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ORCHIDOMETRIC, SEMINAL AND SPERM DIMENSIONAL CHARACTERISTICS: A STUDY ON INTERRELATIONSHIPS IN NILI RAVI BUFFALO (*BUBALUS BUBALIS*) BULLS

Sajid HAMEED[1], Saima MASOOD[2], Hafsa ZANEB[2], Muhammad Sarwar KHAN[3], Muhammad YOUNIS[4], Muhammad AVAIS[3], Zahid KAMRAN[5], Umer FAROOQ[6*], Mirza Imran SHAHZAD[7], Moazzam JAVED[4], Muhammad QUMAR[8]

[1]Department of Anatomy and Histology, Faculty of Veterinary and Animal Sciences, Islamia University of Bahawalpur, Pakistan
[2]Department of Anatomy and Histology, University of Veterinary and Animal Science, Lahore, Pakistan
[3]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Science, Lahore, Pakistan
[4]Semen Production Unit, Qadirabad, Sahiwal, Livestock and Dairy Development Department, Punjab, Pakistan
[5]Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, Islamia University of Bahawalpur, Pakistan
[6]Department of Physiology, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan
[7]Department of Biochemistry, Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur
[8]Department of Animal Nutrition, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

Corresponding author email: umer.farooq@iub.edu.pk

Abstract

Animal conservation and improvement programs for any species or breed require the basic knowledge of its reproductive anatomy, histology and physiology. The present study has been devised with an objective to determine interrelationships between body weight, various orchidometric, fresh seminal, and post thaw seminal attributes for Nili-Ravi buffalo bulls. Materials, Methods & Results: Adult Nili-Ravi buffalo bulls (n = 07) being reared for breeding at Semen Production Unit (SPU), Qadirabad, Sahiwal, Pakistan were used in the present study. Orchidometric attributes included scrotal circumference, testicular length, testicular width and paired testicular volume (PTV), whereas fresh seminal parameters included the assessment of ejaculatory volume, color, pH, mass motility and individual sperm motility, and sperm. The sperm dimensional characteristics included measurements of head length (HL), head breadth (HB), head shape (HS), midpiece length (MPL), tail length (TL) and total sperm length (TSL). Results revealed a significantly positive correlation ($P \leq 0.01$) between body weight and all the studied orchidometric attributes. Similarly, correlation coefficients between all the studied sperm dimensional characteristics were also statistically significant ($P \leq 0.01$). Semen color showed significantly positive correlation with sperm HL, HB, TL and TSL, being negative with HS and MPL. Semen pH showed significantly positive correlation with sperm HS and MPL and significantly negative correlation with other sperm dimensional characteristics. The individual sperm motility had significantly positive correlation with HL, TL and TSL being negatively correlated with MPL. The sperm count showed significantly positive correlation with HL, HB, TL and TSL. Various studies on males of livestock have reported a positive correlation between body weight and various testicular attributes especially SC and PTV. Similarly, semen parameters, both for fresh and post thaw, have also been shown to have interrelationships among each other. The present study has added sperm dimensional characteristics in the study. The perplexing diversity of sperm structure has always been an interest of research world-wide which has resulted in reports of its structure associated with its adaptive function i.e. appropriate fertility. The novelty of this study is that the work has been conducted on Nili-Ravi buffalo bulls, for which the results of this study endorse that body weight, orchidometric, seminal and sperm dimensional characteristics show positive correlation with each other and may be utilized for selection of breeding buffalo bulls as they are readily measurable and reliable indicators of their reproductive status. We recommend the incorporation of sperm morphological attributes in the Breeding Evaluation protocols of the SPUs of Pakistan for selection of reproductively sound breeding bulls.

Key words*: buffalo bulls, correlation coefficient, orchidometry, sperm morphology.*

INTRODUCTION

In Pakistan, livestock sector has a share of 60.54% in agriculture and 11.22% in GDP. This sector showed an annual growth of 4% as per the Economic Survey of Pakistan (ESP, 2019). With a population of 40.0 million buffalo (*Bubalus bubalis*) heads in Pakistan, its gross milk production is highest as compared to milk from other livestock species. From the total global buffalo population, Asia harbors about 98% with small landless farmers owing 3-4 animals per family (Hameed et al., 2016). The Nili, Ravi, Nili-Ravi and Kundi are four main buffalo breeds prevalent in Pakistan amongst which the Nili-Ravi is considered as the mainstay and the backbone of Pakistani rural livestock economy.

In order to attain maximum reproductive performance and fertility of breeding bulls, various readily measurable reproductive parameters such as body weight and orchidometric attributes *viz*. scrotal circumference (SC), testicular length, testicular width, paired testicular volume (PTV) are being utilized and validated for various tropical livestock breeds (Mahmood et al., 2014b; Shrestha et al., 1983; Gage and Freckleton, 2003; Lunstra and Cundiff; 2003; Siddiqui et al., 2008). The morphometric examination of testes provides a reliable estimate of spermatogenesis process of the testes which are dependable predictors of normal process of spermatogenesis (Gage & Freckleton, 2003). The SC is associated with body weight of animal (Devkota et al., 2008) and is highly correlated with weight of testes, while consistency of testes is correlated with bull fertility (Waldner et al., 2010). A significantly positive correlation between SC and spermatozoa concentration has been elucidated for Sahiwal cattle bulls (Ahmad et al., 2005).

Along with superior genetic potential, the selected bulls should also be superior in quality semen production which is ascertained through various seminal attributes assessed through fresh and post-thaw semen quality tests (Farooq et al., 2013a; Farooq et al., 2013b; Farooq et al., 2015). Similarly, sperm morphology has attained avid attention as main predictor of fertility in various species and breeds of livestock (Hameed et al., 2016; AL-Sahaf & Ibrahim, 2012; Pant & Mukherjee, 1972; García-Vázquez et al., 2016).

Animal conservation and improvement programs for any species or breed require the basic knowledge of its reproductive anatomy, histology and physiology. The studies on assessment and interrelationships between various orchidometric, seminal and sperm dimensional characteristics have been reported for various livestock species. Similarly, our group has earlier reported seasonal variations in seminal and sperm dimensional characteristics of buffalo bulls (Hameed et al., 2017; Hameed et al., 2016). However, there is dearth of literature regarding correlation between these attributes for Nili-Ravi buffalo bulls. The present study has, therefore, been devised with an objective to determine interrelationships among and between body weight, various orchidometric (SC, testicular length, testicular width and PTV), fresh seminal (ejaculatory volume, color, pH, mass motility and individual sperm motility), and sperm dimensional characteristics (head length, head breadth, head shape, midpiece length, tail length and total sperm length) for Nili-Ravi buffalo bulls.

MATERIALS AND METHODS

The **s**tudy was carried over 12-month tenure at Semen Production Unit (SPU), Qadirabad, Sahiwal, Pakistan located at latitudes 30º and 31.15º North and longitudes 73º and 74º East, and at an altitude of 564 feet above the sea level.

Nili-Ravi buffalo bulls (n = 07) donating acceptable quality semen were selected for the study. The age of breeding bulls was 5-8 years and all the bulls were ensured to have clinically normal reproductive tract before the start of the study. Throughout the study tenure, the bulls were observed for their normal health, standard management and feeding practices.

Body weight and orchidometric attributes *viz*. SC, testicular length, testicular width and testicular volume were recorded fortnightly during the study period. All the measurements were performed by only one person and with same method of restraint in order to minimize stress. A total of 24 observations were noted for each parameter per bull. The SC was measured by using a measuring tape (Ahmad et al.,

2005). Testicular length (proximal-distal) and width (medio-lateral) of both testes were measured using digital vernier caliper (Shrestha et al., 1983), and the averages were calculated. The PTV was deduced as per the formula given below (Lunstra & Cundiff, 2003):

$$PTV = 0.0396\ (ATL)\ (SC)^2$$

Semen from each bull was collected on weekly basis using artificial vagina (42°C). At each collection, two ejaculates per bull were attained. Resultantly, 48 collections per bull were carried out with a total of 670 ejaculates. After collection, each semen sample was processed for physical seminal attributes such as ejaculatory volume, color, pH, mass motility (score 0-5), individual sperm motility (40X, phase contrast microscope; Olympus BH-2, Tokyo, Japan) and sperm count (photometrically at 560 nm wavelength using Bovine Photometer n° 1119, IMV, France). Details of initial semen evaluation and relevant data have been presented elsewhere (Hameed et al., 2017).

The dimension al characteristics of spermatozoa *viz*. head length (HL), head breadth (HB), head shape (HS), midpiece length (MPL), tail length (TL) and total sperm length (TSL) were measured after staining with Eosin-Nigrosin stain (Ciftci & Zulkadir, 2010) using a software PixelPro. From each sample, 12 morphologically normal sperm were selected for micrometry. The detailed methodology and relevant data have been presented elsewhere (Hameed et al., 2016).

Statistical analyses were conducted through Statistical Package for Social Science (SPSS for Windows V 17.0, SPSS Inc., Chicago, IL, USA).Kolmogorov Smirnov test was employed to test the normal distribution of data. Interrelationships within and between various orchidometric, seminal and sperm dimensional characteristics were deduced through Pearson's correlation coefficient.

RESULTS

Significantly positive correlation (P≤0.01) between body weight and all the studied orchidometric attributes was noticed in the present study (Table 1).

Regarding the correlation among various seminal attributes, it was revealed that the ejaculatory volume of buffalo bulls had a negative correlation with semen color (r = -0.036), pH (r = -0.089), mass motility (r = -0.036) and sperm count (r = -0.035), and positive with individual sperm motility (r = 0.106), though statistically non-significant (P≥0.05).

Table 1. Pearson's correlation coefficients between body weight and various orchidometric attributes of Nili-Ravi buffalo bulls (n = 07)

Parameters	r value
Body weight × Scrotal circumference	0.870**
Body weight × Average testicular length	0.831**
Body weight × Average testicular width	0.868**
Body weight × Paired testicular volume	0.877**
Scrotal circumference × Average testicular length	0.895**
Scrotal circumference × Average testicular width	0.930**
Scrotal circumference × Paired testicular volume	0.990
Average testicular length × Average testicular width	0.954**
Average testicular length × Paired testicular volume	0.947**
Average testicular width × Paired testicular volume	0.957**

**Significant correlation (P ≤0.01, 2 tailed)

Semen color had significantly negative correlation with pH (r = -0.636; P≤0.01), and positive with mass motility (r = 0.957; P≤0.01), individual sperm motility (r = 0.740; P≤0.01) and sperm count (r = 0.679; P≤0.01). Similarly, semen pH showed significantly negative correlation with mass motility (r = -0.717; P≤0.01), individual sperm motility (r = -0.587; P≤0.01) and sperm count (r = -0.895; P≤0.01). The correlation between individual sperm motility and sperm count was also significantly positive (r = 0.633; P≤0.01) (Table 2).

Table 2. Pearson's correlation coefficients among various seminal attributes of Nili-Ravi buffalo bulls (n = 07)

Parameters	r value
Ejaculatory volume × Semen color	-0.036^{NS}
Ejaculatory volume × Semen pH	-0.089^{NS}
Ejaculatory volume × Mass motility	-0.036^{NS}
Ejaculatory volume × Individual sperm motility	0.106^{NS}
Ejaculatory volume × Sperm count	-0.035^{NS}
Semen color × Semen pH	-0.636**
Semen color × Mass motility	0.957**
Semen color × Individual sperm motility	0.740**
Semen color × Sperm count	0.679**
Semen pH × Mass motility	-0.717**
Semen pH × Individual sperm motility	-0.587**
Semen pH × Sperm count	-0.895**
Mass motility × Individual sperm motility	0.799**
Mass motility × Sperm count	0.767**
Individual sperm motility × Sperm count	0.633**

^{NS}Non-significant, **Significant correlation (P ≤0.01, 2 tailed)

Results regarding correlation coefficients between all the studied sperm dimensional

characteristics were statistically significant (P≤0.01) in the present study as given in Table 3. The sperm HL had positive correlation with HB (r = 0.935; P≤0.01), TL (r = 0.885; P≤0.01) and TSL (r = 0.883; P≤0.01), and negative with HS (r=-0.866; P≤0.01) and MPL (r = -0.835; P≤0.01). Similarly, sperm HB was negatively correlated with HS (r = -0.987; P≤0.01) and MPL (r = -0.769; P≤0.01), and positively correlated to TL (r = 0.916; P≤0.01) and TSL (r = 0.916; P≤0.01).

Table 3. Pearson's correlation coefficients among various sperm dimensional characteristics of Nili-Ravi buffalo bulls (n = 07)

Parameters	r value
Head length × Head breadth	0.935**
Head length × Head shape	-0.866**
Head length × Midpiece length	-0.835**
Head length × Tail length	0.885**
Head length × Total sperm length	0.883**
Head breadth × Head shape	-0.987**
Head breadth × Midpiece length	-0.769**
Head breadth × Tail length	0.916**
Head breadth × Total sperm length	0.916**
Head shape × Midpiece length	0.711**
Head shape × Tail length	-0.890**
Head shape × Total sperm length	-0.891**
Midpiece length × Tail length	-0.756**
Midpiece length × Total sperm length	-0.743**
Tail length × Total sperm length	1.000**

**Significant correlation (P ≤ 0.01, 2 tailed)

All the correlations between body weight, orchidometric, seminal and sperm dimensional characteristics were statistically non-significant (P≥0.05) (Table 4).

The results regarding correlation coefficients between various seminal and sperm dimensional characteristics are given in Table 5. Semen color showed significantly positive correlation with sperm HL (r = 0.616; P≤0.01), HB (r = 0.595; P≤0.01), TL (r = 0.594; P≤0.01) and TSL (r = 0.595; P≤0.01), being negative with HS (r = -0.561; P≤0.01) and MPL (r = -0.482; P≤0.01). Semen pH showed significantly positive correlation with sperm HS (r = 0.505; P≤0.01) and MPL (r = 0.344; P≤0.01) and significantly negative correlation with other sperm dimensional characteristics. The mass motility revealed correlation with sperm dimensional characteristics similar to those for semen color. The individual sperm motility had significantly positive correlation with HL (r = 0.376; P≤0.05), TL (r = 0.381; P≤0.05) and TSL (r = 0.375; P≤0.05) being negatively correlated with MPL (r = -0.446; P≤0.01). The sperm count showed significantly positive correlation with HL (r = 0.480; P≤0.01), HB (r = 0.536; P≤0.01), TL (r = 0.621; P≤0.01) and TSL (r = 0.625; P≤0.01).

DISCUSSIONS

The present study is a novel one being reported from Pakistan on interrelationships among and between various reproductive attributes (body weight, orchidometric, seminal and sperm dimensional characteristics) of Nili-Ravi buffalo bulls. In case of lack of prior studies on buffalo bulls, the comparisons of our results have been made with other livestock species.

In the present study, significantly positive correlation was noticed between body weight and all the studied orchidometric attributes (SC, testicular length, testicular width and testicular volume). Earlier studies have reported positive correlation between body weight and SC for Murrah buffalo bulls (da Silva SANTOS et al., 2013; Singh et al., 2014) and Nili-Ravi buffalo bulls (Javed, 1998). The positive correlations of the present study are also in agreement with other studies conducted on river buffalo (Viana, 2008), swamp buffalo (McCool et al., 1985) and Murrah buffalo (Pant et al., 2003). Findings of an earlier work (da Silva SANTOS et al., 2013) regarding the correlation between body weight, testicular length and testicular width are in line with the findings of the present study revealing the significantly positive correlations. Similar correlations have also been observed for *Bos taurus* cattle (Jain et al., 2008). A study on Cholistani breeding bulls (a Zebu indigenous cattle breed of Pakistan) has documented significantly positive correlations between body weight, SC and PTV (Mahmood et al., 2014b). Correlation results documented in Tho Tho bulls (Perumal, 2014) and in Cholistani bulls (Mahmood et al., 2014a) are also in agreement with present results. The PTV is gaining avid attention since last decade as a reliable indicator of reproductive status in breeding bulls (Unanian et al., 2000).

Regarding the correlation among various seminal attributes, the ejaculatory volume had non-significant correlation with all other studied seminal attributes *i.e.* color, pH, mass

motility, individual sperm motility and sperm count. These results are in close agreement with the earlier findings on Nili-Ravi buffalo bulls (Younis, 1996; Javed, 1998). This shows that in buffalo bulls, semen volume does not predict the semen quality (Javed et al., 2000). Similar correlations have also been documented for Cholistani bulls (Mahmood et al., 2014b). In contrary, significantly positive correlation of volume with mass motility and sperm count have been reported for Horro bulls (Galmessa et al., 2005). In present study, semen pH showed negative correlation with mass motility, individual sperm motility and sperm count which is supported by an earlier study on Nili-Ravi buffalo bulls (Javed et al., 2000). Semen color had a positive correlation with mass motility, individual sperm motility and sperm count which is supported another prior work (Javed, 1998).

Table 4. Pearson's correlation coefficients between body weight, orchidometric, seminal and sperm dimensional characteristics of Nili-Ravi buffalo bulls (n = 07)

Parameters	Seminal Attributes						Sperm Dimensional Characteristics					
	Ejaculatory Volume	Color	pH	Mass Motility	Individual Sperm Motility	Sperm Count	Head Length	Head Breadth	Head Shape	Midpeice Length	Tail Length	Total Sperm Length
Body weight	-0.172	0.120	-0.120	0.077	-0.027	0.199	0.119	0.206	-0.234	0.167	0.232	0.244
Scrotal circumference	-0.015	0.151	-0.214	0.121	0.183	0.234	0.195	0.254	-0.267	-0.070	0.303	0.308
Average testicular length	-0.029	0.157	-0.169	0.128	0.198	0.210	0.079	0.110	-0.120	0.034	0.136	0.141
Average testicular width	-0.075	0.127	-0.167	0.103	0.138	0.186	0.102	0.117	-0.120	0.059	0.160	0.166
Paired testicular volume	-0.010	0.154	-0.208	0.126	0.192	0.234	0.163	0.218	-0.231	-0.043	0.260	0.264

Table 5. Pearson's correlation coefficients between seminal and sperm dimensional characteristics of Nili-Ravi buffalo bulls (n = 07)

Parameters	Head length	Head breadth	Head shape	Midpiece length	Tail length	Total Sperm length
Ejaculatory volume	-0.263^{NS}	-0.230^{NS}	0.204^{NS}	0.045^{NS}	-0.244^{NS}	-0.251^{NS}
Color	0.616**	0.595**	-0.561**	-0.482**	0.594**	0.595**
pH	-0.480**	-0.512**	0.505**	0.344*	-0.536**	-0.539**
Mass motility	0.592**	0.566**	-0.530**	-0.480**	0.589**	0.589**
Individual sperm motility	0.376*	0.301^{NS}	-0.254^{NS}	-0.446**	0.381*	0.375*
Sperm count	0.480**	0.536**	-0.534**	-0.313^{NS}	0.621**	0.625**

**Significant correlation ($P \leq 0.01$, 2 tailed); *Significant correlation ($P \leq 0.05$, 2 tailed); ^{NS}Non-significant

Similarly, a positive correlation between mass motility and individual sperm motility in the present study is in line with other findings for Cholistani breed of cattle (Mahmood et al., 2013). A positive correlation between individual sperm motility and sperm count in the present study is also in agreement with prior studies (Galmessa et al., 2005).

The perplexing diversity of sperm structure has always been an interest of research world-wide which has resulted in reports of its structure associated with its adaptive function *i.e.* appropriate fertility. Regarding results of correlation coefficients between all the studied sperm dimensional characteristics, the sperm HL had positive correlation with HB, TL and TSL, and negative with HS and MPL in the present study. A negative correlation between MPL and HL has been reported earlier (Humphries et al., 2008) which is supporting the finding of present study. It has been previously reported that the correlation exists between MPL and sperm head (Piasecka & Kawiak, 2003; Cardullo & Baltz, 1991). No relationship between sperm head size and mid-piece in Iberian red deer has been reported (Malo et al., 2006). In contrary to the findings of present study, positive correlation of MPL with HL, HB, TL and TSL in cattle bulls have been reported (Sarder, 2005). It has been elucidated that the sperm dimensional characteristics are variable in different species, and in various breeds of specie. Furthermore, these attributes tend to vary as per general

health status of the animal and seasons (Hameed et al., 2016; Pant and Mukherjee, 1972; Sarder, 2005).

In the present study, the correlations were deduced between readily measurable reproductive traits (body weight and orchidometric attributes), and seminal and sperm dimensional characteristics. All the correlations were found to be non-significant. Body weight and orchidometric attributes were negatively correlated with ejaculatory volume and semen pH, and positively correlated with semen color, mass motility, individual sperm motility and sperm count. Similar results have been presented for crossbred rams (Moghaddam et al., 2012). According to another study on Cholistani breeding bulls (Mahmood et al., 2014a), there was a non-significant correlation between SC, testicular length, testicular width and PTV with mass motility and individual sperm motility as observed in the present study for Nili-Ravi buffalo bulls. In present study, body weight and orchidometric parameters showed non-significantly negative correlation with ejaculatory volume which has been reported positive in earlier study on Cholistani bulls (Mahmood et al., 2014a). A plausible justification for these variations in results of correlation may be the difference in species, breed, and season of study. Regarding sperm dimensional characteristics, it has earlier been demonstrated that the morphological features of sperm lose their relationship with body mass after phylogenetic controls.

The seminal and sperm dimensional characteristics of the presented study were studied for correlation coefficients in the present study. Semen color showed significantly positive correlation with sperm HL, HB, TL and TSL, being negative with HS and MPL. Semen pH showed significantly positive correlation with sperm HS and MPL and significantly negative correlation with other sperm dimensional characteristics. The individual sperm motility had significantly positive correlation with HL, TL and TSL being negatively correlated with MPL. The sperm count showed significantly positive correlation with HL, HB, TL and TSL. Comparing these results with prior studies, a wide variability has been reported while studying importance of sperm morphology for its transport in female reproductive system and its fertilizing ability (García-Vázquez et al., 2016). The TSL and MPL have been demonstrated to be positively correlated to fertilizing ability in various primates (Humphries et al., 2008). Swimming velocity of sperm enhances sperm competitiveness and ultimate fertilizing ability. Extensive studies have reported a positive correlation between sperm length and its swimming velocity for many mammals as in line with results of present study (Humphries et al., 2008; Gomendio and Roldan, 2004).

CONCLUSIONS

In a nutshell, the present study demonstrates that body weight, orchidometric, seminal and sperm dimensional characteristics show positive correlation with each other for Nili-Ravi buffalo bulls. These attributes may be utilized for selection of breeding buffalo bulls as alternate to expensive methods of bull selection as they are readily measurable and reliable indicators of their reproductive status. The sperm dimensional characteristics are not a part of evaluation in Pakistani SPUs as yet. We recommend that these sperm morphological parameters may be incorporated in Breeding Soundness Evaluation protocols of breeding buffalo bulls for attaining an optimal fertility rate.

REFERENCES

Ahmad, M., Asmat, M., Rehman, N. (2005). Relationship of testicular size and libido to age and season in Sahiwal bulls. *Pakistan Veterinary Journal,* 25, 67.

AL-Sahaf, M.M., Ibrahim, N.S. (2012). Monthly changes in testes and epididymis measurements with some semen characteristics of tail epididymis for Iraqi buffalo. *The Iraqi Journal of Veterinary Medicine,* 36, 204-208.

Cardullo, R.A., Baltz, J.M. (1991). Metabolic regulation in mammalian sperm: mitochondrial volume determines sperm length and flagellar beat frequency. *Cell motility and the cytoskeleton,* 19, 180-188.

Ciftci, H., Zulkadir, U. (2010). NIitochondrial Helix Length. *Journal of Animal and Veterinary Advances,* 9, 1169-1172.

da Silva Santos, P.R., Andrighetto, Jorge, A.M. (2013). The correlation between age, body weight and testicular parameters in Murrah buffalo bulls raised in

Brazil. *Journal of Reproduction and Development,* 59, 14-17.

Devkota, B., Koseki, T., Matsui, M., Sasaki, M., Kaneko, E., Miyamoto, A., Montoya, C.A., Miyake, Y.A. (2008). Relationships among age, body weight, scrotal circumference, semen quality and peripheral testosterone and estradiol concentrations in pubertal and postpubertal Holstein bulls. *Journal of Veterinary Medical Science,* 70, 119-121.

E.S.P. (2019). Pakistan Economic Survey 2018-19. *Pakistan Economic Survey.* Ministry of Finance, Government of Pakistan, Islamabad, Pakistan.

Farooq, U., Ijaz, A., Ahmad, N., Rehman, H., Zaneb, H. (2013a). Investigations on semen quality and freezability of Cholistani breeding bulls-A preliminary study from Cholistan desert of Pakistan. *J Anim Plant Sci,* 23, 359-363.

Farooq, U., Ijaz, A., Ahmad, N., Rehman, H., Zaneb, H. (2013b). Seasonal Variations in Certain Physical and Biochemical Attributes of Semen from Cholistani Bulls. *Pakistan Veterinary Journal,* 33.

Farooq, U., Mahmood, S.A., Ahmad, I., Ahmad, N., Idris, M., Abbas, M.T. (2015). Evaluation of postthaw sperm parameters and fertility of Cholistani service bulls. *Turkish Journal of Veterinary and Animal Sciences,* 39, 472-479.

Gage, M.J., Freckleton, R.P. (2003). Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 270, 625-632.

Galmessa, U., Kebede, M., Gebreyohannes, G., Kebede, G., Dessaleng, J., Tola, A. (2005). Age and season related changes in semen quality of Horro bulls. *Ethiopian Journal of Animal Production,* 5, 43-54.

García-Vázquez, F.A., Gadea, J., Matás, C., Holt, W.V. (2016). Importance of sperm morphology during sperm transport and fertilization in mammals. *Asian journal of Andrology,* 18, 844.

Gomendio, M., Roldan, E.R. (2004). Implications of diversity in sperm size and function for sperm competition and fertility. *International Journal of Developmental Biology,* 52, 439-447.

Hameed, S., Masood, S., Zaneb, H., Khan, M., Younis, M., Avais M., Khan, M. (2017). Semen characteristics as influenced by seasonal and climatic variations in Nili-Ravi buffalo breeding bulls. *Journal of Animal & Plant Sciences,* 27.

Hameed, S., Masood, S., Zaneb, H., Khan, M.S., Younis, M., Avais, M., Khan, M.U.R. (2016). Dimensional characteristics of spermatozoa of Nili-Ravi buffalo bulls: seasonaland climatic influences. *Turkish Journal of Veterinary and Animal Sciences,* 40, 207-213.

Humphries, S., Evans, J.P., Simmons, L.W. (2008). Sperm competition: linking form to function. *BMC Evolutionary Biology,* 8, 319.

Jain, R., Mohanty, T., Pankaj, P. (2008). Study of relationship of age, testicular biometry and semen characteristics in bulls of Sahiwal and Friesian crosses. *Journal of Dairying, Foods and Home Sciences,* 27, 175-180.

Javed, M. (1998). Study on buffalo testes with special reference to scrotal circumference, hormonal profile, sperm morphology and associated lesions. *PhD, University of Agriculture, Faisalabad, Pakistan*.

Javed, M.T., Khan, A., Kausar, R.(2000). Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Veterinarski Arhiv,* 70, 83-94.

Lunstra, D., Cundiff, L. (2003). Growth and pubertal development in brahman-, boran-, tuli-, belgian blue-, hereford-and angus-sired f1 bulls. *Journal of Animal Science,* 81, 1414-1426.

Mahmood, S., Ijaz, A., Ahmad, N., Rehman, H., Zaneb, H., Farooq, U. (2013). Studies on libido and serum testosterone concentration of Cholistani AI bulls under stress free and stressful seasons. *Journal of Animal and Plant Science,* 23, 1491-1495.

Mahmood, S., Ijaz, A., Ahmad, N., Rehman, H., Zaneb, H., Farooq, U., Karaniwala, S.P.U. (2014a). A study on relationships among age, body weight, orchidometry and semen quality parameters in adult Cholistani breeding bulls. *Journal of Animal and Plant Science,* 24, 380-384.

Mahmood, S., Ijaz, A., Ahmad, N., Rehman, H., Zaneb, H., Farooq, U., Karaniwala, S.P.U., Bahawalpur, L. (2014b). A study on relationships among age, body weight, orchidometry and semen quality parameters in adult cholistani breeding bulls. *Journal of Animal and Plant Science,* 24, 380-384.

Malo, A.F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A.J., Roldan, E.R. (2006). Sperm design and sperm function. *Biology letters,* 2, 246-249.

McCool, C., Entwistle, K., Gilham, M. (1985). Testis growth rates in the Australian swamp buffalo. *Buffalo Journal,* 1, 29-36.

Moghaddam, G., Pourseif, M., Asadpour, R., Rafat, S.A., Jafari-Jozani, R. (2012). Relationship between levels of peripheral blood testosterone, sexual behavior, scrotal circumference and seminal parameters in crossbred rams. *Acta Scientiae Veterinariae,* 40, 1-8.

Pant, H., Sharma, R., Patel, S., Shukla, H., Mittal, A., Kasiraj, R., Misra, A., Prabhakar, J. (2003). Testicular development and its relationship to semen production in Murrah buffalo bulls. *Theriogenology,* 60, 27-34.

Pant, K., Mukherjee, D. (1972). The effect of seasons on the sperm dimensions of buffalo bulls. *Reproduction,* 29, 425-429.

Perumal, P. (2014). Scrotal circumference and its relationship with testicular growth, age, and body weight in Tho Tho (*Bos indicus*) bulls. *International Scholarly Research Notices,* 20: 113.

Piasecka, M., Kawiak, J. (2003). Sperm mitochondria of patients with normal sperm motility and with asthenozoospermia: morphological and functional study. *Folia Histochemica et Cytobiologica,* 41, 125.

Sarder, M. (2005). Study on dimensional characteristics of different breeds of bull spermatozoa. *Journal of Animal and Veterinary Advances*.

Shrestha, J., Fiser, P., Langford, G., Heaney, D. (1983). Influence of breed, birth date, age and body weight on testicular measurements of growing rams

maintained in a controlled environment. *Canadian Journal of Animal Science,* 63, 835-847.

Siddiqui, M., Bhattacharjee, J., Das, Z., Islam, M., Haque, M., Parrish J., Shamsuddin, M. (2008). Crossbred bull selection for bigger scrotum and shorter age at puberty with potentials for better quality semen. *Reproduction in Domestic Animals,* 43, 74-79.

Singh, A., Brar, P., Cheema, R. (2014). Relationships among frozen-thawed semen fertility, physical parameters, certain routine sperm characteristics and testosterone in breeding Murrah buffalo (*Bubalus bubalis*) bulls. *Veterinary World,* 7.

Unanian, M.M., Silva, A.E.D.F., McManus, C., Cardoso, E.P. (2000). Características biométricas testiculares para avaliação de touros zebuínos da raça Nelore. *Revista Brasileira de Zootecnia,* 29, 136-144.

Viana, R. (2008). Effect of mineral supplementation minerals level in seminal. in Proceedings of the Proceedings of the XXV Jubilee World Buiatrics Congress; Budapest, Hungary, p. 213.

Waldner, C.L., Kennedy, R.I., Palmer, C.W. (2010). A description of the findings from bull breeding soundness evaluations and their association with pregnancy outcomes in a study of western Canadian beef herds. *Theriogenology,* 74, 871-883.

Younis, M. (1996). Studies on semen quality, freezability and fertility of buffalo bulls during low and peak breeding seasons. *University of Agriculture Faisalabad Pakistan*.