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The Effect of Sperm Morphology and Viability on the Quality of Bovine Semen: Andrological Examination and Seminal Evaluation of Holstein-Friesen and Jersey Bulls in Hawassa Artificial Insemination Center, Sidama, Ethiopia.

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Abstract

Semen production data of Holstein-Friesian and Jersey breeds of bulls collected over a period of four years (from November, 2016 to October, 2020) was analyzed to examine factors affecting the quality and quantity of bovine semen. The bulls were growing ones and they were tested for andrological examination and seminal evaluation before they were ready to start producing semen. The study was done in Hawassa semen production and processing center, Sidama, Ethiopia. Purebred bulls of Holstein-Friesian and Jersey breeds (n = 26) supposed to produce semen which were maintained under artificially ventilated open-sided sheds, were used for semen collection and analysis. The semen was collected in a weekly interval and a total of 78 ejaculates were analyzed. Eosin-Negrosin semen staining technique was used and on average 283 sperm cells per ejaculate was counted. Defective and dead sperm cells were identified in a warm stage compound microscope. Morphological evaluation findings were used to predict the quality of fresh semen from the study bulls. Of the total 22,099 sperm cells studied, 5,065 (23.0%) of them were found defective, and 8,517(38.5%) of them were found dead. Furthermore, of the total 5,065 defective sperm cells, 1,339 (26.4%) of them were found to be with bent tails. The progressive forward motility of fresh spermatozoa in both breeds of bulls was highly affected by the defective morphology of spermatozoa. The defective sperm cells were found to affect significantly ($P<0.05$) the quality of fresh semen in both breeds of bulls. The results of this study indicated that bent tailed sperm abnormality was the most important type of sperm cell defect which significantly ($p<0.05$) affected the individual forward motility of spermatozoa. This type of sperm cell abnormality mostly arises just after ejaculation due to inadequate temperature, pH or osmotic pressure control. As this type of sperm cell defect is encountered during and after semen collection, great care and precautions is needed in collection and handling of fresh semen. Hence, semen collectors, sterilization room personnel, semen laboratory technologists and semen quality control experts at large should focus on the origin of the problem.

Keywords: Bulls, Ethiopia, Hawassa, Holstein-Friesian, Jersey, Morphology, Quality, Semen.

Introduction

Artificial Insemination (AI) is the oldest and currently most common assisted reproductive technology and an important tool in animal production [1]. Originally AI was introduced as a means of preventing spread of venereal diseases. Today AI represents a much more cost-effective means of disseminating superior genes [2]. AI has been most widely used for breeding dairy cattle; 253 million frozen AI doses and 11.7 million liquid doses are produced worldwide every year [3].
Cattle breeding is mostly uncontrolled in Ethiopia making genetic improvement difficult and an appropriate bull selection criteria have not yet been established, applied and controlled [4]. Although artificial insemination, the most commonly used and valuable biotechnology, has been in operation in Ethiopia for over 30 years, the efficiency and impact of the operation has not been well-documented [5, 6].
The total cattle population for the rural sedentary areas of Ethiopia is estimated to be 43.12 million, of which 55.41% are females [7]. The number of crossbred and hybrid cattle breeds in Sidama National Regional State is estimated to be only 0.3% (42,322) of the total cattle population of the country. The average daily cattle milk production is on average 1.7 liters over a lactation period of 180 days with average lactation period of six months. The annual total cattle milk yield and per capita milk consumption is estimated to be 182,662,292 liters and 19 liters, respectively. This is much lesser than that of the sub-Sahara African average [8]

Among the major constraints for the low number of crossbred and hybrid cattle breed as well as the low milk yield in the region is mainly poor genetic potential of the indigenous cattle breeds. The genetic potential of local breeds of cattle is limited due to lower variability. Cattle breed improvement through crossbreeding endeavors has been in place for decades. One of such endeavors includes grading up of indigenous cattle breed through AI service to improve the local cattle breeds [9, 10]. For more than half a century, conception and calving rates of cattle has been enhanced by AI services.

As envisioned in the country's LMP [11] (Barry I Shapiro, 2015), the number of crossbred dairy cattle was aimed to reach from 233,000 to 1,213,000 [12]. The regional domestic cow milk production would have been increased from 84 million liters to 437 million liters between 2015 and 2020 [12]. This was expected to improve the traditional family cow dairy production as well as expand and improve specialized dairy production units. This plan of grading up local cattle breed and milk production was not achieved mainly due to constraints in the field of reproductive biotechnologies. Among the constraints, inadequate supply and delivery of quality bovine semen, liquid nitrogen, limited number of skilled manpower (especially AI technicians) are to mention but a few [13].

In a regional level, it was proposed to supply 500,000 doses of bovine semen from 2015 to 2020 [12]. However, about half of it is made available in the above mentioned period of time. This indicates that the supply was only 50 % of the demand which has been resulted from inefficient production and supply of bovine semen [14]. Although bovine semen is one of the crucial inputs in the crossbreeding activities of cattle, the supply and quality of it is at its lowest rate [15]. Some of the factors affecting quality of semen are morphology and viability of spermatozoa [16, 17, 18]. Proper analytical evaluation enhances the quality and quantity of semen in the processing and distribution (Bekele, 2005) [9] n value chains. To solve the quality problems and scarcity of bovine semen, the production, processing, storage and utilization trend has to be improved [9, 10, 13]. Furthermore, the already produced semen should be utilized properly maintaining its quality from production to the end users. Hence, proper evaluation of morphological and viability characteristics of bovine sperm is a crucial step in the improvement of the quality of bovine semen.

Therefore, the present study was undertaken to examine the effect of sperm morphology on the quantity and quality of semen produced by the Holstein Friesian and Jersey breeds of bulls maintained under climatic conditions of Ethiopia, Sidama.

Materials and Methods

The Study Area

This study was undertaken in the capital city of Sidama National Regional State, Hawasa. The city is located at a distance of 275 km south of Addis Ababa. It has got its name from Lake Hawassa which is located at western part of the city. The total human population of the city is estimated to be 450,000 and the surface area of the city is estimated to be 157.2

square killo meters. Administratively, the city is subdivided into 6 sub-cities and 24 kebeles. According to the World Meteorology Agency [19] (WMA**),** it is found at a coordinates of 7.3 N Latitude and 38.28 E longitudes, respectively. Agro-ecologically, the city is situated at an elevation range of 1,500 meter to 1,750 meters above sea level. The average rainfall is 1091 mm whereas the average rainy days are 160. The record high temperature in degree Celsius is 33 with the average high being 27.3 and the average low being 12.6 degree Celsius. The record low temperature was taken to be minus 2 in degree Celsius.

Urban agriculture especially livestock production is known in the study area for the purpose of food and income generation. Rearing of cattle, shoats, equines and poultry and beekeeping are practiced in the city. The livestock production system is intensive and urban dairy production type. Milk and butter are the common livestock products consumed by the people of the city.

HAIC-Hawassa is established in 2015 by the then regional agricultural bureau (BoA) with the help of Agricultural Growth Program (AGP). The total surface area of the center is 22,100 meter square. The center started semen production activity on November, 2016. Including the semen processing laboratory, the center has got different kinds of infrastructures .

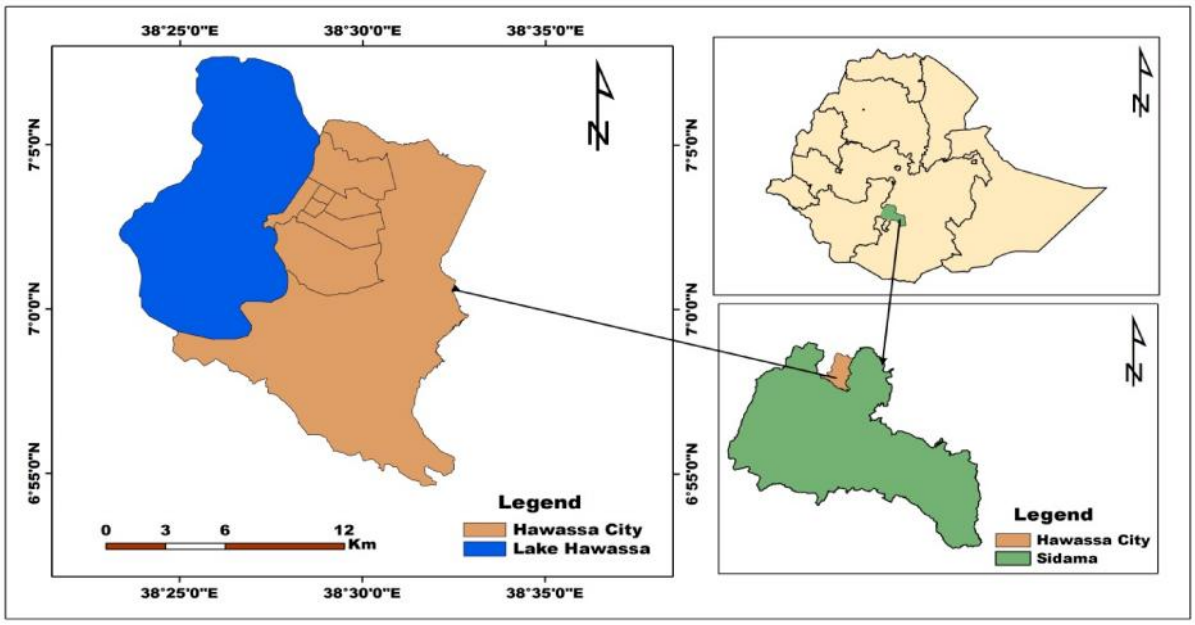

Fig 1: Topographic map of the study area (Source: [20])

Study Design

Semen production data of 26 bulls (15 Holstein-Friesian and 11 Jersey) in Hawasas bovine semen production and distribution center collected over a period of four years (2016-2020) was analyzed to examine the effect of sperm morphology and viability on the quantity and quality of bovine semen. Before semen collection, andrological examination was undertaken on the bulls. After andrological examination, the first, second and third ejaculates of the bulls' semen were undergone seminal evaluation test for morphology and viability.

Study Bulls

There were 15 Holstein-Friesian and 11 Jersey breeds of bulls with 100% exotic blood level. Their age ranged from 19 months to 34 months during their first seminal ejaculation. They were selected and brought to the center through a series of breeding and health check for meeting selection criteria of bulls for AI purpose. They were fed with commercially made concentrate, with hay and green feed according to their body weight [21]. The bulls were kept in a concrete made standard bull house with separate pens. Their health condition was followed and checked regularly

with a veterinarian. After a continuous assessment of their health and body condition, they were allowed to be evaluated for andrological examination as well as semen evaluation. Under the andrological examination, the bulls were examined for the body condition score and body weight using measuring tape. Their scrotal circumference, width and length of each testis were measured using measuring tape. Besides, the accessory sex organs of each bull were examined through rectal palpation.

Study Procedures

Semen Collection Procedures

The bulls were washed and cleaned before semen collection. The semen collection time was 9:00 PM to 4:00 PM at a weekly interval. Their sexual desire was aroused by using dummy bulls for a better libido. Semen was collected using an already prepared artificial vagina (AV) in a weekly interval for three consecutive weeks from the two breeds of bulls. The semen was examined visually for its physical appearance to observe color and presence of extraneous substances. The color of the semen was recorded as creamy, milky and watery. The volume of the semen was measured in the semen collecting test tube in ml. and its pH with the help of pH meter. After recording all the physical parameters, the semen was assessed for its density with the help of integrated haemocytometer. 40 micro liter of semen and 4,000 micro liter of saline water was mixed and let into the spectrophotometry. The quantitative semen analysis result was printed on the spectrophotometry printer paper.

Throughout the semen evaluation process, the temperature of the semen was maintained with the help of water bath as that of the body temperature of the bulls. The mass and individual motility of sperm was evaluated in warm stage compound microscope. 20 micro liter of the semen was taken using micropipette and put in pre-warmed slide for evaluation of total motility under low magnification [22]. The same amount of semen was put in the pre-warmed slide and covered with cover slip to assess the forward progressive movement of the sperm. Mass motility was recorded as very god (4), good (3), poor (2) and very poor (1) depending on the intensity of the forward movement of the sperm where 4 and 3 grades were considered as satisfying the total motility criteria. On the other hand, the individual sperm motility was recorded as percentage of the forward movement of each sperm out of the total sperm evaluated and below 70% was rejected [23].

Sperm Staining Techniques

Eosin-Nigrosin semen staining method was used to evaluate sperm morphology (Swanson, 1951). From stock solution of Eosin 5% and Nigrosin 10%, three drops and five drops, respectively were taken and mixed in a small glass test tube maintained in a water bath at $37^{o}_{C.}$ Two drops of mixed stain and a small drop of semen were taken on a pre-warmed slide and mixed gentl8y. Two smears were prepared and allowed to dry in an open air. Random fields (diagonally) were counted over the stained slide to obtain a representative figure. On average 300 sperm cells per slide were counted to find out the percentage of defective and dead sperm cells and the following formula was used to calculate the proportion of the defective and dead spermatozoa: No. of defective sperm cells counted per total No. of sperm cells counted x 100%. The death percentage per slide was taken as not exceeding 30% [23].

Data Entry and Analysis

The data was double entered in Microsoft excel (Microsoft Corp. Redmond, USA, 2016) and it was validated before it was being imported into SPSS version 21. The semen was considered fulfilling standard quality if its test result was >=70% for progressive forward individual motility.

Explanatory variables were cross-tabulated using Pearson's chi-square test or Fisher's exact test if cells with less than five of expected frequencies occur. The following explanatory variables were added in the analysis: andrological examination results, semen color, volume and its concentration, morphological abnormalities and viability determination. The raw data collected was condensed and summarized with the help of tables and graphs. Moreover, summary statistical quantities such as means, standard errors and measure of dispersion such as variance were included. Then the population parameter was estimated and inferred from the sample statistical quantities. The study result was analyzed using general linear model (GLM).

Results and Discussion

This study was conducted on 26 breeding bulls (15 Holstein-Friesian and 11 Jersey) at Hawassa artificial insemination center, Sidama, Southern Ethiopia, to

evaluate the macroscopic and microscopic characteristics of bull semen. Proper evaluation of freshly collected bull semen before it goes into further processing and it reaches to the end user is important in artificial insemination services. Assuring the quality of semen before use enhances in achieving optimum reproductive efficiency such as conception and calving rates of cows. Therefore, the volume & color, concentration, motility, morphology and viability of the semen have been investigated in this study.

Table 1: Pre-clinical andrological examination results of Jersey and Holstein-Friesian bulls

Breed	Age (Months)	Body weight (Kg)	Scrotal circumference (CM)	Length of right testicle	Length of right testicle	Width of right testicle (cm)	Width of right testicle (cm)
H-F*	27	604	36	14	13	7	7
Jersey	25	432	33	13	13	6	6
H-F*	26	518	34	14	13	6	6

* Holstein-Friesian

Under andrological pre-clinical examination, the average age of the bulls was 26 months while the maximum age was 34 months for Jersey and Holstein-Friesian bulls and the minimum was 19 ages for Holstein-Friesian bull. The average scrotal circumference was measured to be 34 cm. (Table 1). [25] Coe, (1999) indicate that scrotal circumference is positively correlated with the volume of semen ejaculated. However, in this study, there was no significant relationship between volumes ejaculated by the bulls. There was no relationship between the andrological examination results and forward motility of semen. However, Vale Filho, (1997), [26] found a significant relationship between body weight, scrotal circumference, quality and quantity of bovine semen.

The semen of the two breeds of bulls was examined for ejaculate color, volume, concentration, morphology, viability, pH and mass and individual motility of sperm before freezing. Out of the total 78 ejaculates, 57 (73.1%) of them were creamy in color and the rest were milky. There was a significant relationship ($p<0.05$) among No. doses of semen produced, color and concentration of semen in that creamy colored semen was more concentrated and with higher number of semen doses than milky colored semen.

It was observed that the maximum semen average ejaculate was obtained from Jersey breed of bulls and the mean value was 9.0 ml. However, the minimum average ejaculate was obtained from both Jersey and Holstein-Friesian breeds of bulls and the mean value was 2.7 ml. The low volume of the semen could be attributed to the younger age of the bulls. In previous study, ME Hossain, (2012), [27] found the ejaculate volume of 12.9 ml for Holstein Friesian cross Zebu cattle. Moreover, the ejaculate volume for Holstein Friesian cross Zebu cattle was 4.1-7.6 ml. [28, 29, 30]. In fact, volume of semen varies from breed to breed and influenced by a number of factors such as age, breed, weight and season [30, 31]. Laing JA (1988), [32] reported that a bull of high fertility produced greater semen volume than that in a in a lower fertility bull. Thus, volume of an ejaculate may be a good indicator of quality and fertility of bovine semen. The maximum average pH was obtained from both breeds and the mean value was 7.0. This was in agreement with [27].

Average concentrations of fresh sperm were varied ($p<0.01$) from 476.7-1,733.4 million/ml (Table 3). The minimum average sperm concentration was obtained from Jersey bull and the mean value was 476.7 million/ml. The maximum average sperm concentration was obtained from Holstein-Friesian and the mean value was 1,733.4 million/ml. Sperm concentration in ejaculate is one of the important criteria of semen characteristics to qualify fertile males for breeding purposes bulls [33].

Table 2: Macroscopic and microscopic evaluation of unfrozen semen from H-F and Jersey breeds of bulls

Breed	Variables							
	Concentration (Million/ml.)	Mass motility	Individual motility (%)	Total sperm count	Dead Sperm	Defective Sperm	Bent tail	Other defects
H-F*	916.1	3	74	10537	5131	3807	1156	2151
Jersey	728.9	3.3	71	11562	3386	1258	183	575
Average	822.5	3.2	73.5	22099	8517	5065	1339	2726

* = Holstein-Friesian

The number of viable bovine spermatozoa deposited in the female reproductive tract influences the fertilizing ability of the cow up to an upper threshold level [34]. This result differs from that of Shaha SP (2008) [28] who found comparatively low (1000 million/ml) sperm concentration from Friesian cross Zebu cattle. Significant differences in sperm concentration have been shown in semen from different bulls [35]. Sperm concentration in semen could be considered as an initial indicator of semen quality [35, 36]. A positive correlation between sperm concentration at semen collection and motility has been reported which relies on over estimation of motility in more concentrated samples [37, 38]. Nevertheless, in literature regarding whether sperm concentration at the time of semen collection is an indicator of fertilization among normal fertility sire is quite scarce [36].

The arbitrary cut value for mass motility of semen in the center is >=3 which means very good. On the other hand, the arbitrary cut value for individual forward motility of fresh semen in the center is 70%. In this study, the maximum average mass motility of sperm cell was obtained from both breeds of bulls and the mean value was 3.2%. However, the minimum average mass motility was obtained from Jersey breed bulls and the mean value was 1.0%. The average forward motility of sperm was obtained from both breeds of bulls and the mean value was 73.5.0% (Table 3). However, the minimum average motility was obtained from Jersey breed bulls and the mean value was 40.0%. Forward progressive motility of semen is one of the most important requirements of fertile semen, and it is the only applied measure for the quality of fresh and frozen semen [39]. It was found that semen below normal motility (≥ 90 %) was less than half as effective in producing optimum conception rate [40]. It was also reported that motility of spermatozoa was taken as one of the best single evidence for the viability of spermatozoa [40, 41].

Table 3: Number and propprtion of dead and defective sperm cells studied

Parameter	Type of sperm cells					
	Dead	Defective	Bent	Abnormal	Normal	Total
No. sperm cells	8517	5065	1339	13582	8517	22099
Percentage (%)	38.5	23.0	26.4	61.5	38.5	100.00

The average proportion of dead sperm cells was found 8,517 (38.5%). This was beyond the acceptable range and it might have affected the quality of semen. A value of above 30% total dead sperm cell is sufficient to reject a bull for semen production [23]. Nevertheless, JF, (1943) [42] found no significant difference in fertility of semen containing 55 to 95 per cent live sperm. However, semen containing 20 per cent of live sperm was infertile [42].

The core finding of this study indicated that the quality of semen was affected by the morphology of spermatozoa. Of the total 22,099 sperm cells studied, 5,065 (23.0%) of them were found defective. A value of above 20% total abnormalities is sufficient to reject a bull for semen production [43, 44]. Although not significant, the defective sperm cell count was higher in Holstein-Friesian bulls (36.1%) than Jersey bulls (29.3%). In this study, the proportion of minor sperm cell defect (41.3%) exceeded the major sperm cell defect (6.0%). Besides, out of the total 5,065 defective sperm cells counted, 1,339 (26.4%) of them were found to be bent tailed.

Adugna, (2016) [43], indicated that the proportion of bent tail out of the total defective spermatozoa in bovine semen should not exceed 8%. Furthermore, the amount of bent tailed spermatozoa in fertile bull should lie between 8-12% out of the total defective sperm cells [44]. There has been a significant relationship (P<=0.05) between the forward motility of sperm and bent tail type of sperm abnormality. This minor sperm cell defect proportionated 6.1% out of the total sperm cells studied. This type of sperm abnormality is encountered during and just after the ejaculation and collection of semen and during processing it. Therefore, great care should be given during collection and handling of fresh semen before it is frozen [39, 45].

This study revealed that the progressive forward motility of bovine sperm was affected with the defective sperm cells (R^2 = 77.02%). Moreover, the tail defective abnormalities significantly (p<0.05) accounted for the quality of semen. Therefore, it can be summarized that the quality, in this case the forward progressive motility of bovine semen could be improved by proper handling of pre and post semen collection and processing activities. As sperm motility is markedly influenced by temperature, so temperature control in the pre and post collection stages of semen examination is critical.

Conclusion and Recommendations

Assessment of sperm morphology is, by contrast, a useful and important aspect of semen examination. The quality of bovine semen is highly correlated with the morphology and viability of spermatozoa (Pearson, 2008).

The morphological evaluation findings of this study revealed that the quality of fresh bovine semen, in this case the forward progressive motility of bovine semen is highly affected by the morphological abnormalities of spermatozoa. Of the total 22,099 sperm cells studied 5,065 (23.0%) of them were found defective. Furthermore, of the total 5,065 defective sperm cells, 1,339 (26.4%) of them were found to be with bent tails. The progressive forward motility of fresh spermatozoa in both breeds of bulls was highly affected by the defective morphology of spermatozoa. The defective sperm cells were found to affect significantly (P<0.05) the quality of fresh semen in both breeds of bulls. Besides, bent tailed sperm abnormality was the most important type of sperm cell defects which significantly (p<0.05) affected the individual forward motility of spermatozoa. Based on the above conclusion, the following points are recommended:

- Defective sperm cells are one of the major factors affecting the quality of semen which can be minor or major in type. The findings of this study indicated bent tail being the main type of sperm abnormality. Therefore, attention should be given for a close examination of the morphology of semen.
- Since bent type of sperm abnormality arises just after ejaculation due to inadequate temperature, pH or osmotic pressure control, great emphasis should be given in controlling these factors
- As bent type of sperm cell defect is encountered during and after semen collection, great care and precautions is needed in collection and handling of fresh semen
- Semen collectors, sterilization room personnel, semen laboratory technologists and semen quality control experts at large should focus on the proper handling of materials necessary for the production of semen in the center production and processing laboratory.
- Much care is needed in the handling of semen if the results of its examination are to be meaningful. Spermatozoa are very sensitive to cooling, so the semen must be maintained at temperatures close to that of the body (above 30°C) prior to and during assessment, which should be undertaken as promptly as possible after collection.
- Further study on factors affecting the quality of fresh and frozen semen should be undertaken to determine the forward progressive motility of sperm thereby to assure its quality.

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