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RESEARCH ARTICLE

The effects of different egg yolk concentrations used with soy bean lecithin-based extender on semen quality to freeze bull semen

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Özet

Sarıözkan S, Tuncer PB, Bucak MN, Büyükleblebici S, Kinet H, Bilgen A. Boğa spermasının dondurulmasında soya lesitin temelli sulandırıcı ile birlikte kullanılan farklı yumurta sarısı konsantrasyonlarının sperma kalitesi üzerine etkileri. ***Eurasian J Vet Sci, 26, 1, 45-49***

Amaç: Boğa spermasını dondurmak için soya lesitin temelli sulandırıcıya (Bioxcell®) %5 ve 10 konsantrasyonlarında santrifüj edilmiş yumurta sarısı (SYS) katıldı ve bunların çözüm sonu sperm motilite, morfolojik anormallikler ve membran bütünlüğü üzerindeki sinerjistik etkileri değerlendirildi.

Gereç ve Yöntem: Her bir Simental boğadan alınan ejakulatlar (n=12) üç eşit miktara ayrıldı ve sırasıyla %5 (B5), %10 (B10) SYS eklenmiş ve hiç SYS (B0) içermeyen soya lesitin temelli sulandırıcı ile sulandırıldı. Ardından standart protokollere göre donduruldu ve çözdürüldü. Spermatozoa kryocanlılığı, in vitro çözüm sonu motilite (CASA), akrozomal ve diğer anormallikler ve plasma membran bütünlüğü (HOST) yönünden değerlendirildi.

Bulgular: Simental boğalarda, dondurma ve çözdürme sonrası B5 ile sulandırılan grupta, diğer sulandırıcılarla sulandırılan gruplardan önemli derecede daha yüksek CASA motilite ve CASA progresif motilite oranı elde edilmiştir (p<0.001). Gruplar arasında, VAP, VCL ve ALH yönünden önemli bir farklılık bulunmamıştır (p>0.05). En yüksek VSL (p<0.01) ve LIN değeri (p<0.001) B10 grubundan elde edilmiştir. Membran bütünlüğü oranı, B5 grubunda, diğer gruplara göre önemli derecede yüksek bulunmuştur (p<0.001).

Öneri: Soya lesitinle kombine olarak sulandırıcıya %5 SYS eklenmesi boğa spermasının dondurulabilirliğini önemli derecede artırmıştır.

Abstract

Sariozkan S, Tuncer PB, Bucak MN, Buyukleblebici S, Kinet H, Bilgen A. The effects of different egg yolk concentrations used with soy lecithin-based extender on semen tuality to freeze bull semen. ***Eurasian J Vet Sci, 26, 1, 45-49***

Aim: The centrifuged egg yolk (CEY) was added at concentrations of 0.5 or 10% to a defined soy lecithin-based extender (Bioxcell®) used to freeze bull semen and their synergistic effects on post-thaw sperm motility, morphological abnormalities and membrane integrity assessed.

Materials and Methods: Ejaculates obtained from each Simmental bull (n:12) divided in three equal aliquots and diluted in CEY 5% (B5), 10% (B10) supplemented, and without any CEY (B0) in soy bean lecithin - based extender, respectively. Thereafter, they were frozen and thawed following a standart protocol. Sperm cryosurvival was evaluated in vitro by microscopic assessments of post-thaw sperm motility (by means of the CASA), acrosomal and other abnormalities (head, mid-pieces, and tail) and plasma membrane integrity (evaluated by HOST).

Results: In Simmental bulls, semen extended with B5 had significantly higher CASA motility and CASA progressive motility than those extended with the rest of extenders after freezing and thawing (p<0.001). There was no significant difference in VAP, VCL, and ALH among the three groups (p>0.05). For VSL (p<0.01) and LIN (p< 0.001), the highest values were obtained from B10 group. The highest percentages of acrosomal and other abnormalities were found in semen diluted in B10 (p<0.001). In the group frozen B5, the percentage of membrane integrity was significantly higher than that of the other groups (p<0.001).

Conclusion: The use of CEY 5% in combination with soy bean lecithin significantly improved bull semen freezability.

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Keywords: Soy bean lecithin, egg yolk, cryopreservation, semen parameters, bull

Introduction

In cattle productivity, artificial insemination plays a major role by means of frozen semen. Microscopic quality based on motility, functional and morphologic properties is very important after thawing. However, the nucleus, plasma, acrosome and mitochondria membranes of sperm cells are susceptible to freezing/thawing processes (Chatterjee et al 2001, Aires et al 2003, Amirat et al 2004). Cold shock is the major reason of reduced motility after the freezing-thawing. It generally leads to swelling and the blebbing of the acrosomal membrane and disruption or increased permeability of the plasma membrane (Watson 1976, White 1993). Thereby, the use of frozen semen is limited by this unfavorable condition. The irreversible damages can be reduced by using proper extenders and cryoprotectant additives (Gil et al 2003, Jeyendran et al 2008). Egg yolk depends on containing cholesterol, phospholipid and low density lipoprotein prevents the formation ice cristal formation thus, protects integrity of sperm plasma membranes against cold shock during the freeze-thaw process (Hu et al 2010). Although new extenders without animal ingredients are available for example soy bean lecithin based extender, egg yolk-containing extenders have been still widely used to freeze semen (Aires et al 2003, Santiago-Moreno et al 2008). However, it was stated that EY, an animal originated product, is carried microbial sanitary risk (Van Wagtendonk-de Leeuw et al 2000, Vishwanath and Shannon 2000), and it also reduces respiration and motility of sperm cells (Kampshmidt et al 1953) and consequently might affect artificial insemination success (Aires et al 2003). Besides, EY has been complicated to evaluate biochemical, metabolic and microscopic investigations, but this may be accomplished by removing some components in EY by centrifugation (Yassen and Foote 1967, Wall and Foote 1999). Lecithin of plant origin (soy bean) has successfully replaced egg yolk in laboratory and field trials for livestock semen (Thun et al 2002, Fukui et al 2008, Forouzanfar et al 2010). The base influential compounds of soy bean lecithin is the low density lipoprotein fraction like egg yolk, which protects the membrane phospholipid integrity during cryopreservation (Moussa et al 2002, Amirat et al 2004). In recent years, it has been declared that the soy bean lecithin-based extender was used successfully on cryopreservation of both for buck (Sarıözkan et al 2010) and ram semen (Watson 1976, Forouzanfar et al 2010) as well as bull semen freezing (Stradaioli et al 2007, Sarıözkan et al 2009).

The improvement of semen cryopreservation technologies requires in-depth knowledge of the properties of the current extender. However, no studies have been carried out the check the effects of centrifuged EY in combination with soy bean lecithin on bull semen freezability.

We investigated the effects of adding CEY at two different concentration in soy bean lecithin-based extender on CASA motility, motility characteristics, acrosomal and other abnormalities (head, mid-pieces, tail) and membrane integrity during cryopreservation.

Material and Methods

Extender preparation

Clarified egg yolk was prepared as described by Holt et al (1996). Briefly, fresh hen eggs were manually broken. Yolks were separated from the albumen and were carefully rolled on a filter paper to remove chalazes and traces of albumin adhering to the vitellin membrane. The latter was then disrupted with a scalpel blade and yolk was diluted in distilled water (1:3) and centrifuged in sterile tubes at 4.000 x g for 40 min at room temperature. After centrifugation, the lipid material at the top of each tube was removed, the water-soluble clear fraction was saved, and the pellet at the bottom of the tube was discarded.

Animals and semen collection

Semen samples were obtained randomly from three Simmental bulls, aged 3-4 years, from the Lalahan Livestock Central Research Institute (Ankara, Turkey), and maintained under uniform feeding and housing conditions. Two ejaculates were obtained from each bull using an artificial vagina twice a week. Immediately after collection, the ejaculates were immersed in a warm water bath at 34°C until their assessment in the laboratory. Semen assessment was performed in approximately 20 min. The ejaculates with 75% motility and concentrations higher than $1.0X10^9$ spermatozoa/ml were used for this study. Consequently, 36 ejaculates from three bulls were subjected to the freezing protocol.

Semen processing

The sperm motility was estimated using phase-contrast microscopy (X200).The volume of ejaculates was measured in a conical tube graduated at 0.1 ml intervals and sperm concentration was determined by means of an Accucell photometer (IMV, L'Aigle, France). After the evaluation of quality, the fresh semen was then split into three equal experimental groups and diluted to a final concentration of $60x10^6$/ml spermatozoa with a soy bean lecithin-based extender containing CEY 5% (B5), 10% (B10), and CEY %0 (B0) (as a control). We used Bioxcell® (IMV Technologies, L'Aigle, France), whose cryoprotective effect is based on soybean lecithin. After dilution, semen sample were loaded into 0.25 ml French straws and cooled down to 4°C in 2 hours, frozen in a digital freezing machine (Digitcool 5300 ZB 250, IMV, France). Thereafter, the straws were plunged into liquid nitrogen.

Sperm evaluation

Sperm motility, morphological abnormalities, and plasma membrane integrity were assessed for each

sample to determine in vitro sperm quality. The quality of sperm motility and sperm motility characteristics were evaluated objectively using a computer-assisted sperm motility analysis (CASA; IVOS version 12; Hamilton-Thorne Biosciences, MA, USA). Thawing was performed in a 37°C water bath for 20 sec. The thawed semen samples were immediately transferred into plastic tubes of 1 ml and incubated at 37°C for 5 min. A 4-mL sample of diluted semen was put onto a prewarmed chamber slide (20 mm; Leja 4; Leja Products B.V., Holland), and sperm motility characteristics were determined with a X10 objective at 37°C and 10 microscopic fields were analyzed to include at least 300 cells. The following motility values were recorded: motility (%), progressive motility (%),VAP (average path velocity, µm/sec), VSL (straight linear velocity, µm/sec), VCL (curvilinear velocity, µm/sec), ALH (amplitude of lateral head displacement, µm), LIN (linearity index; LIN = [VSL/VCL]x100). Morphological abnormalities were assessed by phase-contrast microscopic examination (X1000 magnification, oil immersion). At least three drops of each sample were added to Eppendorf tubes containing 1 ml of Hancock solution (Schafer and Holzman, 2000). One drop of this mixture was put on a slide and covered with a cover slip. The percentages of acrosome and other abnormalities were determined by counting a total of 400 spermatozoa. Plasma membrane integrity was assessed by by means of the hypo-osmotic swelling test as described previously. Briefly, 5 µl of semen was diluted 50 µl of a hypo-osmotic solution (100 mOsm) and incubated at 37°C for 60 min. After incubation, smear was prepared and two hundred spermatozoa were evaluated (x1000) under bright-field microscopy. Sperm with swollen or coiled tails were recorded (Revel and Mrode 1994, Buckett et al 1997).

Statistical analysis

Results were expressed as the mean±SEM. Means were analyzed by analysis of variance (ANOVA), followed by Duncan test to determine significant differences in all the parameters between groups using the SPSS/ PC computer programme (version 12.0, SPSS, Chicago, IL). Differences with values of P<0.01 were considered to be statistically significant.

Results

The influence of two different CEY concentrations in a defined soy lecithin based extender on semen parameters after the freeze-thawing is shown in Table 1. Regarding to Simmental bulls, semen extended with B5 had significantly higher post-thaw CASA motility and CASA progressive motility than those extended with the rest of extenders after freezing and thawing (p<0.001). There was no significant difference in VAP, VCL, and ALH among the three groups (p>0.05). For VSL (p<0.01) and LIN (p<0.001), the highest values were obtained from B10. The highest percentages of acrosomal and other abnormalities were found in semen diluted in B10 (p<0.001). In the group frozen B5, the percentage of membrane integrity was significantly higher than that of the other groups (p<0.001).

Table 1. Sperm parameters in frozen-thawed Simmental bull semen (mean±SE).

Parameters	Extenders			
	B5	B10	B	P
CASA motility %	54.2±2.13^{c}	30.4±0.88^{a}	43.6±1.64^{b}	p<0.001
Progressive motility %	12.3±0.71^{c}	5.3±0.65^{a}	9.6±0.80^{b}	p<0.001
VAP µm/s	109±2.98	100±2.19	101±3.29	p>0.05
VSL µm/s	80.2±3.44^{ab}	90.9±2.29^{b}	69.1±5.96^{a}	p<0.01
VCL µm/s	219±6.53	211±5.44	207±6.55	p>0.05
ALH µm	8.8±0.36	7.9±0.21	7.8±0.60	p>0.05
LIN %	38.9±2.06^{b}	44.5±1.41^{c}	32.2±1.94^{a}	p<0.001
Acrosome abnormality %	8.7±0.75^{a}	19.7±1.10^{b}	11.3±0.65^{a}	p<0.001
Other abnormalities %	12.3±0.57^{a}	22.2±0.74^{c}	15.6±0.58^{b}	p<0.001
Membrane integrity %	55.7±1.19^{c}	31.8±0.60^{a}	43.8±0.46^{b}	p<0.001

a, b, c: Different superscripts within the same row demonstrate significant differences among groups.

Discussion

All the processes of cryopreservation, including the addition of cryoprotectants, cooling, freezing, and thawing that also create cold shock on the sperm membrane, resulting in the reduction of semen parameters, are important factors (Aitken et al 1989, Chatterjee et al 2001). As sperm cells contain high concentrations of polyunsaturated fatty acids, are highly susceptible to freezing. In this sense, a source of lipoprotein and high molecular weight compound such as EY or soy bean lecithin has been routinely used in most cryopreservation protocols to protect sperm membrane phospholipids against cold shock (Watson 1976, Vishwanath and Shannon 2000, Forouzanfar et al 2010). However, previous studies have demostrated that EY significantly diminish the percentage of intact acrosomes and motility in bull (Aires et al 2003), ram (Watson and Martin 1973), goat (Aboagla and Terada 2004, Sarıözkan et al 2010) semen during cryopreservation. The results of current study indicated that the effect of CEY concentration were statistically significant for sperm motility (CASA), membrane integrity (HOST) and functional integrity after thawing. Thus, based on our results,

the post-thaw motility, membrane and functional integrity of bull semen frozen in diluents with B10 were significantly lower than that of the B5. The concentration of egg yolk (10%) in the soy bean lecithin-based extender increased the viscosity of the extender, which might also have complicated motility and cell membrane integrity. These datas confirm the results of earlier studies (Watson and Martin 1973, Aires et al 2003, Sarıözkan et al 2010), where a similar observation was found with frozen/thawed semen that was extended in EY containing extender. Besides, it has been also indicated that the concentrations of EY or EY constituents could be reduced by centrifugation without decreasing sperm cryosurvival (Yassen and Foote 1967, Wall and Foote 1999) and centrifuged egg yolk provided a higher protection than whole egg yolk during the freeze-thaw process (Fernandez-Santos et al 2006). In this study, 5% CEY provided a higher protection than B0 and B10 extender after freezing-thawing.

It can be implied that egg yolk contains some substances harmful to semen quality parameters. Therefore, additives may be prefered as external cryoprotectant in extenders include soy bean lecithin, which is also present in Bioxcell extender, having less viscous fluid, in combination with centrifuged egg yolk supplementation for cryopreserving bull semen. Since the eliminating of some harmfull components from EY by centrifugation, it is possible that CEY 5% provided the best cryoprotective effect on post-thaw motility, membrane and morphologic integrity during the freeze-thaw process. In addition, it was also found that centrifuged CEY 5% was suitable for conducting on CASA studies.

► Conclusion

The results of this study indicate that the concentration of egg yolk is important on bull semen freezing. Because, when CEY supplemented at 5% concentration to the soy bean lecithin-based extender, a synergistic effect between egg yolk and lecithin afforded even higher post-thaw survival of sperm cells such as motility, morphology, functional integrity compared with that of B0 and B10 during freezing/thawing. Whereas the positive effect of B5 was demonstrated on semen quality, the CEY concentration 10% (B10) leads to deterioration on motility and membrane and functional integrity. Besides, the defined soy bean lecithin-based extender with centrifuged CEY 5% facilitates motility evaluating in CASA due to having less viscous fluid. Furthermore, the results show that freezability of bull semen can be affected by egg yolk concentration and type (centrifuged versus whole). Finally, the present results suggest that the use of low concentration of CEY (5%) and soy bean lecithin in combination significantly improved reproductive traits of bull semen, indicating its beneficial effect during the freeze-thaw process. Nevertheless, further studies should be carried out in order to confirm with artificial insemination trials of this presented results.

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