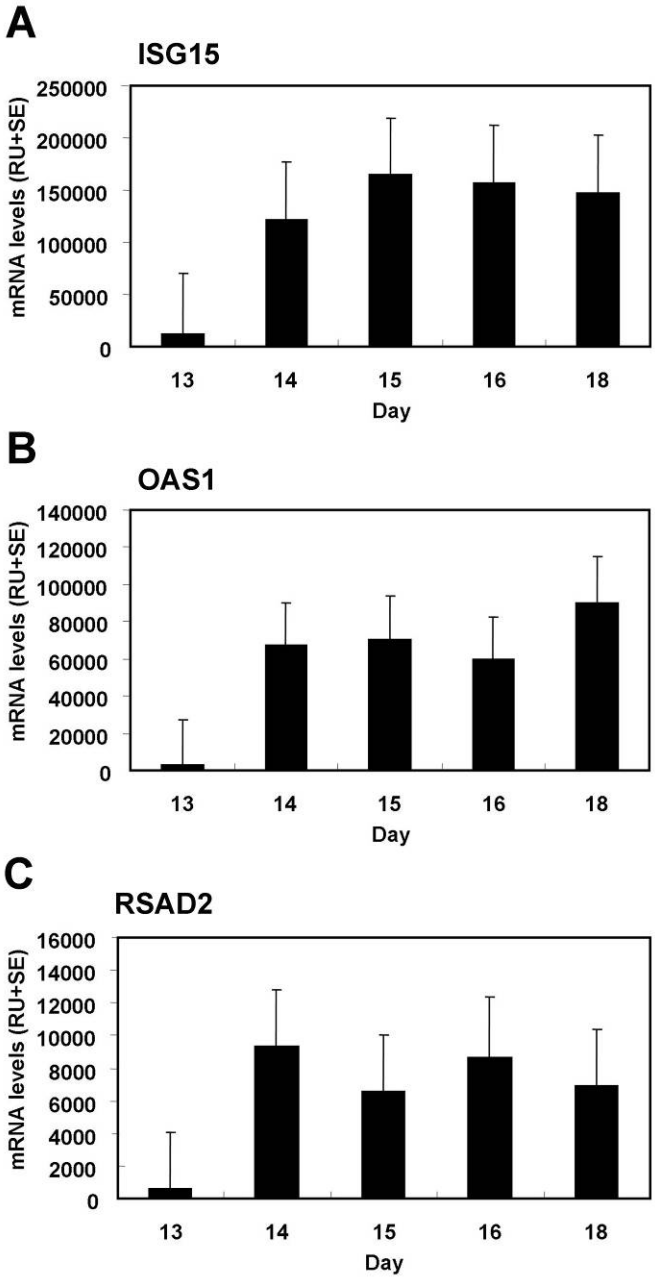

Figure 4.2. Steady-state levels of *ISG15* (A), *OAS1* (B), and *RSAD2* (C) mRNAs in CL on Days 13, 14, 15, 16 and 18 of pregnancy. *ISG15, OAS1, and RSAD2* mRNAs in CL increased from Day 13 to Day 14 and were maintained thereafter (linear, $P<0.05$). Data are presented as least-square mean relative units (RU) with standard error (SE).

Discussion

Steady-state levels of the ISG mRNAs in CL increased on Days 14 and 15, the time of high levels of IFNT produced by ovine trophectoderm, and maintained until Day 18 of pregnancy. Increase in abundance of ISG mRNAs around Days 14 and 15 of pregnancy in ewes occurs in uterine endometrium (Johnson *et al.*, 1999, Ott *et al.*, 1998, Song *et al.*, 2007b), and peripheral blood mononuclear cells (Yankey *et al.*, 2001). Systemic effect of IFNT has been shown in uterus, CL, hypothalamus-pituitary-adrenal axis and blood (Ott *et al.*, 1997, Spencer *et al.*, 1999b, Yankey *et al.*, 2001) but ISG did not repond to IFNT in hypothalamus, pituitary and liver (Chen *et al.*, 2006). IFNT acts as the antiluteolytic signal due to its unique expression pattern by conseptuses within the uterine lumen (Green *et al.*, 2005). Interestingly, intrauterine administration of IFNA, a member of type I IFNs, does not increase interestrous intervals while IFNT does (Davis *et al.*, 1992). Therefore, it appears that IFNT from the conceptus trophectoderm induced ISG expression in CL.

In this study, *MX2* mRNA decreased on Day 16 while other ISGs remained abundant until Day 18. In the ovine conceptus, *IFNT* mRNA rapidly increases on Day 14, decreases on Day 16 and remains low until Day 22 of pregnancy (Hansen *et al.*, 1988). This indicates that MX2 is more sensitive to changes in *IFNT* than other ISGs. Similar results were observed in dairy cows as *MX2* mRNA showed earlier and greater induction and more abundantly in the peripheral blood leukocytes (PBLs) of pregnant cows (Gifford *et al.*, 2007).

How IFNT acts on the CL, either locally or systemically, directly of indirectly, is not clear. High doses of intramuscular (i.m.) IFNT increased CL lifespan as well as intrauterine (i.u.) infusion, indicating both paracrine and endocrine effects of IFNT (Chen *et al.*, 2006). IFNT also activates gene expression in components of the circulating immune system (Gifford *et al.*, 2007, Yankey *et al.*, 2001). However, changes in gene expression in response to IFNT were not observed in

liver (Chen *et al*., 2006), suggesting local actions. IFNT produced from the conceptus may enter either uterine vasculature or lymphatics to enter the general circulation and exert an effect. Also, chemotactic factors might recruit immune cells from the uterus to CL. Chemokine (C-C motif) ligand 2(CCL2) (Alias: Monocyte chemotectic protein 1) is a candidate found in both the uterus and CL of sheep (Asselin *et al.*, 2001, Haworth *et al.*, 1998, Tsai *et al.*, 1997) and regulated by P4 and IFNT. Other unknown factors (IFNT dependent or independent) should be considered.

Collectively, results of the present study confirmed induction of ISGs in CL by IFNT during the peri-implantation period of pregnancy in sheep. Results of the present study support the hypothesis that IFNT affects on uterine blood cells, uterine mucosa, circulating immune cells and CL as suggested by Spencer and colleagues (Spencer *et al.*, 1999b) and others. Future studies will be directed to elucidating the function of immune cells in the CL in response to IFNT during the peri-implantation period of pregnancy. Subsequent mRNA localization and proteomic studies in CL or ovary must be done to define cell-specific expression of ISGs. Also, concentrations of IFNT in blood (local vs peripheral) and lymphatics during the peri-implantation of pregnancy must be determined.

CHAPTER V
CONCLUSIONS

As noted in Chapter III, inconsistences between results from microarray analyses (Gray *et al.*, 2006) and corroboration analyses for some genes (*ACTA2, COL3A1, SPARC, STAT5A, TAGLN*) indicated the need to further examine regulation of expression, This was done in the present studies. *STAT5A* was below the limits of detection by *in situ* hybridization. *ACTA2* mRNAs were abundant primarily in myometrium, but not in LE, GE or stroma, and no significant changes in mRNA were found either in myometrium or blood vessels. *TAGLN* mRNA was high on Day 16 of the estrous cycle in uterine stroma, which was different from *ACTA2* mRNA in the ovine uterus on the same day. *TAGLN* and *ACTA2* mRNA had similar patterns of localization in myometrium, stroma and blood vessels, but not conceptus or uterine epithelia. Furthermore, expression of *COL3A1* in cyclic and pregnant ewes indicated that it was down-regulated by pregnancy but not affected by either P4 or IFNT. Changes in *COL3A1* and *SPARC* mRNAs were corresponded mainly to proestrus in cyclic ewes suggesting effects of estrogen in the ovine uterus, but this must be tested. Neither P4 nor IFNT regulated expression of the five genes examined in the present study. This indicates that comparing differential gene expression in one specific day of the estrous cycle vs pregnancy or among treatment groups using microarray analysis is insuffieicnt to assess regulation given the many hormones and growth factors affecting the uterus during early pregnancy. However, it is acknowledged that microarray analysis is indeed a useful tool to select candidate genes for study.

The *S100A2* and *POSTN* genes were confirmed to be IFNT- and P4-regulated, respectively. *S100A2* was expected to be downregulated by IFNT (Gray *et al.*, 2006) and it was confirmed to be regulated by IFNT with P4 being permissive. *S100A2* appears to regulate *PTGS2* expression and proteomics/

functional studies of the role(s) of S100A2 should be continued. *POSTN* was expected to be up-regulated by P4, down-regulated by IFNT and up-regulated by pregnancy (Gray *et al.*, 2006). P4 was confirmed to up-regulate *POSTN* mRNA, but neither IFNT nor pregnancy enhanced effect of P4 *in vivo*. *POSTN* mRNA was localized in the stratum compactum stroma and caruncle of ovine uteri. Abundant POSTN protein was accumulated along the apical side of the stratum compactum stroma and sites of conceptus attachment on Days 18 and 20 of pregnancy. As this was the first study of POSTN in the uterus, functional studies indicated that POSTN stimulated attachment and migration of ovine trophectoderm cells *in vitro,* as well as an ECM component. Results from studies of POSTN showed the necessity of validation studies extending to proteomic, functional, and/or metabolic analyses to understand the ultimate roles of genes in endometrial-conceptus interactions (Spencer *et al.*, 2007a).

Chapter IV described increases in steady-state levels of ISG mRNAs in ovine CL on Day 14 of pregnancy, suggesting induction of these genes by IFNT. Steady-state levels of ISG mRNAs were maintained in the CL for most of ISGs from Days 14 to 18 of pregnancy, although *MX2* declined after Day 14. This study supports the hypothesis that IFNT has an endocrine action on adjacent reproductive organs during early pregnancy, although the mechanism of action remains to be elucidated. Future studies will explore routes of IFNT transport from the conceptus to CL during the peri-implantation period of pregnancyand ISG expression in CL and/or ovary to discover function of these ISGs in CL.

REFERENCES

Abrahamson M, Barrett AJ, Salvesen G & Grubb A 1986 Isolation of six cysteine proteinase inhibitors from human urine. Their physicochemical and enzyme kinetic properties and concentrations in biological fluids. The Journal of Biological Chemistry **261** 11282-11289.

Afonso S, Romagnano L & Babiarz B 1997 The expression and function of cystatin C and cathepsin B and cathepsin L during mouse embryo implantation and placentation. Development **124** 3415-3425.

Allen WR & Stewart F 2001 Equine placentation. Reproduction, Fertility, and Development **13** 623-634.

Ashworth CJ & Bazer FW 1989 Changes in ovine conceptus and endometrial function following asynchronous embryo transfer or administration of progesterone. Biology of Reproduction **40** 425-433.

Asselin E, Johnson GA, Spencer TE & Bazer FW 2001 Monocyte chemotactic protein-1 and -2 messenger ribonucleic acids in the ovine uterus: regulation by pregnancy, progesterone, and interferon-tau. Biology of Reproduction **64** 992-1000.

Aumailley M & Gayraud B 1998 Structure and biological activity of the extracellular matrix. Journal of Molecular Medicine **76** 253-265.

Austin KJ, Ward SK, Teixeira MG, Dean VC, Moore DW & Hansen TR 1996 Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. Biology of Reproduction **54** 600-606.

Austin KJ, Bany BM, Belden EL, Rempel LA, Cross JC & Hansen TR 2003 Interferon-stimulated gene-15 (Isg15) expression is up-regulated in the mouse uterus in response to the implanting conceptus. Endocrinology **144** 3107-3113.

Barja F, Coughlin C, Belin D & Gabbiani G 1986 Actin isoform synthesis and mRNA levels in quiescent and proliferating rat aortic smooth muscle cells in vivo and in vitro. Laboratory Investment **55** 226-233.

Bartol FF, Roberts RM, Bazer FW, Lewis GS, Godkin JD & Thatcher WW 1985 Characterization of proteins produced in vitro by periattachment bovine conceptuses. Biology of Reproduction **32** 681-693.

Bauersachs S, Ulbrich SE, Gross K, Schmidt SE, Meyer HH, Wenigerkind H, Vermehren M, Sinowatz F, Blum H & Wolf E 2006 Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. Reproduction **132** 319-331.

Bazer FW 1992 Mediators of maternal recognition of pregnancy in mammals. Proceedings of the Society for Experimental Biology and Medicine **199** 373-384.

Bazer FW & First NL 1983 Pregnancy and parturition. Journal of Animal Science **57 Suppl 2** 425-460.

Bazer FW & Johnson HM 1991 Type I conceptus interferons: maternal recognition of pregnancy signals and potential therapeutic agents. American Journal of Reproductive Immunology **26** 19-22.

Bazer FW, Spencer TE & Ott TL 1996 Placental interferons. American Journal of Reproductive Immunology **35** 297-308.

Bazer FW, Spencer TE & Ott TL 1997 Interferon tau: a novel pregnancy recognition signal. American Journal of Reproductive Immunology **37** 412-420.

Bekisz J, Schmeisser H, Hernandez J, Goldman ND & Zoon KC 2004 Human interferons alpha, beta and omega. Growth Factors **22** 243-251.

Benech P, Mory Y, Revel M & Chebath J 1985 Structure of two forms of the interferon-induced (2'-5') oligo A synthetase of human cells based on cDNAs and gene sequences. The EMBO Journal **4** 2249-2256.

Boos A 2000 Immunohistochemical assessment of collagen types I, III, IV and VI in biopsy samples of the bovine uterine wall collected during the oestrous cycle. Cells Tissues Organs **167** 225-238.

Boos A, Stelljes A & Kohtes J 2003 Collagen types I, III and IV in the placentome and interplacentomal maternal and fetal tissues in normal cows and in cattle with retention of fetal membranes. Cells Tissues Organs **174** 170-183.

Bornstein P & Sage EH 2002 Matricellular proteins: extracellular modulators of cell function. Current Opinion in Cell Biology **14** 608-616.

Boucheron C, Dumon S, Santos SC, Moriggl R, Hennighausen L, Gisselbrecht S & Gouilleux F 1998 A single amino acid in the DNA binding regions of STAT5A and STAT5B confers distinct DNA binding specificities. The Journal of Biological Chemistry **273** 33936-33941.

Brekken RA & Sage EH 2001 SPARC, a matricellular protein: at the crossroads of cell-matrix. Matrix Biology **19** 816-827.

Burgeson RE & Nimni ME 1992 Collagen types: Molecular structure and tissue distribution. Clinical Orthopaedics and Related Research **282** 250-272.

Cammas L, Reinaud P, Dubois O, Bordas N, Germain G & Charpigny G 2005 Identification of differentially regulated genes during elongation and early implantation in the ovine trophoblast using complementary DNA array screening. Biology of Reproduction **72** 960-967.

Carroll SL, Bergsma DJ & Schwartz RJ 1986 Structure and complete nucleotide sequence of the chicken alpha-smooth muscle (aortic) actin gene. An actin gene which produces multiple messenger RNAs. Journal of Biological Chemistry **261** 8965-8976.

Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA & Yoshinaga K 2000 Embryo implantation. Developmental Biology **223** 217-237.

Cavaille F 1985 The contractile proteins of the human myometrium. Acta Physiologica Hungarica **65** 453-460.

Cavaille F, Janmot C, Ropert S & d'Albis A 1986 Isoforms of myosin and actin in human, monkey and rat myometrium. Comparison of pregnant and non-pregnant uterus proteins. European Journal of Biochemistry **160** 507-513.

Chang KS, Zimmer WEJ, Bergsma DJ, Dodgson JB & Schwartz RJ 1984 Isolation and characterization of six different chicken actin genes. Molecular and Cellular Biology **4** 2498-2508.

Chebath J, Benech P, Hovanessian A, Galabru J & Revel M 1987 Four different forms of interferon-induced 2',5'-oligo(A) synthetase identified by immunoblotting in human cells. The Journal of Biological Chemistry **262** 3852-3857.

Chen Y, Antoniou E, Liu Z, Hearne LB & Roberts RM 2007 A microarray analysis for genes regulated by interferon-tau in ovine luminal epithelial cells. Reproduction. **134** 123-135.

Chen Y, Green JA, Antoniou E, Ealy AD, Mathialagan N, Walker AM, Avalle MP, Rosenfeld CS, Hearne LB & Roberts RM 2006 Effect of interferon-tau administration on endometrium of nonpregnant ewes: a comparison with pregnant ewes. Endocrinology **147** 2127-2137.

Chin KC & Cresswell P 2001 Viperin (cig5), an IFN-inducible antiviral protein directly induced by human cytomegalovirus. Proceedings National Academy of Sciences USA **98** 15125-15130.

Choi Y, Johnson GA, spencer TE & Bazer FW 2003 Pregnancy and interferon tau regulate MHC class I and beta-2-microglobulin expression in the ovine uterus. Biology of Reproduction **68** 1703-1710.

Choi Y, Johnson GA, Burghardt RC, Berghman LR, Joyce MM, Taylor KM, Stewart MD, Bazer FW & Spencer TE 2001a Interferon regulatory factor-two restricts expression of interferon- stimulated genes to the endometrial stroma and glandular epithelium of the ovine uterus. Biology of Reproduction **65** 1038-1049.

Choi Y, Johnson GA, Burghardt RC, Berghman LR, Joyce MM, Taylor KM, Stewart MD, Bazer FW & Spencer TE 2001b Interferon regulatory factor-two restricts expression of interferon- stimulated genes to the endometrial stroma and glandular epithelium of the ovine uterus. Biology of Reproduction **65** 1038-1049.

Colamonici O, Yan H, Domanski P, Handa R, Smalley D, Mullersman J, Witte M, Krishnan K & Krolewski J 1994 Direct binding to and tyrosine phosphorylation of the alpha subunit of the type I interferon receptor by p135tyk2 tyrosine kinase. Molecular and Cellular Biology **14** 8133-8142.

Cross SS, Hamdy FC, Deloulme JC & Rehman I 2005 Expression of S100 proteins in normal human tissues and common cancers using tissue microarrays: S100A6, S100A8, S100A9 and S100A11 are all overexpressed in common cancers. Histopathology **46** 256-269.

Darnell JE, Jr. 1997 STATs and gene regulation. Science **277** 1630-1635.

Davis M, Ott T, Mirando MA, Moser MT & Bazer FW 1992 Effects of recombinant alpha interferons on fertility and interestrous intervals in sheep. Theriogenology **38** 867-875.

De Paepe A & Malfait F 2004 Bleeding and bruising in patients with Ehlers-Danlos syndrome and other collagen vascular disorders. British Journal of Haematology **127** 491-500.

Demmers KJ, Derecka K & Flint A 2001 Trophoblast interferon and pregnancy. Reproduction **121** 41-49.

Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T & Wang H 2004 Molecular cues to implantation. Endocrine Reviews **25** 341-373.

Donato R 2001 S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. The International Journal of Biochemistry & Cell Biology **33** 637-668.

Duband J, Gimona M, Scatena M, Sartore S & Small JV 1993 Calponin and SM 22 as differentiation markers of smooth muscle: spatiotemporal distribution during avian embryonic development. Differentiation **55** 1-11.

Dunlap KA, Palmarini M & Spencer TE 2006 Ovine endogenous betaretroviruses (enJSRVs) and placental morphogenesis. Placenta **Suppl A** S135-140.

Dunphy JL, Balic A, Barcham GJ, Horvath AJ, Nash AD & Meeusen EN 2000 Isolation and characterization of a novel inducible mammalian galectin. The Journal of Biological Chemistry **275** 32106-32113.

Elkins T, Hortsch M, Bieber AJ, Snow PM & Goodman CS 1990 Drosophila fasciclin I is a novel homophilic adhesion molecule that along with fasciclin III can mediate cell sorting. The Journal of Cell Biology **110** 1825-1832.

Farin CE, Imakawa K & Roberts RM 1989 In situ localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. Molecular Endocrinology **3** 1099-1107.

Farmer JL, Burghardt RC, Jousan FD, Hansen PJ, Bazer FW & Spencer TE 2008 Galectin 15 (LGALS15) functions in trophectoderm migration and attachment. The FASEB Journal **22** 548-60.

Farrell PJ, Broeze RJ & Lengyel P 1979 Accumulation of an mRNA and protein in interferon-treated Ehrlich ascites tumour cells. Nature **279** 523-525.

Finn CA 1971 The biology of decidual cells. Advances in Reproductive Physiology **5** 1-26.

Fleming JA, Choi Y, Johnson GA, Spencer TE & Bazer FW 2001 Cloning of the ovine estrogen receptor-alpha promoter and functional regulation by ovine interferon-tau. Endocrinology **142** 2879-2887.

Fleming JG, Spencer TE, Safe SH & Bazer FW 2006 Estrogen regulates transcription of the ovine oxytocin receptor gene through GC-rich SP1 promoter elements. Endocrinology **142** 2879-2887.

Flint AP, Stewart HJ, Lamming GE & Payne JH 1992 Role of the oxytocin receptor in the choice between cyclicity and gestation in ruminants. Journal of Reproduction and Fertility. Supplement **45** 53-58.

Foser S, Redwanz I, Ebeling M, Heizmann CW & Certa U 2006 Interferon-alpha and transforming growth factor-beta co-induce growth inhibition of human tumor cells. Cellular and Molecular Life Sciences **63** 2387-2396.

Frankel FR, Hsu CY, Myers JC, Lin E, Lyttle CR, Komm B & Mohn K 1988 Regulation of alpha 2(I), alpha 1(III), and alpha 2(V) collagen mRNAs by estradiol in the immature rat uterus. DNA **7** 347-354.

Giancotti FG & Ruoslahti E 1999 Integrin signaling. Science **285** 1028-1032.

Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, Davies CJ & Ott TL 2007 Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. Journal of Dairy Science **90** 274-280.

Gillan L, Matei D & Fishman DA 2002 Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha(V)beta(3) and alpha(V)beta(5) integrins and promotes cell motility. Cancer Research **62** 5358-5364.

Gimona M, Sparrow MP, Strasser P & Herzog M 1992 Calponin and SM 22 isoforms in avian and mammalian smooth muscle. European Journal of Biochemistry **205** 1067-1075.

Godkin JD, Bazer FW, Moffatt J, Sessions F & Roberts RM 1982 Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at day 13-21. Journal of Reproduction and Fertility **65** 141-150.

Gray CA, Dunlap KA, Burghardt RC & Spencer TE 2005 Galectin-15 in ovine uteroplacental tissues. Reproduction **130** 231-240.

Gray CA, Burghardt RC, Johnson GA, Bazer FW & Spencer TE 2002 Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. Reproduction **124** 289-300.

Gray CA, Adelson DL, Bazer FW, Burghardt RC, Meeusen EN & Spencer TE 2004 Discovery and characterization of an epithelial-specific galectin in the endometrium that forms crystals in the trophectoderm. Proceedings National Academy of Sciences USA **101** 7982-7987.

Gray CA, Abbey CA, Beremand PD, Choi Y, Farmer JL, Adelson DL, Thomas TL, Bazer FW & Spencer TE 2006 Identification of endometrial genes regulated by early pregnancy, progesterone, and interferon tau in the ovine uterus. Biology of Reproduction **74** 383-394.

Green MP, Spate LD, Bixby JA, Ealy AD & Roberts RM 2005 A comparison of the anti-luteolytic activities of recombinant ovine interferon-alpha and -tau in sheep. Biology of Reproduction **73** 1087-1093.

Guesdon FM, Stewart HJ & Flint AP 1996 Negative regulatory domains in a trophoblast interferon promoter. Journal of Molecular Endocrinology **16** 99-106.

Guillomot M 1995 Cellular interactions during implantation in domestic ruminants. Journal of Reproduction and Fertility Supplement **49** 39-51.

Guillomot M, Flechon JE & Wintenberger-Torres S 1981 Conceptus attachment in the ewe: an ultrastructural study. Placenta **2** 169-182.

Guillomot M, Flechon JE & Leroy F 1993 Blastocyst development and implantation. In *Reproduction in Mammals and Man*, pp 387-411. Eds C Thibault, MC Levasseur & RHF Hunter. Paris: Ellipses.

Haas AL, Ahrens P, Bright PM & Ankel H 1987 Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. The Journal of Biological Chemistry **262** 11315-11323.

Hafez ESE 1993 Fertilization, cleavage, and implantation. In *Reproduction in Farm Animals*. **8** pp 188-212. Lea & Febiger, PA.

Hall A, Håkansson K, Mason RW, Grubb A & Abrahamson M 1995 Structural basis for the biological specificity of cystatin C. The Journal of Biological Chemistry **270** 5115-5121.

Haller O, Frese M & Kochs G 1998 Mx proteins: mediators of innate resistance to RNA viruses. Revue Scientifique et Technique **17** 220-230.

Han H, Austin KJ, Rempel LA & Hansen TR 2006 Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. Journal of Endocrinology **191** 505-512.

Hansen TR, Imakawa K, Polites HG, Marotti KR, Anthony RV & Roberts RM 1988 Interferon RNA of embryonic origin is expressed transiently during early pregnancy in the ewe. Journal of the Biological Chemistry **263** 12801-12804.

Hansen TR, Austin KJ, Perry DJ, Pru JK, Teixeira MG & Johnson GA 1999 Mechanism of action of interferon-tau in the uterus during early pregnancy. Journal of Reproduction and Fertility **54** 329-339.

Haworth JD, Rollyson MK, Silva P, McIntush EW & Niswender GD 1998 Messenger ribonucleic acid encoding monocyte chemoattractant protein-1 is expressed by the ovine corpus luteum in response to prostaglandin F2 alpha. Biology of Reproduction **58** 169-174.

Hayashi K, Burghardt RC, Bazer FW & Spencer TE 2007 WNTs in the ovine uterus: potential regulation of periimplantation ovine conceptus development. Endocrinology **148** 3496-3506.

Helmer SD, Hansen PJ, Thatcher WW, Johnson JW & Bazer FW 1989 Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend corpus luteum lifespan in cyclic cattle. Journal of Reproduction and Fertility **87** 89-101.

Heyman Y, Camous S, Fevre J, Meziou W & Martal J 1984 Maintenance of the corpus luteum after uterine transfer of trophoblastic vesicles to cyclic cows and ewes. Journal of Reproduction and Fertility **70** 533-540.

Hicks BA, Etter SJ, Carnahan KG, Joyce MM, Assiri AA, Carling SJ, Kodali K, Johnson GA, Hansen TR, Mirando MA 2003 Expression of the uterine Mx protein in cyclic and pregnant cows, gilts, and mares. Journal of Animal Science **81** 1552-1561.

Horcajadas JA, Pellicer A & Simon C 2007 Wide genomic analysis of human endometrial receptivity: new times, new opportunities. Human Reproduction Update **13** 77-86.

Horisberger MA 1992 Interferon-induced human protein MxA is a GTPase which binds transiently to cellular proteins. Journal of Virology **66** 4705-4709.

Horisberger MA & Gunst MC 1991 Interferon-induced proteins: identification of Mx proteins in various mammalian species. Virology **180** 185-190.

Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF & Kudo A 1999 Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. Journal of Bone and Mineral Research **14** 1239-1249.

Howe CC, Overton GC, Sawicki J, Solter D, Stein P & Strickland S 1988 Expression of SPARC/osteonectin transcript in murine embryos and gonads. Differentiation **37** 20-25.

Imakawa K, Anthony RV, Kazemi M, Marotti KR, Polites HG & Roberts RM 1987 Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. Nature **330** 377-379.

Ishiwata H, Katsuma S, Kizaki K, Patel OV, Nakano H, Takahashi T, Imai K, Hirasawa A, Shiojima S, Ikawa H 2003 Characterization of gene expression profiles in early bovine pregnancy using a custom cDNA microarray. Molecular Reproduction and Development **65** 9-18.

Iwahashi M, Ooshima A & Nakano R 1997 Effects of oestrogen on the extracellular matrix in the endometrium of postmenopausal women. Journal of Clinical Pathology **50** 755-759.

Johnson GA, Austin KJ, Van Kirk EA & Hansen TR 1998 Pregnancy and interferon-tau induce conjugation of bovine ubiquitin cross-reactive protein to cytosolic uterine proteins. Biology of Reproduction **58** 898-904.

Johnson GA, Burghardt RC, Bazer FW & Spencer TE 2003a Osteopontin: roles in implantation and placentation. Biology of Reproduction **69** 1458-1471.

Johnson GA, Spencer TE, Burghardt RC, Joyce MM & Bazer FW 2000 Interferon-tau and progesterone regulate ubiquitin cross-reactive protein expression in the ovine uterus. Biology of Reproduction **62** 622-627.

Johnson GA, Joyce MM, Yankey SJ, Hansen TR & Ott TL 2002 The Interferon Stimulated Genes (ISG) 17 and Mx have different temporal and spatial expression

in the ovine uterus suggesting more complex regulation of the Mx gene. Journal of Endocrinology **174** R7-R11.

Johnson GA, Spencer TE, Hansen TR, Austin KJ, Burghardt RC & Bazer FW 1999 Expression of the interferon tau inducible ubiquitin cross-reactive protein in the ovine uterus. Biology of Reproduction **61** 312-318.

Johnson GA, Burghardt RC, Joyce MM, Spencer TE, Bazer FW, Pfarrer C & Gray CA 2003b Osteopontin expression in uterine stroma indicates a decidualization-like differentiation during ovine pregnancy. Biology of Reproduction **68** 1951-1958.

Johnson GA, Bazer FW, Jaeger LA, Ka H, Garlow JE, Pfarrer C, Spencer TE & Burghardt RC 2001a Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. Biology of Reproduction **65** 820-828.

Johnson GA, Stewart MD, Gray CA, Choi Y, Burghardt RC, Yu-Lee LY, Bazer FW & Spencer TE 2001b Effects of the estrous cycle, pregnancy, and interferon tau on 2',5'- oligoadenylate synthetase expression in the ovine uterus. Biology of Reproduction **64** 1392-1399.

Joyce MM, White FJ, Burghardt RC, Muniz JJ, Spencer TE, Bazer FW & Johnson GA 2005 Interferon stimulated gene 15 conjugates to endometrial cytosolic proteins and is expressed at the uterine-placental interface throughout pregnancy in sheep. Endocrinology **146** 675-684.

Kang DC, Gopalkrishnan RV, Wu Q, Jankowsky E, Pyle AM & Fisher PB 2002 mda-5: an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. Proceedings National Academy of Sciences USA **99** 637-642.

Kang DC, Gopalkrishnan RV, Lin L, Randolph A, Valerie K, Pestka S & Fisher PB 2004 Expression analysis and genomic characterization of human melanoma differentiation associated gene-5, mda-5: a novel type I interferon-responsive apoptosis-inducing gene. Oncogene **23** 1789-1800.

Kim JE, Kim SJ, Lee BH, Park RW, Kim K & Kim IS 2000 Identification of motifs for cell adhesion within the repeated domains of transforming growth factor-beta-induced gene, beta ig-h3. The Journal of Biological Chemistry **275** 30907-30915.

Kim JJ, Jaffe RC & Fazleabas AT 1998 Comparative studies on the in vitro decidualization process in the baboon (Papio anubis) and human. Biology of Reproduction **59** 160-168.

Kim KI & Zhang DE 2003 ISG15, not just another ubiquitin-like protein. Biochemical and Biophysical Research Communications **307** 431-434.

Kim S, Choi Y, Spencer TE & Bazer FW 2003a Effects of the estrous cycle, pregnancy and interferon tau on expression of cyclooxygenase two (COX-2) in ovine endometrium. Reproductive Biology and Endocrinology **1** 58.

Kim S, Choi Y, Bazer FW & Spencer TE 2003b Identification of genes in the ovine endometrium regulated by interferon tau independent of signal transducer and activator of transcription 1. Endocrinology **144** 5203-5214.

King GJ, Atkinson BA & Robertson HA 1982 Implantation and early placentation in domestic ungulates. Journal of Reproduction and Fertility Supplement **31** 17-30.

Kirschke H, Barrett AJ & Rawlings ND 1998 Lysosomal Cysteine Proteases. Oxford, UK: Oxford University Press.

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

Kobayashi R, Kubota T & Hidaka H 1994 Purification, characterization, and partial sequence analysis of a new 25-kDa actin-binding protein from bovine aorta: a SM22 homolog. Biochemical and Biophysical Research Communications **198** 1275-1280.

Kruzynska-Frejtag A, Wang J, Maeda M, Rogers R, Krug E, Hoffman S, Markwald RR & Conway SJ 2004 Periostin is expressed within the developing

teeth at the sites of epithelial-mesenchymal interaction. Developmental Dynamics **229** 857-868.

Kumar S, Mitnik C, Valente G & Floyd-Smith G 2000 Expansion and molecular evolution of the interferon-induced 2'-5' oligoadenylate synthetase gene family. Molecular Biology and Evolution **17** 738-750.

Lane TF & Sage EH 1994 The biology of SPARC, a protein that modulates cell-matrix interactions. The FASEB Journal **8** 163-173.

Lee SW, Tomasetto C, Swisshelm K, Keyomarsi K & Sager R 1992 Down-regulation of a member of the S100 gene family in mammary carcinoma cells and reexpression by azadeoxycytidine treatment. Proceedings National Academy of Sciences USA **89** 2504-2508.

Lees-Miller JP, Heeley DH & Smillie LB 1987 An abundant and novel protein of 22 kDa (SM22) is widely distributed in smooth muscles - purification from bovine aorta. The Biochemical Journal **244** 705-709.

Li G, Oparil S, Sanders JM, Zhang L, Dai M, Chen LB, Conway SJ, McNamara CA & Sarembock IJ 2006 Phosphatidylinositol-3-kinase signaling mediates vascular smooth muscle cell expression of periostin in vivo and in vitro. Atherosclerosis **188** 292-300.

Liu X, Robinson GW, Gouilleux F, Groner B & Hennighausen L 1995 Cloning and expression of Stat5 and an additional homologue (Stat5b) involved in prolactin signal transduction in mouse mammary tissue. Proceedings National Academy of Sciences USA **92** 8831-8835.

Liu X, Robinson GW, Wagner KU, Garrett L, Wynshaw-Boris A & Hennighausen L 1997 Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes & Development **11** 179-186.

Logan CY & Nusse R 2004 The Wnt signaling pathway in development and disease. Annual Review of Cell and Developmental Biology **20** 781-810.

Marenholz I, Heizmann CW & Fritz G 2004 S100 proteins in mouse and man: from evolution to function and pathology. Biochemical and Biophysical Research Communications. **322** 1111-1122.

Marié I & Hovanessian AG 1992 The 69-kDa 2-5A synthetase is composed of two homologous and adjacent functional domains. The Journal of Biological Chemistry **267** 9933-9939.

Martal JL, Chêne NM, Huynh LP, L'Haridon RM, Reinaud PB, Guillomot MW, Charlier MA & Charpigny SY 1998 IFN-tau: a novel subtype I IFN1. Structural characteristics, non-ubiquitous expression, structure-function relationships, a pregnancy hormonal embryonic signal and cross-species therapeutic potentialities. Biochemie **80** 755-777.

McCracken J, Schramm W & Okulicz WC 1984 Hormone receptor control of pulsatile secretion of PGF2a from the ovine uterus during luteolysis and its abrogation in early pregnancy. Animal Reproduction Science **7** 31-55.

McHugh KM & Lessard JL 1988 The development expression of the rat alpha-vascular and gamma-enteric smooth muscle isoactins: isolation and characterization of a rat gamma-enteric actin cDNA. Molecular and Cellular Biology **8** 5224-5231.

McHugh KM, Crawford K & Lessard JL 1991 A comprehensive analysis of the developmental and tissue-specific expression of the isoactin multigene family in the rat. Developmental Biology **148** 442-458.

Meyer MD, Drost M, Ott TL, Bazer FW, Badinga L, Hansen PJ & Thatcher WW 1992 Recombinant ovine trophoblast protein-1 extends corpus luteum lifespan and reduced uterine secretion of prostaglandin F2a in cattle. Journal of Animal Science Supplement **70** 270.

Mirando MA, Short EC, Jr., Geisert RD, Vallet JL & Bazer FW 1991 Stimulation of 2',5'-oligoadenylate synthetase activity in sheep endometrium during pregnancy, by intrauterine infusion of ovine trophoblast protein-1, and by intramuscular

administration of recombinant bovine interferon-alpha I1. Journal of Reproduction and Fertility **93** 599-607.

Mohamed OA, Jonnaert M, Labelle-Dumais C, Kuroda K, Clarke HJ & Dufort D 2005 Uterine Wnt/beta-catenin signaling is required for implantation. Proceedings National Academy of Sciences USA **102** 8579-8584.

Nair RR, Solway J & Boyd DD 2006 Expression cloning identifies transgelin (SM22) as a novel repressor of 92-kDa type IV collagenase (MMP-9) expression. The Journal of Biological Chemistry **281** 26424-26436.

Niklaus AL & Pollard JW 2006 Mining the mouse transcriptome of receptive endometrium reveals distinct molecular signatures for the luminal and glandular epithelium. Endocrinology **147** 3375-3390.

Novick D, Cohen B & Rubinstein M 1994 The human interferon alpha/beta receptor: characterization and molecular cloning. Cell **77** 391-400.

Ott TL, Yin J, Wiley AA, Kim HT, Gerami-Naini B, Spencer TE, Bartol F, Burghardt RC & Bazer FW 1998 Effects of the estrous cycle and early pregnancy on uterine expression of Mx protein in sheep (Ovis aries). Biology of Reproduction **59** 784-794.

Ott TL, Fleming JG, Spencer TE, Joyce MM, Chen P, Green CN, Zhu D, Welsh TH, Jr., Harms PG & Bazer FW 1997 Effects of exogenous recombinant ovine interferon tau on circulating concentrations of progesterone, cortisol, luteinizing hormone, and antiviral activity; interestrous interval; rectal temperature; and uterine response to oxytocin in cyclic ewes. Biology of Reproduction **57** 621-629.

Owens GK 1995 Regulation of differentiation of vascular smooth muscle cells. Physiological Reviews **75** 487-517.

Owens GK & Thompson MM 1986 Developmental changes in isoactin expression in rat aortic smooth muscle cells in vivo. Relationship between growth and cytodifferentiation. The Journal of Biological Chemistry **261** 13373-13380.

Passey RJ, Williams E, Lichanska AM, Wells C, Hu S, Geczy CL, Little MH & Hume DA 1999 A null mutation in the inflammation-associated S100 protein

S100A8 causes early resorption of the mouse embryo. The Journal of Immunology **163** 2209-2216.

Perry JS 1981 The mammalian fetal membranes. Journal of Reproduction and Fertility **62** 321-335.

Polakis P 2000 Wnt signaling and cancer. Genes and Development **14** 1837-1851.

Pontzer CH, Ott TL, Bazer FW & Johnson HM 1990 Localization of an antiviral site on the pregnancy recognition hormone, ovine trophoblast protein 1. Proceedings National Academy of Sciences USA **87** 5945-5949.

Renfree MB 1982 Implantation and placentation. In *Reproduction in Mammals and Man*, pp 26-69. Eds CR Austin & RV Short. Cambridge, UK: Cambridge University Press.

Reynolds LP, Borowicz PP, Vonnahme KA, Johnson ML, Grazul-Bilska AT, Wallace JM, Caton JS & Redmer DA 2005 Animal models of placental angiogenesis. Placenta **26** 689-708.

Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, Rogers R, Chen Z, Maeda M, Kruzynska-Frejtag A 2005 Periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. Molecular and Cellular Biology **25** 11131-11144.

Roberts RM 1989 Conceptus interferons and maternal recognition of pregnancy. Biology of Reproduction **40** 449-452.

Roberts RM, Cross JC & Leaman DW 1992 Interferons as hormones of pregnancy. Endocrine Reviews **13** 432-452.

Roberts RM, Ealy AD, Alexenko AP, Han CS & Ezashi T 1999 Trophoblast interferons. Placenta **20** 259-264.

Rosenfeld CS, Han CS, Alexenko AP, Spencer TE & Roberts RM 2002 Expression of interferon receptor subunits, IFNAR1 and IFNAR2, in the ovine uterus. Biology of Reproduction **67** 847-853.

Rowson LE & Moor RM 1966 Development of the sheep conceptus during the first fourteen days. Journal of Anatomy **100** 777-785.

Rowson LE & Moor RM 1967 The influence of embryonic tissue homogenate infused into the uterus, on the life-span of the corpus luteum in the sheep. Journal of Reproduction and Fertility **13** 511-516.

Salama I, Malone PS, Mihaimeed F & Jones JL 2008 A review of the S100 proteins in cancer. European Journal of Surgical Oncology **34** 357-64.

Sandra O, Bataillon I, Roux P, Martal JL, Charpigny G, Reinaud PB, Bolifraud P, Germain G & Al-Gubory KH 2005 Suppressor of cytokine signalling (SOCS) genes are expressed in the endometrium and regulated by conceptus signals during early pregnancy in the ewe. Journal of Molecular Endocrinology **34** 637-644.

Sasaki H, Sato Y, Kondo S, Fukai I, Kiriyama M, Yamakawa Y & Fuji Y 2002 Expression of the periostin mRNA level in neuroblastoma. Journal of Pediatric Surgery **37** 1293-1297.

Sasaki H, Dai M, Auclair D, Fukai I, Kiriyama M, Yamakawa Y, Fujii Y & Chen LB 2001a Serum level of the periostin, a homologue of an insect cell adhesion molecule, as a prognostic marker in nonsmall cell lung carcinomas. Cancer Reserach **92** 843-848.

Sasaki H, Yu CY, Dai M, Tam C, Loda M, Auclair D, Chen LB & Elias A 2003 Elevated serum periostin levels in patients with bone metastases from breast but not lung cancer. Breast Cancer Research and Treatment **77** 245-252.

Sasaki H, Dai M, Auclair D, Kaji M, Fukai I, Kiriyama M, Yamakawa Y, Fujii Y & Chen LB 2001b Serum level of the periostin, a homologue of an insect cell adhesion molecule, in thymoma patients. Cancer Letters **172** 37-42.

Sasaki H, Lo KM, Chen LB, Auclair D, Nakashima Y, Moriyama S, Fukai I, Tam C, Loda M & Fujii Y 2001 Expression of Periostin, homologous with an insect cell adhesion molecule, as a prognostic marker in non-small cell lung cancers. Japanese Journal of Cancer Reserach **92** 869-873.

Satterfield MC, Bazer FW & Spencer TE 2006 Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. Biology of Reproduction **75** 289-296.

Satterfield MC, Dunlap KA, Hayashi K, Burghardt RC, Spencer TE & Bazer FW 2007 Tight and adherens junctions in the ovine uterus: differential regulation by pregnancy and progesterone. Endocrinology **148** 3922-3931.

Schildmeyer LA, Braun R, Taffet G, Debiasi M, Burns AE, Bradley A & Schwartz RJ 2000 Impaired vascular contractility and blood pressure homeostasis in the smooth muscle alpha-actin null mouse. The FASEB Journal **14** 2213-2220.

Schmitt RA, Geisert RD, Zavy MT, Short EC & Blair RM 1993 Uterine cellular changes in 2',5'-oligoadenylate synthetase during the bovine estrous cycle and early pregnancy. Biology of Reproduction **48** 460-466.

Semov A, Moreno MJ, Onichtchenko A, Abulrob A, Ball M, Ekiel I, Pietrzynski G, Stanimirovic D & Alakhov V 2005 Metastasis-associated protein S100A4 induces angiogenesis through interaction with Annexin II and accelerated plasmin formation. The Journal of Biological Chemistry **280** 20833-20841.

Senger PL 2005 Early embryogenesis and maternal recognition of pregnancy. In *Pathways to Pregnancy and Parturition*. **13** pp 284-303. Current Conceptions, Inc. Pullman, WA.

Shanahan CM, Weissberg PL & Metcalfe JC 1993 Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells. Circulation Research **73** 193-204.

Shang W, Doré JJ & Godkin JD 1997 Developmental gene expression of procollagen III in bovine extraembryonic membranes during early pregnancy. Molecular Reproduction and Development **48** 18-24.

Shao R, Bao S, Bai X, Blanchette C, Anderson RM, Dang T, Gishizky ML, Marks JR & Wang XF 2004 Acquired expression of periostin by human breast cancers promotes tumor angiogenesis through up-regulation of vascular

endothelial growth factor receptor 2 expression. Molecular and Cellular Biology **24** 3992-4003.

Shapland C, Hsuan JJ, Totty NF & Lawson D 1993 Purification and properties of transgelin: a transformation and shape change sensitive actin-gelling protein. The Journal of Cell Biology **121** 1065-1073.

Shynlova O, Tsui P, Dorogin A, Chow M & Lye SJ 2005 Expression and localization of alpha-smooth muscle and gamma-actins in the pregnant rat myometrium. Biology of Reproduction **73** 773-780.

Skonier J, Neubauer M, Madisen L, Bennett K, Plowman GD & Purchio AF 1992 cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta. DNA and Cell Biology **11** 511-522.

Song G, Spencer TE & Bazer FW 2005 Cathepsins in the ovine uterus: regulation by pregnancy, progesterone, and interferon tau. Endocrinology **146** 4825-4833.

Song G, Spencer TE & Bazer FW 2006 Progesterone and interferon-tau regulate cystatin C in the endometrium. Endocrinology **147** 3478-3483.

Song G, Bazer FW & Spencer TE 2007a Differential epression of cthepsins and cstatin C in oine ueroplacental tssues. Placenta **28** 1091-1098.

Song G, Bazer FW & Spencer TE 2007b Pregnancy and interferon tau regulate RSAD2 and IFIH1 expression in the ovine uterus. Reproduction **133** 285-295.

Spencer TE & Bazer FW 1995 Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biology of Reproduction **53** 1527-1543.

Spencer TE & Bazer FW 2004 Conceptus signals for establishment and maintenance of pregnancy. Reproductive Biology and Endocrinology **5** 49.

Spencer TE, Sandra O & Wolf E 2007 Genes involved in conceptus-endometrial interacitons in ruminants: insignts from reductionism and thoughts on on holistic approaches. Reproduction **135** 1-16.

Spencer TE, Johnson GA, Burghardt RC & Bazer FW 2004a Progesterone and placental hormone actions on the uterus: insights from domestic animals. Biology of Reproduction **71** 2-10.

Spencer TE, Johnson GA, Bazer FW & Burghardt RC 2004b Implantation mechanisms: insights from the sheep. Reproduction **128** 657-668.

Spencer TE, Bartol FF, Bazer FW, Johnson GA & Joyce MM 1999a Identification and characterization of glycosylation-dependent cell adhesion molecule 1-like protein expression in the ovine uterus. Biology of Reproduction **60** 241-250.

Spencer TE, Johnson GA, Bazer FW, Burghardt RC & Palmarini M 2007b Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. Reproduction, Fertility, and Development **19** 65-78.

Spencer TE, Becker WC, George P, Mirando MA, Ogle TF & Bazer FW 1995 Ovine interferon-tau regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. Biology of Reproduction **53** 732-745.

Spencer TE, Stagg AG, Ott TL, Johnson GA, Ramsey WS & Bazer FW 1999b Differential effects of intrauterine and subcutaneous administration of recombinant ovine interferon tau on the endometrium of cyclic ewes. Biology of Reproduction **61** 464-470.

Spiess K & Zorn TM 2007 Collagen types I, III, and V constitute the thick collagen fibrils of the mouse decidua. Microscopy Research and Technique **70** 18-25.

Stary M, Schneider M, Sheikh SP & Weitzer G 2006 Parietal endoderm secreted S100A4 promotes early cardiomyogenesis in embryoid bodies. Biochemical and Biophysical Research Communications **343** 555-563.

Stewart DM, Johnson GA, Vyhlidal CA, Burghardt RC, Safe SH, Yu-Lee LY, Bazer FW & Spencer TE 2001 Interferon-tau activates multiple signal transducer and activator of transcription proteins and has complex effects on interferon-

responsive gene transcription in ovine endometrial epithelial cells. Endocrinology **142** 98-107.

Takeshita S, Kikuno R, Tezuka K & Amann E 1993 Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. The Biochemical Journal **294** 271-278.

Tsai SJ, Juengel JL & Wiltbank MC 1997 Hormonal regulation of monocyte chemoattractant protein-1 messenger ribonucleic acid expression in corpora lutea. Endocrinology **138** 4517-4520.

Tsai WC, Tsai ST, Jin YT & Wu LW 2006 Cyclooxygenase-2 is involved in S100A2-mediated tumor suppression in squamous cell carcinoma. Molecular Cancer Research **4** 539-547.

Vallet JL, Bazer FW & Roberts RM 1987 The effect of ovine trophoblast protein-one on endometrial protein secretion and cyclic nucleotides. Biology of Reproduction **37** 1307-1316.

van der Rest M & Garrone R 1991 Collagen family of proteins. The FASEB Journal **5** 2814-2823.

Van Heeke G, Ott TL, Strauss A, Ammaturo D & Bazer FW 1996 High yield expression and secretion of the ovine pregnancy recognition hormone interferon-tau by Pichia pastoris. Journal of Interferon & Cytokine Research **16** 119-126.

Wakao H, Gouilleux F & Groner B 1994 Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. The EMBO Journal **13** 2182-2191.

White CA & Salamonsen LA 2005 A guide to issues in microarray analysis: application to endometrial biology. Reproduction **130** 1-13.

White CA, Robb L & Salamonsen LA 2004 Uterine extracellular matrix components are altered during defective decidualization in interleukin-11 receptor alpha deficient mice. Reproductive Biology and Endocrinology **2** 76.

Wicki R, Franz C, Scholl FA, Heizmann CW & Schafer BW 1997 Repression of the candidate tumor suppressor gene S100A2 in breast cancer is mediated by site-specific hypermethylation. Cell Calcium **22** 243-254.

Wintenberger-Torres S & Flechon JE 1974 Ultrastructural evolution of the trophoblast cells of the pre-implantation sheep blastocyst from day 8 to day 18. Journal of Anatomy **118** 143-153.

Wooding FB 1982 The role of the binucleate cell in ruminant placental structure. Journal of Reproduction and Fertility Supplement **31** 31-39.

Wooding FB 1992 Current topic: the synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. Placenta **13** 101-113.

Wooding FB, Staples LD & Peacock MA 1982 Structure of trophoblast papillae on the sheep conceptus at implantation. Journal of Anatomy **134** 507-516.

Yan W & Shao R 2006 Transduction of a mesenchyme-specific gene periostin into 293T cells induces cell invasive activity through epithelial-mesenchymal transformation. The Journal of Biological Chemistry **281** 19700-19708.

Yankey SJ, Hicks BA, Carnahan KG, Assiri AM, Sinor SJ, Kodali K, Stellflug JN & Ott TL 2001 Expression of the antiviral protein Mx in peripheral blood mononuclear cells of pregnant and bred, non-pregnant ewes. The Journal of Endocrinology **170** R7-11.

Yiu GK, Chan WY, Ng SW, Chan PS, Cheung KK, Berkowitz RS & Mok SC 2001 SPARC (secreted protein acidic and rich in cysteine) induces apoptosis in ovarian cancer cells. The American Journal of Pathology **159** 609-622.

Zhang JC, Kim S, Helmke BP, Yu WW, Du KL, Lu MM, Strobeck M, Yu Q & Parmacek MS 2001 Analysis of SM22alpha-deficient mice reveals unanticipated insights into smooth muscle cell differentiation and function. Molecular and Cellular Biology **21** 1336-1344.

Zhang X, Christenson LK & Nothnick WB 2007 Regulation of MMP-9 expression and activity in the mouse uterus by estrogen. Molecular Reproduction and Development **74** 321-331.

Zhou XD, Xiong MM, Tan FK, Guo XJ & Arnett FC 2006 SPARC, an upstream regulator of connective tissue growth factor in response to transforming growth factor beta stimulation. Arthritis and Rheumatism **54** 3885-3889.

Zhu L, Vranckx R, Khau Van Kien P, Lalande A, Boisset N, Mathieu F, Wegman M, Glancy L, Gasc JM, Brunotte F 2006 Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nature Genetics **38** 343-349.

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