Chronicling the discovery of interferon tau

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Abstract

It has been 38 years since a protein, now known as interferon tau (IFNT), was discovered in ovine conceptus-conditioned culture medium. After 1979, purification and testing of native IFNT revealed its unique antiluteolytic activity to prevent the regression of corpora lutea on ovaries of nonpregnant ewes. Antiviral, antiproliferative and immunomodulatory properties of native and recombinant IFNT were demonstrated later. In addition, progesterone and IFNT were found to act cooperatively to silence expression of classical interferon stimulated genes in a cell-specific manner in ovine uterine luminal and superficial glandular epithelia. But, IFNT signaling through a STAT1/STAT2-independent pathway stimulates expression of genes, such as those for transport of glucose and amino acids, which are required for growth and development of the conceptus. Further, undefined mechanisms of action of IFNT are key to a servomechanism that allows ovine placental lactogen and placental growth hormone to affect the development of uterine glands and their expression of genes throughout gestation. IFNT also acts systemically to induce the expression of interferon stimulated genes that influence secretion of progesterone by the corpus luteum. Finally, IFNT has great potential as a therapeutic agent due to its low cytotoxicity, anti-inflammatory properties and effects to mitigate diabetes, obesity-associated syndromes and various autoimmune diseases.

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Embryonic death, uterine factors and pregnancy

A number of historical events set the stage for research leading to the discovery of pregnancy recognition signals in pigs and ruminant species. [Bazer and coworkers \(1969\)](#) noted that hormone-induced superovulation and feeding high-energy feeds, to increase the number of ovulations in polytocous animals, led to greater rates of embryonic death due to inherent limitations within individual embryos and to maternal limitations that restrict litter size or both. Embryonic death losses occur during the peri-implantation period of pregnancy ([Boyd 1965](#)) and increase following hormonal superovulation ([Fowler & Edwards 1957](#), [Adams 1960](#), [Hafez 1964](#)) or the transfer of additional embryos to uterine horns of mice ([McLaren & Michie 1959](#), [Adams 1960](#), [Hafez 1964](#)). [Bazer et al. \(1969\)](#) postulated that a biochemical factor in the uterine lumen limited the number of blastocysts that undergo implantation and placentation. This idea supported results of [Krishnan and Daniel \(1967\)](#) who discovered blastokinin (also known as uteroglobin). Blastokinin was reported to induce the development of morulae to blastocysts in rabbits. The fact that most embryonic deaths in pigs occur during the peri-implantation period of pregnancy ([Gossett & Sorensen 1959](#)) suggested that the major causes were failure of blastocysts to develop and/or uterine capacity,

both of which limited litter size at term ([Bazer et al. 1968, 1969](#)).

Concept of pregnancy recognition signaling

While attention was being given to embryonic death, uterine factors and pregnancy in pigs, [Short \(1979\)](#) clarified the terms luteotrophic, luteolytic, antiluteolytic and pregnancy recognition signaling. Prostaglandin F_{2α} (PGF) was identified as the uterine-derived luteolytic hormone responsible for regression of the CL and cessation of secretion of progesterone by luteal cells in nonpregnant subprimate mammals ([Schramm et al. 1983](#)). The antiluteolytic signals from pig and ruminant conceptuses had not been identified in 1969, but it was assumed that those signals inhibited luteolytic actions of PGF or abrogated the mechanism(s) for PGF-induced luteolysis. Pregnancy recognition signals were diverse ([Flint et al. 1979](#)). For example, available results indicated the lack of need for pregnancy recognition signals for dogs, ferrets and marsupials, but systemic effects of estrogens on functions of corpora lutea (CL) in pregnant pigs had been reported ([Kraeling et al. 1975](#)). [Short \(1979\)](#) reported that abnormal conceptuses failed to signal pregnancy recognition. Thus, the stage was set for research to identify pregnancy recognition signals in mammals. This challenge was addressed in our

laboratories at the University of Florida through research with pigs, sheep and cows.

Pregnancy recognition signaling in pigs

Research on proteins in uterine secretions, their endocrine regulation and their influence on embryonic survival in pigs (see [Bazer 1975](#)) led to research on mechanisms for establishment and maintenance of pregnancy in pigs, sheep and cows. Studies of the oestrous cycle and pregnancy in pigs revealed that PGF was released into the uterine venous system in a pulsatile manner during the period of luteolysis in cyclic, but not pregnant gilts (see [Bazer & Thatcher 1977](#)). Furthermore, it was discovered that the CL of pigs were not responsive to luteolytic effects of PGF until about Day 14 of the oestrous cycle; therefore, PGF was ineffective as a hormone to synchronize estrus in pigs (see [Bazer et al. 1982](#)).

Our research on early pregnancy in pigs revealed that blastocysts hatch from the zona pellucida between Days 6 and 7, expand to large spherical blastocysts by Day 10, and transition to tubular and filamentous forms by Day 12 (1 mm diameter $\times$ 200 mm length) and Day 15 (800–1000 mm in length) of gestation ([Geisert et al. 1982a,b,c](#)). It was later established that elongating pig conceptuses secrete oestrogens ([Perry et al. 1973](#), [Fischer et al. 1985](#)), which are the pregnancy recognition signal in pigs ([Bazer & Thatcher 1977](#)). The endocrine-exocrine theory for pregnancy recognition in pigs was reported by [Bazer and Thatcher \(1977\)](#). The theory of pregnancy recognition signaling in pigs was based on results indicating that oestrogens, acting alone or in concert with prolactin, change the direction of secretion of PGF from uterine epithelia from an endocrine to an exocrine direction. PGF is released from uterine epithelia into the vasculature of the uterus (endocrine secretion) in a pulsatile manner between Days 15 and 16 of the oestrous cycle to induce luteal regression. However, PGF is secreted by uterine epithelia into the uterine lumen (exocrine direction) of pregnant pigs where it is sequestered and metabolized (see [Bazer & Thatcher 1977](#)). This theory was confirmed and modified (see [Zeicik et al. 2011](#)).

Pregnancy recognition signaling in ewes

[Rowson and Moor \(1967\)](#) provided the first evidence that ovine conceptuses secrete a substance(s) that extends CL life-span in ewes and that the conceptus must be present in the uterine horn ipsilateral to the ovary bearing the CL in order to prevent the regression of the CL. Those results indicated that the conceptus factor acted locally to prevent CL regression. The 'antiluteolytic factor' was thermolabile, degraded by proteases and produced by ovine conceptuses between Days 12 and 21 of pregnancy

([Moor & Rowson 1966a,b](#), [Rowson & Moor 1967](#), [Moor 1968](#)). They also found that a blastocyst must be transferred into the uterine horn ipsilateral to the ovary bearing a CL by Day 12 or Day 13 of the oestrous cycle for establishment of a successful pregnancy. This was confirmed by a study in which they found that length of the oestrous cycle of ewes was only affected when conceptuses were flushed from the uterus on or after Day 13 of pregnancy ([Moor & Rowson 1966a](#), [Moor et al. 1969](#)). [Martal \(1979\)](#) and [Ellinwood and coworkers \(1979\)](#) reported an antiluteolytic factor in homogenates of sheep conceptuses collected on Days 14–15, but not Days 21–25 of pregnancy and homogenates of Day 14 and Day 15 conceptuses extended CL lifespan and inter-oestrous intervals when infused into the uterine lumen of cyclic ewes ([Rowson & Moor 1967](#), [Ellinwood et al. 1979](#), [Martal et al. 1979](#)). The antiluteolytic factor in the conceptus homogenates was confirmed to be inactivated by heat and proteases. [Martal et al. \(1979\)](#) named the antiluteolytic factor trophoblastin. Thus, it was established that maternal recognition of pregnancy signaling in ewes involved a protein secreted by the conceptus between Days 12 and 21 after onset of estrus and mating.

Discovery of interferon tau as the pregnancy recognition signal in ruminants

Our research on the estrogen-dependent endocrine-exocrine theory of pregnancy recognition in pigs ([Bazer & Thatcher 1977](#)) biased our thinking toward the concept of oestrogen being the pregnancy recognition signal in other livestock species, e.g., sheep and cows. Therefore, we studied steroid metabolism by uterine endometria and conceptuses from sheep and cows. Results of those studies provided no evidence for conceptus-derived oestrogens being the antiluteolytic factor secreted by conceptuses from sheep or cow conceptuses during the period of pregnancy recognition signaling (see [Eley et al. 1979](#)). Therefore, we cultured sheep conceptuses in medium containing radiolabeled amino acids and analyzed culture medium to detect radiolabeled proteins that were synthesized *de novo*. We were using this method to identify proteins synthesized *de novo* and secreted into culture medium by explants of pig endometria (see [Basha et al. 1980](#)) (see [Fig. 1](#)).

Ovine conceptus conditioned culture medium was first subjected to Sephadex G200 gel filtration chromatography, and we discovered a low-molecular-weight radiolabeled protein that is now known as ovine interferon tau (IFNT) ([Wilson et al. 1979](#)). Later, bovine IFNT was identified using the same methods ([Lewis et al. 1979](#)). This research was supported by a United States Department of Agriculture, Cooperative States Research Service Grant No. 61615162 titled 'Increasing Embryo Survival In Cattle' awarded to Drs Thatcher and Bazer.

Interferon Tau: the pregnancy recognition signal in ruminants

Figure 1 This figure illustrates the sequence of events that led to the discovery of interferon tau (IFNT) in ovine conceptus conditioned culture medium and identified in this figure of a two-dimensional polyacrylamide gel by green arrows. Details of how embryo transfer experiments contributed to discovery of pregnancy recognition signaling are provided in the text. Protein X, trophoblastin, ovine trophoblast protein 1 and type 1 trophoblast interferon were names given to the protein, which was finally named interferon tau.

The discovery of ovine IFNT was by Dr Bazer's graduate student, Michael Wilson. After that, studies were led by Dr Gregory Lewis, a postdoctoral fellow working with Drs Thatcher and Bazer, and Dr James Godkin, a postdoctoral fellow working with Drs R Michael Roberts and Bazer. Wilson switched majors to Agricultural Economics and Dr Lewis focused his research on prostaglandins secreted by the uterus and conceptus. Thus, much of the early research on the protein originally called 'Protein X' and later ovine trophoblast protein one (oTP1) was led by Dr James Godkin. As noted previously, [Martal et al. \(1979\)](#) purified the same protein as protein X/oTP1 and called it 'trophoblastin.'

In order to identify when during early pregnancy the antiluteolytic protein(s) was secreted, sheep conceptuses from Days 10–25 of pregnancy were cultured in the presence of radioactive amino acids. This allowed detection of *de novo* synthesized and secreted proteins in culture medium using two-dimensional polyacrylamide gel electrophoresis and fluorography ([Godkin et al. 1982](#)). One of the radiolabeled proteins in culture medium had a molecular weight of 17–20 kDa, which was similar to the estimated size of the protein(s) originally identified using gel filtration chromatography. Because the 17–20 kDa protein was the first major protein secreted by the ovine conceptus, it was initially designated protein X, which was renamed ovine trophoblast protein one (oTP-1) ([Godkin et al. 1984](#)). oTP-1 was not synthesized and secreted by ovine conceptuses beyond Day 21 of pregnancy, which agreed

with findings of [Moor and Rowson \(1966a\)](#) and [Moor and Rowson \(1966b\)](#). Intrauterine injections of semi-purified oTP-1 between Days 12 and 14 of the oestrous cycle extended inter-estrous intervals and maintenance of CL that continued to produce progesterone ([Godkin et al. 1984](#)). Those results strongly suggested that oTP-1 was the antiluteolytic factor secreted by ovine conceptuses. However, [Vallet and coworkers \(1988\)](#) provided clear evidence that highly purified oTP-1 alone, in the total array of proteins secreted by ovine conceptuses, abrogated the luteolytic mechanism.

Immunocytochemical and *in situ* hybridization analyses revealed that oTP1 was expressed by mononuclear trophoblast cells ([Godkin et al. 1984](#), [Farin et al. 1990](#), [Guillomot et al. 1990](#)) with maximal production between Days 14–16 of pregnancy. Day 15 ovine conceptuses released greater than 100 µg of the protein in culture in a 24-h period ([Ashworth & Bazer 1989b](#)), and expression of oTP-1 mRNA by the conceptus is greatest around Day 14 of pregnancy in sheep and Day 20 of pregnancy in cattle ([Hansen et al. 1985](#), [Stewart et al. 1987](#)). However, low amounts of IFNT are secreted by ovine conceptuses as early as Days 8 and 10 of pregnancy ([Ashworth & Bazer 1989a](#)). Expression of oTP1 mRNA is detectable on Days 10 and 11, maximum after Day 13 and declines after Day 17 of pregnancy ([Farin et al. 1990](#), [Guillomot et al. 1990](#)). The decrease in IFNT gene expression occurred after attachment of conceptus trophoblast to the uterine luminal epithelium during implantation. Thus, IFNT

is produced transiently by ovine conceptuses, with expression being greatest prior to the expression of mRNAs for estradiol (ESR1) and oxytocin (OXTR) receptors in uterine epithelia between Days 13 and 14 of the oestrous cycle in ewes.

Parallel studies with bovine and ovine oTP1 confirmed that they are antiluteolytic signals for pregnancy in both species (Knickerbocker *et al.* 1986, Helmer *et al.* 1987, 1989, Thatcher *et al.* 1989, Meyer *et al.* 1995, Tysseling *et al.* 1997). Using ovine IFNT, Newton and coworkers (1996) demonstrated that it is antiluteolytic when injected into the uterine lumen of goats.

Cloning the gene for oTP1

Dr James Lauderdale, a colleague of Drs Thatcher and Bazer, obtained permission for Dr Russell Anthony, a postdoctoral fellow working with Drs Roberts and Bazer, to work in the laboratories of Drs K R Marotti and H G Polites at the Upjohn Company to clone the gene for oTP1. However, with Dr Roberts' move from the University of Florida to the University of Missouri, cloning of the oTP1 gene was continued by Dr Kaz Imakawa, a graduate student in Dr Roberts' laboratory. Imakawa and coworkers (1987) and Stewart and coworkers (1987) cloned the gene for oTP-1, and it was found to be a member of the Type I interferon alpha (IFNA) family of proteins. The identity between the 172 amino acid ovine oTP-1 and the 165 amino acid bovine IFNA1 mRNA and protein was found to be 63% and 50%, respectively (Roberts 1991, 1993). However, homology between oTP1 and bovine IFN omega 1 (IFNW1) mRNAs and proteins was 85% and 72%, respectively. Thus, oTP1 is in a distinct subgroup of the Type I IFN family (Imakawa *et al.* 1987). Because of its unique developmental expression by trophectoderm and its relatedness to other Type I IFNs (alpha, beta, omega), oTP1 was given the name interferon tau (IFNT) by the International Interferon Society (see Roberts 1991).

Production of recombinant ovine interferon tau and its biological activities

Using a highly purified native IFNT, we demonstrate its potent antiviral (Pontzer *et al.* 1988, 1990), antiproliferative (Pontzer *et al.* 1991) and immunosuppressive (Pontzer *et al.* 1994, Newton *et al.* 1996) properties, as well as insight into the structural motif of IFNT (Jarpe *et al.* 1994). Subsequently, we produced and expressed a synthetic gene for ovine IFNT using the *Pichia pastoris* yeast system. The recombinant IFNT has immunosuppressive, antiviral, antiproliferative and antiluteolytic properties equivalent to native IFNT (Ott *et al.* 1991, Van Heeke *et al.* 1996).

Mechanisms for the antiluteolytic effects of interferon tau

Our research on mechanisms of action of IFNT as the antiluteolytic pregnancy recognition signal in ruminants was conducted at the University of Florida and at Texas A&M University. Dr John McCracken's model of the 'progesterone block' for regulation of the oestrous cycle in ewes (see Schramm *et al.* 1983) provided the basis for our hypotheses on mechanisms whereby IFNT signals pregnancy recognition in sheep and other ruminant species. We tested the hypothesis that progesterone (P4) blocks the expression of receptors for estradiol (ESR1) and oxytocin (OXTR) for a finite period of time, 10–12 days, after which time progesterone (P4) downregulates the expression of its own receptor (PGR) to allow upregulation of expression of *ESR1* and *OXTR* genes. Then, pulsatile release of oxytocin from the posterior pituitary gland and CL elicit secretion of luteolytic pulses of PGF from uterine epithelia on Days 15 and 16 to cause regression of the CL and return to oestrus by the ewe. The following evidence indicates that IFNT alone is the pregnancy recognition component in ewes and other ruminant species: (1) IFNT directly silences transcription of the *ESR1* gene and, indirectly, the E2-induced expression of *OXTR* in uterine luminal and superficial glandular epithelia to abrogate development of the endometrial luteolytic mechanism involving oxytocin-induced luteolytic pulses of PGF (Spencer *et al.* 1995a,b, 1998, Fleming *et al.* 2001, 2006); (2) basal concentrations of PGF and PGE2 in maternal blood are greater in pregnant than cyclic ewes due to continued expression of prostaglandin endoperoxide synthase 2 (*PTGS2*) (Charpigny *et al.* 1997b, Simmons *et al.* 2010); (3) IFNT silences the expression of *ESR1*, which prevents oestradiol (E2) from upregulating the expression of PGR in endometrial epithelia (Fincher *et al.* 1986, Vallet *et al.* 1988, Mirando *et al.* 1990, Spencer *et al.* 1995b) and (4) downregulation of expression of PGR in uterine epithelia is required for expression of P4-induced and IFNT-stimulated genes that support the development of the conceptus (Spencer *et al.* 1998, Choi *et al.* 2001, Bazer *et al.* 2014).

Caprine IFNT, secreted between Days 16 and 21 of gestation, is the pregnancy recognition signal that prevents pulsatile release of luteolytic PGF by uterine epithelia and extends CL lifespan in goats (Newton *et al.* 1996). Bovine IFNT, secreted between Days 12 and 38 of pregnancy, prevents the secretion of luteolytic pulses of PGF by uterine epithelia and blocks effects of exogenous E2 and oxytocin to stimulate pulsatile release of PGF by bovine uterine epithelia. Expression of *ESR1* and *OXTR* mRNAs are either downregulated or *ESR1* and *OXTR* are less abundant or less responsive to E2 and oxytocin in endometria of pregnant compared to cyclic cows or cows receiving intrauterine injections of either ovine or bovine IFNT (Knickerbocker *et al.* 1986,

Helmer *et al.* 1987, 1989, Thatcher *et al.* 1989, Meyer *et al.* 1995, Tysseling *et al.* 1997).

Interferon tau, the sole factor produced by the conceptus that prevents development of the endometrial luteolytic mechanism in sheep (Vallet *et al.* 1988), does not stabilize expression of *PGR* in uterine epithelia during pregnancy (Spencer & Bazer 1995, 1996, Spencer *et al.* 1995b). Rather, IFNT acts on uterine luminal and superficial glandular epithelia to inhibit transcription of *ESR1* and *OXTR* genes and abrogate development of the endometrial luteolytic mechanism (Spencer & Bazer 1996, Spencer *et al.* 1998, Fleming *et al.* 2001). The increases in expression of *ESR1* and *OXTR* mRNAs in uterine luminal and superficial glandular epithelia between Days 11–17 post-oestrus in cyclic sheep do not occur in pregnant ewes (Spencer & Bazer 1995) or in cyclic ewes in which recombinant ovine IFNT is injected into the uterine lumen (Spencer *et al.* 1995c). Thus, oxytocin is unable to induce secretion of luteolytic pulses of PGF. However, basal production of PGF is greater in pregnant than cyclic ewes as IFNT does not inhibit the expression of *PTGS2* in uterine LE/sGE (Charpigny *et al.* 1997b, Simmons *et al.* 2010). The molecular mechanisms involved in IFNT silencing of expression of *ESR1* is likely due to IFNT-inducing expression of IFN regulatory factor two (IRF2), a potent repressor of transcription, in uterine luminal and superficial glandular epithelia (Spencer *et al.* 1998, Choi *et al.* 2001, Fleming *et al.* 2001). In the absence of *ESR1*, ovine uterine epithelia do not express *OXTR* (Fleming *et al.* 2006). The promoter region of the *OXTR* gene does not contain an estrogen response element, rather *ESR1* binds to SP1 elements and interacts with two GC-rich SP1-binding sites, located within 140 bp of the translational start site for *OXTR* (Fleming *et al.* 2006). Mutation analyses of the *OXTR* promoter revealed that the proximal SP1 sites mediated *ESR1* action as well as basal activity of the promoter (Fleming *et al.* 2006).

In contrast to the ovine *OXTR* gene, the bovine *OXTR* gene lacks a classical palindromic oestrogen response element (Bathgate *et al.* 1998), and there is no change in *ESR1* expression in uterine epithelia between pregnant and nonpregnant cows (Robinson *et al.* 1999). Thus, pregnancy and IFNT may alter expression of *OXTR* mRNA independent of *ESR1* in the endometrium of cattle. IRF2 can regulate the expression of the bovine *OXTR* gene (Telgmann *et al.* 2003) suggesting its common role as an effector of the antiluteolytic actions of IFNT in bovine endometria for the establishment of pregnancy.

Progesterone-induced and interferon tau-stimulated genes in the ovine uterus

In ewes, progesterone (P4) induces and IFNT further stimulates the expression of many genes. However, a fundamental unanswered question is whether actions of

progestagens (e.g., fibroblast growth factors 7 and 10, and hepatocyte growth factor) from uterine stromal cells and IFNT act on uterine epithelia or other uterine cell types via novel non-classical cell signaling pathways, independent of PGR and STAT1. Progestagens and IFNT can signal via mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) to affect gene expression and uterine receptivity to implantation (Platanias 2005). All Type I IFNs bind the same receptor, but may activate novel cell-specific signaling pathways. Thus, IFNT may differentially affect gene expression in uterine luminal and superficial glandular epithelia vs uterine glandular epithelium and stromal cells. This cell-specific gene expression in the ovine uterus is due to expression of IRF2 only by uterine luminal and superficial glandular epithelial cells, which is likely responsible for the silencing expression of classical interferon stimulated genes by uterine luminal and superficial glandular epithelia (Spencer *et al.* 1998, Choi *et al.* 2001).

In addition to signaling pregnancy recognition in ruminants, IFNT limits expression of classical interferon-stimulated genes to ovine uterine glandular epithelium and stromal cells, which do not express IRF2. However, there are many P4-induced and IFNT-stimulated genes expressed by uterine luminal and superficial glandular epithelia that lack both PGR and STAT1 (signal transduction and activator of transcription 1). This is because IFNT induces expression of IRF2 in uterine luminal and superficial glandular epithelia in ewes to silence expression of ISGs, but induces expression of many novel genes that support growth and development of the conceptus. Those genes include: wingless-type MMTV integration site family member 7A (*WNT7A*), *LGALS15* (galectin 15), *HIF2A* (hypoxia-inducible factor 2A), *CTSL* (cathepsin L), *CST3* (cystatin C), *SLC5A1* (solute carrier family 5, sodium/glucose cotransporter, member 1), *GRP* (gastrin releasing peptide), *HSD11B1* (11 beta hydroxysteroid dehydrogenase type 1), *IGFBP1* (insulin-like growth factor binding protein 1) and *SLC7A2* (solute carrier family 7, cationic amino acid transporter, y+ system, member 2) (Bazer *et al.* 2009, 2015a,b). Inhibition of the classical Janus kinase (JAK):STAT cell signaling pathway by IRF2 likely allows expression of the P4-induced and IFNT-stimulated genes to increase in response to both the progestagens and IFNT via MAPK and PI3K cell signaling in uterine luminal and superficial glandular epithelia. These include genes for transport of glucose (*SLC2A1* and *SLC5A11*) and amino acids (*SLC7A1* and *SLC7A2*) into the uterine lumen to stimulate proliferation of trophoblast cells by activating the glutamine:fructose-6-phosphate amidotransferase (GFAT)-mediated FK506-binding protein 12-rapamycin-associated protein 1 (FRAP1, formerly mTOR) signaling pathway. Arginine also affects fetal-placental growth and development through effects on synthesis of nitric oxide (NO) and polyamines

that stimulate vascular functions, as well as DNA and protein synthesis for proliferation and differentiation of cells, respectively. However, expression of major histocompatibility complex class I molecules and $\beta 2$ -microglobulin genes that regulate immune rejection responses is silenced in ovine uterine luminal and superficial glandular epithelia to protect the conceptus allograft from rejection by the maternal immune system.

Interferon tau and the servomechanism for maintenance of pregnancy

Interferon tau is a component of the servomechanism for the maintenance of pregnancy in ewes. The servomechanism provides for reciprocal communication between the conceptus and endometrium during implantation and synepitheliochorial placentation in ewes (Spencer *et al.* 1999, 2007, Stewart *et al.* 2000, 2001, Noel *et al.* 2003). This concept is based on evidence that IFNT extends lifespan of CL initially and that placental lactogen (CSH1) and/or placental growth hormone (GH1) secreted by the trophectoderm/chorion from Day 15 to term, and Days 35–65 of pregnancy, respectively, reinforce the antiluteolytic effects of IFNT. However, there were no independent or interactive effects of CSH1 and GH1 on luteal maintenance and function in ewes. Importantly, both CSH1 and GH1 affected the development and function of uterine glands, but only if the uterus was first exposed to intra-uterine IFNT between Days 11 and 21 and then intra-uterine CSH1, GH1 or CSH1 and GH1 between Days 16 and 29 after onset of estrus. During gestation, endometrial gland hyperplasia occurs between Days 15 and 50 of pregnancy and hypertrophy of the uterine glands to increase surface area for maximal production of histotroph occurs after Day 60 of gestation. Sequential exposure of the ovine endometrium to E2, P4, IFNT, CSH1 and GH1 constitutes a 'servomechanism' responsible for activating and maintaining endometrial remodeling, secretory function and uterine growth during gestation. Downregulation of PGR in uterine epithelial cells is a prerequisite for P4-induced expression of genes in uterine GE that include *SPP1* (secreted phosphoprotein 1), *UPT1* (uterine plasmin trypsin inhibitor) and *STC1/STC2* (stanniocalcin). Treatment of ewes with a combination of P4 and E2 increases the expression of *ESR1* and *PGR* in uterine GE, and the increase in *ESR1* inhibits expression of *SPP1* and *UPT1*. Thus, IFNT is a prerequisite to the developmentally programmed sequence of events, mediated by specific paracrine-acting factors at the conceptus-endometrial interface, responsible for both remodeling of the inter-caruncular endometrium and differentiated functions of uterine GE to increase production of histotroph for fetal-placental growth during gestation.

Interferon tau induced effects distal to the conceptus/uterine complex

In addition to the paracrine effects of IFNT secreted by the trophectoderm on the underlying endometrium, IFNT-stimulated genes (ISGs) are expressed in blood cells and cellular components of the CL. IFNT secreted (Oliveira *et al.* 2008) or infused into the uterine vein (Bott *et al.* 2010) of sheep initiated a peripheral antiviral response (e.g., increased expression of interferon stimulated gene 15 (ISG15) in immune cells in maternal blood) to possibly protect the pregnancy from maternal viral infection. The endocrine action of IFNT impacts the CL to induce luteal resistance to PGF (Antoniazzi *et al.* 2013), enhances expression of ISG15 in luteal cells (Oliveira *et al.* 2008, Yang *et al.* 2010) and/or alters functions of immune cells within the CL to maintain CL function and pregnancy (Shirasuna *et al.* 2015).

A bovine animal model was developed to further understand the integration of signalling between IFNT and immune cells that contribute to maintenance of progesterone secretion by large luteal cells in pregnancy (Shirasuna *et al.* 2015). Compared to non-pregnant cows, the CL of pregnant cows on Day 16 after insemination have greater expression of mRNAs for *ISG15* and interleukin 8 (*IL8*), as well as IL8 protein, while expression of *TNF α* mRNA is reduced in pregnant compared to non-pregnant cows. Furthermore, CL of pregnant cows have greater numbers of neutrophils.

Although IFNT does not directly affect the secretion of progesterone (P4) by large luteal cells or migration of neutrophils, IFNT increased expression of *ISG15* and *IL8* mRNAs in large luteal cells. Both *IL8* and *ISG15* stimulate neutrophil migration *in vitro*, but IFNT was ineffective. If neutrophils were pre-treated with IFNT, then IL8 induced a greater neutrophil migration response. When large luteal cells were co-cultured with neutrophils, secretion of P4 increased in response to IFNT compared to amounts of P4 secreted when large luteal cells were cultured alone or co-cultured with neutrophils, but not treated with IFNT. IL8-stimulated secretion of P4 by large luteal cells cultured alone. The increase in neutrophils and IL8 within the CL of pregnant cows suggests additional complementary involvement of IFNT within the CL to maintain secretion of P4 and pregnancy in cows. This model indicates that IFNT stimulates expression of IL8 in luteal cells that activates and induces migration of neutrophils, which stimulate large luteal cells to increase or sustain secretion of P4 in cows during early pregnancy. Thus, IFNT appears to induce both antiluteolytic and complementary intra-ovarian effects that ensure maintenance of a functional CL for maintenance of pregnancy. Further investigations into the integration of the immune and endocrine systems of early pregnancy, via IFNT acting distal to the conceptus/endometrial unit are warranted to advance

understanding of mechanisms for maintaining the CL of early pregnancy.

Potential therapeutic effects of oral interferon tau

The effects of IFNT are not limited to ruminant species and pregnancy. There are reports that IFNT induces immunological tolerance in several animal models. Matings between DBA/2 × C57B mice result in high rates of fetal resorptions, but treatment of dams with exogenous IFNT prevents this pregnancy wastage in an IL10-dependent manner (Chaouat *et al.* 1995, 2004). In the experimental allergic encephalomyelitis (EAE) mouse model, IFNT suppresses EAE by stimulating IL10, i.e., a Th2 response (Soos *et al.* 2002). Treatment of NOD mice with IFNT either orally, intraperitoneally or subcutaneously delays or inhibits development of diabetes (Sobel *et al.* 2008). During pregnancy and in response to IFNT, MHC class I and $\beta 2$ microglobulin gene expression is silenced in uterine epithelia, but stimulated by IFNT in uterine stroma and glandular epithelium as a component of the mechanism(s) to prevent immune rejection of the conceptus allograft (Choi *et al.* 2003). These results indicate that IFNT affects the relative abundance of Th1 and Th2 cytokines produced by immune cells to favour a Th2 anti-inflammatory phenotype of tolerance expected to abrogate the progression of inflammatory diseases in humans and animals. IFNT inhibits human immunodeficiency virus (HIV) replication *in vitro* more effectively than human IFNA, particularly in human macrophages wherein it decreased intracellular HIV RNA and inhibited reverse transcription of viral RNA into proviral DNA (see Maneglier *et al.* 2008). Interferon tau increased synthesis of cellular antiviral factors including 2',5' oligoadenylate synthetase 1, ribonuclease L and myxovirus resistance 1, mouse, homolog of proteins, as well as the anti-inflammatory cytokines interleukin 10 and interleukin 6.

Interferon tau and obesity

Recognizing the link between inflammation, obesity and the anti-inflammatory properties of IFNT, studies were initiated to determine the effects of oral IFNT on time of onset of Type 2 diabetes (T2D), oxidation of glucose and fatty acids in insulin-sensitive tissues, energy expenditure, body mass and tissue weights in Zucker diabetic fatty (ZDF) rats, an animal model of T2D and obesity (see Tekwe *et al.* 2013). Oral administration of IFNT (8 μ g/kg body weight/day) reduced oxidative stress, increased oxidation of energy substrates to CO₂ in insulin-sensitive tissues, decreased white adipose tissue by 40%, increased brown adipose tissue by 46%, increased whole-body energy expenditure and ameliorated oxidative stress in insulin-sensitive tissues (i.e., decreased ratios of oxidized to reduced glutathione), and increased concentrations

of the antioxidant tetrahydrobiopterin. Collectively, metabolic profiles were improved (Tekwe *et al.* 2013). Because IFNT stimulates oxidation of energy substrates and reduces obesity in ZDF rats, IFNT has important implications for treating obesity-related diseases.

Using a diet-induced obesity mouse model treated orally with IFNT in water (8 μ g/kg/day), insulin sensitivity in obese mice was enhanced. This was due to a significant decrease in secretion of proinflammatory cytokines and increases in anti-inflammatory macrophages (M2) and T-reg cells in white adipose tissue (Ying *et al.* 2014, 2016). Thus, IFNT can significantly mitigate obesity-associated systemic insulin resistance and tissue inflammation by controlling macrophage polarization and the population of anti-inflammatory T-reg cells.

Summary

The discovery of IFNT as the pregnancy recognition signal in ruminants and understanding of its mechanism of action have been established over the past few decades. This knowledge had led to translational research to explore its use for pregnancy diagnosis in ruminants by measuring it directly in maternal blood or assessing increases in the expression of interferon-stimulated genes by immune cells in maternal blood. Further, the anti-inflammatory roles of IFNT in treatment of autoimmune diseases such as obesity, diabetes, multiple sclerosis, psoriasis and others remain to be explored through clinical trials to evaluate the potential therapeutic roles of the novel IFNT in treatment of human and animal conditions.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review..

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in pigs and ruminants. They have not cited each paper relevant to the history of IFNT, but additional papers can be obtained by querying PubMed. Between 1970 and 1985, the order in which authors were listed was the order of contribution to the work. After 1985, the corresponding author is the last author listed. Trainees were first author on most papers published based on results of their research for their respective graduate degree.

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