RESEARCH ARTICLE

Biometry and Storage Ability at 4°C of Slaughtered Buck Testis and Correlations of Various Epididymal Seminal Attributes

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Abstract

The present study was conducted on 48 pairs of fresh testicles obtained from mature bucks, irrespective of breed, slaughtered at local abattoir and AL-Nafees Proteins Pvt. Ltd., Mewat, Haryana. Various testicular and epididymal biometric parameters, epididymal seminal attributes and correlation coefficients between testicular volume and seminal epididymal attributes were studied. The testicles with epididymis were put randomly into six groups (plastic bags) each of eight pairs and were stored at 4°C for 96 hours in a refrigerator. One bag/group each was taken out at 6, 12, 24, 48, 72, and 96 hours of cold storage to study the effect of preservation at 4°C on the seminal epididymal attributes. The sperm concentration, total sperm output, progressive sperm motility, viability, HOS reactivity, and sperm with droplets decreased significantly ($p < 0.05$) and progressively increased sperm head and tail abnormalities after 12 hrs of storage until 96 h. The testicular volume was significantly and positively correlated with sperm concentration ($r = 0.76$), total sperm output ($r = 0.95$), initial motility ($r = 0.65$), live sperm ($r = 0.63$), HOS reactive sperm ($r = 0.66$) and sperm with abnormal droplets ($r = 0.60$), however, it was negatively correlated with abnormal sperm head ($r = -0.64$) and sperm tail ($r = -0.69$).

Keywords: Biometry, Buck, Epididymis, Slaughtered testis, Seminal attributes, Storage effect.

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Introduction

Age of onset of puberty and male fertility are important factors in caprine reproduction as single buck breeds to many does (Chaudhari *et al.*, 2018[a,b]). Scrotal/testicular biometry is a good indicator of the sperm-producing ability of a sire. It is one of the important aspects of breeding soundness of males. The biometric study of testes is also helpful in diagnosing, controlling, and treating sub-fertility or infertility in males (Baldaniya *et al.*, 2020). Testicular traits suggest level of sexual activity and daily sperm production potential in buck (Leal *et al.*, 2004). Postmortem recovery of epididymal semen offers a valuable option for preserving male gametes, particularly of high genetic merit sires of endangered species, thus preserving biodiversity and maintaining germplasm bank. Recovery of epididymal sperm in bull, rabbit, human, and dog has been well documented. Blash *et al.* (2000) reported successful recovery of epididymal sperm from dead buck and used for IVF. Kundu *et al.* (2001) and Martins *et al.* (2007) reported cryopreservation of epididymal sperm from dead bucks and bulls and its use in *in vitro* embryo production (IVEP). Yu and Leibo (2002) successfully recovered motile sperm and membrane intact sperm from canine epididymis stored at 4°C for 8 days. Very meager literature is available on the quality of goat epididymal sperm following cold storage of testes after animals' death (Mir *et al.,* 2012; Turri *et al.,* 2013). Hence, the present study was planned to evaluate testicular and epididymal biometry of slaughtered bucks, and the effect of refrigeration storage time on the quality of epididymal sperm, and correlate testicular volume with seminal epididymal attributes.

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Materials and Methods

The experiment was conducted in 2014 at deep-frozen semen laboratory (DFS lab) of Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, ANDUAT, Kumarganj, Ayodhya, UP. Forty-eight pairs of testis (total 96) were collected

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immediately after slaughter of mature bucks, irrespective of breed, from local abattoir and AL-Nafees Proteins Pvt. Ltd., Mewat, Haryana. The testes were collected under strict hygienic conditions, stored in plastic bags in an icebox at 4°C, and immediately transferred to the DFS lab. After arrival at lab, testes were cleaned with physiological saline solution; fascia, blood vessel, and sheath were removed using BP blade and thumb forceps without damaging epididymis. Various biometric parameters, *viz.,* testicular length (TL), testicular diameter at widest portion (TD) and epididymal length (EL) were measured using scrotal tape; weight of testis (TW) with and without epididymis were measured using digital weighing scale, and testicular volume (TV) was calculated using formula: $TV = TD^2 \times TL \times 0.5$. The collected epididymis were then randomly divided into six equal groups, each of 8 pairs, and stored at 4°C in refrigerator up to 96 h. Epididymal seminal attributes were studied at 6, 12, 24, 48, 72 and 96 hr of cold storage by thawing the organs of one group at each interval.

Semen was collected separately from right and left epididymis by slicing and squeezing technique in 15 ml plastic vials (Martins *et al.*, 2007). Collected semen samples were analyzed for volume, sperm concentration, total sperm output, percent progressive motility, live sperm, abnormal sperm and HOS reactive spermatozoa. The volume of semen was measured by direct reading of graduated conical tubes; sperm concentration was estimated using the hemocytometer method. The progressive sperm motility was assessed at 37°C under high power (40X) magnification by putting a drop of epididymal semen on pre-warmed glass slide and covering it with a coverslip. Live sperm percent was estimated using differential staining technique with eosin-nigrosin stain. To count percent abnormal sperm, semen suspended in formal saline solution (wet smear) was put on the slide and covered with coverslip. The percent abnormal sperm (head, tail, and droplets) were determined by counting total 200 spermatozoa under a phase contrast microscope (100 X magnification with oil immersion).

Data were presented as mean and standard error (mean ± SE). Analysis of variance was used to assess differences among the buck testicles and storage treatments. Tukey's HSD test was used to compare treatment means by Graphpad InSat Version 5 Soft. Pearson's correlations were worked out between fresh testicular volume with epididymal seminal attributes.

Results and Discussion

Testicular and Epididymal Biometry

All testicular parameters, *viz.,* length (TL), circumference (TC), diameter (TD), volume (TV), and weight (TW) were significantly ($p < 0.05$) higher in left testicles as compared to right contemporaries overall and in testes of all the groups (Table 1). Like present findings, higher values for left TL, TC, TD and/or TV were reported by different workers in musk deer (Choudhary *et al.*, 2008), rams (Bhattacharya *et al.*, 2010; Al-Mahmodi *et al.*, 2017), bucks (Yaseen *et al.*, 2010; Oyeyemi *et al.*, 2012ll; Al-Mahmodi *et al.*, 2017; Chaudhari *et al.*, 2018[b]; Baldaniya *et al.*, 2020), camel (Abdullahi *et al.,* 2012) and Murrah bulls (Saurabh *et al.*, 2018).

Further, testicular weight with or without epididymis was significantly higher in left testicles as compared to right one (Table 1). A similar trend was reported for testicular weight in bucks (Yaseen *et al.*, 2010; Oyeyemi *et al.*, 2012; Baldaniya *et al.*, 2020), rams (Bhattacharya *et al.*, 2010), and Murrah bulls (Kumar and Srivastava, 2017; Saurabh *et al.,* 2018). Conversely, significantly higher testicular weight in the right testicle against the left testicle was reported in bucks and rams by Al-Mahmodi *et al.* (2017) and in camel by Abdullahi *et al.* (2012).

In the present study, the epididymal length (EL), width (EW), circumference (EC), and weight (EW) for caput, corpus, and cauda epididymis of left testicles were significantly higher than those of right ones (Table 1). Similar heavier left testes and epididymis were recorded by earlier workers in bucks (Yaseen *et al.*, 2010; Oyeyemi *et al.*, 2012; Baldaniya *et al.*, 2020), rams (Bhattacharya *et al.*, 2010) and Murrah bulls (Kumar and Srivastava, 2017; Saurabh *et al.,* 2018).

Epididymal Seminal Attributes at Different Storage Intervals

The seminal epididymal attributes observed at different

Table 1: Biometry, weight and volume (Mean ± SE) of left and right testicle of slaughtered bucks

Attribute	*Left testis (n = 48)*	*Right testis (n = 48)*
Testicular length with epididymis (cm)	9.72 ± 0.18**	9.28 ± 0.20
Testicular length without epididymis (cm)	7.91 ± 0.17**	7.65 ± 0.17
Testicular circumference (cm)	10.83 ± 0.20**	10.41 ± 0.18
Testicular diameter (cm)	3.43 ± 0.06**	3.31 ± 0.05
Testicular volume (cm^3)	59.89 ± 3.13**	53.50 ± 2.84
Caput length (cm)	4.02 ± 0.09**	3.78 ± 0.08
Caput width (cm)	3.33 ± 0.09**	3.00 ± 0.07
Corpus length (cm)	6.29 ± 0.14**	6.00 ± 0.14
Corpus width (cm)	0.50 ± 0.01**	0.44 ± 0.01
Cauda length (cm)	2.57 ± 0.06**	2.01 ± 0.05
Cauda circumference (cm)	4.36 ± 0.07**	4.07 ± 0.07
Weight of caput (gm)	3.47 ± 0.09**	3.17 ± 0.08
Weight of corpus (gm)	2.56 ± 0.06**	2.29 ± 0.07
Weight of cauda (gm)	3.70 ± 0.09**	3.39 ± 0.08
Weight of testis (gm)	59.51 ± 2.18*	55.04 ± 1.01
Weight of testis with epididymis (gm)	68.66 ± 3.13*	63.91 ± 1.11

*$p < 0.05$, **$p < 0.01$ between left and right testes.

refrigeration storage intervals of epididymes are presented in Table 2.

Among different storage groups of the epididymis, significantly ($p < 0.05$) higher semen volume was observed after 24 h (G_3, 0.25 ± 0.01 ml) and lowest after 48 hours (G_4, 0.21 ± 0.01 ml) of storage, whereas other groups were intermediary. Contrary to this, little higher initial semen volume was reported by Kabiraj *et al.* (2011). The sperm concentration per ml and total sperm output decreased significantly ($p < 0.05$), increasing cold storage time from 6 h till 96 h. The initial sperm concentration of the current study compared well with Kabiraj *et al.* (2011) and Chaudhari *et al.* (2018[b]). In the present study, a grouping of gonads was done at random from the slaughtered bucks without ascertaining their actual age, scrotal biometry, breeding potential and testicular health etc, hence the variation in semen volume and sperm concentration observed in different groups and in different studies might be due to difference in age, sexual maturity, nutritional status, general health, endocrine balance, season and soundness of sex organs (Karagiannidis *et al.*, 2000; Blash *et al.*, 2000). Further, the decrease in sperm concentration and sperm output with increase in storage time at refrigeration temperature observed concurred with Blash *et al.* (2000), Mir *et al.* (2012) and Turri *et al.* (2013) in buck epididymal studies due to process of progressive decomposition of tissues of preserved cauda epididymis.

Initial motility, viability, and HOS reactivity of epididymal sperm did not differ significantly ($p < 0.05$) during the first 6 and 12 hours of storage (G_1 and G_2), but thereafter the values were reduced significantly ($p < 0.05$) and gradually with increasing cold storage interval, *i.e.,* at 24, 48, 72 and 96 hours (Table 2). Martins *et al.* (2009) and Kumar *et al.* (2018) also observed a gradual reduction in sperm motility of bull epididymis stored at refrigerator temperature till 96 hours. Hoseinzadeh-Sani *et al.* (2013), however, recorded higher initial motility of buck epididymal sperm. The non-significant change in viability and HOS reactivity of epididymal sperm up to 12 hours of refrigeration storage (G_1 and G_2), and thereafter, significant reduction noted at 24, 48, 72, and 96 hours (G_3, G_4, G_5, and G_6) collaborated well with Kamal *et al.* (2005) and Kabiraj *et al.* (2011). Loss of energy during the postmortem period might compromise membrane integrity, thus increase permeability which in turns results in reduced viability and motility (Fernandez-Santos *et al.*, 2011). Elongation of postmortem time to epididymal sperm retrieval alters the chemical composition and reduces pH of the epididymal lumen, which in turn deteriorates sperm quality (Kaabi *et al.*, 2003). However, cold storage can protect epididymal spermatozoa to some extent by delaying postmortem epididymal changes compared to room temperature storage (Kaabi *et al.*, 2003; Hori *et al.*, 2009; Turri *et al.,* 2013).

The sperm head and tail abnormalities during cold storage of first 6 to 24 h did not differ significantly, but

Table 2: Epididymal seminal attributes (mean ± SE) from slaughtered buck after different cold storage (4°C) intervals

Group/ Storage time	*Volume (ml)*	*Sperm conc/ml (million)*	*Total sperm output (million)*	*Sperm motility (%)*	*Live sperm (%)*	*HOS (%)*	*Sperm abnormality (%)*		
							Head	*Tail*	*Droplet*
G_1 (6 h)	0.23 ± 0.01^{ab}	3656 ± 41.6^{a}	872.5 ± 75.61^{a}	76.25 ± 0.56^{a}	82.38 ± 0.49^{a}	34.88 ± 0.74^{a}	0.62 ± 0.82^{a}	10.25 ± 0.49^{a}	46.13 ± 0.89^{a}
G_2 (12 h)	0.24 ± 0.01^{ab}	3313 ± 29.5^{b}	857.3 ± 66.16^{a}	74.63 ± 0.32^{a}	80.25 ± 0.40^{a}	33.00 ± 0.73^{a}	1.00 ± 0.18^{a}	10.75 ± 0.62^{a}	43.75 ± 1.38^{a}
G_3 (24 h)	0.25 ± 0.01^{a}	3169 ± 23.02^{c}	828.1 ± 63.07^{a}	66.63 ± 0.60^{b}	73.63 ± 0.90^{b}	25.75 ± 0.80^{b}	1.00 ± 0.32^{a}	15.37 ± 0.44^{b}	37.88 ± 0.77^{b}
G_4 (48 h)	0.21 ± 0.01^{b}	3169 ± 23.02^{c}	672.5 ± 37.02^{b}	59.88 ± 0.44^{c}	69.00 ± 0.76^{c}	17.63 ± 0.80^{c}	2.38 ± 0.18^{b}	21.13 ± 0.64^{c}	29.63 ± 0.86^{c}
G_5 (72 h)	0.22 ± 0.05^{ab}	2906 ± 31.96^{d}	656.3 ± 53.46^{b}	49.13 ± 1.02^{d}	57.75 ± 0.86^{d}	11.88 ± 0.35^{d}	3.12 ± 0.22^{c}	21.13 ± 0.61^{c}	21.63 ± 0.53^{d}
G_6 (96 h)	0.23 ± 0.05^{ab}	2550 ± 26.73^{e}	603.8 ± 43.21^{b}	36.63 ± 0.63^{e}	44.75 ± 0.70^{e}	8.12 ± 0.64^{e}	3.50 ± 0.18^{d}	25.5 ± 0.53^{d}	19.13 ± 0.44^{e}

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

Table 3: Correlation coefficient (r = value) of different cytomorphological and seminal attributes in fresh epididymal semen of bucks

Parameters	*Testicular Vol.*	*Semen Vol.*	*Sperm conc./ml*	*Sperm Output*	*Sperm Motility*	*Live Sperm*	*HOS positive*	*Abn. Head*	*Abn. Tail*
Semen Vol.	0.76**								
Sp. conc./ml	0.68**	0.09							
Total sperm output	0.95**	0.85**	0.58*						
Sperm Motility	0.65**	0.08	0.97**	0.56*					
Live Sperm	0.63**	0.07	0.97**	0.55*	0.99**				
HOS positive	0.66**	0.10	0.94**	0.57*	0.97**	0.95**			
Abn. Head	-0.64**	-0.17	-0.86**	-0.59*	-0.88**	-0.87**	-0.90**		
Abn. Tail	-0.69**	-0.15	-0.92**	-0.59*	-0.94**	-0.92**	-0.97**	0.87**	
Abn. Droplets	0.60**	0.03	0.90**	0.47*	0.93**	0.90**	0.94**	-0.79**	-0.89**

*Significant at 5%; **Significant at 1%.

afterward increased significantly as cold storage time increased, *i.e.,* at 48, 72 and 96 h of storage, whereas sperm with droplets during cold storage of first 6 and 12 h did not differ significantly, but afterward reduced significantly as cold storage time increased. There was a progressive and significant rise in sperm head and tail abnormalities whereas decline in droplets with advancing storage time due to postmortem changes in the organ tissues and maturation of sperm with extrusion of cytoplasmic droplets in the preserved epididymis, respectively. These changes in cytomorphology of sperm observed could also be due a battery of etiopathogenesis suggested for sperm abnormalities by earlier workers (Pant *et al.*, 2002; Cooper, 2011). The present findings suggest that if semen cannot be collected immediately after death/slaughter of bucks, their epididymis can be stored at 4°C for 12 hours without compromising semen quality for use from valuable sires.

Correlation Coefficient of Testicular Volume with Epididymal Seminal Attributes

Testicular volume was significantly ($p < 0.01$) and positively correlated with semen volume (r = 0.76), sperm concentration per ml (r = 0.68), total sperm output (r = 0.95), initial motility (r = 0.65), live sperm (r = 0.63), HOS reactive sperm (r = 0.66) and sperm with abnormal droplets (r = 0.60). However it was negatively ($p < 0.01$) correlated with percent abnormal sperm head (r = -0.64) and tail (r = -0.69) (Table 3). Similar trends were reported earlier in Murrah bulls (Kumar and Srivastava, 2017). The volume of epididymal semen was significantly and positively correlated only with total sperm output (r = 0.85). Similarly, Kabiraj *et al.* (2011) recorded significant positive correlation between semen volume, testicular weight and testicular length. Sperm concentration per ml was significantly ($p < 0.01$) correlated with the total sperm output (r = 0.58), initial motility and live sperm (r = 0.97 each), HOS reactive sperm (r = 0.94) and sperm with droplets (r = 0.90), and it was negatively correlated with abnormal sperm head (r = -0.86) and tail (r = -0.92). The total sperm output was significantly ($p<0.05$) correlated with initial motility, live sperm, HOS reactive sperm, sperm with droplets (r = 0.47 to 0.57), and negatively correlated with abnormal sperm head and tail (r = -0.59). Initial motility was significantly ($p < 0.01$) correlated with live sperm (r = 0.99), HOS reactive sperm (r = 0.97) and sperm with droplets (r = 0.93), and negatively with abnormal sperm head (r = -0.88) and tail (r = -0.94). The live sperm was significantly ($p < 0.01$) correlated with HOS reactive sperm (r = 0.95) and abnormal droplets (r = 0.90), and negatively with abnormal sperm head (r = -0.87) and tail (r = -0.92). The HOS reactive sperm was significantly ($p < 0.01$) correlated with sperm with droplets (r = 0.94), and negatively with abnormal sperm head (r = -0.90) and tail (r = -0.97). The abnormal sperm head and tail were positively ($p < 0.01$) correlated (r = 0.87), and had negative correlations with abnormal droplets (r = -0.79; -0.89) (Table 3). Similar

correlations were also reported earlier in Surti bucks by Chaudhari *et al*. (2018[a]). The correlations observed signified the quality of various seminal attributes and their association in the epididymal reserve.

Conclusion

From the study, it can be concluded that the bucks have significantly larger left testicles and epididymes than the right ones, and the sperm quality harvested from epididymis up to 12 h of refrigeration storage is as good as ejaculated semen. Hence fresh epididymis of slaughtered high merit bucks can be conserved for future use of sperm by a suitable technique. Further, the desirable traits that have high correlations should be considered as assisting tools while selecting bucks for breeding purposes and reducing wastage of valuable resources.

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