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Interferon Tau in the Ovine Uterus

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ABSTRACT

The peri-implantation period in mammals is critical with respect to survival of the conceptus (embryo/fetus and associated extraembryonic membranes) and establishment of pregnancy. During this period of pregnancy, reciprocal communication between ovary, conceptus, and endometrium is required for successful implantation and placentation. At this time, interferon tau (IFNT) is synthesized and secreted by the mononuclear trophectodermal cells of the conceptus between days 10 and 21~25. The actions of IFNT to signal pregnancy recognition and induce or increase expression of IFNT-stimulated genes (ISGs), such as *ISG15* and *OAS*, are mediated by the Type I IFN signal transduction pathway. This article reviews the history, signaling pathways of IFNT and the uterine expression of several IFNT-stimulated genes during the peri-implantation period. Collectively, these newly identified genes are believed to be critical to unraveling the mechanism(s) of reciprocal fetal-maternal interactions required for successful implantation and pregnancy.

(**Key words :** Interferon tau, Progesterone, Sheep, Uterus, Implantation)

INTRODUCTION

In eutherian mammals, including sheep, implantation of the blastocyst is a most important developmental event associated with viviparity (Spencer and Bazer, 2002; Spencer et al., 2004). During the peri-implantation period in the ovine uterus, the spherical blastocyst elongates to a tubular and then a filamentous form, and develops into a conceptus. At this time, interferon tau (IFNT) is synthesized and secreted by the mononuclear trophectodermal cells of the conceptus between days 10 and 21~25 (maximally on days 14 to 16) (Ashworth and Bazer, 1989; Farin et al., 1989; Bazer, 1992; Roberts et al., 1999). In the ovine uterus, IFNT acts directly on the endometrial luminal epithelium (LE) and superficial ductal glandular epithelium (sGE) to suppress transcription of estrogen receptor alpha (*ESR1*) and oxytocin receptor (*OXTR*) genes (Spencer and Bazer, 1996; Fleming et al., 2001), thereby preventing production of luteolytic pulses of prostaglandin F2α (PGF) (Fig. 1).

During the estrous cycle, *ESR1* expression increases and progesterone receptor (*PGR*) expression decreases on days 11 to 13, and subsequently estrogen (E2) induces *OXTR* expression on days 13 to 14 (Wathes and Hamon, 1993; Spencer and Bazer, 1995). Thus, oxytocin from the posterior pituitary and/or corpus luteum (CL) can then induce release of luteolytic pulses of PGF on days 15 and 16 (Hooper et al., 1986). During early pregnancy, IFNT produced by the elongating ovine conceptus suppresses *ESR1* expression which then prevents ESR1-induced *OXTR* expression (Spencer and Bazer, 1996; Stevenson et al., 1994; Lamming et al., 1995; Spencer et al., 1995). Collectively, these indicate that the antiluteolytic actions of IFNT are to prevent increases in epithelial expression of E2-responsive *ESR1*, *PGR*, and *OXTR* gene by directly inhibiting transcription of the *ESR1* gene and maintaining secretion of progesterone (P4) by the CL (Fleming et al., 2001; Fleming et al., 2006; Spencer et al., 2004).

In the ovine uterus, establishment and maintenance of pregnancy requires reciprocal communication between the ovary, conceptus, and endometrium by means of endocrine and paracrine signals during implantation and synepitheliochorial placentation (Spencer and Bazer, 2002). P4, the hormone of pregnancy, plays an important role in the establishment and maintenance of a uterine environment that supports conceptus development. Endometrial gland secretions, including growth factors, cytokines, and ions, are predominantly regulated by P4 (Spencer et al., 1999) and are required for peri-implantation

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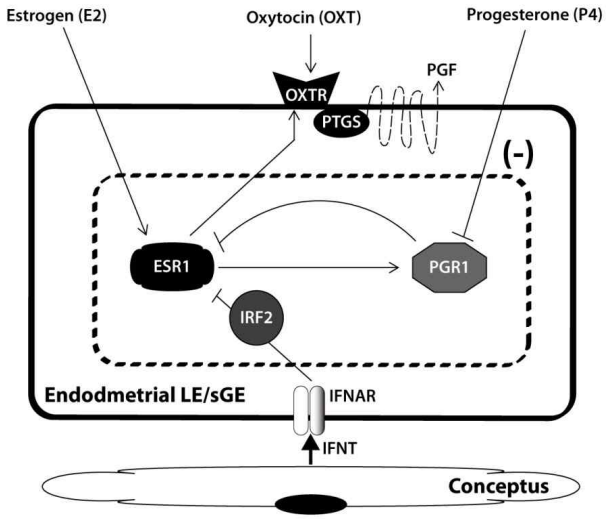

Fig. 1. Schematic illustration of the current working hypothesis on hormonal regulation of the endometrial antiluteolytic mechanism and cross-talk between the conceptus and the maternal endometrium. During the estrous cycle, *ESR1* expression increases and *PGR* expression decreases and then E2 induces *OXTR* expression, thereby allowing oxytocin from the posterior pituitary and/or CL to induce release of luteolytic pulses of *PGF*. In contrast, during early pregnancy, secreted IFNT from fully elongated conceptus silences *ESR1* expression which prevents E2-induced *OXTR* expression. Legend: IFNT, interferon tau; IFNAR, Type I IFN receptor; PGR, progesterone receptor; ESR1, estrogen receptor alpha; OXTR, oxytocin receptor; IRF2, interferon regulatory factor 2; LE, luminal epithelium ; sGE, superficial ductal glandular epithelium. (Adapted from Spencer *et al*., 2007.)

conceptus survival, elongation, and development (Gray et al., 2001; Gray et al., 2000). P4 acts via its cognate receptor, *PGR*. In the ovine endometrium, *PGR* are expressed in epithelia and stroma and allow P4 to directly regulate a variety of genes in the uterus. However, *PGR* expression is down-regulated by continuous exposure to P4 in ovine endometrial LE and GE after days 11 and 13 of pregnancy, respectively (Spencer and Bazer, 1995). The paradigm of loss of *PGR* in endometrial epithelia immediately before implantation is common to sheep (Spencer and Bazer, 2002; Spencer and Bazer, 1995), cattle (Kimmins and MacLaren, 2001), and pigs (Geisert et al., 1994), and other mammals studied to date including humans and mice (see review by (Spencer et al., 2004)).

During the peri-implantation period, uterine epithelial cell functions might be regulated by interactions between reprogrammed epithelial cells following down-regulation of *PGR* and specific factors produced by *PGR*-positive stromal cells in response to P4, and/or products of the conceptus such as IFNT, placental lactogen, and placental growth hormone (see review by Spencer et al., 2004). A large number of genes are induced by IFNT throughout the width of the uterine wall. These IFNT-stimulated genes (ISGs) are proposed to have biological roles in pregnancy recognition and uterine receptivity (Spencer and Bazer, 2002). In addition, induction of an antiviral state in the endometrium during early pregnancy may be beneficial by inhibiting sexually transmitted viruses as well as modulating local immune cells to promote tolerance of the allogeneic conceptus and stimulating production of cytokines beneficial for conceptus survival and growth (Hansen, 1995; Tekin and Hansen, 2002; Croy et al., 2003).

Collectively, knowledge of the complex, precisely orchestrated interaction between P4 and IFNT during the implantation period should provide new insights to improve fertility in humans and domestic animals, and provide key knowledge for interpreting cross-talk mechanisms between maternal endometrium and conceptus.

OVERVIEW OF IFNT IN SHEEP

1. History of IFNT

The developing conceptus must signal its presence to the mother in order to ensure successful establishment and maintenance of pregnancy, a process termed maternal recognition of pregnancy (Short, 1969). In the 1960s, Moor and Rowson reported extension of the inter-estrous interval following a transfer of day 13 sheep blastocysts into recipient ewes on day 12 of the estrous cycle (Moor and Rowson, 1964), and that removal of blastocysts after day 13 significantly extended CL life-span (Moor and Rowson, 1966). In addition, infusion of the homogenates of the sheep conceptus collected between days 14 and 15 into the uterine lumen of recipient ewes (on or before day 12 of their cycle) extend CL life-span and the inter-estrous interval of cyclic ewes. However, infusion of pig conceptus homogenates had no effects on estrous cycle length in ewes (Rowson and Moor, 1967). The transfer of trophoblastic vesicles from blastocysts collected between days 11 and 13, without the embryonic disc, to recipient ewes on day 12 of the estrous cycles maintained CL function (Heyman et al., 1984). The first report of secretion of low molecular weight acidic proteins by day 16 ovine conceptuses was by

Wilson *et al*. (1979). Later, in 1982, Godkin and colleagues characterized secretion of the low molecular weight acid protein by cultured ovine conceptuses collected between days 13 and 21 of pregnancy and termed it protein X (Godkin et al., 1982). In a later study, protein X from ovine trophectoderm was termed ovine trophoblast protein 1 (oTP-1) (Godkin et al., 1984). Subsequently, native purified or recombinant oTP-1 was shown to extend the inter-estrous interval of ewes and to attenuate oxytocin-induced PGF release in sheep (Fincher et al., 1986; Vallet et al., 1988; Ott et al., 1993).

oTP-1 has been found to have an amino acid sequence homologous to that of bovine IFN alpha (Imakawa et al., 1987) and also to possess the antiviral and antiproliferative properties (Pontzer et al., 1988; Pontzer et al., 1991; Roberts et al., 1989). Therefore, oTP-1 has been renamed IFNT and designated as a member of the Type I IFN family (Roberts et al., 1992; Bazer et al., 1996) by the International Interferon Society. In the conceptus, *IFNT* mRNA increases from day 12 to 14 and then declines to day 22 and is localized to mononuclear trophetodermal cells (Farin et al., 1989; Hansen et al., 1988). Like other Type I IFN family members such as IFN alpha, -beta, -delta, -epsilon, -kappa and -omega, IFNT possesses potent antiviral (Pontzer et al., 1988; Pontzer et al., 1991; Roberts et al., 1989), antiproliferative (Roberts et al., 1989; Fillion et al., 1991), and immunomodulatory biological activities and effects (Fillion et al., 1991; Tennakoon et al., 2001). IFNT is most closely related to IFN omega encoding the 172 amino acid sequence (Ealy et al., 1998). Even though IFNT shares a high-degree of DNA and amino acid sequence identity in ruminants (sheep, cattle, goats) (Imakawa et al., 1987; Roberts et al., 1992), the precise biochemical structure of IFNT is different among species because of different post-translational modifications. Ovine IFNT is not glycosylated, bovine IFNT is glycosylated, and caprine IFNT is found in both glycosylated and non-glycosylated forms (Roberts et al., 1992; Nephew et al., 1993; Baumbach et al., 1990).

2. Type I IFN Signal Transduction Pathway

The actions of IFNT to signal pregnancy recognition and induce or increase expression of IFNT-stimulated genes (ISGs) are mediated by the Type I IFN signal transduction pathway (Fig. 2). Type I IFNs bind to a common Type I IFN receptor (*IFNAR*), a heterodimer consisting of two subunits, IFNAR1 and IFNAR2, associated with janus kinase 1 (*JAK1*) and tyrosine kinase 2 (*TYK2*) (Novick et al., 1994; Domanski et al., 1995). The receptor is present in all endometrial cell types, but is highest in endometrial LE (Rosenfeld et al., 2002). IFNAR classically activate the JAK/STAT (signal transducers and activators of transcription) signaling pathway (Darnell, 1997;

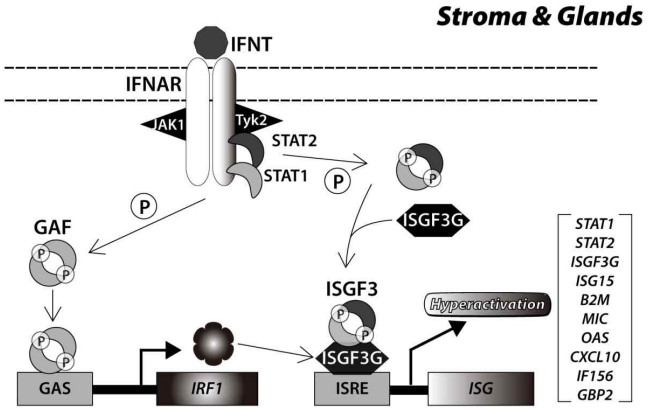

Fig. 2. Schematic illustration of the current working hypothesis on IFNT signaling in the ovine endometrial stroma and glandular epithelium. IFNT binds to a common Type I IFN receptor, IFNAR1 and IFNAR2 containing tyrosine kinase such as JAK1 and TYK2, and activates the JAK/STAT signaling pathway. Phosphorylated STAT1 binds the phosphorylated STAT2 to form a heterodimer and translocates to the nucleus after forming a heterotrimeric transcriptional complex by binding with ISGF3G, collectively termed ISGF3. In addition to STAT1/2 heterodimerization, Type I IFN also induces formation of phosphorylated STAT1 homodimers, termed GAF. In the nucleus, ISGF3 binds to the IFN-stimulated response element (ISRE) in promoter regions of ISGs and activates their transcription in cooperation with several coactivators. Similarly, GAF enters the nucleus, binds to GAS elements, and stimulates the transcription of ISGs. Legend: IFNT, interferon tau; IFNAR, Type I IFN receptor; JAK1, janus kinase 1; TYK2, tyrosine kinase 2; STAT, signal transducers and activators of transcription; GAF, gamma IFN activation factor; GAS, GAF activation sequence; IRF1, interferon regulatory factor 1; ISGF3G, IFN-stimulated transcription factor 3, gamma 48-kDa; ISGF3, IFN-stimulated transcription factor 3; ISRE, IFN-stimulated response element; ISG, IFNT-stimulated gene; ISG15, IFNT-stimulated gene 15; B2M, beta-2-microglobulin; MHC, major histocompatibility complex; OAS, oligoadenylate synthetase; CXCL10, chemokine (C-X-C motif) ligand 10; IFI56, interferon-induced protein 56; GBP2, guanylate binding protein 2, interferon-inducible. (Adapted from Spencer *et al*., 2007.)

Darnell et al., 1994; Stark et al., 1998). Upon cognate ligand binding, IFNAR1 and IFNAR2 heterodimerize, change their conformation, and activate TYK2 and JAK1 by tyrosine phosphorylation (Gauzzi et al., 1996; Muller et al., 1993). The activated TYK2 phosphorylates STAT2 through its SH2 (src homologous 2) domain and then recruits signal transducers and activators of transcription-1 (STAT1) (Colamonici et al., 1994; Yan et al., 1996). Phosphorylated STAT1 binds phosphorylated STAT2 to form a heterodimer. The STAT1-STAT2 dimer is subsequently released from the receptor and binds ISGF3G (IFN-stimulated transcription factor 3, gamma 48 kDa) to form a heterotrimeric transcriptional complex, collectively termed ISGF3, which translocates to the nucleus (Silvennoinen et al., 1993; Shuai et al., 1994). In addition to STAT1/2 heterodimerization, Type I IFN also induces formation of phosphorylated STAT1 homodimers, termed GAF (gamma IFN activation factor) (Haque and Williams, 1994). In the nucleus, ISGF3 binds to an IFN-stimulated response element (ISRE) in promoter regions of ISGs to activate transcription in cooperation with several coactivators, such as the cAMP response element binding protein (CREB)-binding protein (CBP)/p300 (Bhattacharya, et al., 1996). Similarly, GAF enters the nucleus, binds to GAS (GAF activation sequence) elements to stimulate transcription of ISGs (Pine et al., 1994).

3. IFNT-Stimulated Genes (ISGs)

Most IFNT-stimulated genes (ISGs) are expressed by endometrial stroma and middle to deep GE of the ovine uterus (Choi et al., 2001; Johnson et al., 1999; Johnson et al., 2001). These ISGs include *STAT1* and *STAT2* (Johnson et al., 1999; Stewart et al., 2001), *IRF1* (Johnson et al., 1999; Stewart et al., 2001; Spencer et al., 1998), *ISG15* (Johnson et al., 1999; Johnson et al., 2000; Stewart et al., 2001), *Mx* (Ott et al., 1998), 2',5'-oligoadenylate synthetase (*OAS*) (Mirando et al., 1991; Johnson et al., 2001), major histocompatibility complex (*MHC*) class I (Choi et al., 2003), and beta-2-microglobulin (*B2M*) (Choi et al., 2003; Vallet et al., 1991).

IRF2, a known transcriptional repressor of Type I ISGs in the ovine uterus is constitutively expressed in the endometrial LE and sGE, increases during early pregnancy, and prevents induction or increases in transcription of ISGs by IFNT (Fig. 3) (Choi et al., 2002; Kim et al., 2003). *WNT7A* (Kim et al., 2003) and *LGALS15* (also known as galectin-15) (Gray et al., 2004) are the only genes known to be induced in LE and sGE by IFNT utilizing an unknown non-classical signaling pathway that is independent of the classical STAT transcription factors.

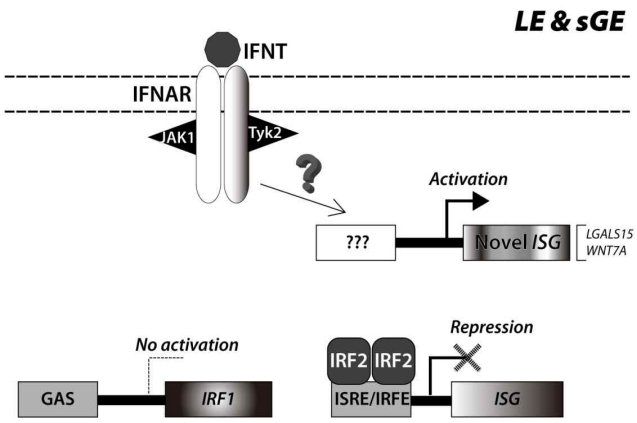

Fig. 3. Schematic illustration of the current working hypothesis on IFNT-signaling in the ovine luminal and superficial ductal glandular epithelium. IRF-2, a known transcriptional repressor of Type I ISGs in the ovine uterus constitutively expressed in the endometrial LE and sGE, increases during early pregnancy, and prevents induction or increases in transcription of ISGs by IFNT. At present, *LGALS15* and *WNT7A* are the only genes known to be induced in LE and sGE by IFNT via an unknown non-classical signaling pathway that does not involve the classical STAT transcription factors. Legend: IFNT, interferon tau; IFNAR, Type I IFN receptor; JAK1, janus kinase 1; TYK2, tyrosine kinase 2; GAS, GAF activation sequence; IRF, interferon regulatory factor; ISG, IFNT-stimulated gene; LGALS15, galectin 15; WNT7A, wingless-type MMTV integration site family, member 7A; LE, luminal epithelium; sGE, superficial ductal glandular epithelium. (Adapted from Spencer *et al*., 2007).

4. Representative STAT1-Dependent ISGs

(1) Ubiquitin Cross-Reactive Protein / IFN-Stimulated Gene 15/17.

In humans, IFN-stimulated gene 15 (*ISG15*) encoding a 15-kDa protein, which has been identified in tumor and lymphoblastoid cells, and is induced by Type I IFNs (IFN alpha and beta) to a greater extent than by Type II IFN (IFN gamma) (Farrell et al., 1979; Korant et al., 1984). However, *ISG15* was renamed ubiquitin cross-reactive protein (*UCRP*) because its sequence is highly homologous to a tandem diubiquitin repeat, and antibodies raised to ISG15 cross-react with ubiquitin (Haas et al., 1987). In bovine

endometrium, a 17 kDa precursor form of UCRP was detected as a 16 kDa form that might have undergone proteolytic cleavage in the endometrium (Johnson et al., 1999; Austin et al., 1996). During early pregnancy, in sheep, *ISG15* mRNA abundance increases only in stroma and GE from days 11 to 15 and then declines thereafter (Johnson et al., 1999). This period is coincident with peak production of IFNT by the ovine conceptus and *ISG15* expression increases in immortalized ovine LE, GE, and stromal cells treated with IFNT (Johnson et al., 1999).

(2) 2',5'-Oligoadenylate Synthetase.

2',5'-oligoadenylate synthetase (*OAS*) is induced by Type I and -II IFNs and polymerizes ATP into 2'-5' linked oligomers in order to bind and activate RNase L which can destroy intracellular viral RNAs. Further, *OAS* is involved in antiviral activity, cell growth, differentiation, and apoptosis (Samuel, 1991; Lengyel, 1993; Salzberg et al., 1997). In the ovine uterus, *OAS* is expressed only in the stroma and deep GE in response to IFNT and P4 during early pregnancy (Johnson et al., 1999; Mirando et al., 1991).

(3) RSAD2.

Radical S-adenosyl methionine domain containing 2 (*RSAD2*), also known as viperin, is a cytoplasmic antiviral protein that consists of 361 amino acids, and is encoded for by a gene which contains putative IRF binding sites in the promoter region (Chin and Cresswell, 2001; Sun and Nie, 2004). In humans, stable expression of *RSAD2* in fibroblasts inhibits human cytomegalovirus infection (Chin and Cresswell, 2001). RSAD2 is also a potential antiviral effector expressed in patients with atherosclerosis (Olofsson et al., 2005) and chronic hepatitis C virus (Helbig et al., 2005). Chin *et al*. reported that *RSAD2* expression is greater in response to Type I than Type II IFN (IFN gamma) and that RSAD2 may have an antiviral function (Chin and Cresswell, 2001).

(4) MDA5 (IFIH1).

Melanoma differentiation associated gene 5 (*MDA5* also known as *IFIH1*) is a dsRNA-dependent ATPase that responds predominantly to Type I IFNs and is known to be induced during differentiation, cancer reversion, and programmed cell death (Kang et al., 2002; Kang et al., 2004). The *IFIH1* gene contains both CARD and RNA helicase motifs and acts as a positive regulator to sense intracellular viral infection and stimulate innate antiviral responses including the production of Type I IFN (Kang et al., 2002; Yoneyama et al., 2005). The V proteins of a wide variety of paramyxoviruses bind IFIH1 and inhibit its ability to activate the IFNB promoter (Andrejeva et al., 2004). Further, IFN beta promoter stimulator 1, which can induce Type I IFN and IFN-inducible genes through activation of IRF3, IRF7 and NF-κB transcription factors, is known as an adaptor during IFIH1-mediated antiviral immune response (Kawai et al., 2005).

5. Representative STAT1-Independent ISGs

(1) Wingless-Type Mouse Mammary Tumor Virus Integration Site Family, Member 7A (WNT7A).

Most members of the WNT family are involved in embryonic cell growth, development, and differentiation and also in maternal-fetal interactions during implantation (Mohamed et al., 2005). In the ovine uterus, *wingless-type mouse mammary tumor virus integration site family, member 7A* (*WNT7A*) was first identified and the gene is induced by IFNT during early pregnancy and expressed only in LE and sGE (Kim et al., 2003). Ovine endometrial *WNT7A* may activate the canonical WNT signaling pathway to stimulate proliferation and differentiation of conceptus trophectoderm may also regulate important genes for uterine receptivity for implantation and conceptus survival (Spencer et al., 2007).

(2) Galectin-15 (LGALS15).

Galectins are widely distributed in a variety mammalian species, as well as non-mammalian species including birds, fish, and amphibians (Cooper and Barondes, 1999). They are members of a superfamily of binding lectins that bind β-galactoside via a CRD (carbohydrate recognition domain) (Barondes et al., 1994). In sheep, LGALS15 was identified as the novel 14 kDa form of a P4-modulated protein associated with crystalline inclusion bodies in endometrial LE and conceptus trophectoderm (Kazemi et al., 1990). In the ovine uterus, *LGALS15* mRNA is expressed only in endometrial LE and sGE where it is induced by P4 and stimulated by IFNT. In addition, LGALS15 protein has a nucleocytoplasmic distribution within the LE and sGE and is also concentrated near and on the apical surface (Gray et al., 2004). Therefore, LGALS15 is secreted into the uterine lumen by the LE and sGE, where it may promote adhesion during implantation, as well as is phagocytosed by the trophectoderm and formed intracellular crystals (Gray et al., 2004; Gray et al., 2005).

(3) Cathepsin L (CTSL).

Cathepsins (*CTS*) are a family of lysosomal proteinases that are active in an acidic environment (Kirschke et al., 1998). They can degrade extracellular matrix (ECM) molecules, including collagens, laminin, fibronectin and proteoglycans and are also involved in catabolism of intracellular proteins and processing of pro-hormones. Available evidence supports the concept that a variety of proteases, as well as their specific inhibitors regulate trophoblast invasion in many species (e.g. mouse, rat, cat, pig, and human) during conceptus implantation (Afonso et al., 1997; Elangovan and Moulton, 1980; Li et al., 1992; Verhage et al., 1989; Geisert et al., 1997; Roberts et al., 1976; Jokimaa et al., 2001). CTSL is normally localized in lysosomes where it plays a major role in intracellular protein catabolism. In rodents, interactions between Ctsb, Ctsl, and Cst3 (Ctsb and Ctsl inhibitors) are important for implantation and placentation, because inhibition of endometrial *Ctsb* and *Ctsl* results in abnormal embryonic development and uterine decidualization during the peri-implantation period (Afonso et al., 1997). In cats, CTSL is localized to the GE and can be detected in the uterine lumen where it is implicated in blastocyst invasion (Li et al., 1992). In pigs, *CTSL* is expressed in the endometrial GE and it is a P4-regulated component of the uterine lumen during implantation and placentation (Geisert et al., 1997).

(4) Cystatin C (CST3).

Cystatin C (*CST3*) is a secreted inhibitor of lysosomal cysteine proteases CTSB and CTSL (Abrahamson et al., 1986; Abrahamson et al., 1990; Hall et al., 1995; Grubb and Lofberg, 1982). In mice, *Ctsb* and *Ctsl* are necessary for normal embryonic development and uterine decidualization. The decidua coordinately expresses *Cst3* to control *Ctsb* and *Ctsl* actions within the implantation site (Afonso et al., 1997). A variety of proteases, as well as their inhibitors, regulate endometrial remodeling and trophoblast invasion in many species (e.g. mouse, rat, cat, sheep, pig, and human) during conceptus implantation and placentation.

DISCUSSION

What are the molecular mechanisms and signal transduction pathways activated by IFNT to regulate transcription of the novel epithelial genes, such as *WNT7A, LGALS15, CTSL,* and *CST3*, only in LE and sGE in the ovine uterus during the peri-implantation period? The current working hypothesis is that IFNT utilizes STAT1-independent signaling pathway(s) to stimulate transcription of those genes in the LE and sGE (Fig. 4). In the ovine endometrial LE and sGE, the essential components of the JAK/STAT signal transduction, such as STAT1, -2, and ISGF3G, are not expressed, but IRF2, a potent transcriptional repressor of ISGs, was identified specifically in those cells, where it could repress or suppress the transcriptional acitivity of the promoter regions of ISGs that contain ISREs and IRF-Es (see bottom panel) (Choi et al., 2001; Stewart et al., 2001). Further, in our *in silico* study, the enhancer/promoter regions of bovine *WNT7A, LGALS15, CTSL,* and *CST3* genes had conserved transcription factor(s) binding sites for AP-1, CEBPB, CREB, ELK1, GATA, and LEF1/TCF7, but not for STATs or IRFs. Are there unknown non-classical JAK/STAT signaling pathways that are independent of STAT1? Recently, Platanias *et al.* reported that the generation of responses to Type I IFN requires the coordination and cooperation of multiple distinctive signaling cascades including the mitogen-activated protein (MAP) kinase p38 pathway and the phosphatidylinositol 3-kinase (PI3K) pathway (see review by Platanias, 2005). The p38 MAP kinase is phosphorylated and activated in several IFN-sensitive cell lines in response to Type I IFN such as IFN alpha and its inhibitor (SB203580) blocks IFN-inducible transcription (Platanias, 2005; Uddin et al., 2000). Inhibition of p38 MAP kinase has no effects on the phosphorylation of STAT1 or -2, and formation of the ISGF3 transcriptional complex (Uddin et al., 1999; Platanias et al., 2005). In addition, Type II IFN (IFN gamma) did not activate p38 MAP kinase in several cell lines (Katsoulidis et al., 2005; Uddin et al., 2000). Further, in the bovine uterus, IFNT activates the p38 MAP kinase pathway for induction of PTGS2 in myometrial cells (Doualla-Bell and Koromilas, 2001). These results indicate that p38 MAP kinase may play a role in Type I IFN-mediated signal transduction that is independent of STATs. Therefore, IFNT activation of p38 MAP kinase may be one signaling pathway whereby IFNT stimulates transcription of certain genes independent of STAT1 in the ovine uterus. Meanwhile, PI3K is activated in response to Type I or II IFNs. In the case of the Type I IFN signaling pathway, Type I IFNs activate the PI3K-signal pathway downstream of JAKs, in an insulin receptor substrate (IRS)-dependent but STAT-independent manner (Platanias, 2005; Kaur et al., 2005). The proposed model for the IFNT signal transduction cascade that is STAT1-independent in the ovine LE and sGE is illustrated in the upper panel of Fig. 4. IFNT-activated JAK1/TYK2 may

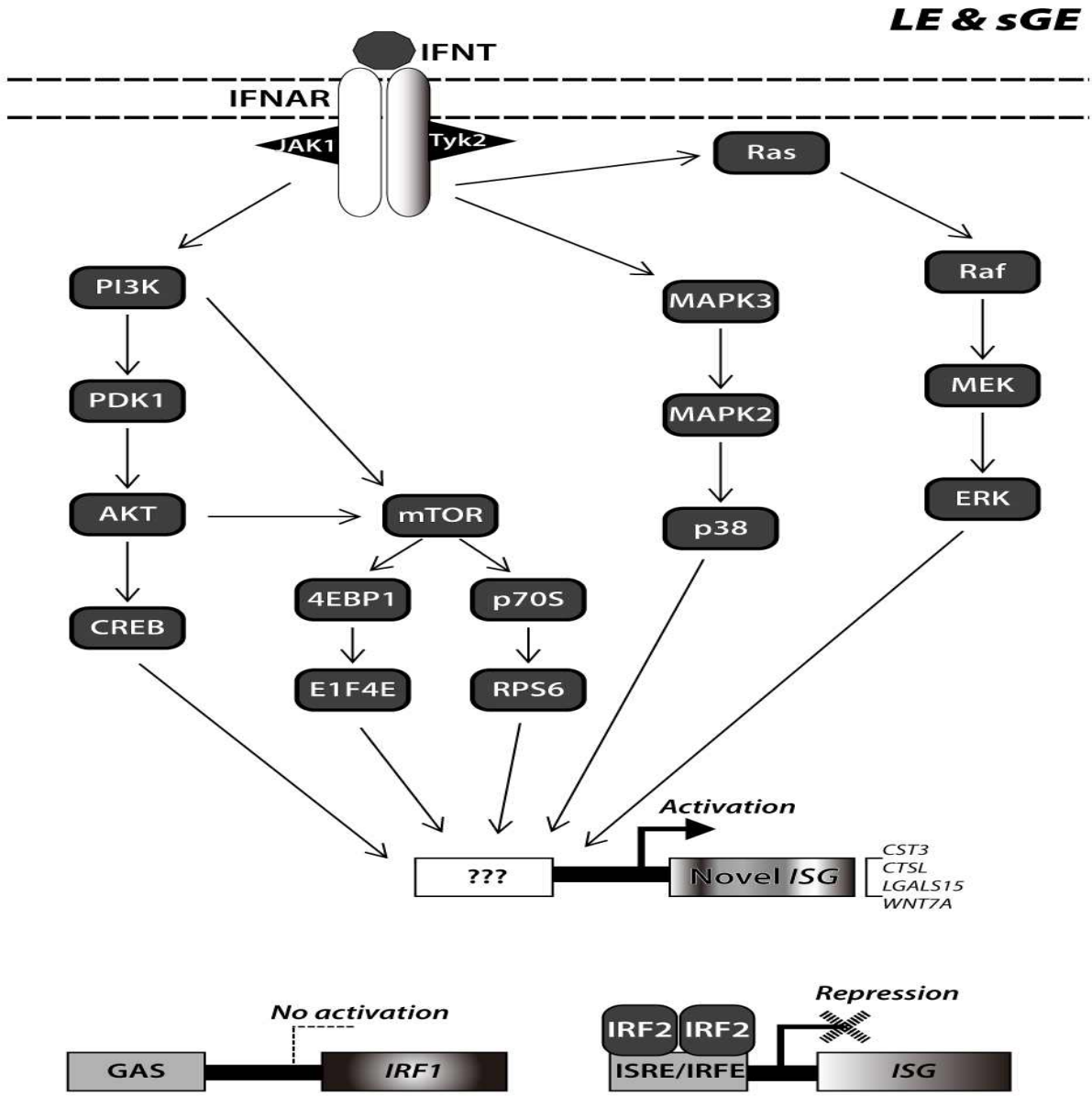

Fig. 4. A proposed model of IFNT signal transduction cascades that is independent of STAT1 in the ovine LE and sGE. IFNT-activated JAK1/TYK2 may regulate the phosphorylation of PI3K, resulting in the downstream activation of PDK1 and AKT. The activated AKT translocate into the nucleus and phosphorylate a variety of target proteins such as CBP/p300 or NF-κB. Also, IFNT may activate MAPKKK or Raf which is activated by activated Ras. Activated MAPKKK and/or Raf subsequently regulate activation of downstream effectors including MAPKK, p38 MAPK, or MEK, ERK, respectively. In addition, the mTOR-p70S6K pathway which is activated by PI3K or AKT, may be involved in mRNA translation of ISGs by phosphorylated RPS6 and translational respressor 4EBP1. Legend: IFNT, interferon tau; IFNAR, Type I IFN receptor; JAK1, janus kinase 1; TYK2, tyrosine kinase 2; GAS, GAF activation sequence; IRF, interferon regulatory factor; ISG, IFNT-stimulated gene; LGALS15, galectin 15; WNT7A, wingless-type MMTV integration site family, member 7A; CTSL, cathepsin L; CST3, cystatin C; PI3K, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent protein kinase 1; AKT, proto oncogenic protein kinase Akt; CREB, cAMP-response element binding protein; mTOR, mammalian target of rapamycin; 4EBP1, EIF4-E-binding protein 1; E1F4E, eukaryotic translation-initiation factor 4 E; p70S, p70 ribosomal protein S6 kinase; RPS6, ribosomal protein S6; $MAPK^3$, mitogen-activated protein kinase (MAPK) kinase kinase; $MAPK^2$, MAPK kinase; ERK, extracellular signal-regulated kinase; LE, luminal epithelium ; sGE, superficial ductal glandular epithelium.

regulate the phosphorylation of PI3K, resulting in the downstream activation of phosphoinositide-dependent protein kinase 1 (PDK1) and proto oncogenic protein kinase Akt (AKT). The activated AKT translocates into the nucleus and then phosphorylates a variety of target proteins such as CREB (cAMP-response element binding protein)-binding protein (CBP)/p300 or NF-κB. Also, IFNT may activate MAPK kinase kinase (MAPKKK) or Raf which is activated by activated Ras. Activated MAPKKK and/or Raf subsequently regulate activation of downstream effectors including MAPK kinase (MAPKK), p38 MAPK, MEK, or extracellular signal-regulated kinase (ERK). In addition, the mammalian target of rapamycin (mTOR)-p70 ribosomal protein S6 kinase (p70S6K) pathways which are activated by PI3K or AKT, may be involved in mRNA translation of ISGs by phosphorylated ribosomal protein S6 (RPS6) and translational respressor 4EBP1 (eukaryotic translation-initiation factor 4 E (EIF4-E)-binding protein 1). This hypothesis is supported by available results that IFNT and growth factors including insulin-like growth factor 2 stimulate PI3K-AKT and MAPK signal transduction cascades in ovine trophectodermal and LE cells. Meanwhile, another possible scenario in the ovine uterus during the peri-implantation period is that IFNT may induce WNT7A using the canonical WNT signaling pathway between days 12 and 16 of pregnancy and then WNT7A acts in an autocrine or paracrine manner to stimulate the *LGALS15, CTSL*, and *CST3* genes in endometrial LE and sGE. Because *WNT7A*is the only gene truly induced by IFNT, its expression is not detected on day 12 of pregnancy, but is induced by IFNT between days 14 and 16 (Farrell et al., 1979). In fact, *LGALS15, CTSL,* and *CST3* genes are stimulated by IFNT between days 14 and 16 of pregnancy,

Interestingly, the ovine placenta expresses large numbers of aspartic proteinase inhibitor genes, termed pregnancy-ssociated glycoproteins, and the endometrial glands also express large amounts of serine protease inhibitors, termed serpins or uterine milk proteins, that could regulate the activity of endometrial CTS identified in these studies. Therefore, the molecular control of expression of CTS in the ovine endometrium may play an important role in establishing a regulatory network of multiple proteolytic enzymes responsible for ECM remodeling during implantation and placentation. Futher, coordinated increases in CTSL and CTSB with CST3 occur in endometrial LE and sGE as well as in conceptus trophectoderm during early pregnancy. Thus, one biological role of CST3 may be to inhibit the actions of cysteine proteases produced by the conceptus and endometrial epithelia in order to limit the invasive activity of the trophoblast. These results support the general idea that proteases and their inhibitors expressed at the maternal-fetal interface are important for uterine receptivity, endometrial remodeling and conceptus implantation during pregnancy in mammals.

CONCLUSIONS

During the peri-implantation period in sheep, *CTSL* and *CST3* are novel P4-induced and IFNT-stimulated genes in endometrial LE and sGE. The majority of ISGs including *RSAD2* and *IFIH1* are expressed in endometrial stroma and middle to deep glands as well as immune cells in response to cell signaling involving the classical STAT1-dependent JAK/STAT signal transduction pathway without a requirement for P4 in the ovine uterus. It has been reported and hypothesized that Type I IFNs and many common ISGs are upregulated for the implanting conceptus in the endometrium during pregnancy in humans, rodents, and domestic animals. Recent evidence that ISGs are among the most upregulated genes in human decidualized stromal cells by trophoblast conditioned medium supports the hypothesis that a lack of ISG expression would compromise pregnancy. In contrast, IFNT induction of several non-classical ISGs, such as *LGALS15*, *WNT7A*, *CTSL*, and *CST3* in endometrial LE and sGE is dependent on P4, which is hypothesized to involve P4-induced down-regulation of PGR in those epithelia, as well as induction of an unknown STAT1-independent signaling pathway. Thus, knowledge of mechanisms whereby IFNT stimulates *CTSL* and *CST3* gene expression in endometrial LE and sGE may elucidate a non-classical signaling pathway for Type I IFNs.

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