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TESTICULAR BIOMETRY AND CERTAIN ASPECTS OF EPIDIDYMAL SEMEN OF ABATTOIR DERIVED MURRAH BULL TESTES

Kumar Ashotosh[1], Sushant Srivastava[1], Rajesh Kumar[1,*] and Pramod Kumar[2]

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ABSTRACT

The aim of this study to assess the biometric parameters of abattoir derived testicles of Murrah buffalo bull. Thirty-six pair of Murrah buffalo bull testicles were collected immediately after slaughter from different abattoir, packed in ice chest (4°C) and transferred to DFS Laboratory, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Ayodhya, UP, India. The tests were processed within six hours of collection. Various biometric parameters testes (testicular length, circumference, diameter, volume, and weight) epididymis (weight, length and width of caput and corpus as well as length and circumference of cauda) was measured. The epididymal semen was harvested and measured; Volume (0.43±0.02 to 0.52±0.05 ml), concentration (1708±105.2 to 1958±126.8 million/ml) and total sperm output (804.2±30.73 to 931.7±103.2 million) was estimated. All biometric parameters were significantly higher in left testicles than those of right contemporaries. Present findings suggest that aforementioned biometric parameters can be used to judge the normality of testis, furthermore, epididymal semen can be harvested from meritorious Murrah buffalo bulls with sudden or unexpected death.

Keywords: *Bubalus bubalis*, buffaloes, biometry, Murrah buffalo bull, seminal attributes, testis

INTRODUCTION

Selection of elite bull at an early age is the most important decision in sound breeding programme (Varghese *et al.*, 2019). Testis size is a good indicator of fertility potential of bulls. Testicular and epididymal biometry is a valuable tool to assess normality as well as sperm production capacity of male animals. Moreover, Biometric study of testes is also helpful in diagnosis, control, and treatment of sub-fertile or infertile males (Baldaniya *et al.*, 2020). Scrotal circumference can be used as predictor of testosterone concentration and certain attributes of seminal vesicles (weight

[1]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Narendra Deva University of Agriculture and Technology, Uttar Pradesh, India
[2]Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Uttar Pradesh, India, *E-mail: drrajesh25@gmail.com

and secretions) (Mahmood *et al*., 2018). Ultrasound imaging (El-Khawaga *et al*., 2012; Ranga *et al*., 2020), also can be used to detect anatomic and functional soundness of the testes. Furthermore, Ali *et al.* (2011) uses ultrasound scanning of testes and epididymis to detect normal and infertile breeding bulls and suggest that diagnostic ultrasound should be used as assisting tool in breeding soundness examination of any bulls selected for breeding purpose. Moreover, Yadav *et al.* (2019) reported that scrotal infra-red thermography and testicular biometry can be used as an indicator of semen quality in Murrah bull. Kang *et al.* (2018) observed that flushing and mincing methods are equally effective and can be used in epididymal sperm harvesting in Hanwoo bulls.

Fertility of cauda epididymal spermatozoa is comparable to spermatozoa from the normal ejaculate (Hafez and Hafez, 2000). Cauda epididymal semen can be successfully used for artificial insemination and *in vitro* embryo production (IVP) in several species (Hori *et al*., 2004; Hori *et al*., 2005). Motile sperm have been isolated from cool stored epididymis of cattle (Nichi *et al*., 2007) and African buffalo (*Syncerus caffer*) (Bartels *et al*., 1999). Epididymal sperm are obtained immediately after death; the gamete remains alive for 24 to 48 h and is viable for fertilization (Dong *et al*., 2008). There is paucity of literature on biometry of testes and caput corpus and cauda epididymis of Murrah buffalo bull. Hence, the present study was aimed to evaluate testicular and epididymal biometry and certain semen characteristics at different cold storage (5°C) interval in slaughtered Murrah buffalo bull testis.

MATERIALS AND METHODS

This study was conducted at Deep Frozen Semen Laboratory (DFS), Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Uttar Pradesh, India.

Total 36 pair of slaughtered Murrah buffalo bull testicles were procured from Allana, slaughterhouse, Arrora, and Mass Agro Food Pvt. Ltd. Unnao Uttar Pradesh, India and placed into sterilized ice chest (4°C) keeping each pair of testis separate for each bull testis and brought to laboratory. Testis were cleaned under tap water and sterilized with normal saline. Various biometric parameters were estimated. Testicular length (cm), measured along the longitudinal axis of the testis from one pole of the testes to other, testicular diameter (cm) measured around the widest point at an area that is equidistant to the testicular poles, epididymal length (cm), measured using a non-stretchable tape placed along the longitudinal axis of the epididymis running from one pole to other. Testicular volume was measured by water displacement technique. Weight (gm) of right and left testes and epididymis was measured by using sensitive electronic weighing scale.

A total of 36 pair of slaughtered buffalo bull testicle randomly divided in six groups and as G1, G2, G3, G4, G5 and G6, 6 pair in each group and stored at refrigerated temperature (4°C) for 6, 12, 24, 36, 48 and 72 h respectively till semen collection. The surrounding connective tissue and blood vessels were removed from the tail of each cauda epididymis to expose the tubules of the cauda epididymis. A small segment of the cauda epididymis was removed with ophthalmic

scissors and placed in a small Petri dish (35 mm). Epididymal semen was collected at 6, 12, 24, 36, 48, and 72 h after slaughter from group G1, G2, G3, G4, G5 and G6 respectively. The spermatozoa were obtained by slicing and squeezing the epididymis, in 15 ml plastic tube (Martins *et al*., 2007).

Semen samples were immediately estimated for volume, sperm concentration and total sperm output. Semen volume was measured by direct reading of graduated collecting tube, sperm concentration by hemocytometer method and total sperm output (in million) obtained by multiplying volume (ml) and semen concentration (million/ ml).

Statistical analysis

Data were presented as mean and standard error of the mean (SEM). Analysis of variance (ANOVA) was used to assess differences among the bulls and treatments when the F ratio is significant (P<0.05). Tukey's HSD test was used to compare treatment means by Graph Pad Instat Version 5 Soft.

RESULTS AND DISCUSSIONS

Testis length

The pooled mean (±SE) of testicular length with epididymis was 11.33±0.21 and 10.34±0.21cm; and without epididymis was 9.78±0.22 and 8.72±0.23 cm in left and right testicles respectively (Table 1) and the corresponding values were significantly (P<0.05) higher in left side as compared to right contemporaries. Unlike present observations higher values were reported by Siqueira *et al.* (2007); Al-Sahaf and Ibrahim (2012), furthermore, lower values were recorded by Saurabh *et al.* (2018) in Murrah bull. Contrary to present observations, no significant difference was observed between left and right testicular length by Saurabh *et al.* (2018) in Murrah bull. The variation might be due to differences in age (Younis *et al.*, 2003), weight, health status and nutrition etc. In present study the age of animals could not be ascertained as testes were collected from slaughterhouse.

Circumference of testis

The circumference of left testicle (mean ±SE) was significantly higher than right one (12.62±0.20 vs 11.59±0.20 cm; P<0.05; Table 1). The significant difference between left and right testicular circumference was also reported by Saurabh *et al.* (2018) but they recorded little higher values (left : right vs 14.06±0.21 vs 13.54±0.21 cm; P<0.05). The difference in present study might be due to difference in age, weight, health status etc.

Testicular volume (TV)

The mean (±SE) of testicular volume of left testicle was significantly higher as compared to right one (93.77±4.62 vs 72.74±3.97 cm^3; P<0.05; Table 1). Likewise, Saurabh *et al.* (2018) observed significantly higher TV in left testicle (83.59±3.67 vs 73.49±3.30 cm^3), however, Siqueira *et al.* (2007) reported higher values, which might be due to difference in age, weight, and breed.

Epididymal length and width

Epididymal biometry (caput, corpus, and cauda) were depicted in Table1. A significant difference in caput length Mean ± SE) of left and right epididymis was noted in present study with left being higher (4.57±0.11 vs 4.30±0.11 cm; P<0.05). Likewise, width of left caput epididymis was significantly more (2.76±0.10 vs 2.56±0.07 cm; P<0.05). A significant difference in corpus

length (mean ± SE) was observed in present study with left being higher (6.09±0.21 vs 5.78±0.20 cm; P<0.05). Similarly, width of left corpus epididymis was significantly more (0.56±0.02 vs 0.54±0.02 cm; P<0.05) as compared to right contemporaries. A significant difference in cauda length (pooled Mean ± SE) of left and right epididymis was recorded in this study with left being higher (2.29±0.07 vs 2.11±0.08 cm; P<0.05). Furthermore, there was significant difference in testicular cauda circumference (Mean ± SE) between left and right testis with left being higher (3.78±0.26 vs 3.60±0.27 cm; P<0.05).

Weight of epididymis and testes

Weight of caput, corpus and cauda and testes (Mean ± S.E.) of slaughtered Murrah buffalo bull testes are depicted in table1. Left parts of caput, corpus, cauda epididymis and testicles were significantly heavier (P<0.05) in comparison to right one of buffalo testes. Similar trends were also reported by Saurabh *et al.* (2018).

Volume

The volume of semen (Mean ± SE) was doing not differ significantly (P<0.05) in different groups varies between 0.43±0.02 to 0.52±0.05 ml. (Table 2). Present findings are in agreement with Yulnawati *et al.* (2009) who recorded comparable semen volume in buffalo. Variation in semen volume in different reports might be due to variation in age (Pant *et al*., 2003), breed, health status of animal under study. Similar to present findings, no significant difference was observed in epididymal semen volume up to 15 to 17 h of storage at 5 to 8°C in Philippine native water buffalo (Valete *et al*., 2015). Furthermore, Ouennes *et al.* (2019) reported that epididymal semen volume did not differ significantly during cold storage at 4°C up 72 h in Algeria breeds of buck.

Concentration/ml

The sperm concentration (Mean ± SE) was did not differ significantly (P<0.05) in different groups varies between 1708±105.2 to 1958±126.8 million per ml (Table 2). Similarly, Yulnawati *et al.* (2009) who recorded comparable sperm concentration in buffalo. Variation in sperm concentration was ascribed to testes size, age, season, environmental mental factors, exogenous agents, anabolic steroids, execise, individuality, health status, sexual excitement, frequency of collection etc. (Tomar *et al*., 1997; Bendtson *et al*., 2014). Likewise, no significant difference was recorded in epididymal semen concentration up to 15 to 17 h of storage at 5 to 8°C in Philippine native water buffalo (Valete *et al*., 2015). Furthermore, Chima *et al*. (2017) did not observed any significant difference in epididymal sperm concentration up 48 h of cold storage at 4°C in Nigerian local dogs and Ouennes *et al*. (2019) reported that epididymal semen concentration did not differ significantly during cold storage at 4°C up 72 h in Algeria breeds of buck.

Total sperm output

The total sperm output (Mean ± SE) was did not differ significantly (P<0.05) in different groups varies between 804.2±30.73 to 931.7±103.2 million (Table 2). Contrary to present findings, lower and higher values were mentioned in previous reports (Yulnawati *et al*., 2009; Al-Sahaf and Ibrahim, 2012). Differences might be attributed to breed, nutritional status, age, sexual maturity, endocrine balance, soundness f sex organs and season of collection (Karagiannidis *et al*., 2000).

In conclusion, testicular and epididymal parameters was significantly higher in left side

Table 1. Testicular and epididymal biometry (Mean ± S.E.) of abattoir derived Murrah bull testes.

Attributes	Left testicle (n=36)	Right testicle (n=36)
Testicular length with epididymis (cm)	11.33±0.21*	10.34±0.21
Testicular length without epididymis (cm)	9.78±0.22*	8.72±0.23
Testicular circumference (cm)	12.62±0.20*	11.59±0.20
Testicular diameter (cm)	4.01±0.09*	3.68±0.06
Testicular volume (cm^3)	93.77±4.62*	72.74±3.97
Caput Length (cm)	4.57±0.11*	4.30±0.11
Caput Width (cm)	2.76±0.10*	2.56±0.07
Corpus Length (cm)	6.09±0.21*	5.78±0.20
Corpus Width (cm)	0.56±0.02*	0.54±0.02
Cauda Length (cm)	2.29±0.07*	2.11±0.08
Cauda Circumference (cm)	3.78±0.26*	3.60±0.27
Weight of Caput (gm)	3.53±0.15*	3.14±0.27
Weight of Corpus (gm)	2.22±0.07*	1.68±0.07
Weight of Cauda (gm)	4.12±0.15*	3.40±0.18
Weight of Testicles with epididymis (gm)	73.36±2.27*	67.56±2.23

*Significant at 5% level (P<0.05).

Table 2. Epididymal semen volume, concentration and total sperm output (Mean ± S.E.) of abattoir derived Murrah bull testes.

Group	CST (h)	Volume (ml)	Sperm Concentration (million/ml)	Total Sperm Output (million)
G1	6	0.43±0.02	1958±126.8	838.8±34.68
G2	12	0.47±0.02	1742±106.8	804.2±30.73
G3	24	0.48±0.03	1708±105.2	816.7±46.38
G4	36	0.50±0.04	1883±163.6	931.7±103.2
G5	48	0.52±0.05	1775±94.65	911.7±86.98
G6	72	0.43±0.02	1867±44.10	811.7±51.15

CST: cold storage time; Values did not differ significantly within a column.

of testicles compared to right contemporaries and testes can be stored at refrigerator temperature (4°C) to harvest semen with satisfactory volume, concentration, and total sperm output from Murrah bull, if there is sudden or unexpected death of animal. However, the effect of cold storage on other seminal attributes remains to be explored.

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