

Increasing fertilization rate of boars: Influence of number and quality of spermatozoa inseminated¹

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ABSTRACT: The influence of the number and quality of spermatozoa inseminated on litter size in swine is examined in this paper. There is evidence to support the following observations. Litter size varies among boars when insemination doses contain the same numbers of spermatozoa. Increasing the number of sperm inseminated generally has a positive effect on the number of pigs born alive, especially between the range of 1 to 3×10^9 cells. The manner in which litter size responds to increasing the number of spermatozoa inseminated varies among boars. These relationships between the number of sperm inseminated and the resulting litter size provide credence to the idea that boars exhibit unique fertility patterns. These divergent fertility patterns probably reflect variability in the ability

of spermatozoa from different boars to fertilize ova. A number of semen quality tests have been developed to estimate the fertility of semen. Several of these have documented that increases in estimates in sperm quality are associated with increases in litter size. However, the relative effectiveness of each of these for determining the optimal number of spermatozoa that should be included in insemination doses remains to be elucidated. In summary, increasing the fertilization rate of boars should be possible by improving semen quality, increasing the number of spermatozoa inseminated, and adjusting using estimates of sperm quality to adjust number of sperm inseminated. However, the magnitude of changes in litter size resulting from these strategies is likely to vary considerably among boars.

Key Words: Boars, Litter Size, Spermatozoa

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Introduction

A spermatozoon that ultimately results in the birth of a live piglet is transported from the cervix through the uterus and enters the uterotubal junction (Hunter, 1990). At this point it attaches to an oviductal cell until it is released and, eventually, encounters an ovum after ovulation (Sirard et al., 1993). During interaction with the ovum, a spermatozoon must bind to and then move through the zona pellucida (Miller, 2000). This allows it to enter the perivitelline space and fuse with the plasma membrane, which eventually leads to the union of its genetic information with that of the ovum.

Presumably, a boar associated with a high farrowing rate and large litters consistently produces inseminations that contain sufficient numbers of spermatozoa capable of completing all of these tasks. Therefore, it

is physiologically reasonable to assume that there are two basic characteristics that are directly responsible for a boar's influence on litter size: the number of spermatozoa inseminated and the proportion of these that can successfully engage ova. The latter of these is often referred to as the quality of spermatozoa and can be estimated in a variety of different ways, including the monitoring of several physical and biochemical traits that allow spermatozoa to fertilize ova.

Whether or not increases in the number of spermatozoa can compensate for decreases in sperm quality is a question that is often posed but has yet to be resolved. This is due to the fact that most studies have examined the effect of these variables on litter size independently or have used a limited range of sperm numbers or quality ratings (Flowers, 1997; Xu et al., 1998). As a result, information about the effect of their interactions on litter size is limited. The purpose of this paper is to discuss recent information examining the relative importance of the number and quality of spermatozoa inseminated in terms of the manner in which they affect the ability of boars to produce live pigs.

Litter Size Based on Numbers of Spermatozoa Inseminated

Salisbury and Vandermark (1961) proposed a theoretical relationship between male fertility and semen

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characteristics that resembles a positive, asymptotic equation. Initially, when the number of spermatozoa is low, male fertility is poor. Increasing the number of sperm in the insemination dose results in a positive increase in fertility. The magnitude of this response gradually diminishes as the number of sperm cells inseminated is increased until a plateau is reached. At this point, insemination of additional numbers of spermatozoa does not affect male fertility. Salisbury and Vandermark (1961) referred to this relationship as the male fertility pattern or curve.

The physiological rationale for male fertility patterns is based on the concept that a finite number of competent spermatozoa must be present in the oviduct to successfully fertilize ova. Improvements in fertility are observed until this threshold level is achieved. After this point, there is not a concomitant increase in fertility when the number of sperm cells inseminated is increased because the population of spermatozoa in the oviduct needed to optimize fertilization has already been achieved.

It has been difficult to demonstrate the existence of fertility patterns for boars. Some of the problems encountered are physiological in nature, whereas others are associated with constraints inherent to the normal management systems in which boar fertility is assessed. Physiological limitations include the inability to store boar semen for long periods without significant reductions in its fertilizing capacity (Johnson et al., 2000) and the need to use large numbers of sperm cells in insemination doses compared with other livestock species (Flowers, 1998a). Both of these effectively limit the number of sows that can be inseminated from a single ejaculate. This, in turn, presents challenges in separating the effect of the boar on litter size from those of the sow, the production environment, and their interaction. Constraints associated with management systems include the high replacement rate for boars and the diverse nature of mating regimens used (Flowers, 1998b). These also make estimation of boar fertility difficult for reasons similar to those mentioned previously.

Nevertheless, there is evidence to support the concepts that boars exhibit fertility patterns based on the number of spermatozoa inseminated and that these differ among individuals. Two assumptions central to the existence of fertility patterns are that males differ in their fertility when the same number of sperm are inseminated and that increasing the number of spermatozoa inseminated increases fertility within some portion of the fertility curve. Johnson et al. (1981, 1982) conducted two comprehensive studies that clearly demonstrated that the fertility of boars is different when insemination doses contain equal numbers of sperm. In both of these studies, insemination doses of 3×10^9 spermatozoa were used to inseminate sows once between 12 and 24 h after estrus. Collectively, there were 3,300 sows used on at least 36 different farms. The range in mean litter sizes among the 24 boars evaluated

was between 8.8 and 12.2 pigs. These data provide evidence that boar fertility, as measured by litter size, can differ considerably among individuals when identical numbers of spermatozoa are inseminated. It is interesting to note that estimates used to evaluate sperm quality in these studies were high. The percentages of sperm cells that exhibited progressive forward motility and had normal morphology were greater than 71 and 91%, respectively. Consequently, it is unlikely that the observed differences in litter size were due to individual variations in these characteristics.

In contrast, Xu et al. (1998) failed to observe differences among boars but did show a positive effect of the number of spermatozoa inseminated on litter size. In their study, insemination doses of 2 or 3×10^9 spermatozoa were produced from ejaculates collected from six boars and used to inseminate sows three times during a 2-d estrus. A total of 444 sows housed on a single farm were used. The range in mean litter size was between 10.2 and 11.5 pigs when the insemination dose contained 3×10^9 spermatozoa and between 9.1 and 10.1 pigs when 2×10^9 sperm cells were used. There was a main effect of insemination dose on litter size, but no interaction between insemination dose and boar. Collectively, these studies provide evidence that several of the assumptions involving relationships between the number of sperm inseminated and litter size central to the fertility pattern concept of Salisbury and Vandermark (1961) are fulfilled.

However, what is lacking is the demonstration that the manner in which litter size changes in response to increasing sperm numbers differs among boars. Results from a study conducted with boars in a commercial stud provide evidence for this (Flowers, 2002). In this study, 40 to 45 ejaculates were collected from 200 crossbred boars (Duroc \times Pietran \times Large White, 2 to 4 yr of age) over a period of 2 yr. The sperm-rich fraction of each ejaculate was used to make insemination doses consisting of 1, 3, 5, 7, or 9×10^9 total spermatozoa in 80 mL of Androhep (Minitube of America, Verona, WI) semen extender. Only ejaculates with greater than 70% motility were extended and processed for delivery to farms. At least two crossbred sows (Landrace \times Large White \times Yorkshire) were bred with each insemination dose from each ejaculate. This resulted in a minimum of 75 sows bred with each insemination dose from each boar. Insemination doses were stored between 16 and 18°C and used within 48 h of collection during the study.

Sows received one insemination each day of estrus. It is important to note that sows used in the study were housed on four different farms that were located within a 40-km radius of one another. Each farm consisted of two 4,000-sow units under the same management. As a result, sows on a single farm were inseminated with semen from only 40 of the 200 boars during individual collection periods. Insemination doses were allocated to farms in a manner such that boars were used on the same farm and in combination with one another an equal number of times during the duration of the experi-

ment. Each farm had similar standard operating procedures, farrowing rates ($84.7 \pm 5.1\%$), and number of pigs born alive (11.2 ± 0.6) before and during the study.

Due to the experimental design of this study, boar and farm effects on litter size were partially confounded during any individual collection period. However, due to the allocation process used for boars, it was possible to estimate the effects of boars and farms. This was done with a repeated measures analysis within an incomplete block design using mixed model methodologies (Cochran and Cox, 1957; Littell et al., 1996). The incomplete block in the model consisted of the combinations of boars and farms that occurred during the study due to the allocation procedures. The statistical model included block, farm, boar, number of sperm inseminated, week, and appropriate interactions. When significant interactions between boar and number of sperm inseminated were present, differences among the number of sperm inseminated within boars were determined.

Analyses of litter size data from this study revealed several different fertility patterns as the numbers of sperm inseminated were increased from 1 to 9×10^9 . One pattern was similar to the asymptotic relationship proposed by Salisbury and Vandermark (1961) in which there was an increase in litter size initially before a plateau was reached. This type of relationship was present in 133 of 200 boars. In contrast, a second fertility pattern was linear. This type of pattern occurred in 32 of 200 boars. It is possible that the linear pattern observed for some boars was simply a function of the experimental design. Their fertility pattern may have actually reached a plateau if more than 9×10^9 spermatozoa were inseminated. If this speculation is correct, then the range from 1 to 9×10^9 sperm cells may represent the portion of their fertility curve in which litter size increases as the number of sperm inseminated increases. Finally, there were 35 boars for which a significant farm \times boar interaction was observed for the relationship between litter size and number of spermatozoa inseminated. The interaction occurred because the fertility pattern for these boars reached a plateau on some farms but remained linear on others. Because the farms on which this occurred differed among boars, explanations for this interaction were not apparent.

In addition, there were distinct differences among boars in the shape of their individual fertility patterns. For those that reached a plateau, individual boar variations occurred in both the insemination dose at which the plateau occurred and the mean litter size that resulted. Similarly, the slope of the linear responses differed among boars. Selected examples of the variation in the relationship between litter size and number of spermatozoa inseminated are illustrated in Figure 1.

In summary, based on the results from these four studies, three general conclusions concerning the relationships among litter size, individual boars, and the number of sperm inseminated seem warranted. First, litter size can differ among boars when equal numbers

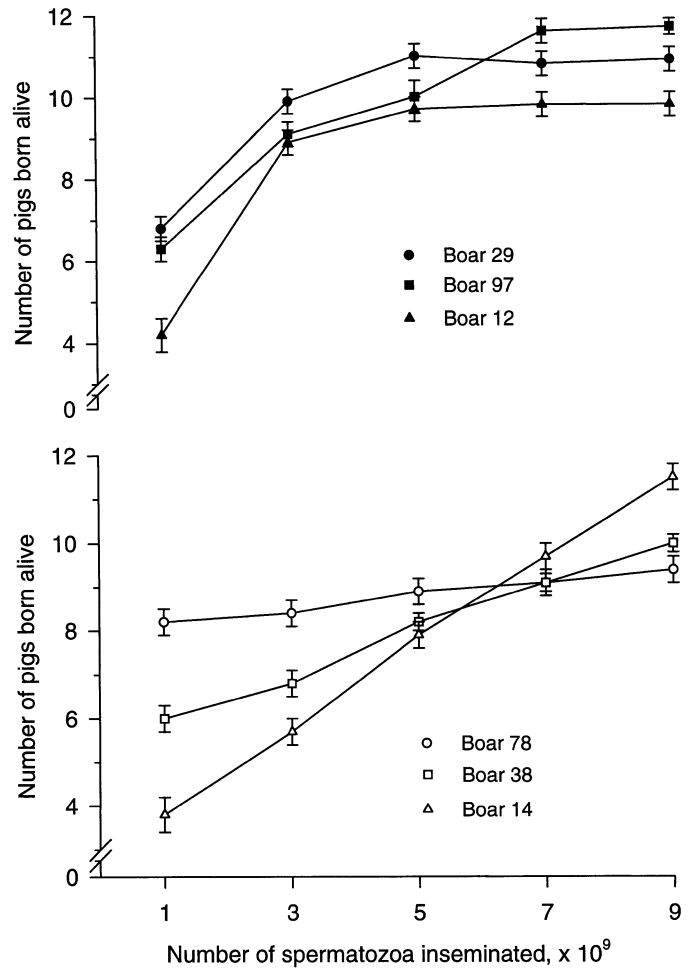


Figure 1. Representative examples of different relationships between number of pigs born alive (mean \pm SEM) and number of spermatozoa inseminated for boars in a commercial stud. The top panel illustrates examples of variation among boars with fertility patterns that reached a plateau. The lower panel illustrates examples of variation among boars with fertility patterns that were linear.

of spermatozoa are inseminated. Second, increasing the number of sperm cells in the insemination dose results in an increase in litter size for some boars. Finally, the magnitude of the response in litter size to an increase in sperm numbers is not the same for all boars. From a practical perspective, these observations provide some important opportunities for improving litter size via boar management on commercial swine operations. Due to the variation observed among boars, it is reasonable to speculate that development of practical and cost-effective methods for determining the optimal number of spermatozoa for insemination doses would have a positive effect on litter size. For example, it would be interesting to know whether spermatozoa from boars that exhibited a linear fertility pattern were different from those with a pattern that reached a plateau in terms of key morphological and biochemical characteristics that are related to their ability to successfully

Table 1. Summary of selected measurements used to estimate sperm quality in boars^a

Measurement	Rationale	References
Florescent stains	Some florescent dyes (Hoechst 33258) enter if the membrane is damaged and stain only dead cells. Others (SYBR-14) enter cells with a membrane potential and stain only live cells.	Johnson et al., 1996
Macroscopic morphology	Proportion of sperm with visual morphological defects is inversely related to ability to fertilize eggs in vitro.	Xu et al., 1998
Hypoosmotic swelling test	When exposed to hypoosmotic conditions the head of sperm with damaged membranes increase in size (begin to swell). Proportion of these sperm in an ejaculate is inversely related to fertility.	Vazquez et al., 1997
Computer-assisted motility analyses	Number and characteristics of motile sperm using computer-assisted semen analysis (CASA) are correlated with fertility.	Holt and Medrano, 1997
Hemizona binding assay	Oocytes are bisected and the number of sperm from different boars that bind to each half is correlated with their fertility.	Fazeli et al., 1995
Sperm plasma membrane protein profile	Proportion of three plasma membrane proteins present on sperm cells is positively correlated with fertility.	Ash et al., 1994 Berger et al., 1996
Sperm chromatin structure	When exposed to acidic conditions and stained with metachromatic dyes, double-stranded DNA emit a green	Evenson et al., 1994
fluorescence, but single-stranded DNA	(damaged) emit a red fluorescence. Ratio of green to red sperm is correlated to fertility of a boar.	

^aAdapted from Flowers (1997).

fertilize ova. If these differences are present and can be identified prospectively, then appropriate adjustments in the number of spermatozoa in insemination doses on an individual basis could be performed.

Estimates of Semen Quality and Their Relationship to Litter Size

A number of procedures have been used to assess semen quality. Selected examples of these are summarized in Table 1 along with a brief synopsis of the rationale for each one. For the purpose of understanding how these tests might be used to identify boars with superior fertility, it is useful to divide them into two categories. One category includes tests that focus on determining the proportion of spermatozoa in an ejaculate that possess certain characteristics that have been shown to be involved with a sperm cell's ability to fertilize ova. In essence, for these tests, the assumption is made that the proportion of spermatozoa with the property being measured is either positively or negatively related, depending on the characteristic, to the number of pigs born alive when the boar is used for breeding. The majority of techniques outlined in Table 1 fit into this classification, including evaluations of normal mor-

phology (Xu et al., 1998), sperm membrane swelling (Vazquez et al., 1997), viability (Johnson et al., 1996), binding to oocytes (Fazeli et al., 1995), and sperm chromatin structure (Evenson et al., 1994).

Two of these tests, normal morphology and sperm chromatin structure, merit additional consideration because their usefulness for determining the effect of individual boars on litter size has been examined. The proportion of spermatozoa with normal morphology explained a large part ($R^2 = 0.59$) of the variance in litter size in the study of Xu et al. (1998). As mentioned earlier, this study involved semen from six boars that was used to breed 444 sows on a single farm. What is impressive about this study is that a single measure of semen quality accounted for more than one-half of the variation in litter size in a commercial setting. Consequently, these results should be encouraging for others attempting to develop prospective methodologies for estimating boar fertility.

Studies evaluating the influence of sperm chromatin structure used heterospermic inseminations composed of equal amounts of spermatozoa from two boars (Evenson et al., 1994). One of the boars used in the mixture historically produced a high number of pigs born alive, and his counterpart routinely sired small litters. The

ratio of sperm with normal DNA to those with damaged DNA was more than 90% effective in predicting the paternity of the pigs resulting from the heterospermic inseminations. Traditionally, heterospermic inseminations have been viewed as a way to minimize the influence of the sow and the production environment on estimates of male fertility (Dziuk, 1996) because spermatozoa from different individuals literally compete with one another simultaneously during fertilization. However, it is important to acknowledge that fertility studies conducted in this way are comparative, or qualitative, in nature. Their results can be used to rank boars in terms of fertility, but it is difficult to translate the rankings into a mean value for the number of pigs born alive or other measures of fertility. As a result, additional work is necessary in order to equate the proportion of spermatozoa with normal DNA in an ejaculate with actual litter size data resulting from homo-spermic inseminations.

The second category for semen quality tests involves procedures that quantify the degree to which individual spermatozoa exhibit a given characteristic, which, also, is involved with fertilization. For these tests, the assumption is made that sperm cells express varying levels of the characteristic. Boars whose spermatozoa exhibit increased amounts produce larger litters compared with individuals whose sperm cells have low values. Computer-assisted motility analyses (Holt and Medrano, 1997) and the protein composition of sperm membranes (Berger et al., 1996) are examples of these types of semen quality tests. In a series of studies conducted on commercial swine farms, changes in the velocity of the motion of spermatozoa during an *in vitro* incubation period explained 20% of the normal variation in litter size (Holt et al., 1997). Furthermore, boars whose spermatozoa exhibited increased straight-line velocity and track linearity were associated with large litter sizes. Similarly, concentrations of three proteins isolated from the plasma membrane were positively correlated ($r = 0.38$ to 0.53) with the ability of sperm from individual boars to bind to egg membranes (Ash et al., 1994) and the proportion of pigs they sired when heterospermic inseminations were used (Berger et al., 1996). Consequently, both of these measures of semen quality seem to be good candidates for explaining some of the variation in fertility patterns among boars.

However, what is difficult to decipher, at the present time, is which of the characteristics outlined in Table 1 simply reflect shifts along an individual fertility pattern compared with those that are involved with changing the level at which the plateau occurs or the slope of a linear response. Shifts along a given fertility curve optimize litter size for individual boars. In contrast, increasing the litter size at which the maximal response is achieved has the potential, in theory, to improve the fertility of boars regardless of their inherent pattern.

Another question that awaits resolution is the degree of dependency inherent among the characteristics measured by each of these procedures. For example, it is

reasonable to speculate that spermatozoa judged to be nonviable by fluorescent stains probably also are non-motile. In this situation, viability estimates should have a high positive correlation with motility and results from either procedure should be able to identify boars that produce small or large litters. In contrast, it is conceivable that sperm cells with excellent morphology could be deficient in the protein composition of their plasma membranes. If this were to occur, then a fertility test based on normal morphology would predict the production of large litters, whereas its counterpart using sperm binding assays would not. In order to address these possibilities, studies that examine the effectiveness of using several of these tests in sequence are needed.

Interactions Between Number and Quality of Spermatozoa Inseminated

As mentioned previously, whether or not changes in the number of spermatozoa inseminated can compensate for variability in sperm quality is an interesting question that has important implications for increasing litter size in pigs. If one accepts the argument that a critical number of competent spermatozoa in the oviduct are required to optimize fertility, then it seems reasonable that this critical number could be achieved via various combinations of the quality and number of spermatozoa inseminated. However, in order for this to be realistic physiologically, three things must occur: 1) increases in the number of spermatozoa inseminated should increase the number of sperm entering the oviduct, 2) entry into and retention of spermatozoa in the oviducts need to differ among boars, and 3) transport and entry of spermatozoa into the oviduct should be independent of sperm quality.

There is evidence for the existence of each of these events. First, Baker and coworkers (1968) inseminated gilts with 1, 5, or 10×10^9 spermatozoa and determined the number remaining in the oviducts 12 to 16 h later. Even though the recovery rate was low, a positive relationship between the number of spermatozoa inseminated and the number of sperm cells recovered from the oviducts was present. Second, significant effects of boars on the number of spermatozoa recovered from the lower isthmus of the oviduct around ovulation have been observed (Mburu et al., 1996). Differences among boars varied from 461 to 1,972 spermatozoa. Finally, a series of experiments by First et al. (1968) demonstrated that dead spermatozoa are transported and enter the oviduct with efficiencies similar to those of live sperm. Consequently, based on what is known about the dynamics of spermatozoa in the oviduct, adjusting sperm numbers at insemination based on some assessment of their quality seems to be a physiologically plausible way to increase litter size for some boars.

Even though critical evaluations of this strategy involving the birth of live pigs are lacking, results from a field study conducted by Johnson et al. (1988) are

encouraging. In this study, insemination doses consisting of 3×10^9 spermatozoa were used to breed sows between 0 and 72 h after collection. In contrast, if the collection-to-insemination interval was greater than 72 h, 6×10^9 spermatozoa were inseminated. No effect of semen age on litter size was observed. Although a contemporary treatment consisting of insemination doses with 3×10^9 , aged (> 72 h) spermatozoa was not included in the design, it is commonly accepted that quality estimates and fertility of fresh semen decrease significantly as storage time increases. This is particularly relevant considering that the semen extenders used in their study, BTS and MR-A, are classified as short-term extenders because fertility of semen is reduced significantly after 3 d of storage (Johnson et al., 2000). Consequently, the speculation that sperm numbers can be adjusted to compensate for reduced semen quality seems to be supported indirectly by the results from field studies.

However, there are several caveats associated with this strategy that should be considered. The best estimates of sperm quality upon which adjustments should be made are not known. Nevertheless, it is logical to speculate that tests that are categorical in nature, such as the percentage of spermatozoa with normal morphology, probably are the best candidates to study initially. Based on the studies discussed previously, insemination of additional numbers of normal sperm should result in additional normal sperm in the oviduct. However, as mentioned previously, estimates based on a sequence of several different tests probably deserve equal consideration.

In addition, it is unlikely that quality adjustments for the number of spermatozoa inseminated would have similar effects for all boars in terms of increasing litter size. For example, boars whose fertility pattern reached a plateau at 3×10^9 spermatozoa would not be expected to elicit the same response as their counterparts with linear patterns or those with patterns that reached a plateau at 7×10^9 spermatozoa. Presumably, the level at which the plateau occurs reflects differences among boars in terms of the critical number of competent spermatozoa in the oviduct needed for optimal fertilization. As a result, it is reasonable to speculate that this strategy for improving litter size is better suited for boars with high rather than low requirements.

Implications

The number and quality of spermatozoa inseminated determine the boar's impact on litter size. The relationship between these traits and litter size tends to be unique for boars and is best described as a fertility pattern or curve. Increasing litter size on operations using natural service is most likely to result from improvements in the quality of spermatozoa. This is due to the fact that most boars contain sufficient spermatozoa in their ejaculate to maximize sperm numbers in the oviduct. In contrast, on operations using artificial

insemination, increasing litter size probably can occur in several different ways, including improving semen quality, increasing the number of spermatozoa inseminated, and using estimates of sperm quality to adjust number of sperm inseminated. However, the magnitude of changes in litter size resulting from these strategies is likely to vary considerably among boars.

Literature Cited

- Ash, K. L., T. Berger, C. M. Horner, and T. R. Famula. 1994. Boar sperm plasma membrane protein profile: correlation with the zona-free hamster ova assay. *Theriogenology* 42:1217–1226.
- Baker, R. D., P. J. Dziuk, and H. W. Norton. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. *J. Anim. Sci.* 27:88–93.
- Berger, T., D. L. Anderson, and M. C. T. Penedo. 1996. Porcine sperm fertilizing potential in relationship to sperm functional capacities. *Anim. Reprod. Sci.* 44:231–239.
- Cochran, W. G., and G. M. Cox. 1957. *Experimental Designs*. 2nd ed. John Wiley & Sons, New York.
- Dziuk, P. J. 1996. Factors that influence the proportion of offspring sired by a male following heterospermic insemination. *Anim. Reprod. Sci.* 43:65–88.
- Evenson, D. P., L. Thompson, and L. Jost. 1994. Flow cytometric evaluation of boar semen by the sperm chromatin structure assay as related to cryopreservation and fertility. *Theriogenology* 41:637–651.
- Fazeli, A. R., C. Holt, W. Steeneg, M. M. Bevers, W. V. Holt, and B. Colenbrander. 1995. Development of a sperm hemizona binding assay for boar semen. *Theriogenology* 44:17–27.
- First, N. L., R. E. Short, J. B. Peters, and F. W. Stratman. 1968. Transport and loss of boar spermatozoa in the reproductive tract of the sow. *J. Anim. Sci.* 27:1037–1040.
- Flowers, W. L. 1997. Management of boars for efficient semen production. *J. Reprod. Fertil. Suppl.* 52:67–78.
- Flowers, W. L. 1998a. Artificial insemination in animals. In: E. Knobil and J. D. Neill (ed.) *Encyclopedia of Reproduction*. p 296. Academic Press, San Diego, CA.
- Flowers, W. L. 1998b. Insemination programs for swine to increase fertility. *J. Anim. Sci.* 76(Suppl. 3):39–46.
- Flowers, W. L. 2002. Fertility patterns of boars in a commercial stud. *Anim. Reprod. Sci.* (In press).
- Holt, C., W. V. Holt, H. D. M. Moore, H. C. B. Reed, and R. M. Curnock. 1997. Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations. Results of two fertility trials. *J. Androl.* 18:20–31.
- Holt, W. V., and A. Medrano. 1997. Assessment of boar sperm function in relation to freezing and storage. *J. Reprod. Fertil. Suppl.* 52:213–222.
- Hunter, R. H. F. 1990. Fertilization of pig eggs *in vivo* and *in vitro*. *J. Reprod. Fertil. Suppl.* 40:211–226.
- Johnson, L. A., J. G. Aalbers, C. M. T. Willems, and W. Sybesma. 1981. Use of boar spermatozoa for artificial insemination. I. Fertilizing capacity of fresh and frozen spermatozoa in sows on 36 farms. *J. Anim. Sci.* 52:1130–1136.
- Johnson, L. A., J. G. Aalbers, C. M. T. Willems, J. H. M. Rademaker, and C. E. Rexroad, Jr. 1982. Use of boar spermatozoa for artificial insemination III. Fecundity of boar spermatozoa stored in beltsville liquid and kiev extenders for three days at 18 C. 1982. *J. Anim. Sci.* 54:132–136.
- Johnson, L. A., J. G. Aalbers, and H. J. G. Grooten. 1988. Artificial insemination in swine: Fecundity of boar semen stored in Beltsville TS (BTS), Modified Modena (MM) or MR-A and inseminated on one, three and four days after collection. *Zuchthygiene (Berl.)* 23:49–55.
- Johnson, L. A., W. M. C. Maxwell, J. R. Dobrinsky, and G. R. Welch. 1996. Staining sperm for viability assessment. *Reprod. Domest. Anim.* 31:37–47.

- Johnson, L. A., K. F. Weitze, P. Fiser, and W. M. C. Maxwell. 2000. Storage of boar semen. *Anim. Reprod. Sci.* 62:143–172.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® System for Mixed Models. SAS Inst. Inc., Cary, NC.
- Mburu, J. N., S. Einarsson, N. Lundheim, and H. Martinez Rodriguez. 1996. Distribution and membrane integrity of spermatozoa in pig oviduct in relation to ovulation. *Reprod. Domest. Anim.* 31:57–68.
- Miller, D. J. 2000. A sperm's perspective of fertilization. *Proc. Am. Soc. Anim. Sci.*, 1999. Available at <http://www.asas.org/jas/symposia/proceedings/0918.pdf>. Accessed Aug. 1, 2001.
- Salisbury, G. W., and N. L. Vandermark. 1961. *Physiology of Reproduction and Artificial Insemination in Cattle*. Freeman and Co., San Francisco, CA.
- Sirard, M. A., A. Dubuc, D. Bolamba, Y. Zheng, and K. Coenen. 1993. Follicle-oocyte-sperm interactions *in vivo* and *in vitro* in pigs. *J. Reprod. Fertil. Suppl.* 48:3–16.
- Vazquez, J. M., E. A. Martinex, P. Martinez, C. Garcia-Artiga, and J. Roca. 1997. Hypoosmotic swelling of boar spermatozoa compared to other methods for analyzing sperm membrane. *Theriogenology* 47:913–922.
- Xu, X., S. Pommier, T. Arbov, B. Hutchings, W. Sotto, and G. R. Foxcroft. 1998. In vitro maturation and fertilization techniques for assessment of semen quality and boar fertility. *J. Anim. Sci.* 76:3079–3089.