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Large White Yorkshire Boar Semen Preservation at Refrigeration Temperature

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A B S T R A C T

Preservation of boar semen at 18°C temperature requires climatic box which is costlier offence. Present study investigated seminal status at refrigeration (5°C) temperature in comparison to commonly used storage temperature (18° C). A total of 72 semen ejaculates were collected, diluted with KIEV extender and incubated at 2 different storage temperatures (18°C and 5°C) for 24, 48 and 72 hours, and seminal attributes were analyzed after each period. Percentage of motile sperm, live sperm, sperm with intact acrosome and HOSST positive sperms decreased significantly ($p<0.01$) as incubation intervals increases at both 18°C and 5°C storage temperature. However, these parameters showed a significantly ($p<0.01$) higher values at 18°C compared to 5°C. It can be concluded that semen of Large White Yorkshire boar extended with KIEV show better seminal attributes for short term storage at 18 °C in comparison to 5°C and this study provides further opportunities to explore the cause and effects for detrimental seminal attributes at 5°C.

Introduction

Piggery is an emerging area of business in India. It is considered as most important source of livelihood for economically weaker particularly tribal sections of country. Artificial insemination (AI) is a technique by which the profits in piggery can be maximized. The preservation of boar semen is a worldwide problem for optimum fertility. Boar semen differs in several aspects from the semen of other domestic animals, for instance, the semen is produced in a large volume and highly sensitive to cold shock and the viability of the sperm cells is dramatically reduced when exposed to temperatures below 15^0C (López Rodríguez *et al.*, 1996). In most cases, liquid stored semen or fresh semen is used for AI in commercial swine herds (Wagner and Thibier, 2000). Therefore, the manipulation of boar semen requires special consideration during preservation process (Johnson *et al.*, 2000). One of the obstacles to the use of artificial insemination (AI) in swine is the short storage life of boar spermatozoa (Johnson *et al.*, 2000). Further, maintenance of 18° C storage temperature requires sophisticated climatic box which is costly and

imported item in India. Utilization of preserved semen for AI in pigs has increased approximately threefold in the past 15 years, and more than 99% of the estimated 19 million inseminations conducted worldwide are made with semen that has been extended in the liquid state and used on the same day, or stored at 15-20°C for 1 to 5 days (Johnson *et al.*, 2000). However, as a general rule, the farrowing rates were 65% to 70% when the semen was used in the first 2 days after collection but they reduced to about 50% with 5-day-old semen (Johnson, 1988). In order to exploit genetically superior boars under production conditions, it has always been need of the hour to store semen for longer periods with acceptable semen quality to ensure optimum fertility. The seminal parameters like sperm motility (%),live sperm (%), sperm with intact acrosome (%) and HOSST (%) positive sperms are among the some parameters used for fertility measurement. It is therefore necessary to further study methods for longer storage of boar semen in liquid state.

A previous study (Pursel *et al.*, 1973) has shown that the fertility potential of extended boar semen stored at 15°C yields acceptable fertilization rates, while exposure to temperatures below 15°C would result in cold-shock and cell death. Over the period of time, some scanty reports on semen preservation (18°C) has been found in the literature, however, reports are meager on preservation of semen of large white Yorkshire boar in India especially under tropical climatic condition. In India, very limited work on preservation of boar semen has been undertaken evaluating effects of different preservation temperature on seminal attributes of Large White Yorkshire boar. Therefore, present investigation was undertaken to study boar semen preservation at refrigeration temperature (5° C) in comparison to 18°Cat different time interval.

Materials and Methods

Six Large White Yorkshire boars aged 2 years maintained at Animal Farm, Bihar Veterinary College, Patna were used for present study. All the boars were kept on approved ration schedule with identical managemental conditions. Semen samples were collected at weekly interval by gloved hand technique adopting the procedure given by Zavos and Liptrap (1987). A total of 72 semen ejaculates, 12 from each boar, were studied in present study. Sperm rich fraction was collected in thermos flask of 500 ml capacity. The sperm rich fraction of boar ejaculate was evaluated for pH, progressive motility of spermatozoa to determine the percentage of motile sperm by examination of a drop of semen on glass slide under coverslip at 37°C under high power of magnification (40X). Live sperm percentage was evaluated by Eosin-Nigrosin staining technique under 100X. Concentration of spermatozoa (millions/ml) was determined using Neubauer's haemocytometer (Salisbury, 1985). Abnormal spermatozoa were assessed by Rose Bengal staining technique under 100X. Percent intact acrosome was assessed by staining the semen smears with Giemsa stain under 100X. Percentage of hypo-osmotic swollen sperms were recorded by incubating semen with a hypo-osmotic solution at 37°C for 60 min and examining swelling of sperm tail under 40X.Semen samples having 70% or more progressive motility were diluted with KIEV extender based on concentration of spermatozoa in the semen. The extended semen were kept in 100 ml flask and filled up to brim and capped to maintain an anaerobic condition. Then these samples were preserved in B.O.D. incubator at two different storage temperatures of 18°C and 5°C for durations of 24, 48 and 72 hours. Soon after completion of each incubation intervals at different preservation temperature, seminal attributes like percent motile sperm, percent live sperm, percent abnormal sperm,

percent sperm with intact acrosome and percent HOSST positive sperm were analyzed.

Statistical analysis

All data were statistically analyzed by SPSS 16.0 using one way ANOVA. Data were expressed as Mean±S.E and $p<0.01$ was considered significant.

Results and Discussion

The mean value (Mean±S.E) of different physico-morphological characteristics of fresh semen of Large White Yorkshire boar have been presented in Table-1. Recorded values of seminal attributes of fresh boar semen are in agreement with the findings of Pandey and Singh (1998), Zou and Yang (2000) and Kansi (2007). However our values differ with Bhuyan *et al.,* (1992). Seminal characteristics have been found to be affected by various intrinsic and extrinsic factors like genetic makeup, agro-climatic difference, age, body weight, feeding, management and functional status of reproductive organs. This might be the reason of variation in our recorded values.

Mean values of seminal attributes of Large White Yorkshire boar during different hours of preservation at different storage temperature are shown in Table-2. Progressive sperm motility is important in evaluating fertilizing potential of spermatozoa and prerequisite for fertilization (Amelar *et al.,* 1980). Sperm motility depends on number of factors, including temperature at which the semen is kept between the time of ejaculation and the time of evaluation. Temperatures lower than 5^0C and higher than 37^0C have been shown to affect sperm functions negatively. In present study, percent sperm motility and livability were significantly ($p<0.01$) higher when stored at 18°C compared to storage at 5°C at all stages of preservation. However, these parameters decline significantly with progress of preservation time with lowest values at 72 hours.

Table.1 Mean value of different seminal characteristics of Large White Yorkshire Boar semen at 37°C

S. No	Seminal characteristics	Mean values (Mean ± S.E.)
1.	Pre-sperm fraction volume (ml)	17.43 ± 0.11
2.	Strained volume (ml)	164.17 ± 1.49
3.	Gel volume (ml)	45.98 ± 0.29
4.	Total volume (ml)	227.58 ± 1.44
5.	pH (strained volume)	7.51 ± 0.029
6.	Progressive motility(%)	64.05 ± 0.267
7.	Sperm concentration (million /ml)	281.67 ± 1.25
9.	Live sperm (%)	67.29 ± 0.274
10.	Sperm abnormality (%)	7.67± 0.225
11.	Intact acrosome (%)	87.59±0.47
12.	HOSST (%)	60.17 ± 0.322

Table.2 Mean value of seminal characteristics of Large White Yorkshire Boar during different hours of preservation at different storage temperature

Sl.No.	Seminal characteristics	Preservation temperature	Preservation hours		
			24 Hrs	48 Hrs	72 hrs
1	Motility (%)	18°C	67.75 ^{Aa} ± 0.41	61.83 ^{Ab} ± 0.405	52.42^{Ac}± 0.514
		5°C	59.92 ^{Ba} ± 0.452	44.92^{Bb} ± 0.26	34.17 ^{Bc} ± 0.423
2	Live sperm (%)	18°C	64.12 ^{Aa} ± 0.372	58.80 ^{Ab} ± 0.192	54.64^{Ac}± 0.313
		5°C	45.62 ^{Ba} ± 0.269	38.60^{Bb} ± 0.266	33.51 ^{Bc} ± 0.32
3	Abnormal sperm (%)	18°C	7.75 ^{Aa} ± 0.141	8.25 ^{Ab} ± 0.132	13.09 ^{Ac} ± 0.291
		5°C	7.84 ^{Ba} ± 0.112	11.52 ^{Bb} ±0.211	17.22^{Bc} ±0.141
4	Intact acrosome (%)	18°C	83.32 ^{Aa} ± 0.55	76.22 ^{Ab} ± 0.63	71.25 ^{Ac} ± 0.41
		5°C	79.66 ^{Ba} ± 0.28	72.21 ^{Bb} ±0.61	69.27^{Bc} ±0.32
5	HOSST (%)	18°C	35.67 ^{Aa} ± 0.074	28.58 ^{Ab} ± 0.26	20.17 ^{Ac} ± 0.322
		5°C	29.75 ^{Ba} ± 0.217	20.25^{Bb} ± 0.217	17.58 ^{Bc} ± 0.26

Mean with different superscripts in a row (a, b, c) and in a column (A,B) differ significantly at $p<0.01$.

Over 50 percent of motile and live spermatozoa with intact acrosome were maintained when stored at 18°C up to 72 hours of preservation in KIEV dilutor compared to storage at 5°C. Our findings are in close agreement with Zou and Yang (2000) and Lalrintluanga *et al.,* (2002). However, abnormal sperm percentage showed increasing trend with increase of preservation time at both storage temperature. Both storage temperature and preservation time were having significant (p<0.01) effect on sperm abnormality percentage. Our findings are in agreement with Pandey and Singh (1998) and Kansi (2007).

In concerned to sperm function tests, the percent sperms with intact acrosome and percent HOSST positive sperm shown a significantly ($p<0.01$) higher values when stored at 18°C compared to storage at 5°C at all stages of preservation. However, these parameters decline significantly (p<0.01) with progress of preservation time with lowest values at 72 hours. Our findings are in close agreement with Lalrintluanga *et al.,* (2002). In our study, HOSST score of each group was lower than percentage of motile sperms. The difference may be attributed to the fact that some spermatozoa with membrane damage may remain motile. In contrast, boar spermatozoa are particularly sensitive to temperature lower than 15°C due to cold shock injuries as compared to spermatozoa of other domestic species (Parks and Graham, 1992). Dilution and cooling render boar sperm membrane more permeable (Ortman and Rodriguez-Martinez, 1994). The sperm plasma membrane suffers an irreversible damage at 5°C (Perez-Llano *et al.,* 2001). This cold shock sensitivity is characterized by an irreversible loss of selective permeability and integrity of the sperm plasma membrane, all of which leads to perturbation of cell and death (Watson, 2000). Low fertilizing ability of spermatozoa at low temperature is

associated with many factors including a highly sensitive plasma membrane of boar spermatozoa against the change in temperature during cooling, freezing and thawing process (Holt, 2000). This problem is related to the lipid composition of the sperm plasma membrane. The plasma membrane of the boar spermatozoa contains a high level of polyunsaturated fatty acids (PUFAs) i.e., docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), and had a low cholesterol to phospholipids ratio. DPA and DHA are dominant fatty acids in the plasma membrane of boar spermatozoa (Johnson, 1985). Although efforts have been made to improve viability of frozen-thawed sperms (Cheon*et al.*, 2002; Yi *et al.*, 2004).

It is evident from this study that all seminal parameters considered in this study except percent abnormal spermatozoa decreased significantly as incubation intervals increases at both 18°C and 5°C storage temperature. Likewise, these parameters showed a significantly ($p<0.01$) higher values when preserved at 18°C compared to 5°C at different incubation intervals. It can be concluded that semen extended with KIEV, maintained the fertilizing ability of spermatozoa (in terms of seminal attributes) better for short term storage at 18°C in comparison to 5°C. therefore, preservation at refrigeration temperature (5 C) needs to be further standardized for economic storage of boar semen.

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