

Effect of Ejaculation Intervals on Semen Quality in Cats

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ABSTRACT. The relationship between ejaculation intervals and semen quality in 4 male cats aged 3–5 years was investigated in this study. Semen was collected 10 times at intervals of every day, every other day, and every three days using an artificial vagina. Semen was collected consecutively twice on the day of semen collection, and the semen quality was examined. In semen collected every day, the number of sperm in the first collection decreased, and the frequency of immature sperm rapidly increased after the 4th day. In semen collected every other day and every three days, although the semen volume markedly varied among the animals on both first and second collections, the volume remained stable for each animal, the number of sperm was similar in the first and second collections, but was clearly larger in the first collection ($p < 0.01$). Sperm motility and abnormality were stable among the various intervals and between the first and second collections in each animal.

KEY WORDS: artificial vagina, ejaculation interval, feline, semen quality.

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Feline semen used in reports of the semen quality has generally been collected by electro-ejaculation (EJ) [1, 3, 4, 6–9, 11–14]. However, EJ is very stressful for animals because it is performed under general anesthesia, and frequent application is difficult [2]. Only Sojka *et al.* [15], Platz *et al.* [14], and Dooley and Pineda [3] and Dooley *et al.* [4] have reported the quality of semen collected using an artificial vagina (AV), some of their reports have compared semen quality between the two collection methods [3, 4, 14]. According to these reports, there are problems related to decreased numbers of sperm, increased semen volume, and contamination by urine in semen collected by EJ compared to those of semen collected using an AV. Because of these problems, semen collection by the AV method is necessary to observe spermatogenic activity in cats. Although the number of animals was only two, Sojka *et al.* [15] reported on ejaculation intervals and semen quality in cats. According to their report [15], the semen quality was not changed by a few times of ejaculation per week, but the number of sperm decreased by daily ejaculation. However, their report did not draw conclusions regarding the feline spermatogenic activity because of the small number of cats and experimental cases used in the experiment. Therefore, we decided to clarify the relationship between the ejaculation intervals and semen quality of semen collected using an AV. We performed this study with the consideration that generally, cats mate multiple times.

MATERIALS AND METHODS

Animals: Male cats bred and maintained in our colony were used in this experiment. Four 3- to 5-year-old cats weighing 3.5–4.4 kg were used. These cats had copulation capability and fertility. The animals were maintained in a room in which the temperature was adjusted to $23 \pm 2^\circ\text{C}$, and

were individually kept in cages measuring $60 \times 90 \times 120$ (h) cm. The animals were given commercial dry food (Hill's Feline Maintenance, U.S.A.) and water *ad libitum*. The female estrous cats maintained in our colony were appropriately used for semen collection.

Semen collection: The AV used in this study was prepared referring to the report by Sojka *et al.* [15]. As shown in Fig. 1, a 1.5 ml plastic sample tube (Fisher Co., Ltd., U.S.A.) and a 1.5 ml silicon gum pipette bulb (Fisher Co., Ltd., U.S.A.) were combined and fixed with parafilm (Can Co., Ltd., U.S.A.). The cover for warming the AV used by Sojka *et al.* [15] was not set in this study.

In semen collections, while a male cat mounted an estrous female cat and was thrusting, the penis was advanced into the AV held between the forefinger and middle finger of the person collecting semen, and ejaculation was induced (first collection). The AV was exchanged for a new one, and collection was repeated after 5–10 min (second collection).

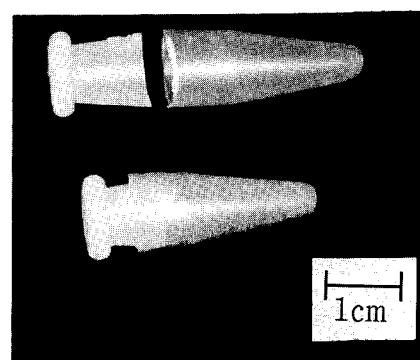


Fig. 1. The artificial vagina used in this study. The upper photograph shows a pipette bulb and sample tube, and the lower photograph shows the device fixed with parafilm.

Semen was collected twice consecutively as described above, and the quality of each semen specimen was examined. To avoid drying, 100 μ l of egg yolk Tris-fructose citrate solution was added to the semen immediately after collection. The experiments were performed between March and July.

Intervals between semen collections: The intervals between semen collections for observation of semen quality were one day (every day), two days (every other day), and three days (every three days). The experiment proceeded in the order of one-day, three-day, and two-day intervals, and a one-month or longer resting period was set between the experimental periods. Furthermore, as a preliminary study, semen was collected three times with three-day intervals before the experiment, and the experiment was initiated three days after the last semen collection. Semen was consecutively collected twice a day on 10 days with various intervals (2 times per day \times 10 days = 20 times in total), and the quality of each semen sample was examined. Accordingly, the durations of the experimental periods were 10 days with every day semen collection, 19 days with every other day semen collection, and 28 days with every three days semen collection. When any abnormal semen quality was observed during the experiment, the experiment was discontinued.

Examination of semen quality: The semen volume was measured using a sample tube with 10 μ l graduations. Sperm motility was represented by % ratio of sperm actively moving forward using a test plate for semen property examination (FA225, Fujihira Industry, Co., Ltd., Japan). The number of sperm, sperm viability, sperm abnormality, and frequency of immature sperm were examined as previously reported [10]. The significance of semen quality was analyzed by Student's *t*-test after analyzing the homogeneity of variance. A *p* value less than 0.05 was regarded as significant.

RESULTS

Semen was consecutively collected twice a day with various intervals from 4 experimental cats. In the semen collected every day, the frequency of immature sperm increased with the progression of the experiment, and the experiment was stopped after the 6th day. In the other experimental periods, there were no abnormalities of the semen quality in either the first or second semen collection. Sexual libido of the male cats did not change during the study period.

Semen volume: The quality of semen in the first and second collection from each animal with each interval is shown in Tables 1–3. The changes in semen volume in the first and second collection with various intervals are shown in Fig. 2. In semen collection every other day, the volume in the first and second collection showed almost the same changes but they were large, within a range from 45 to 80 μ l (Table 3, Fig. 2). Therefore, the changes in the semen volume were almost the same in the first and second collections at all the various intervals.

The number of sperm: Changes in the number of sperm in the semen collected at various intervals are shown in Fig. 3.

The number of sperm in the first collection largely varied within a range of $25\text{--}55 \times 10^6$ in all experimental periods (Fig. 3). In the second collection, the number of sperm changed within a range of $20\text{--}40 \times 10^6$ in semen collected every other day and every three days, but the number of sperm in the semen collected every day clearly decreased compared to those in the other two groups ($p < 0.01$) (Tables 1–3, Fig. 3). The number of sperm clearly decreased in the second collection compared to that in the first collection in all experimental periods ($p < 0.01$). There was no difference in the number of sperm in the first collection between three-day intervals and two-day intervals, nor was there a difference in the second collection between the same two groups. However, in only T36, the numbers of sperm were almost the same in the first and second semen collections every other

Table 1. The quality of cat semen collected every day

Tom No.	Age (years)		Vol. of semen (μ l)		No. of sperm ($\times 10^6$)		Sperm motility (%)		Sperm viability (%)		Sperm abnormality (%)		Immature sperm (%)	
			1st*	2nd*	1	2	1	2	1	2	1	2	1	2
T32	5	Mean	41.7	38.3	70.5	14.8	88.3	77.5	92.7	88.1	4.7	3.7	18.2	11.1
		SE	5.2	4.2	16.4	2.2	2.3	4.6	0.7	2.1	1.1	1.2	3.3	3.0
T44	4		70.8	84.2	25.2	22.8	90.8	85.0	95.5	92.9	5.0	3.3	8.1	10.3
			10.2	7.5	6.6	9.1	1.7	1.4	1.0	0.7	1.2	1.3	0.8	2.5
T36	3		50.0	44.0	44.0	11.4	87.5	76.7	92.6	88.7	3.6	6.5	8.4	7.3
			6.9	6.9	8.0	3.2	1.2	3.9	1.2	3.4	0.5	2.1	2.1	2.9
T40	3		60.0	30.0	24.5	12.3	80.0	75.0	88.3	89.0	5.8	15.5	5.5	2.0
			10.2	12.6	12.0	8.6	5.3	7.1	3.1	4.3	1.8	5.6	0.3	0.9
Total***			55.6	49.1	41.1	15.3**	86.7	78.6	92.3	89.7	4.8	7.3	10.1	7.7
			7.3	13.9	12.5	3.0	2.7	4.4	1.7	1.3	0.5	3.3	3.2	2.4

* Semen was consecutively collected twice and these are data of the first and second collections.

** Significantly different from 1st collection ($p < 0.01$).

*** The values are the means of six semen collections from four experimental cats.

Table 2. The quality of cat semen collected every other day

Tom No.		Vol. of semen (μ l)		No. of sperm ($\times 10^6$)		Sperm motility (%)		Sperm viability (%)		Sperm abnormality (%)		Immature sperm (%)	
		1st	2nd	1	2	1	2	1	2	1	2	1	2
T32	Mean	49.0	40.5	50.7	35.3	89.5	84.0	92.3	90.4	8.5	9.8	5.3	5.2
	SE	6.4	3.3	9.5	5.4	7.1	1.1	0.6	1.1	1.0	1.3	1.1	1.2
T44		73.0	86.0	45.0	34.9	85.0	83.0	92.9	91.3	5.4	2.9	2.6	2.2
		7.7	5.5	5.8	4.7	1.4	2.2	0.7	0.9	0.5	0.6	0.4	0.6
T36		35.6	58.0**	38.4	39.1	77.0	84.5	87.8	92.2	11.8	7.2	3.9	2.2
		5.6	7.0	9.0	4.9	1.6	2.1	1.4	0.8	2.1	1.5	0.6	0.6
T40		61.5	52.5	39.6	32.9**	82.0	82.0	89.8	90.4	8.5	7.6	1.6	1.2
		4.7	3.8	4.4	4.1	1.8	2.6	1.3	1.2	1.0	0.9	0.4	0.3
Total*		54.8	59.3	43.4	35.6**	82.0	82.8	90.7	91.1	8.5	6.9	3.4	2.7
		9.3	11.1	3.3	1.5	2.1	0.8	1.4	0.5	1.5	1.7	0.9	1.0

* The values are the means of ten semen collections from four experimental cats.

** Significantly different from 1st collection ($p < 0.01$).

Table 3. The quality of cat semen collected every three days

Tom No.		Vol. of semen (μ l)		No. of sperm ($\times 10^6$)		Sperm motility (%)		Sperm viability (%)		Sperm abnormality (%)		Immature sperm (%)	
		1st	2nd	1	2	1	2	1	2	1	2	1	2
T32	Mean	39.5	33.5	48.7	28.9*	82.0	83.1	88.7	87.9	9.2	7.0	4.7	6.0
	SE	3.7	2.8	6.6	5.0	3.0	1.0	1.6	3.1	1.4	1.0	1.1	0.9
T44		79.0	92.0	36.3	30.2	83.5	84.0	90.0	89.6	5.7	4.4	3.6	4.5
		8.4	8.6	3.2	6.1	0.8	2.0	0.8	1.2	0.6	1.7	0.7	0.6
T36		36.7	63.1	46.5	30.5	86.0	80.5	90.5	89.4	4.9	5.2	6.0	3.9
		6.6	12.7	6.5	5.7	1.3	3.5	1.2	1.8	1.2	1.1	1.6	1.6
T40		49.5	59.0	40.0	24.8	81.5	76.9	90.2	86.3	7.4	5.8	3.3	1.8
		3.4	5.5	5.1	4.7	1.9	3.5	1.0	1.9	1.2	1.0	0.6	0.5
Total***		51.2	61.9	42.9	28.6**	83.3	81.1	89.9	88.3	6.8	5.6	4.4	4.1
		11.2	13.8	3.3	1.5	1.2	1.8	0.5	0.9	1.1	0.6	0.7	1.0

* Significantly different from 1st collection ($p < 0.05$).

** Significantly different from 1st collection ($p < 0.01$).

***The values are the means of ten semen collections from four experimental cats.

day (Table 2).

Sperm motility: No difference was observed in sperm motility among semen collected at any interval in all animals. The motility ranged from 75.0 to 90.0% (Tables 1–3).

Sperm viability: There was no difference in sperm viability among the experimental periods and animals. Viability ranged from 86.3 to 95.5% (Tables 1–3).

Sperm abnormality: There was no difference in the sperm abnormality between the first and second collections or among the intervals, and the rate was mostly 10% or below (Tables 1–3). However, high rates were detected in some animals. The mean abnormality in the first collection of semen collected every other day was $11.8 \pm 2.1\%$ in T36 (Table 2), and that in semen in the second collection collected every day was $15.5 \pm 5.6\%$ in T40 (Table 1). The abnormal sites were mostly the tail region.

Immature sperm: The frequency of immature sperm in semen collected at each interval is shown in Fig. 4.

The frequency rapidly increased on the 3rd or 4th day in semen collected every day, and reached approximately 15% on the 6th day (Fig. 4). T32 showed a particularly high frequency of immature sperm. In semen collected every other day and every three days, the frequency of immature sperm did not differ between the first and second collections, ranging from 1.2 to 8.0% (Fig. 4). The mean frequency of immature sperm in the first and second collections were $3.4 \pm 0.9\%$ and $4.4 \pm 0.7\%$ in semen collected every other day, and $2.7 \pm 1.0\%$ and $4.1 \pm 1.0\%$ in semen collected every three days, respectively (Tables 2, 3).

DISCUSSION

The semen volume and number of sperm in semen collected using an AV in cats reported by Sojka *et al.* [15], Platz *et al.* [14], Dooley and Pineda [3], and Dooley *et al.* [4] were 40μ l and 57×10^6 , 33.8μ l and 60.7×10^6 , 60μ l and $61.0 \times$

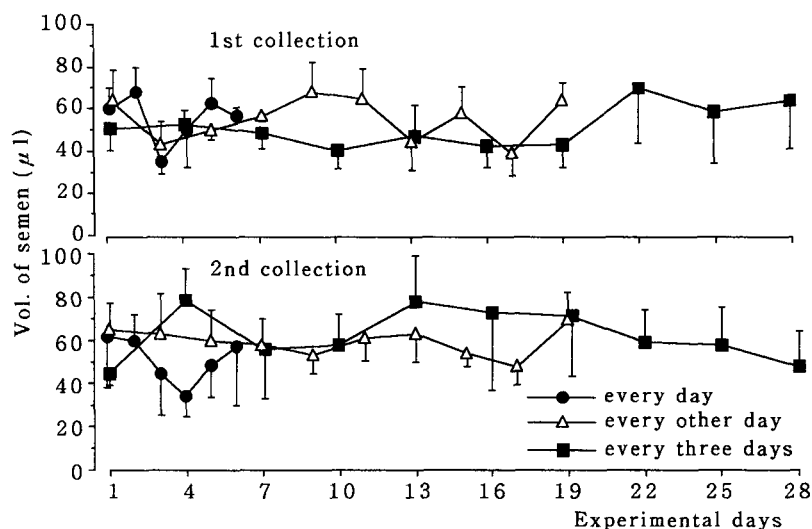


Fig. 2. Volume of semen collected at different time intervals in cats (Mean \pm SE).

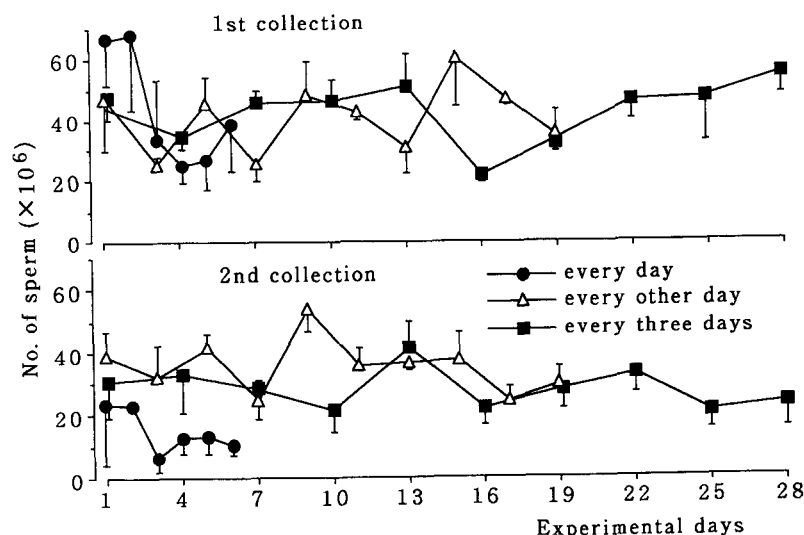


Fig. 3. Number of sperm collected at different time intervals in cats (Mean \pm SE).

10^6 , and $20 \mu\text{l}$ and 30.2×10^6 , respectively. The mean semen volume in each of the four reports showed a large variation. In our study, the semen volume in the first collection with three-day intervals ranged from 36.7 to $79.0 \mu\text{l}$, and the mean volume was $51.2 \mu\text{l}$ (Table 3). The number of sperm ranged from 36.3×10^6 to 48.7×10^6 , and the mean was 42.9×10^6 , showing that the semen volume was slightly larger than those in the previous reports, while the number of sperm tended to be lower in this study. However, the reasons for these differences are unknown.

The cats used in this study were aged 3–5 years when sperm formation is most active [6]. The ages of the cats in the other studies was not disclosed [3, 4, 14, 15], and thus, semen quality cannot be discussed. No comparison of semen

quality could be made either, because there have not been any previous reports on ejaculation intervals and semen quality in cats. The data reported by Sojka *et al.* [15] were obtained from only 2 cats from which semen was collected for 11 consecutive days. According to their report, the number of sperm decreased as the frequency of semen collection increased, but sperm motility, sperm abnormality, and sexual libido did not change. In this study, the number of sperm decreased (Fig. 3) and the frequency of immature sperm increased (Fig. 4) with semen collection twice a day for 6 consecutive days. However, the sperm motility and sperm abnormality did not change as in the study reported by Sojka *et al.* [15]. Although cats copulate several times, the number of sperm was significantly smaller ($p < 0.01$, Tables 1–3) in

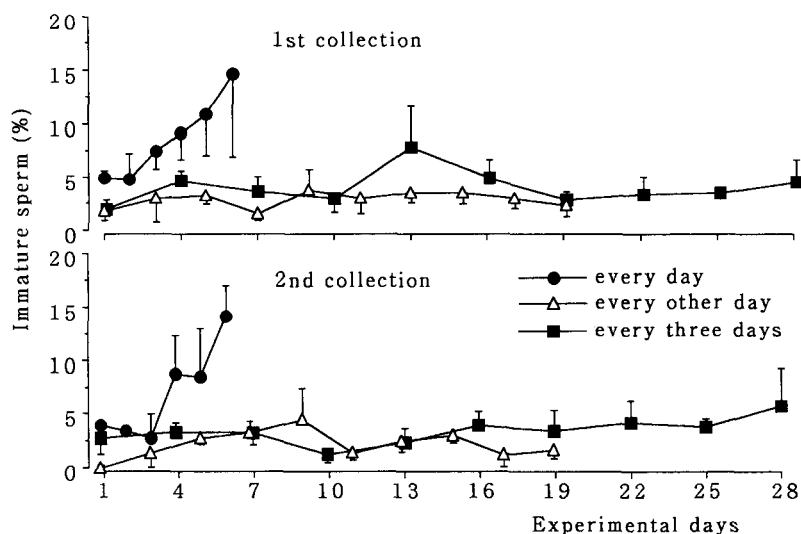


Fig. 4. The rate of immature sperm collected at different time intervals in cats (Mean \pm SE).

the second collection than in the first collection even when semen was collected every three days in this study. Because the number of sperm required for fertilization is not clear in cats, the necessity of multiple copulation cannot be discussed.

As described above, we repeated semen collection with various intervals from 4 male cats aged 3–5 years using an AV, and examined the quality. The above findings show that the semen quality was stable in the first and second collections for up to 10 days with one-day intervals and three-day intervals.

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