Journal of Animal and Poultry Production

Journal homepage: www.japp.mans.edu.eg
Available online at: www.jappmu.journals.ekb.eg

Influence of Dried Egg Yolk with or without Taurine in Extender on Sperm Characteristic of Cryopreserved Buffalo Semen

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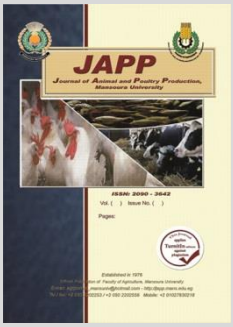

ABSTRACT

The objective of this study is to determine the influence of dried egg yolk (DEY) powder on freezability of semen buffalo as an alternative of fresh egg yolk (FEY) with or without taurine. Semen was collected from five buffalo bulls with total motility (≥70% mass motility), pooled, extended (1: 10) with different tris extenders containing 20% FEY versus 5, 10, 15 and 20% DEY (1st experiment). Tris-EY extender contained 15% DEY was supplemented with taurine (0, 25, 50, 75 and 100 mM) in 2nd experiment. Results showed that increases in progressive motility at concentration 10 , 15% than 20% in DEY and FEY respectively, after dilution, equilibration, and thawing, Also, acrosome integrity percentage in post-equilibrated and thawed semen was improved (P <0.05) by 5, 10, and 15% DEY as compared with FEY, being the highest with 15% DEY. Adding 25 or 50-Mm taurine improved progressive motility after dilution, and acrosome integrity after equilibration, and membrane integrity percentage after all stages of cryopreservation. the powdered egg yolk at concentration of 10 or 15% may be used, as an alternative of fresh egg yolk in the diluents of buffalo sperm freezing. Addition of taurine at a level of 25 and 50 mM has possible protective effects as an antioxidant to improve semen quality of buffalo semen extended with 15% dried egg yolk.

Keywords: Buffalo, sperm extender, dried egg, taurine, freezability.

INTRODUCTION

The defensive influence of egg yolk maybe due to low-density lipoprotein portion current. Egg yolk has been involved in dilutor at concentrations between1.5% and 50% (Salamon *et al.*, 1995). Egg yolk consider a public additive to cryopreservation diluents of sperm. Morever, its animal origin, which represent a potential risk of microbiological contamination in the diluent. On the other hand, using of powdered egg yolk, instead of fresh egg yolk for eschew possible contamination, which it is pasteurized (Marco-Jime´nez *et al*., 2004). While, Andrabi *et al.* (2008) reported that, harvesting hen egg yolk to usage on semen extender was confuse process, concerning suitable disease screening, decontamination, clever flouting of the external shell and internal membrane to isolated yolk from albumin.

Ultracentrifuge egg yolk before using in semen extender, consequently, powdered egg yolk of laboratory grade may be a substitute for used in semen extender of bovine (Ansari *et al*., 2010). Moreover, Thibier and Guerin (2000) evaluated that, powdered egg yolk could be an alternative for fresh egg yolk, as this produce experiences a sterilization procedure to abolish bacteria, in contract with the laws recognized for the human ingesting.

A method of cryopreservation considers as storing cells and tissues in liquid nitrogen, which used in diverse arenas, also, decelerates the cellular metabolic motion then resumes it later thawing (Andrabi and Maxwell, 2007).

Devi (2009) mentioned that, taurine consider as organic acid, comprises sulphur while, molecular structure very similar to γ-aminobutyric acid regarding to taurine has cytoprotective aptitudes which arise from the aptitude to detoxicate, osmo-regulate and maintain calcium homeostasis. (Sinha *et al*., 2008) noticed that, the taurine can be supposed as an antioxidant unpaid to its efficacy in efflux of free extremists along with preserving cell membrane permeability. In contrast, Bucak and Tekin (2007) detected, the trial extenders augmented with different concentration of taurine exhibited, taurine donates to formation of idyllic environment for spermatozoa, at assessment with conservative TRIS-based egg yolk extender .The chief of this study was examine influence of dried egg yolk as an substitute of fresh egg yolk (EY) in case of with taurine or without at freezability spermatozoa of buffalo.

MATERIALS AND METHODS

Materials

The study was carried out at international livestock management training Center (ILMTC), Sakha Station, Animal Production Research Institute, Ministry of Agriculture. All materials ustlized in our study were abounding from Sigma Company.

Animals

Five sexually matured fit buffalo bulls with 3-4 years old were utilized as semen givers. Bulls were housed in individual containers with fed on the optional portion according to Animal Production Research Institute.

Semen collection

Collection of semen was performed weekly by artificial vagina for a period of 10 weeks from April to June 2019. Immediately following every collection, ejaculates from

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DOI: 10.21608/jappmu.2021.151792

5 bulls were designated on the foundation of mass motility (≥70%). Ejaculates pooled to eliminate the bull effect, then diluted with different types of Tris-extender at a rate of 1:10.

Egg yolk powder preparation

According to Wei *et al.* (2019), intact eggs were washed, then the yolk was separated from the white manually, liquid egg yolk was standardized with rousing previous to more dispensation. Egg yolk was decanted into a ampule (width of sample not extra than 1.0 ± 0.5 Cm) and treated with Hydrogen peroxide solution. Subsequently, pouring into trays, while, oven trays drying at 44 ºC for four hours, then cooled and the dried egg was packed into flakes posteriorly, milling and sieving finally, powder was obtained.

Semen processing

After initial examination of each ejaculate, samples separated and diluted with four types of tris-extenders with 20% fresh egg yolk and 5- 10- 15- 20% (w/v) egg yolk powder, individually (1st experiment). Semen diluted with 15% EY powder (based on the results of 1st experiment) was utilized without or with 25- 50- 75- or 100-mM taurine (2nd experiment. Also, dried tris-egg yolk-glycerol extender was tailed to confirm that each 0.25 ml French straw controlled about 20 million progressively motile spermatozoa at each treatment.

The collected semen was pooled and diluted with simple extenders at 37°C associated with diverse ranks of EY powder or taurine. Semen samples diluted was exposed to mutual cooling with an equilibration time of 4 h at 5°C in a refrigerated unit previously existence placed into straws (0.25 ml French straws). Filled straws were sealed with polyvilin alcohol and detained for 10 min at the surface of the liquid nitrogen vapor (˜-120°C) before being absorbed then stowed in liquid nitrogen .

Semen evaluation

Progressive motility

Research microscope with high power magnification (x400) and warmed stage (37°C) were utilized to estimate the ratio of progressive motility of sperm according to Amman and Hammerstedt(1980).

Acrosome integrity

Acrosome integrity was examined by using a Giemsa stain procedure which described by Watson (1975). Giemsa stock solution was prepared as following: ground 3.8g with 375 ml absolute methanol in pounder and grout, then 125 ml Glycerol added and stain mixture which, stored at 37°C for one week before utilized, with surprised for rare minutes all day.

Studied dried smears under a light microscope at magnification of 1000x using oil immersion lens. The percentage of normal acrosome calculated for about 200 spermatozoa randomly selected from at least four microscopic fields.

Sperm membrane integrity

Sperm membrane integrity, hypo-osmotic swelling test (HOST) was showed by solution at osmolarity equal of 100 mOsm/kg . Examine done with socializing 30 µl semen with 300 µl of HOST solution and incubated at 37 ◦C for 60 min, then 10 µl of the mixture was positioned on microscope slide and mounted with a coverslip. 200 spermatozoa was calculated per sample below phase-contrast microscopy with 400×.

Afterward one month, as of all group, 5 straws was designated arbitrarily and thawed for 30 second at 37°C in a water bath. Motility percentage was assessed using a phase-contrast microscope (x40). Afterward supravital staining, feasibility was evaluated by microscope as another directory of sperm eminence. Moreover, valuation of acrosome health and natural head structure, were assessed through a phase-contrast microscope.

Statistical analysis

To evaluated the effect of 4 levels of DEY versus FEY (1st experiment) or 4 levels of taurine versus free 15% DEY, data analysis of variance (one way-ANOVA) with using SAS (1985) after arcsine transformation. Duncan Multiple Range test were utilized to distinct significantly dissimilar means Duncan (1955).

RESULTS AND DISCUSSION

Results:

1st experiment: Effect of dry egg yolk on semen characteristics

Progressive motility:

Results in Table 1 showed higher significantly progressive sperm motility at 10, 15% dried EY when compared with 5 and 20% dried EY and 20% fresh EY after dilution, equilibration, and thawing.

Table 1. Influence of dried egg yolk on sperm progressive motility (%) at buffalo bull spermatozoa with different stages of cryopreservation. (Mean±SE)

Stage	Progressive motility (%)				
	G1 20%(FEY)	G 2 5%(DEY)	G3 10%(DEY)	G4 15%(DEY)	G5 20%(DEY)
Post-dilution	70.5^{b} ±1.37	2.5^{ab}±1.1	76.0^{a}±1.8	74.5^{a}±1.9	71.0^{ab}±2. 1
Post-equilibration	53.5^{b}±1.1	57.0^{a}±1.5	59.0^{a}±0.8	60.0^{a}±1.7	51.5^{b}±0.7
Post-thawing	30.5^{c}±1.5	35.5^{b}±1.8	40.0^{a}±1.1	42.5^{a}±1.8	27.5^{c}±0.8

a, b, and c: **Means with different superscripts within the same row are significantly different (P<0.05).**

FEY: fresh egg yolk. DEY: Dried egg yolk.

Sperm acrosomal integrity:

Results in Table 2 revealed that acrosome integrity percentage in post-equilibrated and thawed semen were significantly (P<0.05) improved by 5, 10, and 15% of dried EY as compared with fresh EY in tris-extender, being the highest with 15% dried EY. The addition of 20% DEY had no effect on sperm motility as compared to 20% fresh EY at all stages. However, dried EY at levels 10 and 15% significantly improved percentage acrosome integrity as compared to 5% dried EY or fresh EY.

Table 2. Influence of dried egg yolk on acrosome integrity of buffalo bull spermatozoa at different cryopreservation stages (Mean±SE)

Stage	Acrosome integrity (%)				
	G1 20%(FEY)	G2 5% (DEY)	G3 10%(DEY)	G4 15%(DEY)	G5 20%(DEY)
Post-dilution	76.6^{b}±2.3	78.7^{b}±1.6	80.1^{a}±1.4	82.4^{a}±1.2	77.3^{b}±2.0
Post-equilibration	64.6^{c}±1.8	72.7^{b}±2.2	75.1^{b}±2.3	78.4^{a}±1.6	59.3^{c}±2.1
Post-thawing	53.0^{c}±3.09	63.9^{b}±3.37	68.4^{ab}±2.91	73.1^{a}±2.55	50.9^{c}±4.86

a, b, and c: **Means with different superscripts within the same row are significantly different (P<0.05).**

FEY: fresh egg yolk. DEY: Dried egg yolk.

2nd experiment: Effect of taurine addition on semen characteristics.

Progressive motility:

Data presented in Table 3 explained that adding 25 and 50 mM of taurine to extender of buffalo bull semen, significantly increased (P<0.05) progressive motility only after dilution as compared with supplementation of other levels or free extender.

Table 3. Influence of taurine on sperm progressive motility (%) of buffalo bull spermatozoa at different stages of cryopreservation. (Mean±SE)

	Progressive motility (%)				
Stage	**G1 0 (taurine)**	**G2 25mM(TAU)**	**G3 50mM(TAU)**	**G4 75mM(TAU)**	**G5 100mM(TAU)**
Post-dilution	71.0 ±2.0	74.8±2.3	75.9±1.5	73.5±0.8	74.0±1.4
Post-equilibration	54.5^{b}±1.5	60.0^{a}±1.2	59.0^{a}±1.4	48.1^{ab}±1.8	48.3^{ab}±2.0
Post-thawing	37.5^{ab}±1.5	47.4^{a}±1.8	47.0^{a}±1.1	34.5^{b}±1.8	35.6^{ab}±0.8

a, and b: Means with different superscripts within the same row are significantly different (P<0.05).
TAU: Taurine.

Sperm acrosomal integrity:

Only in post-equilibrated semen, the acrosome integrity in case of addition of 25 and 50 mM taurine increased significantly with comparing 75 and 100 mM and free extender (Table 4).

Table 4. Effect of taurine on acrosome integrity (%) of buffalo bull spermatozoa at different stages of cryopreservation. (Mean±SE)

	Acrosome integrity (%)				
Stage	**G1 (0 taurine)**	**G2 (25mMTAU)**	**G3 (50mMTAU)**	**G4 (75mMTAU)**	**G5 (100mMTAU)**
Post-dilution	78.6±1.4	78.2±0.9	79.4±1.7	80.4±1.1	76.8±1.6
Post-equilibration	70.0^{b}±1.9	74.2^{a}±1.2	75.6^{a}±1.8	72.6^{ab}±2.3	72.8^{ab}±2.1
Post-thawing	63.0±2.5	64.3±3.1	67.3±2.8	63.7±3.4	62.2±3.8

a, and b: Means with different superscripts within the same row are significantly different (P<0.05).
TAU: Taurine.

Plasma membrane integrity (HOS-t):

At all cryopreservation stages, the percentage of membrane integrity significantly (P<0.05) increased by adding taurine at levels of 25 or 50 mM as compared to other levels of taurine or free extender (Table 5).

Table 5. Effect of taurine on plasma membrane integrity (%) of buffalo bull spermatozoa at different stages of cryopreservation. (Mean±SE)

	Plasma membrane integrity (%)				
Stage	**G1 (0 taurine)**	**G2 (25mMTAU)**	**G3 (50mMTAU)**	**G4 (75mMTAU)**	**G5 (100mMTAU)**
Post-dilution	70.6^{b}±1.1	78.2^{a}±0.7	79.4^{a}±1.3	72.4^{ab}±1.4	71.8^{ab}±1.7
Post-equilibration	58.0^{b}±1.7	69.2^{a}±1.5	68.6^{a}±1.5	60.6^{ab}±2.1	61.8^{ab}±2.4
Post-thawing	45.0^{b}±2.3	56.3^{a}±3.1	57.3^{a}±2.1	50.7^{ab}±2.9	49.2^{ab}±2.8

a, and b: Means with different superscripts within the same row are significantly different (P<0.05).
TAU: Taurine.

Discussion:

Quality of diluted semen with egg yolk extender significantly compact next alarming and freezing-thawing. Strategy at present study was to progress superiority of sperm during storage, freezing and thawing procedures, which it improvement semen extenders by verdict an egg yolk auxiliary. Previously studied have declared purpose and diverse additives which used for reduction sperm damage during storage in state cold liquid or frozen semen (Taghilou *et al.*, 2017& Zarei *et al.* ,2018). Detected some structural, purposeful features of sperm, as motility, viability, and integrity of membrane, may replicate the achievement proportion of storage and may be measured as suitable pointers for usability of semen (Kumar *et al.*, 2003).

The results of the 1st experiment in the current study showed that the adding 15% DEY instead of 20% FEY of semen extender significantly increased sperm motility and acrosome integrity of spermatozoa after dilution, equilibration, and thawing. However, increasing level of DEY to 20% showed insignificant differences in sperm properties with tris-fresh egg yolk extender at all freezing stages. This impact may be attributed to peak in viscosity of medium when egg yolk powder was recycled at highest level (20%). The obtained results are nearly in with study of Marco-Jiménez *et al.* (2004), who showed highest temperatures stretched during process of pasteurizations denatured egg yolk proteins, encouraging them to a more gel-like constancy in the medium once reconstruction important to great diluent viscosity.

The present results disagreed with findings that sperm motility and plasma membrane integrity after dilution not affected (Ansari *et al.*, 2010 and Mahak *et al.*, 2015), because these characteristics were lower in equilibrated than in diluted semen. The present results also showed that DEY provided better cryoprotection at levels of 10 and 15% as compared to 5% DEY, which may indicate a lack in level of protectant of cold shock in the extender. In agreement with our results, several authors found important peaked in percentage of total motile sperm when it frozen at diluent encompassing powdered egg yolk when compared to fresh egg yolk (Macro- Jimenez *et al.*, 2004; Mehdipour *et al.*, 2016).

Effects of adding antioxidants to semen diluents during cryopreservation with only slight useful influence or level injurious impacted on frozen-thawed sperm factors (Mata-Campuzano *et al.*, 2014). The recent studies stated that antioxidants, such as taurine, added to semen extenders appeared to increase the frozen-thawed sperm features. Also, consider as sulfonic amino acid, which irritated the sperm plasma membrane, constrain lipid peroxidation and therefore protect spermatozoa (Balao da Silva *et al.*, 2010; Zarei *et al.*, 2018). Results in the 2nd experiment in the present study declared that, the addition of taurine as an antioxidant at levels of 25 or 50 mM to extender before freezing caused different effects during freezing stages. These levels improved sperm motility and acrosome integrity only in semen post-equilibrated, but had positive effect on improving membrane integrity after dilution, equilibration, and thawing as compared to free extender or other levels of taurine. The insignificant increase in sperm motility in post-thawed semen agreed with several authors (Bucak *et al.*, 2007; Atessahin *et al.*, 2008), whom

concluded an expansion of sperm motility after thawing in various animals. The noticed improvement in quality of cryopreserved semen by addition of taurine, especially membrane integrity may be attributed to that taurine has a protecting influence alongside lipid peroxidation, behind motility, optimistic properties on viability and membrane endurance of sperm after thawing (Chen *et al.,* 1993; Sanchez *et al.,* 1997; Pena *et al.,* 1998; Mehraban *et al.,* 2019).

CONCLUSION

In conclusion, the powdered egg yolk at concentration of 10 or 15% may be used, as an alternative of fresh egg yolk in the diluents of buffalo sperm freezing. Addition of taurine at a level of 25 and 50 mM has possible protective effects as an antioxidant to improve semen quality of buffalo semen extended with 15% dried egg yolk.

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تأثير صفار البيض المجفف فى مخفف السائل المنوى للجاموس مع أو بدون التورين على خصائص الحيوانات المنوية المجمدة

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يهدف هذا البحث إلى دراسة تأثير البيض المجفف (DEY) كبديل للبيض الطازج (FEY) مع أو بدون التورين على تجميد الحيوانات المنوية للجاموس. تم جمع السائل المنوي (≤70 ٪ حركة جماعية) من 5 طلائق جاموس ، مجمعة ومخففه بنسبة (1: 10) بمخففات التريس المختلفة والتى تحتوي على 20٪ FEY مقابل 5 ، 10 ، 15 و 20٪ DEY بالنسبة للتجربة الأولى. بينما يحتوي مخفف التريس على 15٪ من مادة DEY على التورين (0 ، 25 ، 50 ، 75 أو 100 ملي مولار) في التجربة الثانية. تم تخفيف السائل المنوي وحفظه بالتجميد وتقييمه بعد التخفيف والموازنة والذوبان. أظهرت النتائج أن النسبة المئوية للحركة التقدمية كانت أعلى ($P<0.05$) في تركيزات 10 و 15٪ DEY مقارنة بتركيز 20٪ FEY بعد التخفيف و حدوث التوازن والذوبان. تم تحسين نسبة سلامة الأكروسوم في السائل المنوي بعد الاسالة والذوبان ($P<0.05$) 5 و 10 و 15٪ DEY مقارنة مع FEY ، حيث كانت أعلى فى نسبة 15٪ DEY. أدت إضافة تركيزات تورين 25 و 50 مم للمخفف إلى زيادة الحركة التقدمية بعد التخفيف ، وسلامة الغشاء بعد الموازنة ، ونسبة سلامة الغشاء بعد جميع مراحل الحفظ بالتبريد.مما سبق ، يمكن استخدام مسحوق صفار البيض بنسبة 10 و 15٪ ، كبديل عن صفار البيض الطازج ، في مواد المخفف المستخدمه لتجميد الحيوانات المنوية للجاموس. إضافة التورين بمستوى 25 و 50 ملي له تأثيرات وقائية محتملة كمضاد للأكسدة لتحسين جودة السائل المنوي للجاموس المخفف ب15٪ من صفار البيض المجفف.