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Effect of soya-milk based extender on the physico-morphological parameters of Murrah bull semen during cryopreservation

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Abstract

Present investigation was undertaken on three buffalo bulls (Murrah) to study the effect of cryopreservation using Soya-milk extender. A total of 18 collections from three bulls (six collections per bull) were utilized for the study. After initial evaluation, each semen sample was diluted in two different extenders viz. Tris egg yolk extender which acted as control and Soya-milk extender as experimental. Various sperm parameters were studied at equilibration and post-thaw stages. There was no significant difference for percent head abnormalities, percent mid-piece abnormalities and acrosome integrity of spermatozoa among extenders at different stages of freezing. The progressive motility and live sperm percentage was significantly higher for Tris egg yolk extender at dilution, equilibration and post-thaw stages, but the values for Soya-Milk were also within acceptable limits at all stages of freezing as compared to Tris egg yolk extender. Furthermore the percent tail abnormalities and total sperm abnormalities were significantly higher for soya-milk extender at post thaw. In conclusion the experimental extender could be used as an alternate plant based extender in the cryopreservation of bubaline semen.

Keywords: Buffalo semen, cryopreservation, Murrah, soya-milk, Tris egg-yolk

Extenders used in semen extension and cryopreservation provide suitable media for longer survival of spermatozoa. Egg-yolk based extenders are largely used in cryopreservation of semen (Kulaksiz *et al*., 2010; Akhter *et al*., 2012). However with egg yolk there is an increased risk of microbial contamination which may lead to production of certain endotoxins which reduce the potential fertilizing capacity of spermatozoa (Bousseau *et al*., 1998; Aires *et al*., 2003). Moreover Egg yolk contains a diverse and variable composition that makes

it very difficult for quality control (Bousseau *et al*., 1998). Considering this, a plant origin, well defined and pathogen-free substitute of egg yolk would obviously be preferable. Soybean contains large proportion of low-density lipoprotein called soya lecithin similar to egg yolk lecithin and fatty acids such as stearic, oleic, and palmitic acid which have membrane protecting potential during cryopreservation of semen (Chaudhari *et al*., 2015). Because of these properties the soya-milk based extender has been used by several researchers for cryopreservation of bull (Aires *et al*., 2003; Munoz *et al*., 2009) and buffalo semen (Akhter *et al*., 2010; Chaudhari *et al*., 2015). But the studies on buffalo semen available in literature are very limited and quite variable. Keeping in consideration the above facts, the present study was undertaken to assess the freezability of bubaline semen in soya-milk based plant origin extender.

Materials and Methods

The present study was undertaken at the Central Artificial Breeding Station, Hakkal, Jammu (Dept. of Animal Husbandry, Govt. of J & K). Three Buffalo bulls (Murrah) were randomly selected to study the physico-morphological characteristics of spermatozoa during cryopreservation of Murrah bull semen in order to assess the efficacy of Soya based extender as compared to Tris-egg yolk extender on diluted, equilibrated and post thaw semen characteristics. The bulls were housed in individual pens and maintained under scientific and uniform conditions of feeding and management throughout the experimental period.

Semen was collected from three Buffalo bulls under study twice in a week between 8.00 AM and 9.00 AM with the standard artificial vagina using a male partner as dummy. Soon after the collection, the semen collection tubes were numbered and transferred to a water bath at 37°C. All the ejaculates were evaluated for various physic-morphological parameters. After initial evaluation, semen sample was divided into two equal fractions; one fraction was diluted in TRIS egg-yolk extender (TEY) which acted as control, second fraction was diluted in soya based extender (SBE). Semen diluted in both extenders (TEY and SBE) was assessed for progressive motility, live sperm percentage, abnormal sperm and acrosome integrity. The diluted semen was filled in 0.25ml poly vinyl chloride straws using an automatic filling and sealing machine (IS-4, IMV Technologies, France). Different coloured straws were used to fill the two different fractions of semen extended with two diluents. Sealed straws were arranged on a rack and equilibrated for 4 hours in cold handling unit (GO-38, IMV Technologies, France). At the end of equilibration period, the semen samples in straws with both diluents were subjected to same

evaluation tests as those of diluted semen. Immediately after equilibration the rack containing filled semen straws was transferred to a bio-freezer (Digit cool-5300, IMV Technologies, France) where the temperature was brought down from 4 to -140 °C in 7 minutes. The straws were then transferred to pre-cooled plastic goblets and plunged in to liquid nitrogen. After 24 hours of preservation in liquid nitrogen, the frozen semen straws were thawed at 37 °C for 30 seconds and evaluated for various parameters.

Preparation of soya-milk extender

The soya-milk extender was prepared as per method of Nelson *et al*. (1976) with a slight modification that before processing soya beans were baked in microwave oven until turned golden brown. In brief, 50 grams of soya beans were weighed, baked and then soaked overnight in 0.5% $NaHCO_3$ solution. Soaked beans were grounded in high-speed mixer-grinder and filtered through muslin cloth. Final volume of stock soya milk was adjusted to 300 ml with distilled water. Soya milk was autoclaved and stored at 4 °C.

Statistical analysis

All the observations recorded were analysed by using statistical package (SPSS 16). Data on various physico-morphological parameters was analysed by using one way ANOVA. Differences between parameters were tested by post hoc tests.

Results and Discussion

The sperm quality parameters at different stages of semen cryopreservation in the two extenders were assessed and results are shown in Table 1. The percent head abnormalities, percent mid-piece abnormalities and acrosome integrity of spermatozoa did not show any significant difference among extenders at different stages of freezing. This indicates the positive effect of soya milk on semen preservation. The results in the present study reveal a significant difference in the progressive motility and live sperm percentage in between the extenders at dilution, equilibration and post-thaw stages. Similarly Chaudhari *et al*. (2015) also reported significantly higher progressive motility and live sperm percentage in semen extended in Tris egg yolk based extender as compared to Boxcell (Soya-lecithin based) extender at pre freeze and post thaw stages. As explained by Forouzanfar *et al*. (2010), fall in sperm motility is possibly due to the presence of higher concentration of soya lecithin in the extender. Along with this, higher soya concentration in semen diluents was also responsible for low visibility as more lipid globules

Table 1: Various Physico-morphological parameters of bubaline semen at different stages of semen processing in Tris egg yolk and Soya-milk extenders

Parameter	Fresh semen (Mean±SE)	Type of diluents	Diluted stage (Mean±SE)	Equilibrated stage (Mean±SE)	Post-thaw stage (Mean±SE)
Progressive Motility (%)	77.69±0.96	TEY	72.70±0.86 [a]	68.34±0.79 [a]	44.65±1.25[a]
		SBE	67.12±0.84 [b]	63.52±0.85 [b]	38.19±1.15[b]
Live Sperm (%)	82.69±0.42	TEY	76.37±0.36 [a]	71.69±0.53 [a]	51.98±0.71[a]
		SBE	72.89±0.42 [b]	68.20±0.49 [b]	47.26±1.05 [b]
Head abnormalities (%)	2.76±0.32	TEY	3.82±0.32 [NS]	4.11±0.42 [NS]	4.33±0.58 [NS]
		SBE	4.17±0.38 [NS]	4.27±0.39 [NS]	4.66±0.48 [NS]
Mid-piece abnormalities (%)	2.93±0.27	TEY	3.36±0.31 [NS]	4.03±0.33 [NS]	4.69±0.45 [NS]
		SBE	3.83±0.36 [NS]	4.79±0.41 [NS]	5.33±0.45 [NS]
Tail abnormalities (%)	3.86±0.30	TEY	5.86±0.32 [NS]	6.63±0.23[a]	7.84±0.31 [a]
		SBE	6.59±0.44 [NS]	7.18±0.44 [b]	8.59±0.47 [b]
Total sperm abnormalities (%)	9.56±0.47	TEY	13.05±0.70[NS]	14.77±0.85[NS]	16.86±0.69 [a]
		SBE	14.60±0.80[NS]	16.25±0.98[NS]	18.55±0.78 [b]
Acrosome integrity (%)	89.26±0.49	TEY	85.52±0.52[NS]	82.41±0.81[NS]	78.35±1.21 [NS]
		SBE	84.44±0.52[NS]	81.25±0.98[NS]	77.53±1.21 [NS]

Means bearing different superscripts within a column within a characteristic differ significantly (P<0.05).

were observed in it (Singh *et al*., 2012). According to Hirai *et al*. (1997), the reduction in sperm velocity at higher soya lecithin concentration is likely due to high viscosity. The results also reveal a significant difference in the percent tail abnormalities and total sperm abnormalities in between the extenders at post-thaw stage. This may be attributed to the presence of some phyto-toxic chemicals and protein inhibitors in the soyabean which may be responsible for damage to sperm membrane during the cryopreservation. Furthermore, the Soya extender conferred acceptable decrease in sperm motility and live sperm count at all stages of freezing as compared to tris egg yolk extender. So the experimental extender could be used as an alternate plant based extender in the cryopreservation of bubaline semen.

Summary

On the basis of the results obtained in our study, we conclude that though there were differences in some of the parameters between Tris egg yolk (TEY)

and Soya based extender (SBE), Tris egg yolk (TEY) exhibited better results. But soya based extender also performed within the acceptable norms and hence, proposed extender could be recommended for use in cryo-preservation of buffalo-bull semen.

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