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A STUDY ON RELATIONSHIPS AMONG AGE, BODY WEIGHT, ORCHIDOMETRY AND SEMEN QUALITY PARAMETERS IN ADULT CHOLISTANI BREEDING BULLS

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ABSTRACT

The present study was designed to 1) assess preliminary non-invasive, readily measurable reproductive attributes of Cholistani breeding bulls (n=06) such as age, body weight (BW) and orchidometric parameters viz. scrotal circumference (SC), scrotal skin fold thickness (SSFT), average testicular length (Avg L), average testicular width (Avg W), and paired testicular volume (PTV); 2) assess the baseline semen attributes such as volume (mL), mass motility (Score 1-5), individual motility (%), sperm concentration (million/mL), viability (%), morphology (%) and acrosome integrity (%); and 3) determine relationship among dependent and independent variables mentioned in objectives 1 and 2.. The overall means for age, BW, SC, SSFT, Avg L, Avg W, and PTV for these bulls were 92.83±28.15 months, 520.±42.31 kg, 36.02±2.25 cm, 1.10±0.15cm, 16.91±0.70 cm, 7.05±0.35 cm, and 874.37±137.07 cm^3, respectively. Similarly, the overall means for semen volume, mass motility, individual motility, sperm concentration, viability, normal morphology and acrosome integrity were 4.46±1.65mL, 1.95±0.25, 63.46±4.91%, 903.22±182.04 million/mL, 85.35±2.29%, 85.66±2.51% and 84.74±2.62%, respectively. The results of correlation analysis amongst the independent variables revealed positive correlations between BW:Avg L (r=0.920), SC: Avg W (r=0.922) and SC:PTV (r=0.957) at P<0.01; and between BW:PTV (r=0.856) and Avg W:PTV (r=0.851) at P<0.05. The inter relationship between independent and dependent variables showed maximum correlation of age with other semen attributes. The results of this study demonstrate that the reproductive indices of Cholistani breeding bulls are at par with those of Sahiwal bulls. Furthermore, the orchidometric measurements along with age and body weight remain reliable and readily measurable indicators of reproductive potential of breeding bulls.

Key words: Cholistani bulls, age, body weight, orchidometry

INTRODUCTION

In tropical breeds of bulls, the readily measurable reproductive parameters have been reported to include body weight (BW), scrotal circumference (SC), testicular size and sperm morphology (Lunstra and Cundiff, 2003). Scrotal circumference and testicular measurements are superior of all testicular parameters in estimating testicular size and sperm output (Brito *et al.,* 2004; Ahmad *et al.,* 2005). Scrotal circumference is an indirect but reliable indicator of onset of puberty, semen quality, total semen production, testicular pathology, testicular weight and fertility of bulls (Ahmad *et al.,* 2005); whereas, testicular measurements provide reproductive and spermatogenic ability in post pubertal period of bulls (Siddiqui *et al.,* 2008). Likewise, age and BW of the breeding bulls are significantly correlated with each other and with SC as well (Raji *et al.,* 2008). Some researchers suggest paired testicular volume (PTV) as a more adequate selection parameter for reproductive performance in Zebu cattle as compared to SC (Unanian *et al.,* 2000).

Cholistani breed is a zebu *(B. indicus)* or humped breed of cattle being reared by the nomadic pastoralists of Cholistan desert, Pakistan. It has immense potential of thermo-tolerance and tick-resistance and can tolerate severe heat stress (Farooq *et al.,* 2010). Our group has recently reported data on seasonal variations in libido, serum testosterone concentration and physico-chemical attributes of semen from Cholistani breeding bulls (Farooq *et al.,* 2013a; Mahmood *et al.,* 2013). The present study is directed specifically to determine: 1) preliminary baseline data regarding non-invasive, readily measurable reproductive attributes of Cholistani breeding bulls such as age, BW and orchidometric parameters viz. SC, scrotal skin fold thickness (SSFT), average testicular length (Avg L), average testicular width (Avg W) and PTV; 2) preliminary baseline data on semen quality parameters (ejaculatory volume, mass motility, individual sperm motility, sperm count, viability, normal morphology and acrosome integrity); and 3) relationships among dependable and independent variables mentioned in objectives 1 and 2.

MATERIALS AND METHODS

Experimental Site: The present study was carried out at the Semen Production Unit (SPU), Karaniwala, Bahawalpur, located in the Cholistan Desert of Pakistan from October 2011 to January, 2012. This area has an arid, subtropical and monsoonal climate with an average annual rainfall of 180 mm (Farooq *et al.*, 2011). The inconsistency in rainfall results in periodic droughts in the area. Detail regarding geographical location of Cholistan Desert has been described elsewhere (Farooq *et al*., 2013b).

Experimental Animals: Six adult Cholistani breeding bulls with clinically normal reproductive tract and donating semen of acceptable quality, aged 5-11 yrs were used in this study. Information regarding feeding and management of these bulls has been geven elsewhere (Farooq *et al*., 2013b).

Age, Body Weight and Orchidometry: Age of each bull was recorded from the data maintained at the SPU, while BW was taken fortnightly. Orchidometry was carried out fortnightly during the study period while bulls were restrained in standing position in a metallic crush. A total of 08 observations were taken for each parameter per bull. The time of orchidometry (0400 pm) and animal handling personnel were kept constant in order to maintain monotony. The SC was measured after pushing the testicles to the bottom of the scrotum and then measuring the greatest circumference with a flexible tape (Ahmad *et al.,* 2005). The length (proximal-distal) and width (medio-lateral) of each testis was measured with the help of calipers, keeping care not to modify the normal shape of the organ (Shrestha *et al.,* 1983) and the averages for two testes of each bull were calculated. The testis length also included the epididymis. The SSFT was also measured with a vernier caliper after pushing the testicles upwards and holding the scrotal skin fold between the fingers (Edwards *et al.,* 1955). The PTV was calculated using the following formula (Lunstra and Cundiff, 2003):

$$PTV = 0.0396 (Avg\ L) (SC)^2$$

Semen Collection and Evaluation: Semen from each experimental bull was collected at weekly intervals during the experimental period, at homosexual mount using artificial vagina (42°C). Two ejaculates were collected from each bull on each collection. However, occasionally some bulls refused to give the second ejaculate. Thus, a total of 174 ejaculates, with an average of 29 ejaculates per bull were collected and further processed. Immediately after collection, each ejaculate was kept at 37°C and evaluated for physical characteristics including ejaculatory volume, mass activity, individual sperm motility, sperm concentration, percentages of viable, morphologically normal sperm and those with intact acrosome (acrosome integrity). The detail of semen evaluation has been given earlier (Farooq *et al*., 2013b).

Statistical Analysis: Statistical analysis was conducted with the Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). Mean values (± S.D) for each parameter were computed. Step wise regressions were applied with dependable variables including semen volume, mass motility, individual motility, sperm concentration, viability, morphology and acrosome integrity. Independent variables included age, body weight, SC, SSFT, Avg L, Avg W and PTV. Correlations were determined between dependent and independent variables and only those approaching significance ($P<0.05$) were considered for inclusion in regression analysis.

RESULTS AND DISCUSSION

Age, Body Weight and Orchidometry: Mean values (± SD) of age, BW and various orchidometric parameters of Cholistani breeding bulls are given in Table 1.

In the present study, overall mean values for age, BW and SC of Cholistani breeding bulls were 92.83 ± 28.15 months, 520.00±42.31 kg and 36.02±2.25 cm, respectively. A lower value of 32.15±1.02 cm for SC was recorded for Sahiwal bulls of above 5 years of age (Ahmad *et al.,* 2005). Similarly, a lower value of 28.5±0.9 cm for Nelore *(B. indicus)* bulls of 2 years of age has been reported (Brito *et al.,* 2004). These variations may be due to the differences in age and breed of the experimental animals. Scrotal circumference has been shown to increase more rapidly in young than in mature bulls; afterwards, it declines in old bulls owing to senile atrophy (Siddiqui *et al.,* 2008). *B. indicus* bulls are found to be heavier and older at puberty as compared to the *B. taurus* bulls (Nogueira, 2004). Hence, breed characteristics coupled with physiological and anatomical adaptations of Cholistani bulls to the harsh environments of Cholistan desert may also be attributed to these variations.

The SSFT in the present study was found to be 1.10±0.15 cm (Table 1), the range was 0.98 to 1.32 cm. The SSFT has been reported to directly influence the semen quality in terms of spermatozoan morphology (Siddiqui *et al.,* 2008). Elevation of testicular temperature because of thermal insulation of scrotum results in impaired detoxification of reactive oxygen species because of reduction in superoxide dismutase/catalase activities. Ultimate consequence is a hampered normal spermatogenesis and a decreased semen quality (Setchell, 1998).

Measurements of the Avg L and Avg W are important orchidometric parameters which provide information about the reproductive efficacy and

physiological status of bulls (Watson-Whitmyre and Stetson, 1985). Overall Avg L and Avg W of testes in the present study were 16.91±0.70 and 7.05±0.35 cm, respectively.

The PTV has been considered as a better selection marker for reproductive assessment of Zebu cattle as compared to SC (Unanian *et al.*, 2000). The present study revealed an overall PTV of 874.37±137.07 cm^3 (Table 1). This is in line with the work of Das *et al.* (2005), who reported a mean PTV of 835.29±52.39 cm^3 for yak bulls *(Poephagus grunniens L.)*. However, lower mean PTV of 618±24 and 550±18 cm^3 were reported for Angus and Brahman bulls, respectively by Lunstra and Cundiff (2003). These differences in results can be attributed to breed differences.

Semen Attributes: Mean values (± SD) of various semen attributes of Cholistani bulls recorded in this study are presented in Table 2. The overall means for ejaculatory volume, mass motility, individual motility, sperm concentration, viability, normal morphology and acrosome integrity were 4.46±1.65mL, 1.95±0.25, 63.46±4.91%, 903.22±182.04 million/mL, 85.35±2.29%, 85.66±2.51% and 84.74±2.62%, respectively.

The Relationships: The results for correlation analysis of various testicular morphometric parameters with age and BW (independent variables) are presented in Table 3. The results revealed that age of the animals did not show any significant correlation with any of the parameters. Body weight showed a significant positive correlation with Avg L (r=0.920, P<0.01) and PTV (r=0.819, P<0.05). Raji *et al.* (2008), while working on indigenous goats of Nigeria, also reported a positive correlation between BW and Avg L (r= 0.82, P<0.01). Scrotal circumference also showed significant positive correlations with Avg W (r= 0.929, P<0.01) and PTV (r= 0.970, P<0.01) in the present study. Average testicular width also showed positive correlation with PTV (r=0.872; P<0.05). Previous literature indicates a linear (positive) correlation of age and body weight with testicular measurements (Siddiqui *et al.*, 2008). The positive correlation of PTV with BW, SC and average testicular width shows its role as a reliable parameter for prediction of reproductive efficacy in Cholistani bulls.

The correlation coefficients between semen attributes and independent variables are presented in Table 4. Age of the bull showed significant correlations with most of the semen attributes. It had a positive correlation with semen volume (r=0.928, P<0.01) and significant negative correlations with sperm concentration (r=-0.943, P<0.01), sperm viability (r=-0.873, P<0.05), sperm morphology (r=-0.884, P<0.05) and acrosome integrity (r=-0.813, P<0.05). Previous studies have elaborated that age of the breeding bulls has significant effect on semen quality which improves with increasing age but declines after a specific age owing to the senile atrophy (Khan *et al.*, 2007; Bhakat *et al.*, 2011). Similar correlations were reported for 129 beef bulls (Kastelic *et al.*, 2001).

Table 1. Various readily measurable reproductive traits of Cholistani bulls.

Trait	Mean	SD	Min.	Max
Age (months)	92.83	28.15	70	131
BW (kg)	520.00	42.31	483.75	600.63
SC (cm)	36.02	2.25	33.5	38.63
SSFT (cm)	1.10	0.15	0.98	1.32
Avg L (cm)	16.91	0.70	16.1	18.06
Avg W (cm)	7.05	0.35	6.68	7.44
PTV (cm^3)	874.37	137.07	738.06	1069.26

BW=Body weight, SC= Scrotal circumference, SSFT= Scrotal skin fold thickness, Avg L= Average testicular length, Avg W= Average testicular width, PTV= Paired testicular volume.

Table 2. Semen attributes of Cholistani bulls

Trait	Mean	SD	Min.	Max
Ejaculatory volume (mL)	4.46	1.65	2.13	6.73
Mass Motility (Score 1-5)	1.95	0.25	1.55	2.25
Individual Motility (%)	63.46	4.91	57.31	68.50
Sperm concentration (million/mL)	903.22	182.04	678.64	1094.71
Sperm Viability (%)	85.35	2.29	82.29	88.06
Sperm Morphology (%)	85.66	2.51	81.07	87.25
Acrosome Integrity (%)	84.74	2.62	79.80	86.77

In this study, SC showed a significant negative correlation (r=-0.839, P<0.05) with sperm viability. Similarly, significant negative correlation was recorded between Avg W and sperm viability (r=-0.831; P<0.05).

However, Kastelic *et al.* (2001) reported that bulls with larger testes generally produce more morphologically normal sperm compared to those having smaller testes.

The results of regression models between dependent and independent variables are presented in Table 5. Age had a positive linear regression with semen volume and negative with sperm concentration, viability, morphology and acrosome integrity. The SC presented a negative linear regression with sperm viability, being inconsistent to previous published reports (Kastelic *et al.*, 2001). Extensive studies have been done computing regression models for various characteristics (SC, orchidometry, semen attributes, backfat thickness, libido *etc.*) to predict bull fertility; however, a precise conclusion is yet to be achieved.

The selection criteria for bulls of adequate reproductive potential are seeing newer avenues with the advent of ultrasonography and many other laborious, skilful techniques. The results of this study, however, clearly demonstrate that orchidometric measurements along with SC, age and body weight are still reliable and readily measurable indicators of reproductive potential in breeding bulls. Furthermore, it is verified that quantitative aspects of these parameters coupled with estimates of correlations among them can strength the reliability of breeding soundness examination of bulls.

Table 3. Pearson's correlation coefficients between age, body weight and orchidometric parameters in Cholistani bulls

Traits	Age	BW	SC	SSFT	Avg L	Avg W	PTV
Age (months)	1						
BW (kg)	-0.116	1					
SC (cm)	0.590	0.685	1				
SSFT (cm)	0.379	-0.389	0.163	1			
Avg L (cm)	-0.177	0.920**	0.608	-0.195	1		
Avg W (cm)	0.630	0.660	0.929**	-0.112	0.467	1	
PTV (cm^3)	0.405	0.819*	0.970**	0.065	0.781	0.872*	1

BW=Body weight, SC= Scrotal circumference, SSFT= Scrotal skin fold thickness, Avg L= Average testicular length, Avg W= Average testicular width, PTV= Paired testicular volume.
** Correlation is significant at P<0.01 (2-tailed).
* Correlation is significant at P<0.05 (2-tailed).

Table 4. Pearson's correlation coefficients between semen attributes and independent variables

Traits	Age	BW	SC	SSFT	Avg L	Avg W	PTV
Volume	0.928**	0.059	0.687	0.452	0.019	0.675	0.536
Mass Motility	-0.614	0.364	-0.123	0.031	0.552	-0.304	0.063
Individual Motility	0.019	0.466	0.501	0.524	0.669	0.206	0.589
Count	-0.943**	0.299	-0.457	-0.608	0.331	-0.445	-0.253
Viability	-0.873*	-.253	-0.839*	-.252	-0.174	-.831*	-.714
Morphology	-0.884*	0.222	-0.383	-0.097	0.436	-.570	-0.167
Acrosome integrity	-0.813*	0.210	-0.401	-0.205	0.459	-.559	-0.176

BW=Body weight, SC= Scrotal circumference, SSFT= Scrotal skin fold thickness, Avg L= Average testicular length, Avg W= Average testicular width, PTV= Paired testicular volume. ** Correlation is significant at P<0.01 (2-tailed).
* Correlation is significant at P<0.05 (2-tailed).

Table 5. Regression models for semen volume, sperm concentration, viability, normal morphology and acrosome integrity.

Dependent / independent variables	Slope	R^2	Probability
Semen Volume (mL; y-intercept =0.579)			
Age	0.928	0.860	0.008
Sperm Count (10^6/mL; y-intercept = 1469.00)			
Age	-0.943	0.889	0.005
Sperm Viability (%; y-intercept =108.41)			
Age	-0.873	0.761	0.023
SC	-0.839	0.704	0.037
Avg W	-0.831	0.691	0.040
Sperm Morphology (%; y-intercept = 92.964)			
Age	-0.884	0.781	0.020
Acrosome Integrity (%; y-intercept = 91.78)			
Age	-0.813	0.661	0.049

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