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# Relationship between sperm quality traits and field-fertility of porcine semen

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**An investigation involving seven boars, active in artificial insemination, and 1,350 multiparous sows was conducted at a private farm and aimed at examining the relationship between sperm quality traits and boar fertility in terms of farrowing rate and litter size. This experiment was done for 6 months. The semen samples were evaluated for subjective sperm motility and concentration. Ejaculates with at least 1 × $10^8$ sperm/mL and 70% sperm progressive motility were extended with a commercial medium to 30 × $10^6$ sperm/mL and used for artificial insemination (AI). AI dose was 100 mL semen containing 3 × $10^9$ spermatozoa. Aliquots of diluted semen were assessed for live morphologically normal spermatozoa (LMNS, eosin-nigrosin stain exclusion assay) and sperm chromatin instability (SCI, acridine orange assay). Farrowing rates according to different boar sperm varied ($p < 0.001$) from 59.3 to 88.92%. The mean values of LMNS (47.2～76.5%) and SCI (0.16～4.67%) differed significantly among boars. LMNS (r = 0.79, $p < 0.05$) and SCI (r = −0.90, $p < 0.02$) accounted for 62.2 and 81.7% of the variability in farrowing rates, respectively. After the combination of sperm traits, the relationship between percentage of LMNS with stable chromatin structure and farrowing rate was significant (r = 0.86, $p < 0.05$). The number of live piglets per parturition was not significantly correlated with sperm quality attributes. In conclusion, boar fertility after AI with freshly diluted semen can be predicted based on the evaluation of sperm morphology and chromatin integrity.**

**Keywords:** acridine orange, chromatin integrity, field fertility, porcine semen, sperm motility

## Introduction

Artificial insemination (AI) contributes highly to the development of worldwide swine production, making the impact of the male in reproductive efficiency of the pig herds more crucial [14]. In commercial farms, routine examination of boar semen is performed aiming to predict the male's fertility. In general terms, boar sperm assessment includes the determination of concentration, viability, motility and spermatozoa's morphology. In addition, a number of semen manipulation techniques are available to improve the spermatozoa's fertilizing ability [15]. Since fertilization is a complex process involving a huge number of events, fertility research must not only devise more predictive laboratory tests, but also properly combine the results of different assays aiming to predict male fertilizing ability, as spermatozoa should satisfy many requirements for successful fertilization [17]. However, studies in domestic animals showed that these semen characteristics were often not significantly correlated to fertility, while the most valid assessment of boar semen quality is to obtain viable pregnancies and normal offspring following AI. In some cases, characteristics such as having a normal sperm head or tail morphology [10] and progressive forward motility [9,12] had a positive relationship with boar fertility, but not in other cases [24]. Furthermore, sperm DNA chromatin integrity has recently been studied as a cause of male subfertility is [1]. Sperm chromatin is a compact formation which differs from somatic cells in structure and composition. It is the most tightly condensed eukaryotic DNA, at least six times more condensed than the DNA in mitotic chromosomes [22]. Moreover, a high incidence of semen nuclear chromatin instability is associated with reduced breeding efficiency of the boars [8]. Previous studies reported that the evaluation of classical seminal parameters, under commercial conditions, allows the identification of ejaculates with poor fertility potential, but does not have high efficiency in predicting field fertility. We hypothesize that the combined evaluation with a non routine assessed sperm characteristic could present a higher predictive value. Because of no trials have evaluated the predictive value of combining sperm morphology parameters and chromatin instability on

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farrowing rate and litter size, the aim of the present study was to investigate the relationship between these sperm quality traits and boar field fertility.

## Materials and Methods

### Chemical reagents

Sperm nuclear chromatin integrity was evaluated by acridine orange dye (Sigma Aldrich, USA). Sperm morphology was evaluated by eosin (Sigma Aldrich, USA) - nigrosin stain (Merck, Germany).

### Semen collection and processing

Seven boars (8～26 months old) were used in this study, which was conducted in a commercial farm, over a period of six months. The boars were housed under the same conditions, according to European Commission Directive for pigs' welfare (http://ec.europa.eu/food/animal/welfare/farm/pigs_en.htm), and were routinely employed for AI. A sperm-rich fraction of ejaculates was collected by the 'gloved hand' technique and was assessed in the farm sperm laboratory. Ejaculates with sperm concentrations greater than $1 \times 10^8$ sperm/mL and 70% sperm progressive motility were extended with a commercial medium (M III; Minitüb, Germany) to $30 \times 10^6$ sperm/mL and used for AI.

### Evaluation of semen parameters

Aliquots of diluted semen were assessed for live morphologically normal spermatozoa (LMNS) by eosin stain exclusion assay and sperm chromatin instability by the acridine orange test (AOT), as described by Chalah and Brillard [6] and Tejada *et al*. [20], respectively. Spermatozoa with normal head shape, tail and no cytoplasmic droplets were classified as spermatozoa with normal morphology. AOT measures the susceptibility of sperm nuclear DNA to acid-induced denaturation *in situ* by quantifying the metachromatic shift of acridine orange fluorescence from green (native DNA) to red (denatured DNA). Acridine orange dye stains normal double-stranded DNA green and denatured single-stranded DNA red [19]. Slides were examined under a fluorescence microscope (BX41; Olympus, Japan) equipped with a digital camera and image analyzer computer software (U-TV 0.35. C-2, Imaging Software System GmbH for Windows; Olympus, Japan). Two hundred spermatozoa per slide were assessed in ten different optical areas for determination of percentage of sperm chromatin instability (SCI).

### Artificial insemination

In this study 1,350 multiparous sows were involved. Each sow was checked for heat twice a day. Once detected in standing heat, sows were further stimulated by back pressure and inseminated twice, 12 h and 24 h after the standing heat. Each AI dose was 100 mL of semen containing $3 \times 10^9$ spermatozoa.

### Fertility data analysis

The farrowing rate (% of bred sows that farrowed) and the litter size (total number of piglets born alive in a litter) of the pig farm were recorded and analyzed.

### Statistical analysis

Data were analysed according to the statistical procedures described by Petrie and Watson [16] in addition to a statistical package (SPSS 11.01.1; SPSS, USA). One-way ANOVA models were used to test the effect of boar on sperm quality traits and number of live piglets per parturition. If ANOVA showed significant differences among means (main effects), a planned multiple comparison of means was examined by Duncan's multiple range test. The variation among farrowing rates of boars was examined by chi-squared analysis. Sperman's rank correlation coefficients were used to examine the relationships between sperm traits and boar fertility. Differences were considered significant at $p < 0.05$.

## Results

Table 1 shows the *in vivo* performance of tested boars. Farrowing rates following AI with different tested boar sperm varied ($p < 0.001$) from 59.3 to 88.92%. However, the number of live piglets produced by AI with different boar sperm did not differ significantly among boars.

The mean values of LMNS (47.2～76.5%) and SCI (0.16～4.67%) differed significantly among boars (Table 2). LMNS (r = 0.79, $p < 0.05$) and SCI (r = −0.90, $p < 0.02$) accounted for 62.2 and 81.7% of the variability in farrowing rates, respectively.

**Table 1.** Chi-squared analysis for the effect of boar on farrowing rate and one-way ANOVA for the effect of boar on number of live piglets per parturition

| Boar | Farrowing rate (%) | Live piglets per parturition (mean ± SE) |
|---|---|---|
| 1 | 88.92 (369/415)$^{a}$ | 11.21 ± 1.22 |
| 2 | 82.05 (64/78)$^{ab}$ | 10.61 ± 1.34 |
| 3 | 75.88 (151/199)$^{be}$ | 10.56 ± 1.50 |
| 4 | 59.30 (51/86)$^{c}$ | 10.60 ± 1.32 |
| 5 | 83.57 (117/140)$^{ab}$ | 9.30 ± 1.01 |
| 6 | 74.00 (35/50)$^{bcd}$ | 12.25 ± 1.99 |
| 7 | 65.91 (19/44)$^{ce}$ | 12.00 ± 1.17 |

$^{a,b,c,d,e}$Values with different letters in the same column are significantly different at $p < 0.05$ ($\chi^2 = 3.90$) or $p < 0.001$ ($\chi^2 = 10.83$). Figures in parentheses denote number of inseminated sows/number of parturient ones.

**Table 2.** One-way ANOVA and Duncan's multiple range test for inter-boar variation on sperm traits (%) of fresh semen ejaculates

| Boars | Live morphologically normal sperm* | Sperm with chromatin instability† |
|---|---|---|
| 1 | $76.50 \pm 3.97^{ac}$ | $0.16 \pm 0.05^{a}$ |
| 2 | $71.50 \pm 3.83^{ac}$ | $0.60 \pm 0.09^{bd}$ |
| 3 | $48.50 \pm 5.12^{b}$ | $0.55 \pm 0.09^{b}$ |
| 4 | $47.20 \pm 7.40^{bd}$ | $3.78 \pm 1.12^{ce}$ |
| 5 | $74.29 \pm 2.80^{a}$ | $0.92 \pm 0.13^{d}$ |
| 6 | $64.00 \pm 4.28^{ad}$ | $2.09 \pm 0.83^{bcd}$ |
| 7 | $62.53 \pm 1.89^{cd}$ | $4.67 \pm 0.82^{e}$ |

$^{a,b,c,d,e}$Means ± SE with different letters in the same column are significantly different at $^{*}p < 0.01$ and $^{\dagger}p < 0.05$.

**Table 3.** Sperman's rank correlation coefficients (r) between sperm quality traits and fertility parameters (number of pairs = 8)

| Fertility parameters | LMNS | SCI | LNS DNP complex |
|---|---|---|---|
| Farrowing rate | $r = 0.79^{*}$ | $-0.90^{\dagger}$ | $0.86^{*}$ |
| Live litter size | $r = 0.12^{NS}$ | $-0.44^{NS}$ | $0.13^{NS}$ |

NS: non-significant, LMNS: live morphologically normal sperm, SCI: sperm with chromatin instability, LNS DNP complex: live normal sperm with stable deoxyribonucleic acid complex. $^{*}p < 0.05$, $^{\dagger}p < 0.02$.

When sperm traits were combined, the relationship between the percentage of LMNS with stable chromatin structure and farrowing rate was significant ($r = 0.86$, $p < 0.05$). Conversely, the number of live piglets per parturition was not significantly correlated with sperm quality attributes (Table 3).

## Discussion

The results of this study showed that the farrowing rate differed among boars used for AI, but not the litter size. The weak relation between these two parameters (farrowing rate and litter size) were in agreement with the findings of previous studies [14], indicating that these characteristics may be affected differently by the boars' semen quality. Moreover, the high farrowing rate is not always relative to high litter size, as observed in boar number 5 and number 7 of the present study. This suggests that farrowing rate and litter size are important indicators for herd's productivity, but cannot evaluate boar's fertility without the quantification and assessment of sperm characteristics. On the other hand, Gadea *et al*. [11] reported that the evaluation of seminal parameters allows the identification of ejaculates with low fertility potential, but could not efficiently predict field fertility. The aforementioned causes support the theory that probably no classical sperm parameters could predict the farrowing rate and litter size.

Our results showed significant differences of live morphologically normal sperm among boars, in agreement with a previous study [3] in which some boars were excluded from the experiment due to the high percentage of sperm morphological abnormalities. Concerning the relationship between sperm morphology and field fertility, previous studies reported significant correlations in bulls [4,13]. Alm *et al*. [3] concluded that boar semen morphology has a limited positive predictive value for field fertility, while it could be useful for the routine examination of young boars before they are placed into the regular AI collection scheme. Although an inverse correlation was observed between fertility and morphological abnormalities, Waberski *et al*. [21] addressed that morphology could facilitate boar selection for AI because it provided information for the efficiency of spermatogenesis. Moreover, Xu *et al*. [23] found a significant correlation between normal morphology and litter size, but no effect on farrowing rate. However, the litter size was considered to be the most sensitive indicator, which had a strong relation with sperm morphology in the study by Alm *et al*. [3].

According to previous reports, pregnancy rate and litter size are also affected by sperm DNA damage [7]. Moreover, sperm DNA damage has been associated with infertility, early pregnancy loss and genetic abnormalities in the offspring [18], and stopping embryo development at the 4 or 8 cell division stage [2]. Boe-Hansen *et al*. [5] studied the relationship between boar sperm chromatin structure integrity with field fertility and reported that differences in litter size can be observed with thresholds as low as 2.1 or 3.0% for sperm DNA fragmentation in liquid stored boar semen. The study of Boe-Hansen *et al*. [5] was the first study that tried to correlate DNA fragmentation with litter size using a flow cytometric method, while the farrowing rate was not determined. In our study, we confirmed a strong correlation between the farrowing rates with live normal sperm and stable deoxyribonucleic acid complex after the combination of sperm traits.

In order to improve the reproductive performance of the swine herd, many studies support including sperm DNA evaluation to required examinations. Due to the difficulty of assessing sperm chromatin integrity, it is almost impossible to involve this in the routine seminal tests in commercial farms. However, it could be applied periodically in swine herds in cooperation with a laboratory or more frequently in the boars of the AI centers.

In conclusion, boar fertility after AI with freshly diluted semen can be predicted based upon the evaluation of sperm morphology and chromatin integrity.

## References


1. **Agarwal A, Said TM.** Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update 2003, **9**, 331-345.
2. **Ahmadi A, Ng SC.** Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool 1999, **284**, 696-704.
3. **Alm K, Peltoniemi OA, Koskinen E, Andersson M.** Porcine field fertility with two different insemination doses and the effect of sperm morphology. Reprod Domest Anim 2006, **41**, 210-213.
4. **Amann RP, Seidel GE Jr, Mortimer RG.** Fertilizing potential *in vitro* of semen from young beef bulls containing a high or low percentage of sperm with a proximal droplet. Theriogenology 2000, **54**, 1499-1515.
5. **Boe-Hansen GB, Christensen P, Vibjerg D, Nielsen MB, Hedeboe AM.** Sperm chromatin structure integrity in liquid stored boar semen and its relationships with field fertility. Theriogenology 2008, **69**, 728-736.
6. **Chalah T, Brillard JP.** Comparison of assessment of fowl sperm viability by eosin-nigrosin and dual fluorescence (SYBR-14/PI). Theriogenology 1998, **50**, 487-493.
7. **Evenson DP.** Loss of livestock breeding efficiency due to uncompensable sperm nuclear defects. Reprod Fertil Dev 1999, **11**, 1-15.
8. **Evenson DP, Thompson L, Jost L.** Flow cytometric evaluation of boar semen by the sperm chromatin structure assay as related to cryopreservation and fertility. Theriogenology 1994, **41**, 637-651.
9. **Flowers WL.** Management of boars for efficient semen production. J Reprod Fertil Suppl 1997, **52**, 67-78.
10. **Gadea J, Matás C.** Sperm factors related to *in vitro* penetration of porcine oocytes. Theriogenology 2000, **54**, 1343-1357.
11. **Gadea J, Sellés E, Marco MA.** The predictive value of porcine seminal parameters on fertility outcome under commercial conditions. Reprod Domest Anim 2004, **39**, 303-308.
12. **Ivanova M, Mollova M.** Zona-penetration *in vitro* test for evaluating boar sperm fertility. Theriogenology 1993, **40**, 397-410.
13. **Johnson WH.** The significance to bull fertility of morphologically abnormal sperm. Vet Clin North Am Food Anim Pract 1997, **13**, 255-270.
14. **Juonala T, Lintukangas S, Nurttila T, Andersson M.** Relationship between semen quality and fertility in 106 AI-Boars. Reprod Domest Anim 1998, **33**, 155-158.
15. **Lee HL, Kim SH, Ji DB, Kim YJ.** A comparative study of Sephadex, glass wool and Percoll separation techniques on sperm quality and IVF results for cryopreserved bovine semen. J Vet Sci 2009, **10**, 249-255.
16. **Petrie A, Watson PF.** Statistics for Veterinary and Animal Science. 2nd ed. pp. 1-240, Blackwell, Oxford, 2006.
17. **Quintero-Moreno A, Rigau T, Rodríguez-Gil JE.** Regression analyses and motile sperm subpopulation structure study as improving tools in boar semen quality analysis. Theriogenology 2004, **61**, 673-690.
18. **Sailer BL, Jost LK, Evenson DP.** Mammalian sperm DNA susceptibility to *in situ* denaturation associated with the presence of DNA strand breaks as measured by the terminal deoxynucleotidyl transferase assay. J Androl 1995, **16**, 80-87.
19. **Silva PF, Gadella BM.** Detection of damage in mammalian sperm cells. Theriogenology 2006, **65**, 958-978.
20. **Tejada RI, Mitchell JC, Norman A, Marik JJ, Friedman S.** A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertil Steril 1984, **42**, 87-91.
21. **Waberski D, Dirksen G, Weitze KF, Leiding C, Hahn R.** Field studies of the effect of sperm motility and morphology on the fertility of boars used for insemination. Tierarztl Prax 1990, **18**, 591-594.
22. **Ward WS, Coffey DS.** DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. Biol Reprod 1991, **44**, 569-574.
23. **Xu X, Pommier S, Arbov T, Hutchings B, Sotto W, Foxcroft GR.** *In vitro* maturation and fertilization techniques for assessment of semen quality and boar fertility. J Anim Sci 1998, **76**, 3079-3089.
24. **Xu X, Seth PC, Harbison DS, Cheung AP, Foxcroft GR.** Semen dilution for assessment of boar ejaculate quality in pig IVM and IVF systems. Theriogenology 1996, **46**, 1325-1337.