EFFECT OF THE EXTENDERS ON MOTILITY AND FUNCTIONAL MEMBRANE INTEGRITY OF BUCK SPERMATOZOA SUBJECTED TO POST- THAW INCUBATION

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Abstract:

The objective of the study was to compare the effect of egg yolk based and soyabean lecithin based extenders on the motility and functional membrane integrity of Malabari buck semen after post-thaw incubation. Twelve ejaculates with more than 80 percent initial motility were equally split into two groups. Group I and II were extended at the rate of 400 million spermatozoa/ ml with soyabean lecithin based extender (Andromed) and egg yolk based extender, respectively. At the time of post thaw evaluation (zero hour) and at 30 min of incubation at 37° C, spermatozoa of group I showed significantly higher motility ($p<0.05$) than group II, whereas progressive motility of spermatozoa in group I and II did not differ significantly at one hour of incubation. There was significantly ($p < 0.05$) higher percent of functional membrane integrity for group I at the time of thawing, but no significant difference ($p < 0.05$) was observed between the groups during other stages of incubation. Quality of frozen, thawed buck semen in terms of motility and functional membrane integrity after one hour of incubation is not significantly different in soyabean lecithin based extender and egg yolk based (5 per cent) extender.

Key words: *Malabari buck semen, freezing, egg yolk based, soyabean lecithin based, extender, functional membrane integrity*

Cryopreservation causes greater damages to spermatozoa resulting in lower fertilizing ability compared to fresh and refrigerated semen. Composition of semen extenders is one of the most important aspects affecting the degree of spermatozoan cryodamage. Eventhough egg yolk is proved to be beneficial for sperm preservation of many species, goat semen needs special attention during its cryopreservation as the seminal plasma of this species contains an egg yolk coagulating enzyme (EYCE) of bulbourethral gland origin which hydrolyses egg yolk lecithin into fatty acids and lysolecithin resulting in detrimental effects to the spermatozoa. This demands removal of seminal plasma in goats, which is time consuming and may affect sperm viability and cause sperm loss (Gracitua and Arav, 2005).

The wide variability of constituents and high chance of microbial contamination of egg yolk also support the need of replacing egg yolk with a suitable agent. The plant derived lecithin from soybean offers promise for buck semen cryopreservation in this regard.

As the maintenance of sperm progressive motility during incubation has a direct correlation with the survivability of the spermatozoa in the female reproductive tract, the study was undertaken to assess the effect of egg yolk based and soybean lecithin based extenders

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on the progressive motility and functional membrane integrity of frozen buck spermatozoa after post-thaw incubation.

Materials and methods

The study was conducted with twelve ejaculates collected from adult, healthy Malabari bucks, maintained at the Artificial Insemination centre, Dept. of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur.

Ejaculates with more than 80 percent initial motility were divided into two groups Soybean lecithin based extender, Andromed (Minitub, Germany) prepared as per the directions of the manufacturer was used to extend group I at the rate of 400 million sperms/ml. Group II was extended with Tris egg yolk based extender at the same dilution rate. The composition of the Tris-egg yolk based extender is shown below.

Tris hydroxy methyl amino methane -3.028 g

Citric acid -1.175 g

Fructose -1.25 g

Egg yolk -5 ml

Glycerol -6 ml

Streptomycin - 100 mg

Benzyl penicillin -1 lakh IU

Tripple distilled water - Up to 100 ml

The extended semen of each group was filled in pre-cooled 0.5 ml sterile French straws manually. An air space of 1 cm was created at the open end. The laboratory seal was prepared by sealing the open end with polyvinyl alcohol powder. The straws were allowed to equilibrate at 5ºC for two hours. Conventional mode of freezing was adopted in which the straws in the rack were exposed to liquid nitrogen vapour for 10 minutes at four centimeter above the liquid nitrogen level and then plunged into liquid nitrogen.

Post-thaw evaluation was done after 24 hours of freezing. The contents of four thawed semen straws from each group, taken in Eppendorf's tubes were incubated at 37ºC for a period of one hour after thawing. Percent of progressive motility and functional membrane integrity were assessed at zero hour, thirty minutes and one hour. Progressive sperm motility was assessed at 400× objective of the light microscope.

Hypoosmotic sperm swelling test (HOST) was carried out to assess the functional membrane integrity of spermatozoa as per the method used for human spermatozoa (Jeyendran *et al*., 1984). The osmolarity of hypo osmotic solution (4.9 g Trisodium citrate dihydrate and 9.9 g fructose /L) was 100mOsm/L. Control solution (14.7 g Trisodium citrate dihydrate and 29.7 g fructose /L) was prepared with the osmolality of 300 mOsm/L.

Test solution (0.9 ml) was mixed with 0.1 ml semen and incubated at 37ºC for 30 minutes. Similarly, 0.1 ml semen was mixed with 0.9 ml control solution and incubated at 37ºC for 30 minutes. Smears were prepared from both the suspensions after mixing one drop of diluted sample with one drop of 1% eosin. A minimum of 200 spermatozoa were counted from both test and control smears at 1000× objective of light microscope. The spermatozoa with varying degree of coiled tails were considered as positive. Percent of spermatozoa with coiled tail in control solution was subtracted from percent of test positive spermatozoa in order to get the actual percent of spermatozoa reacted to the HOST solution.

The data were statistically analysed (Snedecor and Cochran, 1994) using SPSS (Statistical package for social studies) software. Percent data were transformed using Arcsine prior to analysis. Treatment means were compared using one way ANOVA. Wherever the treatments were found to be different, Duncan's multiple range test (DMRT) was used for comparing the treatment means.

Results and discussion

Progressive motility

The mean percent of post thaw sperm progressive motility at zero hour, thirty minutes and one hour of incubation at 37^0C were 60.17 ± 1.014, 41.00± 3.454 and 25.50± 1.544 in group I and 41.33 ± 0.955, 31.67 ± 1.382 and 25.00 ± 0.931 in group II, respectively (Table1; fig1).

At zero hour and 30 min of incubation, spermatozoa of group I showed significantly higher ($p < 0.05$) percent of motility than group II, whereas progressive motility of spermatozoa in group I and II did not differ significantly at one

Table 1. Effect of the extenders on progressive motility (per cent, Mean ± SEM) of frozen Malabari buck spermatozoa after post thaw incubation (n=12)

Stage of incubation	Group I (extended with Andromed)	Group II (extended with egg yolk based extender)
0h	60.17 ± 1.014[a]	41.33 ± 0.955[b]
30 min	41.00± 3.454[a]	31.67 ± 1.382[b]
1h	25.50± 1.544[a]	25.00 ± 0.931[a]

Values having different superscripts in the same row differ significantly (p<0.05)

hour of incubation.

Functional membrane integrity

Percent of HOS positive sperms at zero hour, thirty minutes and one hour were 58.28 ± 1.572, 44.25 ± 1.548 and 34.83 ± 1.759 in group I and 48.93 ± 2.074, 40.33 ± 3.955 and 31.67 ± 2.552 in group II, respectively. The mean percent values of HOS positive spermatozoa at different time intervals of incubation have been presented in Table 2: Fig 2.

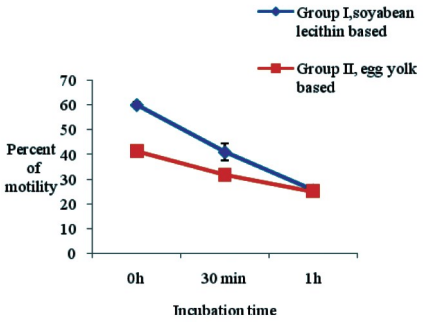

Fig.1. Effect of the extenders on progressive motility (per cent, Mean ± SEM) of frozen Malabari buck spermatozoa after post thaw incubation (n=12)

lecithin to lysolecithin (Iritani and Nishikawa, 1963) and made the sperm membrane more fusogenic (Upreti *et al.,* 1999). Perhaps, the soybean derived lecithins might be resistant to the actions of EYCE.

Lack of significant difference observed in the quality of semen in later stages of incubation test indicates that lecithin in egg yolk also protects sperm membrane phospholipids and increases tolerance of spermatozoa to the freezing and thawing process as reported by Moussa *et al*. (2002). EYCE mediated detrimental effects were insignificantly low in the present study which might be due to the lower

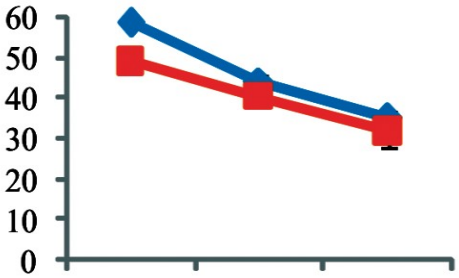

Fig.2. Effect of the extenders on HOS response (per cent, Mean ± SEM) of frozen Malabari buck spermatozoa after post thaw incubation (n=12)

percent of egg yolk (five percent) used.

However, further studies are required to investigate both *in vitro* and *in vivo* fertility aspects to prove the efficacy of soybean lecithin based extender for cryopreservation of buck semen. It is also suggested to develop a cost effective method for preparing soybean lecithin based extender for its acceptability and practical utility in the field level.

Acknowledgment

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Table 2. Effect of the extenders on HOS response (per cent, Mean ± SEM) of frozen Malabari buck spermatozoa after post thaw incubation (n=12)

Stage of incubation	Group I (extended with Andromed)	Group II (extended with egg yolk based extender)
0h	58.28 ± 1.572[a]	48.93 ± 2.074[b]
30 min	44.25 ± 1.548[a]	40.33 ± 3.955[a]
1h	34.83 ± 1.759[a]	31.67 ± 2.552[a]

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