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Breeding soundness of semen producing bulls, their semen quality and inter relationship of functional tests with spermatozoa motility percentage

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This work was conducted to study the breeding soundness of semen producing bulls, their semen quality to functional tests and the inter relationship of these functional tests with spermatozoa motility percentage for the semen produced at National Animal Genetic Improvement Institute (NAGII). A total of 14 breeding bulls (Boran = 4, Crosses of 75% Holstein Frisian × 25% Boran = 4 and Holstein Frisian = 6) with respective ejaculates of 35, 33 and 57 for semen quality evaluations were considered. After physical examination of bulls for breeding soundness, semen samples were tested for functional and structural (spermatozoa acrosome integrity, viability and hypo osmotic swelling reactivity) tests and spermatozoa motility percentage using subjective and objective (computer assisted semen analysis; CASA) evaluation methods, to see the correlation of functional tests with spermatozoa motility percentage. And in the study, nevertheless, significant differences between Cross and HF breeds were not recorded for the breeding soundness evaluation parameters and for most of the semen quality parametric test values; significantly ($P < 0.05$) minimal and highest parametric values of the breeding soundness evaluation and semen quality were recorded in Boran and HF breeds, respectively. All the functional semen quality evaluation tests (HOST, acrosome integrity, sperm morphological defect and viability) were related in one or the other to the objective CASA individual spermatozoa motility evaluation; which were not true with subjective individual spermatozoa motility estimation. Therefore, conducting stringent breeding soundness evaluation for these breeds and evaluating their individual spermatozoa motility percentage using CASA system instead of subjective motility estimation can screen good quality semen and subsequently make possible to predict their fertility potential.

Key words: Boran, correlation, cross-breed, Holstein-Frisian, semen.

INTRODUCTION

A bull has a larger impact on the herd productivity than a single female especially when used for artificial insemination (AI). Artificial insemination has an abundant role for the genetic improvement programs, mainly due to well-established methods for identifying males with the highest genetic merit (Leboeuf et al., 2000; Aynalem et

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al., 2011). At present, frozen-thawed semen is extensively used for AI in animal breeding program throughout the world (Ball and Peters, 2004). Likewise, because of its relative simplicity for application and its contribution to speed up genetic improvement, it is also the first and most commonly used assisted reproductive technology (ART) in Ethiopia.

In Ethiopia, there is insufficient information on the reproductive potential and parameters of zebu and crossbred bulls, on the effect of genetic factors which limits the reproductive and fertility potential of bulls (Tegegne et al., 1995). National Animal Genetic Improvement Institute (NAGII) also uses only conventional semen evaluation method to assess the semen quality; which is not quite enough to predict the fertility potential of bulls. So far many studies (Hunderra, 2004; Sinishaw, 2005; Desalegn et al., 2009; Demeke, 2010; Engidawork, 2018) also conducted subjective semen quality assessment to predict the fertility potential of bovine semen produced from NAGII; but no one used advanced technologies and functional tests at a time to evaluate the semen quality objectively and see the association among evaluation methods which can predict the fertility potential of bulls. In addition to these limitations, currently though there is a huge demand by the government for Boran semen, anecdotal data indicate a dismally low (<30%) pregnancy rate which has hampered its wider use both for AI and Embryo Transfer (ET) programs at universities and research centers. Based on these background ideas, the study hypothesized that employing stringent breeding soundness examination, objective spermatozoa motility evaluation using CASA and functional tests invariably improve semen quality and hence fertility potential of bulls.

MATERIALS AND METHODS

Study animals and management

A total of 14 breeding bulls (Boran = 4, 75% Holstein Frisian × 25% Boran cross = 4 and Holstein Frisian/HF/ = 6) were used in this study. All the bulls were kept in indoor and under identical conditions of management, feeding and watering throughout the study period. They received hay, green forage and concentrate fortified with vitamins and minerals. Water was given *ad libitum*. They are allowed to exercise on running track on weekly basis. A total of 125 ejaculates (Boran = 35, Cross = 33 and HF = 57) were collected once per week over three and half months period.

Breeding soundness evaluation

At time of breeding soundness evaluation of the study bulls; integrity of genital system, libido and semen quality were assessed. The genital organs particularly the testicles were assessed for their size, volume, weight, symmetry, form, consistency and movability inside the scrotal sac. Testicular parameters (length, width, thickness and scrotal circumference) were measured using caliper and flexible scrotal tape to the nearest 0.1 value after restraining each bull in the chute. Procedurally.

Testicular length was measured by placing the fixed arm of the caliper at the proximal end and the sliding arm at the distal end of the testis being taking care to exclude the epididymis.

Testicular width of each testis was measured by sliding the other testis up in the scrotum and placing one arm of the caliper at the medial aspect and the other at the lateral at the point of maximum width.

Testicular thickness was measured by placing the fixed arm of the caliper at the anterior aspect and the sliding arm at the posterior aspect of each testis at the point of maximum thickness.

Scrotal circumference measurement was conducted by pushing the testes firmly into the bottom of the scrotum while placing the thumb and fingers laterally on the side of the neck of the scrotum to make them completely within the lowest point in the scrotum and lying side by side with no evidence of wrinkling of the scrotum. The testes were then held firmly in the scrotum with left hand and measurement of scrotal circumference was taken with right hand by taking care for the thumb of the hand holding the neck of the scrotum not to cause any pressure on the middle of the scrotum. The scrotal tape was then looped around the testes and was drawn firmly in contact with the entire circumference to cause moderate indentation of the scrotum at the level judged to have the largest circumference.

Volume of testes was calculated using a formula described by Love et al. (1991):

$$V = \frac{4(\pi abc)}{3};$$

where a = thickness/2, b = width/2 and c = length/2.

Weight of the testes was also calculated by multiplying volume of each testis with the expected testicular tissue density (1.038) in cattle (Amann, 1990).

Accessory sex glands were assessed for their development through rectal palpation. Libido was evaluated based on a 1-4scale (1- being shy or has no desire to move towards a teaser, 2- being dull or very reluctant to reach the teaser, 3- being active or willingly moves towards the teaser and 4- being aggressive or moves towards teaser in an uncontrolled manner).

Semen collection, preparation and evaluation

Bulls were given bath to remove dung from their prepuce 30 min before collection of semen. Semen was collected with the help of bovine artificial vagina (IMV, France) early in the morning between 09:00 and 10:00 AM as per the method described in Salisbury et al. (1978) which was also the routine practice at NAGII. Immediately after collection, the semen was subjected to initial examination of volume, color, concentration and motility; after which they were further processed as per the laboratory's standard procedure. The minimum initial standards of the laboratory were a volume of greater than or equal to 2 ml, color ranging from milky white to creamy, concentration of greater than or equal to 500 million/ml and an initial motility of above or equal to 70%.

Ejaculates were diluted using OptiXcell extender (IMV Technologies, France) to attain a final concentration of spermatozoa 142.86 million/ml; after which they were filled and sealed into labeled mini straws (IMV Technologies, France). The semen in the straws were allowed to equilibrate at 4°C for about 4 h and then shifted to programmable bio-freezer where the temperature was further brought down to -140°C using liquid nitrogen vapor. These straws were finally shifted to liquid nitrogen containers and stored till distribution and use at field level.

Thawing of chilled and frozen semen straws for the respective individual spermatozoa motility evaluations was done in water bath at 37°C for 30 s.

Motility evaluations

After the subjective semen quality assessments were conducted and approved for further processes, the fresh diluted semen samples were subjected to evaluations of integrated semen analysis system. Integrated semen analysis system (ISASv1®) set-up was pre-adjusted for bovine semen analysis as per the manufacturer's (prosier, Spain) recommendation. Aliquot of 100 µl of fresh diluted semen was placed into a pre warmed micro-centrifuge tube and re-diluted at a rate of 1:3 (semen to extender) to bring the concentration of spermatozoa at 20 to 50 millions/ml; then spermatozoa percent total motility was recorded for each sample. Similarly, evaluations of motility percentages after chilling and biological freezing stages of processing were conducted from 0.25 ml mini straw packs for both subjective and objective methods of evaluations and rate of spermatozoa motility reduction percentages at different stages of processing were calculated as: Rate of motility reduction percentage at chilled = (Fresh individual motility percentage - Chilled individual motility percentage) × 100 / Fresh individual motility percentage; Rate of motility reduction percentage at frozen = (Chilled individual motility percentage - Frozen individual motility percentage) × 100 / Chilled individual motility percentage; Overall rate of motility reduction percentage = (Fresh individual motility percentage - Frozen individual motility percentage) × 100 / Fresh individual motility percentage.

Plasma membrane integrity

It was assessed using hypo-osmotic swelling (HOS) assay as per the method described by Correa and Figueroa Ortíz (2003). Briefly, hypo-osmotic solution of 150 mOsm/L was prepared by dissolving 0.735 g of sodium citrate and 1.351 g of fructose in 100 ml distilled water. Similarly, a control solution of 300 mOsm/L was prepared by dissolving 1.47 g of sodium citrate and 2.702 g of fructose in 100 ml distilled water and both the solutions were maintained at 37°C for 5 min before use. Then 1 ml of each solution was mixed with 0.1 ml frozen thawed semen in a test tube and incubated for 60 min at a temperature of 37°C. Immediately after incubation, each sample was fixed with 0.1 ml of 10% formaldehyde to retain the shape for subsequent observation. A drop of each well mixed semen sample (for the control and HOST) was placed on a glass slide, covered with cover slips and a total of 500 sperm cells in two classes [normal (non-reactive) and coiled or swollen tail (reactive)] for each of the control and HOST were counted in at least 5 different fields of vision at 400X magnification. The proportion of HOST reactive spermatozoa was determined by deducting the number of coiled or swollen tail spermatozoa in the control from the number in hypo-osmotic solution and the resultant figure was taken as percentage of HOS-reactive spermatozoa.

Acrosome integrity

Evaluation of acrosome integrity for frozen thawed semen was conducted using Trypan blue-Giemsa dual stain as described in Didion et al. (1989). Procedurally, the frozen semen was thawed in a water bath at 37°C for 30 s and diluted in 0.9% sodium chloride saline water at a dilution of 1:9 ratio for semen to saline. After taking and gentle mixing of one drop of diluted semen with one drop of 0.27% Trypan blue, smear was made on a slide and allowed to air dried almost vertically at room temperature. Then the smear was fixed in a solution of 86 ml 1 N HCl, 14 ml 37% formaldehyde and 0.2 g of neutral red for 2 min and rinsed with water. The smear was again stained with 7.5% (v/v) Giemsa overnight (16-20 h). After washing and drying of the smears, microscope evaluation was conducted with 1000X oil immersion and based on the stain characteristics acrosomal status was recorded as acrosome intact live (AIL), acrosome intact dead (AID), acrosome lost live (ALL) and acrosome lost dead (ALD). A spermatozoon was considered as live if and only if the sperm cell displayed both head and tail viability and as dead if either the head or the tail or both became unviable. In each observation, the evaluation was conducted at least for 200 spermatozoa.

Live: Dead ratio

As eosin-nigrosin is one of the vital stains suitable for bright field microscopy evaluation, viability of sperm cells was evaluated using eosin-nigrosin staining as per the method described in Danilda et al. (2015). Briefly, microscope slides and eosin-nigrosin stain (1.67 g of eosin and 10 g of nigrosin in 100 ml distilled water) were pre-warmed at a temperature of 37°C and a 15 µl of stain was pipetted onto the edge of a grease free slide followed by 5 µl of frozen-thawed semen loaded next to the stain. The stain and semen were mixed and then smeared on the surface of the slide. The slide was allowed to dry by waving it in air and was examined using a bright field microscope (40X objective lens). Lastly, sperm cells not taking the eosin stain and appeared as white in color were considered as live and those absorbing the eosin and appeared pinkish in color as a result of loss of their membrane integrity were considered as "dead". In each observation, a total of at least 200 sperm cells to calculate the live:dead ratio were counted and recorded.

Statistical analysis

The data obtained from each assessment and evaluation parameters were entered to Microsoft excel sheet and SPSS computer statistical package for windows (Version 16, USA) was used for analysis. For non-parametric data, ratios and percentages were considered. Descriptive statistics, percentages and correlations for semen quality parametric data: spermatozoa acrosome integrity, live:dead ratio, HOST and individual motility for both subjective and CASA were calculated and compression among breeds was done using Analysis of Variances (ANOVA). Duncan's Multiple Range Test (DMRT) was used to compare significant difference for those mean and percentage values at a probability level of 5%. In the analysis, $P< 0.05$ was set for level of significance.

RESULTS

Breeding soundness evaluation

At the time of breeding soundness evaluation, testicular size, symmetry, form, consistency and movability inside the scrotal sac, semen quality, seminal vesicle development, pre seminal fluid drop, protrusion of penis and its complete erection and intensity of contraction for seminal vesicle reflex were assessed and evaluated accordingly.

In all the three breed bulls, the testes were symmetric and movable in the scrotal sac. Seminal vesicles were well developed in all the breeds though the relative sizes were smaller in Boran breed. Protrusion and erection of penis for seminal vesicle reflex were seen and complete in 3 bulls of each breed (75% for each of Boran and Cross breed and 50% for HF breed). Intensity of contraction for seminal vesicle reflex was strong in all Boran and Cross breed bulls but weak in 2 (33.3%) HF

Table 1. Breeding soundness evaluation (M ± SE) in relation to breed type of the bulls.

Parameter	Breed type			*F*-value	Sig.
	Boran (N=4)	Cross (N=4)	HF (N=6)		
Libido	2.94±0.06	2.97±0.03	3.00±0.00	-	0.404
Scrotal circumference (cm)	33.2± 0.5^{a}	37.1±1.4^{b}	38.4±1.3^{b}	4.88	0.030
Left testis length (cm)	12.4±0.4^{a}	13.6±0.5^{b}	13.3±0.2^{ab}	3.38	0.072
Right testis length (cm)	13.0±0.3	14.1±0.6	13.2±0.4	1.54	0.256
Left testis thickness (cm)	6.10±0.1	6.70±0.5	6.90±0.2	2.70	0.111
Right testis thickness (cm)	6.40±0.2	7.30±0.5	7.20±0.2	2.42	0.135
Left testis width (cm)	6.50±0.2^{a}	7.00±0.3^{ab}	7.60±0.1^{b}	8.18	0.007
Right testis width (cm)	6.60±0.1^{a}	7.10±0.3^{ab}	7.60±0.2^{b}	5.25	0.025
Left t testis volume (g/cm^{3})	254±8.7^{a}	342±52.4^{ab}	368±16.8^{b}	4.13	0.046
Right testis volume (g/cm^{3})	287±9.4	388±5.7	378±2.0	2.75	0.107
Left testis weight (g)	264±9.0^{a}	355±5.4^{ab}	381±1.7^{b}	4.13	0.046
Right testis weight (g)	298±9.8	392±5.9	403±2.1	2.75	0.107
Body weight (kg)	459±21.1^{a}	813±50.4^{b}	852±55.4^{b}	17.6	0.000
Age (month)	34.4±0.1^{a}	63.5±8.0^{b}	66.3±4.4^{b}	11.0	0.002

Mean±SE values across rows with different super scripts are significantly different (*P < 0.05*), N= number of bulls.

bulls. The pre seminal fluid drop for seminal vesicle reflex was observed in all Boran and HF bred bulls but not in 3 (75%) Cross breed bulls.

Though, significant differences for all the breeding soundness evaluation parameters (scrotal circumference, testicular measurements, body weight and age) were not recorded among Cross and HF breeds; parameters of scrotal circumference, right testis width, left testis (length, width and volume and weight which are functions of other measurable traits), body weight and age were significantly (*P < 0.05*) minimal for Boran breed (Table 1).

Hypo-osmotic swelling test

Though spermatozoa hypo-osmotic swelling reactive percentage was not significantly different for Crosses in comparison to Boran and HF breeds; significantly (*P < 0.05*) minimal (39.6%) and highest (49.5%) spermatozoa hypo-osmotic swelling reactive percentages were recorded in Boran and HF breeds, respectively (Table 2).

Live: Dead spermatozoa ratio

The mean live:dead spermatozoa ratio of frozen semen were 2.16, 1.96 and 2.21 for Boran, Cross and HF breeds, respectively. Nevertheless, no significant difference was observed for live:dead spermatozoa ratio among the three breeds; in line with spermatozoa HOST reactivity percentage, the highest (2.21) live:dead spermatozoa ratio were recorded for HF breed.

Spermatozoa acrosome integrity

Alike to HOS test significant (*P < 0.05*) difference for acrosome intact live spermatozoa percentage was observed between Boran and HF breeds (Table 2). Though live spermatozoa percentages of 22.2, 18.1 and 17.7% were also additionally recorded for Boran, Cross and HF breeds; however, the acrosomes of these percentages of spermatozoa for each breed were not intact. Similarly, though there was no significant difference among the breeds, in line with spermatozoa HOST reactivity percentage, minimal (67.6%) and highest (72.9%) total acrosome integrity percentages were recorded for Boran and HF breeds, respectively.

Morphological defects

The fresh semen illustrated in Table 2 showed significant differences (P < 0.05) between breeds recorded for morphological defects (head, tail and proximal droplets). Sperm head abnormality was significantly higher (*P < 0.05*) in Boran breed. Significantly higher (*P < 0.05*) tail abnormality was observed for Boran and HF breeds. In contrast, the highest proximal abnormality was noted in crosses. In general, though the total sperm morphological abnormality for Boran and HF breeds was nearly similar; it was significantly higher in Crosses.

Spermatozoa motility percentage and rate of reduction

As illustrated in Table 3, the mean subjective individual spermatozoa motility percentage estimation for fresh, chilled and frozen stages of production were 77.3, 73.7 and 45 for Boran; 77.3, 74.7 and 48.0 for Cross and 77.5, 77.1 and 48.4 for HF breed. The respective objective

Table 2. Incidence of functional spermatozoa percentage (M±SE) among breeds for frozen semen.

Traits of semen quality	Breed type			F-value	Sig.
	Boran (N=35)	Cross (N=33)	HF (N=57)		
HOST reactive	39.6± 2.2[a]	45.2±2.4[ab]	49.5±2.2[b]	4.63	0.011
Live : Dead spermatozoa ratio	2.16±0.15	1.96±0.09	2.21±0.08	1.36	0.26
Acrosome integrity					
Acrosome intact live	43.3±1.92[a]	46.9±1.75[ab]	49.7±1.30[b]	4.15	0.02
Acrosome intact dead	24.3±2.34	24.8±1.84	23.3±1.27	0.21	0.81
Acrosome lost live	22.2±2.15	18.1±2.00	17.7±1.32	1.94	0.15
Acrosome lost dead	10.2±0.93	10.3±0.94	9.35±0.64	0.46	0.64
Total acrosome intact	67.6±2.98	71.7±2.78	72.9±1.81	1.34	0.27
Morphological defects (fresh semen)					
Head	5.02 ± 0.67[b]	4.66 ± 0.55[ab]	3.42 ±0.28[a]	3.60	0.030
Mid piece	0.31 ± 0.10	0.35 ± 0.09	0.18 ± 0.05	1.74	0.181
Tail	4.70 ± 0.60[b]	3.16 ± 0.40[a]	4.91 ± 0.45[b]	3.36	0.038
Proximal droplet	0.53 ± 0.10[a]	8.63 ± 2.62[b]	1.01 ±0.21[a]	12.2	0.000
Distal droplet	1.01 ± 0.21	1.30 ± 0.31	0.93 ± 0.21	0.61	0.547
Total abnormality	11.6 ± 1.05[a]	18.1 ± 2.31[b]	10.4 ± 0.63[a]	9.65	0.000

Mean±SE values across rows with different super scripts are significantly different (*P < 0.05*), N= number of ejaculates.

Table 3. Individual spermatozoa motility and rate of reduction percentages across semen production stages among breeds.

Method of evaluation	Breed	Spermatozoa individual motility percentage (M±SE)			Rate of reduction percentage (M±SE)		
		Fresh	Chilled	Frozen	at chilled	at frozen	Overall
Subjective estimation	Boran	77.3±0.75	73.7±0.83[a]	45.0±0.71[a]	3.57±0.81[b]	28.7±1.01	32.3±0.90[b]
	Cross	77.3±0.76	74.7±0.87[a]	48.0 ± 1.04[b]	2.58±0.76[b]	26.7±1.22	29.2±1.19[a]
	HF	77.5±0.55	77.1±0.59[b]	48.4 ± 0.74[b]	0.35±0.21[a]	28.7±0.90	29.0±0.89[a]
CASA evaluation	Boran	82.5±1.4[b]	57.9±2.8[a]	33.9±2.6[a]	22.8±2.62[b]	24.2±3.56	47.0±2.90[b]
	Cross	76.8±1.5[a]	67.0±1.7[b]	33.1±3.1[a]	8.27±1.47[a]	31.9±3.42	40.2±3.50[ab]
	HF	78.9±1.2[a]	70.2±2.0[b]	42.9±2.3[b]	7.87±1.53[a]	26.9±2.29	34.7±2.23[a]

Column values for each method of evaluation bearing different superscripts are statistically significant (P < 0.05).

CASA motility percentages were 82.5, 57.9 and 33.9 for Boran; 76.8, 67 and 33.1 for Cross and 78.9, 70.2 and 42.9 for HF breed.

The overall rate of reduction for individual spermatozoa motility percentage in both methods of evaluation was high in the case of Boran breed as compared to the other two breeds (Cross and HF). This rate of reduction in particular at the stage of stabilization was significantly (P < 0.05) high for this breed (Table 3).

Relationship of semen evaluation tests

As shown in Figures 1 and 2, high correlation coefficients of CASA individual spermatozoa motility percentage in relation to the semen quality evaluation tests of HOST (R = 0.71) and acrosome intact live spermatozoa percentage (R = 0.31) were observed for the overall frozen semen evaluations. As illustrated in Figures 3 and 4, though, not significantly high; positive associations were also seen among CASA motility percentage with subjective motility estimation (R = 0.26) and subjective individual motility estimation with HOST (R = 0.15). Unexpectedly, though it was low, estimation of subjective individual spermatozoa motility percentage exhibited positive association (R = 0.16) with spermatozoa morphological defects percentage; which was negatively associated (R = -0.08) for CASA evaluation, in which case, validating the expected fact; hence as sperm morphological defect has negative impact on motility (Figures 5 and 6). Similarly, subjective individual spermatozoa motility estimation displayed unexpected negative association (R = -0.0027)

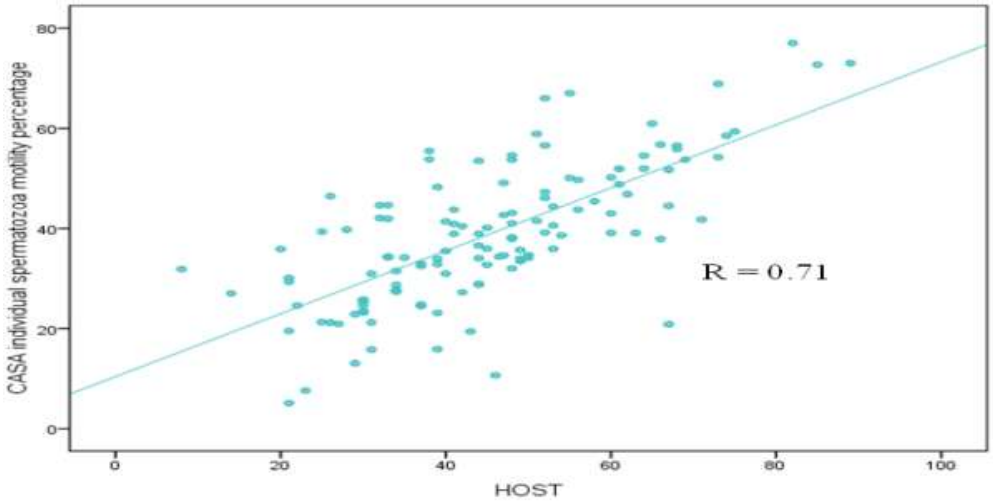

Figure 1. Correlation between CASA individual spermatozoa motility evaluation and HOS test.

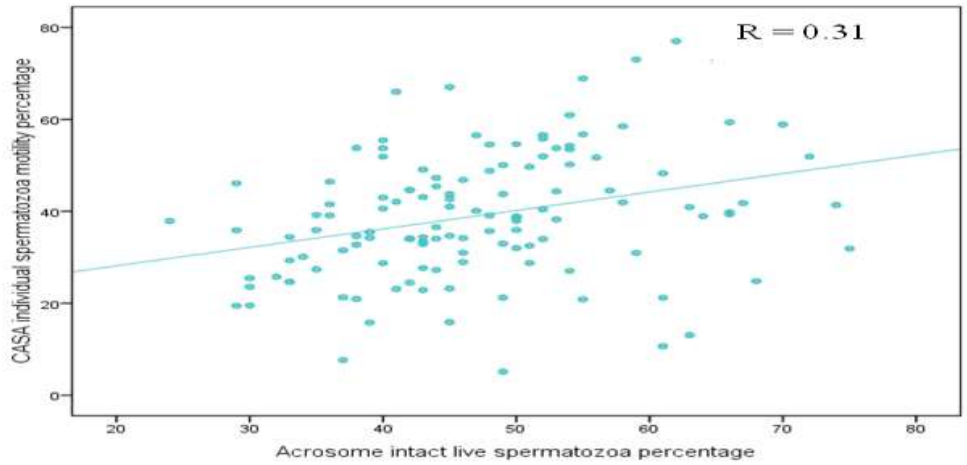

Figure 2. Correlation between CASA individual spermatozoa motility and acrosome integrity percentages.

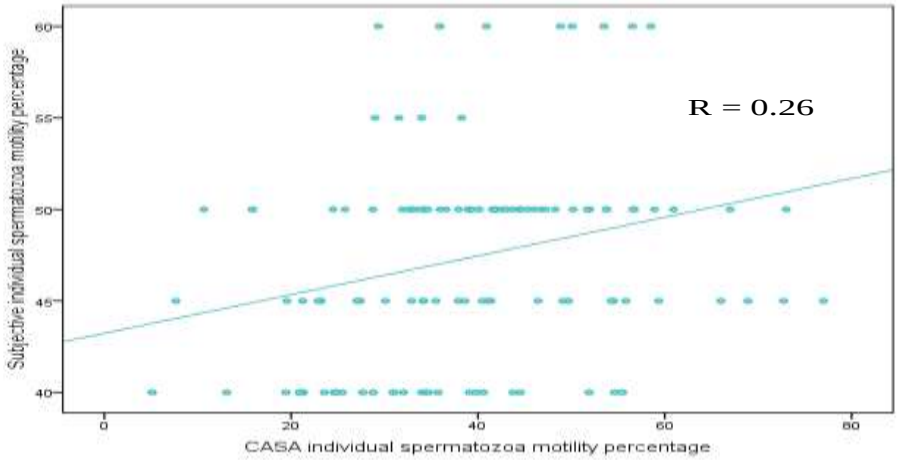

Figure 3. Correlation between subjective and CASA individual spermatozoa motility percentages.

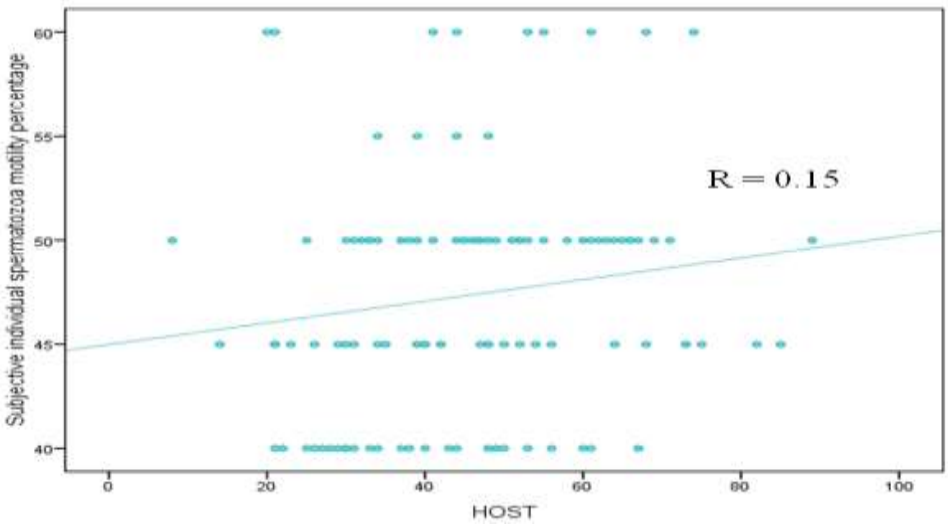

Figure 4. Correlation between subjective individual spermatozoa motility estimation and HOS test.

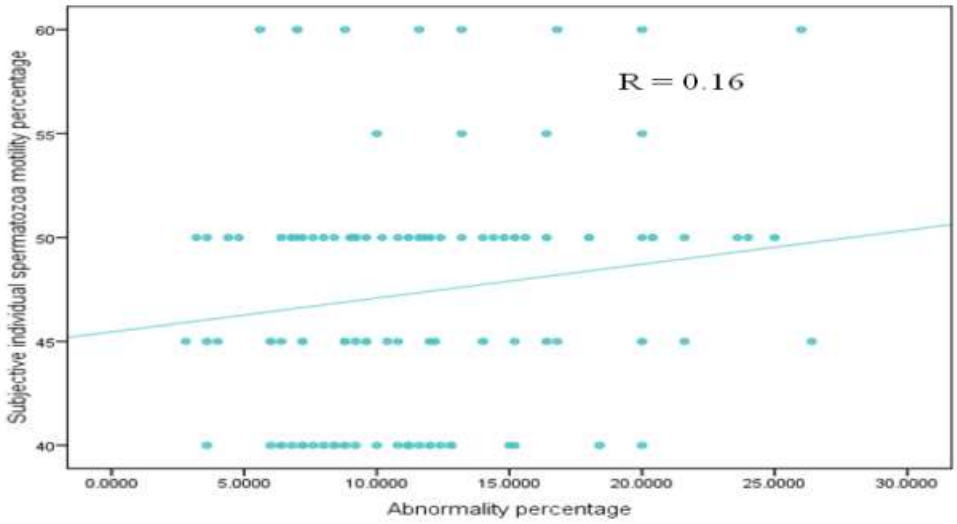

Figure 5. Correlation between subjective individual spermatozoa motility estimation and spermatozoa morphological abnormality percentage.

for acrosome intact live spermatozoa percentage, which was not true for CASA evaluation (Figures 2 and 7).

DISCUSSION

As breeding soundness evaluation is the most practical and useful system for the estimation of bull's fertility potential (Lone et al., 2017); in this study also, a systematic and thorough bull breeding soundness evaluations were conducted to relate them with fertility potential of bulls. Nevertheless, significant differences for all the breeding soundness evaluation parameters were not recorded between Crosses and HF breeds; the study finding revealed as most of the breeding soundness evaluation parameters (Scrotal circumference, Left testis length, width, weight and volume, Right testis width, and Body weight) were significantly ($P < 0.05$) minimal for Boran as compared to the other two breeds. However, this significant ($P < 0.05$) difference for left testis volume and weight might come from the significant difference for its length and width those function the testis volume which in turn also determines the testis weight. In this study mean values of scrotal circumference for Boran,

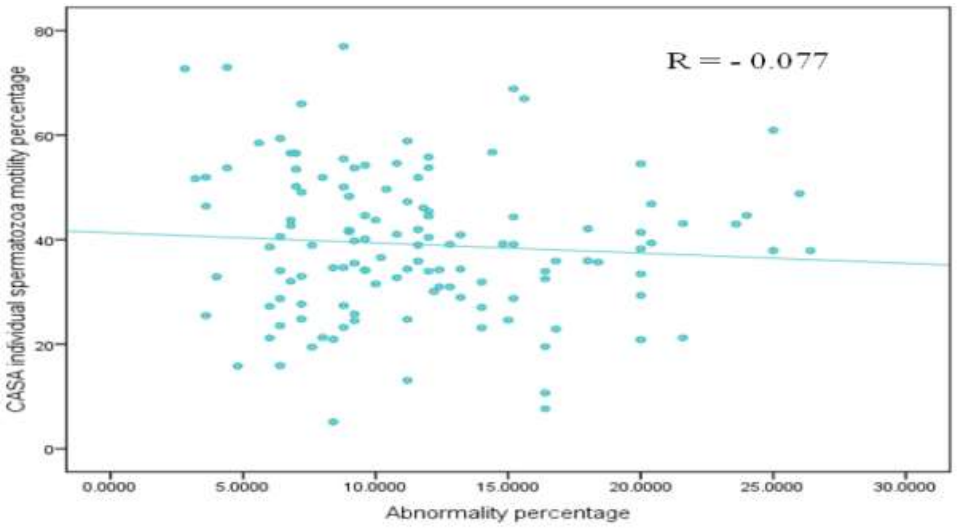

Figure 6. Correlation between CASA individual spermatozoa motility and spermatozoa morphological abnormality percentages.

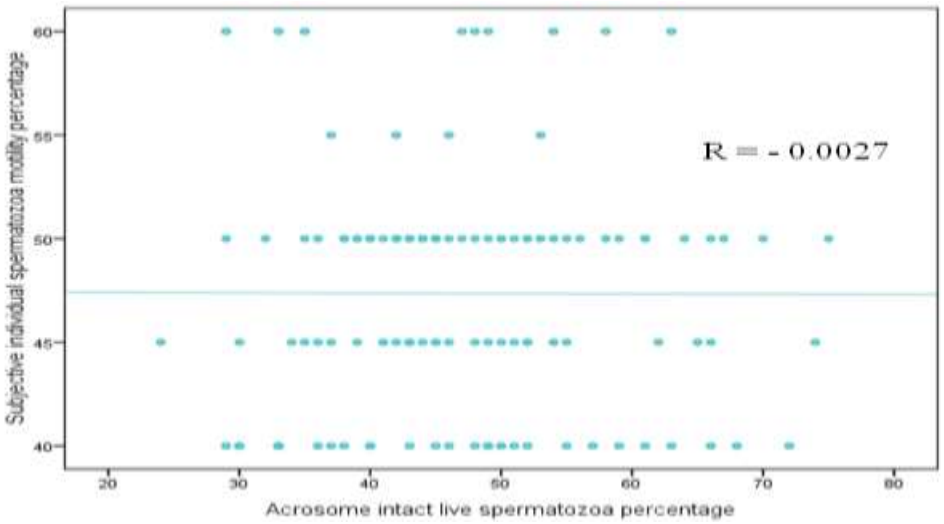

Figure 7. Correlation between subjective individual spermatozoa motilityestimation and acrosome integrity percentage.

Cross and HF breeds were 33.2, 37.1 and 38.4 cm at their respective average age of 34.4, 63.5 and 66.3 months. These values in particular that of Boran were in agreement with the finding reported by Lemma and Shemsu (2015) for pre-service young bulls in the same AI center.

Regardless of the semen quality assessment and evaluation, initial semen quality assessments of spermatozoa morphology and subjective individual spermatozoa motility percentage were accompanied before the actual respective CASA objective motility evaluation and functional tests were conducted. In this study, significant ($P < 0.05$) differences among breeds for some conventional subjective semen quality measures (individual sperm motility percentage at chilled and frozen stages of production, morphological abnormalities of head, tail and proximal droplets) were recorded. In line with the breeding soundness evaluation parametric values, low individual spermatozoa motility percentage under both subjective and objective spermatozoa motility evaluations and high percentages of head abnormalities were recorded for Boran breed, indicating the importance of breeding soundness evaluation along with semen quality for the estimation of bull's fertility potential.

The overall rate of reduction for individual spermatozoa motility percentage in both methods of evaluation was also high for Boran breed as compared to the other two breeds (Cross and HF). This rate of reduction in particular at the stage of stabilization was significantly ($P < 0.05$) high for this breed; implicating the stabilization protocol followed by the laboratory might not be appropriate for this breed semen and may need further optimization. As it was stated by Bang (2008), Holstein Friesian breed is known to give best results compared to other breeds, which was also in close agreement with the current finding. Breed differences on several semen parameters have been previously reported by different authors (Hafez, 1993; Brito et al., 2002). Similarly, Hunderra (2004), Sinishaw (2005), Desalegn et al. (2009), Demeke (2010), Lemma (2011), and Lemma and Shemsu (2015) have also reported the semen characteristics difference among cattle breeds at the same AI centre. However, Engidawork (2018) did not state this breed difference which might be due to short-term study period and a smaller number of observations in his study. Moreover, such variability between studies on semen quality parameters might be attributed to difference in age, nutritional status, season of the year the study covers, method of the semen collection procedure and frequency (Hafez, 1993; Blezinger, 1999; Andrabie et al., 2002). Hence, in this study age of the bulls might also be the other factor to which better semen quality was recorded in mature HF and Cross breed bulls as compared to younger Boran breeds which might probably be due to scrotal circumference and the heat regulation mechanisms, that can increase linearly with age until the rate of broken down for testicular tissues become faster than being replaced (King, 1993; Brito et al., 2002). Moreover fat deposition in the scrotum that could vary due to breed and age of bulls also attributed to variation for efficiency of scrotal thermo-regulation which in turn has impact on semen quality (Barth and Oko, 1989; Coulter et al., 1997; Kommisrud and Berg, 1996). Furthermore, factors such as degree of sperm maturation, stores (ATP), presence of surface-active agents in the cell membrane (agglutinins and detergents), viscosity of the fluids negotiated by the sperm, osmolarity, ionic composition of seminal plasma and possibly substances (Cu, Zn, Mn, Hg, hormones, kinins and prostaglandins) that stimulate or inhibit motility may affect semen quality and bring variation among breeds (Blasco, 1984).

According to WHO (2010) recommendation, when percentage of immotile spermatozoa exceeds 40%, it is clinically important to verify the spermatozoa live: dead ratio of that sample. At the national animal genetic improvement institute, where this study was conducted, post freezing minimum threshold motility percentage is 40%; implicating high chance of getting more than 40% immotile spermatozoa; therefore this situation enforces the institute to assess the proportion of live spermatozoa for the semen produced by it. Moreover, in addition to spermatozoa motility percentage estimation, accurate motility evaluation can also be verified by sperm vitality tests, as the percentage of dead spermatozoa should not be higher than the percentage of immotile spermatozoa (Chemes and Rawe, 2003). In this research, in agreement with breeding soundness evaluation parametric values and individual motility percentage higher live: dead spermatozoa ratio was recorded in HF breed; this also again implicates and supports the evidence described by Bang (2008) for the better quality of HF semen.

An intact and functionally active spermatozoa membrane is required for cell metabolism, capacitation, acrosome reaction, attachment and penetration of the oocyte (Jeyendran et al., 1984). Thus, assessment of the sperm membrane functional status appears to be a significant marker for the fertilizing capacity of spermatozoa (Zaneveld et al., 1990). Exposing spermatozoa to hypo osmolar solution which has the capacity for creation of best result without killing the spermatozoa allows movement of liquid into the sperm cell via the cell membrane; as a result functionally active spermatozoa membrane swells at the tail region to achieve the balance for concentrations of solutes of extra and intracellular space (Vazquez et al., 1997). Due to swelling of the membrane, curling of tail is the sequel (Jeyendran et al., 1984). HOS test can estimate the fertilizing capacity of sperm plasma membrane; hence it has the ability to sign whether the plasma membrane of sperm is biochemically active or not. In this study, though the spermatozoa HOST reactive percentage of Cross bred bulls was not significantly different to both breeds (Boran and HF); significantly ($P < 0.05$), minimal and highest spermatozoa HOST reactive percentages were noted in Boran and HF breeds, respectively. The results of this test also were linked with other semen evaluation tests stated before for the better quality of HF semen. The low HOST reactive percentage in Boran breed might be due to the high incidence of tail abnormality; hence, as the membrane is already damaged, there will be no swelling of membrane that in turn decreases the percentage of HOST reactive spermatozoa. Moreover, as spermatozoa with higher membrane cholesterol content is expected to resist destabilization of membrane following cryopreservation, this spermatozoa HOST reactive percentage difference among the two breeds might also be attributed to varying concentration of their membrane cholesterol (Srivastava et al., 2013). Muhammad et al. (2013) reported mean HOST reactive spermatozoa percentages of 47, 40 and 27 in Sahiwal, HF and Crossbred semen, respectively. On the other hand, Zodinsanga et al. (2015) reported HOST reactive spermatozoa percentages of 32.6 and 48.4 for frozen semen pure and Cross breed bulls, respectively. Their findings in particular the HOST reactive spermatozoa percentages of Sahiwal and HF breeds in the study of

Muhammad et al. (2013) and Cross breed spermatozoa HOST reactivity percentage in the study of Zodinsanga et al. (2015) were in close agreement with this study result.

Likewise to other tests percentage of acrosome intact live spermatozoa was significantly ($P < 0.05$) high for HF breed; which was also an indication for the good quality of this breed semen. This study finding also revealed significant high association of CASA individual spermatozoa motility to functional tests of HOST and acrosome integrity which was not found significant for subjective individual spermatozoa motility estimation. The CASA individual spermatozoa motility evaluation also showed negative association with sperm morphological defect percentage, which unexpectedly showed positive association with the subjective individual spermatozoa motility estimation. This controversy might be due to high incidence (8.63%) of proximal droplets for cross breed spermatozoa and might also be due to frequently used subjective estimation values (40, 45, 50, 55 and 60%) by the lab personnel for frozen semen to which CASA was evaluated and gave the average motility percentage based on the individual spermatozoon kinematic values that can validate the exact motility percentage. Moreover, CASA also filters and omits those sperm cells with low efficiency of motility (curvilinear velocity less than 10 µm/s) and considered them as static cells. Moreover, correlation between motility, viability, acrosomal integrity and HOST was expected since they are all related to plasma membrane integrity (Brito et al., 2003), because of this in some earlier studies, association has been established among semen quality parameters of sperm motility, viability, acrosome integrity and HOST reactive spermatozoa percentage (Lodhi et al., 2008; Kirk et al., 2005; Kumar et al, 2015) and the results of the present study in particular the associations of objective CASA motility evaluation with other functional tests were also in agreement with those earlier studies.

CONCLUSION AND RECOMMENDATION

In this study, nevertheless, significant differences between Cross and HF breeds were not recorded for most of the breeding soundness evaluation parameters and semen quality parametric test values; significantly ($P < 0.05$) minimal and highest parametric values of the breeding soundness evaluation and semen quality were recorded in Boran and HF breeds, respectively; implicating the relative good quality of semen for HF as compared to Boran and Cross breed bulls. Regardless of the semen quality evaluation tests relationship, all the functional semen quality evaluation tests (HOST, acrosome integrity, sperm morphological defect and viability) were related in one or the other to the objective CASA individual spermatozoa motility evaluation; which were not true with subjective individual spermatozoa motility estimation. Based on these conclusions, conducting stringent breeding soundness evaluation together with functional semen quality tests for semen producing bulls and evaluating individual spermatozoa motility percentage using CASA system can screen good quality semen and subsequently it is possible to predict its fertility potential.

CONFLICT OF INTERESTS

The authors declared that as they have no any conflict of interests.

REFERENCES

Amann R (1990). Can the fertility potential of a semen sample be predicted accurately? Journal of Andrology 10(2):89-98.

Andrabi S, Naheed S, Khan L, Ullah N (2002). Semen characteristics of cross bred (Friesian x Sahiwal) bulls at live-stock research station, national agricultural research center. Islamabad: Pakistan Veterinary Journal 22(4):181-187.

Aynalem H, Workneh A, Noah K, Tadelle D, Azage T (2011). Breeding strategy to improve Ethiopian Boran cattle for meat and milk production.IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 26.Nairobi, Kenya, ILRI.https://www.ilri.org/publications/breeding-strategy-improve-ethiopian-boran-cattle-meat-and-milk-production

Ball PJH, Peters AR (2004). Reproduction in Cattle, Blackwell Publishing Ltd., Oxford 248 p, 3250_soft, ISBN: 1405115459https://www.researchgate.net/publication/257491318

Bang (2008). Evaluation of semen and non-return rate of bulls in Artificial Insemination center. Journal of Animal Science 37(2):1-7

Barth A, Oko R (1989). Abnormal morphology of bovine spermatozoa. Iowa State University Press, Ames.

Blasco L (1984). Clinical tests of sperm fertilizing ability. Fertility and Sterility 41(2):177-192.

Blezinger S (1999). Cattle Today: Many Factors Affect Bull Performance.

Brito L, Silva A, Rodrigues L, Vieira F, Deragon A, Kastelic J (2002). The effect of environmental factors, age, and genotype on sperm production and semen quality of *B. indicus*and *B. taurus*AI bulls in Brazil. Theriogenology 70(3-4):181-190.

Brito C, Rodella R, Deschamps F (2003). Chemical profile of cell wall and its implications on *Brachiariabrizantha* and *Brachiariahumidicola* digestibility. Revista Brasileira de Zootecnia 32(6):1835-1844.

Chemes E, Rawe Y (2003). Sperm pathology: a step beyond descriptive morphology. Origin, characterization and fertility potential of abnormal sperm phenotypes in infertile men. Human Reproduction Update 9:405-428. https://www.researchgate.net/publication/8990191

Correa RA, Figueroa Ortíz,JA (2003). Observation of Aggressiveness and Territoriality Among Species of Rhinocryptidae in a Rain Forest Fragment in Southern Chile. https://repositorio.uc.cl/xmlui/bitstream/handle/11534/18400/Observation%20of%20Aggressiveness%20and%20Territoriality%20Among%20Species%20of%20Rhinocryptidae%20in%20a%20Rain%20Forest%20Fragment%20in%20Southern%20Chile.pdf

Coulter G, Cook R, Kastelic J (1997). Effects of dietary energy on scrotal surface temperature, seminal quality and sperm production in young bulls. Journal of Animal Science 75(4):1048-1052.

Danilda H, Peregrino D, Hilarion K, Dianne C, Charisse M, Madison P (2015). Non-invasive clinical diagnosis of estrus for AI synchronization using vaginal cytology in three bubaline breeds in the Philippines. Research Journal of Pharmaceutical, Biological and Chemical Sciences 6(1):562-567.

Didion B, Dobrinsky J, Giles J, Graves C (1989). Staining procedure to detect viability and the true acrosome reaction in spermatozoa of various species. Gamete research 22(1):51-57.

Demeke N (2010). Study on the efficiency of conventional semen evaluation procedure employed at kaliti national artificial insemination center and fertility of frozen thawed semen. M.Sc. thesis, Addis

Ababa University, FVM, DebreZeit, Ethiopia.
Desalegn G, Merga B, Azage T, Belaye B (2009). Status of Artificial Insemination Service in Ethiopia. A paper presented at the 17th Annual Conference of the Ethiopian Society of Animal Production (ESAP), held at the Head Quarters of the Ethiopian Institute of Agricultural Research (EIAR), A.A, Ethiopia pp. 87-104.
Engidawork B (2018). Artificial Insemination Service Efficiency and Constraints of Artificial Insemination Service in Selected Districts of Harari National Regional State, Ethiopia. Open Journal of Animal Sciences 8:239-251.
Hafez E (1993). Reproduction in Farm Animals. 6th ed. Lea and Febiger Philadelphia P 465.
Hunderra S (2004). Evaluation of semen parameters in Ethiopian indigenous bulls kept at kaliti, artificial insemination center. Thesis submitted to CVMA, Ethiopia pp. 1-7.
Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld, LJD (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. Reproduction 70(1):219-228.
King G (1993).Reproduction in domesticated animals. Elsevier Science Publisher B.V. London, New York.
Kirk E, Squires E, Graham J (2005). Comparison of in vitro laboratory analyses with the fertility of cryopreserved stallion spermatozoa. Theriogenology 64:1422-39.
Kommisrud E, Berg K (1996). Influence of duration of sexual preparation on bovine semen characteristics and fertility ratehttps://doi.org/10.1111/j.1439-0531.1996.tb00087.
Kumar U, Gawande A, Sahatpure S, Patil M, Lakde C, Bonde S, Borkar P, Poharkar A, Ramteke B (2015). Assessment of semen quality in pure and crossbred Jersey bulls. Veterinary World *8*(10):1266-1272.
Leboeuf B, Restall B, Salamon S (2000). Production and storageof goat semen for artificial insemination. Animal Reproduction Science 62(1-3):113-141.
Lemma A (2011). Effect of cryopreservation on semen quality and fertility. In: Manafi, M ed. Artificial Insemination in farm animals. InTec, Open Access publisher, Croatia, pp. 191-216.
Lemma A, Shemsu T (2015). Effect of Age and Breed on Semen Quality and Breeding Soundness Evaluation of Pre-Service Young Bulls. Journal of Reproduction and Infertility 6(2):35-40.
Lodhi L, Zubair M, Qureshi Z, Ahmad I, Jamil H (2008). Correlation between hypo-osmotic swelling test and various conventional semen evaluation parameters in fresh Nili-ravi buffalo and Sahiwal cow bull semen. Pakistan Veterinary Journal 28(4):186-188
Lone S, Paray A, Mir S, Ganaie B, Sinha R, Singh P (2017). Breeding Soundness Evaluation in Bulls: A Review. Biomed Journal of Scientific and Technical Research 1(5).
Love C, Garcia M, Riera F, Kenney R (1991). Evaluation of measurestaken by ultrasonography and caliper to estimate testicular volumeand predict daily sperm output in the stallion. Journal of reproduction and fertility. Supplement 44:99-105.
Muhammad Z, Laeeq A, Lodhi E, Ghulam M (2013). Hypo osmotic swelling test as screening for evaluation of semen of bull Journal of Entomology and Zoology Studies 1(6):124-128.
Salisbury G, Demark N, Lodge J (1978). Physiology of reproduction and artificial insemination of cattle. 2ed. ed. San Francisco: W. H. Freeman pp. 385-479.
Sinishaw W (2005). Study on semen quality and field efficiency of AI bulls kept at the National Artificial Insemination Center. MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debrezeit, Ethiopia.
Srivastava N, Srivastava S, Ghosh S, Amit K, Perumal P, Jerme A (2013). Acrosome membrane integrity and cryocapacitation are related to cholesterol content of bull spermatozoa. *A*sian pacific Journal of Reproduction 2(2):126-131.
Tegegne A, Kassa T, Mukasa-Mugerwa E (1995). Aspect of bull production with emphasis on cattle in Ethiopia. In: Proceedings of Third National Conference of Ethiopian Society of Animal Production, Addis Ababa, Ethiopia pp. 43-55.
Vazquez J, Martinez E, Martinez P, Garcia-Artiga C, Roca J (1997). Hypoosmotic swelling of boar spermatozoa compared to other methods for analysing the sperm membrane. Theriogenology 47(4):913-922.
World Health Organization (WHO) (2010). World Health Organization laboratory manual for the examination and processing of human semen. WHO Press, Geneva
Zaneveld L, Jeyendran R, Krajeski P, Coetzee K, Ktuger T, Lombard L (1990). Hypoosmotic swelling test. In: Acosta AA, Swanson RJ, Ackerman SB, editors. Human Spermatozoa in Assisted Reproduction. Baltimore: Williams and Wilkins, pp. 223-227.
Zodinsanga V, Mavi P, Cheema R, Kumar A, Gandotra V (2015).Relationship Between Routine Analysis/Sperm Function and Fertility Tests of Cattle Bull Semen. Asian Journal of Animal Sciences 9:37-44. 10.3923/ajas.2015.37.44.