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RESEARCH ARTICLE

Evaluation of post-thaw quality of Brown-Swiss and Holstein bull semen diluted with different extenders

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Özet

Sarıözkan S, Tuncer PB, Bucak MN, Büyükleblebici S, Kinet H, Bilgen A. Farklı sulandırıcılarla sulandırılmış İsviçre-Esmeri ve Holştayn boğa spermasının çözdürme sonrası kalitesinin değerlendirilmesi. ***Eurasian J Vet Sci, 2010, 26, 1, 51-55***

Amaç: Tris + yumurta sarısı, Bioxcell® ve Optidyl® sulandırıcıları ile sulandırılan İsviçre-Esmeri ve Holştayn boğa spermasının çözdürme sonrası kalitesini değerlendirmektir.

Gereç ve Yöntem: Holştayn (n=36) ve Esmer (n=36) boğalardan alınan ejakulatlar üç kısma ayrıldı ve sırasıyla Tris, Optidyl® ve Bioxcell® sulandırıcılarıyla sulandırıldı. Standart protokollere göre dondurulduu ve çözdürüldü. Sulandırıcıların etkinliği, çözüm sonu sperm motilitesi, akrozomal ve toplam anormallikler ve plazma membran bütünlüğü değerlendirilerek belirlendi.

Bulgular: Holştayn ırkı boğa spermasında, dondurma-çözdürme sonrası en yüksek subjektif ($p<0.01$), ilerleyen ($p<0.001$) ve CASA motilite ($p<0.001$) oranları Optidyl® ile sulandırılan grupta saptanmıştır. Optidyl® sulandırıcısının spermatozoa akrozom ve membran bütünlüğünü diğer sulandırıcılara kıyasla en iyi şekilde koruduğu saptanmıştır ($p<0.001$). Motilite karakterlerinden VAP, VSL ve LIN yönünden en yüksek değerler Optidyl ve Tris sulandırıcılarından elde edilmiştir ($p<0.05$). Esmer ırk boğa spermasında ise, sulandırıcılar arasında en düşük çözüm sonu subjektif ($p<0.01$), CASA ($p<0.001$) motiliteleri ve membran bütünlüğü ($p<0.001$) oranı Bioxcell® ile sulandırılmış grupta elde edilmiştir. Optidyl® sulandırıcısı kullanılan grupta, Bioxcell® sulandırıcısına kıyasla daha yüksek ilerleyen motilite oranı elde edilmiştir ($p<0.01$). Bioxcell® ve Tris sulandırıcısı kullanıldığında daha yüksek oranda akrozomal ve toplam anormallikler saptanmıştır. ALH yönünden, en yüksek değer diğer gruplarla karşılaştırıldığında Optidyl sulandırıcısından elde edilmiştir ($p<0.05$).

Öneri: Holştayn ve İsviçre-Esmeri boğa spermasının dondurulması amacıyla Optidyl® sulandırıcısı Tris + yumurta sarısı ile Bioxcell® sulandırıcılarına tercih edilebilir.

Abstract

Sariozkan S, Tuncer PB, Bucak MN, Buyukleblebici S, Kinet H, Bilgen A. Evaluation of post-thaw quality of Brown-Swiss and Holstein bull semen diluted with different extenders. ***Eurasian J Vet Sci, 2010, 26, 1, 51-55***

Aim: To evaluate post-thaw guality of Brown-Swiss and Holstein bull semen diluted with different extenders.

Materials and Methods: Ejaculates obtained from Holstein (n=36) and Brown-Swiss (n=36) bulls were divided into three aliquots and diluted with Tris-based, Optidyl® and Bioxcell® extenders, respectively. Thereafter they were frozen and thawed following a standard protocol. The effectiveness of freezing extenders was assessed according to post-thaw sperm motility, acrosomal and total abnormalities and plasma membrane integrity.

Results: With respect to Holstein bull semen, the highest percentages of sperm subjective ($p<0.01$), CASA progressive ($p<0.001$), and CASA motility ($p<0.001$) were found in semen diluted with Optidyl®. Optidyl® extender also provided best protection in terms of acrosome and plasma membrane integrity compared to other extenders ($p<0.001$). For motility motion including VAP, VSL and LIN values, the highest values were obtained from Optidyl and Tris ($p<0.05$). With respect to, the lowest percentages of post-thaw subjective ($p<0.01$), CASA motility ($p<0.001$) and membrane integrity ($p<0.001$) were obtained in the semen samples diluted with Bioxcell®. The percentage of progressive motility was found to be higher in Optidyl® than Bioxcell® ($p<0.01$). The highest percentages of acrosomal and total abnormalities were found in semen diluted with Bioxcell and Tris extenders. The highest ALH value was obtained from Optidyl® extender compared to the other groups ($p<0.05$).

Conclusion: Optidyl® extender may be preferred rather than Bioxcell® or Tris + egg yolk extenders for freezing the Holstein and Brown-Swiss bull semen.

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Keywords: Bull semen, cryopreservation, extender, membrane integrity, seminal parameters

Introduction

The freezing and thawing processes are complex and have adversely affected the nucleus, plasma, acrosome and mitochondrial membranes of spermatozoa (Chatterjee et al 2001, Aires et al 2003, Amirat et al 2004). The complex processes generally lead to loss of motility, swelling and the blebbing of the acrosomal membrane and disruption or increased permeability of the plasma membrane of spermatozoa (Watson 1976, White 1993). The detrimental damages can be prevented by suitable extenders and cryoprotectant additives (Gil et al 2003, Jeyendran et al 2008). Egg yolk (EY) is a strong cryoprotectant agent that has been widely used in the extender. Egg yolk protects integrity of sperm plasma membranes and prevents the formation of ice cristals against cold shock depends on containing cholesterol, phospholipid and low density lipoprotein thus has been usually used at the concentration of 20% (w/v) (Hu et al 2010). Since its strong cryoprotection, commercial extenders have been producted by the incorporation of egg yolk for example Optidyl® (Biovet, France). However, EY, an animal originated product, possesses microbial sanitary risk and it also reduces respiration and motility of spermatozoa and consequently might affect the success of artificial insemination (Kampshmidt et al 1953, Van Wagtendonk-de Leeuw et al 2000, Vishwanath and Shannon 2000). Besides, EY has made difficult biochemical, metabolic and microscopic investigations (Yassen and Foote 1967, Wall and Foote 1999). In recent years, new extenders containing soy lecithin in replacement of egg yolk are widely used to freeze the semen (Thun et al 2002, Fukui et al 2008, Forouzanfar et al 2010). The base influential compounds of soy 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). The soy lecithin-based extender was successfully used to freze the semen of farm animals (Gil et al 2003, Stradaioli et al 2007, Sarıözkan et al 2009, Sarıözkan et al 2010). The improvement of semen cryopreservation technologies requires in-depth knowledge of the properties of the current extender. However, no studies have been enforced to compare the effects of egg yolk Tris-based, Bioxcell® and Optidyl® extenders on Holstein and Brown-Swiss bull semen freezability.

We investigated the effects of egg yolk Tris-based extender, and two commercial extenders (Bioxcell® and Optidyl®) on CASA motility, motility characteristics, acrosomal and total abnormalities and membrane integrity of Holstein and Brown-Swiss bull semen during cryopreservation.

Material and Methods

Animals and semen collection

Semen samples were obtained randomly from three Holstein and three Brown-Swiss bulls 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, ejaculates obtained from Holstein (n=36) and Brown-Swiss (n=36) 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 of each bull was divided into three aliquots and diluted with Tris+egg yolk (Trisma base 254 mM, citric acid 78 mM, fructose 70 mM, egg yolk 15% (v/v), glycerol 6% (v/v), pH 6.8), Optidyl® and Bioxcell® extenders, respectively. After dilution, semen samples were loaded into 0.25-ml French straws and cooled down to 4°C in 2 hours, frozen at a programmed rate of 3 °C /min from +4 to –10 °C; 40 °C/min from –10 to –100 °C; 20 °C/min from –100 to –140 °C in a digital freezing machine (Digitcool 5300 ZB 250, IMV, France). Thereafter, the straws were plunged into liquid nitrogen.

Sperm evaluation

Post-thaw 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] x 100). 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 contain-

ing 1 ml of Hancock solution (62.5 ml formalin (37%), 150 ml saline solution, 150 ml buffer solution and 500 ml double distilled water (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 means of the hypo-osmotic swelling (HOS) 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 computer programme (SPSS 12.0, Chicago, IL, USA). Differences with values of $p<0.05$ were considered to be statistically significant.

Results

With respect to Holstein bull semen, the highest percentages of subjective (53.1±1.78%, $p<0.01$), CASA progressive (22.7±1.48%, $P<0.001$), and CASA motility (64.7±0.87%, $p<0.001$) were found in semen diluted with Optidyl®. Optidyl® extender also provided best protection in terms of acrosome (4.1±0.46%) and plasma membrane integrity (60.4±1.70%) compared to other extenders ($p<0.001$). For VAP, VSL and LIN values, the highest values (117±6.13 µm/sec, 84.8±3.46 µm/sec, 41.6±2.17% and 123±3.97 µm/sec, 79.9±4.22 µm/sec, 35.4±2.25%, respectively) were obtained from Optidyl® and Tris ($p<0.05$).

With respect to Brown-Swiss bull semen, the lowest percentages of post-thaw subjective (28.6±1.60%, $P<0.01$), CASA motilities (36.2±1.05%, $p<0.001$) and membrane integrity (34.6±1.23%, $p<0.001$) were obtained in the semen samples diluted with Bioxcell®. The percentage of progressive motility was found to be higher in Optidyl® (17.7±3.13%) than Bioxcell® (7.2±1.14%) ($p<0.01$). The highest percentages of acrosomal (11.2±0.62%; 10.6±1.33%) and total abnormalities (20.1±1.40%; 16.8±1.56%) were found in semen samples diluted with Bioxcell and Tris extenders. The highest ALH value (9.29±0.30 µm) was obtained from Optidyl compared to the other groups ($p<0.05$).

Discussion

Spermatozoa contain high concentrations of polyunsaturated fatty acids, are highly susceptible to freezing/thawing. In this sense, a source of lipoprotein and high molecular weight compound such as EY or soy 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). In this study, regarding to Holstein bulls, the highest percentages of subjective, progressive and CASA motilities were found in semen diluted with Optidyl® as compared to the other extenders. Besides, Optidyl® shows significant cryoprotective effect in protecting functional integrity of acrosome and membranes of sperm against cold shock. The commercial extender, Optidyl® has been producted by the incorporation of egg yolk, thus it has strong cryoprotector agent. Current motility findings are higher than reported by Amirat et al (2004) who demonstrated a lower motility rate of bull semen following the freeze-thawing process. These contradictory results may be due to used bull strain and environment conditions.

Previous studies have demostrated that egg yolk sig-

Table 1. Functional parameters in frozen-thawed Holstein bull semen according to extenders (Mean±SEM).

Parameters	Extenders			
	Bioxcell	Optidyl	Tris	P
Subjective motility %	42.4±1.56^{a}	53.1±1.78^{b}	43.6±4.27^{a}	P<0.01
Progressive motility %	7.0±1.23^{a}	22.7±1.48^{c}	13.2±1.20^{b}	P<0.001
CASA motility %	46.4±1.79^{a}	64.7±0.87^{b}	51.6±4.75^{a}	P<0.001
VAP µm/sec	102±4.37^{a}	117±6.13^{ab}	123±3.97^{b}	P<0.05
VSL µm/sec	65.9±6.07^{a}	84.8±3.46^{b}	79.9±4.22^{ab}	P<0.05
VCL µm/sec	208±7.41	218±15.5	241±7.48	P>0.05
ALH µm	8.20±0.68	9.44±0.47	9.82±0.29	P>0.05
LIN %	32.0±1.98^{a}	41.6±2.17^{b}	35.4±2.25^{ab}	P<0.05
Acrosome abnormality %	9.29±0.61^{b}	4.1±0.46^{a}	9.60±1.08^{b}	P<0.001
Total abnormalities %	17.6±0.57^{b}	12.4±0.61^{a}	14.2±1.63^{a}	P<0.01
Membrane integrity %	45.3±1.39^{a}	60.4±1.70^{b}	49.2±0.97^{a}	P<0.001

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

Table 2. Functional parameters in frozen-thawed Brown-Swiss bull semen bull according to extenders (mean±SEM).

Parameters	Extenders			
	Bioxcell	Optidyl	Tris	P
Subjective motility %	28.6±1.60[a]	42.6±3.52[b]	38.2±2.08[b]	P<0.01
Progressive motility %	7.2±1.14[a]	17.7±3.13[b]	13.0±1.38[ab]	P<0.01
CASA motility %	36.2±1.05[a]	53.1±2.60[b]	43.2±1.77[c]	P<0.001
VAP µm/sec	108±2.43	115±6.34	122±3.05	P>0.05
VSL µm/sec	94.6±2.99	85.9±2.07	87.0±1.78	P>0.05
VCL µm/sec	210±4.97	219±15.30	213±11.10	P>0.05
ALH µm	8.01±0.24[ab]	9.29±0.30[b]	7.82±0.73[a]	P<0.05
LIN %	44.9±1.19	41.4±1.80	45.0±2.07	P>0.05
Acrosome abnormality %	11.2±0.62[b]	7.14±0.51[a]	10.6±1.33[b]	P<0.01
Total abnormalities %	20.1±1.40[b]	13.9±1.32[a]	16.8±1.56[ab]	P<0.05
Membrane integrity %	34.6±1.23[a]	51.7±0.75[c]	43.6±3.16[b]	P<0.001

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

nificantly diminish the percentage of intact acrosomes and motility in bull (Aires et al. 2003), ram (Watson and Martin 1973), and goat (Aboagla and Terada 2004, Sarıözkan et al 2010) semen and consequently might affect the success of artificial insemination (Aires et al 2003) during cryopreservation. In the current study, regarding to Brown-Swiss bull, Bioxcell® played the lowest cryoprotective role in maintaining post-thaw subjective, CASA motilities and membrane integrity, compared to the other extenders. The percentage of progressive motility was also found to be lower in Bioxcell® than Optidyl® (p<0.01). Furthermore, the highest percentages of acrosomal and total abnormalities were obtained when Bioxcell and Tris extender were used in Brown-Swiss bull semen freezing. In this study, semen quality results obtained from Bioxcell® was found to be lower than those obtained by egg yolk Tris-based extender. Conversely, Aires et al (2003) have been reported to gain better motility rate through soy lecithin-based extender than egg yolk Tris extender. These contradictory results might be arised from the differences of semen extender composition.

The results of this study indicate that the incorporation of egg yolk is influential on bull semen freezing. Because, Optidyl and egg yolk Tris based extender afforded higher post-thaw survival of spermatozoa such as motility, morphology, functional integrity compared with that of Bioxcell during freezing/thawing. The soy lecithin-based extender (Bioxcell) and a commercial egg yolk extender (Optidyl) more facilitates the motility evaluating in CASA due to having less viscous fluid than the egg yolk Tris-based extender. Furthermore, the results show that freezability of bull semen can be affected by extender type containing soy lecithin or egg yolk.

Conclusion

Finally, the present results suggest that the commercial egg yolk extender (Optidyl) significantly improved reproductive traits in both two different strain 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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