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Evaluation of specific fractions of Large White Yorkshire boar semen#

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Abstract

The current study was conducted to evaluate the quality of specific fractions of three Large White Yorkshire (LWY) boar semen samples. Fifteen semen samples were collected by gloved hand method as an initial 10 mL of sperm rich fraction (SRF- F1), remaining SRF (F2) and whole semen. Samples were evaluated for colour, volume, pH, sperm concentration and progressive motility. The first 10 mL of SRF of boar semen contained 25 per cent of the total ejaculate spermatozoa and was able to withstand cooling, freezing and thawing better than the spermatozoa of the bulk ejaculate. A significant difference between the fractions was observed in the volume of semen ejaculated, pH and sperm concentration across different fractions of the same ejaculate. The differences in semen fractions were attributed to the differences in the composition of seminal plasma of the initial fraction of SRF.

Keywords: *Boar semen, sperm rich fraction, semen evaluation*

The global swine industry is experiencing steady growth annually, driven by increasing demand for animal protein. The success in pig breeding using artificial insemination (AI) is attributed to advancements in fertility, labour efficiency, genetics, productivity, and its pivotal role in mitigating disease transmission within the herd through live animals. Boar sperm exhibits heightened sensitivity to low temperatures due to its high content of unsaturated phospholipids and low cholesterol in the plasma membrane.

The first 10 mL of sperm rich fraction (SRF) of boar semen was found to contain 25 per cent of the total ejaculate spermatozoa (Rodriguez-Martinez and Wallgren, 2011). Bicarbonate in the seminal plasma triggers cholesterol efflux, leading to plasma membrane reorganization characterized by changes in lipid raft composition and protein phosphorylation, resembling capacitation. Research suggests that the initial 10 mL of SRF has reduced bicarbonate levels, which may contribute to improved cryotolerance (Saravia, 2008). Hence, the present research was formulated to evaluate the quality of boar semen fractions with colour, pH, volume, motility and sperm concentration.

Materials and methods

Three adult healthy LWY boars, aged between one to three years possessing acceptable semen quality and maintained at the Centre for Pig Production and Research (CPPR), Mannuthy were selected for study. A total of 15 semen fractions and 15 complete ejaculates for the control study were collected by gloved hand method at an interval of three to four days between collections from the same boar. The initial 10 mL of SRF (F1) was collected in sterile 15 mL

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centrifuge tube. The rest of SRF (F2) and whole semen were collected in glass conical flasks, allowing it to pass through a Buchner funnel to separate the gel mass at the time of collection. The collected semen was immediately transferred to an insulated container and transported to the laboratory for preliminary evaluation. The fresh semen was evaluated for volume, pH, concentration and progressive motility. The volume of semen fractions was assessed using a graduated measuring cylinder. Sperm concentration was evaluated using a Neubauer counting chamber after diluting the semen to 1:200 using eosin-formal-saline. The progressive motility was assessed after mixing 10 µL of neat semen with 100 µL of PBS. Twenty-five microlitres of the diluted semen were taken on a clean, grease-free glass slide, covered with a clean cover slip and examined under 400× magnification of a phase contrast microscope. The pH of semen was assessed by taking a drop of the semen to the designated well of a hand-held pH meter. Statistical analysis was done using SPSS 24 software using Two-way ANOVA.

Results and discussion

The colour of F1 was thick milky to milky whereas F2 and whole semen was milky to thin milky. Out of 15 ejaculates collected as fractions, 11 (73.33 %) and four (26.67 %) numbers of F1 fractions were thick milky and milky, respectively, while 12 (80.00%) and three (20.00%) numbers of F2 were milky and thin milky, respectively. Colour in the rest of the ejaculate fractions varied from thin milky to watery. Out of 15 complete ejaculates collected, 10 (66.67%) were milky and five (33.33%) were thin milky in colour. Similar observations were recorded by Naik (2019) and Ambily (2021), who reported the colour of F1 as thick milky to milky, F2 as milky to thin milky and whole semen as milky to watery. The colour of semen reflects the concentration of sperm, the thick milky colour of SRF is due to a higher concentration of sperm and the watery colour of the post-sperm fraction was due to the lower number of sperm and higher amount of accessory sex gland secretions.

The mean volume of the gel-free F2 fraction and the whole semen was recorded as 91.33 ± 3.28 mL and 207.00 ± 3.28 mL. The volume of F1 fraction was fixed as 10 mL. There was a statistically significant difference between F2 and whole semen ($p< 0.05$) but not between the boars (Table 1). The higher volume of whole semen was due to higher amount of accessory sex gland secretions. Sebastian-Abad *et al.* (2021) recorded similar values for SRF, whereas Ambily *et al*. (2021) recorded lower values. The differences in semen volume reported by different authors could be due to differences in breed, method of collection, frequency of collection, season of collection,

Table 1. Ejaculate volume (Mean ± SE) in Large White Yorkshire boar in phase I of the study

Boar No.	Mean Ejaculate volume (mL)		F - Value P - value (Between the fractions)
	F2	Whole semen	
1	93.00 ± 5.68	197.00 ± 5.68	911.20* 0.00
2	92.00 ± 5.68	215.00 ± 5.68	
3	89.00 ± 5.68	209.00 ± 5.68	
Overall Mean (n= 15)	91.33 ± 3.28[a]	207.00 ± 3.28[b]	

n= total number of semen collections, * - significant 0.05 level

Means having different superscripts within a row (a, b) differ significantly

Table 2. Hydrogen ion concentration (Mean ± SE) in Large White Yorkshire boar semen in phase I of the study

Boar No.	Mean pH of semen			F - Value p-value (Between the fractions)
	F1	F2	Whole semen	
1	7.30 ± 0.04	7.40 ± 0.04	7.36 ± 0.04	2.58 * (0.00)
2	7.36 ± 0.04	7.44 ± 0.04	7.42 ± 0.04	
3	7.40 ± 0.04	7.44 ± 0.04	7.44 ± 0.04	
Overall Mean (n=15)	7.35 ± 0.02[a]	7.42 ± 0.02[b]	7.40 ± 0.02[ab]	

n= total number of semen collections, * - significant at 0.05 level

Means having different superscripts within a row (a, b) differ significantly

Table 3. Sperm concentration (Mean ± SE) of different fractions of ejaculate in phase I of the study

Boars	Mean sperm concentration (Million/mL)			F -Value P- Value (Between fractions)
	F1	F2	Whole semen	
1	894.00 ± 33.25^{A}	316.00 ± 33.25^{A}	216.00 ± 33.25^{A}	596.03* (0.00)
2	1318.00 ± 33.25^{B}	601.00 ± 33.25^{B}	295.00 ± 33.25^{B}	
3	1288.00 ± 33.25^{B}	581.00 ± 33.25^{B}	277.00 ± 33.25^{B}	
Overall Mean ± SE (n=15)	1166.66 ±19.20^{a}	499.33 ± 19.20^{b}	262.66 ± 19.20^{c}	

n= total number of semen collections, * - significant at 0.05 level

Means having different superscripts within a row (a, d) differ significantly

Means having different superscripts within a column (A, B) differ significantly

Table 4. Sperm progressive motility (Mean ± SE) of different fractions of ejaculate in phase I of the study

Boars	Mean sperm progressive motility (Per cent)			F - Value p-value (Between the fractions)
	F1	F2	Whole semen	
1	86.20 ±1.76	82.60 ± 1.76	87.20 ± 1.76	1.79 ^{ns} (0.18)
2	87.60 ±1.76	87.00 ± 1.76	83.60 ± 1.76	
3	87.00 ±1.76	83.60 ± 1.76	83.60 ± 1.76	
Overall Mean ± SE (n = 15)	85.03 ±1.01	84.40 ± 1.01	84.80 ± 1.01	

F1 – First 10 mL of SRF; F2 – Rest of SRF; Whole semen – Collected as complete ejaculate, n=number of semen collections, ns – non significant at 0.05 level

age and size of the boars and environmental associations including feeding and managemental practices responsible for modifying the semen ejaculation (Frangez*et al*., 2005).

The mean pH of F1, F2 and whole semen was 7.30 ± 0.04, 7.40 ± 0.04 and 7.36 ± 0.04, respectively. There existed a statistically significant difference between fractions ($p< 0.05$), but not between boars (Table 2). The alkaline pH of boar semen was recorded by Wilson (2018). Similar lower pH of F1, compared to F2 and whole semen was recorded by Naik *et al*. (2019) and Ambily *et al.* (2021). The F1 has less bicarbonate than the remaining SRF (Saravia, 2008) and higher sperm concentration produced more metabolic end products, particularly lactic acid, leading to lower pH in F1 than other fractions.

The overall mean sperm concentration of F1, F2 and whole semen was 1166.66 ± 19.20, 499.33 ± 19.20 and 262.66 ± 19.20 million/mL, respectively. Significant differences ($p < 0.05$) were observed between boars and between fractions ($p < 0.05$) in the sperm concentration of semen ejaculates (Table 3). The F1 represented the portion of sperm from the sperm reservoir in the epididymis which had less accessory gland secretions, while F2 had more accessory gland secretion and thus lowered sperm concentration (Rodriguez-Martinez *et al*., 2009). A higher concentration of F1 (1860 ± 0.20 million/ mL) and F2 (1250 ± 0.14 million/mL) were recorded by Siqueira *et al.* (2011) whereas, lower concentrations of 873.12 ± 91.34 million/ mL for F1 and 460 ± 35.24 million/ mL for F2 was recorded by Naik *et al*. (2019). The sperm concentration can be influenced by various factors such as nutritional status, overall health, season and managemental practices.

The overall mean sperm progressive motility in F1, F2 and whole semen were not significantly different between the fractions or between the boars ($p < 0.05$), with values of 85.03 ± 1.01, 84.40 ± 1.01 and 84.80 ± 1.01 per cent, respectively (Table 4). Pena *et al.* (2003) noted a more linear motility pattern in the initial 10 mL of seminal fluid compared to the rest of the ejaculate, possibly attributed to variations in bicarbonate levels or protein components in the seminal plasma. However, in the current study, no distinctions in progressive motility were detected between fractions, potentially because the sperm had a brief exposure to seminal plasma.

According to Saravia (2008), as the bicarbonate level in the initial 10 mL was low, the cholesterol efflux from the plasma membrane would be low in the first 10 mL of SRF and hence it could maintain the architecture of the

plasma membrane during storage period compared to other fractions in low temperature. This accounts for the high preservability of SRF. Hence, the initial 10 mL of SRF could be stored for the long term and the rest could be utilised for the short term as chilled semen.

Conclusion

The study revealed that there were significant differences in the pH and sperm concentration between fractions. Considering F1, F2 and whole semen, it was observed that variations existed in the composition and characteristics of the semen in these fractions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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