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Chemically Well-Defined Extender for Preservation of Black Bengal Buck Semen

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Abstract

This experiment was carried with the aim to prepare a chemically well defined semen extender with soybean lecithin (SL) for cryopreservation of black Bengal buck semen. Semen samples were collected from five black Bengal bucks. The semen samples were extended in Tris buffer with 5% glycerol containing either 15% egg yolk (control group) or SL at different concentrations (1, 1.5 and 2% SL). The semen samples were cooled gradually to 5°C and equilibrated for 3 h at 5°C and frozen in static liquid nitrogen vapor and stored in liquid nitrogen. Semen samples were evaluated after initial dilution, after completion of equilibration and after freeze thawing for sperm motility. Semen sample preserved in extender containing 1% SL was able to maintain sperm motility at par with extender containing egg yolk. However, reduction in motility was observed as the concentration of soybean lecithin increased above 1% level. Artificial insemination of goats with frozen semen preserved with 1% SL gave 51.95% kidding rate. It is concluded that an extender containing soybean lecithin @ 1% can be used as a chemically well defined extender for cryopreservation of black Bengal buck semen.

Keywords: *Buck, semen, preservation, soybean lecithin, motility*

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INTRODUCTION

Black Bengal goat is famous for its adaptability and delicacy of meat. But in recent times, availability of breeding bucks has become more difficult due to castration of male kids at early age for meat purposes [1]. Cryopreservation of semen is an efficient technique to save sperm cells, however despite years of research, the cryopreservation of goat sperm still cannot be carried out efficiently [2]. The composition of the extender in which semen is diluted before freezing is one of the most important factors that influence the success of cryopreservation. The presence of cold shock resistant and cryoprotectant is necessary to preserve sperm during freezing and thawing. Egg yolk (EY) has long been used as a cold shock protectant/cryoprotectant in mammalian semen extender since long time ago. Demands for replacement of egg yolk in extenders have increased in recent years due to the risk of microbial contamination. Furthermore, the wide variability of egg yolk composition makes it difficult to prepare standard extenders. On other hand an enzyme from bulbourethral gland of buck, namely egg yolk coagulating enzyme (EYCE), coagulates the egg yolk which can be harmful to the sperm cells [2]. Recently, research by Akhter *et al.* has shown that using proteins of plant

origin like soya-lecithin as semen extenders lack various risks from egg yolk [3]. The egg yolk phospholipids such as phosphatidylcholine are very important for the maintenance of sperm membrane in freezing-thawing process. So, the same component such as soybean lecithin could be replacement with egg yolk in cryopreservation of spermatozoa. Keeping all these information in view, the present study was designed to study the effect of replacing egg yolk with soybean lecithin on cryopreservation of black Bengal buck semen.

MATERIALS AND METHODS

Five black Bengal bucks (*Capra hircus*) aged 18–42 months, weighing 18–28 kg were used in the study. Semen ejaculates were collected using artificial vagina. The basic extender was prepared by mixing 300 mM Tris, 28 mM glucose, 95 mM citric acid, 5% glycerol (v/v), and 500 µg/ml gentamicin in distilled water respectively. Three different treatment extenders were prepared by adding 1, 1.5 and 2% Soybean Lecithin (w/v) with 100 ml of basic extender and control extender was prepared by adding 15% egg yolk [10]. After preparation, extender was kept in water bath at 37°C for semen dilution.

Semen ejaculates having ≥4% mass activity and ≥80% progressive forward motility were selected and pooled together. Pooled ejaculates were then split into four equal aliquots and diluted at 37°C for extension in extenders containing increasing concentrations of soya lecithin (1, 1.5 and 2.0%) and control extender containing 15% egg yolk at 1:5 dilution rates in a single step. Filling and sealing of straws was done manually at room temperature (25 to 27°C) in 0.25 ml French medium straws. The straws were kept for 3 h at 5°C temperature for equilibration. After equilibration, straws were held on plastic rack in foam box (35×17×20 cm) in static liquid nitrogen vapor, 4 cm above the liquid nitrogen. After freezing for 10 min, the straws were then immediately plunged into liquid nitrogen and stored at –196°C for at least 2 days prior to thawing. Semen straws from the liquid nitrogen container were picked and thawed immediately in a water bath at 37°C for 30 sec. After thawing, semen samples were dropped into a pre-warmed appendroff tube and kept in a water bath at 37°C for further evaluation.

Semen samples were evaluated for sperm motility at three stages namely, immediate after extension with semen extenders, after completion of equilibration period and finally after freeze thawing. Sperm motility was assessed subjectively by using a phase contrast microscope at 400×magnification. Frozen semen straws preserved with 1% SL were used for artificial insemination of goats in the field through Sanjevani Khamar, Harit, Hooghly, West Bengal, to ascertain the kidding rate in the field. The collected data were coded, compiled, tabulated and subjected to statistical analysis by using 'Least Square Analysis of fitting constant' [5].

RESULTS AND DISCUSSION

A total of 120 semen ejaculates were collected from black Bengal bucks, out of which, 96 ejaculates where found suitable for preservation. Motility of sperm cells (%) preserved in different extenders during different phases of semen preservation is presented in Table 1. Significant loss of sperm motility was observed from initial dilution to equilibration and from equilibration time to post freeze thawing of semen samples extended in control, and 1 and 1.5% SL extenders. While in 2% SL group, though there was no significant reduction in motility from initial dilution (66.72±0.04) to equilibration (62.47±0.04), significant reduction in sperm motility was observed after post-thaw evaluation (7.94±0.04) as compared to equilibration (62.47±0.04). There was no significant difference observed in sperm motility among the semen samples diluted in control (78.84±0.04), 1% SL (77.96±0.04) and 1.5% SL (74.24±0.04) extenders immediately after initial dilution, the samples extended in 2% SL had significantly low sperm motility (66.72±0.04) than the control, and 1 and 1.5% SL extenders during the time. After completion of 3 h of equilibration at 5°C, no significant difference in the sperm motility was observed between the samples preserved in control (70.37±0.04) and 1% SL (67.67±0.04), while the samples extended in 1.5% SL (63.8±0.04) and 2% SL (62.47±0.04) had significantly low motile cells than the samples preserved in control (70.37±0.04). The frozen semen straws were thawed at 37°C

for 30 sec and evaluated for sperm motility. There was no difference in the post thaw motility of the semen samples preserved in 1% SL based extender (30.79±0.04) and egg yolk based extender (30.70±0.04). However, the sperm motility was significantly lower in samples preserved in 1.5% SL (20.95±0.04) and 2% SL (7.94±0.04) extenders when compared to control and 1% SL.

Table 1: *Sperm Motility during Different Stages of Cryopreservation in Soybean Lecithin Based Extenders (%).*

Treatment	Dilution	Equilibration	Freeze Thawing
Control	78.84±0.04 $^{a\,P}$	70.37±0.04 $^{b\,P}$	30.70±0.04 $^{c\,P}$
1% SL	77.96±0.04 $^{a\,P}$	67.67±0.04 $^{b\,P\,Q}$	30.79±0.04 $^{c\,P}$
1.5% SL	74.24±0.04 $^{a\,P}$	63.88±0.04 $^{b\,Q}$	20.95±0.04 $^{c\,Q}$
2% SL	66.72±0.04 $^{a\,Q}$	62.47±0.04 $^{a\,Q}$	7.94±0.04 $^{b\,R}$

Data shown all mean ±SEM (n=24).
Means with different superscripts a, b, c in a row differ significantly (P<0.05).
Means with different superscripts P, Q, R in a column differ significantly (P<0.05).

No significant difference in the post thaw motility of the semen samples preserved in 1% SL based extender and egg yolk based extender was observed in the present study. These results are supported by the results of Futino *et al.* who reported that lecithin may have played a protective role during cryopreservation due to its low viscosity, lower presence of debris, improves the kinematics of sperm cells and rearrangements structure of the plasma membrane [5]. Moreover, Papa *et al.* explained that lecithin maintains progressive motility and plasma membrane integrity in stallion sperm samples that were similar to samples frozen in egg yolk [6]. The reduction in motility parameters in extenders containing higher (1.5 and 2% SL) levels of lecithin compared to 1% SL may be related to high viscosity [7].

Out of 77 artificial inseminations carried out in the villages of West Bengal state during the year 2016 with frozen semen straws containing 1% SL based extender, forty goats delivered kids with the kidding rate of 51.95%. Our experiment shows that across the range of tested concentrations of lecithin, optimum level of soybean lecithin for cryopreservation of black Bengal goat sperm is 1%. In similar to our findings, optimum level of soybean lecithin for cryopreservation of ram and human semen were also reported as 1% [8–10]. Salmani *et al.* observed increase of soybean lecithin level above 1.5% in the extender leads to reduce sperm quality after freezing- thawing process [11]. Forouzanfar *et al*. reported that high concentration of soybean lecithin was toxic for sperm motility and viability [9]. Moreover, Wagtendonk-de Leeuw *et al*. reported that high concentrations of lecithin increased viscosity of extenders and this effect could be related to the osmotic pressure of the extender that decreased when soy lecithin concentration increased in the extender [7]. Moussa *et al*. reported that when LDL concentration increases in the extender, osmotic pressure declined due to the precipitation of fructose and salts included in the extender that decrease osmotic pressure leading to damage to sperm cells [12]. Based on the results, it can be concluded that soybean lecithin can be an alternative to egg yolk in extender preparation for freezing black Bengal buck semen without adverse effects on post thaw *in vitro* characters. More investigations need to be accomplished for further evaluation of *in vitro* and *in vivo* fertilizing ability of black Bengal buck semen cryopreserved in extender containing soybean lecithin in place of egg yolk as a chemically defined extender.

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