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A COMPARISON OF DUCK AND CHICKEN EGG YOLK FOR CRYOPRESERVATION OF EGYPTIAN BUFFALO BULL SPERMATOZOA

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Abstract
Cryopreservation of domestic animal spermatozoa has been widely used for artificial insemination and egg yolk is one of the most commonly used cryoprotectants during the freezing- thawing process. The aim of the present study was to compare the effectiveness of different duck egg yolk (DEY) concentrations (10, 15 and 20% DEY) with chicken egg yolk (20% CHEY) on the cryopreservation of Egyptian buffalo spermatozoa following dilution, equilibration and freezing-thawing processes. For this purpose, one ejaculate of semen from each of three Egyptian buffalo bulls were collected twice each week for 4 weeks with artificial vagina (42ºC). Pooled ejaculates were divided into four parts and were diluted in Tric citric acid glycerol extender containing either 10 or 15 or 20% DEY or 20% CHEY at 37ºC. Extended semen was equilibrated for 4h at 5ºC and then was filled in 0.5 ml straws and frozen in liquid nitrogen. Thawing of semen was performed at 37ºC for 30s. Progressive sperm motility, live sperm % and plasma membrane integrity after different stages of cryopreservation were assayed. Values of these parameters of post-thaw semen quality were higher for extender containing 15% DEY as compared with 20% CHEY (control extender), being 59.6% vs. 47.9%, 72.3% vs. 55.0% and 80.7% vs. 69.1% for progressive motility, live sperm and plasma membrane integrity, respectively. Using a post-thawing semen containing 15% DEY yielded comparatively highest conception rate (65.8%) followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%). In conclusion, DEY compared to chicken egg yolk in extender improves the frozen-thawed quality of Egyptian buffalo bull spermatozoa and fertility rate.

***Key words**: Cryopreservation, Duck egg yolk, Buffalo bull semen, Fertility.*

INTRODUCTION

Currently, egg yolk is a common component of most semen cryo-preservation extenders for domestic animals. It has been shown to have a beneficial effect on sperm cryopreservation as a protectant of the plasma membrane and acrosome against cooled shock [1]. Recently several investigators have substituted chicken egg yolk with egg yolk from other a vain species as a component of media used for the cryopreservation of spermatozoa livestock [2,3,4,5,6,7] Results obtained from these trials have been conflicting. Egg yolk from the duck proved superior to that of chicken for the cryopreservation of both stallion and buffalo spermatozoa[3,4]. In contrast opposite results, [8] obtained when a similar study was conducted using boar spermatozoa. In stallion, [9] reported that chukar (***Alectoris chukar***) egg yolk yielded higher post-thaw motility than chicken yolk for cryopreservation of stallion spermatozoa. The objective of the current study was to determine if substitution of chicken egg yolk with different concentration of duck egg yolk would improve post-thaw motility, percentage of plasma membrane integrity and fertility of Egyptian buffalo bull spermatozoa.

MATERIALS AND METHODS

1-Animals and semen collection

The experiment was carried out at the international livestock Management Training Center (ILMTC), Sakha Station belonging to the Animal Production Research Institute, Ministry of Agriculture, Egypt. Semen was collected from three adult and healthy Egyptian buffalo bulls (*B. bubalis*) of similar age group maintained under uniform managemental conditions. Semen was collected by artificial vagina at 42ºC twice weekly for a period of 4 weeks (2 ejaculates x 3 bulls x 4weeks,

replicates; n = 8). Ejaculates possessing more than 70% visual motility on each day collection were pooled in order to have sufficient semen for a replicate and to eliminate the bull effect. The semen was given a holding time of 10 min at 37ºC in water bath before dilution.

The pooled semen was diluted with the Tric extender concerting 3.025 Tris (hydroxymethyl amino methane), 1.675 g citric acid, 0.75 g glucose, 7 ml glycerol, 0.005g streptomycin, 0.25g lincospectin and 20 ml chicken egg yolk or 10; 15, 20 ml duck egg yolk and completed with bi-distilled water up to 100 ml. after dilution, the semen was cooled and equilibrated for 4h at 5ºC.

Semen was then filled in 0.25 ml French straws using a semen filling machine. Straws were than plunged into liquid nitrogen (-196ºC) and stored. After 24h storage, semen straws were thawed at 37ºC for at least 30s in water bath and then incubated at same temperature for 6 h to assess post thaw quality.

2- Semen Evaluation

Sperm motility, live spermatozoa and plasma membrane integrity were determined after different stages of cryopreservation (post-dilution, post equilibration and post-thaw). Plasma membrane integrity of buffalo bull spermatozoa assay is described by [10]

3- Fertility trail

A group of 58 buffalo-cows were artificially inseminated with randomly frozen- thawed semen extended with Tris 20% EY and 62, 76 and 54 buffalo-cows were randomly AI with frozen semen extended with 10%, 15% and 20% DY, respectively. Each female was inseminated with a single frozen-thawed straw at detected estrus using the recto-vaginal technique. Pregnancy rate was performed per-rectum at two months after AI.

4- Statistical analysis

Results were statistically analyzed according to SAS system (1985). The differences among means were tested using Duncan's new multiple range test [11]. The data on in vivo fertility rates were analyzed using Chi-square test.

RESULTS AND DISCUSSION

1- Progressive sperm motility (%)

Mean values (±SE) of progressive sperm motility for four experimental extenders at different stags of cryopreservation are given in Table 1. Post dilution sperm motility did not differ among extender containing 20% CHEY (control) and extenders containing 10% DEY and 15% DEY, the values being 76.7± 1.12, 74.6± 0.74 and 77.1± 0.14, respectively. This shows that DEY has no beneficial effects over CHEY immediately after dilution of semen. Moreover, increasing DEY to 20% significantly decreased sperm motility to 72.1± 0.97 when compared with other extenders. Similarly, post equilibration percentage of sperm motility did not differ among extenders containing 20% CHEY, 10%. DEY and 20% DEY, the values being 67.6%, 65.8% and 66.7%, respectively, while extender containing 15% DEY had a higher ($P < 0.05$) percentage of sperm motility, being 73.8 ± 0.65.

However, when post-thaw sperm motility after 24h storage in liquid nitrogen was considered, all extenders containing DEY showed significantly higher values (52.1, 59.6 and 52.5% for 10, 15 and 20% DEY, respectively) compared to 47.9% for control extender containing 20% CHEY ($P < 0.05$). We attempted to optimize the concentration of duck egg yolk in extender. The progressive sperm motility showed that the 15% DEY in extender was the best concentration to provide the best cryoprotective action for Egyptian buffalo sperm among other three concentrations of tested. These findings are supported by those [3] and [12], who recorded improved sperm motility parameters when the stallion and Nili-Ravi buffalo semen were frozen in extenders containing DEY as compared to CEY. Also, [4] found the highest forward motility of buffalo bull spermatozoa at 6 h post-thaw in DEY extender compared to those having egg yolk from other avian species including guinea fowl, indigenous hen and commercial chicken.

Table 1. Effect of different concentrations from duck egg yolk and 20% hen egg yolk in extenders on motility, live and plasma membrane integrity of buffalo bull spermatozoa at different stage of cryopreservation

Test	D.S.	CEY	DEY		
		20%	10%	15%	20%
Motility Sperm %	PD	76.7 ^{A} ± 1.12	74.6 ^{AB}± 0.74	77.1 ^{A} ±0.14	72.1 ^{B} ± 0.97
	PE	67.9 ^{B} ± 0.97	65.8 ^{B} ± 0.83	73.8 ^{A} ±0.65	66.7 ^{B} ± 0.71
	PT	47.9 ^{C} ± 0.96	52.1 ^{B} ± 0.97	59.6 ^{A} ±0.42	52.5 ^{B} ± 0.75
Live Sperm %	PD	81.0 ^{A} ± 1.24	76.3 ^{AB} ± 2.1	82.3 ^{A} ± 4.0	70.9 ^{B} ± 3.4
	PE	70.7 ^{B} ± 1.15	70.9 ^{B} ± 1.69	78.4 ^{A} ±1.03	65.8 ^{B} ± 3.4
	PT	55.0 ^{C} ± 0.95	61.5 ^{B} ± 0.89	72.3 ^{A} ±0.50	58.7 ^{BC} ±2.46
Plasma Membrane%	PD	88.4 ^{A} ± 0.82	84.0 ^{B} ± 1.35	89.0 ^{A} ±0.67	82.5 ^{B} ±1.75
	PE	79.8 ^{B} ± 1.31	81.4 ^{B} ± 1.13	85.8 ^{A} ± 0.78	77.8 ^{B} ±1.84
	PT	69.1 ^{C} ± 0.70	73.8 ^{B} ± 1.30	80.7 ^{A} ± 0.54	71.2 ^{BC} ±1.82

- A, B, C: Means within the same row with different superscripts are significantly different at P<0.01.
- DEY: Duck Egg Yolk, CEY: Chicken Egg Yolk, DS: Different Stage PD: Post-dilution, PE: Post Equilibration, PT: Post Frozen –thawing

The improvement in the post-thaw semen motility of Egyptian buffalo bull due to replacement of 20% CHEY with different concentration of DEY in the extender can be attributed to differences in the composition of egg yolk from the two a vain species [12].

2- Live spermatozoa and plasma membrane integrity.

According to the results, 15% DEY had the best cryoprotective effect in terms of the highest live spermatozoa and plasma membrane integrity compared to the other three extenders evaluated at different stages of cryopreservation (Table, 1). Post extension live sperm percentage and plasma membrane integrity did not differ between extender containing 15% DEY and control (20% CHEY) extender. The values being 82.3 vs. 81.0% and 89.0 vs. 88.41%, respectively. However, Post equilibration and post-thaw live sperm and plasma membrane integrity were significantly ($P < 0.05$) higher in 15% DEY extender as compared with extender containing 20% CHEY, the values being 78.4 vs. 70.7% and 85.8 vs. 79.8%, respectively, Table (1). The present findings are supported by [12], who recorded improved post-thaw livability when the Nili-Ravi buffalo semen was frozen in extender containing DEY as compared to CEY, the values were 7.03± 0.10 and 6.5 ± 0.15h, respectively ($P < 0.05$). This supports the idea that low density lipoproteins present in the egg yolk stick to the sperm plasma membrane during freeze-thaw process, preventing loss of phospholipids through improving membrane tolerance for the freezing process [13].

According to [2], the basic components of the yolks from chicken and duck eggs did not differ, but the ratios of fatty acids and phospholipids classes were different. Yolk from duck eggs had more monounsaturated fatty acid than yolk from chicken eggs. Moreover, yolk from duck eggs contained more phosphotidy-lionsitol than CEY. [6] found that karayaka ram frozen semen extended in chicken egg yolk recorder lower percentages regarding sperm motility (35 ± 1.6 vs. 47± 2.5%), viability (50± 3.4 vs. 58± 2.6%) and membrane integrity (44± 3.3 vs. 49.2± 5.0%) as compared to DEY extender.

On contrast, [8] found that frozen-thawed bull sperm progressive motility and sperm viability were significantly higher in extender containing chicken egg yolk as compared to DEY, the values being 48.9 vs. 37.1 and 53.3 vs. 42.6%, respectively. It is suggested that the improvement or decline in post-thaw quality of mammalian spermatozoa with EY of different avian species in freezing extender is attributed to the differences in biochemical composition of the yolk [14,2,15].

3- Fertility of frozen buffalo Semen.

A total of 58, 62, 76 and 54 buffalo females were artificially inseminated with frozen-thawed semen extended in 20% CHEY, 10% DEY, 15% DEY and 20% DEY, respectively, Table 2. High conception rate was recorded with the use frozen-thawed semen containing 15% DEY, being 65.8% followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%), but the differences were not significant. It should be mentioned that achieved more 50% in the current study is satisfactory as compared to the previous studies using various freezing and thawing techniques. In the Nili-Ravi buffalo bulls, [16] obtained that fertility rates were higher with semen cryopreserved in extender containing 12% LDLs compared with the control (egg yolk 20%) (72.7% vs. 50%, respectively). According to [17] suggested that a pregnancy rate higher than 50% can be regard as a good result after insemination with frozen- thawed semen.

In conclusion, our results showed that 15% duck egg yolk provided the best cryoprotective action to Egyptian buffalo bull sperm between the two avian egg yolks during the freezing-thawing process in terms of progressive motility, live spermatozoa, plasma membrane integrity and fertility rate. This conclusion is based on sperm characteristics and a full fertility trail which confirmed the beneficial effects of the inclusion of duck egg yolk in Egyptian buffalo semen cryopreservation protocols.

Table 2. Conception rate of buffalo-cows inseminated with frozen semen cryopreserved in different concentrations of duck egg yolk in extenders compare with 20% chicken egg yolk

Item	Chicken Egg Yolk 20%	Duck Egg Yolk		
		10 %	15 %	20 %
No. of inseminated females	58	62	76	54
No. of conceived females	34	36	50	32
Conception rate (%)	58.6	58.1	65.8	59.3

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