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MORPHOMETRIC STUDY OF SLAUGHTERED MURRAH BUFFALO BULL TESTIS AND ITS CORRELATION WITH SEMINAL ATTRIBUTES OF EPIDIDYMAL SEMEN

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ABSTRACT

Present study was conducted to study the morphometric characteristics of slaughtered Murrah buffalo bull testis and its correlation with seminal attributes of epididymal semen. Total 56 pair of slaughtered Murrah buffalo bull testicles were procured from Allana, slaughterhouse, Arrora, and Mass agro food Pvt. Ltd. Unnao Uttar Pradesh, India; maintained at Deep Frozen Semen Laboratory, College of Veterinary science, and animal Husbadry, Ayodhya, Uttar Pradesh, India. Various morphometric parameters of testes and epididymis were analyzed and their correlations with attributes of epididymal semen were evaluated. No significance difference in seminal attributes between 6 h and 12 h after collection of testicles is clearly indicated that semen can be collect up to 12 h after death of animal without any significance loss in quality of semen in Murrah buffalo bull. Significant strong positive correlation among important characteristics (motility, livability and HOS positive sperm) suggests that these test may be used as one of the test for predicting fertility of epididymal semen Murrah buffalo bull.

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

INTRODUCTION

Testicular morphometry is a useful tool to gauging normality as well as sperm production potential of male animals. Cauda epididymal semen has been successfully used for AI and *in vitro* production of embryos (IVP) in several species (Hori *et al*., 2004; Hori *et al*., 2005), moreover fertility is comparable to spermatozoa from the normal ejaculate (Hafez and Hafez, 2000). 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). Motile sperm have been isolated from cool stored epididymis of cattle (Nichi *et al*., 2007) and African buffalo (*Syncerus caffer*) (Bartels *et al*., 1999). Spermatozoa collected from cauda epididymis is then stored in the form of liquid and/or frozen sperm, can be use for assisted reproductive technology such as artificial insemination (AI),

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in vitro embryo production (IVEP) or intra cytoplasmic sperm injection (ICSI). Hence, the present study was aimed to evaluate testicular morphometric characteristics and its correlation with seminal attributes of epididymal semen of slaughtered Murrah buffalo bull testis.

MATERIALS AND METHODS

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

Total 56 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 bags keeping each pair of testis separate for each bull testis and brought to laboratory in ice box. Testis were cleaned under tap water and sterilized with normal saline. Various morphometric parameters were estimated. Testicular length (cm), measured along the longitudinal axis of the testis from one pole of the testis 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 weight (gm), measured by using sensitive electronic weighing scale. Epididymal weight (gm), caput, corpus and cauda measured separately.

A total 56 pair of slaughtered buffalo bull testicle randomly divided in seven groups as G1, G2, G3, G4, G5, G6 and G7, 8 pair in each group and stored at refrigerated temperature (4^{o}C) till semen collection. The testes from each animal were removed immediately after slaughter and transported on ice (4^{o}C) to a laboratory for further processing. 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, 72 and 96 h after slaughter from Group G1, G2, G3, G4, G5, G6 and G7 respectively. The spermatozoa were obtained by slicing and squeezing the epididymis, in 15 ml plastic tube (Martins *et al*., 2007). The spermatozoa collected from each pair of testes were pooled and diluted with extender in a single step (40x10^{6} motile spermatozoa/ml).

Semen samples were immediately estimated for volume, colour, consistency, sperm concentration (photo electric colorimeter method, Paulenz *et al*., 1995), per cent progressive motility at the initial and post thaw stage, per cent live spermatozoa count by differential staining technique using Eosin-Nigrosin stain (Campbell *et al*., 1953), sperm abnormalities were studied by examination of wet smear and dry smear. Wet smears were prepared by suspending semen in formal saline solution and dry smears were stained with Eosin-Nigrosin stain percentage of abnormal sperm was recorded, and percent HOS reactive spermatozoa in fresh as well as frozen-thawed epididymal semen. HOST solution prepared by using fructose 1.35 gm, tri sodium citrate 0.73 gm, distilled water (up to) 100 ml, with final Osmolarity 150 mOs mole. The hypo-osmotic swelling test (HOST) was used to evaluate the functional integrity of the sperm membrane and was performed by incubating 0.1 ml of semen with

1 ml of a 150 mOs mole hypo osmotic solution at 37°C for 60 minutes. After incubation, one drop of the mixture was spread with a cover slip on a pre-warm slide. A total of 200 spermatozoa were counted in at least five different microscopic fields. The per cent of swollen sperm was calculated by the following formula:

Per cent of swollen sperm = (Number of sperm swollen/total sperm counted) x 100

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

Morphometry of testis

Testis length

The pooled mean (±SE) of testicular length with epididymis and without epididymis is depicted in Table 1. The pooled mean (±SE) of testicular length with epididymis (cm) of left testicle was significantly more (P<0.05) than that of right one. Present findings are in accordance with the observations of Yassen *et al. (*2010), but no significant difference was reported by Bansal *et al.* (2003). Unlike present findings Siqueira *et al. (*2007), AL-Sahaf and Ibrahim (2012); Luz *et al.* (2013) reported lower corresponding values. These variations might be due to variation in weight and age of animal (Kumar and Srivastava, 2017). Similarly, the pooled mean (±SE) of testicular length without epididymis (cm) of left testicle was significantly more (P<0.05) than that of right one.

Circumference of testis

The circumference of left testicle (mean±SE**)** was significantly higher than right of same buffalo bull (12.74±0.1 vs 11.75±0.01 cm; P<0.05). Present findings are in agreement with Saurabh *et al.* (2018). Likewise, the overall pooled mean (±SE) of diameter of left testicle was significantly higher than those of right of same buffalo bull (4.02±0.06 vs 3.70±0.06 cm; P<0.05) (Table 1).

Testicular volume

The overall pooled mean (±SE) of testicular volume of left testicle was significantly more than right one (96.64±4.74 vs 73.74±4.26 cm^3; P<0.05; Table 1). Similar findings are reported by Pant *et al*. (2003) but Siqueira *et al.* (2007) reported higher values, which might be due to difference in age, weight, and breed. Testicular volume was significantly (P<0.05) positively correlated with concentration/ml, total sperm count /ejaculate, initial motility, live count, and HOS reactive sperm, however it was significant (P<0.05) negatively correlated with sperm abnormality. However, Siqueira *et al. (*2007) reported no correlation between testicular biometry and sperm production.

Epididymal length and width

Epididymal morphometry are shown in Table 2. A significant difference in caput length (pooled mean ± SE) of left and right epididymis was observed in present study with left being higher (4.73±0.10 vs 4.41±0.09 cm; P<0.05). Similarly, width of left caput epididymis was significantly more (2.81±0.07 vs 2.64±0.06 cm; P<0.05). A significant difference in corpus length

(pooled mean ± SE) of left and right epididymis was observed in present study with left being higher (6.08±0.15 vs 5.73±0.15 cm; P<0.05). Similarly, width of left corpus epididymis was significantly more (0.57±0.01 vs 0.54±0.01 cm; P<0.05). A significant difference in cauda length (pooled mean ± SE) of left and right epididymis was observed in present study with left being higher (2.35±0.0.06 vs 2.12±0.06 cm; P<0.05). Furthermore, there was significant difference in testicular cauda circumference (pooled mean ± SE) between left and right testis with left being higher (3.88±0.21 vs 3.67±0.21 cm; P<0.05). Present findings are in agreement with the report of Osinowo (2006); Bitto and Okpale (2006); Ahemen and Bitto, 2007; Ugwu, 2009.

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 Table 3. 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 also reported by Saurabh *et al.* (2018).

Semen evaluation

Volume

The mean (±SE) volume in fresh epididymal semen in defferent groups was depicted in Table 4. Volume of semen was differed significantly (P<0.05) in different groups. Present findings are in agreement with Yulnawati *et al. (*2009). The volume was significantly (P<0.05) negatively correlated with concentration per ml (r = -0.55). Furthermore, negative correlation between semen volume and sperm concentration is reported in cattle (Field *et al*., 1979) and Buffalo bull (Srivastava, 2011) but Younis *et al.* (1996) reported no correlation with sperm concentration per ml.

Concentration/ml

The mean (±SE) of concentration/ml of epididymal semen among different groups was shown in Table 4. Concentration of semen was differed significantly (P<0.05) among groups which was in agreement with observation of Sule *et al.* (2007) in Buck and lower than the observation of Yulnawati *et al.* (2009). These variations might be due to seasonal influence, individuality, age and breed of the bull (Tomar *et al*., 1986). The concentration per ml was significantly positively correlated with total sperm count /ejaculation (r=0.71), initial motility (r=0.71), live count (r=0.71) and HOS (r=0.72) but significantly negatively correlated with seenvolume (r=-0.55) and sperm abnormality. (r=-0.69). Strong correlations reported by Linford *et al.* (1976); Hirao (1975) whereas, Deibel *et al.* (1976) found poor relationship between their traits.

Total sperm count per ejaculate

The mean (±SE) of total sperm count per ejaculate of buffalo bull semen were shown in Table 4. Variation in the total sperm count per ejaculate might be attributed to the fact that the concentration of sperm varies with bull feeding regimen, reproductive health, size of testis, season of year and different geographical localities (Salisbury *et al*., 1985). Total sperm count per ejaculate was significantly (P<0.01) positively correlated with initial motility (r=0.67), live count (r = 0.68) and HOS positive sperms (r=0.66) and significant (P<0.01) negatively correlated (r=-0.07) with sperm abnormality.

Initial motility

Initial motility of the mean (±SE) of percentage motility in fresh epididymal semen were differed significantly (P<0.05) among the groups (Table 4). Caudal epididymal spermatozoa has been preserved for variable length of time at 4 to 5°C in many species (Martinez-Pastor, 2005; Dong *et al*., 2008), motilily recovered after cool storage within epididymis or out of epididymis (Braun *et al*., 1994; Kikuchi *et al*., 1998; Stilley *et al*., 2000; Bruemmer *et al*., 2002; Yu and Leibo, 2002; Nichi *et al*., 2007) and able to fertilize ova (James *et al*., 2002). Present findings are in agreement with previous reports on dog epididymal semen which did not change up to Days 2 (Stilley *et al*., 2000), 5 (Ponglowhapan *et al*., 2006) or 8 (Bruemmer *et al*., 2002) after cool storage within epididymis. Initial motility was significantly (P<0.01) positively correlated with percent live count (r = 0.98), HOS reactive sperm (r = 0.98), whereas initial motility was significantly (P<0.01) negatively correlated (r = -0.97) with sperm abnormalities.

Live count

The overall live count (mean ± SE) in fresh epididymal semen were differed significantly (P<0.05) among groups (Table 4). The present findings were comparable to the observation of Ansari *et al.* (2011) but higher than the findings of Singh *et al.* (2007); Yulnawati *et al.* (2009). Reduction in the live count percent after prolong storage of epididymal at 4°C might be due to histo-pathological changes in the cauda epididymis which, directly or indirectly affect the livability of spermatozoa during storage. Diluted epididymal sperm could survive for 20 minutes at 37°C (Carr and Acott, 1984) and 60 h when stored in tris diluent (Igboeli and Foote, 1968) and even longer duration, if diluted with extenders (Julie *et al*., 2004; Uttam *et al*., 2009). Live count was significantly (P<0.01) correlated with percentage HOS reactive sperm (r = 0.98) but significantly (P<0.01) negatively correlated (r = -0.97) with sperm abnormalities.

Hypo osmotic swelling test

The mean (±SE) HOS reactive sperm in the epididymal semen was significantly differed (P<0.05) among groups (Table 4). Plasma membrane integrity of the epididymal sperm is relatively lower than the ejaculation sperm. This could be due to presence of seminal plasma in the ejaculate. Like present findings, Yu and Leibo (2002) reported 50% progressive motility and 80% membrane integrity in canine epididymal spermatozoa collected six hours after postmortem and preserved for up to 8 days at 4°C. Percent HOS positive sperm was significantly highly positively correlated (r=0.98; P<0.01) with motility and live per cent but highly negatively correlated (r=-0.95) with per cent abnormal sperms. Intact cytoplasmic membrane is a prerequisite for normal metabolisms (Rizal *et al*., 2003) and fertilization process such as capacitation, acrosomal reaction and adsorption of the sperms to the surface of the ovum (Jeyendran *et al*., 1994).

Sperm abnormality

The means (±SE) sperm abnormality in epididymal semen differed significantly (P<0.05) among groups (Table 4). The sperm abnormality was significantly (P<0.01) negatively correlated (r=-0.95) with HOS reactive sperms. Sperm abnormalities (percent) are one of the most significant indicators of subsequent fertility in a bull (Saacke, 1990). Bull fertility depends upon morphologically normal spermatozoa (Tharwat, 1998). Abnormal sperm morphology has been correlated with reduced fertility in cattle

Table 1. Testicular morphometry of slaughrtered Murrah buffalo bull testes.

Parameters		G1	G2	G3	G4	G5	G6	G7	Over all
Testicular length with Epi (cm)	Left testis	12.00±0.43	11.35±0.44	11.33±0.43	11.68±0.44	12.31±0.50	11.4±0.70	10.08±0.39	11.40±0.19^{A}
	Right testis	11.00±0.43	10.48±0.44	10.33±0.43	10.48±0.43	11.35±0.51	10.0.4±0.69	9.20±0.45	10.41±0.19^{B}
Testicular length without Epi. (cm)	Left testis	9.78±0.40	8.95±0.28	9.87±0.43	10.23±0.42	10.70±0.58	9.87±0.73	8.42±0.38	9.68±0.19^{A}
	Right testis	8.77±0.40	8.07±0.31	8.75±0.46	8.90±0.46	9.57±0.53	8.87±0.73	7.55±0.45	8.64±01.19^{B}
Testicular circumference (cm)	Left testis	13.43±0.46	12.93±0.44	12.81±0.36	12.81±0.36	13.24±0.57	12.58±0.63	11.38±0.36	12.74±0.18^{A}
	Right testis	12.45±0.46	12.18±0.46	11.70±0.40	11.83±0.39	12.11±0.52	11.61±0.63	10.38±0.36	11.75±0.0.18^{B}
Diameter of testis (cm)	Left testis	4.28±0.14	4.11±0.14	4.08±0.11	4.08±0.11	4.21±0.18	4.00±0.20	3.62±0.11	4.02±0.06^{A}
	Right testis	3.94±0.14	3.87±0.14	3.72±0.12	3.76±0.12	3.85±0.16	3.69±0.20	3.30±0.11	3.70±0.06^{B}
Testicular volume ($cm^{3)}$	Left testis	112.8±11.80	98.73±10.89	96.11±9.03	99.04±9.26	113.0±14.23	93.98±14.96	67.58±6.34	96.64±4.74^{A}
	Right testis	89.05±10.06	82.57±10.52	73.40±8.04	76.17±8.03	87.23±11.31	73.67±12.70	51.70±5.64	73.74±4.26^{B}

Mean bearing different superscript (A, B) in a column significantly ($P<0.05$) differed; repeatedly for each attribute.

Table 2. Epididymal morphometry of slaughrtered Murrah buffalo bull testes.

Parameters		G1	G2	G3	G4	G5	G6	G7	Over all
Caput length (cm)	Left testis	4.92±0.31	4.95±0.24	4.97±0.23	5.17±0.23	4.71±0.18	4.21±0.28	4.18±0.19	4.73±0.10^{A}
	Right testis	4.7±0.31	4.58±0.18	4.57±0.23	4.70±0.22	4.51±0.18	3.88±0.27	3.96±0.21	4.41±0.09^{B}
Caput width (cm)	Left testis	2.73±0.12	2.76±0.13	3.038±0.25	3.15±0.25	3.08±0.21	2.55±0.28	2.36±0.12	2.81±0.07^{A}
	Right testis	2.62±0.13	2.52±0.15	2.97±0.20	2.97±0.20	2.83±0.14	2.33±0.16	2.25±0.12	2.64±0.06^{AB}
Corpus length (cm)	Left testis	7.00±0.40	6.81±0.28	6.32±0.39	6.41±0.40	4.92±0.036	2.55±0.16	5.75±0.42	6.08±0.15^{A}
	Right testis	6.88±0.25	6.45±0.25	6.05±0.39	6.06±0.40	4.77±0.036	5.07±0.50	4.88±0.22	5.73±0.15^{AB}
Corpus width (cm)	Left testis	0.65±0.037	0.66±0.28	0.600±0.046	0.600±0.046	0.43±0.018	5.36±0.38	0.55±0.042	0.57±0.01^{A}
	Right testis	0.65±0.026	0.600±0.032	0.57±0036	0.57±0.036	0.43±0.018	0.48±0.038	0.46±0.026	0.54±0.01^{B}
Cauda length (cm)	Left testis	2.63±0.11	2.68±0.15	2.36±0.21	2.46±0.21	2.21±0.09	2.18±0.16	2.17±0.15	2.35±0.063^{A}
	Right testis	2.42±0.10	2.31±0.14	2.23±0.21	2.36±0.19	2.038±0.018	1.83±0.22	1.65±0.14	2.12±0.06^{B}
Cauda circumference (cm)	Left testis	5.46±0.17	4.73±0.21	4.80±0.19	4.88±0.19	3.93±0.056	2.00±0.36	1.38±0.16	3.88±0.21^{A}
	Right testis	5.35±0.21	4.42±0.16	4.60±0.20	4.70±0.21	2.03±0.08	1.85±0.38	1.06±0.11	3.67±0.21^{AB}

Mean bearing different superscript (A, B) in a column significantly ($P<0.05$) differed, repeatedly for each attribute.

Table 3. Weight of caput, corpus & cauda and testes (Mean ± S.E.) of slaughtered Murrah buffalo bull testes.

Parameters		G1	G2	G3	G4	G5	G6	G7	Over all
Weight of caput (gm)	Left testis	3.38±0.22	3.49 ± 0.27	4.25 ± 0.28	4.30 ± 0.28	3.02 ± 0.20	2.92 ±0.17	3.01± 0.21	$3.48±0.11^{A}$
	Right testis	3.00±0.18	3.20 ± 0.25	3.80 ± 0.17	3.83 ± 0.18	2.14 ± 0.14	3.22 ±1.15	2.41± 0.14	$3.09±0.18^{B}$
Weight of corpus (gm)	Left testis	2.67±0.21	1.90 ± 0.24	2.17 ± 0.14	2.27 ± 0.12	2.21 ± 0.13	1.98 ±0.23	1.87 ±0.11	$2.15±0.07^{A}$
	Right testis	2.30±0.22	1.65 ± 0.23	1.56 ± 0.13	1.66 ± 0.16	1.60 ± 0.09	1.30 ±0.09	1.52 ±0.12	$1.65±0.069^{B}$
Weight of cauda (gm)	Left testis	3.97±0.30	3.38 ± 0.33	4.38 ± 0.40	4.46 ± 0.42	4.23 ± 0.37	3.80 ±0.23	3.75 ±0.16	$4.00±0.12^{A}$
	Right testis	3.53±0.22	2.87 ± 0.24	3.98 ± 0.41	4.019±0.41	3.26 ± 0.33	2.88 ±0.29	2.95 ±0.33	$3.35±0.13^{B}$
Weight of testis (gm)	Left testis	65.65±7.09	68.52±3.45	78.60 ±7.04	78.58±7.04	69.87± 2.61	79.33±2.91	78.66±1.65	$74.17±1.95^{A}$
	Right testis	38.70±0.22	62.56±2.87	75.14 ±6.94	75.45± 6.8	63.91 ±5.44	70.1 ± 2.96	72.34±2.16	$68.98±0.060^{B}$

Mean bearing different superscript (A, B) in a column significantly (P<0.05) differed; repeatedly for each attribute.

Table 4. Cyto-morphological epididymal seminal attributes (Mean ± S.E.) in fresh collected slaughtered Murrah buffalo bull testes.

Group	Volume (ml)	Concentration (million/ml)	Total sperm Count/eja. (million)	Motility %	Live count %	HOS %	Abn. %
G1	0.42±0.16	$1775±88.14^{a}$	$723±17.11^{a}$	$81.63±0.77^{a}$	$85.13±1.25^{a}$	$31.38±0.62^{a}$	$18.38±0.37^{a}$
G2	0.43±0.18	$1635±98.34^{b}$	$703.4±18.28^{b}$	$75.75±0.72^{b}$	$80.00±0.70^{b}$	$28.50±0.50^{b}$	$20.88±0.47^{b}$
G3	0.42±0.16	$1531±56.65^{c}$	$645.0±10.35^{c}$	$71.13±0.78^{c}$	$75.75±0.80^{c}$	$26.00±0.56^{c}$	$24.25±0.36^{c}$
G4	0.42±0.16	$1375±40.09^{d}$	$580.0±16.54^{d}$	$63.25±0.88^{d}$	$68.00±0.92^{d}$	$21.50±0.56^{d}$	$27.00±0.56^{d}$
G5	0.43±0.18	$1331±26.62^{e}$	$580.0±17.63^{e}$	$56.50±0.56^{e}$	$62.13±0.85^{e}$	$19.75±0.86^{e}$	$29.25±0.25^{e}$
G6	0.43±0.018	$1275±31.34^{f}$	$580.0±25.07^{f}$	$49.38±0.60^{f}$	$56.13±0.83^{f}$	$15.63±0.67^{f}$	$32.50±0.77^{f}$
G7	0.43±0.018	$1275±21.13^{g}$	$581.9±29.09^{g}$	$44.88±0.63^{g}$	$53.25±0.52^{g}$	$15.00±0.70^{g}$	$32.38±0.82^{g}$

Mean bearing different superscript (a, b, c, d, e, f, g,) in a column significantly (P<0.05) differed, repeatedly for each attribute.

(Thundathil *et al*., 2000) and buffalo (Sengupta and Bhela, 1988) but bull fertility hardly affected if abnormal spermatozoa do not exceed 15 to 20% (Pant *et al*., 2002). In the present study the increase of abnormality (percent) is mainly in the tail which suggests that osmolality change in the media may be responsible for this increase as suggested by Joshi *et al.* (2006).

In conclusion, epididymal semen can be used as the alternative source for production of storage semen straw and further utilize for breed improvement in Murrah buffalo bull. No significance difference in seminal attributes between 6 h and 12 h after collection of testicle is clearly indicate that semen can be collect up to 12 h after death of animal without any significance loss in quality of semen in Murrah buffalo bull. Significantly strong positive correlation among important characteristics (motility, livability and HOS positive sperm), these tests may be used as one of the test for predicting fertility of epididymal semen Murrah buffalo bull.

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