The Haryana Veterinarian (June, 2024) **63(1),** 41-43 ISSN 0033-4359 (P) **Research Article**

EFFICACY OF SEMINAL PLASMA REMOVAL AND CLARIFIED TRIS EGG YOLK CITRATE DILUTER ON SPERM MORPHOLOGY FOR CRYOPRESERVATION OF SURTI BUCK SEMEN

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ABSTRACT

The study's goal was to assess the impact of seminal plasma removal and clarified Tris egg yolk citrate (TEYC) diluter on sperm morphology for frozen thawed Surti buck semen. By using an artificial vagina, a total of 32 semen samples (16 ejaculates/buck) from two Surti bucks older than one year were collected. The semen obtained from both bucks was pooled in each replicate. The pooled semen sample was split into four identical portions. The seminal plasma was removed from two portions by centrifugation, leaving the other two intact. Semen that had not been washed was diluted with non-clarified and clarified TEYC diluter in groups T1 and T2, respectively. On the other hand, washed semen was diluted with non-clarified and clarified TEYC diluter in groups T3 and T4. Afterward, the expanded semen was put into 0.5 ml French medium straws, according to groups and allowed to acclimate to 4º C for four hours. This was followed by cryopreservation. T1 (16.5±1.24), T2 (14.69±1.26), T3 (18.31±1.18), and T4 (15.38±1.19) groups had non-significantly different post-thaw abnormal sperm counts (%), with T2 having the lowest abnormal sperm count and T3 having the highest. In conclusion, the lowest post-thaw abnormal sperm count observed in the group using only clarified Tris egg yolk citrate diluter in comparison to all other protocols indicates that seminal plasma removal has adverse effect on the morphology for cryopreservation of Surti buck semen, while egg yolk citrate diluter clarification has a beneficial effect.

Keywords: Abnormal sperm, Clarification, Cryopreservation, Seminal plasma, Surti buck

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Goats and sheep with high genetic value are frequently bred using artificial insemination (Foote, 2002). Spermatozoa and seminal plasma are the two components that make up goat semen. When egg yolk or milk extender was added to diluter for buck semen, the seminal plasma deteriorated and produces toxic effect.

Egg Yolk Coagulating Enzymes (EYCE), Phospholipase A2 and SBUIII from the bulbourethral glands secretions are present in the seminal plasma of buck semen. These enzymes interact with the skimmed milk and egg yolk and leads to production of toxic elements which affect spermatozoa (Purdy, 2006; Gangwar *et al.*, 2016) and decrease their freezability (Purdy, 2006; Gangwar *et al.*, 2016). EYCE and SBUIII are thought to be the same molecule (Leboeuf *et al.*, 2000). Phospholipase A (Iritani and Nishikawa, 1963) was identified as the EYCE, and SBUIII (now known as BUSgp60) was identified as a 55-60 kDa glycoprotein lipase from the goat bulbourethral gland. Its triacylglycerol hydrolase activity causes the release of oleic acid from milk triglyceride, which in turn causes a decline in the proportion of motile sperm, a decline in movement quality, acrosome damage, and spermatozoa death (Leboeuf *et al.*, 2000). However, Arjun *et al.* (2021), described non-significant role of seminal plasma in the cryopreservation of buffalo semen.

Furthermore, whole egg yolk contains granules that are similar in size and form to spermatozoa and because they decrease sperm motility and respiration, it has been claimed that they can interfere with microscopic inspection or biochemical assays (Moussa *et al.*, 2002). The two main portions of egg yolk, plasma and granules can be separated by centrifugation (Pillet *et al.*, 2011). Therefore, to achieve superior post-cryopreserved sperm quality, it is imperative to eliminate big constituent part from the egg yolk by centrifugation and to employ only cleared plasma. According to several studies, the freezing and thawing characteristics of buck semen were positively impacted by the seminal plasma removal (Kozdrowski *et al.*, 2007). However, sperm quality may suffer if seminal plasma is removed, according to another team (Azeredo *et al.*, 2001).

Thus, the existing experiment was directed to assess the efficacy of seminal plasma removal and clarified Tris egg yolk citrate diluter on sperm morphology in frozen, thawed Surti buck semen in order to explore the detrimental properties of seminal plasma and egg yolk interaction.

MATERIAL AND METHODS

Preparation of clarified TEYC diluter: On day of the experiment, a sterile flask containing TRIS-citric acid-fructose buffer was added with 10% egg yolk to make the TEYC diluter. The clarified and non-clarified TEYC

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Table 1. Effect of clarified Tris egg yolk citrate dilutor and seminal plasma removal on sperm abnormality count (%) in Surti buck semen (Mean±SE)

Groups	Abnormal sperm count (%) (n=16)			Overall (n=48)	F value	P value
	Initial	Pre-freezing	Post thaw			
T1	10.75±1.22[b]	13.81±1.09[ab]	16.5±1.24[a]	13..69±0.75	5.87*	0.01
T2	8.69±0.97[b]	11.5±1.14[ab]	14.69±1.26[a]	11.63±0.73	7.06**	0.00
T3	10.13±1.49[b]	13.13±1.28[b]	18.31±1.18[a]	13.85±0.90	9.81**	0.00
T4	8.88±1.26[b]	11.25±1.25[b]	15.38±1.19[a]	11.83±0.80	7.08**	0.00
Overall (n=64)	9.61±0.62[c]	12.42±0.60[b]	16.22±0.62[a]	—	29.34**	0.00
F value	0.63	1.09	1.69	2.20	—	—
P value	0.60	0.36	0.18	0.09	—	—

[a-c]Means with different superscripts between columns (between various stages of cryopreservation) at p<0.05; p<0.01. ** p<0.01; * p<0.05. T1-Non clarified & non washed semen; T2- Clarified & non washed semen; T3- Non clarified & washed semen; T4 -Clarified & washed semen

dilutor was prepared as per the method described by Karthik *et al.* (2023) and stored at 37º C.

Collection of semen: Two healthy Surti bucks, over one year old were chosen from LFC, COVS & AH, Navsari. The particular chosen bucks were fed properly and kept in good health and hygiene on a regular basis. The chosen bucks were kept apart from the goats and kept in a shared covered pen. Using a female dummy, semen was collected two times a week using AV, from each buck up to two months. A total of 32 semen samples were obtained, 16 from each buck. The buck was given a false mount on a female dummy before every semen collection. To prevent the contamination of buck penis from dummy's hind quarter a buck apron was placed on it. The semen samples were collected into sanitized glass graduated tubes and brought to the workplace within 10 minutes. The ejaculates from both the bucks were combined during each semen collection to reduce individual buck variability and enhance semen volume.

Removal of Seminal Plasma: The neat semen was evaluated immediately and then allocated in four equal parts. Two parts were left unaltered, whereas the other two underwent centrifugation for washing to remove the seminal plasma in accordance with the protocol described by Karthik *et al.* (2023).

Experimental groups: Groups T1 and T3 were prepared by adding non-clarified TEYC diluter to non-washed and washed semen, respectively whereas, groups T2 and T4 were prepared by adding clarified TEYC diluter to non-washed and washed semen, respectively. Following grouping, the sperm morphology was assessed from each group by microscopic examination of semen samples. A total of four straws were prepared for every group and freezing was done as per method designated by Karthik *et al.* (2023). For each group, the pre-freezing and post thaw sperm morphology was assessed.

Statistical analysis: R-3.3.2 software was used to appropriately tabulate and analyze the data related to different aspects. DNMRT (Duncan's New Multiple Range Test) and ANOVA, two suitable statistical techniques, were used to compare the means between and within the diverse groups. At p<0.05 and p<0.01, the mean differences were deemed significant.

RESULTS AND DISCUSSION

Table 1 shows the initial pre-freezing and post-thaw abnormal sperm count for each group. The initial, pre-freezing, and post-thaw abnormal sperm count (%) did not substantially differ across the groups. At all three stages of cryopreservation, the T2 and T4 groups had a lesser percentage of abnormal sperm counts than the T1 and T3 groups. Additionally, the T2 group had a lowest post-thaw abnormal sperm count (14.69±1.26%), whereas T3 group had the highest (18.31±1.18%). Furthermore, the overall mean values of abnormal spermatozoa count (%), regardless of the different phases of cryopreservation, varied non-significantly and were lowest in the T2 group (11.63±0.73) followed by T4 group (11.83±0.80), T1 group (13.69±0.75) and T3 group (13.85±0.90).

The current results were in close covenant with those of Coloma *et al.* (2010), who found that in fresh and frozen thawed semen from Spanish ibex (Capra pyrenaica) bucks, non-washed semen had non-significantly lower morphologic abnormalities (38.9±6.2 and 32.9±5.3%) compared to washed semen (47.9±8.1 and 37.5±7.2%). Similarly, Bhagel *et al.* (2016) found that in non-washed (5.07±0.20, 5.67±0.15 and 6.83±0.24%, respectively) as opposed to washed (6.73±0.22, 8.03±0.15 and 8.80±0.20%, respectively) semen from Barbari bucks, the percentage of initial, pre-freezing, and post-thaw abnormal spermatozoa was significantly (P≤ 0.01) lower. Sen *et al.* (2015) also found that the post-thaw sperm abnormalities in non-washed (22.65±3.70%) compared to washed (30.65±4.01%)

Norduz buck semen was considerably ($p<0.05$) lower. Similarly, Sariozkan *et al.* (2010) found that for Angora bucks, non-washed semen had a significantly ($p<0.001$) lower proportion of post-thaw sperm abnormalities (19.4±0.8%) than did washed semen (26.6±1.5%). The present findings of our study closely aligned with all of the above mentioned findings.

In divergence to the current outcomes, Sharma *et al.* (2018) observed that in fresh and post-thaw semen from Gaddi bucks, there were non-significantly more morphological abnormalities in non-washed (12.7±0.9 and 14.3±0.8%) as compared to washed (11.7±0.8 and 9.7±0.9%) semen, respectively.

Based on the results of this investigation, it was determined that, in order to minimize sperm abnormalities during cryopreservation of Surti buck semen, using clarified Tris egg yolk citrate diluter is preferable over removing seminal plasma.

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