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Effect of Age and Breed on Semen Quality and Breeding Soundness Evaluation of Pre-Service Young Bulls

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Abstract: This study was conducted at the National Artificial Insemination Center, Ethiopia with an objective of evaluating breeding soundness, effect of age and breed on semen quality and fertility of bulls. The evaluation involved 45bulls (36 breeding and 11 pre-service young bulls) consisting of Holstein Friesian, Jersey, Borana and Cross breeds (Borana X Holstein Frisian). Semen was collected using artificial vagina and the raw ejaculates were evaluated for gross and microscopic parameters. The overall mean (±SD) of libido, volume, concentration, mass motility, pre- and post freezing motilities of the breeding bulls were 3±0.0, 7.71±2.4ml, 1.03±0.3bill/ml, 3.47±0.5, 78.35±4.2% and 55.98±5.9%, respectively. Borana bulls produced small volume but highly concentrated semen with the highest mass motility of sperm ($p<0.001$) whereas Holstein Friesian bulls produced the largest volume. Pre-freeze motility of sperm was lowest in crosses ($p<0.05$) than other breeds. The mean (±SD) scrotal circumference was 34.5±1.7cm with a relatively higher percentage of dead sperm (34.2±19.5%) in the pre-service young bulls. Both breed and age were known to be important factors influencing semen parameters. A rigorous selection of pre-service bulls for artificial insemination program will do away age related defects in the semen while breed attributes can be used to individualize semen evaluation procedures.

Key words: Bull · Age · Breed · Breeding Soundness Evaluation · Semen Quality

INTRODUCTION

Over 99% of cattle population in Ethiopia is composed of indigenous cattle, which has an average milk production capacity of 213 kg/cow/lactation [1]. This production level is much lower when compared with production potential of breeds of cattle in other countries. The low productivity are often attributed to a number of factors among which are qualitative deficiency in feed resource base, high prevalence of disease and poor animal performance [2].

In Ethiopia, effort have been made so far by governmental and non-governmental institutions to increase the production performance of indigenous cattle through importation of high producing temperate breeds for direct production and cross breeding [3]. However the cheapest way of increasing production is crossing the indigenous cattle with exotic types. Concerning the breeding technique artificial insemination (AI) is the best solution from several points of view [4]. AI is the first generation reproductive biotechnology that has made a profound contribution to the genetic improvement, particularly in dairy cattle. The major advantages of AI being genetic improvement, controls of venereal diseases, improved record keeping, more economical than natural service when genetic merit is considered, safe to eliminating injury during natural service and availing geographical restrictions [5].

Although there are some encouraging achievements of AI, the success rate was not as expected and the application of AI has been constrained by a number of factors including technical, system related, managemental and financial problems. Among these constraints are poor heat detection skills, communications and transport problems, poor semen collection, storage and handling facilities and inefficiency of AI technicians [6-9].

Semen quality is critically important for the success of AI. Semen can be most useful for AI if it can be cryopreserved, since this method of preservation ideally enables the semen to be stored for an unlimited period

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and availability of genetic material from superior bulls without geographical limitations [4]. Hence, failure to properly evaluate the semen would render the risk of passing over poor genetic material to a larger population. Further, the process of cryopreservation and later on thawing for AI are known to adversely affect the survival of the sperms even under the best technique available today [10]. The success of AI program, therefore, largely depends on the quality and fertility of semen that has been rigorously evaluated. Though there are different methods of semen evaluation but gross and microscopic evaluation only are basically employed at NAIC [8, 9]. However the efficiency of these techniques under the current working condition of NAIC have not been objectively elucidated. Factors from selection technique of the bulls to semen processing and storage are believed to have different level of influence on the quality of semen produced and its fertility during application. Therefore, this study was designed for evaluation techniques employed for selection of pre-service young bulls and to study the effect of age and breed on semen parameters

MATERIALS AND METHODS

Study Area: The study was conduct at Kaliti National Artificial Insemination Center (NAIC) situated 17 km east is located within the sub-city of Addis Ababa. The city lies between 8°55' and 9°07' North and 38°51' East. The mean annual rain fall ranges from 750-15000 milliliters and with the altitude ranging from 2000 meters to 3000 meters above sea level and the average daily temperature about 16°C. NAIC apart from being the sole producer of bull semen, is also responsible for coordination of the overall AI activities within the country. NAIC was also responsible for the production and distribution of liquid nitrogen throughout the country until recently however, because of the government development program, there are now over 10 liquid nitrogen plants situated within the different regional states that also coordinate AI delivery in conjunction with NAIC.

Study Animals: A total of 36 adult bulls that are currently producing semen and 11 pre-service young bulls recently introduced into semen production after training were considered in this study. The breeds of the bulls included Jersey, Borana, Holstein Friesian (HF) and Cross breeds (50% and 75% exotic blood). All the animals were kept indoor, daily fed on hay and concentrate. Water was given *ad libitum*. Individual animal record such as age, body weight, body condition etc is taken for all bulls. All the bulls regularly receive vaccination against common infectious diseases and their health, including hoof condition, is meticulously observed and managed. They are allowed to exercise on weekly basis on running track. While no female animals are present in the premise, teaser bulls are used during the collection of semen using Artificial Vagina (AV). The evaluation of semen production from these bulls was carried out for six months.

Breeding Soundness Evaluation of Pre-Service Young Bulls: The main objective of these examinations was to eliminate infertile bulls and improve the genetic base for fertility within breeds. Thus prior to the acceptance of bulls for semen production, all bull were subjected to various breeding soundness examinations such as integrity of the genital system, mating behavior and semen quality. The genital organs particularly the testicles were examined for their size and palpated for their symmetry, form, movability inside the scrotal sac and consistency. Testicular size was measured using ordinary tape for its length, width and circumference. The integrity of the accessory reproductive organs were assessed through rectal palpation. Libido was evaluated during teasing and was recorded based on a 1-4 scale (with 4 being the highest score). Bulls considered fit during the evaluation were passed to the next step of training on artificial vagina for further semen collection and evaluation.

Semen Collection and Evaluation: Semen from both pre-service young bulls and breeding bulls were collected using AV twice a week. Sexual preparation of the bull prior to semen collection is used to improve the quality of semen obtained and libido of each bull (1 poor through 4 excellent) was scored during this period. This was done through false mounting and allowing the bull to watch other bulls mounting. Semen parameters were evaluated based on gross and microscopic appearance.

Gross Evaluation: The semen was evaluated for its color, volume [ml], pH and presence of contaminants such as blood cells, puss cells, hair or other debris. Watery samples and those with obvious contaminants were rejected.

Microscopic Evaluation: The raw semen was subjected to routine tests such as mass activity (0 to 4 grades with 4 being the highest score) and individual motility [%]; morphological abnormality of sperm cells [%], percent live or dead sperms [%] was estimated after counting 200 sperm cells, concentration [Billion/ml] and

total count [billion]. When the proportion of abnormal sperm exceeded 20%, the semen was rejected. All the data regarding the semen evaluation was recorded on a predesigned record format.

Semen Processing and Handling: Immediately after collection of semen, the raw ejaculates was grossly evaluated and immediately kept in a water bath at 37°C throughout the processing period until the cooling process begins. Semen passing the gross evaluation was then extended using a commercially available egg yolk free semen extender (AndroMed; Minitube, Germany). The amount of extender added was determined based on concentration of sperm cells in the ejaculate and the number of straws to be produced which was in turn determined by the photometer reading.

The extended semen was cooled to +6°C for one hour in a refrigerator. Afterwards, the cooled diluted semen was brought to the cold cabinet at +4°C and final dilution was performed by adding an extender containing glycerol. The extended semen was allowed to stand for 3-4 hours for equilibration after which the semen was filled and sealed in to a 0.25 ml straw that was carefully marked with the proper identification. The straws were then transferred from cold cabinet to a bio freezer that gradually cools the semen to -140°C. Finally, before the semen is plunged into liquid nitrogen for long-term storage, a post freezing individual motility was checked by randomly thawing few straws. Any sample showing below 40% individual motility was discarded. The semen/straws passing this evaluation were kept in goblets and stored in liquid nitrogen at -196°C in different sized dewars until dispatched for AI.

Statistical Analysis: Data were entered on Microsoft excel sheet and analyzed using computer statistical package (SPSS for windows Version 15, USA). Descriptive statistics (Means and standard deviations, percents etc) was used to describe variables. Fixed effects of breed and age were compared using student t-test, one way ANOVA and Chi square test. Correlation among semen parameters was computed using Pearson correlation (r). In the analysis, confidence level was held at 95% and $p<0.05$ was set for level of significance.

RESULTS

A total of 716 collection of raw ejaculate from 36 adult production bulls was evaluated (Table 1). There was no significant difference in libido among the bulls.

Table 1: Evaluation of semen parameters from production bulls (N=36)

Evaluation parameters	Minimum	Maximum	Mean (±SD)
Libido	2	3	3.0±0.0
Volume of semen[ml]	1.5	17	7.7±2.43
Total No of straw produced	29	570	245.06±98.81
Concentration [Bill/ml]	0.4	2.1	1.03±0.34
Mass motility	3	4	3.47±0.50
Pre freezing IM [%]	70	80	78.44±3.33
Post freezing IM [%]	30	60	55.92±5.82

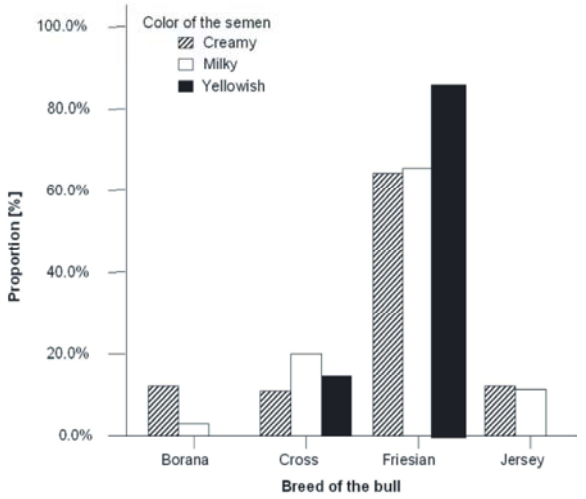

Fig. 1: Distribution of the semen color among the different breeds of breeding bulls

In general, 31.2% of the breeding bulls produced semen with creamy color, 60.3% with milky color and 8.5% produced yellowish semen. Semen with watery color were rejected and were not further processed as they were categorized as semen with poor concentration of sperm. Similarly the incidence of semen with blood or pus giving different combination of the natural color was 0.02%. Breed had a significant effect ($P<0.05$) on semen color (Fig. 1).

Comparison of semen volume showed a significant difference ($p<0.001$) among the different breeds. Borana breeds produced the smallest semen volume while Friesian bulls gave the largest volume of semen (Table 2).

Volume, concentration of sperm per unit, total count, mass motility and pre-freeze individual motility were significantly affected ($p<0.001$) by breed. Borana bulls produced the most concentrate semen with the highest mass motility while crosses produced the lowest in both parameters.

Young bulls that passed the first screening tests were allowed to go to the next step of pre-service examination.

Table 2: Semen parameters of different breeds of bulls used for semen production

Breed	No of collections	Volume[ml]	Concentration [Bill/ml]	Total count[Bill]	Mass motility[Score]	Individual motility[%]
Borana	39	6.51±2.69	1.35±0.39	8.29±3.17	3.77±0.43	79.23±2.16
Cross	125	6.94±2.13	0.93±0.31	6.42±3.22	3.2±0.4	76.12±4.61
Friesian	475	8.01±2.44	1.03±0.33	8.10±3.19	3.5±0.5	78.69±4.33
Jersey	77	7.73±2.19	1.00±0.31	7.74±3.08	3.51±0.5	79.41±2.14

Table 3: Breeding soundness evaluation of pre-service young bulls (N=11)

Parameters	Mean (±SD)	Minimum	Maximum
Age [month]	21.09±3.42	18	29
Body wt [kg]	430.45±91.65	324	610
Scrotal circumference [cm]	34.45±1.69	31	37
Libido (score)	3.42±0.56	2	4
Volume of semen [ml]	7±2.63	2	14
Mass motility (score)	3.48±0.64	2	4
Individual motility [%]	78.18±3.71	70	80
pH of semen	6.84±0.13	6.4	7.2
Concentration [bill/ml]	1.17±0.43	0.24	1.8
Percent of live sperm [%]	65.77±19.44	9.0	87.5
Live / dead ratio	2.75±1.81	0.1	7
Minor sperm defects [%]	5.06±4.57	0.5	20.5
Major sperm defects [%]	0.04±0.19	0	1
Total defects [%]	5.76±5.71	0.5	25

Table 3 shows breeding soundness evaluation of the young bulls. There was a significant correlation ($r=0.727$, $p<0.05$) between body weight and scrotal circumference. Young bulls were further tested on different semen parameters before they were used for semen production. Young bulls with higher libido scores were known to show higher score of mass motility and individual motility. There was a significant correlation ($r=0.512$, $p< 0.001$) between libido and mass motility.

DISCUSSION

The most common semen color observed in mature breeding bulls in the present study is different from previous findings in the same center [8, 9, 11] or what has been reported elsewhere [12]. The finding of 0.02% abnormal color however, is much lower than the 11.7 previously reported by Gebremedhin [8]. Semen color may be affected by the type of feed and is further influenced by the subjective nature of color determination technique. The average semen volume (7.7ml) found in the present study is slightly higher than report by Demeke [9] but was closely similar to previous reports for the same center [11] and elsewhere [13]. However, the record is much closer to the lower range than the mean probably explained by the relatively younger age of the breeding bulls. Young bulls and those of small sized within the species produce smaller volume of semen [14]. Other factors that influence the volume and concentration of semen are frequency of ejaculation, [12], breed, nutritional status, geographic locations, seasons of year, method of semen collection and handling of bulls during collection [13, 15, 16].

Sperm concentration also agrees with other reports [5, 14,]. However, the concentration found in Borana breeds is higher whereas that of the cross is far lower than the same reports. Apart from breed differences, minor variations in concentration may exist between the different techniques of sperm counting like haemocytometer counts, spectrophotometer and electronic counting systems [17].

The mass motility in the breeding bulls is closely similar with the 3.43 report by Dhami *et al.* [18] for Friesian bulls. Pre-freezing individual motility and post-freezing motility of frozen semen were both closely similar to previous studies [5,19]. A post freezing individual motility as low as 30.1-38% have been reported in previous studies carried out at the same center [8, 9] and far lower than the previously recommended marginal value by Zewdie *et al*. [20]. Though freezing semen provides an efficient and successful means of storage, several adverse effects are very common on the spermatozoa usually manifested as a depression depressed of motility and poor fertility [21-23].

The live sperm percentage found in the present study agrees with the recommendation for a normal fertile bull [18]. Live sperm as high as 87.35% are not uncommon in Friesian bulls. However, sperm viability is dramatically reduced after freezing due to different factors such as cryopreservation [10]. The cryopreservation process is known to reduce sperm viability by 50-60% [24, 25]. According to Hafez [13] total abnormal sperm defect in normal fertile bull spermatozoa does not exceed 20% which is consistent with the present finding.

Breed differences on several semen parameters have been previously reported. Holstein Friesian breeds are known to give best results compared to other breed [26], which was also in agreement with the current finding.

There was no difference in almost all of the semen parameters between young pre-service bulls and breeding bulls. Body weight in the young bulls was also known to be positively correlated with scrotal circumference which was also consistent with previous reports [27-29].

CONCLUSION

Most of the semen parameters in both breeding and young pre-service bulls are within the range of previous studies. However, the influence of breed was clearly demonstrated in some semen parameters. These differences seemed to also represent uniqueness of the individual breed of bulls currently used for semen production at the center. Advanced freezing technique and additional meticulous semen evaluation are required to further improve post-thaw quality of semen. Equally important is the need for a rigorous method of selection of breeding bulls as many of the semen attributes are known to be affected by age. Repeated testing of pre-service young bulls is also mandatory to maintain a high-grade selection procedure to retain only bulls that will produce the best quality semen.

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