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Quality Evaluation of Cryopreserved Semen Used in Artificial Insemination of Cattle in Selected Districts of Western Gojjam Zone of Amhara Region, Ethiopia

[1]Tadesse Amare Sisay, [2]Abadi Amare and [3]Zeleke Mekuriaw

[1]College of Agriculture, Department of Animal Science, Wollo University, Dessie, Ethiopia
[2]School of Veterinary Medicine, Wollo University, Dessie, Ethiopia
[3]Bahir Dar University, Bahir Dar, Ethiopia

Abstract: A study was conducted in three purposively selected districts of western Gojjam zone of Amhara National Regional State (ANRS), viz., Bahir Dar Zuria, Mecha and Debub Achefer. The objective of this study was to evaluate post thawed semen quality. Semen characteristics like semen motility, live-dead percentage and morphological abnormalities of frozen semen were assessed. Data were analysed by descriptive statistics and one way ANOVA (analysis of variance) using SPSS version-16. The motility of frozen semen between regional Artificial Insemination Center (AIC) and districts level not showed significant difference. From the total amount of 200 straws sampled frozen semen, 67.07% have been live and 32.93% were dead. The total major morphological defects showed significant difference ($p<0.05$) between the region and districts. The regional AIC frozen semen has highly significant ($p<0.05$) lower defects than districts. But the overall average percentage of major morphological defects (3.7%) was not more than the recommended level 4%. The minor morphological defects of 75% Holstein blood level of frozen semen significantly ($p<0.05$) was lower than 100% Holstein blood level. Finally, it was concluded that large numbers of spermatozoa were lost due to bad handling, transportation, storage and environmental effects, hence, demanding further investigation on factors which affect semen quality.

Key words: Ethiopia % Live-Dead % Motility % Morphological Defects %Post Thawed Semen Quality

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]. The NAIC produced and distributed about 100, 000L of liquid nitrogen in Ethiopia between 1984 and 1993. Whereas the Amhara region AIC produced, 23,590L of liquid nitrogen in 2001. All bull semen used for AI is cryopreserved; allowing long storage times and easy distribution and inseminations are generally done by trained inseminators [4-7].

The process of cryopreservation represents an artificial interruption of the progress of the spermatozoa towards post-ejaculation maturation and fertilization. The major disadvantage is that procedures involved in the cryopreservation process are harmful to spermatozoa and even the best preservation techniques to date result in about half of the sperm population that survive the freezing and thawing procedures. Change in temperature imposes changes on the composition and structure of various sperm plasma membrane domains [8], thereby modifying their function. As it has been demonstrated, the cryopreservation makes damage on sperm membranes, cytoskeleton, motile apparatus and nucleus, alter cell metabolism. Moreover, freezing and subsequent thawing procedures render surviving spermatozoa different from spermatozoa before cryopreservation. They become very sensitive to any stresses by their environment *in vivo* as well as *in vitro* [9, 10]. As a result, fertility from the AI

Corresponding Author: Abadi Amare, School of Veterinary Medicine, Wollo University, P.O. Box, 1145, Dessie, Ethiopia.

with frozen thawed semen is poorer than that obtained with fresh semen, which can be partially compensated by inseminating greater numbers of live spermatozoa. For this reason, proper assessment of the post-thaw quality of spermatozoa is of highest interest for AI industry, since it can provide insights upon the fertilizing capacity of the cryopreserved spermatozoa. Cryopreserved semen management for artificial insemination is a crucial step towards obtaining acceptable pregnancy rates. There by the main goal of semen evaluation is to predict its fertilizing ability [11]. The objective of this research was, therefore, to evaluate post frozen semen quality and to investigate potentially harmful factors for semen quality and subsequent fertility in cattle in the study areas.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in three districts, viz., Bahir Dar Zuria, Debub Achefer and Mecha and regional artificial insemination center located in Western Gojjam zone of the Amhara Regional State. All the study areas were purposively selected because it is believed that these areas were the ones where an AI service is widely exercised and constitute wide range of agro- ecology, located in northwestern part of Ethiopia. The agro-ecological zone of the study area is characterized by lowland (11.2%), mid-altitude (72%), highland (16.6%) and frost *(wurch*) (0.2%) [12].

Study Population and Sampling Procedure: The frozen semen samples collected from the study area of the three districts (Bahir Dar Zuria, Debub, Achefer and Mecha) and regional artificial insemination center were used for assessment of semen quality. The sub-total of eight samples (four sample from 75% and four sample from 100% blood level) of frozen semen have been taken from 75 and 100% blood level of Holstein Friesian breed, from the three districts and regional artificial insemination center. In addition to the above eight samples of frozen semen, two samples (one from each) of frozen semen was taken from 75 and 100% blood level of Jersey breed from regional artificial insemination center. Finally, 10 samples of frozen semen were collected purposively for the laboratory examination for percentage of motility, dead-live and morphological defects. Each of the 10 samples of frozen semen contains 20 straws per sample (10x20=200). Generally, the sample size for semen assessment was 200 straws collected for laboratory examination.

Post Frozen Semen Quality Evaluation: For evaluations of semen quality, physio-morphological assessment of frozen semen was conducted in laboratory at National Artificial Insemination Centre (NAIC). Semen characteristics like semen motility, live-dead percentage and morphological abnormalities of frozen semen were assessed following standard procedures described by Britto *et al.* [11], Rodriguez-Martinez [13], Zewdie *et al.* [14], Hafez [15], Bamba [16], Herman *et al.* [17], Arthur [18] and Januskauskas *et al.* [19] in the laboratory of National Artificial Insemination Center, Kality, Addis Ababa.

Statistical Model

Model 1: Factors affecting semen quality at districts and regional AIC level

$$Yij = \mu + Ei + Fj + eij$$

Where:

- Yij = Semen quality (Motility, dead-Live and Morphological defects)
- μ = Overall mean
- Ei = Effect of i^{th} breed type of bull (Holstein and Jersey)
- Fj = Effects of j^{th} frozen semen blood level (75% and 100% Holstein and Jersey)
- eij = Random error associated with Yij^{th} observation

RESULTS AND DISCUSSION

Motility Evaluation of Post Thawed Frozen Semen: The current study showed that post-frozen semen motility was varying from 35 to 60% however, during fresh state these samples were 75 to 80% for the same bull and the same blood level of the sample as shown in Table 1. The stored frozen semen motility which was taken from regional AIC had relatively better motility than frozen semen at districts level. It may be due to transportation, handling and environmental factors (bad weather conditions) that resulted to lower motility of semen at district level. Blood level of 100% Jersey breed has below the recommended levels of post-thawed semen motility (40%) at regional AIC storage, which was 35% and needs serious attention to deliver this semen to the AI beneficiaries. Frozen semen motility from Holstein Friesian breed was better and withstands weather condition and poor handling and above the recommended levels (40%) of post frozen semen motility.

Table 1: Motility comparison between fresh and frozen semen in Regional AI center and Bahir Dar Zuria, Mecha and Debub Achefere Districts, West Gojjam

Semen sample	N	Frozen semen (%)	Fresh semen (%)	Difference (%)	Motility %	Date of semen production
75-114	20	45	80	35	62.5 (17.5)	26/2/2000
11-157	20	45	80	35	62.5 (17.5)	5/6/2001
11-134	20	60	80	20	70. 0 (10)	30/1/2002
10-185	20	60	75	15	67.5 (7.5)	25/1/2002
75-114	20	35	80	45	57.5 (22.5)	25/5/2001
10-189	20	50	80	30	65. 0 (15)	9/6/2001
75-114^{F}	20	60	80	20	70. 0 (10)	9/6/2001
75-114^{F}	20	60	80	20	70. 0 (10)	15/8/2002
10-171^{F}	20	60	80	20	70. 0 (10)	21/1/2000
10-173^{F}	20	40	80	40	60. 0 (20)	9/6/2000
Average	20	51.5	79.5	28	65.5 (14)	-

75-114=75% Holstein Frisian crossbreed with 25% Boran (from regional AIC), 11-157= 100% Jersey, 11-134= 75% Jersey, 10-185=100% Holstein Frisian breed (from regional AIC), 10-189=100% Holstein Frisian breed and 10-173=100% Holstein Frisian breed, Jersey breed blood level taken from only regional AIC, N= number of straws and the first four sample (75-114A to 10-185) from regional AIC and the rest from districts.

Table 2: One-Way analysis of variance of post frozen semen motility of Regional AIC and Bahir Dar Zuria, Mecha and Debub Achefer districts, West Gojjam

Parameter	Place of frozen semen collected	N	Motility %	95 % CI	P-value
Motility of post frozen semen	Districts	120	50.83 (17.23)	45-60	0.125
	Region	80	52.50 (14.00)	35-60	
	overall	200	51.66 (15.62)	35-60	
Motility of post frozen semen	Breed type and blood level				
	100% Holstein	80	52.50 (13.0)	40-60	0.037
	75% Holstein	80	50.00 (17.6)	35-60	
	Overall	160	51.25 (15.3)	35-60	
	100% Jersey	20	45.00 (8.4)		0.041
	75% Jersey	20	60.00 (10.1)		
	Overall	40	52.50 (9.3)	45-60	

CI= confidence interval, CI for Jersey is not presented due to single sample per blood level.

Similarly, Desalegn [20] in Amhara Region indicated that the average motility of post frozen semen was 51.67% which is in line with the result of average motility of post frozen semen in this study (51.5%). The semen motility dropped maximum difference from 80 to 35% (45%) and the minimum difference was from 75 to 60% (15%) in this study. In this investigation, the results of motility of post frozen semen at field and store level were different. It was believed to be due to dilution, chilling, freezing and storing and transportation of semen from NAIC to regional AI sub centers and to districts AI centre or to Kebele caused further reduction in the semen motility. According to IAEA and FAO [21] post frozen semen bellow minimum recommended motility of 40% should be discarded, because it affects the efficiency of semen fertility. As the result, the reduction of motility of the post thawed semen has its own impact on conception rate.

The post frozen semen examination assesses both the ability of the semen to withstand freezing, thawing and the efficiency of the processing itself. If there are 40% or more of semen moving actively forward after freezing and thawing the quality is acceptable for AI [21].

The result of one- way ANOVA on the post frozen semen characteristics of at districts and regional AIC is presented in Table 2. The motility of frozen semen between regional AIC and districts level not showed significant difference ($p>0.05$). The highest percentage motility was observed in regional AIC (52.5%). It was observed that 100% blood level of Holstein Friesian frozen semen motility significantly ($p<0.05$) higher than 75% blood level of Holstein Friesian frozen semen. Even though statistically significant differences ($p<0.05$) between the two was found the value for both was not lower than the recommended lower values of 40% [14]. However, 100% Holstein frozen semen motility in this study nearly similar to Desalegn [20], who reported (54.50 ± 3.83) for Holstein Friesian but slightly differ with 100% Jersey of Desalegn's report but greater than Kelay [22], overall motility of frozen semen who reported that, (44.99) at Selale and Addis Ababa. Whereas the 100% blood level of Jersey frozen semen motility significantly ($p<0.05$) lower than 75% blood level of Jersey breed frozen semen. It may be the result of 100% of Jersey blood level was more sensitivity to lose its motility by bad handling, frequent exposure and other environmental factors than 75% blood level of Jersey.

Table 3: Dead and live percentage of frozen semen in Regional AI center and Bahir Dar Zuria, Mecha and Debub Achefere districts, west Gojjam

Semen sample	N	TCCPS	TLCC	%LCC	TDCC	%DCC
75-114	20	250	141	56.4	109	43.6
11-157	20	160	109	68.1	51	31.9
11-134	20	227	145	63.9	82	36.1
10-185	20	313	251	80.2	62	19.8
75-114	20	328	222	67.7	106	32.3
10-189	20	254	157	61.8	97	38.2
75-114^{F}	20	234	145	62	89	38
75-114^{F}	20	266	163	61.3	103	38.7
10-171^{F}	20	258	182	70.5	76	29.5
10-173^{F}	20	476	375	78.8	101	21.2
Average	20	276.6	189	67.07	87.6	32.93

75-114=75% Holstein Frisian crossbreed with 25% Boran (from regional AIC), 11-157= 100% Jersey, 11-134= 75% Jersey, 10-185=100% Holstein Frisian breed (from regional AIC), 10-189=100% Holstein Frisian breed and 10-173=100% Holstein Frisian breed, Jersey breed blood level taken from only regional AIC, TCCPS=.total cell counted per sample, TLCC= total live cell counted per sample, %LCC=percentage of live cell counted, TDCC= total dead cell counted per sample, %DCC= percentage of dead cell counted.

Even though, there was difference in frozen semen motility among the region and districts due to difference in handling, there was no significant effect on semen motility due to handling differences. However, the low motility observed in one sample of semen obtained from districts was as low as 35% on the average. The reason for this condition was believed to be that the semen was produced from 75% Holstein and distributed without demand to the region and was kept for many years and frequently exposed to light and air to longer time without being utilized, transportation, bad handling and shortage of liquid nitrogen. Similarly, the mean frozen semen motilities for sample semen obtained from region was as low as 45%. The other possible reason assumed to cause the difference was also the shifting of the long-experienced laboratory technicians by new ones during that time.

Dead and Live Evaluation of Post Thawed Semen: From the total amount of 200 straws sampled semen, 67.07% have been live and 32.93% were dead. It might be due to chilling, freezing and storage at different AI sub centre and transportation of semen from region AI sub centers to district AIC and to Kebeles sub centre caused further lose of viability. Live cells had no pink eosin stain in them and appeared uniformly white. When the semen was dead, the membrane was damaged and it was permeable to eosin. Between districts and regional AIC both Holstein Friesian and Jersey breeds of frozen semen sample were not as such different in live-dead percentage in this study (Table 3).

According to IAEA and FAO [21], for normal reproduction function, at least 50% of the post-thawed spermatozoa should be live, because each straw initially pack with 30 million spermatozoa and at least half of this figure should be live or 15 million sperm cells per straw expected to be live. Even though the live-dead post thawed spermatozoa may not as such affect the AI efficiency in the study area, large numbers of spermatozoa was damaged and need great attention.

Morphological Defect Evaluation of Post Thawed Spermatozoa: The result of one- way ANOVA on the morphological characteristics of frozen semen at region and districts is presented in (Tables 4-6). The total major morphological defects showed significant difference ($p<0.05$) between the region and districts. Hence the highest proportion of major defects was observed in districts (4.3±0.76) which was less than Desalegn [20], who reported for Jersey (6.5± 3.08) and greater than Holstein (2.27± 093). The regional AIC frozen semen has highly significant ($p<0.05$) lower defects than districts. Even though the highest morphological defects registered in districts, the overall major morphological defects not more than the recommended level 4% IAEA and FAO [21]. The average major morphological defects of 100% Holstein Friesian frozen semen (3.842 ±1.23) almost similar with 75% blood level of Holstein frozen semen (3.915 ±0.12). However, 100% blood level of Jersey frozen semen significantly ($p<0.05$) higher than average major morphological defects of (4.06± 0.45) both 75% and 100% of Holstein frozen semen and 75% blood level of Jersey. But the overall major morphological defects of Holstein breed of frozen semen (3.899 ± 0.675) greater than overall average of Jersey breed (3.23± 0.63).

Table 4: One-Way analysis of variance to asses and see effect of handling of semen between Region and Districts of frozen semen morphological defects

Parameter	Place of frozen semen collected	N	Motility %	95 % CI	P-value
Major morphological defect	Districts	120	4.30 (0.76)	2.40-6.50	0.011
	Region	80	2.93 (0.04)	2.04-4.06	
	Overall	200	3.61 (0.40)	2.04-6.50	
Major morphological defect	Blood level and breed type				
	100% Holstein	80	3.84 (1.23)	2.40-4.97	0.618
	75% Holstein	80	3.91 (0.12)	2.04-6.50	
	overall	160	3.89 (0.67)	2.04-6.50	
	100% Jersey	20	4.06(0.45)	4.06	0.043
	75% Jersey	20	2.40 (0.81)	2.4	
	Overall	40	3.23 (0.63)	2.40-4.06	
Minor morphological defect	Districts	120	56.51 (18.60)	43.5-65.6	0.002
	Region	80	30.38 (12.00)	15.4-51.3	
	overall	200	43.45 (15.30)	15.4-65.6	
Minor morphological defect	Blood level and breed type				
	100% Holstein	80	57.27 (6.20)	51.3-65.6	0.037
	75% Holstein	80	46.91 (13.50)	26.3-60.3	
	overall	160	52.09 (9.85)	26.3-65.6	
	100% Jersey	20	15.40 (1.50)	15.4	0.083
	75% Jersey	20	28.50 (0.43)	28.5	
	Overall	40	21.95 (0.86)	15.4-28.5	

S.E = standard error, CI= confidence interval, CI for Jersey is not presented due to single sample per blood level.

Table 5: Major morphological defects of post frozen semen Regional AI center and Bahir Dar Zuria, Mecha and Debub Achefere districts, West Gojjam

Semen sample	N	Counted major defect per sample	% of major defects per sample
75-114	404	8.3	2
11-157	416	17	4.1
11-134	427	9.9	2.4
10-185	472	15	3.2
75-114	412	15	3.7
10-189	408	20	5
75-114^{F}	413	27	6.5
75-114^{F}	424	15	3.5
10-171^{F}	424	20.4	4.8
10-173^{F}	425	10.2	2.4
Average	422	15.8	3.76

75-114=75% Holstein Frisian crossbreed with 25% Boran (from regional AIC), 11-157= 100% Jersey, 11-134= 75% Jersey, 10-185=100% Holstein Frisian breed (from regional AIC), 10-189=100% Holstein Frisian breed and 10-173=100% Holstein Frisian breed, Jersey breed blood level taken from only regional AIC,N=number of counted spermatozoa per sample (see Appendix 1, Table 35).

Table 6: Minor morphological defects of post frozen semen Regional AI center and Bahir Dar Zuria, Mecha and Debub Achefere districts, West Gojjam zone

Semen sample	N	Number of minor defects	% of minor defects
75-114	404	98	26
11-157	416	47	15
11-134	427	112	29
10-185	472	227	51
75-114	412	222	58
10-189	408	202	54
75-114^{F}	413	222	60
75-114^{F}	424	170	44
10-171^{F}	424	258	65.6
10-173^{F}	425	236	58
Average	422.5	179.4	46.06

75-114=75% Holstein Frisian crossbreed with 25% Boran (from regional AIC), 11-157= 100% Jersey, 11-134= 75% Jersey, 10-185=100% Holstein Frisian breed (from regional AIC), 10-189=100% Holstein Frisian breed and 10-173=100% Holstein Frisian breed, Jersey breed blood level taken from only regional AIC,N=number of counted spermatozoa per sample and the first four sample (75-114A to 10-185) from regional AIC and the rest from districts (see Appendix 1, Table 36).

The most common major sperm defects in this study were double forms (3.4%), pear shaped hair (2.8%), abnormal counter (1.6%), small abnormal head (5.65%), detached head (3.36%), abnormal head (1.6%), acrosome defects (4.8%), middle piece defects tail stump (5.95%), strongly folded tail (3.16%), hairpin (3.4%), corkscrew defects (1.6%) and narrow at the base (2.2%).

In general, the average major and minor percentages of morphological defects of the frozen semen samples were 3.76 and 46.04%, respectively. According to IAEA and FAO [21], the major morphological defects affect the AI delivery system through decreasing fertilizing ability of the post-thawed semen and by increasing the numbers of service per conception.

The minor morphological defects of 75% Holstein blood level significantly ($p<0.05$) lower than 100% Holstein blood level. The assumption is that the 100% blood level of Holstein breed is pure exotic and highly affected by or sensitive to harsh conditions, whereas the 75% Holstein has hybrid blood level with other breed and that makes less minor morphological defects. Unlike 100% of Holstein the 100% of Jersey breed shown, lowest minor defects (15.4 ± 1.50) than 75% Jersey (28.5± 0.43) and 100% (57.275± 6.20) and 75% (46.915 ±13.50) of Holstein Frisian. Here is the overall mean minor morphological defects of Jersey breed frozen semen (21.95± 0.86) has lower than Holstein Frisian breed (52.092± 9.85).

Even though the average major morphological defects of post thawed semen observed in this study, was not more than the maximum limit (4%) of defects, however, need a great attention. Especially the acrosome defects were more than the average limit of major morphological defects and it determine the fertility of post thawed semen. The average minor morphological defects of post-thawed semen were more than the maximum limit of 20% [21]. According to Bamba [16] morphological defects were necessary to examine the structural detail of spermatozoa and to assess fertility potential (an indication of fertilising capacity of the frozen sperm) of semen sample. Similarly, Bamba [16] shown that the fertilizing ability of spermatozoa is related to the morphological features and decreased fertility in cattle.

In conclusion, results of this study entailed that large numbers of spermatozoa were lost due to bad handling, transportation, storage and environmental effects. Hence, further investigation on factors which affect semen quality and subsequent fertility of cryopreserved semen in cattle should be done. Buying semen is buying genetics and the semen should be from progeny-tested bulls as possible. The advantages of using particular bulls in particular areas and for specific purposes need to be well known to farmers so that they can make an informed decision when they purchase semen from the AI service.

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