Artificial Insemination with Frozen Epididymal Sperm in Cats

Toshihiko TSUTSUI¹⁾, Miho WADA¹⁾, Mizuho ANZAI¹⁾ and Tatsuya HORI¹⁾

¹⁾Department of Reproduction, Nippon Veterinary and Animal Science University, 7-1 Kyonan-cho 1 chome, Musashino-shi, Tokyo 180-8602, Japan

(Received 12 February 2002/Accepted 3 December 2002)

ABSTRACT. Artificial insemination with frozen cauda epididymal sperm was performed in cats. Sperm were transmigrated from the epididymides in 10 male cats. The mean sperm motility and viability were 67% and 82.5%, respectively, and 11.6×10^7 sperm were recovered. The mean sperm motility after thawing was 24.0%. Eleven female cats received unilateral intrauterine insemination of 5×10^7 sperm, and the conception rate was 27.3% (3/11). This was the first case of conception obtained with frozen epididymal sperm in cats.

KEY WORDS: artificial insemination, epididymal sperm, feline.

J. Vet. Med. Sci. 65(3): 397–399, 2003

It has been demonstrated in many species that epididymal sperm have fertility in *in vitro* fertilization and artificial insemination (AI) [4, 8, 11–15, 25], and fertilization with frozen epididymal sperm was confirmed in pigs [11] and dogs [9].

Freeze-storage of epididymal sperm is an important technique for gamete preservation of species, particularly for preservation of animals becoming extinct. As wild animals in the cat family are becoming extinct, we selected cats as an animal model.

We have performed studies of reproductive techniques such as *in vitro* fertilization [5, 6], embryo transfer [6, 24], and AI [18, 20–23] in cats.

With regard to feline epididymal sperm, studies of the qualities [1,2], state of fertility acquisition [2,3,12], and freeze-storage method [4,8,17] are progressing, and fertility of fresh epididymal sperm [12] and frozen semen [4,8] has been shown, but no *in vivo* study with frozen epididymal sperm has been performed. With frozen feline ejaculated semen, Platz *et al.* [16] and we [23] have succeeded in obtaining newborns by intravaginal and intrauterine insemination, respectively.

Therefore, we froze epididymal sperm by the same method as for freezing ejaculated sperm and investigated the possibility of inducing conception by intrauterine insemination. It has been shown that frozen semen supplemented with Orvus ES Paste (OEP, Nova Chemical Sales Scituate, Inc., MA, U.S.A.) has superior properties after thawing semen without OEP in dogs [9] and pigs [7]. In this way, the usefulness of OEP was also investigated in this study.

Animals: Ten male crossbred cats aged 1.0–3.3 years (mean: 1.9 ± 0.3 , SE) were used. For AI, 11 female cats aged 2–4 years were used. The cats were kept in groups in an animal room measuring 4×7 m with temperature controlled to $23 \pm 2^\circ\text{C}$. The animals were given commercial dry cat food (Hill's Feline Maintenance Dry, U.S.A.).

Transmigration of sperm from epididymis: Surgically excised testis and epididymis were weighed. The epididymis was cut at the corpus into two equal parts. Each part was minced in 1 ml of egg yolk Tris-fructose citrate solution

(EYT-FC) [23] to transmigrate the sperm. The solution was filtered through a stainless steel mesh (80 μm) and sperm were recovered. The time from excision of the epididymis to transmigration of the sperm was within 1 hr.

Sperm quality test and freezing: The recovered sperm were examined by the general semen quality test [18]. The motility, viability, abnormality and number of sperm were measured. The abnormality of sperm was examined only in the cauda epididymis. As for frozen sperm, sperm recovered from the caudal part were frozen by the method we previously reported for ejaculated sperm [23]. The glycerin concentration was 7%, and 2.5×10^7 sperm filled a 250 μl straw. Sperm from 6 animals were supplemented with 1% OEP. When there were no major differences in sperm quality between the right and left epididymides, sperm from bilateral epididymides were combined and frozen.

Artificial insemination: Ovulation was induced by subcutaneous injection of 100 IU hCG on the third or fourth day of estrus [23]. Semen was inseminated in a unilateral uterine horn with more mature ovarian follicles 20 hr after hCG administration before ovulation [23]. Two semen straws (5×10^7 sperm) were thawed in warm water at 37°C for 30 sec. After the semen quality test, semen were centrifuged at 2,000 rpm for 5 min and about 40 μl at the tube bottom was used for AI. Immediately after insemination, 100 IU hCG was additionally injected intravenously to ensure ovulation.

Determination of pregnancy: Animals were examined with an ultrasonographic diagnostic system (ECHOVISION SSD-500 EV 7.5 MHz, Aloka Co., Japan) 15–20 days after AI. In animals determined to be non-pregnant, the blood progesterone (P_4) level was measured 20 days after insemination to determine the success or failure of ovulation. When the blood P_4 level was 3 ng/ml or higher, it was determined that ovulation was induced. The blood P_4 level was measured by EIA [10].

Statistical analysis: Data obtained in this study were analyzed by Student's *t*-test and a significance level lower than 5% was regarded as significant.

Weights of the testes and epididymides and the qualities of sperm recovered from the epididymides are shown in

Table 1. Qualities of feline epididymal sperm

Tom number	Age (year)	BW (kg)	Testis (mg)		Epididymis (mg)		Sperm quality												Number of sperm (× 10 ⁷)			
							Sperm motility (%)				Sperm viability (%)				Sperm abnormality (%)							
			Left	Right	Left	Right	Left		Right		Left		Right		Left	Right	Left		Right			
							Head ^(a)	Tail ^(b)	Head	Tail	Head	Tail	Head	Tail			Head	Tail	Head	Tail	Head	Tail
33	3.3	5.1	2009	2300	543	582	5	40	10	50	56.2	78.7	67.3	78.6	3.4	2.5	3.6	4.7	4.7	5.0		
42	2.0	3.2	1544	1325	230	323	10	70	30	70	52.4	71.2	58.0	72.4	3.6	4.9	0.6	5.3	1.1	4.7		
M1	1.0	4.5	1430	1351	394	233	20	70	10	75	91.9	95.3	81.4	88.9	4.8	3.0	0.1	3.9	0.1	10.6		
M2	1.2	3.8	1335	1202	272	215	30	40	30	40	68.3	79.2	60.4	69.9	3.5	2.5	0.1	5.4	0.5	7.0		
48	2.8	4.4	1290	1366	522	540	20	75	10	80	66.7	79.9	64.7	86.2	5.6	2.1	0.5	6.5	0.8	6.8		
49	2.2	4.1	1048	1087	652	511	10	75	10	80	75.7	93.2	92.0	81.1	6.6	7.8	0.6	2.6	1.1	7.6		
50	1.7	4.3	1300	962	680	515	5	75	5	75	79.3	78.5	74.3	78.5	5.9	8.0	0.6	3.6	2.2	2.1		
51	1.1	4.2	1654	1379	373	398	40	65	50	60	74.3	82.0	86.1	81.5	7.2	5.0	2.2	9.0	1.1	10.0		
53	2.6	3.5	1290	1523	451	354	30	80	30	75	88.3	81.1	92.5	93.3	5.1	4.3	0.2	5.5	0.8	5.0		
M3	1.0	3.9	1253	1210	514	499	5	75	5	65	77.0	85.5	92.5	94.3	2.3	1.2	10.0	11.3	6.2	4.7		
Mean	1.9	4.1	1415.3	1370.5	463.1	417.0	17.5	66.5	19.0	67.0	73.0	82.5	76.9	80.9	4.8	4.1	1.9	5.8	1.9	6.4		
±SE	±0.3	±0.2	±88.8	±121.3	±49.6	±43.8	±4.2	±4.8	±5.0	±4.5	±4.2	±2.5	±4.6	±2.6	±0.5	±0.8	±1.0	±0.9	±0.7	±0.9		

a) Head of epididymis b) Tail of epididymis

Table 2. Conception rate when using frozen epididymal sperm

Queen number	Sperm quality (%)				Number of follicles		Number of kits	Conception rate
	Tom number	Storage (month)	Sperm motility	Sperm viability	Left	Right		
129	33	11	20	39.2	3 ^{b)}	2	5	
130	42	11	25	41.1	3 ^{b)}	3	0	
131	M1	4	15	51.3	4 ^{b)}	1	0	
117	M2	12	10	51.5	1	3 ^{b)}	0	
141	48 ^{a)}	5	50	72.5	2 ^{b)}	2	0	
D29	49 ^{a)}	4	15	40.7	3 ^{b)}	1	1 (Abortion) ^{c)}	3/11
D28	50 ^{a)}	5	40	72.7	3 ^{b)}	2	0	(27.3)
170	51 ^{a)}	1	15	52.9	2	3 ^{b)}	0	
D30	51 ^{a)}	1	15	52.9	2	2 ^{b)}	1	
D55	53 ^{a)}	1	15	38.9	3 ^{b)}	2	0	
D33	M3 ^{a)}	8	35	67.9	4 ^{b)}	1	0	
Mean ± SE		5.7 ± 1.3	24.0 ± 4.4	52.9 ± 4.6	2.7 ± 0.2	2.0 ± 0.2		
					4.7 ± 0.2			

a) The sperm motility and viability in the group supplemented with 1% OEP and the group without OEP were: 28.3 $\pm$ 5.8% and 57.6 $\pm$ 5.9%; and 17.5 $\pm$ 3.7% and 45.8 $\pm$ 3.8%, respectively.

b) Artificially inseminated side.

c) Abortion occurred on day 30 of gestation.

Table 1. Sperm abnormality is presented as total abnormality.

The motility of sperm from the caput epididymis varied widely among animals. Cauda epididymal sperm motility was 40–80% (mean: 65% or higher), which was significantly higher than that of the caput epididymal sperm ($P < 0.01$). The mean viability of sperm was 73% or higher in the caput part, but it was significantly higher in the caudal part ($P < 0.01$). There were no significant differences in sperm quality between the right and left epididymides in each animal.

The number of recovered sperm varied widely between the left and right epididymides in some animals. The mean number of sperm recovered from the caput epididymis was

1.9×10^7 in the left and right, respectively. The mean number of sperm recovered from the cauda epididymis was 5.8×10^7 and 6.4×10^7 in the left and right, respectively, showing a significantly higher number in the caudal part ($P < 0.01$).

Semen quality after thawing and conception: Since there were no significant differences in sperm quality between the bilateral cauda epididymides, sperm from the bilateral cauda epididymides were combined and frozen. Sperm quality after thawing and conception results is shown in Table 2. Sperm was frozen for 1–12 months (mean: 5.7 ± 1.3 months).

The sperm motility was 10–50% (mean: $24.0 \pm 4.4\%$) and the mean viability was $52.9 \pm 4.6\%$. Sperm quality in EYT-FC with or without OEP supplementation was not signifi-

cantly different.

The conception rate obtained by the AI was 27.3% (3/11). Among the fertilized animals, two animals delivered 5 and one kittens on 64 days and 59 days of gestation, respectively, but the other one aborted on 30 days of gestation. In eight non-fertilized animals, ovulation was induced based on the blood P₄ level.

This is the first case of obtaining newborns by using frozen epididymal sperm. Nevertheless, the conception rate was lower than that obtained with ejaculated sperm, even though the sperm motility after thawing was similar to that of ejaculated sperm reported previously [23] and the number of inseminated sperm was the same. To improve the conception rate, increasing the number of sperm (5×10^7) may be one method. Sperm will be inseminated in the bilateral uterine horns in consideration of the number of recovered sperm, but the numbers of mature ovarian follicles and newborns were the same in queen No. 129 despite the fact that sperm were inseminated in unilateral uterine horn. Although this phenomenon was observed in a few animals after unilateral intrauterine insemination of fresh semen in our previous study [23], this was the first observation of the use of frozen semen. If the reason for this phenomenon is clarified, the number of newborns obtained by unilateral intrauterine insemination will be increased. In our dog study with fresh semen, this problem was solved by increasing the number of sperm inseminated in a unilateral uterine horn [20], but it has not been applied to cats. Among the qualities of feline cauda epididymal sperm, the number of recovered sperm was higher in our study than in the study reported by Goodrowe and Hay [2], but sperm motility was similar. The frequency of immature sperm could not be compared because it was not measured because some sperm may physically lose cytoplasmic droplets during the transmigration method.

Although Axner *et al.* [1] reported epididymal sperm quality in detail, comparison of equally divided parts of the epididymis was not possible. The cut region may be the site at which sperm acquire motility, as reported by Axner *et al.*, and this may have been the cause of the large variation in the motility of caput epididymal sperm among animals.

The qualities of frozen epididymal sperm supplemented with OEP were slightly better than those of sperm without OEP, but the number of cases was small and significance of differences was not clear. It is necessary to perform further insemination studies, and to clarify the state of sperm acrosomes in OEP-supplemented sperm.

REFERENCES

1. Axner, E., Linde-Forsberg, C. and Einarsson, S. 1999. *Theriogenology* **52**: 767–778.
2. Goodrowe, K.L. and Hay, M. 1993. *Theriogenology* **40**: 967–975.
3. Hay, M.A. and Goodrowe, K.L. 1993. *J. Reprod. Fertil. (Suppl.)* **47**: 297–305.
4. Jewgenow, K., Penfold, L.M., Meyer, H.H.D. and Wildt, D.E. 1998. *J. Reprod. Fertil.* **112**: 39–47.
5. Kanda, M., Miyazaki, T., Kanda, M., Nakao, H. and Tsutsui, T. 1998. *J. Vet. Med. Sci.* **60**: 423–431.
6. Kanda, M., Oikawa, H., Nakao, H. and Tsutsui, T. 1995. *J. Vet. Med. Sci.* **57**: 641–646.
7. Kato, S., Miyano, T., Nanjo, I., Yasui, T. and Kanda, S. 1990. *Jpn. J. Anim. Reprod.* **36**: 26–30.
8. Lengwinat, T. and Blottner, S. 1994. *Anim. Reprod. Sci.* **35**: 291–301.
9. Marks, S.L., Dupuis, J., Mickelsen, W.D., Memon, M.A. and Platz, C.C.Jr. 1994. *J. Am. Vet. Med. Assoc.* **204**: 1639–1640.
10. Munro, C. and Stabenfeldt, G.H. 1983. *J. Endocrinol.* **101**: 41–49.
11. Nagai, T., Takahashi, T., Masuda, H., Shioya, Y., Kuwazama, M., Fukushima, M., Iwasaki, S. and Hamada, A. 1988. *J. Reprod. Fertil.* **84**: 585–591.
12. Niwa, K., Ohara, K., Hosoi, Y. and Iritani, A. 1985. *J. Reprod. Fertil.* **74**: 657–660.
13. Orgebin-Crist, M.C. and Fournier-Delpech, S. 1982. *J. Androl.* **3**: 429–433.
14. Ogawa, S., Satoh, K., Hamada, M. and Hishimoto, H. 1972. *Nature (Lond.)* **238**: 270–271.
15. Pavlok, A. 1974. *J. Reprod. Fertil.* **36**: 203–205.
16. Platz, C.C., Wildt, D.E. and Seager, S.W.J. 1978. *J. Reprod. Fertil.* **52**: 279–282.
17. Schafer, S. and Holzmänn, A. 2000. *Anim. Reprod. Sci.* **59**: 201–211.
18. Tanaka, A., Takagi, Y., Nakagawa, K., Fujimoto, Y., Hori, T. and Tsutsui, T. 2000. *J. Vet. Med. Sci.* **62**: 1163–1167.
19. Tsutsui, T., Hase, M., Hori, T., Ito, T. and Kawakami, E. 2000. *J. Vet. Med. Sci.* **62**: 533–535.
20. Tsutsui, T., Shimizu, T., Ohara, N., Shiba, Y., Hironaka, T., Orima, H. and Ogasa, A. 1989. *Jpn. J. Vet. Sci.* **51**: 257–263.
21. Tsutsui, T., Tanaka, T. and Hori, T. 2001. *J. Reprod. Fertil. (Suppl.)* **57**: 347–351.
22. Tsutsui, T., Tanaka, A., Takagi, Y., Nakagawa, K., Fujimoto, Y., Murai, M., Anzai, M. and Hori, T. 2000. *J. Vet. Med. Sci.* **62**: 1241–1245.
23. Tsutsui, T., Tanaka, A., Takagi, Y., Nakagawa, K., Fujimoto, Y., Murai, M., Anzai, M. and Hori, T. 2000. *J. Vet. Med. Sci.* **62**: 1247–1251.
24. Tsutsui, T., Yamane, I., Hattori, I., Kurosawa, N., Matsunaga, H., Murao, I., Kanada, M. and Hori, T. 2000. *J. Vet. Med. Sci.* **62**: 1169–1175.
25. Williams, R.M., Graham, J.K. and Hammerstedt, R.H. 1991. *Biol. Reprod.* **44**: 1080–1091.