—Review—

# Molecular Mechanisms Associated with Conceptus-endometrium Interactions during the Peri-implantation Period in Ruminants

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**Abstract:** *In mammals, the establishment of pregnancy is dependent upon coordinated biochemical signaling and physical interactions between the developing conceptus and uterine endometrium. These essential forms of communication between the conceptus and its maternal environment result in continued production of progesterone from the corpus luteum (CL) and the initiation of implantation/placentation. During the peri-implantation period, conceptuses in ruminant ungulates secrete interferon-tau (IFNT), which acts on uterine endometrium and attenuates endometrial production of the luteolysin, prostaglandin $F_{2\alpha}$, resulting in the maintenance of CL function. Expression of the ovine IFNT (oIFNT) genes is restricted to the mononuclear cells of the trophoblast and the protein is produced for only a relatively short and discrete window of time during early pregnancy. This review deals with identification, characterization and regulation of IFNT gene transcription, and uterine responses associated with pregnancy establishment in ruminants.*

**Key words:** *Conceptus, Endometrium, IFNT, Transcription, Implantation, Ruminants*

## Estrous Cycles and Maternal Recognition of Pregnancy in Ruminants

As female animals reach puberty, they experience follicular growth, ovulation, corpus luteum (CL) formation and its regression, the processes of which are repeated regularly until the cessation of reproductive life (menopause). Progesterone, produced and secreted by CL, is involved directly and/or indirectly in numerous uterine functions through endometrial secretions, alteration of blood flow at implantation sites and promotion of suitable physiological and/or immune environments for normal embryonic development. In ruminants, the estrous cycle is regulated by uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced demise of the CL. $PGF_{2\alpha}$ is released from the endometrial luminal and superficial glandular epithelium in an episodic fashion toward the end of the estrous cycle. Oxytocin, of neurohypophyseal and luteal origin, binds oxytocin receptors and initiates pulsatile $PGF_{2\alpha}$ secretion, which in turn, stimulates release of luteal oxytocin and creates a positive feedback loop that results in short-duration pulses which are effective in causing luteolysis [1]. Progesterone exposure during the early to mid-luteal phase of the estrous cycle is essential for initiation of uterine $PGF_{2\alpha}$ production. In sheep, $PGF_{2\alpha}$ begins to be secreted in a pulsatile manner on day 12 (day 0 = day of estrus) of the 16 day-estrous cycle, but if the pregnancy was established, episodic production of $PGF_{2\alpha}$ is attenuated [2, 3]. This endocrinological phenomenon, the maintenance of CL function beyond a period of the normal luteal phase, along with changes in maternal immunology and metabolism, has been described as the process of the maternal recognition of pregnancy [4]. It is thought that maternal-embryonic communication initiates this process, in which CL regression is inhibited by a signal(s) from the conceptus, and progesterone secretion is sustained and subsequently pregnancy is established.

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## Physiological Events Associated with Implantation

Implantation, a critical step for mammalian species in establishing pregnancy, requires tightly regulated completion of sequential events such as maternal uterine development, conceptus development and attachment/invasion to the endometrium, and placental formation. Importantly, conceptus and uterine development must be synchronized and coordinated through physical and/or biochemical communications. Although the initiation and completion (placental formation) of implantation processes are similar between species, the time frame in which these physiological events proceed differs among mammalian species. In ruminant ungulates, the blastocyst hatches from zona pellucida on day 8, however, it does not immediately attach to the uterine epithelium. The spherical blastocyst remains unattached to the uterine lumen for several days before its elongation begins on days 12–13. On day 16, the ovine conceptus starts to attach to the endometrium when trophoblast elongation (up to 25 cm in length) slows down or subsides. Adhesion of the conceptus to the endometrium starts on day 18, and placenta formation is initiated around day 20 [5].

Embryonic and fetal mortality in both farm animals and humans occurs most frequently during the first few weeks after conception, the period corresponding to peri-implantation processes. Although it occurs at a much lower rate, embryonic lethality in rodents invariably occurs most frequently during the early implantation period [6]. In cattle, it is estimated that the fertilization rate is around 90% and the average calving rate is approximately 55%. This suggests that embryonic/fetal mortality is about 35%, of which 70–80% of embryonic losses occur between days 8 and 16 after artificial insemination [7]. Recently, the technology to clone animals was developed; however, the efficiency of production has been very low. In fact, only 2–3% or less of reconstructed eggs results in live offspring, thus the development of this technology has not reached its potential for improving livestock production. The low success rate can be attributed to abnormalities in early developmental processes during embryogenesis that include implantation, maternal recognition of pregnancy, and formation of placenta and initial organogenesis.

## Identification of Anti-luteolytic Factor, oTP-1

In the 1960s, numerous observations determined that the presence of an embryo within the uterus was required for the maintenance of the CL. Removal of the conceptus from the ovine uterus on day 12 or before resulted in CL regression (luteolysis) and the return to estrus at the normal 16–17 day interval. But if the conceptus was removed from the uterus on or after day 13, the CL was maintained in a pseudopregnant state [8]. When embryos were transferred to the uterus on days 12, 13 or 14 of the estrous cycle into synchronized non-pregnant ewes, only day 12 ewes became pregnant [9]. The uterine infusion of conceptus homogenates prepared from day 14 or 15 pregnant ewes inhibited luteolysis, however, the conceptus homogenates from day 25 pregnant ewes did not increase the length of the estrous cycle [9]. This evidence suggests that a signal(s) required for the establishment of pregnancy is secreted from the embryo to mother around day 12 and the timing of signal production is very critical. In 1979, Martal and coworkers observed that the extract of day 14–16 conceptuses, but not day 25 conceptuses actively produced an antiluteolytic factor which extended CL life span [10]. This factor was inactivated by heat or proteinase treatment, thus this protein activity was termed “trophoblastin”. In 1982, Godkin and coworkers then identified and purified a secretory conceptus protein with antiluteolytic activity and named the protein as ovine trophoblast protein-1 (oTP-1). Its mass is about 19 kDa, which includes several isoforms with isoelectric points ranging from 5.3 to 5.7 [11, 12]. Moreover, a protein cross-reacting with oTP-1 antiserum was identified in bovine conceptus cultured medium and termed bovine trophoblast protein-1 (bTP-1) [13, 14]. The molecular mass of bTP-1 (22 to 24 kDa) is slightly greater than that of oTP-1 due to N-glycosylation [15, 16]. Likewise, caprine conceptus was found to possess trophoblast proteins similar to oTP-1 and bTP-1 [17, 18].

Infusion of purified oTP-1 directly into the uterus causes the extension of luteal function, and attenuates uterine $PGF_{2\alpha}$ production [19, 20]. Conceptus homogenates, from which oTP-1 was removed, do not prolong CL lifespan [21]. In addition, oTP-1 binds to the endometrial receptor with high affinity and alters uterine protein expression, but is not readily detected in the blood or any other tissues [12]. Therefore, these observations suggest that oTP-1 is secreted from the trophectoderm of conceptus, acts as an anti-luteolysin, and is thereby recognized as a factor which could elicit the process of maternal recognition of pregnancy [22].

## Structure of Interferon-tau (*IFNT*) Genes

It was demonstrated through an analysis of cDNA and protein sequences that oTP-1 produced by the conceptus was an interferon (IFN) [23–25]. The deduced amino acid sequence of oTP-1 cDNA indicated that it shares a high degree of similarity to that of type I interferons (IFNs). IFNs are cytokines, induced by viral infection, double stranded RNA or malignant growth, and have antiviral and antiproliferative activities. These are divided into two groups, Type I IFN and Type II IFN [26]. Type I IFNs are induced by viral infection and include several subfamilies, IFN$\alpha$/IFN$\omega$ and IFN$\beta$, which are produced by leukocytes and fibroblasts, respectively. IFN$\gamma$ is the only member in type II IFN and produced by T cells or NK cells following mitogen treatment. This conceptus IFN possesses a high degree of structual similarity to IFN$\omega$, consists of four cysteine residues, which are conserved across Type I IFN, and therefore this IFN belongs to a family of type I IFN. However, the observations that this IFN is not secreted by blood cells and more importantly, it can be serologically distinguished from other Type I IFNs, lead to a new classification, ovine IFN-tau (oIFNT) [5, 22]. In the subsequent studies, *oIFNT* genes were identified and several of which were characterized for their nucleotide sequences and were named as *oIFNT* o2, o7, o8, o9 and o10 [27]. Furthermore, Southern blot analysis indicated that the *IFNT* gene is limited to ruminant ungulates [28]. It is considered that *IFNT* may have diverged from IFN$\omega$ and its divergence has occurred along with the evolution of ruminant ungulates [29]. Structural similarity between IFNT and other IFNs has been well described elsewhere [22, 30].

## Biological Properties of IFNT

CL regression is induced by endometrial secretion of $PGF_{2\alpha}$, the production of which is regulated by progesterone, estrogen and oxytocin [31, 32]. IFNT was found to prevent estrogen receptor expression and subsequently estrogen-induced oxytocin receptor expression [33–35], and this is thought to be the mechanism exhibited by IFNT for the prevention of luteolysis. In addition to the observation that the amino acid sequence of IFNT shows high homology among ruminant species, its anti-luteolytic activity is effective across ruminant species. Trophoblastic vesicles from sheep or recombinant oIFNT could extend CL lifespan in the bovine species [36, 37]. Recombinant oIFNT is also effective in the extension of CL function in goats [38].

IFNT possesses antiproliferative effects and strong antiviral activity, and it exhibits $1 \times 10^8$ unit antiviral activity/mg protein like other IFNs [39, 40]. It was shown that oIFNT exhibits strong antiviral activity to HIV and FIV, but it is less cytotoxic than human IFN$\alpha$ [41]. Commonly, it is believed that antiviral activity of IFN exhibits high species specificity and its effect is reduced remarkably when administered between species [26]. However, this is not the case for oIFNT since it possesses a strong antiviral activity to human or feline retrovirus.

## Interferon-stimulated Genes in the Endometrium

IFNT was originally identified as a conceptus factor implicated in the process of maternal recognition of pregnancy. It was found that Type I IFNs bind to a common receptor complex with two polypeptide subunits (IFNAR1 and IFNAR2) [42], both of which are present in ovine uterine epithelial cells [43]. The surface epithelium of the uterine endometrium is undoubtedly the primary target for IFNT [44], but accumulated evidence suggests that IFNT can reach the stroma [45, 46] and even the myometrium [46, 47]. It is well characterized that Type I IFNs upon binding to the receptor activate the JAK-STAT-IRF (janus kinase-signal transducer and activator of transcription-interferon regulatory factor) signaling pathway [48], causing the activation of genes so called interferon-stimulated genes (ISGs). A list of ISGs induced by IFNT and/or progesterone can be found elsewhere [49, 50].

One of the most studied ISGs is ISG15 (interferon-stimulated gene 15) expression during the peri-implantation period. ISG15, a ubiquitin-like protein, is conjugated to intracellular proteins such as phospholipase C-$\gamma$1, JAK1, STAT1 and extracellular regulated kinase 1 [51]. An increase in endometrial production of ISG15 with the addition of recombinant IFNT was shown in tissue and cell culture systems, the observation of which is well supported by parallel expression of conceptus IFNT and ISG15 *in vivo* [45, 51].

WNT7A (wingless-type MMTV integration site family, member 7A) is also an endometrial factor induced by IFNT between days 12 and 14 of ovine pregnancy [52]. The WNT family (19 genes in humans) consists of secreted glycoproteins that regulate cell and tissue growth and differentiation during embryonic

development [53]. WNT family members have been shown to play a critical role in synchronizing uterine-conceptus interactions required for implantation in mice [53]. It was shown that WNT7A exhibits autocrine effects on the ovine luminal epithelium to regulate uterine receptivity and conceptus implantation [54].

Expression of endometrial LGALS15 (galectin 15) is induced by progesterone and is further enhanced by IFNT [55]. Galectins possessing a conserved carbohydrate recognition domain bind $\beta$-galactosides, resulting in cross-linking between glycoproteins and glycolipid receptors such as integrins on the cell surface [56]. It was recently found that LGALS15 stimulates migration and adhesion of ovine trophectoderm cells via activation of Jun N-terminal kinase and integrin signaling, respectively [57].

IFNT induces several chemokines in endometriual tissues including chemokine ligand 10 [CXCL10, interferon-inducible protein-10 kDa (IP-10)] and CXCL9 [monokine induced by interferon $\gamma$ (Mig)] [58–60]. Chemokines are small, secreted proteins possessing chemotactic activity and leading to selective attraction of inflammatory cells. The chemokine superfamily can be subdivided according to the organization of the N-terminal conserved cysteine (C) motif into four groups, which are designated as C-, C-C-, C-X-C, and C-$X_3$-C-chemokines [61]. The minor differences in the N-terminal structure result in differential actions depending on cell type [62]. C-X-C-chemokines generally attract neutrophils and T cells, C-C-chemokines are chemoattractive for monocytes, eosinophils, basophils and T cells [63, 64]. Whereas the C-X-C and C-C-subfamilies constitute several members, the C- and C-Xs-C-chemokines subfamilies are represented by only one member so far, designated lymphotactin (Lptn) also known as activation-induced T cell-derived and chemokine-related molecule (ATAC) or single C motif-1 (SCM-1) and fractalkine/neurotactin, respectively [65–68].

It was found that endometrial expression of CXCL10 was induced by conceptus IFNT [59]. Endometrial CXCL10 in turn attracts immune cells, particularly NK cells, to the implantation site of the endometrium [69], and through CXCL10 receptor, CXCR3, this cytokine regulates trophectoderm cell migration and its integrin expressions [70]. These changes result in conceptus migrations, apposition and initial attachment to the uterine epithelial cells [70].

## Early Conceptus Development

In mice, blastocyst formation marks the segregation of the first two cell lineages in the mammalian preimplantation embryo: the inner cell mass (ICM) that will form the embryo proper and the trophectoderm (TE) that gives rise to the trophoblast lineage. Commitment to ICM lineage is attributed to the function of the two transcription factors, Oct4 (encoded by *Pou5f1*) and Nanog. However, a positive regulator of TE cell fate has not been well described. The caudal-type homeodomain protein Cdx2 and the T-box protein eomesodermin (Eomes) are expressed in the TE, and both *Cdx2* and *Eomes* homozygous mutant embryos die around the time of implantation. A block in early TE differentiation occurs in *Eomes* mutant blastocysts. However, blastocysts of *Eomes* mutant implant, and Oct4 and Cdx2 expression are correctly restricted to the ICM and TE, respectively. Blastocoel formation in *Cdx2* mutants is initiated but epithelial integrity is not maintained and embryos fail to implant [71]. Loss of *Cdx2* results in failure to down-regulate *Oct4* and *Nanog* in outer cells of the blastocyst and subsequent death of these cells. Thus, *Cdx2* is essential for segregation of the TE from ICM lineages at the blastocyst stage by ensuring the repression of *Oct4* and *Nanog* in the TE [72, 73]. In ruminant ungulates, however, OCT4 expression in the TE can be detected up to day 10 of pregnancy, even after blastocyst formation and hatching [74, 75]. It was shown that CDX2 is expressed in ovine and bovine trophoblasts during the conceptus elongation period [75, 76].

## Regulation of IFNT Production

The expression of IFNT is unique in at least three aspects: a lack of viral inducibility, restricted expression to the embryonic trophectoderm and sustained synthesis for more than several days [22]. *IFNT* is not induced by double stranded RNA or viruses [77], but produced by the early trophoblast at a very high level, approaching 100 $\mu$g per cultured conceptus from day 16 pregnant ewe during 24 hours [11, 78]. Minute expression of oIFNT can be detected on day 8 of pregnancy, near the time of blastocyst hatching [79]. The production of oIFNT increases remarkably on day 13, while the blastocyst starts to elongate [80] and reaches the maximum level on day 16 of pregnancy [11, 78]. Expression of oIFNT decreases rapidly as the process of implantation proceeds and at day 22, oIFNT is no longer detected [11]. By contrast, IFN$\alpha$ and IFN$\beta$

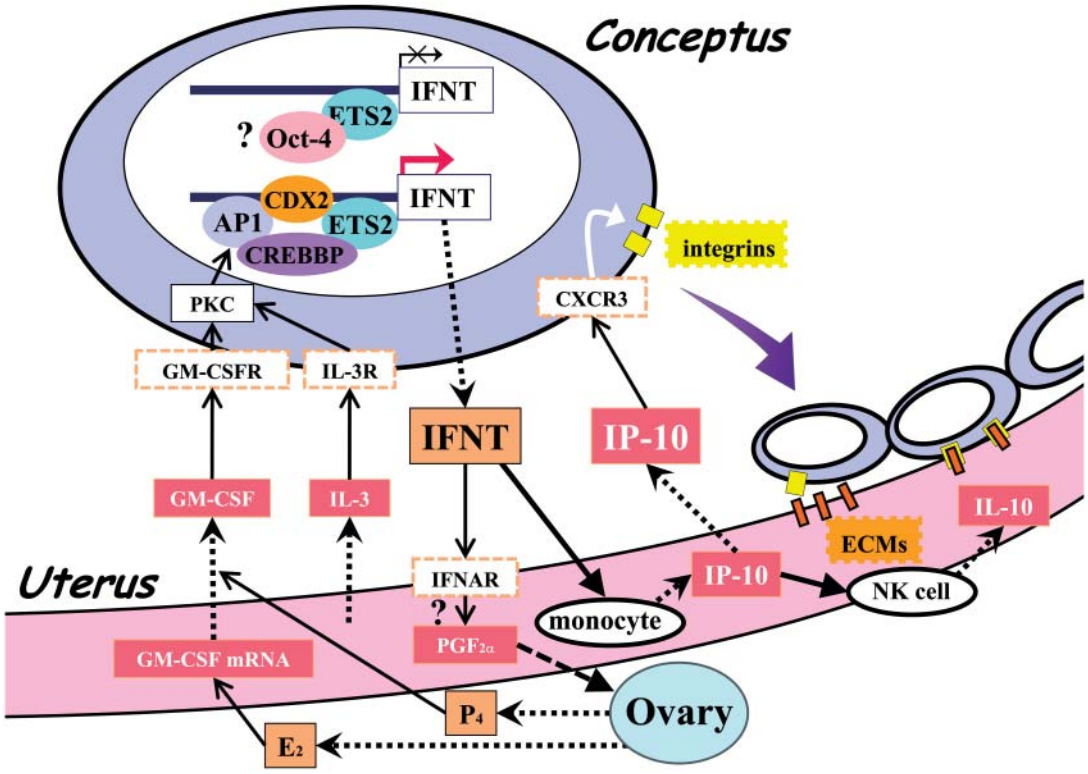


**Fig. 1.** Gene expression associated with the process of implantation in ruminants. Maternal cytokines influence gene expressions in the conceptus, which in turn secretes a pregnancy recognition hormone IFNT. This IFN affects endometrial gene expressions, resulting in attenuation of pulsatile $PGF_{2\alpha}$ release, increase in chemokine expression and immune cell migration, and conditioning uterine environments for conceptus implantation to the uterus.

are induced by double stranded RNA, virus or other pathogens in a variety of tissues, and their expression is generally short-lived, just a few hours after viral infection. These observations indicated that the expression of *IFNT* gene is obviously regulated in a different manner from that of IFN$\alpha$ and IFN$\beta$.

It was thought that the onset of *IFNT* expression is genetically programmed, which is independent of the maternal uterine environment. This was supported by the observation that IFNT production could be initiated after *in vitro* fertilization and maturation [81, 82]. However, substantial production of IFNT seen *in utero* could not be achieved without interaction with the uterine environment [81]. GM-CSF and IL-3 are known to be the factors that significantly enhance the production of oIFNT [78, 83–85]. In addition, GM-CSF expression is found to be higher in pregnant endometrial tissues than in cyclic animals [78], and promotes development of *in vitro* produced bovine embryo [86]. These data indicate that the presence of the conceptus and/or conceptus secretory proteins increases endometrial expression of GM-CSF, which enhances conceptus IFNT production during the peri-implantation period. Recently, Ealy and coworkers demonstrated that endometrial fibroblast growth factor 2 (FGF2) is capable of stimulating IFNT in bovine and ovine species [85, 87]. These investigators found that high quantity of FGF2 mRNA and immunoreactive FGF2 exist in the endometrium. More importantly, supplementation of bovine FGF2 increases not only *IFNT* mRNA levels but biologically active IFNT release from bovine trophoblasts and the trophoblast cell line, CT-1 [85, 87]. These results suggest that conceptus production of IFNT is regulated at least in part by maternal factors. However, the timing or degree to which maternal factors regulate or contribute to IFNT production has not been fully established.

## Transcriptional Regulation of *IFNT* Genes

The effect of GM-CSF and IL-3 on *oIFNT* production in the *in vitro* culture system is mimicked by the addition

of a protein kinase C (PKC) activator, phorbol 12-myristate 13-acetate (PMA) [88]. In addition, the PKC inhibitor, calphostin, was observed to abolish the increase in *oIFNT* mRNA induced by GM-CSF [88]. Prior to the establishment of ruminant trophoblast cell lines in the year 2000 [89, 90], human choriocarcinoma JAR and JEG3 cells were commonly used for the analysis of *IFNT* gene transcription. Both GM-CSF and PMA transactivated the *oIFNT*-CAT reporter constructs, but PMA was more effective in this transient transfection system [88, 91]. These results suggest that endometrial GM-CSF enhances *IFNT* gene transcription through PKC mediated signaling pathways.

A transcription factor, JUN, was also shown to activate *oIFNT*-CAT in JEG3 cells [91]. JUN, a protooncogene, is known to constitute an activator protein-1 (AP-1), which is a target of PKC. Using the upstream region of *oIFNT* gene in JEG3 cells, Yamaguchi and co-workers identified an enhancer region (distal enhancer) between –654 and –555 bases (transcription-initiation site is +1). This enhancer region included AP-1 and GATA recognition sites to which nuclear proteins extracted from JEG3 cells bound [92, 93]. In addition, silencer elements were also thought to exist on both sides of the enhancer region of the *oIFNT* gene [94]. Ezashi *et al.* demonstrated that transcription factor ETS2 binds to the sequences (proximal promoter) from –70 to –79 bases of *bIFNT* gene [95]. ETS2, localized in the ovine embryonic trophectoderm, transactivated *bIFNT*-luciferase reporter construct in JAR cells [95]. These investigators also demonstrated that OCT4 represses the ETS2-induced transcription of *bIFNT* promoter construct [74]. As the expression of OCT4 subsides in the trophectoderm, ETS2 becomes effective in increasing *IFNT* gene transcription.

The question of whether either AP-1, ETS2 or both is required for *IFNT* gene transcription has been debated. Experiments performed earlier indicated that although the proximal promoter, to which ETS2 binds, is undoubtedly required for expression, the far upstream AP-1 (JUN) binding site of the distal enhancer region is necessary for *oIFNT* gene transcription [92, 93, 96]. If in fact, both of AP-1 and ETS2 are required for *IFNT* gene transcription, but it is unclear how these factors work cooperatively on *IFNT* gene expression. A transcription co-activator, cAMP-response element binding protein-binding protein (CREBBP) [97], which is a bridging factor with histone acetyltransferase (HAT) activity, was shown to regulate *oIFNT* gene expression [98, 99]. It was found that CREBBP controls *oIFNT* gene expression through direct binding to ETS2 protein and to JUN [99, 100].

A TE lineage-specific transcription factor, CDX2, is expressed in the ovine and bovine trophoblasts during the time in which IFNT is produced [75, 76]. More importantly, over-expression of *Cdx2* along with *Jun* and *Ets2* in JEG3 cells was very effective in increasing the level of transcription of an *oIFNT* reporter construct [76]. Recently, another factor, a homeodomain factor distal-less 3 (DLX3) which is required for placental development in mice [101], was found to be important for *IFNT* gene transcription [102]. DLX3 expression was found in CT-1 cells and it acts cooperatively with Ets2 in JAR cells. These results suggest that CDX2 and DLX3 could be key elements, determining trophoblast cell-specific activation of *IFNT* gene expression.

In mice, trophectoderm expression of *Cdx2* is regulated through FGF signaling from the ICM, which is in turn essential for the maintenance of trophoblast stem cells [103-105]. FGF4 is expressed by the ICM at the blastocyst stage and subsequently in the epiblast (embryonic ectoderm) whereas the FGF receptor, FGFR2, is expressed in trophoblast cells immediately adjacent to the epiblast (chorion or extraembryonic ectoderm) [106]. If FGF signaling is removed, murine trophoblast stem cells become terminally differentiated. In ruminant ungulates, however, CDX2 could not be regulated solely by FGF signaling, because CDX2 and the ICM specific transcription factor OCT4 are both expressed in bovine trophoblasts [107], not in agreement with those in mice [72]. It was found that transcripts for CREBBP, JUN and ETS2 transcription factors, all of which are involved in *IFNT* gene transcription, are expressed to a similar extent in days 15 and 21 ovine trophoblasts. Finding that the endometrium produces FGF2 may reveal another dimension of transcriptional regulation of *IFNT* genes by the maternal factor [85]. However, to determine whether or not endometrial FGF2 is actively involved in the transcriptional regulation of *IFNT* genes *in utero* requires further investigation.

## Intra-cellular Signaling for *IFNT* Transcription

CREBBP could recruit many nuclear factors including those exhibiting HAT activities such as ATF-2 and P/CAF [108, 109]. However, it remains to be determined whether HAT activity of CREBBP is required for the basal and increased transcription of the *IFNT* gene. During a period of pregnancy, various cytokines and growth factors are known to be present at maternal-fetal annexes [110]. It was demonstrated that GM-CSF and

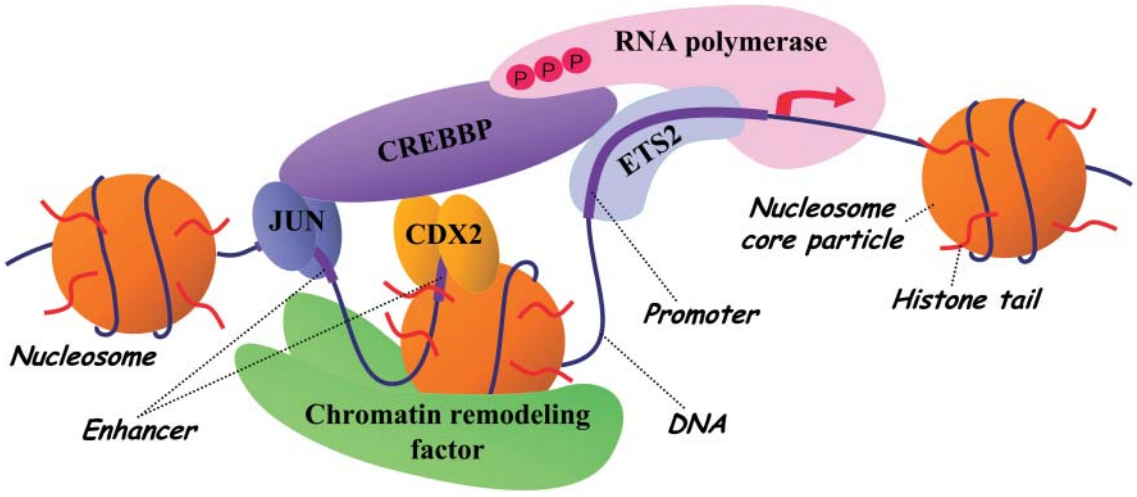


**Fig. 2.** Transcriptional regulation of *IFNT* gene expression. Transcription factors JUN, ETS2 and CREBBP are constitutively expressed during the period of IFNT expression. Trophoblast specific factor CDX2 along with epigenetic regulation creates the euchromatin state where nucleosome structures are loosened and transcription factor assembly becomes possible, resulting in active *IFNT* gene transcription.

IL-3 of the maternal origin activated *IFNT* gene and the effect of GM-CSF on *IFNT* production appears to be mediated through PKC pathway [78, 83, 88, 111]. Since PKC is known to induce AP-1 activation and *ETS2* is also under the PKC signal cascade [112, 113], GM-CSF/PKC/AP-1 cascade is a reasonable hypothesis for *IFNT* gene activation. It has been demonstrated that Ras/Raf/MAPK signaling pathway, which is independent of the PKC pathway, is activated by GM-CSF [114–116]. In addition, AP-1 and ETS2 are also activated via Ras/Raf/MAPK signaling pathway [108, 117–123]. If in fact *IFNT* gene expression is activated by this cascade, several possible signaling cascades could be functioning: 1) The PKC signal cascade is initially activated by maternal GM-CSF, resulting in the activation of AP-1 and ETS2. The transcription of *IFNT* gene is initiated at this time. Subsequent to the activation of PKC signal cascade, Ras/Raf/MAPK signaling pathway is activated by GM-CSF and/or other uterine factor(s), and *IFNT* gene expression is further activated. 2) The Ras/Raf/MAPK signaling pathway is initially activated by maternal GM-CSF (and/or FGF2). Following the activation of this signal cascade, PKC signaling pathway is then activated and the transcription of *IFNT* gene increases further. 3) Both PKC and Ras/Raf/MAPK signal cascades are activated by maternal GM-CSF (and/or FGF2) at the same time, which cause the initiation and maintenance of active *IFNT* gene transcription. 4) Alternatively, CDX2 is activated by phosphorylation via MAPK signaling pathway, resulting in modulation of its transcriptional activity. Because the presence of phosphrylated CDX2 has been detected in the blastocyst, it is probable that Ras/Raf/MAPK signal transduction is indicative of CDX2 expression, which in turn activates *IFNT* gene transcription.

## Epigenetic Regulation of *IFNT* Transcription

Epigenetic alterations such as variation in covalent histone modification and DNA methylation regulate gene expression by altering chromatin conformation. It is known that IFNT production is limited to the trophectoderm. Quite recently, Sakurai and coworkers investigated whether or not *IFNT* gene transcription could be induced in a cell type not related to trophoblast cells [124]. These investigators demonstrated that significant increase in endogenous *IFNT* transcription in a bovine kidney epithelial MDBK cells (which do not normally express IFNT) can be induced through CDX2 over-expression and high H3K18 acetylation. They also noted that lowering H3K9 methylation could be required for the degree of *IFNT* transcription seen in trophoblast cells. These findings suggest that induction of endogenous *IFNT* transcription in bovine trophoblast cells results from partial decondensation of chromosomal domains by histone acetylation and sufficient CDX2 expression, allowing other transcription

factor bindings to the upstream region of *IFNT* genes.

Genomic DNAs extracted from uterine endometrium, white blood cells (WBC), day 14 trophoblasts and day 20 trophoblasts were subjected to bisulfite sequencing analysis and examined for the methylation status between nucleotides –980 and –1 of the *oIFNT*-o10 gene, a stretch containing 14 CpG sites [125]. There are 10 CpG sites in Region 1 (–980 to –450), and the genomic DNA from uterine endometrium and WBC, both of which do not express *oIFNT*-o10, displayed higher methylation than day 14 and 20 trophoblasts. Day 14 trophoblasts, which had highest transcription of the *oIFNT*-o10 gene, was less methylated in the upstream region of *oIFNT*-o10 gene than day 20 trophoblasts, which possessed minute amounts of *oIFNT*-o10 mRNA. Among 10 CpG sites in the Region 1 of day 14 trophoblasts, 5 CpG dinucleotides at –806, –799, –774, –769 and –628 were not methylated. Of 4 CpG sites in Region 2 (–450 to –1), those in day 14 trophoblasts were least methylated, with a methylation score of less than 20% and the CpG site at –7 completely non-methylated. Among the 4 CpG sites, uterine endometrium, WBC and day 20 trophoblasts were far more methylated, with methylation scores of more than 50%. Changes in the degree of DNA methylation in the upstream sequences of the *oIFNT* gene could be one of the major mechanisms leading to down-regulation of its expression and possibly its silencing in non-conceptus tissues [125].

## Conclusion

For a pregnancy to succeed, CL life-span needs to be maintained beyond the normal luteal phase of the estrous cycle. Under progesterone dominant uterine environments, the ruminant conceptus produces IFNT in substantial amounts during the peri-attachment period. The endometrium must then respond to IFNT signaling by producing numerous factors including ISGs. However, it is still unclear why IFNT is expressed only in trophoblasts and how its production is initiated and terminated within a short period of development. Likewise, proper endometrial responses supportive of conceptus development, attachment/invasion and placental formation are not definitively characterized. Further efforts are therefore required to elucidate molecular mechanisms associated with conceptus-endometrial interactions, resulting in proper placental formation.

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