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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effect of buffers and egg yolk concentrations on chilled and frozen-thawed Boer goat spermatozoa

Akeel Ahmed Memon[1,3*], H.Wahid[1*], Y. Rosnina[1], Y.M. Goh[2] and M. Ebrahimi[2]

[1]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia; [2]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia; [3]Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh agriculture University Tandojam Pakistan

Abstract

This experiment was carried out with the aim to compare the effect of buffers and egg yolk concentrations on chilled and frozen-thawed spermatozoa characteristics of Boer goat. Semen was collected using an artificial vagina. After collection and evaluation, ejaculates qualifying the standard criteria were pooled and selected for extension and freezing. In experiment I, pooled semen was split into three parts and each part was mixed with Tris, citrate and skim milk based extender in two step dilution method and cooled at 4°C for 2.5 h. It was observed that chilled spermatozoa in Tris citric acid fructose egg yolk and skim milk buffers provided significantly ($P<0.05$) better results in terms of motility, membrane integrity, normal spermatozoa and viability as compared to citrate egg yolk buffer. Furthermore, Numerically, Tris was superior in all sperm parameters than the skim milk but the difference was non-significant ($P>0.05$). Significant ($P<0.05$) improvement was observed with Tris followed by skim milk and citrate buffers after freezing. This was observed by significant improvement of motility and acrosome integrity compared to citrate and skim milk. Furthermore, Tris, skim milk and citrate provided the best results in terms of membrane integrity and viability respectively. In experiment II, pooled semen was split into three parts and each part was mixed with Tris based extender containing different concentrations of egg yolk. The highest egg yolk level at 18% improved ($P<0.05$) chilled frozen thaw spermatozoa quality of Boer goat. Significantly better results ($P<0.05$) were also observed in all sperm characteristics at 18% egg yolk after freezing. In conclusion, Tris citric acid fructose with 18% egg yolk concentration can be used to improve the chilled and frozen-thaw spermatozoa quality of Boer goat.

Keywords: Goat; semen; freezing; extender

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Introduction

In Malaysia, goat population has increased over the past ten years (Jesse et al., 2011). Boer goats from South Africa and Australia were imported in substantial numbers in the past several years to improve the productivity of local goats and to fulfill the demand of mutton in Malaysia (Ariff et al., 2010). Exploiting this species for augmentation of goat production and accelerating the rate of genetic selection in Malaysia to begin on a commercial basis, Aartificical insemination (AI) could possibly be the choice of breeding methods in these species. Several researchers examined different extenders for freezing goat semen. Amongst them the most commonly used are skim milk with and without egg yolk (Corteel, 1974), sodium citrate egg yolk and Tris egg yolk addition (Salamon and Ritar, 1982).

According to the limited number of studies on comparison of diluents in which the post-thawing viability and fertility were the criteria for evaluation

Corresponding author: Dr. Akeel Ahmed Memon, Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh agriculture University Tandojam, Pakistan; Tel.: +00 92 312 3084776

(Paulenz et al., 2005; Dorado et al., 2007; Mahmood et al., 2011), no clear preference can be established among the diluents examined.

The effect of egg yolk concentration in the preservation of goat semen is far more important as variable results have been reported by various researchers regarding concentration of egg yolk in extenders. The non-washed spermatozoa which were diluted in an extender with 11% egg yolk showed significant deterioration in post-thaw cell survival (Memon et al., 1985). Daskin and Tekin (1996) showed that extender with 20% egg yolk was better than that without egg yolk during the breeding season for the non-washed post-thaw sperm motility. Ustuner et al. (2009) showed that high concentration (18%) of egg yolk did not properly increase the post-thaw motility of non-washed semen regardless of the season. Cabrera et al. (2005) reported that low egg yolk concentration did not properly protect spermatozoa against damage by freeze thawing. Furthermore, Cabrera et al. (2005) stated that the negative effect of egg yolk on buck sperm cryosurvival seems to be more important for goat in temperate countries during the non-breeding season. Therefore, this effect may not be important in tropical environmental conditions, where reproductive activity is less affected due to very little change in temperature and day light (Fatet et al., 2011). The toxicity of egg yolk coagulating enzyme in seminal plasma (Leboeuf et al., 2000), season of semen production, breed, and buck (Karatzas et al., 1997; Purdy, 2006) can be explained the differences in the response of egg yolk concentrations.

Therefore, this study was conducted to compare the ability of most common diluents (Tris, sodium citrate and milk based) (Experiment I), and effect of various egg yolk concentrations (Experiment II) to maintain sperm viability after chilling and freezing with the aim to establish and validate the existing diluents to optimize the storage of Boer goat buck semen in tropical environment of Malaysia.

Materials and Methods

Place of work

Study was conducted at the ar-Raudhah Bio Tech Farm Sdn. Bhd. Kampung Bung Raya 48050 Kuang, Selangor, Malaysia.

Management of bucks

A total of 06 bucks were used as semen donor in this study. These animals were raised on the farm as semen donor for AI purpose since 2009. They were housed in individual pens having good ventilation and fed oil palm fronds (OPF) with hay and pelleted concentrate twice daily in order to achieve a predetermined feed intake of 2.5% body weight (on dry matter basis) per goat per day. Bucks had access to mineral blocks and clean water *ad libitum.* Regular vaccination and deworming was under taken according to the standard protocols.

Collection and evaluation of semen

Semen was collected in an artificial vagina (AV) twice a week between December 2010 to February 2011. Directly after collection, each ejaculate was submerged into a 37°C water bath before evaluation. The semen samples were examined for consistency, color, sperm motility, volume, mass activity, sperm morphology and sperm concentration. The ejaculation volume was determined by collecting semen into a graduated tube. Color was evaluated by visual observation. The consistency was scored as 1 = watery-cloudy, 2 = milky, 3 = thin creamy, 4 = creamy and 5 = creamy-grainy (Shamsuddin et al., 2000). To evaluate the mass activity (wave motion) a drop (20 µl) of undiluted semen was placed on a pre-warmed slide at 37°C without a cover slip and examined under phase contrast microscope (100× magnification) (Nikon, Eclipse, E200, Japan). The mass activity was scored 0 = no motility, 1 = few sperm with weak movement (<20%), 2 = some motile spermatozoa (20–40%) without wave movement, 3 = slow wave movement (40–60%) with motile spermatozoa, 4 = rapid wave movement without whirlpool (60–80%) with motile spermatozoa and 5 = vary rapid wave movement with clear whirlpools (>80%) motile spermatozoa (Avdi et al., 2004). The sperm motility was estimated subjectively by preparing a wet mount of diluted semen (normal saline) by placing a 5 µl drop of fresh semen under coverslip with magnification of 200× using a phase contrast microscope (Nikon, Eclipse, E200, Japan). The final motility was examined at least with 200 spermatozoa, selected randomly from a minimum of four microscopic fields. The sperm concentration was determined by using a haemocytometer. The nigrosin–eosin stain was used to examine the morphologically normal spermatozoa (Evans and Maxwell, 1987). Sperm viability of the samples was assessed by means of the nigrosin–eosin staining (Evans and Maxwell, 1987). The acrosome integrity percentage (normal apical ridges) was determined from sperm smears stained with nigrosin–eosin examined under phase contrast microscope at 1000x magnification under oil immersion objective and bright field (Yildiz et al., 2000). A total of 200 spermatozoa were counted in at least four microscopic fields. The sperm membrane integrity was assessed by hypo-osmotic swelling test (Revell and Mrode, 1994; Buckett et al., 1997).

The ejaculates with volume 1 and 2 ml, concentration of $>2.5 \times 10^9$ sperm/ml, having >75% progressive motility and >85% of sperms with normal

morphology were pooled and selected for extension and freezing.

Experiment I

After collection and evaluation, ejaculates qualifying the standard criteria stated above were processed for cryopreservation. The ejaculates were diluted with normal saline at 1:1 and centrifuged at 1500x g for 3 minutes to remove the seminal plasma (Naing et al., 2010). The supernatant were discarded and sperm rich fractions were split into three parts according to experimental design in two step dilution methods. Briefly each aliquots of semen was mixed with one extenders Tris, citrate and skim milk base cooling extenders at 37ºC. Composition of the treatment extenders is given in the Table 1. Diluted semen sample were cooled at 4 ºC for 2 hours. Each extended semen sample was diluted with freezing extender. Final concentration was adjusted to 120×10^6 sperm/straw. Filling and sealing of the straws was done by automatic filling and sealing machine (MRSI-CE, IMV, France). The straw were equilibrated in a horizontal position in cold cabinet for 30 minutes and then placed in contact with liquid nitrogen ($LN_{2)}$ vapor 3 cm above the surface of LN_2 for 10 minutes in an expandable polystyrene box, then immersed into liquid nitrogen for storage. Immediately, after cooling semen samples were evaluated for motility, membrane integrity, acrosome integrity, morphology and viability. For post thaw examination, after 24hrs of freezing, thawing of the frozen straws was carried out. Four straws were thawed at 37°C for 30 seconds and pooled to perform evaluation.

Experiment II

After first evaluation, the pooled semen was diluted with normal saline as washing solution and centrifuged (Sigma 2-16 P, Sartorius) at 1500 × g for 3 min to remove the seminal plasma. The supernatant were discarded and sperm rich fraction was divided into three groups and mixed with Tris based extender contains different concentration of egg yolk (6%, 12%, or 18%). Further extension and processing were done similarly as in experiment I.

Statistical Analysis

Results were expressed as mean ± S.E.M. Means were analyzed using the one-way analysis of variance (ANOVA) followed by least significant difference (LSD) comparison procedures to determine the significant difference in all the parameters among groups using the SAS version 9.1 (SAS Institute, USA). All statistical tests were conducted at 95% confidence level.

Table 1: Composition of extender

Extender Components	Tris	Citrate	Skim Milk
Tris (g)	2.42	00	00
Citric acid (g)	1.34	00	00
Trisodium citrate dehydrate (g)	00	2.94	00
Fructose (g)	1.00	00	1.00
Skim Milk (g)	00	00	0.90

Each extender supplemented with 0.06 (g) Penicillin, 0.10 (g) Streptomycin, 18ml egg yolk. The freezing extender contains 7ml Glycerol per 100ml.

Results

In experiment I, effect of Tris egg yolk glycerol (TEYG), citrate egg yolk glycerol (CEYG) and skim milk egg yolk glycerol (SMEYG) extenders were investigated on the chilled and frozen sperm cytological characteristics (motility, membrane integrity, morphology, acrosome integrity and viability) in Boer goat buck semen.

Table 2 shows the results of chilled sperm characteristics of Boer goat in different diluents. The findings of this study showed that chilled stored spermatozoa in Tris citric acid fructose egg yolk and skim milk buffers provided significantly ($P<0.05$) better results in terms of motility, membrane integrity, morphology and viability as compared to citrate fructose egg yolk buffer. Furthermore, Tris was superior in all sperm parameters than the skim milk but the difference was non-significant ($P>0.05$).

Effect of Tris, citrate and skim milk buffers on sperm quality of Boer goat semen after freezing are summarized in Table 3. Significant ($P<0.05$) improvement was observed with Tris followed by skim milk and citrate buffers. This is evidenced by significant improvement of motility and acrosome integrity compared to citrate and skim milk. Furthermore, Tris, skim milk and citrate provided the best results in terms of membrane integrity and viability respectively. Slight improvement was observed in morphology of the spermatozoa in Tris and skim milk than citrate. However, no significant difference was detected in the morphology of spermatozoa between the buffers.

In experiment II, effect of different levels/ concentrations of egg yolk on sperm characteristics of Boer goat semen before and after freezing are shown in Table 4 and 5. Significantly better motility and acrosome integrity were observed in 18% egg yolk level followed by 12% and 6% egg yolk level. The highest egg yolk level improved ($P<0.05$) membrane integrity as compared to other tested egg yolk levels. Non-significant ($P>0.05$) differences were observed in morphology of 12 and 18% egg yolk level but still both concentrations shown significantly better morphology of chilled Boer goat spermatozoa than lowest 6% egg yolk concentration. Difference in viability parameter

Table 2: Mean (±S.E.M) percentages of motility, membrane integrity, morphology, acrosome integrity and viability of Boer goat spermatozoa before freezing in different extenders

Extenders	Motility %	Membrane Integrity %	Normal Spermatozoa%	Acrosome Integrity %	Viability %
Tris	62.00±1.76[a]	71.00±1.92[a]	73.80±1.35[a]	57.00±0.94[a]	62.20±1.01[a]
Citrate	47.60±2.76[b]	59.40±1.36[b]	67.80±1.49[b]	54.20±0.86[b]	57.60±0.87[b]
Skim Milk	57.20±1.39[a]	68.00±0.89[a]	72.00±1.14[a]	56.60±0.60[ab]	61.60±0.74[a]

Values with different superscripts within column differ significantly at P<0.05.

Table 3: Mean (±S.E.M) percentages of motility, membrane integrity, morphology, acrosome integrity and viability of Boer goat spermatozoa after freezing in different extenders

Extenders	Motility %	Membrane Integrity %	Normal Spermatozoans %	Acrosome Integrity %	Viability %
Tris	56.80±1.11[a]	64.50±1.80[a]	67.00±2.72	51.40±1.50[a]	56.20±0.86[a]
Citrate	45.00±1.78[c]	54.70±1.42[b]	64.80±1.93	43.00±0.94[b]	44.80±2.00[b]
Skim Milk	51.40±1.56[b]	62.60±1.53[a]	65.80±2.08	47.00±0.83[c]	53.60±0.67[a]

Values with different superscripts within column differ significantly at P<0.05; ns: No significant difference.

Table 4: Mean (±S.E.M) percentages of motility, membrane integrity, morphology, acrosome integrity and viability of Boer goat spermatozoa before freezing in different egg yolk levels in Tris citric acid fructose egg yolk glycerol extender

Egg yolk levels %	Motility %	Membrane Integrity %	Normal Spermatozoa %	Acrosome Integrity %	Viability ns %
6	53.6±1.56[b]	54.2±1.15[b]	52.2±0.80[b]	54.6±0.97[b]	55.4±1.16
12	55.6±0.67[ab]	56.4±0.67[b]	55.6±1.16[a]	57.0±0.83[ab]	58.2±1.39
18	57.6±1.12[a]	61.0±1.81[a]	58.0±1.26[a]	58.8±1.15[a]	58.2±0.91

Values with different superscripts within column differ significantly at P<0.05; ns: No significant difference.

Table 5: Mean (±S.E.M) percentages of motility, membrane integrity, morphology, acrosome integrity and viability of Boer goat spermatozoa after freezing in different egg yolk levels in Tris citric acid fructose egg yolk glycerol extender

Egg yolk levels%	Motility %	Membrane Integrity %	Normal Spermatozoa %	Acrosome Integrity %	Viability %
6	41.6±0.92[c]	44.6±1.16[c]	46.6±1.02[b]	47.8±0.86[b]	47.2±0.73[b]
12	46.4±1.32[b]	48.4±0.67[b]	47.0±0.70[b]	48.2±1.28[ab]	52.4±0.92[a]
18	53.2±0.96[a]	59.2±1.15[a]	57.8±1.01[a]	51.0±0.63[a]	54.6±1.12[a]

Values with different superscripts within column differ significantly at P<0.05.

were not different (P>0.05) before freezing. Furthermore, significantly better results (P<0.05) were observed in all sperm characteristics at 18 % egg yolk after freezing.

Discussion

Mammalian semen in AI programs is commonly extended with diluents containing a buffer. A variety of buffers have been developed (Evans and Maxwell, 1987; MacKenzie, 1994). A buffer for biological use should have pKa between 6-8, maximum water solubility and minimum solubility in all other solvents. It should pass biological membranes with difficulty and cause minimum salt effect. Buffer should be stable and not resemble enzyme substrates, it should not absorb light in the visible or ultraviolet region of the spectrum and it should be easily prepared from inexpensive materials.

In the present study, significantly better (P<0.05) results with Tris is in conformity with that of Drobnis et al., (1980) who observed higher progressive motility in goat semen frozen in Tris yolk glycerol than Tris yolk and skim milk. Similarly Deka and Rao (1987) reported higher progressive motility and intact acrosome in goat semen frozen in Tris citric acid than skim milk egg yolk citrate and raffinose egg yolk glycerol extender. Tuli and Holtz (1992) observed higher progressive motility and live spermatozoa in Tris than Zwitterion buffer based extender. In contrast, Shamsuddin et al. (2000) reported better motility in glucose citrate egg yolk followed by Tris fructose egg yolk and skim milk.

In temperate countries, during the non-breeding season, the negative effect of egg yolk on buck sperm cryosurvival seems to be more important for goat. In the tropics, goats are continuous breeders (Luna-Orozco et al., 2008) therefore, this effect may not be important in tropical environmental conditions, where reproductive activity is less affected due to very little change in temperature and day light (Fatet et al., 2011). In the present study, significantly better motility and acrosome integrity were observed in 18% egg yolk level followed by 12% and 6% egg yolk level. The highest egg yolk level improved (P<0.05) membrane integrity as compared to other tested egg yolk levels. The results of the current study are in agreement with Cabrera et al. (2005) they stated that low egg yolk concentration did not properly protect spermatozoa against damage by freeze–thawing. Similarly, Methew et al. (1984) also reported highest motility at increased egg yolk concentration at 3-5°C storage. The effect of egg yolk concentration in the freezing extender was far more important. The quality of frozen–thawed semen

tended to improve as egg yolk concentration increased Cabrera et al. (2005). In the present study, significantly better results (P<0.05) were observed in all sperm characteristics at increased 18% egg yolk after freezing. Similarly, in non-washed (Daskın and Tekin, 1996) and washed (Islam et al., 2006) goat semen, highest egg yolk concentration tended to be more effective for chilled and frozen storage goat semen. Present findings concur with the reports of Ustuner et al. (2009) who described that egg yolk concentrations had no effect on any semen parameters (P>0.05) other than motility.

In conclusion, our findings indicates that Tris based extender with 18% egg yolk concentration is better for the cooled and frozen Boer goat spermatozoa in tropical environment of Malaysia.

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References

Ariff, O.M., Hifzan, R.M., Zuki, A.B.M., Jiken, A.J. and Lehan, S.M. 2010. Maturing pattern for body weight, body length and height at withers of Jamnapari and Boer goats. *Pertanika Journal of Tropical Agriculture and Science*, 33: 269- 276.

Avdi, M., Leboeuf, B. and Terqui, M. 2004. Advanced breeding and buck effect in indigenous Greek goats. *Livestock Prodoction Science*, 87: 251-257.

Buckett, W.M., Luckas, M.J., Aird, I.A., Farquharson, R.G., Kingsland, C.R. and Lewis-Jones, D.I. 1997. The hypo-osmotic swelling test in recurrent miscarriage. *Fertility and Sterility*, 68: 506–509.

Cabrera, F., Gonzalez, F., Batista, M., Calero, P., Medrano, A. and Gracia, A. 2005. The effect of removal of seminal plasma, egg yolk level and season on sperm freezability of Canary buck (*Capra hircus*). *Reproduction in Domestic Animals*, 40: 191-195.

Corteel, J.M. 1974. Viabilité des spermatozoïdes de bouc conservés et congelésavec ou sans leur plasma séminal: Effet du glucose Annales de Biologie Animale Biochimieet. *Biophysique*, 14: 741-745.

Daskin, A. and Takin, N. 1996. The effect of egg yolk on the quality of frozen Angora buck semen. *Tropical Journal of Veterinary and Animal Sciences*, 20: 395-398.

Deka, B.C. and Rao, A.R. 1987. Effect of extenders and thawing method on post thawing preservation of goat semen. *Indian Veterinary Journal*, 64: 591-594.

Dorado, J., Rodrıguez, I. and Hidalgo, M. 2007. Cryopreservation of goat spermatozoa: Comparison of two freezing extenders based on post-thaw sperm quality and fertility rates after artificial insemination. *Theriogenology*, 68: 168-177.

Drobnis, E.Z., Nelson, E.A. and Burrill, M.J. 1980. Effect of several processing variables on motility and glutamic oxalacetic transaminase levels for frozen goat semen, I. Diluents. *Journal of Animal Science*, 51: 439.

Evans, G. and Maxwell, W.M.C. 1987. Handling and examination semen. p. 93-106. In: Maxwell, W.M.C. (Ed.), Salamon's Artificial Insemination of Sheep and Goat. Butterworths, Sydney.

Fatet, A., Pellicer-Rubio, M.T. and Leboeuf, B. 2011. Reproductive cycles of goats. *Animal Reproduction Science*, 124: 211-219.

Islam, R., Ahmed, K. and Deka, B.C. 2006. Effect of holding and washing on the quality of goat semen. *Small Ruminant Research*, 66: 51-57.

Jesse, F.F.A., Sang, S.L., Saharee, A.A. and Shahirudin, S. 2011. Pathological changes in the organs of mice model inoculated with *Corynebacterium pseudotuberculosis* organism. *Pertanika Journal of Tropical Agriculture and Science*, 34: 145-149.

Karatzas, G., Karagiannidis, A., Varsakeli, S. and Brikas. 1997. Fertility of fresh and frozen-thawed goat semen during the nonbreeding season. *Theriogenology*, 48: 1049-1059.

Leboeuf, B., Restall, B. and Salamon, S. 2000. Production and storage of goat semen for artificial insemination. *Animal Reproduction Science*, 62: 113-141.

Luna-Orozco, J. R., Fernandez, I.G., Gelez, H. and Delgadillo, J.A. 2008. Parity of female goats does not influence their estrous and ovulatory responses to the male effect. *Animal Reproduction Science*, 106: 352-360.

MacKenzie, C. 1994, A comparison of buffers for ram semen, M.Sc. thesis, University of Wales, Bangor, UK.

Mahmood**, S.,** Wasim, A**.** and Hassan, M.J. 2011. Autosomal recessive primary microcephaly (MCPH): clinical manifestations, genetic heterogeneity and mutation continuum. *Orphanet Journal of Rare Diseases***,** 6: pp. 39.

Mathew, J., Raja, C.K.S.V. and Nair, K.P. 1984. Preservation of buck semen in Tris yolk diluent. *Indian Veterinary Journal*, 61: 964-968.

Memon, M.A., Bretzlaff, K.N., and Ott, R.S. 1985. Effect of washing on motility and acrosome morphology of frozen-thawed goat spermatozoa. *American Journal of Veterinary Research*, 46: 473-475.

Naing, S.W., Wahid, H., Azam, K.M., Rosnina, Y., Zuki, A.B., Kazhal, S., Bukar, M.M., Thein, M., Kyaw, T. and San, M.M. 2010. Effect of sugars on characteristics of Boer goat semen after cryopreservation. *Animal Reproduction Science,* 122: 23-28.

Paulenz, H., Soderquist, L., Adnoy, T., Soltun, K., Saether, P.A. and Fjellsoy, K.R. 2005. Effect of cervical and vaginal insemination with liquid semen stored at room temperature on fertility of goats. *Animal Reproduction Sciences,* 86: 109–117.

Purdy, P.H. 2006. A review on goat sperm cryopreservation. *Small Ruminant Research,* 63: 215-225.

Revell, S.G. and Mrode, R.A. 1994. An osmotic resistance test for bovine semen. *Animal Reproduction Science,* 36: 77-86.

Salamon, S. and Ritar, A.J. 1982. Deep freezing of Angora goat semen: Effects of diluent composition and method and rate of dilution on survival of spermatozoa. *Australian Journal of Biological Sciences,* 35: 295-303.

Shamsuddin, M., Amiri, Y. and Bhuiyan, M.M.U. 2000. Characteristics of buck semen with regard to ejaculate numbers, collection intervals, diluents and preservation periods. *Reproduction in Domestic Animals,* 35: 53-57.

Tuli, R.K. and Holtz, W. 1992. The effect of zwitterions buffers on the freezability of Boer goat semen. *Theriogenology,* 37: 947-951.

Ustuner, B., Gunay, U. and Nur, Z. 2009. Effect of seminal plasma, egg yolk, and season on the freezability of saanen buck semen. *Bulletin of Veterinary Insttitute Pulawy,* 53: 369-374.

Yildiz, C., Kaya, A., Aksoy, M. and Tekeli, T. 2000. Influence of sugar supplementation of the extender on motility, viability and acrosomal integrity of dog spermatozoa during freezing. *Theriogenology,* 54: 579-585.