Relationship between the Sperm Count and the Fertilization Rate of Ova Ovulated from the Contralateral Ovary in Intrauterine Horn Insemination in Cats

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ABSTRACT. Unilateral intrauterine horn insemination (UIUI) was carried out in cats, and we investigated the fertilization rate of ova ovulated from the contralateral ovary. Various numbers of sperm were used to inseminate the uterine horn on the side where ovulation was inhibited. The rates of conception were 1/11 (9.1%), 2/11 (18.2%), and 5/7 (71.4%) in the 2×10^6 , 4×10^6 , and 8×10^6 groups, respectively. Furthermore, the fertilization rate was 70.7% in the 8×10^6 group. Thus, ova ovulated from the contralateral ovary were not fertilized or the fertilization rate was low in some cats even when UIUI was performed with a large number of sperm.

KEY WORDS: feline, fertilization rate, unilateral artificial insemination.

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With respect to artificial insemination (AI) in cats, 3 studies each have reported intravaginal insemination (IVI) [4, 6, 8] and intrauterine horn insemination (IUI) [1, 12, 13], and one study, tubal insemination [11]. In dogs and cats, the sperm count required for conception when IUI is used is one-tenth of that when IVI is used [8, 10, 12, 14], but IUI must be surgically performed under general anesthesia or with a fibroscope. Concerning IUI, it remains to be clarified whether insemination in the left and right uterine horn is needed to improve the fertilization rate. We previously performed unilateral IUI (UIUI) with various sperm counts in cats [12]. Ova ovulated from the contralateral ovary achieved fertilization in some animals with a low inseminated sperm count. Nevertheless, we could not accurately show the inseminated sperm count or fertilization with ova ovulated from the contralateral ovary, because ovulation occurred in both the left and right ovaries. We also reported that when the number of sperm for UIUI was increased in dogs with a uterine morphology similar to that in cats, fertilization with ova ovulated from the contralateral ovary was frequently achieved [10].

In this study, we performed UIUI in cats, and clarified the inseminated sperm count and fertilization with an ovum ovulated on the contralateral side.

Animals: We used 20 female cats ranging from 2 to 6 years in age bred at Nippon Veterinary and Animal Science University. Some animals were repeatedly used for the experiment. Nine of these cats underwent unilateral ovariectomy two years ago. Furthermore, we used 4 male cats with coitus capacity and fertility, ranging from 6 to 7 years in age. These cats were acclimated in an animal room at $23 \pm 2^\circ\text{C}$. Female cats were housed as a group in a cat room measuring 4×7 m. Furthermore, male cats were individually housed in cages measuring $60 \times 90 \times 120$ (height) cm. Dry food (Hill's feline maintenance, U.S.A.) and water were given *ad libitum*. Female cats were brought into contact with male cats in the morning and evening everyday, and the

presence or absence of estrus was examined.

All the animals were maintained according to the guidelines of the Animal Care and Use Committee of the Nippon Veterinary and Animal Science University.

Collection of semen and examination of semen qualities: Semen for AI was collected with an artificial vagina for cats, as we previously reported [7]. With respect to semen quality, the semen volume, sperm count, sperm motility, and sperm viability were investigated [2].

Ovulation-inducing method: To induce ovulation in female cats, 100 IU of hCG was subcutaneously administered on day 3 or 4 of estrus. Directly after UIUI, 100 IU of hCG was additionally administered [11]. To evaluate the success of induction of ovulation, the peripheral progesterone (P) level was measured 9 days after hCG administration by EIA [3], and when the P level was increased, induction of ovulation was judged to have been successful.

Artificial insemination: UIUI was surgically performed under general anesthesia 20 hr after administration of hCG [12]. For anesthesia, pretreatment with atropine sulfate (0.05 mg/kg) and acepromazine maleate (0.025 mg/kg) was administered. For induction of anesthesia, ketamine hydrochloride (5 mg/kg) was administered. Anesthesia was maintained with isoflurane and O_2 .

The inseminated sperm count was established as 2×10^6 , 4×10^6 , and 8×10^6 , as we previously reported [12]. To prepare a semen volume of $30 \mu\text{l}$ in each group, semen was adjusted with egg-yolk Tris-fructose-citrate solution [13]. UIUI was performed within 30 min after collection of semen. AI was performed in 11, 11, and 7 animals in the 3 groups, respectively. In cats undergoing unilateral ovariectomy, AI was performed in the uterine horn on the removed side. Furthermore, in cats with bilateral ovaries, follicular ova on the side on which the number of mature ovarian follicles was smaller were suctioned with a 1-ml syringe with a 20G needle and inseminated. The suctioned ova were examined under a microscope. Ovarian follicles with a

diameter of 3 mm or greater were regarded as mature follicles.

Diagnosis of pregnancy: Pregnancy was diagnosed using an ultrasonograph for diagnostic imaging (ALOKA ECHO VISION SSD-500EV) 15 to 20 days after insemination. In cats in which conception was achieved, the state of fetal growth was investigated at 5-day intervals thereafter. Newborns were counted on the day of delivery.

When fetuses were unable to be confirmed after imaging diagnosis of pregnancy by ultrasonography, it was judged as abortion.

Statistical analysis: The rate of conception was compared with the χ^2 test. $p < 0.05$ was regarded as significant.

Induction of ovulation: The state of conception when various counts of sperm were used to inseminate the anovulatory-side uterine horn is shown in Table 1.

Of 31 cats that underwent AI, ovulation was not induced in 2 cats (Queen No. 149 and D28, 6.5%) without an increase in the plasma P_4 level (1.25, 1.68 ng/ml). On UIUI 20 hr after administration of hCG, none of the cats showed

completion of ovulation.

In 11 animals in which ova were suctioned from 1–2 mature ovarian follicles, an identical number of ova to that of suctioned ova was recovered from all animals (Table 1).

Results of conception: Anovulatory-side IUI was performed with 3 sperm counts. The rates of conception were 1/11 (9.1%), 2/11 (18.2%), and 5/7 (71.4%) in the 2×10^6 , 4×10^6 , and 8×10^6 groups, respectively so that, there was no significant difference between the 2×10^6 group and the 4×10^6 group, but there were significance differences between these groups and the 8×10^6 group ($p < 0.01$, $p < 0.05$). In 2 of 8 cats in which conception was achieved, abortion occurred between day 20 and day 25 of pregnancy. The remaining 6 cats delivered 1 to 4 kittens (mean: 2.8 ± 0.7) 62 to 70 days after insemination, with a mean of 66.8 ± 1.2 days (SE). Fertilization rates were 100% (4/4) in one cat in the 4×10^6 group and 20.0 to 100% (mean: $70.7 \pm 20.2\%$) in 5 cats in the 8×10^6 group.

The properties of semen used for IUIU were: sperm motility, 80–95%, and sperm viability, 84.4–98.0%.

Table 1. The results of conception when various counts of sperm were inseminated to the anovulatory-side UIU

No. of sperm ($\times 10^6$)	Queen No.	No. of follicle		P_4 (ng/ml)	Semen quality			No. of kits (Gestation period)	Conception rate (%)
		L	R		Tom No.	Motility (%)	Viability (%)		
2	144	3	(1) ^{b)}	14.2	36	95	93.5	0	
	149	— ^{a)}	4	1.25 ^{c)}	44	95	98.0		
	155	—	4	18.8	44	90	93.2	0	
	156	—	4	12.0	36	80	89.0	0	
	157	3	(1)	8.0	44	85	88.8	0	
	158	4	0	14.5	32	80	90.4	0	1/11 ^{e)}
	159	—	5	10.1	32	90	90.6	0	(9.1%)
	173	—	5	22.5	36	90	85.6	0	
	176	—	4	10.3	44	95	98.0	0	
	180	—	6	8.4	36	95	94.5	0	
	D42	3	(1)	20.9	32	80	90.4	0	
	D52	4	—	8.1	32	90	84.4	ab ^{d)}	
	155–2	—	4	18.8	36	90	98.0	ab ^{d)}	
	156–2	—	3	19.6	32	85	95.0	0	
4	159–2	—	4	14.7	32	80	88.5	0	
	172	(1)	3	23.3	40	90	95.0	0	
	173–2	—	4	18.9	44	95	95.5	0	
	173–3	—	5	16.1	32	80	94.0	0	2/11 ⁵⁾
	176–2	—	2	15.7	32	85	96.4	0	(18.2%)
	186	3	(2)	9.4	44	95	96.5	0	
	D29	3	(2)	18.2	44	90	92.8	0	
	D36	(1)	3	13.4	32	90	94.8	0	
	D54	(1)	4	9.4	44	95	97.3	4 (67)	
	122	(1)	3	10.24	44	95	94.3	1 (68)	
	149–2	—	5	20.4	40	95	92.5	0	
8	155–3	—	5	16.9	36	90	93.3	1 (70)	
	156–3	—	4	20.0	32	90	87.4	4 (68)	5/7 ⁵⁾
	158–2	(2)	4	8.1	40	95	92.5	4 (62)	(71.4%)
	162	(1)	3	8.7	44	95	94.3	3 (67)	
	180–2	—	4	9.8	32	95	94.8	0	
	D28	—	3	1.68 ^{c)}	40	90	95.0		

a) ovary removed b) collected ova c) anovulation

Significantly different between e) and f) at $p < 0.01$

Significantly different between f) and g) at $p < 0.05$

d) abortion (day 20–25 after mating)

Two animals (6.5%) did not ovulate in this experiment. Shille *et al.* [5] reported that ketamine hydrochloride may inhibit ovulation in cats. Various numbers of sperm were used to inseminate the anovulatory-side uterine horn. The rate of conception was noticeably higher in the 8×10^6 group than in the 2×10^6 and 4×10^6 groups, but the results of a previous study [12] and this study showed that ova which ovulated from the contralateral ovary despite a small number of inseminated sperm achieved fertilization in some animals. Furthermore, the results of this study showed that the mean fertilization rate in cats in which conception was achieved after insemination with 8×10^6 sperm was 70.7%. The percentage was lower than the mating-associated fertilization rate, $83.9 \pm 19.5\%$ [9]. Since the maximum number of inseminated sperm was 8×10^6 in this experiment, results of insemination with a higher number of sperm attract attention. In conclusion, ova ovulated from the contralateral ovary were not fertilized or the fertilization rate was low in some cats even when UIUI was performed with a large number of sperm.

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