Short communication

Relationship between sperm plasma membrane integrity and morphology and fertility following artificial insemination

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Abstract

Sperm quality plays an important role in determining fertility. The aim of the study was to examine the relationship between sperm plasma membrane integrity and morphology, and fertility following artificial insemination (AI). A total of 16 ejaculates were collected from three Large White boars using the gloved hand technique. The semen was extended with a commercial extender. The AI dose contained 80 mL semen sample (3×10^9 sperm/mL). Aliquots of diluted semen were assessed for sperm plasma membrane integrity (synthetic binding CD-14 ($SYBR^+$)/propidium iodide (PI^-) and sperm morphology (eosin nigrosin). A total of 73 Duroc-type, Large White and nondescript multiparous sows from smallholder farms were inseminated with extended semen samples. Boar sperm plasma membrane integrity and morphology were subjected to one-way analysis of variance (ANOVA). The average boar sperm plasma membrane integrity and normal sperm morphology were 78.6% and 77.2%, respectively. The average conception and farrowing rates following artificial insemination (AI) were 78.1 and 57.5%, respectively. A negative correlation was observed between sperm plasma membrane integrity and fertility. There was a weak positive correlation between normal sperm morphology and conception rate (r = 0.11). Additionally, a relationship was observed between normal sperm morphology and litter size (r = 0.37) and total number born alive (r = 0.03), although relatively low. In conclusion, a negative relationship was found between sperm plasma membrane integrity and fertility. Moreover, there was a relationship between morphologically normal sperm and litter size, as well as number of piglets born alive, although relatively low.

Keywords: Boar, Eosin Nigrosin, Semen quality, SYBR14/PI
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Improving the qualitative and quantitative analysis of boar semen samples to estimate fertility potential of males is critical for a successful breeding programme. Although much progress has been made, the ability to predict the fertility of semen with traditional laboratory tests is still limited, owing mainly to the complexity of sperm morphological damage and their fertilization potential (Brito *et al*., 2003). In addition, the prediction of sperm fertilizing ability is of great economic importance to breeding of sows as it leads to the selection of boars with better semen fertility, which results in good reproductive performance. Semen fertility trait assessments play a crucial role in the early detection of developmental disorders in male animals (Smital *et al*., 2004). As well as traditional methods of semen assessment, biochemical tests and morphological analyses of sperm are performed. Disturbances in spermatogenesis give rise to morphological sperm defects (Kavak *et al*., 2004). Moreover, recent studies have indicated a correlation between normal morphological

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traits, conception rate, and litter size, which are particularly sensitive to normal head morphology (Gadea, 2005).

Another reliable approach is to use a combination of tests to evaluate various sperm attributes, thereby increasing the accuracy of the prediction (Amann & Hammerstedt, 1993). Assessment of sperm plasma membrane integrity is one of the key parameters in the evaluation of sperm quality in relation to fertility (Pintado *et al*., 2000). One of the major features discriminating dead cells from live ones is loss in physical integrity of their plasma membranes and loss of motility (Burks & Sailing, 1992). Sperm outer membrane integrity and proper function are vital to sperm metabolism, capacitation, ova binding, and acrosome reaction (Brito *et al*., 2003). Hence, assessment of sperm morphology and plasma membrane integrity traits may be useful in predicting the fertilizing ability of sperm. The objective of the study was therefore to determine the relationship between boar sperm morphological traits and fertility after AI.

All procedures in the study that involved animals were performed in accordance with the ethical standards of the Agricultural Research Council (reference APIEC15-046).

The study was conducted at the Pig Research Unit and Germplasm Conservation and Reproductive Biotechnologies Unit in Agricultural Research Council (ARC), South Africa. The ARC campus is located at 25° 55‟ south; 28° 12‟ east. The institute is located in the Highveld region of South Africa and situated at an altitude of 1525 m above sea level.

Three Large White boars, aged 18–24 months, which were genetically different, were used in the study to avoid inbreeding. A total of 16 ejaculates were collected from the boars. The boars were housed individually in pens and routinely used for AI purposes. Sperm rich fractions were collected using the gloved-hand technique in a 300 mL glass beaker. The filtered semen fraction was sealed with a gauze filter inside a pre-warmed (39 ºC) insulated thermos flask. Ejaculates with 70% sperm motility were extended with a commercial extender, Beltsville Thawing Solution, to 3 x 10^9 sperm/ml and stored at 17 ºC until AI.

Plasma membrane integrity was assessed using synthetic binding CD-R 14/propidium iodide (SYBR-14/PI) tests (Garner & Johnson, 1995). Briefly, semen samples were incubated at 38 ºC for 10 minutes with SYBR-14 at a final concentration of 100 µM, and then with PI at a final concentration of 10 µM for 5 minutes at the same temperature. A fluorescent microscope was used at 100 x magnification to count 200 sperm per stained slide and the results were recorded. After this assessment, two sperm populations were identified: live green-stained sperm (SYBR-14^+/PI^-); and dead red-stained sperm (SYBR-14^-/PI^+).

Sperm morphology was determined microscopically after staining the semen samples with eosin nigrosin stain on a slide (Tsakmakidis *et al*., 2010). Briefly, 7µL of boar semen was added to 20µL eosin nigrosin staining solution in a 0.6 mL micro-centrifuge graduated tube and mixed gently. A drop of 5µL boar semen and Eosin Nigrosin stain was placed on a clear end of a microscope slide and smeared. Fluorescent microscope was used at 100 x magnification to count 200 sperm per stained slide and the results were recorded. Morphological defects were recorded and were categorized into major and minor sperm morphological defects according to the classification system by Salisbury *et al*. (1978). Briefly, the major morphological defects included loose head, proximal cytoplasmic droplets, and midpiece reflexes, whereas minor defects included distal cytoplasmic droplets and bent tails.

A total of 73 Duroc-type, Large White and nondescript multiparous sows from nine smallholder farms in Gauteng were used in this study, namely Winterveldt (17), Cullinan (6), Rooival (8), Zuurbekom (26), Randfontein (5), Midvaal (2), Meyerton (2), Brakpan (5), and Bendaro Park (2). The boar to sow ratio was 1 : 33, 1 : 25 and 1 : 15. Before AI, the recipient sows were synchronised by administering 400 IU equine chorionic gonadotropin and 200 IU human chorionic gonadotropin intramuscularly in the neck. Each sow was checked for heat twice a day. Sows were further stimulated by back pressure and inseminated twice, 12 and 24 hours after standing heat. Each AI dose consisted of 80 mL semen containing 3 × 10^9 sperm/ml. Pregnancy diagnosis was done 42 days after artificial insemination with an ultrasound scanner. Conception rate, farrowing rate, litter size and total of piglets born alive were recorded.

Data were analysed using one-way analysis of variance (ANOVA) using Statistical Analysis System® (SAS®) program. Sperm morphology and plasma membrane integrity values are presented as means ± standard error and were considered statistically significant when P <0.05. Pearson correlations were used to examine the relationship between sperm plasma membrane integrity and morphology and fertility.

The summary of boar sperm plasma membrane integrity and morphology with fertility data is illustrated in Table 1. Boar sperm stained with SYBR14 and PI viewed under a fluorescence microscope at 100x magnification is indicated in Figure 1. The live sperm fluoresces green and the red colour indicates dead sperm. There were no differences (P >0.05) between the boars for sperm plasma membrane integrity. On average, the sperm plasma membrane integrity was 75.6%. The proportion of normal morphological sperm varied significantly among the boars (P <0.05). Furthermore, there was a variation in the prevalence of morphological defects (P <0.05). The prevalence of major morphological defects was low in Boar 2 (7.7 ± 2.2) in comparison with Boar 1 (16.4 ± 1.6%) and Boar 3 (13.2 ± 2.1%). It was also found that Boar 2 (9.6 ±

2.3%) and Boar 3 (7.1 ± 4.0%) had the lowest minor morphological defects compared with Boar 1 (14.3 ± 1.2%). As a result, the higher the proportion of morphologically normal boar sperm, the higher the chance of conceiving. Conception rate differed significantly among the boars: Boar 3 yielded the highest conception rate (93.3 ± 0.1%) compared with Boar 1 (63.6 ± 0.1%) and Boar 2 (88.0 ± 0.1%). Farrowing rate was significantly higher in Boar 3 (80.0 ± 0.1%) than in Boar 1 (48.8 ± 0.1%) and Boar 2 (55.3 ± 0.1%). On average, the conception and farrowing rates following oestrus synchronization and AI were 78.1 ± 0.1% and 57.5 ± 0.1%, respectively. The artificial insemination resulted in an acceptable fecundity (i.e., 11.8 ± 0.8 litter size and 10.0 ± 0.6 number of piglets born alive). Similar results were achieved after AI under smallholder production systems in north-eastern India (Kadirvel *et al*., 2013). An average litter size of 11.7 was achieved in the present study. In contrast, lower average litter sizes were achieved at north-eastern India after AI (Kadirvel *et al*., 2013).

Table 1 Summary of boar sperm plasma membrane integrity, morphological traits and fertility (mean ± SE)

Variable	Boar 1	Boar 2	Boar 3	Mean ± SE
Number of ejaculates	6	6	4	16
Sperm plasma membrane integrity (%)	77.7 ± 3.7	71.8 ± 4.9	71.8 ± 6.1	75.6 ± 2.7
Normal sperm (%)	69.3^{a} ± 3.7	82.7^{b} ± 6.1	79.7± 6.1^{b}	77.2 ± 2.9
Major morphological defects (%)	16.4^{b} ± 1.6	7.7^{a} ± 2.2	13.2^{b} ± 2.1	12.8 ± 1.5
Minor morphological defects (%)	14.3^{b} ± 1.2	9.6^{a} ± 2.3	7.1^{a} ± 4.0	11.2 ± 1.5
Number of sows inseminated	33	25	15	73
Conception rate (%)	63.6^{a} ± 0.1	88.0^{b} ± 0.1	93.3^{c} ± 0.1	78.1 ± 0.1
Farrowing rate (%)	48.4^{a} ± 0.1	55.3^{b} ± 0.1	80.0^{c} ± 0.1	57.5 ± 0.1
Litter size	11.6 ± 1.1	10.9 ± 1.2	13.0 ± 2.1	11.8 ± 0.8
Number of piglets born alive	10.1 ± 1.0	9.4 ± 1.1	11.0 ± 1.5	10.0 ± 0.6

^{abc} Different superscripts in a row indicate significant differences (P <0.05)

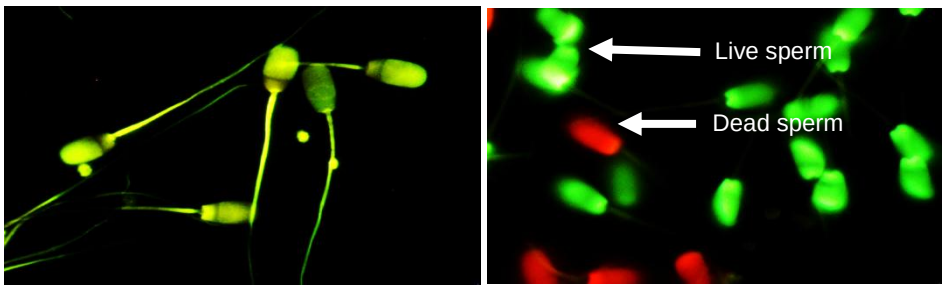

Figure 1 Boar sperm stained with SYBR14 and PI viewed under a fluorescence microscope at 100 x magnification

Pearson's correlation analysis was performed between boar sperm morphological traits and fertility. There was a strong positive correlation (P <0.01) between SYBR-14 (live) sperm plasma membrane integrity and major morphological defects (r = 0.61) and minor morphological defects (r = 0.10). Moreover, negative correlations were found between live sperm using SYBR staining and fertility. Similarly, it was reported that sperm membrane structure is not closely related to fertility (Gadea *et al*., 2004; Gadea, 2005). This may perhaps be because it provides information about the plasma membrane integrity of the sperm, but not about its functionality, such as the capacitation process, acrosome reaction, sperm binding, etc. In contrast, Januskauskas *et al*. (2003) detected significant correlations between field fertility and plasma membrane integrity. In the present study, a relatively low negative correlation was found between boar sperm plasma membrane integrity and litter size, as well as number of piglets born alive. Similarly, boar sperm plasma membrane integrity was not much related to fertility when eosin nigrosin staining were used to assess it (Gadea *et al*., 2004; Lima *et al*., 2015). In contrast, sperm plasma membrane integrity of microscopically assessed Calcein AM and PI stained sperm correlated significantly with litter size (Sutkeviciene *et al*., 2009).

Several studies have reported significant correlations between fertility and incidence of specific morphological defects (Amann *et al*., 2000; Johnson, 1997). In the present study, there was a positive correlation (P >0.05) between normal sperm morphology and conception rate (r = 0.11), although relatively low and statistically not significant. Additionally, a relationship (P >0.05) was observed between normal sperm morphology with litter size (r = 0.37) and total number born alive (r = 0.03). However, negative correlations (P >0.05) were observed between conception rate and major sperm defects as well as minor morphological ones. Conversely, boar sperm morphology has been reported to have limited positive predictive value for field fertility (Alm *et al*., 2006). Although negative correlations were found between fertility and morphological defects, Waberski *et al*. (1990) reported that sperm morphology may assist with boar selection for AI as it provided information about spermatogenesis. In the present study, a weak positive correlation (P >0.05) was found between normal sperm and litter size (r = 0.37). In contrast, a significant correlation was found between normal sperm morphology and litter size (Xu *et al*., 1998). Moreover, in the present study, a strong positive correlation (P <0.01) was found (r = 0.89) between farrowing rate and litter size. However, the high farrowing rate may not always be relative to high litter size. Moreover, weak correlations between farrowing rate and litter size were found in other studies, indicating that these traits may be affected differently by the semen quality of the boars (Tsakmakidis *et al*., 2010; Juonala *et al*., 1998).

In conclusion, there was a negative relationship between sperm plasma membrane integrity and fertility following AI. However, there was a relationship between morphologically normal sperm and litter size as well as number of piglets born alive, although relatively low. A negative relationship was observed between major and minor morphological defects with conception rate. Conversely, a positive relationship was found between major and minor defects with litter size and number of piglets born alive, although relatively low.

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Conflict of Interest Declaration

The authors confirm that there is no known conflict of interest associated with the publication of this manuscript. This manuscript has been read and approved by all authors and that the order of authors listed in the manuscript has been approved by all of us.

Authors' Contributions

MBM, TLN, AKN, and JWN were in charge of project design and writing the manuscript. MBM, RT, and TRN were in charge of project implementation. All co-authors participated in results, statistics and interpretation of the study.

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