

Increasing Sperm Production in Mature Boars via Manipulation of their Neonatal Environment

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Introduction

Most management strategies for increasing the number of spermatozoa per ejaculate have focused on adult boars and have not been very successful (Flowers, 1997). One possible explanation for this is that the biological framework for sperm production as an adult is established early in a boar's life. In swine, as is the case with most mammalian species, sertoli cells can only support the development of a finite number of germ cells during spermatogenesis (Sharpe et al., 2003). Consequently, the number of sertoli cells typically is thought to be the "rate-limiting" factor in terms of sperm production levels in adults (Amann and Schanbacher, 1983). Sertoli cell proliferation in pigs begins during the prenatal period (McCoard et al., 2002) and continues after birth (Swanlund et al., 1995; Franca et al., 2000). There is some debate over when mitotic activity ends postnatally; however, it is generally accepted that a very active and, probably, critical period occurs during the first 3 weeks after birth (McCoard et al., 2003). Consequently, it is possible that a boar's potential for sperm production as an adult might be established by the time it is weaned from its mother.

Recently, a retrospective study was conducted with a limited number of boars ($n=20$) in order to begin to look for possible reasons why some boars consistently produce more spermatozoa than others (Flowers, unpublished). It is of interest to note that there were several pairs of littermates in this data set and in several instances they were on opposite ends of the spectrum in terms of number of spermatozoa produced per ejaculate as adults. One of the factors that was highly correlated ($r = 0.79$) with adult sperm production was a boar's weaning weight, which, in turn, exhibited a strong inverse relationship ($r = -0.85$) with the size of the litter from which the boar was weaned. These relationships seem logical since boars weaned in small litters would experience less competition and have the opportunity to consume larger quantities of milk than their counterparts in large litters. The additional nutrition that they received during lactation would coincide with the active period of sertoli cell mitosis and key developmental periods of other male reproductive organs. Thus, it seems physiologically plausible that manipulation of the litter size in which boars nurse may be a way to enhance their sperm production and other aspects of adult reproductive function.

Experimental Approach

In order to examine the influence of the neonatal environment on adult reproductive performance, 40 terminal-line, crossbred boars were crossfostered at one day of age in such a way that littermates were raised in litters of 6 ($n=20$) or in litters of 9 or more pigs ($n = 20$). Boars were selected from birth litters that has equal numbers of gilts and boars and crossfostering was done in such as way that potential milk production difference among sows

were minimized. For example, if sow A gave birth to 5 boars and 5 gilts and she was randomly selected to nurse a litter of 9 or more piglets, then 4 of her sons were fostered off to four different sows and she received 4 new boars from other sows, thus, creating a situation in which she nursed 5 different genotypes of boars. The study was conducted with a group of boars born in October and another group born in April creating a Fall and Spring replicate (n = 10 boars / treatment / season). The same sires were used to produce the experimental animals in each replicate.

Litters were weaned at 18 days of age and boars were managed according to normal industry practices through the nursery and finishing phases of production. The only exception was that boars were given 4 and 10 square feet of floor space per pig during the nursery and finishing phases, respectively. An important component of the experimental design was that boars from the small (6 pigs) and large litters (≥ 9 pigs) were co-mingled at weaning. This created a situation in which animals from both treatments were in same pens from weaning through finishing. At 5 months of age, boars were moved from pens and housed in individual crates. At 5.5 months of age boars were trained for collection with a dummy sow and collected once per week until they were at least two years of age.

Body weight and testicular size were measured at birth, weaning, and every three weeks thereafter for the duration of the study. The length of time and the number of training session required to train boars for collection was recorded. Total number of spermatozoa and semen quality estimates including computer-assisted motility analyses, head/tail morphology, acrosome morphology, and acrosin activity were recorded for each ejaculate. Finally, when boars were 10 months of age, equal numbers of spermatozoa from two, non-littermate, boars, one each treatment, were pooled to make heterospermic insemination doses. The pooled semen was used to breed sows ($\sim n=10$ per week per combination) and the paternity of the offspring was determined with DNA fingerprinting techniques. Analysis of variance procedures for repeated measures were used to analyze both categorical (Rosner, 1989) and continuous (Snedecor and Cochran, 1989) variables. The statistical model consisted of treatment (small or large litter), season (Fall or Spring), time, and all appropriate interactions.

Results and Discussion

The effect of neonatal litter size on body weight and testes size are shown in Figures 1 and 2. For these two variables, there was a season by treatment interaction. In essence, boars raised in small litters weighed more and have larger testicles than boars reared in large litters. However, the difference between the two treatments is much greater in the spring-born than the fall-born replicate. One interpretation of this is that under less than ideal conditions, as is the case for boars that mature during the summer environment (litters born in the spring), the advantage of being raised in a small litter increased exponentially.

Training for semen collection began when boars were 24 weeks of age (~ 155 days of age). There were no differences in the number of boars successfully being collected by the end of the training period (Figure 3). However, the overall training period was significantly reduced for boars from small (10 days) than large litters (30 days). These data indicate that boars allowed to nurse in litters of 6 pigs or less have larger testicles and greater libido than boars nursing in litters

of 9 or more pigs. The two observations probably are related. Boars raised in small litters had increased testicular size at relatively young ages compared with boars raised in large litters. One interpretation of these data is that testicular maturation and thus testosterone production began earlier. This, in turn, should result in attainment of puberty at a younger age as measured by their desire to mount a dummy sow and be collected. It is particularly impressive that all 20 boars that nursed in small litters mounted and were collected during the first 5 days of the training period. In contrast, only 5 of the 20 boars that nursed in large litters were trained for semen collection during the first 5 days of the training period.

Numbers of spermatozoa per ejaculate are also greater in boars raised in small versus large litters. It is important to remember that there is a 6 month difference in age between the fall-born and spring-born replicates, so these data have been analyzed and presented separately (Figure 3). In the spring-born replicate, boars raised in small litters produced about 10 billion more spermatozoa per ejaculate about 75% of the time (61 weeks) between 42 and 112 weeks of age. In contrast, for those born in the fall, boars raised in small litters consistently had 20 billion more spermatozoa per ejaculate than their counterpart raised in large litters beginning at 39 weeks of age until the end of the study ended when they were 2 years of age. From a practical perspective, the collective advantage of being raised in a small litter was an additional 200 insemination doses (600 billion spermatozoa) for boars born in the Spring and an extra 567 insemination doses (1700 billion spermatozoa) for boars born in the Fall. No significant differences among treatments in motility, morphology, acrosome morphology, acrosin activity, or capacitation status were observed (Table 1).

Finally, boars raised in small litters sired, on average, around 65% of the piglets resulting from heterospermic inseminations. Consequently, they appear to be more fertile than boars raised in large litters (Table 1). It is difficult to translate this relative advantage into differences in farrowing rate and numbers of pigs born alive at the present time. This is due to the fact that use of heterospermic inseminations and paternity testing of the resulting offspring is a relative assessment of fertility. In other words, it can be used to rank boars from most to least fertile. However, this technique cannot really establish whether the most fertile boar produces farrowing rates of 95% or 85%. Nevertheless, these data do indicate that regardless of what the actual fertility level, boars raised in small litters would be higher than those reared in large litters.

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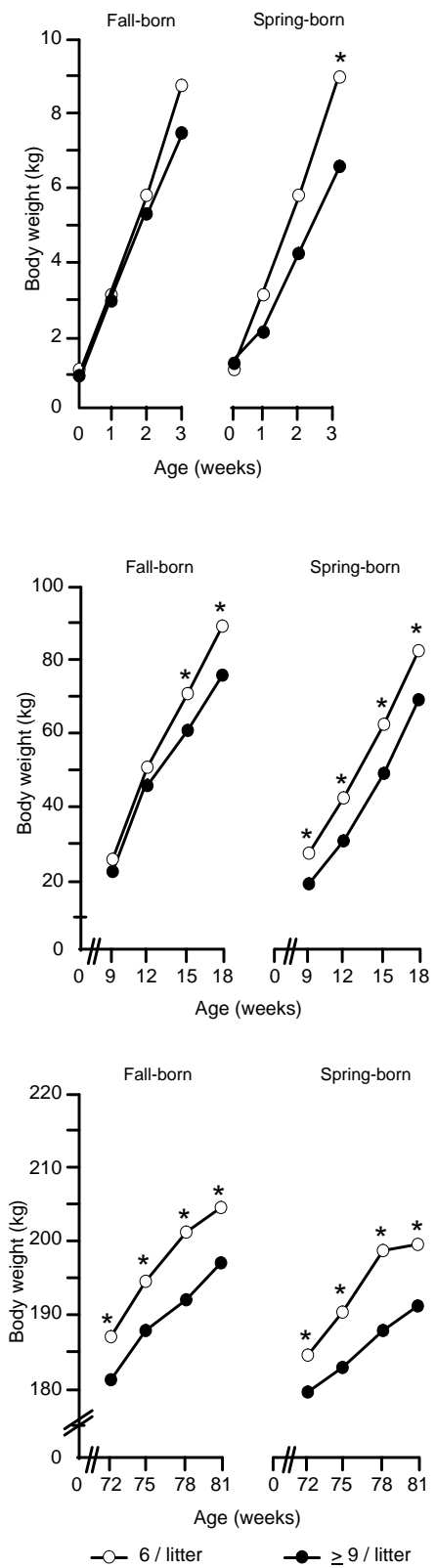


Figure 1. Effect of neonatal litter size on body weight. * Boars raised in litters of 6 weighed more compared with boars raised in litters of ≥ 9 ($P < 0.05$).

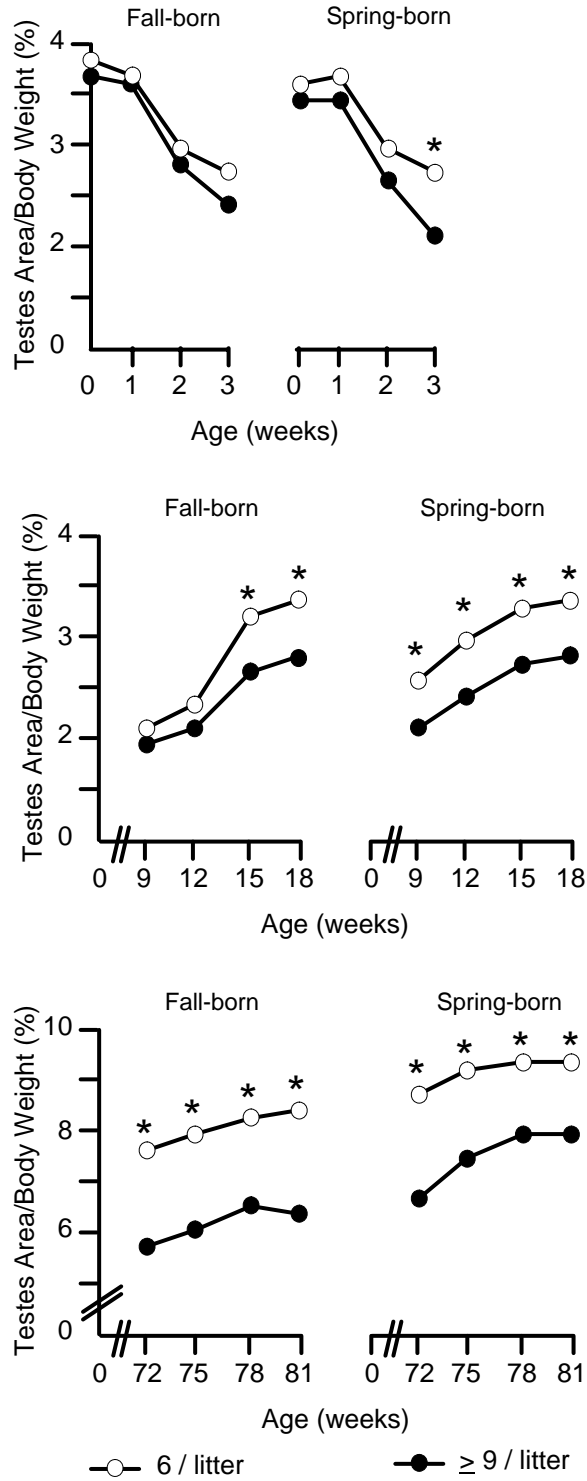


Figure 2. Effect of neonatal litter size on testes size. * Boars raised in litters of 6 had larger testes relative to body size compared with boars raised in litters of ≥ 9 ($P < 0.05$).

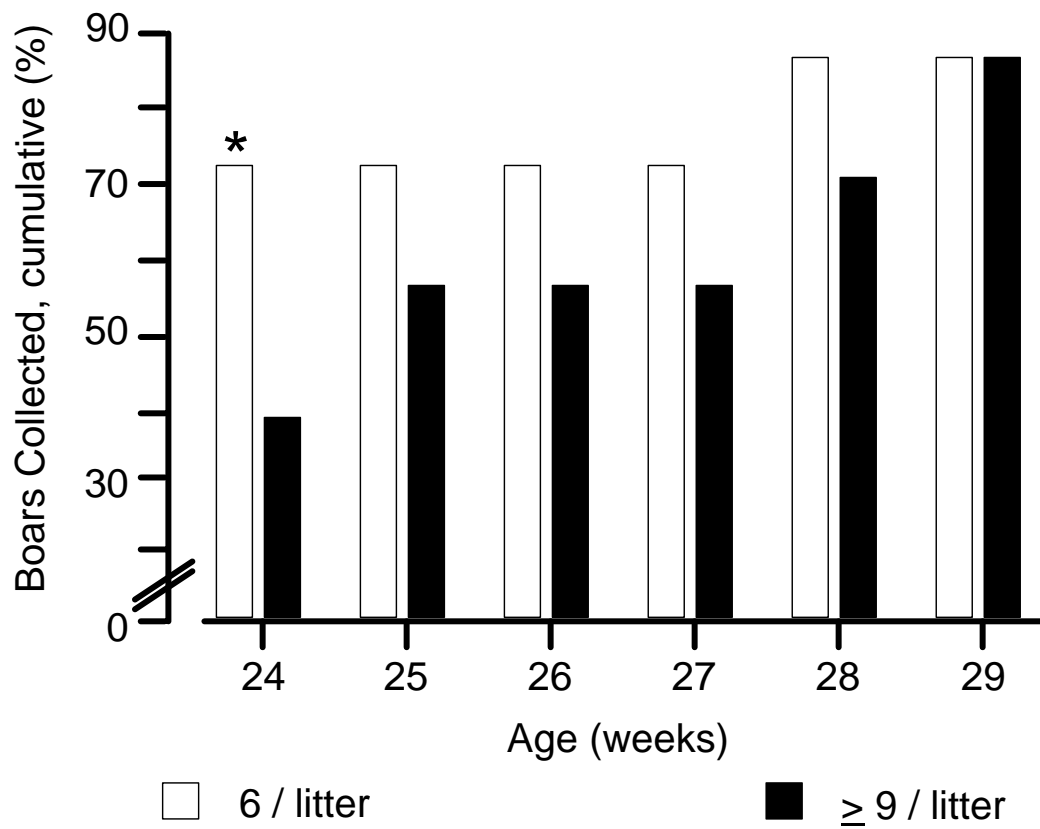


Figure 3. Effect of neonatal litter size on boars trained for semen collection on a dummy sow.
 *More boars raised in litters of 6 were trained to collect from a dummy sow compared with boars raised in litters of ≥ 9 ($P < 0.05$).

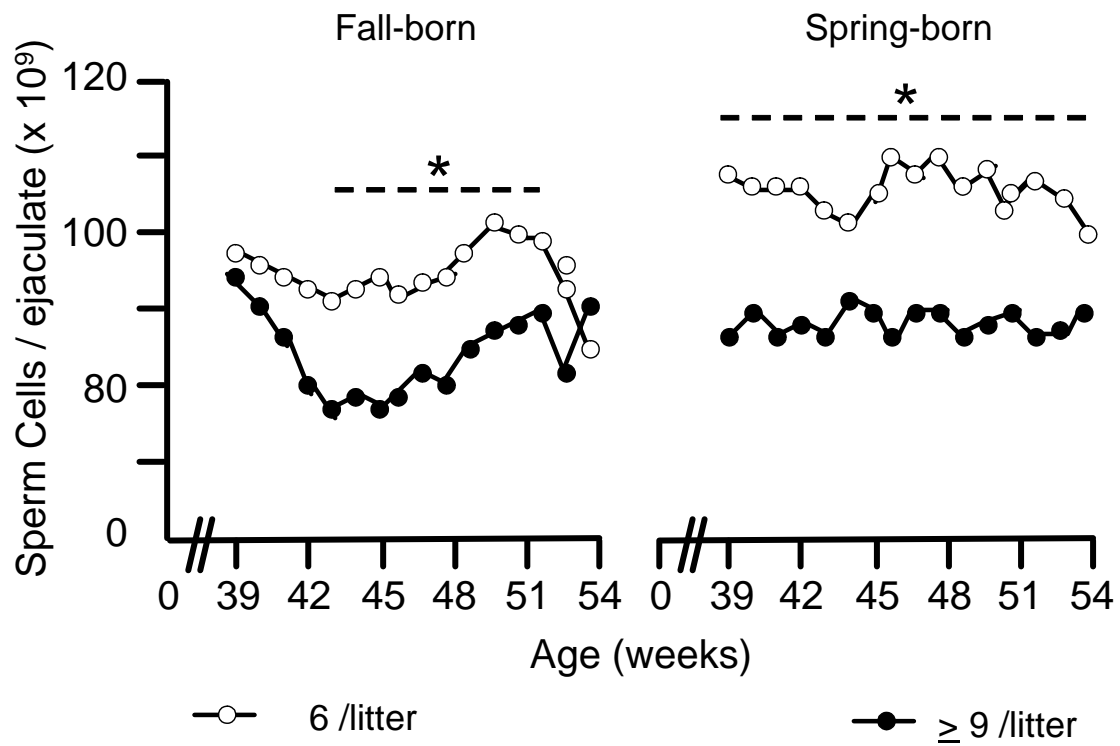


Figure 4. Effect of neonatal litter size on number of spermatozoa per ejaculate between 39 and 54 weeks of age. * Boars raised in litters of 6 produced ejaculates with more spermatozoa compared with boars raised in litters of ≥ 9 ($P < 0.05$).

Table 1. Semen Quality and Fertility Estimates from Boars raised in Small or Large Litters during Lactation (mean \pm s.e.).

Variable	Winter		Summer	
	6 / litter	≥ 9 / litter	6 / litter	≥ 9 / litter
Motile spermatozoa (%)	85.3 \pm 5.7	86.8 \pm 6.5	88.4 \pm 4.3	80.8 \pm 5.7
Normal morphology (%)	91.3 \pm 3.4	84.6 \pm 4.5	88.3 \pm 5.1	82.1 \pm 6.1
Normal acrosome morphology (%)	90.4 \pm 4.7	83.2 \pm 3.6	90.6 \pm 6.1	80.3 \pm 4.2
Acrosin activity (%)	95.3 \pm 4.5	90.3 \pm 3.2	92.8 \pm 4.1	93.4 \pm 4.6
Normal capacitation (%)	80.2 \pm 7.8	70.3 \pm 6.3	85.3 \pm 6.9	79.7 \pm 4.2
Seminal plasma proteins (relative units per ejaculate)	12.2 \pm 2.4	10.1 \pm 2.0	12.9 \pm 2.1	10.7 \pm 1.4
Proportion of piglets sired in heterospermic matings (%)*	67.3 \pm 5.7	32.7 \pm 5.4	63.5 \pm 4.8	36.5 \pm 4.3

* Boars raised in litters of 6 sired more pigs than boars raised in litters of ≥ 9 (P = 0.02)