TRIENNIAL REPRODUCTION SYMPOSIUM: Limitations in uterine and conceptus physiology that lead to fetal losses
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ABSTRACT: Conceptus losses in livestock occur throughout gestation. The uterus and the embryo–placenta–fetus play interconnected roles in these losses, the details of which depend on the period of gestation and the species. Studies in sheep and pigs have indicated that the uterine glands are essential for full fertility, based on experiments where gland development was reduced through the use of exogenous hormones. In sheep and cattle, normally the uterus is well able to support more than a single fetus although these species differ in the consequences of multiple births. When 2 conceptuses are present, the placentas of cattle often anastomose, putting 1 fetus at risk if the other is lost. One likely reason this does not occur in sheep is because sheep embryos undergo intrauterine migration, similar to pigs. In pigs, the relatively equidistant separation of conceptuses is likely to be essential for optimizing conceptus survival as is the simultaneous and uniform elongation of blastocysts that occurs during the time of maternal recognition of pregnancy. Other studies in pigs have indicated that the size of the uterus influences litter size and therefore fetal losses. In response to crowded intrauterine conditions in the pig, increased conceptus losses begin to occur between d 30 and 40 of pregnancy, and further losses occur sporadically during later gestation. There is evidence that improved fetal erythropoiesis can reduce these losses. Other studies indicated that profound changes in placental development occurred under crowded intrauterine conditions that may contribute to losses during late gestation. Reductions in placental stroma formation may compromise the ability of the pig placenta to adapt to reduced uterine space. Consistent with this, both hyaluronan and hyaluronidase activity are decreased in the placentas of small compared with large fetuses. These results indicate that improvements in placental stroma formation could improve placental ability to compensate for reduced intrauterine space, resulting in increased placental function and reduced fetal losses during late gestation.

Key words: erythropoiesis, hyaluronan, placenta

INTRODUCTION

Fertility of livestock species influences the profitability of livestock production. All livestock species experience embryonic and fetal losses, which reduce fertility. However, the extent and timing of losses and the factors responsible display both commonalities and differences among species. This review focuses...
primarily on embryonic and fetal losses in swine but also highlights similarities and differences that occur in ruminant species. Various aspects of uterine and placental physiology that are known to influence the incidence of embryonic and fetal losses are presented and provide some suggestions for future research to further understand physiological mechanisms that might be manipulated to reduce embryonic and fetal loss in livestock.

**OOCYTE EFFECTS**

If one equates fertility with the capacity of individuals to give birth to some number of offspring in a given parity, then fertility decreases as one goes from litter bearing species (e.g., pigs and some breeds of sheep) to monotypic species (e.g., cattle and other sheep breeds). Regardless of the species, the number of offspring per parity is influenced by the number of ova during an ovulatory event and by the maturation level or quality of ova shed during each ovulatory event. The number of ova represents the upper limit to the number of offspring produced in each pregnancy (Johnson et al., 1985, 1999). The level of maturity or developmental competence of a single ovum or the relative competencies among a group of ova also contributes to the success rate of further embryonic and fetal development (Zuccotti et al., 2011). Various reports in cattle indicate that ova shed from follicles of reduced size, either naturally or during synchronization of ovulation, have reduced ability to form blastocysts and generate calves (Perry et al., 2005, 2007; Echternkamp et al., 2009). Similarly, variation in the time of ovulation within a group of ova during an ovulatory event in pigs influences the ability to form viable embryos and fetuses (Pope, 1988; Pope et al., 1988). It has been suggested that this is due to relative asynchrony of development between littermate embryos caused by differences in time of ovulation. However, another report indicated that differences in timing of ovulation among individual ova in a litter are small (3 h) and are, therefore, not extensive enough to explain the losses of later ovulated ova (Soede et al., 1992). However, the small difference in time of ovulation could be associated with differences in oocyte competence to be fertilized and develop into viable embryos. A few hours in time of ovulation may be associated with significant changes in the maturational state of ova, leading to a reduced rate of development in the later ovulating ova and eventual asynchrony with littermate embryos (Pope, 1992). It would be interesting to assess possible differences in oocyte maturation between early and late ovulating ova in the pig to determine whether this timing is associated with differences in competence and what the cause of those differences might be. One useful approach might be to use the one described by Pope (1992), where he compared oocytes from follicles just before any had ovulated with oocytes from follicles that remained after a proportion of the follicles had ovulated, either normally or in response to hCG. Oocytes collected just before ovulation would be a mixture of approximately 70% normal oocytes and 30% delayed oocytes whereas oocytes remaining after a majority of oocytes had been released would be expected to be enriched for oocytes that develop more slowly. Transcriptomic analyses, both mRNA and microRNA (Miles et al., 2012), and proteomic analyses could yield valuable clues to differences between these 2 populations.

Once the number of ova is established, the next limiting factor to fertility becomes fertilization rate. Polge (1978) indicated that fertilization rates in most livestock were high; therefore, fertilization has typically been suggested to be a factor that does not contribute substantially to differences in fertility. However, a recent report by Flowers (2008) revisited this topic and clearly demonstrated that insemination of females by different boars resulted in differences in the number of piglets born alive, even when the number of sperm used per insemination was not a limiting factor. Another paper from this symposium by Flowers (2013) deals with this topic in detail; therefore, fertilization rate will not be further discussed here.

**CONCEPTUS MIGRATION AND ELONGATION**

The next factor affecting fertility is embryonic survival, which is not only ill defined but also likely to be influenced by a variety of physiological factors. This paper will focus on the role of 2 factors during the peri-implantation period: the migration and elongation of conceptuses and the role of the uterus in conceptus survival. In sheep, cattle, and swine, embryo migration allows for a more equitable allocation of uterine resources when multiple embryos are present. In pigs, embryos enter the uterus by d 4, and migration occurs from d 5 to d 10 or 11 of pregnancy (Dhindsa et al., 1967; Waite and Day, 1967). Pig blastocysts are extremely motile and may even pass by neighboring littermates during migration. Migration in the pig results in the relatively uniform distribution of embryos within the available uterine environment. Uniform spacing contributes to the equal distribution of uterine resources to each individual conceptus. This likely optimizes the individual survival chances of each embryo. Embryo migration in pigs has been reported to be controlled by conceptus estrogen secretion during this same time period (Pope et al., 1982, 1986). More recent studies indicate a possible role for lysophosphatidic acid.
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(LPA) in uterine migration. Knockout studies in mice indicate that elimination of the LPA receptor (LPAR3) disturbs embryonic migration (all embryos migrate to the cervical end of the uterine horn) and reduces litter size (Ye et al., 2005). Seo et al. (2012) indicated that the enzyme responsible for generating LPA is present in pig endometrium during the peri-implantation period.

Sheep conceptuses also migrate but for a much shorter time period (Nephew et al., 1989). Migration begins and ends within approximately 24 h before the initiation of elongation of the sheep blastocyst on d 13 of pregnancy. Evidence indicates that conceptus estrogen also controls blastocyst migration in sheep because estrogen secretion increases simultaneously with migration (Nephew et al., 1989). Similar to pigs, the ability of sheep conceptuses to migrate into the other uterine horn when multiple ovulations occur from the same ovary ensures that the entire uterus is available to the conceptuses and contributes to the ability of the sheep uterus to support multiple conceptuses. By contrast, cattle embryos appear to display a reduced ability to migrate within the uterus because twin bovine conceptuses resulting from the same ovary often remain in a single uterine horn (Rowson et al., 1971; Scanlon, 1972). This likely contributes to twin fetuses becoming entangled during the birth process, resulting in increased dystocia in twin births (Echternkamp et al., 2007). The reduced migratory ability of bovine blastocysts also likely contributes to the incidence of placental anastomoses (Plante et al., 1992), which results in infertility of female offspring born co-twin to a bull due to transfer of male-determining factors to the female twin (Padula, 2005). Placental anastomoses also likely contribute to the increased incidence of loss of all conceptuses as a consequence of the loss of one of the conceptuses due to transfer of necrotic tissues or toxins from the dead conceptus (Echternkamp et al., 2007). Curiously, although migration of cattle conceptuses is limited, cattle conceptuses are capable of migration. Conceptus migration in cattle appears to be influenced by the number of conceptuses present. Consistent migration is observed when many conceptuses are introduced into the uterine horns using embryo transfer (McMillan and Peterson, 1999; Berg et al., 2010). Secretion of estrogen by bovine conceptuses is highly variable during the period when migration would potentially take place (Wilson et al., 1992). The lack of estrogen secretion by some bovine conceptuses could explain the relative poor ability of blastocysts to trigger migration. Migration may require multiple conceptuses to secrete enough estrogen to stimulate uterine motility. This possibility indicates that one might improve cattle conceptus migratory ability by selecting for embryos that secrete more estrogen during early pregnancy. To accomplish this, one might select different bulls on their ability to sire conceptuses with high in vitro estrogen secretion when collected and cultured on d 15 of gestation. Then one might mate these bulls with dams selected for twinning. If estrogen truly controls migration, this strategy would improve the distribution of twin embryos within the uterine environment of dams carrying twins. If successful, this could improve outcomes of twinning in cattle.

Whether they migrate or not, sheep, cattle, and swine conceptuses undergo elongation. Ruminant and swine embryos transform from a spherical to a filamentous form within 24 h of initiation of elongation (Perry and Rowlands, 1962; Geisert et al., 1982; Betteridge and Fléchon, 1988). Elongation increases the portion of the uterus available to the conceptus for interactions that support pregnancy. Very little is known regarding factors that trigger elongation or influence the extent to which conceptuses elongate (Hue et al., 2012). Although all livestock conceptuses elongate, there are subtle differences among species. In cattle, when 2 adjacent conceptuses come into contact, placental anastomosis occurs resulting in a shared blood supply between them (Plante et al., 1992). This seldom occurs in pigs, where elongation ceases when adjacent elongating conceptuses come into close proximity with each other, because conceptuses that either touch or overlap with each other during the period of elongation are rare (Perry and Rowlands, 1962; Crombie, 1972). During later gestation, although placetas become attached to each other, adjacent conceptuses do not share blood supplies. This arrangement allows individual pig conceptuses to be relatively independent of each other. This is likely to be a key adaptation to multiple births because it allows the independent loss of individual pig conceptuses (a relatively frequent occurrence in pigs) without causing the loss of neighboring conceptuses, as has been reported to occur in multiple births in cattle (Echternkamp et al., 2007). It also prevents exchange of primordial germ cells and male-determining factors that ultimately result in freemartinism and infertility in female cattle born co-twin to a male.

**UTERINE DEVELOPMENT AND FUNCTION**

The capacity of conceptuses to migrate and elongate clearly indicates the importance of the uterus for the survival of livestock conceptuses. There are several uterine factors that influence conceptus survival, including uterine length, uterine blood flow, and uterine gland development. In pigs reducing uterine length available to conceptuses, either by restricting the length of the uterus using ligation or surgical removal of portions of the uterus, reduces conceptus survival (Knight et al., 1977; Christenson et al., 1987; Chen and Dziuk, 1993; Freking et al., 2007). These studies have indicated that approximately 20 cm of initial uterine length per conceptus are needed to support conceptus development (Chen and Dziuk, 1993). Uterine
length is also known to be highly variable among pigs, indicating an opportunity to improve reproductive capacity by selecting for increased uterine length (Chen and Dziuk, 1993). Unilateral hysterectomy–ovariectomy (UHO), which results in normal ovulation rate but half the normal uterine space available for conceptus development, has been used to measure uterine capacity in pigs (Christenson et al., 1987). After UHO, litter size is no longer correlated with ovulation rate and, therefore, reflects the capacity of the uterus to support conceptus development. Using this surgical modification, it has been possible to select for increased uterine capacity. Eleven generations of selection resulted in increased uterine capacity of approximately one additional conceptus per uterine horn compared with an unselected control (Christenson and Leymaster, 2002). During selection, the length of the excised uterine horn was measured when the UHO surgeries were performed at 160 d of age. From these data, Young et al. (1996) reported that uterine lengths were genetically correlated ($r = 0.69$) with subsequent measurements of uterine capacity, strongly supporting the concept that genetic differences in uterine length contribute to genetic differences in uterine capacity. However, after 11 generations of selection for litter size after UHO, differences in uterine length at 160 d of age were compared among the line selected for uterine capacity, a simultaneously randomly selected control line, and a line selected for increased ovulation rate. Results confirmed that selection for uterine capacity was associated with increased uterine length at 160 d of age, but an even greater increase in uterine length resulted after selection for ovulation rate (Leymaster and Christenson, 1999). This occurred despite other results indicating that selection for ovulation rate resulted in no change in uterine capacity (Christenson and Leymaster, 2002; Freking et al., 2007). This apparent paradox can be explained by the known effects of ovarian development on uterine development. After 90 d of age, the uterus grows more rapidly in intact pigs compared with ovariectomized pigs, demonstrating that uterine growth is influenced by the presence of the ovary (Bartol et al., 1993). Thus, on d 160, uterine weight is influenced by ovarian development. Selection for ovulation rate influences ovarian development, and these changes also likely accelerate ovary-dependent uterine growth, compromising the value of uterine measurements during this period. Taken together, these results indicate that if one were to select for uterine length, measurements must be made before ovary-dependent growth begins (i.e., d 90 of age or earlier) to ensure that differences are uterine specific.

Differences in uterine length do not necessarily translate into differences in the functional capacity of the uterus. Beyond uterine length, factors that are more likely to be related to functionality include blood flow and uterine gland function. Comparisons of blood flow with uterine horns carrying different numbers of fetuses indicate that uterine blood flow increases as the number of fetuses present in the uterus increases, but increased blood flow in response to conceptus number reaches a limit above which no further increases occur (Père and Etienne, 2000). These results are based on observations in a small number of pigs, and presumably the limit of blood flow differs among individual sows. Measurement of the limits of blood flow in a large number of sows could be used to select for improved uterine function but would be complex and expensive. Nevertheless, combining UHO with uterine blood flow measurements would be one approach to making these measurements. This could provide valuable insights into factors ultimately responsible for blood flow limitations in individual sows.

The importance of uterine glands to conceptus loss has been demonstrated in both pigs and sheep. In livestock species, uterine gland development begins very shortly after birth and continues into adulthood (Bartol et al., 1993). Treatment of neonatal lambs with a synthetic progestin completely blocks uterine gland development, creating a uterine gland knockout (UGKO) phenotype (Bartol et al., 1988, 1999). Subsequent studies indicated that conceptus development fails at approximately d 14 of pregnancy in UGKO sheep (Gray et al., 2001). Curiously, progesterone does not have the same effect on uterine gland development in pigs (Vallet et al., 1995). However, treatment of piglets with estrogen during the neonatal period greatly accelerates early uterine gland development but paradoxically reduces gland development in adulthood (Tarleton et al., 1999). Further studies indicated that this impairment in gland development is also associated with reduced fertility (Tarleton et al., 2003). Despite these results from both sheep and pigs, evidence that naturally occurring deficits (vs. hormonally induced deficits) in gland development are related to fertility in either species is sparse. However, naturally occurring impairment of fertility is associated with low “immunoglobulin immunocrit” values, which are indicative of insufficient colostrum ingestion (Bartol et al., 2013; Vallet et al., 2013). This impairment in fertility is likely due to impaired gland development, which has been shown to occur in piglets that do not receive colostrum (Bartol et al., 2013). This surprising effect of colostrum on uterine gland development emphasizes the potential impact of naturally occurring variation in uterine gland development on subsequent fertility.

**PLACENTAL DEVELOPMENT AND FUNCTION**

One of the key outcomes of conceptus elongation is that it influences the size of the subsequent placenta and the uterine surface area available for placental interactions. Livestock species diverge in terms of placentation, with
pigs having a diffuse epitheliocorial placenta and sheep and cattle having a placentomal, syndesmochorial placenta (Van Tienhoven, 1983). In sheep and cattle, most nutrient exchange occurs within the placentomes (Mott, 1982). Results indicate that the average size of placentomes increases in response to experimental reduction in their number, demonstrating flexibility in the ability of the ruminant placenta to adapt to the intraterine environment (Meyer et al., 2010). However, under normal circumstances the capacity of the uterus and placentas in ruminants to provide support for fetuses appears to exceed the typical number of fetuses present, particularly for cattle (Echternkamp, 1992; Echternkamp et al., 2007). In contrast, in litter bearing species such as pigs, the capacity of the uterus and placenta to maintain fetuses is often exceeded, resulting in conceptus losses later in gestation (d 30 to 40) in addition to embryonic losses (Vonnahme et al., 2002; Town et al., 2005). Recent genetic selection for litter size by the swine industry has likely made this problem worse, resulting in larger litters of low birth weight piglets with poor preweaning survival. To reverse this trend, it will be necessary to more fully understand how the pig placenta functions. More importantly, it will be necessary to develop economically viable methods to improve the growth, development, and function of the placenta.

One approach to potentially improve placental function was the use of the fetal weight to placental weight ratio as a measure of placental efficiency (Wilson et al., 1999). This was based on the concept that if placentas differ in their ability to support a fetus, this difference would be reflected in the weight of the fetus that could be supported by a given weight of placenta. However, this concept relied on the assumption that changes in the weight of the placenta resulted in proportional changes in the weight of the fetus. In other words, the fetus and placenta display proportional growth. This assumption is not true, as indicated in the plot of log fetal weight versus log placental weight illustrated in Fig. 1. In a log-log plot, the linear slope of the relationship is the ratio of growth rate of the fetus divided by the growth rate of the placenta (Huxley, 1932; Vallet and Freking, 2006). A slope of 1 indicates proportional growth. A slope <1 indicates that the growth of the fetus is relatively unaffected by changes in the growth of the placenta. As illustrated in Fig. 1, the slope of the log fetal weight vs. log placental weight is <1 throughout gestation. As gestation advances, the slope increases, indicating that the growth of the fetus becomes more and more influenced by the growth of the placenta, but even at the end of gestation, the slope is <1. Slopes <1 are indicative of “sparing” mechanisms that preserve the growth of the fetus when the growth and development of the placenta is reduced (Vallet and Freking, 2006). Thus, the fetal weight to placental weight ratio depends on the growth (and therefore the size) of the placenta and is not a fixed characteristic of a given placenta. The ratio instead varies with placental size, which depends largely on the amount of uterine space that the conceptus was able to acquire during elongation. Because of intraterine migration and conceptus elongation, the amount of uterine space depends on the total number of embryos present and the elongation of neighboring embryos, both of which would not be determined by the genetics of the individual conceptus. This explains why selection for the fetal weight to placental weight ratio over several generations had no effect on conceptus survival (Mesa et al., 2005).

The log fetal weight vs. log placental weight plot (Fig 1) indicates that the fetal weight to placental weight ratio is not useful as a measure of placental efficiency. However, it also indicates that compensatory mechanisms exist that spare the growth of the fetus when the size of the placenta is reduced. These mechanisms are likely not confined to the placenta, as changes in uterine or fetal function that spare the growth of the fetus may also exist. Elucidation of these mechanisms could provide an opportunity to reduce fetal losses and improve fetal growth independent of changes in the size of the placenta. Therefore, a useful place to start is with the placenta, and a first step is to understand how the placenta works.

There are likely 2 primary components to placental function. The first component is global. How does the structure of the placenta result in the transfer of nutrients from the dam to the fetus? The second component is nutrient specific. What mechanisms exist within the
pig placenta that promote or influence the transfer of specific nutrients? Clues to answer the first question were provided by the work of MacDonald (1976) and Leiser and Dantzer (1988). They reported that maternal and fetal capillaries are arranged on either side of the endometrial epithelial-placental trophoblast bilayer in a cross-countercurrent arrangement. The location of the capillaries, combined with the folded arrangement of the epithelial bilayer, creates a physical unit that would efficiently exchange substances between the maternal and fetal blood supplies. Following on this theme, the efficiency of exchange in such a system is influenced by the proximity of the maternal and fetal capillaries, blood flow through each capillary, and the size of the interacting surface between the two blood supplies. In an interacting surface created by folds, the size of the interacting surface is controlled by the number of folds and the depth of each fold. Previous studies have indicated that the distance between the 2 capillaries is reduced to as little as 2 µm by the end of gestation. This is due to the invagination of both capillaries into their respective epithelial surfaces although the capillaries remain separated by both maternal and fetal epithelial cells and their respective basal laminas (Friess et al., 1980). Nothing is known regarding how this invagination of capillaries is accomplished. Blood flow is another factor that can influence transfer and, as previously indicated, maternal blood flow is responsive to litter size, but this response is limited. Very little is known regarding the control of blood flow through fetal placental capillaries of the pig. To our knowledge, the number of folds per length of the folded bilayer has never been assessed in the pig nor is information available regarding factors influencing the number of folds per unit of placental length. One might speculate that the number of folds is controlled by the number of maternal arterioles on the endometrial surface because the structure reported by Leiser and Dantzer (1988) indicates that maternal blood enters at the top of each fold. However, an alternative is that the vasculature develops in response to folding rather than folds being a result of the vasculature. Turning to the width of the folded bilayer, Vallet and Freking (2007) measured this width and reported that it was greater in placentas associated with small compared with large fetuses. These results indicate that increased width of the folded bilayer is a potential compensatory mechanism that occurred in the placenta of small fetuses.

If the width of the folded bilayer compensates for reduced placental size, what factors control the width of the folded bilayer? In the same report, Vallet and Freking (2007) measured the width of the placental stromal tissue above the folded bilayer, with the idea that this region represents an area for further expansion of the folded bilayer. Results indicated that the stromal layer was less in placenta of small fetuses, coincident with wider folds, and consistent with this concept. However, the placenta of the smallest fetus collected on d 105 of gestation had no remaining stroma above the folded bilayer, and this fetus was in clear distress. This suggested that the compensatory mechanism of wider folds was limited by the thickness of the fetal stroma above the folds. Exceeding this limit could provide an explanation for fetal losses due to intrauterine crowding that occur during late gestation (Freking et al., 2007). Given this scenario, two aspects of placental development that may impinge on placental efficiency include factors that 1) control fold development and 2) influence the amount of placental stroma.

As indicated previously, little is known regarding control of epithelial bilayer folding. Folds develop between d 25 and 45 of gestation (Friess et al., 1980). Once developed, examination of trophoblast cells within the folds indicates that cells at the top of the folds (oriented with fetal side of the placenta as “up”) are tall and columnar in appearance whereas those along the sides and bottom are cuboidal (Friess et al., 1980; Vallet and Freking, 2007). Therefore, it is possible that migration of either the tall columnar trophoblast cells upward into the stroma, or of the short cuboidal trophoblast cells downward, or both contribute to fold development. There is very little information available regarding the function of these 2 cell types that can be used to distinguish between these 2 possibilities. Friess et al. (1980) proposed that the tall columnar cells at the top of the folds participate in transport of macromolecules, but there is little supporting evidence of this being a specific function of these cells. This represents a key gap in knowledge regarding function of the pig placenta.

Much of the placental stroma is made up of extracellular matrix, primary components being hyaluronan and heparan sulfate (Steele and Froseth, 1980; Vallet et al., 2010). Enzymes participating in the degradation of these components are likely to be involved in fold development. The placenta contains both hyaluronidase (Vallet et al., 2010) and heparanase (Miles et al., 2009). Hyaluronidase activity is greater in the placenta of small fetuses compared with large fetuses, indicating greater turnover of placental hyaluronan, which is consistent with the greater fold development in placentas of small fetuses. However, hyaluronidase activity has not been localized to specific placental cells. In situ hybridization analysis of heparanase indicated that it is produced by the short cuboidal cells on the sides and troughs of the folded bilayer. This indicates that these cells participate in fold development by degrading the heparan sulfate in the matrix surrounding them (Miles et al., 2009). To generate further information regarding functions of these 2 cell types, each cell type was isolated using laser capture microdissection followed by comprehensive sequencing of the RNA present in each.
cell type (RNA-seq). The transcriptomes of the 2 cell types were compared to provide clues to their different functions. While analysis of data from this experiment is ongoing, Table 1 indicates the top 5 genes for which functions could be identified and that are most highly differentially expressed by the tall columnar and short cuboidal trophoblast cells. Interestingly, the top gene identified in short cuboidal cells was heparanase, which agrees with our previous work (Miles et al., 2009) and provides a validation of the laser capture microdissection method. The two greatest differentially expressed genes in tall columnar cells, the A disintegrin and metalloprotease domain 28 gene, and the transmembrane 4 L six family member 5 gene, are both known to participate in cell migration (Mochizuki and Okada, 2007; Lee et al., 2010), indicating that these cells also participate in fold development. However, full elucidation of the roles of these 2 cell types in the development of the folded epithelial bilayer will require a more complete analysis of the RNA-seq data generated and follow up experiments to test hypotheses suggested by the results.

Along with degradation, synthesis of components of the placental stroma also potentially contributes to increased placental efficiency as results indicate that the placental stroma limits fold development. As indicated previously, 2 primary components of the placental stroma are hyaluronan and heparan sulfate (Steele and Froseth, 1980; Vallet et al., 2010). Hyaluronan is composed of repeating disaccharide units of glucuronate and N-acetyl-glucosamine (Girish and Kemparaju, 2007). Heparan sulfate is composed of more or less sulfated forms of glucuronic acid, iduronic acid, and N-acetyl-glucosamine (Lopes et al., 2006; Esko and Lindahl, 2001). Therefore, major components of the placental stroma are essentially synthesized from forms of glucose. This makes glucose transport and the conversion of glucose into the oligosaccharide components of hyaluronan and heparan sulfate of potential interest.

Glucose transport across the pig placenta occurs down a concentration gradient and, therefore, the amount of transport is dependent on glucose concentrations in maternal and fetal blood (Randall and L’Ecuyer, 1976; Randall, 1977). The placentas of livestock species are also fructogenic, meaning that a substantial portion of the glucose that enters the fetal compartment is converted to fructose (Rama et al., 1973; White et al., 1979; Meznarich et al., 1987). Fructose is not transported back to the maternal blood, so converting glucose to fructose allows fructose to be sequestered within the fetal compartment and reduces the concentration of glucose in the fetal circulation (Huggett et al., 1951). This increases the glucose gradient between dam and fetus and facilitates glucose transport into the fetal circulation.

Because sugars such as glucose are polar, they do not diffuse through cell membranes and their transport is reliant on the existence of facilitated transporters. In pigs, both maternal and fetal epithelial cell layers remain intact throughout gestation. Therefore, transporters are required on both cell types in order for glucose to reach the fetal blood stream. Two types of glucose transporters are known, facilitated glucose transporters (GLUT) and Na dependent glucose transporters (GLNT). The two transporters differ with respect to whether the transport of glucose is passive (i.e., GLUT, down a concentration gradient) or active [i.e., GLNT, facilitated by sodium ion or other solute exchange (Olson and Pessin, 1996; Zhao and Keating, 2007)]. The reliance of glucose transport on a concentration gradient indicates that GLUT proteins are the primary transporters of glucose in livestock. The GLUT transporter family is large, with well over a dozen known members. The GLUT proteins differ in their affinities for various sugars, in their capacities for transport, and in their control by hormones such as insulin (Burant et al., 1992; Olson and Pessin, 1996; Colville et al., 1993; Uldry et al., 2002).

Very little is known regarding the concentration and localization of GLUT proteins within the pig placenta. Bazer et al. (2009, 2012) reported on localization of GLUT Solute carrier 2A1 (SLC2A1) through Solute carrier 2A4 (SLC2A4) mRNA by in situ hybridization.
The mRNA for GLUT 1 protein (SLC2A1) was present on both endometrial epithelial and trophoblast cells throughout gestation. The mRNA for GLUT 2 (SLC2A2) was found in trophoblast cells of the areolae (i.e., epithelial cell structures covering the openings of the uterine glands) and on endometrial epithelial cells located at the tops of the microscopic folds but nowhere in trophoblast cells of the folded bilayer. The mRNA for GLUT 3 (SLC2A3) was not found. The mRNA for GLUT 4 (SLC2A4), an insulin responsive GLUT, was localized to the endometrial epithelial cells until d 30 but not later and was not found on trophoblast cells. So minimally, GLUT 1 protein on the endometrial epithelial and trophoblast cells during gestation in the pig provide transport of glucose from the maternal to the fetal blood supplies of the pig. However, because so many other GLUT proteins exist, it will be necessary to assess them all before a complete picture of glucose transport across the pig placenta is available.

As previously mentioned, the pig placenta, like the placenta of other livestock species, is fructogenic (White et al., 1979). In fact, fructose concentrations in pig fetal plasma are 2 to 4 times greater than those of glucose (Randall and L’Ecuyer, 1976; Père, 1995). Paradoxically, studies have shown that fructose is poorly metabolized to CO₂ (Meznarich et al., 1987), and it has been concluded, therefore, that fructose does not represent a significant source of energy for the developing pig fetus. The fact that the placenta converts large amounts of glucose to fructose, only to have the fructose be unavailable to the fetus for energy, does not make immediate sense physiologically. This physiological paradox may be resolved in two ways. First, it is not necessary to convert fructose to CO₂ in order for the fetus to gain energy from it. Second, the use of fructose as an energy substrate is not the only possible use of fructose by the fetus.

Sugars are converted to CO₂ and water by aerobic metabolism, liberating energy in the form of ATP. However, anaerobic glycolysis also generates ATP and the end result of this process for mammalian cells is typically lactate. Curiously, along with increased fructose concentrations, lactate concentrations in fetal plasma are also very high (Père, 1995; Fowden et al., 1997). Lactate is also present in greater concentrations in umbilical venous compared with umbilical arterial blood, indicating that the placenta is a net producer of lactate and that the fetus is a net consumer of lactate. These relationships indicate that the placenta is engaging in substantial anaerobic metabolism. It seems possible that the high fructose concentrations in fetal fluids might be needed to support energy generation by anaerobic metabolism, but the use of fructose for lactate production by the placenta has not been studied. Also, anaerobic metabolism would be more likely to take place in tissues with poor capillary infusion. The placental capillaries are primarily located on either side of the epithelial cell bilayer (Leiser and Dantzer, 1988; Dantzer and Leiser, 1994), making the epithelial cells relatively well oxygenated, whereas the stromal tissue would be some distance away. If other placental capillaries exist that perfuse stromal tissue, they would be fed with relatively deoxygenated blood returning from the fetus (Kiserud, 2005). Taken together, this indicates that the stroma may exist in a relatively anaerobic environment that might rely on high concentrations of fructose. Further work is needed to define the source of lactate in placental tissue, the location of fructose specific transporters, and the role of anaerobic metabolism in placental physiology.

Alternatively, fructose need not be used solely for production of energy. White et al. (1982) reported incorporation of fructose carbon into nucleic acids, which explains previous reports of localization of fructose carbon to nuclei of cells (Huggett and Pelc, 1964). Also, glucosamine is produced from fructose via the hexosamine pathway, which also uses glutamine as the source of the amine group (Buse, 2006), and glucosamine represents one-half the sugar molecules making up both hyaluronan and heparan sulfate (Moussian, 2008). Because glucosamine contributes to glycosaminoglycan synthesis, high fructose concentrations could provide glucosamine substrate for adequate synthesis of hyaluronan, heparan sulfate, and other stromal glycosaminoglycans that are required for development of stromal tissue.

Interestingly, the hexosamine pathway also regulates cell proliferation by activation of the mammalian target of rapamycin (mTOR) pathway (Wen et al., 2005). This was subsequently confirmed using d 12 pig trophoblast cells (Kim et al., 2012). In addition, fragments of both hyaluronan and heparan sulfate are known to be angiogenic (West et al., 1985; Ilan et al., 2006; Jakobsson et al., 2006), indicating that turnover of these glycosaminoglycans could play a role in the development of capillaries on either side of the epithelial bilayer. Therefore, glucose passage from maternal blood through the endometrial epithelial cell and into the trophoblast followed by conversion of fructose to glucose by placental trophoblast cells may regulate numerous aspects of placental function and development.

**SUMMARY AND CONCLUSIONS**

In summary, conceptus and uterine factors contribute to fetal losses and these can occur throughout the life of the animal. Uterine factors such as length, uterine gland development, uterine gland function, and uterine blood flow can all contribute to fetal losses. Failure of conceptus migration and elongation and variation in...
conceptus migration and elongation contribute to fetal losses by reducing access to uterine space and therefore access to uterine resources. During later pregnancy, compensatory mechanisms exist within the placenta and fetus that can partially remediate lack of uterine space and reduced placental size, but the compensatory ability of the placenta is limited. One compensatory mechanism is likely to be increased folding of the maternal–fetal interface within the placenta, which is limited by the thickness of the stromal tissue in which the folded interface is embedded. Glycosaminoglycans are primary components of the placental stroma, and these are composed almost entirely of forms of glucose. Glucose is transported from the maternal to the fetal blood supply, mediated by GLUT 1 and potentially other GLUT proteins. Substantial amounts of glucose are converted to fructose by the placenta. Fructose can be used for glucosamine synthesis for the production of hyaluronan and other glycosaminoglycans, which would contribute to stromal extracellular matrix. Placental GLUT proteins that are specific to fructose may provide a key regulatory element for fructose metabolism in the fetal compartment. Current results also indicate that both tall columnar and short cuboidal trophoblast epithelial cells interact with the stromal matrix and also contribute to folding of the epithelial bilayer. The liberation of glycosaminoglycan fragments by hyaluronidase and heparanase during remodeling of the placental matrix during fold development contribute to angiogenesis of the placenta. This scenario suggests a central role for fructose in development of the pig placenta. Moreover, because placentas of other livestock species are also fructogenic, knowledge gained regarding the role of fructose in placental and fetal development will provide information that could improve reproductive outcomes for sheep and cattle.

**LITERATURE CITED**


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