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Relationships among frozen-thawed semen fertility, physical parameters, certain routine sperm characteristics and testosterone in breeding Murrah buffalo (*Bubalus bubalis*) bulls

A. K. Singh, P. S. Brar and R. S. Cheema

Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana - 141 004, Punjab, India.
Corresponding author: A. K. Singh, e-mail: assengar2001@yahoo.co.in, PSB: parkashbrar@gmail.com, RSC: ranjna.cheema@gmail.com

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Abstract

Aim: The present study was carried out to examine the relationships among frozen-thawed semen fertility, physical parameters, seminal quality, and testosterone concentration in Murrah buffalo bulls.

Materials and Methods: A total of 30 breeding Murrah buffalo bulls (either progeny tested or under progeny testing program) were randomly selected from two government bull farms in Punjab. None of the bulls selected for this study had any preceding physical abnormality. A field fertility trial was conducted to determine the first service conception rate (FSCR). The number of females inseminated per bull semen was 10. All the bulls were inspected for structural soundness, measurement of scrotal circumference, testicular biometry, and internal pelvic area (IPA). Frozen-thawed semen was evaluated for total motility, progressive motility, viability, concentration, abnormality, and hypo-osmotic swelling test (HOST). Testosterone was estimated in blood plasma, seminal plasma as well as frozen-thawed semen extracts for establishing relationship.

Results: The FSCR was 48% in the bulls having a scrotal circumference of ≥44 cm, although, there was no significant correlation between FSCR and scrotal circumference. Similarly, no consistent relationship existed between sperm concentration and scrotal circumference. A positive correlation was observed between IPA and FSCR (r=0.294). Of the six post-thaw seminal components (total motility, progressive motility, viability, HOST (%), total abnormality and concentration) only total motility had a high significant ($p<0.01$) correlation with FSCR (r=0.694). Varied correlations existed between other seminal parameters and fertility. Using a simple regression analysis, the post-thaw motility, IPA, prepuce length and testosterone (independent variables) combined to explain approximately 62% of the variation in the FSCR (dependent variable).

Conclusion: The present study indicated that despite low to high correlations between seminal characteristics, physical parameters, fertility, and testosterone; the observations support the importance of these components and their function in maintaining semen quality and subsequent fertility.

Keywords: buffalo spermatozoa, fertility, pelvic area, scrotal circumference, testosterone.

Introduction

Artificial insemination (AI) has made possible the effective use of best breeding males, thus greatly improving the genetic quality of breeding herds [1]. The ability to consistently select the bulls with high semen freezability is essential to obtain viable pregnancies following AI. Previously, differences in post-thaw semen fertility between bulls have been reported [2]. A number of semen manipulation techniques are available to improve freezability of spermatozoa [3]. Correspondingly, the knowledge of basic biometric characteristics of the reproductive organs has been found to provide valuable information for the evaluation of breeding and fertility potential of the animals [4]. The determination of scrotal circumference is a good indicator of semen production and eventually volume, sperm motility, viability, and morphology [5]. Similarly, pelvic area is a desirable and highly heritable trait in breeding bulls that has been linked to scrotal circumference, masculinity, semen quality and ultimately inherent fertility [6]. Furthermore, endocrine control of reproductive potential in males is mainly governed by testosterone.

Preliminary studies have indicated that the testosterone levels also influenced the semen quality and fertility although the results were not consistent [7]. Previous studies have found relationships of classical seminal parameters with physical parameters [5], *in vivo* fertility [2] and testosterone concentration [8] individually. However, no report to date exhibited a combination of all variables, which could present a higher predictive value.

The relationship of frozen-thawed semen fertility to scrotal and testicular biometry was sparse and limited in the buffalo bulls; the present study was, therefore, conducted with the objective to help determine

the relationships among fertility of frozen-thawed semen, physical parameters, some spermiogram parameters and testosterone in breeding Murrah buffalo bulls.

Materials and Methods

Animals

Thirty breeding Murrah buffalo bulls (4-8 years) were randomly selected from two government bull farms in Punjab. They were maintained under loose housing system (covered area - 12 ft. × 10 ft. and uncovered area - 25 ft. × 10 ft.) and standard feeding schedule along with ad lib green fodder and water availability and standard management conditions. The bulls were either progeny tested or under progeny testing program. Semen was being collected twice a week through artificial vagina method. None of the bulls selected for this study had any preceding physical abnormalities. All the bulls were examined for breeding soundness, semen evaluation, and blood collection during the month of September, 2012.

Ethical approval

The physical parameters of the bulls were measured and the frozen semen straws were collected from the semen banks. Since, the present study being a part of a larger study for doctorate thesis, approval had been obtained from the Institutional Animal Ethics Committee.

Andrological examination

All the bulls were inspected for structural soundness viz. height, length, width and height of crest, overall body confirmation, the prepuce, the penis, measurement of scrotal circumference, testicular biometry and internal pelvic area (IPA) as per the standard measures. The height (cm) of the bull (distance from ground to the midway point between the tubercoxae), length (cm) of the bull (distance from the spinous process of the fifth thoracic vertebrae to the anterior tail head), sheath length (cm; distance from front of scrotum to preputial orifice) and prepuce length (cm; distance form ventral abdomen to preputial orifice) were recorded. Masculinity score was determined by measuring the width of shoulders, width of crest, height of crest, height, length and weight of the bull and scored from 1 to 10 indicating the least masculine to most masculine bull, respectively.

Scrotal circumference

Scrotal circumference (cm) was measured by holding the testicles firmly into the lower part of the scrotum so as to minimize the scrotal wrinkles. A looped measuring tape was taken transversely at the greatest diameter of the scrotum and tape was pulled in such a way that it was in close contact with the entire circumference.

Testicular biometry

It was calculated by measuring testicular length and width. Testicular length was measured from the distance between caput and cauda epididymis (dorsal to ventral direction) and its width at the widest testicular point (lateral to medial direction) with the help of Vernier caliper and a scale. Both the right testicular volume (RTV) and the left testicular volume (LTV) were calculated using Harriet's formula:

$V\ (cm^3) = L \times W^2 \times 0.522$

Where L being the testicle length and W the testicle width and 0.522 the constant [9].

IPA

Distance between hook bones, between hip bones and between point of croup to hip joint were measured using external pelvimeter to calculate the IPA by the Arloing's formula [10]:

$$\text{Transverse diameter of pelvis outlet (a)} = \frac{\text{Distance between hook bones} + \text{Distance between hip bones}}{4}$$

Vertical diameterof pelvis outlet (b) = $\frac{3}{4}$ × Distance between point of croup and hip joint

Transverse diameter of pelvis inlet = a × 1.22

Vertical diameter of pelvis inlet = b × 1.3

IPA (cm^2) = 1.22a × 1.3b

Where 1.22 and 1.3 are constants.

Semen samples

Thirty mini straws (0.25 mL) from each bull frozen on the same date and of the same batch were collected and earmarked for the study from 30 bulls.

Semen evaluation

Frozen-thawed semen was evaluated for total motility, progressive motility, viability and hypo-osmotic swelling test (HOST). Both total motility and progressive motility were determined through computer assisted semen analysis (CASA; Hamilton-thorn IVOS 12.2). Frozen-thawed semen (10 μL) from each straw was mounted on a disposable CASA slide (Leja-8; IMV Technologies, France) to analyze the total motility and progressive motility. Five randomly selected fields were scanned per straw and five straws per bull semen were evaluated to denote the total motility and progressive motility, obtaining 25 scans for each bull. Semen concentration was determined through photometer (Hamilton Microlab 500 series). Sperm viability and abnormality were established by analyzing the slide stained through nigrosin-eosin staining method [11]. Briefly, a semen sample (one straw) was washed twice in phosphate buffer solution (PBS). One drop of semen was mixed with one drop of stain and a thin smear was prepared using a pre-warmed, clean and grease free glass slide from the semen stain mixture and examined under oil immersion lens of light microscope to determine sperm viability. Bright field microscopy (×400) was used to detect head, mid piece and tail

abnormalities and location of cytoplasmic droplets on spermatozoa. Morphologic defects detected in the spermatozoa included both primary and secondary abnormalities. Functional integrity of the sperm was evaluated by HOST using the hypo-osmotic solution (100 mOsm/L) [12]. Frozen-thawed semen (100 μL) was mixed with 1.0 mL of HOST solution and incubated at 37°C for 1 h. One drop of incubated semen was placed on a slide, coverslip applied and examined under bright field microscope (×400) for curled tail spermatozoa. Similarly, 100 μL of semen was incubated in normal saline under similar conditions and number of curled tail spermatozoa was counted. The number of curl tailed sperm in normal saline was deducted from the number in HOST solution and the resultant figure was taken as the HOS-reactive sperm. A total of 200 sperms each were counted under different fields and percent viability, percent abnormality and HOS reactive sperms were calculated separately.

The mean of 25 scans for the total motility and progressive motility and the mean of three replicates (straws) for the percent viability, percent HOST, abnormality and concentration per bull semen were used for the statistical analysis.

Testosterone estimation

Blood was collected in heparinized vials from bulls after semen collection, plasma harvested and stored at −20°C. Similarly, seminal plasma was separated from fresh semen and stored at −20°C. Frozen-thawed semen was centrifuged at 3000 rpm for 10 min to separate out dilutor and spermatozoa. Sperm pellet was washed twice with PBS (pH 7.4) containing protease inhibitor, suspended in 1.0 mL of 2% sodium dodecyl sulfate (62.5 mM Tris-HCl, pH 8.0) and sonicated at 4°C (20 W, thrice for 20 s each) to remove the dilutor. Finally, the sperm suspension was centrifuged at 15000 rpm for 30 min and the sperm extracts were stored in 0.5 mL fractions at −20°C pending analysis. Blood plasma, seminal plasma and sperm extract testosterone concentration was determined using a Testosterone Microwell ELISA Kit (Tanya Biotech, Mohali, Punjab). The intra- and inter-assay coefficients of variations for low and high control samples were 6.7 and 5.3% and 4.1 and 7.9%, respectively. The detection limit of the assay was 0.3 ng/mL.

Field fertility trial

The number of females inseminated per bull semen was ten. Therefore, 10 mini straws from each bull were used for the field fertility trial. All the buffaloes (n=300) enrolled for fixed time insemination program (October-April) from 4 private organized farms had calved 60-80 days earlier. They were healthy, multiparous (2nd-5th parity) and maintained under standard feeding and management systems. Prior to the start of breeding program, the clinical assessment of genitalia was done ultrasonographically using a B-mode linear array trans-rectal transducer with 5/7.5 MHz interchangeable frequency (AGROSCAN, ECM, France) to visualize a cyclic CL and rule out reproductive tract infections, if any. The buffaloes were synchronized using double ovsynch protocol ($PGF2_{\alpha}$-GnRH-$PGF2_{\alpha}$-GnRH on day - 2, 0, 7 and 9, respectively) followed by fixed time inseminations at 16 and 40 h after last GnRH injection, respectively. The pregnancy diagnosis was done on day 60 post-insemination and confirmed after day 90 using ultrasonography. The first service conception rate (FSCR) was calculated using the following formula [13]:

$$\text{FSCR (\%)} = \frac{\text{Number of buffaloes conceived after first insemination}}{\text{Total number of buffaloes inseminated}} \times 100$$

Statistical analysis

Data were presented as mean±standard error of mean and all analyses were performed with Statistical Package for Social Sciences (SPSS 16.0, Chicago, IL; 2003) program. Duncan's multiple range test and student's *t*-test were used to compare mean values of total treatments with minimum significant interaction at 5% level. The significance of differences among the variables was tested through one-way analysis of variance, and comparison of the variables was done using Karl Pearson's correlation and mixed linear regression analysis. Measurements of post-thaw sperm parameters were entered into the regression model as independent variables with FSCR as dependent variable.

Results and Discussion

Physical parameters and fertility measurements

The bulls used in the present study differed markedly in physical parameters and fertility measurements (Table-1). The overall FSCR was low (37.0±3.2%). The FSCR in estrus synchronized buffaloes depends upon semen handling, semen quality, number of sperms deposited, site of insemination, season of breeding, fertilization status, embryo quality, bull effect and time of AI [14]. In the present study, attempt was made to minimize the variations due to the insemination technique, time of insemination, semen handling and site of semen deposition. Hence, the variation in the FSCR might probably be due to a combination of the post-thaw semen quality and the bull effect. Bull fertility varied widely even after using good quality semen [15]. Correlations between physical measurements of reproductive soundness and fertility in general were low and variable (Table-2). While body weight ($r=-0.237$, $p>0.05$) and masculinity score ($r=-0.176$, $p>0.05$) were negatively correlated with FSCR, sheath length (0.128, $p>0.05$) was positively correlated with FSCR. Prepuce length was the only physical parameter to be significantly and positively correlated ($r=0.391$, $p<0.05$) with FSCR, body weight ($r=0.443$, $p<0.01$) and masculinity score ($r=0.429$, $p<0.01$). Further, masculinity score was highly correlated ($r=0.728$, $p<0.001$) to body weight.

As the body weight, increased, masculinity score increased ($p<0.05$); however, a concomitant increase in the FSCR was not observed (Table-3). Similar observations in Australian Santa Gertrudis bulls were recorded by Bertram [16] who demonstrated inconsistent relationships between live-weight, body condition score, masculinity scores, sheath and prepuce measurements and bull fertility. They further reported that a longer prepuce may result in more serves during collection of semen through an artificial vagina method. Although, physical defects may diminish a bull's ability to produce quality semen and the consequent fertility, the data indicate that the evaluation of bulls solely on the basis of physical traits as means of predicting fertility was questionable.

Table-1: Physical parameters, fertility, semen evaluation and testosterone concentration of buffalo bulls (mean±SEM).

Parameters	Unit	Bulls (*n*=30)	Range
Age	Years	6.1±0.3	4.0-8.0
Body weight	kg	592.8±10.9	512.3-693.5
Height	cm	142.4±1.3	132.0-152.0
Length	cm	104.6±1.8	90.0-120.0
Masculinity score	-	7.2±0.3	5.0-9.0
Width of shoulders	cm	35.2±0.6	30.0-40.0
Width of crest	cm	28.1±0.7	22.0-35.0
Height of crest	cm	25.7±0.9	18.0-32.0
Scrotal circumference	cm	40.2±0.6	36.1-46.1
LTV	cm^3	311.3±7.3	263.1-400.9
RTV	cm^3	315.0±8.0	253.7-417.6
Sheath length	cm	39.9±0.5	35.0-46.0
Prepuce length	cm	10.1±0.4	7.0-14.0
Distance between hook bones	cm	53.1±0.9	45.0-60.0
Distance between pin bones	cm	20.8±0.4	18.0-24.0
Distance between point of croup to hip joint	cm	34.7±0.5	30.0-38.0
IPA	cm^2	760.2±9.0	700.3-856.5
FSCR	%	37.0±3.2	10.0-70.0
Semen			
Total motility	%	55.5±1.6	40.4-72.7
Progressive motility	%	29.5±1.2	17.5-40.0
Viability	%	69.3±1.7	55.8-82.0
HOST	%	65.1±2.2	53.8-80.3
Total abnormality	%	14.9±1.3	2.3-24.7
Concentration	million/mL	921.4±31.0	638-1379
Testosterone			
Blood plasma	ng/mL	14.7±0.5	10.4-20.5
Seminal plasma	ng/mL	2.3±0.2	1.6-3.3
Sperm extract	ng/mL	6.7±0.2	5.6-7.6

HOST: Hypo-osmotic swelling test, FSCR: First service conception rate, IPA: Internal pelvic area, LTV: Left testicular volume, RTV: Right testicular volume, SEM: Standard error of mean

Scrotal circumference and testicular biometry

Data on scrotal circumference and testicular volume revealed a non-significant ($p>0.05$) difference for both LTV and RTV in the bulls. As the body weight increased, the scrotal circumference increased; however, there was no parallel increase in testicular volume, conception rate and sperm concentration ($p>0.05$), which was in accordance with the findings of Costa [17]. Both scrotal circumference (36.1-46.1 cm) and testicular volume (LTV, 263.1-400.9 cm^3; RTV, 253.7-417.6 cm^3) of all the bulls were within the range indicating that sperm cell output did not vary in bulls with differential fertility. Nevertheless, the bulls having a scrotal circumference of ≥44 cm and ≥42 cm had high fertility and high sperm concentration, respectively (Table-4). The probability of a bull to qualify as a satisfactory potential breeder increased until about a scrotal circumference of 38 cm was achieved [18]. In addition, scrotal circumference can serve an indicator of fertility [19]. Although low, yet a positive correlation ($r=0.089$, $p>0.05$) between scrotal circumference and fertility was in agreement with the observations of Peixoto [20] who reported that in AI the bulls are not directly exposed to estrus females for extra-gonadal sperm reserves to be depleted. When synchronized heifers were serviced naturally by mature bulls, the correlation between scrotal circumference and pregnancy rate was relatively high [21]. A positive association of testicular volume with sperm concentration was also observed. The bulls with larger testicular volume (both LTV and RTV) had more sperm concentration. Previous studies have reported that the relative testis size was positively associated with a number of sperm stored in it and negatively with sperm motility and total spermatozoal abnormalities [8,22]. Similarly, larger testes without any abnormality have been reported to produce more sperm than smaller testes [4].

IPA

The IPA recorded in bulls was 760.2±9.0 cm^2. Correlation between IPA and fertility was low. IPA

Table-2: Correlation between physical parameters and fertility in buffalo bulls

Parameter	Body weight	Masculinity score	Scrotal circumference	Sheath length	Prepuce length	IPA
FSCR	−0.237	−0.176	0.089	0.128	0.391*	0.294
IPA	0.314*	0.280	0.205	−0.076	−0.173	
Prepuce length	0.443**	0.429**	0.117	−0.064		
Sheath length	0.147	0.158	−0.261			
Scrotal circumference	0.392**	0.245				
Masculinity score	0.728***					

*p<0.05, **p<0.01, ***p<0.001, FSCR: First service conception rate, IPA: Internal pelvic area

Table-3: Relationship between physical parameters and fertility (Mean±SEM)

Masculinity score	Number of bulls	Body weight (kg)	Width of crest (cm)	Sheath length (cm)	Prepuce length (cm)	FSCR (%)
5	4	518.3±2.3^{a}	24.0±0.8^{a}	37.3±0.9^{a}	8.5±0.7^{a}	37.5±8.5
6	7	556.0±9.4^{b}	26.6±1.2^{b}	38.6±0.7^{ac}	9.5±0.7^{ad}	38.6±5.9
7	5	549.6±9.3^{b}	26.7±1.3^{b}	42.3±1.0^{bd}	9.9±0.9^{ad}	46.0±8.7
8	7	619.1±6.1^{c}	29.5±1.2^{c}	39.9±1.1^{ce}	11.6±0.6^{bc}	34.3±6.5
9	7	676.9±6.1^{d}	31.4±0.7^{c}	41.1±1.1^{deb}	10.4±0.7^{cd}	32.9±8.4

Values with different superscripts in the same column differ significantly (p<0.05), FSCR: First service conception rate, SEM: Standard error of mean

Table-4: Relationship between scrotal circumference, testicular volume, sperm concentration, body weight and fertility (mean±SEM)

Scrotal circumference (cm)	Number of bulls	Testicular volume		Body weight (kg)	Sperm concentration (million/mL)	FSCR (%)
		LTV (cm^3)	RTV (cm^3)			
36.0-37.9	11	338.6±13.1^{a}	320.0±14.8	566.7±18.3^{a}	901.7±65.9^{ab}	34.5±4.9^{a}
38.0-39.9	5	286.4±11.7^{b}	304.3±19.2	595.5±19.7^{ab}	859.0±40.0^{a}	38.0±8.0^{ab}
40.0-41.9	4	282.0±15.4^{b}	327.5±23.0	574.8±38.9^{ab}	863.8±53.7^{ac}	32.5±7.5^{ab}
42.0-43.9	5	312.5±9.8^{c}	296.5±11.5	634.5±22.7^{b}	1013.4±64.3^{b}	34.0±10.3^{ab}
≥44.0	5	306.1±14.8^{bc}	323.5±24.3	620.6±21.5^{b}	981.2±74.9^{bc}	48.0±8.0^{b}

Values with different superscripts in the same column differ significantly (p<0.05), LTV: Left testicular volume, RTV: Right testicular volume, SEM: Standard error of mean, FSCR: First service conception rate

was negatively correlated to the prepuce length (r=−0.173) and sheath length (r=−0.076) and positively correlated to FSCR (r=0.294) and scrotal circumference (r=0.205; Table-2). In addition, a significant positive correlation (r=0.314, p<0.05) between IPA and body weight was observed, which was consistent with the findings of Rusk [23] who recorded a similar correlation (r=0.321, p<0.05) of pelvic area with body weight and body condition score. Pelvic area is an important desirable trait affecting bull fertility [24]. The bulls with smaller pelvic areas were classified as substandard potential breeders. Furthermore, the bulls (>2 years of age) should have a pelvic area in the range of 600-700 cm^2. Singh [25] recorded a pelvic area of 748.15±26.56 cm^2 in mature breeding buffalo bulls (>6 years). They further reported a positive correlation of the pelvic area with fertility (r=0.45) and scrotal circumference (r=0.87). Likewise, the size of the pelvis was positively correlated with scrotal circumference, semen production and subsequent fertility in bulls [6]. A higher IPA in the current observations could be a species and age difference, which was consistent with the results of other workers [26]. Clearly, IPA involved a complex interplay of body development, age and other physical cues.

Post-thaw spermiogram parameters

The correlation between total semen parameters and measures of fertility exhibited a variable and inconsistent response (Table-5). While the progressive motility (r=0.387, p<0.05) had significant moderate correlation with FSCR, the post-thaw motility was highly correlated with FSCR (r=0.694, p<0.01). This conclusion was in accordance with the results of other workers [27] who demonstrated a similar correlation (r=0.791, p<0.01) between the total motile spermatozoa and fertilization rate. Moreover, no relationship between the percent motile spermatozoa and the scrotal circumference could be established; although, a significant (p<0.05) relationship between FSCR and percent motile spermatozoa was observed (Table-6). As the percent motile spermatozoa increased, a simultaneous increase in the FSCR was noticed. This was in agreement to an earlier report by [28] who indicated a significant and consistent association between the percent motile spermatozoa and fertilization rate. The post-thaw sperm viability and FSCR were negatively correlated (r=−0.005). Some workers [29] have reported a low correlation (r=0.06) between live spermatozoa and fertility while others [30] have observed a high correlation (r=0.96). The sperm abnormalities in the current study were well within the permissible limits. Morphologic defects detected in the spermatozoa included both primary (5.7±0.7%) and secondary abnormalities (9.3±0.8%). The prevalence of head, mid-piece, tail abnormalities and cytoplasmic droplets (both proximal and distal) were 0.9%, 0.9%, 4.9% and 1.0%, respectively. Barth and Oko [31] have reported that virtually every bull sound for breeding produced a small percentage of spermatozoa with abnormality in all the ejaculates. Sperm abnormality was negatively correlated with total motility (−0.331; p<0.05) and progressive motility (−0.528; p<0.01). This was in consonance to an earlier report in *Bos indicus* bulls [28]. A poor non-significant correlation (r=0.081) of the total sperm abnormality was found with FSCR. A similar correlation (r=0.074) was reported when synchronized heifers were inseminated artificially [32]. In the present study, a significant positive correlation (r=0.463; p<0.01) was observed between sperm concentration and progressive motility. Although weaker,

Table-5: Correlation between post-thaw semen measurements and fertility in bulls

Parameter	FSCR	Total motility	Progressive motility	Viability	HOST	Total abnormality
Concentration	−0.210	0.304*	0.463**	0.024	−0.007	0.046
Total abnormality	0.081	−0.331*	−0.528**	0.152	−0.013	
HOST	0.419*	0.623**	0.279	0.471*		
Viability	−0.005	0.143	−0.238			
Progressive motility	0.387*	0.582**				
Total motility	0.694**					

*p<0.05, **p<0.01, FSCR: First service conception rate, HOST: Hypo-osmotic swelling test

Table-6: Relationship between percent motile sperm cells and scrotal circumference (mean±SEM)

Percent motile sperm cells	Number of bulls	Scrotal circumference (cm)	FSCR (%)
50.0-59.9	14	40.2±0.9	30.7±4.1[a]
60.0-69.9	10	40.5±1.2	40.0±6.7[ab]
≥70.0	6	39.4±1.3	46.7±4.2[b]

Values with different superscripts in the same column differ significantly (p<0.05), FSCR: First service conception rate, SEM: Standard error of mean

yet a significant positive correlation (r=0.304; p<0.05) of sperm concentration was observed with total motility [5]. The correlation between HOST and viability was moderate (r=0.471) but significant (p<0.05) due to the fact that both analyzed the functional integrity of sperm plasma membrane which is of fundamental importance for capacitation, acrosome reaction and binding of spermatozoa to oocyte. A similar correlation of HOST with fertility (r=0.419, p<0.05) was in agreement to the findings of Rogers and Parker [33]. This correlation was even stronger (p<0.01) between HOST and total motility (r=0.623) that could be attributed to the fact that motility is a function of intra-cellular adenosine triphosphate (ATP). Any leakage of intracellular ATP through the damaged sperm plasma membrane due to anisosmotic condition is certain to affect sperm motility [34].

Testosterone levels

Higher percentage of bulls (70%) had blood plasma testosterone concentrations more than 15 ng/mL. The seminal plasma and sperm extract testosterone concentrations ranged between 1.6-3.3 ng/mL and 5.6-7.6 ng/mL, respectively. The present study is apparently the first to report testosterone levels in frozen-sperm extracts and seminal plasma of buffalo bulls. Sauerwein [35] obtained testosterone concentrations in the seminal plasma of simmental bulls ranging from 5.9-6.8 ng/mL. Individual and species differences, age, target organ responsiveness and interference of seminal proteins may be the possible factors causing divergence in endocrine profile in the current study. The overall regression analysis revealed in approximately 62% variation of the post-thaw motility, IPA, prepuce length and testosterone together in the FSCR. Previously, a similar variation (58%) of libido, semen motility and testosterone with fertility had been reported in breeding bulls [36]. A significant positive correlation (r=0.785; p<0.05) between blood plasma testosterone levels and fertility was in consonance with the results of Kasimanickam [37] who postulated that bulls required threshold level of testosterone to maintain fertility because testosterone is involved in the expression of some fertility associated proteins in the seminal plasma that contributed towards fertility. Further, a highly significant correlation of seminal plasma testosterone (r=0.886; p<0.01) and sperm extract testosterone (r=0.891; p<0.01) levels with fertility was observed. Steroid determination directly in the sperm extracts and seminal plasma gives a more legitimate picture, owing to the site of androgen production, binding to androgen protein secreted by sertoli cells and confined action for sperm production [7].

Conclusion

Variable correlations existed between seminal characteristics, physical parameters, fertility and testosterone concentration. Therefore, a combination of factors taken together must be used to predict the bull fertility. The selection of structurally sound and physically normal bulls having large scrotal circumference, pelvic area and better semen freezability may be considered to have better fertility.

Authors' Contributions

The present study was part of AKSs PhD dissertation. PSB approved the experimentation protocol and provided facilities in performing ultrasound scanning of animals as well as in the estimation of steroids using ELISA. AKS and RSC conducted the experiment. AKS performed statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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