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# Efficacy of soy-lecithin for replacing egg yolk in tris extender on quality of frozen buck semen

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ABSTRACT

The present study undetaken to find the efficacy of incorporating soy-lecithin in tris extender for replacing egg yolk, a widely used animal component. Pooled ejaculates (40), 10 from each of four bucks maintained at Goat Research Station, Burnihat were used for freezing in tris extender containing 1% and 1.5% soy-lecithin and 20% egg yolk by adopting split sample technique following conventional method. The post thaw sperm motility in 1.5% soy-lecithin-tris extender (60.20±0.45%) was comparable with that of 20% egg yolk-tris (61.20±0.45%), the difference being non-significant. However, the post thaw values for live sperm, intact acrosome and hypo-osmotic swollen sperm were significantly lower in 1.5% soy-lecithin-tris as compared to that in 20% egg yolk-tris. The post thaw values were significantly higher for 1.5% than that for 1% soy-lecithin for all the parameters studied. It could be concluded that 1.5% soy-lecithin-tris extender has similar efficacy with that of 20% egg yolk-tris extender in respect of post thaw sperm motility; however, significantly lower post thaw values for remaining sperm qualities obtained with soy-lecithin in tris-based extender necessitate further trials comprising higher number of ejaculates from more bucks to find a suitable level of soy-lecithin for replacing egg yolk in tris extender for freezing of goat semen.

**Key words**: Egg yolk, Freezing, Goat semen, Post thaw quality, Soy-lecithin, Tris

The success of AI depends on quality of frozen semen which in turn depends on extenders. Currently egg yolk based extender is extensively used for semen extension and storage, because of its low density lipoprotein (LDL) which protects the sperm phospholipids during cryopreservation. However, the fertilizing capacity of spermatozoa is negatively affected by the risk of microbial contamination associated with egg yolk (Bousseau *et al.* 1998). Moreover, the World Organization for Animal Health recommended in its terrestrial animal health code of 2003 that animal origin products used in semen processing should be free of any biological risk (Marco-jimenez *et al.* 2004). Hence the search for non-animal origin, well-defined and contamination-free medium for extension of semen is highly desirable. On the other hand, the problem about using extenders containing egg yolk in goat semen has been attributed to an enzyme from bulbourethral gland called egg yolk coagulating enzyme (EYCE) later identified as phospholipase A. The interaction between this enzyme and egg yolk can be harmful to the sperm cells. Therefore, during processing, centrifugation and removal of seminal plasma (washing) is often recommended to improve the quality of frozen thawed goat semen. Demands for replacement of egg yolk in extenders have increased due to the concern that it contains substances that increase the viscosity of extenders, impede respiration of spermatozoa, and diminishes sperm motility (Sharafi *et al.* 2009). Soy-lecithin, a natural mixture of phosphatidylcholine and several fatty acids such as stearic, oleic and palmitic, could serve as an alternative replacing the animal based component in an extender. Previous studies suggested that addition of soy-lecithin to semen extender improved post-thaw sperm motility, viability, acrosome integrity and sperm membrane structure in human (Reed *et al.* 2009), boar (Zhang *et al.* 2009), stallion (Papa *et al.* 2011), bull (Akhter *et al.* 2012), ram (Emamverdi *et al.* 2013), buffalo (Chaudhari *et al.* 2015) and goat (Lekshmi bhai *et al.* 2015). Therefore, the present study was planned to find the efficacy of Tris extender containing 1% and 1.5% soy-lecithin as compared to that containing 20% egg yolk on quality of frozen Beetal and Sirohi buck semen.

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## MATERIALS AND METHODS

Beetal and Sirohi adult bucks (2 each) maintained at Goat Research Station, Assam Agricultural University, Burnihat

Table 1. Sperm motility, live sperm, intact acrosome and HOST-reacted sperm (mean*±SE) after equilibration (AE) and after freezing (AF) of buck semen in tris extender containing soy-lecithin or egg yolk

| Tris extender | Sperm motility (%) | | Live sperm (%) | | Intact acrosome (%) | | HOST (%) | |
|---|---|---|---|---|---|---|---|---|
| | AE | AF | AE | AF | AE | AF | AE | AF |
| Soy-lecithin (1%) | $70.32^{b}$±0.30 | $57.77^{b}$±0.54 | $80.75^{c}$±0.50 | $65.40^{c}$±0.56 | $73.12^{b}$±0.73 | $61.30^{c}$±0.74 | $66.12^{c}$±0.50 | $57.35^{c}$±0.50 |
| Soy-lecithin (1.5%) | $71.87^{a}$±0.41 | $60.20^{a}$±0.45 | $82.05^{b}$±0.46 | $67.07^{b}$±0.56 | $75.45^{b}$±0.71 | $63.80^{b}$±0.58 | $63.82^{b}$±0.63 | $60.17^{b}$±0.46 |
| Egg yolk (20%) | $74.05^{a}$±0.39 | $61.20^{a}$±0.45 | $84.20^{a}$±0.43 | $72.32^{a}$±0.47 | $77.85^{a}$±0.72 | $68.42^{a}$±0.43 | $70.95^{a}$±0.57 | $64.35^{a}$±0.63 |

*40 observations. Means bearing different superscripts in a column under each parameter differ significantly (P<0.05).

were used in the study. The bucks were thoroughly examined for sexual and general health before being selected. The animals were maintained under uniform feeding and managemental practices. Semen was collected from each buck once/twice a week with the help of a standard artificial vagina using a restrained doe as a mount. Immediately after collection, each ejaculate was evaluated for volume, mass activity (Zemjanis 1970) (based on the numerical scale 0–4) and initial sperm motility. Only ejaculates having volume 0.8 ml or more, mass activity 3+ or more and initial sperm motility 70% or more were used for the study. Pooled ejaculates (40), 10 from each buck were used. Each pooled ejaculate was diluted (1:5) with warm (35ºC) tris buffer, split into three equal parts and centrifuged for 7 min at 3,000 rpm to remove the seminal plasma. The pellets so formed were diluted (1:15) separately considering the original volume with tris extender containing 1% soy-lecithin, 1.5% soy-lecithin and 20% egg yolk which served as control. The extended semen was cooled gradually to 5°C @ 1°C/3 min and equilibrated for 4 h at 5°C, and frozen in liquid nitrogen using French mini straws (0.25 ml) following standard method. After 24 h of storage in liquid nitrogen, the frozen semen was thawed in warm (37°C) water for 30 sec. The semen was evaluated for sperm motility, live sperm (Blom 1977), live intact acrosome (Watson 1975) and hypo osmotic swelling test (HOST)- reacted sperm (Revel and Mrode 1994) after equilibration and after freezing on thawing. The statistical analyses of the data were performed with one way ANOVA using the Statistical Analysis Systems (enterprise Guide 4.2 version) and Duncan's Multiple Range Test (DMRT) was used to compare the differences between mean values.

## RESULTS AND DISCUSSION

Analysis of variance revealed that per cent sperm motility, live sperm, intact acrosome and HOST-reacted sperm both after equilibration and after freezing differed significantly (P<0.01) between different tris-based extenders. The overall mean sperm motility after equilibration and after freezing was significantly (P<0.05) higher in tris extender containing 20% egg yolk (control) and 1.5% soy-lecithin than that in 1% soy-lecithin while there was no significant difference in the parameter between the former two. The present findings of non-significant difference in post-thaw sperm motility between 1.5% soy-lecithin and 20% egg yolk in tris extender find support from the report of Khalifa and Abdel-hafez (2014) and Yodmingkwan *et al.* (2016) who found nonsignificant difference in sperm motility after thawing between soy-lecithin and egg yolk containing tris extender in Rahmani ram and Boer goat respectively. Murphy *et al.* (2018) reported non-significantly higher progressive motility in plant-based Optixcell (45.7±4.09%) as compared to egg yolk-based Bullxcell (41.8±1.82%). Salmani *et al.* (2013 and 2014) observed that the post thaw total and progressive sperm motility did not differ significantly between 1% soy-lecithin and 15% egg yolk in tris extender in Mahabadi buck. Significantly higher post-thaw sperm motility in soy-lecithin-based commercial extender (Andromed) than that in tris-based extender was reported by Lekshmi *et al.* (2015) in Malabari bucks. Fatty acids contained in soy-lecithin may be resistant to the action of egg yolk coagulating enzyme and act similar to phospholipids which reduce the freezing point and thus avoid the formation of large ice crystals, and minimize the replacement of plasmalogens and thus the mechanical damage to the sperm membrane (Waterhouse *et al.* 2006). The exogenous phospholipids present in extenders can replace some of the sperm membrane phospholipids to maintain plasma membrane structure and function. Soy-lecithin may form a protective film around the cell to prevent the formation of intracellular ice crystals and thus the mechanical damage during freezing/thawing (Zhang *et al.* 2009). Further, the antioxidant component in soy-lecithin like glutathione may protect the sperm during freezing by inhibiting the lipid peroxidation in the sperm membrane by scavenging the free radicals (Salmani *et al.* 2013). The active fraction of egg yolk that gives protection is believed to be a low density lipoprotein (Watson and Martin 1975). Lecithin in soybean and egg yolk protects sperm membrane phospholipids and increases the tolerance of spermatozoa to freezing process (Moussa *et al*. 2002).

In the present study, the overall mean live sperm both after equilibration and after freezing was significantly (P<0.05) higher in tris extender containing 20% egg yolk (control) than in that containing 1% and 1.5% soy-lecithin and with that containing 1.5% soy-lecithin registering more line sperms than that containing 1% soy-lecithin. Similar to the present findings, Salmani *et al*. (2013) in Mahabadi goat semen recorded significantly lower sperm viability in tris extender with 10% soy-lecithin than that containing 15% egg yolk. On the contrary, no significant difference in sperm

viability after equilibration and after freezing was reported between with and without soy-lecithin supplementation in tris extender by Khalifa and Abdel-hafez (2014) in ram semen. No significant difference in sperm viability after freezing was observed between tris extender containing 15% egg yolk and 1% soy-lecithin by Salmani *et al.* (2013) and between tris extender containing 15% egg yolk, 1% soy-lecithin and 1.5% soy-lecithin by Salmani *et al.* (2014) in Mahabadi buck semen. Chaudhari *et al.* (2015) reported that sperm viability after equilibration and after freezing was significantly higher in commercial Optixcell extender than that in tris-citrate-fructose-egg yolk-glycerol extender which was attributed to phosphatidylcholine from soy-lecithin that restored phospholipids of membranes, thereby preserving the integrity of the membrane and maintaining viability at low temperature.

The overall mean intact acrosome both after equilibration and after freezing was significantly ($P<0.05$) higher in tris extender containing 20% egg yolk (control) than that containing 1% soy-lecithin and 1.5% soy-lecithin. Significantly lower percentage of intact acrosome in tris containing 1% and 1.5% soy-lecithin than that containing 20% egg yolk was in accordance with the report of Veerabramhaiah *et al.* (2012) in bull semen who found significantly lower incidence of intact acrosome in commercial Biociphos plus extenders than in tris extender after freezing. On the other hand, Chaudhari *et al.* (2015) observed significantly higher incidence of intact acrosome after equilibration and after freezing in commercial soybean based Optixcell extender as compared to tris extender. The finding of significantly higher mean intact acrosome after freezing in extender containing 1.5% soy-lecithin than 1% soy-lecithin in the present study was at contrary to that reported by Vidal *et al.* (2013) who found no significant difference in the acrosomal integrity of Saanen goat spermatozoa post thaw when extended in tris extender supplemented with soy lecithin at different concentrations (0.04%, 0.08% and 0.16%). However, the concentrations of soya-lecithin used in the work of Vidal *et al.* (2013) were at wide variance from that of the present study.

The overall mean HOST-reacted sperm both after equilibration and after freezing was significantly ($P<0.05$) higher in tris extender containing 20% egg yolk (control) than that containing 1% soy-lecithin and 1.5% soy-lecithin, and also that containing 1.5% soy-lecithin than 1% soy-lecithin. The present findings of significantly lower proportion of HOST-reacted sperm in soy-lecithin as compared to egg yolk in tris extender were in concurrence with that recorded by Chaudhari *et al.* (2015) in frozen Surti buffalo semen who obtained significantly lower percentage of Host-reacted sperm in commercial Bioxcell extender than in tris extender containing 20% egg yolk. However, no significant difference in sperm membrane intrigrity was observed between tris buffer supplemented with 1% soy-lecithin and tris extender containing 15% egg yolk in frozen Mahabadi buck semen (Salmani *et al.* 2013, 2014). In the present investigation, HOST-reacted sperm after equilibration and after freezing was significantly higher in tris buffer supplemented with 1.5% soy-lecithin than 1% soy-lecithin which could be due to insufficiency of lower concentration of soy-lecithin in conferring necessary protection to sperm membrane (Vidal *et al.* 2013). However, Salmani *et al.* (2014) recorded no significant difference for incidence of HOST-reacted sperm in Tris buffer added with 1% and 1.5% soy-lecithin.

It could be concluded that although the quality of frozen goat semen based on live sperm, intact acrosome and HOST-reacted sperm was significantly higher in tris extender containing egg yolk than that containing soy-lecithin in the present study, an equivalent percentage of post thaw sperm motility in extender containing soy-lecithin as with that of egg yolk suggests that soy-lecithin holds a possibility of replacing egg yolk in tris following modified use and further trials.

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