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Seminal plasma fractions of Large White Yorkshire boar semen differentially influence sperm preservability at 15°C

V.S. Swathish[1], Hiron M. Harshan[2*], C. Jayakumar[3], M. P. Unnikrishnan[4] and A. P. Usha[5]
Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University
Kerala, India

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Abstract

The study evaluated the effect of specific seminal plasma fractions on the in vitro fertility parameters of Large White Yorkshire (LWY) boar sperm. Semen collected as separate fractions from four adult LWY boars as F1 (first 10 mL of sperm rich fraction), F2 (rest of the sperm rich fraction), F3 post sperm rich fraction, was used to harvest seminal plasma. The effect of these fractions on sperm preservability at 15°C was studied using cauda epididymal sperm. The sperm incubated in the seminal plasma of F1 had better in vitro fertility parameters during the stages of preservation. Thus, it could be inferred that the sperm of the F1 fraction are more suitable to preservation than sperm of other fractions.

Keywords: *Seminal plasma fractions, F1, SRF, cauda epididymal sperm*

Among the domestic animals, semen of boar is unique as it is voluminous and is ejaculated as jets in broadly three fractions as (i) pre-sperm fraction (PSF, dominated by the secretions of accessory glands except seminal vesicles), (ii) the sperm-rich fraction (SRF, containing the major portion of sperm, bathed in epididymal fluid, diluted step wise with fluids derived from the seminal vesicles and prostate) and (iii) the post-sperm-rich fraction (PSRF, containing progressively lower sperm over time, suspended in fluid from the seminal vesicles, prostate and, by the end of the ejaculation, the bulbourethral glands (Mann and Lutwak-Mann, 1981).

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1. *MVSc. scholar*
2. *Associate Professor,*
3. *Associate Professor and Head*
4. *Assistant Professor, Centre for Pig Production and Research, Mannuthy*
5. *Director of Farms, KVASU and Professor and Head, Centre for Pig Production and Research, Mannuthy*

**Corresponding author: hiron@kvasu.ac.in, Ph. 9495661032*

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The seminal plasma of the corresponding fractions differs in their composition. The initial 10 mL of SRF (designated as F1) having lower levels of bicarbonate and higher levels of acrosin inhibitors (Jonakova *et al.,* 2007; Saravia, 2008). The post SRF had ions like Na+, Cl^-, Ca^{++}, Zn^+ and bicarbonate (Einarsson, 1971); of which Ca^{++} and bicarbonate were considered as initiators of capacitation (Harrison *et al.,* 1997). The bicarbonate in seminal plasma brings about a cholesterol efflux resulting in plasma membrane rearrangement with change in lipid raft and phosphorylation of proteins mimicking the capacitation (Harrison and Gadella, 2005). In fact, it has been reported that sperm in the initial 10 mL of SRF could withstand cryotolerance better when compared to sperm fortuitously present in other fractions (Saravia, 2008).

Taking into account the above findings, it could be inferred that seminal plasma influenced the sperm characters and as the seminal plasma fractions in boar semen differed in composition, their influence on sperm could also differ. Hence, this study was designed to investigate the effect of seminal plasma from different ejaculate fractions on boar sperm preservability at 15°C. As the cauda epididymal sperm are not exposed to accessory gland secretions, they can be considered as ideal candidates for the study.

Materials and methods

The study was conducted during the period of May 2021 to September 2022 using semen ejaculates collected from four adult, healthy Large white Yorkshire (LWY) boars aged one to three years, maintained under uniform feeding, housing and managemental conditions at the Centre for Pig Production and Research in Mannuthy, Thrissur.

Semen was collected using gloved hand method as per Wilson *et al.* (2019). The initial 10 mL of the SRF (F1), rest of the SRF (F2) and post SRF (F3) were collected separately, after allowing the semen to pass through a Buchner funnel to separate the gel mass. A total of 16 selected semen ejaculates (four from each of the four boars) having at least 70 per cent progressive motility were used for harvest of seminal plasma. The ejaculate fractions were centrifuged immediately (2000 g for 15 min at room temperature) and supernatant plasma was re-centrifuged (10000 g for 30 min at 15°C) to obtain clear seminal plasma. The corresponding seminal plasma fractions were pooled, further half of harvested seminal plasma from F1, F2 and F3 were pooled and designated as F4, which corresponded to seminal plasma of whole ejaculate. The harvested seminal plasma was stored in Eppendorf tubes at -80°C until used.

Cauda epididymal sperm were used to assess the effect of seminal plasma fractions on sperm preservability. The cauda sperm are not exposed to seminal plasma and hence are devoid of the influence of seminal plasma components, at the same time they are morphologically and physiologically similar to the ejaculated sperm. Testes were obtained from mature LWY boars slaughtered at Meat Technology Unit Mannuthy for the harvest of cauda epididymal sperm within 30 min of slaughter. The method of Lasley and Bogart (1944) was used for harvest of sperm from the cauda epididymides and sperm were initially extended with Beltsville thawing solution (BTS) at a ratio of 1:10, split into five aliquots and extended with seminal plasma or BTS so as to contain 300×10^6 progressive motile sperm per mL as, Group A: extended with pooled seminal plasma of all F1; Group B: extended with pooled seminal plasma of all F2; Group C: extended with pooled seminal plasma of all F3; Group D: extended with pooled seminal plasma of all F4 and Group E: Semen extended with BTS. These fractions were incubated in the seminal plasma or BTS for a period of three hours at 17°C. Subsequently, they were further extended to 50×10^6 sperm/mL with BTS and stored at 15°C for 72 h.

The extended sperms were evaluated for progressive motility, viability (Campbell *et al*., 1953), sperm abnormality (employing the smear prepared for viability), acrosome integrity (Giemsa staining technique, Watson, 1975) and sperm response to rapid hypo-osmotic swelling (HOS) test (Perez-Llano *et al.,* 2001) at 24, 48, and 72 h interval of preservation at 15°C.

Six such replicates were carried out.

Table 1. *In vitro* fertility parameters in cauda epididymal sperm of Large White Yorkshire boar incubated for three hours in seminal plasma fractions or BTS at 17°C and subsequently preserved at 15°C post extension with BTS (n=6).

Parameter	Preservation time	Mean in per cent ± SE (range)					F-value
		Group A	Group B	Group C	Group D	Group E	
Progressive motility	24 h	75.83 ± 3.27[a]	73.33 ± 1.67[a]	65.83 ± 2.01[b]	72.50 ± 1.12[a]	77.50 ± 1.70[a]	4.615**
	48 h	58.33 ± 2.79[b]	48.33 ± 2.79[c]	46.67 ± 2.47[c]	50.83 ± 2.01[c]	67.50 ± 1.71[a]	12.949**
	72 h	43.33 ± 3.07[b]	32.50 ± 2.14[c]	28.33 ± 1.67[c]	33.33 ± 1.67[c]	52.50 ± 3.10[a]	16.464**
Sperm viability	24 h	83.71 ± 1.56[a]	78.96 ± 1.45[a]	72.63 ± 2.45[b]	79.31 ± 1.69[a]	83.45 ± 0.93[a]	7.063**
	48 h	71.59 ± 2.16[ab]	65.41 ± 2.42[bc]	61.45 ± 2.24[c]	63.34 ± 2.48[c]	77.17 ± 1.64[a]	8.647**
	72 h	58.66 ± 2.73[b]	48.97 ± 1.41[c]	45.04 ± 1.30[c]	50.22 ± 2.26[c]	66.90 ± 3.00[a]	15.286**
Sperm abnormality	24 h	8.16 ± 0.36[cd]	9.22 ± 0.42[ab]	9.99 ± 0.32[a]	8.92 ± 0.30[bc]	7.59 ± 0.10[d]	8.599**
	48 h	10.46 ± 0.37[cd]	12.38 ± 0.53[ab]	13.26 ± 0.32[a]	11.58 ± 0.34[bc]	9.70 ± 0.53[d]	11.088**
	72 h	12.10 ± 0.45[bc]	13.85 ± 0.62[a]	14.55 ± 0.51[a]	13.45 ± 0.34[ab]	11.59 ± 0.36[c]	6.988**
Acrosome integrity	24 h	81.65 ± 1.30[a]	77.78 ± 1.20[b]	76.47 ± 1.14[b]	78.05 ± 1.40[b]	82.86 ± 0.95[a]	5.145**
	48 h	74.85 ± 1.14[ab]	71.19 ± 0.97[cd]	69.36 ± 0.65[d]	72.62 ± 1.00[bc]	76.99 ± 0.89[a]	10.127**
	72 h	65.51 ± 1.41[ab]	61.05 ± 1.14[cd]	58.14 ± 1.04[d]	62.09 ± 1.26[bc]	68.67 ± 1.21[a]	11.267**
HOS response	24 h	40.49 ± 1.17[ab]	36.56 ± 1.59[bc]	35.15 ± 1.45[c]	36.53 ± 1.78[bc]	41.34 ± 1.05[a]	3.615*
	48 h	35.27 ± 1.09[ab]	30.95 ± 1.01[cd]	28.72 ± 1.07[d]	32.80 ± 1.31[bc]	36.86 ± 0.91[a]	9.059**
	72 h	29.26 ± 0.68[b]	25.99 ± 0.55[c]	23.86 ± 0.53[d]	26.23 ± 0.87[c]	31.25 ± 0.40[a]	21.830**

***Significant at 1 per cent level, *Significant at 5 per cent level*

Means having different lower case alphabet as superscript differ significantly within a row

Group A: Cauda sperm incubated with pooled F1 seminal plasma (first 10 mL of sperm rich fraction).

Group B: Cauda sperm incubated with pooled F2 seminal plasma (rest of sperm rich fraction) Group C: Cauda sperm incubated with pooled F3 seminal plasma (post-sperm-rich fraction)

Group D: Cauda sperm incubated with pooled F4 seminal plasma (pooled F1, F2 and F3)

Group E: Extended with BTS

The treatment means were compared with one way ANOVA using SPSS software version 24.0.

Results and discussion

The cauda epididymal sperm *in vitro* fertility parameters recorded during the 72 h of preservation are represented in Table 1. It was noted that among the seminal plasma fraction treated groups (group A, F1; group B, F2; group C, F3; and group D, F4), sperm of group A had the better parameters during the period of storage. During the same period, sperm of group C, treated with F3 (seminal plasma of post SRF) exhibited the lowest *in vitro* fertility

parameters. On comparing the *in vitro* fertility parameters of sperm incubated and extended in BTS with those of sperm incubated in seminal plasma prior to preservation at 15°C, the values recorded in group E (incubated in BTS alone) were better or equivalent to the best recorded values in seminal plasma treated groups.

Pena *et al*. (2003) had recorded better progressive motility in sperm of F1 than rest of ejaculate. Saravia (2008) recorded better cryotolerance in sperm of F1 when compared to those of F2, which was attributed to the different protein composition of F2. Even though proteins with favourable effects were not identified at the plasma membrane level, a larger number of proteins in F2 were discovered to be related with a reduction in motility. Saravia (2008) had also discovered variations in pH, bicarbonate, ions, and proteins between F1 and F2.

The seminal plasma contributed by accessory sex glands are attributed to increase the fertility potential of the sperm, thus resulting in rapid deterioration of quality. Among the seminal plasma fractions, F1 has been reported to have the highest amount of cauda epididymal fluid and thus conversely, the least amount of accessory gland fluids (Rodriguez-Martinez *et al.,* 2009). Sperm of F1 had limited exposure of bicarbonate, fructose and zinc in comparison to sperm in F2 (Rodriguez-Martinez *et al.,* 2009). The different environment of sperm in F1 was thus believed to have positive effect on their function. Dziekoska (2017) found that the fraction F1 (the first 20 mL of SRF) showed stronger motility than the rest of the SRF, even before semen extension.

The higher progressive motility observed in group A could also be a reflection of the higher proportion of viable spermatozoa recorded in the group. Sellés *et al.* (2001) had recorded that the boar sperm in the earliest fractions were more resistant to chilling and freezing-thawing stress than those in the bulk ejaculate. Saravia (2008) reported that P1 spermatozoa (equivalent to group A treated with F1 in the current investigation) were better able to withstand handling stress and did not display capacitation-like sperm instability during controlled chilling. This aspect was ascribed to the low bicarbonate level (non-capacitating media) of F1. The lower bicarbonate resulted in reduced changes to plasma membrane architecture of sperm in F1 during the chilling process, thus helping them retain higher viability for a longer period of time compared to other fractions. The initial fractions have epididymal proteins like lipocalins and inhibitors of acrosin and trypsin, which could protect cells from proteolytic degradation from acrosin released from damaged sperm (Jonakova *et al.,* 2007). Epididymal sperm were reported to be more resistant to cold shock than ejaculated sperm cells (Malo *et al.,* 2011), which again suggests deleterious effects of seminal plasma on membrane stability.

The enzymes SOD, catalase, and GPx activity were shown to be higher in cauda epididymal fluid and prostatic fluid by Koziorowska (2011). The antioxidant capacity of F1 thus is much higher as it is composed mainly of cauda epididymal fluid. Barranco *et al.* (2015) confirmed this aspect and stated that the total antioxidant capacity (TAC) in F1 was the highest among the ejaculate, thus having the best preservability.

Naik (2019) reported that sperm abnormality was significantly lower in F1 from the 24th h of storage, when compared to all other fractions. This was attributed to the superior capacity of sperm in the first 10 mL of SRF to tolerate cold shock. Bicarbonate ions, more predominant in seminal plasma fractions excluding F1 are reported to induce scrambling of plasma membrane phospholipids (Harrison *et al.,* 1996), which could result in membrane alterations leading to abnormal forms (Saravia *et al.,* 2007). The relatively higher bicarbonate in F3 fraction of seminal plasma is expected to bring about increased changes in membrane and thus acrosome integrity when compared to the lower bicarbonate in F1 fraction. Saravia (2008) had described that the sperm in F1 fraction were less prompt to depict capacitation like changes as it contained low bicarbonate levels. Additionally, the sperm of F1 were reported to have a higher amount of cholesterol (Naik, 2019; Ambily *et al.,* 2021). Cholesterol has been described to play significant role in thermotropic behaviour of phospholipids, reducing the damages induced by cold shock (Parks and Lynch, 1992).

Druart *et al.* (2009) suggested that the sperm binding proteins from accessory sex glands decrease the fluidity of sperm plasma membrane thus leading to reduced HOS response. As the proportion of accessory gland fluids was higher in F2, F3 and F4, a lower HOS response in sperm treated with these fractions was expected when compared to those treated with F1 or BTS.

The low total antioxidant capacity of boar seminal plasma coupled with high PUFA content in sperm membrane phospholipids made boar sperm more vulnerable to peroxidative damage (Brezezinska-Slevbodzinska *et al.*, 1995). The changes in membrane lipids are expected to affect the HOS response of boar sperm.

The accessory sex gland secretions increase the fertility potential of the sperm resulting in a concomitant rapid deterioration of quality. Thus, sperm extended in BTS (group E) represented a population, which are the least exposed to accessory gland secretions and hence the better *in vitro* fertility parameters recorded in this group during preservation are along expected lines.

Conclusion

Summarising the results, F1 fraction of boar seminal plasma had least deleterious effects on sperm keeping quality under refrigeration at 15°C. Thus, it could be suggested that sperm from F1 fraction of boar semen were better suited for preservation.

Conflict of interest

The authors declare that they have no conflict of interest.

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