NOTE *Theriogenology*

Intratubal Insemination with Fresh Semen in Dogs

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ABSTRACT. The number of spermatozoa required to obtain conception by intratubal insemination in dogs was examined. Three groups consisting of 5, 8 and 8 dogs received 0.5×10^6, 2.0×10^6 and 4.0×10^6 spermatozoa, respectively, into each uterine tube. No conception occurred in the 5 animals inseminated with 0.5×10^6 spermatozoa, but conception occurred in 6/8 (75.0%) and 3/8 (37.5%) dogs inseminated with 2.0×10^6 and 4.0×10^6 spermatozoa, respectively. Among the pregnant animals, three aborted (33.3%) and the mean number of newborns was small, 2.5 ± 0.5 (SE). One acardiacus anceps was observed with normal fetus in one animal with a Caesarean delivery.
KEY WORDS: acardiacus anceps, canine, intratubal insemination.

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Although it has been reported that the number of spermatozoa required to obtain conception by intrauterine horn insemination is 1/10 of that required by intravaginal insemination in dogs [8, 10, 12, 13], 1.0×10^7 spermatozoa are required for insemination into one uterine horn in dogs [9]. It is generally considered that only a small number of spermatozoa inseminated into the uterus reaches the uterine tubes and fertilizes the ova. Most spermatozoa inseminated into the vagina and uterus are not involved in fertilization. Therefore, the number of spermatozoa to be infused may be reduced by inseminating spermatoza at a site close to the fertilization site. It has been reported that conception could be obtained by intratubal insemination with 1.0×10^5 spermatozoa in rabbits [4] and 2.5×10^5 spermatoza in rats [6]. However, we previously reported that the conception rate was 42.9%, when 4.0×10^7 spermatoza were inseminated in cats [11]. Therefore, the number of spermatozoa required for intratubal insemination varies among animal species and this may be due to variation in conditions for capacitation of spermatoza among the species.

Canine intrauterine insemination requires surgical techniques. For insemination performed by laparotomy, a method of insemination into the oviduct close to the fertilization site is considered. To efficiently use spermatozoa, we investigated whether conception can be obtained by intratubal insemination with fresh semen in dogs and examined the number of spermatozoa needed for intratubal insemination

Animals: Nineteen female beagles aged 2–6 years used in the experiment. Since two dogs (Bitch Nos. 315 and 330) were used twice, the number of cases was 21. Semen was collected at appropriate times from 6 male beagles aged 5–10 years with proven fertilizing ability. Animals for the experiment were kept one per cage measuring $160 \times 75 \times 65$ cm. Animals were given commercial dog food in the morning and water in the morning, afternoon, and evening. Food for pregnant dogs was given twice a day (morning and evening) from 35 days of gestation.

Collection of semen and test of semen quality: Semen was manually collected in three fractions immediately before artificial insemination. The semen quality in the second fraction containing spermatoza was examined as previously reported [4]. Sperm motility was examined by a semen quality examination plate (Fujihira, Co., Ltd., Japan) and presented as the ratio of most active spermatozoa. Sperm viability was assessed by means of eosin-nigrosin stain.

Determination of ovulation day: To determine the ovulation day in the dogs tested, blood was collected from the cephalic vein daily starting 6 days after the beginning of bleeding of estrus and the ovulation day was estimated from the blood progesterone (P_4) level. The initial day with a P_4 level of 2 *n*g/m*l* or higher was defined as the ovulation day [1]. The P_4 level was measured by EIA [5].

Intratubal insemination: Intratubal insemination was performed during the mating period, 3–5 days after ovulation. Artificial insemination was performed under general anesthesia. Animals were pretreated with atropine sulfate (0.05 mg/kg) and acepromazine maleate (0.025 mg/kg), and anesthesia was induced using ketamine hydrochloride (7 mg/kg) and maintained with halothane. Intratubal insemination was performed using a glass capillary in the ampulla of the uterine tubes at a site approximately 2 cm from the fimbriate tubae. Intratubal insemination was performed in both uterine tubes. Spermatozoa at 0.5×10^6, 2.0×10^6, and 4.0×10^6, were inseminated into 5, 8, and 8 animals, respectively. The semen volume was adjusted to 20 μl with egg yolk Tris-fructose citric acid [8]. Corpus luteum was counted in the right and left ovaries.

Diagnosis of pregnancy: Pregnancy was determined 25 days after insemination using an ultrasonographic imaging diagnosis system (ECHOVISION SSD-500EV, Aloka Co., Japan). Pregnant animals were observed every 5 days until delivery to confirm normal fetal development. The number of newborns was counted on the delivery day.

The conception results of bilateral intratubal insemination with various numbers of spermatozoa performed during the correct mating period are shown in Table 1.

As for the quality of semen used in the artificial insemi-

Table 1. Conception rates after intratubal insemination of individual bitches

Number of spermatozoa	Conception rate (%)	Bitch number	Semen quality			Number of corpora lutea	Number of pups (duration of gestation in days)
			Dog number	Sperm motility (%)	Sperm viability (%)		
		321	276	95	98.8	7	0
		271	271	95	94.0	11	0
0.5×10^6	0/5	298	266	95	95.5	5	0
	(0)	327	271	95	94.9	7	0
		326	271	95	94.9	8	0
		315–1	271	95	94.1	6	2 (61)
		293	253	90	91.1	8	3 (60)[a)]
		331–1	273	90	90.0	6	4 (59)
	6/8	328	275	95	97.4	7	0
2.0×10^6	(75.0)	325	273	95	92.2	7	0[b)]
		289	266	95	88.6	10	3 (61)
		307	266	95	88.6	7	0[b)]
		313	275	95	95.6	6	0
		315–2	271	95	94.0	6	0
		333	271	95	94.0	10	2 (60)
		329	271	95	94.0	6	0
	3/8	304	276	95	97.0	7	0[c)]
4.0×10^6	(37.5)	332	271	90	96.2	7	1 (60)
		309	276	95	96.1	8	0
		319	276	95	88.7	9	0
		331–2	271	95	94.6	9	0
Mean ± SE				94.3±0.4	93.8±0.7	7.2±0.5	2.5 ± 0.5[d)] (60.2±0.3)

a) Acardiacus anceps was excluded from the number of newborns. b) Aborted on day 35 of gestation. c) Aborted between days 26 and 29 of gestation. d) The mean number of newborns for six bitches.

nation, the mean sperm motility and viable rate were 94.3 ± 0.4 (SE) and 93.8 ± 0.7%, respectively.

No conception occurred in the 5 animals inseminated with 0.5×10^6 spermatozoa. Of 8 animals inseminated with 2.0×10^6 spermatozoa, 6 animals (75%) became pregnant. However, abortion occurred in 2 animals (Bitch Nos. 325 and 307) after 35 days of gestation. One and 5 fetuses were lost, respectively. The other 4 animals delivered 2–4 newborns, 2.5 ± 0.5 newborns on average. The ratio of the number of newborns to the number of corpora lutea in the 4 animals was 30.0–71.4%, 45.0 ± 9.7 on average. Since Bitch No. 293 did not deliver despite severe birth pains, Caesarean section was performed. As shown in Fig. 1, acardiacus anceps was observed on the tip of the left uterine horn. This acardiacus anceps was oval shaped and rich in fat, and a flat structure, looking like a tongue, was observed in the center. The weight was 65 g. No placenta, fetal membrane, or umbilical cord was observed in this acardiacus anceps, and there was no adhesion to neighboring fetuses. This acardiacus anceps was excluded from the number of newborns.

Three of 8 animals inseminated with 4.0×10^6 spermatozoa became pregnant and the conception rate was 37.5%, which was lower than that rate obtained by inseminating 2.0×10^6 spermatozoa. One of them (Bitch No. 304) aborted one fetus between 26 and 29 days of gestation. Among the 2 animals that delivered, the number of newborns was small and the ratios of the number of newborns to the number of corpora lutea were 20.0% and 14.3%. There was no external abnormality observed in puppies delivered in this experiment.

This in the first report of delivery of newborns obtained by intratubal insemination in dogs.

A conception rate of 75.0% was obtained by inseminating 2.0×10^6 spermatozoa into the uterine tubes in dogs. The conception rate decreased to 37.5% when the number of spermatozoa was increased to 4.0×10^6, suggesting that the increase in inseminated spermatozoa impaired fertilization. Three of 9 impregnated animals (33.3%) had abortion, the number of newborns was small, and an acardiacus anceps was observed in Bitch No. 293, suggesting that there was a problem in embryonic development after fertilization.

The number of spermatozoa required for fertilization by intratubal insemination in dogs was higher than those in rabbits [4] and rats [6] but similar to that in cats [11].

The mean ratio of the number of fetuses to the number of corpora lutea was high, 88.7%, in dogs mated [7], but the ratio was low in this study. We also observed a reduction of fertilization rate in intratubal insemination in cats [11]. Hunter [2] compared embryogenesis after intratubal insem-

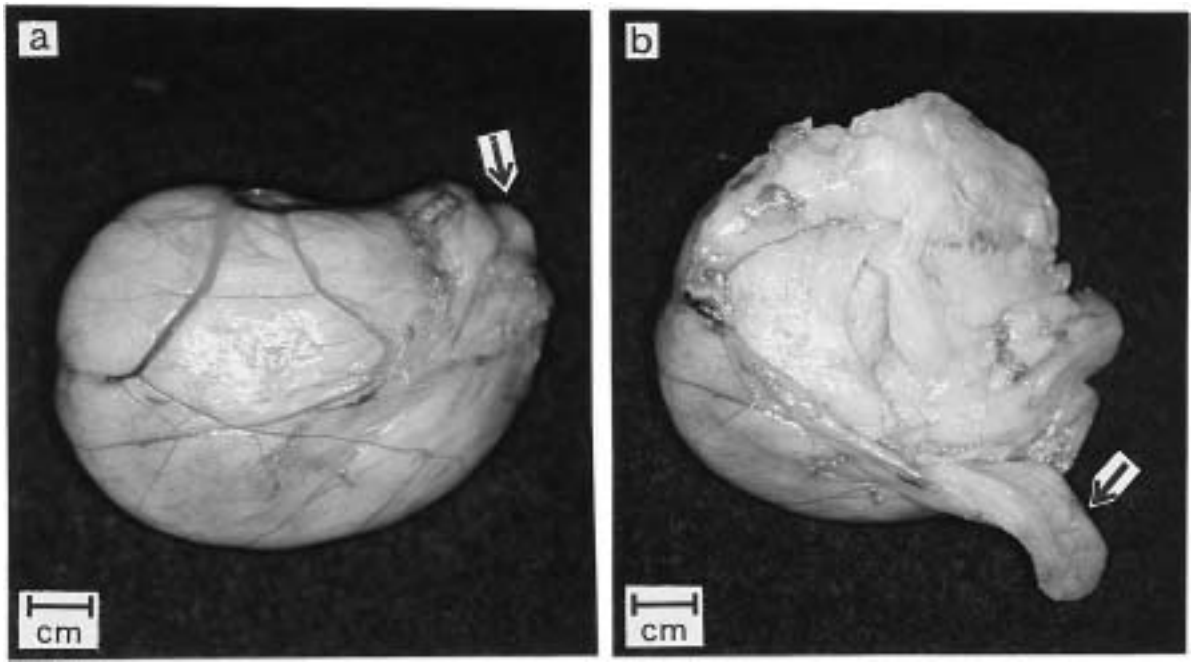

Fig. 1-a. Acardiacus anceps observed on the tip of the left uterine horn of Bitch No. 293. The photo shows the excised acardiacus anceps. The structure indicated by an arrow may be a tongue.

Fig. 1-b. The surface thin membrane of the acardiacus anceps was removed and the tongue-like structure, indicated by an arrow, was exposed.

ination and intrauterine insemination in pigs and observed a high incidence, 33.8%, of polyspermic fertilization in intratubal insemination. In contrast, Kanayama *et al.* [3] observed normal embryogenesis after intratubal insemination in rabbits. Therefore, fertilization and embryogenesis after intratubal insemination may vary among animal species. A phenomenon similar to that observed in pigs may have occurred in dogs, resulting in a decrease in the number of fetuses. Since to our knowledge, canine acardiacus anceps has not previously been reported, it may be very rare. It is not clear whether acardiacus anceps developed due to intratubal insemination.

A conception rate of 75% was obtained by intratubal insemination of fresh semen containing 2.0×10^6 spermatozoa in dogs, but the number of fetuses was small. It is necessary to investigate the incidence of polyspermic fertilization in intratubal insemination.

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