Original Research

Effect of Soybean Lecithin Based Extender and Ovixcell on Quality of Frozen Semen in Beetal, Sirohi and Assam Hill Goat

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Abstract

A total of 45 pooled ejaculates comprising, three Beetal, three Sirohi and three Assam hill goat, 15 from each breed were evaluated for live sperm, sperm concentration, cold shock resistance index, intact acrosome and sperm abnormalities. Each pooled ejaculate was split into three parts and then extended in three extenders i.e., Tris extender containing 20 % egg yolk, Tris extender containing 1.5 % soya lecithin and Ovixcell and frozen using liquid nitrogen vapour. Each semen sample was evaluated after equilibration and after freezing. Semen after freezing was also evaluated for extracellular release of ALT and AST. The fertility rate of semen frozen using three extenders was recorded. In Beetal, Sirohi and Assam hill goat bucks all the seminal attributes studied immediately after collection and after pooling were within normal ranges. The mean post-thaw sperm motility, live sperm, intact acrosome, HOST-reacted sperm, extracellular ALT and AST irrespective of breed in Tris extender containing 20 % egg yolk, 1.5 % soya lecithin and in commercial Ovixcell extender was 62.11 ± 0.65, 57.67 ± 0.52 and 35.33 ± 0.64 %; 64.94 ± 0.30, 61.39 ± 0.14 and 38.77 ± 0.30 %; 44.60 ± 0.40, 42.84 ± 0.28 and 29.83 ± 0.28 %; 49.59 ± 0.32, 46.72 ± 0.38 and 30.82 ± 0.18 %; 41.75 ± 2.07, 45.04 ± 1.94 and 115.92 ± 12.81 U/L; and 70.56 ± 3.38, 84.07 ± 3.58 and 109.89 ± 7.11 U/L respectively. The post thaw values in Tris extender with 20 % egg yolk were significantly (P<0.05) higher than in that containing 1.5 % soya lecithin and in commercial Ovixcell extender, and also in Tris extender containing 1.5 % soya lecithin than that in Ovixcell extender for sperm motility, live sperm, intact acrosome and HOST-reacted sperm. The fertility rate was found to be the highest in 1.5 % soya lecithin.

Key words: Assam Hill Goat, Beetal, Sirohi, Soybeanlecithin and Ovixcell

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Introduction

Goat rearing is an integral part of livelihood, particularly for women, landless labours and marginal farmers. Goat husbandry is ideally suited for the poorest, because of short gestation period, low capital investment, low cost of maintenance and growing demand for its milk and meat. Goat was probably the first domesticated animal. The world population of goats was recorded to be 861.9 million which registered an increase of 146 per cent over the total population (590.1 million) encountered in 1990 (FAOSTAT, 2008). 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 helps to protect the spermatozoa against cold shock (Beccaglia *et al*., 2009). 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 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. But sperm washing is a time consuming and cumbersome step that results in lost or damaged sperm. Currently several commercial vegetable origin extenders are available with promising results which is considered to be alternative to egg-yolk based extenders. However, the high cost of such extenders makes their use inconvenient in developing countries. Like egg yolk, soybean contains a high amount of low-density lipoprotein lecithin which may be used to replace egg yolk in the extender and the process of washing may not be necessary for freezing of goat semen. Moreover, there is lack of information on the fertility rate of buck semen frozen in soybean lecithin based extender. Keeping in view the above facts, the present study has been planned with the following objectives to study the effect of soybean lecithin based extender on quality of frozen semen in Beetal, Sirohi and Assam hill goat.

Materials and Methods

Three Beetal bucks aged one to three years, three Sirohi bucks aged one to four years and three Assam hill goat bucks aged two to three years maintained at Goat Research Station, Assam Agricultural University, Burnihat were used in the study. The bucks were thoroughly examined for sexual and general health before being selected for the present study. The bucks were stall fed and maintained under uniform feeding and

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managemental practices throughout the period of study. Semen was collected from each buck once or twice a week with the help of a standard artificial vagina using a restrained doe in oestrus as a mount for Sirohi bucks and Assam hill goat bucks. The Beetal bucks were trained to donate semen using another buck as a mount. A total of 135 ejaculates comprising 15 ejaculates from each buck were used in the study. Immediately after semen collection the glass graduated centrifuge tube containing semen was placed in a beaker containing warm water (35ºC). The semen was then evaluated for volume, mass activity and initial sperm motility as follows.

Evaluation of Fresh Semen

Semen was evaluated immediately after collection, during equilibration and after freezing. Immediately after collection, semen was evaluated for ejaculate volume, mass activity, initial sperm motility, sperm concentration, hypo osmotic swelling test, cold shock resistance, intact acrosome and different sperm abnormalities.

Evaluation of Extended Semen during Equilibration and Freezing

Each semen sample was evaluated for sperm motility, live sperm, intact acrosome and HOST-reacted sperm after equilibration and after freezing. Sperm motility, live sperm and intact acrosome were evaluated as in case of fresh semen. Semen after freezing was also evaluated for Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) by standard methods. The fertility rate of semen frozen using three extenders was studied.

Processing of Semen

A total of 45 pooled ejaculates, comprising 15 pooled ejaculates each of Beetal, Sirohi and Assam hill goats were used for studying the effect of three extenders on quality of frozen semen. The extenders were: (i) Tris extender containing 20 per cent egg yolk, (ii) Tris extender containing 1.5 per cent soya lecithin and (iii) Ovixcell. After evaluation each pooled ejaculate was split into three parts. Two parts were extended (1:15) with two extenders i.e. Tris extender containing 1.5 per cent soya lecithin and Ovixcell extender. In the remaining part seminal plasma was removed prior to extension. For removal of seminal plasma it was diluted (1:5) with Tris buffer (Deka, 1984) maintained at 35ºC having pH 6.8 and consisting of 2.422 g Tris, 1.36 g citric acid, 1.0 g fructose and 73.6 ml distilled water. The tubes containing diluted semen samples were placed in a beaker containing water at 35ºC and brought to the frozen semen laboratory. The seminal plasma was separated by centrifugation at room temperature for seven minutes at 3000 rpm. The supernatant fluid was discarded and the centrifugate was extended (1:15) with Tris extender containing 20 per cent egg yolk. The pH of the extender was adjusted to 6.8 using 5 per cent citric acid solution before addition of glycerol and egg-yolk. All the constituents of the extender except egg yolk were mixed and kept

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overnight at 5ºC. On the following morning, prior to semen collection it was warmed up to room temperature and egg yolk was added.

Composition of Ovixcell Extender

It contained ultra-pure water, salts, sugar, electrolytes, glycerol, antibiotics and animal free protein whose concentrations were not revealed as a trade secret. Further 5 per cent glycerol was added to it as the original extender was developed specifically for preservation of ram semen at 4°C for 24 hours. The extended semen was cooled gradually to 5˚C @ 1˚C / 3 minutes and equilibrated in cold handling cabinet for 4 hours at 5˚C. French mini straws (0.25 ml) and polyvinyl alcohol powder of different colours were used for filling and sealing the extended semen. A particular colour of the straw was used for a particular breed and a particular colour of polyvinyl alcohol powder was used for a particular extender. The straws, polyvinyl alcohol powder, a clean towel, a tray containing water and freezing racks were maintained at 5ºC in a cold handling cabinet. The straws were filled in by suction inside the cold handling cabinet. The open ends of the straws were then emptied using a Bubbler's comb. The ends were then wiped with a clean towel and sealed by tamping against 10 mm layer of polyvinyl alcohol powder. The sealed straws were then placed in water to ensure proper hardening of the seal. Thirty minutes before the end of 4 hours of equilibration period the straws were taken out from the water and wiped dry using pre-cooled (5ºC) towel. After drying, the straws were arranged in a freezing rack horizontally and frozen in liquid nitrogen vapour (4 cm above liquid nitrogen) for 10 minutes inside a thermocol box. Immediately after freezing, the frozen semen straws were directly plunged in liquid nitrogen and collected in a goblet containing liquid nitrogen and transferred to liquid nitrogen container for storage. On the following day the frozen semen was thawed in warm water at 37°C for 30 seconds for evaluation.

Each semen sample was evaluated for sperm motility, live sperm, intact acrosome and HOST-reacted sperm after equilibration and after freezing. Sperm motility, live sperm and intact acrosome were evaluated as in case of fresh semen. Semen after freezing was also evaluated for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by standard methods. The fertility rate of semen frozen using three extenders was studied. In a limited fertility trial carried out in the field, the number of successful pregnancies based on 60-day non-return rate on insemination of goats using Beetal semen frozen in three extenders, *viz.* Tris extender containing 20 per cent egg yolk; Tris extender containing 1.5 per cent soya lecithin and commercial Ovixcell extender were recorded and calculated in percentage. A total of 48 goats were inseminated with frozen thawed Beetal buck semen comprising 16 goats for each extender. Data obtained were analyzed in SAS Enterprise Guide-4.3 software.

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Result and Discussion

The 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). The major cause of variation in semen quality is the environment. Quality of semen is determined by a combination of factors operating at two levels; at the level of the bull, conditions such as nutrition and temperature are considered to exert important influence on semen quality while at the level of the spermatozoa, environment relates to those conditions to which the spermatozoa are exposed after ejaculation and in the process of cryopreservation (Dorji *et al*., 2014). Sperm motility is essential for normal fertilization, and it is still currently the most common parameter of "sperm quality" acting as an indirect measure of metabolic activity and sperm viability (Chelucci *et al*., 2015). In the present study, the overall mean post-thaw sperm motility of Beetal, Sirohi and Assam hill goat buck semen was the highest in Tris extender containing 20 per cent egg yolk (62.11 ± 0.65 %) which served as control followed by Tris extender containing 1.5 per cent soy-lecithin (57.67 ± 0.52 %) and commercial Ovixcell extender (35.33 ± 0.64 %). Sperm motility in semen samples extended with Tris extender containing 20 per cent egg yolk, 1.5 per cent soya lecithin and Ovixcell extender differed significantly ($P<0.01$) after equilibration and after freezing. This is in agreement with the findings of earlier workers in South African Indigenous (Matshaba *et al*., 2013), Mahabadi (Salmani *et al*., 2014), Sarda (Chelucci *et al*., 2015) and Malabari bucks (Lekshmi Bhai *et al*., 2015), bull (Moussa *et al*., 2002; Aires *et al*., 2003; Amirat *et al*., 2004; Veerabramhaiah *et al*., 2012) and buffalo semen (Akhter *et al*., 2010; Chaudhari *et al*., 2015). On the other hand, no significant difference in post-thaw sperm motility was observed between Tris containing 1.5 per cent soy-lecithin and Tris egg yolk (15 %) by Salmani *et al.* (2014) in Mahabadi and Kalita (2016) in Beetal and Sirohi goat semen, L1G7 (lecithin 1 % , glycerol 7 %) and commercial Bioxcell extenders by Sharafi *et al.* (2009) in Bakhtiari ram, soy-lecithin and Tris extenders by Khalifa and Abdel-Hafez (2014) in Rahmani ram, and commercial Bioxcell and Tris extenders by Akhter *et al*. (2010) in Nili-Ravi buffalo semen.

Sperm motility after equilibration and after freezing was significantly ($P<0.05$) higher in Tris extender containing 20 per cent egg yolk (control) than in that containing 1.5 per cent soya lecithin and Ovixcell extender, and also in Tris extender containing 1.5 per cent soya lecithin than that in Ovixcell. This finding supports the observation of Chelucci *et al.* (2015) who found using CASA significantly higher sperm motility in adult Sarda buck semen cryopreserved in Tris-based extender containing 20 per cent egg yolk than in Tris extender supplemented with 1 per cent soybean lecithin and in commercial extender Ovixcell and also in that containing 1 per cent soy-lecithin than that in Ovixcell. Kalita (2016) also found after equilibration and after freezing in Beetal and Sirohi goat semen significantly higher sperm motility in Tris

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extender containing 20 per cent egg yolk (control) than in that containing 1 per cent soy-lecithin. Akhter *et al*. (2012) in buffalo-bull also observed after equilibration significantly higher sperm motility in Tris extender containing 20 per cent egg yolk (control) than in that containing 5 per cent and 15 per cent soy-lecithin and Veerabramhaiah *et al.* (2012) in Punganur bull who observed after equilibration and after freezing significantly higher sperm motility in Tris extender than that in commercial Biociphos Plus extender. Similarly Chaudhari *et al.* (2015) in Surti buffalo found after equilibration and after freezing significantly higher sperm motility in TYFG than in commercial Bioxcell extender. Salmani *et al*. (2013 and 2014) also reported that the post-thaw total and progressive sperm motility in Mahabadi buck semen was higher in Tris extender containing 15 per cent egg yolk (control) than that in 1 per cent soy-lecithin although the difference was non-significant. Further Rastegarnia *et al.* (2015) observed that in buffalo, soybean-based extenders *viz.* Andromed and Bioxcell could not improve post-thaw motility as compared with Tris-citric egg yolk extender.

On the contrary, no significant difference in sperm motility after equilibration and after freezing was observed by Kalita (2016) between Tris extender containing 20 per cent egg yolk (control) and 1.5 per cent soy-lecithin in Beetal and Sirohi buck semen. However, significantly higher post-thaw sperm motility in soy-lecithin based commercial extender than that in Tris based extender was reported by Lekshmi Bhai *et al.* (2015) in Malabari buck with Andromed extender, Moussa *et al*. (2002) in Holstein bull with Biociphos extender and Chaudhari *et al.* (2015) in Surti buffalo with Optixcell extender. Akhter *et al.* (2012) also found that the post-thaw sperm motility was significantly higher in Tris extender containing 10 per cent soy-lecithin than that containing 20 per cent egg yolk (control) in buffalo bull. Although the mechanism by which soybean lecithin protects sperm during freezing/thawing remains unknown, there are two hypotheses to explain the phenomenon. Phospholipids, being the major component of sperm membrane, play important physiological functions in reducing the freezing point, thus avoiding the formation of large ice crystals, and in minimizing the replacement of plasmalogens to reduce the possible mechanical damage to the sperm membrane (Graham and Foote, 1987; Giraud *et al*., 2000; Waterhouse *et al*., 2006). Therefore, the exogenous phospholipids present in extenders can replace some of the sperm membrane phospholipids to maintain plasma membrane structure and function (Graham and Foote, 1987; Trimeche *et al*., 1997; Zhang *et al*., 2009).

Another possibility, also widely accepted by many researchers, is that the egg yolk phospholipids or soy lecithin do not enter the membrane to alter the phospholipids concentration but may form a protective film around the cell to prevent the formation of intracellular ice crystals and to protect the sperm membrane from mechanical damage during freezing/thawing (Quinn *et al*., 1980; Simpson *et al*., 1987; Zhang *et al*., 2009). Further, the influential antioxidant component in soy-lecithin like glutathione may protect the sperm viability by inhibiting the lipid peroxidation that occur during cooling or freezing process in the sperm

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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). However, there are several differences in the structure of lecithin derived from egg yolk and those derived from plants such as soy-lecithin (Lekshmi Bhai *et al.*, 2015). Perhaps, the soybean-derived lecithin might be resistant to the action of egg yolk coagulating enzyme. In the present study, sperm viability after equilibration and after freezing was observed to differ significantly ($P<0.01$) between semen samples extended with Tris extender containing 20 per cent egg yolk (control), 1.5 per cent soya lecithin and Ovixcell extender. This finding supports the observations of Salmani *et al.* (2013, 2014), Hernandez-Corredor *et al*. (2014) and Kalita (2016) in buck. Veerabramhaiah *et al.* (2012) in Punganur bull and Chaudhari *et al*. (2015) in Surti buffalo semen also found after equilibration and after freezing significant difference in sperm viability between the concentrations of soy-lecithin supplemented in Tris extender or between commercial soybean-based extenders and control.

On the contrary no significant difference in sperm viability after equilibration and after freezing was reported between soy-lecithin supplemented in Tris extender and control by Khalifa and Abdel-Hafez (2014) in ram semen and between commercial Bioxcell (soya-lecithin based) and Tris (control) extenders by Akhter *et al.* (2010) in buffalo semen. Overall live sperm percentage of Beetal, Sirohi and Assam hill goat semen both after equilibration and after freezing was significantly ($P<0.05$) higher in Tris extender containing 20 per cent egg yolk (74.76 ± 0.21 and 64.94 ± 0.30) as compared to that containing 1.5 per cent soya lecithin (71.38 ± 0.29 and 61.39 ± 0.14) and Ovixcell extender (50.87 ± 0.26 and 38.77 ± 0.30), and also in that containing 1.5 per cent soya lecithin than that in Ovixcell extender. The significantly ($P<0.05$) higher live sperm percentage after equilibration and after freezing in Tris extender containing 20 per cent egg yolk (control) than in that containing 1.5 per cent soya lecithin and Ovixcell extender recorded in the present study was in agreement with the findings of Salmani *et al.* (2013) in Mahabadi goat semen who found after freezing significantly higher sperm viability in Tris extender containing 15 per cent egg yolk than in that supplemented with 10 per cent soy-lecithin. Chelucci *et al*. (2015) also reported that in Sarda buck of Italy Tris extender supplemented with 20 per cent egg yolk led to highest viability after freezing and thawing as compared to Tris with 1 per cent soy-lecithin and Ovixcell extender.

Similarly, other workers also obtained significantly higher live sperm percentage in Tris extender containing egg yolk (control) than in commercial soybean-based extender for Punganur bull (Veerabramhaiah *et al.*, 2012 with Biociphos Plus) and Surti buffalo semen (Chaudhari *et al*., 2015 with Bioxcell). On the contrary no significant difference in sperm viability after freezing was observed in Mahabadi buck semen by Salmani *et al*. (2013) between Tris extender containing 15 per cent egg yolk (control) and 1 per cent soy-lecithin and by Salmani *et al.* (2014) among Tris extender containing 15 per

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cent egg yolk (control), 1 per cent soy-lecithin and 1.5 per cent soy-lecithin. Rastegarnia *et al*. (2014) also observed no significant difference in post-thaw percentage of live sperm in buffalo semen frozen in Andromed, Bioxcell and Tris-citric egg yolk extenders. However, Chaudhari *et al*. (2015) reported that sperm viability after equilibration and after freezing was significantly higher in commercial Optixcell extender (soybean-based) than that in Tris-citrate-fructose-egg yolk-glycerol extender. Higher sperm viability observed with commercial soybean-based extenders might be caused by phosphatidylcholine from soy-lecithin that restore phospholipids of membranes, thereby preserving the integrity of the membrane and maintaining viability at low temperature. The variations might be due to differences in concentration of egg yolk and soy-lecithin used in different studies involving different animals. The higher percentage of live sperm observed after equilibration and after freezing in Tris extender containing 20 per cent egg yolk affirmed the observations on sperm motility in the present study.

Table 1: Sperm motility (mean*±se) after equilibration and after freezing of Beetal, Sirohi and Assam hill goat (AHG) buck semen in Tris-egg yolk, Tris-soya lecithin and commercial ovixcell extenders

Extender	Sperm Motility (%)							
	After Equilibration				After Freezing			
	Beetal	Sirohi	AHG	Overall**	Beetal	Sirohi	AHG	Overall**
Tris-20 % Egg yolk	71.00±1.00	70.67±0.96	70.33±1.03	70.67^{a} ±0.56	63.00±1.07	62.67±1.37	60.67±0.83	62.11 ^{a} ± 0.65
Tris-1.5 % Soya lecithin	67.00±0.95	65.33±0.77	66.00±0.87	66.11^{b} ±0.50	59.00±0.87	57.67±0.83	56.33±0.91	57.67^{b} ± 0.52
Ovixcell	49.00±1.21	48.67±1.14	47.33±1.18	48.33^{c} ±0.67	36.00±1.11	36.00±1.21	34.00±1.00	35.33^{c} ± 0.64

*Means bearing different superscripts in a column differ significantly (P<0.05); **15 observations ** 45 observations*

It was observed that incidence of intact acrosome in semen samples extended with Tris extender containing 20 per cent egg yolk, 1.5 per cent soya lecithin and commercial Ovixcell extender differed significantly (P<0.01) after equilibration and after freezing (Table 3). This finding gains support from the observation of Chelucci *et al*. (2015) in Sarda bucks who observed significant difference in post-thaw incidence of intact acrosome between Tris egg yolk (20%), Tris soy-lecithin (1%) and Ovixcell extenders. Veerabramhaiah *et al*. (2012) using Biociphos Plus extender in bull and Chaudhari *et al.* (2015) using Bioxcell extender in buffalo bull semen also found after equilibration and after freezing significant differences in the incidence of intact acrosome between commercial soybean-based extender and control (Tris extender containing 20 % egg yolk). On the contrary no significant difference in the post-thaw incidence of intact acrosome was observed between commercial Bioxcell and Tris-citric-egg yolk extender by Akhter *et al.* (2010) in Nili-Ravi buffalo and between Andromed, Bioxcell and Tris-citric-egg yolk extenders by Rastegarnia *et al*. (2014) in buffalo semen.

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Table 2: Live sperm (mean*±se) after equilibration and after freezing of Beetal, Sirohi and Assam hill goat (AHG) buck semen in Tris-Egg Yolk, Tris-Soya lecithin and commercial Ovixcell extenders

Extender	Live Sperm (%)							
	After Equilibration				After Freezing			
	Beetal	Sirohi	AHG	Overall**	Beetal	Sirohi	AHG	Overall**
Tris-20 % Egg yolk	76.20±0.19	74.10±0.31	73.97±0.23	74.76^{a}±0.21	65.00±0.48	65.00±0.58	64.83±0.52	64.94^{a}±0.30
Tris-1.5 % Soya lecithin	72.17±0.46	71.83±0.48	70.13±0.42	71.38^{b}±0.29	61.43±0.32	61.60±0.23	61.13±0.13	61.39^{b}±0.14
Ovixcell	51.23±0.45	50.70±0.43	50.67±0.48	50.87^{c}±0.26	39.43±0.48	38.57±0.59	38.30±0.47	38.77^{c}±0.30

*Means bearing different superscripts in a column differ significantly (P<0.05); **15 observations ** 45 observations*

In the present study, overall mean percentage of intact acrosome after equilibration and after freezing was significantly (P<0.05) higher in Tris extender containing 20 per cent egg yolk (control) than in that containing 1.5 per cent soya lecithin and Ovixcell extender, and also in Tris containing 1.5 per cent soya lecithin than that in Ovixcell. The present finding of significantly (P<0.05) higher overall percentage of intact acrosome recorded with control as compared to 1.5 per cent soya lecithin and Ovixcell extender was in agreement with the finding of Kalita (2016) in Beetal and Sirohi buck semen who observed after equilibration and after freezing significantly higher percentage of intact acrosome in Tris extender containing 20 % egg yolk than in that containing 1 per cent soy-lecithin and 1.5 per cent soy-lecithin. Veerabramhaiah *et al*. (2012) in bull semen also found significantly higher percentage of intact acrosome in Tris extender than in commercial Biociphos Plus extender after freezing. Chaudhari *et al.* (2015) also reported significantly (P<0.05) higher incidence of intact acrosome after equilibration and after freezing in Tris extender than in commercial Bioxcell extender.

On the other hand Chaudhari *et al.* (2015) in the same experiment observed significantly higher incidence of intact acrosome after equilibration and freezing in commercial soybean-based Optixcell extender than in Tris extender (control). Chelucci *et al*. (2015) also observed that acrosome membrane integrity of frozen Sarda buck spermatozoa was better preserved in Tris extender containing 1 per cent soybean lecithin and in commercial Ovixcell extender than that in Tris extender containing 20 per cent egg yolk. According to Singh *et al*. (2013) and Rehman *et al*. (2014) optimal soya lecithin concentration in the extender is prerequisite for protection of spermatozoa during temperature variations. Concentration of soybean below or above the optimal may be harmful and this might be the reason for poor performance of the Boixcell extender in the study. In the present study overall incidence of intact acrosome after freezing was significantly (P<0.05) higher in extender containing 1.5 per cent soya lecithin than that in soya lecithin-based commercial Ovixcell extender. On the contrary Vidal *et al.* (2013) reported that post-thaw there was no significant difference in the acrosomal integrity of Saanen goat spermatozoa when extended in Tris extender supplemented soya lecithin at different concentrations (0.04 %, 0.08 % and 0.16 %).

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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 (1.5 %). The higher incidence of intact acrosome obtained after equilibration and after freezing in Tris extender containing 20 per cent egg yolk affirmed the observation on live sperm in the present study. HOST-reacted sperm in the semen samples extended with Tris extender containing 20 per cent egg yolk, 1.5 per cent soya lecithin and Ovixcell extender differed significantly (P<0.01) after equilibration and after freezing. This is in agreement with the findings of earlier workers in Mahabadi (Salmani *et al.*, 2013, 2014), Malabari (Lekshmi Bhai *et al.*, 2015), Beetal and Sirohi bucks (Kalita, 2016) and in Surti buffalo semen (Chaudhari *et al.*, 2015). On the contrary, no significant difference in sperm membrane integrity was reported by Lekshmi Bhai *et al.* (2015) after equilibration in Malabari buck semen extended in Tris and Andromed extenders. Similarly, Akhter *et al.* (2010) also did not observe any significant difference in post-thaw percentage of HOST-reacted sperm in Nili-Ravi buffalo semen diluted in commercial Bioxcell extender and Tris-citric-egg yolk extender. In the present study, overall mean HOST-reacted sperm both after equilibration and after freezing was significantly (P<0.05) higher in Tris extender containing 20 per cent egg yolk (control) than in Tris extender containing 1.5 per cent soya lecithin and Ovixcell extender, and also in Tris containing 1.5 per cent soya lecithin than that in Ovixcell.

Table 3: Incidence of intact acrosome (mean*±se) after equilibration and after freezing of Beetal, Sirohi and Assam Hill Goat (AHG) buck semen in Tris-Egg yolk, Tris-Soya lecithin and commercial Ovixcell extenders

Extender	Intact Acrosome (%)							
	After Equilibration				After Freezing			
	Beetal	Sirohi	AHG	Overall**	Beetal	Sirohi	AHG	Overall**
Tris-20 % Egg yolk	72.23±0.93	71.97±0.54	71.47±0.50	71.89^{a}±0.39	45.07±0.74	44.27±0.62	44.47±0.73	44.60^{a}±0.40
Tris-1.5 % Soya lecithin	70.13±0.60	69.27±0.73	69.70±0.48	69.70^{b}±0.35	42.77±0.56	42.53±0.43	43.23±0.44	42.84^{b}±0.28
Ovixcell	49.50±0.48	49.23±0.64	49.77±0.33	49.50^{c}±0.28	30.97±0.39	29.30±0.40	29.23±0.53	29.83^{c}±0.28

Means bearing different superscripts in a column differ significantly (P<0.05); ***15 observations*** ** *45 observations*

The significantly (P<0.05) higher percentage of HOST-reacted sperm after equilibration and after freezing in Tris extender containing 20 per cent egg yolk than that in extender containing Tris buffer supplemented with 1.5 per cent soya lecithin and Ovixcell extender recorded in the present study finds the support in the observation of Kalita (2016) in Beetal and Sirohi buck semen who found after equilibration and after freezing significantly higher incidence of HOST-reacted sperm in Tris extender containing 20 per cent egg yolk (control) than in that containing 1 per cent soy-lecithin and 1.5 per cent soy-lecithin. Chaudhari *et al.* (2015) in frozen Surti buffalo semen also found significantly (P<0.05) higher percentage of HOST-reacted sperm in Tris extender containing 20 per cent egg yolk than that in commercial Bioxcell extender. On the contrary Salmani *et al.* (2013, 2014) in frozen Mahabadi buck semen observed no significant difference in

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sperm membrane integrity between Tris extender containing 15 per cent egg yolk and Tris buffer supplemented with 1 per cent soybean lecithin.

Table 4: HOST-reacted sperm (mean*±se) after equilibration and after freezing of Beetal, Sirohi and Assam hill goat (AHG) buck semen in Tris-Egg yolk, Tris-Soya lecithin and commercial Ovixcell extenders

Extender	Host-Reacted Sperm (%)							
	After equilibration				After freezing			
	Beetal	Sirohi	AHG	Overall**	Beetal	Sirohi	AHG	Overall**
Tris-20 % Egg yolk	69.57±0.32	69.67±0.26	69.77±0.29	69.67^{a}±0.16	49.57±0.49	49.17±0.74	50.03±0.36	49.59^{a}±0.32
Tris-1.5 % Soya lecithin	65.07±0.75	65.13±0.69	64.00±0.47	64.73^{b}±0.37	47.27±0.68	46.97±0.72	45.93±0.55	46.72^{b}±0.38
Ovixcell	36.87±0.59	36.37±0.57	36.57±0.58	36.60^{c}±0.33	31.73±0.33	30.63±0.19	30.10±0.24	30.82^{c}±0.18

*Means bearing different superscripts in a column differ significantly (P<0.05); **15 observations ** 45 observations*

In the present study, after equilibration and after freezing HOST-reacted sperm was significantly (P<0.05) higher in Tris buffer supplemented with 1.5 per cent soya lecithin than in Ovixcell extender. On the other hand, Salmani *et al.* (2014) recorded no significant difference for incidence of HOST-reacted sperm in Tris buffer added with 1 per cent and 1.5 per cent soy-lecithin. Higher soya lecithin concentrations might be toxic to the integrity of sperm membrane, whereas lower concentration might be insufficient to provide the necessary protection (Vidal *et al.*, 2013). Futino *et al.* (2010) reported that, despite having a protective effect, soy-lecithin (and other substances) at high concentrations could become harmful to sperm due to their potential toxicity, thus reducing fertilizing capacity of sperm. It may be stated that the concentration of soy-lecithin used in the present work was based on the studies of Salmani *et al.* (2014) and Kalita (2016). It was observed that Beetal, Sirohi and Assam Hill Goat semen frozen in Tris extender containing 20 per cent egg yolk led to significant (P<0.05) rise in sperm motility, livability, incidence of intact acrosome and sperm membrane integrity. This is in agreement with the observations of earlier workers (Leite *et al*., 2010; Beran *et al*., 2012; Chaudhari *et al*., 2015) who found better post-thaw motility, viability, acrosomal as well as plasma membrane integrity in egg yolk-based extender than in commercial soybean-based Bioxcell extender. The better post thaw semen quality, in respect of all the parameters studied in buck semen frozen with Tris extender containing 20 per cent egg yolk indicated better maintenance of plasma membrane and internal structures of spermatozoa by egg yolk.

The ALT and AST activities in frozen Beetal, Sirohi and Assam Hill Goat buck semen differed significantly between extenders for extracellular release of ALT (P<0.05) and AST (P<0.01) (Table 5). The more extracellular activities of ALT and AST indicate more sperm cell damage. Hence, assay of enzymes like ALT and AST was used as a method of evaluation of semen quality (Saikia, 2006; Borah, 2009; Veerabramhaiath *et al*., 2011; Sharma *et al*., 2013). It was found that the mean values of extracellular ALT

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and AST activities in frozen Beetal, Sirohi and Assam hill goat buck semen were significantly (P<0.05) lower in Tris extender containing 20 per cent egg yolk (control) than that in Ovixcell extender. The ALT and AST are intracellular enzymes and their release into extracellular medium indicates sperm cell damage incurred during the process of cryopreservation (Graham and Pace, 1967; Singh *et al*., 1996; Ingale *et al*., 2000). Presence of higher intracellular enzymes (ALT, AST) in the extracellular medium of frozen thawed semen is due to increased permeability or breakage of plasma membrane caused by cryopreservation (De Rauck and Knight, 1964; Tuli *et al*., 1982). Lower enzyme activities in extracellular medium recorded in the present study with 20 per cent egg yolk indicated that egg yolk afforded better protection to sperm plasma membrane during processing and cryopreservation of buck semen.

The fertility rate found to be the highest in the present study with Tris extender containing 20 per cent egg yolk (68.75 %) was higher than that (45.45 %) reported by Singh *et al*. (1995) in does inseminated with buck semen frozen in Tris extender containing 20 per cent egg yolk and 8 per cent glycerol. The rate of pregnancy on artificial insemination in does using semen frozen with Tris egg yolk, Tris soya lecithin and Ovixcell extender did not differ significantly. The finding could not be compared due to paucity of literature on fertility trials in goat using semen frozen with lecithin-based extender.

Table 5: ALT and AST activities (mean*±se) in extracellular fluid of Beetal, Sirohi and Assam hill goat (AHG) buck semen frozen in Tris-egg yolk, Tris-soya lecithin and commercial Ovixcell extenders

Extender	ALT (U/L)				AST (U/L)			
	Beetal	Sirohi	AHG	Overall**	Beetal	Sirohi	AHG	Overall**
Tris-20 % Egg yolk	44.67±3.97	43.95±4.16	36.63±2.11	41.75^{a}±2.07	72.09±7.06	72.43±5.37	67.17±5.31	70.56^{a}±3.38
Tris-1.5 % Soya lecithin	47.64±3.85	44.92±2.82	42.55±3.38	45.04^{ab}±1.94	86.15±6.76	85.67±6.34	80.40±5.80	84.07^{a}±3.58
Ovixcell	51.26±4.88	51.42±3.00	49.24±4.57	50.64^{b}±2.40	115.92±12.81	113.67±13.66	100.07±10.74	109.89^{b}±7.11

*Means bearing different superscripts in a column differ significantly (P<0.05); **15 observations ** 45 observations*

The reports on the effect of soybean lecithin-based extender in freezing semen as compared to semen frozen by using conventional extender like Tris egg yolk on conception rate following insemination in sheep and cattle were replete with inconsistent results. Khalifa and Abdel-Hafez (2014) reported significantly (P<0.05) higher pregnancy rate on insemination in ewes with semen frozen using Tris-glucose-citric acid-soy lecithin-glycerol extender (63.64 %) as compared to that in Tris-glucose-citric acid-egg yolk-glycerol extender (54.55 %). Non-significant difference in pregnancy rate for frozen semen in ewes was obtained by utilizing Bioxcell, a soya lecithin-based extender and milk egg yolk extender for freezing of semen by Gil *et al*. (2003), the respective rate of pregnancy being 28.4 and 27.2 per cent for the two extenders. Similarly, Fukui *et al*. (2008) recorded non-significant difference in pregnancy rate in ewes between Tris-based extender containing egg yolk (64.5 %) and Andromed, a commercial soybean lecithin-based extender (56.7 %) on insemination using the extended frozen semen. Masoudi *et al*. (2017) could not find significant

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difference in pregnancy and parturition rates in ewes using semen frozen in Tris-based extender containing 20 per cent egg yolk (32 and 22 % resp.) and 1 per cent soybean lecithin extender (30 and 22 % resp.). The non-significant difference in the rate of pregnancy on insemination in ewes with soybean lecithin-based extender and Tris / milk egg yolk extender obtained by different workers could imply that soya lecithin could be used in lieu of Tris / milk egg yolk as extender of frozen semen without affecting fertility. Of the two soybean lecithin-based extenders Bioxcell was reported (Khalifa *et al*., 2013) to improve conception rate in ewes to 71 per cent as compared to 46.2 per cent with Andromed extender. In cattle, Aries *et al*. (2003) reported significantly ($P<0.01$) higher conception rate on 56-day non-return basis when Holstein-Friesian bull semen was frozen using Andromed extender (70.45 %) than with Tris egg yolk extender (67.85 %) on insemination involving a large number of cows. On the contrary significantly ($P<0.05$) lower conception rate on 56-day non-return basis was obtained with Biociphos plus extender (60.2-66.7 %) when compared with Tris standard (67.0 – 70.1 %) and Tris concentrate (67.5 – 69.9 %) extenders in frozen semen (Van Wagtendonk-de Leeuw*et al*., 2000). Crespilho *et al*. (2012) recorded significantly ($P<0.05$) lower pregnancy rate on the basis of 40-day ultrasound scanning using Botu-Bovs-1% soya lecithin extender (36.45 %) in comparison with Tris-fructose-20% egg yolk (59.26 %) and Botu-Bovs-20 % egg yolk (62.37 %) extenders. It could be inferred from the above reports on conception rate that there was no unanimity in the efficacy of frozen semen extended with a soybean lecithin-based extender in attaining fertility on impregnation so as to serve it as a replacement for Tris-based extender.

Conclusion

A comprehensive study in goat on fertility of frozen semen extended in soybean lecithin-based extender rather than in Tris-based extender will have to be undertaken to ascertain the efficacy of a non-egg yolk-based extender in achieving fertility rate comparable with that obtained using a conventional extender. Hence, semen frozen in the conventional Tris extender containing egg yolk can be used successfully in A.I. with better anticipated conception rates. The advantages of lecithin based extender over milk and/ or egg yolk regarding sanitary issues are unquestionable. However, the hypothesis that cryoprotective capacity of soy-lecithin is similar to that provided by egg yolk was not confirmed in the present study and the efficacy of lecithin-based extender in preserving goat semen with acceptable fertility is yet to be determined unequivocally.

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