The Thai Journal of Veterinary Medicine

Volume 48
Issue 1 *March, 2018*

Article 1

3-1-2018

Evaluation of two cryopreservation protocols on cauda epididymal spermatozoa characteristics in domestic cats

Kanokwan Thammapradit

Suppawiwat Ponglowhapan

Original Article

Evaluation of two cryopreservation protocols on cauda epididymal spermatozoa characteristics in domestic cats

Kanokwan Thammapradit Suppawiwat Ponglowhapan*

Abstract

Various protocols of feline spermatozoa cryopreservation have been reported and used in clinical practice. To compare the quality of post-thawed epididymal spermatozoa subjected to two different sperm freezing protocols, sperm samples were collected from domestic cats (n=7) after castration and divided into 2 aliquots as for Protocol 1 (P1) or Protocol 2 (P2). In P1, after centrifugation, sperm pellets were extended with Tris-based egg yolk extender (TEY) containing 3% glycerol and held for 60 min (5°C) prior to the second dilution (1:1) with 7% glycerol and 1% Equex STM Paste. The straws were frozen by lowering the goblets in three steps into a LN_2 tank. In P2, sperm pellets were re-suspended in TEY free of glycerol, left for 15 min at room temperature before the addition (1:1) of TEY containing 8% glycerol and 1% Equex. After loading into 0.25-m*l* straws at room temperature, the sperm were cooled to 5°C for 25 min and subsequently frozen in a Styrofoam box at 4 cm above LN_2 for 10 min. Thawing was done at 37°C for 1 min. No significant differences (P>0.05) between the two protocols (P1 vs P2) in all parameters were observed; total motility (30±8.1 vs 30±3.4; mean±SEM), progressive motility score (3±0.2 vs 3±0.2), percentage of spermatozoa with intact plasma membrane (38.5±4.5 vs 44.5±6.9) and intact acrosome (34±3.2 vs 38±4.7), respectively. In conclusion, both protocols used in the present study yielded similar post-thawed sperm characteristics.

Keywords: epididymides, feline, freezing protocol, spermatozoa

Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand

***Correspondence**: sponglowhapan@gmail.com*

Introduction

Gamete preservation in the Felidae family is considered an essential tool of assisted reproductive technology not only for wildlife conservation but also breeding pedigree cats. In dogs and cats, semen freezing is usually set in a clinic and requires low operating expense, investment and freezing instruments, compared with the commercial production of frozen semen available in farm animals. Various techniques of freezing-thawing and the composition of freezing and thawing medium for either ejaculated or epididymal cat spermatozoa have been published. Moreover, supplementation of detergents and antioxidants in semen extender to improve survival of cryopreserved cat spermatozoa has been extensively studied in recent years; however, the results are controversial among the studies to draw a final conclusion (Thuwanut et al., 2008; Thiangtum et al., 2009).

Cryopreservation of testicular tissue (Chatdarong et al., 2016) and epididymal sperm harvested from either corpus or caudal parts (Kunkiti et al., 2016) are good alternatives to rescue the valuable genetic materials when the animals or sperm donors were castrated for medical reasons or died suddenly. Successful pregnancy in domestic cats following unilateral intrauterine artificial insemination with cryopreserved sperm cells obtained from caudal epididymides has been published by Tsutsui et al. (2003). Although the post-thaw sperm motility (24%) and conception rate were low (27.3%) (Tsutsui et al., 2003), the results prove that the *in vivo* fertilizing ability of feline epididymal spermatozoa is retained after the freeze-thaw process, thus encouraging the use of frozen-thawed epididymal sperm in assisted reproduction.

Since the first report of sperm freezing in domestic cats documented in 1978 (Platz et al., 1978), many protocols for feline sperm cryopreservation have been developed by many research groups (Tsutsui et al., 2000; Zambelli et al., 2002; Axnér et al., 2004; Tebet et al., 2006; Jiménez et al., 2013; Vizuete et al., 2014), causing difficulty in the comparison of results of sperm quality post-thawing among studies and in the selection of a suitable protocol to use in practice. The aim of this study was to compare the post-thaw quality of epididymal cat spermatozoa subjected to two different cryopreservation protocols previously published (Zambelli et al., 2002; Axnér et al., 2004). There are some differences in the procedures between these two cryopreservation protocols such as the percentage of final glycerol concentration, sperm exposure time to glycerol, duration of pre-freezing equilibration period and freezing techniques. However, the two protocols were selected for evaluation in this study because (i) both are widely used in clinical practice (ii) results of sperm motility after thawing obtained from both studies were satisfactory and, most importantly, (iii) due to the small volume of feline semen, both techniques are simple and highly reproducible to perform without using an expensive commercial programmable controlled-rate freezer.

Materials and Methods

Semen collection: This study was conducted under the stipulations of University Animal Care and Use Committee (Protocol No. 13310068). Following routine orchidectomy, the testicles and epididymides were collected from 7 healthy tomcats (1-4 years old, 2.5-4 kg body weight). Tissue samples were placed immediately in a sterile 0.9% NaCL solution supplemented with 50 μg/m*l* gentamicin. In the laboratory, both caudal epididymides were dissected free from visible blood vessels and connective tissues, placed in 700 µ*l* of warm (37°C) Tris buffer and incised repetitively into 4-6 small pieces to allow the spermatozoa to disperse in the surrounding medium. After 10 min in the incubator (37°C), the tissue segments were removed. An aliquot of 20 µ*l* of fresh sperm sample was assessed for total motility (%TM), progressive motility (PM), membrane integrity, acrosome integrity, sperm concentration and total number of sperm cells. Sperm concentration was assessed using a Bürker Chamber. The fresh sperm sample was equally divided into 2 aliquots subjected to 2 different sperm treatments, placed in 0.5 m*l* Eppendorf tubes and then centrifuged at 700×g for 6 min. The supernatant was discarded.

Cryopreservation protocols: It is suggested that epididymal cat spermatozoa are better cryopreserved when two-step dilution and addition of a detergent like Equex STM Paste are applied to the freezing protocol (Buranaamnuay, 2015). In this study, sperm freezing was carried out by two-step dilution methods as for Protocol 1 (P1) (Axnér et al., 2004) or Protocol 2 (P2) (Zambelli et al., 2002). The freezing process in P2 was slightly modified from the technique described by Zambelli and colleagues (Zambelli et al., 2002) because freezing semen in a Styrofoam box is simple and practically performed by practitioners; straws were frozen by placing horizontally above liquid nitrogen. Semen extenders and thawing media were prepared in a single batch according to the previously reported protocols shown in Tables 1 and 2. The first dilution was done by re-suspending the resultant sperm pellet with Ext-I (P1, Table 1) or Ext-A (P2, Table 2).

In P1, sperm pellets were extended with Tris-based egg yolk extender (TEY) containing 3% glycerol at room temperature (25°C) and held for 60 min in a bench cooler that reached 5°C in 45 min. To get a final sperm concentration of 50×10^6 spermatozoa/m*l*, 5% glycerol and 0.5% Equex, the second dilution (1:1) was done at 5°C with another TEY containing 7% glycerol and 1% Equex STM Paste (Nova Chemical Sales, Scituate, Inc., MA, USA). After loading into 0.25-m*l* straws at 5°C, the straws were frozen by lowering the goblets vertically in three steps into a tank prior to plunging the straws in liquid nitrogen (Apollo SX-18 LN2 tank; MVE Cryogenetics®, New Prague, MN, USA). The top of the canister was held at 7, 13 and 20 cm below the opening of the tank for 2, 2 nd 1 min, respectively (Rota et al., 1997; Axnér et al., 2004).

In P2, sperm pellets were re-suspended in TEY free of glycerol (0%), left for 15 min at room temperature before further addition (1:1, vol/vol) of TEY containing 8% glycerol and 1% Equex STM Paste

(Nova Chemical Sales, Scituate, Inc., MA, USA), giving a final sperm concentration of 50×10^6 spermatozoa/m*l*, 4 % glyceol and 0.5% Equex. After loading into 0.25-mL straws at room temperature, the sperm were immediately cooled in a refrigerator at 5°C for 25 min. Subsequently, the straws were transferred into a Styrofoam box and frozen by placing horizontally in vapour at 4 cm above the surface of liquid nitrogen for 10 min and then immersed into liquid nitrogen until further analysis.

Semen thawing: The straws were kept for at least 1 month before being thawed at 37°C for 1 min and emptied into Eppendorf tubes containing 50-µ*l* thawing medium (37°C) (Tables 1 and 2). After thawing, the sperm samples were left on a bench top for 5 min before evaluation.

Table 1 Composition of semen extenders for Protocol 1 (P1)

	Ext-I	Ext-II	Thawing medium
Tris (wt/vol)	2.4%	2.4%	2.4%
Citric acid (wt/vol)	1.4%	1.4%	1.4%
Fructose (wt/vol)	0.8%	0.8%	0.8%
Sodium benzyl penicillin (wt/vol)	0.06%	0.06%	0.06%
Streptomycin sulfate (wt/vol)	0.1%	0.1%	0.1%
Egg yolk (vol/vol)	20%	20%	-
Glycerol (vol/vol)	3%	7%	-
Equex STM Paste (vol/vol)	-	1%	-
Distilled water	up to 100 m*l*	up to 100 m*l*	up to 100 m*l*

Table 2 Composition of semen extenders for Protocol 2 (P2)

	Ext-A	Ext-B	Thawing medium
Tris (wt/vol)	2.4%	2.4%	2.4%
Citric acid (wt/vol)	1.4%	1.4%	1.4%
Glucose (wt/vol)	0.8%	0.8%	0.8%
Sodium benzyl penicillin (wt/vol)	0.06%	0.06%	0.06%
Streptomycin sulfate (wt/vol)	0.1%	0.1%	0.1%
Egg yolk (vol/vol)	20%	20%	-
Glycerol (vol/vol)	-	8%	-
Equex STM Paste (vol/vol)	-	1%	-
Distilled water	up to 100 m*l*	up to 100 m*l*	up to 100 m*l*

Sperm evaluation: Subjective sperm motility (%TM) was determined on a warm plate (37°C) under a phase contrast microscope at 400×magnification. Forward progressive motility (PM) was recorded on a 0-5 grading scale; 0/5 = no forward sperm movement and 5/5 = rapid, steady forward progression (Platz and Seager, 1978).

Evaluation of sperm membrane integrity was evaluated with a combination of fluorophores EthD-1 and SYBR-14 (Molecular probes Inc., OR, USA) (Axnér et al., 2004). A total of 200 spermatozoa were evaluated for each sample; live sperm (intact plasma membrane) stained green with SYBR-14, dead sperm stained red with EthD-1 and some moribound sperm stained with both red and green.

Acrosome status was determined according to a staining procedure using FITC-PNA staining and propidium iodide (PI) was used as a counterstain to give a better visualization of the cell under epifluorescent microscope (Axnér et al., 2002). Two hundred spermatozoa were evaluated and classified into 3 categories (intact, reacted or loss). Only percentage of sperm with intact acrosome is presented in the result.

Statistical analysis: Differences between fresh and frozen-thawed samples were analysed with a paired-*t* test. Mean percentage of total sperm motility, percentage of sperm with intact plasma membrane, percentage of sperm with intact acrosome and progressive motility score of P1 and P2 were analysed with paired-*t* tests or a Wilcoxon test depending on the normal distribution of the data. All data are shown as mean±SEM. Significant differences were considered when *P*-values ≤ 0.05.

Results

Mean (±S.D.) sperm concentration and total sperm count of the fresh samples (n=7) was $412.7\pm149.3\times10^6$ spermatozoa/m*l* and $282.6\pm101.5\times10^6$ spermatozoa, respectively. Comparing the values of sperm parameters observed before and after freezing-thawing, significant declines in %TM (P<0.01), the percentages of sperm with intact plasma membrane (P<0.01) and intact acrosomal membrane (P<0.05), but not PM (P>0.05), were observed after cryopreservation of both protocols (Table 3). The post-thawed qualities of spermatozoa determined by %TM, PM, intact plasma membrane and intact acrosome did not differ significantly (P>0.05) between the P1 and P2 freezing protocols (Table 3).

Discussion

Under similar controlled conditions, e.g. the source of sperm samples, chemicals, extender preparation and sperm analysis, the effect of the two cryopreservation protocols on epididymal cat spermatozoa evaluated in this study were reliably comparable and clearly demonstrated. Freezing-thawing compromised the post-thaw quality of cat epididymal spermatozoa. However, the score of

progressively motile spermatozoa was not significantly affected by the freeze-thaw process. The results also showed that the sperm characteristics post-thaw (%TM, membrane integrity and acrosome integrity) were not significantly different between the P1 and P2 protocols, suggesting that differences in extender composition, cooling, freezing and thawing conditions between the two protocols caused damages to the epididymal cat spermatozoa to the same extent.

Table 3 Percentages (mean±SEM) of total motility, progressive motility, intact sperm membrane, and intact acrosome of fresh sperm samples and post-thawed samples (n=7) subjected to two cryopreservation protocols (P1 versus P2)

	Fresh	P1	P2
Total motility (%)	50±5.1[a]	30±8.1[b]	30±3.4[b]
Progressive motility	3±0.2 [a]	3±0.2 [a]	3±0.2 [a]
Intact plasma membrane (%)	63±1.6 [a]	38.5±4.5 [b]	44.5±6.9 [b]
Intact acrosome (%)	50±1.7 [a]	34±3.2 [b]	38±4.7 [b]

Means within a row with different superscript letters (a,b) differ significantly (P<0.05).

In feline reproductive practice, choosing a protocol for freezing semen that offers superior results is somehow struggling because the semen quality of cat displays large individual variations (Axnér et al., 2008). Moreover, variations in semen quality between/within individual cats is challenging for clinicians to conserve feline spermatozoa (Axnér et al., 2008). In our study, the two cryopreservation protocols were tested because the techniques are simple and convenient to perform in practice and both protocols require less investment and freezing instruments. Although there are some differences in the freezing processes between P1 and P2, the main composition of the cryopreservation medium (TEY extender) is almost similar except for the pre-freezing final concentration of glycerol and the type of sugar (fructose and glucose). In addition, TEY has been proved to be superior to egg-yolk free extender for cryopreservation of cat spermatozoa (Jiménez et al., 2013). Glycerol is frequently used for freezing cat semen as a penetrating cryoprotectant to reduce the physical and chemical stresses resulting from the freeze-thaw process of sperm cells, and glycerol concentrations ranging between 3-8% (vol/vol) are almost universal in semen extenders (Hay and Goodrowe, 1993; Tsutsui et al., 2003; Vick et al., 2012; Buranaamnuay, 2015). A slight difference in the final glycerol concentrations between the two protocols after the second dilution (5% for P1 and 4% for P2) should not have any significant impact on the post-thawed sperm quality observed in this study. In fact, final concentrations of glycerol ranging between 4-5% are commonly applied in the freezing of cat semen (Thuwanut et al., 2010; Villaverde et al., 2013).

The time interval the spermatozoa remain in contact with glycerol (equilibration period) before freezing is essential to allow the cryoprotectant to penetrate cell membrane, enabling equilibrium between intra- and extracellular concentrations of glycerol and other osmotically active extender elements (Evans and Maxwell, 1987). On the other hand, toxic effects of glycerol may negatively result in alterations in the structure and/or biochemical integrity of sperm cells including induced acrosome reaction (Barbas and Mascarenhas, 2009). The optimal times for equilibration as well as glycerol concentrations are, therefore, the key issues for developing the cryopreservation protocol. In P1, the exposure of sperm to a glycerol-containing medium was in the first dilution step and the equilibration time lasted about 60 min. In P2, the glycerol containing an extender was added in the second step and it took 25 min for equilibration. An equilibration period of about 20 min at 5°C has been recommended for cryopreservation of feline spermatozoa (Luvoni et al., 2003). However, a recent study has shown that a shorter equilibration time (a few minutes) before freezing is sufficient because no significant differences in %TM, viability, membrane integrity and acrosome integrity of epididymal cat spermatozoa were noted when compared with 60-min equilibration time (Buranaamnuay, 2015). In dogs, time intervals for equilibration varying between 0 and 4 hr have been suggested (Okano et al., 2004). These findings indicate that the penetration of glycerol across sperm membrane in dogs (Okano et al., 2004) and cats (Buranaamnuay, 2015) may not take long.

Sugars are added to semen extenders to protect intracellular metabolic reserves and cell components. In small animals, monosaccharides such as fructose and glucose are commonly used in sperm cryopreservation medium rather than disaccharides or trisaccharides. In dogs, the addition of fructose in freezing (Yildiz et al., 2000) or chilled semen extenders (Ponglowhapan et al., 2004) yields significantly better results of sperm motility and velocity parameters. However, glucose supplemented in TEY extender has been suggested for chilled dog semen because a 50% conservation rate of motile spermatozoa can be sustained longer than extenders containing fructose (Iguer-ouada and Verstgen, 2001). Comparing the benefit of fructose and glucose on preserved feline spermatozoa, no differences were detected for either of the monosaccharides (fructose versus glucose) added to extenders for freezing (Jiménez et al., 2013) or chilled semen (Glover and Watson, 1987). Taken together, the different composition of sperm cryopreservation medium between P1 and P2 as well as the initial exposure of sperm to glycerol in the dilution steps are unlikely to affect the quality of cryopreserved cat spermatozoa.

The positive effect of a water-soluble anionic detergent like Equex STM Paste as a supplement to freezing extenders for cryopreserved spermatozoa (ejaculated versus epididymal) has been documented in many species including dogs and cats (Axnér et al., 2004; Ponglowhapan and Chatdarong, 2008; Zambelli et al., 2010). Although a cryoprotective effect of Equex on the acrosome of epididymal sperm of dogs (Ponglowhapan and Chatdarong, 2008) and cats

(Axnér et al., 2004) was pronounced, increased exposure time of Equex to feline spermatozoa either before or after freezing-thawing significantly reduced the quality of sperm cells. A previous study has demonstrated that, prior to freezing, shorter exposure to Equex (10 min) significantly improved epididymal cat sperm characteristics in terms of the integrity of plasma membrane and acrosome status in comparison to longer exposure (70 min) (Buranaamnuay, 2015). In the same line, prolonged exposure of Equex after thawing also deteriorated longevity of feline epididymal spermatozoa (Axnér et al., 2004). In our study, Equex-containing semen extenders were added in the second dilution step of both P1 and P2 with different exposure periods. In P1, the extender containing 1% Equex was added just before loading the semen samples into mini straws and subsequently frozen (approximately 5 min of exposure period), whereas the sperm exposure time to Equex STM Paste in P2 was longer, being 25 minutes. Because no significant differences between P1 and P2 in any post-thawed sperm parameters were observed, our findings indicate that the longer sperm exposure time to Equex in P2 protocol during cooling process was acceptable.

In this study, the spermatozoa were frozen in straws by different freezing techniques, vertically in P1 (Tank) and horizontally in P2 (Styrofoam box). The two methods of freezing were tested for ejaculated dog spermatozoa and differences in freezing rates were shown in a previous study (Peña and Linde-Forsberg, 2000). Although the results were not conclusive, the quality of dog spermatozoa frozen horizontally in a Styrofoam box (moderately fast freezing rate) seemed to be better than of those frozen vertically in a tank when Equex STM paste was present, regardless of thawing rates (Peña and Linde-Forsberg, 2000). Similarly, the results obtained in our study showed no significant differences in sperm motility (%TM and PM) and plasma and acrosomal membrane integrity between the P1 and P2 protocols. The non-significant differences found in this study imply that the variation of freezing rates according to the vertical and horizontal methods causes minute differences in sperm parameters post-thawing. Generally, different freezing techniques may require different thawing rates to achieve optimal sperm survival. When the 2 thawing rates (70°C, 8 sec versus 37°C, 15 sec) were performed in cryopreserved dog semen, it is clear that dog spermatozoa survive better after thawing at 70°C for 8 sec (Peña and Linde-Forsberg, 2000). Unlike dog sperm, frozen cat spermatozoa can be thawed either at 70°C or 37°C (Chatdarong et al., 2010). In this study, thawing was done at 37°C for 1 min due to convenience in practice.

In conclusion, our findings showed that both cryopreservation protocols (P1 and P2) used for caudal epididymal cat spermatozoa yielded similar post-thaw sperm characteristics in terms of sperm motility, plasma membrane integrity and acrosome integrity. Both freezing protocols are good alternatives to perform in clinical practice.

Acknowledgements

This study was financially supported by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). We thank the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University for providing tissue materials used in this study.

References

Axnér E, Hermansson U and Linde-Forsberg C 2002. Capacitation time of feline epididymal spermatozoa. Proceedings of the 3rd Annual Symposium of the European Veterinary Society for Small Animal Reproduction (EVSSAR). Liége, Belgium. p. 115.

Axnér E, Hermansson U and Linde-Forsberg C 2004. The effect of Equex STM paste and sperm morphology on post-thaw survival of cat epididymal spermatozoa. Anim Reprod Sci. 84(1-2): 179-191.

Axnér E 2008. Updates on reproductive physiology, genital diseases and artificial insemination in the domestic cat. Reprod Domest Anim. 43(Suppl. 2): 144-149.

Barbas JP and Mascarenhas RD 2009. Cryopreservation of domestic animal sperm cells. Cell Tissue Bank. 10(1): 49-62.

Buranaamnuay K 2015. Determination of appropriate cryopreservation protocols for epididymal cat spermatozoa. Reprod Domest Anim. 50(3): 378-385.

Chatdarong K, Thuwanut P and Morrell JM 2016. The development of cat testicular sperm cryopreservation protocols: Effects of tissue fragments or sperm cell suspension. Theriogenology. 85(2): 200-206.

Chatdarong K, Thuwanut P, Manee-in S, Lohachit C and Axnér E 2010. Effects of thawing temperature and post-thaw dilution on the quality of cat spermatozoa. Reprod Domest Anim. 45(2): 221-227.

Evans G and Maxwell W 1987. Short-term (liquid) storage of semen. In: Salamon's artificial insemination of sheep and goats. Sydney: Butterworth-Heinemann Ltd. p.194.

Glover TE and Watson PE 1987. The effects of egg yolk, the low density lipoprotein fraction of egg yolk, and three monosaccharides on the survival of cat (*Felis catus*) spermatozoa stored at 5°C. Anim Repord Sci. 13(3): 229-237.

Hay MA and Goodrowe KL 1993. Comparative cryopreservation and capacitation of spermatozoa from epididymides and vasa deferentia of the domestic cat. J Reprod Fertil Suppl. 47: 297-305.

Iguer-ouada M and Verstegen JP 2001. Long-term preservation of chilled canine semen: effect of commercial and laboratory prepared extenders. Theriogenology. 55(2): 671-684.

Jiménez E, Pérez-Marín CC, Vizuete G, Millán Y and Agüera EI 2013. Effect of different extenders on in vitro characteristics of feline epididymal sperm during cryopreservation. Reprod Domest Anim. 48(2): 665-672.

Kunkitti P, Bergqvist AS, Sjunnesson Y, Johannisson A and Axnér E 2016. The tolerance of feline corpus and cauda spermatozoa to cryostress. Theriogenology. 85(3): 502-508.

Luvoni GC, Kalchschmidt E, Leoni S and Ruggiero C 2003. Conservation of feline semen. Part I: cooling and freezing protocols. J Feline Med Surg. 5(4): 203-208.

Okano T, Murase T, Asano M and Tsubota T 2004. Effects of final dilution rate, sperm concentration and times for cooling and glycerol equilibration on post-thaw characteristics of canine spermatozoa. J Vet Med Sci. 66(11): 1359-1364.

Peña A and Linde-Forsberg C 2000. Effects of Equex, one- or two-step dilution, and two freezing and thawing rates on post-thaw survival of dog spermatozoa. Theriogenology. 54(6): 859-875.

Platz CC and Seager SWJ 1978. Semen collection by electroejaculation in the domestic cat. JAVMA. 173(10): 1353-1355.

Platz CC, Wildt DE and Seager SWJ 1978. Pregnancy in the domestic cat after artificial insemination with previously frozen spermatozoa. J Reprod Fertil. 52(2): 279-282.

Ponglowhapan S and Chatdarong K 2008. Effects of Equex STM Paste on the quality of frozen-thawed epididymal dog spermatozoa. Theriogenology. 69(6): 666-672.

Ponglowhapan S, Essén-Gustavsson B and Linde Forsberg C 2004. Influence of glucose and fructose in the extender during long-term storage of chilled canine semen. Theriogenology. 62(8): 1498-1517.

Rota A, Ström B, Linde-Forsberg C and Rodriguez-Martinez H 1997. Effects of equex STM paste on viability of frozen-thawed dog spermatozoa during in vitro incubation at 38 degrees C. Theriogenology. 47(5): 1093-1101.

Tebet JM, Martins MI, Chirinea VH, Souza FF, Campagnol D and Lopes MD 2006. Cryopreservation effects on domestic cat epididymal versus electroejaculated spermatozoa. Theriogenology. 66(6-7): 1629-1632.

Thiangtum K, Pinyopummin A, Hori T, Kawakami E, Tsutsui T 2009. Effect of catalase and superoxide dismutase on motility, viability and acrosomal integrity of frozen-thawed cat spermatozoa. Reprod Domest Anim. 44(Suppl. 2): 369-372.

Thuwanut P, Chatdarong K, Techakumphu M, Axnér E 2008. The effect of antioxidants on motility, viability, acrosome integrity and DNA integrity of frozen-thawed epididymal cat spermatozoa. Theriogenology. 70(2): 233-240.

Thuwanut P, Chatdarong K, Johannisson A, Bergqvist AS, Söderquist L and Axnér E 2010. Cryopreservation of epididymal cat spermatozoa: effects of in vitro antioxidative enzymes supplementation and lipid peroxidation induction. Theriogenology. 73(8): 1076-1087.

Tsutsui T, Wada M, Anzai M and Hori T 2003. Artificial insemination with frozen epididymal sperm in cats. J Vet Med Sci. 65(3): 397-399.

Tsutsui T, Tanaka A, Takagi Y, Nakagawa K, Fujimoto Y, Murai M, Anzai M and Hori T 2000. Unilateral intrauterine horn insemination of frozen semen in cats. J Vet Med Sci. 62(12): 1247-1251.

Vick MM, Bateman HL, Lambo CA and Swanson WF 2012. Improved cryopreservation of domestic cat sperm in a chemically defined medium. Theriogenology. 78(9): 2120-2128.

Villaverde AI, Fioratti EG, Penitenti M, Ikoma MR, Tsunemi MH, Papa FO and Lopes MD 2013. Cryoprotective effect of different glycerol concentrations on domestic cat spermatozoa. Theriogenology. 80(7): 730-737.

Vizuete G, Jiménez E, Agüera EI and Pérez-Marín CC 2014. Impact of ultra-rapid freezing on the motility, morphology, viability and acrosome integrity of epididymal cat sperm diluted in sucrose-based extenders. Reprod Domest Anim. 49(1): e5-8.

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(4): 579-585.

Zambelli D, Caneppele B, Castagnetti C and Belluzzi S 2002. Cryopreservation of cat semen in straws: comparison of five different freezing rates. Reprod Domest Anim. 37(15): 310-313.

Zambelli D, Iacono E, Raccagni R and Merlo B 2010. Quality and fertilizing ability of electroejaculated cat spermatozoa frozen with or without Equex STM Paste. Theriogenology. 73(7): 886-892.

บทคัดย่อ

ผลของวิธีการแช่แข็งน้ำเชื้อ 2 วิธี ต่อคุณภาพอสุจิที่เก็บจากอิพิดิไดมิสส่วนท้ายในแมว

กนกวรรณ ธรรมประดิษฐ์ ศุภวิวัธน์ พงษ์เลาหพันธุ์*

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบคุณภาพน้ำเชื้อแมวที่เก็บจากอิพิดิไดมิสส่วนท้ายซึ่งผ่านการเก็บรักษาด้วยวิธีการแช่แข็งที่แตกต่างกัน ภายหลังการผ่าตัดทำหมันแมว (n=7) ตัว ทำการแบ่งอสุจิที่เก็บจากอิพิดิไดมิสส่วนท้ายของแมวแต่ละตัวออกเป็น 2 ส่วนเพื่อนำไปแช่แข็งด้วยวิธีที่แตกต่างกัน 2 วิธี วิธีที่ 1 ภายหลังการปั่นเหวี่ยงเพื่อเอาน้ำเลี้ยงเชื้อออก ทำการเติมสารละลายน้ำเชื้อ (TEY) ที่มีความเข้มข้นกลีเซอรอลร้อยละ 3 แล้วตั้งไว้ที่อุณหภูมิ 5 องศาเซลเซียสเป็นเวลา 60 นาที จากนั้นจึงเติมสารละลายน้ำเชื้อชนิดที่ 2 ที่มีความเข้มข้นกลีเซอรอลร้อยละ 7 และความเข้มข้น Equex STM ร้อยละ 1 แล้วจึงนำน้ำเชื้อบรรจุลงหลอดขนาด 0.25 มิลลิลิตรเพื่อแช่แข็งโดยวางหลอดในแนวตั้งเหนือไอไนโตรเจนเหลว วิธีที่ 2 เติมสารละลายน้ำเชื้อครั้งแรกโดยไม่มีส่วนผสมของกลีเซอรอล แล้วตั้งไว้ที่อุณหภูมิห้องนาน 15 นาทีก่อนเติมสารละลายน้ำเชื้อชนิดที่ 2 ซึ่งมีความเข้มข้นของกลีเซอรอลร้อยละ 8 และ Equex STM ร้อยละ 1 จากนั้นจึงบรรจุอสุจิในหลอดเพื่อแช่แข็งโดยวางหลอดในแนวนอนเหนือไอไนโตรเจนเหลว ทำการละลายน้ำเชื้อแช่แข็งในอ่างน้ำที่อุณหภูมิ 37 องศาเซลเซียสเป็นเวลานาน 1 นาทีเพื่อประเมินคุณภาพ การศึกษาพบว่า ภายหลังการแช่แข็ง คุณภาพของน้ำเชื้อที่ผ่านการแช่แข็งทั้ง 2 วิธีลดลงเมื่อเปรียบเทียบกับคุณภาพก่อนการแช่แข็ง และไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างวิธีการแช่แข็งน้ำเชื้อทั้ง 2 วิธี ทั้งในด้านอัตราการเคลื่อนไหว คะแนนการเคลื่อนที่ไปข้างหน้า ความสมบูรณ์ของผนังเซลล์ และความสมบูรณ์ของอะโครโซม

คำสำคัญ: อิพิดิไดมิส แมว การแช่แข็งน้ำเชื้อ อสุจิ

ภาควิชาสูติศาสตร์ เธนุเวชวิทยาและวิทยาการสืบพันธุ์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330 ประเทศไทย

****ผู้รับผิดชอบบทความ** E-mail: sponglowhapan@gmail.com*