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Effects of bovine serum albumin during preservation of boar semen at 17 °C

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Abstract
Bovine serum albumin (BSA) has been reported to improve sperm quality, primarily by enhancing sperm motility, viability, membrane integrity and acrosome integrity. In this study, crossbred (75% LWY x 25% Zovawk) boars' semen samples were collected and diluted with GEPS extender containing different concentration (control, 5, 10 and 15%) of BSA at 24 hours of preservation and recorded for sperm motility, per cent live sperm, membrane integrity and acrosome integrity. The results showed that supplementation of BSA showed significantly (P<0.01) higher in sperm parameters compared to control. Interestingly we have found that 10% BSA level group was significantly (P<0.01) higher sperm motility, per cent live sperm, membrane integrity and acrosome integrity which was followed by 5% BSA, 15% BSA and control group at 24 hours of preservation at 17°C. Taken together these results suggested that BSA had a positive role in the regulation of crossbred (75% LWY x 25% Zovawk) boar sperm quality.

Keywords: Boar semen, bovine serum albumin, GEPS, liquid storage

Introduction
Numerous, studies have demonstrated that the addition of protectants can provide an effective defence against the detrimental effects of oxidative stress [1]. Bovine serum albumin (BSA), a highly soluble macromolecular protein complex naturally occurs in mammalian semen and also can be isolated from bovine plasma [2]. BSA is common semen protective agent that requires high production costs and complex productive technology. Previous studies have reported that BSA could decrease the lipid peroxidation in the plasma membrane caused by ROS and protects the plasma membrane efficiently.
Most previous studies about semen quality evaluation mainly focus on sperm motility, membrane integrity and acrosome integrity, which are the common macro- indexes of spermatozoa. Numerous studies have indicated that BSA could effectively maintain boar sperm motility during liquid storage at 17°C. Very high concentration of BSA may decrease sperm motility, plasma membrane integrity and acrosome integrity [3].
The concentration of BSA usually ranges from 1-30 mg/ml or more which may carry a large amount of fatty acids can be utilizes as a source of energy by spermatozoa [5]. Therefore, with the goal of contributing to this subject, we have comprehensively assessed the sperm quality by evaluating different parameters of sperm to explore the effects of BSA supplementation on sperm function. Our study contributes guidelines for used of different concentration of BSA as a semen extender supplementation.

Materials and methods
Experimental animals
Three mature healthy cross bred boars (75% Large White Yorkshire X 25% Zovawk) of about 2.5-3 years age group from ICAR- All India Co-ordinated Research Projects on Pig (ICAR-AICRP), College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, Mizoram with normal reproductive parameters were used for the present study.
Before semen collection collector hands was washed thoroughly with diluted potassium permanganate (1:1000) solution and dried. The dummy was adjusted to proper height and position. The boar was then brought to the collection site and allowed to mounting over the dummy. After the boar becomes sexually excited, the erected penis was grasped firmly over the corkscrew end using sterilized latex free blue nitrile glove and an intermittent pulsatile pressure was applied on the penis for obtaining complete ejaculation of the semen.

The collected semen was then allowed to pass through the Buchner funnel (to separate the gel mass) into a pre-warmed (37°C) thermo- flask of 750 ml capacity where it is stored until further processing has been done.

Diluent preparation

Ringer-Tyroide's solution was prepared by adding 8.0 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$, 0.1-0.2 g $MgSO_4$, 0.5-1.0 g $NaHCO_3$ and 1.0 g Glucose were mixed with 1000 ml of triple glass distilled water and then kept in refrigerator.

GEPS (Glucose sodium salt of EDTA-potassium sodium tartrate-sodium citrate dehydrates) was prepared by adding glucose 3.5 g, Tris sodium citrate 0.30 g, disodium- EDTA 0.20 g, potassium sodium tartrate 1.0 g, triple glass distilled water up to 100 ml, Strepto-penicilin 180 mg, gentamicin 20 mg and pH were maintained at 6.8.

Semen processing

The fresh semen samples were concentrated by centrifugation at 1000 rpm for 5 minutes at 25˚C temperature. The supernatant was discarded and the concentrated sperm were processed to make the desired concentration of sperm cells depending upon the experiment.

The fresh semen samples were evaluated for Motility [4], live sperm [4], Membrane integrity by HOSST test [5] and acrosome integrity by Giemsa staining [6].

The statistical analysis was done using one way ANOVA to determine whether there was significant difference between the means of different percentage of bovine serum albumin during preservation period. The Tukey's Post Hoc test was used to find out specific group means difference.

Results and Discussion

The mean percentage of sperm motility during preservation for 24 hours at 17˚C in GEPS extender containing 5%, 10% and 15% BSA and control were found to be 74.38±1.55, 77.46±1.42, 73.61±1.32 and 69.54±1.42 per cent respectively. The significant difference (P<0.01) in mean sperm motility between different percentages of albumin and also between preservation periods in the present study indicates that the main effects were not independent. This could be due to the optimum concentration of albumin which protects the sperm from damage during preservation [7]. The present finding was in agreement with the report of Wall *et al.* [8] and Matsuoka *et al.* [7]. The significantly higher sperm motility in 10 per cent BSA than that in 0, 5 and 15 per cent at 24 hours of preservation indicates that 10% BSA in extender preserved sperm motility better than that of BSA level studied. The present finding of sperm motility in 10 per cent BSA during 24 hours of preservation was found to be higher than that reported by Dixon *et al.* [9]. These differences might be due to breed, age, preservation temperature and extender.

The mean percentage of live sperm during preservation for 24 hours at 17˚C in GEPS extender containing 5% BSA, 10% BSA, 15% BSA and control were found to be 73.85±1.00, 80.15±1.40, 81.92±1.31 and 77.69±1.68 per cent respectively. The present finding of live sperm with 5, 15% BSA at 24 hours and 10% BSA at 24 hours of preservation are in close agreement with the report of Lee *et al.* [10]. The significant difference (P<0.01) in mean sperm motility between different percentages of albumin and also between preservation periods in the present study indicates that the main effects were not independent. The possible effects of BSA on sperm parameters may be due to the prevention of lipid peroxidation [11]. The present finding was in agreement with the report of Matsuoka *et al.* [7] and Hossain *et al.* [12]. The significantly higher live sperm in 10 per cent BSA than that in 0, 5 and 15 per cent at 24 hours of preservation indicates that 10% BSA in extender preserved live sperm better than that of BSA level studied.

The mean percentage of HOSST reacted sperm during preservation for 24 hours at 17˚C in GEPS extender containing 5% BSA, 10% BSA, 15% BSA and control were found to be 64.00±1.28, 70.38±1.73, 73.54±1.39 and 68.69±1.22 per cent respectively. The critical difference test revealed that there is significant difference (P<0.01) between control and treatment with different BSA levels during preservation period and also differ significantly (P<0.01) in 10% BSA from 5% and 15% BSA group at 24 hours of preservation. The present finding of HOSST reacted sperm with 5% BSA at 24 hours of preservation is higher than the report of Lee *et al.* [10]. This difference might be due to the effect of breed, additives, extender and preservation temperature.

The mean percentage of acrosome intact sperm during preservation at 17˚C in GEPS extender containing 5% BSA, 10% BSA, 15% BSA and control were found to be 75.85±1.07, 80.77±0.94, 83.30±0.77 and 78.69±1.03 per cent at 24 hours respectively. The present finding of intact acrosome with different levels of BSA was found to be higher than that of reported by Lee *et al.* [13]. This difference might be due to the effect of breed, additives, extender and preservation temperature. The significant difference (P<0.01) in mean acrosome intact sperm between different percentages of albumin and also between preservation periods in the present study indicates that the main effects were not independent. This could be due to the antioxidant property of BSA that can protect the sperm from free radicals during preservation period [14]. The present study was in close agreement with that reported by [7]. The significantly higher acrosome intact sperm in 10 per cent BSA than that in 0, 5 and 15 per cent at 24 hours of preservation indicates that 10% BSA in extender preserved acrosome integrity better than that of BSA level studied.

Table 1: Sperm parameters (Mean ± SE) in Crossbred (75% LWY X 25% Zovawk) boar semen in different percentages of BSA during preservation at 17˚C

Parameter (N=13)	Control	5% BSA	10% BSA	15% BSA	F-Value
Motility (%)	69.54^{a}±1.42	74.38^{b}±1.55	77.46^{b}±1.42	73.61^{b}±1.32	5.21**
Live sperm (%)	73.85^{a}±1.00	80.15^{bc}±1.40	81.92^{c}±1.31	77.69^{ab}±1.68	6.501**
Plasma membrane integrity (%)	64.00^{a}±1.28	70.38^{bc}±1.73	73.54^{c}±1.39	68.69^{bc}±1.22	7.837**
Acrosomal integrity (%)	75.85^{a}±1.07	80.77^{bc}±0.94	83.30^{c}±0.77	78.69^{bc}±1.03	10.811**

**P<0.01

Control = Extender without bovine serum albumin (BSA)

^{a,b,c,}Mean bearing different superscript of lower case alphabets in row differed significantly.

Conclusion

Sperm quality of crossbred boars (75% LWY x 25% Zovawk) semen with 10% BSA in the extender was found to be significantly higher in compare to control, 5% and 15% BSA in terms of sperm motility, live, membrane integrity and acrosome integrity during preservation at 17 °C.

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