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Evaluation of motility and morphology of frozen bull semen under different thawing methods used for artificial insemination in North Shewa zone, Ethiopia

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ABSTRACT

The frozen bull semen straws were gathered from three specifically chosen locations in the North Shewa Zone, namely Baso, Chacha, and Debre-Birhan town, and the laboratory work was carried out at the Debre Birhan agricultural research center. The researchers wanted to see how different thawing methods affected the mass motility of frozen bull sperm. The thawing protocols used for mass motility evaluation were 37 °C for 30 s, 35 °C for 40 s, 40 °C for 40 s, body temperature using a hand for 15 s and body temperature using a hand for 30 s by using a water bath. The mass motility of the frozen bull semen was evaluated subjectively using a phase contrast microscope with 10x magnification. The frozen straws were taken from six bulls and two breeds (Holstein Friesian and 75% Holstein Friesian). Motility, morphology, and storage time length of frozen bull semen were analyzed using the general linear model of SAS (9.0). The mass motility of spermatozoa varied from 31 to 41.6 percent in different thawing protocols. Among the thawing protocols, 37°C/30 s (thawing at 37°c for 30 s) was found to have a better motility percentage, which was about 41.6 percent of the frozen bull semen being motile. The frozen sperm cell motility using the 37 °C for 30 s thawing protocol from the national artificial insemination center was 47%, Baso 46.6% and Chacha 39.6%. There was no significant difference ($p > 0.05$) in the percentage of mass motility of frozen bull spermatozoa between Holstein Friesian and 75% Holstein Friesian cross breeds. There was no significant difference ($p > 0.05$) in the mass motility and morphology of frozen bull spermatozoa at different storage time length (58–204 days). The current study indicated that the major problems with low semen mass motility were thawing protocols which were used by artificial insemination technicians. Finally, the study recommended that thawing at 37 °C for 30 s had better frozen bull semen mass motility than other methods, so, artificial insemination technicians should use this recommended thawing method to have better frozen bull semen mass motility when they are doing artificial insemination (AI).

1. Introduction

Artificial insemination, which involves manually placing sperm in the female's reproductive system instead of natural, mating by a bull, has become a common procedure for improving the genetics of farm animals such as cattle. It has been widely used for breeding dairy cattle as the most valuable management practice available to the cattle producer and has produced bulls of superior genetic available to all producer (Webb, 2003; Bearden, 2004). According to Webb (2003) artificial insemination was introduced in 1938 in Asmara which is the current capital city of Eritrea, then that was part of Ethiopia. It was again dropped due to unaffordable expenses of importing semen, liquid nitrogen and other related inputs requirement. In 1972, an independent service was started in the North Shewa Zone under ministry of agriculture. In general the technology of AI for cattle has been introduced at the farm level in the Ethiopia over 38 years ago as a tool for genetic improvement. The current National Artificial Insemination Center (NAIC) was begun in 1984 to coordinate the overall artificial insemination (AI) operation at national level.

The efficiency of the service in the country, however, has remained at a very low level due to infrastructure, managerial, and financial constraints, as well as poor heat detection, improper timing of inseminations and embryonic death (Shiferaw et al., 2003). Dairy cattle's breeding is mostly uncontrolled in Ethiopia, making genetic improvement difficult and an appropriate bull selection criteria has not yet been established,

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applied and controlled. Even if artificial insemination is the most commonly used and valuable biotechnology tool and has been in operation in Ethiopia for many years, the achievement and impact of the operation has not been well-documented and evaluated.

Reproductive problems related to crossbreed dairy cows under farmer's situation were vast, including management. It is widely believed that the artificial insemination service in Ethiopia has not been successful to improving the reproductive performance of the dairy industry (Sinishaw, 2005). According to previous studies (Desalegn et al., 2008; Dekeba et al., 2006) AI service is weak and even declining due to unreliable service in the smallholder livestock production systems of the Ethiopian highlands. The problem is more aggravated by lack of recording scheme, wrong selection procedures, and poor management of AI bulls associated with poor motivation and skills of inseminators (Shiferaw et al., 2003).

Artificial Insemination (AI) practiced as a cattle genetic improvement program for about three decades in Ethiopia, but with little success. The most important constraints associated with artificial insemination were the loss of structural linkage between the AI Center and service giving units, absence of collaboration and regular communication between the National Artificial Insemination Center (NAIC) and stakeholders, lack of breeding policy and herd recording system, inadequate resources in terms of inputs and facilities, and the absence of incentives and rewards to motivate AI technicians (Desalegn et al., 2008). But related constraints like thawing technique, heat detection, handling of liquid nitrogen container and motility of frozen bull semen were not evaluated. The cross breeding of dairy cattle program in Ethiopia is facilitated by using frozen bull semen organized by the national artificial insemination center, but there is no well organized evaluation conducted to assess distributed frozen semen motility as well as morphology and its handling and thawing methods by different AI technicians. Fertility from artificial insemination with frozen/thawed bull semen is lower when compared with fresh bull semen. That can be somewhat compensated for using a huge number of viable sperm cells. For this reason, proper assessment of frozen bull semen motility and morphology with recommended thawing methods and storage time is of highest interest for artificial insemination dairy technology, as it should provide hopes or insights into the fertilizing ability of the frozen bull sperm cell. Frozen bull semen handling for artificial insemination is a crucial phenomenon towards having an acceptable number of calves. Frozen semen motility and morphology evaluation is important to predict its fertilizing capacity. Therefore, the objective of this research was to evaluate the motility and morphology of frozen bull semen under different thawing methods and different storage length which was used for artificial insemination in North Shewa Zone.

2. Material and methods

2.1. Description of the study area

The study was conducted at the laboratory of livestock research directorate of Debre Birhan Agricultural Research Center (DBARC) in the cool highland of central northern of Ethiopia which is located at latitude of 9° 36′N and longitude 39° 38′E. The area is located at 120 km northeast from Addis Ababa with the altitude of 2780m above sea level and the mean annual rainfall of the area ranges from 781-1279mm. The maximum and minimum annual temperatures of the area are 17.8 °Celsius and 8.83 °Celsius, respectively. Similarly, the mean monthly maximum and minimum temperatures of the area are 18.6 °Celsius and 8.20 °Celsius, respectively and relative humidity 68% (center data). The climate characterized by bimodal rainfall consists of long rain season (June–September), short rain season (February–May) and dry season (October–January) (Fekadu, 2015).

2.2. Animals and sampling procedures

The study districts were selected purposely based on the potential of artificial insemination and milk-shade districts (Baso and Chacha) and proximate to Debre Birhan City. A questionnaire survey was conducted on artificial insemination technicians to collect data on the methods of thawing of frozen semen, data recording, arrival of frozen semen to the AI site, management of liquid nitrogen container and how they could top up the liquid nitrogen container, how AI technicians contact the farmers when a cow is in estrus, how they could travel to the estrus cow and how they detect the estrus status of the cow.

Frozen bull semen was collected from three districts and one research center in North Shewa Zone to evaluate different semen quality parameters. A total of 68 straws of frozen semen from six Holstein Friesian and 75% Holstein Friesian cross breed bulls were obtained and evaluated in the present study. Samples were then transported to Debre Birhan agricultural research laboratory in a small -196°c liquid nitrogen container for semen quality evaluation.

2.3. Frozen semen quality evaluation

Examine the mass motility and morphology of frozen bull sperm using various thawing techniques and storage times. For motility testing, the following thawing protocols were used: 37 °C for 30 s, 35 °C for 40 s, 40 °C for 40 s, body temperature by hand for 15 s, and body temperature by hand for 30 s using a water bath. Studies by Desalegn et al. (2008) and (Amare and Reda, 2012) claim that the normal thawing process was 37 °C for 30 s, although AI technicians used 35 °C for 40 s and 40 °C for 40 s. The length of storage time (from collection to evaluation date) is also taken into account. Three researchers independently assessed the motility of the sperm, and the average motility was calculated. The frozen bull semen was then examined by extracting a frozen semen straw from a -196 °C container, wiping it immediately with a towel, and placing it in a 37 °C water bath for 30 s. Frozen bull sperm motility was estimated subjectively by using a phase contrast microscope (Scope Technology Scope Photo 3.0.12.) and a micropipette to drop frozen semen on the slide and cover it with a cover slip and seen with a magnification of 10X on the objective lens. The frozen bull semen motility is graded or leveled zero to five (0–5) score and percentage based on the intensity of motility as described by (Evans and Maxwell, 1987).
According to these methods:

1. Very poor: very few active spermatozoa, with weak movement around (around 10% of mass motility)
2. Poor: few active spermatozoa (20–40% mass motility),
3. Fair: (slow moving spermatozoa (40–70% of mass motility)
4. Good: (dense, vigorous movement of about 75–90% of sperm cells were active)
5. Very good: (cloudy, dense and rapidly moving more than 90% of sperm were active.

The morphological abnormalities of sperm was assessed using stained slides with Eosin Nigrosin stain or dye product. The abnormality of the head, mid and tail had been evaluated using phase contrast microscope at the magnification of 100X. Using pipette and putting a drop of frozen sperm was placed on a pre-warmed microscope slide (35°c) and then stained with Eosin-Nigrosin staining methods and products (Rege et al., 2000).

A drop of Eosin, four drops of Nigrosin and a drop of semen are placed on a clean, grease free slide then mix the frozen semen first with eosin and then immediately with Nigrosin stain. Again the mixture is taken on the edge of a slide and pulled across the top of another slide leaving a smear, thereafter it was air dried 100 numbers of spermatozoa are counted under oil immersion at a magnification of 100X in different areas of smear and classify them as normal and abnormal sperm cell (Rege et al., 2000)

$$Normal\ \ Morphology\ \ percentage = total\ \ normal\ \ counted / total\ \ numbers\ \ counted \times 100$$

2.4. Statistical analysis

The effect of different thawing method breeds (Holstein Friesian and 75% Holstein Friesian), individual bull, storage length and location were analyzed by the general linear model using SAS 9.0 program. The significance of fixed effects (thawing method, breeds, storage length and individual bull) was tested by least squares under Tukey' HSD.

The model for motility and morphology is as follows,

$$Y_{ijkm} = \mu + B_i + S_j + W_k + E_{ijkm}$$

Y_{ijkm} = Dependent variable/motility or morphology of frozen bull semen

μ = Overall mean

B_i = Effect of i^{th} blood levels

S_j = Effect of j^{th} thawing methods

W_k = Effect of k^{th} day length

E_{ijkm} = Residual error

3. Results and discussion

3.1. Motility of frozen bull semen in different thawing techniques and genotypes

According to the current investigation, the mass motility of frozen bull semen ranged from 31 to 41.6 percent, as shown in Table 1. At national artificial insemination center (NAIC), however, the average mass motility of fresh semen was greater than 60% across bulls and breeds of various blood levels. According to Desalegn et al. (2008), the average mass motility of frozen bull semen in the Amhara region was 51.7 percent and 53.18 percent at NAIC, this is higher than the current average. In the current investigation, there was no significant difference ($p > 0.05$) between the thawing method and genotypes. With a 37°C/30 s thawing procedure, the preserved frozen bull semen mass motility taken straight from NAIC exhibited considerably greater individual semen motility. It could be explained by the fact that different thawing procedures affect the mass mobility of frozen bull sperm (Amare and Reda, 2012, Bekana, Tegeg). The recent findings suggest that thawing methods have an impact on frozen sperm motility. Due to shipping, filling, thawing methods, frequency of topping up liquid nitrogen to the handling and management of liquid nitrogen containers, harsh weather circumstances, and the effectiveness of artificial inseminator thawing ability, frozen bull semen mobility may be low/poor. The mass motility of frozen sperm may be reduced as a result of the ambient temperature. Other than frozen semen motility, breed, age, and season might affect semen quality characteristics (Hirwa Claire et al., 2017). The frozen semen mass motility was not affected by the bull's genotype/blood level, which was directly accepted by the author (Hirwa Claire et al., 2017) in their study on the role of breed on the quality of bovine semen utilized for artificial insemination using Friesian and Jersey cattle. The frozen bull semen motility, in particular, was very low, even below the recommended frozen semen motility, at hand/30 s and 40°C/40 s. As a result, delivering a uniform and standard thawing approach and procedures requires special care. Friesian Frisian

Table 1. Motility of frozen bull semen in different thawing method and breed.

Parameters	N	Motility (%)	Standard deviation
Thawing method			
35°c/40 s	10	36.23	7.9
37°c/30 s	38	41.6	10.4
40°c/40 s	5	33.4	2.9
Hand/15 s	11	39	11
Hand/30 s	4	31	3.3
Genotype			
Pure Holstein Friesian	52	38.4	10.2
75% Holstein Friesian cross with local	16	39.9	9.8

3.2. Morphology and motility of frozen bull semen under different locations

The average mass motility of frozen bull semen from a different site was 44.27 percent (Table 2), which was lower than the results from Desalegn et al. (2008) and Amare and Reda (2012). In the Amhara region, the average mass motility of frozen bull semen was 51.67 percent (Birhanemeskel and Kide, 2018), while it was 52 percent in Adigrat. The current study showed that frozen semen mass motility from the NAIC was 47%, which is a bit comparable with Desalegn et al. (2008) and (Amare and Reda, 2012) which was 51.67% and 51.5% in the Amhara region respectively, while the chacha was under the recommended level of frozen semen mass motility of 39.4%. Similarly, Desalegn et al. (2008) the average mass motility of post frozen semen was 50.83%, which is in contrast with the results of the mass motility of frozen semen in this study, which was 47%, which is from NAIC. The currently frozen bull semen mass motility was better than the (Amare and Reda, 2012) post-freeing motility of the long rain season and short dry season, which was 38.51% and 39.68% respectively. Similarly, Engidawork (2018) the average percentage of frozen bull semen motility in Harar regional state was 49.6%, which is in line with the current results of motility of frozen semen of 46.6% in Baso district and 47% in NAIC of this study. According to Amare & Reda, (2012), the mass motility of frozen bull semen at field and store-level was different due to dilution, chilling, freezing, and storing and transportation of semen from NAIC to different regions of the artificial center, zone, and districts. Except for Chacha, which had 39.4 percent frozen bull semen motility, the current results showed that three locations were above the acceptable amount. The current study evaluated frozen bull semen mass motility collected from four locations using the suggested thawing methodology. The morphology of the frozen bull semen was 92.17% of the sperm cells were morphologically normal. According to (Chapman et al., 2016), 75% of the sperm cells are morphologically normal. It is recommended for artificial insemination. The present study showed that there was a contrary to (Engidawork, 2018), which was about 72.6% of the sperm cells were morphologically normal in the Harar Region, while the current study indicated that 92.17% of the sperm cells were normal. In similar, the report indicated that major morphological defects can influence the artificial insemination delivery system by minimizing the fertilizing capacity of frozen-thawed bull semen and enhancing the number of services per conception. In the present study, 92.17% of morphologically normal spermatozoa, in line with Desalegn et al. (2008), 91.5% of sperm cells were normal morphology on Holstein Friesian. The present study showed that there was no significant ($p > 0.05$) difference in storage length and another study (Amare and Reda, 2012) low motility of frozen semen believed to be that the semen was stored or kept for a time, light, no top-upping of liquid nitrogen without being utilized and poor handling.

Table 2. Percent of motility and morphology of frozen bull semen under different location and storing date using standard thawing methods 37°c/for 30 s.

Parameters	N	Motility % (SE)	Normal morphology (SE)
Location		44.27	92.17
Baso	12	46.6 (13)	94.2 (2.3)
Debre Birhan	6	44.1 (12.4)	93.1 (2.9)
Chacha	5	39.4 (7.5)	91.8 (3.03)
NAIC	15	47 (12.3)	89.6 (7.9)
Storage length (days)			
58	10	37 (5.6)	91.9 (2.7)
102	5	37 (8.1)	94.2 (3.27)
153	6	44.1 (12.4)	93.1 (2.9)
214	6	39.1 (10.3)	94.6 (1.8)

Table 3. Motility and morphology of different bulls.

Bull ID	N	Motility%	Normal spermatozoa %
1733	11	41.8 (10.2)	89.8 (4.7)
1818	6	54.1 (7.3)	94.6 (1.8)
1873	5	39.4 (7.5)	91.8 (3)
1879	5	39 (13.4)	93.8 (2.7)
2664	5	37 (8.1)	94.2 (3.2)
9766	5	36 (4.1)	80 (8.4)

3.3. Motility and morphology of different superior bulls

The current study showed that individual frozen bull semen motility and morphology varied from 36 to 54.1% and 80–94.6%, respectively, as indicated in Table 3. The individual bull frozen semen motility and morphology are shown in Table 3. The current study showed that frozen semen motility from 1818 a pure Holstein Friesian breed, was 54.1% and 94.6% of normal morphology, which contrasts with Desalegn et al. (2008) and (Amare and Reda, 2012) which was 53.2% at NAIC. The current 1818 pure Holstein Friesian breed frozen bull semen motility and morphology was better than the (Amare and Reda, 2012) post freeing motility of the long rain season and short dry season, which was 38.51% and 39.68% respectively.

4. Conclusion and recommendation

Using the same thawing protocol which is 37°c/30 s, the frozen bull semen motility from the NAIC was better (47%), followed by Baso with 46.6%, while the chacha was 39.4% under the recommended level of frozen semen motility of 39.6%. The motility of frozen bull semen between different thawing techniques varied from 31 to 41.6 percent. From the five frozen bull semen thawing techniques, 37°c/30 s is better and about 41.6 percent of frozen bull semen is motile. The average motility of frozen bull semen across locations was 44.27 percent. The motility and morphology of frozen bull semen in the same thawing technique, which is 37°c/30 s, varied from 39 to 47 percent and 89.6 to 94.2 percent respectively in the location of different artificial insemination centers. The storage time had an influence on the motility of frozen semen and varied from 37% to 44.1%. There was variation in individual bulls for semen motility, which varied from 36-54.1%. The major problem with low semen motility was thawing methods which were used by AI technicians. Finally, the study recommended that thawing at 37°c for 30 s had better frozen bull semen motility than other methods. So, the AI technician should use this recommended thawing method to have better frozen bull semen motility when they are doing artificial insemination.

Declarations

Author contribution statement

Shenkute Goshme; Tadiws Asfaw; Chekol Demiss; Shanbel Besufekad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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