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Fertility of Friesian Bull's Semen Diluted with Low Fat Cow Milk

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Abstract: *This study compared semen traits of Friesian bulls reared in subtropical area.Furthermore,the study compared semen's fertilityafterdilution, cooling and freezing inlow fat milk and Tris diluent. A number of 136 semen samples were collected fromthreematureFriesian bullsby artificial vagina over a period ofnine months.The semen volume, mass motility, individual motility and sperm cell concentrations were evaluated immediately after collection. Each semen sample was equally divided into two tubes and extended either withTris buffer with 5% egg yolk or low fat milk toa concentration of 5 ×10^7sperm cells per ml.The diluted semen was packed into 0.5ml straws, equilibrated at 5^{o}C, froze to -196^{o}C and stored.Sperm individual motility was assessed after equilibration and freezing.Fertility was assessed after inseminating65cowsin standing heat withsemen frozen in low fat milk and inseminating 38 cows withsemen frozen in Tris diluent. The semen's fertility rate was determined based on the rate of conception.*

The results of semen evaluation showed that the semen traits did not vary (p>0.05) with the month of collection.The mean volume of semen samples was 4.48 ± 1.39 ml; the mean mass motility was 3.23±0.54; the mean individual motility was 68.17± 5.01% and the mean sperm cell conc. was 1.34 ± 0.47×10^9. In addition, the post thawing individual motilities did not differ (p>0.05) with diluent type.Furthermore, fertility rates do not differ (p>0.05) with diluent type. The number of cows conceived after insemination with semen frozen in low fat cow milk was 64 (96.9%) while the number of cows conceived after insemination with semenfrozen inTriswas 33(88.7%). In conclusion, the fertility of Friesian bulls' semen frozen in low fat milk is comparable tothat of semen frozen inTris egg yolk buffer.

Keywords: *Friesian bull, semen, freezing, cow milk, fertility*

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I. Introduction

Semen diluents are aqueous solutions that consist of extenders, protectors and implementers (Salisbury et al. 1941; Philips and Lardy, 1941; Dunn et al. 1953; Chaudari and Mshelia, 2003; Gadea, 2003; Elsheikh 2017). Extenders are chemical solutions composed of electrolytes (salts), and non-electrolytes that extend the semen volume without preserving it. Protectors are substances that protects sperms from cold shock (egg yolk), ice crystals (glycerol) and from active substances that aid the process of fertilization microorganisms (antibiotics). The implementers are physiologically by acting directly on sperms or on the female genital tract such as mucinase enzyme, catalase enzyme, oxytocin and vitamin B_1& B_{12} (Elsheikh 2017). Additionally, supplementation of some amino acids such as taurine, hypotaurine, glutamine, glycine, proline and histidine to semen diluent is good for semen preservation (Chen et al. 1993; Chaudari and Mshelia, 2003; Elsheikh 2017).Manysemen diluents that can be used to increase the semen volume to the desired insemination dose in AI practices are available (Chaudari and Mshelia 2003; Gadea, 2003; Elsheikh 2017).These commercially available semen diluents consist of many chemical ingredients that are purchased in most cases from certain overseas companies. The prices of thesesemen diluents are non-affordable especially for researchers from under developing countries. Furthermore, these diluents are vulnerable to damage and spoilage during storage especially in tropical and subtropical areas. This damage, which might be encountered due to high temperature and storage problems, will lead to loss of huge amounts of chemical and consequently an increment in diluents costs. The cow whole milk that is a natural product, which consists of minerals, amino acids, vitamins, proteins, fats, and sugar (lactose), is an excellent candidate for semen dilution. Besides the cow milk's excellent formula, it is relatively cheap and available for use at any time. Many researchers have used whole milks a semen diluent (Ahamed and Foote 1985; Foote and Arriola1987**;** Chen et al. 1993;Foote et al.1993; Elhammali,1996; Footeet al. 2002;Akhter et al 2015).Thus, the current study compared the fertility of Friesian bulls' semen frozen in low fat milk with that of semen frozen in Tris diluent.

II. Materials and methods

2.1. Friesian bulls' management

This study was conducted at the Center for Artificial Insemination in Kuku, Khartoum North, Sudan, in the period from June 1995 to February 1996.This studyused threemature Friesian bulls (age range 4-6 years)and weighing between 650-700 kg. The bulls were housed in half-closed pens where each bull was kept in a separate pen of 9× 5 meters. Every year the bulls were vaccinated against infectiousdiseases. Bulls regularly received 200-mcg ivermectin/kg (BoehingerIngelheim) to combat internal parasite and the penswere regularly sprayed with acaroids to combat external parasites. The bulls were offered ad-libitum dry and/or green Sudangrass cultivar locally named "Abu Sab'een"and each bull was fed 10kg of a ration based on soybeans, sesame cakes and groundnut cakes. The water for drinking was continuously supplied.

2.2. Preparation of low fat Milk diluent

The whole cow milk was placed into a water bath, allowed to reach 95 oC, and left therein for 10 minutes. The whole milk samples were left at room temperature to cool and were poured into sterile containers. The fat layer formed on the surface of milk after cooling was removed manually and the milk was filtered with sterile gauze to ensure the removal of fat (low fat milk). Then the milk samples were divided to equal samples into two containers (A and B). Glycerol was added to a concentration of 20% to container A, while the milk in container B was not supplemented with glycerol. Antibiotics were added to the milk samples and the containers were kept at 5oC until thesecond day (the day of semen collection).

2.3. Preparation of Tris diluent

Tris diluent was prepared as described by Elhammali(1996)and warmed until it reached 75^{o}C. Then the Tris diluent was supplemented with5% egg yolk and antibiotics and divided into equal portions (A and B), portion A was supplemented with 15% glycerol while portion B was left without glycerol. The two portions were kept at 5 ^{o}C until the second day (the day of semen collection).

2.4. Semen collection, dilution and cooling

One hundred thirty six semen samples were collect over nine monthsby artificial vagina; theirvolumes were recorded and kept in water bath at 38 ^{o}C. Thereafter, the mass motility, individual motility, sperm abnormalities and sperm cell concentration were assessed as described elsewhere (Blom, 1950; Bao, 1971; Nadaraja, 1976; Elhammali, 1996). After evaluation, the 136 semen samples were divided into equal volumes, diluted with an appropriate volume of the prepared diluents that do not contain glycerin at 38 ^{o}C.Afterwards the diluted semen samples were cooled to5 °C (Foote and Arriola1987**;** Chen et al. 1993; Foote et al.1993).After one hour of semen cooling, the pre-cooled glycerol containing diluent portions were added gradually to the diluted semen so that the final glycerol concentration in low fat milk was 10 % and in Tris was7.5%.

2.5.Semen processing and thawing

The cooledsemen samples of each bull were packed into 0.5 ml straws to have an insemination dose of 2.5×10^{7} sperm cell/straw. The straws were labeled with bulls' number, type of diluent, date of processing. The straws were left to equilibrate at 5^{o}Cfor5 hours. The straws were then placed onto a metal shelf placed at 5 cm above the liquid nitrogen surface in special container. This shelf allowed the straws to be in the liquid nitrogen vapor(-80 ^{o}C). After 10 minutes, the straws were stored in liquid nitrogen and kept frozen until used for insemination. Before using semen for insemination, some straws were placed in warm water at 37^{o}C for 30 seconds to assess post thaw individual motilities for the different semen samples.

2.6. Cows' insemination and pregnancy diagnosis

The semen straws of the three different bulls were distributed to highly qualified inseminators to inseminate cows in heat in private farms near the artificial insemination center where the study was conducted. The inseminators recorded the cows inseminated, date of insemination and type of straws used in special insemination record. After 45 days post insemination, the inseminated cows were examined for pregnancy and pregnant cows were recorded.

2.7. Statistical method

Semen data were analyzed with repeated measure ANOVAusing the SAS (ver. 9.2; Institute Inc., Cary, NC).The data of semen traits are presented as means's. Fertility results were compared with Chi $\times^{2}$test and are presented as percentages**.**

Differences between treatments were considered statistically significant at $p < 0.05$.

III. Results

3.1.Semen traits

As in table (1). The semen traits of the three Friesian bulls were similar ($p>0.05$) throughout the collection period. The mean ejaculate volume was 4.48 ± 1.39ml, the mean mass motility was 3.23 ± 0.54, the mean of individual motility percent was68.17 ± 5.01% and the mean sperm cell concentration was 1.34 ± 0.47 $\times 10^9$/sperm cells per ml.

Table (1). Friesian bulls semen's traits before cooling to 5° C[a].

Month /Year	Samples No.	Ejaculate vol. (ml)	Mass motil. (rate)	Individual motility (%)	Sperm cell con.($\times 10^9$)
06/95	08	5.81 ± 1.99	3.18 ± 0.53	67.50 ± 4.16	1.22 ± 0.36
07/95	22	4.82 ± 1.63	3.18 ± 0.66	65.90 ± 1.19	1.29 ± 0.57
08/95	11	4.72 ± 1.42	3.19 ± 0.44	69.09 ± 3.45	1.81 ± 0.21
09/95	17	3.53 ± 0.33	3.24 ± 0.45	70.90 ± 5.28	1.16 ± 0.58
10/95	11	4.36 ± 1.26	3.16 ± 0.70	64.16 ± 9.17	1.23 ± 0.43
11/95	18	4.47 ± 1.18	3.25 ± 0.36	70.88 ± 8.61	1.83 ± 0.77
12/95	17	4.76 ± 1.88	3.35 ± 0.86	68.09 ± 5.07	1.04 ± 0.45
01/96	25	4.16 ± 1.40	3.24 ± 0.49	68.57 ± 4.39	1.18 ± 0.49
02/96	07	3.71 ± 1.44	3.26 ± 0.37	68.47 ± 3.77	1.29 ± 0.39
Overall mean	136	4.48 ± 1.39	3.23 ± 0.54	68.17 ± 5.01	1.34 ± 0.47

[a] Data of semen's traits are presented as means ± SD.

*No differences were recorded ($p>0.05$).

3.2.Post cooling and thawing individual motilities

Table 2 shows that the post cooling and thawing individual motilities of semen cooled or frozen in low fat milk did not differ ($p>0.05$) from thatcooled orfrozen in Tris diluents. The overall mean of post cooling individual motilitiesof semen frozen in milk and Tris were 61.15 ± 5.15 and60.52 ± 6.0 in the same respective; while the post thawing motilities were 46.78 ± 7.12 and 47.39 ± 7.75 in the same respective as above.

Table (2). Friesian bulls semen's individual motilities after cooling and freezing [a].

Month/Year	Individual motilities (%) at 5° C		Post thaw individual motilities (%)	
	Low fat milk	Tris	Low fat milk	Tris
06/95	61.25 ± 6.67	60.70 ± 6.40	40.62 ± 6.23	46.25 ± 12.17
07/95	61.42 ± 3.89	61.39 ± 4.72	41.59 ± 8.36	46.59 ± 05.50
08/95	62.43 ± 2.16	60.90 ± 4.86	45.90 ± 8.89	47.27 ± 09.04
09/95	58.82 ± 6.25	58.23 ± 8.46	47.05 ± 7.08	45.29 ± 08.56
10/95	62.72 ± 7.53	59.54 ± 5.56	50.10 ± 8.00	44.09 ± 06.03
11/95	59.45 ± 5.39	59.54 ± 7.56	42.77 ± 6.23	47.77 ± 08.26
12/95	62.05 ± 5.01	64.70 ± 4.49	53.23 ± 5.57	53.82 ± 04.51
01/96	60.80 ± 5.71	60.40 ± 6.60	49.80 ± 7.28	47.60 ± 07.08
02/96	61.42 ± 3.77	59.28 ± 5.34	50.00 ± 6.45	47.85 ± 08.59
Overall mean*	61.15 ± 5.15	60.52 ± 6.00	46.78 ± 7.12	47.39 ± 7.75

[a] Data are presented as means ± SD.

*No differences were found ($p>0.05$).

3.3.Semen fertility

As from table 3 the diluent type did not affected($p>0.05$) semen's fertility. However, fertility rate was higher when cows were inseminated with semen diluted and frozen in low fat cow milk. The fertility rate of semen diluted and frozen in low fat milk was 96.9% while that of semen diluted and frozen in Tris diluent was 88.7%.

Table (3). Effect of type of diluent on Friesian bulls semen's fertility [a].

Diluent type	Bull's no.	Cows inseminated	No. (%) conceived	Fertility rate (%) *
Low fat milk	26	23	22 (095.0)	095.0
	43	19	19 (100.0)	100.0
	56	23	22 (95.00)	095.0
Total	-	65	63 (96.90)	096.9
Tris diluent	26	08	08(100.0)	100.0
	43	15	14 (93.00)	093.0
	56	15	11 (73.00)	073.0
Total	-	38	33 (88.70)	88.70

[a] Semen's fertility rates are based on conception rates.

*Fertility rates do not differ with diluent type ($p>0.05$).

IV. Discussion

The current study demonstrates clearly that the low fat cow milk is a good semen diluent and the fertility of semen frozen in low fat milk is comparable to that of semen frozen in Tris diluent. Furthermore, the values of semen's traits of Friesian bulls reared under subtropical conditions arelowerthan thoseof semen traits of Friesian bulls reared intemperate zones; however, these semen traits are acceptable.

The semen volume reported in this study is similar to that reported in crossbred bulls (Elsharif, 1989),Friesian bulls (Selah et al., 1992), and Butana bulls "a local zebu breed "reared in the same environment(Makawi,1994). The semen volume reported in this study is less than that of Friesian bulls reared under temperate conditions(Everett and Bean, 1982; Almoquist, 1982; Everett et al.1978). The difference is probably due to high temperature in subtropical environment; however, nutritional and/or bulls' age differences cannot be ignored.

Individual motility percent reported in this study agrees with that reported in Butana bulls(Makawi, 1994), crossbred bulls**(**Elsharif, 1989),Friesian bulls(Almoquist, 1982;Hammali, 1996) and an Indian zebu bulls (Visitation, 1987). However, this individual motility percent isless than that reported in Holstein Friesian bulls(Everett and Bean, 1982; Everett et al., 1978).This difference is probably due to environmental differences; type of diluent used and breed difference. Furthermore, the post thawing individual motilitiesof semen frozen in low fat milk and Tris were similar and in accordance with those reported elsewhere (Ahmed and Foot, 1985, Foot and Arriola, 1987, Chen, 1987, Makawi**,** 1994).

The sperm cells concentration recorded in the current study is in accordance with that of Butana bulls reared under the subtropical environment of Sudan (Makawi, 1994) and that of HolsteinFriesian bulls reared under the semi-arid environment of Saudi Arabia(Salah et al., 1992) and Sudan (Hammali, 1996).

The results of this study showed that the fertility of semen diluted and frozen in low fat milk is comparable to that of semen diluted and frozen in Tris. Despite the similarity of the semen characteristics, the pregnancy rate of cows inseminated with semen diluted and frozen in low fat milk (96.0%)was better than that of semen diluted and frozen in Tris (88.7%).The fertility rate findings of this study match with that reported by other researchers (Majeed et al., 1993; Das and Yongolo, 1987;Hammali, 1996). However, these fertility results are higher than that of some other studies (Foot et al., 19 87; Mickelsen, 1993;Sankhi andCapitan1993; Johnson and Memon, 1995)This difference may be due to the low number of cows compared to the number of cows in previous studies.

V. Conclusion

In conclusion, the values of semen traits of Friesian bulls reared under subtropical conditions do not differ with the month of collection. Furthermore, the fertility of Friesian bulls' semen frozen in low fat cow milk is comparable to that of semen frozen in Tris egg yolk buffer.

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