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Short-Term Chilled Storage of Cat Semen Extended with and Without Taurine Containing Milk Extenders

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Abstract: The aim of this study was to observe the effects of taurine containing and non-containing milk extenders on the spermatological characteristics of chilled stored male cat semen. Semen was collected from 4 cats of 2-3 ages under general anesthesia (xylazine-ketamine HCl) by an electro-ejaculator during the study. Total 5 ejaculates with a weekly basis were collected from each male cat. The semen was split into 2 portions and one was extended with taurine containing Skimmed Milk-Glucose (SMG-T) extender at 26°C (egg yolk (10 mL)-D (+) glucose (1.00 g)-taurine (50 mM)-skimmed milk (to 100 mL)-penicillin-G (1000 IU mL^{-1})-streptomycin sulphate (1000 μg mL^{-1})) the other portion was extended with Skimmed Milk-Glucose extender (SMG) (egg yolk (10 mL)-D (+) glucose (1.00 g)-skimmed milk (to 100 mL)-penicillin-G (1000 IU mL^{-1})-streptomycin sulphate (1000 μg mL^{-1})). The extended semen samples were chilled to 5°C in an hour (0.35°C min^{-1}). Spermatological investigations such as motility (%) and abnormal spermatozoon rates (%) (acrosome, head, mid-piece, tail and total) were done at dilution, cooling to 24 h, 48 h and 72 h at 5°C. Morphological defect rates were examined by staining with Spermac® stain by a light microscope at 1000 magnification. The mean motility of cooled semen in the SMG-T extender at the 24 h was the highest with 75.50±5.82% and this value was 66.25±12.86% in the SMG extender ($p<0.05$). During the above time period, the mean acrosome and total abnormal spermatozoon rates in the SMG-T extender were lowest with 18.55±3.15 and 32.70±4.59%, respectively, these averages were highest in the SMG extender with 19.60±5.67 and 40.15±9.10%, respectively ($p<0.05$). The motility and morphologic defect rates at the 48 h were similar in the SMG-T and SMG extenders. At the 72 h, acrosome and tail defect rates in the SMG-T extender were lowest with 33.50±4.36 and 15.65±6.26%, respectively whilst, these values were highest in the SMG extender with 40.45±8.76 and 17.65±3.84%, respectively ($p<0.05$). At the conclusion of the study, it can be said that SMG-T and SMG extenders can be successfully employed to store cat semen for shorter periods (24-48 h), also, SMG-T extender is superior to the SMG extender in cooled storage of cat semen for 24 h.

Key words: Cat, semen, taurine, extender, cooling, storage

INTRODUCTION

Domestic cat seems to be an excellent research model in wild cats. Preservation of liquid cat semen is an important technique in feline reproduction. Maintaining the motility and fertility of spermatozoa during short-term storage at 5°C is an important consideration in the use of liquid semen in cats.

Studies dealing with the effects of low density lipoprotein fraction of egg yolk, adding various monosaccarids (fructose, glucose and galactose) to the test extender and lactose, citrate and test extenders on short time storage of cat semen at 5°C have been conducted (Glover and Watson, 1985). Also, comparative studies on storing cat semen at 4°C with tris and test extenders are present (Sánchez and Tsutsui, 2002).

The lipid composition of the sperm membrane is a major determinant of the viability, lipid peroxidation and cold shock experienced in spermatozoa (Hammerstedt, 1993). The mammalian sperm cell contains a high proportion of polyunsaturated fatty acids and is therefore particularly susceptible to peroxidative damage, with a subsequent loss in membrane integrity; impaired cell function and decreased motility of spermatozoa (Alvarez *et al*., 1987; Griveau *et al*., 1995). Oxidative stress is one factor associated with decline in fertility during

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semen storage. The Reactive Oxygen Species (ROS) is a normal consequence of oxidative metabolism and appears to be involved in damage to spermatozoa even under hypothermic storage conditions (Maxwell and Stojanov, 1996).

Antioxidants have been used in attempts to block or prevent oxidative stress in a variety of cell systems. These antioxidants act a variety of steps to either scavenge ROS directly or to prevent propagation of lipid peroxidation in cell membranes (Ball *et al*., 2001). Taurine is a sulphur containing β-amino acid present in the reproductive tract of several mammals (Meizel *et al*., 1980) and found high concentrations in spermatozoa, seminal plasma and epididymal fluid of domestic cats (Buff *et al*., 2001).

In recent years, studies have also been conducted on rabbit, ram, bull and stallion semen diluents, including anti-oxidants such as taurine to improve during storage chilling motility, viability and membrane integrity of spermatozoa (Maxwell and Stojanov, 1996; Ball *et al*., 2001; Alvarez and Storey, 1983; Foote *et al*., 2002). The uses of taurine with skim-milk extender in cat semen chilling have not previously been reported. Therefore, the present study was undertaken to investigate the effects of anti-oxidants such as taurine on sperm motility and morphology during storage (24, 48 and 72 h) at 5°C.

MATERIALS AND METHODS

Four male cats of mixed breed aged 2-3 (average 3.5-4.5 kg live weight) served as semen donors in this study. The male cats were kept in individual cages measuring 60×90×120 (h) cm in an animal room in which the temperature was adjusted to 24±2°C. The experiments were performed during the breeding season between March and April. The male cats were supplied with dry food (IAMS Company, USA) and fresh drinking water *ad libitum* daily.

Semen collection and examination of semen quality: The animals were restrained with xylazine 2 mg kg^{-1} (Alfazyne®, Alfasan Int. Netherlands) in combination (all injections given im) with ketamine-HCl 10 mg kg^{-1} (Alfamine® 10%, Alfasan Int. Netherlands). Food and water were withheld from male cats for 12 and 2 h, respectively, before anesthesia. Semen was collected from the male cats by means of electro-ejaculator (P-T Electronics, Model 302, USA). Anesthetized male cats were fixed horizontally and lubricated rectal probe was placed into the rectum. Electrical stimulus was administrated following the Platz and Seager (1978) procedure. The set of administrations was between 2-7 V and each set contained 60 stimulus, with a total of 180 electrical stimulus. The emissions were collected in pre-warmed Eppendorf tubes. Total 5 ejaculates with a weekly basis were collected from each male cat. All procedures dealing with the animals have been controlled and confirmed by the Ethical Comity of the Faculty of Veterinary Medicine.

Table 1: Preparation of skim-milk extender with and without containing taurine

Ingredients	SMG extender	SMG-T extender
Glucose D (+) $C_6H_{12}O_6$	1.0 g	1.0 g
Taurine $C_2H_7NO_3S$	-	50 mM
Streptomycin Sulfate	1000 mg mL^{-1}	1000 mg mL^{-1}
Penicillin G	1000 IU mL^{-1}	1000 IU mL^{-1}
Egg yolk	10 mL	10 mL
Skim-milk	-100 mL	-100 mL

Evaluation of spermatozoa: The ejaculate volume was measured with variable pipette (10-100 μL) and 10 μL of the ejaculate was placed on a microscope slide and evaluated subjectively for the percentage of progressive motile spermatozoa in a hot plate phase-contrast microscope at ×200 magnification. Three or four microscopic fields were evaluated for each ejaculate. Spermac® stain kit (Stain Enterprises, Republic of South Africa) was employed in morphologic observations (Schäfer and Holzman, 2000). The preparation was examined by light microscope at ×1000 magnification by counting 200 spermatozoa (acrosome, head, mid-piece, tail and total).

Sperm processing and cooling: The semen was split into 2 portions and one was extended with taurine containing Skimmed Milk-Glucose extender (SMG-T) at 26°C, the other portion was extended with Skim-Milk-Glucose extender (SMG) (Table 1). The extended semen samples were cooled to 5°C in 60 min. Spermatological investigations such as motility (%) and abnormal spermatozoon rates (%) (acrosome, head, mid-piece, tail and total) were done at dilution, cooling to 5°C, after 24, 48 and 72 h at 5°C.

Statistical analysis: Results were expressed as the mean±SD mean were analyzed using a one-way Analysis of Variance (ANOVA). Differences with values of $p<0.05$ were considered to be statistically significant.

RESULTS

No siginificant difference was observed among the male cats with regard to fresh semen (volume, motility and morphologic defect rates) ($p>0.05$). The results for fresh semen are presented in Table 2 and motility and morphologic defect rate results after dilution at 26°C and cooling to 5°C are presented in Table 3 ($p>0.05$).

Table 2: Spermatological characteristics in fresh semen

			Morphologic defects (%)				
Male cat	Volume (μL)	Motility (%)	Acrosome	Head	Mid-piece	Tail	Total
A	82.00±13.03	88.00±4.47	7.60±2.40	4.00±2.23	2.80±1.48	4.40±0.89	18.80±6.10
B	121.00±45.05	89.00±4.18	8.40±3.78	4.20±2.49	3.80±1.78	6.60±4.16	23.00±5.10
C	112.00±41.62	88.00±5.70	8.60±3.91	5.00±1.87	3.80±2.68	4.80±1.09	22.20±3.56
D	115.00±28.28	86.00±4.18	8.40±2.88	6.60±2.40	2.60±2.19	6.60±4.45	24.20±4.55
Means	107.50±35.15	87.75±4.43	8.25±3.05	4.95±2.32	3.25±1.99	5.60±3.05	22.05±4.95

Table 3: Motility and morphologic defect values after dilution and cooling

			Morphologic defects (%)				
Stage	Extender	Motility (%)	Acrosome	Head	Mid-piece	Tail	Total
Dilution	SMG-T	87.25±4.72	10.65±3.20	3.40±2.37	2.90±2.51	4.80±3.15	21.75±5.50
	SMG	85.50±5.35	12.35±4.39	3.95±2.26	2.80±1.76	7.00±4.57	26.10±8.55
Cooling	SMG-T	84.00±5.03	13.10±4.51	3.70±3.09	1.75±1.29	7.05±5.40	25.60±7.91
	SMG	82.25±6.78	14.80±4.38	3.85±1.95	2.40±1.53	8.45±5.85	29.50±8.25

Means±SD, p>0.05

Table 4: Mean (±SD) values for sperm motility and morphology, at different times relative to semen cooling (24, 48 and 72 h after cooling)

			Morphologic defects (%)				
Duration	Extender	Motility (%)	Acrosome	Head	Mid-piece	Tail	Total
24 h	SMG-T	75.50±05.82[a]	18.55±03.15[a]	4.25±2.45	1.25±1.86	08.60±5.50	32.70±04.59[a]
	SMG	66.25±12.86[b]	25.60±05.67[b]	3.75±2.40	1.85±1.50	14.95±8.73	46.15±09.10[b]
48 h	SMG-T	64.25±07.30	23.70±05.28	5.60±2.91	2.00±2.10	14.35±4.27	45.65±06.93
	SMG	55.25±11.18	30.70±08.58	4.00±2.25	1.90±1.62	13.40±4.65	50.00±10.29
72 h	SMG-T	49.75±10.94[a]	33.50±04.36[a]	5.15±2.39	1.60±1.67	15.65±6.26	55.90±07.55[a]
	SMG	36.50±12.47[b]	40.45±08.76[b]	5.55±2.23	2.30±1.87	17.65±3.84	66.15±10.49[b]

[a, b]Different superscripts within the same column demonstrate significant differences (p<0.05) by SPSS and ANOVA

The mean motility of cooled semen in the SMG-T extender at the 24 h was the highest with 75.50±5.82% and this value was 66.25±12.86% in the SMG extender (p<0.05). During the above time period, the mean acrosomal and total abnormal spermatozoa rates in the SMG-T extender were lowest with 18.55±3.15 and 32.70±4.59%, respectively, these averages were highest in the SMG extender with 25.60±5.67 and 46.15±9.10%, respectively (p<0.05). The motility and morphologic defect rates at the 48 h were similar in the SMG-T and SMG extenders. The mean motility of cooled semen in the SMG-T extender at the 72 h was the highest motility with 49.75±10.94% and this value was 36.50±12.47% in the SMG extender (p<0.05). During the above time period, the mean acrosomal and total abnormal spermatozoa rates in the SMG-T were lowest with 33.50±4.36 and 55.90±7.55%, respectively, these averages were highest in the SMG extender with 40.45±8.76 and 66.15±10.49%, respectively (p<0.05) (Table 4).

DISCUSSION

In the present study, we have provided the effects of taurine containing and non containing skimmed milk-glucose extenders on the spermatological characteristics of chilled stored male cat semen. The mean spermatological traits of 20 fresh electro-ejaculates semen from four male cats have been in accordance with most researchers (Glover and Watson, 1985; Baran *et al*., 2004a). For acrosomal and total abnormal spermatozoa rates our results were similar to some researchers (Baran *et al*., 2004a, b) lower than some (Glover and Watson, 1985). The different techniques in morphological examination of semen samples and the breed of male cats used could have caused the difference.

The motility value of both groups after cooling was higher than that 51 and 62.2% motility values of Harris *et al*. (2001) and Glover and Watson (1985) with TesT extender. Cat semen is quite sensitive to cold shock after diluting and chilling. Especially if chilling is rapid (>0.4°C min^{-1}) cat semen is easily deteriorated. For this, the low cooling rate (0.35°C min^{-1}) that we have employed in the study can be claimed to keep the motility achieved. According to Glover and Watson (1985), cat ejaculated spermatozoa are intermediately susceptible to cold shock. The susceptibility of spermatozoa to cold shock is related to the composition of the plasma membrane and differs between species and individuals.

The cooling rate also influences sperm survival. According to Pukazhenti *et al*. (1999), rapid cooling harms spermatozoa more than slow cooling does. Long-term cold storage in both ejaculated (Glover and Watson, 1985; Pukazhenti *et al*. 1999) and epididymal (Harris *et al*., 2001) cat spermatozoa has been reported,

the epididymal spermatozoa showing >50% motility after 14 days storage. In many species, it has been demonstrated that epididymal spermatozoa are more resistant to cold shock than are ejaculated spermatozoa. Similarly, it was reported by Harris *et al*. (2001) that in the cat, epididymal spermatozoa may have beter motility than ejaculated spermatozoa (69 vs. 51.4%) after 5 days of cold storage, although, differences were not tested for statistical significance. Whether cat ejaculated and epididymal spermatozoa differ in their sensitivity to cold shock has, obviously, not yet been fully explored. Glover and Watson (1987) have compared various concentrations of egg yolk in storing cat semen at 5°C for 24-72 h and reported the best motility with 10% egg yolk rate and stored semen for 72 h. The motility value of cat semen kept at 4°C for 72 h in Tris extender by Sánchez and Tsutsui (2002) and Siemieniuch and Dubiel (2007) was similar to our value with SMG-T extender. However, the first researchers have collected the semen via artificial vagina and the others directly from the epidydimidis of vasectomised cats.

Under natural system of mating, sperm are exposed primarily to anaerobic conditions, thus reducing potential damage by ROS. In addition, oviduct fluids contain substantial concentrations of taurine (Miller and Shultz, 1987) an important protector of cells against accumulation of ROS when they are exposed to aerobic conditions (Alvarez and Storey, 1983; Holmes *et al*., 1992). When sperm are used for artificial insemination of domestic animals; they are exposed to oxygen and visible light radiation during various processing procedures. These could lead to formation of ROS and damage to sperm cell motility and genomic integrity (Aitken *et al*., 1998). Under these conditions additional taurine helped to sperm motility (Alvarez and Storey, 1983). In our study also, taurine containing (50 mM) SMG extender, had a protective effect on both motility and abnormal spermatozoa rate.

Egg yolk has been shown to have a protective effect on the motility and fertility of cooled bull spermatozoa. It is commonly included in semen extenders used for cold storage and cryopreservation in a number of species. Egg yolk exerts its effect through lipoproteins included in the Low-Density Fraction (LDF), the protection apparently taking place on the surface of the membrane, although, we still do not know how the lipoproteins exert this protection. There are some conflicting results on whether egg yolk is beneficial or not for cold storage of cat spermatozoa. According to Glover and Watson (1987), the survival time of cat spermatozoa stored at 5°C is not prolonged in the presence of egg yolk and suggested that a simple buffer without egg yolk and sugars might be the best cold storage media for cat spermatozoa. By contrast, Pukazhenti *et al*. (1999), reported that motility was maintained for longer intervals in the presence of egg yolk. The effect of EY on sperm membrane integrity and acrosomal integrity was not reported in the study by Pukazhenti *et al*. (1999). Similarly, it was reported by Sánchez and Tsutsui (2002) that ejaculated cat spermatozoa could be cold stored for up to 72 h in an EYT extender.

CONCLUSION

It is observed that in short term storage of cat semen at 5°C, addition of an antioxidant substance taurine to skimmed milk-glucose extender makes a cell protective effect in relation to motility and abnormal spermatozoa rate. Further studies are required for a better understanding of the biochemical changes and obtain more information on the determination of lipid peroxidation and antioxidant capacities in cooled male cat semen.

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