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Evaluation Of Different Crossbreed Beef Bulls Based On Physical And Bio-Chemical Properties Of Semen At BLRI Cattle Research Farm

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Abstract:*Development of livestock is a crying need at present because global demand for animal proteins is increasing day by day.A crucial component of efficient calf production is high quality bull semen for successful fertilization and improved herd health. The investigation was aimed to evaluate the bulls for breeding soundness by assessing their fresh semen. 15 different genotypes beef bulls of (pure breed BCB-1, Limousin cross, Charolais cross, Simmental cross and Brahman cross) were examined physically with a special emphasis to the palpation of the scrotum and testicles and rectal palpation of the pelvic genitalia were carried out once every two weeks. Semen quality parameters (SQPs) such as physico-morphological parameters (motility, viability, total sperm abnormality, sperm concentration) vanguard distance travelled by sperm and biochemical parameters [aspartate amino transaminase (AST), alanine amino transaminase (ALT) and lactate dehydrogenase (LDH), Alkaline Phosphatase (ALP)] were studied in these five genotypes of beef bulls. Semen ejaculates were collected and SQPs analyzed by Hamilton-thorn computer assisted semen analyzer and seminal plasma was separated and preserved at -20^{0}C until analysis. Mean value of semen ejaculate volume among five different genotypes of beef bulls varied from 4.25-9.33 ml. Range of mean value of pH among the bulls were 6.5-6.85. Highest mean value of sperm concentration 362.76 mil/ml was found in Limuosin cross and highest percent motility (53.48%) was also found in Limousin cross. Among these biochemical parameters of seminal plasma, the highest level of ALT (35.67 ±1.52) U/I was found in Simmental cross and the highest level of AST (51 ±4.12) U/I was found in Charolaisbutthe lowest level of ALT (25.7±2.11)U/I and AST (22.8±2.7)U/I both was found in Limousin cross. The highest level of ALP (2116 ±1.52) U/I in seminal plasma among five different breeds of beef bulls was found in Simmental cross and the lowest level of ALP (987.7±2.36)U/Iwas found in Brahman cross. These three parameters are negatively correlated with semen quality. The mean highest level of LDH was found in Limousin (97.2±3.92)U/I. Thus the semen of Limousin cross was better than others and can be used for crypreservation and artifical insemination which would facilitate the development of the beef bulls kept in BLRI.*

Key words*: bull, artificial insemination, semen, sperm.*

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I. Introduction

Livestock are a vital source of economic and social support for millions of poor people throughout South Asia. Global demand for animal proteins is increasing day by day, necessitating increased efficiency of global food production. Improving reproductive efficiency of beef cattle, especially bull fertility, is one of the effective way to meet this ever growing demand of beef in Bangladesh.The cattle supply milk and meat for human consumption, thus play vital role in meeting protein demand**.** The livestock population in Bangladesh has been estimated to be about 49 millions of which the population of cattle is about 29 million (Directorate of Livestock Services, 2012-13). And this is not sufficient to meet the whole demand of beef in Bangladesh. So increasing production and development of beef cattle is one of the most important issues now a day. The contribution of the bull either through the natural mating or artificial insemination (AI) where each bull represents half of the genetic composition of its progeny and many cows can be inseminated with the semen of a single bull and its contribution in the production of meat and milk is of great importance which necessitates careful scrutinization of the productive traits of bulls before extensive use [1, 2, 3]. Artificial insemination allows for maximum use of the most valuable breeders and, at the same time, for significant increase of breeding advance[4].

It is reported that low reproductive rates in tropical location are often blamed on female cattle, although it may be assumed that climate and poor health may also influence the fertility of bulls, especially semen quality and libido[5]. One important factor which limits the reproductive performance and fertility of bulls have been reported to be the quality of semen they produce, which can be affected by numerous exogenous and endogenous factors[1,3]. In Bangladesh, comprehensive work has not been done with regard to quality of semen and fertility of cattle by AI. So extensive study also needed on quality of semen and its correlation to herd fertility.In order to increase the predictive power of assessment, simultaneous analysis of multiple sperm attributes, or outcomes of several laboratory assessments must be combined to look for the overall effect of several independent sperm parameters. Physical and chemical properties of bull semen particularly ejaculate volume, density, pH and motility may influence herd fertility and play important role in successful dairy operation[6]. It is well established that characteristics of bull semen vary widely, not only between the bulls, but also between the ejaculates within the bulls and from time to time or season to season[7]. Semen volume, concentration of spermatozoa, proportion of dead and abnormal spermatozoa, and motility of spermatozoa are recognized as important indices of semen quality and significantly correlated with freezability and/or fertility of bovine semen[8]. Semen producing ability and quality of individual bull are essentials to ensure the supply of superior quality germplasm for maintaining the production performance in future progeny of individual breed in the country[9]. In case of artificial insemination, a number of physical and chemical changes occur in these processes, which may alter properties of bull semen and impair herd fertility[10]. However, artificial insemination allows for maximum use of the most valuable breeders, at the same time, for significant increase of breeding advance. Moreover, using semen of proved quality reduces the spread of sexually transmitted diseases[11]. Some Biochemical parameter such as concentration of glucose, fructose, Na, K, Aspartate aminotransferase, Alanine aminotransferase, Heparin binding protein, creatinine also related to the fertilization capability of bull semen[12]. A close correlation was found between fertility and P, K, vitamin C, Glutamate oxaloacetate transaminase (GOT), alkaline phosphatase and total protein. Subsequently, attention is now being directed towards the assessment of other aspects of semen quality as predictors of bull fertility. Proteins present in the seminal plasma and sperm have been reported as markers of bull fertility. Seminal plasma, a complex mixture of secretions from testis, epididymis and accessory sex glands contained factors that modulated the fertilizing ability of sperm. Proteins such as osteopontin, prostaglandin D, heparin-binding protein (HBP) synthase and bovine seminal plasma proteins (BSP A1,A 2,A 3) have been reported as indicators of bull fertility[13,14,15,16]. 28 - 30 kDa HBP of sperm membrane was considered as one of the genetic markers for male fertility and heritable character.

Concerning the quality of semen in cross-bred bulls, the information available was not sufficient to be used as the basis of genetic development. The main objective of the present study were to evaluate the physico-morphological and biochemical characteristics of semen of the crossbred bulls, to rank up the bulls based on semen quality cryopreservation of the evaluated semen if found suitable of specific bulls, use of cryopreserved semen for artificical insemination and to develop the genotypes of more beef producing beef at the Bangladeh Livestock Research Institute (BLRI), Savar, Dhaka.

II. Materials and methods

Study site

The experiment was carried out on 15 breeding bull of five types of genotypes at the Central Cattle Breeding Station and Dairy Farm, Savar, Dhaka. The farm was surrounded by plain land with plenty of green vegetation. The highest and lowest ambient temperature of the experimental area was recorded as 37-38^{0}C and 15-21^{0}C, respectively. Average humidity was 76.6%.

Experimental bulls

Experimental bulls were BCB-1 pure breed, BCB-1 × Limousin (Limousin), BCB-1 × Simental (Simmental), BCB-1 × Charolais (Charlaise), BCB-1 × Brahman (Brahman). Among those bulls that are being utilized for the semen production at BLRI, 15 bulls of five types of genotypes were selected for this particular study. All the bulls were kept intensively under the same management conditions being given 2 kg concentrate and 9 to 10 kg hay per day, mineral lick every 1.5 to 2 months during dry period (1.25 kg/bull) and green feeds at the time of availability. The bulls were weighed every month and the scrotal circumference was taken once during the study period. The age of the bulls were determined by the date of birth from the record book maintained by AI center and ranged from 36 to 90 months. Detailed information on each bull such as age, body weight, health status, vaccination was obtained from the record books of the bull station.

Examination of bulls

Before introduction to the collection schedule, the bulls were examined physically with a special emphasis to the palpation of the scrotum and testicles and rectal palpation of the pelvic genitalia were carried out once every two weeks. The sexual behavior of the bulls was observed, including libido, erection, mounting

and grasping of the dummy, protrusion of penis and thrust and ejaculation. The scrotum was inspected with respect to size, shape and freedom from skin disease.

Physical examination

The examination was based on internal and external component. A trans-rectal examination was used to evaluate the health of secondary sex organs - urethra, prostate, seminal vesicles, ampullae and vas deferens. Palpation of the testicles and epididymis and examination of the sheath and penis were done to detect abnormalities that could affect breeding performance.

Clinical Examination of Bulls

The bulls were thoroughly examined especially with regard to the locomotion body condition score, rectal temperature, breathing rate and pulse rate. The nutrition condition of the bulls was scored into 1-5 scale[17]. The bulls used to be weighed routinely once in a month. The scrotum of bulls was inspected with respect to size, symmetry and any visible skin diseases. The consistency of testes was assessed on the basis of firmness and resilience into 1-5 scale.

Collection of semen

The semen was collected by artificial vagina[18]. Before collection of semen, all parts of artificial vagina (AV) set were cleaned, sterilized and assembled. The inner liner was put into the cylinder and both ends of inner liner were reflected over the cylinder forming water like space between them. The cone along with vial was slipped over one of the ends of the cylinder and then tightened with rubber band. Two third of the outer jacket of vagina was filled with warm water. The temperature inside the artificial vagina was (110-115) ^{0}F. An air screw was used for blowing air between two layers to create desired pressure. Required amount of lubricant was applied inside the artificial vagina with a glass rod. Component of an artificial vagina includes a glass container for semen with a water jacket and a volume scale, a thin flexible latex sleeve, a latex cone joining the end of the vagina with the collection tube, a rubber cylindrical casing with a valve for pouring water and blowing air, a bag and a thermal protector and a mechanical container. When the bull was sufficiently excited to jump over the dummy, the penis of the bull was directed into the artificial vagina by holding the sheath to collect the semen in a vial. After collection, the vial containing semen was put into hot water at 37^0C for preventing cold shock. It was closed with cotton and labeled. In all of the cases semen was collected by using the artificial vagina, and only the first ejaculate was used for the study purpose and a total of 30 semen samples were collected and analyzed for physico-morphological analysis.

Examination of the collected sample

Physical examination of the semen and the spermatozoa

Immediately following collection, the semen was kept at 34^0C[19] in water bath (IMV, L'AIGLE, France) and examined grossly (for appearance, volume, and presence of foreign materials such as dust or pus), microscopically (for mass activity and individual motility, live/dead count and morphology of the spermatozoa) and concentration, sperm total count, viable number (percent motile multiplied by total count) of the spermatozoa following the recommended procedures[3,19,20,21,22].

Gross examination was made as rapidly as possible after collection for the presence of any dust, hair, or any foreign body, and to note the color of the semen for each animal in each breed and was recorded as creamy, white, yellow, watery, or brown. The volume of the ejaculate from each animal in each group was recorded in graduated collection tubes to the nearest 0.1 ml.

A large drop of undiluted semen was placed on warmed slide (37^0C) and examined on a stage warmed thermostatically set (at 37^0C) phase contrast microscope (Nikon, Japan) at magnification of 100 times (100x) and will be scored for mass activity from 0 to 5 according to the intensity of the wave motion. The individual motility of sperm cells was estimated as a percentage by examining undiluted semen placed on warmed slide covered under warmed cover slip and then those sperm cells which exhibited progressive movements under stage warmed (37^0C) microscope at a magnification of 200x and scored 0 to 100 % according to the estimated percentage of spermatozoa which move in a progressive forward direction.

Sperm cell concentration was determined by using calibrated spectrophotometer (IMV, Technologies France) and Hamilton-Thorne Computer Assisted Semen Analyzer. The sperm concentration obtained was multiplied by the total volume of the ejaculate (to get total count of spermatozoa) the total count was multiplied by progressively forward moving percentages (individual motility) to get viable number of the spermatozoa[23].

For morphological examination of the spermatozoa, one milliliter of Hancock solution (Buffered formole saline) was kept in 34 ^{0}C in water bath to which one drop of fresh semen was added with warm (37^0C) Pasteur pipette and gently mixed for preservation of the spermatozoa. Later morphological abnormalities mainly head, mid-piece and tail defect, proximal and distal droplets were examined by placing small drop of the sample

on clean grease free glass slide and covering by cover slide under phase contrast microscope (200x) (Hancock's technique). By this technique 500 spermatozoa were counted and the abnormalities were visualized and recorded as head, mid-piece (body) and tail abnormalities[20, 21]. Characterization of head abnormality (including acrosome defect) was made on 500 spermatozoa stained by the William's technique from the sample preserved by using Hancock solution.

The proportion of live spermatozoa was determined on smears prepared from fresh semen by the Eosin-Nigrosin technique during which 500 spermatozoa were counted under 1000x or oil immersion lens (light microscope). In this technique: two drops of mixed stain and one drop of fresh semen were mixed and smears were made on glass slide (by using spreader glass), allowed to dry in air, and finally examined under bright field light microscope (1000x). Cells which accepted Eosin subsequently stain pink, against dark background of Nigrosin and were taken as dead. Similarly, sperm cells which exhibited such property to at least half of the sperm head were taken as dead. On the other hand, those sperm cells that didn't accept the stain and remained white were taken as alive. Sperm dimension measurements were done by measuring the head, midpiece and tail of the spermatozoa from those stained by the William stain technique (using Bright field microscope and micrometer) and five spermatozoa were measured from each slide and a total of 50 spermatozoa for each bull breed were measured.

Biochemical analysis of enzymes, in seminal plasma

Following collection Immediately after the analysis of physico-morphological properties, remaining semen was centrifuged at 3000 rpm for 10 minutes and the supernatant, seminal plasma was separated from spermatozoa and the procedure was repeated with the supernatant and spermatozoa was decanted, preserved at -20^0C until analysis for enzymes (GOT, GPT, ALP, AST).

Alanine aminotransferase (ALT/GPT) analysis

Analysis were carried out by Spectrophotometric measurement, colorimetric method, using commercially available kit (Randox, UK) and the ALT activity was measured at 37 ^{0}C and wavelength 400 nm, which is based on the optimized method according to the recommendations of the Deutsche Gesellschaft fur KlinischeChemie. In these tests a 10 µl drop of the seminal plasma was deposited on the Vitros ALT slide and was evenly distributed by the spreading layer (containing the ALT substrate L-alanine and sodium α-ketoglutarate) to the underlying layers. This method is based on the principle that ALT/ GPT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate forming glutamate and pyruvate. The concentration of pyruvate is measured by taking optical density using spectrophotometer and the optical density is converted to enzyme activity according to kit protocol. The enzyme activity was given in units per liter (U/L). One U has been known to be the amount of enzyme which converts one micro mole of the substrate per minute.

Aspartate aminotransferase (AST/GOT) analysis

Analysis were carried out by spectrophotometric measurement using commercially available kit (Randox UK) and the AST activity was measured at 37 ^{0}C and wavelength of 305 nm, which is based on the optimized procedure IFCC, method modified to 37 ^{0}C. This method is based on the principle that AST/GOT catalyzes the transfer of an amino group from L-aspartate to α ketoglutarate in the presence of Pyridoxal-5-phosphate (P-5-P) forming glutamate and oxaloacetate. The final reaction step involves the peroxidase catalyzed oxidation produce a colored dye. The rate of change in reflectance density is measured over a linear region then converted to enzyme activity in units per liter (U/L).

Alkaline phosphatase analysis (ALP)

Analysis were carried out by Spectrophotometric measurement, colorimetric method, using commercially available kit (Randox, UK) and the ALP activity was measured at 37 ^{0}C and wavelength 400 nm, which is based on the optimized method according to the recommendations of the Deutsche Gesellschaft fur KlinischeChemie. The alkaline phosphatase present in the sample catalyzes the hydrolysis of the p-nitrophenyl phosphate to p-nitrophenol at alkaline P^H, 37^{0}C. The p-nitrophenol that absorbs light at wavelength in the region of 400 nm, and it is measured by spectrophotometer. The optical density is then converted to enzyme activity measured in units per liter (U/L) according to kit protocol.

Lactate dehydrogenase (LDH)

Analysis were carried out by Spectrophotometric measurement using commercially available kit (Randox, UK) and the LDH activity was measured at 37 ^{0}C and wavelength of 305 nm using spectrophotometer. The optical density is then converted to enzyme activity measured in units per liter (U/L) according to kit protocol.

Data analysis

For each parameter the mean value was calculated for weekly values in six bulls (pooled mean) which was then analyzed for its overall mean, median, minimum, maximum and standard error of semen characteristics by using the descriptive statistics and bivariate correlation coefficient of those semen characteristics were analyzed using SPSS (2002) statistical package, and the 95 % confidence interval of semen characteristics were analyzed using STATA (2001) statistical package. Proportion calculation was used to see the proportion of different semen colors and to see the relation between the color of semen and its spermatozoa concentration. A single sample t-test was used to compare the mean values of the present semen parameters with the literature values.

III. Results

Physical examination

The general health conditions of bulls were found to be good. The confirmation of the locomotory organs of all bulls was found to be normal. Sexual behaviour of the bulls like libido, erection and protrusion of the penis, mounting and grasping of the dummy, seeking movement, thrust and ejaculation were found to be satisfactory. The bulls were all free from venereal diseases. Sexual behaviour of all the bulls was satisfactory. Britoet al., found that scrotal circumference was a good predictor of sexual maturity in Bosindicus bulls[23]. As scrotal circumference increases, age at puberty decreases and productivity is improved in daughters[24].

Semen quality analysis

The results of the semen analysis based on a twice per week collection for 12 consecutive weeks for the 15 bulls of five genotypes kept at BLRI and total 29 semen samples examined for physico-morphology and enzyme analysis are given as follows:

Physico-morphological properties of semen

Data of Color, consistency, opaqueness given below show that creamy colored are generally thick and cloudy. Yellowish and watery colored semens are thin and transparent.

Table 1: Result of physical test fresh semen of five different crosbred beef bulls

Animal tag	Color	Consistency	Opaqueness	Uniformity	Odour
Purebred BCB-1	Watery-white	Thin White	Cloudy	Uniform	Characteristics semen odour
Limousine cross	Creamy-milky	Thick creamy	Opaque	Uniform	Characteristics semen odour
Charolais cross	Yellowish	Thin watery	Transparent	Uniform	No
Simmental cross	Creamy-watery	Thin White	Cloudy	Uniform	No
Brahman cross	Creamy	thick creamy	Cloudy	Uniform	Characteristics semen odour

Observations on semen color in bulls in this study (Table 2) showed that 37.93 % and 24.24 % and 24.13% of the semen collected during the study period had creamy, milky and watery colors respectively, the remaining portion being yellow, yellowish (13.13%).

Table 2: Proportions of different semen colors in six indigenous bulls

No	Color	Unit	Frequency	%
1	Creamy	PCS	11	37.93
2	Milky	PCS	7	24.14
3	Yellowish	PCS	4	13.8
4	Watery	PCS	7	24.13
Total		PCS	29	100.00

Here: % = Percent

As it is given in Table 3, the creamy colored semen had spermatozoa concentration which ranged from 0.1-0.3 to 1.2-1.5×10^3 million/ml to 1.2-1.5×10^3 million/ml, the greatest part (45.45%) being those which had spermatozoa concentration between 0.3-0.6 million/ml. The largest proportion (57.14%) of the milky semen had spermatozoa concentration which lies between 57.14 million/ml. The largest proportion (71.34%) of the watery semen had spermatozoa concentration which lies 0.1-0.3million/ml. The largest proportion (75%) yellow semen had spermatozoa concentration of 0.1-0.3 1.1million/ml.

Table 3: Relation of semen colors and spermatozoa concentration in six indigenous bulls

No	Sperm conc. (10^3mil/ml)	Unit	Frequency	%
Creamy	0.1-0.3	PCS	4	36.37
	0.3-0.6	PCS	5	45.45
	0.6-0.9	PCS	0	0
	0.9-1.2	PCS	0	0
	1.2-1.5	PCS	2	18.18
	Total		11	100.00
Milky	0.1-0.3	PCS	3	42.86
	0.3-.0.6	PCS	4	57.14
	Total		7	100
Watery	0.1-0.3	PCS	5	71.43
	0.3-0.6	PCS	2	28.57
	Total		7	100
Yellowish	0.1-0.3	PCS	3	75
	0.3-.6	PCS	1	25
	Total		4	100

Volume, pH and Sperm concentration in fresh semen five different genotypes of bulls

During study, the mean semen volume was 4.79±0.65ml, in four different breeds of bulls and ranged from 2.00ml to 8.50ml. The mean value of semen motility was found 66.64± 0.50 ml, 5.16±0.92 ml, 9.33±2.52 ml, 5.75±4.83 ml, 4.25±1.41ml in Purebred BCB-1, Limousine cross, Charolais cross, Simmental cross,Brahman cross, respectively and the motility was ranged from 55% to 75%. The mean semen concentration of five different breeds of bulls was observed 219.72±84.75million/ml, 362.76±272.76 million/ml, 228.81±157.91 million/ml, 195.95±117.0 million/ml, 290.64±35.88 million/ml and which ranged from 800 to 1775 million/mm3. The mean value of pH of semen was 6.59±0.57, 6.62±0.09, 6.80±0.05, 6.85±0.07, 6.71±0.30 in five different breeds of bulls and ranged from 6.50 to 6.90.

Table 4: Physical properties and pH of fresh semen of different beef genotypes

	Genotypes (Mean±SD)				
Parmeters	Purebred BCB-1	Limousine cross	Charolais cross	Simmental cross	Brahman cross
Volume(ml)	4.79±0.65[a]	5.16±0.92[b]	9.33±2.52[c]	5.75±4.83	4.25±1.41
pH	6.59±0.57[a]	6.62±0.09[a]	6.80±0.05[b]	6.85±0.07[b]	6.71±0.30[c]
Sperm conc.(mil/ml)	219.7±84.75[a]	362.76±272.7[b]	228.81±157.9[c]	195.9±117.0[d]	290.64±35.8[e]

Results are expressed as mean± SEM (Standard error of mean). Values in the same row that do not share common superscripts are significantly different at P<0.05. SD = Standard Deviation, M/ml = Million per milliliter.

Motility analysis

As regards to the motility of semen, the highest (53.48±11.75%) value of motility of semen was found in Limousine cross breeds of bulls and lowest (22.38±31.65%) was found in Red Purebred BCB-1 of bulls. The value of motility of semen in other three breeds was 15.52±25.40, 23.02±4.69 and 47.57±5.69 for Charolaise cross, Simmental cross, Brahman cross breeds of bull respectively.

Table 5: Motility of fresh semen of different cross bred beef cattle genotypes

Motility(%)	Genotypes(Mean±Sd)				
	Purebred BCB-1	Limousine cross	Charolais cross	Simmental cross	Brahman cross
Static	77.62±31.65[a]	41.62±8.34[b]	85.52±74.60[c]	76.95±4.74[a]	52.1±5.23[d]
Progressive	11.37±16.07[a]	31.29±13.28[b]	9.30±9.23[c]	5.67±2.92[d]	22.45±16.76[e]
Motile	22.38±31.65[a]	53.48±11.75[b]	15.52±25.40[c]	23.02±4.69[b]	47.57±5.69[d]
Slow	1.98±2.80[a]	5.48±9.31[b]	0.0[c]	1.67±0.61[d]	1.42±0.67[e]

Results are expressed as mean± SEM (Standard error of mean). Values in the same row that do not share common superscripts are significantly different at P<0.05.

Highest amount (85.52±74.60%) of static sperm was found in Charolaiscross, and the lowest concentration (41.62±8.34%) of static sperm was found in Limousine cross. In case of progressiveness of sperm, which is important for fertility, highest concentration (31.29±13.28%) was found in Limousin cross and the lowest (5.67±2.92%) concentration was found in Simmental cross. The highest percent of slow sperm (5.48±9.31%) was found in Limousin and the lowest was in Charolaise cross (0%).

Relation between sperm concentration and motility of semen of five breeds

Comparasion between motility and sperm concentration of semen of five breeds showed that, there was a strong relation between this. The highest amount of both sperm concentration (363mil/ml) and motility ~50% was found in Limousin and the lowest motility was found ~19% in Charolaise cross and lowest sperm conc. ~196% was found in Simental cross.

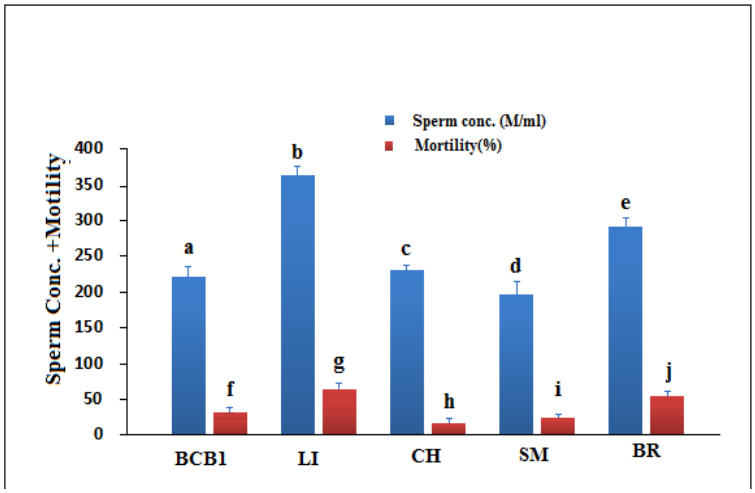

Figure 1: Graphical representation of sperm conc. and Motility of fresh semen of five genotypes of breef cattle. The bars represent the mean ± SEM (Standard error of mean). Data were analyzed by one-way ANOVA followed by Fisher's PLSD for post hoc comparison. Bars with different alphabets are significantly different at P< 0.05. Here, BCB-1= Pure Breed BCB(Pabna bull); LI = Limousin cross, CH = Charolaise cross, SM = Simental cross; BR= Brahman cross.

Morphometric analysis

Highest amount of bent tail **(**26.33±5.14) was found in simmental cross and the lowest (5.9±2.25) in Limousine. Coiled tail was highest (4.63±4.07) in Charolais and lowest (0.45±0.64) in Brahman cross. DMR was highest in BCB-1 and Lowest in Brahman. Distal Droplet was highest (3.92±1.77) in simmental cross and lowest (0) in Charolais cross. Proximal Droplet was highest (62.67±6.09) in Limousin and lowest (25.45±5.87) in Brahman cross.

Table 6: Morphometric parameters of fresh semen of different cross bred beef cattle

Parameters			Genotypes		
	Purebred BCB-1	Limousine cross	Charolais cross	Simmental cross	Brahman cross
Bent tail	19.38±7.39	5.9±2.25	6.42±7.17	26.33±5.14	19.97±7.32
Coiled tail	1.93±2.7	1.33±1.07	4.63±4.07	3.73±3.02	0.45±0.64
DMR	4.74±5.69	1.78±1.67	4.73±3.80	4.30±3.58	1.175±0.11
Distal Droplet	1.43±2.03	0.78±0.75	0	3.92±1.77	1.675±2.37
Proximal droplet	49.33±18.05	62.67±6.09	7.96±11.20	42.75±13.03	25.45±5.87

Values are shown as (Values±Sd), Here Sd = standard deviation

Biochemical analysis

Bull semen has of various types of enzymes such as Aspartate Aminotransferase (AST), Alanine Amiotransferases (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH) etc. Result of the analysis of this this enzyme are given below:

Alanine Aminnotransferase (ALT)

Values of ALT level in among five genotypes of beef bulls significantly differ at P<0.05. The highest level of ALT (35.67 ±1.52) in seminal plasma among five different breeds of beef bulls was found in Simmental cross and the lowest level of ALT (25.7±2.11) was found in Limousin cross. Level of ALT in other genotypes of beef bulls was 30.3±2.13 34.2±90.97 27.2±2.36 in pure breed BCB-1, Charolaise and Brahman respectively.

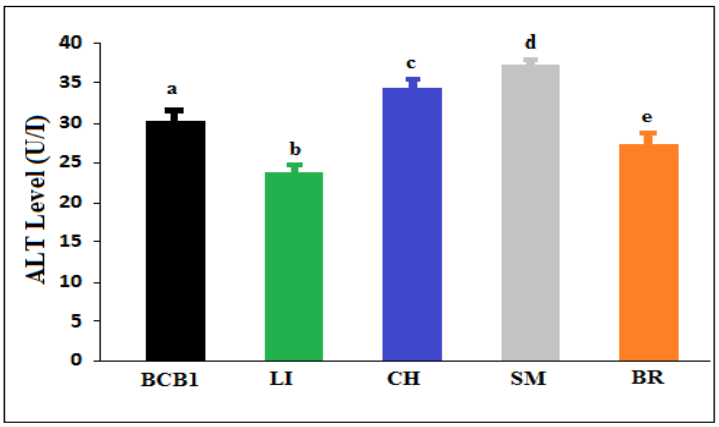

Figure 2: Graphical representation of Aspartate Aminotransferases(ALT) of seminal plasma of five genotypes of breef cattle. The bars represent the mean ± SEM (Standard error of mean). Data were analyzed by one-way ANOVA followed by Fisher's PLSD for post hoc comparison. Bars with different alphabets are significantly different at P< 0.05. Here, BCB-1= Pure Breed.

Alkaline Phosphatase (ALP)

The highest level of ALP (2116 ±1.52) in seminal plasma among five different breeds of beef bulls was found in Simmental cross and the lowest level of ALP (987.7±2.36) was found in Brahman cross. Level of ALP in other genotypes of beef bulls was 1384±2.13, 1446±2.11, 1467±0.97 in pure breed BCB-1, Limousin and Charolaise respectively. Values of ALP level in among five genotypes of beef bulls significantly differ at P<0.05.

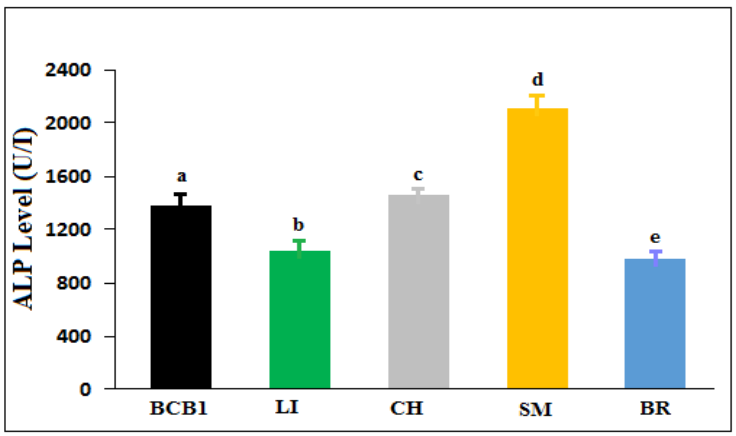

Figure 3: Graphical representation of Alkaline Phosphatase (ALP) of seminal plasma of five genotypes of breef cattle. The bars represent the mean ± SEM (Standard error of mean). Data were analyzed by one-way ANOVA followed by Fisher's PLSD for post hoc comparison. Bars with different alphabets are significantly different at P< 0.05. Here, BCB-1= Pure Breed BCB (Pabna bull); LI= Limousin cross, CH= Charolaise cross, SM=Simental cross; BR=Brahman cross.

Aspartate Aminotransfarases (AST)

The highest level of AST (51 ±4.12) in seminal plasma among five different breeds of beef bulls was found in Charolais and the lowest level of AST (22.8±2.7) was found in Limousin cross. Level of AST in other genotypes of beef bulls was 37±3.1,29.8±2.82, 41.5±4.12, in pure breed BCB-1, Charolaise and Simmental respectively.

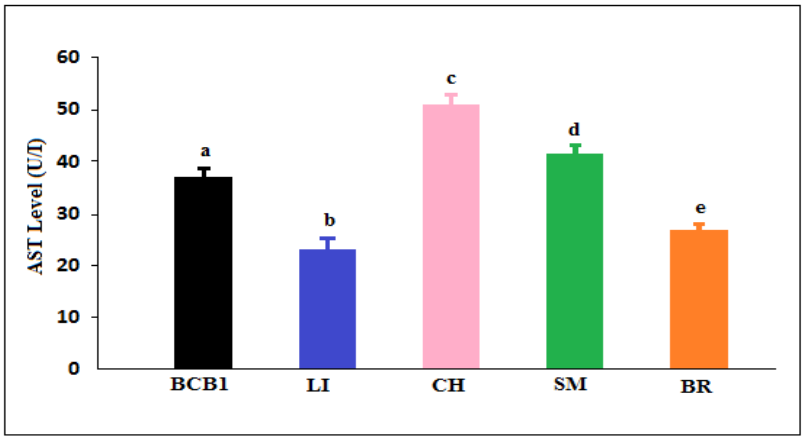

Figure 4: Graphical representation of Aspartate amino transferases(AST) of seminal plasma of five genotypes of breef cattle. The bars represent the mean ± SEM (Standard error of mean). Data were analyzed by one-way ANOVA followed by Fisher's PLSD for post hoc comparison. Bars with different alphabets are significantly different at P< 0.05. Here, BCB-1= Pure Breed BCB (Pabna bull); LI= Limousin cross, CH= Charolaise cross, SM=Simental cross; BR=Brahman cross

Lactate Dehydrogenase (LDH)

The highest level of LDH (97.2 ±3.92) in seminal plasma among five different breeds of beef bulls was found in Limousin cross and the lowest level of LDH (41±1.99) was found in Simmental cross. Level of LDH in other genotypes of beef bulls was 69.5±4.12, 51±1.74, 86.41±3.19 in pure breed BCB-1, Charolaise, Brahman respectively. Values of ALP level in among five genotypes of beef bulls significantly differ at P<0.05.

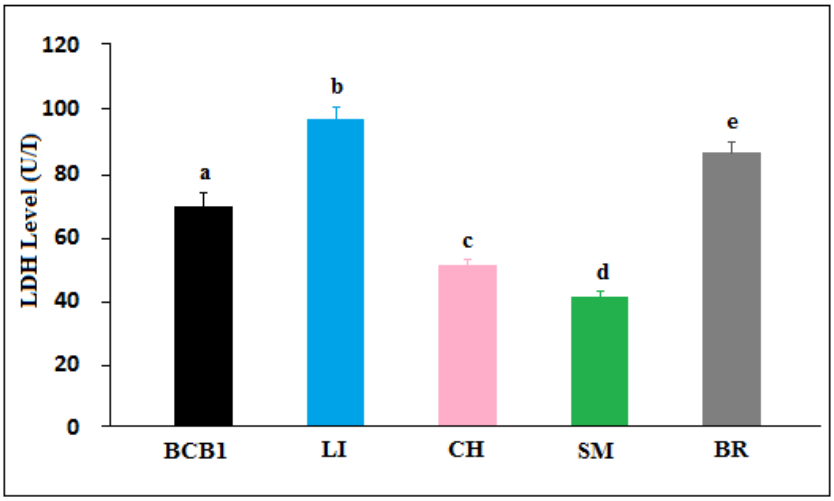

Figure 5: Graphical representation of Lactate Dehydrogenase analysis (LDH) of seminal plasma of five genotypes of breef cattle. The bars represent the mean ± SEM (Standard error of mean). Data were analyzed by one-way ANOVA followed by Fisher's PLSD for post hoc comparison. Bars with different alphabets are significantly different at P< 0.05. Here, BCB-1= Pure Breed BCB (Pabna bull); LI= Limousin cross, CH= Charolaise cross, SM=Simental cross; BR=Brahman cross.

IV. Discussion

In this study it was observed that 36.37%, 45.45%, 18.18 % of the semen with creamy color had spermatozoa concentration of 0.1 - 0.3, 0.3 - 0.6, 1.2 - 1.5, million/ml respectively. The observed milky colored semen also had spermatozoa concentration of 0.1 - 0.3, 0.3 - 0.6, million/ml in 42.86%, 57.14 %, of the cases respectively. In case of creamy colored semen, this result agrees with former reports. It has been reported that the semen with spermatozoa concentration greater than or equal to 1.0 - 1.2 million/ml has been reported to have light creamy to creamy color. Semen with spermatozoa concentration of 500,000 to 600,000 per ml has been reported to be milky, and that has spermatozoa concentration less than 300,000 per ml has been known to be watery. The present observation of milky (with spermatozoa concentration ranging from 0.1 - 0.3 to 1.2 - 1.5 million/ml) and watery semen (with spermatozoa concentration of 0.51 billion/ml) is higher than that reports of Roberts[21]. This variation might be associated to breed or due to subjectivity of the color determination technique.

In this study the experimental bulls had mean semen volume of pure breed BCB1, Limousin cross, Charolaise cros, Simmental crss and Brahman cross were (4.79±0.65), 6.62±0.09, 6.80±0.05, 6.85±0.07, 6.71±0.30 respectively. This value is significantly lower ($P < 0.01$) than the semen volume reported in Bostaurusbulls (6.9 ml and 8.2 ml) in different years in Brazil and similar in Bosindicus in Brazil[23], the value for the latter being 6.6 ml and 6.7 ml in different years. The semen volume reported by Ahsanet al., in Sahiwal bulls (3.64 ml) is significantly lower than this value. The same author reported semen volume of 5.62 (0.14) in Friesian- Sahiwal cross which is significantly lower than the present value ($P < 0.01$)[25]. Such variability between reports on semen volume might be attributed to difference in age, breed, nutritional status, geographic location, season of the year the study covers, method of the semen collection procedure and frequency[1, 3, 20, 26, 27, 28]. However, the range of values given for semen volume in the literature agrees well with the present result[3, 19]. Considering the breed effect, it was found that the volume of semen varied among the breeds in this study. Shaha et al., found significantly ($p<0.05$) highest (7.6 ml) volume of semen was in Holstein-Friesian × Zebu crosses and lowest (4.0 ml) in Sahiwal × Zebu[29]. Sane et al., found that the mean volume of the ejaculate in adult dairy and buffalo bull was 5.4 - 6.5 ml and 1.5 - 3.7 ml (range: 0.5 - 6.0 ml) respectively[30].

Semen pH is a measure of the acidity or alkalinity of semen. pH value of semen of different breeds of bull showed that, there was no significant differences between BCB-1 pure breed, Limousim cross and among Charoliase cross, Simmental cross, Brahman cross breeds of beef (table 4). The result was found in accordance with references found that average pH about 6.1 - 6.5[29, 31]. Lowering of pH with lactic acid was demonstrated to immobilize bull sperm [32]. The pH of seminal plasma ranges from 6.5 - 7.4, which is common in the domestic species and has the potential to neutralize vaginal acid[33]. The pH value did not differ significantly between breeds and season. In a study, Shahafound that the pH of Friesian cross Zebu varied from 6.1-6.5[29]. Therefore, the result of the current study and other relevant findings indicate that pH of semen is not markedly influenced by the variation due to breed. Motility is one of the most important requirements of fertile semen. Donhamet al., found that semen below normal motility (≥ 90 %) was less than half as effective in producing optimum conception rate[34]. Davis reported motility of spermatozoa as one of the best single evidence of viability[35]. Duration of motility in stored semen was reported by Comstock as another reliable index of fertility[36]. Lasley found no significant difference in fertility of semen containing 55 - 95 % live sperm, however, semen containing 20 % of live sperm was infertile[37]. In this study it was observed that the mean (SE) individual motility of spermatozoa of BCB-1 pure breed, Limousin, Charolaise, Simmental cross and Brahman cosss was (22.38±31.65)%, (46.48±11.75)%, (15.52±25.40)%, (23.02±4.69)%, and (47.57±5.69)% respectively. The individual motility of spermatozoa reported by Ahsanet al., as 50.5 % and 60.55 % respectively in Friesian-Sahiwal cross and Sahiwal bulls [25]. Individual motility reported by Andrabiet al., as 55.0 % in Friesian-Sahiwal cross bulls are significantly lower than the present value[38]. The highest motility among the five genotypes of bulls was found in Limousin cross (53.48%) and the lowest motility was found in Charolaise cross. In Brahman cross motility was found 47.57%. According to Hossainet al., the semen of Limousin cross and Limousin are very good and fair grade respectively[10].

Sperm concentration in semen could be considered as an initial indicator of semen quality in semen used for cryopreservation [39]. Significant differences in sperm concentration have been shown in semen from different bulls [40, 41]. Wide ranges have been known for normal fertile bull spermatozoa concentration as 800 - 2000, 1000 - 3000 million per milliliter of semen[3, 42]. The variability of spermatozoa concentration with different works report could be due to variation in genotype, nutrition, age, management, semen collection frequency and technique[1, 3, 20, 26, 27, 38]. Present study found that the mean spermatozoa concentration of semen was 219.7±84.75, 362.76±272.7, 362.76±272.7, 195.9±117.0,290.64±35.8 million/ml in BCB-1 pure breed, Limousin, Charolais, Simmental cross and Brahman cosss respectively. Highest amount of sperm concentration (362.76±272.7) was found in Limousin cross and the lowest (195.9±117.0)insimmental cross. The motility of semen was also found highest in Limousin cross. A positive correlation between sperm concentration at semen collection and motility has been reported, in respective to motility and sperm concentration Limousin cross is the best among the five genotypes of beef bulls[43, 44]. The spermatozoa of normal fertile bull has been recommended not to contain more than 20 % total abnormality, and individual head, midpiece and tail abnormality of 10 % or more [2, 3]. Highest amount of bent tail **(**26.33±5.14) was found in simmental cross and the lowest (5.9±2.25) in Limousine. Coiled tail was highest (4.63±4.07) in Charolais and lowest (0.45±0.64) in Brahman cross. DMR was highest in BCB-1 and Lowest in Brahman. Thus study showed Limousin cross and Brahman cross are good in regards to physical abnormality.

In the present study, enzymes studied include - Alanine Aminotransferases, Aspartate Aminotransferase, alkaline phosphatase and lactate dehydrogenase, these enzymes are used as good indicators of semen quality because they measure PM stability of spermatozoa [45]. AST and ALT are essential for metabolic processes which provide energy for survival, motility and fertility of spermatozoa and these transaminase activities in semen are good indicators of semen quality because they measure sperm membrane stability [46]. Thus, increasing the percentage of abnormal spermatozoa in ejaculate causes high concentration of

transaminase enzyme in the extra cellular fluid due to sperm membrane damage and ease of leakage of enzymes from spermatozoa [47]. Analysis of these enzyme profiles revealed that good quality ejaculates have significantly lower AST and ALT than poor quality ejaculates[48]. The possible source of these enzymes is thought to be the testes or epididymides because they show a positive correlation with sperm concentration and a negative correlation with semen volume [49].Higher activity of AST and ALT in poor quality ejaculates seminal plasma clearly indicated that much of the enzyme leaked out in the extracellular fluid following ejaculation due to structural damage, increase cell membrane permeability, destabilize the membrane integrity of acrosome, plasma, mitochondria and flagella of the sperm. A positive correlation of AST/GOT activities in post thaw semen, with acrosomal damage in ruminant spermatozoa and a negative correlation with fertility, were also reported by Zhao-Qi et al.,[50]. Results show that the highest level of ALT (35.67 ±1.52) in seminal plasma among five different breeds of beef bulls was found in Simmental cross and the lowest level of ALT (25.7±2.11) was found in Limousin cross. The highest level of AST (51 ±4.12) in seminal plasma among five different breeds of beef bulls was found in Charolais and the lowest level of AST (22.8±2.7) was found in Limousin cross. In regards to ALT and AST enzymes the semen of limousine cross is best among five the genotypes of beef bulls. Seminal ALP activity is observed on sperm head, midpiece and tail fragments and it is known to regulate phosphorylation of proteins by the cAMP-dependent protein kinase necessary for spermatozoal motility [51].

Dhami and Kodagalistated that ALP and GOT could be used as markers for fertility in bull because they observed a negative correlation between ALP leakage and motility of bull spermatozoa[52]. Results show the highest level of ALP (2116 ±1.52) in seminal plasma among five different breeds of beef bulls was found in Simmental cross and the lowest level of ALP (987.7±2.36) was found in Brahman cross and ALP level in Limousin cross also lowest than other three genotypes of beef bulls. In case of ALP parameter semen of Brahman cross and Limousin cross are better than others. Research revealed that LDH could be responsible for driving glycolysis when O_2 is limited, by carrying NADH-mediated reduction of pyruvate to lactate, and reduced LDH activity in SP might indicate disturbed spermatozoal function and metabolism [53]. So the presence of high conc. of LDH in SP is important for quality of semen. The highest level of LDH (97.2 ±3.92) in seminal plasma among five different breeds of beef bulls was found in Limousin cross and the lowest level of LDH (41±1.99) was found in Simmental cross. Thus results show Limousin cross are better in respective of LDH level.

V. Conclusion

This study evaluated the semen physico-morphological and biochemical characteristics of five types of genotypes of beef bulls kept at the BLRI AI center. Based on the physico-morphological and biochemical parameters of fresh semen analyzed from these bulls, it was observed that most of the parameters are best in Limousin cross. Brahman cross also showed better than other types of genotypes beef bulls except Limousin cross. Semen of Limousin cross can be used for crypreservation and artificial insemination. Parameters (motility, sperm conc. pH, morphometric attribute, ALT, AST, ALP, LDH) in other genotypes are poor and cannot be used for artificial insemination. This can due to management, food habit, lack of exercise, diseases and/or other abnormal health condition. After completing preliminary studies it was found that the seminal attributes were encouraging with few abnormalities. However, due to constraints of low numbers of semen producing bulls, the present results need further validation in a larger bull population. Future studies should be planned which can address the field fertility of semen of these beef bulls breed in association with the laboratory results.

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