EFFECT OF BOVINE SERUM ALBUMIN IN EXTENDER ON POST-THAW QUALITY AND IN VIVO FERTILITY OF BUFFALO BULL SEMEN

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ABSTRACT

Excessive amounts of reactive oxygen species during cryopreservation can overwhelm the antioxidant defense mechanisms of semen. The present study determined the effect of bovine serum albumin (BSA) as antioxidant in Tris citric acid (TCA) extender on post thaw quality and in vivo fertility of buffalo semen. Suitable ejaculates from Nili-Ravi buffalo bulls (n=3) were split-pooled, extended in TCA containing 0.0 (control), 0.5 or 1.0% BSA and cryopreserved in liquid nitrogen vapours. Post thaw progressive motility, integrity of plasmalemma and acrosome of spermatozoa in 0.5% BSA was better than that in 1.0% BSA (P<0.05). Acrosome integrity of spermatozoa frozen in 0.5% BSA was also better than that in control semen (P<0.05). In vivo fertility did not differ (P = 0.12) between 0.5% BSA and control although it was higher in the group inseminated with semen containing 0.5% BSA (78.3%) than that with control semen (56.5%). In conclusion, 0.5% BSA seemed to be beneficial for inclusion in TCA-based buffalo semen extender.

Keywords: buffalo, Bubalus bubalis, cryopreservation, extender, bovine serum albumin, fertility

INTRODUCTION

Freezing mammalian spermatozoa to subzero temperatures and then thawing causes irreversible injuries brought about by osmotic changes, intracellular and extracellular ice formation, excessive dehydration, denaturation of proteins and membrane damage (Said et al., 2010). Exposure of spermatozoa to oxygen and visible light radiation during processing of semen can also result in formation of reactive oxygen species (ROS) (Bilodeau et al., 2000). Small physiological levels of free radicals are essential for the regulation of normal sperm functions; however, production of excessive amounts of ROS during cryopreservation can overwhelm the antioxidant defense mechanisms of semen (El-Tohamy, 2012). Bovine serum albumin (BSA), the most abundant circulating protein in the plasma, has important antioxidant activities through its multiple-binding sites and free radical-trapping properties (Roche et al., 2008). BSA has been shown to improve post thaw quality of bovine (Ashrafi et al., 2013), ovine (Uysal and Bucak, 2007) and caprine (Anghel et al., 2011) semen. To our knowledge, the effect of adding BSA in semen extender on cryopreserved buffalo bull spermatozoa has not previously been assessed. The present work evaluated the effect of BSA in Tris citric acid (TCA) extender on progressive motility, integrity of plasmalemma and
acrosome of buffalo spermatozoa at post thawing and compared in vivo fertility of semen extended in the better BSA concentration.

**MATERIALS AND METHODS**

All the chemicals used in this study were from Merck Millipore, Germany.

**Extenders preparation**

Tris citric acid extender (pH 7, osmotic pressure 320 mOsmol/kg) was prepared with Tris (hydroxymethyl)-aminomethane (3 g) citric acid (1.56 g) fructose (0.2 g) glycerol (7 mL) streptomycin sulphate (100 μg) egg yolk (20 mL) and distilled water (73 mL). This extender was supplemented with two concentrations of BSA (0.5 or 1.0% w/v). The control extender was not supplemented with BSA. These three extenders were split into three batches each (for three replicates) and were stored at -20°C.

**Collection and processing of semen**

Collection of semen was made from three healthy bulls of the Nili-Ravi breed maintained at the National Agricultural Research Centre, Islamabad, Pakistan. The bulls were 6-7 yr of age and maintained under uniform management conditions. Semen was collected once a week using an artificial vagina (42°C) for three weeks (three replicates). Two consecutive ejaculates were harvested with 10 minutes interval from each bull on the collection day after proper washing of bulls and false mounts. Semen was held at 37°C in a water bath for 10-15 minutes while evaluation for motility and sperm concentration were made. Ejaculates with >1.0 mL volume, >60% visual progressive motility and >0.5 billion per mL sperm concentration were used for the present study. Pooled semen was apportioned between three extenders. One portion was diluted with control extender and the two other portions with extenders containing 0.5% or 1.0% BSA. Cooling of extended semen to 4°C was done in 2 h. Equilibration was carried out at the same temperature for 4 h. Semen was packed in half mL French straws and cryopreserved by keeping on a rack, 6 cm above liquid nitrogen (LN₂) for 15 minutes. Semen straws were kept in LN₂ for 24 h before post thaw evaluation.

**Post thaw evaluation**

Three straws were thawed from each treatment in a water bath at 37°C for 30 s. Post thaw sperm progressive motility, integrity of plasmalemma and acrosome were compared. Progressive motility was evaluated at 400 X by placing 5 μL thawed semen on a pre-warmed slide. For functional integrity of plasmalemma, hypo-osmotic (HOS) solution with osmolarity ~190 mOsmol/kg, was made by mixing sodium citrate (0.735 g) and fructose 1.35 g/100 mL of distilled water. A 500 μL of HOS solution and 50 μL of semen sample were mixed and incubated at 37°C for 40 minutes. A drop of this mixture was used to examine 200 sperm at 400 X. Spermatozoa (%) with intact membrane (i.e. with coiled tails) were determined.

For acrosome integrity, thawed semen (100 μL) was fixed in 1% formal citrate (500 μL) (2.9 g tri-sodium citrate dihydrate and 1 mL of 37% formaldehyde in 99 mL of distilled water). Intactness of acrosome was characterized by the presence of normal apical ridge. Two hundred spermatozoa were studied using a phase contrast microscope at 1000 X.
In vivo fertility

Out of two BSA concentrations, one giving better in vitro quality of semen at post thawing was compared for in vivo fertility with control by inseminating 46 buffaloes (23 / treatment) maintained by farmers in Islamabad. Healthy buffaloes in their 2nd to 5th lactation, 3-9 month post calving were used for this purpose. All the inseminations were performed by a single technician. Conception rate was determined by palpation per rectum 60 d after insemination.

Statistical analysis

Progressive motility, plasmalemma and acrosome integrity were expressed as mean ± SE. ANOVA (general linear model) was used to compare the three treatments. Significant differences (with P<0.05) were compared by the least significant difference test. In vivo fertility between two groups of buffaloes was compared by the chi square method. Minitab statistical software (release 12.22) was used for data analysis.

RESULTS AND DISCUSSION

Effect of adding BSA to buffalo semen extender is shown in Table 1. Post-thaw sperm progressive motility and integrity of plasmalemma were significantly better in the semen sample containing 0.5% BSA than that in semen containing 1.0% BSA (P<0.05). Neither of these parameters differed significantly between control and semen containing 0.5% BSA. Acrosome integrity of spermatozoa was significantly better in the semen sample containing 0.5% BSA than in the one containing 1.0% BSA and control (P<0.05). Sperm acrosome integrity is vital for the acrosomal reaction leading to fertilization and it has been shown to be one of the useful parameters to predict the fertilization potential of buffalo bull spermatozoa (Kumar et al., 2012). It has been documented that supplementation of semen extender with BSA improved post thawing motility, and protected acrosome and membrane integrity of ram spermatozoa (Uysal and Bucak, 2007). BSA also had beneficial effects on the motility, viability, normal morphology and plasmalemma integrity in (Holstein) bull spermatozoa after a freeze-thaw process (Ashrafi et al., 2013) and these protective effects of BSA on spermatozoa were associated with an increment in antioxidant enzymes activity, total thiols and total antioxidant capacity. The mode of action of BSA for frozen thawed buffalo semen might be the same as described for bull. BSA was not equally beneficial at the two concentrations tested in the present study.

Addition of BSA at a concentration of 0.5% of TCA extender resulted in higher post-thaw motility, integrity of plasmalemma and acrosome of buffalo sperm compared with BSA at a concentration of 1.0%. It has been noted that small physiological levels of ROS are essential for the regulation of normal sperm functions such as sperm capacitation, the acrosome reaction, and sperm-oocyte fusion (Cocuzzza et al., 2007). An inappropriate increase in antioxidant could alter the production of required ROS for physiological processes of spermatozoa (Hu et al., 2010). Other studies have also shown that BSA is not equally effective at various concentrations. Ashrafi et al. (2013) observed that out of four concentrations of BSA tested 1.0% was most beneficial for bull semen in citrate-egg yolk extender. For buck semen, supplementation of Tris-based extender with 5.0% BSA gave better post thaw motility and membrane integrity than that with 10.0% BSA (Anghel et al., 2011). For ram semen, Tris-
Table 1. Effect of bovine serum albumen (BSA) in Tris citric acid (TCA) extender on progressive motility, integrity of plasmalemma and acrosome of buffalo bull sperm at post-thawing.

<table>
<thead>
<tr>
<th>BSA in TCA extender</th>
<th>Progressive motility (%)</th>
<th>Plasmalemma integrity (%)</th>
<th>Acrosome integrity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>38.33±1.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.00±3.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>31.67±2.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5%</td>
<td>48.33±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.00±2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.00±2.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1%</td>
<td>30.00±5.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.33±3.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.67±2.96&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

P –value

0.0188  
0.0407  
0.0018

Values are mean ±SE
Means with different superscripts within the same column vary significantly (P<0.05).

Figure 1. Effect of bovine serum albumen (BSA) in Tris citric acid (TCA) extender on in vivo fertility of buffalo bull semen.
based extender supplemented with 20.0% of BSA was better than that with 5.0% (Uysal and Bucak, 2007). These reports and the findings of the present study indicate that concentration of BSA to protect sperm from ROS may differ with the species.

In vivo fertility in buffaloes inseminated with control semen (13/23, 56.5%) and semen containing 0.5% BSA (18/23, 78.3%) did not differ (Figure 1, P=0.12). Although the number of inseminations was not large, a conception of 78.3% with 0.5% BSA seems very promising for buffalo. The values did not differ significantly but there was trend for higher conception rate with semen supplemented with BSA than that with control.

In conclusion, 0.5% BSA seemed to be beneficial for inclusion in TCA-based buffalo semen extender.

REFERENCES


