Reproduction (2001) **121**, 41–49

Review

Trophoblast interferon and pregnancy

Kristina J. Demmers[1], Kamila Derecka[1,2] and Anthony Flint[1*]

[1]*School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK; and* [2]*Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, 10-747 Olsztyn, Poland*

The maternal recognition of pregnancy in ruminants requires the production of interferons by the preimplantation blastocyst. These proteins, the trophoblast interferons (IFN-τ), are the products of a number of similar genes, the expression of which is controlled by characteristic promoter regions. They are expressed for a short period in high concentrations, and have antiluteolytic, antiviral, antiproliferative and immunomodulatory effects, through receptors on the endometrial epithelium. The antiluteolytic effects of IFN-τ result from inhibition of endometrial expression of the oxytocin receptor, through which circulating oxytocin stimulates episodic prostaglandin $F_{2\alpha}$ production. Some of the properties of IFN-τ differ from those of other type I interferons, and they may have novel therapeutic effects. Because of their central role in early gestation, these proteins have excited the interest of reproductive physiologists. However, their other properties, and the fact that their expression is controlled so precisely, have made them of interest to a wide range of biologists.

The trophoblast interferons (IFN-τ) were discovered in 1987 through the purification of extracts of ovine blastocyst-secreted proteins with antiluteolytic properties. The active principles were monitored by biological assays involving administration into the uterus of cyclic sheep. After purification to homogeneity, the antiluteolytic proteins were identified by N-terminal sequencing (Stewart *et al.*, 1987) and cDNA cloning (Imakawa *et al.*, 1987). At the time of the identification of IFN-τ, there was already a large body of information on the role of blastocyst-secreted proteins in the maternal recognition of pregnancy, and on the physiological processes underlying luteolysis and the establishment of pregnancy. There was also an extensive literature on related interferons, and several type I interferons were immediately available in quantities sufficient for experiments *in vivo*. As a result, the identification of the antiluteolysin led to rapid progress, particularly in relation to the control of its synthesis, its mechanism of action and its immunomodulatory and possible therapeutic properties. These advances and the background to them have been reviewed elsewhere (Bazer *et al.*, 1996; Martal *et al.*, 1998; Roberts *et al.*, 1999). Research in this area has also benefited from the subsequent production of large quantities of recombinant IFN-τ. Different isoforms have been expressed in yeast and in baculovirus systems, and a number of mutant sequences have been expressed and compared functionally and structurally with other recombinant type I interferons (Alexenko *et al.*, 1997; Radhakrishnan *et al.*, 1999). There have also been advances in assay methodology, and an ELISA is now available in addition to the cytopathic effect inhibition assay, a cell culture assay dependent on IFN-induced protection against viral infection.

The IFN-τ isoforms, which are expressed only in ruminant trophoblasts, represent one of five families of related type I interferons, named IFN-α, -β, -δ, -ω and -τ. Within the type I interferons, there have been many gene duplications, and nucleotide substitution analysis has allowed a description of their evolution (Roberts *et al.*, 1998). The IFN-α and -β genes diverged through duplication of a common progenitor gene about 250 million years ago, after the first appearance of birds. IFN-δ (so far identified only in pigs) arose from IFN-α about 180 million years ago and, thereafter, IFN-ω diverged from IFN-α approximately 130 million years ago. IFN-τ arose from IFN-ω in the Ruminantia about 36 million years ago. Most of the IFN gene families have undergone extensive duplications, so that there are now multiple isoforms of IFN-α, -ω and -τ. Because they evolved so recently, IFN-τ genes would be expected to be found only in ruminants arising since that time, that is, in the cervids (which first appeared about 30 million years ago) giraffes (15 million years ago) and bovids (10–15 million years ago). All these genes retain the common characteristic of having no introns (although it is not known why this should be) and anti-viral and anti-proliferative activity. IFN-τ and -ω both contain 172 amino acids (other type I interferons usually contain 166 amino acids).

*Correspondence
Email: anthony.flint@nottingham.ac.uk

1470-1626/2001

Maternal recognition of pregnancy

The antiluteolytic effects of the IFN-τ are responsible for the maternal recognition of pregnancy, which is the term used to describe how a mother responds (physiologically) to the presence of a conceptus in her reproductive tract. In domestic ruminants, the developing embryo does not implant until relatively late in development, although the conceptus is clearly capable of communicating with the mother well before implantation occurs, and before the conceptus has access to the maternal circulation. Failure of the conceptus to signal its presence at the appropriate time leads to pregnancy loss.

The pioneering experiments of Moor and Rowson (1964, 1966a,b) revealed that the maternal recognition of pregnancy in ewes takes place at about day 12–13 of pregnancy. Transfer of blastocysts to the non-pregnant uterus before days 11–12 in sheep prolonged luteal function, whereas removal of blastocysts from the uterus of pregnant animals before this time did not extend the life of the corpus luteum. Furthermore, infusing homogenized day 14 or 15 conceptuses into the uterine lumen extended luteal function in non-pregnant sheep, although this antiluteolytic effect was absent when day 25 embryonic homogenates were infused. It was evident that a substance released by preimplantation conceptuses prevents luteolysis. Isolated trophoblastic vesicles transferred into the uterus extended the life of the corpus luteum in both sheep and cattle, so the antiluteolysin (termed 'trophoblastine' at this time) was known to be a product of the embryonic trophoblast (Martal *et al.*, 1979).

Discovery of trophoblast interferon

Godkin *et al.* (1982) purified a protein secreted by the sheep conceptus that seemed to bear the hallmarks of the antiluteolysin. This protein consisted of several isoforms with a molecular weight of approximately 18 000, and was the major secretory product of the conceptus trophoblast tissue between day 13 and day 15 of pregnancy, the time of maternal recognition of pregnancy. This protein, initially called 'ovine trophoblast protein 1' (oTP-1), is now known as ovine interferon–tau (oIFN-τ).

The trophoblast protein was produced maximally for only a few days of pregnancy, at a time when the trophoblast was attached only loosely to the uterine wall. Production of oIFN-τ increased over three orders of magnitude from day 12 to day 16, when synthesis was maximal. When injected into the uterine lumen of cyclic ewes, purified oIFN-τ mimicked the effect of conceptus homogenates by delaying luteal regression, and immunoneutralized extracts did not, indicating that IFN-τ alone is sufficient for maternal recognition of pregnancy.

The trophoblast signal was not detectable in the peripheral circulation of pregnant sheep, indicating that it does not leave the uterus to act on the corpus luteum. Instead, oIFN-τ acts directly on the uterine endometrium, where it alters protein and $PGF_{2\alpha}$ production. Endometrial cells express type I interferon receptor subunits IFNAR1 and IFNAR2 (Kaluz *et al.*, 1996) and, although the ruminant receptor subunits differ from those in man (with 67 and 58% sequence identity, respectively; Han *et al.*, 1997), they have the same structural features.

A protein with similar properties, produced between day 16 and day 24 of gestation, was characterized subsequently as a secretory product of bovine conceptuses. This protein, bIFN-τ, was shown to be antiluteolytic and to alter endometrial $PGF_{2\alpha}$ output. bIFN-τ has molecular masses of 22 000 and 24 000 kDa, each with multiple isoforms, and is glycosylated with *N*-linked oligosaccharides. In contrast, oIFN-τ is not glycosylated. A caprine IFN-τ has been identified, which crossreacts with antiserum to oIFN-τ and is secreted between day 16 and day 21 of pregnancy. The proteins of this complex consist of a mixture of both glycosylated and non-glycosylated polypeptides.

Isoforms and structures

The IFN-τ genes expressed by ruminant conceptuses share approximately 70% homology with IFN-ω (also known as IFN-α_{II}). A 595 bp open reading frame encodes a 195 amino acid pre-protein containing a 23 amino acid signal sequence which is cleaved to yield the mature protein. IFN-τ shows remarkable homology of cDNA nucleotide sequence across ruminant species. Bovine, ovine and caprine IFN-τ are more similar in sequence to each other than bIFN-τ is to bovine IFN-ω. In the coding region, the nucleotide sequences exhibit approximately 90% identity, and their inferred amino acid sequences about 80% identity.

Eighteen IFN-τ cDNA or genomic sequences have been described in sheep and 11 in cattle, although not all are transcribed. Parsimony analysis indicates that the ovine isoforms may be placed into five related groups. Within each species, IFN-τ sequences are similar, as a result of the gene duplication events by which they arise having occurred relatively recently, and it is difficult to distinguish differences among individual genes from allelic variation. In sheep, these genes appear to display distinct patterns of expression in the trophectoderm and are subject to different developmental regulation during pregnancy. Why there should be multiple isoforms of IFN-τ is unclear since individual ovine IFN-τ isoforms extend corpus luteum function when injected into the lumen of cyclic ewes. Ealy *et al.* (1998) have demonstrated that isoforms of ovine IFN-τ vary in their biological potency, including their ability to extend corpus luteum lifespan in non-pregnant ewes.

The additional six carboxy terminal amino acids in IFN-τ (relative to IFN-α or -β) do not affect the antiviral or antiluteolytic properties of the molecule, as shown by comparing the wild-type and mutated molecules (Ealy *et al.*, 1998). However, other mutations at the carboxy terminus of the molecule do alter both antiviral and antiluteolytic properties. These experiments show that the antiluteolytic

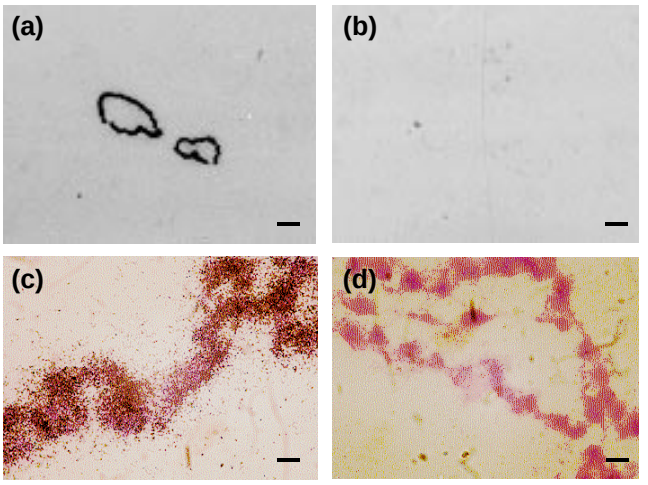

Fig. 1. The localization of interferon τ (IFN-τ) mRNA in day 16 bovine embryonic trophoblast. Sections of trophoblast hybridized with antisense oligonucleotide (a,c) and corresponding sense sections (b,d). Counterstaining with haematoxylin and eosin is used in (c) and (d). IFN-τ mRNA expression in the trophectoderm is shown in (a) and (c). Scale bars represent 940 μm (a,b); 36 μm (c,d) (Picture courtesy of R. Robinson and D. C. Wathes, Royal Veterinary College.)

activity of IFN-τ is related to its antiviral and antiproliferative properties and not necessarily to its receptor affinity (Niswender *et al.*, 1997).

Mechanisms controlling IFN-τ synthesis

The transient nature of IFN-τ secretion

Stewart *et al.* (1989) showed by northern blotting of blastocyst mRNA, and Farin *et al.* (1990) demonstrated by *in situ* hybridization, that oIFN-τ is expressed transiently during a limited period of development, and that its expression is localized to the extra-embryonic trophectoderm of bovid conceptuses (Fig. 1). The mRNA is detectable in low concentrations in embryos from day 10 to day 12 of pregnancy, increases from day 13 to day 15, coincident with the time of maternal recognition of pregnancy, and decreases shortly afterwards. Peak production occurs at day 16 in sheep (Godkin *et al.*, 1982) and day 17 in cattle (Bartol *et al.*, 1985), and IFN-τ mRNA is detectable in the trophectoderm until about day 20 in sheep or day 25 in cattle. At the time of peak production, IFN-τ mRNA is present in blastocysts at a higher concentration than any other mRNA (Fig. 2). Ashworth and Bazer (1989) demonstrated by radioimmunoassay that IFN-τ is detectable in the media of cultured sheep blastocysts shortly after hatching from the zona pellucida, which is at least 1 week before peak production at the time of conceptus elongation, but the concentrations involved are unlikely to elicit a physiological response at this stage. The marked increase in the expression of the IFN-τ gene on day 13 in

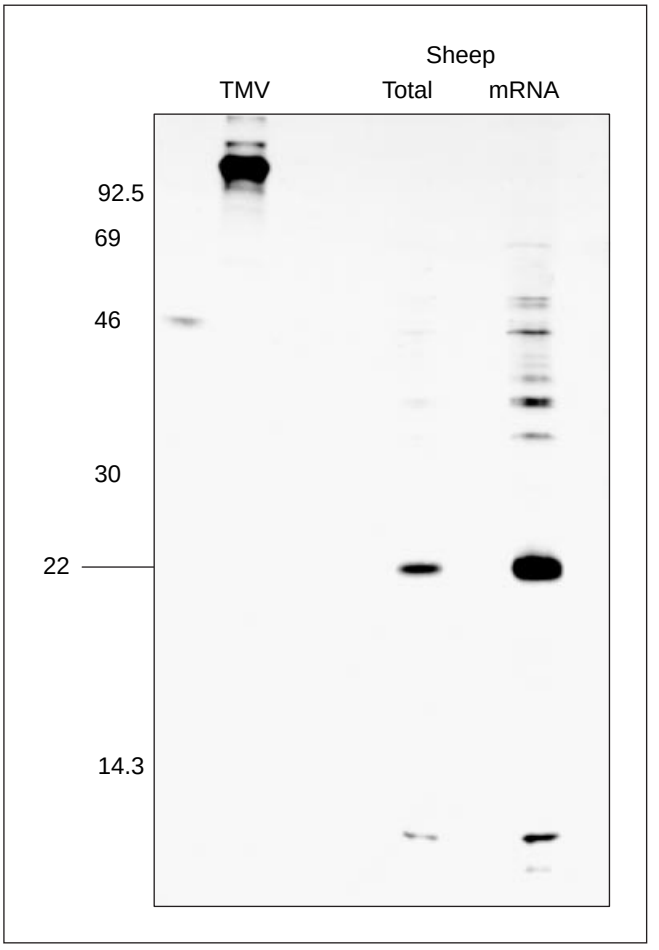

Fig. 2. Sheep blastocyst RNA translated *in vitro* using rabbit reticulocyte lysate in the presence of [^{35}S]methionine, indicating that interferon τ (IFN-τ) (22 kDa) is the major mRNA species present at the blastocyst stage. Total RNA was extracted from a blastocyst on day 16 after oestrus and either translated unchanged or after purification of poly A$^+$ RNA to give mRNA. The quality control standard was from tobacco mosaic virus (TMV) to show that the lysate translated large transcripts. M_r markers are on the left. Note the M_r of the major product, IFN-τ at 22 kDa, reflects the lack of post-translational processing to remove the signal peptide (C terminal amino acids 1–23) which leads to an M_r of 18 kDa in the secreted protein. (Figure from Stewart *et al.*, 1989.)

sheep and day 15 in cattle coincides with the morphological transition of the blastocyst from a spherical to a filamentous form, rather than strictly correlating with the day of pregnancy.

The onset of IFN-τ expression appears to be genetically programmed independently of the maternal uterine environment, since IFN-τ is expressed *in vitro* after *in vitro* fertilization and maturation. However, conceptus IFN-τ production is clearly affected by the uterine environment, as IFN-τ production by ovine conceptus tissue *in vitro* is higher when cultured in the presence of endometrial tissue. Furthermore, the maternal plasma progesterone

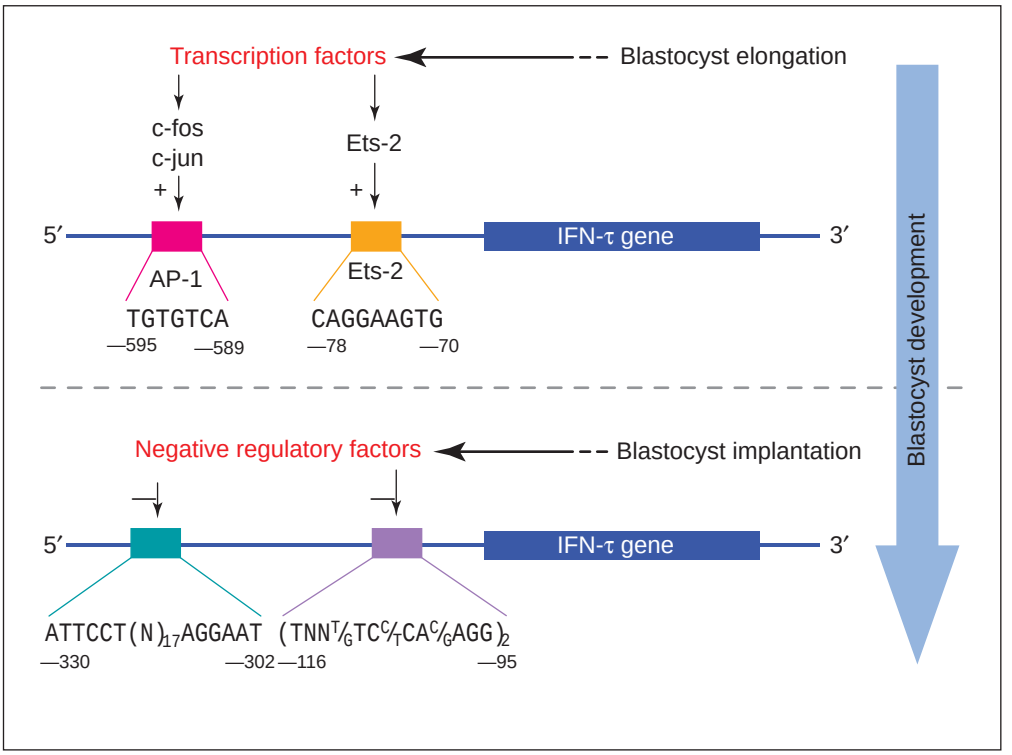

Fig. 3. Control of interferon τ (IFN-τ) secretion by the developing conceptus. Expression of several IFN-τ genes, which code for different isoforms, is induced and repressed through the actions of transcription factors. Both activatory and negative regulatory sequences have been identified at different points on the DNA sequence upstream of the genes. The activatory sequences respond to the transcription factors Ets-2 and c-fos–c-jun. Since the onset and cessation of IFN-τ gene expression coincide with blastocyst elongation and implantation, respectively, it is possible that the factors controlling IFN-τ expression are involved in changes in trophectoderm metabolism associated with these processes.

concentration, which controls uterine glandular secretion, is correlated with IFN-τ production by the conceptus in cattle (Kerbler *et al.*, 1997; Mann *et al.*, 1999). Termination of IFN-τ expression is dependent on implantation, since cessation of oIFN-τ expression occurs in the regions of the trophoblast that have established cellular contacts with the uterine epithelium during the implantation process.

Control of IFN-τ gene expression

The rapid onset and cessation of IFN-τ gene expression in the trophectoderm of the elongating blastocyst makes this family of genes of particular interest in the study of transcriptional regulation (Fig. 3). Furthermore, unlike other groups in the type I interferon family, the IFN-τs are not induced by viruses (Guesdon *et al.*, 1996). The enhancer and promoter sequences upstream of the IFN-α and -β genes are well characterized and contain sequences known to confer viral induction. In contrast, the upstream sequences 5′ to the IFN-τ genes, which are conserved among members of the group, are quite distinct, and do not contain viral induction sequences. In searching for the sequences responsible for controlling IFN-τ expression, Ezashi *et al.* (1998) emphasized the role of the Ets family of transcription factors. They used yeast one-hybrid analysis of a day 13 conceptus cDNA library and electromobility shift assay, and suggested that Ets-2 activates gene transcription through a specific enhancer sequence, CAGGAAGTG, located between –78 and –70 bp upstream of the transcription start site. On co-transfection of an IFN-τ promoter (–126 to +50 bp) luciferase reporter construct and an Ets-2 expression plasmid into human choriocarcinoma (JAR) cells, luciferase expression was increased up to 30-fold by concurrent Ets-2 expression. A mutated Ets-2 motif found in inactive IFN-τ pseudogenes (with the central sequence TGAA in place of GGAA) was not activated by Ets-2 co-transfection. Ets-2 was shown to be present in day 15 trophectoderm nuclei by immunocytochemistry and, taken together, these data implicate Ets-2 in the onset of IFN-τ gene expression. It is presumed that Ets-2 controls IFN-τ expression since the gene duplication event leading to the evolution of the IFN-τ group resulted in the new gene being placed immediately downstream of the Ets-2 enhancer sequence.

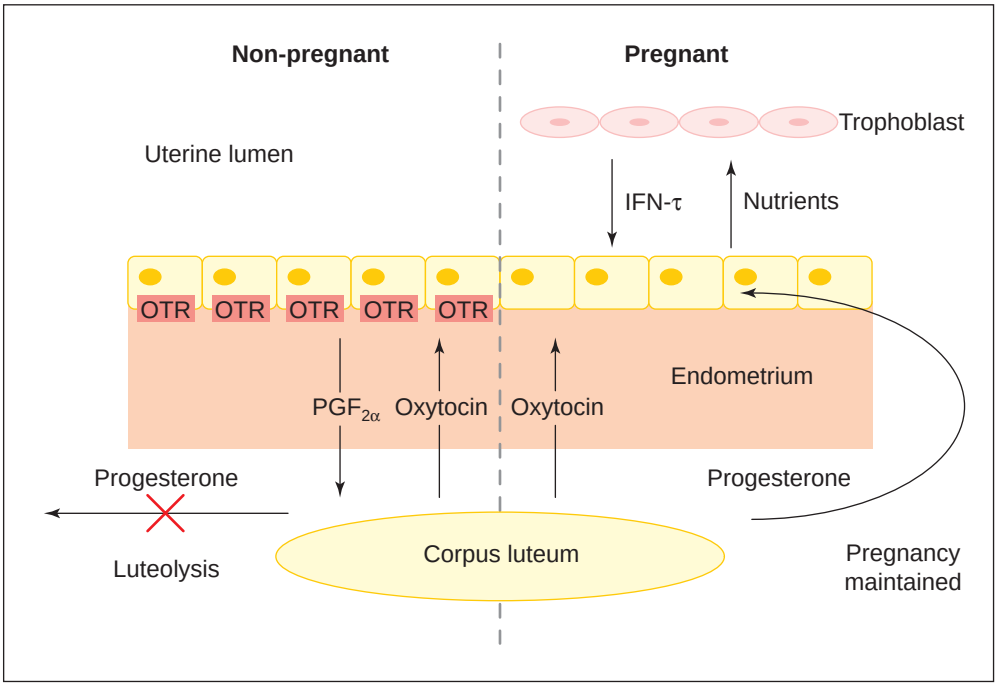

Fig. 4. Tissues, hormones and hormone receptors involved in the maternal recognition of pregnancy. In non-pregnant polyoestrous ruminants, luteolysis occurs at the end of each ovarian cycle as a result of uterine secretion of the luteolysin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Secretion of $PGF_{2\alpha}$ is episodic, and driven by oxytocin secreted by the corpus luteum. As luteal oxytocin, in turn, stimulates $PGF_{2\alpha}$ secretion, a positive feedback loop generates each secretory episode. The timing of $PGF_{2\alpha}$ secretion is determined by the onset of oxytocin receptor (OTR) expression by endometrial epithelial cells. In pregnancy, oxytocin receptor expression is blocked by interferon τ (IFN-τ) secreted into the uterine lumen by the trophoblast cells of the conceptus. This secretion occurs during a precise window in time, and commences before the blastocyst is attached to the endometrium. By blocking luteolysis, the conceptus ensures continued exposure of the endometrium to high circulating concentrations of progesterone, which, in turn, maintains the secretory activity of the endometrial glands, which provide nutrients required for blastocyst growth. Receptors for IFN-τ and for $PGF_{2\alpha}$ are expressed constitutively on endometrial and luteal target cells.

Other factors that may activate IFN-τ gene expression include granulocyte–macrophage colony-stimulating factor (GM-CSF), acting via the proto-oncogene c-*jun* and an AP-1 site at –654 to –555 bp (Imakawa *et al.*, 1993; Yamaguchi *et al.*, 1999). It is not surprising that Ets-2 and AP-1 pathways are involved in an event (IFN-τ gene expression) so closely associated with a rapid phase of blastocyst growth, as both these factors control genes responsible for cell proliferation. However, Ets-2 and AP-1 are expressed in a wide variety of tissues, while IFN-τ is expressed only in the trophoblast, and it is unlikely that IFN-τ expression is controlled by these factors alone. Guesdon *et al.* (1996) and Yamaguchi *et al.* (1999) have identified negative regulatory domains in the bovine IFN-τ promoter that may be involved in the precisely timed cessation of gene expression.

Mechanisms of IFN-τ action

Antiluteolytic properties

IFN-τ suppresses the normal pattern of pulsatile release of uterine $PGF_{2\alpha}$ leading to luteolysis at the end of the oestrous cycle (Fig. 4). In sheep, basal $PGF_{2\alpha}$ production is not eliminated, and circulating concentrations of the $PGF_{2\alpha}$ metabolite, 15-keto-13,14-dihydro-PGF (PGFM), are higher in pregnancy than during the oestrous cycle. Nevertheless, in pregnant animals, pulses of $PGF_{2\alpha}$ are diminished, and the corpus luteum remains functional.

Generation of luteolytic episodes of $PGF_{2\alpha}$ secretion requires luteal secretion of oxytocin and the interaction of circulating oxytocin with its receptor, which is located principally on endometrial epithelial cells. IFN-τ modulates

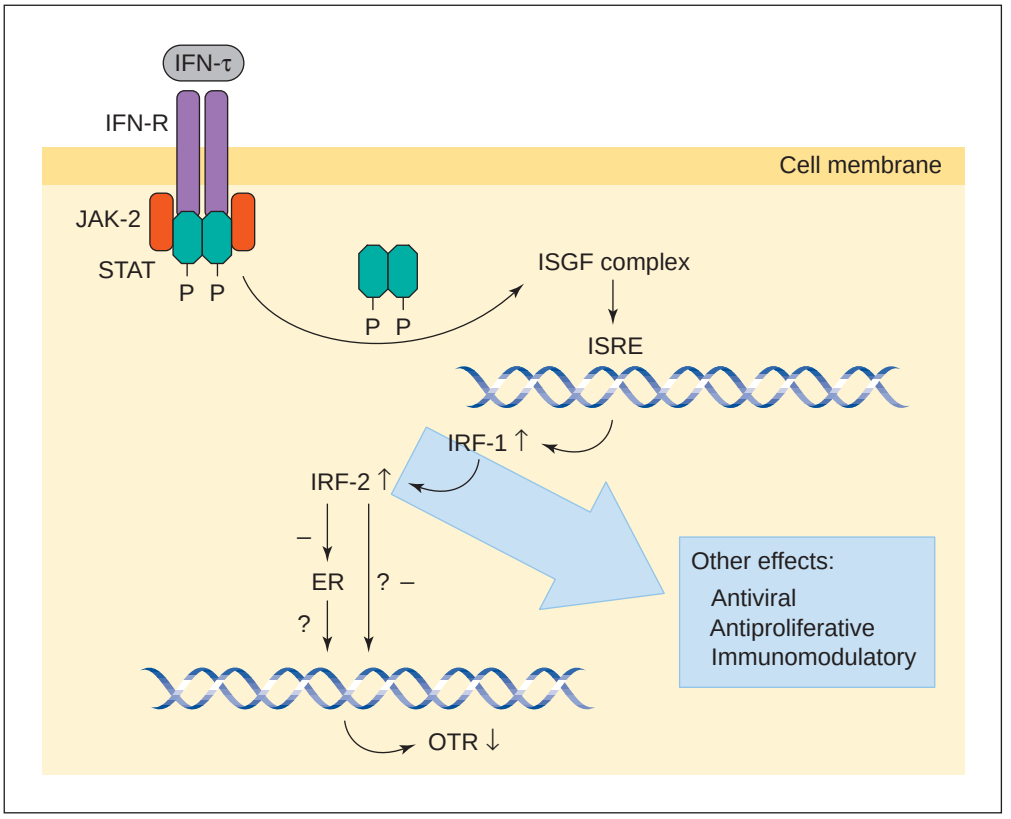

Fig. 5. Proposed mechanism of action of interferon τ (IFN-τ) on endometrial cells. IFN-τ binds to a dimeric interferon receptor (IFN-R) spanning the cell membrane. The intracellular domain of the receptor binds tyrosine kinases (Janus kinases, JAKs), which are activated after interferon binding, and subsequently phosphorylate other proteins named signal transducers and activators of transcription (STATs). The STATs dimerize and bind two other proteins to form a trimeric interferon-stimulated gene factor (ISGF) complex, which is translocated to the nucleus, where it binds an interferon-stimulated regulatory element (ISRE), resulting in the expression of the IRF-1 gene. The product of this gene, in turn, activates expression of IRF-2, which interacts with other regulatory elements to control the expression of interferon-responsive genes, including the oxytocin (OTR) and oestrogen (ER) receptors. At present, it is uncertain whether IRF-2 controls oxytocin receptor expression directly, or via the oestrogen receptor, or both. The effects of the IRFs include the induction of antiviral, antiproliferative and immunomodulatory responses to interferons.

uterine $PGF_{2\alpha}$ release by inhibiting endometrial oxytocin receptor expression (Flint *et al.*, 1992). Northern blotting shows that this effect is exerted at the level of gene transcription (Stewart *et al.*, 1993).

In sheep, the endometrial oxytocin receptor is not expressed before day 10 after mating. After day 20, luteal concentrations of oxytocin mRNA are low and the corpus luteum is incapable of secreting oxytocin. Therefore, the time at which IFN-τ is expressed coincides with the period over which luteal oxytocin is available to stimulate $PGF_{2\alpha}$ secretion (Flint *et al.*, 1992).

Since, in sheep and cattle, IFN-τ blocks episodes of $PGF_{2\alpha}$ secretion driven by luteal oxytocin, it might be expected that IFN-τ would not be expressed by the blastocyst in those species not expressing oxytocin in the corpus luteum. This expectation raises the question of whether expression of oxytocin at high concentrations in the corpus luteum and trophoblast secretion of IFN-τ have arisen in the same species, that is, in artiodactyls that have evolved during the last 36 million years? It appears they have: high concentrations of luteal oxytocin and trophoblast secretion of IFN-τ are both present in the cervids (and are also involved in the maternal recognition of pregnancy in red deer *Cervus elaphus*; Demmers *et al.*, 1999, 2000) but neither is present (at least in physiologically relevant concentrations) in equids, suids or camelids. The giraffids have IFN-τ (Liu *et al.*, 1996), but it is not known whether they have luteal oxytocin. The only exception to this rule appears to be roe deer *Capreolus capreolus* in which luteal oxytocin is present, but the blastocyst enters a long period of preimplantation diapause before elongation, and does not express IFN-τ (Flint *et al.*, 1994). IFN-τ is not required in the roe deer because oxytocin does not cause $PGF_{2\alpha}$ release, so luteolysis does not occur and there is no preimplantation maternal recognition of pregnancy.

Because oxytocin receptor gene expression is induced by oestrogen (McCracken *et al.*, 1984), it has been suggested that IFN-τ affects the oxytocin receptor through an inhibitory action on the oestrogen receptor (Fig. 5). There is a precedent for this mechanism, as type I interferons activate protein kinase C and, in many cells, phorbol esters, which also activate protein kinase C, increase the turnover of oestrogen receptor mRNA and reduce concentrations of oestrogen receptor protein (Martin *et al.*, 1995). However, IFN-τ may also reduce the transcriptional activity of the oestrogen receptor without affecting receptor concentrations, as Robinson *et al.* (1999) have shown that the downregulation of oxytocin receptor expression precedes any change in oestrogen receptor concentration in bovine endometrial epithelium. Surprisingly, in the short term, IFN-τ increases oestrogen receptor transcriptional activity (Flint *et al.*, 2000).

Bazer *et al.* (1997) proposed that the mechanism by which IFN-τ suppresses oxytocin receptor expression involves the type I interferon receptor signal transduction system and several members of the interferon-induced transcription factor family (Fig. 5). This family includes interferon-stimulated gene factor-3 (ISGF3), interferon regulatory factor 1 (IRF-1), IRF-2, interferon consensus sequence binding protein (ICBSP) and lymphoid-specific IRF. Binding of a type I interferon to its receptor immediately activates the latent tyrosine kinases, JAK1 and tyk2, which phosphorylate tyrosine residues of signal transducers and activators of transcription 1 (STAT1), STAT1A and STAT2 (for review, see Stark *et al.*, 1998). These three phosphoproteins then bind a fourth DNA-binding protein and the resulting interferon-stimulated gene factor binding complex is transported to the nucleus, where it binds to an IFN-stimulated responsive element (ISRE) present in the promoter–enhancer region of interferon-responsive genes. This activates transcription of interferon-responsive genes such as IRF-1, which, in turn, activate expression of the negative acting transcription factor IRF-2. Bazer *et al.* (1997) suggest that an IFN-τ-induced regulatory factor (possibly IRF-2) suppresses expression of the oestrogen receptor directly by binding to an IFN-τ-responsive element in the oestrogen receptor gene. The same factor also blocks, either directly or indirectly, the expression of the oxytocin receptor gene, preventing the uterine luteolytic mechanism and ensuring the establishment of pregnancy.

Immune effects of IFN-τ

In addition to controlling oxytocin receptor gene expression in the endometrium, IFN-τ affects the synthesis of other cytokines that contribute to the immunomodulation required to prevent rejection of the conceptus and stimulate blastocyst growth. IFN-τ increases expression of IFN-γ and interleukin 4 (IL-4) by bovine lymphocytes *in vitro* (Tuo *et al.*, 1999) and reduces the proliferative responses of lymphocytes to IL-2 (Niwano *et al.*, 1989). IFN-τ increases endometrial cyclo-oxygenase 2 (COX-2) concentrations and PGE_2 production (Dannet-Desnoyers *et al.*, 1994), which may contribute to the reduction in IL-2 expression observed in bovine lymphocytes and endometrium (Emond *et al.*, 1998; Leung *et al.*, 2000). IFN-τ may also affect immunomodulatory cell–cell interactions through increased expression of MHC class I molecules on endometrial cells (Todd *et al.*, 1998) and by decreasing the expression of transforming growth factor β (TGF-β) and retinol-binding protein (Godkin *et al.*, 1997). Pregnancy is thought to involve a shift from a Th1 towards a Th2 immune environment, with a reduction in inflammatory, cytotoxic T-cell responses in favour of B-cell activation and a decrease in proinflammatory interleukins such as IL-1α, IL-2 and IL-6 (Wegmann *et al.*, 1993). Leung *et al.* (2000) found no evidence for a change in lymphocyte populations at the time of the maternal recognition of pregnancy and so this shift presumably occurs principally after the cessation of IFN-τ production. However, an increase in PGE_2 production together with a decrease in IL-2 IFN-τ may initiate these changes.

PGE_2 also enhances GM-CSF production by peripheral lymphocytes and endometrium and, as described above, GM-CSF may induce further IFN-τ synthesis and blastocyst growth. Other endometrial cytokines induced by IFN-τ include bovine granulocyte chemotactic protein 2 (Hansen *et al.*, 1999). In addition, IFN-τ induces the ubiquitin crossreactive protein or interferon-stimulated gene 17 product (ISG-17; Hansen *et al.*, 1999), which controls cytosolic protein processing through the proteosome; osteopontin (Johnson *et al.*, 1999), which promotes cell–cell attachment and may be involved in attachment of the blastocyst to the endometrial epithelial surface; and the antiviral Mx protein (Ott *et al.*, 1998).

Antiviral and antiproliferative properties

The antiviral and antiproliferative properties of type I interferons have been reviewed by Stark *et al.* (1998). The antiproliferative effects of IFN-τ, which in Daudi cells (a human Burkitt lymphoma cell line) are less marked than those of IFN-α, result in cell cycle blockade at G1, probably through inhibition of the cyclin-dependent kinase, cdk2 (Subramaniam and Johnson, 1997). The differential cytotoxicity is due to different affinities of IFN-α and -τ for the type I receptor. In Madin Darby bovine kidney cells, IFN-α has a higher affinity than IFN-τ for the receptor (K_d of 4.45×10^{-11} versus 3.90×10^{-10} mol l^{-1}; Subramanian *et al.*, 1995). Antiviral activity depends on low receptor occupancy, but the antiproliferative effect is manifested only at higher occupancy, and so requires higher concentrations of IFN-τ than of IFN-α. Consistent with the lack of involvement of the carboxy terminal six amino acid extension in IFN-τ, the differences in binding affinity reflect differences in the interactions of the N-terminal ends of the molecules with the receptor.

Because IFN-τ has a reduced cytotoxicity compared with IFN-α, -β or -γ (Soos and Johnson, 1999), it may be useful therapeutically. IFN-τ causes fewer side effects than IFN-β at

effective doses in experimental murine allergic encephalomyelitis, an autoimmune animal model for multiple sclerosis (Soos and Johnson, 1999) and has potent antiviral activity against the human and feline immunodeficiency retroviruses and against ovine lentivirus and human papilloma virus. However, it does have acute effects after systemic administration on T-cell populations, and can cause symptoms of acute cytokine poisoning in some species (for example, red deer; Demmers *et al*., 2000).

Pathology resulting from lack of IFN-τ

Because of its central role in the maternal recognition of pregnancy, failure to produce sufficient IFN-τ, or production of appropriate quantities at an inappropriate time, would be expected to lead to pregnancy loss. Attempts to use IFN-α or IFN-τ to improve pregnancy rates have been inconclusive; Nephew *et al*. (1990) and Schalue-Francis *et al*. (1991) both showed that IFN-α treatment increased lambing rate after natural mating, but the study of Schalue-Francis *et al*. (1991) could not be repeated with a group of animals with more normal fertility. Systemic administration of IFN-α decreased pregnancy rates in heifers, possibly due to the decrease in serum concentration of progesterone seen in this species after IFN-α administration (Barros *et al*., 1992) but IFN-τ improved calving rates in red deer after asynchronous embryo transfer (Demmers *et al*., 2000). Developments in slow-release technology (L'Haridon *et al*., 1995) raise the possibility that IFN-τ may be introduced into the uterus at the time of embryo transfer.

As reviewed above, the onset of IFN-τ synthesis is closely linked to blastocyst development, particularly to the stage at which elongation occurs. Therefore, any condition delaying blastocyst development to this stage compromises pregnancy. The preimplantation blastocyst depends for nutrients and other support on secretions of the endometrium, and the secretory endometrium is, in turn, dependent on progesterone. This relationship leads to a positive feedback loop at the time of IFN-τ synthesis, whereby IFN-τ maintains luteal progesterone secretion, and progesterone supports the blastocyst. However, at earlier stages of blastocyst development, other factors, such as the quality of the ovulated follicle and the extent of luteinization, determine the rate of luteal progesterone secretion and these factors may be influenced, in turn, by genetic and environmental factors including nutrition. There is currently considerable interest in these questions because of the high rate of subfertility in high-yielding dairy cattle.

Direct evidence for an effect of progesterone concentration on IFN-τ production comes from cows supplemented with progesterone. In cows with low plasma progesterone on day 5 after insemination, concentrations of IFN-τ in uterine flushings on day 16 are low, and blastocyst development is impaired. Progesterone supplementation improves blastocyst development, increases uterine IFN-τ concentrations and improves pregnancy maintenance (Mann *et al*., 1999). Because of the difficulty of administering IFN-τ to its site of action (the uterus), and the cost of the large quantities required, progesterone supplementation may be a more effective treatment for embryo loss in cattle than IFN-τ itself.

References

Key references are identified by asterisks.

Alexenko AP, Leaman DW, Li JZ and Roberts RM (1997) The antiproliferative and antiviral activities of IFN-tau variants in human cells *Journal of Interferon and Cytokine Research* **17** 769–779

Ashworth C and Bazer FW (1989) Changes in ovine conceptus and endometrial function following asynchronous embryo transfer or administration of progesterone *Biology of Reproduction* **40** 425–433

Barros CM, Newton GR, Thatcher WW, Drost M, Plante C and Hansen PJ (1992) The effect of bovine interferon-$\alpha_1$1 on pregnancy rate in heifers *Journal of Animal Science* **70** 1471–1477

Bartol FF, Roberts RM, Bazer FW, Lewis GS, Godkin JD and Thatcher WW (1985) Characterisation of proteins produced *in vitro* by periattachment bovine conceptuses *Biology of Reproduction* **32** 681–693

Bazer FW, Spencer TE and Ott TL (1996) Placental interferons *American Journal of Reproductive Immunology* **35** 297–308

Bazer FW, Spencer TE and Ott TL (1997) Interferon tau: a novel pregnancy recognition signal *American Journal of Reproductive Immunology* **37** 412–420

Dannet-Desnoyers G, Wetzels C and Thatcher WW (1994) Natural and recombinant bovine interferon tau regulate basal and oxytocin induced secretion of prostaglandin $F_{2\alpha}$ and E_2 by epithelial cells and stromal cells in the endometrium *Reproduction, Fertility and Development* **6** 193–202

Demmers KJ, Kaluz S, Deakin DW, Jabbour HN and Flint APF (1999) Production of interferon by the conceptus in red deer *Cervus elaphus*. *Journal of Reproduction and Fertility* **114** 59–65

Demmers KJ, Jabbour HN, Deakin DW and Flint APF (2000) Production of interferon by red deer (*Cervus elaphus*) conceptuses and the effects of roIFN-τ on the timing of luteolysis and the success of asynchronous embryo transfer *Journal of Reproduction and Fertility* **118** 387–395

Ealy AD, Alexenko AP, Keisler DH and Roberts RM (1998) Loss of the signature six carboxyl amino acid tail from ovine interferon-tau does not affect biological activity *Biology of Reproduction* **58** 1463–1468

Emond V, Fortier MA, Murphy BD and Lambert RD (1998) Prostaglandin E-2 regulates both interleukin-2 and granulocyte–macrophage colony-stimulating factor gene expression in bovine lymphocytes *Biology of Reproduction* **58** 143–151

***Ezashi T, Ealy AD, Ostrowski MC and Roberts RM** (1998) Control of interferon-τ gene expression by Ets-2 *Proceedings National Academy of Sciences USA* **95** 7882–7887

Farin CE, Imakawa K, Hansen TR, McDonnell JJ, Murphy CN, Farin PW and Roberts RM (1990) Expression of trophoblastic interferon genes in sheep and cattle *Biology of Reproduction* **43** 210–218

Flint APF, Stewart HJ, Lamming GE and Payne JH (1992) Role of the oxytocin receptor in the choice between cyclicity and gestation in ruminants *Journal of Reproduction and Fertility Supplement* **45** 53–58

Flint APF, Krzywinski A, Sempere A, Mauget R and Lacroix A (1994) Luteal oxytocin and monoestry in the roe deer *Capreolus capreolus*. *Journal of Reproduction and Fertility* **101** 651–656

Flint APF, Abayasekara DRE, Wheeler-Jones CPD, Riley PR, Kaluz S, Kaluzova M, Sheldrick ELR and Fisher PA (2000) Acute effects of interferon on estrogen receptor function do not involve the extracellular signal regulated kinases $p42^{mapk}$ and $p44^{mapk}$ *Journal of Interferon and Cytokine Research* **20** 233–241

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

***Godkin JD, Smith SE, Johnson RD and Dore JJE** (1997) The role of trophoblast interferons in the maintenance of early pregnancy in ruminants *American Journal of Reproductive Immunology* **37** 137–143

Guesdon F, Stewart HJ and Flint APF (1996) Negative regulatory domains in a trophoblast interferon promoter *Journal of Molecular Endocrinology* **16** 99–106

Han CS, Mathialagan N, Klemann SW and Roberts RM (1997) Molecular cloning of ovine and bovine type I interferon receptor subunits from uteri, and endometrial expression of messenger ribonucleic acid for ovine receptors during the estrous cycle and pregnancy *Endocrinology* **138** 4757–4767

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

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

Imakawa K, Helmer SD, Nephew KP, Meka CSR and Christenson RK (1993) A novel role for GM–CSF: enhancement of pregnancy specific interferon production, ovine trophoblast protein-1 *Endocrinology* **132** 1869–1871

Johnson GA, Burghardt RC, Spencer TE, Newton GCR, Ott TL and Bazer FW (1999) Ovine osteopontin II. Osteopontin and alpha(v)beta (3) integrin expression in the uterus and conceptus during the preimplantation period *Biology of Reproduction* **61** 892–899

Kaluz S, Fisher PA, Kaluzova M, Sheldrick EL and Flint APF (1996) Structure of an ovine interferon receptor and its expression in endometrium *Journal of Molecular Endocrinology* **17** 207–215

Kerbler TL, Buhr MM, Jordan LT, Leslie KE and Walton JS (1997) Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle *Theriogenology* **47** 703–714

Leung ST, Derecka K, Mann GE, Flint APF and Wathes DC (2000) Uterine lymphocyte distribution and interleukin expression during early pregnancy in cows *Journal of Reproduction and Fertility* **119** 25–33

L'Haridon RM, Huynh L, Assal NE and Martal J (1995) A single intrauterine infusion of sustained recombinant ovine interferon-τ extends corpus luteum life span in cyclic ewes *Theriogenology* **43** 1031–1045

Liu LM, Leaman DW and Roberts RM (1996) The interferon-τ genes of the giraffe, a nonbovid species *Journal of Interferon and Cytokine Research* **16** 949–951

McCracken JA, Schramm W and Okulicz WG (1984) Hormone receptor control of pulsatile secretion of $PGF_{2\alpha}$ from the ovine uterus during luteolysis and its abrogation during early pregnancy *Animal Reproduction Science* **7** 31–55

Mann GE, Lamming GE, Robinson RS and Wathes DC (1999) The regulation of interferon-tau production and uterine hormone receptors during early pregnancy *Journal of Reproduction and Fertility Supplement* **54** 317–328

Martal J, Lacroix M-C, Loudes C, Saunier M and Wintenberger-Torres S (1979) Trophoblastin, an antiluteolytic protein present in early pregnancy in sheep *Journal of Reproduction and Fertility* **56** 63–73

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

Martin MB, Garcia-Morales P, Stoica A, Solomon HB, Pierce M, Katz D, Zhang S, Danielsen M and Saceda M (1995) Effects of 12-*O*-tetradecanoylphorbol-13-acetate on estrogen receptor activity in MCF-7 cells *Journal of Biological Chemistry* **270** 25 244–25 251

Moor RM and Rowson LEA (1964) Influence of the embryo and uterus on luteal function in the sheep *Nature* **201** 522–523

Moor RM and Rowson LEA (1966a) The corpus luteum of the sheep: effect of the removal of embryos on luteal function *Journal of Endocrinology* **34** 497–502

Moor RM and Rowson LEA (1966b) The corpus luteum of the sheep: functional relationship between the embryo and the corpus luteum *Journal of Endocrinology* **34** 233–239

Nephew KP, McClure KE, Day ML, Xie S, Roberts RM and Pope WF (1990) Effects of intra-muscular administration of recombinant bovine interferon-α_1 during the period of maternal recognition of pregnancy *Journal of Animal Science* **68** 2766–2770

Niswender KD, Li J, Powell MR, Loos KR, Roberts RM, Keisler DH and Smith MF (1997) Effect of variants of interferon-tau with mutations near the carboxyl terminus on luteal life span in sheep *Biology of Reproduction* **56** 214–220

Niwano Y, Hansen TR, Kazemi M, Malathy P-V, Johnson HD, Roberts RM and Imakawa K (1989) Suppression of T-lymphocyte blastogenesis by ovine trophoblast protein-1 and human interferon-α may be independent of interleukin-2 production *American Journal of Reproductive Immunology* **20** 21–26

Ott TL, Yin JY, Wiley AA, Kim HT, GeramiNaini B, Spencer TE, Bartol FF, Burghardt RC and Bazer FW (1998) Effects of the estrous cycle and early pregnancy on uterine expression of Mx protein in sheep (*Ovis aries*) *Biology of Reproduction* **59** 784–794

Radhakrishnan R, Walter LJ, Subramaniam PS, Johnson HM and Walter MR (1999) Crystal structure of ovine interferon-tau at 2.1 angstrom resolution *Journal of Molecular Biology* **286** 151–162

***Roberts RM, Liu LM, Guo QT, Leaman D and Bixby J** (1998) The evolution of the type I interferons *Journal of Interferon and Cytokine Research* **18** 805–816

***Roberts RM, Ealy AD, Alexenko AP, Han CS and Ezashi T** (1999) Trophoblast interferons *Placenta* **20** 259–264

Robinson RS, Mann GE, Lamming GE and Wathes DC (1999) The effect of pregnancy on the expression of uterine oxytocin, oestrogen and progesterone receptors during early pregnancy in the cow *Journal of Endocrinology* **160** 21–33

Schalue-Francis TK, Farin PW, Cross JC, Keisler D and Roberts RM (1991) Effect of injected bovine interferon-$\alpha_1$1 on oestrous cycle length and pregnancy success in sheep *Journal of Reproduction and Fertility* **91** 347–356

Soos JM and Johnson HM (1999) Interferon-tau – prospects for clinical use in autoimmune disorders *Biodrugs* **11** 125–135

Stark GR, Kerr IM, Williams BRG, Silverman RH and Schreiber RD (1998) How cells respond to interferons *Annual Review of Biochemistry* **67** 227–264

Stewart HJ, McCann SHE, Barker PJ, Lee KE, Lamming GE and Flint APF (1987) Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein *Journal of Endocrinology* **115** R13–R15

Stewart HJ, McCann SHE, Northrop AJ, Lamming GE and Flint APF (1989) Sheep antiluteolytic interferon: cDNA sequence and analysis of mRNA levels *Journal of Molecular Endocrinology* **2** 65–70

Stewart HJ, Stevenson KR and Flint APF (1993) Isolation and structure of a partial sheep oxytocin receptor cDNA and its use as a probe for northern analysis of endometrial RNA *Journal of Molecular Endocrinology* **10** 359–361

Subramaniam PS and Johnson HM (1997) A role for the cyclin-dependent kinase inhibitor p21 in the G91 cell cycle arrest mediated by the type I interferons *Journal of Interferon and Cytokine Research* **17** 11–15

Subramaniam PS, Khan SA, Pontzer CH and Johnson HM (1995) Differential recognition of the type I interferon receptor by interferons tau and alpha is responsible for their disparate cytotoxicities *Proceedings National Academy of Sciences USA* **92** 12 270–12 274

Todd I, McElveen JE and Lamming GE (1998) Ovine trophoblast interferon enhances MHC class I expression by sheep endometrial cells *Journal of Reproductive Immunology* **37** 117–123

Tuo WB, MacMillan H, Gunter N, Bazer FW and Brown WC (1999) Upregulation of interleukin-4 and IFN-gamma expression by IFN-tau, a member of the type I IFN family *Journal of Interferon and Cytokine Research* **19** 179–187

Wegmann TG, Lin H, Guilbert L and Mosmann TR (1993) Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunology Today* **14** 353–356

Yamaguchi H, Ikeda Y, Moreno IJ, Katsumura M, Miyazawa T, Takahashi E, Imakawa K, Sakai S and Christenson RK (1999) Identification of a functional transcriptional factor AP-1 site in the sheep interferon τ gene that mediates a response to PMA in JEG3 cells *Biochemical Journal* **340** 767–773