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A PRELIMINARY STUDY ON LIBIDO AND SEMINAL ATTRIBUTES OF DAJAL BREEDING BULLS

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Abstract

This study aims to deduce a baseline data regarding seminal attributes (fresh and post thaw semen) and libido of indigenous Dajal and Cholistani breeding bulls. Data were collected on weekly basis for three months and a total of 138 ejaculates were attained for analyses. Among fresh semen attributes, mass motility was significantly higher for Dajal bulls as compared to Cholistani bulls being 2.7±0.2 and 2.1±0.1, respectively. Dajal proved to bear the brunt of cryopreservation in a better way in terms of percentage of live spermatozoa (14.7±0.2% damage). The values of libido index score, reaction time and Time Lapsed between Two Ejaculates were also significantly higher for Dajal breeding bulls. This preliminary study is the first of its kind, which furnishes baseline data regarding various reproductive attributes of Dajal breeding bulls. It envisions future studies on Dajal bulls with a larger sample size related to effect of age, season, feeding regimen, various types of extenders on semen quality, and assessment of fertility rate in order to attain an enhanced productivity.

Key words*: Breeding bulls; Dajal cattle breed; libido estimation; semen analysis*

INTRODUCTION

The uninterrupted crossing of local/indigenous livestock with exotic bulls/semen has led to loss of indigenous genetic resources, extinction of native varieties and disturbance of eco-system (Mathias & Mundy, 2005; Tempelman and Cardellino, 2007). The ultimate consequence has resulted in replacement of indigenous local breeds with exotic ones (Köhler-Rollefson et al., 2009).

A terrifying loss of one indigenous livestock breed in two weeks has been reported by the FAO (Rischkowsky & Pilling, 2007; Köhler-Rollefson et al., 2009). The South-Asian countries have lately realized the dire need to conserve the indigenous genetic resources as a landmark to sustainable rural/sub rural livelihoods (Ruto et al., 2008).

Consequently, extensive research work has started pouring in, regarding the productive and reproductive aspects of indigenous livestock from Pakistan in order to conserve and propagate these breeds.

Pakistan is endowed with 15 indigenous cattle breeds which constitute 43% of total cattle population (Khan et al., 2008). Extensive research work has been conducted on Sahiwal and Red Sindhi breeds of cattle which have acclaimed them both at national and international level for being thermo-tolerant, hardy and tick-resistant breeds. Last decade has witnessed substantial work conducted and reported on productive and reproductive attributes of the formerly neglected Cholistani breed of indigenous cattle being reared by the nomads of Cholistan desert (Farooq et al., 2010; Farooq et al., 2012; Farooq et al., 2013a; Farooq et al., 2013b; Farooq et al., 2015). Resultantly, national conservation and propagation programs for this breed are under way.

Dajal is a locally-famed indigenous cattle breed of Pakistan mostly being used for draught purposes. It's utility and hence, its number has declined over the years owing to mechanization in agriculture and livestock sectors. It is a native breed of a small town, Dajal, located in District Dera Ghazi Khan (DGK), Punjab,

Pakistan (Figure 1). It has a white or greyish body color with distinct deep grey areas over the neck, shoulder and hump in mature males. It has long legs, a sturdy torso, compact body and well-built structure which give it a peculiar look of 'half bull and half horse'. Head is almost medium sized with short strong neck, black muzzle and eye lashes. Small dewlap, stumpy horns, small pointed ears, moderately developed hump, straight back, tight sheath and black tuft of tail are prominent points of Dajal animals (Figures 2 and 3) (Ward, 2003).

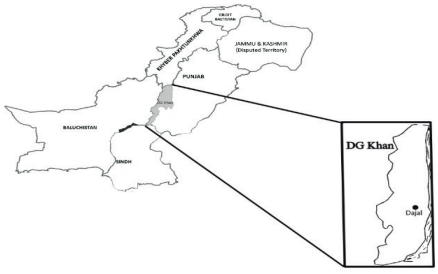

Figure 1. Geographical location of Dajal area, District Dera Ghazi Khan (DGK), Punjab, Pakistan

Figure 2. An elite specimen of Dajal breeding bull being incorporated in the present study reared at the Semen Production Unit, Karaniwala, Cholistan, Punjab, Pakistan

Figure 3. An elite specimen of Dajal Cow being reared at a private farm of Fazilpur, Dera Ghazi Khan, Punjab, Pakistan

Average body weight of Dajal breed is 550 kg to 650 kg of weight in mature bulls and 350 to 450 kg in cows. Cows are low yielder of milk and 1121±92 liters of milk per lactation have been reported (Aslam et al., 2002; Kenyanjui et al., 2009). A rough estimate of 72 thousand heads of Dajal has been reported (Khan et al., 2008).

It is not yet an established breed as it has not been depicted in the Livestock Census of Pakistan of 2006. There are unconfirmed reports attained from the native inhabitants and old-timer veterinarians of the Dajal area (native to Dajal breed of cattle) that an Assistant Commissioner, Francis, back in 1940s had initiated a Dajal Cattle Breeding Scheme as per an Inspection Note emanated from his office. However, the scheme waned off after independence of Pakistan in 1947 (Saeed, 2020). Later in 1972, the government of Pakistan established a Dajal Cattle Breeding Farm at state land of Fazilpur village of Dajal. Dajal cattle breed was reared here for two years and again in 1974, it was replaced by the Sahiwal cattle as per the provincial government's orders. The farm was designated for managing purebred Sahiwal cattle only and consequently Dajal vanished from the government attention till late 2016.

Scanty work on paternal lineage (Anwar et al., 2014) and diseases (Sohail et al., 2019) in Dajal have been reported from Pakistan. However, no work has yet been conducted on reproductive attributes of this neglected breed. Lately, few Dajal breeding bulls, selected on the basis of phenotype, have been brought to the Semen Production Unit (SPU), Karaniwala, Bahawalpur (Punjab, Cholistan, Pakistan) and are being utilized for semen collection in an attempt of its conservation and propagation. The demand of its frozen semen is confined to all the districts of DGK Division. Furthermore, the Livestock and Dairy Development Department, Punjab, Pakistan has initiated highlighting this breed at various local/national livestock shows known as 'Kissan Mailas' in local language. The present study is the first of its kind, designed with a general objective to highlight the indigenous Dajal breed of cattle from Pakistan. In specific, it aims to deduce a preliminary baseline data regarding libido and seminal attributes (fresh and post thaw semen)

of Dajal breeding bulls being reared at SPU Karaniwala, Punjab, Pakistan in comparison to Cholistani bulls.

MATERIALS AND METHODS

The present study was conducted for three months (April to June, 2019) at a state SPU located in the Cholistan Desert, Punjab, Pakistan. Geographical location of this area has been described earlier (Farooq et al., 2012). This is an arid tropical area with May and June (dry summer) being the hottest months of the year with the maximum temperature exceeding 45ºC. Negligible rains of monsoon are witnessed in July and August (wet summer). Winters are seen in December and January with temperature of 17-20ºC. Adult (5-6 years of age) breeding Dajal (n = 03) and Cholistani bulls (n = 03) having clinically healthy reproductive tract and donating semen of acceptable quality were selected for the study. The age of the study animals was attained from the records of the SPU, Karaniwala and their profile is presented in Table 1.

Table 1. Profile of Dajal and Cholistani breeding bulls incorporated in the study, being harbored at Semen Production Unit, Karaniwala, Punjab, Pakistan*

Bull #	Date of Birth	Age at Start of Research	Date of Entering SPU
DAJAL			
KWC-56	May, 2013	05 Years, 11 Months	03-07-2014
KWC-73	October, 2013	05 Years. 06 Months	02-08-2015
KWC-86	March, 2014	05 Year, 01 Month	17-06-2017
CHOLISTANI			
KWD-1	October, 2013	05 Years. 06 Months	17-06-2016
KWD-2	December, 2013	05 Years. 04 Months	17-06-2016
KWD-3	March, 2013	05 Years. 01 Months	17-06-2016

*Data Retrieved from the Records of SPU, Karaniwala

The body weight was taken fortnightly during the study period. They were kept under naturally prevailing climatic conditions, fed good quality seasonal fodder at the rate of 10% of body weight per bull and 2-3 kg of concentrate per bull per day. Vaccination against Hemorrhagic 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. The details regarding feeding and management of these animals have been described elsewhere (Farooq et al., 2013).

At the time of semen collection, libido of each donating bull was estimated in terms of reaction time, libido index and time-lapsed-between-two-ejaculates (TLTE). A dummy bull (mostly Sahiwal bull and occasionally Cholistani bull) was used for bulls to mount on. All bulls were given sexual preparation before entry into the test area. 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 (Crichton & Lishman, 1985).

Semen from each experimental bull was collected at weekly intervals, using an artificial vagina (AV). The final temperature of AV before semen collection was in the range of 41-44°C. Two ejaculates were collected from each bull on each collection and a total of 12 collections were carried out. Infrequently, some bulls did not give the second ejaculate and hence, a total of 138 ejaculates with 23 ejaculates per bull were attained and processed further. 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), percentages of live and morphologically normal sperm and those with intact acrosome (Eosin-Nigrosin Staining) as prescribed (Jainudeen et al., 1982). Details of initial semen evaluation and relevant data have been presented elsewhere (Farooq et al., 2013).

The collected ejaculates were pooled and One-step slow method of dilution was adopted for each semen sample using Tris-fructose-egg-yolk glycerol extender, being used in the SPUs of Pakistan. Final sperm concentration of 30×10^6spermatozoa per insemination dose of 0.5 mL straws was resultantly, attained. Details of semen extension and freezing have been given elsewhere (Farooq et al., 2015). Frozen straws were stored in liquid Nitrogen until used for post thaw analyses.

The frozen straws were thawed at 37°C for 30 sec and subjected to post thaw seminal attributes *viz*. individual sperm motility (40X, phase contrast microscope; Olympus BH-2, Tokyo, Japan), percentages of live, morphologically normal sperms and those with normally intact acrosomes as per recommended protocols (Rasul et al., 2001). Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS V. 21 for Windows, Chicago, IL, USA). Data were presented as mean (± SE) values for libido, fresh seminal and post thaw seminal attributes. Independent sample t-test was implied for deducing differences between these attributes in Dajal and Cholistani bulls at the significance level of P≤0.05.

RESULTS AND DISCUSSIONS

The results regarding age, body weight and libido estimation parameters for Dajal and Cholistani breeding bulls are presented in Table 2.

Results indicated that though the age of bulls of each breed was almost same, yet there was significant (P≤0.05) difference in body weight being higher for Dajal (595.0±11.7 kg) as compared to Cholistani bulls (486.6±11.4 kg) (Table 2).

Table 2. Comparative overall mean values (± SE) for age, body weight and libido estimation parameters of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal	Cholistani
Age (months)	62.6±0.6	65.0±1.2
Body weight (kg)	595.0±11.7	486.6±11.4*
Libido index (Score 1-6)	4.3±0.2	3.9±0.1*
Reaction Time (min)	0.3±0.07	0.8±0.1*
Time elapsed between two ejaculates (min)	2.0±0.1	2.7±0.2*

*Significant within rows (P≤0.05)

In Pakistan, Dhanni, Dajal and Lohani are considered as draught indigenous cattle breeds whereas Cholistani and Red Sindhi are considered as milch breeds. While assessing birth weight as affected by various factors in a study, it has been reported that the birth weight, weaning weight and yearling weight in calves of Dajal cattle is higher as compared to other indigenous livestock breeds of Pakistan (Moaeen-ud-Din & Bilal, 2017). This study also endorsed the need of improving Dajal as a beef breed in the wake of the fact that Pakistan does not have a beef breed as yet. Genetic predisposition, environment and feeding regimen mainly maintain the body weight of farm animals as elucidated earlier.

The mean (± SE) value of libido index score for Dajal breeding bulls in present study was significantly (P≤0.05) higher being 4.3±0.2 as compared to 3.9±0.1 for Cholistani bulls (Table 2). Libido is considered as the most vital reproductive attribute for a breeding bull. It is directly related with the serum testosterone level and hence fertility rate. The sexual behavior, normally, is weaker in *Bos indicus* bulls as compared to *Bos taurus* bulls (Higdon et al., 2000). However, the score attained by the Dajal bulls in present study seems beyond satisfactory. Comparing the results with other studies conducted on Zebu cattle breed, slightly higher libido score of 4.8±0.07 has been reported from indigenous cattle bulls of Ethiopia (Abera et al., 2006) whereas a lower score of 3.2±0.17 has been reported for Sahiwal breeding bulls (Ahmad and Asmat, 2005). Keeping in view the same season and feeding regimes in this study, better libido score in Dajal breeding bulls may be attributed as an inherited character of this breed.

Regarding reaction time and TLTE, relevant mean values (± SE) for Dajal breeding bulls were 0.3±0.07 mins and 2.0±0.1 mins, respectively being significantly (P≤0.05) different and better as compared to those for Cholistani bulls (0.8±0.1 and 2.7±0.2 mins). The values for reaction time for both breeds are lower than those reported earlier for Cholistani bulls (4.5±1.1 mins) (Mahmood et al., 2013) and Sahiwal bulls (3.2±1.0 mins) (Ahmad & Asmat, 2005). Even higher value of 5.0±1.0 mins has been reported as a reaction time for Horroro indigenous bulls of Ethiopia (Abera et al., 2006). Similarly, the values for TLTE in both breeds of present study were lower than 9.2±1.5 mins reported for Sahiwal breeding bulls. Lower values for reaction time and TLTE for both breeds under study as compared to previous literature on Zebu bulls may depict effect of appropriate management skills, season or feeding regimen which influenced enhance

sexual activity in them resulting in a lower reaction time.

The results for physical attributes of fresh semen in Dajal and Cholistani breeding bulls of present study are given in Table 3.

Table 3. Comparative overall mean values (± SE) for fresh seminal attributes of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal	Cholistani
No. of ejaculates per bull	1.96±0.01	1.97±0.01
Ejaculatory volume (mL)	5.1±0.4	5.7±0.2
pH	6.1±0.09	6.0±0.04
Mass motility (Score 1-5)	2.7±0.2	2.1±0.1*
Individual sperm motility (%)	58.7±2.0	62.0±2.4
Sperm Concentration (million/mL)	739.1±17.2	892.0±21.0*
No. of dozes frozen per bull	155.2±3.0	187.5±4.3*
Live sperm (%)	67.2±2.0	79.9±1.5*
Morphologically normal sperm (%)	75.0±2.0	83.1±2.1*
Acrosome integrity (%)	75.6±1.6	82.0±1.8
No. of proximal cytoplasmic droplets	1.5±0.2	1.2±0.1
No. of distal cytoplasmic droplets	0.3±0.1	0.2±0.07

*Significant within rows (P≤0.05)

Mass motility was the only parameter significantly (P≤0.05) higher for Dajal bulls as compared to Cholistani bulls being 2.7±0.2 and 2.1±0.1, respectively. Other studies have also reported lower values of mass motility in Cholistani (Farooq et al., 2013a; Farooq et al., 2013b) and Sahiwal breeding bulls (Ahmad et al., 2003; Sarder, 2003). Similarly, Holstein-Friesian bulls when reared in subtropical environment of Pakistan also gave a lower mass motility score of 1.7±0.2 (Fiaz et al., 2010), than those recorded in our study. It has been well elaborated that the indigenous zebu (humped) breeds of cattle are tick-resistant, disease-resistant, thermo-tolerant and well-adapted to harsh environments (Farooq et al., 2010). Higher score in present study could be an inherent adaptability pattern of the breeds under study. As the mass motility score has been proven to be positively correlated to the libido index, hence better results of mass motility in Dajal bulls may be attributed to a higher libido score as seen in the present study.

The mean values (± SE) for remaining fresh seminal attributes *i.e.*, sperm concentration, number of dozes frozen per bull, and live and morphologically normal spermatozoa were significantly (P≤0.05) lower in Dajal bulls as compared to Cholistani (Table 3). Sperm concentration of 739±17.2 million/mL in Dajal bulls for present study is lower than the value of 918.0±65.7 million/mL reported earlier for Cholistani bulls (Farooq et al., 2013). Similarly, even higher concentration of 2541.9±1699.0 million/mL for Indian zebu bulls (Siddiqui et al., 2008) and 1471.0±37.0 million/mL for Sahiwal bulls (Sarder, 2003) has been reported. Difference in breed, geo-location and age could be a plausible justification for these differences. The live and morphologically normal spermatozoa in Dajal bulls (67.2±2.0 and 75.0±2.0%) were though significantly (P≤0.05) lower than those for Cholistani bulls (79.9±1.5 and 83.1±2.1%) (Table 3), yet the values were in the range reported elsewhere for various Zebu bulls (Sarder, 2003; Ahmad & Asmat, 2005; Farooq et al., 2013).

Results regarding mean (± SE) values of post thaw seminal attributes for Dajal and Cholistani breeding bulls are given in Table 4.

The individual sperm motility, morphologically normal sperm and those with intact acrosomes were significantly (P≤0.05) lower for Dajal breeding bulls as compared to Cholistani. Live spermatozoa, number of proximal droplets and distal droplets were however, non-significantly (P≥0.05) different between two study breeds. Values of all the studied post thaw attributes were at par with those reported for Cholistani (Farooq et al., 2013) and Sahiwal (Ahmad et al., 2003; Jain et al., 2008) breeding bulls elsewhere.

Table 4. Comparative overall mean values (± SE) for post thaw seminal attributes of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal	Cholistani
Individual sperm motility (%)	37.0±1.0	54.1±1.2*
Live sperm (%)	57.3±2.3	66.8±2.6
Morphologically normal sperm (%)	52.4±2.1	67.3±2.2*
Acrosome integrity (%)	54.3±1.5	68.0±2.2*
No. of proximal cytoplasmic droplets	1.0±0.3	0.6±0.2
No. of distal cytoplasmic droplets	0.5±0.1	0.4±0.1

*Significant within rows (P≤0.05)

Extension, freezing, cooling and thawing are few components of cryopreservation for bovine

semen which renders extensive physiological and morphological stress on spermatozoa. The present study also caters an objective of assessing the degree of cryodamage caused to the spermatozoa of Dajal and Cholistani breeding bulls during the intricate cryopreservation process. The effect of freezing on various post thaw attributes in terms of increase or decrease (%) is presented in Table 5.

Table 5. Comparative effect of freezing on post thaw seminal attributes of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal			Cholistani		
	Fresh Semen	Post thaw semen	Decrease / Increase (%)	Fresh Semen	Post thaw semen	Decrease / Increase (%)
Individual sperm motility (%)	58.7±2.0	37.0±1.0	36.9±2.5*	62.0±2.4	54.1±1.2	12.7±0.2*
Live sperm (%)	67.2±2.0	57.3±2.3	14.7±0.2	79.9±1.5	66.8±2.6	16.3±0.3
Morphologically normal sperm (%)	75.0±2.0	52.4±2.1	30.1±2.0*	83.1±2.1	67.3±2.2	19.0±0.3*
Acrosome integrity (%)	75.6±1.6	54.3±1.5	28.7±1.2*	82.0±1.8	68.0±2.2	17.0±0.2*
No. of proximal cytoplasmic droplets	1.5±0.2	1.0±0.3	33.3±1.9*	1.2±0.1	0.6±0.2	50.0±1.2*
No. of distal cytoplasmic droplets	0.3±0.1	0.5±0.1	40.0±1.2*	0.2±0.07	0.4±0.1	50.0±1.1*

*Significant within rows for Cholistani and Dajal breeding bulls (P≤0.05)

All the studied attributes, except live spermatozoa, were significantly (P≤0.05) decreased in post thaw semen, both for Dajal and Cholistani breeding bulls. The mean (± SE) values for damage/decrease in live spermatozoa were 14.7±0.2% for Dajal and 16.3±0.3% for Cholistani bulls. Hence, Dajal proved to bear the brunt of cryopreservation in a better way in terms of percentage of live spermatozoa. Remaining values were within the range presented for other Zebu bulls (Ahmad et al., 2003; Sarder, 2003; Jain et al., 2008; Farooq et al., 2013; Farooq et al., 2015).

The present study is a preliminary one which furnishes baseline data regarding various reproductive attributes of Dajal breeding bulls. It envisions future studies on these bulls with a larger sample size related to effect of age, season, feeding regimen, and various types of extenders on semen quality, and assessment of fertility rate in order to attain an enhanced productivity. We recommend that a directional approach and positive strategies may be devised by all the stakeholders to conserve and propagate this dying breed considering it an indigenous asset. In addition, this breed, owing to its resemblance to Bhag Nari breed of cattle, may be propagated through genetic selection as a beef breed for Pakistan. It will not only fulfill the ever-increasing beef demand of the country but can also become an export item in future.

CONCLUSIONS

The present study, in a nutshell, revealed that Dajal showed promising results regarding libido index, reaction time, TLTE and fresh seminal mass motility as compared to Cholistani.

Furthermore, the degree of damage incurred upon the spermatozoa of Dajal breeding bulls during the cryopreservation process is substantially less.

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