In vitro Sperm Function Tests and Testicular Biometry for Fertility Prediction in Boar

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Abstract The study was conducted on six porcine males and thirty six semen ejaculates for its fertility assessment. The average length of the left and right testicle of the boars measured by ultrasonography was 9.47 ± 0.73 cm and 9.09 ± 0.65 cm, respectively. The sperm concentration/ml increased significantly as testicular diameter increased in size. The average length of right and left of boar testicle measured by Vernier caliper was 10.4 ± 0.57 cm and average width was 4.3 ± 0.14 cm. The average volume of boar semen was 115.00 ± 11.83 ml with milky colour. Thick consistency was observed in 83.3 % semen samples whereas 16.6 % semen samples were having thin consistency. Out of 36 semen ejaculates, 16.6 % semen samples had density of DD whereas 83.3 % had a density of DDD. The Mass activity, live percentage, percentage of morphologically abnormal spermatozoa and total sperm concentration in boar semen were 3 ± 0, 75.41 ± 2.07 %, 0 %, and 523 ± 60.07 million/ml, respectively. Mean percentage of hypo osmotic swelling test (HOS-Test) of semen found in the present study was 73.47 ± 2.26. The average time for reduction of resazurin dye from blue to violet was 1.805 ± 0.163 and from violet to pink was 9.944 ± 0.890. None of the sample change colour from pink to white. In the present study 36 ejaculates of boar semen were subjected for TVC of bacteria. In order to differentiate the bacterial species contaminating semen raw semen was placed on different agar plates. The species isolated with higher frequency in boar semen were of Staphylococcus species-83.3% (30 samples) followed by E.coli -63.8% (23 samples). Mean TVC obtained in this study was 45.13 × 10³.

Keywords Porcine; semen; sperm function tests; testicular biometry

1. Introduction

The challenges faced by the country in securing food as well as nutritional security to fast growing population need an integrated approach for livestock farming. Among the various livestock species, piggery is the most potential source of meat production and more efficient feed converters after broilers. Apart from meat it also provides bristles and manure. But total pig population of India is only 11134. Therefore it is a need of hour that pig farmers should be trained for scientific rearing of the pigs to retain pig fecundity and production. Pigs farrow two times in a year with minimum eight piglets
at each time. They mostly deliver sixteen piglets/annum. Piglets are saleable at the age of six months as they achieve the weight on an average 50 kg.

To mitigate the deficit between demands and supply of quality pig and pork products, the focus should be made on the basic and applied research output through genetic improvement of indigenous pigs, selective breeding and cross breeding. It is important to promote breeding soundness examination (BSEs) on boars, which can help to identify poor or questionable breeders/males before they affect herd fertility. Improvement of physiological and reproductive efficiency of pig production can be achieved through utilization of fertile boar bearing excellent semen quality. In last decade range of in vitro tests have been developed to examine structural characteristics and monitor crucial aspects of sperm function. Keeping in view the present scenario of sperm function tests for fertility prediction in boar, the present study was planned to perform the in vitro sperm function tests along with testicular biometry and bacterial total viable count of boar seminal plasma.

2. Materials and Methods

Selection of Animals

Fertile boars of White Yorkshayer breed belonging to Pig Farm, KNP College of Veterinary Science, Shirwal Dist Satara Maharashtra state (India) were selected for present research pursuit.

Physical Examination of Reproductive Organs

All selected boars (n=6) were subjected to complete physical examination of reproductive organs to determine location, shape, consistency and discomfort on palpation of the testicles, prepuce, penis, scrotum. Physical examination of the reproductive organs was done only once during the first semen collection.

Biometry of Testis

A real time B-mode, portable ultrasonography machine with 5-mHz sector transducer was used for scanning of testis in boars. During scanning the relevant image was frozen and measurements were taken by electric calipers. All the relevant pictures were printed by using electronic printer. The measurements were also taken in standing position by using a digital Vernier Caliper.

Semen Collection

Collection of semen of each boar was done by Gloved Hand Method. Digital pressure was exerted intermittently on the corkscrew end of the penis and semen was collected in collection flask. After the collection semen was filtered by using muslin cloth to remove the gel fraction.

Semen Evaluation

Semen samples were evaluated for various macroscopic, microscopic and sperm function tests. The former consisted of volume, color, consistency and density, while later consisted of mass activity, initial motility, total sperm count, live sperm percentage and abnormal sperm percentage. Additional sperm function tests were performed to evaluate functional ability of sperm, which included Resazurin test, Hypo-osmotic swelling test.

Sperm Function Test

Hypo-Osmotic Swelling Test (HOS test)

Equal volumes of 2.7% aqueous solution of fructose and 1.47% aqueous solution of sodium citrate (0.5 ml each) were mixed and kept in an incubator at 37°C for 10mins. 0.1 ml of semen was added in
above hypo-osmotic solution and incubated at 37˚C for 30 minutes. 10µl of this mixture was taken on glass slide and covered with cover glass. Slide was observed under 40x objective lens to determine the number of spermatozoa showing swollen head and coiled tail indicating sperms with intact plasma membrane.

**Resazurin Reduction Test**

Resazurin solution was prepared by mixing 50 mg Resazurin (7-hydroxy-3H, phenoxzin-3, one-10-oxide mixed in 100 ml of 0.9 % normal saline. This resazurin solution was used for further study. 250 μl porcine semen was taken in a test tube and 25μl resazurin solution was added. This solution was layered with small quantity of mineral oil. Test tube was kept in incubator set at 37˚C for colour change. The time required for color change from dark blue to violet, violet to pink, pink to white and was noted in minutes.

### 3. Results and Discussion

**Physical Examination of Reproductive Organs of Boars**

On examination it was found that, in all 6 boars, both the testes were symmetrical, firm and slightly resilient. Epididymides on palpation was found to be symmetrical from left to right, head firm and tail were tense. The prepuce and preputial diverticulum were free from signs of irritation or infection. Penis was found to be normal and free from persistent frenulum, lacerations, ulcers and scars. Scrotum was free of scarring, abscesses, thickening, irritation and from evidence of mange. Testes were freely movable within scrotum and no excess fluid was palpable. These observations were in accordance with those of Shipley (1999).

**Biometry of Boar Testis**

On placing the transducer on the scrotal skin after fixation of the testicle, an image with a relatively homogenous echogenic parenchyma with a centrally located hyperechoic mediastinum testis was seen. The tunica albuginea appeared as a distinct hyperechoic line, which surrounded the testicular parenchyma. The mediastinum testis was used as a reference point for higher accuracy and complete measurement. Clark et al. (2003) reported similar findings in boars. Testicular measurements of boars were taken after freezing the images when the mediastinum testes was identified as having largest diameter, testicular diameter was measured by use of electronic cursor points for all selected boars.

**Mean Ultrasonic Measurements of Testis in cm**

| Left Testicular Diameter- (LTD) | Right Testicular Diameter- (RTD) | Average Diameter (cm)(n=6) |
|---------------------------------|---------------------------------|-----------------------------|
| 9.475 ± 0.736                  | 9.09 ± 0.65                    | 9.285 ± 0.676               |

**Comparison between Paired Testicular Diameter (PTD) (var 1) and Sperm Concentration per ml(Var1) using Students T test**

| Correlation Matrix | Var1 | Var2 | Var3 | Var4 | Var5 | Var6 | Var7 |
|--------------------|------|------|------|------|------|------|------|
| Var1               | 1.000| 0.866| 0.864| 0.879| 0.909| 0.830| 0.872|
Students T Test

| Variables Tested | T Value | T Table | Significance at 5% |
|------------------|---------|---------|-------------------|
| Var1 - Var2      | 3.485   | 2.776   | Significant       |
| Var1 - Var3      | 3.474   | 2.776   | Significant       |
| Var1 - Var4      | 3.575   | 2.776   | Significant       |
| Var1 - Var5      | 4.331   | 2.776   | Significant       |
| Var1 - Var6      | 3.089   | 2.776   | Significant       |
| Var1 - Var7      | 3.548   | 2.776   | Significant       |

* (P<0.05)

Significant differences were found between PTD and Sperm concentration/ml (P<0.05). The study indicates that as testicular diameter increased in size, the sperm concentration/ml increased significantly. However, the present observations are higher and significant than those observed by Clark et al. (2003) who found no significant difference between PTD and average total sperm numbers (ATSN), since this study was limited due to the small number of observation in boars this may account for the fact that differences were not detected.

All the experimental boars were subjected to testicular biometry by using Vernier calipers to evaluate length, width once during the study.

Mean Vernier Caliper Measurements of Testis

| Parameter (n=6) | Right          | Left           | Average         |
|----------------|----------------|----------------|-----------------|
| Length (cm)    | 10.40 ± 0.76   | 10.68 ± 0.75   | 10.4 ± 0.57     |
| Width (cm)     | 4.3 ± 0.21     | 4.4 ± 0.2      | 4.3 ± 0.14      |

Semen Evaluation

Semen samples of 6 boars were evaluated for various macroscopic and microscopic tests. Total 6 ejaculates from each boar (total 36 ejaculates) were collected at weekly interval. Immediately after collection volume, colour, consistency and density were evaluated by visual observations and semen was placed in a water bath at 37°C, till further evaluation was carried out.

Macroscopic Evaluation of Boar Semen

Mean ± SE values of Macroscopic Semen Evaluation

| Parameter          | Result          |
|--------------------|-----------------|
| Volume (ml) (n=6)  | 115.00 ± 11.83  |

Mean semen value reported by Masenya et al. (2011) in Kolbrek and Large White boar semen was reported as 140.4 ± 48.6 & 177.5 ± 60.4 which is higher than that observed in the present study. This difference may be attributed to hot environmental condition since collection was done in afternoon session. Decreased nutrition and hot environmental conditions also cause decrease in semen volume (Foote, 1978). The difference in collection time volume may be attributed to a decrease in estradiol-17β levels. Estradiol-17β is involved in maintenance of libido and it has been demonstrated that this hormone is responsible for maintaining semen volume in castrated boars. The consistency was recorded as thick and thin with 83.3% showing thick consistency of semen while 16.6% having thin
semen consistency. 16.6% semen samples had density of DD where as 83.3% had a density of DDD. Finding of milky colour of semen is same as described by Shipley (1999) in boars. When the literature was scanned specific references pertaining to consistency and density in porcine were not available for comparison. However present findings corroborate with those reported in dogs. Maximum numbers of semen samples were milky, thick with density DDD.

**Microscopic Evaluation of Boar Semen**

| Parameter (n=36)                      | Result |
|--------------------------------------|--------|
| Mass activity                        | 3 ± 0  |
| Live sperm %                         | 75.41 ± 2.07 |
| Morphologically abnormal sperm %     | 0      |
| Sperm concentration (million/ml)     | 523 ± 60.07 |

The Mass activity of spermatozoa was 3 ± 0, which is nearby to observation made by Vyt et al. (2004) for boars and Kunbhar et al. (2011) for kundhi buffalo bull. The average percentage of live spermatozoa in the present study was 75.41 ± 2.07 which is lower than observations made by Kumaresan et al. (2009) (90.92 ± 1.56 %) and Masenya et al. (2011) (84.6 ± 6.1 %), and Borg et al. (1993) in boars. Morphologically no abnormality in spermatozoa in any experimental boars was found in the samples during study. In the present study the average sperm concentration in boars found was 523 ± 60.07 million/ml as compared to 727 ± 340.8 million/ml by Masenya et al. (2011) in boars and 133 ± 4, 86 ± 32, 100 ± 22, 117 ± 25 million/ml in Duroc, Landrace, Duroc/Landrace and Yorkshire boars reported by Kommisrud et al. (2002). It can be concluded that conventional microscopic characteristics of semen are good indicators of sperm quality and are simple and rapid semen evaluation tests.

**Sperm Function Tests in boars**

**Plasma Membrane Integrity by Hypo-Osmotic Swelling Test (HOST)**

The clinical use of the hypoosmotic swelling test (HOS-Test) to identify spermatozoa with a functional intact membrane has been reported for humans and domestic species, including the boar. This test evaluates response of spermatozoa to hypo-osmotic stress. The basis of this assay is that, when viable sperms are exposed to hypo-osmotic medium, the intact membrane swells and their tail curls and bulges due to influx of fluid. Sperm undergoing coiling reaction are considered to have positive reaction and sperm with damaged membrane will not swell because solution will not enter. Total 36 samples of boars were subjected to hypo-osmotic swelling test.

**Mean percentage of Hypoosmotic Swelling Test**

| Result (Samples n=36) | Average (%) |
|-----------------------|-------------|
| 73.47±2.26            |             |

Samardzija et al. (2008) reported a mean percentage as 16.96 ± 4.27 in boars which is lower than that observed in the present study.

**Resazurin Reduction test**

Resazurin is a chemical indicator and its reduction by mitochondrial enzymes of metabolically active sperm cells offer an assessment of the reducing capacity of semen, which is manifested by a spectrum of colours. Workers have reported a significant correlation between Resazurin reduction test (RRT) and fertility, as RRT evaluates the metabolic status of active spermatozoa and it is
associated with the concentration of motile sperms (Erb et al., 1952; Dart et al., 1994). RRT has been used successfully in assessing fertility potential in boars (Zrimsek et al., 2004).

*Mean Values of Resazurin Reduction Test (Time in minutes)*

| Semen collected from 6 boars | Blue to violet | Violet to pink |
|-------------------------------|----------------|---------------|
| Result                        | 1.805 ± 0.163  | 9.944 ± 0.890 |

None of the sample change colour from pink to white; it could be attributed to the lower concentration of spermatozoa in porcine semen as compared to bull and rams. When the literature was scanned specific references pertaining to RRT in porcine were not available for comparison. However present findings corroborate partly with those reported by Erb et al. (1952) for bulls (blue to pink) and Pathak et al. (1989) in cross bred bulls (violet to pink). Present findings are also similar to those reported by Erb et al. (1950) in bulls (Blue to pink), El Battawy (2008) and Dart (1994) in Limousin bulls (blue to pink).

**Bacterial Total Viable Count in Boar Semen**

| Semen collected from 6 boars | Average (10⁶ CFU/mL) |
|-------------------------------|---------------------|
| Result                        | 45.13               |

Mean TVC of boar semen obtained in this study was 45.13 x 10³ which is lower than that reported by Ciornei et al. (2012) in boars. In order to differentiate the bacterial species contaminating semen raw semen was placed on different agar plates. The species isolated with higher frequency in boar semen were of Staphylococcus species-83.3% (30 samples) followed by E.coli -63.8% (23 samples), this findings corroborate with those observed by Ciornei et al. (2012) in boars.

4. Conclusion

Ultrasonographic testicular biometry in porcine can be carried out by excluding epididymis and scrotal skin thickness, which cannot be avoided when testicular biometry is done by using Vernier calipers. Significant differences were found between paired testicular diameter (PTD) and sperm concentration per ml of semen sample. This indicates that as testicular diameter increases in size, the sperm concentration/ml increase significantly. Even collected in the strictest aseptic condition boar semen contains bacteria.

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