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Study on effect of hair coat color on various reproductive attributes of Cholistani service bulls

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Abstract

The objective of the present study was to assess the possible effect of hair coat color on various orchidometric, seminal and andrological attributes of black-coated and brown-coated Cholistani service bulls. Adult Cholistani bulls (n= 8) were grouped as black-spotted (n= 4) and brown-spotted (n= 4). Semen collection was performed once weekly (2 ejaculates/collection) using artificial vagina (AV) throughout the study period (October-May). Semen samples were evaluated for various physical attributes. Body weight and orchidometric attributes of the bulls were attained using calipers. Blood sampling was performed twice weekly and serum testosterone level was deduced through automated analyzer. The scrotal circumference, Scrotal Skin Fold Thickness and Paired Testicular Volume were significantly higher (P≤0.05) in black-spotted Cholistani bulls as compared to brown-spotted bulls. Similarly, the ejaculatory volume (5.8±0.1 mL) and individual sperm motility (63.3±1.4 %) were significantly higher (P≤0.05) in black-spotted bulls. Percentage of live spermatozoa, and spermatozoa with normal morphology and acrosome was also higher in black-spotted bulls though statistically non-significant (P≥0.05). Amongst post thaw seminal attributes, percentage of live spermatozoa, and spermatozoa with normal morphology and acrosome were significantly higher (P≤0.05) in black-spotted bulls. Similarly, serum testosterone level in black-spotted Cholistani bulls (6.9±0.3 ng/mL) was significantly higher (P≤0.05) compared to brown-spotted bulls (5.4±0.3 ng/mL). The black-spotted Cholistani service bulls showed substantially better orchidometric and seminal attributes allied with higher serum level of testosterone.

Introduction

Coat color in livestock is a highly variable and heritable trait which has attained substantial importance in the last decade or so. Furthermore, its impact on productive and reproductive attributes and longevity has also been reported for various breeds of cattle [1,2] goats [3] and sheep [4].

Elevated temperatures and humidity in tropical/subtropical geographic entities of the world (including Pakistan) highly affect the productive and reproductive attributes of cattle as documented extensively [5,6]. However, minimizing the effect of heat stress on production attributes in terms of milk and meat is still a perplexing question which needs elaborated research. To address this issue, one practical approach being implied is the selection of cattle based on hair coat color. The color and type of hair plays a pivotal role in transferring of heat from skin of the animal to the environment and vice versa ultimately resulting in thermo-regulation [1,7]. Furthermore, recent shift in weather patterns around the world makes it inevitable that different relationships between hair color and type with various productive and reproductive attributes of cattle be revisited. Most of these effects have been studied for *Bos Taurus* cattle breeds and the *Bos indicus* still remain un-navigated.

Amongst the 15 recognized indigenous cattle breeds of Pakistan, the Cholistani breed has gained substantial national and international fame for being a hardy and thermo-tolerant breed [8]. Cholistani cattle are a humped *Bos indicus* breed being reared under pastoralism mainly in desert area of Cholistan in Pakistan. Phenotypically, it has large flabby body with long hears, small horns, dewlap in both sexes and a well-developed hump in male animals [9]. It is commonly termed as 'flea bitten' due to its flecked pattern coat color of black or brown by the local of Cholistan desert, Pakistan (**Fig. 1**). Several studies have already reported various phenotypic, productive and reproductive attributes of this previously ignored breed of cattle in Pakistan [8-10] concluding that it is a thermo-tolerant breed and can maintain most of its physiological parameters in a narrow range without being stressed [11]. It also has reported the effect of age and season on various seminal attributes of the service bulls of this breed [11-13]. However, the effect of hair coat color on various reproductive attributes for this breed has not yet been reported. The present study is, hence, a continuation of research on Cholistani breeding bulls aimed towards assessing the effect of hair coat color (black-spotted and brown-spotted) on various orchidometric, seminal and andrological attributes of Cholistani service bulls.

Materials and Methods

Study area

The present study was carried out at the Semen Production Unit (SPU), Karaniwala (Cholistan desert)- one of the SPUs of Punjab, Pakistan. The desert is sprawled at 26000 km^2 at latitudes 27°42′and 29°45′ North and longitudes 69°52′and 75°24′East and at an altitude of about 112 m above sea level. The region has harsh summer with temperature soaring up till 50°C.

Study animals, semen collection and evaluation

Adult Cholistani service bulls aged 5-11 years (*n*= 08) free of chronic diseases and having clinically normal reproductive tract were selected for the present study. They were grouped as black-spotted (*n*= 04) and brown-spotted (*n*= 04) based on visual identity/phenotype. Management and feeding patterns of these bulls under study was similar to that reported by Farooq et. al. 2013 [12]. Semen of acceptable quality was collected once weekly from these bulls from October to May (08 months) using an artificial vagina (AV) with temperature ranging from 41-44°C. Two ejaculates per collection were taken from each bull. Some bulls, though, denied the second ejaculate at occasional times. A total of 482 ejaculates were available at the end for further analyses.

The analyses of physical attributes of semen samples included ejaculate volume, mass and individual motility (10X and 40X respectively, using phase contrast microscope; Olympus BH-2, Tokyo, Japan), concentration of sperms (photometrically at 560 nm wavelength with Bovine Photometer n° 1119, IMV, France), percentages of live and morphologically normal sperm and those with intact acrosome (100X with phase contrast microscope; Olympus BH-2). Photometer was utilized to deduce the number of doses to be frozen in 0.5 mL straws (40 million sperms/0.5 mL straw). The detailed protocol for semen collection and evaluation has been presented elsewhere [8].

Orchidometry

The body weight and orchidometric attributes of the bulls *viz.* scrotal circumference (SC), scrotal skin

fold thickness (SSFT), average testicular length (AvgTL), average testicular width (AvgTW) and paired testicular volume (PTV) were attained fortnightly by restraining bull in a metallic crush during the study period. Eight observations of each parameter were taken per bull. In order to maintain monotony, animal handling personnel and time (1600 hours) remained same. For the measurement of SC, the testicles were pushed to the bottom and flexible tape was used for measuring greatest circumference, thus available [14]. Calipers were used to attain the proximal-distal length and medio-lateral width of each testis. It was taken care of that the normal shape of the testis does not get distorted [11]. The result was gathered as an average both for length and width. Epididymis was included in the measurement of testicular lengths. For SSFT, the testicles were pushed upwards and grasping the scrotal skin fold, measurement was taken by the help of a vernier caliper [15]. For calculating the PTV, appropriate formula was used [16]: $PTV = 0.0396 (Avg\ L) (SC)^2$

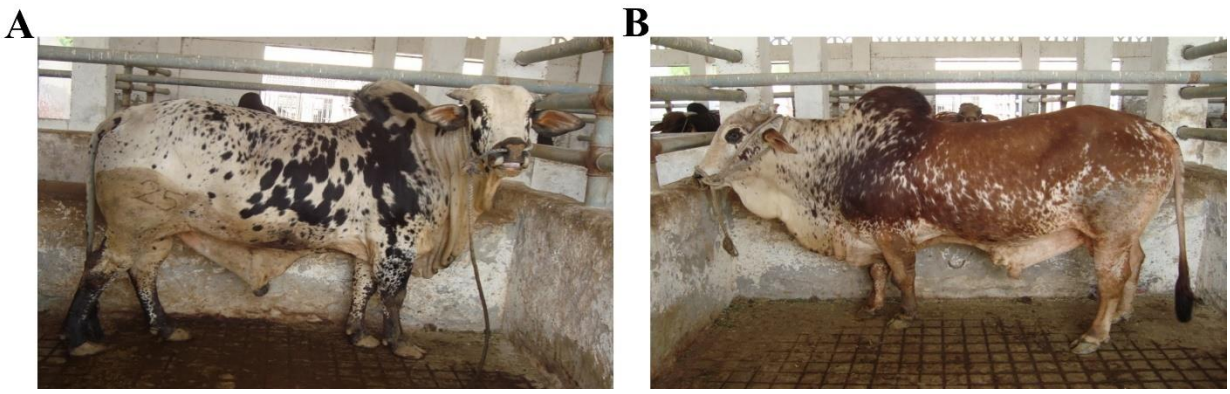

Fig. 2: Elite specimen of black-spotted (a) and brown-spotted (b) Cholistani service bulls being reared for semen collection at the Semen Production Unit, Karaniwala, Bahawalpur, Pakistan (Cholistan).

Blood sampling and serum testosterone concentration

Apropos to the adequate restraint of the animal, blood collection was performed twice in a week throughout the study period in a prescribed aseptic method. Blood sample from each animal was attained in a serum extraction vacutainer (BD Vacutainer, Becton, Dickinson and Company, New Jersey). In this way, sixteen blood serum samples were attained per bull with a total of 96 samples. For minimizing stress to the study animals during blood collection, same personnel and restraining protocol were used. The collection was conducted at the same time (1600 hours) on sampling day. Serum was extracted by centrifugation and serum testosterone level, in ng/mL, was determined using MiniVIDAS 12 Compact Automated Immunoanalyzer using Biomeriux Kit 30418 (CPT Code 84403, Germany). It had a 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.

Statistical analysis

Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA) was used for data analysis. Mean values (±SE) were calculated for all studied parameters. Difference in various study attributes (orchidometric, seminal and andrological) between black-spotted and brown-spotted bulls was ascertained through independent t-test [17] with significance at $P \leq 0.05$.

Results and Discussion

The present work is a novel one being reported for two color variants of indigenous Cholistani breed of cattle being reared under nomadism in Cholistan desert of Pakistan *i.e.* black-spotted and brown-spotted. It takes into account the traditional seminal and orchidometric attributes presently in practice for an appropriate breeding soundness examination (BSE). To the best of our knowledge, there is no such study in literature directed towards the parameters studied in the present work. Hence, the comparison and discussion has been devised on the basis of other parameters, traits and attributes studied for coat colors in various livestock.

The mean (±SE) values for body weight and various orchidometric attributes (SC, SSFT, AvgTL, AvgTW and PTV) for black-spotted and brown-spotted Cholistani service bulls are presented in **Table 1**. The

mean body weight of black-spotted bulls (509.7±2.9 kg) was significantly lower (P≤0.05) compared to brown-spotted ones (545.0±7.7 kg). However, SC, SSFT and PTV were significantly higher in black-spotted Cholistani bulls as compared to their counterpart brown-spotted bulls. No previous study furnishes data on these orchidometric parameters regarding coat color in cattle. However, a study conducted on coat color of bucks has reported a significant influence of coat color on all scrotal parameters. The highest scrotal length and circumference was observed in bucks with brown coat color having white marking [18].

Selection of superior *B. indicus* breeding bulls is a significant in cost management, reducing generation gap, increasing genetic gains which ultimately results in enhancing overall productivity. Several instantly quantifiable attributes such as those reported in present study (BW, SC, SSFT, AvgTL, AvgTW and PTV) at puberty can be effectively implied for suitable selection of breeding bulls particularly in resource feeble settings of the world such as Cholistan desert. The SSFT is reported to have an influence on the semen quality directly in terms of spermatozoan morphology [19]. High temperature in testicles due thermal insulation of scrotum affects detoxification of reactive oxygen species due to decline in superoxide dismutase/catalase activities. This results in impaired spermatogenesis and a compromised semen quality [11].

Table 1: Mean (±SE) values for body weight and various orchidometric attributes of black-spotted (n= 04) and brown-spotted (n= 04) Cholistani service bulls.

Parameters	Black-spotted	Brown-spotted
Body weight (kg)	509.7±2.9*	545.0±7.7
Scrotal Circumference (cm)	36.2±0.3*	34.5±0.4
Scrotal Skin Fold Thickness (cm)	1.1±0.03*	0.9±0.02
Average Testicular Length (cm)	16.5±0.1*	17.5±0.2
Average Testicular Width (cm)	7.1±0.05*	6.9±0.05
Paired Testicular Volume (cm^3)	866.05±19.2*	835.5±26.4

*Values within rows are significant (P≤0.05)

In general, it is a common notion that the pastoralists use their own criteria for identification, selection and kinship assignment for indigenous cattle throughout the world. All the cattle are named at birth and coat color is main factor of identification criterion [20]. Selection criterion is stringently based upon various phenotypic traits (including coat color) and the pastoralists are richly endowed with indigenous knowledge of their native breeds [21,22]. Similar is the case with the Cholistani desert nomads of Pakistan. The ultimate indigenous knowledge of pastoralists allied with incorporation of orchidometric measurements may result in an improved selection procedure for this breed.

Regarding the fresh seminal attributes, the ejaculatory volume (5.8±0.1 mL) and individual sperm motility (63.3±1.4 %) were significantly (P≤0.05) higher in black-spotted bulls as than brown-spotted ones in present study. The percentage of live spermatozoa, and spermatozoa with normal morphology and acrosome was also higher in black-spotted bulls though statistically non-statistically (P≥0.05) (**Table 2**). All the post thaw seminal attributes of the present study were higher in black-spotted Cholistani bulls as compared to brown-spotted ones. However, percentage of live spermatozoa, and spermatozoa with normal morphology and acrosome were significantly higher (P≤0.05), with individual motility and plasma membrane integrity being non-statistically (P≥0.05) higher (**Table 3**). Hence, black-spotted bulls had comparatively better seminal results as compared to their counterpart brown-spotted bulls. Personal communication of the authors with various livestock herders of Cholistan desert revealed that they preferred black-spotted cattle over brown-spotted which strengthen and are indicative through the results of our study. No work has previously been reported for these seminal attributes in context to coat color of service bulls. Some studies have, however, reported effect of coat color on milk yield [23,24], reproductive indices [23], physiological parameters, longevity [2] and fertility rate [1] in differently coat-colored cattle. A study conducted to assess correlation between inseminations per conception (IPC) and coat color of Holstein cattle in tropical environment, significant (P≤0.05) correlation values have been reported for coat color. The number of IPC decreased with an increase in percentage of black coat color revealing a higher fertility rate in black-coated cattle [1]. Similar results have also been reported earlier in which it was noticed that cattle with black hair color had higher conception rates [24]. In another study [23], respiratory rate and panting score did not differ between white and black cows. It was concluded that coat color does not confer any advantage in terms of reproductive attributes if cattle are kept in controlled and thermo-neutral zone. Regarding longevity, an interesting study conducted on black and white Holsteins, it was revealed that black hair-coated cattle have greater longevity as compared to white hair-coated cattle [2]. A study has also revealed that cortisol levels in hair are not affected by pigmentation indicating similar pattern of heat-stress maintenance for both [25].

Table 2: Mean (±SE) values for various fresh semen attributes of black-spotted (n= 04) and brown-spotted (n= 04) Cholistani service bulls

Parameters	Black-spotted	Brown-spotted
Ejaculatory volume (mL)	5.8±0.1*	3.6±0.1
No. of ejaculates/bull	1.8±0.03	1.8±0.05
Mass motility (Score 1-5)	2.1±0.07	2.1±0.1
Individual sperm motility (%)	63.3±1.4*	58.8±2.1
Sperm concentration (mil/mL)	816.7±31.4*	1040.2±50.2
No. of dozes frozen/bull	196.6±10.7	182.2±13.7
Live sperm (%)	83.9±1.6	80.4±2.3
Normal sperm morphology (%)	83.5±1.6	80.0±2.3
Acrosome integrity (%)	83.8±1.6	80.3±2.3

*Values within rows are significant (P≤0.05)

Table 3: Mean (±SE) values for various post-thaw semen attributes of black-spotted (n= 04) and brown-spotted (n= 04) Cholistani service bulls

Parameters	Black-spotted	Brown-spotted
Individual motility (%)	52.4±1.7	51.3±2.1
Live sperm (%)	72.1±2.2*	66.4±2.7
Normal sperm morphology (%)	72.0±2.2*	66.7±2.7
Acrosome integrity (%)	74.3±2.2*	68.8±2.8
Plasma membrane integrity (%)	69.5±2.1	64.4±2.6

*Values within rows are significant (P≤0.05)

In cattle, as for other mammals, the coat color is a phenotype which is because of the presence of two pigments eumelanin (black-brown) and phaeomelanin (red-yellow) released by the melanocyte cells of the skin [26]. The Extension (E) and Aguti (A) loci located on chromosome 18 are, in fact, responsible for regulating the production of both pigments in a controlled fashion [26]. Dominant alleles at E locus usually produce uniform black color in cattle whereas the recessive alleles enhance brown coloration. It has been well established that black hair coat absorbs more solar radiations than white hair coat, though the radiations penetrate deeper into white hair coats. Higher body temperatures in black coat color cattle have also been extensively reported [5,7]. However, black coats are less dense, with thicker and shorter hair, and hence improve heat tolerance in tropical environments [1,27].

The serum testosterone level in black-spotted Cholistani bulls (6.9±0.3 ng/mL) was significantly (P≤0.05) higher compared to brown-spotted bulls (5.4±0.3 ng/mL) (**Fig. 2**). Similar results have been reported for black-coated and brindle-colored cattle from Korea. A strong correlation was reported between coat color and serum testosterone level with the black-coated cattle having higher levels [28]. Serum testosterone level in *Bos taurus* bulls is generally considered to be higher as compared to Zebu bulls [29] causing a reduced sexual activity in bulls of the later species. The results of testosterone level in serum of Cholistani service bulls of the current study are in physiological range reported elsewhere [13]. A higher testosterone level in black-coated Cholistani service bulls in present study is justifiable when seen in perspective to their better results of fresh and post thaw seminal attributes in present study.

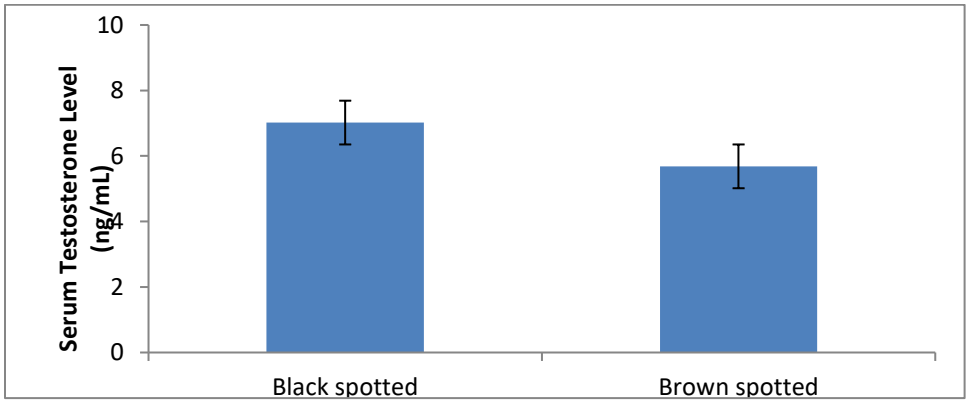

Fig. 1: Serum testosterone level (ng/mL) of black-spotted (n= 04) and brown-spotted (n= 04) Cholistani service bulls. Data are mean ±SE. Bars identified by different letters (a,b) differ (P≤0.05).

Conclusion

In a nutshell, the black-spotted Cholistani service bulls showed higher orchidometric measurements as compared to brown-spotted bulls. This could be correlated to substantially better fresh and post thaw seminal attributes allied with higher serum level of testosterone. The results conjoin with the indigenous perception of Cholistani pastoralists for a liking towards black-spotted Cholistani cattle. It seems

evident to recommend that the rich indigenous knowledge of pastoralists about this breed may be taken into serious account while devising breeding and selection policies to attain appropriate performance parameters. The black-spotted indigenous zebu cattle may perform better than other color variants if kept under better housing, feeding and management regimen. For future horizon, we recommend an analysis of productive attributes, fertility rate, gene mapping and Marker Assisted Selection (MAS) for different color variants of Cholistani breed of cattle.

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Conflict of interest

The authors declare no conflict of interest.

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