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Guoyao Wu *Editor*

Amino Acids in Nutrition and Health

Amino Acids in the Nutrition of Companion, Zoo and Farm Animals

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1 One-Carbon Metabolism and Development of the Conceptus During Pregnancy: Lessons from Studies with Sheep and Pigs

Fuller W. Bazer, Heewon Seo, Gregory A. Johnson, and Guoyao Wu

Abstract

The pregnancy recognition signal from the conceptus (embryo/fetus and associated membranes) to the mother is interferon tau (IFNT) in ruminants and estradiol, possibly in concert with interferons gamma and delta in pigs. Those pregnancy recognition signals silence expression of interferon stimulated genes (ISG) in uterine luminal (LE) and superficial glandular (sGE) epithelia while inducing expression of genes for transport of nutrients, including glucose and amino acids, into the uterine lumen to support growth and development of the conceptus. In sheep and pigs, glucose not utilized immediately by the conceptus is converted to fructose. Glucose, fructose, serine and glycine in uterine histotroph can contribute to one carbon (1C) metabolism that provides one-carbon groups for the synthesis of purines and thymidylate, as well as *S*-adenosylmethionine for epigenetic methylation reactions. Serine and glycine are transported into the mitochondria of cells and metabolized to formate that is transported into the cytoplasm for the synthesis of purines, thymidine and *S*-adenosylmethionine. The unique aspects of one-carbon metabolism are discussed in the context of the hypoxic uterine environment, aerobic glycolysis, and similarities in metabolism between cancer cells and cells of the rapidly developing fetal-placental tissues during pregnancy. Further, the evolution of anatomical and functional aspects of the placentae of sheep and pigs versus primates is discussed in the context of mechanisms to efficiently obtain, store and utilize nutrients required for rapid fetal growth in the last one-half of gestation.

Keywords

Pregnancy · Placenta · One-carbon metabolism · Formate · Glycine · Serine · Glucose · Fructose

Abbreviations

1C	one carbon
AFT4	activating transcription factor 4
GE	glandular epithelium
IFNT	interferon tau
ISG	interferon stimulated gene
LE	luminal epithelium
MTHFD	methylenetetrahydrofolate dehydrogenase
MTOR	Mechanistic target of rapamycin
PFK	phosphofructokinase-1
PHGDH	phosphoglyceride dehydrogenase

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PPP	pentose phosphate pathway
PSAT	phophoserine aminotransferase
PSPH	phosphoserine phosphatase
sGE	superficial glandular
SHMT	serine hydroxymethyltransferase
TCA	tricarboxylic acid
THF	tetrahydrofolate
Tr	trophectoderm
αKG	α-ketoglutarate

1.1 Introduction

Reproduction is essential to the propagation of all species. Accordingly, diverse species employ multiple options regarding mechanisms for pregnancy recognition signaling, implantation, placentation and the initiation of parturition. Livestock species and primates differ in mechanisms for implantation and placentation and these differences impact how nutrients are acquired, stored and utilized. Implantation is invasive in primates, but diffuse and superficial in livestock species (Perry 1981). Before ovine and porcine blastocysts develop into a conceptus, they "hatch" from the zona pellucida on Days 6–7 of pregnancy and then undergo a remarkable transition to filamentous forms in preparation for implantation and placentation (Bazer and Johnson 2014; Johnson et al. 2018). Sheep blastocysts are spherical on Days 4 (0.14 mm) and 10 (0.4 mm), elongate to the filamentous form between Days 12 (1.0 by 33 mm) and 15 (1 by 150–190 mm), and extend through the uterine body into the contralateral uterine horn by Days 16–17 of pregnancy while attaching to the uterine luminal epithelium (LE) to initiate implantation. Pig blastocysts are 0.5–1 mm diameter spheres when they "hatch" from the zona pellucida and increase in size to Day 10 of pregnancy (2–6 mm) before undergoing a morphological transition to large spheres of 10–15 mm diameter and then tubular (15 mm by 50 mm) and filamentous (l by 100–200 mm) forms on Day 11. During the transition from tubular to filamentous forms, pig conceptuses elongate at 30–45 mm/h, primarily by cellular remodeling and proliferation of trophectoderm cells. However, hyperplasia is responsible for subsequent growth and elongation of the conceptus to 800–1000 mm length by Day 15 of pregnancy as implantation progresses. Elongation of ovine and porcine conceptuses is a prerequisite for central implantation that involves the trophectoderm achieving maximum surface area contact with uterine epithelia that secrete and/or transport nutrients into the uterine lumen. As conceptuses elongate they metabolize and are responsive to significant concentrations of molecules supplied in the form of histotroph within the uterine lumen. Histotroph is a complex mixture of molecules either secreted or transported into the uterine lumen and includes hormones, enzymes, growth factors, cytokines, transport proteins, adhesion factors, nutrients and other substances that plays roles in conceptus nourishment, implantation and placentation (Bazer et al. 2015). The invasive implantation for primates results in the spherical blastocyst invading into the uterine stroma wherein it establishes intimate contact with maternal blood vessels that directly supply it with nutrients and other molecules essential for growth and development in preparation for placentation (Huppertz and Borges 2008).

Placentae evolved independently among the livestock species leading to substantial differences in morphology, vascularization, folding of the chorioallantois and associated uterine endometrium, development of placentomes (uterine caruncles and placental cotyledons in ruminants), development of areolae to absorb secretions directly from uterine glands for transport into the fetal-placental circulation, and development of the allantois (Seo et al. 2019, 2020a). The allantois is connected to the fetal bladder via the urachus that allows molecules cleared via the fetal kidney to enter the bladder and then move, via the urachus, into allantoic fluid within the allantoic sac. Those molecules that accumulate in allantoic fluid include nutrients, growth factors and hormones that can be reabsorbed across the allantoic epithelium into the fetal-placental vasculature. Thus, the allantois is a repository from which recirculation of nutrients, hormones, growth factors, cytokines and other molecules

occurs to meet demands for placental development and exponential growth of the fetus. Placental weight in sheep increases from 5 to 435 g between Days 25 and 80 of gestation while fetal weight increases from 0.2 to 257 g during the same period, but fetal weight is tenfold greater on Day 140 (2956 g) of the 147 day period of gestation (Bazer et al. 2012a, b). Similarly, placental weight increases from 0.21 to 250 g between Days 20 and 70 of gestation in pigs, while fetal weight increases from 0.06 to 313 g on Day 70, but increases another threefold to 900 g on Day 100 and about 1500 g at term (Day 114 of gestation) (Knight et al. 1977).

Pigs have a diffuse, epitheliochorial placenta and sheep have a cotyledonary, synepitheliochorial placenta with six and five (within placentomes) layers of cells, respectively, separating maternal and fetal blood. In order to overcome this significant barrier to the transport of nutrients from the uterine vasculature to the placental vasculature, blood flow to the pregnant uteri of pigs increases from about 1.25 L/min on Day 45 of gestation to 2.75 L/min by Day 110 of a 114 day gestation period and this requires considerable maternal heart work by the dam (Pere and Etienne 2000). For ewes, uterine blood flow increases from about 50 ml/min on Day 30 of gestation to 1.4 L/min on Day 140 of a 147 day period of gestation (Metcalfe et al. 1959). In the hemochorial placenta of women, the chorion is in direct contact with maternal blood and only three layers of cells separate maternal and fetal blood allowing for much more efficient transport of nutrients. Accordingly, uterine blood flow in pregnant women increases to a lesser degree than for pigs and sheep, from around 95 ml/min in early pregnancy to 342 mL/min during late gestation (Thaler et al. 1990). This evolution of placental types may reduce maternal heart work as one can appreciate from differences in uterine blood flow at the end of gestation; 2.75 L/min for pigs, 1.4 L/min for sheep and 0.342 L/min for women (Fig. 1.1).

Given the relatively inefficient placentae in sheep and pigs, allantoic fluid serves as a reservoir for a reserve of nutrients that compliments the direct transfer of nutrients across the placenta in support of growth and development of the conceptus. The placentae of sheep and pigs include the yolk sac, amnion, allantois and chorion, but only yolk sac, amnion and chorion are present in the human placenta. The yolk sac provides the initial vascular system, primordial germ cells and hematopoietic stem cells, but it regresses during the first 30–60 days of gestation, depending on species. Retention of the allantois in species with chorioallantoic and synepitheliochorial placentae provides the reservoir for the accumulation of nutrients. In pigs, allantoic fluid volume increases from Day 20 (4 ml) to Day 30 (189 ml), decreases to Day 45 (75 ml) and increases again to Day 58 (451 ml) (Bazer 1989). Thereafter, allantoic fluid volume decreases to term at Day 114 of gestation. Allantoic fluid volume in sheep conceptuses increases from Day 25 (21 ml) to Day 40 (72 ml), decreases to 32 ml on Day 70, and then increases to 450 ml on Day 140 of a 147 day period of gestation (Bazer et al. 2012a, b).

Early anatomical studies suggested that the allantoic sac and its fluid was a reservoir for fetal urine and that the mesonephric glomeruli were the "source" of allantoic fluid (see Bazer 1989). However, the urinary system does not make water, but only redistributes available water. Allantoic fluid is, therefore, of maternal origin. A comparison of concentrations of electrolytes in maternal or fetal plasma and allantoic fluid reveals that allantoic fluid is not a dialysate of plasma since the osmotic gradient favors the exchange of fluids in an allantoic-to-maternal rather than in a maternal-to-allantoic direction. Allantoic fluid and the allantoic epithelium have several key roles. First, increases in allantoic fluid volume expand the chorioallantoic membranes and force them into apposition with the maternal uterine epithelia to maximize placental surface area for nutrient and waste exchange. Second, allantoic fluid contains substantial quantities of electrolytes, water, sugars, proteins and other nutrients that are cleared by the kidney and accumulate in the allantoic sac to be reabsorbed into the fetal-placental circulation across the allantoic epithelium. Third, the allantoic epithelium is derived from the hindgut and is, therefore, an

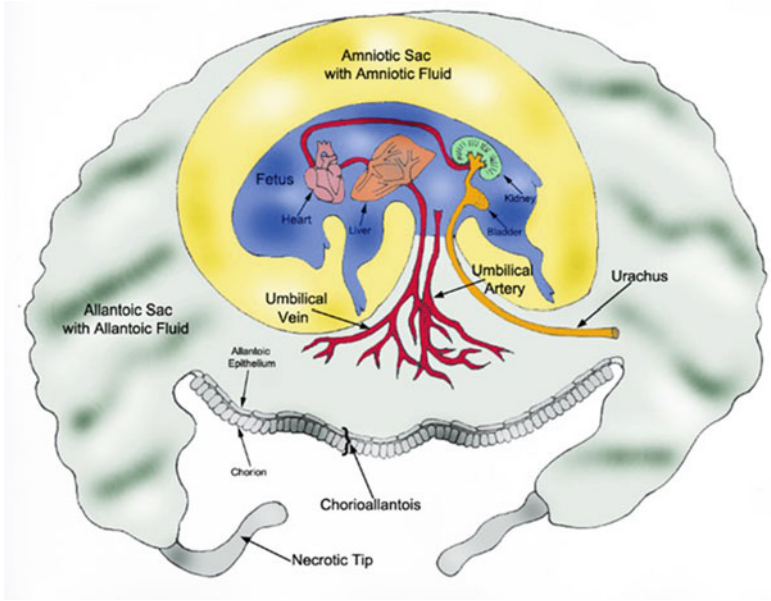

Fig. 1.1 The pig conceptus is representative of animals with epitheliochorial or synepitheliochorial placentae. The chorion is in direct contact with uterine epithelia and transports nutrients and other molecules into the vasculature of the fetal-placental tissues. The allantoic sac contains allantoic fluid that serves as a reservoir for nutrients and other molecules transported into the fetal-placental vasculature. Those nutrients and molecules support development of the conceptus; however, those not utilized are cleared through the kidney, into the bladder and then, via the urachus, transported into the allantoic sac for storage until needed. Subsequently, those nutrients and other molecules are transported across the allantoic epithelium into the fetal-placental circulation to meet metabolic or regulatory functions. This recirculation of nutrients and other molecules provides an efficient means for storage, access, and utilization of nutrients with allantoic fluid. The amnion is filled with amniotic fluid that supports the conceptus and allows it to develop symmetrically. In the latter stages of pregnancy sheep fetuses have been reported to drink amniotic fluid

epithelium capable of absorbing or actively transporting nutrients into the fetal-placental vasculature.

Rapid growth of ovine and porcine conceptuses includes extensive proliferation, remodeling and migration of trophectoderm cells, as well as growth and development of the fetus. Each of these processes consumes and depletes available oxygen and nutrients, resulting in metabolic stress for implanting conceptuses. Rapid development of conceptuses occurs in a hypoxic environment in which aerobic glycolysis provides substrates for the hexoseamine biosynthesis pathway, pentose phosphate pathway, and one-carbon metabolism, as well as production of adenosine triphosphate (ATP) required for rapid proliferation and migration of conceptus Tr cells.

A recent report on the survival of African naked mole-rats was most informative as it revealed how they tolerate hours of extreme hypoxia/anoxia and survive for 18 min under total oxygen deprivation (anoxia). Under those conditions, the Naked Mole rats switch metabolically to aerobic glycolysis fueled by fructose that was metabolized to lactate in the brain (Park et al. 2017). Global expression of the GLUT5 fructose transporter and high levels of expression of ketohexokinase (fructokinase) in tissues of naked mole rats under anoxia resulted in fructose-driven aerobic glycolysis that circumvented the normal feedback inhibition of phosphofructose kinase-dependent glycolysis. This was key to the prolonged viability of naked mole rats under hypoxic or anoxic conditions. Ketohexokinase converts fructose to fructose-1-PO_4 that is metabolized to glyceraldehyde, dihydroxyacetone phosphate and glyceraldehyde 3 phosphate. That pathway is not inhibited by pH, citrate or ATP as occurs when glucose is

metabolized via the hexokinase pathway to glucose-6-PO_4.

Trophectoderm cells of sheep and pigs in their hypoxic environment are metabolically distinct from cells of resting tissues, and reflect characteristics of cancer cells and activated lymphocytes in their ability to enhance aerobic glycolysis (Yang and Vousden 2016). There is evidence that pig trophectoderm cells express the ketohexokinase enzyme (Steinhauser et al. 2016). Utilization of the ketohexose pathway in trophectoderm cells of sheep and pigs under hypoxic conditions during the peri-implantation period of pregnancy and later stages of gestation is clearly advantageous.

L-Lactate, a major metabolic product of aerobic glycolysis, also creates an acidic environment for trophectoderm cells (Gardner 2015) and plays a role in survival of Naked Mole rats (Park et al. 2017). In mice, aerobic glycolysis also provides for a high carbon flux to fulfil biosynthetic demands, increase concentrations of lactate and lower pH around the conceptus (Gardner 2015). Lactate activates cell signaling under hypoxic conditions at implantation sites to: (1) increase expression of hypoxia inducible factor 1-α and down-stream growth factors such as bioactive vascular endothelial growth factor to increase angiogenesis; (2) modulate local immune responses to favor immune tolerance; and (3) modulate expression of enzymes that modify the extracellular matrix of the endometrium in preparation for implantation. The conversion of pyruvate into lactate via lactate dehydrogenase also regenerates NAD^+ required for glycolysis to continue. Maintenance of the NAD^+/NADH redox balance is necessary for conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate, and NADH is a cofactor for the transcriptional regulator C-terminal-binding protein involved in cell growth, differentiation, and transformation (Lunt and Vander Heiden 2011).

In cells that are not dividing and migrating, metabolism of glucose through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation is an efficient way to produce ATPs. However, as noted previously, proliferating and migrating cells are metabolically distinct from resting cells (Pearce et al. 2013; Burton et al. 2017). Cancer cells and activated lymphocytes enhance aerobic glycolysis (also known as the Warburg effect) to produce glycolytic intermediates used as substrates for metabolism via: (1) the pentose phosphate pathway (PPP) for generating a pentose sugar (i.e., ribose 5-phosphate) as a precursor for synthesis of nucleotides, and NADPH for nitric oxide synthesis an anti-oxidative reactions; (2) one-carbon metabolism for *de novo* synthesis of purines and thymidine for synthesis of nucleotides, and *S*-adenosyl methionine for methylation reactions and epigenetic modifications of genes; (3) hexosamine biosynthesis for synthesis of glycosaminoglycans (e.g., hyaluronic acid), uridine diphosphate-N-acetyl glucosamine, a cell signaling molecule, and uridine diphosphate-N-acetyl galactosamine involved in synthesis of glycolipids, glycosaminoglycans and proteoglycans; and (4) the TCA cycle for generation of NADH, FADH2 and ATP. A result of activation of the PPP and 1C metabolism is a decrease in availability of pyruvate for metabolism via the Kreb's cycle. Cancer cells overcome this metabolic restriction by utilizing glutaminolysis to convert glutamine into a TCA cycle metabolite, α-ketoglutarate (αKG), through a process known as anaplerosis. Glutaminolysis-derived αKG is converted into citrate via enzymes of the Krebs cycle, a process known as reductive glutamine metabolism, and citrate is exported into the cytosol where it is cleaved into oxaloacetate and acetyl-CoA. The latter is used for the synthesis of lipids. The active TCA cycle generates ATP that inhibits the enzyme phosphofructokinase-1 (PFK) and, therefore, glycolysis. This inhibition can be circumvented via activation of the polyol pathway to synthesize fructose from glucose, and fructose-driven glycolysis (also called fructolysis) continues to provide glycolytic intermediates. Enzymes required for the polyol pathway are expressed by conceptus trophectoderm cells of pigs and sheep. We propose that in a hypoxic environment, trophectoderm cells of pig and sheep conceptuses: (1) utilize glucose via the glycolytic biosynthetic pathway, and accumulating glycolytic intermediates are shunted into the *de novo* synthesis of nucleotides; (2) utilize glutamine

as an alternate carbon source to maintain TCA cycle flux and provide biosynthetic precursors for the synthesis of lipids; and (3) convert glucose to fructose through the polyol pathway, and fructolysis provides glycolytic intermediates from fructose-1-PO4 metabolism that is not inhibited by ATP, citrate or pH. The synthesis of nucleotides and lipids through these biosynthetic pathways is essential to support extensive proliferation and migration of conceptus Tr cells required for implantation and early placentation.

1.2 Metabolism in Trophectoderm During Peri-Implantation Period

1.2.1 Warburg Effect in a Hypoxic Environment

Implantation and early placentation in humans involves rapid growth of the conceptus that requires extensive proliferation, migration and differentiation of cells, all of which rapidly exhaust available oxygen and nutrients. In humans, the fetal heart does not start beating until the 5th week of pregnancy and an effective circulation through the placental villi is only achieved towards the end of the first trimester (Burton et al. 2017). Therefore, implantation and early placentation in humans take place in a hypoxic environment (Burton et al. 2017; Tayade et al. 2007). During the initial stages of implantation, pig and sheep conceptuses elongate and attach to the uterine LE, processes that also require extensive proliferation, migration, and differentiation of cells in a hypoxic environment. This results in hypoxia inducible factor 1-α expression by trophectoderm cells of pig conceptuses that is upstream of expression of vascular endothelial growth factor and angiopoietins required to initiate angiogenesis and transport of nutrients from the dam into the uterine lumen and or fetal-placental vascular system. Optimal utilization of multiple biosynthetic pathways is likely an essential aspect of early conceptus development for both humans and livestock species including sheep and pigs; however, little is known about the biosynthetic pathways employed by conceptuses of these species. Metabolism may occur through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation to produce ATP (O'Neill et al. 2016). However, proliferating, migrating and differentiating cells are metabolically distinct from cells of resting tissues and reflect characteristics of cancer cells and activated lymphocytes. Cancer cells and activated lymphocytes utilize aerobic glycolysis (also called the Warburg effect) (Andrejeva and Rathmell 2017; Yang et al. 2017) that generates various metabolites required to support multiple metabolic pathways. Those pathways include: (1) the PPP for generating pentoses, ribose 5-phosphate and NADPH; (2) the hexosamine biosynthesis pathway (HBP) for producing UDP-N-acetylglucosamine (UDP-GlcNAc) and glycosaminoglycans such as hyaluronic acid; (3) one-carbon metabolism that generates formate for the de novo synthesis of purine nucleotides, thymidylate, S-adenosylmethionine required for methylation reactions; and (4) generation of NADPH. These metabolic pathways require cooperation between amino acids and glucose (Wu 2018).

1.2.2 Glutaminolysis as a TCA Cycle Anaplerosis

Activation of the PPP, HBP and one-carbon metabolism decreases the generation of pyruvate as substrate for the TCA cycle. Therefore, proliferating cells such as trophectoderm cells, may utilize glutaminolysis to convert glutamine into the TCA cycle metabolite, αKG, a process known as anaplerosis (Yang et al. 2017; Jiang et al. 2016). The creatine kinase pathway is another pathway to generate ATP to support conceptus development (Brosnan and Brosnan 2016). Glutaminolysis-derived αKG can also support synthesis of fatty acids, as noted previously. Glutamine increases in the uterine lumen during the peri-implantation period of pigs and sheep that increases proliferation of porcine trophectoderm cells in vitro. All enzymes required for glutaminolysis are expressed by the Tr cells of pig conceptuses (Seo et al. 2020b).

1.2.3 Polyol Pathway and Fructolysis to Bypass Feedback Inhibition of Glycolysis

An active TCA cycle generates ATP that inhibits PFK and, therefore, glycolysis. This inhibition can be overcome by activation of the polyol pathway to synthesize fructose from glucose (see Park et al. 2017). Fructose-driven glycolysis then provides glycolytic intermediates continuously. Enzymes required for the polyol pathway are expressed by conceptus trophectoderm cells of pigs (Steinhauser et al. 2016). Again, the Naked mole rat, under conditions of anoxia or hypoxia, immediately increases the conversion of glucose to fructose and activation of ketohexokinase to generate fructose-1-PO4 for aerobic glycolysis downstream of phosphofructokinase (Park et al. 2017). The synthesis of nucleotides and lipids through these biosynthetic pathways is essential to support extensive proliferation and migration of conceptus trophectoderm cells required for implantation and placentation.

1.3 Serine as a Major Source of 1C Unit

1.3.1 Serine Biosynthesis from Glucose

Increased serine biosynthesis is one of the metabolic changes occurring in proliferating cells (see Mattaini et al. 2016; Locasale 2013; Ma et al. 2017; Yang and Vousden 2016). Serine is required for several biosynthetic pathways including the synthesis of other amino acids and the production of phospholipids, but its linkage with one-carbon metabolism is particularly relevant to populations of proliferating cells such as cancer cells and trophectoderm cells of elongating ovine and porcine conceptuses. One-carbon metabolism has been referred to as an integrator of nutrient status, an analogy often used for mTOR. Hexose sugars, particularly fructose and glucose, and amino acids enter the pathway, undergo chemical modification and then are out-sourced for diverse cellular functions. Cells can either obtain serine from the outside environment, as we propose here for serine production in uterine LE and uptake by adjacent trophectoderm, or through intracellular synthesis from hexose sugars. The reactions of serineneogenesis are catalyzed by the successive actions of the enzymes phosphoglyceride dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT), and phosphoserine phosphatase (PSPH). PHGDH converts 3-phosphoglycerate to 3-phosphohydroypyruvate which is the committed step into the pathway for serine biosynthesis. PSAT next converts 3-phosphohydroypyruvate to 3-phosphoserine (P-Ser), which is then converted to serine by PSPH. Serine can either remain in the cytosol or be transported to neighboring cells where it can enter mitochondria for incorporation into one-carbon metabolism, a network of interconnected biochemical pathways that facilitate the transfer of one-carbon units for biosynthesis. Within mitochondria, serine hydroxymethyltransferase 2 (SHMT2) catalyzes the reversible reaction of serine and tetrahydrofolate (THF) to glycine and 5,10-methylene tetrahydrofolate (mTHF). mTHF is required for synthesis of formate within the mitochondria. Formate then goes to the cytoplasm for synthesis of thymidine for DNA synthesis, purines for RNA and DNA synthesis and S-adenosyl methionine which is the primary methyl donor for methylation reactions such as those for epigenetic modifications of gene expression. The temporal and cell-specific expression of these genes in uteri and placentae of pigs and sheep indicates that glucose and fructose can be converted to serine within the uterine LE via PHGDH, PSAT1 and PSPH, while serine can also be transported into trophectoderm cells by SLC1A4 (neutral amino acid transporter A). Mechanistic target of rapamycin (MTOR) and hypoxia inducible factor 1-α can then potentially induce expression of SHMT2 and methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) in trophectoderm cells to convert serine to 1 carbon units (unpublished observations) (Fig. 1.2). Because plant porteins contain relatively low content of both serine and glycine (Hou et al. 2019; Li and Wu 2020), *de novo* synthesis of serine is of nutritional and physiological importance for successful pregnancy outcomes in ruminants and swine that typically consume plant-based diets.

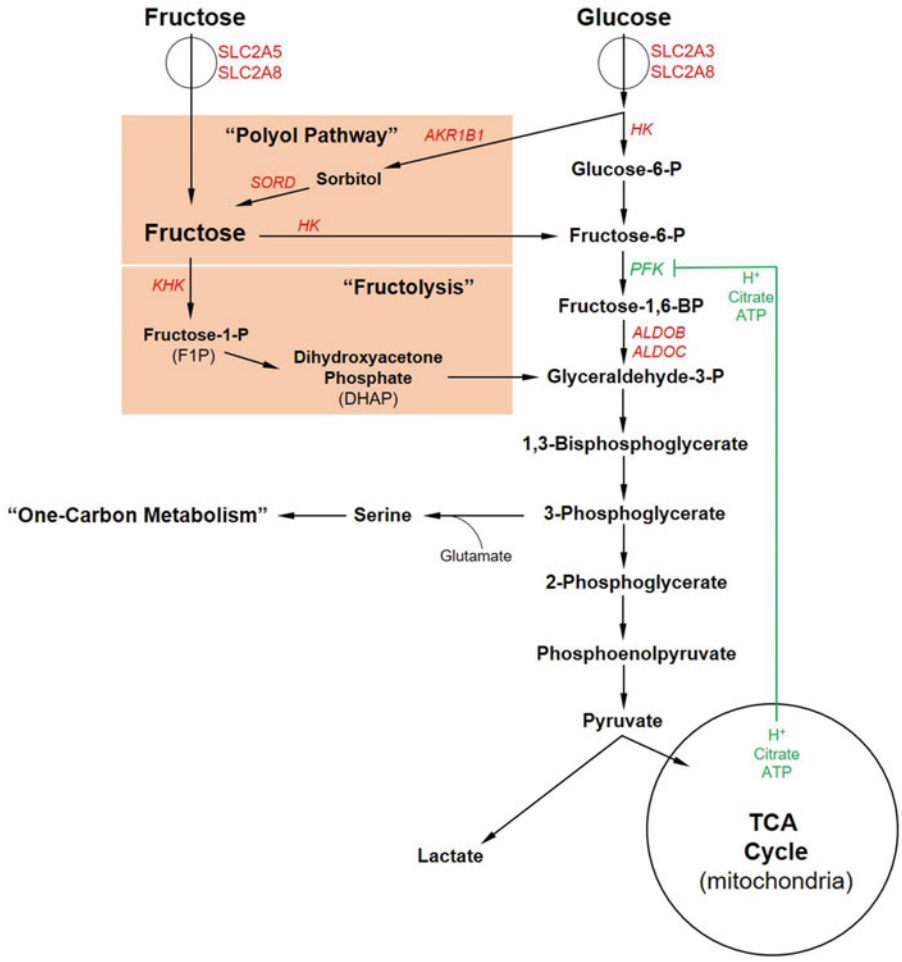

Fig. 1.2 Glucose may enter tissues via glucose transporter 1 (SLC2A1) and be phosphorylated to glucose-6-PO4 and fructose-6-PO4 by hexokinases and then PFK generates fructose-1,6 bisphoshphate that aldolases B and C convert to trioses, dihydroxyacetone phoshphate and glyceraldehyde-3-PO4 for further metabolism to pyruvate and lactate. This pathway is inhibited by increases in ATP, citrate, and decreases in pH. However, fructose produced from glucose via the aldoase pathway can enter cells via SLC2A5 and be phosphorylated by ketohexokinase to fructose-1-PO4. Aldolase B primarily the converts fructose-1-PO4 to trioses (dihydroxyacetone phoshphate and glyceraldehyde-3-PO4) that can be metabolized to pyruvate and lactate. The pathway whereby ketohexokinase yields fructose-1-PO4 at a higher efficiency than by hexokinases and fructose-1-PO4 is directly metabolized into trioses via aldolase B or aldolase C to bypass feedback inhibition by ATP, citrate and pH. However, glyceraldehyde-3-PO4 can also be metabolized via the serineogenesis pathway for one-carbon metabolism

As noted previously, there is a link between serine, one carbon metabolism and rapidly proliferating cells such as cancer cells and trophectoderm cells of elongating ovine and porcine conceptuses. In rapidly dividing cells, it is known that amino acids such as arginine, stimulate MTOR in trophectoderm cells (Bazer et al. 2015), but there is also evidence for a link between MTOR and one-carbon metabolism indicating cross-talk among metabolic pathways in such cells. It should also be noted that MTORC1 activates activating transcription factor 4 (ATF4) that stimulates expression of MTHFD2, but also PHGDH, PSAT and PSP that generates serine for one-carbon metabolism (Ben-Sahra et al. 2016). Glucose and fructose can be metabolized via the hexosamine biosynthetic pathway to activate the Akt-TSC2-MTOR signaling cascade due to glycosylation and activation of those transcription by UDP-N-acetylglucosamine, a primary product of

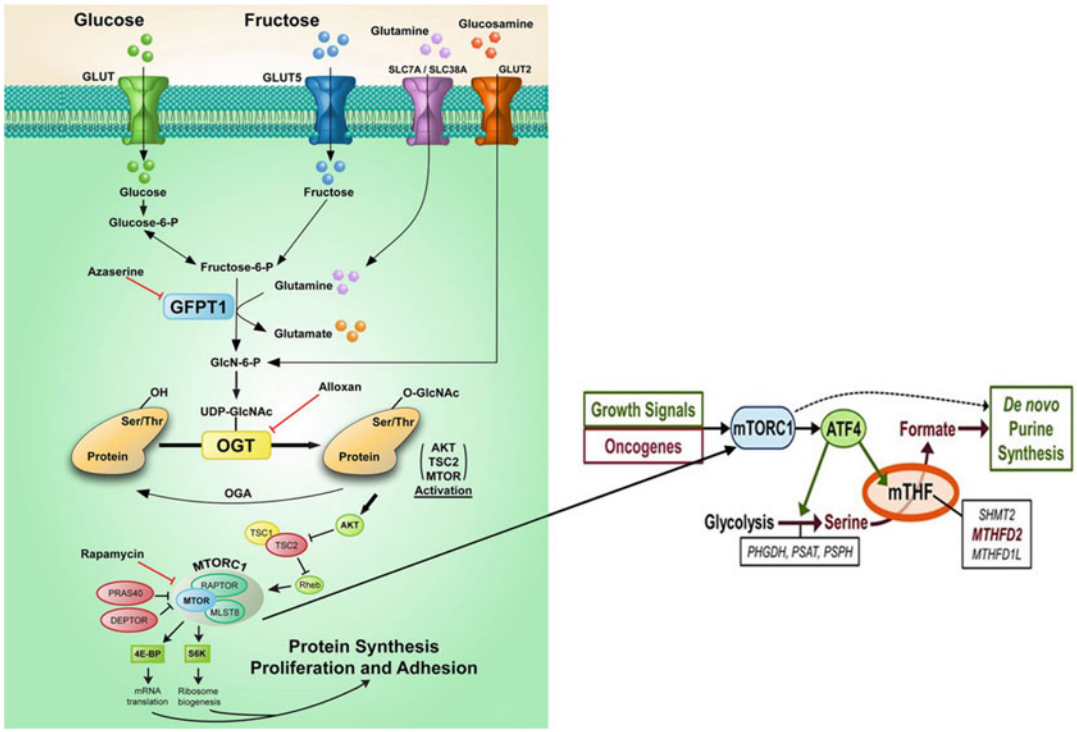

Fig. 1.3 In rapidly dividing cells, it is known that amino acids such as arginine, stimulate the mechanistic target of rapamycin cascade in that stimulates proliferation and expression of mRNAs in ovine and porcine trophectoderm cells (see Bazer et al. 2015). This figure provides evidence for cross-talk between pathways fueled by molecules involved in glycolysis. In this case, there is cross-talk between the hexosamine biosynthesis pathway and the pathway for generating molecules required for one-carbon metabolism. It should also be noted that MTORC1 activates activating transcription factor 4 (ATF4) and ATF4 then stimulates expression of phosphoglyceride dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT), and phosphoserine phosphatase (PSPH) to generate serine for one-carbon metabolism (Ben-Sahra et al. 2016). Glucose and fructose can be metabolized via the hexosamine biosynthetic pathway to activate the Akt-TSC2-MTOR signaling cascade. This results from glycosylation and activation of Akt-TSC2-MTOR transcription factors by actions of UDP-N-acetylglucosamine, a primary product of the hexosamine biosynthesis pathway and O-glycosyltransferase (OGT) (Wang et al. 2016). Thus, the mitochondrial tetrahydrofolate (mTHF) cycle is activated to generate formate for purines, thymidine and s-adenosylmethionine to support rapid proliferation of trophectoderm cells. (This figure is adapted from those published Wang et al. (2016) and Ben-Sahra et al. (2016))

the hexosamine biosynthesis pathway (Wang et al. 2016). The generation of MTOR via this pathway may then activate ATF4 to induce the pathway for generation of serine for one-carbon metabolism (Fig. 1.3).

1.3.2 Serine Biosynthesis from Fructose

Fructose is clearly the most abundant hexose sugar in allantoic fluid and fetal blood of ungulates and cetaceans (Kim et al. 2012). Fructose can be synthesized from glucose via the polyol pathway, also known as the sorbitol-aldose reductase pathway (Steinhauser et al. 2016). Glucose is converted to sorbitol by aldo-keto reductase family 1 member B (AKR1B1), then sorbitol is converted to fructose by sorbitol dehydrogenase (SORD). Cells obtain fructose from their environment through fructose transporters, particularly solute carrier family 2 member 5 (SLC2A5, also known as GLUT5) and SLC2A8 (also known as GLUT 8). Within cells, fructose is phosphorylated by ketohexokinase to fructose-1-phosphate that can be metabolized to dihydroxyacetone phosphate

and then 3-phosphoglycerate for entry into the pathway for synthesis of serine. This switch to fructose-1-PO4 by-passes the key regulatory step that limits glycolytic flux (Park et al. 2017). Glucose metabolism via hexokinases 1 and 2 yields glucose-6-PO4 that is metabolized by the pathway requiring PFK. PFK is subject to feedback inhibition by ATP, hydrogen ions, and citrate. However, fructose-1-phosphate metabolites enter glycolysis downstream of PFK which permits continued metabolic flux through aerobic glycolysis that is not inhibited by ATP, pH or citrate.

Sorbitol is present in high concentrations in porcine, ovine, bovine, and human placentae, especially during early pregnancy and there is expression of SLC2A5 and SLC2A8 by pig conceptuses (Jauniaux et al. 2005; Steinhouser et al. 2016; Bazer et al. 2012a, b). Fructose is transported into trophectoderm cells by SLC2A5 and SLC2A8, converted to fructose-1-phosphate by ketohexokinase and further metabolized via aerobic glycolysis that supports hexosamine biosynthesis, pentose phosphate pathway and one-carbon metabolism, all of which are essential to support fetal-placental development and ensure a successful outcome of pregnancy.

1.3.3 Serine in Biological Fluids During Pregnancy

Serine is abundant in the uterine histotroph of sheep (Gao et al. 2009a) and pigs (Bazer et al. 2012a, b). Concentrations of serine (nmol) in uterine flushings from ewes increase between Days 10 and 15 of pregnancy (542 ± 267 versus 1975 ± 687) and decrease, perhaps due to metabolism, by Day 16 of pregnancy (1708 ± 494). Similarly, concentrations of glycine (nmol) increase in uterine flushings from pregnant ewes between Day 10 (4214 ± 739) and 14 (8598 ± 2308) and then decrease to Day 16 (5805 ± 2004). Concentrations of serine (nmol) are much greater in litter bearing pigs. Concentrations of glycine (nmol) in uterine flushings from pigs increase between Days 10 (93,387 ± 12,435) and 15 (95,282 ± 54,745) and concentrations of glycine (nmol) are 63,8878 ± 6145 and 41,409 ± 25,858 on the same respective days. The decreases in serine and glycine in uterine flushings toward the end of the peri-implantation period of pregnancy likely reflect increased uptake and metabolism by highly active trophectoderm cells. Serine is also very abundant in allantoic fluid of sheep with mean concentrations (μmol/L) of 1636, 19,072 and 16,468 on Days 40, 100 and 140 of gestation, respectively (Kwon et al. 2003). On Day 140 of gestation in sheep, serine accounts for about 60% of total α-amino acids in allantoic fluid (Kwon et al. 2003). Glycine (μmol/L) is also very abundant in ovine allantoic fluid on Days 40 (2449), 100 (5464) and 140 (1132). Mean concentrations (μmol/L) of serine (1218) and glycine (3054) are also very abundant in allantoic fluid on Day 110 of gestation (Wu et al. 1995). Increases in abundances of serine and glycine are coincident with rapid proliferation of trophectoderm cells as the conceptus elongates and mononuclear trophectoderm cells differentiate into trophoblast giant cells that invade the uterine LE to undergo syncytialization at the uterine-placental interface (Seo et al. 2019). Also, there is rapid development of the chorioallantois and amnion during the first one-half of gestation that will support exponential growth of the fetus during the second one-half of gestation. Fetal development does not increase exponentially until after placental development is essentially complete. The trophoblast giant cells have a high level of expression of the serine transporter SLC1A4 mRNA indicating that they take up serine released by adjacent uterine LE cells. The primary membrane transporters for serine are solute carrier family 1 member 4 (SLC1A4) and SLC1A5, both of which are expressed by uterine epithelia and trophectoderm cells of ovine conceptuses during the peri-implantation period of pregnancy (Gao et al. 2009b). Patterns of expression of transporters for glycine, such as GLc6A9, by uterine epithelia and trophectoderm of sheep and pigs are unknown.

1.4 Formate as a Major Output of 1C Metabolism

Brosnan and Brosnan (2016) noted that formate is the neglected member of one-carbon metabolism because it is not linked to a tetrahydrofolate (THF) coenzyme like other molecules involved in one-carbon metabolism. They also noted that formate is more mobile than THF-linked molecules and easily provides inter-organ and inter-organelle shuttling of one-carbon groups due to its presence in blood at considerably higher concentrations than folates. Cancer cells have higher concentrations of formate than cells of healthy tissues (Wang et al. 2013) and over-expression of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), a bifunctional mitochondrial enzyme, is associated with increased proliferation of cancer cells (Gustafsson et al. 2015).

1.4.1 Neural Tube Defects

Neural tube defects occur in Mthfd1l null mice as that gene encodes for mitochondrial 10-formyl-THF synthase that catalyzes the interconversion of 10-formyl-THF to formate (Momb et al. 2013). However, neural tube defects in Mthfd1l null mice are reduced significantly when dams are provided with sodium formate in their drinking water. Dietary supplementation with sodium formate also reduced neural tube defects in Gldc (glycine dehydrogenase (decarboxylating), mitochondrial) null mice (Pai et al. 2015).

Pai et al. (2015) reported that glycine decarboxylase (Gldc) null mice are deficient in folates charged with one-carbon groups. Gldc null mice have two phenotypes. One phenotype was partially penetrant with 25–30% of mice having neural tube defects, particularly exencephaly. The second phenotype of mice exhibited nonketotic hyperglycinemia characterized by elevated concentrations of glycine in their plasma and a high incidence of hydrocephalus. Supplementing the diet with sodium formate in drinking water from Day 1 of pregnancy restored normal concentrations of folate in the plasma and eliminated the neural tube defects in the partially penetrant phenotype, but did not alleviate defects in the hyperglycinemia phenotype. Thus, the glycine cleavage system provides one-carbon groups, in the form of 5,10-methylene-tetrahydrofolate required for normal closure of the neural tube, particularly between embryonic days 8.5 and 10.5 in mice. Alternatively, glycine can be oxidized by glycine oxidase to glyoxylate, which is decarboxylated by NAD-linked glyoxylate dehydrogenase to produce formate (Wu 2013).

1.4.2 Formate During Pregnancy in Sheep and Pigs

There are limited studies in sheep and humans linking formate with the growth and development of conceptuses. For pregnant ewes at Day 120 of gestation, concentrations of format in fetal plasma and in amniotic fluid were six- and ninefold greater than those in maternal plasma, and concentrations of format in plasma from neonatal lambs remained high until around 8 weeks of life (Washburn et al. 2015). Similarly, in pregnant women, concentrations of formate, as well as its precursors (serine, glycine, tryptophan, and methionine) were greater in the plasma from cord blood than maternal plasma. However, babies with variant forms of the MTHFD1 gene (1958 G to A) and MTHFR gene (1298 A to C) had lower concentrations of formate in their blood, but infants with the more common mutation in the MTHFR gene (677 C to T) had concentrations of formate that were similar to those for normal babies.

As noted earlier, serine and glycine are abundant in uterine flushings and in fetal fluids and may be used for synthesis of one carbon groups such as formate. Cetin et al. (1992) reported significant uptake of serine by both liver and hindlimbs of fetal lambs, as well as a net uptake of serine across the placenta. They also obtained umbilical venous and maternal arterial blood from 24 normal (AGA) and 31 intrauterine growth retarded (IUGR) fetuses with 16 AGA pregnancies between 18 and 25 weeks of gestation and 8 AGA and 31 IUGR pregnancies between 27 and 39 weeks of gestation (Cetin et al. 1993). They reported no significant relationship between concentrations of amino acids in maternal arterial blood and

gestational age except for threonine, methionine, serine and glutamic acid. Further, concentrations of glycine, aspartic acid, and glutamic acid increased in umbilical vein plasma and were significantly greater in normal fetuses during the third trimester than in the second trimester, and glycine was the only amino acid in umbilical vein plasma to increase in concert with gestational age. Washburn et al. (2015) suggested that formate is synthesized in the placenta from serine and distributed to fetal tissues as a substrate for use in one-carbon metabolism since the ovine placenta exhibits high activity of mitochondrial SHMT throughout pregnancy (Narkewicz et al. 1999). Also, human placentae express an abundance of mitochondrial bifunctional protein (MTHFD2) mRNA that codes for methylenetetrahydrofolate dehydrogenase and 5,10-methenyl-THF cyclohydrolase (Prasannan et al. 2003). Each of those three enzymes is critical for the synthesis of formate in mitochondria. Thus, Washburn et al. (2015) suggest that formate is not only an intracellular metabolite in one-carbon metabolism, but an inter-organ metabolite that distributes one-carbon groups to rapidly developing tissues.

1.5 Compartmentalization of 1C Metabolism

1.5.1 Mitochondrial 1C Metabolism

The primary route for production of folates begins in mitochondria where serine, glycine, sarcosine and dimethylglycine are converted to 5,10-methylene-tetrahydrofolate (5,10 –CH_2-THF). Brosnan and Brosnan (2016) noted the following key points regarding synthesis of formate in mitochondria: (1) sarcosine is not abundant in tissues and contributes little to the synthesis of formate; (2) serine metabolism is initiated by an isoform of mitochondrial serine hydroxymethyltransferase (SHMT)-2; (3) glycine metabolism is initiated by the mitochondrial glycine cleavage system; (4) sarcosine and dimethylglycine metabolism are initiated, respectively, by sarcosine dehydrogenase and dimethylglycine dehydrogenase; (5) 5,10-methylene-THF produced from these substrates is oxidized to 10-formyl-THF by the sequential actions of the mitochondrial isoforms of 5,10-methylene-HF dehydrogenase and 5,10-methenyl-THF cyclohydrolase; (6) 5,10-methylene-THF dehydrogenase and 5,10-methenyl-THF cyclohydrolase are bifunctional proteins in mammalian mitochondria; (7) the two bifunctional mitochondrial isoforms are methylenetetrahydrofolate dehydrogenase/5,10-methenyl-THF cyclohydrolase (MTHFD2) and MTHFD2L; (8) MTHFD2 is expressed primarily in tumors and embryonic tissues; (9) 10-Formyl-THF synthase produces formate and THF from 10-formyl-THF; (10) formate is transported from the mitochondria into the cytosol by an unknown mechanism and may be incorporated into cytosolic 10-formyl-THF and then other THF-linked one-carbon intermediates; (11) limited amounts of formate may be produced from histidine catabolism to formiminoglutamate and this one-carbon group may be metabolized to yield cytosolic 5,10-methylene-THF to be oxidized to 10-formyl-THF and then to formate in the cytosol; and (12) serine is likely the most important precursor of formate as both carbons 2 and 3 of serine are incorporated into formic acid and formate.

1.5.2 Cytosolic 1C Metabolism

Three canonical functions of one-carbon metabolism are synthesis of purine nucleotides, synthesis of thymidylate, and provision of labile methyl groups to remethylate homocysteine to methionine (Brosnan and Brosnan 2016). Formate functions in the cytoplasm include actions of ATP-dependent 10-formyl-THF synthetase with 5,10-methenyl-THF cyclohydrolase and 5,10-methylene-THF dehydrogenase in the trifunctional protein MTHFD1. The 10-formyl-THF is incorporated into the 2 and 8 positions of the purine ring and may be further reduced to 5,10-methylene-THF and 5-methyl-THF, respectively, for thymidylate synthesis and remethylation of homocysteine to methionine. During folate deficiency, mammalian cells in the S phase of the cell cycle can translocate SHMT1, SHMT2α, thymidylate synthase, dihydrofolate reductase, and MTHFD1 to the nucleus to form a functional metabolon to achieve the synthesis of

thymidylate (Field et al. 2014). Those authors indicated that SHMT1 and SHMT2α function as scaffold proteins rather than as enzymes because catalytically inactive SHMT1 also enhances thymidylate synthesis. Formate produced in mitochondria enters the nucleus for conversion to 5,10-methylene-THF by the three reactions of MTHFD1. Thus, cells deficient in folate and 5,10-methylene-THF are able to achieve de novo synthesis of thymidylate at the expense of remethylation of homocysteine to methionine.

1.6 Summary

This review links morphological and functional aspects of placentae required for transport of nutrients across the placenta and into the fetal-placental vasculature for delivery to those respective tissues. Further, placentae of sheep and pigs must support rapid growth of the fetus in spite of capillaries in the placenta being separated from maternal capillaries by 5 or 6 layers of cells. Accordingly, the allantois serves as a reservoir in which nutrients in excess of metabolic needs can be stored and then reabsorbed, as needed, to compliment the on-going transfer of nutrients from maternal to fetal-placental vasculatures. The focus of the review is on the utilization of available serine and glycine by the rapidly developing placenta, as well as pathways for glucose and fructose to be used to produce 3-phosphoglycerate that can enter into the serinogenesis pathway in the presence of glutamate. It is also noteworthy that fructose in the blood and allantoic fluid of sheep and pigs is at conentrations 11–30 times those of glucose. Fructose can be phosphorylated by ketohexokinase to yield fructose-1-PO4 that is metabolized via a pathway that by-passes phosphofructokinase to assure continuous generation of metabolites via aerobic glycolysis that supports hexosamine biosynthesis, pentose phosphate pathway and one-carbon metabolism, all of which are essential to support fetal-placental development and ensure a successful outcome of pregnancy.

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Cell-Specific Expression of Enzymes for Serine Biosynthesis and Glutaminolysis in Farm Animals

2

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Abstract

During the peri-implantation period, conceptuses [embryo and placental membranes, particularly the trophectoderm (Tr)] of farm animals (e.g., sheep and pigs) rapidly elongate from spherical to tubular to filamentous forms. In concert with Tr outgrowth during conceptus elongation, the Tr of sheep and pig conceptuses attaches to the endometrial luminal epithelium (LE) to initiate placentation. In sheep, binucleate cells (BNCs) begin to differentiate from the mononuclear trophectoderm cells and migrate to the endometrial LE to form syncytial plaques. These events require Tr cells to expend significant amounts of energy to undergo timely and extensive proliferation, migration and fusion. It is likely essential that conceptuses optimally utilize multiple biosynthetic pathways to convert molecules such as glucose, fructose, and glutamine (components of histotroph transport by sheep and pig endometria into the uterine lumen), into ATP, amino acids, ribose, hexosamines and nucleotides required to support early conceptus development and survival. Elongating and proliferating conceptus Tr cells potentially act, in a manner similar to cancer cells, to direct carbon generated from glucose and fructose away from the TCA cycle for utilization in branching pathways of glycolysis, including the pentose phosphate pathway, one-carbon metabolism, and hexosamine biosynthesis. The result is a limited availability of pyruvate for maintaining the TCA cycle within mitochondria, and Tr cells replenish TCA cycle metabolites via a process known as anaplerosis, primarily through glutaminolysis to convert glutamine into TCA cycle intermediates. Here we describe the cell-specific expression of enzymes required for serine biosynthesis, one-carbon metabolism and glutaminolysis at the uterine-placental interface of sheep and pigs, and propose that these biosynthetic pathways are essential to support early placental development including Tr elongation, cell migration, cell fusion and implantation by ovine and porcine conceptuses.

Keywords

Enzymes · Pig/Sheep · Placentation · Glucose/Fructose · Glutamine · Glycolysis · Glutaminolysis

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Abbreviations

3-PG	3-phosphoglycerate
BNC	binucleate cells
GE	glandular epithelium

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GLUD	glutamate dehydrogenase
LE	luminal epithelium
PHGDH	phosphoglyceride dehydrogenase
PHP	3-phosphohydoypyruvate
PSAT	phophoserine aminotransferase
PSPH	phosphoserine phosphatase
SAM	*S*-adenosylmethionine
SHMT	serine hydroxymethyltransferase
SLC	solute carrier
TCA	tricarboxylic acid
THF	tetrahydrofolate
Tr	trophectoderm
α-KG	α-ketoglutarate

2.1 Introduction

Over two-thirds of pregnancy losses in mammals occur during the peri-implantation period of pregnancy. In sheep and pigs, conceptuses [embryos and associated placental membranes, particularly the trophectoderm (Tr)] undergo dramatic morphological changes from spherical to tubular and then filamentous forms, and attach to the uterine luminal epithelium (LE) to initiate placentation (Bazer and Johnson 2014). These processes require that Tr cells expend significant amounts of energy to undergo timely and extensive proliferation and migration at a time when the conceptuses have not yet established a placental connection to the uterus, and are dependent upon limited nutrients either secreted or transported into the uterine lumen by cells of the endometrium (Perry et al. 1973; Fischer et al. 1985). Therefore, optimal utilization of multiple biosynthetic pathways to convert molecules such as glucose, fructose, and glutamine (components of histotroph secreted and/or transported by sheep and pig endometria into the uterine lumen), into ATP, amino acids, ribose, hexosamines and nucleotides is required to support early conceptus development and survival. Studies with swine have shown that glutamine is a highly abundant amino acid in porcine fetal allantoic and amniotic fluids (Wu et al. 1995, 1996), and its adequate provision in diets is critical for conceptus growth and survival (Wu et al. 2011; Zhu et al. 2018). Our previous work has also demonstrated that ovine fetal fluids contain a large amount of glutamine during gestation (e.g., about 25 mM in allantoic fluid on day 60 of gestation; Kwon et al. 2003). However, little is understood about the biosynthetic pathways employed by sheep and pig conceptuses during the peri-implantation period of pregnancy (Bazer et al. 2020).

Cell metabolism primarily occurs through the TCA cycle and oxidative phosphorylation, which is a complex, but efficient, process that requires mitochondrial biogenesis to produce ATP (Wu 2018). However, proliferating cells, such as the Tr of sheep and pigs, are metabolically distinct from cells of resting tissues, and reflect characteristics of cancer cells and activated lymphocytes (Andrejeva and Rathmell 2017). A hallmark of tumors and activated lymphocytes is their ability to enhance glycolysis, even in the presence of oxygen, a phenomenon known as the Warburg effect or aerobic glycolysis which is a classic example of the ability of proliferating cells to reprogram the activation of metabolic pathways (Yang et al. 2017; DeBerardinis and Chandel 2016). Glycolysis is a physiological response to hypoxia in normal tissues, but in the 1920s Otto Warburg observed that tumor slices and cancer cells with ascites fluid constitutively take up glucose and produce lactate regardless of the availability of oxygen, an observation now recognized in many types of cancer cells and tumors. The widely accepted theory is that cancer cells switch from using oxidative phosphorylation to using glycolysis because high glycolytic rates result in more rapid generation of ATP as compared with the oxidation of glucose and activation of the TCA cycle. The glycolytic intermediates that accumulate are then shunted into branching pathways of glycolysis for *de novo* synthesis of nucleotides, amino acids, and fatty acids to fulfill the metabolic demands of proliferating cells (Cruys et al. 2016; Rathmell et al. 2000; Wang et al. 1976; O'Neill et al. 2016).

The proliferating Tr cells of sheep and pig conceptuses appear to use a similar switch from reliance on oxidative phosphorylation to activation of glycolysis. Glucose and fructose are present in the uterine flushings from early pregnant

sheep and pigs (Zavy et al. 1982; Gao et al. 2009a). In pigs, expression of the facilitated diffusion transporter of the solute carrier family 2A1 (SLC2A1, responsible for the basal uptake of glucose into most cells) and SLC2A8, a high affinity glucose transporter that can also transport fructose, are expressed in uterine LE, whereas SLC2A3 (a high affinity and high capacity glucose transporter), and SLC2A8 are present in conceptus Tr cells. In sheep, SLC2A1 and SLC5A1 are expressed by LE while SLC2A1, SLC2A3, SLC2A4, SLC5A1, and SLC5A11 are expressed by conceptus Tr (Gao et al. 2009b). Therefore, sheep and pig conceptus Tr cells have access to glucose and fructose as energy sources during the peri-implantation period of pregnancy. These Tr cells appear to utilize the glucose for glycolytic branching pathways including the pentose phosphate pathway, serine biosynthesis, one-carbon metabolism, and hexosamine biosynthesis (Kim et al. 2012; Wang et al. 2016), because conceptus Tr cells express key enzymes required for those pathways (described in the next sections) and the pentose phosphate pathway is highly active in porcine Tr cells (Lin et al. 2013). Therefore, the Warburg effect appears to be operational in proliferating Tr cells of sheep and pig conceptuses.

Glutamine, another principal growth-supporting substrate, not only contributes carbon, but also reduces nitrogen for the de novo biosynthesis of a number of diverse nitrogen-containing compounds (Pavlova and Thompson 2016). One glutamine molecule is used in the production of uracil and thymine, while cytosine and adenine each require two glutamines, and guanine requires three molecules of glutamine for synthesis (Wu 2013). Thus, glutamine is a critical structural components in the biosynthesis of nucleotides. Accordingly, glutamine levels have been shown to be a rate-limiting factor for cell cycle progression, and glutamine shortage leads to cell proliferation arrest and S-phase accumulation in certain cellular contexts. The concentration of glutamine increases in the uterine lumen during the peri-implantation period of pregnancy, and glutamine affects proliferation of porcine Tr cells *in vitro* (Kim et al. 2013; Gao et al. 2009a). We hypothesize that the Tr cells of ovine and porcine conceptuses utilize glucose and fructose within the uterine lumen via the glycolytic biosynthetic pathway, and that accumulating glycolytic intermediates are shunted into pathways for the *de novo* synthesis of nucleotides and amino acids, and that glutamine within the uterine lumen is used as an alternate carbon source to maintain TCA cycle flux.

2.2 Overview of Serine Biosynthesis, One-Carbon Metabolism, and Glutaminolysis

Glycolysis is classically depicted as a single chain of molecular events that leads to the generation of pyruvate, but a number of glycolytic intermediates can be diverted into branching pathways, generating diverse biosynthetic precursors (Wu 2018). One of the most intensely studied growth-promoting mechanisms that shunts metabolites out of the glycolytic pathway is the use of 3-phosphoglycerate as a precursor for serine biosynthesis (Fig. 2.1). Serine is required for several biosynthetic pathways including the synthesis of other amino acids and the production of phospholipids, but its linkage with one-carbon metabolism or the folate cycle is particularly relevant to proliferating cells such as cancer cells and the Tr of elongating sheep and pig conceptuses. Cells can either obtain serine from the outside environment or through intracellular synthesis from hexose sugars. Serine biosynthesis is catalyzed by the successive actions of the enzymes phosphoglyceride dehydrogenase (PHGDH), phophoserine aminotransferase (PSAT), and phosphoserine phosphatase (PSPH). PHGDH converts 3-phosphoglycerate (3-PG) to 3-phosphohydoypyruvate (PHP) which is the committed step into the pathway for serine biosynthesis. PSAT next converts PHP to 3-phosphoserine (P-Ser), which is then converted to serine by PSPH. Increases in serine biosynthesis is one of the metabolic changes that

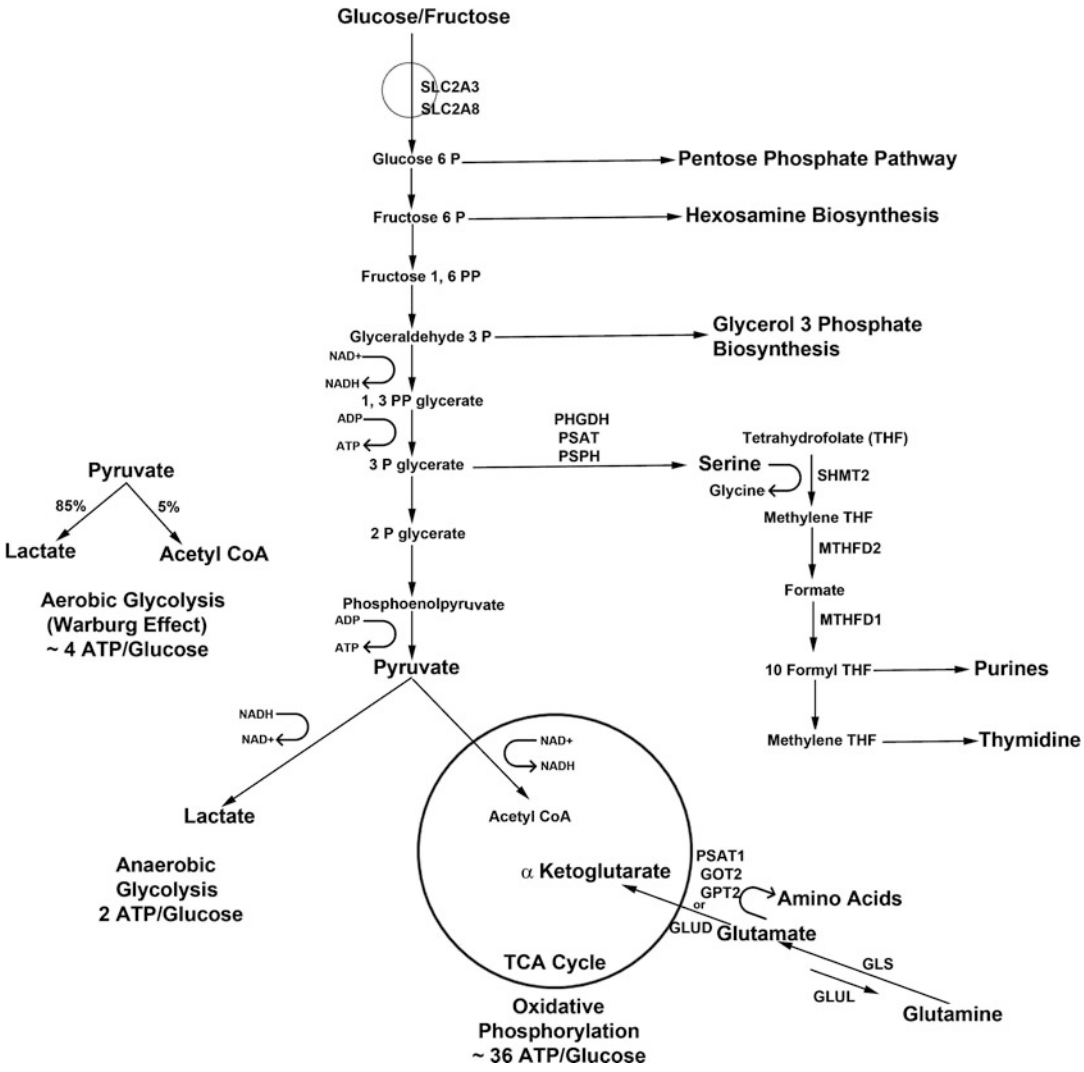

Fig. 2.1 Overview of possible utilization of glucose and fructose through anaerobic and aerobic glycolysis for serine biosynthesis and one-carbon metabolism, and utilization of glutamine via glutaminolysis. Glucose, fructose, and glutamine are abundant nutrients in the conceptuses of farm animals, and glutamine is present in food and animal proteins at relatively high content (Hou et al. 2019; Li and Wu 2020). Glucose is utilized via multiple metabolic pathways (including glycolysis in the presence or absence of oxygen). Similar pathways may exist for fructose metabolism. Phosphate-activated glutaminase (a mitochondrial enzyme in mammals) plays an important role in initiating its catabolism in conceptus, with metabolites including glutamate, aspartate, alanine, pyruvate and lactate. This pathway for partial glutamine catabolism is termed glutaminolysis analogous to glycolysis where glucose is converted into pyruvate and lactate via partial metabolism

occurs in proliferating cells. Enhanced expression of PHGDH, a rate-limiting enzyme of serine biosynthesis, occurs in breast cancer and melanoma cells (Locasale et al. 2011; Possemato et al. 2011).

The newly synthesized serine, or serine transported into the cell can be incorporated into one-carbon metabolism or the folate cycle. The carbon-3 of serine unit can be transferred to a carrier molecule, tetrahydrofolate (THF), in a enzymatic reaction catalyzed by serine hydroxymethyltransferase 2 (SHMT2) in the mitochondria, and SHMT1 in the cytosol, generating 5, 10-methylene-THF and glycine.