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Research Article

Collection of Semen from Van Cats Using Electroejaculation and Freezing of Semen

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ABSTRACT

In this study, the aim was to collect semen from Van Cats using electroejaculation, to perform spermatologic examinations and to freeze the semen. In the research, 7 Male Van Cats were used. Semen was collected from cats at 10 different times at weekly intervals using electroejaculation with general anesthesia. The semen was diluted with 3 diluents (3% Equiex, 6% Equiex, Biosfos). Motility and vitality values were analyzed. Then the semen was frozen in liquid nitrogen vapor. The semen dissolved was analyzed for motility and vitality values. In the study, amount of semen, sperm motility, dead sperm, sperm concentration, total abnormal sperm, stimulation time and stimulation count were found to be 91.92±21.49 µl, %67.42±4.94, %23.91±8.59, 109.20±66.12x10^6/ml, %25.57±4.57, 83.08±4.57 sec and 10.35±0.56, respectively. After dissolution, motility and vitality values were found to be, respectively, 45.79±5.49% and 53.57±6.03% with 3% Equiex diluent, 41.78±5.71% and 52.64±6.35% with 6% Equiex diluent, 40.00±5.45% and 51.50±6.72% with Biosfos diluent. After the dissolution, abnormal sperm rates related to the head, middle piece and tail were 3.74±0.91%, 13.62±2.63%, 21.91±4.03%, respectively. In conclusion, approximately 43% motility achieved with the freezing and dissolution of the semen suggests that the semen of Van Cat can be frozen with 43% success.

Key words: Electroejaculation, Semen Collection, Semen freezing, Van cat

INTRODUCTION

There is a need for the studies of scientists in terms of the continuation of genealogy and the protection of purity of Van Cats. With these scientific studies, both the genealogy of Van Cats will be taken under protection and some problems which occur in breeding may be understood through understanding their physiological properties and therefore, the steps which will have been taken in this field may get result. Along with this study, it was aimed to guarantee the future of a living culture creature by collecting, freezing and thawing semen from Van Cats.

Freezing and storing the semen is a significant technique in terms of the preservation of gamete of species especially for the protection of animals in danger of extinction (Tsustsui *et al*., 2003). In the protection of breeds of Van Cats, the prevention of degeneration of pure genetic structures and storing the genetic material by freezing constitute a significant stage. Based on that, it was aimed to collect and freeze semen from Van Cats by electro-ejaculation method in this study. The semen was collected from the cats under anesthesia with electro-ejaculation method and diluted with 3 diluents and then frozen after necessary assessments were made.

MATERIALS AND METHODS

Animal material

Seven 7 healthy male Van Cats aged between 3-6 years and weighted at 2.5-6 kg in Van Cat Research Center Cat House were used as the research material in the study. The cats were fed with industrial cat food and fresh water at the amounts that they may drink at any moment was kept in front of them. The cats to which application was made were not used in the mating throughout the study.

Tools and materials used

The electro-ejaculator device used in the study was specially designed for the cats and consisted of a rectal roble with 3 electrodes which had 1 cm thickness and 12 cm length, voltmeter and amperemeter. The device had an adjusting knob for adjusting the electric dose to be given

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with the breaker which had been protecting the cat and operator against the electrical current.

Anesthesia of cats

The cats were given 0.3 ml ketamine hydrochloride (Ketalar, Eczacıbaşı, Istanbul, Turkey) and 0.3 ml xylazin hydrochloride (Rompun, Bayer, Istanbul, Turkey) as the study material and general anesthesia was administered within desired period (Table 1) (Samsar, 1977).

Collection of semen from cat with electro-ejaculator

The electro-ejaculator was applied after the cat was taken under anesthesia securely (Platz and Seager, 1978; Axnér and Linde-Forsberg, 2002; Daşkın, 2002; Zambelli and Cunto, 2006). The cats to which anesthesia was applied were laid on the table at which semen would be taken at the lateral position. The long hair at the penis and anus area of cat was cut and the rectum and preputium areas were carefully cleaned. In conformity with the literature data (Platz and Seager, 1978; Axnér and Linde-Forsberg, 2002; Daşkın, 2002), the rectal probe was lubricated and inserted approximately 7-8 cm into the rectum. When difficulties were met during the application, the probe was pushed forward the ventral or dorsal. When there was possibility of feces contamination, the probe was taken out and inserted again after it was cleaned and lubricated (Platz and Seager, 1978; Daşkın, 2002). Then, the eppendorf tube was placed on the penis. The gentle pressures were cranially directed to expose the penis and electrical stimulations were started to be given with the stimulation button. The semen was taken to a pre-heated semen collection receptacle which was placed on the glans penis (Axnér and Linde-Forsberg, 2002).

The voltage application was started with 0 V and the voltage was increased until the hind legs were released. After staying for 2 sec. at maximum level, the voltage was dropped to 0 V in 1 sec. After waiting for two sec., the stimulation voltage was increased again in 3 sec. until the paws of hind legs were shown. After staying for 2 sec. at maximum level, the voltage was dropped to 0 V in 1 sec. again. The electrical stimulations were continued in this way. The lowest voltage value which created contractions at the hind legs of cat even if just a drop was 2 V.

The voltage which was applied in the study varied between 2-8 V. In the limited number of cases at which 8 V was reached, urine mixed to the semen. While the frequency and current were constant at the stimulations, the voltage and number of stimulations varied. The collection of semen from cat with the electro-ejaculation method is given in Figure 1.

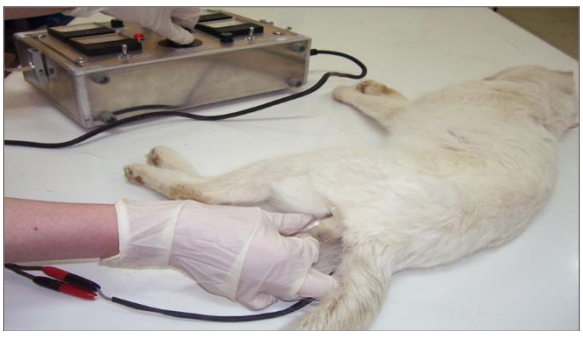

Fig. 1: Sperm retrieval by electro-ejaculation method.

Evaluation of semen

The macroscopic and microscopic examinations of semen were made. In the macroscopic examination, the semen amount and semen color were evaluated. In the microscopic examination, the semen motility, semen concentration, dead semen and abnormal semen ratios were examined.

Semen volume was determined by adjustable automatic pipette (10-1000 µl) and the value was recorded as µl. Motility was estimated by a hot plate phase-contrast microscope at x200 magnification by viewing at least 3 fields and estimating the percentage % rate of processing spermatozoa. Spermatozoon concentration was calculated by the hemocytometric method and was recorded as $x10^6$/ml. To assess the number of abnormal sperm and dead sperm, eosin-nigrosine staining was used. In a light microscope, 300 sperm cells were evaluated at x400 magnifications. When the dead sperm was evaluated, the sperm that took the head part were taken into account.

Dilution and freezing of semen

In this study, semen was collected from each cat at total 10 times as once in a week. 3 pellets were frozen for each semen collected. Therefore, total 210 pellets were frozen throughout the study.

Biofos, 3% Equiex and 6% Equiex diluents at which glycerol was kept ready were left in capped Eppendorf tubes 100 µl with automatic pipettes the day before the semen was collected and they were kept at +4°C until the study was conducted. Then, the semen which was collected with the electro-ejaculation method were divided into 3 equal parts and diluted with 3 diluents at +26°C. The semen which was pre-diluted was drawn to 0.25 ml pellets (Kruuse 340670) after the glycerolisation procedure and left to the equilibration at +4°C for 2.5 hours. The pellets taken from equilibration were aligned on the grill inside the freezing receptacle at which there was liquid nitrogen (6 cm above the liquid nitrogen surface) and exposed to the liquid nitrogen vapor for 7 min. Then, the pellets which were frozen in the liquid nitrogen vapor were plunged into the liquid nitrogen.

Statistical analyses

In order to determine the difference among all values (the post-dilution and thawing motility and vitality values of cat semen where diluted with 3% Equiex, 6% Equiex and Biosfos), χ^2 test was applied. Also, the one-way variance analysis and post-hoc Tukey-HSD test were used in order to determine the sperm properties of cats among themselves and the number and periods of stimulation applied while collecting semen from the cats. The statistical analysis of all data was made by using SPSS software package program. The values obtained were given as the average ± standard deviation (Akgül, 20003).

RESULTS

The weights and ages of cats from which semen was collected with the electro-ejaculation and the ketamine hydrochloride + xylazin hydrochloride dose given for the anesthesia and the anesthesia periods are given in Table 1. The semens at various amounts were collected from each

cat. The semen amount at the ejaculates of cats, semen motility and concentration, dead semen ratio, total abnormal semen ratio, stimulation period and number of stimulation are given in Table 2. The post-dilution and thawing semen motility and vitality values of cat semen diluted with 3% Equiex are given in Table 3; The post-dilution and thawing semen motility and vitality values of cat semen diluted with 6% Equiex are given in Table 4; The post-dilution and thawing semen motility and vitality values of cat semen diluted with Biofos are given in Table 5; The abnormal semen ratios of fresh and post-dilution cat semen are given in Table 6.

It was determined that the best vitality value of semen collected from cats after freezing and thawing was obtained from the semen diluted with 3% Equiex.

DISCUSSION

In the literature, there is no a direct information concerning to the electro-ejaculation period. In this study, the average ejaculation period was determined as 83.08±4.57 sec. within the electro-ejaculation program. While this period was very long compared to average 21 sec. ejaculation period of Platz and Seager (1978) , it was short compared to the ejaculation period (125.53±5.62 sec.) compared to the study of Kaya (2000). In the study of Kaya (2000), such a high electro-ejaculation period may be attributed to that the voltage applied with the electro-ejaculation device was maximum 3 V. Then, Kaya (2000) stated in his study that the urine mixed to the semen in the cases exceeding 3 V. Low electro-ejaculation

Table 1: The ages, weights, and administered anesthetic doses of the cat.

	Cat No	Weight (g)	Age	ketamine hydrochloride + xylazin hydrochloride (ml)	Anesthesia period (min.)
1	160629	3.52	4.5	0.3 + 0.3	55
2	135038	3.44	5.5	0.3 + 0.3	55
3	164333	3.75	3.5	0.3 + 0.3	55
4	127807	3.77	5.5	0.3 + 0.3	50
5	128714	4.50	6	0.4 + 0.4	60
6	163527	2.50	3	0.3 + 0.3	40
7	161142	6	6	0.4 + 0.4	55-60

Table 2: The amount of sperm in the cat, sperm motility and density, dead sperm rate, total abnormal sperm rate, duration of stimulation and number of stimuli.

Cat No	Semen amount (µl)	Semen motility (%)	Dead semen (%)	Semen concentration (…x10^6/ ml)	Total abnormal semen (%)	Stimulus period (sec)	Number of stimulation
1	82.90±12.81^{ab}	68.00±5.37	22.80±8.75	110.66±9.53^{c}	26.40±3.27	84.00±4.21^{ab}	10.30±0.48^{ab}
2	97.20±24.26^{ab}	65.50±3.68	27.70±11.90	139.04±22.78^{d}	24.20±7.52	84.00±4.2^{ab}	10.50±0.52^{ab}
3	91.90±18.18^{ab}	67.50±4.85	23.00±8.45	70.62±9.96^{b}	24.60±3.68	84.00±5.65^{ab}	10.50±0.70^{ab}
4	92.50±14.56^{ab}	67.50±5.40	24.90±8.25	57.36±8.50^{b}	26.70±3.30	81.60±3.37^{ab}	10.20±0.42^{ab}
5	88.70±26.70^{ab}	70.50±4.37	21.40±6.60	127.40±6.85^{d}	24.80±3.15	81.60±3.37^{ab}	10.20±0.42^{ab}
6	80.80±14.56^{a}	67.50±3.53	18.30±5.96	236.94±11.21^{e}	26.40±3.89	86.40±6.31^{b}	10.80±0.78^{b}
7	109.50±26.99^{b}	65.50±6.43	29.30±5.90	22.38±8.14^{a}	25.90±6.02	80.00±0.00^{a}	10.00±0.00^{a}
Average	91.92±21.49	67.42±4.94	23.91±8.59	109.20±66.12	25.57±4.57	83.08±4.57	10.35±0.56

There was a significant difference at the level of P<0.05 between the values marked with different letters at same column.

Table 3: The post-dilution and thawing motility of cat semen diluted with 3% Equiex and average±SEM values of vitality values and the results of χ^2 test made between these values.

				Dilution with 3% Equiex		Significance Level
	Post-dilution motility (%)	Cat No		Significance Level	Post-thawing vitality (%)	
1	65.00±4.71**	44.00±4.59**	**P<0.001	75.50±8.32**	53.50±6.26**	**P<0.001
2	61.00±4.59**	42.00±4.83**	"	70.00±12.02**	49.50±6.85**	"
3	64.50±5.99**	44.00±6.58**	"	74.50±8.32**	53.50±6.26**	"
4	65.00±4.71**	44.50±3.69**	"	72.50±7.55**	51.50±5.80**	"
5	69.50±4.38**	50.50±4.38**	"	75.50±7.25**	55.50±4.38**	"
6	65.50±3.69**	48.00±4.83**	"	80.00±6.24**	58.00±5.37**	"
7	64.50±6.85**	47.50±5.40**	"	69.00±5.68**	53.50±4.74**	"
Average	65.00±5.38**	45.79±5.49**	"	73.86±8.52**	53.57±6.03**	"

Table 4: The post-dilution and thawing motility of cat semen diluted with 6% Equiex after dilution and thawing and average±SEM values of vitality values and the results of χ^2 test made between these values.

Cat No	Dilution with 6% Equiex		Significance Level	Dilution with 6% Equiex		Significance Level
	Post-dilution motility (%)	Post-thawing motility (%)		Post-dilution motility (%)	Post-thawing motility (%)	
1	60.00±4.71**	40.50±5.50**	**P<0.001	75.50±8.31**	53.00±6.32**	**P<0.001
2	58.50±3.37**	38.00±4.83**	"	70.00±12.01**	49.50±6.85**	"
3	62.00±5.86**	41.50±6.68**	"	74.50±8.31**	52.00±6.32**	"
4	62.00±5.37**	42.50±4.24**	"	72.50±7.54**	50.50±5.98**	"
5	64.50±4.37**	45.50±4.37**	"	75.50±7.24**	55.00±5.27**	"
6	60.50±3.68**	43.00±4.83**	"	80.00±6.23**	58.00±5.37**	"
7	59.50±6.85**	41.50±7.47**	"	68.50±6.25**	50.50±5.50**	"
Ort	61.00±5.14**	41.78±5.71**	"	73.78±8.61**	52.64±6.35**	"

Table 5: The post-dilution and thawing motility of cat semen diluted with Biosfos after dilution and thawing and average±SEM values of vitality values and the results of χ^2 test made between these values.

Cat No	Dilution with Biosfos		Significance Level	Dilution with Biosfos		Significance level
	Post-dilution motility (%)	Post-thawing motility (%)		Post-dilution motility (%)	Post-thawing motility (%)	
1	59.00±5.16**	38.50±5.29**	**P<0.001	75.50±8.31**	50.50±6.43**	**P<0.001
2	56.00±4.59**	37.00±4.83**	"	70.00±12.01**	48.50±6.68**	"
3	59.00±4.59**	39.50±6.43**	"	74.00±9.36**	51.50±5.79**	"
4	60.00±4.71**	41.00±4.59**	"	72.50±7.54**	50.50±5.98**	"
5	63.50±4.74**	45.00±4.71**	"	75.50±7.24**	55.00±5.27**	"
6	60.00±3.33**	40.50±2.83**	"	80.00±6.23**	57.00±6.74**	"
7	58.50±6.68**	38.50±6.25**	"	68.50±6.25**	47.50±6.34**	"
Average	59.42±5.14**	40.00±5.45**	"	73.71±8.75**	51.50±6.72**	"

Table 6: Average±SEM values of abnormal semen ratios at fresh semen and post-thawing semen of cats and the results of χ^2 test made between these values.

Cat No	Abnormal semen ratios at fresh semen (%)			Abnormal semen ratios at post-thawing semen (%)		
	Head (%)	Middle piece (%)	Tail (%)	Head (%)	Middle piece (%)	Tail (%)
1	1.90±0.73**	7.90±1.19***	16.60±2.98	3.70±1.05**	11.80±2.61***	23.00±4.29
2	2.30±0.94**	7.10±2.60***	14.80±4.58	4.10±0.99**	15.60±3.34***	23.50±2.27
3	2.00±0.81**	8.20±2.39***	14.40±2.27	3.50±0.97**	13.90±3.78***	23.00±3.91
4	2.10±0.73**	9.40±1.34***	15.20±2.09	3.80±0.91**	14.30±1.82***	22.50±3.37
5	2.20±0.78**	8.60±1.26***	14.00±2.44	3.30±0.48**	13.30±1.56***	19.80±3.15
6	2.10±0.73**	8.60±1.89***	15.70±2.49	3.80±0.78**	12.50±0.97***	19.10±4.58
7	1.90±0.73**	7.20±1.13***	16.80±5.39	4.00±1.05**	14.00±2.00***	22.50±4.88
Average	2.07±0.76	8.14±1.86	15.35±3.39	3.74±0.91	13.62±2.63	21.91±4.03

**A significant difference at the level of P<0.001 was determined between the abnormal semen ratios at fresh and post-thawing semen dependent on the head. ***A significant difference at the level of P<0.001 was determined between the abnormal semen ratios at fresh and post-thawing semen dependent on the middle piece.

period in the study of Platz and Seager (1978) may be attributed to that the cats were different species.

It was determined that the average amount of semen collected from Van Cats with electro-ejaculation method was 91.92±21.49 µl. This amount was in conformity with the value of 100±1.0 µl that Carter *et al.* (1984) collected from the domestic cats with electro-ejaculation method.

The semen at the amount of 0.14±0.1 ml that Tebet *et al*, (2006) collected from 10 domestic cats with electro-ejaculation was 1.5 times more than the semen amount in this study. This difference may be resulted from that the researchers gave additional 10-20 stimulations in the cases that the semen amounts collected were low. As the stimulation period will extend in this case, the seminal plasma will also increase.

In this paper, it was determined that the average motility of semen collected from Van Cats was 67.42± 4.94%. The motility which was averagely 70.6% at the semen collected by Axnér *et al.* (1998) with the electro-ejaculation and the motility value which was averagely 69.7±3.1% at the semen collected by Neubauer *et al.* (2004) from the teratospermic cats showed similarity with the motility in this study.

It was determined that the average semen concentration of Van Cats was 109.20±66.12x10^6/ml. The average semen concentration notified in this study coincides with the value reported as 111.2±75.3x10^6/ml by Tebet *et al.* (2006) who collected semen with same method. Along with that the concentration value which was reported by Kaya (2000) at Ankara Cats as 184.53±26.28x10^6/ml was higher than the average semen concentration in this study, it remains within the range belonging to the individual semen concentrations notified.

As the semen concentration may vary among various semen collected from the animals from the same species and even the individuals and same individual depending on the environmental factors, breeding-feeding and the way of collecting semen, it is normal that the concentration value of this study was lower or higher than the values notified by other researchers.

It was determined that the abnormal semen ratios of semen collected from Van Cats with electro-ejaculation method depending on head, middle piece and tail were 2.07±0.76%, 8.14±1.86% and 15.35±3.39%, respectively and total abnormal semen ratio was averagely 25.57± 4.57%. This value is in conformity with the value of 26.48±1.29% determined by Kaya (2000).

Baran *et al.* (2004a) reported that the total morphological disorder at cats varied between 15.88± 5.41% and19.50±5.93% and again Baran *et al.* (2004b) reported in another study that total morphological disorder was 8.40±4.79%. Total morphological disorder value notified in both studies was slightly lower than the value submitted in this study. This case may be attributed to that cats from different species were used in the studies or the morphology was determined with different methods.

The dead semen ratio determined at semen collected from Van Cats was averagely 23.91±8.59% and this value shows parallelism with the value notified by Kaya (2000) as 20.80±1.30%.

Cocchia *et al.* (2009) determined the alive semen ratio at epidydimal semen as 74.3±8.6% and Siemieniuch and Dubiel (2007) determined the same value at epidydimal semen as 84.9±7.8%. With reference to these values, 36% dead semen ratio was higher than the value determined in this study and 16% dead semen ratio was lower than the value determined in this study. This case may be resulted from different species of cats and semen on which evaluation was made (epidydimal, ejaculated).

In this study, the semen collected with electro-ejaculation were diluted with 3 diluents (3% Equiex, 6% Equiex and Biofos) and their motility and vitality ratios were examined. It was determined that the best motility was obtained from the semen diluted with 3% Equiex among three diluents. The vitality ratios of Van Cat's semen diluted with three diluents were almost same and 73%.

When the post-thawing motility values of semen collected from Van Cats were generally reviewed, the diluent from which highest motility was obtained after thawing was 3% Equiex. In this study, it was seen that the motility value which was 67.42±4.94% during the semen collection was 40-45% after the thawing. Therefore, it may be said that the semen may be frozen with 40-45% success.

Platz *et al.* (1978) determined the post-thawing motility as 53.8±4.8%. While the motility notified by the researchers was higher than the value determined in this study, the value notified by Kaya (2000) as 35% was lower than the value in this study.

Baran *et al.* (2004a) determined the post-thawing average motility of semen collected with the electro-ejaculation method as 53.00±10.85% and 50.50±13.90%. The motility values recorded by the researchers were higher than the motility in this study. This difference may be resulted from the semen diluents that the researchers used.

48% dead semen ratio obtained from the semen collected from Van Cats after freezing and thawing coincides with 48.94±3.90% dead semen ratio determined by Kaya (2000) at Ankara Cats after thawing.

The alive semen ratio after freezing and thawing the epidydimal semen determined by Siemieniuch and Dubiel (2007) as 62±6.9%; Cocchia *et al.* (2009) determined this ratio as 45.2±9.4%. With reference to these values, it may be attributed to different species of cats, different semen diluents and different freezing methods that the dead semen ratios were not in conformity with the ratio in this study.

While 38% total abnormal semen ratio obtained from the semen collected from Van Cats after freezing and thawing coincides with the values that Kaya (2000) determined as 38% and Baran *et al.* (2004a) determined as 40%, it was lower than the value notified by Cocchia *et al.* (2009) as 52.8±31.5%. This case may be attributed to the difference at the semen evaluated (epidydimal, ejaculated).

In conclusion, this study is the first study in terms of both determining the spermatologic properties of Van Cats and freezing the semen of Van Cat. With this study, approximately 43% motility obtained by freezing and thawing the semen collected from Van Cat with electro-ejaculation method indicates that the semen of Van Cat may be frozen with 43% success.

In the future researchers, the continuation of existence of Van Cat as a pure race and the increase of their number may be possible along with the success provided that artificial insemination is made with frozen semen of Van Cat.

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