Endocrine actions of interferon-tau in ruminants

TR Hansen[1], LK Henkes[1], RL Ashley[1], RC Bott[1], AQ Antoniazzi[1,2] and H Han[3]

[1]*Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences and* [2]*BioRep, Departamento de Clínica de Grandes Animais, Centro de Ciências Rurais, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil;* [3]*Department of Animal Sciences, Colorado State University, Campus Delivery 1683, Fort Collins, CO 80523, USA*

The ovine conceptus releases interferon-τ (IFNT), which prevents upregulation of the endometrial estrogen receptor (ESR1) and, consequently, oxytocin receptor (OXTR), thereby disrupting pulsatile release of prostaglandin F2α (PGF) in response to oxytocin. IFNT, through paracrine action on the endometrium, protects the corpus luteum (CL) during maternal recognition of pregnancy. Pregnancy also induces IFN stimulated genes (ISGs) in peripheral blood mononuclear cells (PBMCs), which is interpreted to reflect a "prompted" antiviral and immune cell response peripherally in ruminants. IFNT was recently demonstrated to be released from the uterus in amounts of ~ 200 μg (2 x 10^7 U)/24 h via the uterine vein and to induce ISGs in the CL during maternal recognition of pregnancy. Delivery of recombinant ovine (ro) IFNT into the uterine vein in a location that is upstream of the utero-ovarian plexus from Day 10 to 17 maintained serum progesterone concentrations and extended normal 16-17 d estrous cycles to beyond 32 d. It is concluded from these studies that IFNT is released into the uterine vein and initiates a peripheral antiviral response to protect pregnancy from maternal viral infection. It also may have endocrine action through inducing luteal resistance to PGF and longer-term survival of the CL and maintenance of pregnancy.

Introduction

The timing of release and composition of signals from the conceptus are critical for maintenance of the CL and pregnancy. For example, transferring a Day 5-9 embryo into the uterus of Day 5 estrous cycling ewes extends luteal lifespan (Moor & Rowson 1966b). If a Day 12-13 embryo is transferred into a recipient ewe later than 12 d post-estrus, the chances of the pregnancy surviving to term are drastically reduced (Moor & Rowson 1964, Moor & Rowson 1966a). Therefore, the uterus must receive and respond to a signal from the conceptus by Day 12, after which time it becomes unable to maintain pregnancy. In ewes with one surgically isolated uterine horn, only the CL ipsilateral to the gravid horn survived (Moor & Rowson 1966a), suggesting that the embryo has a local effect on the gravid uterus. Embryos transferred to an isolated horn of ewes with CL on both ovaries only maintained the CL on the side ipsilateral to the embryo (Moor & Rowson 1966a). These experiments were interpreted to indicate that a "local unilateral relationship between the embryo and the corpus luteum" existed and that a systemic response to the conceptus was not necessary to maintain early pregnancy. Hansen et

Corresponding author E-mail: thomas.hansen@colostate.edu

al. reported an interestrous interval of 19 d when conceptuses were flushed on Day 13 and an extension to 35 d when flushed on Day 17 (Hansen *et al.* 1985). These data are interpreted to mean that signals from the conceptus are necessary by Day 12-13 and need to continue until Day 17 to protect the CL from lytic release of uterine-derived PGF.

The major conceptus secretory protein was initially termed protein X and is secreted during Days 10-12 (Godkin *et al.* 1982, Bazer & Roberts 1983). Protein X, was renamed ovine trophoblast protein-1, and later IFNT as reviewed in (Bazer *et al.* 1991, Roberts *et al.* 1992). Peak secretion of IFNT from the ovine conceptus occurs between Days 15-17 of pregnancy (Hansen *et al.* 1985, Ashworth & Bazer 1989, Roberts 1989). IFNT acts through the Type I interferon receptor which shares two subunits, IFNAR1 and IFNAR2. These subunits are expressed in the luminal epithelium, sub-luminal glandular epithelium, and stroma of the ovine uterus in Day 14-15 cyclic and pregnant ewes (Rosenfeld *et al.* 2002). Paracrine action of IFNT on the endometrium has been shown to alter (ewe) (Zarco *et al.* 1988a, Zarco *et al.* 1988b) or attenuate (cow) (Meyer *et al.* 1995) luteolytic pulses of PGF (based on detection of PGFM). Nonpregnant ewes secrete PGF in a pulsatile fashion, while pregnant ewes have a more constant, slowly increasing pattern in the release of PGF (Peterson *et al.* 1976, Zarco *et al.* 1988b). However, more PGF is found exiting the uterus through the uterine vein in Day 13 pregnant vs cyclic ewes (Wilson *et al.* 1972). This antiluteolytic mediation in release of PGF from the endometrium during pregnancy is regulated by conceptus-derived IFNT.

Because the release of PGF from the endometrium is not completely ablated and the CL produces PGF (Silva *et al.* 2000), several groups have described the CL of pregnancy to be more resistant to lytic effects of PGF compared to the CL of the estrous cycle (Inskeep *et al.* 1975, Mapletoft *et al.* 1976, Pratt *et al.* 1977, Silvia & Niswender 1984). Exactly why and how this luteal resistance to PGF occurs during pregnancy is unknown. Also, whether the actions of intraluteal PGF or PGF that continues to be delivered into the uterine vein need to be blocked during early pregnancy is unknown. This review examines endocrine action of pregnancy in ruminants. It is proposed that endocrine release of IFNT into the uterine vein upregulates peripheral antiviral and immune cell responses, which when further challenged with viral infection, immediately respond and protect the pregnancy. A second endocrine action of IFNT during early pregnancy is proposed through induction of interferon-stimulated genes (ISGs) in the CL, which contributes to luteal resistance to PGF.

Luteal cells and luteolysis

Late in the ovine luteolytic process (Day 16), release of progesterone diminishes because of PGF action on large luteal cells. Oxytocin release by large luteal cells and action of oxytocin on small luteal cells further inhibits secretion of progesterone and stimulates intracellular levels of calcium and apoptosis (Niswender *et al.* 2007). PGF also is released from large luteal cells, possibly through a PG transporter (SLCO2A1), and binds PGF receptor (PTGFR) on large luteal cells to further stimulate oxytocin release, increases in intracellular calcium concentrations and, consequently, death of the CL (Davis *et al.* 2010). Luteolysis may also include PGF activation of protein kinase C (PKC) and RAF/MEK1/ERK-mediated increase in early growth response 1 (EGR1) and transforming growth factor B (TGFB1) (Hou *et al.* 2008) as well as repression of insulin like growth factor (IGF-1) and cell-survival responses (phosphoinositide 3-kinase; PI3K and protein kinase B; Akt) (Arvisais *et al.* 2010).

Paracrine action of IFNT

Ruminant conceptuses are free-floating in the uterus during maternal recognition of pregnancy. Thus, paracrine action of IFNT regulates endometrial gene expression and indirectly induces antiluteolytic responses (Godkin *et al.* 1984a, Godkin *et al.* 1984b). In addition to the classical JAK/STAT pathway (Hansen *et al.* 1999, Perry *et al.* 1999, Binelli *et al.* 2001, Pru *et al.* 2001b), Type I IFN also activates PI3K and Akt pathways (Rani *et al.* 2002, Badr *et al.* 2010). IFNT silences up-regulation of ovine *ESR1* (Spencer *et al.* 1995, Spencer & Bazer 1996, Fleming *et al.* 2001) and, consequently, *OXTR* (Spencer & Bazer 1996, Chen *et al.* 2006) in the endometrium. Decreased transcription of the *OXTR* is likely due to the decrease in ESR1 caused by IFNT, as *OXTR* is not directly regulated by IFNT (Fleming *et al.* 2006). Suppressed ESR1 and OXTR in the endometrium causes alteration (ewe) (Zarco *et al.* 1988a, Zarco *et al.* 1988b) or attenuation (cow) (Meyer *et al.* 1995) of luteolytic pulses of PGF (based on detection of PGFM).

Several ISGs have been identified in the ruminant uterus such as 2′, 5′-oligoadenylate synthetase (*OAS*) (Mirando *et al.* 1991, Schmitt *et al.* 1993, Johnson *et al.* 2001), myxovirus (influenza virus) resistance (*Mx*) (Ott *et al.* 1998), and IFN-stimulated gene 15 (*ISG15*) (Naivar *et al.* 1995, Austin *et al.* 1996, Johnson *et al.* 1999b). One conserved primate (Bebington *et al.* 1999a, Bebington *et al.* 1999b, Bebington *et al.* 2000), mouse (Austin *et al.* 2003, Bany & Cross 2006), and bovine (Austin *et al.* 1996, Hansen *et al.* 1997, Johnson *et al.* 1998, Perry *et al.* 1999, Thatcher *et al.* 2001) uterine response to pregnancy is induction of the ubiquitin homolog, ISG15. ISG15 mediates processes such as RNA splicing, chromatin remodeling/polymerase II transcription, cytoskeletal organization and regulation, stress responses, translation and viral replication (Malakhova *et al.* 2003, Giannakopoulos *et al.* 2005, Zhao *et al.* 2005, Takeuchi *et al.* 2006).

Intrauterine delivery of roIFNT delays return to estrus

Because IFNT acts locally on endometrial release of PGF, models were developed in sheep to test effects of intrauterine infusion of IFNT on interestrous interval. Intrauterine infusion of 50 μg native IFNT twice daily extended interestrous interval to 27 d (Vallet *et al.* 1988). Intrauterine infusion of 340 μg roIFNT for 8 d extended return to estrus from 25-64 d in four out of five ewes (Martal *et al.* 1990). Likewise, intrauterine infusion of 1.4×10^7 U/d roIFNT from Day 10-18 delayed return to estrus to 33 ± 14 d (Green *et al.* 2005). These studies were interpreted to mean that IFNT acted in paracrine action to extend the luteal phase by attenuating pulses of PGF and, thereby, protect the CL through antiluteolytic action. Importantly, the potential entry of exogenous IFNT from the intrauterine infusion studies was not evaluated in the context of endocrine action through its potential direct impact on the maintenance of CL function.

Endocrine action of IFNT

Systemic delivery of roIFNT induces hyperthermia, but has varied impact on fertility

Delivery of Type I IFN, via intramuscular or subcutaneous injection also was examined by several groups for capacity to extend interestrous interval and increase fertility (Nephew *et al.* 1990, Martinod *et al.* 1991, Schalue-Francis *et al.* 1991, Davis *et al.* 1992). These studies employed mg quantities of rIFN administered through twice daily injections (subcutaneous), which may not reflect physiological levels of IFNT released by the uterus. These quantities (mg) of rIFN induced hyperthermia, and no effect or a decline in fertility. For example, Ott and colleagues (Ott *et al.* 1997) observed an induction of mild hyperthermia following sub-

cutaneous injections of 2, 4 or 6 mg rIFNT on Day 12 post estrus. These investigators also injected 1, 2 or 4 mg roIFNT/d on Days 11-15 and described a modest delay in onset of estrus after adjusting for previous length of estrous cycle. These doses of roIFNT were subsequently reduced to 2 x 10^7 U (200 μg) in intrauterine deliveries to avoid hyperthermia and high death loss of ewes (Spencer *et al.* 1999).

Subcutaneous (Spencer *et al.* 1999) and intramuscular injection (2 mg) (Chen *et al.* 2006) of roIFNT given between Days 11-17 stimulated ISG15 expression within the ovine CL. Chen and colleagues (Chen *et al.* 2006) reported an inter-estrus interval of 32.7 d in ewes that received intrauterine infusions of 200 μg roIFNT, but an average interval of only 17 and 22 d in ewes that were injected i.m. with 200 μg or 2 mg roIFNT, respectively. These investigators also described an increase in endometrial ISG15 expression in response to infusion of roIFNT and injection of 2 mg roIFNT, but not following injection of 200 μg roIFNT. None of these systemic methods of roIFNT treatment prolonged return to estrus for more than a few days.

Induction of ISGs in blood cells during early pregnancy

Until recently (Oliveira *et al.* 2008, Bott *et al.* 2010), IFNT was thought to be sequestered within the uterine lumen and not present in peripheral circulation in high enough concentrations to be detected. Although, IFN-alpha has been shown to suppress tumor necrosis factor α and IFNγ-stimulated prostaglandin production by cultured luteal cells (Pate 1995). Likewise, culture of luteal cells with IFN-alpha and in concentrations of progesterone similar to those observed during early pregnancy also suppressed IFNγ-induction of MHC class II glycoproteins. Pate (1995) concluded from these studies that a signal similar to trophoblast-derived IFNT might reach the ovary and act directly to protect the CL.

ISGs such as MX1 (Ott *et al.* 1998), ISG15 (Johnson *et al.* 1999a, Johnson *et al.* 1999b) and OAS-1 (Johnson *et al.* 2001) have been shown to be upregulated in uterine cross sections as deep as the myometrium. For this reason, IFNT was suspected to induce a secondary mediator in the myometrium. This secondary mediator of IFNT action was called an "interferon-medin" (Spencer *et al.* 1996).

PBMCs from pregnant sheep have increased concentrations of ISGs mRNA (Yankey *et al.* 2001) compared to nonpregnant sheep, which also is the case in cattle (Han *et al.* 2006, Gifford *et al.* 2007). Presence of ISGs in extrauterine tissues such as jugular PBMCs provoked study of ISGs in uterine vein and uterine artery blood as well as the CL (Oliveira *et al.* 2008, Bott *et al.* 2010). Concentrations of ISG15 mRNA in jugular vein on Day 15 of pregnancy were similar to uterine vein and artery ISG15 concentrations suggesting endocrine induction of ISGs through the presence of the conceptus and release of either IFNT or an interferon-medin from the uterus.

Very little is known about the genes that are regulated in blood cells during early pregnancy and no studies have been done to compare blood and endometrial gene expression in response to pregnancy. We hypothesized that several genes would be upregulated by pregnancy on Day 18 of bovine pregnancy in endometrial and blood cells. Several hundred endometrial (674 genes upregulated and 721 downregulated $\geq$ 1.5 fold; $P < 0.05$) and blood cell (375 genes upregulated and 784 downregulated $\geq$ 1.2 fold; $P < 0.05$) genes were differentially expressed based on pregnancy status on Day 18 of pregnancy (United States Patent Application: 20100035270 and Fig. 1). Upregulated ISGs in endometrium (Fig. 1) were similar to other reports using microarray (Klein *et al.* 2005), (Bauersachs *et al.* 2006, Chen *et al.* 2006, Bauersachs *et al.* 2008) and conventional molecular biology approaches (Johnson *et al.* 1999a, Pru *et al.* 2001a, Rempel *et al.* 2005).

A

Endometrial Genes				Blood Genes			
Fold	P <	Identity	Name	Fold	P <	Identity	Name
9.1	0.0002	BE723335	IRF7	1.5	0.01	BE683272	IRF7
57.6	0.0002	NM_174366	ISG15	3.1	0.01	NM_174366	ISG15
14.1	0.00002	CK966909	UBE1L	1.9	0.002	CK966909	UBE1L
124.7	0.00001	NM_173941	MX2	1.8	0.01	NM_173941.2	MX2
14.7	0.001	CB432365	RIGI	1.7	0.01	CB432365	RIGI
15.4	0.0001	CK960499	OAS	4.5	0.001	CK960499	OAS
1.9	0.03	NM_175827.2	CCL5	2.1	0.02	NM_175827.2	CCL5
48.7	0.001	CB422521	IFI44	1.9	0.03	CB422521	IFI44
10.7	0.0005	NM_174007	CCL8	2.1	0.05	AW659977	TCRb
10.7	0.002	CB419326	GBP5	1.8	0.04	CK945023	CD3D
1.5	0.01	NM_173893.1	B2MG	1.8	0.02	NM_174015	CD8
48.5	0.0001	CB427688	~EIF4E	2.1	0.02	AB008616	MHC-1
-18.4	0.02	NM_174816	OXTR	-2.2	0.04	CK775610	Homer 2

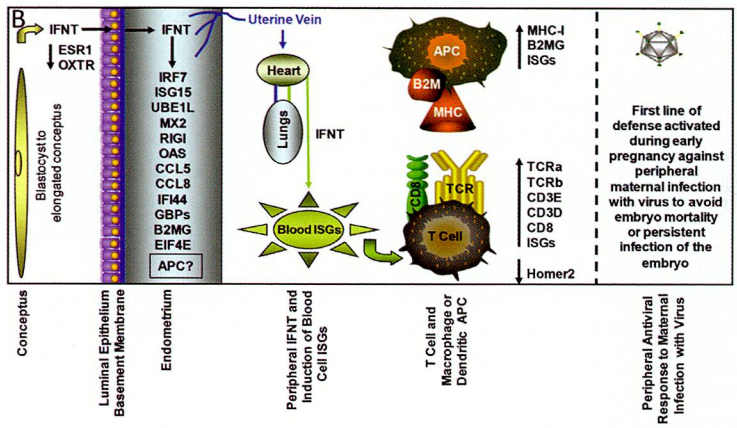

Fig. 1. Hypothetical model of conceptus-induced preemptive maternal resistance to viral infection. Panel A contains fold changes and identities for genes described in Panel B. Cows were artificially inseminated on Day 0. Presence of a conceptus was confirmed on Day 32 post AI by using ultrasound. Blood was collected from lactating Holstein Dairy cows 18 days following AI (4 pregnant and 3 nonpregnant cows) and processed to purify RNA for microarray screening according to the QiaAMP procedure (Qiagen, Inc.). Because of excessive costs in collecting the uterus from lactating dairy cows, Angus-Gelbvieh beef cows were used for endometrial studies. Endometrial RNA was isolated from cows on Day 18 of pregnancy (3 cows; conceptus identified) or the estrous cycle (3 cows; not inseminated) following slaughter and submitted for microarray analysis. The bovine Affymetryx gene chip was screened at the University of Colorado Health Sciences Center (UCHSC) DNA Microarray Core facility. Data were analyzed using GCOS and GeneSifter software. Statistical significance was determined using the t-test calculated from Robust Multichip Average data (Irizarry *et al.* 2003). A 1.5-fold cut off was used to identify all differentially expressed genes in endometrium. A 1.2-fold cut off was used to identify all differentially expressed genes in blood because there were fewer affected genes in the blood and identification of shared gene expression between blood and endometrium was one primary focus of the experiment. Panel B provides hypothesized role of IFNT in activating a peripheral maternal antiviral response. IFNT is released from the conceptus that initiates a local (*i.e.*, paracrine) type I IFN response through up-regulation of ISGs, chemokines, and other genes in the endometrium and myometrium before exiting the uterus by the uterine vein. In endocrine fashion, IFNT then transcriptionally upregulates ISGs and genes involved in activation of T cell and antigen presenting cells (APC) such as macrophages and dendritic cells. The conceptus, therefore, coordinates both local and systemic immunomodulatory events that allow the mother to cope with potential viral infections as more aggressive cytotoxic responses that may be detrimental to the histocompatibly distinct embryo are concomitantly curbed.

Virus induces retinoic acid-induced gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA-5) which contain RNA helicase domains that bind viral dsRNA and response elements that activate interferon regulatory factors (IRF3 and IRF7), which then induce synthesis of Type I IFN. Several other *ISGs* are induced to regulate viral infection such as OAS-1, which activates endoribonuclease to degrade virus; protein kinase R (PKR) to phosphorylate eukaryotic initiation factor (eIF)-2, which in turn inhibits translation and replication of virus; and ISG15 (Lenschow *et al.* 2007) and MX (MacMicking 2004). This pathway of genes is known to be required for cellular resistance to viral infection. Likewise, several indicators of T cell activation in blood cells were identified.

Homer protein homolog 2 (*HOMER2*) was a major downregulated gene in both endometrium and blood in response to pregnancy. In T cells, *HOMER2* is upregulated after T cell activation (Diehn *et al.* 2002), however it is considered a negative regulator of T cell activation and production of IL-2 (Huang *et al.* 2008). A downregulation of *HOMER2* would allow better access for stimulation of nuclear factor of activated T cells (NFAT) by calcineuron (Huang *et al.* 2008). Other indicators that T cells are activated in response to IFNT include upregulation of many T cell receptor (TCRA, TCRB, TCRG) components such as T cell surface glycoproteins CD3D, CD3E and CD8. IRF7 is a key regulator of Type I IFN-dependent activation of CD8 positive T cells (Honda *et al.* 2005). Both *CD8* and *IRF7* were upregulated in blood in response to pregnancy. CD8 is known to function as a co-receptor with TCR when interacting with major histocompatibility complex (MHCI) on target cells.

Type I IFNs also induce MHCI on many cell types and activate not only T cells, but also natural killer cells. Based on the microarray data, *MHCI* was upregulated in blood cells from Day 18 pregnant when compared to nonpregnant cows and is normally upregulated in professional antigen presenting cells such as dendritic cells and tissue macrophages. The apparent upregulation of blood cell MHCI in response to pregnancy is based on hybridization to probe sets in the microarray and may have been caused by different MHC haplotypes that were expressed by the limited number of cows used in this study (Davies *et al.* 1994). However, beta-2-microglobulin (B2MG) was upregulated in endometrium, but not in blood cells, which might reflect expression of this binding partner for MHCI in tissue followed by homing or translocation of pre-antigen presenting blood cells. Following viral infection of these cells, massive upregulation of RIGI and MDA5 RNA helicases would assist in degrading viral RNA. Processing of viral coat proteins and presentation of antigen would be facilitated through existing upregulation of MHCI and B2MG as well as enhancing the presence of activated CD8 T cells.

Upregulation of CD8 and TCR activated T cells and MHCI/B2MG antigen presenting cells could be detrimental because the conceptus expresses foreign antigens that would clearly be recognized by these cells. However, in ruminants at this very early stage of pregnancy, the conceptus has developed only very peripheral adhesion complexes to the uterine lumen with no penetration of the basement membrane of the endometrium. For this reason, the uterine lumen remains isolated from any circulating T cells. Also *B2MG* and MHC class I genes are silenced in ovine endometrial luminal epithelium and trophectoderm even though they are upregulated in other endometrial layers (Choi *et al.* 2003). Very few immune cells are localized to the endometrium during this stage of pregnancy in ruminants (Vander Wielen & King 1984). This might be caused by lack of specific chemokine receptors on these cells and/or lack of endometrial production of chemokines that would specifically recruit these cells to the endometrium. Alternatively, this could be accounted for by increased expression or presence of immune cell repulsive factors.

However, we described macrophage inflammatory protein (CXCL2) (Hansen et al. 1999) and granulocyte chemotactic protein-2 (CXCL6) (Teixeira *et al.* 1997) to be induced by pregnancy

and IFNT in bovine endometrium. Also, other groups demonstrated that CXCL9, CXCL10, CXCL11 and CXCR3 are localized to the maternal-fetal interface in sheep during early pregnancy (Imakawa *et al.* 2006). Likewise, monocyte chemotactic protein (Asselin *et al.* 2001) and IFN-gamma inducible protein 10 (Nagaoka *et al.* 2003) have been shown to be expressed in endometrium in response to pregnancy. Based on enclosed microarray analysis, chemokine receptor mRNA concentrations were not upregulated in blood cells from Day 18 pregnant when compared to nonpregnant cows. Chemokine ligand 5 (*CCL5*) was the only major chemokine mRNA that was significantly upregulated in blood. Likewise, only three chemokine ligands, *CCL8, CXCL10 and CCL5* were extensively upregulated in the endometrium. CCL5 induces recruitment of T cells to the lung following viral lung disease (Culley *et al.* 2006). CCL8 is chemotactic for monocytes and CD8+ as well as CD4+ T cells (Taub *et al.* 1995) and might recruit these cells to the endometrium in the event of infection. However, if the associated chemokine receptors are not differentially expressed on these T cells, then very few of these cells would be actively recruited to the endometrium, unless there was a maternal-peripheral infection with virus. Because these maternal antiviral T cells and antigen presenting cells are activated through exposure to Type I IFN from the conceptus, they would immediately be recruited to the endometrium where a first or last line of defense would be established to inhibit virus from passing through the endometrial basement membrane and infecting the conceptus (Fig. 1).

Direct endocrine action of IFNT on the corpus luteum

Endocrine release of conceptus-derived IFNT into the uterine vein. Lymph nodes draining the uterus (iliac) and the head (submandibular) from Day 15 pregnant ewes were examined and found not to differ in ISG gene expression (Antoniazzi et al., unpublished results); suggesting that IFNT was not released into uterine lymphatic drainage and this was not a pathway through which IFNT induced PBMC ISGs. This also was consistent with previous reports describing no antiviral activity in lymph draining the uterus during pregnancy (Lamming *et al.* 1995). However, Godkin and co-workers demonstrated that infusion of radiolabeled IFNT into the uterine lumen resulted in escape of very low levels into the blood (Godkin *et al.* 1984a). Schalue-Francis (Schalue-Francis *et al.* 1991) described antiviral activity in the uterine vein, although levels reported were very low. For these reasons we re-examined the uterine vein as a source for endocrine delivery of IFNT. Antiviral activity was evaluated in uterine vein blood from Day 15 pregnant sheep, which revealed significant amounts of Type I IFN (~200μg/24h) on Day 15 of pregnancy (Oliveira *et al.* 2008). Also, pre-adsorption of uterine vein blood from Day 15 pregnant ewes with antibody against roIFNT significantly reduced antiviral activity (Bott *et al.* 2010). It was concluded from these studies that IFNT is released into the uterine vein on Day 15 of pregnancy. Other than Day 15, the timing and concentration of IFNT in uterine vein blood is unknown and is a focus of ongoing study.

Endocrine induction of ISGs in large luteal cells. ISG15 mRNA concentrations were uregulated in CL from Day 15 pregnant compared to nonpregnant ewes (Fig. 2) (Oliveira *et al.* 2008, Bott *et al.* 2010). ISG15 protein and conjugation of ISG15 to targeted proteins also were upregulated in CL in response to pregnancy. ISG15 was predominantly localized to large luteal cells on Day 15 of pregnancy, with less, but significant localization to small luteal cells. Also, large luteal cells isolated on Day 10 of the estrous cycle and cultured with roIFNT for 24 h showed significant induction of ISG15 (Oliveira *et al.* 2008). Finally, IFNAR1 and IFNAR2 mRNA are expressed in the ovine CL (Antoniazzi et al., unpublished results). It was concluded from these studies that in addition to extensively characterized paracrine action on

the endometrium, IFNT also likely has direct endocrine action on extrauterine tissues such as blood cells and the CL.

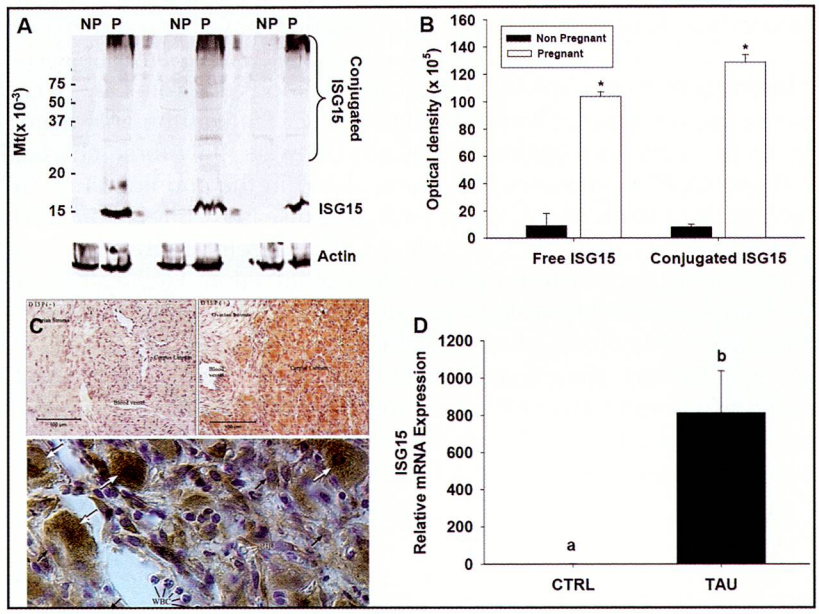

Fig. 2. Western blot of ISG15 (15-kDa) and its conjugates (A, B), immunohistochemical localization of ISG15 in CL (C) and induction of ISG15 following culture of isolated large luteal cells (Day 10 of estrous cycle) with 100 ng/ml roIFNT for 24 h (D). Both free and conjugated ISG15 were induced (*; $P < 0.05$) by pregnancy and in response to culture with roIFNT (a, b; $P < 0.05$). Imunohistochemical staining for ISG15 was upregulated in cross-sections from CL on Day 15 of pregnancy (C: top right panel). Panel C Upper left: no primary antibody control. Localization of ISG15 was most intense in large luteal cells (lower panel in C; white arrows). For more details please see (Oliveira *et al.* 2008). Copyright 2008, The Endocrine Society.

Endocrine delivery of 200 μg roIFNT/d into the uterine vein for 7 d delayed return to estrus. Release of IFNT into the uterine vein on Day 15 of pregnancy was estimated previously to be ~ 200 μg/d (Oliveira *et al.* 2008). For this reason, osmotic pumps loaded to deliver 200 μg/d into the uterine vein for seven consecutive days were surgically installed. Estimated blood volume in sheep was 3.48 L based on average weight of 60 kg and blood volume of 58 ml/kg. Thus, on Day 15 of pregnancy, systemic levels in circulation would stabilize around 2.4 ng/ml/h. This is biologically relevant considering the dissociation constant (Kd) of 3.7×10^{-10} M (Li & Roberts 1994) and estimated 50% occupancy of the receptor at 6.3 ng IFNT/ml.

Osmotic pumps delivering 200 μg roIFNT into the uterine vein/d were surgically installed on Day 10 of the estrous cycle in sheep (Fig. 3). Eighty percent (4/5) of ewes infused with roIFNT for 7 d had extended estrous cycles and luteal phase serum progesterone concentrations through 32 d (Bott *et al.* 2010). In the nonresponder ewe, serum progesterone concentrations were declining at the time of installation of the pump, which was interpreted as onset of luteal regression prior to endocrine delivery of roIFNT. To our knowledge this is the first report of such small endocrine concentrations of IFNT to induce a significant long-term delay in return to estrus.

There was no effect of 24 h endocrine delivery of roIFNT into the uterine vein starting on Day 10 of the estrous cycle on serum progesterone concentrations (Fig. 4) (Bott *et al.* 2010). A

sub-luteolytic dose of PGF (4 mg/58 kg), described previously to cause a significant decline in serum progesterone without complete luteolysis (Silvia & Niswender 1984, Silvia & Niswender 1986, Silva *et al.* 2000, Bott *et al.* 2010), was injected 12 h following delivery of roIFNT into the uterine vein. PGF caused a significant decline in serum progesterone concentrations within 6 h, even in ewes with 12 h pre-exposure to delivery of roIFNT into the uterine vein. However, by 8-12 h after injection of PGF serum progesterone returned to concentrations in roIFNT-infused ewes that were intermediate and not different from BSA- or roIFNT-infused ewes in the absence of PGF injection. Endocrine delivery of IFNT also induced upregulation of ISG15 mRNA in the endometrium and the CL. This "recovery" in serum progesterone concentrations was suggested to reflect induction of luteal resistance to PGF through endocrine delivery of IFNT.

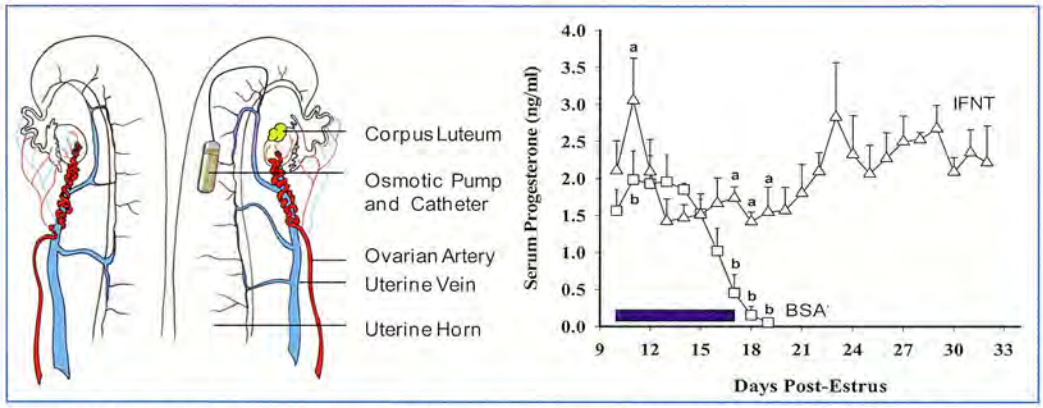

Fig. 3. Osmotic pump delivery (Left Panel) of 200 μg roIFNT (2 x 10^7 IU)/d roIFNT or BSA for 7 d into the uterine vein caused extension of the estrous cycle and maintenance of serum progesterone concentrations (Right Panel) in 80% of ewes. Shaded bar represents time of osmotic pump infusion (Days 10-16). Adapted from Bott *et al.* 2010.

Intraluteal prostaglandins

Concentrations of 15 hydroxyprostaglandin dehydrogenase (PGDH) and prostaglandin-endoperoxide synthase 2 (PTGS2) mRNAs increase in CL on Day 13 of pregnancy compared to the estrous cycle suggesting that the CL of pregnancy is better able to degrade endogenous PGs in the face of increased synthesis via PTGS2 (Silva *et al.* 2000). This upregulation of PTGS2 might contribute to reduced luteal sensitivity to PGF through shifting biosynthesis to PGE2 (Costine *et al.* 2007) through PGE synthase (PTGES).

PGDH, PTGES, PGFS, and PTGS2 mRNA concentrations did not change following 24 h infusion of IFNT into the uterine vein (Bott *et al.* 2010). This was interpreted to suggest that IFNT does not induce luteal resistance through inhibiting mRNAs encoding intraluteal biosynthesis of PGs, at least on Day 10 of the estrous cycle. SLCO2A1 facilitates transport of PGs across membranes (Chan *et al.* 1998, Schuster 1998, Endo *et al.* 2002, Schuster 2002). PGF is transported through SLCO2A1 in the ovine endometrim (Banu *et al.* 2008) and through countercurrent exchange in the utero-ovarian plexus (Lee *et al.* 2010). One action of IFNT might be to downregulate SLCO2A1 in the utero-ovarian plexus and thus disrupt delivery of PGF to the CL. SLCO2A1 may also regulate release of PGF from large luteal cells during autocrine activation of PTGFR to induce luteolysis (Niswender *et al.* 2007). Downregulation of SLCO2A1 in large luteal cells might impair release of PGF and, consequently, autocrine action of PGF on large luteal cell apoptosis. Another target in the CL for development of resistance to luteolysis is downregulation of the PGTFR. However, it has been reported that PTGFR mRNA (Juengel

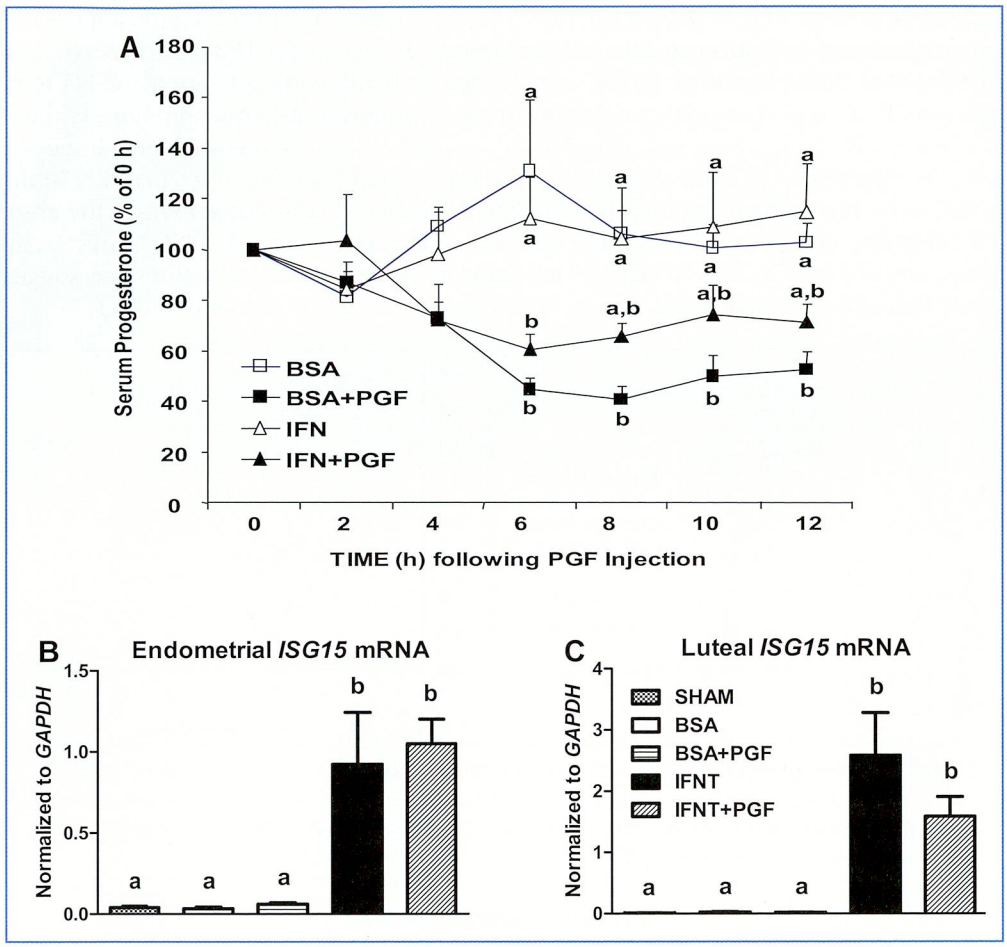

Fig. 4. Endocrine delivery of IFNT into the uterine vein protects the CL from PGF through attenuating the decline in serum progesterone (A) and possibly through induction of ISGs in the endometrium (B) and CL (C). BSA or roIFNT (200 μg/24h) were infused into the uterine vein in 24 ewes (12 ewes per treatment). Half of these ewes were injected with PGF (4mg/58 kg bw) 12 h later. Panel A describes a decrease in serum progesterone concentrations 6 h following PGF regardless of infusion treatment. However, in roIFNT infused ewes, serum progesterone concentration increased after 6 h to levels not different from controls. Data are the mean (n = 6 ewes per treatment) $\pm$ SE. Significant differences ($P < 0.05$) in treatments and time are denoted with different letters. Endometrium and CL (Panel C) were collected and examined for ISG15 mRNA concentrations, which increased following endocrine delivery of roIFNT. Means differ ($P < 0.05$) when designated by different superscripts. Adapted from Bott *et al.* 2010.

et al. 1998), protein (Wiepz *et al.* 1992) and affinity constant (Wiepz *et al.* 1992) are not attenuated during maternal recognition of pregnancy in sheep. These nicely designed studies concluded that the mechanism through which the ovine CL achieves resistance to PGF during early pregnancy does not involve PTGFR. Prostaglandin E2 receptor (PTGER2) is coupled to the cAMP signal transduction pathway, which stimulates steroidogenesis and production of progesterone in cultured human luteinized granulosa (Chandras *et al.* 2007). PTGER4 also is a G-protein receptor coupled to cAMP as reviewed in (Sugimoto & Narumiya 2007), but to our knowledge, functional coupling of this receptor subtype to synthesis of progesterone has not

been studied in the CL. Upregulation of PGE2 receptors coupled to cAMP signaling in the CL during pregnancy might also contribute to luteal resistance to PGF. Regardless of the specific PTGERs involved, the general steroidogenic action of PGE2 has been extensively studied since the first report that it induces adenylate cyclase in bovine CL (Marsh 1971).

Conclusions

IFNs were discovered as antiviral cytokines (Isaacs & Lindenmann 1957). Viral infection of a pregnant cow can result in vertical transmission to the fetus (Casaro *et al.* 1971). A significant upregulation of Type I IFN and ISGs has been described in PBMC following infection of pregnant heifers with bovine viral diarrhea virus prior to and following development of the fetal immune system (Smirnova *et al.* 2008, Shoemaker *et al.* 2009, Smirnova *et al.* 2009). This defense to viral infection also is initiated through release of IFNT from the conceptus during early pregnancy. Through prompting, but not completely activating maternal antiviral responses, peripheral maternal resistance may protect the pregnancy in the event that viral infection occurs.

Why the ruminant conceptus produces IFNT, a Type I IFN, in amounts large enough to induce systemic responses has been proposed to be related to maternal mediation of inflammatory and immune responses that might be detrimental to the "foreign" conceptus (Roberts *et al.* 1992). A variation in this theme is suggested herein, where the local endometrial and peripheral maternal immune responses become primed during early pregnancy through conceptus-derived IFNT to express *ISGs* that could more effectively recognize virus, mount an antiviral response and consequently prohibit transfer of any maternal viral infection to the conceptus or fetus. This antiviral mechanism is important in ruminants in context of the epitheliochorial placenta and the lack of transport of maternal antibodies to the embryo or fetus and would facilitate more rapid maternal defense to spread of viral infection to the unprotected pregnancy. Pregnancy-induced antiviral mechanisms may also exist in other mammalian species despite different modes of maternal recognition of pregnancy and implantation.

The CL required during pregnancy for 50 days in sheep and 6-8 months in cattle (Senger 2003). One critical early mechanism to protect this CL during maternal recognition of pregnancy is the release of IFNT from the conceptus and paracrine action on the endometrium to disrupt upregulation of ESR1, OXTR and luteolytic pulsatile release of PGF. IFNT also is released into the uterine vein and has endocrine action on the CL as well as PBMC. It functions to induce ISGs in the CL which are hypothesized to provide resistance to continued exposure to PGF from the uterus as well as from the CL. The CL becomes resistant to PGF in response to pregnancy (Inskeep *et al.* 1975, Mapletoft *et al.* 1976, Pratt *et al.* 1977, Silvia & Niswender 1984). Mechanisms associated with resistance to PGF might include modification of PGF receptor coupling to G-proteins, activation of PKC and associated apoptotic responses; endometrial (Banu *et al.* 2008), uterine vein (Lee *et al.* 2010) and intraluteal transport of PGF through SLCO2A1 and upregulation of receptors and luteotrophic responses to PGE2 (PTGERs) (Antoniazzi et al., unpublished results). Type I IFN, which are closely related to IFNT, protect immune cells from apoptosis through activating the PI3Kδ, Akt, Rho-A and NFκB (Badr *et al.* 2010). Cell death and apoptotic genes are induced by PGF during luteolysis (reviewed in (Niswender *et al.* 2007)). PGF-mediated induction of the PKC-Raf-MEK1-Erk pathway entails blocking the cell survival Akt pathway (Arvisais *et al.* 2010). We suspect that endocrine action of IFNT might stabilize the cell survival Akt pathway. Endocrine delivery of IFNT into the uterine vein induced a significant extension of estrous cycles (> 32 d) using the lowest amounts of IFNT to date that are relevant in context of the Kd of the IFN type I receptor. Systemic delivery of similar biochemically relevant doses of IFNT might be tested in future experiments to improve embryo survival.

Acknowledgements

We thank the following collaborators: Dr. Russell Anthony, Dr. Jason Bruemmer, Dr. Gordon Niswender, Dr. Natalia Smirnova, Dr. Brett Webb, Dr. Cristina Weiner, and Dr. Ann Hess (Colorado State University); Dr. James Pru (Washington State University), Ms. Kathleen Austin (University of Wyoming); Dr. Fuller Bazer and Dr. Tom Spencer (Texas A&M University), and Dr. João Francisco Oliveira (Universidade Federal de Santa Maria, Brazil). All experiments using animals were reviewed and approved by the Colorado State University and the University of Wyoming IACUC. Research was funded by USDA-NIFA-NRI grant 2006-35203-17258.

References

Arvisais E, Hou X, Wyatt TA, Shirasuna K, Bollwein H, Miyamoto A, Hansen TR, Rueda BR & Davis JS 2010 Prostaglandin F2alpha represses IGF-I-stimulated IRS1/phosphatidylinositol-3-kinase/Akt signaling in the corpus luteum: role of ERK and P70 ribosomal S6 kinase. *Mol Endocrinol* **24** 632-643.

Ashworth CJ & Bazer FW 1989 Changes in ovine conceptus and endometrial function following asynchronous embryo transfer or administration of progesterone. *Biol Reprod* **40** 425-433.

Asselin E, Johnson GA, Spencer TE & Bazer FW 2001 Monocyte chemotactic protein-1 and -2 messenger ribonucleic acids in the ovine uterus: regulation by pregnancy, progesterone, and interferon-tau. *Biol Reprod* **64** 992-1000.

Austin KJ, Bany BM, Belden EL, Rempel LA, Cross JC & Hansen TR 2003 Interferon-stimulated gene-15 (Isg15) expression is up-regulated in the mouse uterus in response to the implanting conceptus. *Endocrinology* **144** 3107-3113.

Austin KJ, Ward SK, Teixeira MG, Dean VC, Moore DW & Hansen TR 1996 Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. *Biol Reprod* **54** 600-606.

Badr G, Saad H, Waly H, Hassan K, Abdel-Tawab H, Alhazza IM & Ahmed EA 2010 Type I interferon (IFN-alpha/beta) rescues B-lymphocytes from apoptosis via PI3Kdelta/Akt, Rho-A, NFkappaB and Bcl-2/Bcl(XL). *Cell Immunol* **263:**31-40.

Banu SK, Lee J, Satterfield MC, Spencer TE, Bazer FW & Arosh JA 2008 Molecular cloning and characterization of prostaglandin (PG) transporter in ovine endometrium: role for multiple cell signaling pathways in transport of PGF2alpha. *Endocrinology* **149** 219-231.

Bany BM & Cross JC 2006 Post-implantation mouse conceptuses produce paracrine signals that regulate the uterine endometrium undergoing decidualization. *Dev Biol* **294** 445-456.

Bauersachs S, Mitko K, Ulbrich SE, Blum H & Wolf E 2008 Transcriptome studies of bovine endometrium reveal molecular profiles characteristic for specific stages of estrous cycle and early pregnancy. *Exp Clin Endocrinol Diabetes* **116** 371-384.

Bauersachs S, Ulbrich SE, Gross K, Schmidt SE, Meyer HH, Wenigerkind H, Vermehren M, Sinowatz F, Blum H & Wolf E 2006 Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. *Reproduction* **132** 319-331.

Bazer FW & Roberts RM 1983 Biochemical aspects of conceptus–endometrial interactions. *J Exp Zool* **228** 373-383.

Bazer FW, Thatcher WW, Hansen PJ, Mirando MA, Ott TL & Plante C 1991 Physiological mechanisms of pregnancy recognition in ruminants. *J Reprod Fertil Suppl* **43** 39-47.

Bebington C, Bell SC, Doherty FJ, Fazleabas AT & Fleming SD 1999a Localization of ubiquitin and ubiquitin cross-reactive protein in human and baboon endometrium and decidua during the menstrual cycle and early pregnancy. *Biol Reprod* **60** 920-928.

Bebington C, Doherty FJ & Fleming SD 1999b Ubiquitin cross-reactive protein gene expression is increased in decidualized endometrial stromal cells at the initiation of pregnancy. *Mol Hum Reprod* **5** 966-972.

Bebington C, Doherty FJ & Fleming SD 2000 Ubiquitin and ubiquitin-protein conjugates are present in human cytotrophoblast throughout gestation. *Early Pregnancy* **4** 240-252.

Binelli M, Subramaniam P, Diaz T, Johnson GA, Hansen TR, Badinga L & Thatcher WW 2001 Bovine interferon-tau stimulates the Janus kinase-signal transducer and activator of transcription pathway in bovine endometrial epithelial cells. *Biol Reprod* **64** 654-665.

Bott RC, Ashley RL, Henkes LE, Antoniazzi AQ, Bruemmer JE, Niswender GD, Bazer FW, Spencer TE, Smirnova NP, Anthony RV & Hansen TR 2010 Uterine vein infusion of interferon tau (IFNT) extends luteal life span in ewes. *Biol Reprod* **82** 725-735.

Casaro AP, Kendrick JW & Kennedy PC 1971 Response of the bovine fetus to bovine viral diarrhea-mucosal disease virus. *Am J Vet Res* **32** 1543-1562.

Chan BS, Satriano JA, Pucci M & Schuster VL 1998 Mechanism of prostaglandin E2 transport across the plasma membrane of HeLa cells and Xenopus oocytes expressing the prostaglandin transporter "PGT". *J Biol Chem* **273** 6689-6697.

Chandras C, Harris TE, Bernal AL, Abayasekara DR & Michael AE 2007 PTGER1 and PTGER2 receptors

mediate regulation of progesterone synthesis and type 1 11beta-hydroxysteroid dehydrogenase activity by prostaglandin E2 in human granulosa lutein cells. *J Endocrinol* **194** 595-602.

Chen Y, Green JA, Antoniou E, Ealy AD, Mathialagan N, Walker AM, Avalle MP, Rosenfeld CS, Hearne LB & Roberts RM 2006 Effect of interferon-tau administration on endometrium of nonpregnant ewes: a comparison with pregnant ewes. *Endocrinology* **147** 2127-2137.

Choi Y, Johnson GA, Spencer TE & Bazer FW 2003 Pregnancy and interferon tau regulate major histocompatibility complex class I and beta2-microglobulin expression in the ovine uterus. *Biol Reprod* **68** 1703-1710.

Costine BA, Inskeep EK, Blemings KP, Flores JA & Wilson ME 2007 Mechanisms of reduced luteal sensitivity to prostaglandin F2alpha during maternal recognition of pregnancy in ewes. *Domest Anim Endocrinol* **32** 106-121.

Culley FJ, Pennycook AM, Tregoning JS, Dodd JS, Walzl G, Wells TN, Hussell T & Openshaw PJ 2006 Role of CCL5 (RANTES) in viral lung disease. *J Virol* **80** 8151-8157.

Davies CJ, Joosten I, Andersson L, Arriens MA, Bernoco D, Bissumbhar B, Byrns G, van Eijk MJ, Kristensen B, Lewin HA & et al. 1994 Polymorphism of bovine MHC class II genes. Joint report of the Fifth International Bovine Lymphocyte Antigen (BoLA) Workshop, Interlaken, Switzerland, 1 August 1992. *Eur J Immunogenet* **21** 259-289.

Davis MA, Ott TL, Mirando MA, Moser MT & Bazer FW 1992 Effect of recombinant alpha interferons on fertility and interestrous interval in sheep. *Theriogenology* **38** 867-875.

Davis TL, Bott RC, Slough TL, Bruemmer JE & Niswender GD 2010 Progesterone inhibits oxytocin- and prostaglandin F2alpha-stimulated increases in intracellular calcium concentrations in small and large ovine luteal cells. *Biol Reprod* **82** 282-288.

Diehn M, Alizadeh AA, Rando OJ, Liu CL, Stankunas K, Botstein D, Crabtree GR & Brown PO 2002 Genomic expression programs and the integration of the CD28 costimulatory signal in T cell activation. *Proc Natl Acad Sci U S A* **99** 11796-11801.

Endo S, Nomura T, Chan BS, Lu R, Pucci ML, Bao Y & Schuster VL 2002 Expression of PGT in MDCK cell monolayers: polarized apical localization and induction of active PG transport. *Am J Physiol Renal Physiol* **282** F618-622.

Fleming JA, Choi Y, Johnson GA, Spencer TE & Bazer FW 2001 Cloning of the ovine estrogen receptor-alpha promoter and functional regulation by ovine interferon-tau. *Endocrinology* **142** 2879-2887.

Fleming JG, Spencer TE, Safe SH & Bazer FW 2006 Estrogen regulates transcription of the ovine oxytocin receptor gene through GC-rich SP1 promoter elements. *Endocrinology* **147** 899-911.

Giannakopoulos NV, Luo JK, Papov V, Zou W, Lenschow DJ, Jacobs BS, Borden EC, Li J, Virgin HW & Zhang DE 2005 Proteomic identification of proteins conjugated to ISG15 in mouse and human cells. *Biochem Biophys Res Commun* **336** 496-506.

Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, Davies CJ & Ott TL 2007 Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. *J Dairy Sci* **90** 274-280.

Godkin JD, Bazer FW, Moffatt J, Sessions F & Roberts RM 1982 Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at day 13-21. *J Reprod Fertil* **65** 141-150.

Godkin JD, Bazer FW & Roberts RM 1984a Ovine trophoblast protein 1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein synthesis. *Endocrinology* **114** 120-130.

Godkin JD, Bazer FW, Thatcher WW & Roberts RM 1984b Proteins released by cultured Day 15-16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. *J Reprod Fertil* **71** 57-64.

Green MP, Spate LD, Bixby JA, Ealy AD & Roberts RM 2005 A comparison of the anti-luteolytic activities of recombinant ovine interferon-alpha and -tau in sheep. *Biol Reprod* **73** 1087-1093.

Han H, Austin KJ, Rempel LA & Hansen TR 2006 Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. *J Endocrinol* **191** 505-512.

Hansen PJ, Anthony RV, Bazer FW, Baumbach GA & Roberts RM 1985 In vitro synthesis and secretion of ovine trophoblast protein-1 during the period of maternal recognition of pregnancy. *Endocrinology* **117** 1424-1430.

Hansen TR, Austin KJ & Johnson GA 1997 Transient ubiquitin cross-reactive protein gene expression in the bovine endometrium. *Endocrinology* **138** 5079-5082.

Hansen TR, Austin KJ, Perry DJ, Pru JK, Teixeira MG & Johnson GA 1999 Mechanism of action of interferon-tau in the uterus during early pregnancy. *J Reprod Fertil Suppl* **54** 329-339.

Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, Shimada N, Ohba Y, Takaoka A, Yoshida N & Taniguchi T 2005 IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* **434** 772-777.

Hou X, Arvisais EW, Jiang C, Chen DB, Roy SK, Pate JL, Hansen TR, Rueda BR & Davis JS 2008 Prostaglandin F2alpha stimulates the expression and secretion of transforming growth factor B1 via induction of the early growth response 1 gene (EGR1) in the bovine corpus luteum. *Mol Endocrinol* **22** 403-414.

Huang GN, Huso DL, Bouyain S, Tu J, McCorkell KA, May MJ, Zhu Y, Lutz M, Collins S, Dehoff M, Kang S, Whartenby K, Powell J, Leahy D & Worley PF 2008 NFAT binding and regulation of T cell activation by the cytoplasmic scaffolding Homer proteins. *Science* **319** 476-481.

Imakawa K, Imai M, Sakai A, Suzuki M, Nagaoka K, Sakai S, Lee SR, Chang KT, Echternkamp SE & Christenson RK 2006 Regulation of conceptus adhesion by endometrial CXC chemokines during the implantation period in sheep. *Mol Reprod Dev* **73** 850-858.

Inskeep EK, Smutny WJ, Butcher RL & Pexton JE 1975 Effects of intrafollicular injections of prostaglandins in non-pregnant and pregnant ewes. *J Anim Sci* **41** 1098-1104.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U & Speed TP 2003 Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4** 249-264.

Isaacs A & Lindenmann J 1957 Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci* **147** 258-267.

Johnson GA, Austin KJ, Collins AM, Murdoch WJ & Hansen TR 1999a Endometrial ISG17 mRNA and a related mRNA are induced by interferon-tau and localized to glandular epithelial and stromal cells from pregnant cows. *Endocrine* **10** 243-252.

Johnson GA, Austin KJ, Van Kirk EA & Hansen TR 1998 Pregnancy and interferon-tau induce conjugation of bovine ubiquitin cross-reactive protein to cytosolic uterine proteins. *Biol Reprod* **58** 898-904.

Johnson GA, Spencer TE, Hansen TR, Austin KJ, Burghardt RC & Bazer FW 1999b Expression of the interferon tau inducible ubiquitin cross-reactive protein in the ovine uterus. *Biol Reprod* **61** 312-318.

Johnson GA, Stewart MD, Gray CA, Choi Y, Burghardt RC, Yu-Lee LY, Bazer FW & Spencer TE 2001 Effects of the estrous cycle, pregnancy, and interferon tau on 2',5'-oligoadenylate synthetase expression in the ovine uterus. *Biol Reprod* **64** 1392-1399.

Juengel JL, Melner MH, Clapper JA, Turzillo AM, Moss GE, Nett TM & Niswender GD 1998 Steady-state concentrations of mRNA encoding two inhibitors of protein kinase C in ovine luteal tissue. *J Reprod Fertil* **113** 299-305.

Klein C, Bauersachs S, Ulbrich SE, Einspanier R, Meyer HH, Schmidt SE, Reichenbach HD, Vermehren M, Sinowatz F, Blum H & Wolf E 2005 Monozygotic Twin Model Reveals Novel Embryo-Induced Transcriptome Changes of Bovine Endometrium in the Pre-Attachment Period. *Biol Reprod*.

Lamming GE, Wathes DC, Flint AP, Payne JH, Stevenson KR & Vallet JL 1995 Local action of trophoblast interferons in suppression of the development of oxytocin and oestradiol receptors in ovine endometrium. *J Reprod Fertil* **105** 165-175.

Lee J, McCracken JA, Banu SK, Rodriguez R, Nithy TK & Arosh JA 2010 Transport of prostaglandin F(2alpha) pulses from the uterus to the ovary at the time of luteolysis in ruminants is regulated by prostaglandin transporter-mediated mechanisms. *Endocrinology* **151** 3326-3335.

Lenschow DJ, Lai C, Frias-Staheli N, Giannakopoulos NV, Lutz A, Wolff T, Osiak A, Levine B, Schmidt RE, Garcia-Sastre A, Leib DA, Pekosz A, Knobeloch KP, Horak I & Virgin HWt 2007 IFN-stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. *Proc Natl Acad Sci U S A* **104** 1371-1376.

Li J & Roberts RM 1994 Interferon-tau and interferon-alpha interact with the same receptors in bovine endometrium. Use of a readily iodinatable form of recombinant interferon-tau for binding studies. *J Biol Chem* **269** 13544-13550.

MacMicking JD 2004 IFN-inducible GTPases and immunity to intracellular pathogens. *Trends Immunol* **25** 601-609.

Malakhova OA, Yan M, Malakhov MP, Yuan Y, Ritchie KJ, Kim KI, Peterson LF, Shuai K & Zhang DE 2003 Protein ISGylation modulates the JAK-STAT signaling pathway. *Genes Dev* **17** 455-460.

Mapletoft RJ, Del Campo MR & Ginther OJ 1976 Local venoarterial pathway for uterine-induced luteolysis in cows. *Proc Soc Exp Biol Med* **153** 289-294.

Marsh J 1971 The effect of prostaglandins on the adenyl cyclase of the bovine corpus luteum. *Ann N Y Acad Sci* **180** 416-425.

Martal J, Degryse E, Charpigny G, Assal N, Reinaud P, Charlier M, Gaye P & Lecocq JP 1990 Evidence for extended maintenance of the corpus luteum by uterine infusion of a recombinant trophoblast alpha-interferon (trophoblastin) in sheep. *J Endocrinol* **127** R5-8.

Martinod S, Maurer RR, Siegenthaler B, Gerber C & Hansen PJ 1991 The effects of recombinant bovine interferon-alpha on fertility in ewes. *Theriogenology* **36** 231-239.

Meyer MD, Hansen PJ, Thatcher WW, Drost M, Badinga L, Roberts RM, Li J, Ott TL & Bazer FW 1995 Extension of corpus luteum lifespan and reduction of uterine secretion of prostaglandin F2 alpha of cows in response to recombinant interferon-tau. *J Dairy Sci* **78** 1921-1931.

Mirando MA, Short EC, Jr., Geisert RD, Vallet JL & Bazer FW 1991 Stimulation of 2',5'-oligoadenylate synthetase activity in sheep endometrium during pregnancy, by intrauterine infusion of ovine trophoblast protein-1, and by intramuscular administration of recombinant bovine interferon-alpha I1. *J Reprod Fertil* **93** 599-607.

Moor RM & Rowson LE 1964 Influence of the Embryo and Uterus on Luteal Function in the Sheep. *Nature* **201** 522-523.

Moor RM & Rowson LE 1966a The corpus luteum of the sheep: functional relationship between the embryo and the corpus luteum. *J Endocrinol* **34** 233-239.

Moor RM & Rowson LE 1966b Local maintenance of the corpus luteum in sheep with embryos transferred to various isolated portions of the uterus. *J Reprod Fertil* **12** 539-550.

Nagaoka K, Sakai A, Nojima H, Suda Y, Yokomizo Y, Imakawa K, Sakai S & Christenson RK 2003 A chemokine, interferon (IFN)-gamma-inducible protein 10 kDa, is stimulated by IFN-tau and recruits immune cells in the ovine endometrium. *Biol Reprod* **68** 1413-1421.

Naivar KA, Ward SK, Austin KJ, Moore DW & Hansen TR

1995 Secretion of bovine uterine proteins in response to type I interferons. *Biol Reprod* **52** 848-854.

Nephew KP, McClure KE, Day ML, Xie S, Roberts RM & Pope WF 1990 Effects of intramuscular administration of recombinant bovine interferon-alpha I1 during the period of maternal recognition of pregnancy. *J Anim Sci* **68** 2766-2770.

Niswender GD, Davis TL, Griffith RJ, Bogan RL, Monser K, Bott RC, Bruemmer JE & Nett TM 2007 Judge, jury and executioner: the auto-regulation of luteal function. *Soc Reprod Fertil Suppl* **64** 191-206.

Oliveira JF, Henkes LE, Ashley RL, Purcell SH, Smirnova NP, Veeramachaneni DN, Anthony RV & Hansen TR 2008 Expression of interferon (IFN)-stimulated genes in extrauterine tissues during early pregnancy in sheep is the consequence of endocrine IFN-tau release from the uterine vein. *Endocrinology* **149** 1252-1259.

Ott TL, Fleming JG, Spencer TE, Joyce MM, Chen P, Green CN, Zhu D, Welsh TH, Jr., Harms PG & Bazer FW 1997 Effects of exogenous recombinant ovine interferon tau on circulating concentrations of progesterone, cortisol, luteinizing hormone, and antiviral activity; interestrous interval; rectal temperature; and uterine response to oxytocin in cyclic ewes. *Biol Reprod* **57** 621-629.

Ott TL, Yin J, Wiley AA, Kim HT, Gerami-Naini B, Spencer TE, Bartol FF, Burghardt RC & Bazer FW 1998 Effects of the estrous cycle and early pregnancy on uterine expression of Mx protein in sheep (Ovis aries). *Biol Reprod* **59** 784-794.

Pate JL 1995 Involvement of immune cells in regulation of ovarian function. *J Reprod Fertil Suppl* **49** 365-377.

Perry DJ, Austin KJ & Hansen TR 1999 Cloning of interferon-stimulated gene 17: the promoter and nuclear proteins that regulate transcription. *Mol Endocrinol* **13** 1197-1206.

Peterson AJ, Tervit HR, Fairclough RJ, Havik PG & Smith JF 1976 Jugular levels of 13, 14-dihydro-15-keto-prostaglandin F and progesterone around luteolysis and early pregnancy in the ewe. *Prostaglandins* **12** 551-558.

Pratt BR, Butcher RL & Inskeep EK 1977 Antiluteolytic effect of the conceptus and of PGE2 in ewes. *J Anim Sci* **45** 784-791.

Pru JK, Austin KJ, Haas AL & Hansen TR 2001a Pregnancy and interferon-tau upregulate gene expression of members of the 1-8 family in the bovine uterus. *Biol Reprod* **65** 1471-1480.

Pru JK, Rueda BR, Austin KJ, Thatcher WW, Guzeloglu A & Hansen TR 2001b Interferon-tau suppresses prostaglandin F2alpha secretion independently of the mitogen-activated protein kinase and nuclear factor kappa B pathways. *Biol Reprod* **64** 965-973.

Rani MR, Hibbert L, Sizemore N, Stark GR & Ransohoff RM 2002 Requirement of phosphoinositide 3-kinase and Akt for interferon-beta-mediated induction of the beta-R1 (SCYB11) gene. *J Biol Chem* **277** 38456-38461.

Rempel LA, Francis BR, Austin KJ & Hansen TR 2005 Isolation and sequence of an interferon-tau-inducible, pregnancy- and bovine interferon-stimulated gene product 15 (ISG15)-specific, bovine ubiquitin-activating E1-like (UBE1L) enzyme. *Biol Reprod* **72** 365-372.

Roberts RM 1989 Conceptus interferons and maternal recognition of pregnancy. *Biol Reprod* **40** 449-452.

Roberts RM, Cross JC & Leaman DW 1992 Interferons as hormones of pregnancy. *Endocr Rev* **13** 432-452.

Rosenfeld CS, Han CS, Alexenko AP, Spencer TE & Roberts RM 2002 Expression of interferon receptor subunits, IFNAR1 and IFNAR2, in the ovine uterus. *Biol Reprod* **67** 847-853.

Schalue-Francis TK, Farin PW, Cross JC, Keisler D & Roberts RM 1991 Effect of injected bovine interferon-alpha I1 on estrous cycle length and pregnancy success in sheep. *J Reprod Fertil* **91** 347-356.

Schmitt RA, Geisert RD, Zavy MT, Short EC & Blair RM 1993 Uterine cellular changes in 2',5'-oligoadenylate synthetase during the bovine estrous cycle and early pregnancy. *Biol Reprod* **48** 460-466.

Schuster VL 1998 Molecular mechanisms of prostaglandin transport. *Annu Rev Physiol* **60** 221-242.

Schuster VL 2002 Prostaglandin transport. *Prostaglandins Other Lipid Mediat* **68-69** 633-647.

Senger PL 2003 *Pathways to pregnancy and parturition*. Pullman, WA: Current Conceptions.

Shoemaker ML, Smirnova NP, Bielefeldt-Ohmann H, Austin KJ, van Olphen A, Clapper JA & Hansen TR 2009 Differential expression of the type I interferon pathway during persistent and transient bovine viral diarrhea virus infection. *J Interferon Cytokine Res* **29** 23-35.

Silva PJ, Juengel JL, Rollyson MK & Niswender GD 2000 Prostaglandin metabolism in the ovine corpus luteum: catabolism of prostaglandin F(2alpha) (PGF(2alpha)) coincides with resistance of the corpus luteum to PGF(2alpha). *Biol Reprod* **63** 1229-1236.

Silvia WJ & Niswender GD 1984 Maintenance of the corpus luteum of early pregnancy in the ewe. III. Differences between pregnant and nonpregnant ewes in luteal responsiveness to prostaglandin F2 alpha. *J Anim Sci* **59** 746-753.

Silvia WJ & Niswender GD 1986 Maintenance of the corpus luteum of early pregnancy in the ewe. IV. Changes in luteal sensitivity to prostaglandin F2 alpha throughout early pregnancy. *J Anim Sci* **63** 1201-1207.

Smirnova NP, Bielefeldt-Ohmann H, Van Campen H, Austin KJ, Han H, Montgomery DL, Shoemaker ML, van Olphen AL & Hansen TR 2008 Acute non-cytopathic bovine viral diarrhea virus infection induces pronounced type I interferon response in pregnant cows and fetuses. *Virus Res* **132** 49-58.

Smirnova NP, Ptitsyn AA, Austin KJ, Bielefeldt-Ohmann H, Van Campen H, Han H, van Olphen AL & Hansen TR 2009 Persistent fetal infection with bovine viral diarrhea virus differentially affects maternal blood cell signal transduction pathways. *Physiol Genomics* **36** 129-139.

Spencer TE & Bazer FW 1996 Ovine interferon tau suppresses transcription of the estrogen receptor and

oxytocin receptor genes in the ovine endometrium. *Endocrinology* **137** 1144-1147.

Spencer TE, Becker WC, George P, Mirando MA, Ogle TF & Bazer FW 1995 Ovine interferon-tau inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. *Endocrinology* **136** 4932-4944.

Spencer TE, Ott TL & Bazer FW 1996 tau-Interferon: pregnancy recognition signal in ruminants. *Proc Soc Exp Biol Med* **213** 215-229.

Spencer TE, Stagg AG, Ott TL, Johnson GA, Ramsey WS & Bazer FW 1999 Differential effects of intrauterine and subcutaneous administration of recombinant ovine interferon tau on the endometrium of cyclic ewes. *Biol Reprod* **61** 464-470.

Sugimoto Y & Narumiya S 2007 Prostaglandin E receptors. *J Biol Chem* **282** 11613-11617.

Takeuchi T, Inoue S & Yokosawa H 2006 Identification and Herc5-mediated ISGylation of novel target proteins. *Biochem Biophys Res Commun* **348** 473-477.

Taub DD, Proost P, Murphy WJ, Anver M, Longo DL, van Damme J & Oppenheim JJ 1995 Monocyte chemotactic protein-1 (MCP-1), -2, and -3 are chemotactic for human T lymphocytes. *J Clin Invest* **95** 1370-1376.

Teixeira MG, Austin KJ, Perry DJ, Dooley VD, Johnson GA, Francis BR & Hansen TR 1997 Bovine granulocyte chemotactic protein-2 is secreted by the endometrium in response to interferon-tau (IFN-tau). *Endocrine* **6** 31-37.

Thatcher WW, Guzeloglu A, Mattos R, Binelli M, Hansen TR & Pru JK 2001 Uterine-conceptus interactions and reproductive failure in cattle. *Theriogenology* **56** 1435-1450.

Vallet JL, Bazer FW, Fliss MF & Thatcher WW 1988 Effect of ovine conceptus secretory proteins and purified ovine trophoblast protein-1 on interoestrous interval and plasma concentrations of prostaglandins F-2 alpha and E and of 13,14-dihydro-15-keto prostaglandin F-2 alpha in cyclic ewes. *J Reprod Fertil* **84** 493-504.

Vander Wielen AL & King GJ 1984 Intraepithelial lymphocytes in the bovine uterus during the oestrous cycle and early gestation. *J Reprod Fertil* **70** 457-462.

Wiepz GJ, Wiltbank MC, Nett TM, Niswender GD & Sawyer HR 1992 Receptors for prostaglandins F2 alpha and E2 in ovine corpora lutea during maternal recognition of pregnancy. *Biol Reprod* **47** 984-991.

Wilson L, Jr., Butcher RL & Inskeep EK 1972 Prostaglandin F2alpha in the uterus of ewes during early pregnancy. *Prostaglandins* **1** 479-482.

Yankey SJ, Hicks BA, Carnahan KG, Assiri AM, Sinor SJ, Kodali K, Stellflug JN & Ott TL 2001 Expression of the antiviral protein Mx in peripheral blood mononuclear cells of pregnant and bred, non-pregnant ewes. *J Endocrinol* **170** R7-11.

Zarco L, Stabenfeldt GH, Basu S, Bradford GE & Kindahl H 1988a Modification of prostaglandin F-2 alpha synthesis and release in the ewe during the initial establishment of pregnancy. *J Reprod Fertil* **83** 527-536.

Zarco L, Stabenfeldt GH, Quirke JF, Kindahl H & Bradford GE 1988b Release of prostaglandin F-2 alpha and the timing of events associated with luteolysis in ewes with oestrous cycles of different lengths. *J Reprod Fertil* **83** 517-526.

Zhao C, Denison C, Huibregtse JM, Gygi S & Krug RM 2005 Human ISG15 conjugation targets both IFN-induced and constitutively expressed proteins functioning in diverse cellular pathways. *Proc Natl Acad Sci U S A* **102** 10200-10205.