Early embryonic survival in the pig: Can it be improved?

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Early embryonic survival in the pig: Can it be improved?[1]

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ABSTRACT: A major limitation for increasing litter size in swine is embryonic loss that occurs during the 2nd to 3rd wk of gestation. High ovulation rates of modern sows have more than supplied the potential number of embryos necessary to improve litter size. The current challenge is determining how early conceptus development affects the ability to maintain the viability through the remaining 90 d of gestation to maximize farrowing house production. To achieve this, it is necessary to identify and understand the possible causes of embryonic death. Because fertilization rates are generally high in swine, early embryonic loss during the first 20 d of gestation is considered to critically effect potential litter size. There are three periods during which early embryonic loss can occur: 1) pre-elongation development, 2) trophoblastic elongation, and 3) placental attachment. The first two periods are related to time of fertilization and subsequent developmental rate for each individual embryo within the litter. Asynchrony in embryonic development relative to uterine development can result in loss of embryos before d 10 of gestation. Competitive acquisition of adequate uterine space between littermate embryos, essential for blood flow delivery of nutrients needed for survival to term, is established during conceptus elongation on d 12 of gestation. Progressive changes in the uterine microenvironment between d 10 and 16 of gestation play a major role in embryonic survival following trophoblast elongation and placental attachment. In current production systems, there can still be sufficient numbers of embryos present after d 30 of gestation to provide improvement in average litter size at farrowing. However, producers are still faced with the challenge of maximizing fetal survival to term. Therefore, fully understanding the biological controls of follicle ovulation rate, synchrony of ovulation, embryonic developmental rate, uniformity of conceptus elongation, uterine horn capacity, uterine glandular and vascular development, and placental vascularization could provide possible clues to improving embryo quality.

Key Words: Embryo, Pigs, Pregnancy, Uterus

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Introduction

Improving litter size would have a substantial impact on the efficiency of swine production; Tomes and Nielson (1982) and Rothschild (1996) have indicated that overall profitability increased as the number of pigs per sow per year increased. Although it has long been recognized that increasing litter size would increase the efficiency of production, significant improvements have not been achieved. There is potential for average litter sizes of 14 or more pigs if all ovulated ova develop into live pigs, but the average number of pigs born per litter in the United States is only 10.5 (USDA, 2001); approximately 30 to 50% of the ova released from the ovary do not survive through gestation (Pope, 1994). Fertilization of ovulated swine ova is generally greater than 95%; therefore, the loss of potential piglets is predominately the result of early embryonic (d 10 to 30) and fetal (d 31 to 70) deaths. A previous review by Pope (1994) indicated that although ovulation rate establishes potential piglet number, an increase in ovulation rate by itself would not result in a marked improvement in litter size. Ovulation rate responds to direct selection in swine, but the returns for improving litter size have been minimal (Cunningham et al., 1979; Lamberson et al., 1991). Eleven generations of selection for uterine capacity using the unilateral-hysterectomy-ovariectomy (**UHOX**) model has also failed to significantly improve overall litter size of gilts (Christenson and Leymaster, 2000). However, index selection for ovulation rate and embryonic survival, designed to maximize response in litter size, has been effective in increasing litter size after 14 generations of selection (Johnson et al., 1999). Therefore, future improvements in litter size may be realized if we gain a clearer understanding

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of the balance of follicular maturation and timing of ovulation, as well as embryonic, uterine, and placental factors associated with conceptus development and survival.

Day 10 to 30 Is a Critical Stage in Conceptus Development

Establishment and maintenance of pregnancy involves a number of closely integrated signals between the ovary, uterus, and conceptus in most mammalian species (Bazer et al., 1982; Roberts et al., 1993). Alterations in the synchronous development of the uterine environment with the growing and differentiating conceptus during early pregnancy can result in a failure to establish pregnancy (Geisert and Yelich, 1997). In monotocous domestic species, failure of the embryo to develop and survive results in termination of the pregnancy, but in the polytocous pig, failure of individual embryo survival within the uterus is a norm that does not necessarily affect continuation of the pregnancy. However, total loss of conceptuses early in development would result in a failure to maintain functional corpora lutea beyond d 15 of gestation (Bazer et al., 1984).

The majority of embryonic mortality usually occurs prior to d 20 to 30 of gestation (Pope, 1994). Data for the gilt, excluding females in which total fertilization failure occurs, have indicated that little embryonic loss takes place before 7 d after the onset of estrus (Polge, 1982). Estimates suggesting that the majority of embryonic mortality occurs between d 10 and 30 of gestation are not surprising given the critical events that occur during this period. The reasons for which early embryonic mortality occurs can be quite extensive and complex (see Dzuik, 1987). Improper timing of conceptus and uterine development, which involves synthesis of nutrients and attachment factors, failure of conceptus signaling, competition of embryos (uterine crowding), and genetic factors all contribute to early conceptus loss. The following review will attempt to bring together the current information available on factors that are proposed to be involved with conceptus development, factors leading to embryonic mortality, and the many approaches taken to improve embryonic survival in the pig.

Conceptus Development

To understand the cause for loss of apparently healthy embryos during the first third of pregnancy, one first needs to understand how pig conceptuses interact with the uterine environment to modulate endometrial function and placental attachment for nutrient exchange from the mother (reviewed by Stroband and Van der Lende, 1990). In the pig, conceptus synthesis and release of estrogen on d 11 to 12 and 15 to 20 of gestation provides the signal to maintain luteal production of progesterone throughout gestation (Geisert et al., 1990). In order to deliver estrogen throughout the majority of the uterine surface, the perimplantation embryos migrate and space themselves throughout both uterine horns between d 8 to 11 of gestation (Pope et al., 1982a; Dzuik, 1985). Following migration within and between the uterine horns, conceptuses undergo a rapid differentiation and expansion of their trophoblastic membranes between d 11 and 12 of gestation (Anderson, 1978; Geisert et al., 1982a,b). Conceptuses change in diameter and morphology as they develop from a 1- to 2-mm sphere to a 3- to 8-mm ovoid and a 9- to 20-mm tubular shape on d 10 to 11 (Geisert et al., 1982b). Once reaching approximately 10 mm in diameter, conceptuses rapidly elongate (2 to 3 h) to a thin, filamentous (> 100 mm) form and initiate attachment to the uterine surface epithelium (Dantzer, 1985; Blair et al., 1991; Burghardt et al., 1997). Rapid expansion of the trophoblast, which is linked to an increase in estrogen synthesis and release, is followed by a continuation of placental growth (Ka et al., 2001), and the trophoblast can exceed 1 m in length by d 16 of gestation (Perry and Rowlands, 1962). Expansion of the conceptus throughout the uterus of the pig is essential, because early pregnancy is not maintained if a substantial portion of each horn is not occupied by conceptuses (see Dzuik, 1987; Geisert et al., 1990). The morphological change in the elongating conceptus is not the result of cellular hyperplasia, but rather of cellular remodeling (Geisert et al., 1982a; Mattson et al., 1990; Pusateri et al., 1990).

Rapid transition in conceptus morphology followed by initiation of placental attachment to the uterine surface represents one of the periods of greatest embryonic loss in the pig (Pope and First, 1985). Conceptus secretion of estrogen and development from a spherical to filamentous morphology occur concurrently with the period of pregnancy establishment in the gilt (see Geisert et al., 1990) and are temporally related to changes in endometrial estrogen (Geisert et al., 1993) and progesterone (Geisert et al., 1994) receptors. The decline in uterine epithelial progesterone receptors on d 12 of pregnancy and the presence of epithelial estrogen receptors in the uterine endometrium during d 10 to 18 relates to the effects of estrogen on the uterine epithelial glycocalyx, conceptus attachment, and alterations in endometrial secretory products (Dantzer, 1985; Blair et al., 1993; Geisert and Yelich, 1997; Ka et al., 2000). Following conceptus elongation and the initiation of estrogen release, trophoblastic attachment to the epithelial lining of the endometrium occurs between d 13 and 18 of gestation (King et al., 1982; Burghardt et al., 1997). The embryo develops from the inner cell mass and the allantoic membrane expands to form the placental vascular network for transport of nutrients and fluid into the allantoic cavity (Bazer et al., 1981; King et al., 1982). Physiological events during early pregnancy are controlled by ovarian progesterone secretion, conceptus estrogen synthesis, and the cellular localization of endometrial steroid receptors (see Geisert and Yelich, 1997; Burghardt et al., 1997).

It is evident that three events, rapid trophoblastic elongation, conceptus estrogen synthesis, and placental attachment to the uterine epithelial surface, are critical for early porcine embryonic survival. Early trophoblast expansion most likely regulates and limits the final size of each embryo's placental surface area throughout gestation. The synthesis and release of growth factors and cytokines by the developing conceptus during the preimplantation period of embryonic development are considered to play potentially critical roles in cellular growth, differentiation, and remodeling of the embryo and extraembryonic tissues. Both embryonic and endometrial production of various growth factors, as well as interactions between the maternal endometrium and developing conceptus, are essential for providing the synergistic environment for endocrinological and immunological signals for establishment of pregnancy (see review by Geisert and Yelich, 1997; Ka et al., 2000). Developmental events that influence embryonic differentiation and growth are most likely regulated through the programmed expression of growth factors and their specific receptors. Both the conceptuses and endometrium must actively participate in balancing the presence of many proteolytic enzymes and their inhibitors to mediate the epitheliochorial type of placental attachment and growth in the pig (see Geisert and Yelich, 1997).

The previous information provides a base to understand the many temporal interactions that occur between the conceptuses and the uterine environment during establishment of pregnancy and placental attachment. The following sections will now try to provide some insight into the cause(s) of early conceptus loss and attempts to alleviate mortality.

Litter Embryonic Diversity

One of the easiest times to observe embryonic developmental diversity within individual litters is to recover embryos from the uterus between d 11 and 12 of pregnancy. Perry and Rowlands (1962) and Anderson (1978) demonstrated the size and morphological differences that can occur within a litter prior to and during the period of trophoblastic elongation. Geisert et al. (1982b) indicated that the conceptuses initiate elongation upon reaching an approximate spherical diameter of 10 mm on d 11 to 12. However, although these conceptuses can elongate, littermates that are 7 to 8 mm or smaller may not elongate for at least another 4 to 8 h. This transitional lapse in development can obviously create a problem in gaining appropriate uterine territory for survival of the less-developed embryos to term if uterine space is limiting. The classic studies of William Pope clearly illustrated that competition between embryos can occur within the uterus. Through embryo transfer, it was demonstrated that asynchrony in development of healthy porcine conceptuses leads to a competitive advantage of the more developmentally advanced littermates (Pope et al., 1982b, 1986b). Wilde et al. (1988) also confirmed that less-developed embryos within a litter are just as capable of surviving when not placed in competition with more-developed littermates.

Possible reasons for the diversity of conceptus development within a litter have been suggested. Considerable information has indicated that the *Ped* (preimplantation embryo development) gene influences the time to cleavage and thus the rate of early development in mice (Warner et al., 1998; Wu et al., 1999). The *Ped* gene product is the major histocompatibility complex (**MHC**) class Ib, Qa-2 protein that is expressed on the cell surface, and it seems to function through a cross-linking mechanism to influence rate of cleavage in early mouse embryo development (McElhinny and Warner, 2000; McElhinny et al., 2000). The lack of inbred lines to discern the presence of a similar relationship in the swine leucocyte antigen (**SLA**) MHC has limited our knowledge of the Ped gene in pigs. There has been some indication for haplotype differences in ovulation rate and number of embryos in inbred miniature pigs (Conley et al., 1988), but its effect on embryo development has not been established. A search for the human homologue to the *Ped* gene is currently underway (Warner et al., 1998); if it is identified this may assist in locating the *Ped* gene in swine.

Although the data for pigs are extremely limited, we must also not overlook the possible paternal contributions to early conceptus development (Dzuik, 1987). The fertilizing sperm can influence variation in fertility; Eid et al. (1994) indicated that the rate and time of entry of the oocyte into the S-phase of the cell cycle is different with sperm from high- vs low-fertility bulls. The transition from maternal to embryonic genome regulation of cell function occurs at the four-cell stage of development in the pig (Prather, 1993). The classic experiments of Surani et al. (1986) first demonstrated the critical function of both the maternal and paternal genome in modulating normal embryonic and placental growth in mice. Genomic imprinting (see John and Surani, 2000) has been demonstrated to have major effects on placental development and fetal survival in mice (Georgiades et al., 2001). Insulin-like growth factor-2 and insulin-like growth factor-2 receptor are two of the genes proposed to be imprinted during early embryonic development (see Moore and Reik, 1996). The presence of insulin-like growth factors in the porcine uterine lumen and insulin-like growth factor receptors in the developing conceptus (Geisert and Yelich, 1997) certainly suggest that if imprinting occurs similarly to that in the mouse, the paternal genome could contribute to the rate of conceptus growth and differentiation in the pig.

Asynchronous oocyte maturation and variation in time of ovulation for a cohort of developing antral follicles have been linked to the diversity of embryos within a litter (Pope et al., 1990; Pope, 1994). Utilizing transrectal utlrasonography to evaluate time of ovulation in pigs, Soede et al. (1992) determined that ovulation of follicles occurred within 1 to 4 h and was not related to embryo diversity. However, a number of stud-

ies have reported that the duration of ovulation can vary from 1 to 9 h between individual animals (see Pope, 1994). Although the majority of follicles ovulate over a short period of time, a few (one to four) ovulate over a more protracted period. Pope et al. (1988) demonstrated that elimination of the late-ovulating follicles on d 1 of the estrous cycle removed the diversity in embryo development at d 11 of gestation. Furthermore, Xie et al. (1990a,b) demonstrated that the later-ovulating follicles become the less-developed embryos in the litter. These later-ovulating oocytes clearly contribute to the pool of embryos that are less-developed on d 11 of gestation and, therefore, maybe the first to be eliminated from the litter when uterine space is limited. Whether it is time of fertilization or genes that control cleavage rate of the embryos, some major and minor differences in embryo development within a litter do exist.

How Can Embryonic Diversity Determine Survival?

It is clear that embryonic diversity can affect survival of littermate embryos (Wilmut et al., 1985; Pope, 1994). When does selection of embryos begin? There are two periods of uterine selectivity during early embryonic development in the pig. The first period of embryonic loss (d 5 to 10) is not related to competition between embryos but to individual embryo asynchrony with its uterine environment. A portion of early selection could be attributed to meiotically immature or genetically abnormal ovulated oocytes that fail to develop at all or at a normal rate in pace with uterine development. Indeed, Hunter (2000) recently reviewed the factors involved in oocyte maturation and follicle heterogeneity, which if suboptimal could compromise subsequent embryo development. Koenig and Stormshak (1993) indicated that immature and chromosomally abnormal ova may represent as many as 30 to 40% of the oocytes ovulated in gilts, and this could account for the lower embryo survival in gilts bred on first vs third estrus. These data suggest that the quality of the oocytes ovulated may play an important role in subsequent development and survival. Asynchronous embryo transfer studies have indicated that porcine embryos become highly sensitive to the changing uterine environment during the blastocyst stage of development. The uterine environment seems to support various stages of development up to d 6 to 7 of gestation. However, Polge (1982) demonstrated that pregnancy rates decrease with transfer of embryos 24 h behind in development with the recipient uterus and decrease to nearly zero if they are 48 h behind. Rapid embryo deterioration within 24 h occured in d-6 embryos transferred to a d-8 uterine environment (Geisert et al., 1991). Thus, if embryos are greatly behind in development, the uterine environment established through corpus luteum progesterone stimulation can have a negative influence on survival.

The second period of uterine selectivity of embryonic survival occurs with conceptus elongation and estrogen synthesis on d 11 to 12 of pregnancy. Vallet et al. (1998) documented that changes in uterine secretion on d 11 to 12 of pregnancy are related to progressive alterations in protein secretion induced by progesterone rather than being an event completely triggered by the conceptus. Precise timing for the changes in uterine secretion most likely occurs with progesterone-induced down-regulation of uterine epithelial progesterone receptors on d 10 of the estrous cycle and pregnancy (Geisert et al., 1994). Loss of progesterone receptor is specific to the endometrial surface and glandular epithelium, because receptor levels are maintained in the stroma. The elegant study of Cunha et al. (1983) first demonstrated the interactions between developing epithelium and mesenchyme. It is clear that epithelial-mesenchyme interactions are involved with hormonal responsiveness of the uterus (Cooke et al., 1998; Kurita et al., 1998). Many effects of progesterone on uterine epithelial function can be attributed to progesterone activation of stromal progesterone receptors and the stimulated release of progestamedins such as keratinocyte growth factor (**KGF**) (Ka et al., 2000). A switch from epithelial progesterone regulation to stromal control would not only allow an alteration in uterine protein and enzyme release into the uterine lumen, but also induce changes in epithelial apical glycocalyx permissive to placental attachment (Dantzer, 1985; Burghardt et al., 1997; Geisert and Yelich, 1997). Uterine release of tissue kallikrein, a serine protease, on d 12 of the estrous cycle in the pig may function to alter the uterine epithelial glycocalyx for attachment (Vonnahme et al., 1999) and activate many growth factors for conceptus growth (Geisert et al., 2001). These studies indicate that the uterus dictates the period of rapid conceptus growth and attachment following hatching from the zona pellucida. However, there are also select changes in uterine release of ions, growth factors, and enzymes that are stimulated by the elongating conceptus (Geisert and Yelich, 1997; Ka et al., 2000). Several studies have indicated that estrogen from the tubular and elongating conceptuses can alter the uterine environment, which may influence the ability of lesser-developed littermates to either elongate or obtain sufficient uterine space to survive (Pope et al., 1990; Xie et al., 1990b; Geisert et al., 1991). Estrogen release from elongating conceptuses may induce a uterine secretory environment that is not conducive to later elongation of less-developed littermates, resulting in their degeneration, as has been proposed by Pope et al. (1990). Certainly, the uterine environment is changed with the synthesis of estrogen and elongation of the conceptuses throughout the uterine horn (see Geisert and Yelich, 1997). Although this seems to be a very plausible hypothesis, a degree of caution must be taken because data have indicated that administration of estrogen to pregnant gilts prior to conceptus elongation does not interfere with development on d 11 and 12 of pregnancy (Morgan

et al., 1987a,b; Geisert et al., 1991; Cardenas et al., 1997). However, administration of estrogen prior to conceptus elongation does act as an endocrine disruptor and affects subsequent survival of embryos after d 16 of pregnancy (Long and Diekman, 1986; Pope et al., 1986b; Morgan et al., 1987a). The loss of embryos seems to be an effect of estrogen on the uterine environment and epithelial glycocalyx rather than a direct effect on the conceptus (Blair et al., 1991); estrogen treatment on d 12 of pregnancy does not interfere with embryo survival (Geisert et al., 1991).

Although uniformity in embryonic development is an attractive model, it does not seem to be the sole basis for increased prolificacy of the Chinese Meishan pig (Wilmut et al., 1992; Ford, 1997). The advantage of the Meishan embryos in litter size seems to be achieved through modulating placental length that can be generated following the initial elongation of the conceptuses coupled with an increase in placental vascularity (see Ford, 1997; Biensen et al., 1999). Meishan embryos have similar numbers of inner cell mass cells but contain fewer trophectoderm cells and produce smaller amounts of estrogen compared to contemporary Yorkshire embryos (Anderson et al., 1993; Rivera et al., 1996; Kaminski et al., 1997). These two key factors would effectively restrict the surface area occupied by each conceptus following expansion of the trophoblast and reduce the effects of estrogen on neighboring littermates. The high concentrations of estrogen from the developing Yorkshire gilts is not necessary to support development; a 57% reduction in conceptus estrogen synthesis with aromatase inhibitors did not affect early conceptus development (O'Neill et al., 1991). So, does the lower estrogen synthesis by the developing conceptuses lead to greater survival? Although exogenous estrogen seems to have an obvious effect on embryo survival, there is a question as to whether even the higher amounts of estrogen produced by Yorkshire conceptuses can have more than a local effect on the uterine microenvironment that it contacts.

The inability of the sow to maintain a unilateral pregnancy early in pregnancy (Anderson, 1966) and the need for at least two embryos in each uterine horn to establish pregnancy in the pig (Polge et al., 1966) suggests that estrogen does not diffuse throughout the uterine horn easily. The presence of sulphotransferase in the porcine endometrium effectively conjugates and inactivates estrogens moving through the endometrium (Pack and Brooks, 1974). Thus, although effects of estrogen cannot be discounted, the Meishan embryos' slowed rate of development (Youngs et al., 1993) and uterine inhibitory effect on embryonic development (Youngs et al., 1994) have provided possible clues for survival through restriction of conceptus elongation and development of a more vascular placenta, as proposed by Ford (1997). Determination of how the decrease in trophectoderm cell number is regulated either in the conceptus or through uterine secretion needs to be evaluated. On d 10 to 12 of gestation, the pig uterus is a rich source for growth factors such as insulin-like growth factor (IGF)-I and -II, epidermal growth factor, leukemia inhibitory factor, KGF, and connective tissue growth factor (see Geisert and Yelich, 1997; Ball et al., 1998; Ka et al., 2000), most of for which their ligand receptor is expressed in the developing pig conceptus (Geisert and Yelich, 1997; Ka et al., 2001). The reported decrease in uterine protein secretion in Meishan compared to commercial pigs (Youngs et al., 1994; Vallet et al., 1998) would support a uterine role in modulating conceptus growth. However, the reciprocal conceptus transfer study of Youngs et al. (1994) indicated that, in addition to the uterine environment, genotype of the conceptus is also involved with preimplantation growth, which links back to the previous conceptus *Ped* gene discussion. Increasing our understanding of the factors modulating conceptus trophoblast elongation and placental angiogenesis following placental attachment to the uterine surface will become increasingly important for future attempts to improve competition among embryos within the pig uterus.

Progress has been made in determining changes in gene expression during the process of conceptus elongation (Yelich et al., 1997a,b; Wilson et al., 2000); however, the key(s) to triggering the process or understanding the pathway is far from established at this time. Recent studies have suggested the presence of a positive feedback loop for conceptus growth (Green et al., 1995); the conceptus seems to stimulate endometrial release of IGF and KGF into the uterine lumen during the developmental period preceding conceptus elongation (Geisert et al., 2001; Ka et al., 2001). Treatment of gilts with estrogen near the time of trophoblast elongation increases uterine protein and can advance the initiation of the cellular remodeling process (Cardenas et al., 1997). Ka et al. (2001) have demonstrated that conceptus estrogen release during pregnancy increases uterine epithelial KGF expression, which in turn stimulates proliferation and differentiation of conceptus trophectoderm. The known role of KGF in stimulating epithelial proliferation, migration, and cellular differentiation (Rubin et al., 1995) makes the interactions between IGF, estrogen, and KGF an attractive model for conceptus growth and remodeling. Interrelationships between these factors can explain conceptus growth regulation by the uterus and the conceptus itself, as previously discussed. These data would also lead to the suggestion that the more developmentally advanced, estrogenic conceptuses gain an even more competitive advantage in enhancing trophectoderm growth.

Is Embryonic Uniformity the Model for Improving Litter Size?

Given all the information on conceptus growth and development, one must ask whether conceptus uniformity is an important factor contributing to litter size in commercial swine herds. Theoretically, in the perfect pregnancy, embryo uniformity would be the most desir-

able situation for the pig. This would require that the number of ovulations did not exceed uterine capacity, all oocytes ovulated were at the same maturity and viability, fertilized synchronously, and had the same genetic potential for rate of development, and equidistant uterine spacing occurred. Asynchrony with the uterine environment would not be a problem because the time of ovulation regulates when the initial increase in luteal progesterone secretion occurs to stimulate the uterine secretory program for conceptus development. In this paradigm, ovulation rate should not exceed uterine capacity because this could in theory lead to uterine crowding and loss of the entire litter if no mechanism for having a competitive advantage between embryos existed. It is difficult enough to control any one of the aforementioned factors, which explains our inability to rapidly improve litter size. With this thought in mind, it would be remiss not to acknowledge the thoughtful and insightful paper Dzuik (1987) published 14 yr ago addressing the same question. Dzuik (1987) championed that asynchrony in conceptus development is actually a survival advantage in the pig and that we may pay too much attention to embryonic loss rather than focusing attention on why some embryos survive. A limited amount and degree of nonuniformity between groups of developing porcine embryos is actually an attractive model. When ovulation rate does not exceed uterine capacity, the majority of embryos that are developmentally competent to survive through d 8 will elongate despite some variation in development rate. Even if these embryos are somewhat variable in development, they should have less difficulty in acquiring sufficient uterine space for placental development. The number of conceptuses present on d 12, not conceptus uniformity, directly affects litter size in this female. If ovulation rate exceeds uterine capacity in a female, then there will be a number of possible scenarios for the potential outcome in litter size. There could be greater early (before d 8) loss of embryos through ovulation of immature and(or) defective oocytes and late-ovulating follicles that places a number of developing embryos in jeopardy of being asynchronous with the uterine environment. Loss of these early embryos could normalize the space available for the surviving conceptuses depending on the number developing to d 12 and set in motion the competition related to rate of growth and elongation. This female would be limited by both uterine capacity and the number of conceptuses initiating elongation, which makes for a variable range in possible outcomes with litter size. For example, if 18 viable embryos were present on d 12 and they all elongated at the same time, crowding would cause fetal survival problems after d 30 of gestation, depending on the individual placental surface area providing nutrients for each fetus. If 10 to 12 embryos elongated first, they would have space available to develop but could restrict lesser-developed littermates from either elongating or having sufficient placental space to develop to term. If a ligation is placed 15 cm from the tip of the uterine horn following fertilization, the restricted conceptuses reach the tubular stage of development but become deformed and do not elongate (J. P. Harney and F. W. Bazer, personnel communication). Thus, during the process of trophoblast elongation, some conceptuses that are lodged between two elongated embryos most likely will not undergo elongation and subsequently become deformed in development. Wu et al. (1988) indicated that an embryo requires more than 20 cm of uterine horn length to survive after the 7th wk of gestation. Therefore, although there will be viable embryos that develop to d 30 of gestation, they may not have sufficient placental surface area to survive to term.

So how are consistent large litter sizes achieved? Because some embryonic loss is inevitable, the ovulation rate needs to be high enough to overcome the loss of early defective embryos but provide a developing cohort of 15 to 16 embryos that can elongate relatively synchronously. Total synchrony of ovulation is not important as long as cohorts of 15 to 16 quality oocytes are present at fertilization (see Pope et al., 1990). The majority of the three to four lesser-developed embryos would either be eliminated (fail to elongate) or, depending on individual positioning of embryos within the horn, fill in space if available. Positioning of conceptuses in relation to one another within the uterus is still an important issue to survival (Dzuik, 1987). Embryo survival in this female would depend on the ovulation rate, number and quality of embryos developing in the uniform cohort, and the uterine capacity of the dam, which leads back to the traits of Meishan embryos. What are the traits of the embryos from more contemporary sows developed with the index selection for ovulation rate and embryonic survival (Johnson et al., 1999) that have increased litter size? Is there balance in ovulation of a cohort of quality embryos and(or) increase in placental efficiency? Although a larger, more complete study is needed, Wilson et al. (1999) suggested that selection based on placental size and efficiency could improve litter size at term. If placental size and vascularity are the important modulators of improved efficiency, research is needed to determine how to effectively detect sows with increased placental efficiency or determine methods to regulate conceptus and uterine development in pigs.

Attempts to Improve Embryonic Survival

The multitude of studies attempting to improve litter size through selective time of breeding, regulation of ovulation, feed intake, steroid therapy, and nutritional supplementation have been previously reviewed (Christenson, 1986; Dzuik, 1987; Ashworth, 1994; Pope, 1994; Foxcroft, 1997). For the most part, studies evaluating nutritional supplementation have suffered from the lack of sufficient animal numbers and have failed to either clearly demonstrate improved fertility or have had no effect (Foxcroft, 1997). Certainly the presence and important role of uterine factors such as riboflavin,

uteroferrin, retinol-binding protein, and folate-binding protein (Malathy et al., 1990; Vallet et al., 1996, 1999) fueled the numerous nutritional supplementation trials attempting to increase embryonic survival (see Dzuik, 1987; Ashworth, 1994; Foxcroft, 1997). Given that the uterine environment of pigs on a nutritionally balanced diet is not significantly altered by additional nutrients or vitamins and that it is difficult to affect conceptus developmental rate, it is not surprising that studies have failed to demonstrate consistent, if any, beneficial effects. Indeed, embryo survival is not significantly affected in gilts subjected to inanition for the first 40 d of gestation (Anderson, 1975). Problems in growth, survival, and maintenance of pregnancy occur if the period of inanition is increased to greater than 40 d, but pregnancy is maintained if the females are supplemented with daily treatments of progesterone and estrogen.

Improvements in embryo survival have been noted when gilts are treated with retinyl-palmitate prior to the time of ovulation (Coffey and Britt, 1993; Whaley et al., 1997). An increase in embryo survival was attributed to advancing resumption of meiosis and improving embryo quality rather than to any direct effect through altering uterine function (Whaley et al., 2000). Feeding above maintenance requirement before mating also improved blastocyst cell numbers and reduced size variability within litters (Ashworth et al., 1999). Cardenas et al. (1997) indicated that treatment of gilts with testosterone from d 13 of the estrous cycle until the following estrus improved blastocyst survival, possibly through improved oocyte quality. These data are consistent with the previous report of Hunter et al. (1993), who indicated that oocytes in Meishan gilts are more mature and uniform than Large-White gilts. Thus, information points to the importance of increasing the number of developmentally mature oocytes before we even progress to the uterine environmental modulation of conceptus development.

So what can be done to improve the interaction between conceptus and uterine environment? Obviously, understanding how placental length and vascularity are modulated will help direct us to studies that may alter early development of the conceptus. Development of differential-display PCR, PCR-based cDNA subtraction, and the forthcoming production of microarrays will accelerate identification of genes involved with conceptus and uterine development. Information concerning genes involved with conceptus and uterine development may improve our search for and use of genetic markers for reproductive efficiency as the pig genome map is completed (Rothschild et al., 1997; Linville et al., 2001). Technologies in transgenesis and cloning are attractive methods to develop increased reproductive efficiency in swine but have been rather difficult to develop, and the low heritability of litter size will still not provide the rapid increase that most might expect (Haley et al., 1988).

It should seem obvious that if we want more uterine space, we need to select for pigs with longer horns. Uterine length seems to be a limiting factor to litter size when ovulation rate increases (Wu et al., 1987). However, although variation in uterine horn length exists, a difference between commercial breeds and Meishan pigs is not apparent (see Ford, 1997). Gama and Johnson (1993) also indicated that there was no significant change in uterine dimensions of cyclic gilts following eight generations of selection for litter size. If the data do not indicate variation in length of the uterine horns as a major factor in embryo survival, what uterine parameter(s) would affect placental surface area necessary for embryo survival? The level of endometrial folding, number of uterine glands, and uterine capillary bed density within the endometrium are likely candidates that cannot be easily evaluated in the live animal. Porcine endometrium is not a flat, smooth surface but contains many macroscopic folds that branch off into numerous primary and secondary ridges to increase surface for the placenta (Bjorkman et al., 1981; Dantzer, 1984, 1985; Keys and King, 1990). Density of endometrial folding cannot be accounted for by only measuring horn length because this folding, along with uterine growth, provides for the expansion of the uterus after d 18 of gestation (Wu et al., 1988). A key component for uterine survival in all species, especially true for epitheliochorial-type placentation in the pig, is nutrient passage to the placenta established through capillary blood flow within the endometrium (Ford, 1995). Alteration of blood flow to the uterus is evident at the time of conceptus elongation and during expansion of the allantois to fill the uterine horn between d 15 and 30 of gestation (Ford, 1995). The conceptus induces an inflammatory response to enhance nutrient flow into the uterine lumen (Keys and King, 1988, 1995). The presence of kinin β2 receptors in the endometrium and the conceptus-induced release of kinins (Allen et al., 2002), potent regulators of blood pressure, indicates the active participation of the conceptus in modulating maternal blood flow to the porcine placenta. Although from a different species and type of placentation, vascular casts of the uterus and maternal cotyledons (Figure 1) in Florida native ewes compared to the more prolific Blackfaced ewes (two to three lambs) illustrates the alteration in vascular density that can be developed to support multiple fetuses in the prolific breed of sheep (F. W. Bazer, F. F. Bartol, and D. H. Barron, unpublished data). The vascular networks of the maternal uterus and fetal placenta in the pig are complementary to one another and become more complex as gestation proceeds (Lesier and Danzter, 1988). Many angiogenic factors are involved with regulating endometrial and placental vascular growth (see Reynolds and Redmer, 1995). However, we currently do not have a clear understanding of the regulation of angiogenesis during pregnancy in the pig.

The superficial attachment and placentation of the pig dictates the critical need for endometrial generation

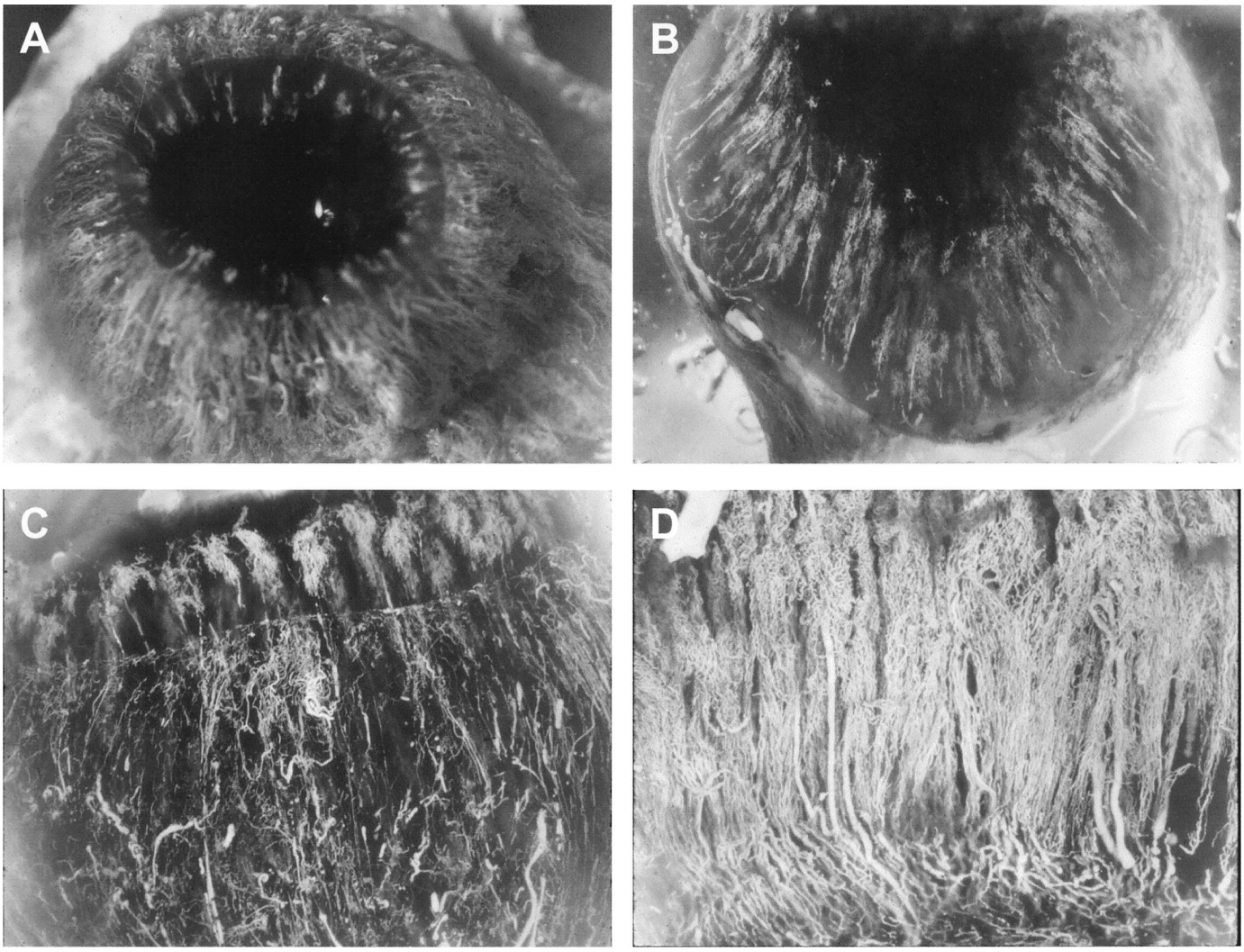

Figure 1. Vascular casts of the maternal cotyledons from a single-lamb-bearing Florida native ewe (B, C) and a prolific, multilamb-bearing Blackface ewe (A, D).

of uterine secretions. The importance of glandular support for the developing porcine conceptus has been made even more evident by the failure of early ovine conceptuses to elongate and attach in uterine gland knockout ewes (Gray et al., 2001a,b). Prolonged neonatal exposure of ewes to progesterone ablates uterine gland morphogenesis and absence of glands in the adult (Bartol et al., 1999). Thus, it is clear that neonatal exposure to steroids or other endocrine disruptors can alter uterine function in the adult. In the pig, uterine glands are absent at birth and uterine adenogenesis in the pig occurs from birth to 21 d postnatally (Bartol et al., 1993; Tarleton et al., 1999). Tarleton et al. (1999) demonstrated that estrogen stimulates but treatment with the estrogen antagonist ICI 182,780 inhibits adenogenesis in the neonatal uterus. Therefore, it may be possible to alter neonatal uterine glandular morphogenesis and development through steroidal and(or) lactogenic hormone administration. Spencer et al. (1999) proposed that sequential exposure of the endometrium to steroids and lactogenic and somatogenic hormones is involved in the endometrial gland remodeling and secretory function in the pregnant ewe. Uterine infusion of growth hormone and placental lactogen increases uterine glandular development and secretory function in steroid-treated, ovariectomized ewes. It is also possible to treat neonatal lambs with prolactin and increase the number of uterine glands in the adult (T. E. Spencer, personal communication). The presence of prolactin receptors in the porcine endometrium (Young et al., 1990) suggests that it may be possible to influence the number of uterine glands in the pig. With the known effects of estrogen and prolactin on uterine and glandular development in females, uterine alterations and litter capacity in pigs need to be evaluated following neonatal treatment with estrogen and prolactin.

Over the past 40 yr of probing the complexity of embryonic development and loss, we have developed more answers for why embryonic loss occurs but provided little in the solution for rapidly improving litter size in the pig. Improvements are not likely to result from any nutritional or steroid therapies aimed at changing

uterine function during gestation but could result through better control of ovulation and oocyte quality or changes in the uterine vascular and secretory capacity, as we should have already learned from the mammary gland. Can we modify uterine and conceptus function in the future? The rapid improvements in technology will certainly allow us to gain more understanding of conceptus and uterine factors involved with development. Only time will tell if we can use the information to develop methods to modulate average uterine capacity beyond the levels of production today.

Implications

The numerous biological factors involved with regulating litter size in pigs have slowed progress for rapidly increasing litter size. Our knowledge of the factors regulating conceptus and uterine development and function has increased but is far from complete. The developing technologies to investigate gene and protein expression will continue to assist in unraveling the mysteries of early embryonic survival in the pig. However, improvements in litter size will only occur when we can use the information to increase placental efficiency and(or) uterine secretory function. It is clear that the uterus in the pig can be modified to alter glandular development. Therefore, a combination of increased endometrial glandular density and placental vascularity may move litter size beyond the current plateau currently achieved in the swine industry.

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