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INVITED REVIEW

The many faces of interferon tau

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Abstract Interferon tau (IFNT) was discovered as the pregnancy recognition signal in ruminants, but is now known to have a plethora of physiological functions in the mammalian uterus. The mammalian uterus includes, from the outer surface to the lumen, the serosa, myometrium and endometrium. The endometrium consists of the luminal, superficial glandular, and glandular epithelia, each with a unique phenotype, stromal cells, vascular elements, nerves and immune cells. The uterine epithelia secrete or selectively transport molecules into the uterine lumen that are collectively known as histotroph. Histotroph is required for growth and development of the conceptus (embryo and its associated extra-embryonic membranes) and includes nutrients such as amino acids and glucose, enzymes, growth factors, cytokines, lymphokines, transport proteins for vitamins and minerals and extracellular matrix molecules. Interferon tau and progesterone stimulate transport of amino acids in histotroph, particularly arginine. Arginine stimulates the mechanistic target of rapamycin pathway to induce proliferation, migration and protein synthesis by cells of the conceptus, and arginine is the substrate for synthesis of nitric oxide and polyamines required for growth and development of the conceptus. In ruminants, IFNT also acts in concert with progesterone from the corpus luteum to increase expression of genes for transport of nutrients into the uterine lumen, as well as proteases, protease inhibitors, growth factors for hematopoiesis and angiogenesis and other molecules critical for implantation and placentation. Collectively, the pleiotropic effects of IFNT contribute to survival, growth and development of the ruminant conceptus.

Keywords Uterus · Amino acids · Proteins · Pregnancy · Uteroferrin · Interferon tau

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Introduction

The establishment and maintenance of pregnancy in eutherian mammals require that the conceptus (embryo/fetus and extra-embryonic membranes or placenta) provide a pregnancy recognition signal. The pregnancy recognition signal is for maintenance of a functional corpus luteum (CL) for production of progesterone which is the essential hormone of pregnancy. Species-specific pregnancy recognition signals for CL maintenance include chorionic gonadotropin (CG) in primates, estrogens in pigs, lactogenic hormones in rodents, and interferon tau (IFNT) in ruminants (Bazer et al. 2010). Those pregnancy recognition signals also act on cells of the uterine endometrium to effect changes in gene expression in a cell-specific manner. In sheep and pigs, IFNT and estrogens, respectively, induce interferon regulatory factor 2 (IRF2) expression only in uterine luminal (LE) and superficial glandular (sGE) epithelia which silences expression of classical interferon-stimulated genes while permitting expression novel genes that support conceptus development such as

transporters for glucose and amino acids (Bazer 2013; Bazer et al. 2009). However, uterine glandular (GE) epithelium and stromal cells do not express IRF2; therefore, they express classical interferon-stimulated genes (ISGs) such as the ubiquitin-like interferon-stimulated gene 15(ISG15), myxovirus resistance 1, mouse, homolog of (MX1) and 2-prime,5-prime-oligoadenylate synthetase 1 (OAS) (see Bazer et al. 2009). The uterine LE/sGE, on the other hand, respond to IFNT in sheep and estrogen in pigs to express genes that increase transport and or secretion of nutrients, growth factors, transport proteins and other components of histotroph that support growth and development of the conceptus. This review focuses on the roles of IFNT for signaling pregnancy recognition in ewes and stimulating expression of genes critical to establishment of a uterine environment that supports conceptus development and pregnancy.

Characteristics of IFNT

Interferon tau has a molecular weight of 19–24 kDa depending on state of glycosylation. Ovine IFNT lacks glycosylation, whereas bovine IFNT is N-glycosylated at ASN78 and caprine IFNT is a mixture of nonglycosylated and glycosylated forms (Bazer et al. 1997; Alexenko et al. 2002). The pI of ovine IFNT is 5.3–5.8 and it is stable at pH 2–3 and at room temperature. Interferon tau has 172 amino acids with two disulfide bridges (1–99, 29–139) and an amino terminus proline. Interferon tau binds the Type I interferon receptor (the 100 kDa IFNAR1 and the 70 kDa IFNAR2) used by interferons alpha, beta, and omega with Kd values between 0.1×10^{-10} and 0.4×10^{-10} M. In sheep, the type I IFN receptors are expressed on essentially all cells of the body and IFNAR1 is expressed by conceptus trophectoderm (see Brooks and Spencer 2014).

Type I IFNs bind IFNAR1 and IFNAR2 and induce cell signaling via the Janus activated kinases (JAKs) and tyrosine kinase 2 (TYK2) pathways, respectively. This leads to formation of phosphorylated STAT1–STAT1 homodimers (gamma-activation factor, GAF) that translocate to the nucleus and bind GAS (gamma-activated site) elements in the promoter region of ISGs. One GAS-regulated gene is interferon regulatory factor 1(IRF1) which binds and activates interferon-stimulated response elements (ISREs) of many ISGs to amplify effects of type I IFNs. Type I IFNs act predominantly via interferon-stimulated gene factor 3 gamma (ISGF3G) rather than GAF, because STAT2 and ISGF3G are ISGs that prolong effects of IFNT by increasing expression of STAT2 and IRF9 that favor formation of ISGF3G rather than GAF. However, classical ISGs, including STAT1 and IRF9, are not expressed by uterine LE/sGE because IFNT-induced IRF2 is a potent transcriptional repressor that silences expression of ISGs (Spencer et al. 1998; Choi et al. 2001).

Mechanism for regulation of the estrous cycle in sheep

In sheep, luteolytic pulses of prostaglandin F2α (PGF) are released by uterine LE/sGE that express receptors for oxytocin (OXTR) and prostaglandin synthase 2 (PTGS2) (Bazer 2013). PTGS2 is the rate-limiting enzyme in synthesis of prostaglandins such as PGF. The uterine luteolytic mechanism requires sequential effects of progesterone (P4), estradiol (E2) and oxytocin (OXT) acting through their respective receptors on uterine LE/sGE to stimulate accumulation of phospholipids, expression of OXTR and release of OXT-induced luteolytic pulses of PGF. At onset of estrus (Day 0), estrogens from mature Graafian follicles increase expression of uterine estrogen receptor alpha (ESR1), PGR and OXTR, respectively. During diestrus, P4 from CL: (1) stimulates accumulation of phospholipids in LE/sGE that liberate arachidonic acid for synthesis and secretion of PGF; (2) acts via PGR to silence expression of ESR1 and OXTR in uterine LE/sGE for 10–12 days; and (3) downregulates PGR between Days 11 and 13 to release the "P4 block" and allow expression of ESR1 and then OXTR by uterine LE/sGE. The OXTR on uterine LE/sGE allow those cells to respond to pulsatile release of OXT from the CL and posterior pituitary with release of luteolytic pulses of PGF from the uterine LE/sGE that results in structural and functional demise of the CL between Days 15 and 16 of the estrous cycle.

Mechanism for pregnancy recognition signaling in sheep

Interferon tau is the pregnancy recognition signal from conceptuses of ruminant species (Bazer et al. 2009, 2010). Secretion of ovine IFNT by conceptus trophectoderm begins on about Day 10, increases to maximum amounts between Days 13 and 16 and then ceases to be secreted after Day 21 of pregnancy (Bazer 2013). IFNT silences transcription of the ESR1 and, therefore, ESR1-dependent expression of OXTR in uterine LE/sGE to prevent development of the endometrial luteolytic mechanism that requires oxytocin-induced pulsatile release of PGF. However, basal production of PGF is higher in pregnant than cyclic ewes due to continued expression of PTGS2 in the uterine LE/sGE, as well as production of prostaglandins by the conceptus (see Fig. 1).

The silencing ESR1 expression by IFNT also prevents E2 from inducing PGR in endometrial epithelia. This is important as the absence of PGR in uterine epithelia is

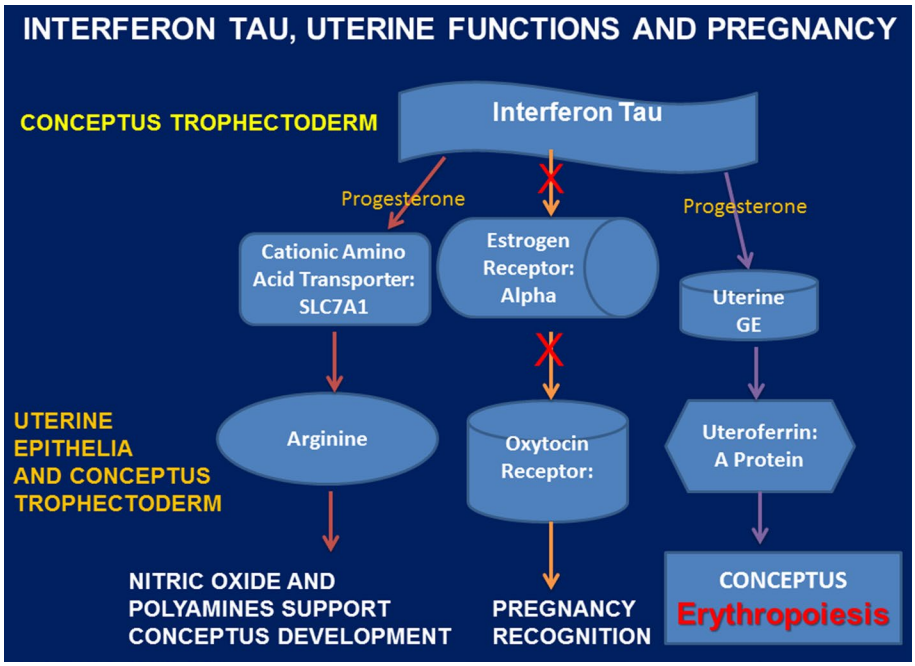

Fig. 1 Interferon tau (IFNT) acts, with progesterone (P4) being permissive to its effects, signals pregnancy recognition by silencing expression of estrogen receptor alpha (ESR1) and oxytocin receptor (OXTR) to block oxytocin-induced pulsatile release of prostaglandin F2α which abrogates the luteolytic mechanism that would otherwise lead to regression of the corpora lutea on the ovaries that produce progesterone. IFNT and P4-induced progestamedins act on uterine glandular epithelia for secretion of uteroferrin that stimulates erythropoiesis, while IFNT and P4-induced progestamedins act via uterine luminal and superficial glandular epithelia to increase transport of arginine that becomes available to conceptus trophectoderm and uterine epithelia for metabolism to nitric oxide and polyamines essential for conceptus growth and development

required for expression of a unique set of P4-induced and IFNT-stimulated genes in ovine uterine LE/sGE during early pregnancy. Progesterone is permissive for most, if not all, actions of IFNT on the uterus. Uterine receptivity to implantation is P4 dependent, but is preceded by loss of expression of PGR and ESR1 by uterine epithelia in all species reported, including primates, and, in ewes, the loss of PGR is a prerequisite for expression of several ISGs. Thus, P4 appears to act via PGR-positive stromal cells to increase expression of a progestamedin(s) in ewes. In ewes, the progestamedins are most likely FGF10 and HGF that exert paracrine effects on uterine epithelia and conceptus trophectoderm that expresses FGFR2IIIb and cMET (proto-oncogene Met), respectively. Both progestamedins and IFNT can act via mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) cell signaling to effect changes in gene expression and uterine receptivity to implantation (Platanias 2005; Spencer and Bazer 2002). In sheep, classical ISGs (e.g., ISG15, MX and OAS) are induced by IFNT only in uterine GE, stroma and immune cells that do not express IRF2. Because ovine uterine LE/sGE lack PGR and STAT1, and IFNT induces IRF2 in uterine LE/sGE, we hypothesize that IFNT activates gene transcription through alternate cell signaling pathways such as MAPK and PI3K that are complimentary to effects of progestamedins such as FGF10. The genes expressed by uterine LE/sGE in immediate contact with conceptus trophectoderm include transporters for amino acids, glucose, growth factors, proteases, protease inhibitors and extracellular matrix proteins that are critical for conceptus development (Spencer et al. 1998, 2007; Spencer and Bazer 2002).

Interferon tau and gene expression by uterine lumenal and superficial glandular epithelia

The ovine uterine LE/sGE cells respond to IFNT which signals via alternative cell signaling pathways that may include MAPK and PIK3 (Platanias 2005); however, an alternate cell signaling pathway in sheep has not been defined. Nevertheless, uterine LE/sGE in direct contact with conceptus trophectoderm express novel genes critical to conceptus development. Those P4-induced and IFNT-stimulated genes include solute carrier family 7 (cationic amino acid transporter, y+ system for arginine), member 2 (SLC7A2 for glucose), cystatin C (CST3), cathepsin L (CTSL), solute carrier family 2 member 1 (SLC2A1, facilitated glucose transporter), hypoxia-inducible factor 1,

alpha subunit (HIF2A. upstream of angiogenic factors such as angiopoietins and vascular endothelial growth factor), and galectin 15 (LGALS15) that encodes for secretory proteins and transporters that deliver molecules into the uterine lumen that are critical to conceptus development (Bazer et al. 2010, 2011; Spencer et al. 2007).

A paradox of pregnancy in all mammals is that cessation of expression of PGR and ESR1 by uterine epithelia is a prerequisite for uterine receptivity to implantation, as well as expression of genes by uterine epithelia for selective transport of molecules into the uterine lumen that supports conceptus development. Thus, effects of P4 are mediated via PGR expressed in uterine stromal and myometrial cells by stromal cell-derived growth factors known as "progestamedins" (Satterfield et al. 2008; Bazer et al. 2011). Cell-specific gene expression of P4-induced and IFNT-stimulated genes in uterine LE/sGE is due to IFNT-induced expression of IFR2 that selectively inhibits classical JAK/STAT cell signaling, but not alternative cell signaling via MAPK- and/or PI3K-induced expression of genes in uterine LE/sGE of sheep.

Interferon tau and gene expression by uterine glandular epithelia and stroma

Interferon tau increases expression of several ISGs in the GE and stroma of the ruminant uterus. Most of these genes have been characterized in the sheep, and include, among others, STAT1 and STAT2, major histocompatibility complex class I and β2-microglobulin, IRF1, IRF9, Mx, OAS, ubiquitin conjugating enzymes 1-8U and Leu-13, cathepsins H and K, ferritin heavy polypeptide 1, prothymosin alpha and ISG15 (see Johnson 2008). Although the temporal and spatial expression within the endometrial stroma of pregnant sheep varies slightly between genes, they generally follow the expression pattern first described for ISG15 (Johnson et al. 1999). ISG15 mRNA is first detectable in uterine LE and stratum compactum stroma on Day 13 of pregnancy which is immediately prior to implantation, but then expression extends to the stratum spongiosum stroma by Day 15 of the period of implantation. Expression of ISG15 is maintained throughout the stroma through Day 25, then declines by Day 30 of pregnancy, after which time expression is limited to patches of the stratum compactum stroma along the maternal-conceptus interface throughout pregnancy (Joyce et al. 2005). At present, we can only speculate on the roles of ISGs within the pregnant endometrium. The best characterized of these genes, ISG15, is a functional ubiquitin homologue that has the C-terminus Leu-Arg-Gly-Gly amino acid sequence common to ubiquitin, allowing conjugation to intracellular proteins (Haas 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 IFNT signaling from the trophectoderm that can temporally target proteins for pregnancy-associated regulation and/or modification.

Effects of IFNT on amino acid transporters in ovine uterine lumenal and superficial glandular epithelia

Pregnancy-associated mechanisms for transport of amino acids from the maternal circulation into the uterine lumen and then into conceptus trophectoderm are required for growth and development of the elongating conceptus (Verrey et al. 2004; Regnault et al. 2002; Grillo et al. 2008). In ewes, P4 and IFNT interact to increase expression of selected amino acid transporters in the uterus, particularly uterine LE/sGE that is in intimate contact with conceptus trophectoderm (Gao et al. 2009a). System y+ (SLC7A1, 2, and 3) cationic amino acid transporters are expressed in uteri of cyclic and pregnant ewes and conceptuses with *SLC7A1* and *SLC7A2* mRNA being abundant in uterine LE/sGE between Days 16 and 20 of pregnancy; however, the abundance of SLC7A3 is not affected by day of the estrous cycle or pregnancy status. *SLC7A1*, *SLC7A2* and *SLC7A3* mRNAs are expressed in both trophectoderm and endoderm of ovine conceptuses. Progesterone alone stimulates SLC7A1 expression in uterine LE and GE, and IFNT tends to increase SLC7A1 abundance in uterine LE/sGE; however, expression of SLC7A2 is induced by P4 and further stimulated by IFNT in uterine LE/sGE (Gao et al. 2009b). Progesterone appears to be permissive to actions of IFNT on gene expression when progesterone induces and IFNT further stimulates gene expression.

Neutral and acidic amino acid transporters expressed at low abundance in uteri of cyclic and pregnant ewes and conceptuses include SLC1A2, SLC1A3, SLC3A1, SLC6A14, SLC6A19, SLC7A6, SLC38A3 and SLC38A6 (Gao et al. 2009c). However, SLC1A1 and SLC7A5 mRNAs are abundant in uterine LE/sGE and GE, while SLC1A3 and SLC38A4 mRNAs are most abundant in uterine stromal cells. SLC1A5 mRNA is expressed primarily in uterine LE/sGE and stromal cells and is more abundant in uteri of pregnant than cyclic ewes. Progesterone induces and IFNT further stimulates expression of SLC1A5 in uterine LE/sGE. Several mRNAs for neutral and acidic amino acid transporters are expressed in ovine conceptus trophectoderm (SLC6A19, SLC7A5, SLC7A6, and SLC43A2) and others are more abundant in extra-embryonic endoderm (SLC1A4, SLC1A5, SLC6A19, SLC7A5, SLC7A6, SLC7A8 and SLC43A2) of ovine conceptuses.

Arginine and conceptus development in sheep

Interactions between the conceptus and various uterine cells, especially uterine LE/sGE and GE significantly affect conceptus development, uterine blood flow, water and electrolyte transport, maternal recognition of pregnancy, transport of nutrients such as amino acids and glucose into the uterine lumen, and secretion or selective transport of molecules required for conceptus for growth and development (Bazer et al. 2012). Here, the focus will be on arginine and interactions between arginine and secreted phosphoprotein 1 [SPP1, also known as osteopontin (OPN)], that activate mechanistic target of rapamycin (MTOR) cell signaling to stimulate migration, hypertrophy and hyperplasia of cells of the conceptus (Kim et al. 2010). Secreted phosphoprotein 1 binds integrins to initiate multiple cell signaling pathways, including FRAP1/mTOR, to support attachment and force-generated migration of trophectoderm cells (Kim et al. 2010; Guertin and Sabatini 2009). Arginine, leucine and glutamine are abundant in the conceptus (Bazer et al. 2013; Wu et al. 2013a) and their concentrations in the uterine lumen increase markedly between Days 10 and 16 of pregnancy, the peri-implantation period, when there is rapid growth and development of the ovine conceptus (Gao et al. 2009d; Kim et al. 2013). Sheep blastocysts are spherical between Days 4 (0.14 mm diameter) and 10 (0.4 mm diameter) and then elongate to the filamentous forms on Days 12 (1.0 × 33 mm), 14(1.0 × 68 mm) and 15 (150–190 mm long × 1 mm diameter) before extending through the uterine body and into the contralateral uterine horn by Days 16–17 (Bazer and First 1983). These dramatic changes in morphology of sheep conceptuses precede attachment of trophectoderm to uterine LE and initiation of implantation (Steven 1975). It is during this period of morphological and functional transition in development that 30–40 % of ovine conceptuses die as many fail to elongate and achieve sufficient contact between trophectoderm and uterine LE/sGE for uptake of nutrients and other components of histotroph.

The concentrations of arginine in uterine fluid (0.46–0.80 mM Arg) on Days 15 and 16 of gestation are greater than in maternal plasma and there is sevenfold more recoverable arginine from the uterine lumen of ewes on Day 15 of pregnancy compared to Day 15 of the estrous cycle. (Gao et al. 2009d). The increase in abundance of arginine in the uterine lumen coincides with rapid growth and development of ovine conceptuses during the peri-implantation period of pregnancy. Arginine plays crucial roles in survival and development of the conceptus. First, arginine is abundant in proteins of animal cells and in physiological fluids of the conceptus (Wu et al. 2013a, b). Second, arginine induces hypertrophy and hyperplasia of trophectoderm cells which are required for conceptus development. Third, arginine stimulates MTOR cell signaling to activate ribosomal protein S6 (RPS6) to induce hyperplasia and hypertrophy of trophectoderm required for conceptus elongation (Wynn and Wynn 1988; Guillomot et al. 1993). Fourth, motility and outgrowth of trophectoderm required for implantation are stimulated by arginine via MTOR cell signaling in mice (Martin and Sutherland 2001; Martin et al. 2003) and sheep (Kim et al. 2010). Finally, Arg is nutritionally essential for fetal–placental growth and development via its role in production of nitric oxide (NO) and NO signaling, as well as synthesis of polyamines, insulin secretion and insulin-mediated anabolic effects (see Wu et al. 2007a, b).

Nitric oxide and polyamines (putrescine, spermidine, and spermine) are products of arginine catabolism that are critical for placental growth (Wu et al. 2009). Arginine stimulates placental NO production by enhancing expression of GTP cyclohydrolase I (GCH1), the first and rate-controlling enzyme for synthesis of tetrahydrobiopterin (BH4) which is an essential cofactor for all isoforms of NO synthase. Along with insulin-like growth factors, vascular endothelial growth factors and other growth factors, NO and polyamines are required for angiogenesis, embryogenesis, placental growth, utero-placental blood flows, and transfer of nutrients from mother to fetuses, as well as fetal–placental growth and development (see Wu et al. 2009). Similarly, members of the arginine family of amino acids are highly abundant in ovine allantoic fluid (e.g., 10 mM citrulline and 25 mM Gln on Day 60 of gestation) (Kwon et al. 2003). The ovine placenta expresses arginase; therefore, citrulline is very abundant in allantoic fluid. Accordingly, rates of NO and polyamine synthesis in ovine placentae are highest during early gestation when placental growth is most rapid (Kwon et al. 2004a, b; Wu et al. 2005, 2012). We hypothesize that impaired placental growth (including vascular growth) or function results from reduced placental synthesis of NO and polyamines, thereby contributing to IUGR in both underfed and overfed dams (Wu et al. 2004). This hypothesis has gained support from experiments involving sheep (Satterfield et al. 2010, 2012, 2013; Lassala et al. 2010, 2011), pigs (Li et al. 2014; Mateo et al. 2007), and rats (Zeng et al. 2008, 2013).

Nitric oxide and trophoblast motility

Nitric oxide generated from conversion of arginine to NO by eNOS and/or iNOS in trophoblast cells activates guanyl cyclase to produce cGMP, stimulates trophectoderm motility perhaps by modifying the extracellular matrix (ECM), induces vasodilation of maternal blood vessels (Guo et al. 2005) and regulates cellular energy metabolism (Dai et al. 2013). During elongation and implantation of ovine conceptuses, P4 increases expression of SPP1 by uterine GE

(Johnson et al. 2003) and NO potentially enhances SPP1 expression to increase cell adhesion and invasion by cultured cells as suggested by results from other in vitro and in vivo models (Cartwright et al. 2002; Saxena et al. 2000). In addition, HGF-induced motility of human trophoblast cells is activated by NO signaling through PI3K/AKT/MTOR cell signaling (Kwon et al. 2004a). Increases in eNOS and iNOS in ovine placentomes that occur between Days 30 and 60 of gestation are sustained to Day 140 of gestation and coordinate increases in NO synthesis which parallels increases in vascular development in the placentae and utero-placental blood flows in ewes (Reynolds et al. 2005).

Polyamines and trophoblast motility

Changes in motility of trophectoderm cells may result from increases in expression of ornithine decarboxylase (ODC1), the rate-limiting enzyme in polyamine synthesis from arginine (Mehrotra et al. 1998). Polyamines associate with DNA and nuclear proteins to produce normal chromatin required for gene transcription, proliferation of trophectoderm and formation of multinucleated trophectoderm cells that give rise to giant cells in the placentae of mice (Kwon et al. 2004a). Cell signaling pathways induced by polyamines include MAPK and proto-oncogenes, c-myc, c-jun, and c-fos (Kwon et al. 2004b). Polyamines also activate MTOR cell signaling to stimulate protein synthesis in porcine trophectoderm cells (Kong et al. 2014). ODC1 is important for motility, integrin signaling via focal adhesion kinases, cytoskeletal organization, and invasiveness of mouse blastocysts. Polyamines also stimulate trophectoderm cell motility through modification of beta-catenin phosphorylation, and changes in uterine epithelial cells required for blastocysts to adhere to uterine LE and undergo implantation (Martin et al. 2003).

Synthesis of polyamines is highest in ovine placentomes and endometria between Days 30 and 60 of gestation when their growth and morphological changes, as well as development of the placental vascular bed, are most rapid for increased uterine blood flow to support fetal growth in the subsequent period of gestation (Kwon et al. 2004b). Knockout of the *Odc1* gene in mice is not lethal until the gastrulation stage of mouse embryogenesis (Pendeville et al. 2001). There is a requirement for polyamines later in embryogenesis as *Odc1* null embryos at the late morula to early blastocyst stages do not survive in vitro due to apoptotic cell loss in the inner cell mass, but this condition can be rescued by providing putrescine (a precursor of spermidine and spermine) in drinking water of the dam up to the early implantation stage, but not beyond that stage of pregnancy (Pendeville et al. 2001).

Loss-of-function of SLC7A1, ODC1 and NOS3 on ovine conceptus development

The in vivo functions of arginine in ovine conceptuses were further elucidated in studies to gain insight into the functional roles of arginine in growth and development of ovine conceptuses during the peri-implantation period of pregnancy. An in utero morpholino anti-sense oligonucleotide (MAO) loss-of-function approach was used to knockdown translation of mRNAs for: SLC7A1, the primary transporter for arginine into conceptus trophectoderm; ODC1, the rate-limiting enzyme for conversion of arginine to polyamines; and NOS3, the primary NOS isoform in ovine conceptus trophectoderm for production of NO and citrulline. The use of MAO in vivo to target knockdown of mRNA translation is specific to trophectoderm cells of the conceptuses because uterine epithelial cells do not take up MAO and are, therefore, unaffected with respect to mRNA translation (Wang et al. 2014a, b, c).

Loss-of-function of SLC7A1 in ovine conceptuses

The in vivo use of MAO-SLC7A1 to knockdown translation of SLC7A1 mRNA in sheep conceptus trophectoderm completely disrupted conceptus development. As compared to MAO control conceptuses the abundance of arginine in MAO-SLC7A1 conceptuses was reduced significantly (Wang et al. 2014a). Evaluation of downstream pathways of arginine effects revealed a decrease in the abundance of both ODC1 and NOS3 proteins in MAO-SLC7A1 conceptuses. Thus, pathways for production of both NO and polyamines were impaired due to deficiencies in transport of arginine into the conceptuses.

Loss-of-function of NOS3 in ovine conceptuses

The MAO knockdown of translation of NOS3 mRNA in trophectoderm of ovine conceptuses inhibited their development (Wang et al. 2014b). The MAO-NOS3 conceptuses were delayed morphologically in development, being elongated, but smaller, thinner and disorganized, compared to MAO control conceptuses. However, production of IFNT was not reduced significantly in MAO-NOS3 conceptuses suggesting that the developmental delay in conceptuses development was not due to failure of pregnancy recognition signaling. These results support the hypothesis that NO is important for normal growth and development of ovine conceptuses and that the stunted phenotype of MAO-NOS3 ovine conceptuses was due to NO insufficiency and downstream adverse effects on pathways such as that for synthesis of polyamines. The abundance of citrulline was greater while the abundance of ornithine was less in the uterine lumen of MAO-NOS3 ewes which suggests

increased transport of citrulline into the uterine lumen and/or reduced uptake of citrulline by the conceptus. It is also possible that decreased transport of ornithine into the uterine lumen increased uptake of citrulline by the conceptus and/or increased catabolism of ornithine through the ornithine aminotransferase pathway by uterine LE/sGE and GE of MAO-NOS3-treated ewes. Citrulline, a co-product of NOS, was measured in conceptuses since NO has a biological half-life of less than 5 s. In MAO-NOS3 conceptuses there was less citrulline and less ornithine (produced from hydrolysis of arginine by arginase) than in MAO control conceptus tissues. Decreases in arginine and ornithine, as well as glutamine and glutamate in MAO-NOS3 conceptuses suggest disruption of pathways for synthesis or transport of those amino acids. NOS3 is localized primarily in membrane-bound caveolae and may affect the transport of basic, neutral, and acidic amino acids by conceptus trophectoderm (Poulin et al. 2012). There were no significant differences in the abundance of NOS1, NOS2, arginine decarboxylase (ADC) or agmatinase (AGMAT) proteins between MAO-NOS3 and MAO control ewes, suggesting that those alternative pathways for production of NO and polyamines were not affected. There was, however, an increase in agmatine in the uterine lumen and conceptuses which may account for the smaller and thinner elongated MAO-NOS3 conceptus phenotype that produced IFNT. The most novel finding was the significant decrease in abundances of arginine, ornithine and polyamines in MAO-NOS3 conceptuses and the reduced amounts of polyamines in the uterine lumen suggesting that NOS3 significantly influences transport and metabolism of amino acids in ovine conceptuses. Guo et al. (2005) reported that NO affects expression of SPP1 (osteopontin) and we (G. Wu, G.A. Johnson, and F.W. Bazer, unpublished results) have evidenced that SPP1 affects placental (trophectoderm) transport properties; therefore, the deficiency in NOS3 may reduce SPP1 which may lead to decreased transport of arginine, ornithine and polyamines into the uterine lumen and or conceptus tissues.

Loss-of-function of ODC1 in ovine conceptuses

Ornithine decarboxylase is the rate-controlling enzyme for classical de novo biosynthesis of polyamines; however, it is also known that arginine can be converted to agmatine via arginine decarboxylase (ADC) and agmatine can be converted to polyamines via agmatinase (AGMAT) (Bernstein et al. 2011; Wang et al. 2014c). We discovered that the ADC/AGMAT pathway is functional in ovine conceptuses for synthesis of polyamines and compensates for loss of ODC1 activity following in vivo MAO knockdown of translation of *ODC1* mRNA. Interestingly, for MAO-ODC1 ovine conceptuses, one-half was morphologically and functionally normal and the other one-half was not morphologically normal or functionally normal. MAO-ODC1 knockdown conceptuses that maintained a normal phenotype had a greater abundance of *ADC* and *AGMAT* mRNAs and proteins that compensated for the loss of ODC1 to support polyamine synthesis. The majority of polyamine synthesis may normally be via the conventional ODC1-dependent pathway; however, the ADC/AGMAT-dependent pathway is, at least in sheep, may be a complimentary or compensatory pathway for production of polyamines for supporting survival and development of mammalian conceptuses. The compensatory effect may be related to genotype of conceptus, perhaps sex linked, but that has not been investigated.

Interactions between arginine and secreted phosphoprotein 1

We have demonstrated that SPP1 binds alphav:beta3 and alpha5:beta1 integrin heterodimers to induce focal adhesion assembly in our ovine trophectoderm cells (oTr1), a prerequisite for adhesion and migration of cells through activation of: (1) P70S6K via crosstalk between FRAP1/MTOR and MAPK pathways; (2) MTOR, PI3K, MAPK3/MAPK1 (Erk1/2) and MAPK14 (p38) signaling to stimulate oTr1 cell migration; and (3) focal adhesion assembly and myosin II motor activity to induce migration of Tr cells. These cell signaling pathways, acting in concert, mediate adhesion, migration and cytoskeletal remodeling of Tr cells essential for expansion and elongation of conceptuses and attachment to uterine LE for implantation (Kim et al. 2010). However, we now have results from in vitro experiments with oTr1 cells indicating individual and combined effects of arginine and recombinant SPP1 (X Wang, G Wu, G A. Johnson and FW Bazer, unpublished results). At physiological concentrations, arginine (0.2 mM) significantly increased oTr1 cell proliferation, but SPP1 had no effect. However, the combination of arginine and SPP1 significantly increased cell proliferation through activation of the PDK1-Akt/PKB-TSC2-MTORC1 cell signaling while SPP1 increased cell spreading, so together, arginine and SPP1 increase the abundance of cytoskeleton proteins, as well as proliferation and size of oTr1 cells. These effects of arginine and SPP1 on proliferation and cytoskeletal reorganization of oTr1 cells, as well as migration and attachment of trophectoderm cells for implantation suggest that MTORC1 and MTORC2 cell signaling stimulates elongation of ovine conceptuses during the peri-implantation period of pregnancy.

Arginine stimulates expression of IFNT by oTr1 cells

Arginine stimulates expression of IFNT (Kim et al. 2011); however, the mechanism of action of arginine has not

been established. Therefore, we investigated secretion of IFNT by oTr1 treated with arginine, putrescine and NO donors, as well as their associated inhibitors (X Wang, G Wu, G A. Johnson and FW Bazer, unpublished results). Arginine-stimulated proliferation, IFNT production and protein synthesis per oTr1 cell via a pathway involving increases in phosphorylated TSC2 and MTOR abundance. N5-[imino(nitroamino)methyl]-L-ornithine, methyl ester, monohydrochloride (L-NAME; NOS inhibitor), α-difluoromethylornithine (DFMO; ODC1 inhibitor), and L-NAME + DFMO significantly reduced effects of arginine on cell proliferation by 11, 16, and 22 % at 48 h, and 15, 27 and 39 % at 96 h of incubation, respectively. Values were greater than for the arginine-free control suggesting that arginine is itself acting as a growth factor. Both putrescine and NO stimulate cell proliferation, but only putrescine increased IFNT production. These results indicate that arginine is essential for oTr1 cell proliferation and IFNT production via the NO/polyamine-TSC2-MTOR signaling pathway while, in turn, IFNT increases expression of SLC7A1 to increase transport of arginine into the uterine lumen for metabolism to NO and polyamines.

Inteferon tau and uteroferrin [also known as tartrate resistant acid phosphatase (TRAP) and phosphatase, acid, type 5, tartrate-resistant (ACP5)]

Uteroferrin (UF) is an iron-containing glycoprotein secreted by uterine GE in response to progesterone and transported across the placental areoalae into the fetal circulation and allantoic fluid to deliver iron and to stimulate hematopoiesis based on results of studies with pigs (see Roberts et al. 1986). Uteroferrin is expressed by uterine GE in ewes between Days 18 and 120 of pregnancy and its expression is progesterone induced and further stimulated by IFNT (Gao et al. 2010). However, expression of UF mRNA in ovine uterine GE was not affected by ovine placental lactogen or ovine growth hormone. The roles of UF in the ovine uterus may include transport of iron across the placenta and stimulation of hematopoiesis as is the case for pigs.

Uteroferrin is secreted by uterine GE of pigs and other ungulates, as well as human placenta, spleen and osteoclasts (see Roberts et al. 1986). Uteroferrin from human placenta and pig endometrium have 82 % amino acid sequence identity. In pigs, UF from pig uterine GE is transported across the placenta and into the fetal–placental circulation and taken up by reticuloendothelial cells of the fetal liver via high mannose receptor-mediated endocytosis (Renegar et al. 1982; Saunders et al. 1985). Uteroferrin is transferred to hematopoietic cells in fetal liver and its iron is incorporated into hemoglobin in erythrocytes (Ducsay et al. 1984). Pig and human UF also exhibit colony forming unit erythroid (CFU-E), granulocyte monocyte colony forming unit (CFU-GM), and granulocyte-erythroid-monocyte/macrophage-megakaryocyte (CFU-GEMM) activities independent of effects of transferrin (Bazer et al. 1991). Thus, UF both transports iron from mother to conceptus for synthesis of hemoglobin and stimulates aspects of hematopoiesis. UF is readily detectable in yolk sac, liver, spleen and bone marrow of fetal pigs, the major sites of hematopoiesis. Of current interest is the fact that UF and SPP1 show remarkable cell type-specific co-localization in uteri and placentae of pregnant pigs, and both are secreted from uterine GE for access to the fetal–placental circulation and sites of hematopoiesis.

Erythropoiesis begins in the yolk sac of the developing conceptus with hematopoietic stem/progenitor cells (HSPC), and extends to liver, spleen and bone as HSPC sequentially colonize hematopoietic niches in those organs during pregnancy (Roberts et al. 1986). Erythroid progenitor cells (EPC) of humans, pigs and mice differentiate and enucleate after entering the circulation. Based on in vivo experiments with myelosuppressed piglets (Laurenz et al. 1997a, b, c), UF reduces the rate of 5-fluorouracil (5-FU)-induced leukocytopenia, enhances recovery of neutrophils and monocytes and attenuates suppression of erythrocytes. Myelosuppressed pigs treated with both UF and colony stimulating factor 2 (CSF2) also have attenuated suppression of erythrocytes, reduced leukocytopenia, and enhanced recovery of erythrocytes and thrombocytes. Young pigs that were not myelosuppressed, but treated with UF and CSF2 also exhibited a protracted increase in leukocytes, particularly monocytes and neutrophils, and they experienced a dramatic eosinophilia as compared to control pigs. Although UF and CSF2 increased CFU-GEMM, CFU-GM and BFU-E progenitor cells to increase erythropoiesis, the molecular mechanism(s) responsible is not known.

UF null mice exhibit severe abnormalities of long bones and the axial skeleton due to deficiencies in endochondral ossification, osteopetrosis, increased amounts of inflammatory cytokines from macrophages and an increase in tartrate sensitive lysosomal acid phosphatase activity (LAP) (Hayman et al. 1996; Suter et al. 2001; Hayman et al. 2001; Hayman et al. 2011). Uteroferrin-lysosomal (UF-LAP) acid phosphatase double knockout mice have even more severe skeletal defects (Hayman et al. 2011). Although, effects of UF:LAP double knockout in mice on hematopoiesis have not been reported, mutations in UF that reduce or eliminate its acid phosphatase activity increase hyperphosphorylated SPP1 which leads to skeletal defects, neurological problems, developmental delays, increased production of interferon alpha and systemic autoimmunity, and impaired erythropoiesis (see Behrens and Graham 2011; Briggs et al. 2011; Lausch et al. 1996; Hare et al. 2013; Zhang et al.

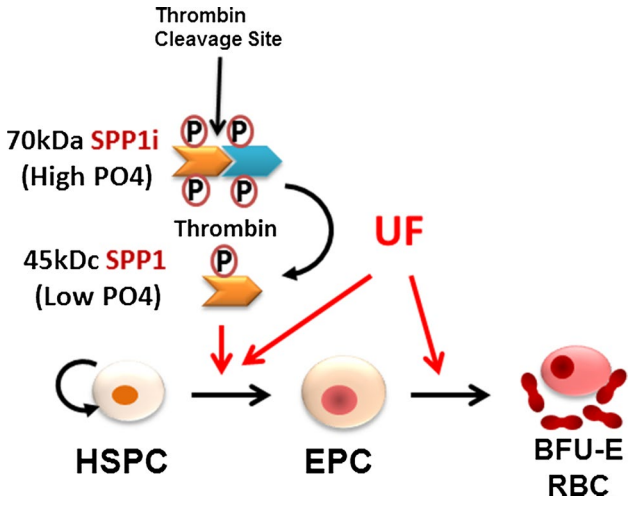

Fig. 2 We propose that secreted phosphoprotein 1 is cleaved to 45 and 25 kDa forms by thrombin and that uteroferrin, an acid phosphatase, then decreases the phosphorylation state of SPP1 to hypophosphorylated subunits of SPP1 to act within hematopoietic niches to modulate erythropoiesis

2003; Hayman et al. 1996, 2001; Suter et al. 2001; Hayman and Cox 2003). Highly phosphorylated SPP1 is considered a natural substrate for UF (Behrens and Graham 2011; Briggs et al. 2011). Our results from studies of mice revealed that UF has significant burst-forming unit erythroid (BFU-E) and colony forming unit erythroid (CFU-E) activities in mice and that UF co-localizes with SPP1 in fetal and adult "hematopoietic niches" of pigs (Ying et al. 2014), but not CFU-GM or CFU-GEMM as reported early from studies of hematopoietic progenitor cells from pigs and humans (Bazer et al. 1991) (see Fig. 2).

Using primary fetal liver hematopoietic cells from mice, UF has a synergistic stimulatory effect with erythropoietin and other growth factors on both burst-forming unit erythroid (BFU-E) and CFU-E (Ying et al. 2014). Uteroferrin, along with erythropoietin, increased expression of transcription factors required for terminal differentiation of erythrocytes and genes required for synthesis of hemoglobin. Thus, UF promotes fetal erythropoiesis at various stages of pregnancy, including BFU-E and CFU-E progenitor cells and terminal stages of differentiation of hematopoietic cells in the erythroid lineage. Terminal differentiation of erythroid cells is controlled by a network of transcription factors effecting changes in expression of genes for enzymes required for the synthesis of hemoglobin and transcription factors regulating final differentiation of erythroid cells. The expression of genes related to hemoglobin synthesis including (hemoglobin) Hbb1 and Hba1, delta-aminolevulinate synthase 2 (ALAS2) and other transcription factors is increased by UF (see Ying et al. 2014). Uteroferrin increases expression of transcription factor gata-binding protein 1 (GATA1) that controls proliferation and maturation of erythroid cells, histone deacytlase 1 (HDAC)1 that forms a complex with GATA1 to regulate chromatin changes during the terminal stage of erythropoiesis and kruppel-like factor 1 that directs differentiation of erythroblasts into reticulocytes. Thus, UF increases production of reticulocytes from erythroblasts by upregulating expression of key transcription factor genes.

Conclusions and perspectives

Interferon tau was first recognized as the pregnancy recognition signal in ruminants. However, as chronicled in this review, that is only one function of IFNT. Interferon tau also has anti-inflammatory effects that may be critical to establishment of a local immune status that protects the semi-allogenic conceptus trophectoderm from the maternal immune system (Choi et al. 2003). Another critical role of IFNT is its effects on uterine LE/sGE to induce expression of IRF2 which silences expression of ESR1 and OXTR to abrogate the luteolytic mechanism. Silencing ESR1 also prevents E2 from inducing expression of PGR in uterine LE/sGE; therefore, progestamedins, e.g., FGF10 and HGF from uterine stromal cells and IFNT from conceptus trophectoderm regulate gene expression by uterine LE/sGE during the peri-implantation period of pregnancy. Further, IFNT-induced IRF2 silences expression of ISGs in uterine LE/sGE while being permissive to their expression of genes for nutrient transport, angiogenesis, tissue remodeling, extracellular matrix molecules, hematopoiesis and other components of histotroph required for conceptus development. The induction of transporters for cationic amino acids by progesterone and further increases in their expression by IFNT markedly increases transport of arginine into the uterine lumen during the peri-implantation period of pregnancy. Arginine, as a precursor for NO and polyamines, plays a critical role in successful elongation of the conceptus and stimulates production of IFNT by trophectoderm which, in turn, acts in concert with progestamedins to enhance transport of arginine. Progesterone also induces and IFNT increases the expression of UF by uterine epithelia in sheep which provides for transplacental transport of iron and for stimulation of hematopoiesis in the conceptus. Thus, the many faces of IFNT include its role in pregnancy recognition signaling and cell-specific expression of components of histotroph including amino acids essential for conceptus development and UF for stimulation of erythropoiesis.

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