

## Mechanism of action of interferon-tau in the uterus during early pregnancy

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Early pregnancy is maintained in ruminants through the actions of conceptus-derived interferon (IFN)-tau on the endometrium. IFN-tau alters uterine release of PGF<sub>2α</sub>, which results in rescue of the corpus luteum and continued release of progesterone. The mechanism of action of IFN-tau includes inhibition of oestradiol receptors, consequent reduction in oxytocin receptors, activation of a cyclooxygenase inhibitor, and a shift in the PGs to favour PGE<sub>2</sub> over PGF<sub>2α</sub>. IFN-tau also induces several endometrial proteins that may be critical for survival of the developing embryo. One endometrial protein induced by pregnancy and IFN-tau has been identified as bovine granulocyte chemotactic protein-2 (bGCP-2). This chemotactic cytokine (chemokine) has been used as a marker to delineate IFN-tau from IFN-alpha responses in the endometrium. A second protein, called ubiquitin cross-reactive protein (UCRP), resembles a tandem ubiquitin repeat. UCRP becomes conjugated to cytosolic endometrial proteins in response to IFN-tau and pregnancy. Proteins conjugated to UCRP are either modulated or targeted for processing through the proteasome. The action of IFN-tau is mediated by induction of signal transducer and activator of transcription 1 (STAT-1), STAT-2 and interferon regulatory factor 1 (IRF-1) transcription factors. Induction of these transcription factors, the alpha chemokines and UCRP is the prelude to maternal recognition of pregnancy in ruminants.

### Introduction

The mechanism through which interferon-tau (IFN-tau) alters release of the prostaglandins (PGs) during early pregnancy in cows and ewes will be discussed in this review. However, the primary focus will be description of induction of endometrial bovine granulocyte chemotactic protein-2 (bGCP-2) and ubiquitin crossreactive protein (bUCRP) by pregnancy and IFN-tau. Bovine UCRP is induced by IFN-alpha and IFN-tau, whereas bGCP-2 is induced only by IFN-tau. The mechanism of IFN-tau action includes induction of signal transducers and activators of transcription (STAT) and IFN regulatory factor 1 (IRF-1) in the bovine endometrium. However, IFN-tau-specific induction of GCP-2 may involve a variant receptor subunit that is associated with the janus kinase (Jak)/STAT/IRF, as well as protein kinase C signal transduction pathways. An overview of function of ubiquitin and human IFN-stimulated gene product 15 (hulSG15) is provided as a basis for understanding the function of bUCRP in conjugating to and regulating endometrial proteins during early pregnancy in cows.

### Luteolysis

The oxytocin receptor is a seven transmembrane, G-protein-associated receptor that induces inositol triphosphate turnover, cytosolic calcium and protein kinase C (reviewed in Flint *et al.*, 1995). In the

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uterus, these events result in activation of cyclooxygenase 2 (COX-2) which is the inducible form of PG synthase (Asselin *et al.*, 1997a). The resulting increase in  $\text{PGF}_{2\alpha}$  causes regression of the corpus luteum. Destruction of the corpus luteum by  $\text{PGF}_{2\alpha}$  results in a decline in circulating progesterone. This decline in progesterone primes the hypothalamus and pituitary to release gonadotrophins that induce ovulation and initiate a new oestrous cycle.

It is currently accepted that the oxytocin receptor becomes uncoupled from associated signal transduction pathways during maternal recognition of pregnancy (reviewed in Bazer *et al.*, 1997). The result is an attenuation of release of  $\text{PGF}_{2\alpha}$ , rescue of the corpus luteum, continued release of progesterone, and uterine support of the developing embryo. The hormone or cytokine implicated in uncoupling the oxytocin receptor during pregnancy in ruminants is conceptus-derived IFN-tau (reviewed in Thatcher *et al.* 1995; Bazer *et al.*, 1997).

### Interferon-tau

Interferon-tau, originally called trophoblastin or trophoblast protein-1, was identified as dominant polypeptides that were released by the conceptus on days 16–24 of pregnancy (reviewed in Roberts *et al.*, 1990). Infusion of these trophoblast proteins into uteri of non-pregnant cows caused an extension of the oestrous cycle that was mediated by the attenuation of release of  $\text{PGF}_{2\alpha}$ . Isolation of the cDNA encoding the bovine trophoblast polypeptide and inferred amino acid sequence revealed 45–70% identity with type I IFNs ( $\alpha$ ,  $\beta$ ,  $\omega$ ) (Imakawa *et al.*, 1990). The trophoblast IFNs were subclassified as IFN-tau in 1990 (Roberts *et al.*) because of differences in cDNA sequence (Imakawa *et al.*, 1990), number of genes (Hansen *et al.*, 1991), binding to receptors (Hansen *et al.*, 1989), organization of promoters on genes (Hansen *et al.*, 1991), trophoblast-specific expression (Farin *et al.*, 1990), and distribution of genes across species.

### Interferon Receptor and Signal Transduction

Interferon-tau binds to type I IFN receptors that are present in the uterine endometrium (Hansen *et al.*, 1989; Han *et al.*, 1997). The subunits of IFN-alpha receptors have been named IFNAR1 and IFNAR2, and co-expression is necessary for functional high affinity IFN binding and signal transduction (Domanski *et al.*, 1995). The extracellular region of IFNAR1 consists of two distinct 200 amino acid domains (D200).

Janus kinase-1 (JAK-1) and tyrosine kinase-2 (Tyk-2) constitutively associate with IFN receptors. Tyrosine kinase-2 associates with the cytoplasmic domains of both IFNAR1 and IFNAR2 (Colamonici *et al.*, 1994), while Jak-1 associates preferentially with IFNAR2 (Novick *et al.*, 1994). It is believed that these tyrosine kinases directly phosphorylate STAT proteins, although other kinases may be involved (Darnell, 1997). STATs undergo tyrosine phosphorylation, assemble into multimeric complexes, of which IFN-stimulated gene factor 3 (ISGF3) is an example, and translocate to the cell nucleus. ISGF3 is composed of a 48 kDa DNA-binding protein termed p48, STAT-1a (p91), STAT-1b (p84), and STAT-2 (p113) (Kessler *et al.*, 1990). After transport to the nucleus, ISGF3 specifically recognizes the IFN-stimulated response element (ISRE) DNA sequence (Levy *et al.*, 1989). Although a consensus ISRE sequence has been defined, there is considerable sequence heterogeneity. Together, differential activation and combinatorial association of these transcription factors regulate IFN-stimulated genes (ISGs).

### IFN-tau and the Oxytocin Receptor

Regulation of the oxytocin receptor is an essential event in the mechanism of action of IFN-tau. In pregnant ewes, it is likely that IFN-tau attenuates release of  $\text{PGF}_{2\alpha}$  by inhibiting formation of oxytocin receptors (Vallet *et al.*, 1990). Progesterone, oestrogen, oxytocin, and their respective

	10	20	30	40	50	60	70	75
bGCP-2	VAAVVRELR	CVCLTTTPG	IHPKTVSDLQ	VIAAGPQCSK	VEVIATLKNG	REVCLDPEAP	LIKKIVQKIL	DSGKN
8K-9	(100%)		TVSDLQ	VIAAGPQ				
8K-13	(92%)					EVCLxPEAP	LIK	
mMIP-2	AVVASELR	RCQCLKTLPG	IDLKNIQSLs	VTPPGPHCAQ	TEVIATLKGG	QKVCLDPEAP	LVQKIIQKIL	NKGKAN
8K-10	(88%)			TTPPGHsgQ	TEVIATL			
8K-12	(67%)	eCLeTLeG	IhLK					

**Fig. 1.** Amino acid sequence of peptides derived from the purified uterine 8 kDa protein and identity with the alpha chemokines: bovine granulocyte chemotactic protein 2 (bGCP-2) and murine macrophage inflammatory protein 2 (mMIP-2). The 8 kDa protein was purified and digested to yield peptides (8K-9, 8K-13, 8K-10 and 8K-12) that were partially sequenced. Peptide amino acids and identities (in parentheses) are shown underneath amino acid sequences for bGCP-2 and mMIP-2. Amino acid sequences have been reported previously for the 8K-9 and 8K-13 peptides (Teixeira *et al.*, 1997).

receptors are involved in this process. The theory is that progesterone blocks oestrogen-induced oxytocin receptor synthesis immediately before luteolysis (McCracken *et al.*, 1984). Interferon-tau continues to inhibit oestrogen receptors and oxytocin receptors during early pregnancy, resulting in attenuation of pulsatile  $\text{PGF}_{2\alpha}$  release. In sheep, IFN-tau suppresses oestrogen receptor gene expression in the endometrial epithelium (Spencer and Bazer, 1996), which in turn inhibits oxytocin receptor formation and oxytocin-induced signal transduction through inositol triphosphate turnover (Mirando *et al.*, 1990; Ott *et al.*, 1992). These effects on the oxytocin receptor, coupled with activation of a PG synthase inhibitor in cows (Thatcher *et al.*, 1995), result in attenuation of luteolytic  $\text{PGF}_{2\alpha}$  pulses. In addition to inhibiting  $\text{PGF}_{2\alpha}$  release, IFN-tau also might stimulate release of  $\text{PGE}_2$  at the expense of  $\text{PGF}_{2\alpha}$  in cows (Asselin *et al.*, 1997b). This concept is intriguing in the context of the luteotrophic effects of  $\text{PGE}_2$  and in regard to how synthesis of PGs might be regulated by IFN-tau during early pregnancy. IFN-tau might induce degradation or activation of an enzyme downstream from COX-2 (i.e.,  $\text{PGE}_2$ -9-ketoreductase) that favours synthesis of  $\text{PGE}_2$  rather than  $\text{PGF}_{2\alpha}$  in endometrial epithelial cells. It also might activate transcription factors that inhibit, or inhibit transcription factors that activate, the oestrogen receptor gene.

## IFN-tau and Uterine Proteins

### Uterine chemokines

IFN-tau induces 8 kDa uterine proteins during early pregnancy in cows (Rueda *et al.*, 1993). Amino acid sequencing of four internal peptides derived from the 8 kDa uterine proteins revealed identity with the alpha chemokine family: 92–100% identity with bovine bGCP-2 (Teixeira *et al.*, 1997), and 67–88% identity with murine (m) macrophage inflammatory protein-2 (mMIP-2; Fig. 1). Antiserum against bGCP-2 peptide was generated and used in western blot studies to describe release of bGCP-2 by the endometrium during early pregnancy and in response to conceptus-derived IFN-tau (Fig. 2). Bovine GCP-2 was not released by endometrium representing the oestrous cycle or in response to the closely related IFN-alpha unless high concentrations were used (Fig. 2b; Staggs *et al.*, 1998). This unique induction of bGCP-2 prompted the hypothesis that it could be used as an index of IFN-tau-specific signal transduction. Another interesting aspect was that phorbol ester mimicked the effects of IFN-tau in inducing the release of bGCP-2 by cultured bovine primary endometrial cells (BEND cells) (Staggs *et al.*, 1998). It was concluded from these experiments that IFN-tau signal transduction was complex in the bovine endometrium and might involve transcription factors other than or ancillary to those described for the Jak/STAT pathway. Type I IFNs compete for binding to a common cell surface receptor. The AR1 subunit of the IFN receptor undergoes ligand-dependent tyrosine phosphorylation. It is possible that AR1 undergoes a specific conformational change in response to IFN-tau to allow phosphorylation of transcription factors that are unique to IFN-tau-specific signal transduction.

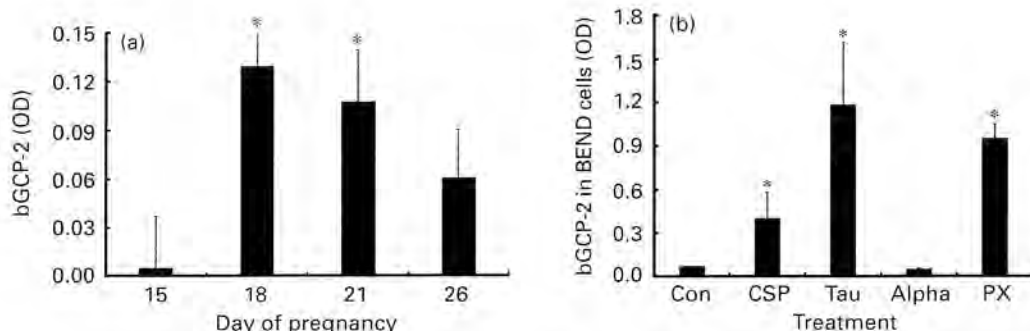


Fig. 2. Release of bovine granulocyte chemotactic protein 2 (bGCP-2) in response to pregnancy (a) and conceptus secretory protein (CSP; 200  $\mu\text{g ml}^{-1}$ ), IFN-tau (Tau; 25  $\text{nmol l}^{-1}$ ), IFN-alpha (Alpha; 25  $\text{nmol l}^{-1}$ ) and phorbol ester (PX; 100  $\text{ng ml}^{-1}$ ), Con: control (b). In (a), explants were collected from pregnant cows on days 15, 18, 21, or 26 of pregnancy ( $n = 3$  cows per day) and cultured for 24 h as described by Austin *et al.* (1996a). In (b), bovine endometrial (BEND) cells ( $n = 3$  replicates) were cultured for 24 h with respective treatments as described by Staggs *et al.* (1998). Bovine GCP-2 was detected using one-dimensional PAGE and western blot analysis (anti-boGCP-2 peptide antiserum; Teixeira *et al.*, 1997). Western blots were scanned using densitometry. \*Optical density (OD) means were significantly different ( $P < 0.05$ ) when compared with day 15 of pregnancy (a) or controls (b). Data in (b) were redrawn from Staggs *et al.* (1998) with permission from Biology of Reproduction.

Several uterine proteins are induced by IFN-tau, but only a few of these proteins have been identified. For example, the interferon-induced protein Mx (Charleston and Stewart, 1993), 2', 5' oligoadenylate synthase (Short *et al.*, 1991), and  $\beta_2$ -microglobulin (Vallet *et al.*, 1991) are induced by IFN-tau. However, the specific function of these proteins during early pregnancy remains elusive. The chemokines, although specific markers for IFN-tau action, also belong to this growing category of identified proteins with no known specific function during early pregnancy.

Pregnancy is dependent upon a receptive uterus that allows attachment and invasion of the conceptus while preventing immunological rejection. Chemokines are potent chemo-attractants for cells of the immune system and have been implicated in cell adhesion, inflammatory, and angiogenic processes (reviewed in Oppenheim *et al.*, 1991). The chemokines may attract the conceptus or cells of the immune system to implantation sites. They also may direct an immunostimulatory phenotype that, coupled with scavenger effects (macrophage, neutrophils), controls inflammation and adhesion associated with limited invasion of the maternal caruncles by the conceptus. Available recombinant bovine uterine chemokines would facilitate experiments designed to examine these hypotheses.

#### Ubiquitin crossreactive protein

A 17 kDa uterine protein was identified through examining proteins released into medium of cultured endometrial explants from day 18 pregnant cows (Naivar *et al.* 1995). This protein was released by endometrial explants in response to both recombinant (r)bIFN-tau and rIFN-alpha. Because the 17 kDa protein was similar in size to huSG15 (also called huUCRP), and immunoreacted with antiserum against ubiquitin, it was called bUCRP (Austin *et al.*, 1996a). Antiserum against ubiquitin was used to demonstrate that bUCRP was released by the endometrium at times coincident with IFN-tau release from the conceptus. Bovine UCRP was first detected in the medium of cultured endometrial explants representing day 15 of pregnancy. Release of bUCRP increased on day 18 and remained high through day 26 of pregnancy. Recombinant bIFN-tau induced release of bUCRP by endometrial explants representing day 12 of the oestrous cycle in a dose-dependent manner. Likewise, bUCRP was found in significant amounts in uterine flushings from day 18 pregnant cows.

IFN induces release of ISG15 by mouse Ehrlich ascites tumour cells (Farrell *et al.*, 1979). In addition, ISG15 is released by several cell lines in response to IFN and during the acquisition of an antiviral state. Human ISG15 is released in response to type I IFNs and it induces release of IFN-gamma by T- and B-lymphocytes (Recht *et al.*, 1991). Through inducing release of IFN-gamma from T-cells, ISG15 may augment natural killer cell proliferation and activate monocytes and macrophages.

Endometrial UCRP may be the bovine counterpart to huISG15. Bovine UCRP is released by the endometrium in response to IFN-tau during early pregnancy. Whether bUCRP induces IFN-gamma remains to be determined. The activation of natural killer cells by bUCRP in the endometrium is intriguing, as these effects might be considered detrimental to the developing conceptus (Robertson *et al.*, 1994). However, an extensive description of cell-mediated and humoral cytokines, and consequences of action in the bovine endometrium during early pregnancy is lacking. Bovine UCRP has limited amino acid sequence identity with ubiquitin and huISG15, but retains the C-terminal amino acids, Arg-Gly-Gly (RGG) that have been implicated in the first step in covalent conjugation to cytosolic proteins (Austin *et al.*, 1996b). Because bUCRP retains functional amino acids of ubiquitin and is released from endometrial explants in response to IFN-tau during early pregnancy, it probably has cytosolic and extracellular roles that are consistent with the maintenance of pregnancy.

*Ubiquitination.* Proteolysis is an important intracellular regulatory mechanism for controlling many biological processes. In eukaryotes, ubiquitin-dependent proteolysis is essential for protein turnover. Ubiquitin is a highly conserved 76 amino acid polypeptide that is involved in a wide array of biological systems including cell cycle regulation, DNA repair, receptor modification, signal transduction, antigen presentation, the stress response, and both proteasomal and non-proteasomal protein degradation (Finley and Chau, 1991). Conjugation of ubiquitin to proteins can result in either polyubiquitination or mono-ubiquitination. Polyubiquitination is believed to target proteins for rapid degradation (Finley and Chau, 1991): for example, it may be involved in the process of luteal regression (Murdoch *et al.*, 1996). Carboxyl terminal amino acids on ubiquitin, via the RGG sequence, link to primary amines on targeted proteins (Hershko and Ciechanover, 1992). Packed multi-ubiquitin chains are then added through isopeptide bonds between carboxyl terminals and Lys-48 residues on successive ubiquitin monomers. This quaternary structure is unique and aids in directing the complex to the proteasome where the targeted protein is degraded and ubiquitin monomers are released and recycled (Finley and Chau, 1991).

Mono-ubiquitinated proteins, such as actin, histones, microtubules and the growth hormone receptor are longer-lived and probably undergo ubiquitin protein modification as well as modest rates of targeted degradation (reviewed in Finley and Chau, 1991). A case in point is the transcription factor nuclear factor kappa B (NF- $\kappa$ B), for which the proteasome proteolytic pathway is required for proper activation (Palombella *et al.*, 1994). This transcription factor regulates a variety of genes involved in immune system function such as those encoding immunoglobulin kappa light chains, interleukin 2 (IL-2) receptor alpha chain, class I major histocompatibility complex (MHC), and a number of cytokines that include IL-2, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IFN-beta. NF- $\kappa$ B also has been implicated in the expression of cell adhesion genes that encode selectin, intercellular adhesion molecule 1 and viral cellular adhesion molecule 1 (Palombella *et al.*, 1994).

*Unique primary structure of bUCRP.* Human ISG15 has 29–31% amino acid sequence identity with two tandem ubiquitin monomers. Inferred amino acid sequence of the bUCRP cDNA and gene have 68% identity with huISG15 and 31% identity with a tandem ubiquitin repeat (Austin *et al.*, 1996b). Only one major bUCRP gene exists (Perry *et al.*, 1997). Thus, it appears likely that bUCRP is the counterpart to huISG15. However, there are significant differences in primary structure between huISG15 and bUCRP. The huISG15 C-terminal amino acids are removed via post-translational processing to yield a 15 kDa protein that ends in RGG (Feltham *et al.*, 1989). The bUCRP cDNA

encodes a 17 kDa protein in which translation stops immediately after RGG amino acids (Austin *et al.*, 1996b). The coding region of the bUCRP gene is identical to that reported for the cDNA. Functional residues of ubiquitin that are retained in bUCRP include the C-terminal RGG residues and Arg-72 of ubiquitin that have been retained as Arg-150 in bUCRP. This residue is involved with binding to ubiquitin conjugating enzyme (E1), and is required for ordered addition of substrate in a complex ligation pathway (Burch and Haas, 1994). Other ubiquitin residues implicated in binding to targeted proteins (i.e., Arg54), degradation through the 26 S proteasome (i.e. His68) (Ecker *et al.*, 1987), and polymerization of monomers (Lys48) (Finley and Chau, 1991) are absent in bUCRP.

*Unique cytosolic role of bUCRP.* It was hypothesized that bUCRP has a cytosolic role in the modification of uterine proteins during early pregnancy. In a manner similar to ubiquitin, bUCRP may become conjugated to cytosolic endometrial proteins. Because bUCRP lacked residues known to form ubiquitin polymers and to target proteins for degradation, it was suspected that the array of proteins conjugated to bUCRP, and the destiny of these proteins might be different from those conjugated to ubiquitin.

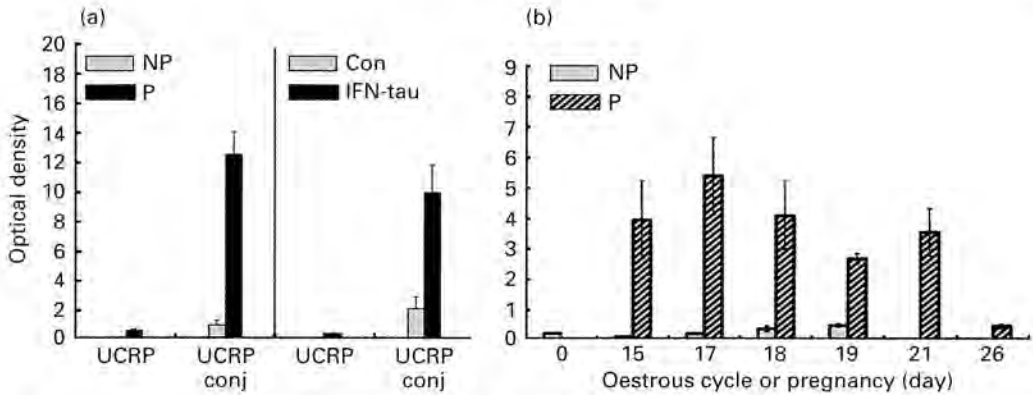
Antiserum was developed against a bUCRP peptide that immunoreacted with bUCRP, but did not immunoreact with ubiquitin on western blots. This antibody also detected bUCRP when conjugated to an array of endometrial proteins (Johnson *et al.*, 1998). These complexes with bUCRP were called conjugates and represented proteins coupled to a single bUCRP molecule (monomer), or to polymers of bUCRP. Co-incubation of antibody against bUCRP with antigenic bUCRP peptide blocked detection of bUCRP and its conjugates. Pre-absorption of bUCRP antibody with ubiquitin had no effect on detection of bUCRP or bUCRP conjugates. Thus, detection of bUCRP and its conjugates by the anti-bUCRP antibody was immunospecific.

Ubiquitin and its conjugates are abundant in the bovine endometrium and are not regulated by IFN-tau (Johnson *et al.*, 1998). Because bUCRP and its conjugates were induced by pregnancy and IFN-tau (Fig. 3a), it was concluded that proteins conjugated to bUCRP were distinct from those conjugated to ubiquitin. The induction of bUCRP and its conjugates in endometrial explants required exposure to rbIFN-tau for 12 h. This appearance of bUCRP and its conjugates was consistent with the time required for transcription, translation, and conjugation of bUCRP to cytosolic proteins.

Phosphorylated STATs become ubiquitinated and degraded within minutes after treatment with IFN-gamma (Kim and Maniatis, 1996). This process is so fast that use of proteasome inhibitors was required to observe an accumulation of ubiquitinated STAT-1. Interestingly, proteins conjugated to bUCRP continued to accumulate through 48 h after treatment with rbIFN-tau (Johnson *et al.*, 1998). This accumulation of proteins that were conjugated to bUCRP might reflect increased protein modulation or stabilization as opposed to degradation. It was inferred from these experiments that regulation or degradation of endometrial proteins by bUCRP was a component of establishing early pregnancy in ruminants. For example, bUCRP might ligate to and modify, or initiate proteasomal degradation of cytosolic uterine proteins that are involved with the release of PGF<sub>2 $\alpha$</sub> .

IFN-tau may induce conjugation of bUCRP to the oxytocin receptor so that it becomes unable to bind to oxytocin during early pregnancy in cows (Fig. 4). Alternatively, bUCRP may conjugate to the oestrogen receptor, or to transcription factors that induce transcription of the oestrogen receptor gene and target these proteins to degradation. Likewise, enzymes involved with the synthesis of PGs may be modified by bUCRP such that PGF<sub>2 $\alpha$</sub>  is attenuated and PGE<sub>2</sub> is stimulated. In addition, IFN-tau may dictate the selective proteolysis or modulation of uterine cytokines by bUCRP to accommodate the changing immune demands of pregnancy. The identification of proteins that are conjugated to bUCRP during early pregnancy and in response to IFN-tau would address these hypothesized roles of bUCRP.

*Induction of bUCRP mRNA in the endometrium by IFN-tau.* Another major difference between huISG15 and bUCRP is the apparent widespread expression of the huISG15 gene in several cell lines and tissues (Lowe *et al.*, 1995). Northern blot analysis revealed that expression of the bUCRP gene was



**Fig. 3.** Induction of bovine ubiquitin crossreactive protein (bUCRP) in endometrial explants. (a) Induction of bUCRP and its conjugates by pregnancy and IFN- $\tau$ . Explants from day 18 pregnant (P;  $n = 4$ ) or non-pregnant (NP,  $n = 4$ ) cows were cultured for 24 h. Cytosolic proteins were separated using one-dimensional PAGE and analysed for the presence of bUCRP and its conjugates using western blotting as described by Johnson *et al.* (1998). Endometrial explants from day 14 non-pregnant cows ( $n = 3$ ) were cultured in the absence (Con) or presence ( $25 \text{ nmol l}^{-1}$ ) of rbIFN- $\tau$  for 24 h. Bovine UCRP and its conjugates were detected as described in Johnson *et al.* (1998). Data in (a) are adapted from Johnson *et al.* (1998) with permission from Biology of Reproduction. (b) Endometrium was collected from non-pregnant and pregnant cows on the days specified ( $n = 4$  cows on each day). RNA was isolated, northern blotted, hybridized with radiolabelled bUCRP cDNA and quantitated as described in Hansen *et al.* (1997); (b) is reprinted from Hansen *et al.* (1997) with permission from The Endocrine Society. \*Optical density means were significantly different ( $P < 0.05$ ) from NP or Con.

restricted to endometria from pregnant cows or from endometria from non-pregnant cows that had been treated *in vitro* with rbIFN- $\tau$  (Hansen *et al.*, 1997).

Bovine UCRP mRNA was not detected using northern blot analysis in endometrium from non-pregnant cows (days 15, 17, 18 or 19) or in spleen, kidney, liver, corpus luteum or muscle (Hansen *et al.*, 1997). The bUCRP cDNA did not hybridize to ubiquitin mRNAs. Both major ubiquitin transcripts UbB (1.2 kb) and UbC (2.5 kb) were present in all tissues examined and were not regulated by IFN- $\tau$ . Bovine UCRP mRNA was detected in endometria from pregnant cows by day 15, reached highest values by day 17, remained high on days 18, 19 and 21, and then declined to amounts that were not detectable on day 26 (Fig. 3b). This pattern of transcription closely paralleled detection of UCRP protein in cultured endometrial explants from cows on the same days of pregnancy (Austin *et al.*, 1996a). The bUCRP mRNA transcript ( $\sim 700$  bp) can be induced in bovine endometrial cells (i.e. BEND cells) derived from non-pregnant cows when cultured with  $25 \text{ nmol rbIFN-}\tau \text{ l}^{-1}$  (Hansen *et al.*, 1997). Although the role of huISG15 may be general to many cell types, transcription of the bUCRP gene appears to be a specific uterine response to conceptus-derived IFN- $\tau$  during early pregnancy in cows.

*The bovine UCRP gene.* The huISG15 promoter contains a functional tandem ISRE at position  $-39$  relative to the mRNA cap site (Reich *et al.*, 1987), but little is known about sequences that are upstream of this tandem ISRE. The TATA box (TATATAA) in the bUCRP gene is located at position  $-31$  from the mRNA cap site (Perry *et al.*, 1997). Both genes contain a single intron after the initiation codon, followed by coding, termination, 3' non-coding, and polyadenylation sequences. The bUCRP gene promoter is similar to the huISG15 gene promoter in the placement of a conserved tandem ISRE at position  $-90$ . However, three additional putative ISREs are present in the bUCRP gene at positions  $-123$ ,  $-332$  and  $-525$ .

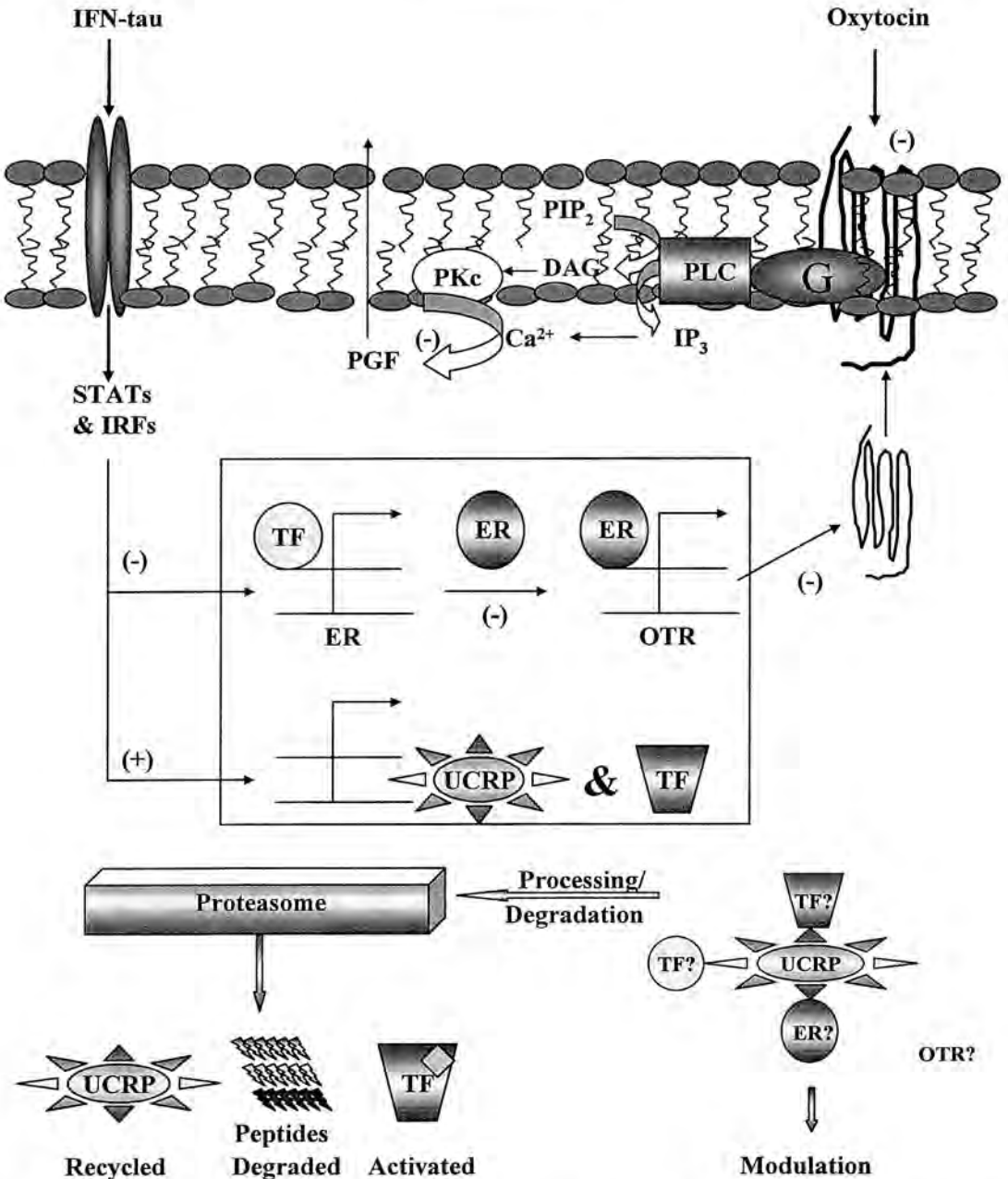


Fig. 4. Hypothetical mechanism of IFN-tau action in bovine uterine endometrium. IFN-tau binds to an endometrial receptor and induces phosphorylation of signal transducers and activators of transcription (STATs). STATs induce transcription and subsequent phosphorylation of interferon regulatory proteins (IRFs). The STATs and IRFs may have direct actions through inhibiting the oestradial receptor (ER), and potentially, the oxytocin receptor (OTR) genes. Disruption of oxytocin receptor gene transcription results in reduced binding to oxytocin. Consequently, G proteins (G), phospholipase C (PLC), second messengers associated with activation of protein kinase C (PKc), and increased calcium become uncoupled resulting in diminished release of PGF<sub>2α</sub>. In addition to inhibiting transcription of the oestradial receptor gene, IFN-tau, through inducing the STATs and IRF-1 also induces transcription of bovine ubiquitin crossreactive protein (bUCRP) and potentially transcription factor (TF)



Interferon receptors exhibit preference for ligand and sort cytosolic responses through tyrosine kinase phosphorylation of at least six STAT transcription factors. Nuclear proteins induced by rbIFN-tau in BEND cells interact with bUCRP ISRE DNA in mobility shift assay (Perry *et al.*, 1998). At least four slower migrating bands were induced by rbIFN-tau. IFN-tau induced appearance of STAT-1, STAT-2 and IRF-1 in nuclear proteins extracted from BEND cells (Perry *et al.*, 1998; Binelli *et al.*, 1998). The temporal relationship between appearance of the STATs (0.5 h) and IRF-1 (2 h) in BEND cells was consistent with induction of these nuclear proteins in other cells by other type I IFNs. The STATs are activated within minutes of IFN treatment. Induction of phosphorylation of cytoplasmic STATs initiates translocation to the nucleus, formation of transcriptional complexes and induction of secondary transcription factors called IRFs. The bUCRP gene may be induced by STATs and IRF-1. The intriguing and more complex issue raised by these experiments is how the STATs and IRF-1 regulate other genes in the endometrium during early pregnancy. Genes involved with the conjugation of bUCRP to targeted cytosolic proteins may be induced by these transcription factors. Likewise, genes encoding enzymes that shunt arachidonic acid to PGF<sub>2 $\alpha$</sub>  may be inhibited by these transcription factors.

### Conclusions

The study of uterine responses to the conceptus during early pregnancy resulted in identification of several uterine proteins that may prepare the uterus for the implanting embryo. One critical event that must be circumvented or altered is uterine release of PGF<sub>2 $\alpha$</sub>  (Fig. 4). Inhibition of transcription of the oestrogen receptor gene is one critical action of IFN-tau in the modulation of PGF<sub>2 $\alpha$</sub>  release (Spencer and Bazer, 1996). Exactly how IFN-tau regulates the oestrogen receptor gene remains to be determined. It may involve direct interaction of the STATs or IRFs (Bazer *et al.*, 1997; Spencer *et al.*, 1998) with negative acting response elements. Alternatively, it may involve removal of positive *trans*-acting transcription factors through conjugation to bUCRP and degradation through the proteasome. Likewise, enzymes involved in the synthesis of PGF<sub>2 $\alpha$</sub>  could be degraded through conjugation to bUCRP, whereas enzymes involved in the synthesis of PGE<sub>2</sub> could be activated or stabilized.

Alpha chemokines have only recently been identified in the uterine endometrium (Teixeira *et al.*, 1997). The function of these chemotactic cytokines in the uterus is unknown, but may involve preparing the endometrium for implantation or changing the immune environment to an environment that nurtures survival of the embryo. Several years ago it was suggested that the IFN-tau receptor was complex based on curvilinear Scatchard plot and cross-linking experiments (Hansen *et al.* 1989). Recent experiments, using cultured endometrial explants and BEND cells, revealed that the uterine endometrium can distinguish between type I IFNs (Staggs *et al.*, 1998). Phorbol ester and IFN-tau, but not the closely related IFN-alpha, induced release of bGCP-2. However, the IFN receptor cloned from ovine endometrium appears to be identical in nucleotide sequence to the IFN-alpha receptor (Han *et al.*, 1997). Because this IFN receptor was cloned using IFN-alpha receptor sequences, it may not be surprising that the receptor identified in ovine and bovine endometrium was identical. It remains unresolved whether there is a unique IFN-tau receptor subunit in the endometrium. Likewise it remains unknown if the IFNAR might change conformation after binding IFN-tau to expose a cytosolic domain that activates IFN-tau-specific STATs or IRFs. IFN-tau signal transduction appears to involve the STATs, and IRF-1. Future experiments using the bUCRP gene promoter and potentially the bGCP-2 gene promoter may delineate exactly which transcription factors are induced in response to IFN-tau. Certainly

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genes. Bovine UCRP becomes conjugated to cytosolic proteins and either modulates these proteins or targets them to the proteasome where they are either degraded into peptides or activated. An example of activation is provided by the transcription factor, NF-kappa B that must be processed through the proteasome prior to regulating transcription of genes (Palombella *et al.*, 1994). DAG: diacyl glycerol; PIP<sub>2</sub>: phosphoinositol-4,5 diphosphate.

development of the BEND cell line will facilitate these experiments as well as those planned to identify endometrial proteins that become conjugated to bUCRP in response to pregnancy and IFN-tau.

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