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Research Article

A STUDY TO EVALUATE THE EFFECT OF FRESH AND POWDERED EGG YOLK ON POST THAW SEMEN QUALITY IN BARBARI BUCK

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ABSTRACT

A study was conducted to determine the effect of fresh and powdered egg yolk on standard semen attributes in pure bred Barbari buck. Ejaculates collected from four Barbari goats were pooled and evaluated at 37^{0}C. The pooled semen sample was divided into three equal parts, later diluted in the semen extender (TRIS) according to the treatments (T-1: 3% fresh egg yolk, T-2: 3% powdered egg yolk and T-3: 20% powdered egg yolk after sperm washing) and cooled to 5^{0}C and frozen in 0.25 ml French straws in liquid nitrogen. Frozen straws were thawed individually at 37^{0}C for 30s in a water bath for evaluation. Post-thaw sperm motility was significantly ($P<0.01$) higher in T-1 followed by T-2 and T-3 treatments. No significant effect was observed on percent abnormal sperms. The mean (±S.E.) values for percent live sperm and HOST positive sperms were significantly ($P<0.01$) higher in the T-1 and T-2 as compared to T-3 treatment. Higher ($P<0.01$) proportion of sperms with intact acrosome was observed in T-1 when compared to T-3 but was non-significantly higher than T-2 treatment. Thus, it can be concluded that the use of fresh egg yolk when incorporated @ 3% in semen extender is a better alternative for semen cryopreservation in buck followed by powdered egg yolk @ 3% and powdered egg yolk @ 20% after sperm washing.

Key words: Barbari goat, cryopreservation, egg yolk, seminal attributes

Egg yolk is a basic ingredient of semen extender utilized during cryopreservation. It is frequently used as a cryoprotective agent and is highly effective for the maintenance of sperm fertility in different species (Sansone *et al.,* 2000; Garde *et al.*, 2003). The egg yolk provides successful protection to the sperm against cold shock and lipid-phase transition effect during the freeze–thaw process (Moussa *et al.,* 2002). It acts as a protectant of the plasma membrane and acrosome against temperature-related injury during sperm cryopreservation (Amirat *et al.*, 2004). The buck seminal plasma contains an enzyme secreted by the bulbo-urethral glands, which in presence of egg yolk leads to the formation of lysophosphatidylcholines, that are toxic to sperms (Roy, 1957; Leboeuf *et al*., 2000). This restricts the use of egg yolk either at low concentrations in extenders for goat semen (Priyadharsini *et al.,* 2011) or cryopreservation after sperm washing (Kozdrowski *et al.,* 2007) that further complicates the process. So, taking into account the hypothesis that the use of powdered egg yolk compared to fresh egg yolk can be a better alternative for the cryopreservation of buck semen, a study was conducted to evaluate the effect of fresh and powdered egg yolk on post thaw semen.

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MATERIALS AND METHODS

Four normal, healthy adult Barbari bucks aged between 2-3 years, weighing 25-35 kg reared at the experimental goat sheds of of the university were used as experimental animals. Semen was collected twice a week during the month of January and February, 2015 at 7:00 am using an artificial vagina (20 cm in length and 4.5 cm in diameter). A non-estrous doe was used for mounting of bucks and the semen was collected in graduated cups. Immediately after collection, the semen was evaluated and pooled to make sufficient volume. The pooled semen sample was divided into three equal parts and subsequently diluted in the semen extender (TRIS) according to the treatments which was based on literature available for goat cryopreservation (T1 as control-3% fresh egg yolk, T2-3% powdered egg yolk and T3-20% powdered egg yolk after sperm washing) and subjected to cryoprocessing. Frozen-thawed sperm samples from each treatment were evaluated for different parameters. The progressive motility of the spermatozoa was observed under high power phase objective (40x) on a thermostatically controlled stage maintained at 37^{0}C. Percentage of live or dead spermatozoa and morphological abnormalities were evaluated using eosin-nigrosin staining technique (Hancock, 1952). HOST was performed according to

the method described by Jeyendran *et al.* (1984). Acrosome integrity was judged by Giemsa staining technique (Watson,1995). The result were analyzed using a one-way analysis of variance, followed by a Duncan's test to determine the significant differences both within and between different treatment groups, using the SPSS/PC computer program (version 14.0; SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

The effect of supplementation of fresh and powdered egg yolk on seminal attributes of frozen-thawed goat spermatozoa is presented in Table 1. Post-thaw sperm motility was significantly (P<0.01) higher in semen frozen with fresh egg yolk (T-1) followed by T-2 and T-3 treatments. The mean (±S.E.) values for percent live sperm significantly (P<0.01) were higher in the T-1 and T-2 treatments as compared to T-3 treatment while a non-significant difference was observed in percent abnormal sperms in all the three groups. Significantly (P<0.01) higher values of HOST positive sperms were observed in T-1 and T-2 treatments as compared to T-3 treatment. The acrosome integrity had a higher (P<0.01) proportion of intact acrosomes in T-1 treatment when compared to T-3 treatment but non-significantly higher than T-2 treatment. During the process of cryopreservation, spermatozoa are exposed to cold shock, which increases their susceptibility to lipid peroxidation due to higher production of reactive oxygen species (ROS) (Bucak *et al*., 2008). Egg yolk is used as a common component of semen extenders during cryopreservation. It has been shown to have a beneficial effect on sperm cryopreservation as a protector of the plasma membrane and acrosome against temperature-related injury due to the presence of phospholipids, in association with others components (Purdy, 2006).

In the present study, a significant difference (P<0.01) in percent progressive motile sperms was observed in T-1 and T-2 treatments. This may be due to differential interaction of fresh and powdered egg yolk with secretion of bulbourethral gland resulting in increased sperm motility. In T-3, significantly lower values (P<0.01) were observed that might be the result of centrifugation and removal of seminal plasma during cryoprocessing indicating primarily the role of seminal plasma over lethal interactive mechanism in buck semen. It may further be supplemented by the fact that cryopreservation induces oxidative stress leading to excessive generation of ROS by immature and abnormal spermatozoa during sperm processing *viz.* extending, freezing, thawing process, and low antioxidant concentrations in seminal plasma (Sikka, 2004). Significantly (P<0.01), lower values of percent live sperms and HOST positive sperms were recorded in T-3 treatment as compared to T-1 and T-2 treatments; it might be due to removal of seminal plasma in T-3 (Bilodeau *et al.,* 2000). Removal of seminal plasma might have lowered the antioxidative capacity leading to reduced viability and membrane integrity (Juyena and Stelletta, 2012). However, Ansari *et al.* (2010) compared powdered egg yolk with fresh egg yolk in an extender for cryopreservation of Zebu bull semen and observed that sperm plasma membrane integrity remained similar at all the stages of cryopreservation while sperm motility and viability were significantly (P<0.01) higher after thawing in the extender containing powdered egg yolk. The probable difference in the findings may be due to the selective interaction of fresh and powdered egg yolk with secretion of bulbourethral gland supplemented with centrifugation and seminal plasma removal (Cabrera *et al.,* 2005).

Cold shock during sperm cryopreservation is associated with oxidative stress that leads to generation of ROS. ROS-induced damage to spermatozoa is mediated by oxidative attack of bis-allylic methylene groups of sperm phospholipid-bound polyunsaturated fatty acids (PUFAs), leading to lipid peroxidation. To counteract the harmful effects of ROS, spermatozoa and seminal plasma possess a number of antioxidant

Table 1

Effect of fresh and powdered egg yolk on post thaw semen quality in Barbari goat

Seminal attributes	Treatments			Overall values
	T-1	T-2	T-3	
Progressive motility (%)	54.00^{a}±0.82	51.57^{b}±0.78	47.71^{c}±0.84	51.10±0.73
Percent live spermatozoa	56.57^{a}±0.72	55.14^{a}±0.59	49.29^{b}±1.13	53.67±0.84
Percent abnormal spermatozoa	5.00±0.31	4.71±0.29	5.43±0.20	5.05±0.16
HOST positive sperm	55.00^{a}±0.65	52.29^{a}±0.94	47.71^{b}±1.15	51.67±0.85
Percent intact acrosome	53.57^{a}±0.61	50.14^{ab}±1.79	47.29^{b}±0.94	50.33±0.88

Values with different superscripts in a row are significantly different (P<0.01)

T1= 3% fresh egg yolk; T2= 3% powdered egg yolk; T3=20% powdered egg yolk after sperm washing

systems that scavenge ROS and prevent internal cellular damage and maintain the integrity of sperm cell (Gurler *et al.,* 2015). The significantly ($P<0.01$) higher values of sperms with intact acrosome in T-1 treatment compared to T-3 treatment might be result of seminal plasma removal rich in antioxidative enzymes leading to reduced antioxidative capacity that in turn disrupted the sperm acrosome in T-3 treatment. Thus, it can be concluded that the use of fresh and powdered egg yolk when incorporated @ 3% in semen extender may be a better alternative for semen cryopreservation in buck compared to 20% egg yolk after sperm washing.

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