

# Prenatal Programming of Postnatal Performance – the Unseen Cause of Variance

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## ■ Introduction

In domestic species like the pig, the number of offspring born is an important economic trait, and the components of litter size (ovulation rate, embryonic survival and uterine capacity) responsive to genetic selection are well established (Johnson et al., 1985). However, as selection for ovulation rate has been associated with selection against early embryonic survival, and because birth weight decreased as litter size increased, it was concluded that selection for uterine capacity might be the most productive approach in genetic selection programs (Johnson et al., 1999). A recent study of associations among within-litter variation in birth weight and pre-weaning survival and weight gain, also led to the conclusion that selection for increased litter size that results in more low-birth-weight piglets may not be beneficial, unless measures are taken to improve the survival of the low-birth-weight offspring (Milligan et al., 2002). ***Thus, both the developmental competence of the pigs born, as well as the size of the litter, need critical consideration.***

In the context of the present session, we intend to summarize existing literature that indicates that the variation in growth performance after birth may be largely determined, and essentially pre-programmed, during fetal development in the uterus. Furthermore, it is likely that these pre-programmed limitations in growth performance will only finally express themselves in the late grower / early finisher stage of production. We will also present preliminary evidence that differences in fetal development that will likely affect postnatal growth performance can even be present without associated effects in birth weight. Thus sorting pigs by weight at the nursery and grower stages will not resolve the variation in growth performance that is still an inherent characteristic of particular pigs or litters. ***Thus we face the conundrum that 13 pigs born in two different litters, with the same average birthweight, may originate***

***from litters with very different numbers of surviving embryos around day 30 of gestation, and this will pre-program these two litters to have very different postnatal growth potential.***

### **Uterine capacity as the ultimate limitation on litter size.**

The concept of uterine capacity was established using different experimental approaches to study effects of uterine crowding in the pig. These included uterine ligation, oviduct resection, unilateral hysterectomy and ovariectomy (UHO), superovulation, and embryo transfer, and led to the conclusion that when the number of embryos exceeded 14, intrauterine crowding was a limiting factor for litter size born (Dziuk, 1968). Bazer et al. (1969 a, b) also concluded that increased embryonic loss, associated with a greater number of embryos *in utero*, was due to maternal limitations and not to inherent limitations of the embryo. They suggested that two physiological mechanisms might be involved.

- Initially, embryo selection might be the result of competition among embryos for some biochemical factor in the uterus necessary for their continued development
- However, in later gestation, intrauterine competition for the establishment of adequate surface area for nutrient exchange between fetal and maternal circulations may act to limit litter size.

Interestingly, in the context of variation in development in utero, the concept has been advanced that mechanisms promoting competition among embryos in the pre-implantation period will act to reduce within-litter variation in development by selectively removing the least developed embryos (van der Lende et al., 1990). Nevertheless, the more recent results of Père et al. (1997) confirm that, even in sows with "normal" ovulation rates, uterine capacity can affect both litter size and the average birth weight of the litter.

### **When does uterine capacity impact fetal survival and development?**

Fenton et al. (1970) determined that uterine capacity only becomes a limiting factor for fetal survival after day 25 of gestation. Knight et al. (1977) further defined day 30 to 40 of gestation as the critical period when uterine capacity exerts its effects, and subsequent studies in both intact and UHO females support this conclusion (see Vallet, 2000). Vallet et al. (2003) suggested that fetal growth rate is less sensitive to intrauterine crowding than placental growth rate and that, as in the prolific Meishan female (Ford and Youngs, 1993), an increase in placental efficiency may initially protect the developing fetus from a limitation in placental size. ***However, conclusions based only on a consideration of fetal weight may overlook critical effects on fetal development that are established early in gestation.***

The study of within-litter variation in prenatal development suggested that the extremes of intrauterine growth retardation (IUGR) or "runting" described previously in the pig (Adams, 1971; Widdowson, 1971; Hegarty and Allen, 1978; Flecknell et al., 1981), were identified within a discrete sub-set of fetuses (Wooton et al., 1983). In subsequent studies of the association between within-litter differences in prenatal development and postnatal survival and growth, van der Lende and de Jager (1991) concluded that the lower pre-weaning growth of the runted pigs born could not be entirely explained on the basis of their lower birth weight. This suggested that IUGR or runting had a more complex effect on the developmental potential of such pigs. Interestingly, data from the same laboratory indicated that the extent of IUGR within a litter was associated with specific patterns of embryonic survival (van der Lende et al., 1990) and the largest litters *in utero* generally included one or more runted fetuses. ***Furthermore, the data from this study supported the conclusion that within-litter variation in development was already established at the early fetal stage (day 27 to 35) of gestation.***

## ■ Patterns of Prenatal Loss and Developmental Potential

Pre-implantation embryonic losses are still considered to be the largest proportion of prenatal loss in the pig, with some lesser loss in the post-implantation period that will ultimately reflect uterine capacity (as reviewed by Ashworth and Pickard, 1998). In commercial practice, this generalization likely reflects the situation in gilts in which ovulation rates of 10 to 15, associated with some degree of embryonic loss, is the primary factor limiting litter size. Weaned, first parity, sows also tend to fall into this category, because although ovulation rate may be higher (15 to 20 ovulations), many sows tend to be in a catabolic state and this decreases embryonic survival to 60 to 65% in many reported studies.

However, the dynamics of prenatal loss in existing commercial dam-lines may be changing (Foxcroft, 1997). In these populations, it appears that several generations of direct selection for litter size has indirectly resulted in a discrepancy between the number of conceptuses surviving to the post-implantation period and uterine capacity. As a consequence of markedly increased ovulation rates, associated with good or even modest embryonic survival in the pre-implantation period, the number of embryos surviving to the immediate post-implantation period (day 25 to 30) initially greatly exceeds uterine capacity. As a result, ***a substantial proportion of prenatal loss is now occurring in the post-implantation period.***

Even in individual gilts with 20 or more ovulations, embryonic survival rate can be 100% at day 28 of gestation (Almeida et al., 2000), whereas average first litter size is still only 10 to 12 piglets. In higher parity females, the situation may

be even more extreme and mean ovulation rates of  $26.6 \pm 0.4$  (Vonnahme et al., 2002) and  $24.7 \pm 0.4$  (Town et al., unpublished) have been reported in commercial dam-line sows, **with 15 to 20% of higher parity sows having greater than 30 ovulations**. Despite relatively poor embryonic survival to day 30 (approximately 60% in both studies), numbers of conceptuses *in utero* at day 30 (approximately 15) still exceeded uterine capacity. Consistent with the literature reviewed earlier, uterine capacity then exerted its effects and a significant reduction in the number of conceptuses occurred by day 45 to 50 of gestation. However, as also reported in earlier literature, even this modest increase in uterine crowding around day 30 of gestation had consequences for placental development, seen as a decrease in placental volume (Almeida et al., 2000) and placental weight (Vonnahme et al., 2002).

Although the size and weight of the embryo was not seen to be affected by crowding up to day 44 of gestation, potential impacts on fetal development need careful study. If placental compensatory mechanisms are not adequate, crowding of the uterus in the early post-implantation period of gestation may affect fetal development of surviving conceptuses, in a manner analogous to IUGR. This raises important questions for both fetal and postnatal development. In the context of commercial grow-finish performance, a specific interest in effects on the development of fetal muscle fibres, which start to differentiate around day 35 of gestation in the pig, is particularly important. In contrast to situations in which the occurrence of IUGR is limited to a discrete subpopulation of "runt" fetuses (Royston et al., 1982; Wooton et al., 1983), **a changing pattern of embryonic loss that results in uterine crowding in early gestation appears to produce a more uniform effect on placental development that will thus affect the development of all surviving fetuses**.

## ■ Factors Affecting Muscle Development in the Pig

A series of studies in the pig have demonstrated that maternal nutrition during gestation has an effect on piglet birth weight, and that low birth weight is primarily associated with a reduced number of secondary muscle fibres (Handel & Stickland, 1987; Dwyer et al., 1994). Consistent with earlier data of Hegarty and Allen (1978) indicating that runts in the litter have reduced muscle growth potential, Dwyer et al. (1993) also established a positive correlation between the total number of muscle fibres and postnatal growth potential, and that littermates with a high numbers of fibres grew faster and more efficiently than littermates with a lower number of fibres. Dwyer et al. (1994) further demonstrated that the effect of maternal nutrition occurred between 25 and 50 days of gestation, the period immediately preceding secondary muscle fibre hyperplasia.

Effects of maternal nutrition during gestation on fetal development are widely reported and this area of literature was the subject of an excellent review by Robinson et al. (1999). Furthermore, Maltin et al. (2001) extensively discussed the impact of manipulating myogenesis by various techniques *in utero* on subsequent muscle development. ***The early period of myogenesis, involving the differentiation of primary muscle fibers seems to be resistant to nutritional manipulation, whereas nutritional effects on differentiation and hyperplasia of secondary fibers have been demonstrated between day 25 to 90 of gestation.***

From the perspective of using nutritional intervention and other treatments to reduce the variation in birth weight and postnatal growth within litters, it is interesting to note that the greatest reported impact of increased maternal nutrition (Dwyer et al., 1994), treatment with exogenous somatotropin during early gestation (Rehfeldt et al., 2001), and breed of sow (Ashworth et al., 1998) was on the smallest pigs within the litter. These results suggest that relative under-nutrition of the smallest fetuses *in utero* is the driver of low birth weight and poor postnatal growth performance. Furthermore, the early data of Widdowson (1976) showed that if limited nutrition initially results in the runting of pigs before and after birth, high subsequent feed intakes do not result in a normal development during compensatory growth, implying that some form of "fetal re-programming" had occurred *in utero*.

Based on the schematic representation of muscle fibre development shown in **Figure 1**, this led to the central hypothesis tested in a number of our recent studies, that "by detrimentally affecting placental size in early gestation, uterine crowding will also affect fetal organ development and the number and type of muscle fibres, analogous to the situation of IUGR in nutritionally challenged sows".

Preliminary data from an initial experiment indicated that even when the number of conceptuses *in utero* does not significantly affect birth weight, "crowding" nevertheless results in measurable IUGR in the fetus (Town et al., 2002). In another study, a surgical approach was used to vary the number of fetuses developing in the uterus, and even though the uterine crowding observed was not at the level that probably occurs in existing commercial dam-line sows, a higher number of fetuses in the uterus resulted in measurable developmental changes. As shown in **Figure 2**, there were again effects of increasing numbers of conceptuses and fetuses *in utero* on placental development (Town et al., unpublished data, University of Alberta, 2003).

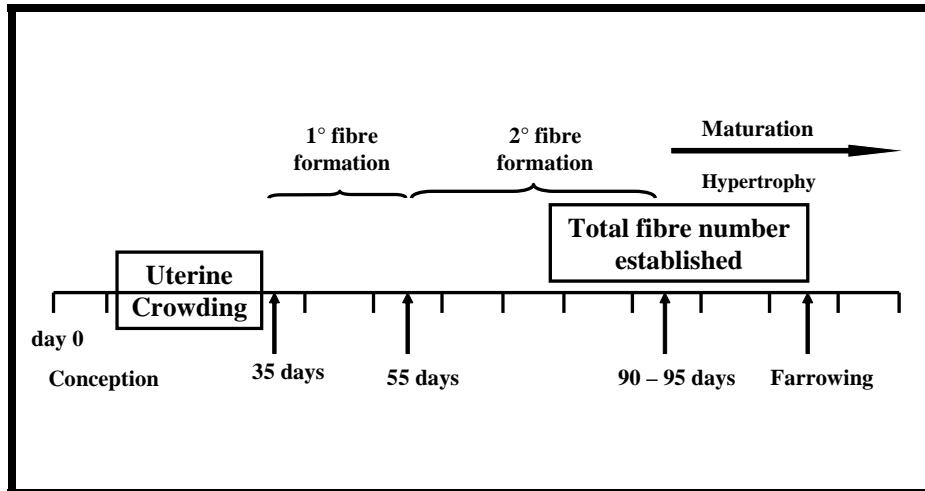


Figure 1. Schematic representation of the time-course of muscle fibre development in the pig, indicating a critical window in early pregnancy when crowding effects limit placental development and set in place detrimental effects on fetal development and lifetime growth performance.

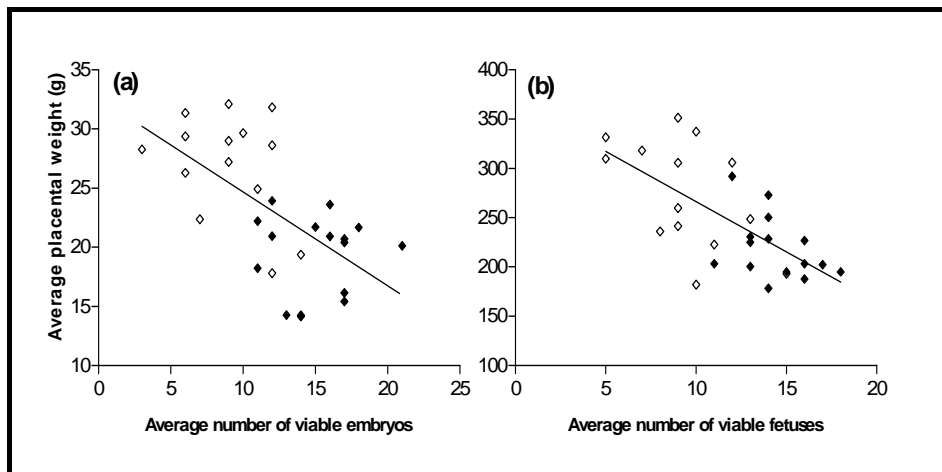


Figure 2. Correlation between average placental weight and (a) number of viable embryos at day 30 of gestation ( $r^2 = -0.37$ ;  $P = 0.0003$ ) and (b) number of viable fetuses at day 90 of gestation ( $r^2 = -0.45$ ;  $P < 0.0001$ ) in CTR animals ( $\diamond$ ) and LIG animals ( $\blacklozenge$ ).

**Table 1. Muscle fibre development data (means  $\pm$  SEM) for day 90 fetuses from control (CTR) and unilaterally oviduct ligated (LIG) sows (N=28). Can you define CSA?**

Parameter	Treatment group <sup>1</sup>	
	CTR (n=14) “Crowded”	LIG (n=14) “Non-Crowded”
Primary fibre no/mm <sup>2</sup>	29.5 $\pm$ 1.5	25.8 $\pm$ 1.3
Primary fibre CSA <sup>2</sup> ( $\mu\text{m}^2$ )	123.5 $\pm$ 5.6	130.4 $\pm$ 4.0
Secondary fibre no/mm <sup>2</sup>	678.7 $\pm$ 16.5	673.3 $\pm$ 18.6
Secondary fibre CSA ( $\mu\text{m}^2$ )	23.1 $\pm$ 1.5	20.2 $\pm$ 0.5
Muscle weight (g)	1.25 $\pm$ 0.06 <sup>a</sup>	1.47 $\pm$ 0.09 <sup>b</sup>
Muscle CSA (mm <sup>2</sup> )	47.71 $\pm$ 2.85 <sup>a</sup>	58.78 $\pm$ 2.65 <sup>b</sup>
Number total primary fibres	1394 $\pm$ 81	1480 $\pm$ 57
Number total secondary fibres	32,691 $\pm$ 2098 <sup>a</sup>	39,628 $\pm$ 2074 <sup>b</sup>
Secondary:Primary fibre ratio	24.01 $\pm$ 1.49	26.80 $\pm$ 0.06

<sup>1</sup> Means  $\pm$  SEM within a row with different superscripts differ (P < 0.05)

<sup>2</sup> CSA = Cross-sectional area

As shown in **Table 1**, among the various measures of IUGR we were able to establish specific effects on the number of secondary muscle fibres. This provides some of the first evidence that the variation in the number of conceptuses surviving to the post-implantation period will affect not only placental but also fetal development.

In the literature cited earlier, comparable effects on muscle fiber development, created by maternal under-nutrition during gestation, resulted in lifetime limitations in growth performance and muscle mass. It is thus reasonable to assume that the observed effects of embryo crowding *in utero* on the number of secondary muscle fibers will be associated with similar limitations in post-natal growth performance.

The extent of uterine crowding that we have managed to create in the above study, and a number of comparable studies in both gilts and higher parity sows, has been less than the crowding we predict in at least a sub-population of higher parity sows in existing commercial dam-lines. Nevertheless, it appears that we are able to demonstrate that differences in what is happening in the pre-natal period in individual females has proven consequences for the pattern of muscle fiber development. Together with the earlier literature reviewed, which suggested that muscle development in the extreme runts within a litter is also compromised, we think that these results provide a good insight into the biological origins of much of the post-natal variability in growth performance encountered in the industry.

### ■ Implications for “Hitting the Grid”

If pre-natal development makes such a profound contribution to post-natal variation in growth performance, is there any practical resolution to this problem? Perhaps one or two suggestions can be considered.

- Firstly, it is likely that certain categories of sows, like high parity sows with increased ovulation rates and few problems with lactational catabolism will produce the greatest incidence of altered developmental potential due to overcrowding *in utero*. In the extreme situations, developmental limitations will also be associated with low birth weights, and at least this population of pigs could be designated to segregated production flows at the nursery and grow-finish stages.
- The information reviewed above, also indicates that the growth potential of runt pigs within a litter will forever be compromised. Therefore, simply mixing these pigs with smaller weight pigs that were not the runts in their litters, will not recognize that the developmental potential of these two sets of pigs is very different. Perhaps the best use of true runts is the barbecue market and realistically they have little potential in traditional grow-finish systems trying to compete unfairly with a succession equal weight pigs with whom they are “sorted” during the production process.
- If we accept that runting and other forms of IUGR actually limits the number of muscle fibres, then it is probably unrealistic to consider that nutritional intervention can do much to alleviate this problem. The muscle mass is simply not there to produce a high level of growth performance and money spent on expensive nutritional programs to try and correct this problem may not be money well spent. However, as other data indicates that the survivability of runt pigs may also be seriously compromised, special attention to the needs of these pigs may be needed to keep them alive through the weaning and nursery stages of production.
- Finally, if our ongoing studies are able to identify specific dam-lines in which the imbalance between ovulation rate and embryonic survival, and

uterine capacity, is a particular problem for fetal development, then improved selection programs may be able to correct this problem. Clearly, these programs will have to more effectively account for the different components of litter size, to produce a dam-line sow that has the potential to produce a uniform finishing pig throughout her reproductive lifespan.

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