Roles of selected nutrients in development of the porcine conceptus during pregnancy

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Conceptus development in mammals depends on an intra-uterine environment filled with histotroph that includes molecules that are secreted by uterine epithelia and/or selectively transported into the uterine lumen. In pigs, total recoverable glucose, fructose, arginine, leucine and glutamine increase in histotroph with advancing days of the peri-implantation period of pregnancy and in allantoic fluid later in gestation. During pregnancy, the uterine luminal epithelium (LE) and trophectoderm of conceptuses each express specific transporters for glucose. The most abundantly expressed amino acid transporters in uterine LE and trophectoderm are those for glutamate, neutral amino acids and cationic amino acids. These nutrient transporters are also expressed in uterine epithelia and placental tissues of pigs throughout gestation and expression of transporters and accumulation of nutrients in the uterine lumen is affected by progesterone and estradiol. Treatment of porcine trophectoderm cells with glucose, arginine and leucine stimulates the mechanistic target of rapamycin nutrient sensing cell signaling pathway to increase phosphorylation of RPS6K, RPS6 and EIF4EBP1 in the nucleus or cytoplasm to stimulate proliferation, mRNA translation and protein synthesis. Glucose and fructose are equivalent in stimulating proliferation of pig trophectoderm cells and in inducing synthesis of hyaluraonic acid via the hexosamine pathway. The results of our research indicate mechanisms whereby select nutrients act differentially to affect translation of mRNAs for cell signaling molecules that affect conceptus growth, development, and survival during pregnancy in pigs.

Introduction

Embryonic mortality and fetal morbidity claim 20-50% of conceptuses (embryo/fetus and extra-embryonic membranes) in pigs (Bazer *et al*. 2009; Bazer *et al*. 2011a). Successful establishment and maintenance of pregnancy requires orchestrated communication between

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conceptus trophectoderm and uterine epithelia mediated by autocrine, paracrine, and endocrine molecules that regulate uterine and/or trophectoderm expression of genes that support conceptus development. The uterine epithelia synthesize and secrete, as well as transport numerous proteins and nutrients, collectively known as histotroph, required for conceptus development, implantation and placentation (see Bazer *et al*. 2011b; Bazer *et al*. 2012a).

Pig conceptuses initiate secretion of estrogens for pregnancy recognition signaling to maintain CL that secrete progesterone (P4) required for an intrauterine environment that supports pregnancy (Spencer *et al*. 2004). Interactions among the conceptus and uterine luminal (LE), superficial glandular (sGE) and glandular (GE) epithelia and stromal cells effect changes in components of uterine histotroph (Bazer *et al*. 2012a) (Figure 1). In the absence of uterine glands, pregnancy fails in ewes (Gray *et al*. 2001) and litter size is reduced in gilts with reduced development of uterine glands (Bartol *et al*. 2006).

Uterine histotroph includes nutrients that increase in the uterine lumen during the peri-implantation period of pregnancy including arginine (Arg), leucine (Leu), glutamine (Gln) and

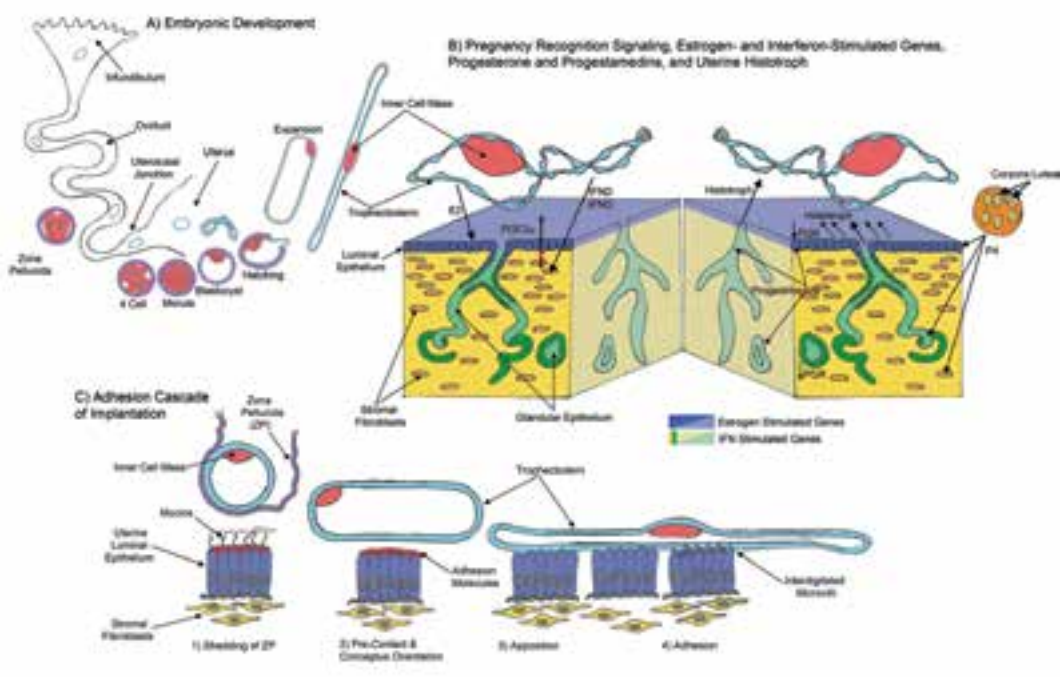

Fig. 1 A. Oocytes fertilized in the oviduct enter the uterus at the 4- to 8-cell stage, advance developmentally to blastocysts and then elongating conceptuses (embryo and its extra-embryonic membranes). B. The endometrial epithelia cease expressing receptors for progesterone (PGR) due to autoregulation by progesterone and estradiol-17β along with prolactin maintains exocrine secretion of prostaglandin $F_{2\alpha}$ (PGF) into the uterine lumen to prevent regression of the corpus luteum. Estradiol-17β also induces interferon regulatory factor 2 (IRF2) that silences expression of classical interferon stimulated genes (ISG). The uterine luminal epithelium (LE) secretes fibroblast growth factor 7 that acts via its receptor (FGFR2IIIb) expressed by uterine LE, uterine glandular epithelium and trophectoderm. With down-regulation of PGR in uterine epithelia the uterine luminal (LE) and superficial glandular epithelia express genes that are induced by progesterone, presumably acting via a progestamedin from uterine stromal cells, and further stimulated by estradiol-17β and perhaps interferons delta and gamma. Collectively, molecules secreted by uterine epithelia or transported into the uterine lumen are referred to as histotroph. C. The adhesion cascade for implantation of the conceptus in the pig occurs between Days 13 and 25 of pregnancy.

glucose (Wu *et al*. 2010). The mechanistic target of rapamycin (MTOR) cell signaling pathway is a key nutrient sensing cell signaling pathway (Kim *et al*. 2002). As shown in Figure 2, the MTOR complexes (MTORC1 and MTORC2) include FRAP1 (FK506 binding protein 12-rapamycin associated protein. MTORC1 includes FRAP1, MTOR associated protein LST8 (MLST8) and regulatory associated protein of MTOR (RAPTOR) associated with cell proliferation, mRNA translation and protein synthesis. MTORC2 includes FRAP1, LST8 and rapamycin insensitive companion of MTOR (RICTOR) associated with cell migration, cytoskeletal organization and cell survival. The combined effects of MTORC1 and MTORC2 influence proliferation and migration of trophectoderm cells, as well as changes in cytoskeletal organization and gene expression required for elongation of porcine conceptuses (Guertin *et al*. 2006). FRAP1, a highly conserved serine-threonine protein kinase, senses and responds to changes in amino acid levels and energy sufficiency, as well as hormones and mitogens (Dennis *et al*. 1996; Gingras *et al*. 1999; Gingras *et al*. 2001; Jacinto *et al*. 2006). Knockout of *Frap1* (Gangloff *et al*. 2004; Murakami *et al*. 2004), *Raptor* and *mLST8* (Guertin *et al*. 2006), *Rictor* (Guerten *et al*. 2006; Shiota *et al*. 2006), and *Mapkap1* (Jacinto *et al*. 2006) genes in mice results in dysfunction of MTORC1 and MTORC2, and fetal lethality at different stages of development.

Amino acids and glucose in cell signaling

Arginine. Arginine is nutritionally essential for conceptus growth and development via its role in nitric oxide (NO) signaling and polyamine synthesis (Wu *et al*. 2004; Figure 3). In mice,

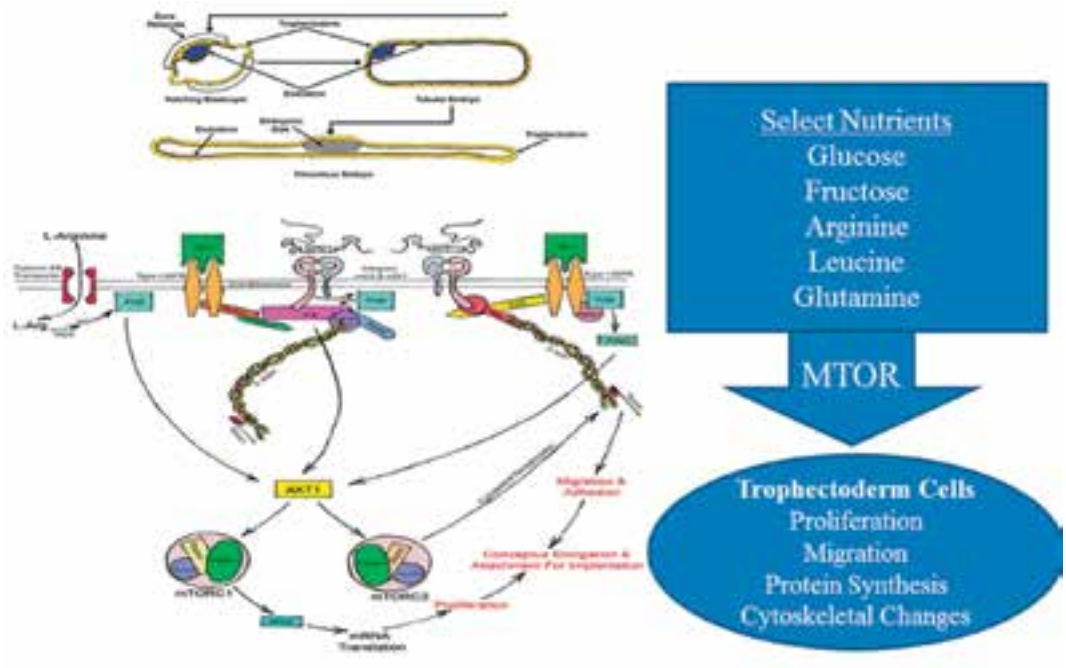

Fig. 2 Select nutrients such as L-arginine, as well as insulin-like growth factor 2 (IGF2) and secreted phosphoprotein 1 (SPP1) that are known to be present in the uterine lumen can induce cell signaling via the AKT1/mTORC1/mTORC2 pathways to affect cell proliferation, cell migration, mRNA translation and cytoskeletal remodeling in trophectoderm cells during elongation of the conceptus that are critical to survival and development of the conceptus.

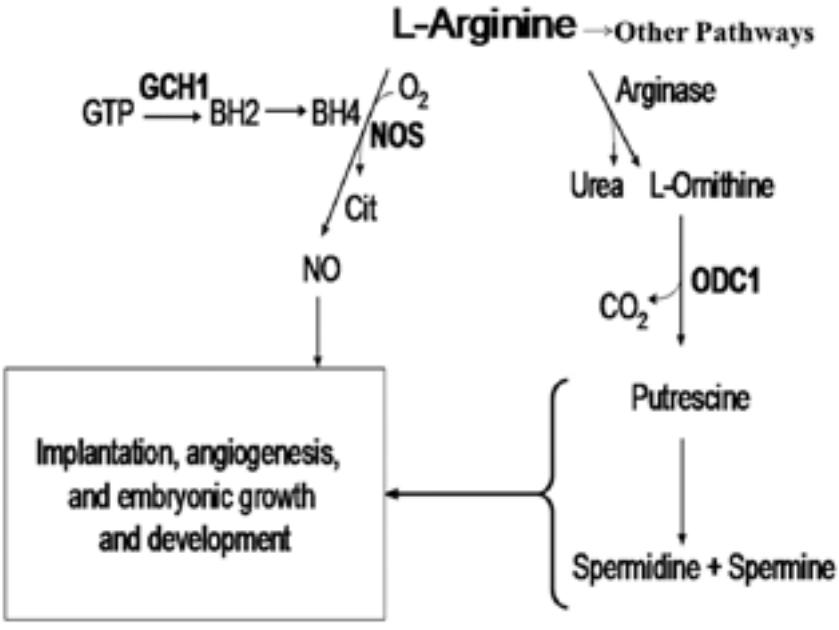

Fig. 3 L-Arginine can affect proliferation and migration of ovine trophectoderm cells by being converted to nitric oxide (NO) by nitric oxide synthase (NOS) or by being converted to L-ornithine by arginase with ornithine then being converted to polyamines by ornithine decarboxylase (ODC1). The activity of all NOS isoforms requires tetrahydrobiopterine (BH4) that is dependent of GTP cyclohydrolase (GCH1) for its synthesis. Arginine stimulates GCH1 expression in ovine conceptus trophectoderm (Kim *et al*., 2011c), but this has not been demonstrated for pig conceptus trophectoderm.

NO promotes blastocyst attachment and trophoblast motility, possibly through modifications of the extracellular matrix and stimulation of vasodilation of maternal blood vessels (Gwatkin 1966). Arg activates MTOR in our established porcine (pTr) and ovine (oTr) trophectoderm cell lines more than any other amino acid (Bazer *et al*. 2012a; Kim *et al*. 2013). NO enhances utero-conceptus blood flow and the transfer of nutrients and oxygen from mother to fetus (Bird *et al*. 2003). Polyamines regulate DNA and protein synthesis, scavenge reactive oxygen species, induce cell proliferation and differentiation (Igarashi & Kashiwagi 2000), and stimulate trophectoderm cell motility, implantation, and conceptus development (Martin and Sutherland 2001; Martin *et al*. 2003; Dey *et al*., 2004; Wu *et al*., 2007; Zhao *et al*. 2008).

Leucine. Leu, the most abundant branched-chain essential amino acid in many dietary proteins, affects protein synthesis and degradation, leptin secretion, energy balance and, in excess, it is an energy substrate to spare glucose (Lei *et al*. 2012). Leu and Arg stimulate MTOR cell signaling in oTr cells (Kim *et al*. 2011a) and these amino acids are required for transition of morulae to blastocysts in rodents (Gwatkin 1996) (Figure 4). Leu is a major donor of the amino group for endogenous synthesis of Gln in pigs (Self *et al*., 2004; Wu *et al*., 2011).

Glutamine. Metabolism of Gln provides reducing equivalents for production of ATP in ovine (Wales & Du 1994) and bovine (Rieger *et al*. 1992) conceptuses to complement glucose metabolism (Rieger, 1992). Gln can be converted into citrulline, the precursor of Arg, in porcine and ovine placentae (Kwon *et al*. 2003) and Gln inhibits NO production from Arg (Wu *et al*. 2001); therefore, Gln and Arg are linked in amino acid catabolism. Gln is critical for the glutamine:fructose-6-phosphate amidotransferase 1 (GFPT1) pathway for conversion of fructose

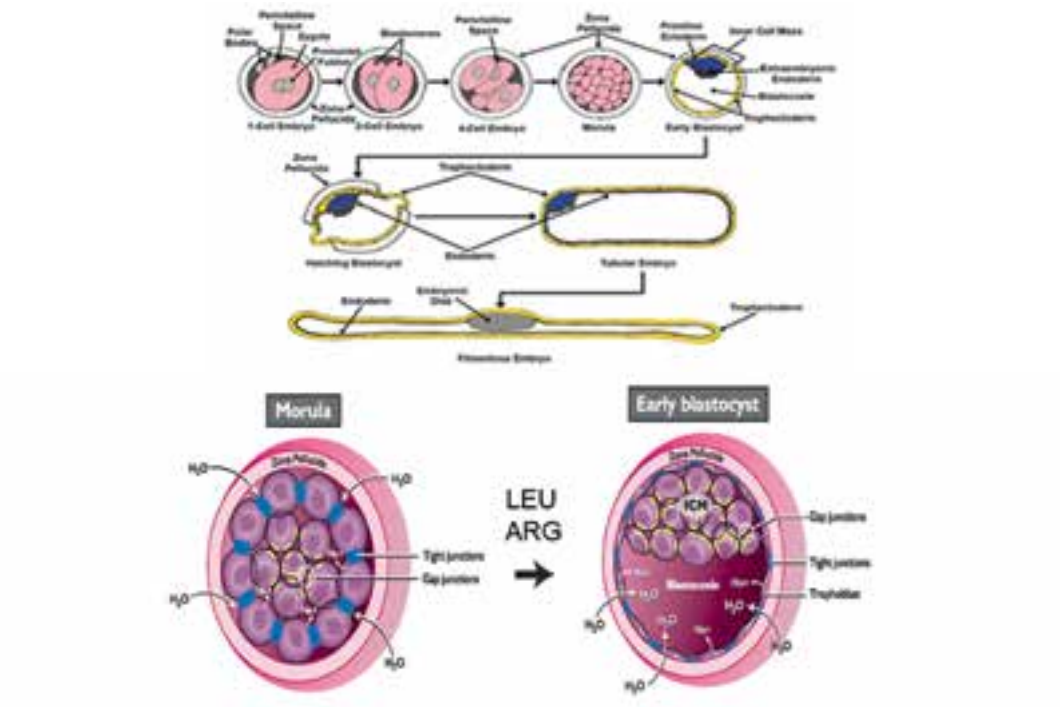

Fig. 4 Studies of mouse blastocysts revealed that develop is arrested when maintained for 5 days in culture medium lacking arginine, lysine and histidine (Gwatkin 1996). This suggests that blastocyst expansion and implantation are controlled *in vivo* by the presence of these specific amino acids in the uterine lumen. Additional research confirmed that mouse blastocysts also require the presence of either leucine or arginine to exhibit expansion, motility and outgrowth of trophectoderm required for formation of the blastocyst and implantation (Martin and Sutherland, 2001; Martin *et al*., 2003). These developmental events are also considered critical for growth and development of porcine embryos and blastocysts that precedes the essential processes of elongation and attachment of conceptus trophectoderm and uterine LE for implantation. The figure is adapted from Senger (2003).

6-PO4 to glucosamine 6-PO4 and the synthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) and glycosaminoglycans such as hyaluronic acid (Flynn *et al*. 2002; Kim *et al*. 2012).

Glucose. Glucose is required for survival and development of embryos during the preimplantation period when its utilization is partially regulated by the PI3K pathway and its metabolism increases from the 2-cell to the blastocyst stages of development (Riley & Moley, 2006). Pyruvate is a primary energy source from fertilization to hatching of blastocysts in the uterus, and then glucose uptake by blastocysts increases and lactate is a major product of glucose metabolism (Wales 1986; Rieger 1992; Wales and Waugh 1993; Gardner *et al*. 1996). There is accumulation of glucose, but little glycogen synthesis by ungulate conceptuses (Pike 1981). Rather, glucose not required to meet demands of the conceptus is converted to fructose (see Kim *et al*. 2012). Glucose-fructose isomerase in uterine flushings and allantoic fluid of pig conceptuses may allow some interconversion of fructose to glucose (Zavy *et al*. 1982; Gu *et al*. 1987; Bazer *et al*. 1991). Wen *et al*. (2005) linked MTORC1 with GFPT1 as a nutrient sensing pathway that stimulates proliferation of trophectoderm cells.

Changes in abundance of Arg, Leu, Gln and glucose in uterine flushings and in allantoic fluid of pigs

Conceptus development in pigs is affected by glucose and select amino acids, particularly Arg (Kim *et al*. 2013). Glucose, Arg, Leu and Gln increase in uterine flushings of pregnant, but not cyclic gilts between Days 10 and 15 after onset of estrus and in uterine flushings from pseudopregnant and ovariectomized gilts treated with P4. Temporal and cell-specific changes in expression of glucose and amino acid transporters in the uterine epithelia and conceptus trophectoderm of pigs are coincident with conceptus elongation. In pigs, *SLC2A1* is the most abundant glucose transporter in uterine LE from Days 15 through 80 of pregnancy, and it is expressed by the allantoic epithelium between Days 20 and 30 of pregnancy. Patterns of expression of *SLC2A4* and *SLC2A1* are similar, but *SLC2A4* is less abundant and not detectable after Day 30 of pregnancy. *SLC2A2* mRNA is abundant in conceptuses from Days 12 to 50, and unique to placental areolae and peaks of folds of the chorion between Days 50 and 85 of pregnancy. The amino acid transporter *SLC7A3* is most abundant in the chorion when Arg transport across the placenta is maximal. *SLC5A1* expression is induced in uterine LE by E2. Treatment of gilts with P4 increases amounts of neutral amino acids in the uterine lumen of pigs.

Additional studies evaluated expression of genes that encode transporters for neutral (*SLC1A1*, SLC1A4, and *SLC1A5*) and cationic amino acids (S*LC7A1*, *SLC7A2*, *SLC7A7* and *SLC7A9*), as well as ornithine decarboxylase (*ODC1*) in the uterine endometrium, peri-implantation conceptus and chorioallantoic placenta of pigs (J.Shim, H. Seo, Y. Choi, J. Kim, F.W. Bazer and H. Ka, unpublished results). In the uterus *SLC1A1* mRNA increases on Day15 of pregnancy and then decreases near term as *SLC1A5* and *SLC1A4* mRNAs increase during mid- to late pregnancy. The abundance of *SLC7A1*, *SLC7A2* and *SLC7A9* mRNAs is highest on Day 12 of pregnancy, but lower throughout the remainder of pregnancy. Expression of *SLC7A7* mRNA is also high during early pregnancy, but low during mid- to late pregnancy. *SLC1A1, SLC7A7, SLC7A9* and *ODC1* mRNAs are localized to uterine LE and GE, and chorion during pregnancy. *SLC1A1, SLC1A4, SLC1A5, SLC7A1, SLC7A7* and *SLC7A9* mRNAs are expressed in conceptuses on Days 12 and 15 of pregnancy. In placental tissues there is biphasic expression of *SLC1A4* mRNA with highest levels on Day 30 and at term, while expression of *SLC7A2*, *SLC7A9* and *ODC1* mRNAs is highest on Day 30 of pregnancy. Expression of *SLC1A1*, *SLC1A5*, *SLC7A1* and *SLC7A7* in the placenta was not affected by day of pregnancy. These changes in expression of amino acid transporters in the endometrium and conceptus are likely critical for development of pig conceptuses.

Arg and ornithine nitrogen account for 40% to 55% of the total free alpha-amino acid nitrogen in allantoic fluid between Days 30 and 45 of gestation which suggests significant roles for those amino acids during placental development that precedes rapid fetal growth later in gestation (Wu *et al*. 1996). Arg is readily converted to NO and polyamines to enhance conceptus development. These nutrients and components of uterine histotroph in allantoic fluid are available to support growth and development of the pig conceptuses (Bazer *et al*. 1991; Kim *et al*. 2013) which have a true epitheliochorial placenta and rely on histotroph for nutrients and other factors throughout pregnancy (Knight *et al*. 1977; Geisert *et al*. 1982).

Endocrine and paracrine signaling for establishment of pregnancy and gene expression in uteri of gilts

Pig conceptuses secrete estrogens as the pregnancy recognition signal during the peri-implantation period of pregnancy (Bazer &Thatcher 1977; Zeicik *et al*. 2011). Pig conceptus

trophectoderm also secretes both interferon delta (IFND) and interferon gamma (IFNG) that stimulate expression of an array of IFN-stimulate genes (ISGs) in a temporal and spatial (cell-specific) manner (Bazer *et al*. 2009; Johnson *et al*. 2009),but the roles of IFND and IFNG are not known (Johnson *et al*. 2009). Uterine receptivity to implantation is dependent on P4 and estradiol-17β (E2) in pigs. The paradox is that receptors for P4 (PGR) are down-regulated after Day 5 and receptors for E2 (ESR1) are not expressed after Days 15 to 16 in uterine LE and GE of pregnant pigs. Loss of PGR and ESR1 in uterine epithelia is a prerequisite for implantation, expression of genes for secretory proteins, and selective transport of molecules into the uterine lumen to support conceptus development (Geisert *et al*. 1993; Geisert *et al*. 1994; Ka *et al*. 2007). Down-regulation of PGR is coincident with loss of expression of proteins such as MUC1 that interfere with interactions between integrins and extra-cellular matrix molecules required for implantation (Carson *et al*. 2002). Then, both E2 and P4 are required to induce expression of fibroblast growth factor 7 (FGF7) by uterine LE initially and, from around Day 20 of pregnancy, uterine GE to effect transcription of genes in trophectoderm that affect conceptus development and in uterine LE and GE to affect secretion and transport of components of histotroph (Ka *et al*. 2000; Ka *et al*. 2001; Ka *et al*. 2007). Changes in uterine histotroph in ewes occurs in response to P4 and interferon tau (IFNT), as well as FGF10 and hepatocyte growth factor (HGF) from uterine stromal cells which regulate gene expression by uterine LE and sGE and trophectoderm (Bazer *et al*. 2012a). Similarly, the composition of histotroph in pigs is likely affected by P4, E2, IFND and IFNG.

Effects of select nutrients on porcine trophectoderm (pTr) cells

During the peri-implantation period of pregnancy, elongation of the conceptus involves proliferation, migration, differentiation and cytoskeletal changes in the pig trophoblast (trophectoderm and extra-embryonic endoderm) (Geisert *et al*. 1982). Research with oTr cells and Day 16 ovine conceptus explant cultures revealed that Arg, Leu and glucose activate AKT1, MTORC1, RPS6K and RPS6 cell signaling to stimulate proliferation and migration, mRNA translation and protein synthesis (Kim *et al*. 2011a; Kim *et al*. 2011b; Kim *et al*. 2011c). Those nutrients differentially increase: 1) NOS isoforms and production of NO from Arg; 2) ODC1 for conversion of ornithine to putrescine; 3) guanosine triphosphate cyclohydrolase 1 (GCH1) for conversion of guanosine triphosphate to tetrahydrobiopterin, an essential cofactor for all NOS isoforms; and 4) IFNT, the pregnancy recognition signal in ruminants (Kim *et al*. 2011b). Arg is most stimulatory to proliferation, migration and protein synthesis in oTr cells (Kim *et al*., 2011a; Kim *et al*., 2011b) and pTr cells (Kong *et al*. 2012; Kim *et al*. 2013) probably due to its metabolism to NO and polyamines (Kim *et al*., 2011a; Kim *et al*. 2011c) (See Figure 3).

Arginine, leucine, glutamine, glucose and fructose activate the nutrient sensing cell signaling pathway in porcine conceptus trophectoderm

Expression of insulin-like growth factor-2 (IGF2), ODC1 and NOS isoforms is stimulated by nutrients via the MTOR- RPS6K-RPS6 pathway (Nielsen *et al*. 1995; Kimball *et al*. 1999; Martin & Sutherland 2001). IGF2 enhances placental and fetal growth (Ohlsson *et al*. 1989) through effects mediated by the IGF1 receptor and RPS6K under the control of MTORC1 (Nielsen 1992; Nielsen *et al*. 1995) and by inducing NO production (Kaliman *et al*. 1999). ODC1 regulates conceptus development and differentiation by catalyzing the synthesis of polyamines required for proliferation and migration of trophectoderm cells in ruminants (Van Winkle & Campione

1983; El-Badry *et al*. 1990; Bachrach *et al*. 2001 and mice (Mehrotra *et al*. 1998; Martin & Sutherland 2001; Martin *et al*. 2003), as well as rapid growth of the conceptus and increases in placental blood flow in pigs and sheep (Wu & Morris 1998; Kwon *et al*. 2004; Wu *et al*. 2005). NO promotes blastocyst attachment and trophoblast motility, possibly through modifications of the extracellular matrix and stimulation of vasodilation of maternal blood vessels (Gwatkin 1966). These are controlled through PI3K/AKT/MTOR cell signaling induced by HGF (Cartwright *et al*. 2002) and/or secreted phosphoprotein 1 (SPP1, also known as osteopontin) which stimulates MTOR cell signaling (Takahashi *et al*. 2000; Guo *et al*. 2005; Kim *et al*.. 2008). When pTr cells are treated with Arg, Leu or Gln there is a significant increase in phosphorylated forms of RPS6K and RPS6 (Kim *et al*. 2013). These nutrients increase the abundance of pRPS6K in nuclei and the abundance of pRPS6 protein in the cytoplasm (Kim *et al*. 2013) as one would expect since pRPS6 increases mRNA translation and protein synthesis. Arg, Leu, and Gln also increase the abundance of phosphorylated EIF4EBP1 protein in the nucleus of pTr cells that is associated with protein synthesis for trophectoderm cell growth, proliferation, and metabolism. Arg and Leu increase proliferation of pTr cells by 8.2-, and 8.1-fold, respectively; however, in the absence of glucose, Gln does not increase proliferation of pTr cells. But, Gln in the culture medium does significantly increases proliferation of pTr cells in response to Arg and Leu (Kim *et al*. 2013).

The combined effects of MTORC1 and MTORC2 account for proliferation and migration of trophectoderm cells, and change the organization of the cytoskeletal elements and gene expression required for elongation and implantation of porcine conceptuses. Kim *et al*. (2013) studied effects of Arg, Leu and Gln on proliferation of pTr cells transfected with either MTOR siRNA, RPTOR siRNA or RICTOR siRNA. MTOR siRNA inhibited effects of Arg, Leu, and Gln on cell proliferation, as did RPTOR siRNA, and RICTOR siRNA at 48 h post-transfection of pTr cells (Kim *et al* 2013). Thus, MTORC1 (MTOR-RPTOR) and MTORC2 (MTOR-RICTOR) signaling pathways are stimulated by Arg, Leu, and Gln in pTr cells (Kim *et al*. 2013).

Histone H3 is phosphorylated during both mitosis and meiosis and affects different phases of cell division/proliferation (Hans and Dimitrov, 2001). Arg, Leu, Gln and glucose stimulated cell proliferation is associated with nuclear localization of phosphorylated histone H3 (J. Kim, G.Song and F.W. Bazer, unpublished results). Further, Leu, but not Arg acts downstream of AKT1 or MTOR to increase beta catenin localization in junctional complexes of pTr cell membranes whereas Arg induces changes in the cytoskeletal architecture of pTr cells that may account for changes in shape of trophectoderm cells during conceptus elongation(J. Kim, G. Song and F.W. Bazer, unpublished results).

In vivo effects of arginine on successful outcomes of pregnancy

Arg is a nutritionally essential amino acid for survival, growth and development of the embryo/fetus and neonate (Wu *et al*., 2010). Dietary supplementation with Arg-HCl from Day 30 of pregnancy to term increases fetal survival in gilts (Mateo *et al*. 2007), and embryonic survival and litter size in rats (Zeng *et al*. 2008). In a subsequent study, dietary supplementation with 0.4% or 0.8% Arg significantly increased concentrations of Arg in maternal plasma, total volume of amniotic fluid, vascularity of chorionic and allantoic membranes, and litter size was increased by two conceptuses while embryonic mortality decreased by 14% (X.L. Li and G. Wu, unpublished results).

In ewe models of intra-uterine growth retardation, intravenous administration of Arg-HCl enhanced fetal growth (Lassala *et al*., 2009; Lassala *et al*., 2011). Also, in women with intra-uterine growth retardation of their fetus at week 33 of gestation, daily intravenous infusions of arginine increased birth weights (Xiao and Li 2005).

Roles of glucose and fructose in trophectoderm cell function

Pig blastocysts undergo morphological changes and differentiation that require select nutrients in the uterine lumen that include glucose and fructose (Bazer *et al*., 1991). Glucose and fructose are equivalent in stimulating proliferation of pTr cells, as well as increasing the abundance of phosphorylated RPS6K, -EIF4EBP1 and -RPS6 proteins, and as substrate for metabolism via the hexosamine pathway via glutamine-fructose-6-phosphate transaminase 1 (GFPT1) (Figure 5). These effects of glucose and fructose on pTr cells were inhibited by azaserine, an inhibitor of GFPT1. GFPT1 siRNA also blocked metabolism of fructose and glucose via the hexosamine pathway for synthesis of hyaluronic acid which is a significant glycosaminoglycan in the developing placenta.

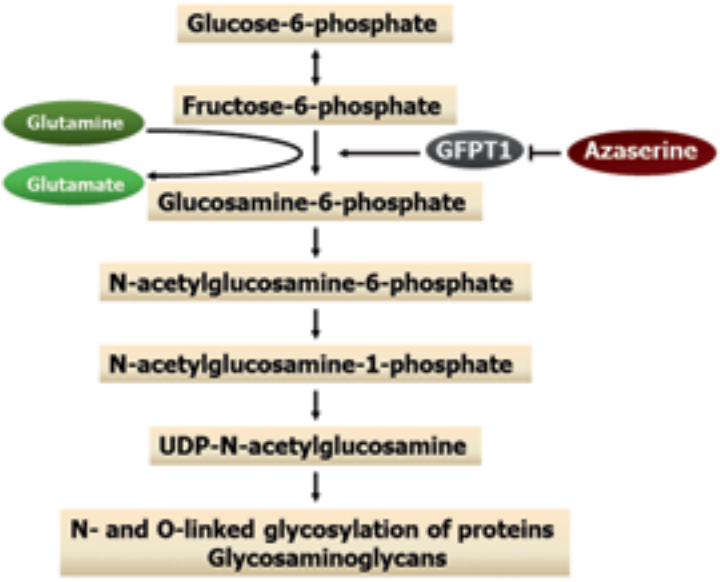

Fig. 5 The hexosamine pathway allows for both glucose and fructose to be metabolized to glucosamine-6-phosphate that lead to activation of the MTOR cell signaling pathway, as well as synthesis of glycosaminoglycans, including hyaluronic acid, that are critical to growth and development of the conceptus. There is also synthesis of UDP-N-acetylglucosamine that may stimulate MTOR cell signaling (Wen *et al*. 2005).

Fructose and glucose can be used for synthesis of neutral lipids and phospholipids in heart, liver, kidney, brain and adipose tissue of fetal lambs (Scott *et al*. 1967) and fructose can enter adipocytes by both insulin-dependent and insulin-insensitive mechanisms (Halperin & Cheema-Dhadli 1982). Fructose is also incorporated into nucleic acids (Huggett and Pelc, 1964; White *et al*. 1979; White *et al*. 1982). In HeLa cells fructose mostly enters the pentose shunt to produce reducing equivalents and nucleic acids necessary for biosynthetic processes (Reitzer *et al*. 1979). Developing pig conceptuses may use fructose in a similar manner to spare glutamine which is required for conversion of fructose-6-PO_4 to glucosamine-6-PO_4 by GPFT1 (Wu *et al*. 2011).

Concentrations of fructose in plasma of fetal pigs are 2- to 4-times higher than those of glucose (Randall and L'Ecuyer, 1976; Pere, 1995), but it is poorly metabolized to carbon dioxide (Meznarich *et al*., 1987) suggesting that it is not a significant source of ATP. The placenta converts large amounts of glucose to fructose; therefore, to consider fructose unavailable for

metabolism by the fetal-placental tissues is not logical. First, it is not necessary to convert fructose to carbon dioxide in order for the fetus to gain ATPs. Second, the use of fructose as an energy substrate is not the only pathway for its utilization by the conceputs (Figure 6).

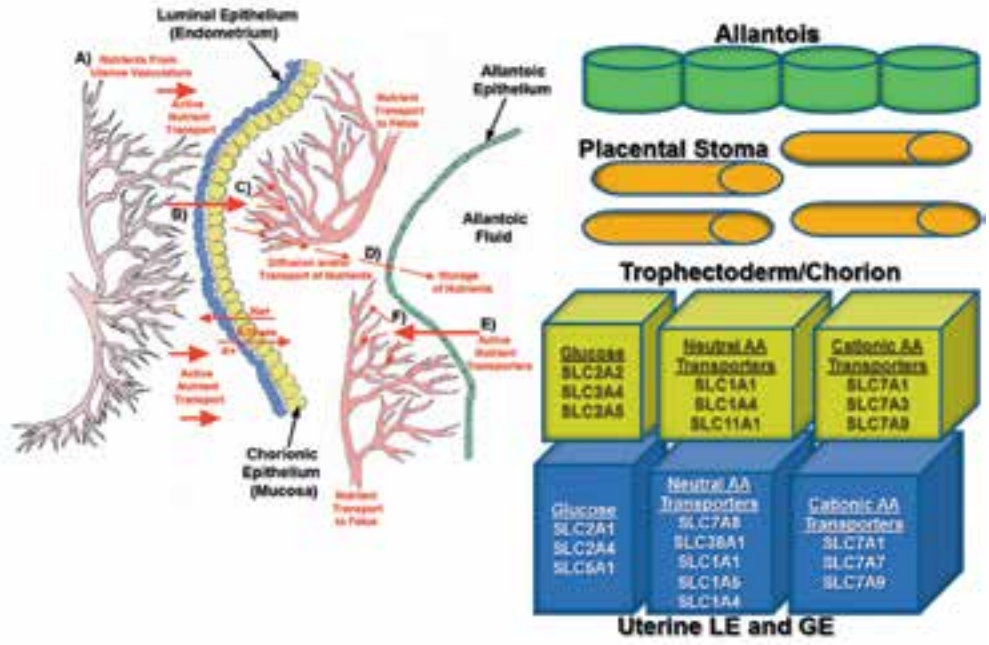

Fig. 6 A. Nutrients are transported from the maternal capillaries across the uterine luminal epithelium towards the apical surface of the chorionic epithelial cells and into the fetal-placental circulation of the conceptus. B. A Na^+/K^+ ATPase pump likely produces an ion gradient across the chorion to mediate active transport of nutrients into the connective tissue of the chorioallantois. C. Nutrients then diffuse or are transported into the vasculature within the allantois and into the general circulation of the conceptus. D. Nutrients may diffuse to the basal surface of the allantoic epithelium and then across to the allantoic sac or be transported by nutrient transporters into the allantoic fluid. E and F. Nutrients can be stored in allantoic fluid for transport across the allantoic epithelium into the fetal-placental vasculature by nutrient transporters by an undefined mechanism. The transporters identified for transporting nutrients from maternal circulation into the uterine lumen and from there into the conceptus are shown for uterine luminal (LE) and glandular (GE) epithelia and trophectoderm/chorion; however, transporters in the placental stromal cells and allantoic epithelium are unknown. There is evidence for transport of nutrients from allantoic fluid into the vasculature of the chorioallantois (Bazer 1989).

Sugars are converted to carbon dioxide and water by aerobic metabolism, liberating energy in the form of ATP. But, anaerobic glycolysis also generates ATP and lactate. There are high concentrations of both fructose and lactate in blood of fetal pigs (Pere, 1995; Fowden *et al.*, 1997) and the placenta is a net producer of lactate while the fetus is a net consumer of lactate. This suggests that the placenta engages in anaerobic metabolism. The placental capillaries are located primarily on either side of the epithelial cell bilayer, i.e., chorion and allantois (Leiser and Dantzer, 1988; Dantzer and Leiser, 1994), making them relatively well oxygenated, while placental stromal cells some distance from those capillaries are relatively poorly oxygenated. Thus, placental stromal cells exist in a relatively anaerobic environment and synthesize lactate, a hallmark of anaerobic metabolism via lactate dehydrogenase A (LDHA) (Draoui and Feron, 2011). Results of a preliminary immunohistochemical analysis localized LDHA to placental stromal cells in pigs and RNA-seq analysis of pig trophoblast cells detected their expression

of LDHB, which converts lactate to pyruvate for aerobic metabolism (J. L. Vallet, unpublished results). These results support the hypothesis that a dichotomy exists in pathways for generation of ATP between epithelial and stromal cells in the pig placenta (Figure 7).

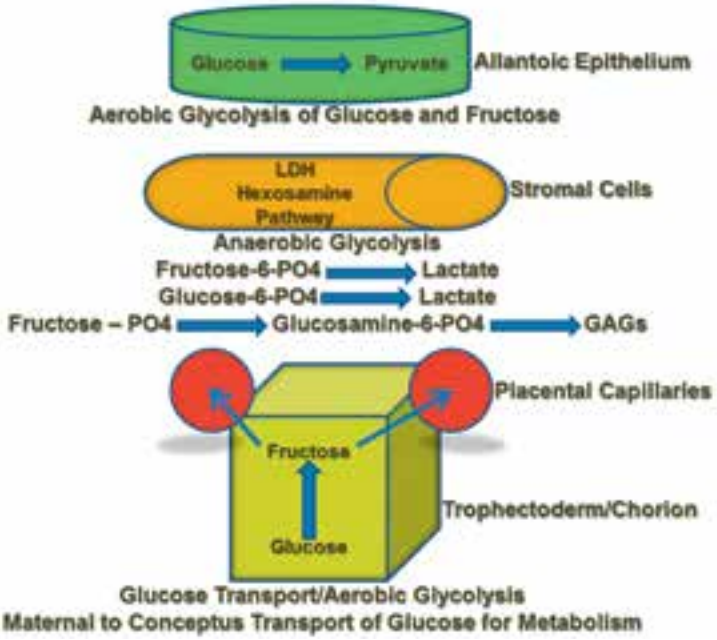

Fig. 7 This figure depicts a model for transport of glucose to the chorion where it is converted to fructose and transported into the placental capillaries to be metabolized by fetal-placental tissues. Glucose can be converted to carbon dioxide and water by aerobic metabolism, liberating energy in the form of ATP. But, anaerobic glycolysis also generates ATP, and the end result for mammalian cells is typically lactate. The placenta is a net producer of lactate and the fetus is a net consumer of lactate. For example, stromal cells, in the placenta are subjected to low oxygen tension and, therefore, engage in anaerobic metabolism whereas the relatively well oxygenated epithelial cells (chorion and allantois) engage in aerobic metabolism. The synthesis of lactate, a hallmark of anaerobic metabolism, is associated with expression of lactate dehydrogenase A (LDHA) (Draoui and Feron, 2011). Preliminary results indicate that LDHA is localized to placental stromal cells whereas trophectoderm expresses the LDHB which converts lactate to pyruvate aerobically (J. L. Vallet, unpublished results). This result supports the hypothesis that a dichotomy exists in pathways for metabolism of glucose for generation of ATP between epithelial and stromal cells in the pig placenta.

The dichotomy in energy metabolism between stromal and epithelial cells of the pig placenta may be due to differences in availability of sugar substrate. This can be controlled by access of fructose and glucose to the two cell types which exhibit differential expression of glucose transporters. SLC2A5, a specific fructose transporter (Burant *et al*., 1992), is most abundant in trophectoderm (Vallet *et al*., 2012) which is the source of fructose generated by the placenta (Huggett *et al*., 1951). SLC2A5 likely transports fructose out of the trophectoderm cells into the fetal circulation. The expression of SLC2A5 by stromal cells is evidence that fructose has access to these cells as a candidate substrate for anaerobic metabolism and metabolism via other metabolic pathways such as the hexosamine pathway. The hexosamine pathway may also regulate cell proliferation by activation of the MTOR pathway (Wen *et al*. 2005; Kim *et al*. 2012).

Glucosamine represents half the sugar molecules making up both hyaluronan and heparan sulfate (Moussian, 2008) and it is produced from fructose via the hexosamine pathway (Buse, 2006). Thus, fructose is a substrate for synthesis of glucosamine-6-PO_4 and glycosaminoglycans including hyaluronan and heparan sulfate required for placental development. Fragments of both hyaluronan and heparan sulfate are angiogenic (West *et al*. 1985; Ilan *et al*. 2006; Jakobson *et al*. 2006), suggesting that turnover of these glycosaminoglycans plays a role in the development of capillaries on either side of the epithelial bilayer of the chorioallantoic placenta of the pig. Hyaluronic acid and hyaluronidase increase in the uterine lumen of pigs in response to progesterone (Ashworth *et al*. 1990), which may stimulate angiogenesis (West *et al*. 1985) and/or angiogenesis, morphogenesis and tissue remodeling of the pig placenta as for the human placenta (Ponting & Kumar 1995). Hyaluronic acid accumulates in the placentae of most mammals and localizes to the umbilical cord and placental blood vessels (Mitchell *et al*. 2003) where it supports fibroblasts and stem cells (Wang *et al*. 2004). It is clear that angiogenesis is critical to conceptus development in all species and that fructose is used for synthesis of hyaluronic acid that supports angiogenesis.

The placental stromal cells in pigs includes the extracellular matrix of which primary components are hyaluronan and heparan sulfate (Steele and Froseth, 1980; Vallet *et al*., 2010), as well as hyaluronidase (Vallet *et al*., 2010) and heparanase (Miles *et al*., 2009) that degrade those glycosaminoglycans. Increases in hyaluronidase activity in placentae of small pig fetuses suggests greater turnover of placental hyaluronan to support more extensive folding of placentae as a compensatory mechanism to increase placental surface area for exchange of nutrients and gases (Vallet *et al*., 2010). Hyaluronidase activity has not been localized to specific placental cells in pigs; however, heparanase is produced by cuboidal cells of the folded chorioallantoic bilayer suggesting that these cells participate in fold development by degrading heparan sulfate in the extracellular matrix (Miles *et al*., 2009). RNA-seq analysis of trophoblast cells identified heparinase as a major gene in the cuboidal cells, while the two most differentially expressed genes in tall columnar cells were for A Disintegrin and Metalloprotease Domain 28 (ADAM) and TransMembrane 4 L Six Family Member 5 (TM4SF). Protein products from both genes participate in cell migration (Mochizuki and Okada, 2007; Lee *et al*., 2010) likely required for chorioallantoic folding. Thus, the unusual abundance of fructose in fetal blood and fetal fluids allows fructose to fulfill novel physiological roles during gestation that must be taken into account in studies of intra-uterine development of ungulate conceptuses.

Declaration of interest

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

Funding

This research was supported by USDA CSREES National Research Initiative Grants 2008-35203-19120 and 2011-67015-20028, as well as the World Class University Program (R31-10056) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology.

Acknowledgement

The authors acknowledge the contributions of current and past members of their laboratories who contributed to the findings reported in this review paper.

References

Ashworth CJ, Fliss MF & Bazer FW 1990 Evidence for steroid control of a putative angiogenic factor in the porcine uterus. *Journal of Endocrinology* **125** 15-19.

Bachrach U, Wang YC & Tabib A 2001 Polyamines: new cues in cellular signal transduction. *News Physiological Science* **16** 106-109.

Bartol FF, Wiley AA & Bagnell CA 2006 Uterine development and endometrial programming. *Society for Reproduction and Fertility Supplement* **62** 113-130.

Bazer FW 1989 Allantoic Fluid: Regulation of Volume and Composition. In *Fetal and Neonatal Body Fluids*, pp 135-157. Ed R A Brace. Cornell, NY: Perinatology Press.

Bazer FW & Thatcher WW 1977 Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F_{2a} by the uterine endometrium. *Prostaglandins* **14** 397401.

Bazer FW, Thatcher WW, Matinat-Botte F, Terqui M, Lacroix MC, Bernard S, Revault M & Dubois DH 1991 Composition of uterine flushings from Large White and prolific Chinese Meishan gilts. *Reproduction Fertility and Development* **3** 51-60.

Bazer FW, Spencer TE, Johnson GA, Burghardt RC & Wu G 2009 Comparative aspects of implantation. *Reproduction* **138** 195-209.

Bazer FW, Spencer TE, Johnson GA & Burghardt RC 2011a Uterine receptivity to implantation of blastocysts in mammals. *Frontiers in Bioscience* **S3** 745-767.

Bazer FW, Wu G, Johnson GA, Kim J & Song G 2011b Uterine histotroph and conceptus development: Select nutrients and secreted phosphoprotein 1 affect MTOR cell signaling in ewes. *Biology of Reproduction* **85** 1094–1107.

Bazer FW, Song G, Kim J, Erikson DW, Johnson GA, Burghardt RC, Gao H, Satterfield MC, Spencer TE & Wu G 2012a Mechanistic mammalian target of rapamycin (MTOR) cell signaling: Effects of select nutrients and secreted phosphoprotein 1 on development of mammalian conceptuses. *Molecular and Cellular Endocrinology* **354** 22–33.

Bazer FW, Spencer TE & Thatcher WW 2012b Growth and development of the ovine conceptus. *Journal of Animal Science* **90** 159-170.

Bird IM, Zhang L & Magness RR 2003 Possible mechanisms underlying pregnancy-induced changes in uterine artery endothelial function. *American Journal of Physiology Regulatory Integrative Comparative Physiology* **284** R245-258.

Burant CF, Takeda J, Brot-Laroche E, Bell GI & Davidson NO 1992 Fructose transporter in human spermatozoa and small intestine is GLUT5. *Journal of Biological Chemistry* **267** 14523-14526.

Buse MG 2006 Hexosamines, insulin resistance, and the complications of diabetes: current status. *American Journal of Physiology Endocrinology and Metabolism* **290** E1-E8.

Carson DD 2002 The glycobiology of implantation. *Frontiers in Biosciences* **7** 1535-1544.

Cartwright JE, Tse WK & Whitley GS 2002 Hepatocyte growth factor induced human trophoblast motility involves phosphatidylinositol-3-kinase, mitogen-activated protein kinase, and inducible nitric oxide synthase. *Experimental Cell Research* **279** 219-226.

Dantzer V & Leiser R 1994 Initial vascularization in the pig placenta: I. Demonstration of nonglandular areas by histology and corrosion casts. *Anatomical Record* **238** 177-190.

Dennis PB, Pullen N, Kozma SC & Thomas G 1996 The principal rapamycin-sensitive p70 (s6k) phosphorylation sites, T-229 and T-389, are differentially regulated by rapamycin-insensitive kinase kinases. *Molecular and Cellular Biology* **16** 6242-6251.

Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T & Wang H 2004 Molecular cues to implantation. *Endocrine Reviews* **25** 341-373.

Draoui N & Feron O 2011 Lactate shuttles at a glance: from physiological paradigms to anti-cancer treatments. *Disease Model Mechanisms* **4** 727-732.

El-Badry OM, Minniti C, Kohn EC, Houghton PJ, Daughaday WH & Helman LJ 1990 Insulin-like growth factor II acts as an autocrine growth and motility factor in human rhabdomyosarcoma tumors. *Cell Growth and Differentiation* **1** 325-331.

Flynn NE, Meininger CJ, Haynes TE, Wu G 2002 The metabolic basis of arginine nutrition and pharmacotherapy. *Biomedical Pharmacotherapy* **56** 427-438.

Fowden AL, Forhead AJ, Silver M & MacDonald AA 1997 Glucose, lactate and oxygen metabolism in the fetal pig during late gestation. *Experimental Physiology* **82** 171-182.

Fukuda H, Iritani N & Tanaka T 1983 Effects of high-fructose diet on lipogenic enzymes and their substrate and effector levels in diabetic rats. *Journal of Nutritional Science and Vitaminology (Tokyo)* **29** 691-699.

Gangloff YG, Mueller M, Dann SG, Svoboda P, Sticker M, Spetz JF, Um SH, Brown EJ, Cereghini S, Thomas G & Kozma SC 2004 Disruption of the mouse mTOR gene leads to early post-implantation lethality and prohibits embryonic stem cell development. *Molecular and Cellular Biology* **24** 9508-9516.

Gardner DK, Lane M, Calderon I & Leeton J 1996 Environment of the preimplantation human embryo in vivo: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. *Fertility and Sterility* **65** 349-353.

Geisert RD, Brookbank JW, Roberts RM & Bazer FW 1982 Establishment of pregnancy in the pig. II. Cellular remodelling of the porcine blastocysts during elongation of day 12 of pregnancy. *Biology of Reproduction* **27** 941955.

Geisert RD, Brenner RM, Moffatt RJ, Harney JP, Yellin T & Bazer FW 1993 Changes in estrogen receptor protein, mRNA expression and localization in the endometrium of cyclic and pregnant gilts. *Reproduction Fertility and Development* **5** 247-260.

Geisert RD, Pratt TN, Bazer FW, Mayes JS & Watson GH 1994 Immunocytochemical localization and changes in endometrial progestin receptor protein during the porcine estrous cycle and early pregancy. *Reproduction Fertility and Development* **6** 749-760.

Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R & Sonenberg N 1999 Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes and Development* **13** 1422-1437.

Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszynska A, Aebersold R & Sonenberg N 2001 Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes and Development* **15** 2852-2864.

Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW & Spencer TE 2001 Developmental biology of uterine glands. *Biology of Reproduction* **65** 1311-23.

Gu W, Jones CT & Harding JE 1987 Metabolism of glucose by fetus and placenta of sheep. The effects of normal fluctuations in uterine blood flow. *Journal of Developmental Physiology* **9** 369-389.

Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ & Sabatini DM 2006 Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKC alpha, but not S6K1. *Developmental Cell* **11** 859-871.

Guo H, Marroquin CE, Wai PY& Kuo PC 2005 Nitric oxide-dependent osteopontin expression induces metastatic behavior in HepG2 cells. *Digestive Disease Science* **50** 1288-1298.

Gwatkin RB 1966 Defined media and development of mammalian eggs in vitro. *Annals of the New York Academy of Science* **139** 79-90.

Halperin ML & Cheema-Dhadli S 1982 Comparison of glucose and fructose transport into adipocytes of the rat. *Biochemistry Journal* **202** 717-721.

Hans F & Dimitrov S 2001 Histone H3 phosphorylation and cell division. *Oncogene* **20** 3021-3027.

Huggett AS & Pelc SR 1964 The utilization of foetal blood fructose in the sheep. *Journal of Physiology* **171(Suppl)** 42P-43P.

Huggett AS, Warren FL & Warren NV 1951 The origin of the blood fructose in the foetal sheep. *Journal of Physiology* **113** 258-273.

Igarashi K & Kashiwagi K 2000 Polyamines: mysterious modulators of cellular functions. *Biochemica Biophysica Research Communication* **271** 559-564.

Ilan N, Elkin M & Vlodavsky I 2006 Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. *International Journal of Biochemisty and Cell Biology* **38** 2018-2039.

Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J & Su B 2006 SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* **127** 125-137.

Jakobsson L, Kreuger J, Holmborn K, Lundin L, Eriksson I, Kjellén L & Claesson-Welsh L 2006 Heparan sulfate in *trans* potentiates VEGFR-mediated angiogenesis. *Developmental Cell* **10** 625-634.

Johnson GA, Bazer FW, Burghardt RC, Spencer TE, Wu G & Bayless KJ 2009 Conceptus-uterus interactions in pigs: endometrial gene expression in response to estrogens and interferons from conceptuses. *Society of Reproduction and Fertility Supplement* **66** 321-332.

Ka H, Spencer TE, Johnson GA & Bazer FW 2000 Keratinocyte growth factor: Expression by endometrial epithelia of the porcine uterus. *Biology of Reproduction* **62** 1772-1778.

Ka H, Jaeger LA, Johnson GA, Spencer TE & Bazer FW 2001 Keratinocyte growth factor in up-regulated in the porcine uterine endometrium and functions in trophectoderm cell proliferation and differentiation. *Endocrinology* **142** 2303-2310.

Ka H, Al-Ramadan S, Johnson GA, Jaeger LA, Burghardt RC, Spencer TE & Bazer FW 2007 Regulation of fibroblast growth factor-7 expression by progesterone and estradiol-17□ and their receptors in the porcine endometrium. *Biology of Reproduction* **77** 172–180.

Kaliman P, Canicio J, Testar X, Palacin M & Zorzano A 1999 Insulin-like growth factor-II, phosphatidylinositol 3-kinase, nuclear factor-kappa B and inducible nitric-oxide synthase define a common myogenic signaling pathway. *Journal of Biological Chemistry* **274** 17437-17444.

Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H. Tempst P & Sabatini DM 2002 mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* **110** 163-175.

Kim J, Song G, Gao H, Farmer JL, Satterfield MC, Burghardt RC, Wu G, Johnson GA, Spencer TE & Bazer, FW 2008 Insulin-like growth factor 2 (IGF2) activates PI3K-AKT1 and MAPK cell signaling pathways and stimulates proliferation and migration of ovine trophectoderm cells. *Endocrinology* **149** 3085-3094.

Kim J, Burghardt RC, Wu G, Johnson GA, Spencer TE & Bazer FW 2011a Select Nutrients in the ovine uterine lumen: VII. Effects of arginine, leucine, glutamine and glucose on trophectodem cell signaling, proliferation and migration. *Biology of Reproduction* **84** 7078.

Kim J, Burghardt RC, Wu G, Johnson GA, Spencer TE & Bazer FW 2011b Select Nutrients in the ovine uterine lumen: VIII. Arginine stimulates proliferation of ovine trophectoderm cells through mTORRPS6KRPS6 signaling cascade and synthesis of nitric oxide and polyamines. *Biology of Reproduction* **84** 6269.

Kim J, Burghardt RC, Wu G, Johnson GA, Spencer TE & Bazer FW 2011c Select Nutrients in the ovine uterine lumen: IX. Differential effects of arginine, leucine, glutamine and glucose on interferon tau, orinithine decarboxylase and nitric oxide synthase in the ovine conceptus. *Biology of Reproduction* **84** 11391147.

Kim J, Song G, Wu G & Bazer FW 2012 Functional roles of fructose. *Proceedings of the National Academy of Science United States of America* **109** E1619-1628.

Kim J, Song G, Wu G, Gao H, Johnson GA & Bazer FW 2013 Arginine, leucine, and glutamine stimulate proliferation of porcine trophectoderm cells through the MTOR-RPS6K-RPS6-EIF4EBP1 signal transduction

pathway. *Biology of Reproduction* (In Review)

Kimball SR, Shantz LM, Horetsky RL & Jefferson LS 1999 Leucine regulates translation of specific mRNAs in L6 myoblasts through mTOR-mediated changes in availability of eIF4E and phosphorylation of ribosomal protein S6. *Journal of Biological Chemistry* **274** 11647-11652.

Knight JW, Bazer FW, Thatcher WW, Franke DE & Wallace HD 1977 Conceptus development in intact and unilaterally hysterectomizedovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. *Journal of Animal Science* **44** 620637.

Kong X, Tan B, Yin Y, Gao H, Li X, Jaeger LA, Bazer FW & Wu G 2012 L-arginine stimulates the mTOR signaling pathway and protein synthesis in porcine trophectoderm cells. *Journal of Nutritional Biochemistry* **23** 1178-1183.

Kwon H, Spencer TE, Bazer FW & Wu G 2003 Developmental changes of amino acids in ovine fetal fluids. *Biology of Reproduction* **68** 1813-1820.

Kwon H, Wu G, Meininger CJ, Bazer FW & Spencer TE 2004 Developmental changes in nitric oxide synthesis in the ovine placenta. *Biology of Reproduction* **70** 679-686.

Lassala A, Bazer FW, Cudd TA, Datta S, Keisler DH, Satterfield MC, Spencer TE & Wu G 2009 Parenteral administration of L-arginine prevents fetal growth restriction in undernourished ewes. *Journal of Nutrition* **139** 660-665.

Lassala A, Bazer FW, Cudd TA, Datta S, Keisler DH, Satterfield MC, Spencer TE & Wu G 2011 Parenteral administration of L-arginine enhances fetal survival and growth in sheep carrying multiple pregnancies. *Journal of Nutrition* **141** 849-855.

Lee SA, Kim TY, Kwak TK, Kim H, Kim S, Lee HJ, Kim SH, Park KH, Kim HJ, Cho M & Lee JW 2010 Transmembrane 4 L six family member 5 (TM4SF5) enhances migration and invasion of hepatocytes for effective metastasis. *Journal of Cellular Biochemistry* **111** 59-66.

Lei J, Feng D, Zhang Y, Dahanayaka S, Li X, Yao K, Wang J, Wu Z, Dai Z & Wu G 2012 Regulation of leucine catabolism by metabolic fuels in mammary epithelial cells. *Amino Acids* **43** 2179-2189.

Leiser R & Dantzer V 1988 Structural and functional aspects of porcine placental microvasculature. *Anatomy and Embryology* **177** 409-419.

Martin PM & Sutherland AE 2001 Exogenous amino acids regulate trophectoderm differentiation in the mouse blastocyst through an mTOR-dependent pathway. *Developmental Biology* **240** 182-193.

Martin PM, Sutherland AE & Van Winkle LJ 2003 Amino acid transport regulates blastocyst implantation. *Biology of Reproduction* **69** 1101-1108.

Mateo RD, Wu G, Bazer FW, Park JC, Shinzato I & Kim SW 2007. Dietary L-arginine supplementation enhances the reproductive performance of gilts. *Journal of Nutrition* **137** 652-656.

Mehrotra PK, Kitchlu S, Farheen S 1998 Effect of inhibitors of enzymes involved in polyamine biosynthesis pathway on pregnancy in mouse and hamster. *Contraception* **57** 55-60.

Meznarich HK, Hay Jr WW, Sparks JW, Meschia G & Battaglia FC 1987 Fructose disposal and oxidation rates in the ovine fetus. *Quarterly Journal of Experimental Physiology* **72** 617-625.

Miles JR, Vallet JL, Freking BA & Nonneman DJ 2009 Molecular cloning and characterisation of heparanase mRNA in the porcine placenta throughout gestation. *Reproduction Fertility and Development* **21** 757-772.

Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T, Troyer D & Medicetty S 2003 Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* **21** 50-60.

Mochizuki S & Okada Y 2007 ADAMs in cancer cell proliferation and progression. *Cancer Science* **98** 621-628.

Moussian B 2008 The role of GlcNAc in formation and function of extracellular matrices. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **149** 215-226.

Murakami M, Ichisaka T, Maeda M, Oshiro N, Hara K, Edenhofer F, Kiyama H, Yonezawa K & Yamanaka S 2004 mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. *Molecular and Cellular Biology* **24** 6710-6718.

Nielsen FC 1992 The molecular and cellular biology of insulin-like growth factor II. *Proceedings of Growth Factor Research* **4** 257-290.

Nielsen FC, Ostergaard L, Nielsen J & Christiansen J 1995 Growth-dependent translation of IGF-II mRNA by a rapamycin-sensitive pathway. *Nature* **377** 358-362.

Ohlsson R, Larsson E, Nilsson O, Wahlstrom T & Sundstrom P 1989 Blastocyst implantation precedes induction of insulin-like growth factor II gene expression in human trophoblasts. *Development* **106** 555-559.

Pere MC 1995 Maternal and fetal blood levels of glucose, lactate, fructose, and insulin in the conscious pig. *Journal of Animal Science* **73** 2994-2999.

Pike IL 1981 Comparative studies of embryo metabolism in early pregnancy. *Journal of Reproduction and Fertilility Supplement* **29** 203-213.

Ponting JM & Kumar S 1995 Isolation and characterisation of a hyaluronan binding protein, hyaluronectin, from human placenta and its colocalisation with hyaluronan**.** *Journal of Anatomy* **186** 131-142.

Randall GC & L'Ecuyer C 1976 Tissue glycogen and blood glucose and fructose levels in the pig fetus during the second half of gestation. *Biology of the Neonate* **28** 74-82.

Reitzer LJ, Wice BM & Kennell D 1979 Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells. *Journal of Biological Chemistry* **254** 2669-2676.

Rieger D 1992 Relationships between energy metabolism and development of early mammalian embryos. *Theriogenology* **37** 75-91.

Rieger D, Loskutoff NM & Betteridge KJ 1992 Developmentally related changes in the uptake and

metabolism of glucose, glutamine and pyruvate by cattle embryos produced in vitro. *Reproduction Fertility and Development* **4** 547-557.

Riley JK & Moley KH 2006 Glucose utilization and the PI3-K pathway: mechanisms for cell survival in preimplantation embryos. *Reproduction* **131** 823-835.

Scott TW, Setchell BP & Bassett JM 1967 Characterization and metabolism of ovine foetal lipids. *Biochemical Journal* **104** 1040-1047.

Self JT, Spencer TE, Johnson GA, Hu J, Bazer FW & Wu G 2004 Glutamine synthesis in the developing porcine placenta. *Biology of Reproduction* **70** 1444-1451

Senger PL 2003 *Pathways to Pregnancy and Parturition*. 2nd Edition. Current Conceptions Inc., Pullman, Washington.

Shiota C, Woo JT, Lindner J, Shelton KD & Magnuson MA 2006 Multiallelic disruption of the rictor gene in mice reveals that mTOR complex 2 is essential for fetal growth and viability. *Developmental Cell* **11** 583-589.

Spencer TE & Bazer FW 2004 Uterine and placental factors regulating conceptus growth in domestic animals. *Journal of Animal Science* **82** E-Suppl E4-13.

Steele VS & Froseth JA 1980 Effect of gestational age on the biochemical composition of porcine placental glycosaminoglycans. *Proceedings of the Society for Experimental Biology and Medicine* **165** 480-485.

Takahashi F, Takahashi K, Maeda K, Tominaga S & Fukuchi Y 2000 Osteopontin is induced by nitric oxide in RAW 264.7 cells. *IUBMB Life* **49** 217-221.

Vallet JL, Miles JR & Freking BA 2010 Effect of fetal size on fetal placental hyaluronan and hyaluronoglucosaminidases throughout gestation in the pig. *Animal Reproduction Science* **118** 297-309.

Vallet JL, Leymaster KA, Johnson GA & Bazer FW 2012 Limitations in uterine and conceptus physiology that lead to fetal losses. *Journal of Animal Science* (Accepted)

Van Winkle LJ & Campione AL 1983 Effect of inhibitors of polyamine synthesis on activation of diapausing mouse blastocysts in vitro. *Journal of Reproduction and Fertility* **68** 437-444.

Wales RG 1986 Measurement of metabolic turnover in single mouse embryos. *Journal of Reproduction and Fertility* **76 717-725.**

Wales RG & Waugh EE 1993 Catabolic utilization of glucose by the sheep conceptus between days 13 and 19 of pregnancy. *Reproduction Fertility and Development* **5 111-122.**

Wales RG and Du ZF 1994 The metabolism of glutamine by the preimplantation sheep conceptus and its interaction with glucose. *Reproduction Fertility and Development* **6** 659-667.

Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC & Chen CC 2004 Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* **22** 1330-1337.

Wen HY, Abbasi S, Kellems RE & Xia Y 2005 mTOR: a placental growth signaling sensor. *Placenta* **26** Suppl A S63-69.

West DC, Hampson IN, Arnold F & Kumar S 1985 Angiogenesis induced by degradation products of hyaluronic acid. *Science* **228** 1324-1326.

White CE, Piper EL & Noland PR 1979 Conversion of glucose to fructose in the fetal pig. *Journal of Animal Science* **48** 585-590.

White CE, Piper EL, Noland PR & Daniels LB 1982 Fructose utilization for nucleic acid synthesis in the fetal pig. *Journal of Animal Science* **55** 73-76.

Wu G & Morris SM Jr 1998 Arginine metabolism: nitric oxide and beyond. *Biochemical Journal* **336** 1-17.

Wu G, Bazer FW, Tuo W, Flynn SP 1996 Unusual abundance of arginine and ornithine in porcine allantoic fluid. *Biology of Reproduction* **54** 1261-1265.

Wu G, Haynes TE, Li H, Yan W & Meininger CJ 2001 Glutamine metabolism to glucosamine is necessary for glutamine inhibition of endothelial nitric oxide synthesis. *Biochemical Journal* **353** 245-252.

Wu G, Jaeger LA, Bazer FW & Rhoads JM 2004 Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. *Journal of Nutrition and Biochemistry* **15** 442-451.

Wu G, Bazer FW, Hu J, Johnson GA & Spencer TE 2005 Polyamine synthesis from proline in the developing porcine placenta. *Biology of Reproduction* **72** 842-850.

Wu G BF, Davis TA, Jaeger LA, Johnson GA, Kim SW, Knabe DA, Meininger CJ, Spencer TE & Yin YL 2007 Important roles for the arginine family of amino acids in swine nutrition and production. *Livestock Science* **112** 8-22.

Wu G, Bazer FW, Burghardt RC, Johnson GA, Kim SW, Li XL, Satterfield MC & Spencer TE 2010 Impacts of amino acid nutrition on pregnancy outcome in pigs: mechanisms and implications for swine production. *Journal of Animal Science* **88** E195-204.

Wu G, Wu G, Bazer FW, Johnson GA, Knabe DA, Burghardt RC, Spencer TE, Li XL & Wang JJ 2011 Triennial Growth Symposium: Important roles for L-glutamine in swine nutrition and production. *Journal of Animal Science* **89** 2017-2030.

Xiao, XM & Li LP 2005 L-arginine treatment for asymmetric fetal growth restriction. *International Journal Gynecological Obstetrics* **88** 15–18.

Zavy MT, Clark WR, Sharp DC, Roberts RM & Bazer FW 1982 Comparison of glucose, fructose, ascorbic acid and glucosephosphate isomerase enzymatic activity in uterine flushings from nonpregnant and pregnant gilts and pony mares. *Biology of Reproduction* **27** 1147-1158.

Zeng XF, Wang FL, Fan X, Yang WJ, Zhou B, Li PF, Yin YL, Wu G & Wang JJ 2008 Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. *Journal of Nutrition* **138** 1421-1425.

Zhao YC, Chi YJ, Yu YS, Liu JL, Su RW, Ma XH, Shan CH & Yang ZM 2008 Polyamines are essential in embryo implantation: expression and function of polyamine-related genes in mouse uterus during peri-implantation period. *Endocrinology* **149** 2325-2332.

Ziecik AJ, Waclawik A, Kaczmarek MM, Blitek A, Jalali BM & Andronowska A 2011 Mechanisms for the establishment of pregnancy in the pig. *Reproduction in Domestic Animals* Supplement **3** 31-41.