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

Murasing DK, Lalrintluanga K, Ahmed FA, Talukdar DJ, Singh NS, Gali JM and Tolenkhomba TC

DOI: https://doi.org/10.22271/j.ento.2020.v8.i6u.8047

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 [4]. 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.

**Dilution preparation**

Ringer-Tyrode’s solution was prepared by adding 8.0 g NaCl, 0.2 g KCl, 0.2 g CaCl₂, 0.1-0.2 g MgSO₄, 0.5-1.0 g NaHCO₃ 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-penicillin 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 was 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% BSA, 10% BSA, 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 antioxidant property of BSA that can prevent 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 live sperm better than that of BSA level studied.

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 acrosome intact 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 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 present finding of HOSST reacted sperm with 5% BSA at 24 hours of preservation is higher than that of 10% BSA 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 that of reported by Lee et al.[10]. This difference might be due to the effect of breed, additives, extender and preservation temperature.

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

~ 1576 ~
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.

Acknowledgement
The authors are thankful to the Dean, VC and PI of ICAR-AICRP on Pig, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram for providing Research facilities.

References
1. Bustamante Filho IC, Pederzolli CD, Sgaravatti AM, Gregory RM, Dutra Filho CS, Jobim MI et al. Skim milk-egg yolk based semen extender compensates for non-enzymatic antioxidant activity loss during equine semen cryopreservation. Anim. Reprod 2018;6(2):392-399.
2. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. FEBS letters 2008;582(13):1783-1787.
3. Zhang XG, Yan GJ, Hong JY, Su ZZ, Yang GS, Li QW, et al. Effects of bovine serum albumin on boar sperm quality during liquid storage at 17 °C. Reprod. Dom. Anim 2015;50(2):263-269.
4. Blom EA. one-minute live-dead sperm stain by means of eosin-nigrosin. Fertil. Steril 1950;1:176-177.
5. Jeyendran RS, Vander Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJD. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. Reprod 1984;70(1):219-228.
6. Tamuli MK, Watson PF. Use of a simple staining technique to distinguish acrosomal changes in the live sperm sub-population. Anim. Reprod. Sci 1994;35(3-4):247-254.
7. Matsuoka T, Imai H, Kohno H, Fukui Y. Effects of bovine serum albumin and trehalose in semen diluents for improvement of frozen-thawed ram spermatozoa. J. Reprod. Dev 2006;52(5):675-683.
8. Wall E, Beal, Lydia M. White and Duane L, Garner. Sex Ratio After Insemination Of Bovine Spermatozoa Isolated Using A Bovine Serum Albumin Gradient J. Anim. Sci 1984;58(6).
9. Dixon KE, Songy Jr EA, Thrasher DM, Kreider JL. Effect of bovine serum albumin on the isolation of boar spermatozoa and their fertility. Theriogenology 1980;13(6):437-44.
10. Lee SH, Park CK. Antioxidative effects of magnetized extender containing bovine serum albumin on sperm oxidative stress during long-term liquid preservation of boar semen. Biochem. Biophys. Res. Comms 2015;464(2):467-472.
11. Perumal P, Nahak AK, Vupuru K, Khate K, Balamurugan TC, Krupakaran RP. Effect of Addition of Bovine Serum Albumin on the Liquid Storage (5 °C) of Mithun (Bosfrontalis) Semen. J Cell. Tissue. Res 2015;15(1):4795.
12. Hossain MS, Hyeong LJ, Miah AG, Tsujii H. Effect of fatty acids bound to bovine serum albumin-V on acrosome reaction and utilization of glucose in boar spermatozoa. Reprod. Med. Biol 2007;6(2):109-115.
13. Lee WH, Kim WH, Cheong HT, Yang BK, Park CK. Effect of Alph0a-Linolenic Acid with Bovine Serum Albumin or Methyl-Beta-Cyclodextrin on Membrane Integrity and Oxidative Stress of Frozen-Thawed Boar Sperm. Dev. Reprod 2019;23(1):11.
14. Uysal O, Bucak MN. Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. Acta. Vet. Brno 2007;76(3):383-390.