

Original Research Article

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Fresh Semen Characteristics of Large white Yorkshire Boar Semen Selected for Liquid Semen Preservation

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ABSTRACT

The laboratory examination of semen quality parameters is an indirect way of assessing male fertility. The objective of this research was to study the semen quality parameters of Large White Yorkshire (LWY) boar ejaculates, which had the minimum sperm progressive motility necessary for being selected for further processing. The pigs were maintained at the Centre for pig production and research, Mannuthy, Kerala. A total of 24 ejaculates were collected from seven boars using gloved hand technique. The collected semen was examined for volume, colour, pH, sperm concentration, progressive motility, viability, abnormality, acrosome integrity and functional membrane integrity. Gel free volume differed significantly between boars. There was no significant difference observed in pH, sperm concentration and progressive motility among the seven boars studied. Significantly higher ($p < 0.05$) viable sperms were observed in few boars. Similar observations were noted with respect to sperm abnormality too. Significant differences ($p < 0.05$) were observed in acrosome integrity and hypo-osmotic sperm swelling response among boars.

Keywords

Progressive
Motility, Viability,
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Introduction

Pigs offer a great support to livestock farmers due to their high prolificacy, feed conversion efficiency, short generation interval and smaller capital investment. For ensuring

revenue from the venture, the breeding efficiency from the pigs has to be ensured. Though artificial insemination (AI) in pig industry plays a major role in upgradation of species, it is not as popular as in cattle because of limitations of semen quantity required for

insemination and poor preservability (especially freezability) of boar semen. The laboratory evaluation of semen quality parameters is a convenient indirect way of assessing male fertility, without conducting an artificial insemination trial on each ejaculate. Hence, the article describes the normal parameters of LWY boar semen reared under Kerala conditions.

Materials and Methods

Twenty four ejaculates from seven LWY boars aged between 12-24 months, maintained at the Centre for Pig Production and Research, Mannuthy, were collected using gloved hand technique and a dummy mount. The semen was collected into a pre-warmed amber coloured insulated conical flask after allowing it to pass through a Buchner funnel to separate the gel mass. The collected semen was immediately transported to the laboratory for preliminary evaluation in an insulated container. The fresh semen collected was evaluated for volume, colour, pH, concentration, progressive motility, sperm viability, sperm abnormality, acrosome integrity and sperm response to hypo-osmotic swelling test (HOST). The volume of the semen ejaculate was measured by using graduated class A glass cylinder of 250 mL capacity. The colour of each ejaculate was visually assessed and pH was measured using hand held pH meter (Horiba, Model – LAQUAtwin pH-11, USA). The sperm concentration in the semen was evaluated by haemocytometer method (Salisbury *et al.*, 1985). Sperm progressive motility was recorded as a per cent of individual motility of the spermatozoa after diluting the semen. Only ejaculates with more than 70 per cent progressive motility were selected for further evaluation. Thus comparison was made between ejaculates found suitable for liquid semen preservation. Sperm viability and abnormality was assessed using eosin-nigrosin

staining technique as described by Campbell *et al.*, (1953) sperm acrosome integrity by Giemsa staining technique as described by Watson (1975) and the functional membrane integrity of the spermatozoa was assessed by HOST response as per the method described by Jeyendran *et al.*, (1984).

Percentage data recorded were transformed using Arcsine prior to analysis. Treatment means were compared using one way Analysis of variance (ANOVA).

Results and Discussion

The colour of the gel free ejaculates ranged from milky to thick milky. Of the 24 ejaculates collected, two had thin milky colour, seventeen had milky colour and five had thick milky colour. The observations of the current study were similar to the findings of Sreekumaran (1974). Frunza *et al.*, (2008) reviewed that the normal colour for boar semen ejaculate was white with bluish shadows. A decrease in spermatozoa concentration (even due to increased frequency of collections) made the semen colour to appear clear.

The volume of the semen ejaculates ranged between 120-280 mL, with a mean volume of 197.33 ± 8.65 mL. The volume of semen was significantly higher ($p < 0.01$) in boar 1 and 3; and significantly lower ($p < 0.01$) in boar 5. The observed gel free ejaculate volume was similar to those observed by Kantharaj (2001) in LWY (209.50 ± 4.63 mL). Hafez (1993) recorded a range of 50-400 mL for semen volume in boars. The semen volume observed in the present study fell within these reported ranges.

The overall average fresh semen pH was found to be on the alkaline side (7.39 ± 0.03), ranging from 7.10-7.60. On an individual boar basis the pH was found to range from $7.30 \pm$

0.09 to 7.50 ± 0.00 . There was no significant difference observed in the pH of semen ejaculates between the boars. According to Johnson *et al.*, (2000), the pH of boar semen ejaculate was in the range of 7.2-7.5, which was in accordance to the findings in the current work. The pH recorded by Strzezek *et al.*, (1995) in Yorkshire boar semen was slightly lower (7.1 ± 0.07). Semen ejaculates of the same animal can differ in its pH. A higher contribution of accessory sex gland secretions to the ejaculate, increased the alkalinity of semen (Mann, 1974).

The overall sperm concentration ranged from 140 to 310.8 million/mL with a mean \pm SE of 236.82 ± 9.49 million/mL. On an individual boar basis the sperm concentration varied from 166.67 ± 13.33 to 276.67 ± 12.02 million/mL. No significant variation was observed among the sperm concentration between the seven boars. This was in accordance with the observations of Labussiere (1990; 200-300 million/mL) and Strzezek *et al.*, (1995; 211 ± 22.07 million sperms/mL) in Yorkshire boar semen. Kantharaj (2001) recorded a sperm concentration of 261-329 million/mL with an average of 288.28 ± 2.73 million/mL in LWY boar semen, which was slightly higher than the concentration observed in the present study. The sperm concentration in the semen decreases as the frequency increases. With increasing frequency, the ejaculate volume is mainly contributed by seminal fluid from accessory glands and there will be a reduction in the concentration.

The overall sperm progressive motility, immediately after collection, ranged from 75.00 – 85.00 per cent with an average of 79.38 ± 0.76 per cent. Individual boar progressive motile sperm per cent varied from 75.00 ± 0.00 to 83.33 ± 1.67 . No significant difference was observed in progressive

motility of sperm in fresh semen between boars, which was in agreement with the findings of Kantharaj (2001). Much lower values of 65.70 per cent were observed by Sreekumaran (1974) with LWY boars. Cerolini *et al.*, (2001) recorded motility of 63.00 ± 2.20 per cent in a study which included boars of different genetic lines (Landrace, Large White and commercial hybrids).

In current study, the mean \pm SE sperm viability in the fresh semen samples was found to be 84.00 ± 0.83 with the range of 77–90 per cent. Individual boar viable sperm per cent varied from 79.00 ± 0.91 to 88.50 ± 1.26 . Significantly higher ($p < 0.05$) viability was found in boar 1 and significantly lower ($p < 0.05$) viability was found in boar 5. The recorded values were found to be much closer to values obtained in LWY boars by Murthy (1974, 84.5 per cent) and slightly higher values was reported by Sreekumaran (1974, 88.8 per cent) and Kantharaj (2001, 87.53 ± 0.56 per cent). The sperm viability observed was also lower to those observed in Hampshire boars (90.92 ± 1.56 per cent, Kumaresan *et al.*, 2009) and LWY boars (92.40 ± 0.28 per cent, Lalrintluanga *et al.*, 2016). The observed differences could be due to variations in season and handling of semen.

The overall mean \pm SE sperm abnormality recorded in the present study of fresh ejaculate obtained from LWY boars was 6.15 ± 0.57 per cent with a range of 2.00 – 12.00 per cent. On an individual boar basis the sperm abnormality varied from 3.17 ± 0.44 to 9.50 ± 1.19 per cent. Significantly higher ($p < 0.05$) abnormality was found in boar 6 and significantly lower ($p < 0.05$) abnormality was found in boar 1. These findings were in close agreement with the values reported by Kantharaj (2001) in LWY boars (6.88 ± 0.40 , ranged from 1.67 – 10.67).

Table.1 Fresh semen characteristics of Large white Yorkshire boars (n=24; Range, Mean ± SE)

Semen characteristics	Animal	Range	Mean ± SE	Overall Mean ± SE and range (n=24)	F-value (p-value)
Gel free volume (mL)	Boar 1 (n=3)	226-280	238.67 ± 21.18 ^a	197.33 ± 8.65 120.00 - 280.00	4.802** (0.005)
	Boar 2 (n=3)	165-243	208.00 ± 22.87 ^{abc}		
	Boar 3 (n=3)	220-270	248.67 ± 14.89 ^a		
	Boar 4 (n=3)	209-225	216.67 ± 4.63 ^{ab}		
	Boar 5 (n=4)	140-200	162.50 ± 13.15 ^c		
	Boar 6 (n=4)	120-210	165.00 ± 19.36 ^{bc}		
	Boar 7 (n=4)	150-200	172.50 ± 11.09 ^{bc}		
pH	Boar 1 (n=3)	7.4-7.5	7.43 ± 0.03	7.39 ± 0.03 7.10 - 7.60	0.787 ^{ns} (0.592)
	Boar 2 (n=3)	7.3-7.5	7.43 ± 0.07		
	Boar 3 (n=3)	7.3-7.6	7.43 ± 0.09		
	Boar 4 (n=3)	7.5	7.5 ± 0.00		
	Boar 5 (n=4)	7.1-7.5	7.30 ± 0.09		
	Boar 6 (n=4)	7.1-7.6	7.30 ± 0.11		
	Boar 7 (n=4)	7.1-7.5	7.38 ± 0.09		
Concentration (millions/mL)	Boar 1 (n=3)	210 – 280	240 ± 20.82	236.82 ± 9.49 140.00 - 310.80	2.270 ^{ns} (0.086)
	Boar 2 (n=3)	260 – 300	276.67 ± 12.02		
	Boar 3 (n=3)	180-260	226.67 ± 24.04		
	Boar 4 (n=3)	140-180	166.67 ± 13.33		
	Boar 5 (n=4)	141.75-271.95	234.46 ± 31.11		
	Boar 6 (n=4)	220-310.8	259.13 ± 19.11		
	Boar 7 (n=4)	210-293	244.83 ± 17.38		

** : Means with different superscript in a column for a given parameter differ significantly ($p < 0.01$), ^{ns} : non-significant

Table.2 Progressive motility, viability, abnormality, acrosome integrity and functional membrane integrity in freshly ejaculated sperm of Large white Yorkshire boars (n=24; Range, Mean ± SE)

Sperm characteristics	Animal	Range	Mean ± SE	Overall Mean ± SE and range (n=24)	F-value (p-value)
Progressive motility (in per cent)	Boar 1 (n=3)	80.00-85.00	81.67 ± 1.67	79.38 ± 0.76 75.00 - 85.00	1.884 ^{ns} (0.142)
	Boar 2 (n=3)	80.00-85.00	83.33 ± 1.67		
	Boar 3 (n=3)	75.00-85.00	80.00 ± 2.89		
	Boar 4 (n=3)	75.00	75.00 ± 0.00		
	Boar 5 (n=4)	75.00-85.00	78.75 ± 2.39		
	Boar 6 (n=4)	75.00-80.00	78.75 ± 1.25		
	Boar 7 (n=4)	75.00-80.00	78.75 ± 1.25		
Sperm viability (in per cent)	Boar 1 (n=3)	86.00-90.00	88.50 ± 1.26 ^a	84.00 ± 0.83 77.00 - 90.00	3.473* (0.020)
	Boar 2 (n=3)	83.50-90.00	86.50 ± 1.89 ^{ab}		
	Boar 3 (n=3)	82.00-87.00	85.17 ± 1.59 ^{ab}		
	Boar 4 (n=3)	83.00-89.00	85.83 ± 1.74 ^{ab}		
	Boar 5 (n=4)	77.00-81.00	79.00 ± 0.91 ^c		
	Boar 6 (n=4)	78.00-86.00	82.25 ± 1.93 ^{bc}		
	Boar 7 (n=4)	78.00-87.00	83.25 ± 2.06 ^{abc}		
Sperm abnormality (in per cent)	Boar 1 (n=3)	2.50-4.00	3.17 ± 0.44 ^c	6.15 ± 0.57 2.00 – 12.00	3.001* (0.035)
	Boar 2 (n=3)	3.00-7.00	5.33 ± 1.20 ^{bc}		
	Boar 3 (n=3)	7.00-9.00	8.00 ± 0.58 ^{ab}		
	Boar 4 (n=3)	4.00-8.00	6.00 ± 1.15 ^{abc}		
	Boar 5 (n=4)	2.00-10.00	5.50 ± 1.71 ^{bc}		
	Boar 6 (n=4)	7.00-12.00	9.50 ± 1.19 ^a		
	Boar 7 (n=4)	2.00-7.00	5.00 ± 1.08 ^{bc}		
Sperm acrosomal integrity (in per cent)	Boar 1 (n=3)	93.00-96.00	95.00 ± 1.00 ^a	91.81 ± 0.86 83.00 – 98.00	4.010* (0.011)
	Boar 2 (n=3)	88.00-90.00	89.00 ± 0.58 ^b		
	Boar 3 (n=3)	93.00-98.00	95.33 ± 1.45 ^a		
	Boar 4 (n=3)	95.00-97.50	96.50 ± 0.76 ^a		
	Boar 5 (n=4)	87.00-95.00	91.75 ± 1.79 ^{ab}		
	Boar 6 (n=4)	86.00-91.00	88.75 ± 1.11 ^b		
	Boar 7 (n=4)	83.00-95.00	88.50 ± 2.75 ^b		
Functional membrane integrity (in per cent)	Boar 1 (n=3)	64.00-74.50	69.50 ± 3.04 ^{abc}	70.71 ± 1.73 55.50 - 87.00	3.864* (0.013)
	Boar 2 (n=3)	69.00-79.00	74.33 ± 2.91 ^{ab}		
	Boar 3 (n=3)	65.00-72.00	68.33 ± 2.03 ^{bc}		
	Boar 4 (n=3)	55.50-63.00	58.83 ± 2.20 ^c		
	Boar 5 (n=4)	74.00-87.00	80.25 ± 2.78 ^a		
	Boar 6 (n=4)	60.00-83.00	66.75 ± 5.45 ^{bc}		
	Boar 7 (n=4)	68.00-80.00	74.00 ± 2.74 ^{ab}		

*: Means with different superscript in a column for a given parameter differ significantly ($p < 0.05$), ^{ns}: non-significant

According to Crabo (1997), sexually immature boars aged around 6–7 months tended to have a higher per cent of abnormalities than older

boars. Acceptable boar semen ejaculate should not cross 20 per cent of total morphological aberrations for further processing (Johnson *et*

al., 2000). Thus, the sperm abnormality values obtained in present study were within the normal permissible levels.

The overall per cent of (mean \pm SE) acrosome intact spermatozoa recorded in the present study of fresh semen ejaculates was 91.81 ± 0.86 per cent with a range of 83.00 to 98.00 per cent. On an individual boar basis the intact acrosome per cent varied from 88.50 ± 2.75 to 96.50 ± 0.76 per cent. Significantly higher ($p < 0.05$) acrosome integrity was observed in boar 1, 3 and 4 and no significant variation was found between them. Significantly lower ($p < 0.05$) values was observed in boar 2, 6 and 7 and no significant difference was noticed between these boars. The findings was found similar to the values reported by Kommissrud *et al.*, (2002, 93.9 per cent) but the results observed in the current study were higher than those reported by Das *et al.*, (2016) in Zovawk boar (71.8 ± 0.29 per cent). Pursel (1979) reported that the damage to acrosome increased with duration of storage rather than the extender used because of per oxidation effect.

The overall per cent of (mean \pm SE) functional membrane integrity tested by HOST in the present study was found to range from 55.50 to 87.00 per cent with an average of 70.71 ± 1.73 per cent. Individual boar sperm response to HOST per cent varied from 58.83 ± 2.20 to 80.25 ± 2.78 . Significantly higher ($p < 0.05$) HOST per cent was found in boar 5 and significantly lower ($p < 0.05$) HOST per cent was found in boar 4. The values observed in the present study were comparable with the results recorded by Das *et al.*, (2016, 69.55 ± 0.45 per cent) in Zovawk breeds of boar. However Kumaresan *et al.*, (2009) recorded as low as 54.67 ± 0.66 per cent of HOS positive sperms in his studies. When spermatozoa were hypo-osmotically stressed, those with a biochemically active membrane will permit the influx of water through an intact membrane until it reaches osmotic

equilibrium. This is suggestive of normal functional activity of the sperm membrane (Saravia *et al.*, 2004). Matson *et al.*, (2008) considered Hypo osmotic sperm swelling response as an indicator of fertility.

It could be concluded that LWY boar ejaculates, found suitable for liquid semen preservation, differed between boars with respect to gel free volume, sperm viability, abnormality, acrosome integrity and HOS response. While no such differences could be observed in the measurements of pH, sperm concentration and progressive motility between ejaculates of different boars.

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