

Factors Affecting Litter Size in Pigs¹

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Litter size in pigs has been defined in a number of ways, depending on the goals of the specific experiment. Number of total piglets, including mummies and stillborns, number of fully formed piglets, excluding mummies but including stillborns, and the number of piglets born alive have all been used as endpoints of litter size research. The end measures are chosen with the idea that the causes of late stage fetal death (resulting in mummies) or factors involved in stillbirth are separate from other pregnancy related factors regulating the total number of piglets born, and should therefore be studied separately. While this strategy is useful, clearly the results generated may not be completely relevant to the endpoint desired by the swine producer, which is more marketable pigs per sow. From the producer's perspective, preweaning loss is also relevant. Because the goal of our research is to improve the efficiency of swine production and therefore increase marketable pigs per sow, we have shifted our research focus to include research to define factors influencing preweaning survival.

The paragraph above alludes to the complexity of the interacting factors influencing the number of piglets weaned. We divided piglets weaned into prenatal factors influencing the number of fully formed fetuses at the end of pregnancy, perinatal factors influencing stillbirth and postnatal factors influencing preweaning survival. Because these divisions occur sequentially, pregnancy factors also affect subsequent stillbirth and preweaning survival, and perinatal factors also affect preweaning survival.

The number of fully formed piglets present at farrowing is influenced by the number of ova shed, the fertilization failure rate, the embryonic mortality rate, and the number of fetuses maintained by the uterus during gestation (uterine capacity; Bennett and Leymaster, 1989). Note that two of these factors are rates of occurrence. The number resulting from these processes depends on the number before the processes took place, and therefore these rates do not actually represent real limitations to the number of fully formed piglets. For example, if one wishes to increase the number of fertilized ova, and fertilization failure rate is relatively fixed (most studies indicate a failure rate of 10% or less, Polge 1978), one only needs to increase the number of ova. In contrast, the number of ova shed and uterine capacity are not rates, and the number of fully formed piglets at farrowing is related to the most limiting of these two factors (the number of ova

shed must be discounted by the fertilization failure rate and embryonic mortality rate, but the number surviving these two rates of occurrence can easily be increased by increasing the number of ova shed). In today's production setting, the number of ova shed, even after discounting for fertilization failure rate and embryonic mortality rate, usually exceeds uterine capacity, and the number of fully formed fetuses is thus primarily limited by uterine capacity (Town et al., 2005).

The term "uterine capacity" suggests that the limitation to the number of fully formed fetuses that it represents is entirely due to uterine factors, but this is not the case. Uterine capacity, or the number of fully formed fetuses that can be maintained by the uterus until farrowing, is actually the result of the interaction between uterine, placental and fetal factors influencing the survival of the fetus during pregnancy. Two primary uterine factors affecting uterine capacity are uterine blood flow and uterine protein secretion. Two primary placental factors affecting uterine capacity are the size of the placenta and the efficiency of nutrient transport. Fetal factors influencing uterine capacity include fetal erythropoiesis and fetal nutrient usage. Each factor displays more or less compensatory behavior as the number of fetuses within the uterus increases.

Studies indicate that uterine blood flow increases throughout gestation (Pere and Estienne, 2000). However, uterine blood flow appears to be maximal at around 5 fetuses per uterine horn, and flow does not increase with further increases in the number of fetuses present. Because maternal blood is the ultimate source of all nutrients for the developing fetus during pregnancy, the failure of blood flow to compensate for increased fetal numbers would seem to be a serious limiting factor. Although it has been suggested that placental estrogens regulate uterine blood flow (Geisert et al., 1990), regulation of uterine blood flow during gestation in the pig is not completely understood.

Uterine protein secretion during early pregnancy and placental size during later pregnancy would seem to be interrelated. Pig blastocysts transform from a spherical to a filamentous form around day 11 of pregnancy, and this transformation has been termed elongation (Geisert et al., 1982). It is likely that the size of the placenta is ultimately determined by the extent of blastocyst elongation during this period. Furthermore, blastocysts do not elongate past each other, and it is likely that substantial elongation of neighboring conceptuses can interfere with the elongation process. The Chinese Meishan breed of pig is reported to have greater uterine capacity (Haley and Lee, 1993), and this is likely due to the fact that both the placenta and the fetus of the Meishan are smaller (Christenson, 1993). The small placenta of the Meishan can be traced to slower growth of the Meishan blastocysts during early pregnancy, resulting in more limited elongation of the Meishan blastocyst on day 11 of pregnancy (Anderson et al., 1993). Subsequent research demonstrated that this phenomenon was primarily uterine in origin (Youngs et al., 1994), and Vallet et al., (1998) reported that this was likely due to decreased uterine protein secretion by the Meishan uterus, limiting Meishan conceptus growth and elongation. Thus it appears that one component of the improved uterine capacity of the Meishan is limitation of blastocyst elongation by the Meishan uterus, mediated in some way by reduced protein secretion, which then results in reduced negative interactions between neighboring blastocysts allowing more uniform placental sizes. We attempted to mimic the reduction of uterine protein secretion and conceptus development in European breed pigs using the progesterone inhibitor RU486, and were successful in reducing both uterine protein secretion and conceptus development on day 11 of pregnancy. However, this did not result in subsequent increased

uterine capacity (Vallet and Christenson, 2004). Nevertheless, these experiments point to the importance of uterine protein secretion in controlling conceptus elongation, placental size and uterine capacity. We subsequently used proteomic technology to identify proteins secreted by the uterus during this period (Kayser et al., 2006). Two glycolipid metabolizing proteins were identified, suggesting that glycolipid metabolism may play a role in the elongation process. Glycolipids play roles in numerous biological processes (Perry and Hannun, 1998; Roviezzo et al., 2004) and we have recently begun exploring changes in the endothelial differentiation gene (EDG) receptors, which respond to byproducts of glycolipid metabolism (Ishii et al., 2004). We have found interesting temporal changes in the expression of some of them, suggesting that glycolipids may play a role in the elongation process.

Placental function also influences the health of the developing fetus, and thus contributes to uterine capacity. The most important placental function that is relevant to uterine capacity is the efficiency with which the placenta transfers nutrients. Placental efficiency has received a great deal of attention in the last decade or so, largely through the work of Steve Ford and coworkers. They introduced the concept of the fetal weight to placental weight ratio as a measure of placental efficiency (Biensen et al., 1998), and went on to report that selection for this trait led to increased litter size (Wilson et al., 1999). However, their selection experiment employed only 12 animals, which were selected for only a single generation. A more recent selection experiment using divergent selection for the fetal weight to placental weight ratio, which employed more sows and 4 generations of selection, did not result in improvements in litter size (Mesa et al., 2005). One of the primary reasons for this might be that other factors affect the weight of the fetus besides placental function, and in fact, above a moderately sized placenta, the size (and therefore the function) of the placenta has no effect on the size of the fetus (Vallet, 2000). Thus, other measures of placental efficiency are needed to begin to effectively select for this trait.

We hypothesized that beyond the actual size of the placenta, the structure of the placenta with relation to the flow of maternal and fetal blood also likely affects efficiency. Exchange by simple diffusion is governed by several factors, including whether blood flows are concurrent, crosscurrent or countercurrent, the length of time the two blood systems are closely apposed to each other, and the actual distance between the two blood systems. The maternal and fetal blood supplies of the pig occur in a cross-countercurrent arrangement (Leiser and Dantzer, 1988) that is at right angles to the plane of the placenta and the exchange of nutrients takes place within microscopic folds of the placenta. These folds become deeper and more complex with advancing gestation, and the distance between the maternal and fetal blood supplies also decreases. Both the changes in the placental folds and the decreased distance between blood supplies play a role in the improvement of placental efficiency with advancing gestation. We have also recently shown that the depth of the folds is greater in placenta of small fetuses, suggesting that deepening of the folds may be one placental mechanism that compensates for inadequate placental size and therefore uterine space. How the microscopic folds develop is completely unknown, but they are embedded in a placental stroma that we have recently shown is partially composed of hyaluronan. Hyaluronan, hyaluronan synthesizing and degrading enzymes, and hyaluronan binding proteins play significant roles in tissue morphogenesis and angiogenesis (Laurent and Fraser, 1992; Knudson and Knudson, 1993; Spicer and Tien, 2004). Thus, hyaluronan synthesizing and degrading enzymes likely play a role in formation and vascularization of the placental folds, and we have recently demonstrated changes in the expression of two forms of

hyaluronidase, 1 and 2, during gestation. We are continuing to explore their role in placental development and efficiency.

Turning to fetal function, we have performed several experiments that suggest a role for the development of the blood supply in uterine capacity and litter size. Our experiments have demonstrated (1) that fetal hematocrits are impaired in small fetuses (Pearson et al., 1998; Vallet et al., 2002), (2) that the development of the blood supply takes place more quickly in Meishan fetuses (Pearson et al., 1998; Vallet et al., 2003), (3) that selection for increased uterine capacity is associated with increased fetal hematocrit (Vallet et al., 2001), (4) that a genetic marker based on the erythropoietin receptor (EPOR) is associated with improved uterine capacity and litter size (Vallet et al., 2005), and (5) that the EPOR marker is associated with increased fetal liver EPOR expression during the development of the fetal blood supply. We have also explored the ability of the fetus to modify the growth of various organs in response to nutrient deficiencies and selection for uterine capacity (Vallet and Freking, 2006). Our results indicate that both the brain and heart possess mechanisms that preserve their growth within the fetus, while the liver and spleen do not. Furthermore, subtle alterations in the growth of the fetal liver were associated with selection for uterine capacity. Taken together, these results confirm that mechanisms within the fetus also contribute to overall uterine capacity and litter size.

Several perinatal factors influence stillbirth, but one of the most important factors would appear to be the process of farrowing itself (Van Dijk et al., 2005). To get a better idea of interrelationships between the farrowing process and the incidence of stillbirth, we began monitoring farrowing and stillbirth in first parity gilts through the use of video surveillance. Cameras were mounted at the rear of farrowing crates and images were obtained at 1 to 5 second intervals until farrowing was completed. Videos were assessed for the birth of each piglet, and whether the piglet was born alive. Individual piglet farrowing intervals were calculated during the farrowing process. Farrowing intervals were longer at the beginning of the process and became shorter in the middle. Curiously, birth of the last piglet in the litter was significantly delayed, and the average farrowing interval for this piglet was 2.5 times that of the piglet before. Turning to stillbirth rate, in contrast to farrowing intervals, stillbirth rate became progressively greater as the farrowing process continued, increasing from about 2% at the beginning of farrowing to about 5% toward the end. Although changes in stillbirth rate did not mirror changes in farrowing interval, the stillbirth rate for the last pig in the litter was estimated to be 15%, 2.5 times that of the piglet before and similar to the difference in farrowing interval. Average farrowing intervals were longer for small litters, especially during July when temperatures were high, and decreased with increasing litter size. The decrease in farrowing interval with increasing litter size, combined with the unusually long farrowing interval of the last piglet, suggests a piglet or placental contribution to the speed of the process. Although cortisol (First and Bosc, 1979), prostaglandin and oxytocin (Gall and Day, 1987) can all initiate farrowing, very little is known of factors that facilitate farrowing in pigs, with the exception of oxytocin and relaxin (Guthrie, 1985; Wathes et al., 1989; Gilbert et al., 1994; Cho et al., 1998;). We collected blood samples on days 110 and 113 of gestation from the same gilts, to measure progesterone, estrogen and relaxin, and will attempt to relate the results to farrowing intervals. Thus far, progesterone does not appear to influence farrowing intervals.

Finally, we turn to preweaning survival. Numerous studies indicate that one of the primary factors influencing preweaning survival is low birth weight (Tuchscherer et al., 2000; Milligan et

al., 2002), which brings us back to uterine capacity, because birth weights are established during gestation. Beyond birth weights, piglets from different breeds or lines of pigs differ widely in preweaning survival. Using data from our USMARC swine database, we could show that for piglets below a birth weight of 1 kg, our lean and obese selected lines differed dramatically in piglet survival. Losses for these low birth weight piglets were 4 times greater in the lean line than the obese line. Similar results were obtained comparing Meishan with our BX population (Occidental, Duroc and primarily white breed, crossbred pigs). For piglets born below 1 kg birth weight, piglet losses in the BX population were twice that in the Meishan. The Meishan and obese line piglets appear to have greater piglet fat stores at birth in common (Stone et al., 1985), and other literature supports this as a factor in preweaning survival (Mersmann, 1974). Using reciprocal transfer techniques, we are currently exploring whether the effect in Meishans originates from the maternal environment generated by the Meishan uterus, or is a characteristic of the piglets themselves. These results suggest that one of the best ways to select for preweaning survival might be to develop noninvasive ways to measure piglet energy stores at birth. Some possibilities we are considering are bioelectric impedance (related to body fat) and piglet body temperature shortly after birth (related to energy stores). In support of this contention, piglet body temperature 1 h after birth has been reported to be a significant risk factor for preweaning loss in a Meishan F2 population of pigs (Casellas et al., 2004).

In conclusion, the number of piglets weaned is influenced by the number of fully formed piglets, the stillbirth rate and preweaning survival. All of these factors appear to be significantly influenced by the size of the fetus/piglet, which is likely controlled by uterine space/placental size, placental efficiency and fetal nutrient usage. Over and above this, the stillbirth rate is strongly influenced by the farrowing process, and preweaning survival is likely modulated by piglet energy stores at birth. Genetic markers associated with these traits, or simple phenotypes that are well correlated with these traits, are needed to identify superior animals.

References

- Anderson, LH, Christenson, LK, Christenson, RK, Ford, SP. 1993. Investigations into the control of litter size in swine: II. Comparisons of morphological and functional embryonic diversity between Chinese and American breeds. *J. Anim. Sci.* 71:1566-1571.
- Bennett, GL, Leymaster, KA. 1989. Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. *J. Anim. Sci.* 67:1230-1241.
- Biensen, NJ, Wilson, ME, Ford, SP. 1998. The impact of either a Meishan or Yorkshire uterus on Meishan or Yorkshire fetal and placental development to days 70, 90 and 110 of gestation. *J. Anim. Sci.* 76: 2169-2176.
- Casellas, J, Noguera, JL, Varona, L, Sanchez, A, Arque, M, Piedrahita, J. 2004. Viability of Iberian x Meishan F2 newborn pigs. II. Survival analysis up to weaning. *J. Anim.Sci.* 82:1925-1930.
- Cho, SJ, Dlamini, BJ, Klindt, J, Schwabe, C, Jacobson, CD, Anderson, LL. 1998. Antiporcine relaxin (antipRLX540) treatment decreases relaxin plasma concentration and disrupts delivery in late pregnant pigs. *Anim. Reprod. Sci.* 52:303-316.

Christenson, RK. 1993 Ovulation rate and embryonic survival in Chinese Meishan and White Crossbred pigs. *J. Anim. Sci.* 71:3060-3066.

First, NL, Bosc, MJ. 1979. Proposed Mechanisms controlling parturition and the induction of parturition in swine. *J. Anim. Sci.* 48:1407-1421.

Gall, MA, Day, BN. 1987. Induction of parturition in swine with prostaglandin F₂ α , estradiol benzoate and oxytocin. *Theriogenology* 27:493-505.

Geisert, RD, Brookbank, JW, Roberts, RM, Bazer, FW. 1982. Establishment of pregnancy in the pig: II. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy. *Biol. Reprod.* 27:941-955.

Geisert, RD, Zavy, MT, Moffatt, RJ, Blair, RM, Yellin, T. 1990. Embryonic steroids and the establishment of pregnancy in pigs. *J. Reprod. Fertil. Suppl.* 40:293-305.

Gilbert, CL, Goode, JA, McGrath, TJ. 1994. Pulsatile secretion of oxytocin during parturition in the pig: temporal relationship with fetal expulsion. *J. Physiol.* 475:129-137.

Guthrie, HD. 1985. Control of time of parturition in pigs. *J. Reprod. Fert. Suppl.* 33:229-244.

Haley, CS, Lee GJ. 1993. Genetic basis of prolificacy in Meishan pigs. *J. Reprod. Fertil. Suppl.* 48:247-259.

Ishii, I, Fulushima, N, Ye, X, Chun, J. 2004. Lysophospholipid receptors: signaling and biology. *Annu. Rev. Biochem.* 73:321-354.

Kayser, JP, Kim JG, Cerny, RL, Vallet, JL. 2006. Global characterization of porcine intrauterine proteins during early pregnancy. *Reproduction.* 131:379-388.

Knudson, CB, Knudson, W 1993. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. *FASEB J.* 7:1233-1241.

Laurent, TC, Fraser, JRE. 1992. Hyaluronan. *FASEB J* 6:2397-2404.

Leiser, R, Dantzer, V. 1988. Structural and functional aspects of porcine placental microvasculature. *Anat. Embryol.* 177:409-419.

Mersmann, HJ. 1974. Metabolic patterns in the neonatal swine. *J. Anim. Sci.* 38:1022-1030.

Mesa, H, Safranski, TJ, Fischer, KA, Cammack, KM, Lamberson, WR. 2005. Selection for placental efficiency in swine: genetic parameters and trends. *J. Anim. Sci.* 83:983-991.

Milligan, BN, Dewey, CE, De Grau, AF. 2002. Neonatal-piglet weight variation and its relation to pre-weaning mortality and weight gain on commercial farms. *Prev. Veter. Med.* 56:119-127.

Pearson, PL, Klemcke, HG, Christenson, RK, Vallet, JL. 1998. Uterine environment and breed effects on erythropoiesis and liver protein secretion in late embryonic and early fetal swine. *Biol. Reprod.* 58:911-918.

Pere, MC, Etienne, M. 2000. Uterine blood flow in sows. Effects of pregnancy stage and litter size. *Reprod. Nutr. Dev.* 40:369-382.

Perry, DK, Hannun, YA. 1998. The role of ceramide in cell signaling. *Biochim. Biophys. Acta* 1436:233-243.

Polge, C. 1978. Fertilization in the pig and horse. *J. Reprod. Fert.* 54:461-470.

Roviezzo, F, Del Galdo, F, Abbate, G, Bucci, M, D'Agostino, B, Antunes, E, De Dominicis G, Parente, L, Rossi, F, Cirino, G, De Palma R. 2004. Human eosinophil chemotaxis and selective in vivo recruitment by sphingosine 1-phosphate. *Proc. Nat. Acad. Sci.* 101:11170-11175.

Spicer, AP, Tien, JYL. 2004. Hyaluronan and morphogenesis. *Birth Defects Research* 72:89-108.

Stone, RT, Champion, DR, Klindt, J, Martin, RJ. 1985. Blood parameters and body composition in fetuses from reciprocal crosses of genetically lean and obese swine. *Proc. Soc. Exp. Biol. Med.* 180:191-195.

Town, SC, Patterson, JL, Pereira CZ, Gourley, G, Foxcroft, GR. 2005. Embryonic and fetal development in a commercial dam-line genotype. *Anim. Reprod. Sci.* 85:301-316.

Tuchscherer, M, Puppe, B, Thchscherer, A, Tiemann, U. 2000. Early identification of neonates at risk:traits of newborn piglets with respect to survival. *Theriogenology* 54:371-388.

Vallet, JL 2000 Fetal erythropoiesis and other factors which influence uterine capacity in swine. *J. Appl. Anim. Res.* 17:1-26.

Vallet, JL, Christenson, RK. 2004. Effect of progesterone, mifepristone, and estrogen treatment during early pregnancy on conceptus development and uterine capacity in swine. *Biol. Reprod.* 70:92-98.

Vallet, JL, Christenson, RK, Trout, WE, Klemcke, HG. 1998. Conceptus, progesterone and breed effects on uterine protein secretion in swine. *J. Anim. Sci.* 76:2657-2670.

Vallet, JL, Freking, BA, 2006. Changes in fetal organ weights during gestation after selection for ovulation rate and uterine capacity in swine. *J. Anim. Sci.* 84:2338-2345.

Vallet, JL, Freking, BA, Leymaster, KA, Christenson, RK. 2005. Allelic variation in the erythropoietin receptor gene is associated with uterine capacity and litter size in swine. *Anim. Genet.* 36:97-103.

Vallet, JL, Leymaster, KA, Cassady, JP, Christenson, RK. 2001. Are hematocrit and placental efficiency selection tools for uterine capacity in swine. *J. Anim. Sci.* 79(Suppl. 2):89(Abstr.)

Vallet, JL, Klemcke, HG, Christenson, RK. 2002. Interrelationships among conceptus size, uterine protein secretion, fetal erythropoiesis and uterine capacity. *J. Anim. Sci.* 80:729-737.

Vallet, JL, Klemcke, HG, Christenson, RK, Pearson, PL. 2003. The effect of breed and intrauterine crowding on fetal erythropoiesis on day 35 of gestation in swine. *J. Anim. Sci.* 81:2352-2356.

Van Dijk, AJ, Van Rens, BTTM, Van der Lende, T, Taverne, MAM. 2005. Factors affecting duration of the expulsive stage of parturition and piglet birth intervals in sows with uncomplicated, spontaneous farrowings. *Theriogenology.* 64:1573-1590.

Wathes, DC, King, GJ, Porter, DG, Wathes, CM. 1989. Relationship between pre-partum relaxin concentrations and farrowing intervals in the pig. *J. Reprod. Fert.* 87:383-390.

Wilson, ME, Biensen, NJ, Youngs, CR, Ford, SP. 1999. Novel insight into the control of litter size in pigs, using placental efficiency as a selection tool. *J. Anim. Sci.* 77:1654-1658.

Youngs, CR, Christenson, LK, Ford, SP. 1994. Investigations into the control of litter size in swine. III. A reciprocal embryo transfer study of early conceptus development. *J. Anim. Sci.* 72:725-731.