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Research article

Hypo-osmotic swelling test discriminates potentially more viable boar spermatozoa diluted using various extenders at different storage times

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Abstract

Boar semen samples are normally tested across multiple parameters to ensure maximum performance. However, conventional semen quality parameters may not provide a robust indication of the fertility potential of spermatozoa. This study examined sperm plasma membrane integrity using the hypo-osmotic swelling test (HOST). It was hypothesized that HOST could effectively select possibly more viable spermatozoa despite normality in other parameters. Semen samples (n=15) from five boars were processed into AI doses (~37.5 x 10^6 spz/mL) using five different commercial extenders: Androstar Plus, BTS, Dilufert Silver, MIII, and VIM. Using Sperm Class Analyzer® CASA system, total and progressive motility, and plasma membrane integrity using dye exclusion test and HOST were evaluated at Day 0, Day 3, and Day 5 of storage. Boar 2 tended to have more HOST-positive spermatozoa over the other boars in Dilufert Silver, VIM, and Androstar Plus extenders in both Day 3 and Day 5 and in BTS at Day 3. Several significant interactions were also observed when storage times, extenders used, and the kind of boars were considered. Moreover, the total and progressively motile spermatozoa did not vary between boars and different extenders across different storage durations, except for MIII between Day 0 and Day 5. Hypo-osmotic swelling test (HOST) could serve as a useful test for detecting the functional integrity of boar sperm and sensitive enough to discriminate potentially more viable spermatozoa particularly when applied in small-scale swine operations without advanced capacity for comprehensive semen analyses.

Keywords: Boar sperm, Hypo-osmotic swelling test, Motility, Vitality

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INTRODUCTION

A comprehensive quality assessment of boar spermatozoa is necessary to ensure that standard performance is achieved for successful fertilization. Thus, raw semen samples are normally tested across multiple parameters to ensure boar sperm maximum performance prior to artificial insemination (AI). With AI in pigs, minimum requirements may include 60%-70% progressive motility, 80% normal sperm morphology, ~40x10^9 total spermatozoa per ejaculate, and making sure that other parameters such as vitality, presence of cytoplasmic droplets, colour, odour, volume, doses produced, DNA damage, possible contaminants, and boar age are also included (Colenbrander et al., 1993; Althouse, 2007; Schulze et al., 2014; Peña et al., 2017; Peña et al., 2023). This is particularly important in pig AI as ejaculates may be processed into semen doses using nutrient-rich medium (Foxcroft et al., 2008; Knox, 2016) and stored at 17°C for later use while at the same time maintaining effective sperm performance (Johnson et al., 2000; Knox, 2016; Pezo et al., 2019). This practice has provided greater flexibility among swine producers as it allows not only the collection of semen from disease-free and superior boars but also the insemination of multiple sows even at the remote locations at the same time. Thus, standard quality in AI doses cannot be taken lightly as the potential drastic negative impact could cost the pig farmers' productivity and profit.

Cold shock and oxidative stress may cause irreversible damage and loss of boar sperm quality which could significantly impact fertility outcomes (Johnson et al., 2000; Watson, 2000; Wiebke et al., 2022). Cold shock, in particular, occurs when boar spermatozoa are rapidly exposed to temperature changes particularly below 12°C (Althouse et al., 1998), causing negative effects on motility and metabolic activity, disruption of lipid and protein interactions and selective membrane permeability, among others (Pursel et al., 1972; reviewed by Yeste, 2018).

Boar management, handling of semen samples, contamination, and other environmental factors could also affect the quality of extended boar semen (Rodriguez et al., 2017) and may be associated with the build-up of free radicals which can predispose spermatozoa to oxidative stress and compromise sperm functions. Very importantly, the reduction in the functional integrity of the sperm plasma membrane could significantly affect the metabolic activities of spermatozoa which in turn impacts its motility performance, capacity to undergo capacitation and acrosome reaction, and interaction with oviduct epithelium and the ova, among others (Rodriguez-Martinez, 2001; Rodriguez-Martinez, 2003). These parameters are all required for successful fertilization. This simply means that disturbances in the plasma membrane functions could predispose spermatozoa to reduced fertility potential.

This study investigated the quality of boar spermatozoa relative to its combined performance in terms of motility, and plasma membrane integrity (dye exclusion test, and hypo-osmotic swelling test or HOST). It was hypothesized that selection of high quality and possibly more viable boar spermatozoa particularly when outsourced from established boar stud may be done by completing a simple and rapid test such as the HOST on top of routine procedures of sperm motility and vitality. As HOST tends to indicate the functional integrity of the sperm plasma membrane over a simple structural damage detected using the usual dye exclusion technique (Vazquez et al., 1997), it has been used to screen sub-viable spermatozoa in men (Vazquez et al., 1997), spermicidal effects of toxins and chemotherapeutic agents, (see also review by Jeyendran et al., 1992), sperm capacitation (Lechniak et al., 2002), and sperm cryosurvival, (Přinosilová et al., 2014). Earlier studies in pigs have reported significant correlation between HOST results and in vivo fertility (Pérez-Llano et al., 2003) with apparently lower values in ejaculates of problematic boars (Samardžija et al., 2008). Moreover, as the country's pig production is mostly on small-scale or semi-commercial in nature, the HOST would be practically valuable to pig farms with limited resources. This study in particular looks at HOST results in boar AI doses prepared from semen samples purchased from a smallhold farm and processed using different commercial extenders at different storage times. Assessment of the plasma membrane integrity, on top of the usual motility and morphology, could serve as a useful indicator of sperm fertility potential.

MATERIALS AND METHODS

Semen Samples and Experimental Design

Semen samples were outsourced from a single boar stud operated by a well-experienced AI operator located about 12 km away from the boar semen laboratory of the College of Veterinary Medicine, Visayas State University, Baybay City, Leyte (10°44'44.5"N 124°47'48.5"E). The boars were housed in individual stalls and fed with about 3 kg/head/day (14% CP) and semen was collected regularly for processing into semen doses and sold for artificial insemination within the locality. For this study, n=15 ejaculates were used from five Large White boars aged ~1.5 yrs old, collected on rotation with two-three boars collected every Friday (between November-December 2022) until three separate ejaculates were collected from each boar. Following the standard collection procedure by its owner, about 100mL of the ejaculate was purchased and placed inside the two sterile 50-mL conical tubes and brought to the semen laboratory using an improvised boar semen shipper (Peña, 2023). Semen samples were then processed using commercial diluents including Beltsville Thawing Solution (BTS), MIII, Dilufert Silver, VIM), and Androstar Plus extenders. Semen quality analyses were then conducted at Day 0, Day 3 and Day 5 of storage.

Sperm Motility and Vitality Evaluations

Sperm quality was assessed using the Sperm Class Analyzer® (SCA® version 6.6.15.0, Microptic S.L., Barcelona, Spain), a computer-aided sperm analysis (CASA) system as described earlier (Van Der Horst et al., 2018; Peña, 2023). Upon arrival, the raw sample was initially filtered using a plastic semen collection bag (US Bag® with filter and sprout, Minitube, Germany) and an aliquot diluted 1:10 in 0.9% NaCl solution was prepared to determine the sperm concentration and initial motility. Thereafter, about 40-mL individual semen doses were prepared (~37.5 x 10^6 spz/mL) for each of the five commercial extenders: Androstar Plus, BTS, Dilufert Silver, MIII and VIM, and respective semen quality analyses were conducted at Day 0, Day 3, and Day 5 of storage. For sperm motility, 2mL semen sample was individually prepared according to each extender preparation in a 4-mL plastic tube and warmed in water bath (38°C) for about 20-30 minutes prior to analysis. Wet mounts were prepared in succession by aliquoting about 10 µL semen on a normal microscope slide, pre-warmed to about 38°C (Goldcyto slide warmer, Microptic S.L., Barcelona, Spain) and covered with a 22mm x 22mm cover slip. Thereafter, about 500 spermatozoa per treatment slide were examined accordingly using the normal slide setting of the SCA® MOT module.

For sperm vitality, an aliquot taken from the 2-mL semen sample prepared earlier was stained using the Eosin-Nigrosin stains (Minitube, Tiefenbach, Germany) and standard smears were prepared (using Hemaprep, J.P. Gilbert Co., USA) in two microscope slides and dried in air. About 200 spermatozoa were examined thoroughly using 400x magnification and classified between live (full opaque/white) and dead (colored head) spermatozoa. Live spermatozoa were considered vitally normal, and the number was divided over 200 then multiplied by 100 to calculate the percent live spermatozoa.

Hypo-osmotic swelling Test (HOST)

In preparing the hypo-osmotic solution, BTS was prepared initially following the recommended dilution and stored at 5°C as stock solution (labelled BTS Stock Solution). On the day of the experiment, an aliquot of the BTS Stock Solution was diluted into 1:3 using distilled water (labelled BTS-HOST Test Solution) for use in the HOST (Jeyendran et al., 1992; Malo et al., 2011; Chung et al., 2013). Thereafter, 30µL semen sample and 100µL ul BTS-HOST Test Solution was mixed thoroughly in a 1.5-ml tube and incubated at 37°C for 30 minutes in warm blocks. Using about 10µL of the sample, a smear was prepared on a clean slide and covered with a coverslip and the edges were sealed with a clear nail polish. The slides were examined immediately by considering about 200 spermatozoa per slide, classified as either HOST-positive or HOST-negative. HOST-positive spermatozoa were identified by the curling/swelling of tails and expressed as percentage of the total number of spermatozoa examined (Jeyendran et al., 1992; Lechniak et al., 2002).

Data Presentation and Statistical Analyses

All data were entered into a spreadsheet using Google Sheet and converted into a Comma Separated Values (CSV) file required by JASP (Version 0.18.3), a free and open-source statistical computer software (JASP Team, 2024) for appropriate statistical analyses. Significant differences (p value of ≤ 0.05) between treatment groups were determined using repeated measures mixed ANOVA with Greenhouse-Geisser sphericity correction applied and Post-hoc using Bonferroni adjustment (Gross-Sampson, 2020).

RESULTS

Figure 1 shows the HOST-positive spermatozoa while Figure 2 presents the comparative results among boars for each of the five extenders. HOST-positive spermatozoa were generally lower on Day 0 and started to increase between Day 3 and Day 5. Boar 2 tended to have more HOST-positive spermatozoa over the other boars in Dilufert, VIM and Androstar extenders (Figures 2B, 2D and 2E) in both Day 3 and Day 5 and in BTS (Figure 2A) at Day 3. Moreover, several significant interactions were observed when the three factors such as storage times, extenders used and the boars were considered. For example, HOST-positive spermatozoa were significantly different between Boar 2, at Day 3 and Day 5, using Androstar than with Boar 1 and Boar 5 at Day 0, using different extenders. Other significant interactions include: VIM, Boar 5, Day 5 vs Androstar, Boar 5, Day 5; Androstar, Boar 2, Day 3 vs Dilufert, Boar 3, Day 3 and VIM, Boar 5, Day 5; VIM, Boar 5, Day 3 vs Androstar, Boar 2, Day 5; MIII, Boar 2, Day 5 vs Androstar, Boar 2, Day 5; Androstar, Boar 2, Day 5 vs Dilufert, Boar 3, Day 5 and VIM, Boar 5, Day 5; BTS, Boar 5, Day 5 vs VIM, Boar 5, Day 5; and VIM, Boar 5, Day 5 vs Androstar, Boar 5, Day 5.

In terms of vitality (eosin-nigrosin staining), boar spermatozoa remained relatively normal across the duration of storage. However, a significant difference can be observed but is limited to using Androstar extender between Day 0 and Day 3 only (Table 1).

Figure 3 presents the motility performance of boar spermatozoa among the five boars across Day 0, Day 3 and Day 5 of storage. Overall, the total and progressively motile spermatozoa did not vary between boars and different extenders across different storage times, except for MIII between Day 0 and Day 5. Moreover, no interaction was observed when all factors were considered for boars, extenders and length of storage.

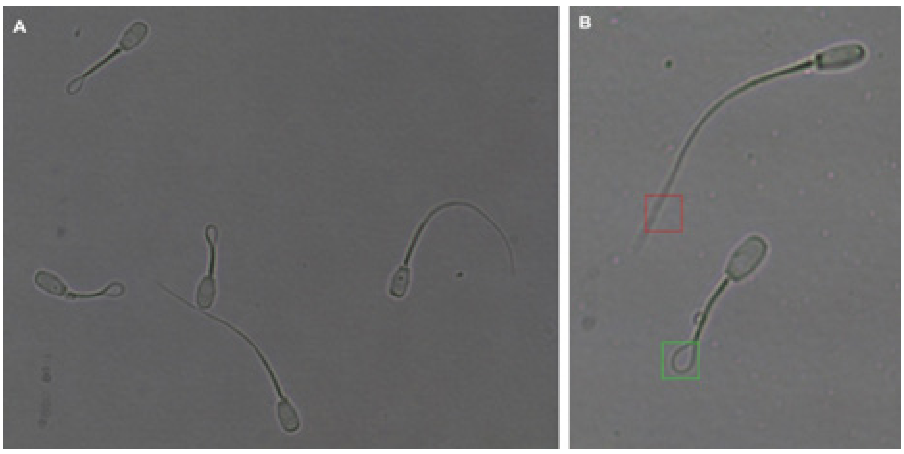

Figure 1 Typical microscopic appearance of HOST-positive spermatozoa (A, with coiled tails; B, coiled tail in green box)

Table 1 Mean (± SEM) percentage of live boar spermatozoa across five different commercial extenders at different durations of storage.

Extender	Live Spermatozoa (%)					
	Day 0 (n=15)	CV	Day 3 (n=15)	CV	Day 5 (n=15)	CV
Androstar	95.00 ± 0.62[a]	0.03	96.60 ± 0.60[b]	0.02	96.73 ± 0.44	0.02
BTS	94.20 ± 0.68	0.03	95.53 ± 0.59	0.02	93.63 ± 0.72	0.03
Dilufert	94.17 ± 0.66	0.03	94.80 ± 0.58	0.02	94.57 ± 0.63	0.03
MIII	94.47 ± 0.53	0.02	94.87 ± 0.51	0.02	94.33 ± 0.68	0.03
VIM	95.40 ± 0.56	0.02	95.40 ± 0.42	0.02	94.23 ± 0.61	0.02

Values with different letters differ significantly between treatments for each parameter ($P \leq 0.05$); Numbers in parentheses indicate sample size. CV, coefficient of variation.

DISCUSSION

Processing liquid-stored boar semen involves various stages, from collection to dilution and packaging into appropriate AI doses for storage until insemination. One crucial step in this process is to make sure that boar spermatozoa meet the industry standard of quality. Thus, raw samples are normally tested across multiple parameters to ensure maximum performance of boar sperm. However, conventional semen quality parameters are generally limited and may not provide a robust indication of the fertility potential of spermatozoa.

Our study looked into the motility (total and progressive) and vitality (dye exclusion test and HOST) of boar spermatozoa with special focus on the HOST, an easy and affordable test focused on the functional integrity of the sperm plasma membrane. It was hypothesized that HOST could effectively identify more viable spermatozoa despite normality in the results of other parameters, regardless of the extender used or duration of storage. For example, unlike the usual eosin-nigrosin test for determining structurally defective spermatozoa (dead sperm), HOST is used to indicate the biochemical status thus, the functional integrity of the sperm plasma membrane. While the HOST itself is not new, it was necessary to feature the simplicity of the procedure and highlight its potential value as a sensitive and reproducible test that could be used in screening sub-viable spermatozoa (Vazquez et al., 1997) particularly in small-scale pig operations where advanced semen analyses are limited.

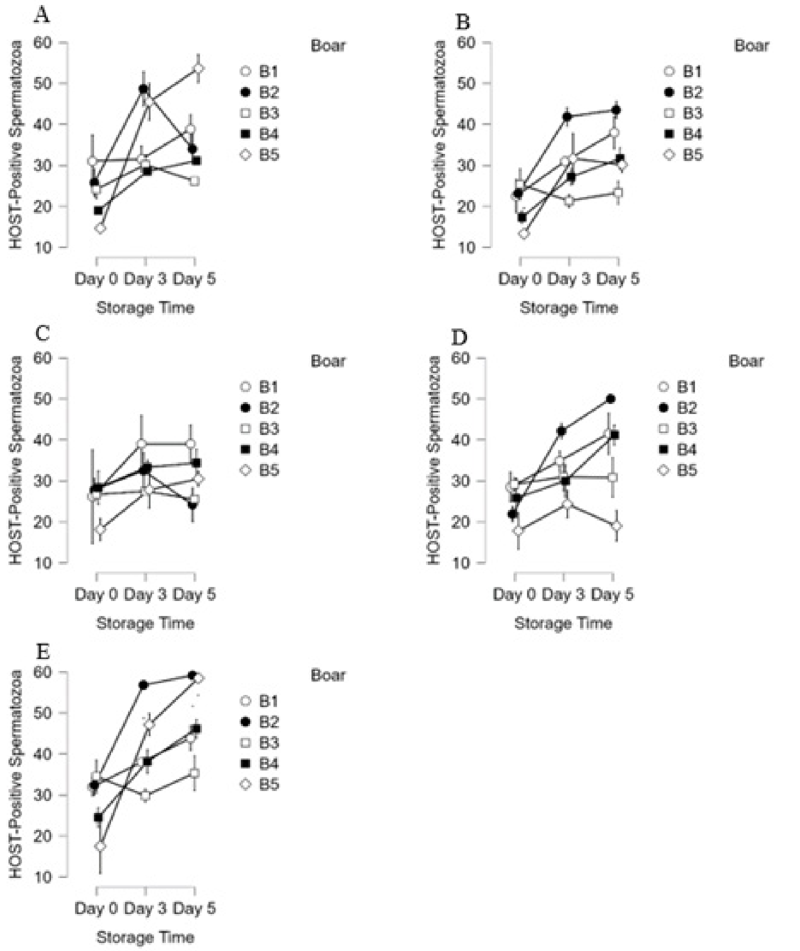

Figure 2 Mean (±SEM) HOST-positive boar spermatozoa at Day 0, Day 3 and Day 5 of the five boars at different extenders – 2A, BTS; 2B, Dilufert; 2C, MIII; 2D, VIM; and 2E, Androstar. N=15 semen samples per boar per extender type per day treatment. Significant interactions include Boar 2, at Day 3 and Day 5, using Androstar than with Boar 1 and Boar 5 at Day 0, using different extenders; VIM, Boar 5, Day 5 *vs* Androstar, Boar 5, Day 5; Androstar, Boar 2, Day 3 *vs* Dilufert, Boar 3, Day 3 and VIM, Boar 5, Day 5; VIM, Boar 5, Day 3 *vs* Androstar, Boar 2, Day 5; MIII, Boar 2, Day 5 *vs* Androstar, Boar 2, Day 5; Androstar, Boar 2, Day 5 *vs* Dilufert, Boar 3, Day 5 and VIM, Boar 5, Day 5; BTS, Boar 5, Day 5 *vs* VIM, Boar 5, Day 5; and VIM, Boar 5, Day 5 *vs* Androstar, Boar 5, Day 5.

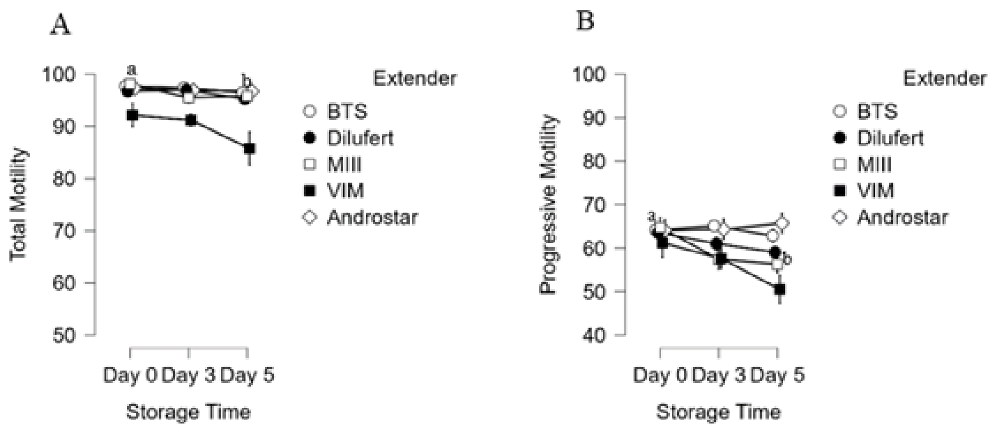

Figure 3 Mean (±SEM) total (3A) and progressively motile (3B) spermatozoa of boars on Day 0, Day 3, and Day 5 of storage. N=15 semen samples/boar per treatment day. Significant difference was limited to MIII extender between Day 0 and Day 5.

The literature provides extensive evidence on the use and clinical value of HOST particularly when used in routine semen analysis. The test employs a straightforward procedure of incubating spermatozoa in hypoosmotic medium which causes spermatozoa with biochemically intact plasma membrane to swell due to the influx of water (Vazquez et al., 1997). Microscopically, part of or the entire tail appears bent resulting to 'tail curling' or swelling. Since only biochemically intact plasma membrane shows tail curling when exposed to hypo-osmotic conditions, it can be deduced that HOST-reactive spermatozoa have maintained the functional integrity of the plasma membrane. Moreover, since a functionally intact plasma membrane is essential on several physiological events required by spermatozoa prior to fertilization such as capacitation, acrosome reaction, and oviduct-binding, the HOST-negative spermatozoa may have deleterious consequences to sperm fertility performance.

The results of this study showed that Boar 2 appeared to indicate more stable and biochemically functional plasma membrane compared to the other boars particularly on Day 3 and Day 5. Interestingly, Boar 2 also consistently showed better results with the eosin-nigrosin staining test although generally, the results were relatively similar across the boars. While this study was not complemented by field fertility trials and that, interpretation should be taken in appropriate context, these results effectively demonstrate the ability of using HOST in selecting potentially more viable spermatozoa and thus, selecting better boars.

Boar 2, showing the highest HOST-reactive spermatozoa (44.40 ± 3.05% at Day 3 and 42.17 ± 3.5% at Day 5), also relates well in some of the results by other studies in fresh or extended semen as summarised in Table 2. Of particular interest is on the results of 72h after storage where these results (27.97 ± 1.52% to 44.40 ± 3.05%) were also closely coincided with earlier reports (Frydrychova et al., 2010;Khan et al., 2006).

However, it was not expected to have a generally lower HOST-reactive spermatozoa at Day 0 than Day 3 or Day 5. It is understood that during the HOST procedures, spermatozoa are subjected to stress conditions which effectively select spermatozoa with the most resistant plasma membrane (Pérez-Llano et al., 2003). However, it was difficult to explain whether the longer duration time or the extenders used in the study provided some enhancing protection to sperm plasma membrane and thus, resulting to higher HOST-reactive results. Unlike those boars in commercial breeding stud farms, the boars used in this study were maintained in seemingly low management practices normally seen in smallholder (backyard) type of pig farming.

While we do not have a complete record to show the fertility performance of the animals included in the study, these boars have been used as sources of semen for processing into AI doses and sold to breeder sow owners within the locality. Nevertheless, at Day 0, HOST-reactive spermatozoa range from 16.30 ± 1.63% to 28.040 ± 3.04% and is albeit higher than the 16.96 ± 4.27% in problematic boars (Samardžija et al., 2008), 24.8% in capacitated boar spermatozoa, and in various results from frozen-thawed boar spermatozoa (Table 2).

Table 2 Different levels of HOST-reactive boar spermatozoa between fresh-extended or frozen-thawed samples.

Treatments	HOST-reactive boar spermatozoa (%)	References
Fresh-extended	59.0 ± 4.8% to 90.0 ± 4.1%	(Pérez-Llano et al., 2001)
	57.5 ± 21.3%	(Buranaamnuay et al., 2009)
	49% to 58% (24h), 34% to 44% (72h), 26% to 37% (96h) results depend upon the extender used	(Frydrychova et al., 2010)
	16.96 ± 4.27% (ranges from 6.18% to 42%; identified as problematic boars)	(Samardžija et al., 2008)
	35.2% in fresh vs 24.8% in capacitated sperm	(Lechniak et al., 2002)
	55.45 ± 2.95% to 57.74 ± 2.85% (0h), 23.35 ± 2.84% to 31.50 ± 3.12% (72h), 19.55 ± 3.45% to 25.60 ± 2.12% (96h) results depend upon the extender used	(Khan et al., 2006)
	54% (21% to 89%)	(Buranaamnuay et al., 2008)
	42.2–68.3%	Vazquez et al. 1997)
Frozen-thawed	37.9 ± 4.7% to 48.7 ± 3.7% (in plastic medium straws)	(Saravia et al., 2005)
	10.5 ± 7.7% (in 0.25mL straws), 11.5 ± 9% (in 0.5mL straws)	(Buranaamnuay et al., 2009)
	11.4 ± 1.3% to 21.78 ± 1.4% (depending upon the type of sugar used)	(Chanapiwat et al., 2012a)
	19% (3% to 45%)	(Buranaamnuay et al., 2008)
	16.4 ± 1.4% to 18.4 ± 1.2% (depending upon the type of freezing extender used)	(Chanapiwat et al., 2012b)

While HOST results did not appear to have sufficiently discriminated semen samples of intermediate fertility in bull semen (Rota et al., 2000), sufficient evidence show that the level of HOST-positive spermatozoa could significantly explain the variation in the number of live born piglets (24.5%) and litter size (7.8%), respectively (Michos et al., 2021). In the study of (Pérez-Llano et al., 2001), demonstrating the significant correlation of HOST results to farrowing rates, such classifications may be possible: 30%-60% (50.2 ± 2.8%), 61%-80% (68.7 ± 4.2%), and 81%-100% (77.3 ± 4.7%), respectively. This further indicates the relevance of performing the HOST in semen analysis.

CONCLUSIONS

This study demonstrated that HOST could serve as a useful test for detecting the functional integrity of boar sperm and sensitive enough to discriminate potentially more viable spermatozoa. The overall effect may be influenced in part by a combination of factors such as the kind of boar, the extender used, and the duration of storage. Moreover, on the basis of some earlier standards, the boars under consideration may not be classified as problematic boars but there appears the need to improve the management to effect increased fertility performance. A follow-up study may be needed to correlate these results with the farrowing performance of these boars.

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AUTHOR CONTRIBUTIONS

STPJ contributed to project administration, funding acquisition, study conceptualization, original manuscript writing, review, and editing, and data consolidation and analysis. BTPY, and MEBJ equitably contributed to methodology, sampling and laboratory works, data management, and review of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Althouse, G.C., 2007. Artificial insemination in swine: boar stud management. In: Youngquist, R.S., Walter R. Threlfall, W.R. (Eds.), Current therapy in large animal theriogenology. Saunder Elsevier, Edinburgh, pp. 731-738.

Althouse, G.C., Wilson, M.E., Kuster, C., Parsley, M., 1998. Characterization of lower temperature storage limitations of fresh-extended porcine semen. Theriogenology. 50, 535-543.

Buranaamnuay, K., Singlor, J., Tummaruk, P., Techakumphu, K., 2008. The establishment of boar semen cryopreservation in Thailand: Postthaw semen quality, sperm concentration and variation among ejaculations. Thai. J. Agri. Sci. 41, 135-141.

Buranaamnuay, K., Tummaruk, P., Singlor, J., Rodriguez-Martinez, H., Techakumphu, M., 2009. Effects of straw volume and Equex-STM® on boar sperm quality after cryopreservation. Reprod. Domest. Anim. 44, 69-73.

Chanapiwat, P., Kaeoket, K., Tummaruk, P., 2012a. Cryopreservation of boar semen by egg yolk-based extenders containing lactose or fructose is better than sorbitol. J. Vet. Med. Sci. 74, 351-354.

Chanapiwat, P., Kaeoket, K., Tummaruk, P., 2012b. Improvement of the frozen boar semen quality by docosahexaenoic acid (DHA) and l-cysteine supplementation. Afr. J. Biotechnol. 11, 3697-3703.

Chung, K.H., Kim, I.C. and Son, J.H., 2013. Effects of different concentrations of Escherichia coli and days of preservation on boar sperm quality. Reproductive and Developmental Biology, 37(4), pp.213-217.

Colenbrander, B., Feitsma, H., Grooten, H., 1993. Optimizing semen production for artificial insemination in swine. J. Reprod. Infertil. 48, 207-215.

Frydrychova, S., Cerovsky, J., Lustykova, A., Rozkot, M., 2010. Effects of long-term liquid commercial semen extender and storage time on the membrane quality of boar semen. Czech. J. Anim. Sci. 55, 160-166.

Gross-Sampson, M., 2020. Statistical analysis in JASP: a guide for students, 4th edition JASP v0.14. Available online: https://doi.org/10.6084/m9.figshare.9980744.v1

JASP Team., 2024. JASP (Version 0.18.3) [Computer software]. Available online: https://jasp-stats.org/.

Jeyendran, R., Van der Ven, H., Zaneveld, L., 1992. The hypoosmotic swelling test: an update. Arch. Androl. 29, 105-116.

Johnson, L., Weitze, K., Fiser, P., Maxwell, W., 2000. Storage of boar semen. Anim. Reprod. Sci. 62, 143-172.

Khan, M., Naskar, S., Das, A., Bardoli, R., 2006. Comparative efficacy of different diluents on liquid preservation of boar semen. Indian J. Anim. Sci. 76, 780.

Knox, R.V., 2016. Artificial insemination in pigs today. Theriogenology. 85, 83-93.

Lechniak, D., Kedzierski, A., Stanislawski, D., 2002. The use of HOST test to evaluate membrane functionality of boar sperm capacitated in vitro. Reprod. Domest. Anim. 37, 379-380.

Malo, C., Gil, L., Cano, R., Martínez, F. and Galé, I., 2011. Antioxidant effect of rosemary (Rosmarinus officinalis) on boar epididymal spermatozoa during cryopreservation. Theriogenology. 75, 1735-1741.

Michos, I., Tsantarliotou, M., Boscos, C.M., Tsousis, G., Basioura, A., Tzika, E.D., Tassis, P.D., Lymberopoulos, A.G., Tsakmakidis, I.A., 2021. Effect of boar sperm proteins and quality changes on field fertility. Animals. 11, 1813.

Peña, S.J.T., Gummow, B., Parker, A.J., Paris, D.B.B.P., 2017. Revisiting summer infertility in the pig: could heat stress-induced sperm DNA damage negatively affect early embryo development? Anim. Prod. Sci. 57, 1975-1983.

Peña, S.J.T., Pagente, M., Ymas, B., Janier, M., 2023. Bacteriospermia among smallholder artificial insemination boars in the Philippines and potential associated factors. Asian Pac. J. Reprod. 12, 35-41.

Pérez-Llano, B., Lorenzo, J.L., Yenes, P., Trejo, A., García-Casado, P., 2001. A short hypoosmotic swelling test for the prediction of boar sperm fertility. Theriogenology. 56, 387-398.

Pérez-Llano, B., Yenes-García, P., García-Casado, P., 2003. Four subpopulations of boar spermatozoa defined according to their response to the short hypoosmotic swelling test and acrosome status during incubation at 37 °C. Theriogenology. 60, 1401-1407.

Pezo, F., Romero, F., Zambrano, F., Sánchez, R.S., 2019. Preservation of boar semen: an update. Reprod. Domest. Anim. 54, 423-434.

Pursel, V., Johnson, L., Rampacek, G., 1972. Acrosome morphology of boar spermatozoa incubated before cold shock. J. Anim. Sci. 34, 278-283.

Rodriguez-Martinez, H., 2001. Sperm function in cattle and pigs: morphological and functional aspects. Archiv Fur Tierzucht. 44, 102-113.

Rodriguez-Martinez, H., 2003. Laboratory semen assessment and prediction of fertility: still utopia? Reprod. Domest. Anim. 38, 312-318.

Rodriguez, A.L., Van Soom, A., Arsenakis, I., Maes, D., 2017. Boar management and semen handling factors affect the quality of boar extended semen. Porcine Health Manag. 3, 15.

Samardžija, M., Dobranić, T., Krušlin, S., Cergolj, M., Karadjole, M., Prvanović, N., Grizelj, J., 2008. The use of the hypoosmotic swelling test and supravital staining in evaluation of sperm quality in boars. Vet. Arh. 78, 279-287.

Saravia, F., Wallgren, M., Nagy, S., Johannisson, A., Rodríguez-Martínez, H., 2005. Deep freezing of concentrated boar semen for intra-uterine insemination: Effects on sperm viability. Theriogenology. 63, 1320-1333.

Schulze, M., Buder, S., Rüdiger, K., Beyerbach, M., Waberski, D., 2014. Influences on semen traits used for selection of young AI boars. Anim. Reprod. Sci. 148, 164-170.

Vazquez, J.M., Martinez, E.A., Martinez, P., Garcia-Artiga, C., Roca, J., 1997. Hypoosmotic swelling of boar spermatozoa compared to other methods for analysing the sperm membrane. Theriogenology. 47, 913-922.

Watson, P.F., 2000. The causes of reduced fertility with cryopreserved semen. Anim. Reprod. Sci. 60–61, 481-492.

Wiebke, M., Hensel, B., Nitsche-Melkus, E., Jung, M., Schulze, M., 2022. Cooled storage of semen from livestock animals (part I): boar, bull, and stallion. Anim. Reprod. Sci. 246, 106822.

Yeste, M., 2018. State-of-the-art of boar sperm preservation in liquid and frozen state. Anim. Reprod. 14, 69-81.

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