

Original Research Article

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## Evaluation of Large White Yorkshire Boar Semen Fractions

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### ABSTRACT

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Boar semen is ejaculated as three uneven fractions. Recent studies have reported that the spermatozoa in initial portion of sperm rich fraction (SRF) of the ejaculates are more resistant to cold shock and survive cryopreservation better than the spermatozoa in the rest of the ejaculate. The present work was carried out to assess the quality of Large white Yorkshire (LWY) boar semen fractions reared at Kerala. Semen collected with gloved-hand technique as fractions of 10 mL each (initial 10 mL of SRF - F1; rest of SRF – F2) were subjected to quality assessments of colour, volume, pH, sperm concentration and sperm progressive motility. Upon statistical analysis, significant difference was noticed in volume of semen ejaculate between boars and in pH and sperm concentration between fractions of the ejaculate.

### Introduction

Commercial pig farming is the one of the best and profitable business, can contribute national income to our country highly. This is especially true in Kerala scenario, where pig production has got acceptance and has gained popularity because of certain inherent traits of pigs like high fecundity, better-feed

conversion efficiency, early maturity and short generation interval, relatively smaller capital investment that can contribute to faster economic return to the farmers.

Artificial insemination (AI) using cryopreserved semen has showed less fertility and it requires high sperm numbers for optimum fertility. Boar sperm is highly

sensitive to low temperature as its plasma membrane contains high levels of unsaturated phospholipids and low cholesterol, which makes the boar sperm highly sensitive to low temperature. The higher sensitivity has led to the use of boar semen preserved at 15°C for routine insemination rather than cryopreserved semen as in other domestic species. Boar ejaculates semen in three uneven fractions as in dog and stallion, which are expelled as jets or spurts. Few studies have reported that the initial 10 mL of sperm rich fraction (SRF) was resilient to cooling, freezing and thawing because of its lower bicarbonate level and presence or absence of specific proteins. Hence a study was framed to assess quality of boar semen fractions (F1 and F2).

### **Materials and Methods**

Fifty five boar semen ejaculates were collected using gloved hand technique from four LWY boars maintained at the Centre for pig production and research, Mannuthy. Ejaculates were collected as fractions of 10 mL each in sterile 15mL centrifuge tubes, allowing it to pass through a Buchner funnel to separate gel mass. The initial 10 mL of sperm rich fraction was considered as F1 and the remaining sperm rich fraction as F2 for the further study. The collected semen fractions were immediately transferred to an insulated container and transported to the laboratory for preliminary evaluation. Semen was collected from the boars once per day at a frequency of twice a week with an interval of three to four days between collections from the same boar.

The fresh semen collected was evaluated for volume, colour, pH, sperm progressive motility and concentration. The volume of semen ejaculate as fractions was assessed by using graduated test tubes. The colour of F1 and F2 of the ejaculate was visually assessed separately and graded as watery, milky or thick milky based on the intensity of the white

colour of the fractions when taken in the graduated test tubes. The pH of the fractions was assessed by taking a drop of the semen to the designated well of a hand held pH meter (Horiba, Model – LAQUA twin pH-11, USA) after calibrating the equipment with a pH 7.0 buffer. The equipment measured pH to an accuracy of one decimal point. The sperm progressive motility in the F1 and F2 fraction was assessed after mixing 10 µL of neat semen with 100 µL of PBS. Twenty five microlitre of diluted semen was taken on a clean, grease free glass slide, covered with a clean cover slip and examined under 400× magnification of a phase contrast microscope (Olympus, Model: Magnus MLX, India) with bio-therm stage facility maintained at 37°C. Semen samples with a minimum of 70 per cent sperm progressive motility were selected for the study. The sperm concentration of each fractions of boar semen ejaculate was determined by using a Neubauer counting chamber after diluting the semen to 1:200 using eosin-formal-saline (Salisbury *et al.*, 1985). The sperm concentration was expressed in millions/mL.

### **Results and Discussion**

Among all the collections of phase I and II (n = 30), the volume of semen in LWY boar ejaculates ranged between 103.5-220.5 mL, with a mean volume of  $144.9 \pm 5.29$  mL. Individual boar semen volume ranged from  $108.5 \pm 1.60$  to  $176.25 \pm 9.16$  mL. The volume of semen differed (p < 0.01) significantly between boar 1, 2 and 4; while boar 3 had a similar ejaculate volume as boar 1. Kantharaj (2001) reported mean ejaculate semen volume of  $209.50 \pm 4.63$  mL in LWY boar. It could vary from 50-400 mL and 125-500 mL as reported by Hafez (1993) and Arthur *et al.*, (1996), respectively. Wilson (2018) recorded the mean total ejaculate volume of  $167.73 \pm 7.95$  mL in LWY boar. The differences in semen volume reported by

the other workers might be attributed to differences in the method of collection, frequency of collection, season of collection, age and size of the boars and other environmental association including feeding and managerial practices responsible for modifying the semen ejaculation.

Out of 55 ejaculates collected, 42 of F1 were thick milky and 13 were milky, while 42 of F2 were milky and 13 thin milky in colour. The colour in the rest of the ejaculate fractions varied from thin milky to watery. Studies of Hancock (1959) observed amorphous watery jelly-like mass in first 10-20 mL (pre sperm) followed by around 40 mL of sperm containing fraction which was densely opaque and similar to bull semen. Succeeding fractions became progressively less opaque due to progressive reduction of sperm concentration. They noted that decrease in sperm concentration would increase the transparency of the semen ejaculate.

The overall mean pH of fresh semen fractions (F1 and F2) was found to be on the alkaline side ( $7.36 \pm 0.01$ ;  $7.45 \pm 0.01$ ) ranging from 7.3-7.5 and 7.4-7.5, respectively. On an individual boar basis the pH of F1 and F2 was found to range from  $7.35 \pm 0.02$  to  $7.37 \pm 0.02$  and  $7.40 \pm 0.00$  to  $7.47 \pm 0.08$ , respectively. Although there was no significant difference between boars, the pH of semen ejaculate differed significantly between fractions ( $p < 0.01$ ) with those in F1 being lower. Roberts (1986) reported the normal pH of boar semen ranged from 7.0 to 7.8 (alkaline side). The alkalinity or acidity of the semen was affected by the higher or lower levels of accessory gland secretions in the ejaculate. Higher the accessory gland secretions to the ejaculate, higher would be the alkalinity of semen (Mann, 1974). The recorded pH values were similar to reports of Aamdal and Hogset (1957) where the pH value of boar semen varied from 7.43 to 7.5 in pre-sperm and

sperm rich fractions and from 7.43 to 7.5 in post spermatic fractions. Saravia *et al.*, (2010) reported lower pH ( $7.07 \pm 0.03$ ) with lower amount of bicarbonate ( $13.71 \pm 0.64$  mM/L) in first 10 mL of SRF (F1) than the rest of SRF (F2). F2 had pH of  $7.32 \pm 0.07$  with significantly higher bicarbonate level ( $20.21 \pm 0.79$  mM/L) than F1. In the present study, it was noted that the pH of boar semen varies in the different fractions of the same ejaculate and variation was also observed from one ejaculate to another. Specifically in F1, the pH was found to be lower compared to F2, which might be due to low bicarbonate level in the F1 and also higher sperm concentration credited to higher metabolic end products consecutively higher lactic acid production.

The overall mean sperm progressive motility in both F1 and F2 fractions, immediately after collection was  $84.06 \pm 0.93$  and  $82.5 \pm 1.02$  per cent, respectively, with a range of 75-90 in both the fractions. In individual boars, sperm progressive motility in F1 varied from  $81.25 \pm 2.39$  to  $86.25 \pm 1.25$  per cent and in F2 it varied from  $80.00 \pm 2.04$  to  $83.75 \pm 2.39$  per cent. No significant variation was observed in sperm progressive motility between boars, or between fractions. Pena *et al.*, (2003b) reported more linear pattern of motility in the first 10 mL SRF than rest of ejaculate, this difference might be due to differences in bicarbonate levels or protein components of seminal plasma in two portions. In present study, no difference in progressive motility was observed between fractions, which might be due to the very short exposure of sperm to the seminal plasma before they were evaluated for motility.

The overall mean  $\pm$  SE sperm concentration in F1 was found to be  $873.12 \pm 91.34$  million/mL with a range of 450.00-1570.00 million/mL and F2 had sperm concentration of  $460.00 \pm 35.24$  million/mL with range of 240-690 million/mL (Table 1 and 2).

**Table.1** Total ejaculate volume in large white Yorkshire boars (Range, Mean ± SE)

	Mean ± SE (Range)	Overall range and Mean ± SE (n=30)	F-value (p-value)
<b>Boar 1 (n=8)</b>	150.06 ± 5.56 <sup>B</sup> (125-167)	144.90± 5.291 (103.5-220.5)	22.339 (0.01)**
<b>Boar 2 (n=8)</b>	176.25 ± 9.16 <sup>A</sup> (150.5-220.5)		
<b>Boar 3 (n=7)</b>	139.57 ± 2.46 <sup>B</sup> (129.5-149)		
<b>Boar 4 (n=7)</b>	108.50 ± 1.60 <sup>C</sup> (103.5-116)		

\*\* Significant at 0.01 level; Means having capital letter as superscript differs significantly within a column.

**Table.2** The fresh semen characteristics of large white Yorkshire boar ejaculates in F1 and F2

Semen characteristics	Animal	Mean ± SE (range)		F-value (p-value)
		F1	F2	Between fractions
<b>pH</b>	Boar 1 (n=4)	7.35 ± 0.02 (7.3-7.4)	7.47 ± 0.02 (7.4-7.5)	45 (0.01)**
	Boar 2 (n=4)	7.37 ± 0.02 (7.3-7.4)	7.47 ± 0.08 (7.4-7.5)	
	Boar 3 (n=4)	7.35 ± 0.05 (7.3-7.5)	7.4 ± 0.00 (7.4)	
	Boar 4 (n=4)	7.37 ± 0.02 (7.3-7.4)	7.47 ± 0.02 (7.4-7.5)	
	Overall Mean ± SE (range) n = 30	7.36 ± 0.01 (7.3-7.5)	7.45 ± 0.01 (7.4-7.5)	
<b>Progressive motility (in per cent)</b>	Boar 1 (n=4)	81.25 ± 2.39 (75-85)	80.00 ± 2.04 (75-85)	1.27 (0.27) <sup>ns</sup>
	Boar 2 (n=4)	86.25 ± 1.25 (85-90)	82.50 ± 3.22 (75-90)	
	Boar 3 (n=4)	85.00 ± 2.04 (80-90)	83.75 ± 2.39 (80-90)	
	Boar 4 (n=4)	83.75 ± 1.25 (80-85)	81.25 ± 1.25 (80-85)	
	Overall Mean ± SE (range) n = 30	84.06 ± 0.93 (75-90)	82.5 ± 1.02 (75-90)	
<b>Sperm concentration</b>	Boar 1	875.00 ± 13.22	495.00 ± 6.45	226.65

(millions/ml)	(n=4)	(850-910)	(480-510)	(0.01)**
	Boar 2 (n=4)	487.50 ± 13.14 (450-510)	280.00 ± 14.71 (240-310)	
	Boar 3 (n=4)	725.00 ± 25.98 (660-780)	415.00 ± 14.43 (380-450)	
	Boar 4 (n=4)	1405.00 ± 121.20 (1050-1570)	650.00 ± 21.60 (590-690)	
	Overall Mean ± SE (range) n = 30	873.12 ± 91.34 (450-1570)	460.00 ± 35.24 (240-690)	

\*\* - significant at 0.01 level; \* - significant at 0.05 level; ns – non significant

On an individual boar basis, the F1 sperm concentration varied from 487.50 ± 13.14 to 1405.00 ± 121.20 million/mL and sperm concentration in F2 varied from 280.00 ± 14.71 to 650.00 ± 21.60 million/mL.

There was significant difference between boars ( $p < 0.01$ ) and between fractions ( $p < 0.01$ ) in the sperm concentration of semen ejaculates. Similar findings were recorded by Siqueira *et al.*, (2011), where researchers found higher sperm concentration in F1 ( $1860 \pm 0.20 \times 10^6$ ) than F2 ( $1250 \pm 0.14 \times 10^6$ ).

Glover and Mann (1954) observed that the sperm concentration varied in different fractions and even in different waves of ejaculation. As the study has shown significant differences in the pH and sperm concentration between the two fractions F1 and F2, it might be postulated that there exists differences in the composition and properties of the semen of this fraction and hence the keeping quality of these fractions may differ.

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