SHORT COMMUNICATION

Effect of Cold Storage on the Quality of Epididymal Semen of Slaughterhouse Derived Buck Testes

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Abstract

The present study was carried out on 48 pairs of testicles from mature bucks, irrespective of breed, slaughtered at local abattoirs of Faizabad and Sultanpur districts, UP and at AL-Nafees Protein Pvt. Ltd., Mewat, Haryana. The testicles were collected and stored in plastic bags in ice chest at 4°C and immediately transferred to the laboratory. The testicles were randomly divided into six storage groups, *i.e.*, 6, 12, 24, 48, 72 and 96 hours; eight pairs of testicles in each group. After completion of storage time, testes of respective storage groups were cleaned with physiological saline solution, and the fascia, blood vessels, and sheath were removed using BP blade and thumb forceps. The various epididymal seminal attributes assessed were semen volume, sperm concentration, total sperm recovery, sperm motility, viability, abnormality, and HOS reactivity using standard procedures. The volume of epididymal semen harvested differed significantly with storage time. The sperm concentration did not vary significantly up to 48 hours of storage; sperm motility and viability did not differ significantly up to 12 hours of cold storage, while there was a progressive increase in abnormality and decrease in HOS reactivity. Thus, cold storage preservation and harvesting of epididymal semen up to 48 hours can be made to conserve the fertility of precious dead/slaughtered buck.

Keywords: Buck, Cold storage, Epididymal semen, Quality, Slaughter.

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Introduction

The cauda epididymis of live animals provides an excellent milieu for sperm storage only for a definite period depending upon species. The interest in preserving endangered species and specially germplasm has resulted in increased attention to the possible recovery of functional spermatozoa from the epididymis of dead animals. Recovery of epididymal semen after death offers a valuable option for preserving male gametes (Strand *et al*., 2016); thus, assists in sustaining biodiversity and maintaining gene bank. Harvesting of live sperms from the epididymis of dead animals have been well documented in bulls (Strand *et al*., 2016; Stephen *et al*., 2019), buffalo bulls (Kumar *et al*., 2018), rams (Lone *et al*., 2011; Mir *et al*., 2012; Sasaf *et al*., 2015), and bucks (Turri *et al*., 2014; Ouennes *et al*., 2019). Indeed, spermatozoa recovered from dead animals (stored at room temp, 22°C) as long as 24 h after death can be used for IVF, zygote production, and the birth of live young (Songsasen *et al*., 1998). Blash *et al*. (2000) and Martins *et al*. (2007) reported successful harvesting of epididymal sperm from dead bucks and used for IVF and *in vitro* embryo production. Furthermore, Martins *et al*. (2009) recorded a decline in total motility of bovine epididymal spermatozoa after 48 h of storage at 5°C, which did not change until 72 hours. The present study was planned to evaluate effect of cold storage on epididymal semen quality of slaughtered buck testes.

Materials and Methods

The experiment was carried out at deep-frozen semen (DFS) laboratory of Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, ANDUAT, Ayodhya, UP, India. Forty-eight pairs of testes (total 96) were collected immediately after slaughter of bucks, irrespective of breed, from local abattoirs of Faizabad and Sultanpur districts of UP and AL-Nafees Proteins Pvt Ltd, Mewat, Haryana. The fresh testicles collected with

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epididymis were placed in plastic bags in ice chest at 4-5°C and immediately transferred to DFS lab. They were randomly divided into six storage groups, *i.e.*, 6, 12, 24, 48, 72 and 96 hours; eight pair of testicles in each group and preserved in the refrigerator at 4°C for 6-96 hours. After completion of storage time, testes of the respective group were cleaned with physiological saline solution; the fascia, blood vessels, and sheath were removed using BP blade and thumb forceps.

Semen was collected separately from the right and left epididymis by slicing and swim-up technique in 15 ml plastic vials (Turri *et al*., 2012; Joram *et al*., 2016). The samples collected at different storage intervals were analyzed for semen volume, sperm concentration, sperm output/recovery, as well as sperm motility, viability, morphology, and HOS reactivity. The volume of semen was measured by direct reading of graduated conical tubes; sperm concentration was estimated using the hemocytometer method. The percent progressive motile sperm was assessed at 37°C under high power (40X) magnification by placing a drop of epididymal semen on a pre-warmed glass slide and covering it with a coverslip. Sperm viability was estimated using a differential staining technique with eosin-nigrosin stain. To estimate sperm abnormality, semen suspended in formal saline solution (wet smear) was put on the slide and covered with a coverslip. The percent total sperm abnormalities were determined by counting total of 200 spermatozoa under a phase contrast microscope at 100 X magnification with oil immersion.

Data were presented as mean and standard error (Mean ± SE). Analysis of variance and Tukey's HSD test were used to assess differences among the storage treatment using Graph pad Instat Version 5 software.

Results and Discussion

Epididymal Sperm Recovery

The epididymal seminal attributes of slaughtered bucks testes observed at different storage intervals at 4°C are presented in Table 1. Among different cold storage groups or intervals, the highest semen volume was harvested at 6 h of storage (0.43 ± 0.02 ml) and the lowest at 96 h storage interval (0.20 ± 0.02 ml), other groups/intervals being intermediate, and the differences were statistically significant ($p < 0.05$) between periods. The epididymal sperm concentration did not differ significantly during 6 to 48 hours of storage, but thereafter the concentration progressively and significantly ($p < 0.05$) reduced, Further, a continuous and significant reduction was noted in total sperm recovery (1410.83 ± 67.58 to 522.6 ± 46.18 million; $p < 0.05$) during 6 to 96 hours of cold storage. In brief, there was a gradual and significant ($p < 0.05$) reduction in semen volume, sperm concentration, and total sperm recovery with increasing storage time of testes at 4°C (Table 1).

Turri *et al*. (2012) recorded comparable epididymal semen volume in buck, however, little higher values were reported by Furstoss *et al.* (2009), Ouennes *et al*. (2019), and Patel *et al.* (2021). Further, Ouennes *et al*. (2019) recorded non-significantly decreased epididymal semen volume at 24 hours of storage at 4°C as compared to 0 hour in bucks. Valete *et al.* (2015) studied the duration of storage (10-12 hours vs 15-17 hours) at 5-8°C and 18-22°C and recorded non-significant reduction epididymal semen volume relative to time. Similarly, Mir *et al*. (2012) recorded a significant and progressive decrease in epididymal sperm output up to 48 h of cold storage. Like the current finding, a decreasing trend of sperm concentration during storage was also reported earlier in bucks (Turri *et al*., 2014) and water buffalo (Valete *et al*., 2015). A similar trend of reduction in total sperm output due to cold storage was also reported by Blash *et al.* (2000), Mir *et al*. (2012), and Turri *et al*. (2014) in bull, ram and buck epididymal semen due to the process of progressive decomposition of tissues of preserved epididymis. In the present study, a significant decrease in sperm concentration after 48 h of storage might be due to a decrease in sperm motility due to cold storage, as sperm was harvested by slicing and swim-up method (Mir *et al.,* 2012). The variation in semen volume and sperm count observed at different storage intervals and in different studies might be due to difference in age/breed, sexual maturity, nutritional status, general health, endocrine balance, season/climatic conditions, soundness of sex organs (Blash *et al.,* 2000; Patel *et al*., 2021) and duration of storage (Valete *et al*., 2015; Ouennes *et al*., 2019; Patel *et al*., 2021).

Table 1: Cyto-morphological characteristics of epididymal semen of slaughtered bucks

Cold storage time	*Semen volume (ml)*	*Sperm concentration (million/ml)*	*Sperm recovery (million)*	*Sperm motility (%)*	*Sperm viability (%)*	*Sperm abnormality (%)*	*HOS (%)*
6 h	0.43 ± 0.02^{a}	3281 ± 106.9^{a}	1410.83 ± 67.58^{a}	75.13 ± 0.58^{a}	81.88 ± 0.67^{a}	23.88 ± 0.98^{a}	36.88 ± 0.58^{a}
12 h	0.33 ± 0.02^{b}	3125 ± 90.14^{ab}	1031.25 ± 67.73^{b}	74.00 ± 0.93^{a}	81.75 ± 1.30^{a}	28.63 ± 0.60^{b}	33.50 ± 0.76^{b}
24 h	0.30 ± 0.00^{c}	3094 ± 96.56^{abc}	928.2 ± 28.05^{bc}	66.25 ± 1.30^{b}	73.00 ± 1.13^{b}	35.88 ± 0.83^{c}	29.25 ± 1.20^{c}
48 h	0.30 ± 0.00^{c}	3123 ± 36.68^{ab}	936.9 ± 11.00^{bc}	59.00 ± 0.50^{c}	68.00 ± 0.76^{c}	40.25 ± 0.56^{d}	27.13 ± 0.30^{dc}
72 h	0.25 ± 0.02^{d}	2975 ± 37.80^{c}	743.75 ± 56.94^{c}	48.25 ± 1.03^{d}	56.63 ± 0.90^{d}	40.63 ± 1.22^{d}	22.88 ± 0.44^{de}
96 h	0.20 ± 0.02^{e}	2613 ± 29.50^{d}	522.6 ± 46.18^{d}	35.63 ± 0.63^{e}	44.00 ± 0.60^{e}	49.25 ± 0.73^{e}	18.88 ± 0.48^{e}

Means bearing common superscript within a column do not differ significantly ($p > 0.05$).

Epididymal Sperm Quality

The sperm motility and viability did not differ significantly up to 12 hours of storage, but thereafter significant ($p<0.05$) reduction was noted at 24, 48, 72 and 96 hours, Sperm abnormality increased significantly and progressively in different storage groups, except between 48 and 72 hours. The HOS reactive epididymal sperms differed significantly ($p < 0.05$) among different cold storage groups and reduced progressively from 36.88 ± 0.58 to 18.88 ± 0.48 % with storage time of 6 hours to 96 hours at 4°C (Table 1).

Unlike current observations much higher initial motility was reported in bucks epididymal sperm (Hoseinzadeh-Sani *et al.,* 2013; Bukar *et al*., 2017). Like present findings, no significant difference in motility was noticed in epididymal buck semen during cold storage for 24 hours (Ouennes *et al*., 2019) and 48 hours (Turri *et al*., 2014) at 4°C, but thereafter continuous and significant reduction was reported. Furthermore, significant and progressive reduction in sperm motility and viability with storage time at 4°C was also recorded in epididymal sperm of ram (Mir *et al*., 2012; Sasaf *et al*., 2015), buck (Hoseinzadeh-Sani *et al.,* 2013), and water buffalo (Valete *et al*., 2015). Elongation of post-mortem time to epididymal sperm retrieval alters the chemical composition and reduces epididymal lumen's pH, which in turn deteriorates sperm quality (Kaabi *et al.*, 2003). Furthermore, cold storage can protect epididymal spermatozoa to some extent by delaying post-mortem epididymal changes compared to room temperature storage (Kaabi *et al*., 2003; Hori *et al*., 2009; Fernandez-santos *et al*., 2011).

Present findings on sperm abnormalities were comparable with Mir *et al*. (2012), who noted a significant increase in percent abnormal sperm with increasing cold storage time of ram semen from 0, 24 to 48 h. However, Hoseinzadeh-Sani *et al*. (2013) and Turii *et al*. (2014) noted a non-significant increase in tail abnormality between 0 vs 24 hours of cold storage, but after 24 hours significant increase was observed in goat semen. Ouennes *et al*. (2019) recorded non-significant decrease in HOS reactivity up to 24 hours, but at 48 hours and 72 hours a progressive and significant reduction was observed in goat spermatozoa.

Conclusion

From the study, it can be inferred that the cold storage temperature (4°C) seems to protect spontaneous decay of epididymal semen quality over a limited time, in particular motility and viability, by virtue of inducing a reduction in metabolism of spermatozoa. When buck semen cryopreservation is not immediately feasible due to sudden or unexpected death or other reasons, goat testicles can be transported and stored at 4°C up to 12 hours post-mortem to preserve epididymal semen, comparable to that of ejaculated semen. The cold storage preservation could be used for post-mortem collection of epididymal semen from animals of high genetic merit and from endangered species when the collection of semen before death is not feasible.

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