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Developing a Gamete Rescue and Cryopreservation Plan for Borana Bulls Semen in and Around Bishoftu Town, Eastern Shoa Zone, Ethiopia

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Abstract: This study was conducted on epididymal spermatozoa of 64 apparently healthy Boran bulls in Bishoftu, Ethiopia from November, 2016 to May, 2017. The current study aimed to assess the sperm parameters (sperm motility and sperm viability) of the cryopreserved epididymal spermatozoa of bulls to set basic information for the cryopreservation plan of epididymal spermatozoa of the Boran bulls. The overall mean values of the sperm mass motility at 0, 1 and 3 hours of post-thaw were 65.08 (0.87) %, 60.52 (0.90) % and 55.95 (0.88) % respectively and the sperm progressive motility at 0, 1 and 3 hours of post-thaw were 62.55 (0.85) %, 57.58 (0.84) % and 52.59 (0.79) % respectively. The overall mean value of the sperm viability at 0 hour of post-thaw was 61.73 (1.18) %. From the animal related factors analyzed, age, body weight and left epididymal weight were seen to be significantly associated ($P<0.05$) with the mean percentage of spermatozoa viability. Except body weight of the bulls, significant differences ($P<0.05$) in the mean percentages of the sperm motility were revealed by the other factors (age, testis weight and epididymis weight). Based on the analyzed sperm parameters, it was observed that the average values of the semen attributes (sperm motility and sperm viability) have almost similar values with that of the values recommended in different literatures for the normal fertile bulls. Therefore, by taking the findings of this study as an initial reference, further studies should be done to develop appropriate protocol for the cryopreservation technique of the epididymal semen of the indigenous bulls, like the Boran bulls.

Key words: Boran Bull · Cryopreservation · Epididymal Semen · Extenders · Sperm Parameters

INTRODUCTION

Artificial insemination (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 [1]. 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 and availability of genetic material from superior bulls without geographical limitations [2].

The unexpected losses of genetically valuable animals, as well as the difficulty to collecting semen from wild species lead to an increase in the use of artificial reproductive techniques, since it proves to be one of the unique possibilities to preserve the genetic material of these animals [3]. So, the recovery and cryopreservation of spermatozoa from the epididymis of deceased animals is a viable option in maintaining the germplasm available for feature use [4].

Studies have shown the efficacy and potential of epididymal spermatozoa to result in fertilization *in vitro* and *in vivo* by using these spermatozoa in artificial insemination, *in vitro* fertilization of embryos and intra-cytoplasmic sperm injection with satisfactory results [5-7]. Similarly, pregnancies and live births from cooled or cryopreserved epididymal sperm have been performed successfully in various domesticated and wild animals including human [8].

Even though chilling semen provides an efficient and successful means of short-term storage; it has yet some adverse effects on the spermatozoa manifested as a

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depression in viability rate, structural integrity and depressed motility in addition to decreased conception rates [9]. For bovine, Tris egg yolk and skimmed milk based extenders have been used to protect sperm from the detrimental effects of cooling and freezing. But milk and milk based extenders are known to be practical and efficient in protecting spermatozoa of various species [10]. Therefore, obtaining caudal epididymal sperm is an important technique in the propagation and conservation of animal specimens with high genetic values after serious injury or from dead animals and endangered species [11]. In addition, when the epididymal sperms are obtained immediately after death, the gametes remain alive for 24 to 48 hours and are viable for fertilization [12].

About cattle, most researches have focused on improving freezing protocols for ejaculated bull sperm. There are limited or few reports from the studies that were done elsewhere in the world, on the use, characteristics, procedures of cryopreservation and fertility of bovine epididymal sperm. In Ethiopia, previously there was not any study which was conducted on bovine epididymal semen, particularly on about its collection techniques, cryopreservation and assessment of the basic semen parameters (sperm motility and sperm viability).

Therefore, the objectives of this study were:

- To assess the basic sperm parameters (sperm motility and sperm viability) on the epididymal semen of Boran bulls those were slaughtered at the Hashim Export Abattoir.
- To evaluate Tris egg yolk and Skimmed milk as sperm extenders for the cryopreservation of Boran bull epididymal spermatozoa.
- To study the potential of the epididymal gametes of Boran bulls to develop appropriate cryopreservation technique protocol in the future.

MATERIALS AND METHODS

Study Area: The study was conducted in Bishoftu from November, 2016 to May, 2017 at Hashim Export Abattoir and at Addis Ababa University, College of Veterinary Medicine and Agriculture. Bishoftu is a town, which is located at 9°N and 40°E with an altitude of 1880 meters above sea level in the central highlands of Ethiopia 47kms South East of Addis Ababa. It has annual rainfall of 1151.6 mm of which 84% falls down during the long rainy season that extends from June to September and the remaining during the short rainy season that extends from March to May. The mean annual minimum and maximum temperatures is 8.5 °C and 30.7 °C, respectively and the mean humidity is 61.3% [13].

The bulls that were slaughtered at the abattoir during this study were mainly originated from Borana areas of Ethiopia. Borana is located at approximately 600 km South of Addis Ababa with an altitude of 970 meters above sea level [14]. The district is affected by recurrent droughts due to disrupted rainfall patterns [15]. It falls in a semi-arid zone and receives an annual rainfall ranging from 400 to 700 mm. Temperature ranges from 29 to 38°C [14].

Study Population: The study was conducted on 64 Boran bulls that were slaughtered at Hashim Export Abattoir. The age of the study animals ranged from 3 to 6 years (yrs) and all of them were appeared apparently healthy during the pre-slaughter inspection and they had good body condition.

Study Design and Sampling: Non-probability sampling [16] with a purposive inclusion of the study animals was conducted. Ante-mortem examination was done on all 64 bulls at the lairage during the visit days and the pairs of their testes were collected immediately after slaughter.

Data Collection

Sample Collection and Transportation: Before slaughter, all bulls at the lairage were physically examined for any abnormalities. Inspection of the bulls was made while at rest or while in motion for any obvious sign of disease. Age (based on dentitions) was recorded according to Johnson [17] and the body condition scoring was determined and recorded as Heinonen [18]. The bulls were weighed and body weight of each bull was recorded. Immediately after slaughter, totally sixty four pairs of testes were collected from the abattoir. Then testes were individually packed in plastic bags and transported to the Multi-purpose laboratory of Addis Ababa University, College of Veterinary Medicine and Agriculture in ice box for the collection of the epididymal sperm [5].

Measuring the Weight of the Testes and the Epididymes: Immediately up on arrival to the laboratory, each pair of testes was kept in refrigerator at 4°C. Then, the weight of the testes was measured with sensitive balance and the results were recorded appropriately. After that the tunica albuginea was carefully removed from each testis and the epididymis was then carefully separated from the testis using the scalpel blade and thumb forceps. Then epididymes were weighed on a sensitive balance and the results were recorded appropriately according to Martins *et al*. [5].

Semen Collection and Processing: According to Abu *et al*. [19], the spermatozoa were collected from the caudal epididymes at room temperature by the incision method. Several incisions were made on the lower end of the epididymis to enable spermatozoa swim out and flushed/diluted by the pre-prepared two types of sperm extenders (Tris egg yolk and Skimmed milk) into the pre-warmed beakers i.e., thirty-two pair of epididymes were flushed by the Tris egg yolk extender and the remaining 32 samples (32 pair of epididymes) were flushed by the Skimmed milk extender. After dilution with the extenders, a 2 ml sample from each suspension was incubated at 37°C in a water bath for one minute and the percentage of spermatozoa viability was evaluated and recorded at 0 hour of post-thaw and the percentage of the epididymal sperm motility (spermatozoa mass motility and spermatozoa progressive motility) was estimated and recorded at 0, 1 and 3 hours of post-thaw of the samples.

Examination of the Semen

Evaluation of Semen Viability: According to Akhter *et al*. [20], to evaluate sperm viability a drop of diluted sperm sample was mixed with a drop of eosin and sodium citrate on clean slide. Then, the smear was air dried and examined under the microscope (40X magnifications). Dead spermatozoa were stained either partially or completely pink or red and live spermatozoa appeared colorless or white. Depending on this the percentage of live sperm cells (sperm viability) was determined for each sample.

Evaluation of Semen Motility: To evaluate the percentage of sperm mass motility a small drop of thawed semen was placed on a warmed (37°C) glass slide and cover-slipped and the visual motility was assessed under a light microscope with a high power magnification and the percentage of motile sperm was estimated and recorded according to Mahmoud *et al*. [21]. Sperm progressive motility in the samples was estimated on the basis of a percentage ratio of progressively (straight line forward movement) moving spermatozoa assessed in several view fields of a light microscope under high power magnification. Briefly, a small drop of thawed sperm sample was placed on a pre-warmed slide. Then, a cover slip was put over the drop and examined under microscope in order to determine the percentage of the sperm progressive motility of each sample based on Mortimer [22].

Data Analysis: All the generated data were entered in Microsoft Excel 2010. The data were analyzed using Statistical Package for Social Sciences (SPSS) software version 20 and the results are expressed as mean values and standard errors for the various parameters taken into consideration. Pearson's chi-square test was used to study if there is association for the percentage of sperm viability in different animal related factors (age, body weight, testicular and epididymal weight). For the determination of the significant differences among the parameters of the experimental versions, the means were analyzed by the t-test. Generalized Linear Model of one way Analysis of Variances (ANOVA) method was used to determine the individual animal or bull related factors and extenders based variations with regard to the seminal attributes (percentage of sperm motility and sperm viability). Multivariate analysis was used to determine correlations between variables and their significance was tested again by using ANOVA. The significance level was set at 95%.

RESULTS

A total of 64 apparently healthy matured Boran bulls slaughtered at the Hashim Export Abattoir were examined for the general assessment of their epididymal spermatozoa quality parameters (sperm motility and sperm viability) in relation with that of rescuing these gametes with the routine cryopreservation technique using two types of sperm extenders (Tris egg yolk and Skimmed milk). At the abattoir and in the laboratory the bulls were recorded with different age, body weight, testicular and epididymal weight measurement values. Those animal related factors (variables) were analyzed and compared for their significance to the percentage of semen viability and thus from the animal related factors analyzed, the mean values of age, body weight and left epididymal weight were seen to be significantly associated ($P<0.05$) with the mean percentage of spermatozoa viability (Table 1).

In this study, body weight significantly influenced ($P<0.05$) total testicular weight and total epididymal weight of the bulls. This means the bulls which had greater than 250 kg body weight had significantly larger testicular and epididymal weight when compared with the bulls having less than 250 kg body weight, as it is shown in Table (2).

The descriptive statistics of the spermatozoa motion characteristics (mean percentages of mass motility and progressive motility at 0, 1 and 3 hours of post-thaw) along with the mean percentages of spermatozoa viability are presented in Table (3). The obtained results of the Mean (Standard error) percentages of spermatozoa mass motility were higher than the percentages of spermatozoa progressive motility and the total mean percentage of spermatozoa viability was calculated as 61.73+ 9.51 %.

Table 1: Summary statistics for animal related factors (variables) and the mean percentages of spermatozoa viability of the Boran bulls

Variables	Minimum	Maximum	Mean (SD)	SEM	P-value
Age (yr)	3	6	4.33 (1.04)	0.13	0.045
Body wt. (Kg)	182	549	250.95 (45.41)	5.67	0.011
Left testicle wt. (g)	110	194	158 (21.25)	2.65	0.626
Right testicle wt. (g)	112	196	159.92 (21.27)	2.66	0.736
Left epididimys wt. (g)	14	26	20.73 (2.69)	0.33	0.030
Right epididimys wt. (g)	15	27	21.28 (3.02)	0.37	0.058

SD: Standard deviation, SEM: Standard error of the mean

Table 2: The influence of bulls' body weight on total testicular and epididymal weight

	Body weight (kg)		
Weight /g	Greater than 250	Less than 250	P-value
Total testicular weight	346.73±26.80	292.50±36.85	0.0001
Total epididymal weight	44.90±4.02	39.47±5.20	0.0001

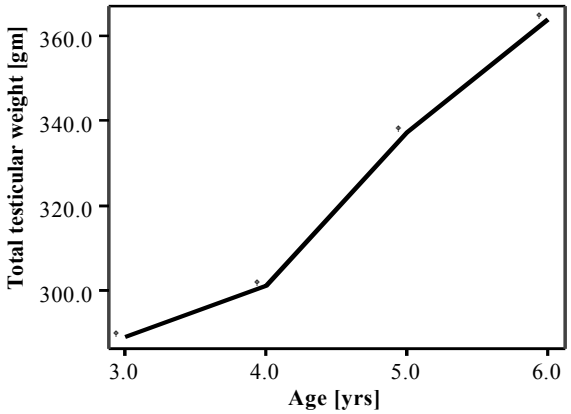

Fig. 1: The relationship between the total testicular weights with the age of the Boran bulls

Table 3: Summary statistic for different epididymal spermatozoa parameters of the Boran bulls at 0, 1 and 3 hours of post-thaw

Spermatozoa Parameters			Range	Mean	SD	SEM
Motility (%)	Mass motility	At 0 Hr.	50-80	65.08	7.00	0.87
		At 1 Hr.	45-75	60.52	7.20	0.90
		At 3 Hr.	40-70	55.95	7.08	0.88
	Progressive motility	At 0 Hr.	45-75	62.55	6.74	0.85
		At 1 Hr.	40-70	57.58	6.71	0.84
		At 3 Hr.	40-65	52.59	6.38	0.79
Viability (%)			45-80	61.73	9.51	1.18

SD: Standard deviations, SEM: Standard error of the mean

The paired mean differences and correlations between the animal related factors and motion characteristics (mass motility and progressive motility) of the epididymal spermatozoa of the bulls are given in Table 4. Except due to body weight of the bulls, significant differences ($P<0.05$) in the mean percentages of the sperm motility were revealed by the other animal related factors (age, testis weight and epididymis weight).

Based on the two types of sperm extenders (Tris egg yolk and Skimmed milk) used in this study, the mean (standard deviations and standard error) percentages of the sperm motility at 0, 1 and 3 hours of post-thaw and the mean (standard deviations and standard error) percentages of the sperm viability are given in Table 5. Except at 0 hour of post-thaw, the outcome of sperm motility at different hours of post-thaw revealed that there was significant difference ($P<0.05$) for the spermatozoa that were diluted in Tris egg yolk and in Skimmed milk but there was no significant difference ($P>0.05$) in sperm viability for the spermatozoa that were diluted in Tris egg yolk and in Skimmed milk.

Table 4: Paired mean differences and correlations between the animal related factors and mass motility and progressive motility of the epididymal spermatozoa of the Boran bulls at 0, 1 and 3 hours of post-thaw

		Mass motility (%)			Progressive motility (%)		
Animal Related Factors		At 0 Hr.	At 1 Hr.	At 3 Hr.	At 0 Hr.	At 1 Hr.	At 3 Hr.
Age (yr)	PMD SEM	-60.75 (0.81)	-56.18 (0.86)	-51.62 (0.84)	-58.21 (0.80)	-53.25 (0.80)	-48.26 (0.75)
	Correlation	0.53	0.36	0.37	0.34	0.35	0.40
	Significance	0.000	0.003	0.002	0.005	0.004	0.001
Body wt. (Kg)	PMD SEM	185.87 (5.44)	190.43 (5.57)	195.00 (5.59)	188.40 (5.63)	193.37 (5.60)	198.35 (5.61)
	Correlation	0.34	0.18	0.17	0.11	0.16	0.14
	P-value	0.007	0.140	0.178	0.350	0.194	0.256
Left testis wt. (g)	PMD SEM	92.92 (2.07)	97.48 (2.32)	102.04 (2.30)	95.45 (2.34)	100.42 (2.35)	105.40 (2.35)
	Correlation	0.76	0.51	0.53	0.50	0.50	0.50
	P-value	0.000	0.000	0.000	0.000	0.000	0.000
Right testis wt. (g)	PMD SEM	94.84 (16.57)	99.40 (2.39)	103.96 (2.38)	97.37 (2.40)	102.34 (2.42)	107.32 (2.43)
	Correlation	0.76	0.44	0.46	0.44	0.43	0.42
	P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.001
Left epididymis wt. (g)	PMD SEM	-44.34 (0.66)	-39.78 (0.77)	-35.21 (0.75)	-41.81 (0.72)	-36.84 (0.72)	-31.85 (0.68)
	Correlation	0.74	0.52	0.53	0.52	0.52	0.51
	P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Right epididymis wt. (g)	PMD SEM	-43.79 (0.68)	-39.23 (0.82)	-34.67 (0.80)	-41.26 (0.79)	-36.29 (0.79)	-31.31 (0.73)
	Correlation	0.66	0.40	0.42	0.35	0.34	0.38
	P-value	0.0001	0.001	0.0001	0.004	0.005	0.002

PMD: Paired mean difference, SEM: Standard error mean

Table 5: Summary statistic for the characteristics of the epididymal spermatozoa of Boran bulls that were diluted in Tris egg yolk and Skimmed milk at 0, 1 and 3 hours of post-thaw

		Mass motility (%)			Progressive motility (%)			
Type of Extenders		At 0 Hr.	At 1 Hr.	At 3 Hr.	At 0 Hr.	At 1 Hr.	At 3 Hr.	Viability (%)
Tris egg yolk (N=32)	Mean	63.64^{a}	58.75^{a}	54.13^{a}	61.19^{a}	55.81^{a}	50.97^{a}	60.03^{a}
	SD	6.17	6.60	6.29	6.51	6.25	5.93	9.31
	SEM	1.09	1.16	1.11	1.15	1.10	1.04	1.64
Skimmed milk (N=32)	Mean	66.72^{a}	62.28^{b}	57.78^{c}	63.91^{a}	59.34^{d}	54.22^{c}	63.44^{a}
	SD	7.48	7.45	7.44	6.80	6.79	6.48	9.54
	SEM	1.32	1.31	1.31	1.20	1.20	1.14	1.68
Total (N=64)	Mean	65.08	60.52	55.95	62.55	57.58	52.59	61.73
	SD	7.00	7.20	7.08	6.74	6.71	6.38	9.51
	SEM	0.87	0.90	0.88	0.84	0.84	0.79	1.18

SD: Standard deviation, SEM: Standard error mean, N: Number of samples,

Different letters (a, b, c, d) within the same column indicate significant differences ($P<0.05$) between the two types of sperm extenders (Tris egg yolk and Skimmed milk) used

Table 6: Pearson's coefficient of correlation between the spermatozoa motility and spermatozoa viability of the semen of the Boran bulls

	Mass motility (%)			Progressive motility (%)		
Parameters	At 0 Hr.	At 1 Hr.	At 3 Hr.	At 0 Hr.	At 1 Hr.	At 3 Hr.
Correlation coefficient	0.570**	0.847**	0.821**	0.825**	0.818**	0.802**

**Correlation is significant at the 0.01 level (2-tailed)

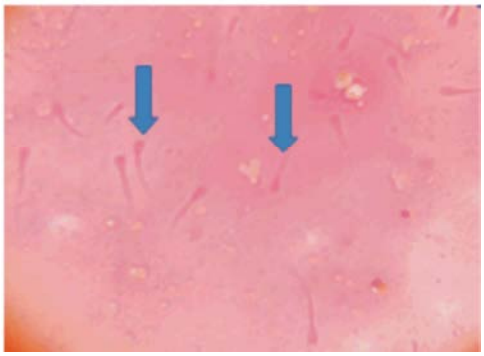

Fig. 2: Dead Sperm cells of the Boran bulls retaining the color of the Eosin stain (arrows)

In this study, the results of correlation analysis showed that spermatozoa motility had significantly ($P<0.01$) large correlation with that of spermatozoa viability (Table 6).

DISCUSSION

Epididymal spermatozoa harvested from the threatened dead animal can be cryopreserved and used for the production of viable embryos for the propagation of species [23]. Cryopreservation is a tool which offers an opportunity to preserve the germplasm from the dead animal either from domestic or non-domestic species. In Ethiopia few cattle breeds have been categorically identified and those identified are not adequately assessed and conserved [24].

In the present study, the mean (standard error (SE)) values of the age, body weight, left and right testicular and epididymal weights of the bulls were recorded. From those animal related factors analyzed, the mean values of age, body weight and left epididymal weight were seen to be significantly associated ($P<0.05$) with the mean percentage of spermatozoa viability (Table 1). Except due to body weight of the bulls, significant differences ($P<0.05$) in the mean percentages of the sperm motility were revealed by the other animal related factors (age, testis weight and epididymis weight) (Table 4).

Zebu cattle in general have been recognized to reach puberty 6-12 months later than the European breeds, the age at puberty for the latter being 12-14 months [25, 26]. Age at first breeding has been found to depend more on body weight than on age and can be delayed by slow growth. Similarly it has been reported that testicular size, scrotal circumference and body weight are positively correlated with age and the semen volume, quality and amount of mature spermatozoa have also been found to be positively correlated with testicular size and scrotal circumference [18, 25, 27].

From a biological point of view, only viable spermatozoa carrying intact genetic information are potentially fertile and therefore, most of the methods used so far focus on sperm viability and DNA integrity. Motility and gross morphology estimated by light microscopy are by now most used parameters for semen quality assessment, especially in AI laboratories. Motility may be divided in quantitative motility (percentage of sperm cells with a progressive motility) and qualitative motility [28].

In the present study, the mean (SE) percentage values for the mass spermatozoa motility were 65.08 (0.87) %, 60.52 (0.90) % and 55.95 (0.88) % at 0, 1 and 3 hours of post-thaw, respectively (Table 3). These values are in line with the mean (SE) percentage value for ejaculated mass spermatozoa motility of exotic bulls as 57.2 (0.11) % of post-thaw [29].

In this study, it was observed that the mean (SE) percentages of the progressive motility of spermatozoa of Boran bulls were 62.55 (0.85) %, 57.58 (0.84) % and 52.59 (0.79) % at 0, 1 and 3 hours of post-thaw, respectively. The epididymal spermatozoa progressive motility reported by Melina *et al.* [30] in Tabapuã bulls of 41.25 % is significantly lower ($P<0.05$) than the present values. The present values are in line with the values reported by Ahsan *et al*. [31] of 50.5 % and 60.55 % respectively in Friesian Sahiwal cross and Sahiwal bulls, the sperm progressive motility reported by Andrabi *et al.* [32] was of 55.0 % in Friesian-Sahiwal cross bulls.

On the other hand the mean values of the progressive motility of spermatozoa of Boran bulls in this study were lower than the values reported by Veeraiah *et al*. [33] of 76.55 % for Ongole bulls, progressive motility reported by Omar [34] of 79.33 % for Zambian short horn zebu, individual motility reported by Hundera [35] as 68.72 (1.37) in Ethiopian indigenous bulls and individual motility reported by Adamou *et al.* [36] was of 75.7 % for Borgou bulls. In this study, there is significant correlation ($P<0.05$) between the mean percentages of sperm motility and the mean age of the Boran bulls. Supporting these results, Abdel–Raouf [37] and Almquist and Cunnigham [38] found an increase in percentage of motile spermatozoa with the advancement of age.

In this study, it was observed that the mean (SE) percentage of viability of spermatozoa of Boran bulls was 61.73 (1.18) %. This value is lower than the ejaculated

spermatozoa live percentage values reported by Shelke and Dhami [39] in Gir as 80.13 %, live percentage of spermatozoa reported by Rana and Dhami [40] was of 71.85 %, live percentage of spermatozoa reported by Ahsan *et al*. [31] for Sahiwal and Friesian-Sahiwal cross bulls was 72.22 % and 74.22 % respectively, live percentage of spermatozoa reported by Hundera [35] for Ethiopian indigenous bulls was 79.73 (0.65) %, the spermatozoa live percentage reported by Dhami *et al.* [41] for Friesian bulls was 87.35 % and Veeraiah *et al*. [33] in Ongole bulls was 82.17 %. Colchen–Bourlaoud and Thibier [42] observed that percentage of dead spermatozoa decreased with increase in age and increase in dead spermatozoa with advancement of age was attributed toimpairment of epididymal function [43]. In this study, there is significant association ($P<0.05$) between the mean percentage of sperm viability and age of the Boran bulls.

In the present study, Tris egg yolk and Skimmed milk sperm extenders were evaluated for their suitability in the cryopreservation of Boran bull epididymal spermatozoa. Skimmed milk had better efficiency than the Tris egg yolk. Except at 0 hour of post-thaw, the outcome of sperm motility at different hours of post-thaw revealed that there was significant difference ($P<0.05$) for the spermatozoa that were diluted in Tris egg yolk and in Skimmed milk but there was no significant difference ($P>0.05$) in sperm viability for the spermatozoa that were diluted in Tris egg yolk and in Skimmed milk (Table 5). Egg yolk has been used as the main component of extender for freezing bull semen due to its availability even in tropical countries [44]. Ahmad *et al*. [45] found that Tris–citric acid based extender is suitable for the cryopreservation of buffalo spermatozoa in terms of post-thaw motility and survivability. Later on, Dhami and Kodagali [46] studied the effects of semen extenders based on Tris or citrate buffer. Egg yolk is a common component of semen freezing extenders for most of the livestock species [47]. Powdered egg yolk may be used as an extender for the cryopreservation of Zebu bull spermatozoa [48].

CONCLUSION

Age, body weight and epididymis weight have significant effect in the viability of the spermatozoa.Skimmed milk had better efficiency than the Tris egg yolk.

Based on the above conclusion the following are recommended:

- By taking the results of this study as an initial reference, the national AI center and the other stakeholders of the livestock sector should involve in the research and development of appropriate protocols in the gamete rescuing and cryopreservation of the epididymal spermatozoa of our indigenous bulls and other animals for the economic and genetic resource benefits of the country.
- Studies should be conducted on the assessment of the fertility levels of the epididymal spermatozoa of our indigenous bulls including the Boran bulls.
- The AI service provision should be restructured by incorporating the cryopreservation technique of the epididymal semen as a genetic source for the genetic improvement of livestock in the country.

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