SEMINAL PLASMA REMOVAL IMPROVES CRYOPRESERVED SEMEN QUALITY IN GADDI BUCKS

A. SHARMA[1]*, P. SOOD[2] AND P.K. DOGRA[3]

Department of Veterinary Gynaecology and Obstetrics, CSKHPKV, Palampur -176062

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ABSTRACT

Forty-eight semen ejaculates, collected in artificial vagina, from eight adult Gaddi bucks were randomly divided into groups viz. freezing the entire semen sample (n=24) or removal of seminal plasma by centrifugation (n=24). Semen was extended in tris citrate egg yolk extender to maintain 150 million sperm / dose (0.25 ml). The filling, sealing, equilibration, vapour freezing and final plunging in liquid nitrogen was done as per the standard norms. The post-thaw results (%) of washed semen samples were better ($p<0.05$) than their non-washed counterparts (progressive motility 30.4±1.6 *vs.* 22.8±2.1; morphological abnormalities 9.7±0.9 *vs.* 14.3±0.8; HOST 61.5±2.0 *vs.* 45.6 ±3.8). In conclusion, the removal of seminal plasma in cryopreservation protocol of Gaddi buck semen appears essential to obtain better post-thaw results.

Keywords: Buck, Gaddi goats, Semen cryopreservation, Seminal plasma, Washing

INTRODUCTION

The bulbourethral glands of goat release Egg Yolk Coagulating enzyme (EYCE, phospholipase A2) in the seminal plasma that interacts with egg yolk, an essential component of semen extender, thus producing substances toxic to sperm cells and reducing its freezing ability (Gangwar *et al.,* 2016). To minimize the deleterious interaction of seminal plasma and egg yolk, the washing of sperms by centrifugation is done to remove the seminal plasma (Tabarez *et al.,* 2017 and Narwade *et al.,* 2017). Others reported that the removal of seminal plasma may have negative effect on sperm quality (Azeredo *et al.,* 2001). Therefore, the present study was designed to investigate the impact of seminal plasma on quality of cryopreserved semen in Gaddi goats.

MATERIALS AND METHODS

The study was conducted on apparently healthy Gaddi bucks (n=8) aged between 1.1 to 4.5 yr (2.25±0.45 yr), weighing 31-57 kg, (40.0±3.43 kg) maintained at 32.6°N, 76.3°E, altitude 1290.8 m between October-December, 2016. All the bucks were maintained under identical management conditions and were screened for Brucellosis and Chlamydial infections. The animals were vaccinated against routine infections at least 2 mo before study and were dewormed using broad spectrum anthelmintics. The bucks were subjected to grazing for 5 h / day and were housed to confinement for the remaining period. The Gaddi bucks were fed as per standards of National Research Council, India and had round the clock access to the clean drinking water.

Semen was collected twice weekly by artificial vagina (AV) and a total of 48 Gaddi ejaculates were included in the study. The female in estrus was used as teaser for semen collection. Immediately after collection, the ejaculates were placed in water bath (34-35°C) and aliquots were taken to assess the semen quality. The fresh semen was evaluated for colour, volume and concentration followed by microscopic examination for mass motility and initial motility. Fresh semen samples with ≥3 mass motility and >70% initial motility were used for cryopreservation. The ejaculates were divided

[1]Assistant Professor; [2]Professor and Head, Department of Teaching Veterinary Clinical Complex; 3Professor and Head, Department of Instructional Livestock Farm Complex; *vet50amy@gmail.com

as non-washed (n=24) and washed (n=24) ejaculates. The washed samples were centrifuged twice at 2500 g for 7 minutes with Ringer's solution. Thereafter, all the samples were extended in tris citrate egg yolk extender, (TRIS 2.42 gm; Citric Acid 1.37 gm; D-Fructose 1 gm; Benzyl penicillin 1000 IU/ml; Streptomycin Sulphate 1mg/ml; Egg yolk 20%; Glycerol 7%; Ultrapure water upto 100 ml; pH - 6.7 to 6.9). Extended semen was filled using micropipette (Minitube, Germany) in 0.25 ml French mini straws (IMV Technologies, L'Aigle, Cedex, France) having final concentration of $150x10^6$ sperms/ dose. The filled straws were sealed with the help of polyvinyl alcohol powder (IMV Technologies). All the straws were laid on a stainless-steel rack and placed in cooling cabinet (Macro Scientific works Pvt. Ltd. India) for 4 h in order to bring down the temperature from 30°C to 4°C by gradual cooling. After equilibration, the semen straws were shifted into styrofoam box filled with LN_2 in a manner so that that LN_2 surface was 4 cm below the rack having straws. Straws were exposed to LN_2 vapours for 7 minutes. Finally, the straws were plunged into LN_2 for storage. A day after LN_2 storage, one straw each from each buck was thawed in water bath at 37°C for 30 sec for post-thaw evaluation.

The results of seminal plasma removal were evaluated in terms of progressive motility, Live and Dead sperm count, morphological abnormalities and HOST. The results are presented as percent motility, liveability, morphological abnormalities and HOST reactive sperms. The data were analysed using one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine significant difference in all parameters between groups using the latest SPSS® 20 level version for windows. The differences with values of $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

In fresh semen of Gaddi goats, the non-washed versus washed sample results for the volume were 0.50±0.05 *vs.* 0.63±0.06 ml, color was creamy white to yellowish with thick consistency, concentration was 2359.4±209.93 *vs.* 3133.8±194.64 ($p<0.05$) and mass motility was 3.90±0.06 *vs.* 4.14±0.04 ($p<0.05$), respectively. Incidentally, a higher volume, concentration and mass motility were reported in washed semen samples which was congruence to earlier findings of Thakur *et al.,* 2005, (Chegu goats; 0.47 ml), Mohan *et al.,* 1980 (pashmina goats, 0.62 ml), and was lesser than Ali and Mustafa, 1986 (Nubian goat, 1.09 ml), Tabrez *et al.,* 2017 (Blanca de Rasquera bucks, 1.0 ml young bucks 1.8 ml older bucks). The average ejaculate volume of buck semen is 1.0 ml (Seremak *et al.,* 1999), but volume varies between 0.2-2.0 ml depending upon the breed and frequency of semen collection. The average concentration of buck semen is 2500-4500 millions/ml and varies with breed, season and age (Purdy, 2006).

Furthermore, between non-washed and washed semen samples, the differences ($p<0.05$) were recorded between post-thaw seminal parameters viz. progressive motility, morphological abnormalities and HOST. Similar results with beneficial effects of seminal washing were earlier observed by Tabrez *et al.,* 2017 (Blanca de Rasquera bucks), Ritar and Salamon, 1982 (Angora bucks). Thus, it was suggested that there is beneficial effect of washing for removal of seminal plasma as it avoids the EYCE and protein SBUIII acts (Purdy, 2006), thus leading to production of toxic substances. Others also reported that the removal of seminal plasma from buck ejaculate is beneficial in preserving the integrity of sperm after freezing (Memon *et al.*, 1985).

In contrast, a study revealed no difference between the washed and non-washed semen samples (Jimenez-Rabadan *et al.,* 2012). Others emphasised washing as a complex time consuming process causing damage of certain percentage of spermatozoa as well the removal of some components of seminal plasma that can be important to protect sperm membrane against freezing-thawing (Azeredo *et al.,* 2001 and Anand *et al.,* 2017). Furthermore, the discrepancy in sperm loss from centrifugation due to variation in efficiency of

Table 1: Effect of seminal washing on seminal parameters (Mean±SEM) in Gaddi buck (n=8)

Sperm parameters	Fresh semen		Post-thaw	
	Non-washed ejaculate (n=24)	Washed ejaculate (n=24)	Non-washed (n=24)	Washed (n=24)
Progressive motility	70.8±0.6	71.5±0.5	22.8±2.1[a]	30.4±1.6[b]
Liveability	77.1±1.5	74.9±1.0	33.9±3.9	38.4±2.3
Morphological abnormalities	12.7±0.9	11.7±0.8	14.3±0.8[a]	9.7±0.9[b]
HOST reactive	76.3±1.9	77.2±1	45.6±3.8[a]	61.5±2.0[b]

[a vs b] $p<0.05$, within row

centrifugation procedure cannot be precluded (Kucuk *et al.,* 2014). In present study, the centrifugation did not increase the rate of abnormal sperms in the washed samples (11.7±0.8 *vs.* 9.7±0.9%, $p>0.05$).

In conclusion, irrespective of the different variables dictating post-thaw semen quality, the removal of seminal plasma considerably improves post-thaw semen quality, and therefore becomes essential for its successful use.

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