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STUDIES ON LIBIDO AND SERUM TESTOSTERONE CONCENTRATION OF CHOLISTANI AI BULLS UNDER STRESS FREE AND STRESSFUL SEASONS

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ABSTRACT

The study was executed with a specific objective to assess baseline data on libido indices (reaction time, libido index and time lapsed between two ejaculates) and serum testosterone concentration of Cholistani AI bulls (n=06) being reared at SPU, Karaniwala, Bahawalpur, Pakistan. Furthermore, influence was noticed on these parameters of stress free and stressful seasons. Four seasons of 2 months duration each were defined as i) stress free autumn (October-November), ii) stressful winter (December-January), stressful dry summer (May-June) and iv) stressful wet summer (July-August). The overall mean (±SEM) values for Reaction Time, libido index and Time Lapsed between Two Ejaculates in the present study were 4.5±1.10 min, 3.48±0.03 and 3.04±0.30 min, respectively. Non-significant (P>0.05) differences were found in all the parameters during four seasons. The overall mean value for serum testosterone concentration was 5.81±0.32ng/mL with no influence of seasons on it. In a nutshell, the Cholistani AI bulls maintain their serum testosterone concentration at a constant level during stress free and stressful seasons, hence keeping their libido indices constant, too. This is indicative of the fact that this breed has an innate ability of being well adapted to the harsh, hot desert climate. This adaptability, in turn, helps them maintain their reproductive abilities at optimum levels even in stressful seasons. This preliminary study envisages for a broader study both on fresh and post thaw seminal indices adjunct with fertility trial in this neglected indigenous cattle breed of Pakistan.

Keywords: Cholistan, stress, libido, reproductive indices

INTRODUCTION

The indiscriminate crossing of our local/indigenous livestock with exotic bulls/semen has merely led to loss of indigenous genetic resources, extinction of native varieties and disturbance of eco-system (Mathias and Mundy, 2005; Singh, 2006; Templeman and Cardellino, 2007). The ultimate consequence has only been a mere replacement of indigenous local breeds with exotic ones (Kohler-Rollerchfson *et al.,* 2009) without any substantial increase in livestock production. A terrifying loss of one indigenous livestock breed in two weeks has been reported by the FAO (Rischkowsky and Pilling, 2007; Kohler-Rollefson *et al.,* 2009). However, times are now changing and taking a swift twist towards the realization of the role of locally adapted indigenous livestock of a region for a sustainable agriculture in rural/suburban livelihoods. The milestone was attained in an International Conference on Animal Genetic Resources held in Switzerland in 2007 (FAO, 2007; Kohler-Rollefson *et al.,* 2009) which pioneered a global perception of conservation and propagation of locally adapted indigenous livestock.

Following the global lead, directions of research in Pakistan are also being focused towards other indigenous cattle breeds apart from Sahiwal. Cholistani is one such breed being focused extensively since the last few years (Farooq, *et al.,* 2010; Farooq *et al.,* 2012; Farooq *et al.,* 2013). It is a one-humped breed of cattle (*Bos indicus*) being reared by the nomads of the Cholistan desert of Pakistan. It gained its entry in the Livestock Census of Pakistan in 2006 to be recognized as a breed since then. Its phenotypic characteristics include a large sized flabby body with small horns, long ears, well-developed hump in males, and large dewlap in both males and females (Farooq *et al.,* 2010). Its body is speckled red, black or brown all over with the switch of the tail being black.

The present study is the first of its kind, designed to deduct a baseline data on libido indices and serum testosterone concentration of Cholistani AI bulls. Furthermore, the study also furnishes the influence of stress free and stressful seasons on these indices.

MATERIALS AND METHODS

Experimental site: The present study was conducted at

the Semen Production Unit (SPU), Karaniwala, Bahawalpur, located in the Cholistan Desert of Pakistan. Sprawling over an area of 26,000 Km^2, this desert is located at latitudes 27º42´and 29º45´North and longitudes 69º52´and 75º24´East and at an altitude of 112m above the sea level. The climate of this area is arid, hot subtropical and monsoonal with the average annual rainfall of 180 mm (Ali *et al.,* 2009). The inconsistency in rainfall results in periodic droughts in the area.

Experimental period and seasons: The study was conducted over an 8-month period during 2012-13 *i.e.* in the months of May, June, July, August, October, November and December 2012, and January 2013. Keeping in view the prevailing climatic conditions of the study area, four seasons of 2 months duration each were defined viz. i) stress free autumn (October-November), ii) stressful winter (December-January), stressful dry summer (May-June) and iv) stressful wet summer (July-August). The data regarding climatic conditions (temperature, relative humidity and rainfall) during the study period were retrieved from Regional Meteorological Centre (RMC), Lahore, Pakistan and the averages are presented in Table 1.

Experimental animals: Six adult Cholistani AI bulls having clinically normal reproductive tract and donating semen of acceptable quality, aged 5-11 years were used in this study. These bulls were maintained under their natural climate and were fed seasonal green fodder at the rate of 10% of body weight and 2-3 kg of concentrate per bull per day. Vaccination against Haemorrhagic Septicemia and Foot and Mouth disease was carried out as per schedule. Preventive measures against worm infestation were undertaken twice in a year or whenever felt necessary.

Estimation of libido: The libido of each bull was judged once a week, at the time of semen collection, in terms of reaction time, libido index and the time lapsed between two ejaculates (TLTE). An intact bull restrained in a metallic service crush was used as a teaser. Before introduction into the test area, each bull was given a sexual preparation for about ten minutes. The time between introduction of bull into the test area and first mount was defined as 'reaction time' (Singh and Pangawkar 1989). A libido index, ranging from 0 to 6 was calculated for each bull depending upon his sexual interest in the teaser, as explained by Chenoweth (1981).

Blood sampling and serum testosterone concentration: The animals were restrained in the cattle crush by trained personnel and fortnightly blood collection was carried out aseptically through a disposable syringe (18 gauge, 5cm long) during the study period. About 5mL blood was collected from each animal and was transferred into vacutainers containing thixotropic gel separator for serum separation. A total of 16 blood/blood serum samples were collected per animal (24 samples per season). In order to minimize the stress in the animal, to standardize the collection procedure, and to remove diurnal variation, all the animals were restrained with the same technique and the collection was made by the same personnel and at the same time of the day *i.e* around 04:00 pm. Serum was harvested through centrifugation.

Serum testosterone level was determined through MiniVIDAS 12 Compact Automated Immunoanalyzer using Biomeriux kit 30418 (CPT Code 84403) having the detection range of 0.1-13ng/mL (within 95% probability). The inter-assay and intra-assay coefficient of variance (CV) were 4.7 and 5.7%, respectively. The concentration was presented in ng/mL.

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 (±SE) were calculated for libido indices and serum testosterone concentration. Influence of stress free and stressful season on various attributes was deducted through ANOVA and the difference between mean values was compared through Duncan's multiple range test ($P<0.05$). Pearson's correlation coefficients were also calculated between serum testosterone concentration and libido indices (Steel *et al.,* 1996).

RESULTS AND DISCUSSION

The South-Asian countries have lately realized the ominous need to conserve/propagate the indigenous genetic resources as a landmark to sustainable rural/sub rural livelihoods (Ruto *et al.,* 2008). Following the lead, an effort has been made through the present study to bring to prominence a formerly neglected indigenous cattle breed of Pakistan- Cholistani- being reared by the nomadic herders of the Cholistan desert. The study represents detailed information regarding baseline data on libido indices and serum testosterone concentration of Cholistani AI bulls along with their variations under stress free and stressful seasons.

Libido estimation: It is widely accepted that 'bull is half the herd" (Abera *et al.,* 2006). Moreover, this is also an established fact that optimum fertility requires four vital reproductive qualities in a bull with the '**libido**' being the foremost. The remaining three are: sound ability to detect estrus; testicular development to produce sufficient viable sperm; and to have sufficient fertile sperm to ensure fertilization and embryo sustainability (Ellis, 2008). However, in AI programs a very few bulls are contributing their genes to larger population of cows and the breeding soundness evaluation does not incorporate a regular assessment of libido and mating/social behavior. The present study presents for the first time an account of

libido estimation (reaction time, libido index and TLTE) in Cholistani AI bulls (Table 2).

The mean value for the reaction time of Cholistani AI bulls in the present study (4.5±1.10 min) is higher than that of 3.28±0.50 min reported earlier (Ahmad *et al.,* 2005) for adult Sahiwal bulls. However, a higher reaction time of 5.0±0.01 min has been recorded for Horro-Friesian bulls (Zebu cross) in Ethiopia (Abera *et al.,* 2006). Difference in breed could be attributed to these variations. Regarding the *Bos taurus* breeds, Belgian Blue and Holstein-Friesian bulls showed a lower Rx Time of 1.2±0.01 and 1.5±0.02 min, respectively (Hoflack *et al.,* 2006). Decreased sexual interest/activity and hence, depressed reproductive indices of Zebu breeds as compared to their taurine counterparts has been termed as a genetic predisposition (Higdon *et al.,* 2000, Molina *et al.,* 2000). Age, season, feeding and management are some of the factors that affect the reaction time (Ahmad *et al.,* 2005).

The mean libido score in the present study (3.48±0.03) is slightly higher than that of 3.26±0.17 reported for Sahiwal bulls (Ahmad *et al.,* 2005). However, a higher score of 4.83±0.07 was recorded for Zebu cross bulls in Ethiopia (Abera *et al.,* 2006). While comparing the sexual behavior of Zebu bulls for single-sire (SSM) versus multiple-sire mating groups (MSM) in Costa Rica it was concluded that the sexual activities were frequent in SSM group compared to the MSM ones. Furthermore, the sexual behavior was found to be weaker in Zebu bulls as compared to taurine counterparts (Molina *et al.,* 2000). Difference in age, breed, feeding/management, type of floor, semen collection regimen and andrological patterns of bulls might have been possible reasons for these variations (Ahmad *et al.,* 2005; Elrabie *et al.,* 2008).

The mean TLTE for the present study (3.04±0.30 min) is lower than 9.28±1.5 min reported for Sahiwal bulls (Sarder *et al.,* 2007). Literature review proved to be outdated on this aspect is quiet old and hence has not been incorporated in discussion.

Regarding the seasonal influence, all the libido estimation parameters (reaction time, libido score and TLTE) were found to be almost same during stress free and stressful seasons with non-significant variation (P>0.05) (Table 2). However, the value of libido index (3.50±0.07) was the highest during stressful winter season. In Pakistan, to date, the libido estimation parameters have been studied for Sahiwal bulls only, and the results have revealed significant effect of season on reaction time and libido score (Ahmad *et al.,* (2005). The former was found to be longer in summer, whereas, the later was lower in summer. Heat stress and shortage of fodder in dry summer was given as a plausible justification for this seasonal variation. However, the same libido indices throughout the year for Cholistani AI bulls, in the present study, might be due to this breed's inherent ability of being fully adapted to the harsh, hot desert climate which helps them maintain their reproductive abilities at optimum levels even in stressful seasons (Farooq *et al.,* 2012; 2013).

Serum testosterone concentration: Serum testosterone level in Zebu bulls is generally considered to be lower than in *Bos taurus* bulls (Gulia *et al.,* 2010; Sekoni *et al.,* 2010) resulting in a decreased sexual activity in animals of the former species. Mean serum testosterone level for Cholistani AI bulls in the present study (5.81±0.32 ng/mL) (Fig 1) revealed a wide range from 00.89 to 14.59ng/mL. Lower mean values of 0.69±0.12 and 0.65±0.11 ng/mL have been reported for buffalo bulls by Sajjad *et al.,* (2007) and Javed *et al.,* (2000), respectively. The difference in species can be attributed to these variations. The mean serum testosterone recorded in the present study is lower than the value of 8.6±0.7ng/mL reported for indigenous Zebu Bunaji bulls (Sekoni *et al.,* 2010). However, they also reported a wide range for serum testosterone concentrations and an episodic pattern of testosterone secretion in study animals. These peaks were related to sexual and behavioral activity of animals in response to photoperiodicity, temperature and postural variations.

Non-significant effect (P>0.05) of stress free and stressful seasons was noticed on blood serum testosterone level in Cholistani AI bulls under study (Fig 1). Godfrey *et al.,* (1990), however, reported significant seasonal variation in serum testosterone concentration of Zebu Brahman bulls, being lower in winter. Depressed hypothalamic-hypophyseal axis owing to cold stress was given as plausible justification. Perry *et al.,* (1991) also reported non-significant effect of season on serum testosterone level in tropical beef bulls. The harmonious endocrine pattern in the present study may be suggestive of an adapted state of the animals under their native environment without being stressed.

Table 1. Average values for climatic data during the seasons of experimental period

Parameter	Stress Free Season (Oct/Nov)	Stressful Seasons		
		Winter (Dec/Jan)	Dry Summer (May/June)	Wet Summer (July/Aug)
Mean Temperature (°C)	23.7	13.4	36.4	33.2
Mean Relative Humidity (%)	58.0	57	47.1	61.2
Mean Rainfall (mm)	2	4.2	4.8	83.5

Table 2. Effect of season on libido estimation parameters of Cholistani AI bulls*

Parameters	Stress Free	Stressful			Overall Mean
	Autumn (Oct/Nov)	Winter (Dec/Jan)	Dry Summer (May/June)	Wet Summer (July/August)	
Reaction time (min)	4.59±0.66	3.82±0.51	5.11±0.66	4.89±0.60	**4.5±0.17**
Libido index (Score 0-6)	3.49±0.07	3.50±0.07	3.47±0.07	3.48±0.08	**3.48±0.03**
TLTE (min)	3.68±0.04	3.0±0.21	2.50±0.82	2.90±0.99	**3.04±0.30**

*Data are mean ±SE. TLTE=Time Lapsed between Two Ejaculates

Table 3. Pearson's correlation coefficient of serum testosterone concentration with libido indices of Cholistani AI bulls

Parameters	r value
Reaction time	0.599
Libido index	0.334
Time lapsed between two ejaculates	0.404

**Correlation is significant (P<0.01; 2 tailed).

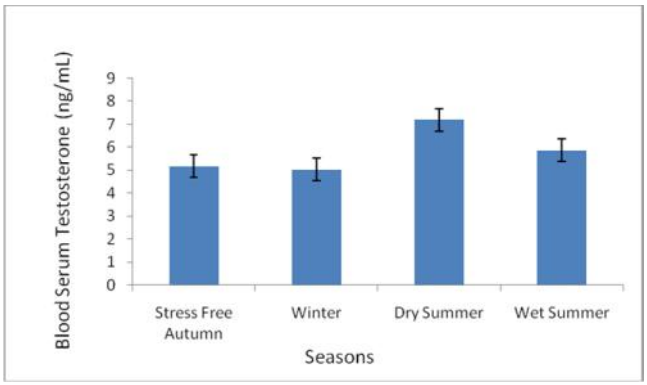

Fig 1. Seasonal variation in serum testosterone concentration of Cholistani AI bulls. Data are mean ±SE. Difference was non-significant (P>0.05)

Correlations: Serum testosterone concentration was found to be positively correlated (P<0.01) with all three libido indices included in the present study (Table 3). This is in line with various previously published data (Gulia *et al.,* 2010). Testosterone produced from the Leydig cells of the testes is vital for spermatogenesis and male characteristics (Sekoni *et al.,* 2010). Furthermore, its vascular distribution throughout the body is a major contributory factor to the libido of males (Sajjad *et al.,* 2007).

Conclusion: In a nutshell, the Cholistani AI bulls maintain their serum testosterone concentration at a constant level during stress free and stressful seasons, hence keeping their libido indices constant, too. This is indicative of the fact that this breed has an innate ability of being well adapted to the harsh, hot desert climate. This adaptability, in turn, helps them maintain their reproductive abilities at optimum levels even in stressful seasons. This preliminary study envisages for a broader study both on fresh and post thaw seminal indices adjunct with fertility trial in this neglected indigenous cattle breed of Pakistan.

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