Erciyes Üniv Vet Fak Derg 8(1) 23-26, 2011
J Fac Vet Med Univ Erciyes 8(1) 23-26, 2011

Ara tırma Makalesi
Research Article

The Effect of Different Dilution Rates of Angora Buck Semen Frozen with Bioxcell® Extender on the Post-thaw Quality*

Ali DA KIN[1], Recai KULAKSIZ[2], Ergun AKÇAY[1], Halil EROL[3]
[1] *Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Ankara-TURKEY*
[2] *Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Kafkas-TURKEY*
[3] *Lalahan Livestock Central Research Institute, Ankara-TURKEY*

Summary: This investigation was designed to evaluate the suitability of Bioxcell® extender for the freezing of goat semen and to evaluate the effect of sperm concentrations using Bioxcell® extender on sperm parameters (motility, acrosome and plasma membrane integrity). Semen from 2 Angora bucks were used in this study. Ejaculates were collected using an artificial vagina twice a week, for three weeks during the breeding season. Semen extended with Bioxcell® extender to a final concentration of 200, 400 and 800 x 10^6 spermatozoa/ml were loaded into 0.25 ml straws and equilibrated at 4°C for 2 hours. Straws were frozen in nitrogen vapour and then stored at -196°C. Semen frozen at 800 x 10^6 spermatozoa/ml, the rates of motility and HOS test (38 %, 39 %, respectively) were significantly (P<0.05) higher than frozen at 200 x 10^6 (20 %, 18 %, respectively) and 400 x 10^6 (14 %, 14 %, respectively) spermatozoa/ml. The lowest percentage of abnormal acrosome was seen in the semen frozen at 800 x 10^6 spermatozoa/ml (32 %) which was significantly different (P<0.05) compared to 400 and 200 x 10^6 spermatozoa/ml (43 %, 50 %). Our results indicate that significant differences exist between low dilution rates, and that high concentrations better protects spermatological properties damages during cryopreservation.

Key Words: Bioxcell, dilution rate, extender, goat semen

Bioxcell® Sulandırıcısıyla Dondurulan Ankara Teke Spermasının Çözüm Sonrası Kalitesi Üzerine Farklı Sulandırma Oranlarının Etkisi

Özet: Bu ara tırma Bioxcell® sulandırıcısının teke spermasının dondurulmasında uygunlu unu de erlendirmek ve sulandırma oranlarının çözüm sonrası spermatolojik parametrelere (motilite, akrozom ve plasma membran bütünlü ü) etkisini ara tırmak amacıyla yapılmı tır. Çalı mada, 2 ba Ankara tekesinden alınan ejakülatlar kullanılmı tır. Ejakülatlar çiftle me mevsiminde haftada iki kez suni vajen kullanılarak alınmı tır. Sperma Bioxcell® sulandırıcısı ile 200 x 10^6, 400 x 10^6, 800 x 10^6 spermatozoa/ml olacak ekilde sulandırılmı ve sulandırılan spermalar payetlere (0.25 ml) çekilerek 4°C' ta 2 saat ekilibrasyona bırakılmı tır. Payetler sıvı azot buharında dondurularak daha sonra de erlendirilmek üzere -196°C'taki sıvı azotta saklanmı tır. Bioxcell sulandırıcısıyla yakla ık 800 x 10^6 spermatozoa/ml olacak ekilde sulandırılıp dondurulan gruptaki motilite ve host de erleri (sırasıyla % 38, % 39) ml'de 400 x 10^6 (% 20, % 18) ve 200 x 10^6 (% 14, % 14) spermatozoa içeren gruplarının motilite ve Host de erleri (sırasıyla % 20, % 18; % 14, % 14) arasındaki fark istatistiksel olarak önemli bulunmu tur (P<0.05). En dü ük akrozom anomalisi oranı yine ml' de 800 x 10^6 spermatozoa olacak ekilde sulandırılan grupta bulunmu ve akrozom anomalisi yönünden gruplar arasında önemli farklar saptanmı tır (P<0.05). Sonuç olarak, sulandırma oranları arasında önemli farklar oldu u ve dü ük dilüsyon oranı ile yüksek spermatozoa konsantrasyonun çözüm sonrası spermatolojik özellikleri daha iyi korudu u belirlenmi tir.

Anahtar Kelimeler: Bioxcell®, sulandırıcı, sulandırma oranı, teke sperması

Introduction

Egg yolk-based extenders have been widely utilized for freezing goat semen. However, freez buck semen with classical cryoprotectans buck semen poses some difficulties (e.g. egg yolk animal lecithin) in relation with the presence of seminal plasma (SP). In goats, therefore, the use of egg-yolk containing extenders requires the removal of most of the seminal plasma by washing before the dilution of spermatozoa (12). However, washing is a complex and time consuming process, and it also causes damage and some loss of spermatozoa (5). Other possible disadvantages of using egg yolk in semen extenders, such as a wide variability of the composition and the risk of contamination by bacteria and mycoplasma, have also been reported (2). Therefore, it would be preferable to use egg yolk free diluents. One of the these egg yolk free extender, Bioxcell® (IMV, L`Aigle, France), which was originally developed for cattle, could be used for goat semen cryopreservation, without the need to centrifuge the semen prior to the addition of the extender (14,18).

Geli Tarihi/Submission Date : 22.11.2010
Kabul Tarihi/Accepted Date : 07.02.2011

* This article was presented in 13 th Congress of ESDAR in Gent- BELGIUM

Goat spermatozoa are still frozen at relatively high concentrations. While there is no clear industry standard, goat semen is commonly frozen after only 4-5-fold (v/v) extension with cryodiluent (approximately 800-1000 million spermatozoa per ml) or may be extended to a specific concentration. This is most likely for convenience and to avoid negative effects associated with high dilution rates of spermatozoa (15). However, there is a lack of information on the pre-freezing rate to which spermatozoa can be diluted without a reduction in their post-thaw survival. Moreover, no studies have been carried out on the dilution effect of Bioxcell® vapour on goat semen freezability.

Cryopreservation of bovine and ovine semen in soybean lecithin based extender Bioxcell® maintained the semen quality (motility, plasma membrane integrity and acrosomal integrity) and produced acceptable fertility rates. The soy lecithin-based extender (e.g Bioxcell®) was successfully used to freeze the semen of farm animals (9, 18, 20).

The aim of the present study was to evaluate the effects of cryopreservation at different dilution rates on ram sperm motility, acrosome and plasma membrane integrity, using Bioxcell® extender.

Material and Methods

The 2 bucks were housed at the Education Research and Practice Farm, Faculty of Veterinary Medicine, University of Ankara, Turkey. The bucks were kept under natural light and maintained under a uniform and constant nutrition regime with each ram being fed on a daily diet of 1 kg concentrate, dried grass, salt lick and water ad libitum. Semen samples were obtained from the 2 mature Angora bucks (aged 2-3 years) and a total of 10 ejaculates collected from the goats with the aid of an artificial vagina, twice a week during the breeding season.

Extender B (Bioxcell®): A commercially available diluent (IMV, L'Aigle, France), this extender contains soy-bean extract with antibiotics (lincomycin, spectinomycin, tylosin, gentamycin) and glycerol (7%) included.

Semen extended with Bioxcell® in diluent to a final concentration of 800 x 10^6 (one part semen: five part extender), 400 x 10^6 (one part semen: ten part extender), 200 x 10^6 (one part semen: thirteen part semen) spermatozoa/ml were loaded into 0.25 ml straws and sealed with polyvinyl alcohol (PVA). Straws were equilibrated at 4°C for 2 hr and after equlibration, the straws were suspended on a styrofoam rack 4 cm above the liquid nitrogen (in a vapour) for 15 min. The straws were then plunged into the liquid nitrogen; where stored until thawing. After storage for a period of 4 weeks, the semen straws were thawed in a water bath (at 37°C for 30 second) for microscopic semen evaluation immediately after thawing.

Sperm motility was assessed using a phase-contrast microscope (x 400 magnification), with a warm stage maintained at 37°C. A wet semen mount was made using 5 µL semen placed directly on a microscope slide and covered by a cover slip. Motility estimations were performed from three different microscopic fields in each sample at 37°C. The mean of the three estimations was used as the final motility score (1).

For the assessment of acrosomal abnormalities, at least three drops of each sample were added to an Eppendorf container containing 1 mL Hancock solution (62.5 mL formalin (37%), 150 mL saline solution, 150 mL buffer solution and 500 mL double-distilled water) One drop of this semen mixture was put on a slide and covered with a cover slip. The percentage of the acrosomal abnormalities was determined by counting a total of 200 sperm under phase-contrast microscopy (x1000, oil immersion) (19).

The hypoosmotic swelling test (HOST) was used to evaluate the functional integrity of the sperm membrane, based on curled and swollen tails, and was performed by incubating 10 µL of semen with 100 µL ml of a 100 mOsM hypoosmotic solution (9 g fructose + 4.9 g sodium citrate per liter of distilled water) at 37°C for 60 min. After incubation, 10 µL of the mixture was spread with a cover slip on a warm slide. Two hundred sperm were evaluated under magnification 1000 x with bright-field microscopy. Sperm with swollen or coiled tails were recorded (9).

Statistical Analyses

The study was repeated 5 times and the results expressed as the mean ± SEM *(X + S x)*. One-way analysis of variance (ANOVA) with a subsequent Tukey's test was used to compare the mean values resulting from the various treatments at a significance level of $P<0.05$. All analyses were carried out using the SPSS 11 for Windows statistical software package (6).

Results

Semen frozen at 800 x 10^6 spermatozoa/ml, the rates of motility and HOS test (38 %, 39 %) were significantly (P<0.05) higher than frozen at 400 x 10^6 (20 %, 18 %) and 200 x 10^6 (14 %, 14 %) spermatozoa/ml. The lowest percentage of abnormal acrosome was seen in the semen frozen at 800 x 10^6 spermatozoa/ml (32 %) which was significantly different (P<0.05) compared to 400 and 200 x 10^6 spermatozoa/ml (43 %, 50 %) (Table 1).

Table 1. Effect of prefreezing of dilution rate of goat semen in Bioxcell® extender on rate of motility, acrosomal abnormality and plasma membrane integrity of spermatozoa after thawing.

Extender	Sperm concentration (x10^6 sperm/ml) And Dilution rate	n	Motility X + S x	Acrosomal Abnormality X + S x	Plasma membrane ntegrity X + S x
Bioxcell	800 (1:5)	5	$38{\pm}4.8^a$	$32{\pm}3.1^a$	$39{\pm}4.1^a$
Bioxcell	400 (1:10)	5	$20{\pm}2.9^b$	$43{\pm}2.0^b$	$18{\pm}2.7^b$
Bioxcell	200 (1:15)	5	$14{\pm}2.2^b$	$50{\pm}3.3^b$	$14{\pm}3.4^b$
Significance			P<0.05	P<0.05	P<0.05

a, b: Different superscripts in the same column indicate significant differences (P<0.05)

Discussion

These results demonstrate that there is a considerable reduction in goat sperm motility and membrane functionality following cryopreservation at high sperm dilution rates or with reduced numbers of spermatozoa per dose. This condition probably results from the lower concentration of seminal plasma at higher dilutions. At the highest dilution these beneficial elements could be diluted, reducing spermatozoal protection. Similar results have been reported by Khalifa and El-Saidy (11), Ansari et al. (2) and Sarıözkan et al. (18).

Various dilution rates have been utilized in the liquid and froze storage of goat semen. Early researchers employed dilution rates ranging from 11- to 26-fold, which are significantly higher than the 2–5-fold dilution rates commonly used today (15). However, there is little information on optimum prefreezing dilution rates for goat spermatozoa. The rate of dilution is usually varied to produce a standardised number of spermatozoa per inseminate dose or is simply based on the number of females to be inseminated per ejaculate (15). Low dilution rates are most likely used to avoid the "dilution effect", described by Mann (13) as a loss of motility and viability when spermatozoa are diluted to high levels. It is thought the "dilution effect" is due to a reduction in the concentration of protective factors in male reproductive tract secretions (10).

Khalifa and El-Saidy (11) reported a positive effect of low pre-freeze extension of (1: 4) goat spermatozoa, with the highest post-thaw motility and acrosome integrity observed not (1:19). As it was stated in the previous study, the present study we could confirm these findings. However, Castillo et al. (4) reported that the highest post-thaw motility obtained with use of 200 x 10^6 spermatozoa/ml. However, no direct comparison of dilution rate has been made. These contradictory reports may result from the use of extenders with different compositions (e.g. Tris based and egg yolk-free extenders) and the concentration of glycerol used in the studies. Thus, it is difficult to compare studies as it is unknown whether the effects on spermatozoa are due to dilution per se, or to variation in the contribution of certain diluent components, such as the concentration of toxic cryoprotectants per sperm cell (8,17). In the present study, a higher concentration of cryoprotectants per sperm cell could have attributed to maintenance of function during freezing and thawing.

In conclusion, the Bioxcell® extender used in the study are suitable for goat semen cryopreservation at low dilution rates. The results from the present study demonstrate that survival of goat semen after 1:4 dilution rate (800 x 10^6 spermatozoa/ml) is superior to a 1:10 (400 x 10^6 spermatozoa/ml) and 1:15 (200 x 10^6 spermatozoa/ml) dilution rates. However, further research is required to confirm this results.

Acknowledgement

This study was supported by grants from TUBITAK, Turkey (KAMAG-106G005). The authors wish to thank Aykut Üner for statistical assistance.

References

1. Ax RL, Dally MA, Lenz RW, Love CC, Varner DD, Hafez B, Bellin ME, 2000. Semen evaluation. Hafez B, Hafez ESE, eds. *Reproduction in Farm Animals*, Seventh Edition. Philadelphia: Lippincott Williams and Wilkins, pp. 365-375.
2. Ansari A, Towhidi M, Moradi SB, Bahreiniz M, 2010. Effects of two egg yolk-free commercial extenders and centrifugation on freezing ability of semen in Mahabadi goat. *J Anim Sci,* 88, E-Suppl. 2: 396.
3. Bousseau S, Brillard JP, Marguant-Le Guienne GB, Guerin B, Camus A, Lechat M,1998. Comparison of bacteriological qualities of various egg yolk sources and the in vitro and in vivo fertilizing potential of bovine semen frozen in egg yolk or lecithin based diluents. *Theriogenology*, 50: 699-706.
4. Castilho EF, Guimarães JD, Martins, LF, Pinho RO, Guimarães, SEF, Espeschit CJB, 2009. Use of propolis and ascorbic acid on goat semen cryopreservation. *R Bras Zootec*, 38: 2335-2345.
5. Corteel JM, 1981. Collection, proceeding and artificial insemination of goat semen. Gall C, ed. *Goat Production*. London: Academic Press, pp. 171-191.
6. Daniel WW, ed., 1991. *Biostatistics: A Foundation for Analysis in the Health Sciences*. Hoboken: John Wiley and Sons, pp. 274-320.
7. Evans G, Maxwell WMC, 1987. *Salamon's Artificial Insemination of Sheep and Goats*. Sydney: Butterworths, pp. 107-141.
8. Gacitua H, Arav A, 2005. Successful pregnancies with directional freezing of large volume buck semen. *Theriogenology*, 63: 931-938.
9. Gil J, Rodiguez-Irazoqui M, Lundeheim N, Soderquist L, Rodriguez- Martinez H, 2003. Fertility of ram semen frozen in Bioexcell and used for cervical artificial insemination. *Theriogenology*, 59:1157-70.
10. Harrison RAP, Dott HM, Foster GC, 1982. Bovine serum albumin, sperm motility, and the "dilution effect". *J Exp Zool,* 222: 81-88.
11. Khalifa TAA, El-Saidy BE, 2006. Pellet-freezing of Damascus goat semen in a chemically defined extender. *Anim Reprod Sci*, 93: 303-315.
12. Leboeuf B, Restall B, Salomon S, 2000. Production and storage of goat semen for artificial insemination. *Anim Reprod Sci*, 62: 113-141.
13. Mann T, 1964. *The Biochemistry of Semen and of the Male Reproductive Tract*. London: Methuen and Co Ltd, pp. 37-78.
14. Muino R, Fernandez M, Pena AI, 2007. Post-thaw survival and longevity of bull spermatozoa frozen with an egg yolk-based or two egg yolk-free extenders after an equilibration period of 18 h. *Reprod Domes Anim*, 42: 305-311.
15. Purdy PH, 2006. A review on goat sperm cryopreservation. *Small Rumin Res*, 63: 215-225.
16. Revell SG, Mrode RA, 1994. An osmotic resistance test for bovine semen. *Anim Reprod Sci*, 36: 77-86.
17. Salamon S, Maxwell WMC, 2000. Storage of ram semen. *Anim Reprod Sci*, 62: 77-111.
18. Sarıözkan S, Bucak MN, Tuncer PB, Ta demir U, Kinet H, Uluta PA, 2010. Effects of different extenders and centrifugation/washing on postthaw microscopic-oxidative stress parameters and fertilizing ability of Angora buck sperm. *Theriogenology,* 73: 316-323.
19. Schafer S, Holzmann A, 2000. The use of transmigration and spermac stain to evaluate epididymal cat spermatozoa. *Anim Reprod Sci*, 59: 201-211.
20. Stradaioli G, Noro T, Sylla L, Monaci M, 2007. Decrease in glutathione (GSH) content in bovine sperm after cryopreservation: comparison between two extenders. *Theriogenology*, 67: 1249-1255.

Yazı ma Adresi:
Recai KULAKSIZ
Kafkas Üniversitesi Veteriner Fakültesi
Dölerme ve Suni Tohumlama Anabilim Dalı
Pa açayırı/KARS
Email:recaikulaksiz@gmail.com