

## **Conceptus-stimulated Signal Transduction Pathway in the Endometrium to Maintain Pregnancy**

### **Contents**

Abstract

Introduction

Maternal Recognition of Pregnancy Associated with CL Maintenance

Effectors of Maternal Recognition of Pregnancy: IFN- $\tau$

The JAK-STAT Pathway

    Type I Interferon Receptors

    JAK Kinases

    Signal Transducers and Activators of Transcription Proteins

    Interferon-directed Gene Activation

    JAK-STAT Pathway Regulation

    Specificity of Interferon Signaling

    Cross-talk with Other Intracellular Pathways

The JAK-STAT Pathway in the Ruminant Endometrium

Bovine bIFN- $\tau$ -stimulated Protein Synthesis in the Endometrium

Uterine-conceptus Interactions and Reproductive Failure in Cattle

Concluding Remarks

References

### **Correspondence**

---

Department of Dairy and Poultry Sciences, University of Florida, Gainesville 32611

PO BOX 110920 - Gainesville, FL - 32611 - USA

phone: (352) 392-5590 - fax: (352) 392-5595

email: [thatcher@dps.ufl.edu](mailto:thatcher@dps.ufl.edu)

## Abstract

Conceptus-stimulated Signal Transduction Pathway in the Endometrium to Maintain Pregnancy. *Ann Rev Biomed Sci* 1999; VOL:PAGES. In cattle, the process of maternal recognition of pregnancy requires that molecules from the conceptus interact with the uterine endometrium to suppress pulsatile secretion of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) and thereby block regression of the corpus luteum (CL). It is hypothesized that the conceptus-secreted cytokine, bovine interferon- $\tau$  (bIFN- $\tau$ ), activates the JAK-STAT pathway of signal transduction in the bovine endometrium. Activation of this signal transduction system results in expression of bIFN- $\tau$ -responsive genes and proteins which could act to inhibit specific steps of the  $PGF_{2\alpha}$  synthetic pathway. In the present review we present evidence from whole animal to molecular studies supporting the antiluteolytic role of bIFN- $\tau$ , describe details of the type I interferons-activated JAK-STAT pathway of signal transduction and attempt to integrate the activation of such a pathway by bIFN- $\tau$  leading to its suppressive effects on uterine  $PGF_{2\alpha}$  production.

**Key Words:** interferon-tau, prostaglandin  $F_{2\alpha}$ , JAK-STAT, endometrium, pregnancy, cattle.

## Introduction

In cattle, it is well established that presence of a healthy conceptus around day 17 of pregnancy is required to block luteolysis. The embryonic factor bovine interferon- $\tau$  (bIFN- $\tau$ ) is a type I interferon and the embryonic factor which interacts with the endometrium to promote such an antiluteolytic effect. However, cellular and molecular mechanisms stimulated within the endometrium by bIFN- $\tau$  are only now being elucidated. Various cellular models involving the molecular actions of cytokines through the JAK-STAT pathway provide a framework for delineating the mechanisms through which bIFN- $\tau$  is able to maintain early pregnancy in ruminants. Objectives of this review are (1) to describe the physiological events occurring during the window of maternal recognition of pregnancy in cattle, (2) to characterize the signal transduction systems stimulated by type I interferons, and (3) to delineate intracellular actions of bIFN- $\tau$  in the endometrium to suppress synthesis of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ).

## Maternal Recognition of Pregnancy Associated with CL Maintenance

In cattle, commitment to pregnancy is only accomplished if adequate signaling exists between maternal and embryonic units. Maternal recognition of pregnancy has been defined as the process by which the periattachment conceptus signals its presence to the maternal unit, as reflected by maintenance of the CL (Short, 1969; reviewed in Hansen, 1991). More specifically, the process of maternal recognition of pregnancy requires that embryonic molecules interact with the uterine endometrium and change its program, so that pulsatile secretion of  $PGF_{2\alpha}$  is blocked and thereby luteolysis is impeded. The net result is continuous secretion of  $P_4$  by the CL, which is required for continuation of

pregnancy. Roles of  $P_4$  include continuous stimulation of uterine secretions and inhibition of smooth muscle contractions (Hafez, 1993). In cattle, the critical period for maintenance of pregnancy is around day 17 of the estrous cycle. Betteridge and others (1980) transferred embryos to synchronized recipients and demonstrated that pregnancy was only maintained if embryos were transferred prior to day 17. Moreover, inter-estrus interval increased from 20 to 25 days when conceptuses were removed on day 17 vs. day 15 of pregnancy (Northey & French, 1980).

Pregnancy effects on suppression of pulsatile  $PGF_{2\alpha}$  could be exerted at several levels: (1) suppression of the  $PGF_{2\alpha}$ -releasing stimulus (i.e., oxytocin, LH,  $E_2$ ), (2) alterations of the  $P_4$ -primed uterus (i.e.,  $PGF_{2\alpha}$ -synthesizing machinery), and (3) decrease in substrate required for  $PGF_{2\alpha}$  synthesis (i.e., AA). This review will focus on intracellular pathways for generation of the pulsatile secretion of  $PGF_{2\alpha}$  in response to oxytocin stimulus (Flint et al., 1986; Burns et al., 1997; Thatcher et al., 1997). Oxytocin receptors start to increase in the  $P_4$ -primed, responsive uterus. Oxytocin originating from the neurohypophyseal lobe of the pituitary gland binds to the seven transmembrane-domain, G protein-coupled receptors and activates phospholipase C (PLC). The PLC cleaves membrane phosphatidylinositol biphosphate, yielding inositol trisphosphate ( $IP_3$ ) and diacylglycerol (DAG). The  $IP_3$  binds to specific receptors in the endoplasmic reticulum resulting in release of calcium from internal stores into the cytosolic compartment. The DAG activates protein kinase C (PKC), leading to serine phosphorylation of cytosolic, calcium-dependent phospholipase  $A_2$  ( $PLA_2$ ), probably through a MAP-kinase dependent pathway (Lin et al., 1993). The  $IP_3$ -stimulated increase in cytosolic calcium acts to further stimulate  $PLA_2$  activity (Clark et al., 1991). Stimulated  $PLA_2$  translocates to the membrane where phospholipid substrates are located (Clark et al., 1991). Activated, membrane-bound  $PLA_2$  cleaves arachidonic acid (AA) from phospholipids. Free AA is converted to prostaglandin  $H_2$  ( $PGH_2$ ) by the enzyme cyclooxygenase-2 (COX-2). Prostaglandin  $F_{2\alpha}$  synthase converts  $PGH_2$  into  $PGF_{2\alpha}$ , which is then released into the uterine circulation. In the endometrium, this process occurs preferentially in epithelial cells, compared to stromal cells (Danet-Desnoyers et al., 1994). The  $PGF_{2\alpha}$  gains access to the ovary through a counter-current mechanism. Binding of  $PGF_{2\alpha}$  to receptors in the CL stimulates release of luteal oxytocin, that can bind oxytocin receptors in the endometrium to elicit further release of  $PGF_{2\alpha}$ , characterizing a positive feedback loop.

Regarding alterations on the  $P_4$ -primed uterus, Arnold and others (1999) incubated endometrial explants obtained from day 17 cyclic or day 17 pregnant cows with intracellular stimulators of  $PGF_{2\alpha}$  synthesis, and measured concentrations of  $PGF_{2\alpha}$  secreted into the culture medium. Melittin, PDBu and calcium ionophore each stimulated release of  $PGF_{2\alpha}$  from explants of cyclic cows compared to control treatment (medium alone). In contrast, all stimulators mentioned above failed to induce release of  $PGF_{2\alpha}$  in explants originating from pregnant cows. This indicated that pregnancy affected the intracellular  $PGF_{2\alpha}$ -generating machinery to suppress its ability to stimulate  $PGF_{2\alpha}$ . Interpretation of these data suggests that pregnancy may have inhibitory effects at each

of the steps stimulated by treatments, which include PKC (PDBu) and PLA<sub>2</sub> (melittin, ionophore). Alternatively, pregnancy may affect a distal, convergence point in the pathway, for example, at the level of COX-2. Effects on the enzymatic machinery can be to decrease expression and/or activity of PKC, PLA<sub>2</sub> and COX-2. In fact, existence of a pregnancy-induced inhibitor of COX-2 activity has been found in the endometrium of cows (Basu & Kindahl, 1987; Gross et al., 1988). Danet-Desnoyers and others (1993) identified linoleic acid as an active molecule in bovine endometrium which acted to decrease COX-2 activity. Moreover, linoleic acid acted as a competitive inhibitor of AA on a PGF<sub>2α</sub> generator assay (Thatcher et al., 1994). It is possible that altered lipid metabolism in the pregnant endometrium increases availability of linoleic acid to inhibit COX-2 activity and thereby decrease PGF<sub>2α</sub> production. Thatcher and others (1995) compared concentrations of free linoleic and free AA in endometrial microsomes from day 17 cyclic and pregnant cows. Pregnancy decreased concentrations of AA and increased concentrations of linoleic acid compared to estrous cycle, to result in a change of the ratio of linoleic to AA of 0.6 to 2.4 in endometrium between cyclic and pregnant cows.

## Effectors of Maternal Recognition of Pregnancy: IFN-τ

A considerable amount of research focused on identification and purification of conceptus products with the PGF<sub>2α</sub>-secretion inhibitory activity required for maintenance of pregnancy. A family of molecules has been identified as the embryonic antiluteolytic factor in ruminants, named IFN-τ (Martal et al., 1979; Godkin et al., 1982; Bartol et al., 1985; Helmer et al., 1987; Imakawa et al., 1989; Roberts et al., 1992; Stewart et al., 1989 and Thatcher, 1999). Isoforms of bIFN-τ are glycosylated, have molecular weights between 22 and 24 kD, and vary in isoelectric forms between PI 6.3 and 6.8 (Helmer et al., 1987; Anthony et al., 1988).

Antiluteolytic effects of bIFN-τ have been examined both in vivo and in vitro. Intrauterine infusions of highly enriched bIFN-τ complex (Helmer et al., 1989) and recombinant bIFN-τ (Meyer et al., 1995) extended lifespan of CL in cows, compared to control infusions. Moreover, PGF<sub>2α</sub> release in response to an oxytocin injection was suppressed in day 17 cyclic cows infused with recombinant bIFN-τ compared to controls (Meyer et al., 1995).

Danet-Desnoyers and others (1994) tested the ability of bIFN-τ to suppress basal and oxytocin-stimulated secretion of PGF<sub>2α</sub> from primary cultures of endometrial epithelial cells obtained from day 15 cyclic cows. Twenty four hour-incubation with bIFN-τ reduced both basal and oxytocin-stimulated secretion of PGF<sub>2α</sub>. This agrees with data from Meyer and co-workers (1996) and Xiao et al., (1999). Meyer and others (1996) reported that endometrial epithelial cells obtained from cows which received intrauterine infusions of bIFN-τ secreted less basal and oxytocin-stimulated PGF<sub>2α</sub> compared with cows infused with a control protein. Moreover, Xiao and co-workers (1999) cultured endometrial epithelial cells obtained from cows of days 1 to 4 of the estrous cycle in

presence of oxytocin or a combination of oxytocin and bIFN- $\tau$ . Similar to the data described above, bIFN- $\tau$  effectively reduced both oxytocin- and phorbol ester-stimulated PGF<sub>2 $\alpha$</sub>  secretion. In contrast, Asselin and others (1998) showed that bIFN- $\tau$  increased secretion of PGF<sub>2 $\alpha$</sub>  from endometrial epithelial cells from days 1 to 5 of the estrous cycle. However this effect was significant only when extremely high doses of bIFN- $\tau$  (20  $\mu$ g/ml) were used. Collectively, these data support the concept that bIFN- $\tau$  interacts with the endometrial epithelium and affects the PGF<sub>2 $\alpha$</sub> -generating machinery to decrease PGF<sub>2 $\alpha$</sub>  production. In an effort to pinpoint specific enzymes that bIFN- $\tau$  altered in the PGF<sub>2 $\alpha$</sub> -generating cascade, Xiao and others (1999) measured messenger ribonucleic acid (mRNA) and protein expression for COX-2 in endometrial epithelial cells treated with oxytocin and with oxytocin in combination with bIFN- $\tau$ . Oxytocin maximally stimulated COX-2 mRNA and protein from 3 to 24 hours compared to controls. Treatment with bIFN- $\tau$  reduced this effect of oxytocin, and this was consistent with a reduction in PGF<sub>2 $\alpha$</sub>  secretion in medium. In contrast, Asselin and co-workers (1997) found that bIFN- $\tau$  actually stimulated expression of COX-2, which would contradict the antiluteolytic role of bIFN- $\tau$ . However, they also reported that bIFN- $\tau$  stimulated expression of an endometrial prostaglandin E<sub>2</sub>-9-ketoreductase, which catalyzes the conversion of PGF<sub>2 $\alpha$</sub>  into PGE<sub>2</sub> (Asselin & Fortier, 1998). Since PGE<sub>2</sub> has been shown to have luteo-protective actions (Pratt et al., 1977), they proposed a model whereby bIFN- $\tau$  actually stimulates the PGF<sub>2 $\alpha$</sub> -generating machinery, but a conversion of PGF<sub>2 $\alpha$</sub>  to PGE<sub>2</sub> at the end of the cascade would support an antiluteolytic effect of bIFN- $\tau$ .

It is expected that in order to stimulate intracellular changes resulting in decreased PGF<sub>2 $\alpha$</sub>  production, bIFN- $\tau$  needs to stimulate a receptor-mediated mechanism of signal transduction. This mechanism should evoke intracellular second messengers to ultimately regulate molecules involved in the generation of PGF<sub>2 $\alpha$</sub> . Such regulation could involve synthesis of proteins inhibitory to the PGF<sub>2 $\alpha$</sub>  production cycle, or, could acutely activate molecules already present in the cell to suppress PGF<sub>2 $\alpha$</sub>  stimulatory actions. There is limited information on the nature of IFN- $\tau$  receptors. Knickerbocker and Niswender (1989) measured numbers of unoccupied binding sites for IFN- $\tau$  in endometrium of cyclic and pregnant sheep. Number of unoccupied binding sites decreased for both cyclic and pregnant ewes from day 4 to day 12. Then it increased for cyclic animals, but was still decreased for pregnant ewes, indicating that bIFN- $\tau$  binding sites were possibly being occupied by conceptus-secreted IFN- $\tau$ . Interestingly, affinity for binding sites increased after day 12 for pregnant ewes but decreased for cyclic ewes. Hansen and co-authors (1989) reported use of cross-linking experiments to characterize association of iodinated ovine IFN- $\tau$  to membrane peptides. They identified binding of IFN- $\tau$  to both 100 and 70 kD membrane polypeptides. Comparison of binding kinetics of IFN- $\tau$  with IFN- $\alpha$  in this experiment suggested existence of different receptors for these two ligands. However, Li and Roberts (1994) showed a reciprocal displacement of IFN- $\tau$  and IFN- $\alpha$  from bovine endometrial cell membranes, suggesting that binding sites for these two molecules were

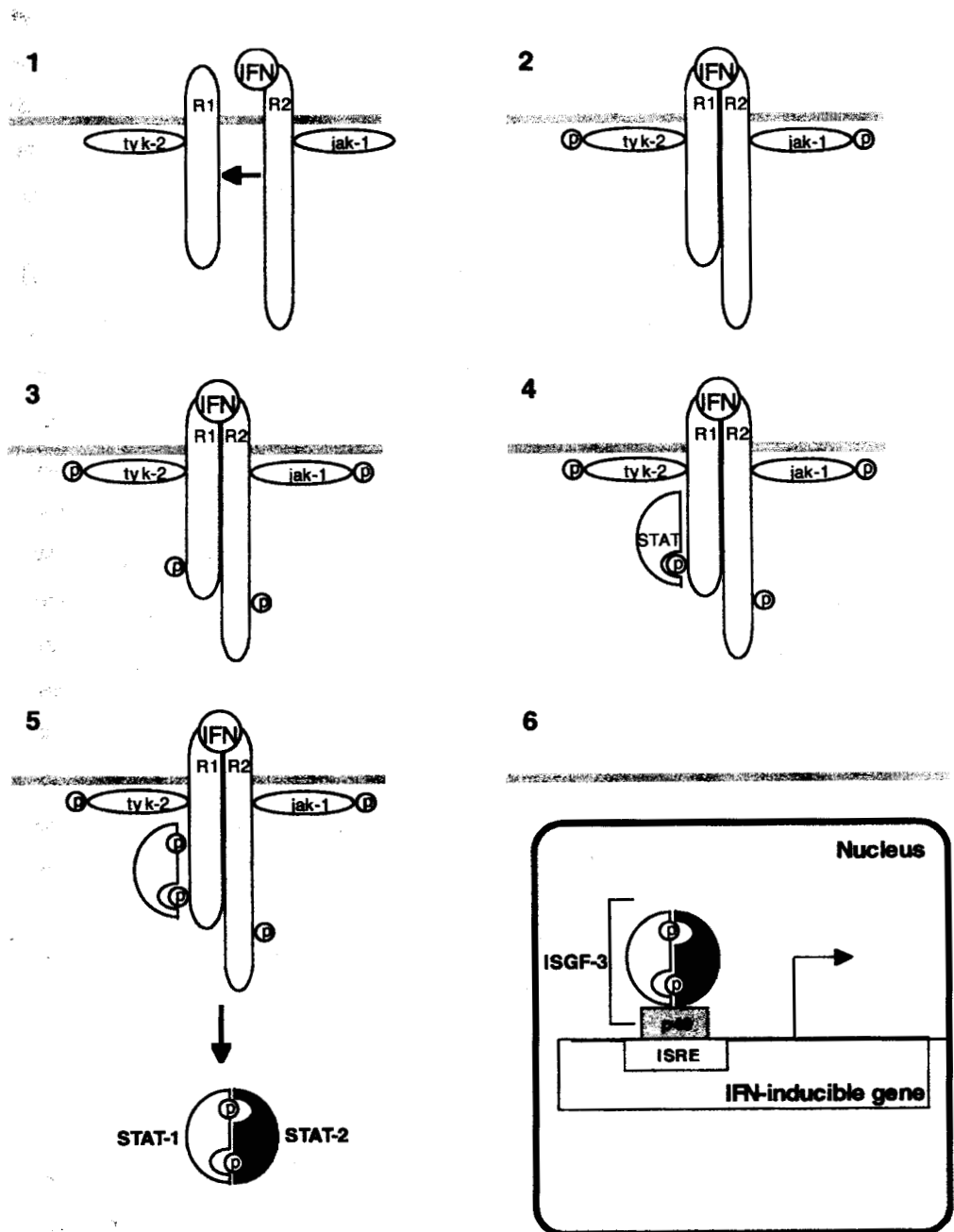
the same. Recently, Han and Roberts (1998) reported cloning and characterization of receptors for IFN- $\tau$  in cattle endometrium. Sequences of receptor subunits IFN $\alpha$ R1 and IFN $\alpha$ R2 are similar to ones utilized by other type I interferons such as IFN- $\alpha$ . However, these receptors were not linked with functional data, to demonstrate that such subunits are necessary and sufficient to suppress PGF<sub>2 $\alpha$</sub>  synthesis.

## The JAK-STAT Pathway

The observations above lead to the assumption that bIFN- $\tau$  stimulated a signal transduction system, the JAK-STAT pathway, similar to other type I interferons (Schindler et al., 1992; Darnell et al., 1994; Darnell, 1997; Figure 1). In this paradigm, interferon receptors do not contain intrinsic kinase activity, but they are physically associated with protein tyrosine kinases from the Janus family (JAK kinases). Binding of interferon to its receptor causes phosphorylation of tyrosine residues in the JAK kinases (Figure 1, panel 2) and in the cytoplasmic tail of the receptor (Figure 1, panel 3). The tyrosine phosphorylated receptor attracts signal transducer and activation of transcription, or STAT, proteins to close contact (Figure 1, panel 4). Members of the STAT family of proteins then become phosphorylated on tyrosine residues and form homo- and hetero-dimers (Figure 1, panel 5). Dimerized STATs migrate to the nucleus where they bind to the specific regulatory elements located in the promoter region of interferon-regulated genes. In this manner, STAT proteins stimulate transcription of genes and synthesis of interferon-specific proteins (Figure 1, pane 6). Characteristics of molecules involved in this pathway, and evidence for existence of this pathway in the bovine endometrium will be examined next.

### Type I Interferon Receptors

Type I interferon receptor consists of two chains, IFN $\alpha$ R1 and IFN $\alpha$ R2, which can be presented in different forms. The IFN $\alpha$ R1 is present as a full chain (IFN $\alpha$ R1a) and as a shorter splice variant (IFN $\alpha$ R1s). The IFN $\alpha$ R2 chain exists in soluble, short and long forms, designated IFN $\alpha$ R2a, IFN $\alpha$ R2b and IFN $\alpha$ R2c respectively. Probably IFN $\alpha$ R1a and IFN $\alpha$ R2c are the predominant forms (Pestka, 1997). Pestka (1997) reviewed a series of experiments where the different IFN $\alpha$ R chains were expressed in Chinese hamster ovary cells, and ability of different type I interferons to signal through the different chain combinations was evaluated. There is a remarkable diversity of such interactions, in which specific interferons can only signal through specific combinations of chains, but not others. Pestka (1997) proposes that differential expression of individual chains and ability of individual interferons to signal through specific chain arrangements confers tissue-specific responsiveness to interferons. For example, Plataniias and co-workers (1996<sup>a</sup>) reported that IFN- $\beta$  signaling requires association of IFN $\alpha$ R1 with p100, a tyrosil phosphoprotein, which was later identified as a particular chain of the interferon receptor complex. These types of experiments have not been conducted in bovine reproductive tissue, to test signaling ability of bIFN- $\tau$ . Instead of the antiviral assays used in the



**Figure 1.** The JAK-STAT pathway of signal transduction and gene activation.

1) Binding of type I interferon (IFN) to the interferon- $\alpha$ -receptor (R)-2 chain causes recruitment of R1; 2) dimerization of IFN receptor complex causes reciprocal tyrosine phosphorylation of associated JAK kinases (tyk-2 and jak-1); 3) JAK kinases phosphorylate receptor subunits in tyrosine residues; 4) unphosphorylated, cytosolic STAT proteins bind to the receptor complex through SH2 domains present in STAT proteins; 5) JAK kinases phosphorylate tyrosine residues in bound STATs, STATs dissociate from receptor complex and associate into a heterodimer (STATs 1 and 2); 6) dimerized STATs translocate to the nucleus, bind to the DNA binding protein p48, forming the ISGF-3 transcription complex, which stimulate synthesis of IFN-inducible genes.

experiments mentioned above, functional assays measuring suppression in synthesis of PGF<sub>2α</sub> from endometrial cells would be in order. Moreover, existence of a bIFN-τ-specific receptor chain remains elusive.

Colamonici and coworkers (1994<sup>a</sup>; 1994<sup>b</sup>) demonstrated that the tyrosine kinase p135<sup>tyk2</sup>, or tyk-2 is associated physically with the IFNαR1 chain of the interferon receptor. Immunoblots revealed the ability of monoclonal antibodies to IFNαR1 and to tyk-2 to reciprocally coimmunoprecipitate both proteins. Association of tyk-2 was mapped to a 46-amino acid juxtamembrane region of the IFNαR1 chain. Furthermore, they demonstrated that tyk-2 could directly phosphorylate tyrosine residues in the IFNαR1 chain after stimulation with IFN-α (Colamonici et al., 1994<sup>b</sup>). Besides binding to extracellular interferons and associating with JAK kinases, the interferon receptor complex also has other functions in the JAK-STAT pathway. The unstimulated IFNαR2 chain may contain associated unphosphorylated STAT proteins (Li et al., 1997). Binding of interferon brings IFNαR2 and IFNαR1, which contains tyk-2, together. Dimerization of receptor chains elicits transfer of STATs to the IFNαR1, where STATs become tyrosine phosphorylated. This confirms previous finding that tyrosine 466 in the chain of IFNαR1 acts as a docking site for association of the SH2 domain of STAT-2, and such binding is required for tyrosine phosphorylation of STAT-2 by tyk-2 (Yan et al., 1996). Similar to STAT-2, STAT-3 activation also requires binding to IFNαR1 (Yang et al., 1996). Furthermore, it has been demonstrated that phosphotyrosine modules (i.e., sequence of amino acids surrounding the tyrosine residue in the receptor chain) play a major role in selecting which STAT binds (Gerhartz et al., 1996). The authors demonstrated that a two point mutation in the phosphotyrosine module changed the specificity of interferon-gamma (IFN-γ) receptor from STAT-1 to STAT-3. Chains of the interferon receptor may also play roles independent of the JAK-STAT pathway. For example, Abramovich and others (1997) reported binding of a protein-arginine methyltransferase to the IFNαR1 chain. This finding suggests that methylation of proteins may be a signaling mechanism complementary to tyrosine phosphorylation, and methylation may be required for full stimulation by interferons. In fact, cells deficient in this methylase activity by antisense become less sensitive to the antiproliferative effect of interferons. Finally, Platanias and co-authors (1996<sup>b</sup>) reported that the interferon receptor mediates tyrosine phosphorylation of insulin receptor substrate 2 (IRS-2). The IRS-2 molecules associate with IFNαR1 and become phosphorylated by tyk-2. Moreover, phosphorylated IRS-2 associates with the p85 regulatory subunit of the phosphatidylinositol 3'-kinase, suggesting that this kinase participates in the interferon signaling cascade downstream from IRS-2. Collectively, the examples presented above illustrate actions of the multifunctional interferon receptor. It is tempting to speculate that some of such actions may be required for the antiluteolytic roles of bIFN-τ in the endometrium.



## **JAK Kinases**

Janus kinases or JAKs *tyk-2* and *jak-1* are associated respectively with IFN $\alpha$ R1 and IFN $\alpha$ R2 and are involved in tyrosine phosphorylation of STAT proteins. The carboxy-terminal domains of the jak kinases share considerable sequence homology with the catalytic domains of other protein tyrosine kinases. The amino-terminal half of the jaks contains regions of sequence homology to other members of the jak family and the extreme amino-terminal domain probably is involved in association with interferon receptor chains (Williams & Haque, 1997). Ligand-mediated dimerization of interferon receptor chains is required for interferon-stimulated signal transduction. Dimerization evokes reciprocal tyrosine phosphorylation and consequent activation of JAKs associated with interferon receptor chains (Ihle et al., 1995). Phosphorylation of the kinase is the first of three tyrosine phosphorylations culminating in STAT activation. Activated JAKs phosphorylate tyrosine residues on the interferon receptor chains, which serve as docking sites for STATs, as mentioned above. Lastly, STATs are phosphorylated by the JAKs (Darnell, 1997). Activated JAKs are not specific for particular STATs. Different receptors can activate the same STATs through different JAKs. Moreover, STAT docking sites can be interchanged between different cytokine receptors, and the STAT specific for the docking site present will be activated by binding of the ligand specific for the extracellular domain of the receptor (Stahl et al., 1995). Therefore, STAT activation is determined more by specific interactions between STATs and their receptors than by specific JAKs associated with receptor chains.

## **Signal Transducers and Activators of Transcription Proteins**

Unlike other common intracellular second messengers, STATs not only convey the extracellular signal to the interior of the cell, but they themselves carry such a signal to the nucleus, acting as transcription factors to activate transcription of genes induced by particular ligands. STATs 1 through 6 have been described (Darnell, 1997). STAT-1 $\alpha$  and 1 $\beta$  are encoded by alternative splicing of a single mRNA transcript. Human STAT-1 $\alpha$  consists of 750 amino acids, while the extreme carboxy-terminal 38 amino acids are missing for STAT-1 $\beta$ . STAT-2 is composed of 851 amino acids. STATs 1, 2 and 3 have significant sequence homology (Fu et al., 1992; Zhong et al., 1994). The domain distribution in the STAT molecule includes a centrally-located DNA-binding domain, a carboxy-terminal transcription activation domain, and SH2 and SH3 domains located in between (Fu, 1992). The SH2 domain allows docking to tyrosine phosphorylated sites in the IFN and cytokine receptors, as discussed above, and also STAT dimerization. SH2 domain sequences are specific for each STAT, but mutant STATs 1 and 3, in which SH2 domains were swapped, completely reversed their specificity for interaction with specific phosphotyrosine motifs (Hemmman et al., 1996). This indicates that the SH2 domain is the sole determinant of specific STAT factor recruitment to receptors. STATs contain a unique tyrosine residue in the carboxy-terminal region (Y701, Y690 and Y705 for STATs 1, 2 and 3 respectively). A recently developed model for STAT activation (Li et al., 1997)

proposes that unphosphorylated STATs 1 and 2 are associated with the IFN $\alpha$ R2 chain. Binding of interferon causes dimerization of this chain with IFN $\alpha$ R1. Tyrosine phosphorylated residue 466 of IFN $\alpha$ R1 binds the SH2 domain of STAT2, which is then phosphorylated on tyrosine 690, providing a docking site for the SH2 domain in STAT-1. STAT-1 is phosphorylated on tyrosine 701, and then dimerizes with STAT-2 through reciprocal binding of tyrosine phosphorylated residues with SH2 domains. However, an unsolved question is what drives SH2 domains of STATs to dissociate from a higher affinity interaction with receptor phosphotyrosine to form dimers which association is mediated by a lower-affinity phosphotyrosil interaction (Greenlund et al., 1995). In light of this question, Gupta and co-authors (1996) proposed an alternative model for STAT binding and dimer formation. After binding to the receptor phosphotyrosine motif, the STAT shifts its target to the tyrosine motif in the tyrosine kinase. Tyrosine phosphorylation of STAT would cause a conformational change to destabilize this interaction with the kinase, and STATs would then be driven to form more energy-stable interactions with other STATs and form dimers. They based this model on the finding that SH2 domains from STATs 1 and 2 bind with high affinity to phosphotyrosine motifs on JAK kinases.

STAT dimers are competent to bind DNA. Known DNA binding heterodimers are STAT 1:2 and STAT 1:3 (strong binding) and STAT 2:3 (weak binding). Homodimers are STAT 1:1 and STAT 3:3 (strong) and STAT 2:2 (form seldom in absence of STAT-1; Darnell, 1997). In variance with the notion that tyrosine phosphorylation is required for STAT dimerization, Stancato and co-workers (1996) demonstrated that STAT complexes exist in the cytosol of unstimulated cells. Moreover, such association was independent of tyrosine phosphorylation, since the Y701F STAT-1 mutant still bound to STAT-2 in reticulocyte lysates. Such an interaction was weak, since it was not observed in extracts obtained with high-salt, detergent-containing buffers.

Current models for the mechanism of STAT activation of gene transcription propose that following dimerization, STAT complexes translocate to the nucleus. However, mechanism of transport to the nucleus remains unclear, since STAT proteins lack a nuclear localization signal (NLS; Johnson et al., 1998<sup>b</sup>), which is required for nuclear transport mediated through the importin mechanism (Gorlich & Mattaj, 1996). Johnson and co-authors (1998<sup>b</sup>) proposed an intriguing model for nuclear translocation of STATs after activation by IFN $\gamma$ . Since the carboxy-terminal domain of the IFN $\gamma$  molecule contain a NLS, they propose that following binding to IFN $\gamma$ , a complex containing the IFN $\gamma$ -receptor, jak kinases, STATs and the bound ligand become internalized by endocytosis. Upon cytoplasmic localization, the NLS sequence in the IFN $\gamma$  molecule could associate with the importin protein complex, which would then catalyze the transport of this complex to the nucleus, where STAT-mediated transcription activation would ensue. They provided evidence for actual nuclear translocation of a peptide containing the carboxy-domain of the IFN $\gamma$  molecule. Although seemingly unique, they provide evidence of over 30 cytokines and/or their receptors, which utilize STATs as signal transducers, that contain NLS in

their sequence, indicating that this ligand-receptor-assisted nuclear translocation is a viable and intriguing mechanism. Among such cytokines and receptors are the human IFN $\alpha$  and the human IFN $\alpha$ R1 molecules. Data in a recent paper is in variance with this concept (Millocco et al., 1999). Those authors engineered a STAT-1-estrogen receptor chimera, in which the estrogen receptor ligand binding domain was fused to the carboxy-terminus of STAT-1 molecules. After transfection to STAT-deficient U3a cells, this "conditionally active STAT" underwent dimerization following estrogen/tamoxifen treatment. Moreover, these chimeras were able to undergo nuclear translocation and activated transcription of interferon-induced genes such as interferon regulatory factor 1 (IRF-1). It was concluded that tyrosine phosphorylation of STAT is probably only a trigger for dimerization, since dimerized, non-phosphorylated STAT chimeras also were able to stimulate interferon-specific gene activation. Furthermore, since the estrogen receptor domain used in the chimera did not contain any NLS, dimerization alone was sufficient to promote nuclear translocation, sequence-specific DNA binding and transcription activation functions of the chimeric STATs. A study conducted by Strehlow and Schindler (1998) indicated that the amino-terminal 100 amino acids of particular STATs mediated their nuclear translocation activity. Chimeric constructs in which those amino acids in STAT-1 were substituted by those of STAT-2 abolished nuclear translocation of STAT-1, while other functions were maintained, such as activation by receptor, dimerization and DNA binding. Collectively, it is fair to say that the mechanism of STAT nuclear translocation remains unclear. Although the work of Johnson et al. (1998<sup>b</sup>) puts forth an exciting proposition for such a mechanism, data from Milocco and others (1999) argues against the requirement of a ligand-receptor-assisted transport mechanism. However, existence of both mechanisms is feasible *in vivo*.

### **Interferon-directed Gene Activation**

After translocation to the nuclear compartment, STAT complexes can act as transcription factors, to direct expression of interferon-induced genes. The best studied transcription activation complex containing STAT dimers is called interferon-stimulated gene factor 3 (ISGF-3), which is composed of a STAT 1:2 dimer and a nuclear DNA binding protein, p48 (Darnell et al., 1994; Bluysen et al., 1996). ISGF-3 was first identified in electrophoretic mobility shift assays as a complex induced by interferon treatment. It was formed independent of protein synthesis, and was found to bind to consensus sequences on the regulatory region of interferon-stimulated genes (Kessler et al., 1988). Consensus sequences are known as interferon-stimulus response elements (ISREs). Williams and Haque (1997) presented a summary of sequences of ISREs of known interferon-induced genes. A second interferon-induced transcription-activation complex also was identified and named ISGF-2 (Kessler et al., 1988). Such a complex is formed contingent on protein synthesis, presents different pattern of migration in mobility

shift assays and was later identified as the transcription factor interferon regulatory factor 1 (IRF-1; Parrington et al., 1993). Interestingly, IRF-1 and p48 are from the same family of proteins and can bind to the same promoter elements (i.e., ISREs) in the regulatory region of interferon-stimulated genes (Kessler et al., 1988; Parrington et al., 1993). The p48 and STAT 1:2 dimer do not associate in a stable manner to form the ISGF-3 complex in the absence of DNA. However, contacts of amino acids 150 to 250 in the STAT-1 molecule with the carboxy-terminal portion of DNA-bound p48 stabilizes ISGF-3 (Horvath et al., 1996). Vickenmeier and co-workers (1996) reported direct binding of recombinant, tyrosine phosphorylated STAT-1:1 dimers to tandem DNA binding sites. STAT-2 also forms homodimers, but requires p48 for strong transactivation of transcription (Bluyssen & Levy, 1997). However, interactions with DNA were not stable. Addition of STAT-1 increased the affinity and altered sequence selectivity of p48-DNA interactions. In this scenario, ISGF-3 assembly involves p48 functioning as an adaptor protein to recruit STAT-1 and STAT-2 to an ISRE, STAT-2 contributes with potent transactivation but is unable to directly contact DNA, while STAT-1 stabilizes the complex by contacting DNA directly. Alternatively to transcription-induction through ISRE binding, interferons also induce genes like IRF-1 which lack ISREs. Such genes are induced through sequences named Inverted Repeats, present in their promoters (Haque & Williams, 1994). Finally, type I interferons can stimulate expression of the c-fos gene through binding of homo- and heterodimers of STATs 1 and 3 to the sis-inducible element, present in the promoter region of that gene (Yang et al., 1996).

### **JAK-STAT Pathway Regulation**

As in other tyrosine-phosphorylation-induced signaling systems, biological responses resulting from activation of the JAK-STAT pathway are transient (Shuai et al., 1992). Although the pathway of activation via the JAK-STAT pathway is well established, few molecules have been identified that switch the signal off (Starr & Hilton, 1999). It is reasonable to predict that regulation of a tyrosine phosphorylation pathway could occur through the actions of phosphatases, to inactivate phosphotyrosil groups on receptors, JAKs and STATs, and proteases to degrade activated complexes. There is evidence for occurrence of both mechanisms of regulation (i.e., phosphatases and proteases) in the JAK-STAT pathway, but more recent data indicate presence of novel regulatory molecules also playing a role. Callus and Mathey-Prevot (1998) showed that treatment of Ba/F3 cells with a specific proteasome inhibitor led to stable tyrosine phosphorylation of the interleukin-3 (IL-3) receptor and STAT-5, after stimulation with IL-3. Further investigation revealed that stable phosphorylation events were due to prolonged activation of JAKs. Moreover, Kim and Maniatis (1996) demonstrated that after activation with interferon- $\gamma$ , STATs became ubiquitinated and quickly degraded. In contrast with data from Kim and Maniatis (1996), but in agreement with data from Callus and Mathey-Prevot (1998), Haspel and others (1996) reported that proteasome inhibitors increased time of activation of STAT-1 by prolonging signals from the receptor (i.e., preventing

degradation of receptor-JAKs complexes), but not by blocking removal of phosphorylated STATs. This was based on the finding that  $^{35}\text{S}$ -labeled STAT-1 translocated to the nucleus upon tyrosine phosphorylation and later returned to the cytoplasm in non-phosphorylated configuration. Data from Strehlow and Schindler (1998) agrees and expands these findings, in that chimeric STATs with mutated amino-terminal domains exhibited defects in nuclear translocation and deactivation, indicating that these two events might be linked (i.e., deactivation may be dependent on previous nuclear localization). Indeed, David and others (1993) demonstrated that a nuclear tyrosine-phosphatase is responsible for deactivation of phosphorylated STATs. To support the existence of a mechanism for regulating activity of STATs based on phosphatases, Haque and co-authors (1995) reported that treatment of cells with orthovanadate, molybdenate and tungstate, which are effective inhibitors of protein-tyrosine phosphatases, resulted in accumulation of interferon- $\gamma$ -induced phosphorylated STATs. Involvement of novel molecules in the regulation of the JAK-STAT pathway was reviewed by Starr and Hilton (1999). They propose a model in which suppressors of cytokine signaling (SOCS) proteins such as SOCS1 bind directly to JAKs to inhibit their catalytic activities. Another protein, CIS, binds to activated receptors to prevent docking of STATs. SH2-domain phosphatase-1 (SHP-1) dephosphorylates JAKs or activated receptors. Finally, a protein inhibitor of activated STAT (PIAS) inactivates STAT dimers. Song and Shuai (1998) demonstrated that SOCS1 and SOCS3 inhibited interferon-mediated antiviral and antiproliferative activities in HeLa cells. This was linked with abolished tyrosine phosphorylation and nuclear translocation of STAT-1 in response to interferon- $\alpha$ . Chung and others (1997<sup>a</sup>) reported that PIAS3 directly interacted with STAT3 and inhibited DNA binding of both STAT-3:3, STAT-1:3 dimers. Binding of STAT-1 homodimers was not affected. Moreover, co-transfections of both STAT-3 and PIAS3 showed a decrease in luciferase activity from an IRF-1 reporter gene in response to increasing amounts of PIAS3.

### **Specificity of Interferon Signaling**

Information presented in previous sections offers several opportunities for occurrence of specific cellular responses to interferons. Such opportunities include: (1) milieu of subtypes of interferons present at the receptor, in which for example, different iso-forms of ovine IFN- $\tau$  have different ability to extend estrous cycle length in ewes (Ealy et al., 1998); (2) composition of the receptor complex, where recruitment of particular receptor subunits may affect which STATs are recruited; (3) amino acid context of the phosphotyrosine module on the receptor chain, and amino acid context of the SH2 domain on STATs will also determine which STAT will dock to which receptor chain; (4) which STATs are present and what dimers will form upon ligand binding; (5) mechanism of nuclear translocation of STATs, since whether STATs translocate as dimers, alone or in combination with ligand-receptor complexes may influence the configuration and specificity of the transcriptional activation complex; (6) formation of single or multiple transcription activation complexes, which will depend

on nature of dimers and interacting nuclear proteins; (7) dynamics of downregulation of JAK-STAT pathway, in which specific branches of the pathway may be inhibited while others may remain active to elicit specific responses; finally (8) there could be interactions with other cellular pathways.

### **Cross-talk with Other Intracellular Pathways**

The best known cross-talk between JAK-STAT and other signaling pathways is that represented by serine and threonine phosphorylation of STAT residues, both constitutively and in response to ligands (see Leaman et al., 1996 for review). Such phosphorylation events are important, since treatment of cells with kinase inhibitors disrupts STAT-3:3 DNA complexes. A mitogen-activated protein kinase (MAPK) may be involved in phosphorylation of serine residues of STAT-1, because the serine 727 lies in a consensus sequence for MAPK phosphorylation. In fact, Stancato and co-workers (1997) proposed a model in which activation of MAPK was dependent on activated JAK kinases. Binding of interferon- $\alpha/\beta$  induced tyrosine phosphorylation of JAK-1, which stimulated activity of membrane bound Raf-1. Activated Raf-1 phosphorylates MEK and activates MAPK. MAPK in turn phosphorylates serine residues on STAT-1, contributing to modulation of activity for this signal transducer. However, modulation of STAT activities by MAPK may be stimulatory or inhibitory. For example, Chung and others (1997b) reported serine phosphorylation of STAT-3 by growth factors, while STAT-1 was poor substrate for several MAPK tested. Interestingly, serine phosphorylation of STAT-3 reduced tyrosine phosphorylation of STAT-3, and consequently inhibited dimerization, nuclear translocation and gene activation.

Signal transducers such as IRS-1 and IRS-2, that are activated in response to insulin, IL-2, IL-4 etc, are tyrosine phosphorylated by JAK-1. Epidermal growth factor (EGF) is able to activate tyrosine phosphorylation of STATs 1 and 3 (David et al., 1996). Interestingly, this does not require presence of JAKs. Moreover, truncated receptor constructs containing the intrinsic kinase activity but lacking the autophosphorylation domains were also effective in phosphorylating STATs. This indicates that an alternative mechanism, where docking through SH2 domain of STATs is not required for phosphorylation, is in place for EGF-induced STAT phosphorylation.

The obligatory intracellular bacterium of macrophages, *Ehrlichia chaffeensis*, blocked tyrosine phosphorylation of STAT-1, JAK-1 and JAK-2 in response to IFN- $\gamma$  within 30 minutes of infection (Lee & Rikihisa, 1998). Also, PKA activity was increased 25 fold after infection. Inhibitors of PKA activity partially abrogated the *E. chaffeensis*-induced inhibition of STAT-1 tyrosine phosphorylation, suggesting negative regulation of the JAK-STAT pathway by the PKA-dependent mechanisms.

Another interesting theme is the occurrence of synergistic effects as a result of co-activation of cellular pathways involving the JAK-STAT system. For example, cooperation of interferon- $\gamma$  and tumor necrosis factor (TNF) during inflammatory responses is a result of cooperation between STAT-1 and the transcription factor NF-

$\kappa\beta$ . Synergistic expression of several genes involved in the inflammatory process was contingent on presence of both transcription factors (Ohmori et al., 1997). Stimulation by oncostatin M (OSM) induces expression of matrix metalloproteinases (MMPs). Analysis of the regulatory region of MMP-1 gene revealed presence of an AP-1 site as well as a STAT binding element. Korzus and co-workers (1997) reported enhancement of MMP expression due to synergistic actions of AP-1 and STAT-1. Such an effect was Ras-dependent, which implies crosstalk between the MAPK and the JAK-STAT pathways of signal transduction.

Yet another example of crosstalk is between the JAK-STAT pathway and the PI 3' kinase, which has both lipid and serine kinase activities. Pfeffer and others (1997) reported that PI 3' kinase is tyrosine phosphorylated through the JAK-STAT pathway. Tyrosine phosphorylated STAT-3 proteins, bound to the IFN $\alpha$ R1 chain of the interferon receptor serves as a docking site for PI 3' kinase, which couples its SH2 domain to tyrosine phosphorylated residues in the STAT molecule. Upon docking, the PI 3' kinase is activated by JAKs, which then promotes serine phosphorylation of STAT-3 to increase STAT-3 activity. In another study (Uddin et al., 1997) interferon- $\alpha$  stimulated serine kinase activity of PI 3' kinase, which in turn activated the signal transducer IRS-1. Moreover, stimulation with interferon  $\beta$  caused activation of MAPK, and such stimulation was inhibited by Wortmannin, an inhibitor of PI 3' kinase activity. This suggests involvement of the PI 3' kinase on MAPK activation. In contrast, with data from Pfeffer and others (1997), Wortmannin failed to inhibit formation of the ISGF3 complex and interferon-mediated induction of ISG-15, indicating that the PI 3' kinase probably is not required for interferon effects.

Finally, the work of Flati and others (1996) indicates that stimulation of cells with interferon- $\alpha$  causes activation of PLA<sub>2</sub>, as measured by release of AA in culture medium. PLA<sub>2</sub> was associated with JAK-1, and inhibitors of PLA<sub>2</sub> activity prevented formation of active ISGF3 transcription complexes. However, such inhibition did not block binding of activated STAT-1 to inverted repeat sequences, such as present in the regulatory region of IRF-1. Moreover, treatment of cells with interferon- $\alpha$  stimulates tyrosine phosphorylation of PLA<sub>2</sub>. The authors argue for a structural role of PLA<sub>2</sub>, which may be required for correct assembly of the ISGF3 transcription complex.

## **The JAK-STAT Pathway in the Ruminant Endometrium**

In addition to the bIFN- $\tau$  receptor data described previously, very little has been done on the elucidation of the signal transduction system activated by bIFN- $\tau$  in the endometrium. Spencer and others (1998) conducted two studies to detect induction of interferon-stimulated transcription factors, IRF-1 and IRF-2. Both factors were absent in cyclic ewes and present in pregnant ewes (days 11 and 13, cyclic and days 13, 15 and 17, pregnant). In cyclic ewes with ligated uterine horns, unilateral infusion of ovine IFN- $\tau$  induced expression of IRF-1 and IRF-2 but not in the uterine horn receiving a

BSA infusion. Since expression of these factors is contingent on a functional JAK-STAT pathway, these data support existence of such a pathway in the endometrium. Bathgate and co-workers (1998) also reported existence of IRFs in endometrium of pregnant cows. Perry and co-authors (1999) reported presence of STATs 1 and 2 and IRF-1 in the nucleus of BEND cells stimulated with bIFN- $\tau$  (Figure 2). Moreover, bIFN- $\tau$  stimulated tyrosine phosphorylation and nuclear translocation of STAT proteins in bovine endometrium (Figure 3; Binelli et al., 1998; Binelli, 1999).

## **Bovine bIFN- $\tau$ -stimulated Protein Synthesis in the Endometrium**

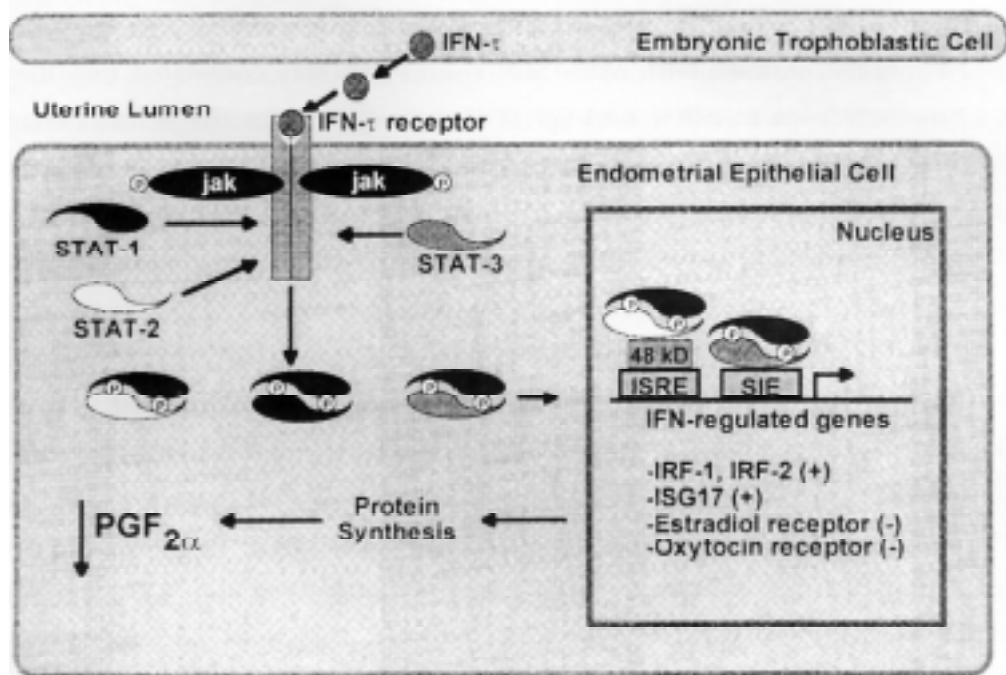
Rueda and co-workers (1993) reported secretion of 12 and 28 kD proteins both from pregnant endometrial explants and cyclic endometrial explants stimulated with bIFN- $\tau$  in vitro. In a subsequent paper, Naivar and others (1995) further characterized those proteins and discovered a novel, 16 kDa secretory protein (P16). Endometrium explants were obtained from day 18 pregnant cows and incubated in presence or absence of bIFN- $\tau$ . Both basal and stimulated secretion of all three proteins increased in culture medium in a time-dependent manner. More importantly, the 12 kD protein (Rueda et al., 1993), now renamed as P8, was induced only in response to bIFN- $\tau$  but not in response to IFN- $\alpha$ , suggesting the possibility of bIFN- $\tau$  eliciting specific signal transduction and protein synthesis. Moreover, P8 but not P16 secretion could be stimulated by phorbol ester (Staggs et al., 1998). Amino acid analysis of the P8 revealed identity with the alpha chemokine family: 92-100% identity with bovine bGCP-2 (Teixeira et al., 1997). Functions of bGCP-2 remain elusive, but it has been suggested (Hansen et al., 1999) that being a chemokine, bGCP-2 may attract conceptus cells to attachment sites in the endometrium. Also, bGCP-2 may attract cells from the immune system, to release cytokines beneficial to embryonic development. P16 was identified as interferon stimulated gene 17 (ISG17; Austin et al., 1996<sup>a,b</sup>; Figure 2). The ISG17 mRNA (Hansen et al., 1997) and protein (Austin et al., 1996<sup>b</sup>) are induced by bIFN- $\tau$ , and sequence analysis of the ISG17 gene revealed presence of a conserved ISRE in the promoter region, indicating putative activation by bIFN- $\tau$  (Perry et al., 1997). Analysis of the primary structure of ISG17 revealed presence of critical amino acids and domains implicated in functions of ubiquitin, such as conjugating with other proteins. However, ISG17 lacked residues required for targeting proteins to proteasomal degradation (Austin et al., 1996<sup>a</sup>). Therefore, it was proposed that a possible role for ISG17 was to modify uterine proteins during early pregnancy (Hansen et al., 1999). In fact, Johnson and others (1998<sup>a</sup>) reported that specific conjugates of ISG17 and endometrial cytosolic proteins were formed in response to treatment with bIFN- $\tau$ . Moreover, such complexes were distinct from complexes containing ubiquitin, indicating a bIFN- $\tau$ -induced, specific action. Although proteins present in the ISG17 conjugates have not yet been identified, an attractive hypothesis is that bIFN- $\tau$  induces conjugation of ISG17 to proteins involved in the cascade of PGF<sub>2 $\alpha$</sub>  production in the endometrium. Such targeting could modify function of such proteins to make them less able to stimulate PGF<sub>2 $\alpha$</sub>  production.



Research from Spencer and co-workers (1998) also showed that endometrial estrogen receptors and oxytocin receptors were reduced in the uterine horns infused with ovine IFN- $\tau$ , and this was correlated negatively with observed increases in IRF-1 and IRF-2 expression. Since IRF-2 has been implicated as an inhibitor of gene transcription (Harada et al., 1994; Figure 2), the authors hypothesized that perhaps interferon-induced IRFs were involved in inhibition of gene transcription for estrogen and oxytocin receptors. In fact, Fleming and co-workers (1998) cloned the ovine estrogen receptor gene and discovered IRF response element (IRE) consensus sequences in the promoter region, further supporting the hypothesis of interferon modulation of estrogen receptor expression. Deletion constructs of the estrogen receptor promoter linked to luciferase reporter gene were transfected into endometrial cells. Treatment of these cells with ovine IFN- $\tau$  caused reduction in luciferase expression only in constructs containing the IREs. Using the same rationale, Bathgate and others (1998) sequenced the bovine oxytocin receptor gene and also found IREs in the regulatory region, and such sites bound bovine IRF-1 and -2. Again, the suggestion is that bIFN- $\tau$ -induced transcription repressors may downregulate expression of oxytocin receptors, to ultimately decrease PGF<sub>2 $\alpha$</sub>  secretion in the pregnant uterus (Figure 2).

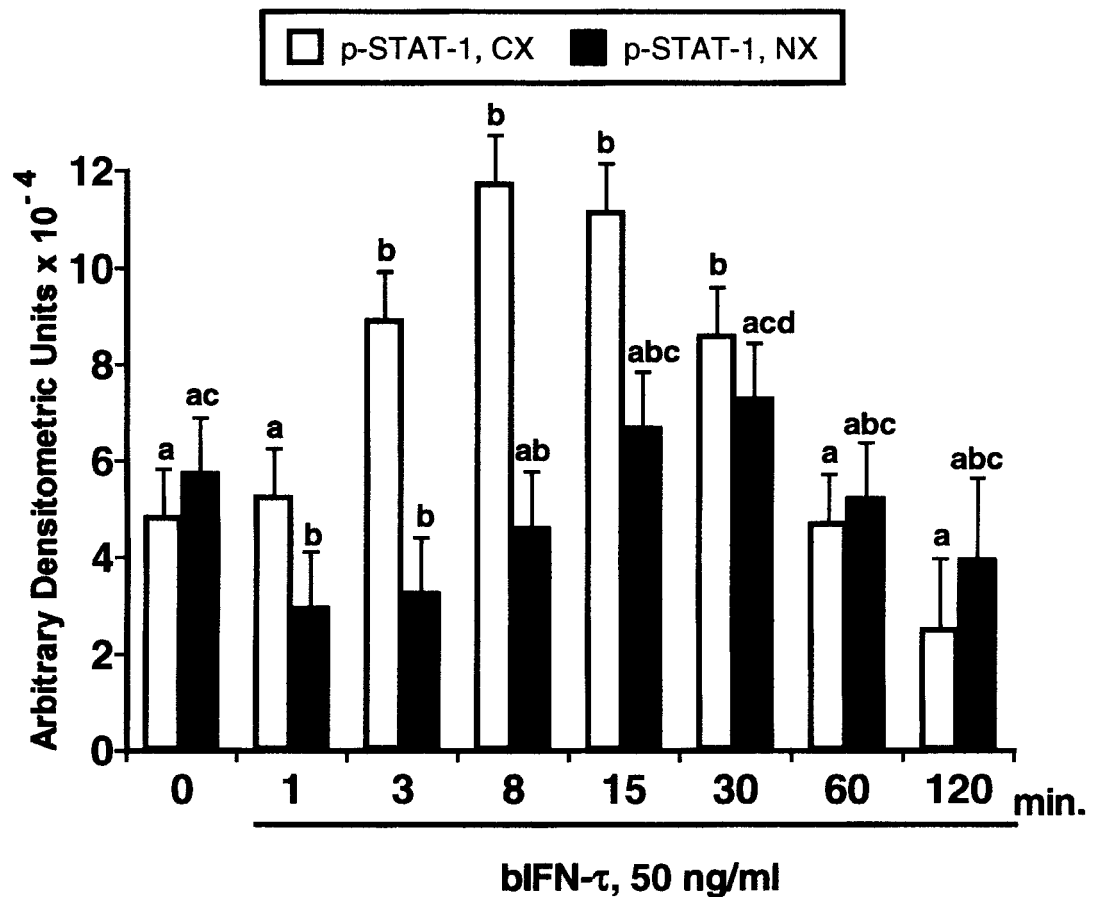
## **Uterine-conceptus Interactions and Reproductive Failure in Cattle**

Thus far, this review has illustrated the enormous amount and intricacy of interactions that need to occur between embryonic and maternal uterine tissues in order for a successful pregnancy to be established. Given the high percentage of embryonic mortality occurring during early pregnancies, it becomes apparent that a precise program of interactions must be followed, and that deviations from such a program may lead to pregnancy termination. Such a program includes both embryonic and maternal components. For example, the embryonic unit must be able to effectively interact with the maternal endometrium, undergo elongation and send antiluteolytic signals to the maternal unit in order to survive. The maternal unit should provide a quiescent and nutritive environment, conducive for embryonic attachment, and should bear intracellular mechanisms to receive and transduce antiluteolytic signals from the conceptus, that ultimately inhibit the default, PGF<sub>2 $\alpha$</sub> -secretory pathway of the uterus. Thatcher and Hansen (1992) reported that day 17 conceptus varied in size from 15 to 250 mm. Since inhibition of PGF<sub>2 $\alpha$</sub>  is probably dependent on total amount of bIFN- $\tau$  secreted and on area of endometrium occupied by the conceptus, smaller conceptuses would have already a smaller chance of survival. Environmental effects such as heat stress decrease conceptus development (Geisert et al., 1988) and apparently compromises ability of the conceptus to secrete bIFN- $\tau$ , leading to failure in pregnancy recognition (Putney et al., 1988). There is also evidence for a role of the uterus to stimulate secretion of bIFN- $\tau$  by conceptus. Hernandez-Ledezma and coworkers (1992) cultured IVF (in vitro fertilization)-produced embryos to blastocyst



**Figure 2.** Hypothetical model for the bIFN- $\tau$ -stimulated JAK-STAT pathway of signal transduction in endometrial epithelial cells. Trophoblastic cells on the conceptus secrete bIFN- $\tau$  into the uterine lumen, and bIFN- $\tau$  interacts with its receptor in the apical aspect of endometrial epithelial cells. Binding of bIFN- $\tau$  elicits tyrosine phosphorylation, homo- and hetero-dimer formation of pre-existing, unphosphorylated STAT proteins. Dimers of STATs translocate to the nucleus where they bind to specific cis-activating elements [sis-inducible element (SIE) and interferon-stimulus response element (ISRE)] present in the regulatory region of interferon-regulated genes to stimulate their transcription. It is hypothesized that proteins induced by bIFN- $\tau$ , such as interferon-stimulated gene 17 (ISG17), interferon regulatory factor (IRF)-1 and IRF-2 may act to suppress synthesis of PGF $_{2\alpha}$  from endometrial cells. Also, the JAK-STAT pathway may suppress synthesis of proteins involved in the PGF $_{2\alpha}$  synthesis machinery, such as receptors for oxytocin and estradiol.

stage and either continued in vitro culture or transferred conceptus to synchronized recipient cows. Embryos were recovered 4 days later, placed in culture dishes and secretion of bIFN- $\tau$  was quantified. Secretion of bIFN- $\tau$  was highly stimulated by exposure to the uterine environment, indicating that optimal production of the antiluteolytic signal by the conceptus is not solely determined by the conceptus. Stojkovic and co-workers (1999) reported that bovine embryos derived by embryo flushing and in vitro production produced more bIFN- $\tau$  in long term culture than embryos derived from nuclear transfer or embryo splitting. Such differences may contribute to lower pregnancy rates following transfer of the latter two embryo classes to recipients. Failure of cows to extend CL lifespan in response to bIFN- $\tau$  have been reported (Helmer et al., 1989; Meyer et al., 1995). This indicates failure in the interferon receptor system, JAK-STAT-mediated signal transduction, post-signaling mechanisms within the endometrium or a combination of these factors. These responses have not been examined in a population of cows and warrant further investigation.



**Figure 3.** Densitometric analysis of tyrosine phosphorylated (p) STAT-1 immunoprecipitated from cytosolic extracts (CX) and nuclear extracts (NX) from BEND cells treated with bIFN- $\tau$  for increasing intervals of time. BEND cells were either left untreated ("0" minutes) or treated with bIFN- $\tau$  (50 ng/ml) from 1 to 120 minutes. Cells were lysed, CX and NX obtained, immunoprecipitated and analyzed by immunoblotting using antibodies against phosphotyrosine. Within each variable, bars with distinct subscripts are statistically different,  $p < 0.1$ . Note the early increase in phosphorylation of STAT-1 in the cytosol and the delayed increase in phosphorylation in the nucleus, which suggests that STAT-1 is initially phosphorylated in the cytosol and then translocates into the nuclear compartment.

## Concluding Remarks

The present review provides a framework for development of hypotheses to improve embryo survival and reproductive efficiency in ruminants.

## List of References

- Abramovich C, Yakobson B, Chebath J, Revel M. A protein-arginine methyltransferase binds to the intracytoplasmic domain of the IFN $\alpha$ R1 chain in the type I interferon receptor. *EMBO J* 1997;16:260-6.
- Anthony RV, Helmer SD, Sharif SF, Roberts RM, Hansen PJ, Thatcher WW, Bazer FW. Synthesis and processing of ovine trophoblast protein-1 and bovine trophoblast protein-1, conceptus secretory proteins involved in the maternal recognition of pregnancy. *Endocrinology* 1988;123:1274-80.

- Arnold DR, Binelli M, Wilcox CJ, Thatcher WW. Regulation of prostaglandin  $F_{2\alpha}$  secretion from endometrium of cyclic and pregnant cattle. Proc 5<sup>th</sup> International Symposium on Reproduction in Domestic Ruminants 1999;(In press).
- Asselin E, Drolet P, Fortier MA. In vitro response to oxytocin and interferon-tau in bovine endometrial cells from caruncular and inter-caruncular areas. Biol Reprod 1998;59:241-7.
- Asselin E, Fortier MA. Molecular cloning of a bovine endometrial 20 $\alpha$ -hydroxysteroid dehydrogenase (20 $\alpha$ -HSD): regulation by bIFN- $\tau$  [Abstract 47]. Biol Reprod 1998;58(suppl 1):86.
- Asselin E, Lacroix D, Fortier MA. IFN- $\tau$  increases PGE<sub>2</sub> production and COX-2 gene expression in the bovine endometrium in vitro. Mol Cell Endocrinol 1997;56:402-8.
- Austin KA, Pru JK, Hansen TR. Complementary deoxyribonucleic acid sequence encoding bovine ubiquitin cross-reactive protein. Endocrine 1996<sup>a</sup>;5:191-7.
- Austin KA, Ward SK, Teixeira MG, Dean VC, Moore DW, Hansen TR. Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. Biol Reprod 1996<sup>b</sup>;54:600-6.
- Bartol FF, Roberts RM, Bazer FW, Lewis GS, Godkin JD, Thatcher WW. Characterization of proteins produced in-vitro by peri-attachment bovine conceptuses. Biol Reprod 1985;32:681-94.
- Basu S, Kindahl H. Prostaglandin biosynthesis and its regulation in the bovine endometrium: a comparison between nonpregnant and pregnant status. Theriogenology 1987;28:175-93.
- Bathgate RAD, Tillmann G, Ivell R. Molecular mechanisms of bovine oxytocin receptor gene regulation [Abstract]. Biol Reprod 1998;58(suppl 1):160.
- Betteridge KJ, Eaglesome MD, Randall GCB, Mitchell D. Collection, description and transfer of embryos from cattle 10-16 days after oestrus. J Reprod Fertil 1980;59:205-16.
- Binelli M. Maternal-embryonic interactions during early pregnancy in cattle. Gainesville, USA: University of Florida, 1999. Dissertation.
- Binelli M, Diaz T, Hansen TR, Thatcher WW. Bovine interferon-tau induces phosphorylation and nuclear translocation of STAT-1, -2 and -3 in endometrial epithelial cells [Abstract]. Biol Reprod 1998;58(suppl 1):214.
- Bluyssen AR, Durbin JE, Levy DE. ISGF3 gamma p48, a specificity switch for interferon activated transcription factors. Cytokine Growth Factor Rev 1996;7:11-17.
- Bluyssen AR, Levy DE. STAT-2 is a transcriptional activator that requires sequence-specific-contacts provided by STAT 1 and p48 for stable interaction with DNA. J Biol Chem 1997;272:4600-5.
- Burns PD, Graf GA, Hayes SH, Silvia WJ. Cellular mechanisms by which oxytocin stimulates uterine PGF<sub>2 $\alpha$</sub>  synthesis in bovine endometrium: roles of phospholipases C and A<sub>2</sub>. Domest Anim Endocrinol 1997;14:181-91.
- Callus BA, Mathey-Prevot B. Interleukin-3-induced activation of the JAK/STAT pathway is prolonged by proteasome inhibitors. Blood 1998;91:3182-92.

- Chung CD, Liao J, Liu B, Rao X, Jay P, Berta P, Shuai K. Specific inhibition of Stat3 signal transduction by PIAS3. *Science* 1997<sup>a</sup>;278:1803-5.
- Chung J, Uchida E, Grammar TC, Blenis J. STAT-3 serine phosphorylation by ERK-dependent and -independent pathways negatively modulates its tyrosine phosphorylation. *Mol Cell Biol* 1997<sup>b</sup>;17:6508-16.
- Clark JD, Lin L-L, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, Knopf JL. A novel arachidonic acid-selective cytosolic PLA<sub>2</sub> contains a Ca<sup>2+</sup>-dependent translocation domain with homology to PKC and GAP. *Cell* 1991;65:1043-51.
- Colamonici OR, Uyttendaele H, Domanski P, Yan H, Krolewski JJ. P135<sup>tyk2</sup>, an interferon- $\alpha$ -activated tyrosine kinase, is physically associated with an interferon- $\alpha$  receptor. *J Biol Chem* 1994<sup>a</sup>;269:3518-22.
- Colamonici OR, Yan H, Domanski P, Handa R, Smalley D, Mullersman J, Witte M, Krishnan K, Krolewski J. Direct binding to and tyrosine phosphorylation of the  $\alpha$  subunit of the type I receptor by p135<sup>tyk2</sup> tyrosine kinase. *Mol Cell Biol* 1994<sup>b</sup>;14:8133-42.
- Danet-Desnoyers G, Johnson JW, O'Keefe SF, Thatcher WW. Characterization of a bovine endometrial prostaglandin synthesis inhibitor (EPSI) [Abstract]. *Biol Reprod* 1993;48(suppl 1):115.
- Danet-Desnoyers G, Wetzels C., Thatcher WW. Natural and recombinant bovine interferon  $\tau$  regulate basal and oxytocin-induced secretion of prostaglandins F<sub>2 $\alpha$</sub>  and E<sub>2</sub> by epithelial cells and stromal cells in the endometrium. *Reprod Fertil Dev* 1994;6:193-202.
- Darnell JE Jr. STATs and gene regulation. *Science* 1997;277:1630-5.
- Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994;264:1415-21.
- David M, Grimley PM, Finbloom DS, Larner AC. A nuclear tyrosine phosphatase downregulates interferon-induced gene expression. *Mol Cell Biol* 1993;13:7515-21.
- David M, Wong L, Flavell R, Thompson SA, Wells A, Larner AC, Johnson GR. STAT activation by epidermal growth factor (EGF) and amphiregulin. Requirement for the EGF receptor kinase but not phosphorylation sites or JAK1. *J Biol Chem* 1996;271:9185-8.
- Ealy AD, Drost M, Hansen PJ. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. *J Dairy Sci* 1993;76:2899-905.
- Flati V, Haque SJ, Williams BRG. Interferon- $\alpha$ -induced phosphorylation and activation of cytosolic phospholipase A<sub>2</sub> is required for the formation of interferon-stimulated gene factor 3. *EMBO J* 1996;15:1566-71.
- Fleming JGW, Spencer TE, Bazer FW. Cloning and analysis of the 5' flanking region of the ovine estrogen receptor alpha gene [Abstract]. *Biol Reprod* 1998;58(suppl 1):402.
- Flint APF, Leat WMF, Sheldrick EL, Stewart HJ. Stimulation of phosphoinositide hydrolysis by oxytocin and the mechanism by which oxytocin controls prostaglandin synthesis in the ovine endometrium. *J Biochem* 1986;237:797-805.
- Fu X-Y. A transcription factor with SH2 and SH3 domains is directly activated by an interferon alpha-induced cytoplasmic protein tyrosine kinase(s). *Cell* 1992;70:323-35.

- Fu X-Y, Schindler C, Improtta T, Aebersold R, Darnell JE Jr. The proteins of ISGF-3, the interferon transcriptional activator, define a gene family involved in signal transduction. *Proc Natl Acad Sci USA* 1992;89:7840-3.
- Geisert RD, Zavy MT, Biggers BG. Effect of heat stress on conceptus and uterine secretion in the bovine. *Theriogenology* 1988;29:1075-82.
- Gerhartz C, Heesel B, Sasse J, Hemmann U, Landgraf C, Schneider-Mergener J, Horn F, Heinrich PC, Graeve L. Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. I. Definition of a novel phosphotyrosine motif mediating STAT1 activation. *J Biol Chem* 1996;271:12991-8.
- Godkin JD, Bazer FW, Moffatt J, Sessions F, Roberts RM. Purification and properties of a major, low molecular weight protein released by the trophoblast sheep blastocysts at day 13-21. *J Reprod Fertil* 1982;65:141-50.
- Gorlich D, Mattaj JW. Nucleocytoplasmic transport. *Science* 1996;271:1513-18.
- Greenlund AL, Morales MO, Viviano BL, Yan H, Krolewski J, Schreiber RD. STAT recruitment by tyrosine phosphorylated receptors: an ordered, reversible affinity driven process. *Immunity* 1995;2:677-87.
- Gross TS, Thatcher WW, Hansen PJ, Helmer SD. Presence of an intracellular endometrial inhibitor of prostaglandin synthesis during early pregnancy in the cow. *Prostaglandins* 1988;35:359-78.
- Gupta S, Yan H, Wong LH, Ralph S, Krolewski J, Schindler C. The SH2 domains of STAT1 and STAT2 mediate multiple interactions in the transduction of IFN- $\alpha$  signals. *EMBO J* 1996;15:1075-84.
- Hafez, ESE. Anatomy of female reproduction. In: Hafez ESE (ed.), *Reproduction in farm animals*, 6<sup>th</sup> ed. Philadelphia: Lea & Febiger; 1993:20-55.
- Han CS, Roberts RM. Cloning and characterization of interferon- $\tau$  receptors [Abstract 19]. *Biol Reprod* 1998;58(suppl 1):108.
- Hansen PJ. Rescue of the corpus luteum from luteolysis by bovine trophoblast protein-1: an example of maternal recognition of pregnancy. *Revista Brasileira de Reprodução Animal* 1991;3(suppl 1):42-65.
- Hansen TR, Austin KJ, Johnson GA. Transient ubiquitin cross-reactive protein gene expression in the bovine endometrium. *Endocrinology* 1997; 138:5079-82.
- Hansen TR, Austin KJ, Perry DJ, Pru JK, Teixeira MG, Johnson GA. Mechanism of interferon-tau action in the uterus during early pregnancy. *Proc 5<sup>th</sup> International Symposium on Reproduction in Domestic Ruminants* 1999; (In press).
- Hansen TR, Kazemi M, Keisler DH, Malathy P-V, Imakawa K, Roberts RM. Complex binding of the embryonic interferon, ovine trophoblast protein-1, to endometrial receptors. *J Interferon Res* 1989;9:215-25.
- Haque SJ, Flati V, Deb A, Williams BRG. Roles of protein-tyrosine phosphatases in STAT1 $\alpha$ -mediated cell signalling. *J Biol Chem* 1995;270:25709-14.
- Haque SJ, Williams BRG. Identification and characterization of an interferon (IFN)-stimulated response element-IFN-stimulated gene factor 3-independent signaling pathway. *J Biol Chem* 1994;269:19523-9.

- Harada H, Takahashi E, Itoh S, Harada K, Hori TA, Taniguchi T. Structure and regulation of the human interferon regulatory factor 1 (IRF-1) and IRF-2 genes: implications for a gene network in the interferon system. *Mol Cell Biol* 1994;14:1500-9.
- Haspel RL, Salditt-Georgieff M, Darnell JE Jr. The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon protein tyrosine phosphatases. *EMBO J* 1996;15:6262-8.
- Helmer SD, Hansen PJ, Anthony RV, Thatcher WW, Bazer FW, Roberts RM. Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1. *J Reprod Fertil* 1987;79:83-91.
- Helmer SD, Hansen PJ, Thatcher WW, Johnson JW, Bazer FW. Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend corpus luteum lifespan in cyclic cattle. *J Reprod Fertil* 1989;87:89-101.
- Hemmann U, Gerhartz C, Heesel B, Sasse J, Kurapkat G, Grotzinger J, Wollmer A, Zhong Z, Darnell jr JE, Graeve L, Heinrich PC, Horn PF. Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. II. Src homology SH2 domains define the specificity of stat factor activation. *J Biol Chem* 1996;271:12999-13007.
- Hernandez-Ledezma JJ, Sikes JD, Murphy CN, Watson AJ, Schultz GA, Roberts RM. Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. *Biol Reprod* 1992;47:374-80.
- Horvath CM, Stark GR, Kerr IM, Darnell JE Jr. Interactions between STAT and non-STAT proteins in the interferon-stimulated gene factor 3 transcription complex. *Mol Cell Biol* 1996;16:6957-64.
- Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Silvennoinen O. Signaling through the hematopoietic cytokine receptors. *Annu Rev Immunol* 1995;13:369-98.
- Imakawa K, Hansen TR, Malathy PV, Anthony RV, Polites HG, Marotti KR, Roberts RM. Molecular cloning and characterization of complementary DNA corresponding to bovine trophoblast protein-1: a comparison with ovine trophoblast protein-1 and bovine interferon- $\alpha_{11}$ . *Mol Endocrinol* 1989;3:127-39.
- Johnson GA, Austin KJ, Van Kirk EA, Hansen TR. Pregnancy and interferon tau induce conjugation of bovine ubiquitin cross-reactive protein to cytosolic uterine proteins. *Biol Reprod* 1998a;58:898-904.
- Johnson HM, Torres BA, Green MM, Szente BE, Siler KI, Larkin J 3<sup>rd</sup>, Subramaniam PS. Hypothesis: ligand/receptor-assisted nuclear translocation of STATs. *Proc Soc Exp Biol Med* 1998b;218:149-55.
- Kessler DS, Levy DE, Darnell JE Jr. Two interferon-induced nuclear factors bind a single promoter element in interferon-stimulated genes. *Proc Natl Acad Sci USA* 1988;85:8521-5.
- Kim TK, Maniatis T. Regulation of interferon- $\gamma$ -activated STAT1 by the ubiquitin-proteasome pathway. *Science* 1996;273:1717-9.
- Knickerbocker JJ, Niswender GD. Characterization of endometrial receptors for ovine trophoblast protein-1 during the estrous cycle and early pregnancy in sheep. *Biol Reprod* 1989;40:361-9.

- Korzus E, Nagaase H, Rydell R, Travis J. The mitogen-activated protein kinase and JAK-STAT signaling pathways are required for an oncostatin M-responsive element-mediated activation of matrix metalloproteinase 1 gene expression. *J Biol Chem* 1997;272:1188-96.
- Leaman DW, Leung S, Li X, Stark GR. Regulation of STAT-dependent pathways by growth factors and cytokines. *FASEB J* 1996;10:1578-88.
- Lee EH, Rikihisa Y. Protein kinase A-mediated inhibition of gamma interferon-induced tyrosine phosphorylation of Janus kinases and latent cytoplasmic transcription factors in human monocytes by *Ehrlichia chaffeensis*. *Infect Immun* 1998;66:2514-20.
- Li J, Roberts RM. Interferon- $\tau$  and interferon- $\alpha$  interact with the same receptors in the bovine endometrium. *J Biol Chem* 1994;269:13544-50.
- Li X, Leung S, Kerr IM, Stark GR. Functional subdomains of STAT2 required for preassociation with the alpha interferon receptor and for signaling. *Mol Cell Biol* 1997;17:2048-56.
- Lin L-L, Wartmann M, Lin AY, Knopf JL, Seth A, Davis RJ. cPLA<sub>2</sub> is phosphorylated and activated by MAP kinase. *Cell* 1993;72:269-78.
- Martal J, Lacroix M-C, Lourdes C, Saunier M, Wintenberger-Torres M. Trophoblastin, an antiluteolytic protein present in early pregnancy in sheep. *J Reprod Fertil* 1979;56:63-73.
- Meyer MD, Danet-Desnoyers G, Oldick B, Thatcher WW, Drost M, Schalue K, Roberts RM. Treatment with recombinant bovine interferon- $\tau$  in utero attenuates secretion of prostaglandin F from cultured endometrial epithelial cells. *J Dairy Sci* 1996;79:1375-84.
- Meyer MD, Hansen PJ, Thatcher WW, Drost M, Badinga L, Roberts RM, Li J, Ott TL, Bazer FW. Extension of corpus luteum lifespan and reduction of uterine secretion of prostaglandin F<sub>2 $\alpha$</sub>  of cows in response to recombinant interferon- $\tau$ . *J Dairy Sci* 1995;78:1921-31.
- Milloco, LH, Haslan JA, Rosen J, Seidel HM. Design of conditionally active STATs: insights into STAT activation and gene regulatory function. *Mol Cell Biol* 1999;19:2913-20.
- Naivar KA, Ward SK, Austin KJ, Moore DW, Hansen TR. Secretion of bovine uterine proteins in response to type I interferons. *Biol Reprod* 1995;52:848-54.
- Northey DL, French LR. Effect of embryo removal and intrauterine infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. *J Anim Sci* 1980;50:298-302.
- Ohmori Y, Schreiber RD, Hamilton TA. Synergy between interferon-gamma and tumor necrosis factor-alpha in transcriptional activation is mediated by cooperation between signal transducer and activator of transcription 1 and nuclear factor kappa  $\beta$ . *J Biol Chem* 1997;272:14889-907.
- Parrington J, Rogers NC, Gewert DR, Pine R, Veals SA, Levy DE, Stark GR, Kerr IM. The interferon-stimulable response elements of two human genes detect overlapping sets of transcription factors. *Eur J Biochem* 1993;214:617-26.



- Perry DJ, Austin KJ, Hansen TR. The uterine bovine ubiquitin cross-reactive protein gene: a comparison with human interferon stimulated gene 15 [Abstract]. *Biol Reprod* 1997;56(suppl 1):181.
- Perry DJ, Austin KJ, Hansen TR. Cloning of interferon stimulated gene 17: the promoter and nuclear proteins that regulate transcription. *Molecular Endocrinology* 1999;13:1197-206.
- Pestka S. The interferon receptors. *Semin Oncol* 1997;24(suppl 9):s918-s940.
- Pfeffer LM, Mullersman JE, Pfeffer SR, Murti A, Shi W, Yang CH. STAT3 as an adapter to couple phosphotydylinositol 3-kinase to the IFNAR1 chain of the type I interferon receptor. *Science* 1997;276:1418-20.
- Platanias LC, Uddin S, Domanski P, Colaminici OR. Differences in interferon alpha and beta signaling. Interferon beta selectively induces the interaction of the alpha and beta subunits of the type I interferon receptor. *J Biol Chem* 1996<sup>a</sup>;271:23630-3.
- Platanias LC, Uddin S, Yetter A, Sun XJ, White WF. The type I interferon receptor mediates tyrosine phosphorylation of insulin receptor substrate 2. *J Biol Chem* 1996<sup>b</sup>;271:278-82.
- Pratt BR, Butcher RL, Inskeep EK. Antiluteolytic effect of the conceptus and of PGE<sub>2</sub> in ewes. *J Anim Sci* 1977;45:784-91.
- Roberts RM, Cross JC, Leaman DW. Interferons as hormones of pregnancy. *Endocrine Rev* 1992;13:432-52.
- Rueda BR, Naivar KA, George EM, Austin KJ, Francis H, Hansen TR. Recombinant interferon- $\tau$  regulates secretion of two bovine endometrial proteins. *J Interferon Res* 1993;13:303-9.
- Schindler C, Shuai K, Prezioso VR, Darnell JE Jr. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science* 1992;257:809-13.
- Short, RV. Implantation and the maternal recognition of pregnancy. In: Wolstenholme GEW, O'Connor M, eds, *Foetal Autonomy*. Ciba foundation symposium. London: J and A Churchill Ltd, 1969;377-86.
- Shuai K, Schindler C, Prezioso VR, Darnell JE Jr. Activation of transcription by interferon- $\gamma$ : tyrosine phosphorylation of a 91kDa DNA binding protein. *Science* 1992;259:1808-12.
- Song MM, Shuai K. The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. *J Biol Chem* 1998;273:35056-62.
- Spencer TE, Ott TL, Bazer FW. Expression of interferon regulatory factors one and two in the ovine endometrium: effects of pregnancy and interferon-tau. *Biol Reprod* 1998;58:1154-62.
- Staggs KL, Austin KJ, Johnson GA, Teixeira MG, Talbott CT, Doosley VA, Hansen TR. Complex induction of bovine uterine proteins by interferon-tau. *Biol Reprod* 1998;59:293-7.

- Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE Jr, Yancopoulos GD. Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* 1995;267:1349-53.
- Stancato LF, David M, Carter-Su C, Lerner AC, Pratt WB. Preassociation of STAT1 with STAT2 and STAT3 in separate signalling complexes prior to cytokine stimulation. *J Biol Chem* 1996;271:4134-7.
- Stancato LF, Sakatsume M, David M, Dent P, Dong F, Petricoin EFF, Krolewski JJ, Silvennoinen O, Saharinen P, Pierce J, Marshall CJ, Sturgill T, Finbloom DS, Lerner AC. Beta interferon and oncostatin M activate Raf-1 and mitogen-activated protein kinase through a JAK1-dependent pathway. *Mol Cell Biol* 1997;17:3833-40.
- Starr R, Hilton DJ. Negative regulation of the JAK/STAT pathway. *Bioessays* 1999;21:47-52.
- Stewart HJ, McCann SHE, Northrop AJ, Lamming GE, Flint APF. Sheep anti-luteolytic interferon: cDNA sequence and analysis of mRNA levels. *J Mol Endocrinol* 1989;2:65-70.
- Strehlow I, Schindler C. Amino-terminal signal transducer and activator of transcription (STAT) domains regulate nuclear translocation and STAT deactivation. *J Biol Chem* 1998;273:28049-56.
- Stojkovic M, Buttner M, Zakhartchenko V, Riedl J, Reichenbach H-D, Wenigerkind H, Brem G, Wolf E. Secretion of interferon-tau by bovine embryos in long-term culture: comparison of in vivo derived, in vitro produced, nuclear transfer and demi-embryos. *Anim Reprod Sci* 1999;55:151-62.
- Teixeira MG, Austin KJ, Perry DJ, Dooley VD, Johnson GA, Francis BR, Hansen TR. Bovine granulocyte chemotactic protein-2 is secreted by the endometrium in response to interferon-tau (IFN- $\tau$ ). *Endocrine* 1997;6:31-7.
- Thatcher WW. Ruminants. In: Knobil E, Neill J, eds. *Encyclopedia of reproduction*, vol 4. San Diego: Academic Press, 1999;302-11.
- Thatcher WW, Binelli M, Burke J, Staples CR, Ambrose JD, Coelho S. Antiluteolytic signals between the conceptus and the endometrium. *Theriogenology* 1997;47:131-40.
- Thatcher WW, Hansen PJ. Systems to alter embryo survival. In: Van Horn HH, Wilcox CJ, eds. *Large dairy herd management*. Champaign: Am Dairy Sci Assoc, 1992;164-75.
- Thatcher WW, Meyer MD, Danet-Desnoyers G. Maternal recognition of pregnancy. *J Reprod Fertil* 1995;49(suppl):15-28.
- Thatcher WW, Staples CR, Danet-Desnoyers G, Oldick B, Schmitt E J-P. Embryo health and mortality in sheep and cattle. *J Anim Sci* 1994;72(suppl 3):16-30.
- Uddin S, Fish EN, Sher DA, Gardziola C, White MF, Plataniias LS. Activation of the phosphotydylinositol 3-kinase serine kinase by IFN- $\alpha$ . *J Immunol* 1997;158:2390-7.
- Vickmeier U, Cohen SL, Moareff I, Chait BT, Kuriyan J, Darnell JE Jr. DNA binding of in vitro activated STAT 1 alpha, STAT 1 beta and truncated STAT 1: interaction between NH2-terminal domains stabilizes binding of two dimers to tandem sites. *EMBO J* 1996;15:5616-26.
- Williams BR, Haque SJ. Interacting pathways of interferon signaling. *Semin Oncol* 1997;24(suppl 9):s9-70-7.

- Xiao CW, Murphy BD, Sirois J, Goff AK. Down-regulation of oxytocin-induced cyclooxygenase-2 and prostaglandin F synthase expression by interferon- $\tau$  in bovine endometrial cells. *Biol Reprod* 1999;60:656-63.
- Yan H, Krishnan K, Greenlund AC, Gupta S, Lim JT, Schreiber RD, Schindler CW, Krolewski JJ. Phosphorylated interferon-alpha receptor 1 subunit (IFN $\alpha$ R1) acts as a docking site for the latent form of the 113 kDa STAT2 protein. *EMBO J* 1996;15:1064-74.
- Yang CH, Shi W, Basu L, Murti A, Constantinescu SN, Blatt L, Croze E, Mullersman JE, Pfeffer LM. Direct association of STAT3 with the IFN $\alpha$ R1 chain of the human type I interferon receptor. *J Biol Chem* 1996;271:8057-61.
- Zhong Z, Wen Z, Darnell JE Jr. STAT-3 and STAT-4: members of the family of signal transducers and activators of transcription. *Proc Natl Acad Sci USA* 1994;91:4806-10.

