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Pure Egyptian Cattle Bulls Show both Individual Variation and Different Interaction with Extender in the Post-Thawing Sperm Parameters

Alaa A Mohammed* **and Wahid M M Ahmed**

Animal Reproduction and Artificial Insemination,Veterinary Research Division, National Research Centre, Giza, Egypt

***Corresponding author:** Alaa A Mohammed, Animal Reproduction and Artificial Insemination,Veterinary Research Division, National Research Centre, Giza, Egypt, Tel: 33371362; E-mail: alaanrc@gmail.com

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Abstract

Objectives: As very few studies were done on the freezability of pure Egyptian cattle bull sperm. So, we designed this study to evaluate the individual variations in freezability of native bull semen extended in Tris based diluent and sodium citrate-based diluent.

Methods: Semen was collected by artificial vagina, examined at once in farm laboratory. Only semen samples fit the minimum parameters are extended in two extenders first the universal one (TRIS) and second modified Sodium citrate extender by adding glycerol (CU-16) which created at Cornell University to evaluate the individual bull variation and interaction between extender and bull.

Results: Post-Thawing individual sperm motility, live, abnormal, acrosome integrity percentage was evaluated in addition to Hypo-Osmotic Swelling test (HOS). The highest value for motility, live abnormal and acrosome percentage were 44.00 ± 1.12, 52.25 ± 1.60, 21.25 ± 0.81 and 62.80 ± 2.58 from bull 2, 1, 3 and 2, respectively for semen extended in TRIS, and 42.25 ± 1.61, 52.00 ± 1.76, 22.15 ± 0.85 and 57.40 ± 3.07, from bull 1, 1, 4, 3, respectively for semen extended in CU-16. The results of HOS were 59.25 ± 1.76 and 55.95 ± 2.13 from bull 1 extended in TRIS and CU-16, respectively.

Conclusion: A significant variation ($p<0.05$) in tested parameter was clear when semen extended in TRIS extender with the absence of such variation when semen extended in CU-16 except in live sperm percentage. With ignoring the extender effect a clear significant variations were detected between bulls in all tested parameters.

Keywords: Egyptian cattle bull; Semen cryopreservation; Extender

Introduction

Semen cryopreservation is a well-developed technique commonly used worldwide; it extends the life of spermatozoa by decreasing sperm metabolism and toxin production, but induces partially irreversible injury to sperm membranes, which may reduce sperm motility, viability and the fertilization rate after artificial insemination [1-5]. Sperm damage during preservation in liquid nitrogen at -196°C has been attributed by many authors to cold shock, ice crystal formation, oxidative stress, osmotic changes, cryoprotectant toxicity and re-organizations of lipid-protein and phospholipid layers within the cell membranes [6-8]. It is proposed that membrane is thought to be a primary target of cold shock or freezing damage in cells, which leads to a loss of integrity and selective permeability of the sperm plasma membrane [9,10]. Cryopreservation damages many sperm structures and is detrimental to the post-thawed sperm characteristics plasma membrane integrity and including motility [11-13].

Bull sperm membrane characteristics by high concentrations of polyunsaturated fatty acids and low cytoplasmic contents so sperm have low concentrations of antioxidant. Therefore, sperm as a cell is highly susceptible to lipid peroxidation by free radicals such as O_2, lacking antioxidant activity lead to the structural damage of sperm membranes during the freezing-thawing process [14,15]. The composition of extender containing the suitable cryoprotectants seems to play a significant role for successful semen cryopreservation [2,16]. Therefore, in last years, different antioxidants have been used to protect spermatozoa from the destructive effects of cryopreservation and free radicals [17].

Therefore, this work planned to evaluate the post-thawing parameters difference between pure Egyptian cattle bull their parent purchased from new valley province in 1986. And the interaction between bull and two type of diluent first one the standard Tris diluent and the other is sodium citrate supplemented with glycine amino acid (CU-16) on the post-thawing sperm parameters.

Materials and Methods

Experimental animals

Five mature native Egyptian bulls aged 3-4 years were used in the present study. Parents of these bulls were purchased from New- Valley Governorate at Upper Egypt during 1986. The bulls were raised under identical management conditions practiced in the National Research Centre Experimental Farm at Abou-Rawash, Giza, where they kept tied in bran provided with a covered shelter, away from cows. All bulls were clinically sound and examination of their genitalia proved to be normal with an absence of any palpable abnormalities. The general

management schedule for disease prevention used by the Egyptian Organization for Veterinary Services was followed.

All animal experiments carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines.

Semen collection

Prior to the experiment, bulls were subjected to routine daily exercise and were trained to mount an anestrous cow as a teaser for at least three months. Semen collection was done using a Danish model artificial vagina (AV). Two successive ejaculates per week were obtained from each bull. The ejaculates were then immediately transferred to the farm laboratory and placed in a warm water bath at 30°C for assessment and processing.

Semen evaluation

Directly after collection, evaluation of semen characteristics was carried out. The following criteria were considered:

Semen volume was estimated to the nearest 0.1 ml using graduated collection tubes.

Individual sperm motility was evaluated in semen samples diluted with 2.9% sodium citrate dihydrate solution, spread almost evenly under a glass cover slide and examined microscopically using adjusted hot-stage microscope at 38°C. Individual sperm motility percent was determined on a personal scale of 0-100% to the nearest 5% after viewing some microscopic fields.

Sperm cell concentration ($X10^6$ /ml) was determined by direct cell count using the standard haemocytometric method and the total sperm per ejaculate was calculated.

Live and abnormal sperm percentages were assayed by staining smears with Eosin-Nirgosin [18]. A total of 200 sperm cells were examined unsystematically. Live sperm percentage and total sperm abnormalities were recorded.

Hypo-osmotic swelling test (HOS) was carried according to Rota et al. with modification considered in the washing process of the sperm [19].

Acrosome integrity was evaluated by staining using trypan blue and Giemsa stains according to Way et al. [20].

Semen processing

Only semen samples with at least 70% sperm motility, 700×10^6 sperm/ml and 80% morphologically normal sperm were used for processing.

Diluents used

consists of Tris (hydroxymethyl-aminomethane) 3.028 g, Citric acid monohydrate 1.675 g, Fructose 1.25 g, Glycerol 8.0 ml, Distilled water 92 ml and Egg yolk 25 ml. Antibiotics were added at rate 1 gm dihydrostreptomycin sulfate and 1×10^6 units of penicillin G sodium to 1000 ml diluent [21].

CU-16 consists of Sodium citrate dehydrate 1.45 g, Glucose 1.25 g, Glycine 0.94 g and Distilled water to 100 ml. The final extender composed of 20 parts egg yolk and 80 parts buffer to which antibiotics were added at rate 1 gm dihydrostreptomycin sulfate and 1×10^6 units of penicillin G sodium to 1000 ml diluent [22].

Semen samples were extended with the first portion of extender contains no glycerol at 30°C and cooled to 5°C within 2 hours. At 5°C, the glycerolized portion of the extender was added. The extended semen samples were equilibrated for 2 hours. Semen packed in 0.25 ml French straws and frozen on the surface of liquid nitrogen for 10 minutes after that embedded in liquid nitrogen.

Statistical analysis

Results are shown as means ± standard error of means (SEM). Data were analyzed using analysis of variance (ANOVA) and Duncan descriptive tests by SPSS software version 14 for Windows (SPSS, IBM, Chicago, IL, USA). Values with $p<0.05$ regarded as statistically significant. Regression analysis was performed using the same software.

Results

The post-thawing sperm parameter of different native bull semen diluted in TRIS diluent was tabulated in Table 1. The best sperm motility (44.00 and 43.75) was observed with bull No. 2 and 1, respectively, while the lowest results obtained from bull No. 4 (38.75) with a significant difference between them. Live sperm percent higher significantly in bull 1 (52.25) than bull number 3 and 4 (46.15 and 46.75, respectively). The lowest abnormal percent obtained from bull No. 1 (18.95) and the highest value with bull No. 3 (21.25). The best result of HOS test obtained from bull No. 1 (59.25) and the lowest value from bull No.3 (50.20). Acrosome integrity percent were higher with bull number 2 (62.80) and lower with bull number 3 (51.15) with a significant variation between bulls. So the better post-thawing sperm parameters were obtained from bull number 1 and 2 without any significant variation between them, while the worst results from bull number 3 and 4 without any significant variation between them except motility % but the difference does not reach the level of significance.

Bull	1	2	3	4	5
Post-thaw	-	-	-	-	-
Sperm motility percent	43.75 ± 2.02^{ab}	44.00 ± 1.12^{a}	43.25 ± 1.93^{ab}	38.75 ± 1.25^{b}	40.00 ± 1.74^{ab}
Live sperm percent	52.25 ± 1.60^{a}	50.30 ± 1.67^{ab}	46.15 ± 1.87^{b}	46.75 ± 1.36^{b}	48.95 ± 1.66^{ab}
Abnormal sperm percent	18.95 ± 1.09	21.20 ± 0.63	21.25 ± 0.81	20.25 ± 0.99	20.80 ± 0.85
HOS test	59.25 ± 1.76^{a}	58.65 ± 2.30^{a}	50.25 ± 1.45^{b}	50.20 ± 1.84^{b}	55.75 ± 2.03^{ab}
Acrosome integrity percent	58.50 ± 1.85^{ab}	62.80 ± 2.58^{a}	51.15 ± 1.54^{c}	51.70 ± 1.98^{c}	55.90 ± 1. 29^{b}
Row with different superscript are significantly differs at (P<0.05)					

Table 1: Effect of individual bull on post-thaw sperm parameters of native bull semen diluted in Tris (Mean ± SE).

The post-thawing sperm parameter of native bull semen diluted in CU-16 was tabulated in Table 2. The lowest result of post-thaw sperm motility was (37.75) for bull No. 4 diluted in CU-16. With the highest results for bull No. 1 (42.25) with an absence of significant difference between bull. The best results for abnormal sperm percentage and HOS were obtained from bull number 1 (19.7 ± 1.04 and 55.95 ± 2.13,

respectively), while the worst results obtained from bull number 4 (22.15 ± 0.83 and 50.10 ± 2.28, respectively). For acrosome integrity percentage highest value obtained from bull number 3 (57.40 ± 3.07) while the lowest value obtained from bull number 1 (51.70 ± 1.75). The only significant variation between bull semen diluted in CU-16 was obtained for live sperm percentage with the best value gained from bull number 1 (52.00 ± 1.76) while the lowest value with bull number 4 (45.3 ± 2.11).

Bull	1	2	3	4	5
Post-thaw	-	-	-	-	-
Sperm motility percent	42.25 ± 1.61	40.75 ± 1.41	41.00 ± 1.65	37.75 ± 2.04	39.00 ± 1.52
Live sperm percent	52.00 ± 1.76^{a}	49.70 ± 1.28^{ab}	49.10 ± 1.74^{ab}	45.30 ± 2.11^{b}	47.15 ± 1.62^{ab}
Abnormal sperm percent	19.70 ± 1.04	20.25 ± 0.94	21.15 ± 0.83	22.15 ± 0.85	21.90 ± 0.88
HOS test	55.95 ± 2.13	51.40 ± 1.89	55.35 ± 2.69	50.10 ± . 2.28	51.55 ± 1.97
Acrosome integrity percent	51.70 ± 1.75	55.30 ± 2.38	57.40 ± 3.07	53.45 ± 1.37	54.60 ± 1.54
Row with different superscript are significantly differs at (P<0.05).					

Table 2: Effect of individual bull on post-thaw sperm parameters of native bull semen diluted in CU-16 (Mean ± SE).

Individual variations between bulls irrespective to diluent use were summarized in the Table 3 the best results of sperm motility, live, abnormal and HOS percentage were found from bull number 1, while the best results for acrosome integrity percentage obtained with bull number 2 with clear significant variations between bulls in tested parameters.

Bull	Motility%	Live %	Abnormal %	HOS	Acrosome
1	43.00 ± 1.28^{a}	52.13 ± 1.18^{a}	19.33 ± 0.74^{a}	57.60 ± 1.39^{a}	55.10 ± 1.37^{ab}
2	42.38 ± 0.93^{a}	50.00 ± 1.04^{ab}	20.73 ± 0.56^{ab}	55.03 ± 1.58^{ab}	59.05 ± 1.83^{a}
3	42.13 ± 1.26^{a}	47.62 ± 1.28^{bc}	21.20 ± 0.57^{ab}	52.80 ± 1.55^{bc}	54.27 ± 1.77^{b}
4	38.25 ± 1.18^{b}	46.03 ± 1.25^{c}	21.20 ± 0.66^{ab}	50.15 ± 1.45^{c}	52.58 ± 1.20^{b}
5	39.50 ± 1.14^{ab}	48.05 ± 1.16^{bc}	21.35 ± 0.59^{b}	53.65 ± 1.44^{abc}	55.25 ± 1.22^{ab}
Total	41.05 ± 0.53	48.77 ± 0.54	20.76 ± 0.28	53.84 ± 0.68	55.25 ± 0.68
Columns with different superscript are significantly differs at (P<0.05).					

Table 3: Post-thawing sperm parameters of native bull semen showing individual variation.

Studying the difference between two used extender regardless to the bull effect, the results tabulated in Table 4. The statistical analysis with independent T-test reveal absences of significant variation between extender in all tests post-thawing sperm parameter.

Extender	Motility%	Live %	Abnormal %	HOS	Acrosome
TRIS	41.95 ± 0.76	48.88 ± 0.75	20.49 ± 0.39	54.82 ± 0.92	56.01 ± 0.98
CU-16	40.15 ± 0.74	48.65 ± 0.79	21.03 ± 0.41	52.87 ± 0.99	54.49 ± 0.95
Total	41.05 ± 0.53	48.77 ± 0.54	20.76 ± 0.28	53.84 ± 0.68	55.25 ± 0.68

Table 4: Post-thawing sperm parameters of native bull semen extended in TRIS and CU-16 extender.

Discussion

Individual variation in the percentage of post-thawing apoptotic sperm between five Portuguese local breeds (Ramo Grande) bulls was observed [23]. The absence of individual variation between three fertile Brangus–Simmental cross-bred bulls from the University Putra Malaysia farm were cleared [24].

Alterations in sperm DNA integrity are induced during the cryopreservation process and during *in vitro* incubation and manipulation of bull semen. The type of semen extender affect the DNA integrity of sperm and may partially explain the observed fertility changes after AI with Norwegian Red semen in skimmed milk-egg yolk (SMEY) and Triladyl®. Additionally, the deviations in sperm DNA quality during *in vitro* incubation of extended semen may be linked to the bull breed [25].

Sperm motility evaluations are important for the evaluation of semen quality and assessments of membrane integrity. Plasma membrane functionality measured by the hypo-osmotic swelling test (HOST), sperm plasma membrane integrity and acrosomal integrity were considered good predictors of conception rate, also confirmed that the combination of Eosin/Negrosin staining test and HOST was highly correlated with *in vitro* fertility; and showed that when sperm plasma and acrosomal integrity (assessed by Trypan/Blue Giemsa staining) a higher correlation coefficient was obtained, also the morphological variables identified as important predictors of fertility [26].

The quality of semen can be influenced by the extender used in dissimilar to our results which may be attributed to the difference in the breed, number of examined bulls the diluent used [27].

The post-thaw sperm motility, intact acrosome and total sperm abnormalities were affected by genetic constituents of bulls so it differs between bulls form same breed [28,29]. On the same line variations between pure and crossbred bulls as between ejaculates from the same bull in the deep freezing quality sperm in the same time, they cleared the effect of diluent in the post-thaw sperm parameters [30]. Same found for crossbred bulls, Tharparkar X Jersey, Sahiwal X Jersey and Jersey X Non-Desoript bulls [31]. And to overcome bad freezer bulls authors suggested overcome by adjusting freezing and thawing protocol for each bull or over packing sperm in the straw [32]. For hypo-osmotic swollen test variation was very clear between 5 bulls from same breed (Piedmontese) which extended in Tris [33]. The sensitivity of sperm to osmotic stress during cryopreservation and thawing and thawing differ between individual bulls can classified bulls as good freezers or bad freezers implies the presence of certain membrane structure characteristics and that differences are genetically

coded [4]. Crossbred of Holstein Friesian X Hariana diluted in Tris exposed to post-thawing hypo-osmotic swollen test results varied significantly between bulls [34].

The discovery of the biological effects of amino acids in the prevention of cell damage during the freezing-thawing process [35]. The published data suggested that addition of amino acids to extender improved post-thawing sperm motility, sperm viability, acrosome integrity and membrane integrity in goat glutamine, different concentrations of Taurine, Cysteine and Trehalose, Cysteine, glycine, L-cysteine DL-alanine Glycine L-glutamine L-tryptophan, addition of glycine to Standard extender Tris with concentration 25 mm to bull semen improve post-thawing sperm motility, viability, decrease abnormality and dramatically improve the conception rate, ram [36-40]. However, by which mechanism these amino acid components protect spermatozoa during the freezing-thawing process, have not clearly understood and are still unclear.

Glycine used in the basic diluent for bull sperm [41]. The role of glycine in the protection of sperm during freezing-thawing process still not clear but many authors explain a suspected role in protection, as free amino acids and quaternary nitrogen containing compounds retard thermal denaturation of enzymes or provide thermal protection or maintain enzyme structure and function [42-44].

Conclusion

Clear variation between post-thawed tested parameters in the native bull semen although they are from the same parents. Such results need more investigation over an extended time to select the best bulls for preservation native breed from extinction. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

None

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