Conceptus-uterus interactions in pigs: endometrial gene expression in response to estrogens and interferons from conceptuses

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This review highlights information on conceptus-uterus interactions in the pig with respect to uterine gene expression in response to estrogens and interferons (IFNs) secreted from elongating conceptuses. Pig conceptuses release estrogens for pregnancy recognition, but also secrete IFNs that do not appear to be antiluteolytic. Estrogens and IFNs induce expression of largely non-overlapping sets of genes, and evidence suggests that pig conceptuses orchestrate essential events of early pregnancy including pregnancy recognition signaling, implantation and secretion of histotroph by precisely controlling temporal and spatial (cell-specific) changes in uterine gene expression through initial secretion of estrogens, followed by cytokines including IFNG and IFND. By Day 12 of pregnancy, estrogens increase the expression of multiple genes in the uterine luminal epithelium including SPP1, STC1, IRF2 and STAT1 that likely have roles for implantation. By Day 15 of pregnancy, IFNs upregulate a large array of IFN responsive genes in the underlying stroma and glandular epithelium including ISG15, IRF1, STAT1, SLAs and B2M that likely have roles in uterine remodeling to support placentation.

Introduction

This review assembles information on the regulation of endometrial gene expression by conceptus estrogens (Geisert *et al.* 1982a), and interferons (IFNs) delta (IFND) and gamma (IFNG) (La Bonnardière *et al.* 1991, Lefèvre *et al.* 1998) during the peri-implantation period of pregnancy. The estrogens and IFNs regulate cell-type specific expression of endometrial genes responsible for the complex interplay between endometrium and conceptus required for pregnancy recognition signaling and implantation.

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Pig conceptus estrogens and interferons

Early pregnancy in pigs is complex and is influenced by the overlapping events of conceptus elongation (Fig. 1A), endometrial remodeling for implantation (Fig. 1B) and pregnancy recognition signaling. In pigs, oxytocin released in a pulsatile manner by the uterus binds oxytocin receptors to stimulate pulsatile release of prostaglandin $F_{2\alpha}$ (PGF) from endometrial luminal epithelium (LE) (Mirando *et al.* 1995). PGF is the uterine luteolysin in pigs as corpus luteum (CL) regression correlates with pulsatile release of PGF into the uterine venous drainage beginning on Day 15 or 16 of the estrous cycle and hysterectomy extends CL lifespan to about 120 days (Mirando *et al.* 1995). However, roles of PGs in the pig uterus remain to be clarified. Inhibitors of PG synthesis fail to protect the CL from luteolysis (Kraeling *et al.* 1985), amounts of PGF and PGE2 in the uterine lumen are greater in pregnant than cyclic pigs (Bazer and Thatcher 1977), uterine PGF is processed into an inactive metabolite through a utero-ovarian countercurrent vascular pathway within the broad ligament (Krzymowski *et al.* 1990), and PGE2 synthase:PGF synthase ratios are higher in CL from pregnant than cyclic pigs, but not between CL ipsilateral or contralateral to the pregnant uterine horn. Therefore, it has been suggested that compounds from the conceptus are transported within the mesometrium to the ovaries to enhance CL maintenance (Wasielak *et al.* 2008).

Pregnancy recognition is the result of conceptus secretion of estrogens on Days 11 and 12 of pregnancy to redirect PGF secretion from the uterine vasculature to the uterine lumen (Fig. 2A & 2B). The theory of estrogen-induced maternal recognition of pregnancy in pigs is based on the following evidence: (i) the uterine endometrium secretes luteolytic PGF; (ii) pig conceptuses secrete estrogens which are antiluteolytic; (iii) PGF is secreted toward the uterine vasculature (endocrine) in cyclic gilts to induce luteolysis; and (iv) secretion of PGF in pregnant gilts is into the uterine lumen (exocrine) where it is sequestered from the corpora lutea and/or metabolized to prevent luteolysis (Bazer and Thatcher 1977). In addition to pregnancy recognition, conceptus estrogens modulate uterine gene expression thought to be required for implantation (Geisert *et al.* 1982b). The importance of estrogen to implantation of pig conceptuses is underscored by the fact that premature exposure of the pregnant uterus to estrogen on Days 9 and 10 results in degeneration of all pig conceptuses by Day 15 (Ross *et al.* 2007). It should be noted that PGE2, as well as lysophosphatic acid (LPA) have proposed roles in pregnancy signaling. Expression of PGE2 synthase by trophoblast and endometrium decreases production of PGF in favor of PGE2 to support CL maintenance (Ziecik *et al.* 2008). In addition LPA: (i) increases in uterine luminal fluids of pigs; (ii) its receptor, EDG7, is expressed by pig conceptuses; and (iii) its expression is increased by estrogen in endometrial epithelia during early pregnancy (So *et al*, 2008). Indeed, LPA3 is critical for embryo migration and spacing in mice (Ye *et al.* 2005), events that are critical to implantation and placentation in pigs.

Pig trophectoderm is unique in secreting both Type I and Type II IFNs during the peri-implantation period (Figs. 2A & 2B). Cultured conceptuses from Day 11 of pregnancy secrete proteins that cross react with antiserum against INF alpha (IFNA) (Cross & Roberts 1989), but peak antiviral activity is not measured in uterine flushings or conceptus culture media until Day 14 of pregnancy (Mirando *et al.* 1990). The major IFN species, constituting 75% of antiviral activity in pig conceptus secretory proteins (CSP), is type II IFNG. The other (25%) is the novel type I IFND (La Bonnardière *et al.* 1991, Lefèvre *et al.* 1998). Abundant IFNG mRNA is detectable in porcine trophectoderm between Days 13 and 20 of pregnancy, whereas IFND mRNA is detectable in Day 14 conceptuses only by RT-PCR analysis (Joyce *et al.* 2007a). On Day 15 of pregnancy, immunoreactive IFNG and IFND proteins are co-localized to peri-nuclear membranes typically occupied by endoplasmic reticulum and Golgi apparatus, as well as cytoplasmic vesicles within clusters of trophectoderm cells along the endometrial LE (Lefèvre

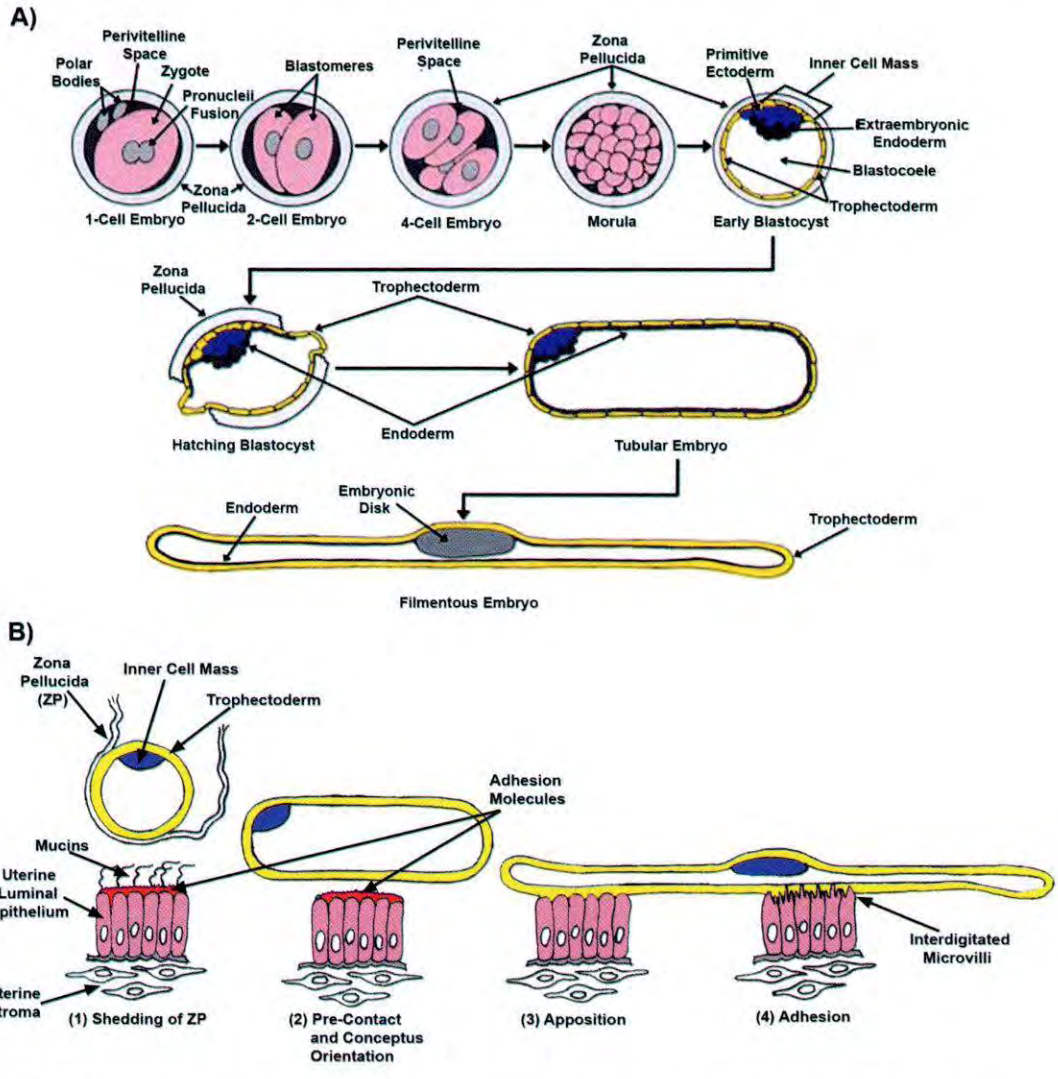

Fig. 1 Panel A. Pre-implantation development of the pig conceptus within the oviducts and uterus. The origin and organization of tissue layers within the conceptus are depicted. In pigs, the 1-cell fertilized ovum or zygote undergoes cleavage to form a 2-cell embryo by 26 h after fertilization, enters the uterus at the 4- to 8-cell stage between 48 to 56 h, differentiates into the blastocyst, "hatches" from the zona pellucida as a 0.5 to 1 mm diameter sphere, increases in size to Day 10 of pregnancy (2-6 mm), then undergoes a morphological transition to a large sphere of 10 to 15 mm diameter and then a tubular (15 mm by 50mm) and filamentous (l mm by 100-200 mm) form on Day 11. Panel B. Implantation Cascade in Pigs. Implantation in pigs extends from Days 13-25 and includes four phases that overlap and involve increasingly complex interactions between trophectoderm and uterine luminal epithelium to achieve true epitheliochorial placentation. Conceptus attachment first requires loss of anti-adhesive molecules in the glycocalyx of LE, comprised largely of mucins that sterically inhibit attachment. This results in "unmasking" of molecules, including selectins and galectins, that contribute to initial attachment of conceptus to uterine LE. These low affinity contacts are then replaced by a more stable and extensive repertoire of adhesive interactions between integrins and maternal ECM, for example secreted phophoprotein 1 (SPP1 or Osteopontin), which appear to be the dominant contributors to stable adhesion at implantation.

et al. 1998). In contrast to sheep conceptuses, in which a Type I IFN (interferon tau) is the signal for maternal recognition of pregnancy (Spencer *et al.* 2007), the IFNs produced by pig conceptuses do not appear to be antiluteolytic. Intrauterine infusion of CSP on Days 12 and 15 of the estrous cycle have no effect on interestrus interval or temporal changes in concentrations of progesterone in plasma (Harney & Bazer 1989). Although pig conceptus IFNs have yet to be shown to influence pregnancy recognition, paracrine effects for IFNs are suggested by localization of IFN receptors on endometrial epithelial cells (Lefèvre *et al.* 1998), increased secretion of prostaglandin E_2 (Harney & Bazer 1989), expression of several known IFN-responsive genes in the endometrium (Hicks *et al.* 2003, Joyce *et al* 2007a, Joyce *et al.* 2007b, Joyce *et al.* 2008), and modulation of uterine stromal and glandular epithelial (GE) gene expression by the IFNs in CSP preparations (Joyce *et al* 2007a, Joyce *et al.* 2007b, Joyce *et al.* 2008).

Estrogen- and interferon-stimulated genes in the endometrium

Estrogens and IFNs regulate endometrial genes that interact to effect communication between endometrium and conceptus during pregnancy recognition and implantation in pigs. Fig. 2 illustrates the fact that timing of estrogen secretion by the conceptus correlates with the induction of *SPP1* expression in the LE, whereas stromal induction of *STAT1* correlates with IFNG and IFND secretion by the conceptus. Indeed administration of exogenous estradiol to ovariectomized pigs induces *SPP1* mRNA in endometrial LE (White *et al.* 2005), while intrauterine infusion of CSP, which contain IFND and IFNG, into cyclic pigs treated with exogenous estrogen increases STAT1 as compared to intrauterine infusion of control proteins (Joyce *et al.* 2007a), similar to expression patterns for these genes during the peri-implantation period of pigs (Fig. 2C). Upregulation of *SPP1* within uterine LE and *STAT1* within stroma and GE in close proximity to the implanting conceptus implies paracrine regulation of genes by conceptus estrogens and IFNs. It is likely that effects of estrogen on the endometrium are restricted to regions near the conceptus due to metabolic activity of trophectoderm. During pregnancy, pig endometrium rapidly converts estradiol to estrone and then converts it to the biologically inactive estrone sulfate which is present in high concentrations within the uterine lumen of pregnant pigs (Flood 1974). The trophectoderm has sulfatase enzyme activity that restores the biological activity of estrogen, allowing for a localized effect of estrogen to up-regulate genes such as *SPP1* in LE. In contrast, it is somewhat surprising that initial increases in expression of *STAT1* in stroma are restricted to sites of contact between the conceptus and uterus, given that *IFNG* synthesis and secretion by pig conceptuses appears to be similar in magnitude to IFNT production by sheep conceptuses (Joyce *et al.* 2007a). Indeed, *STAT1* expression increases universally in the stroma and GE of pregnant sheep independently of conceptus location within the lumen, presumably due to the high levels of secretion of IFNT by conceptuses (Spencer *et al* 2007). One explanation for the spatial pattern of *STAT1* expression in the pig uterus is that IFND and IFNG act synergistically to upregulate expression of ISGs. Interactions between Type I and Type II IFNs have been demonstrated previously (Decker *et al.* 1989). It is plausible that high levels of IFNG act on uterine stroma and GE to increase intracellular stores of interferon-stimulated gene factor 3 (ISGF3) so that the much lower levels of IFND can maximally upregulate STAT1 expression in close proximity to the implanting pig conceptus. To date, only a limited number of estrogen- and IFN-stimulated genes have been localized in the pig endometrium. Table 1 summarizes gene expression in pig uteri during normal pregnancy and in response to i.m. injections of estrogen and/or intra-uterine injections of pig CSP containing IFNG and IFND (Ka *et al.* 2000, Hicks *et al.* 2003, White *et al.* 2005, Joyce *et al* 2007a, Joyce *et al.* 2007b, Ka *et al.* 2007, Ross *et al.* 2007, Joyce *et al.* 2008, So *et al.* 2008, Song *et al.* 2009). Several of these genes are discussed further in the remainder of this review.

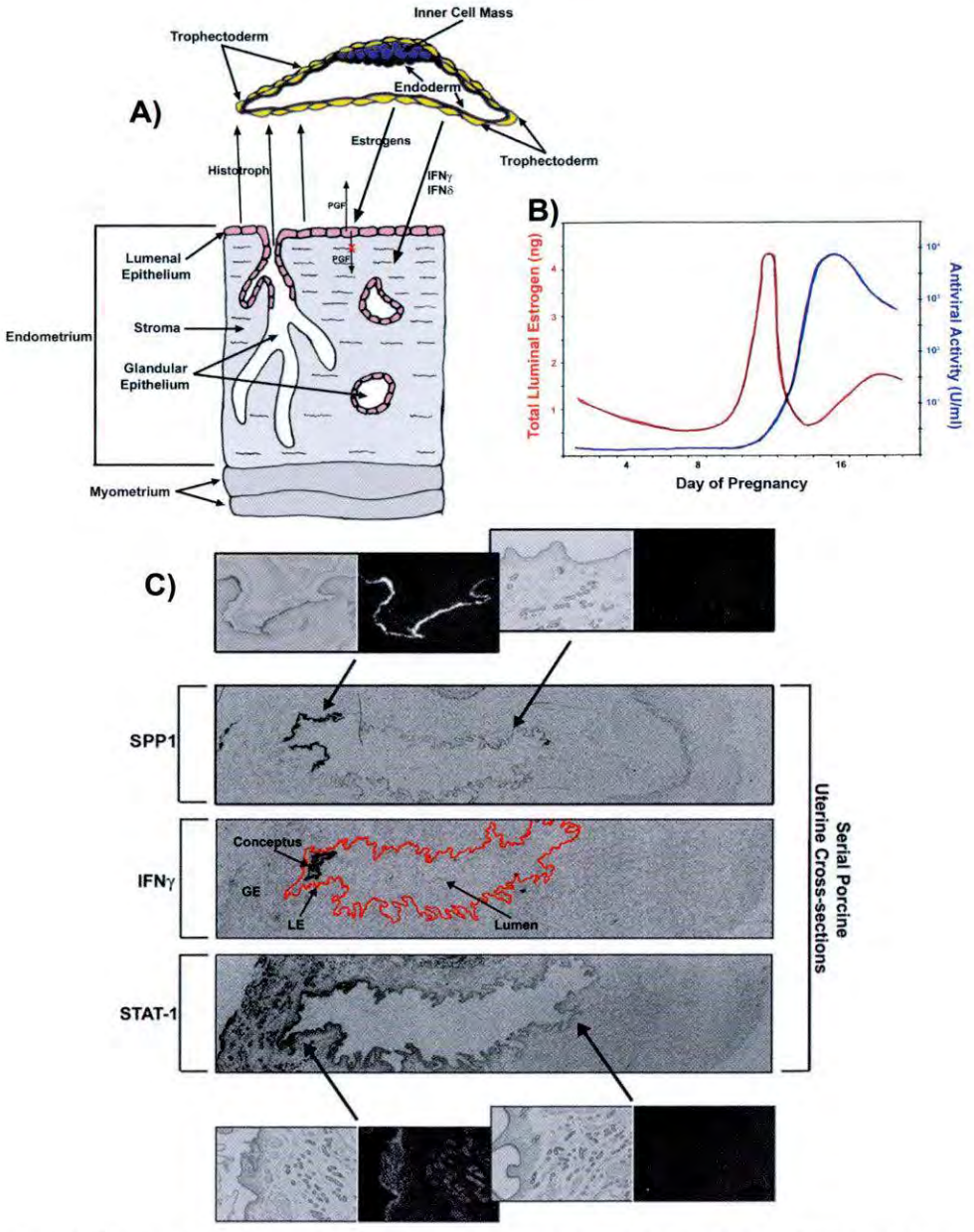

Fig. 2 Panel A. Peri-implantation signaling between the conceptus and uterus in the pig. Secretion of conceptus estrogens and IFNs elicit and support uterine responses including maintenance of the CL, production of histotroph and induction of multiple estrogen- and IFN-stimulated genes. Panel B. Graph depicting temporal changes in uterine luminal levels of conceptus estrogen and IFN antiviral activity in pigs during early pregnancy. Panel C. Conceptus estrogens (E2) induce *SPP1* in endometrial luminal epithelium (LE), and conceptus IFNs induce *STAT1* in endometrial stroma and glandular epithelium (GE) during the periimplantation period of pig pregnancy. In situ hybridization analysis of *SPP1*, *IFNG* and *STAT1* mRNAs in autoradiographic images (Biomax-MR; Kodak) showing entire serial cross-sections of the uterine wall from Day 15 of pregnancy. The luminal epithelium of tissue probed for *IFNG* mRNA has been outlined in red for histological reference. Corresponding brightfield and darkfield images from the same uterus probed with *SPP1* and *STAT1* cRNAs are also shown. Endometrial *SPP1* and *STAT1* increase in close proximity to paracrine release of E2 and IFNG from implanting pig conceptuses. Width of each field of autoradiographic images is 20 mm. Width of each field of brightfield and darkfield images is 940 μm.

Table 1. Temporal and Cell-Type Specific expression of conceptus estrogen- and interferon-regulated genes during the establishment of pregnancy in pigs. Only genes that have been spatially localized in pig endometrium and trophectoderm are listed.

Location	Regulation		Initial Day of Expression	
	By Estrogen	By IFNs	11-13	13-15
Conceptus			Estrogen	*IFNG*
			EDG7	*IFND*
Uterine lumen	FGF7		Estrogen	IFNG
	SPP1		FGF7	SPP1
	STC1		STC1	
LE	*AKR1B1*		*AKR1B1*	
	B2M		*B2M*	
	CD24		*CD24*	
	FGF7		*FGF7*	
	IRF2		*IRF2*	
	MX1		*NMB*	
	NMB		*SLA1,2,3*	
	SLA1,2,3		*SLA6,7,8*	
	SLA6,7,8		*SPP1*	
	SPP1		*STC1*	
	STC1		*EDG7*	
	EDG7			
GE		*B2M*		*B2M*
		IRF1		*IRF1*
		ISG15		*ISG15*
		SLA1,2,3		*MX1*
		SLA6,7,8		*SLA1,2,3*
		STAT1		*SLA6,7,8*
		STAT2		*STAT1*
				STAT2
ST		*B2M*		*B2M*
		IRF1		*IRF1*
		ISG15		*ISG15*
		SLA1,2,3		*MX1*
		SLA6,7,8		*SLA1,2,3*
		STAT1		*SLA6,7,8*
		STAT2		*STAT1*
				STAT2

LE, Luminal epithelium; GE, Glandular epithelium; ST, Stroma

Secreted Phosphoprotein 1 (SPP1)

SPP1, also called osteopontin (OPN), is a secreted ECM protein for which expression is upregulated during the initial stages of pregnancy in uteri of pigs (White *et al.* 2005) and other mammalian species, including humans (Johnson *et al.* 2003). SPP1 contains an Arg-Gly-Asp (RGD) sequence that mediates binding to cell surface integrin receptors, including $\alpha v\beta 3$, $\alpha 5\beta 1$, $\alpha v\beta 1$, $\alpha v\beta 5$, $\alpha v\beta 6$ and $\alpha 8\beta 1$ (Johnson *et al.* 2003). Alternative binding-sequence interactions between *SPP1* and integrins such as $\alpha 4\beta 1$, $\alpha 9\beta 1$ and $\alpha 4\beta 7$ can also occur (Johnson *et al.* 2003). As noted previously, estrogens secreted by the elongating Day 12 conceptus induce synthesis and secretion of *SPP1* specifically in endometrial LE cells in direct apposition to the

implanting conceptus (White *et al.* 2005). SPP1 protein is abundant on both the apical surface of uterine LE as well as conceptus trophectoderm (Tr) coinciding with the attachment phase of implantation in pigs (White *et al.* 2005). Recently, direct binding of αvβ6 trophectoderm and αvβ3 uterine LE integrins to *SPP1* was demonstrated. This binding stimulated trophectoderm cell adhesion and migration, but not proliferation, suggesting that *SPP1* is an excellent candidate to promote trophectoderm migration for conceptus elongation and attachment to endometrial LE for implantation in pigs (G. Johnson and D. Erikson, unpublished observations).

Stanniocalcin 1 (STC1)

STC1 is a homodimeric phosphoglycoprotein that regulates calcium and phosphate homeostasis (Wagner *et al.* 1986, Madsen *et al.* 1998). STC1 is also expressed in uteri of pregnant pigs, sheep and mice (Stasko *et al.* 2001, Song *et al.* 2006, Song *et al.* 2009). Recently, *STC1* mRNA was localized to uterine LE during the period of conceptus attachment to uterine LE for implantation in pigs i.e., Days 12-25 of pregnancy (Song *et al.* 2008). Further, *STC1* expression by uterine LE was induced by progesterone from the CL and further stimulated by estrogen from elongating pig conceptuses (Song *et al.* 2009). The presence of a 25 kDa form of STC1 in uterine luminal fluids from Days 12 through 15 suggests a role(s) in ion transport within trophectoderm and LE cells (Song *et al.* 2008). Indeed, total recoverable calcium in uterine flushings from pigs increases abruptly on Days 11 to 12, coincident with production of estrogen by elongating blastocysts (Geisert *et al.* 1982a). Therefore, estrogen may induce STC1 secretion from LE that then enhances transport of intracellular calcium to the lumen, resulting in increased levels of free calcium that mediate uterine secretion of multiple proteins that compose histotroph (Geisert *et al.* 1982b).

Interferon-Stimulated Gene 15 (ISG15)

In has been known for some time that IFNT increases expression of several IFN-stimulated genes (ISGs) in the stroma of the ruminant uterus. Over the last decade the list of ISGs known to be upregulated in endometrial stroma and GE has grown from one (*ISG15* in both cows and sheep) (Johnson *et al.*1999a, Johnson *et al.* 1999b), to over 20 proteins (see review for complete listing, Spencer *et al.* 2007). The first (Naivar *et al.* 1995) and most thoroughly studied ISG (Rempel *et al.* 2007) is *ISG15*, a functional ubiquitin homologue that has the C-terminus Leu-Arg-Gly-Gly amino acid sequence common to ubiquitin, allowing conjugation to intracellular proteins (Hass *et al.* 1987). Conjugation of proteins either targets proteins for rapid degradation in the proteasome, or stabilizes proteins for long-term modification (Wilkinson 2000). *ISG15* does indeed form stable conjugates with endometrial proteins, indicating a biologically active molecule that is responsive to conceptus IFNs and can target proteins for pregnancy-associated regulation and/or modification (Johnson *et al.* 1998, Joyce *et al.* 2005). Shown in Fig. 3 is the first evidence that *ISG15* is expressed in the stromal stratum compactum of pregnant pigs in response to conceptus IFNs. *ISG15* increases in the stroma between Days 12 and 14, then decreases gradually between Days 15 and 20 to undetectable levels by Day 35 (Data not shown).

Interferon Regulatory Factors (IRFs) 1 and 2

IRF1 is a key intermediate in the induction cascade of many classical ISGs through binding and transactivating IFN-stimulated response elements (ISREs) in their promoters (Floyd-Smith

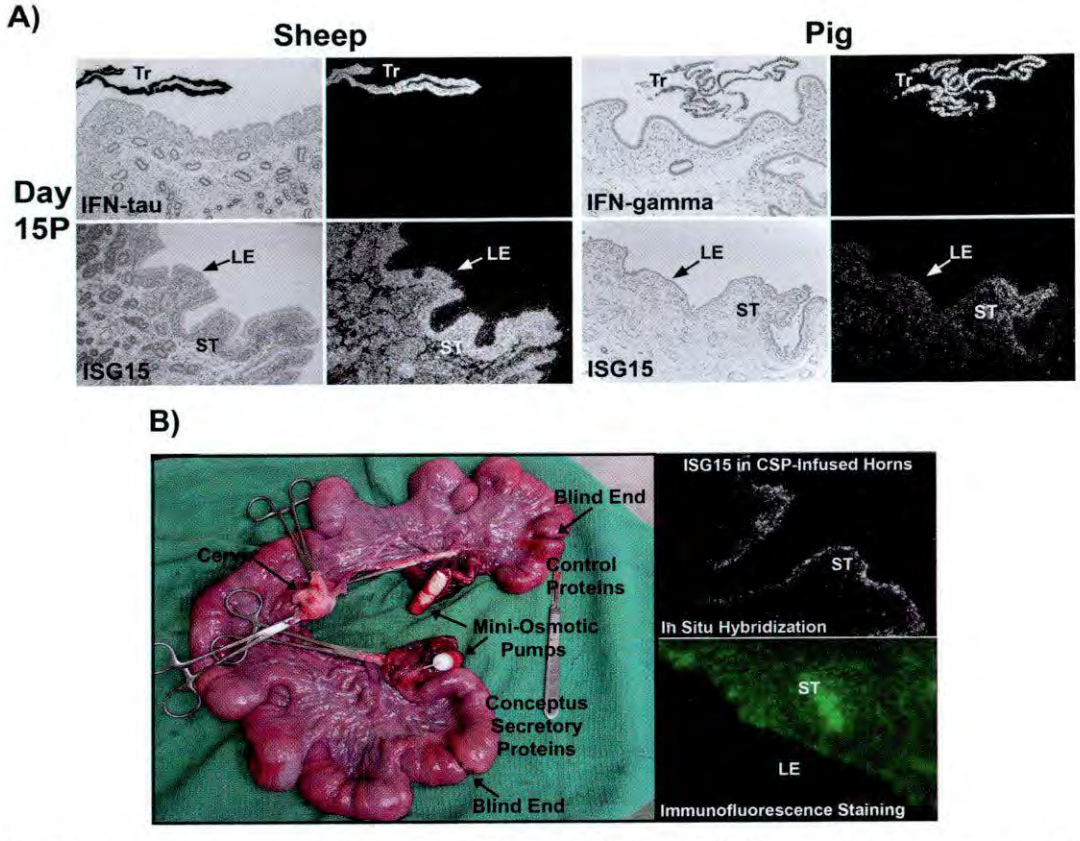

Fig. 3 Trophoblast IFNs increase expression of ISG15 within the endometrial stroma of domestic animals. A) *In situ* hybridization analyses for IFN-tau, IFN-gamma, and *ISG15* mRNA in cross-sections of sheep and pig uterui and placentae. Corresponding brightfield and darkfield images from Day 15 pregnant (P) sheep and pigs are shown. LE, luminal epithelium; ST, stroma; Tr, trophectoderm. Width of each field is 940 μm. B) Induction of *ISG15* in pig endometrium by conceptus secretory proteins containing IFNG and IFND. Left panel: On Day 12 post-estrus pigs were surgically implanted with two indwelling ALZET® osmotic pumps. For each pig, one uterine horn was infused by a pump filled with porcine serum albumin whereas the other uterine horn was infused by a pump filled with porcine conceptus secretory proteins (CSP) containing IFNs. Right panels: In situ hybridization and immunofluorescence detection of *ISG15* within the stratum compactum stroma of horns infused with CSP. Horns infused with control serum albumin did not express *ISG15* (data not shown). The rabbit polyclonal antibody against recombinant bovine ISG15 was kindly provided by Dr Thomas R. Hansen, Colorado State University.

et al. 1999, Chatterjee-Kishore *et al.* 2000, Stewart *et al.* 2002). Both Type I and Type II IFNs induce IRF1 expression (Floyd-Smith *et al.* 1999) which, in the reproductive tract of mice, has a role in placental development (Chatterjee-Kishore *et al.* 2000). In sheep, *IRF1* expression increases in stroma and GE, but not in the LE during early pregnancy. Presumably, this is due to expression of IRF2, a potent transcriptional repressor of ISGs, that is constitutively expressed in the LE and increases during early pregnancy (Stewart *et al.* 2002). Pig and sheep endometria have similar patterns of expression for *IRF1* and *IRF2*. IRF1 expression is upregulated in the stromal stratum compactum between Days 12 and 15 and remains through Day 25 of pregnancy (Joyce *et al.* 2007b). When pigs were implanted with mini-osmotic pumps that delivered conceptus secretory proteins (CSP) containing IFNs to the uterine horn (see Fig. 3)

CSP increased *IRF1* in stroma, indicating upregulation of *IRF1* by conceptus IFNs (Joyce *et al.* 2007b). In contrast, *IRF2* mRNA increased in LE after Day 12 in response to conceptus estrogen (Joyce *et al.* 2007b). The similar temporal and spatial patterns of expression for *IRF1* and *IRF2* in pigs and sheep support the idea that *IRF2* represses expression of ISGs in the LE of pigs and perhaps mammals in general.

Signal Transducer and Activator of Transcription (STAT) 1

Cell-type specific induction of *STAT1* expression in pig endometrium is differentially regulated by conceptus signals. Estrogen secretion by the conceptus on Day 12 increases *STAT1* in the LE (Joyce *et al.* 2007a). Stromal induction of *STAT1* correlates with secretion of IFND and IFNG by the conceptus, and intrauterine infusion of conceptus secretory proteins (see Fig. 3), which contain IFND and IFNG, increases *STAT1* in a manner similar to that observed on Days 15-20 of pregnancy (Joyce *et al.* 2007a). STAT1 activation generally results in transcription of genes that are antiproliferative, proapoptotic and proinflammatory that could profoundly influence endometrial remodeling for implantation and placentation (van Boxel-Dezaire *et al.* 2006). Interestingly, although Type I IFNA and Type II IFNG each induce expression of largely non-overlapping sets of genes, they can also act in concert to reinforce physiological responses (Levy *et al.* 1990). This synergy has been demonstrated for induction of STAT1. Normally relatively non-responsive to IFNG, sequential treatment of cells with IFNG followed by IFNA results in higher magnitude ISG induction (Levy *et al.* 1990). In addition, co-treatment with IFNG and IFNA increases the magnitude and extends the period of ISG expression over IFNA alone (Decker *et al.* 1989). Clearly the pig may be unique among mammalian species studied. In pigs, combined conceptus IFND and IFNG may influence uterine physiology through cooperative induction of cytokine-specific transcription factors, such as *STAT1*, that allow reinforcement of effects of distinct cell-surface ligands while maintaining the specificities of the individual inducing IFNs in their ability to induce effects such as endometrial gene expression.

Swine Leukocyte Antigens (SLA) and Beta 2 Microglobulin (B2M)

MHC class I molecules (swine leukocyte antigens (SLAs) in pigs) and beta 2 microglobulin (B2M) are membrane glycoproteins that present peptide antigens to T cell receptors, and bind to inhibitory and activating receptors on natural killer cells and other leukocytes. They are involved in the discrimination of self from non-self by the immune system, and are essential to graft rejection. Downregulation of the expression of these classical MHC class I molecules and/or expression of nonclassical monomorphic MHC class I molecules by cells of the placenta benefit pregnancy (Hunt *et al.* 1987, Huddleston & Schust 2004). Although known to be interferon response genes, MHC class I and B2M are absent from endometrial LE during the peri-implantation period (Choi *et al.* 2003, Joyce *et al.* 2008). In pigs, expression of classical *SLA1*, *SLA2* and *SLA3*, non-classical *SLA6*, *SLA7* and *SLA8*, and *B2M* increases in endometrial LE between Days 5 and 9 in response to progesterone, then decreases between Days 15 and 20 (Joyce *et al.* 2008). Downregulation of SLA class I and B2M expression in uterine LE, in coordination with a lack of expression of these genes by the placenta (Ramsoondar *et al.*1999), may be important for preventing fetal allograft rejection in species exhibiting epitheliochorial placentation. In contrast to the situation observed for LE, expression of *SLAs* and *B2M* increases in stromal cells by Day 15 of pregnancy in response to conceptus IFNs, and remains detectable through Day 40 (Joyce *et al.* 2008). Cell-type specific regulation of *SLA* and *B2M* expression by progesterone and IFNs suggests that placental secretions control expression of immune

regulatory molecules on uterine cells to provide an immunologically favorable environment for survival of the fetal-placental semi-allograft.

Conclusions and future directions

Collectively, recent evidence from our laboratory and others suggests that pig conceptuses orchestrate precise temporal and spatial (cell-specific) changes in uterine gene expression through initial secretion of estrogens, followed by cytokines including IFNG and IFND. However, only a few differentially expressed genes have been investigated. Further, the pregnancy-specific role(s) of estrogen- and IFN-stimulated genes remains largely conjectural. Researchers in pig reproduction are challenged to incorporate new technologies including discovery-based microarray analyses to determine changes in global gene expression, adenovirus, morpholino and small interference RNAs to perform gain- and loss-of-function studies of specific endometrial and trophoblast genes, and state-of-the-art cell culture and imaging techniques necessary to delineate specific mechanistic functions of conceptus and uterine proteins.

Acknowledgements

This work was supported by National Research Initiative Competitive Grants nos. 2006-35203-17199 and 2008-35203-19120 from the USDA Cooperative State Research, Education, and Extension Service.

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