Relationship between the Number of Sperms and the Rate of Implantation in Bitches Inseminated into Unilateral Uterine Horn

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ABSTRACT. Studies were made on the number of sperms required for fertilization of ova ovulated from the bilateral ovaries in bitches. The experiment was carried out with 44 female beagles, which were divided into four groups of seven to eleven bitches each. The four groups were inseminated with 40×10^6 sperms/0.2 ml, 20×10^6 sperms/0.1 ml, 10×10^6 sperms/0.1 ml, and $3-5 \times 10^6$ sperms/0.1 ml, respectively. All the bitches were laparotomized at an optimum time for mating and inseminated at the tip of the horn of an unilateral uterus. In the four groups conception took place at a rate of 7/7, 8/8, 10/11, and 2/7, respectively. The number of puppies exceeded the number of ova ovulated on the inseminated side at a rate of 5/7, 6/8, 0/10, and 0/2 in the four groups, respectively. Therefore, it was clarified that when more than 20×10^6 sperms/0.1 ml were inseminated in the ipsilateral uterine horn, ova ovulated on the contralateral side were fertilized also. Then a unilateral ovary was ovariectomized in 11 bitches, which were inseminated with 10×10^6 sperms/0.1 ml in the ipsilateral uterine horn. As a result, only one bitch became conceptive and gave birth to only one puppy. From these results, it was considered that 10×10^6 sperms/0.1 ml may be most adequate for the ova on the side of insemination alone to be fertilized.—**KEY WORDS:** artificial insemination, dog, fresh semen, uterus.

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In the studies on artificial insemination in bitches, fresh semen [4–6, 13, 15, 19, 20, 22], cooled semen [6, 7, 9, 10, 13, 19], or frozen semen [1, 2, 4, 6, 14, 16–18, 20, 21] have been used. They were inseminated, however, into the vagina to examine the rate of conception. No report has been made to elucidate the relationship between intrauterine insemination and conception. A few papers have been published to report the longevity of sperms in the uterus after introduction by mating [3] and the duration of fertilization ability [25].

In the present experiment intrauterine insemination was studied as a method of insemination with sperms of different male origin by using ova ovulated from the bilateral ovaries. It was expected that this method would be a means to contribute to the clarification of a mechanism of the

intrauterine transfer of canine embryos.

MATERIALS AND METHODS

Animals: Beagles of a closed colony bred by the authors were used. They consisted of nine breeding males 1–5 years old and 21 parous females 2–6 years old. Since some females were used more than once, it was from a total of 44 female cases that experimental data were collected.

Semen: Semen samples were collected from nine dogs by digital manipulation [8]. Semen was separated into three fractions, as described by Harrop [8]. The second fraction (2nd F) samples were checked for semen volume, sperm count, sperm motility, and sperm viability. Sperm count was determined by use of the hematocytometer. Sperm motility was examined by a semen

quality examination plate and scord according to Harrop [8] and Heywood [10]. Sperm viability rate was determined by means of eosin-nigrosin stain. Semen containing sperms of grade 4 motility [12] was used.

Intrauterine insemination: As already reported, the semen used for artificial insemination was the 2nd F sperm concentration of which had been adjusted by dilution with the third fraction. A small amount of 0.1 ml semen was used although in some individuals it was not enough to contain 40×10^6 sperms, since the sperm concentration of the 2nd F was low. For these individuals 0.2 ml of semen was used. In the insemination experiment four groups of 7–11 bitches each were injected with 40×10^6 sperms/0.2 ml (A), 20×10^6 sperms/0.1 ml (B), 10×10^6 spermes/0.1 ml (C), and $3-5 \times 10^6$ sperms/0.1 ml (D), respectively.

For intrauterine insemination each bitch was laparotomized and a 20G retaining needle connected with a 1 ml injection cylinge was inserted into about the middle portion of the left or right uterine horn and pushed to the tip of the uterine horn. A small number of sperms was injected into the uterine horns in four of the seven bitches of group D. The interval of time between sperm collection and injection was about 30 min. From the results of this insemination experiment it was judged that only groups in which ova ovulated from the inseminated side had been fertilized. Then, such sperm concentration was inseminated into the uterine horn on the side where the ovary had been excised in bitches (E). Establishment of conception was observed. The time of insemination was fixed according to the results of the author's previous experiment;

Table 1. Conception by using 40×10^6 sperms/0.2 ml into unilateral uterine horn

Bitch No.	Semen Quality					Findings on Laparotomy				
	Male No.	2nd Fraction Volume (ml)	Mo- ^{a)} tility Status	Vi-ability (%)	Count ×10 ⁸ /ml	No. of Ovulations	Inseminated Ut. Horn	No. of Implan-tations	B/A ^{b)}	
123	223	0.7	4	98.9	9.2	L 5 R 4	♂	1 3	4/4	
139	223	0.7	4	87.9	6.7	L 3 R 4	♂	4 3	7/3 ^{c)}	
159	221	1.8	4	94.0	2.1	L 1 R 6	♂	3 3	6/1	
171	213	0.8	4	92.5	6.3	L 2 R 4	♂	2 2	4/2	
176	223	0.7	4	90.0	8.5	L 4 R 4	♂	0 1	1/4	
179	218	0.8	4	97.3	5.2	L 4 R 3	♂	3 1	4/3	
181	223	0.6	3	85.0	7.5	L 2 R 5	♂	2 2	4/2	
Mean		0.9	3.9	92.2	6.5	7.3 ^{d)}				
SE		0.1	0.1	1.7	0.8	0.3				

a) Grade of sperm motility: 1=<25%, 2=25–50%, 3=50–75%, 4=75–100%.

b) A: No. of ovulations in ovary of inseminated side, B: No. of implantations of both ut. horns.

c) □: No. of ovulations in ovary of inseminated side<No. of implantations of both ut. horns.

d) Left + right.

that is, from 60 to 108 hr after ovulation, or on the 4th or 5th day after the onset of estrous behavior [23, 24, 26].

The number of ovulations: Ovulation points were counted through the ovarian bursa at the time of laparotomy. When it was impossible to examine ovulation through the ovarian bursa, the bursa was partly incised to expose the ovary.

Judgment of conception: In the experimental bitches subjected to intrauterine insemination, judgment of conception was made by confirming implantation in the uterine horn by laparotomy 25 days after insemination.

Laparotomy: Laparotomy was performed under general anesthesia on bitches which had been treated presurgically with a com-

bination of acepromazine and atropine sulfate. Ketamine and valium were administered intramuscularly to the bitches to induce anesthesia which was maintained by gas anesthesia (1% halothane and oxygen).

RESULTS

Conception of intrauterine insemination: Conception results and semen quality of each four groups used for intrauterine insemination are shown in Tables 1–4, i. e., the mean value for semen quality of the 2nd F used in this experiment for groups A–D were each 0.9, 0.7, 1.0, 0.9 ml in volume, 3.9, 3.9, 3.9, 4.0 in motility, 92.2, 90.8, 93.4, 91.8% in viability and 6.5, 5.2, 4.9, 3.4×10^8 sperms/ml in number, respectively. Also,

Table 2. Conception by using 20×10^6 sperms/0.1 ml into unilateral uterine horn

Bitch No.	Semen Quality					Findings on Laparotomy				
	Male No.	2nd Fraction Volume (ml)	Mo- ^{a)} tility Status	Vi-ability (%)	Count ×10 ⁸ /ml	No. of Ovu-lations	Insemi-nated Ut. Horn	No. of Implan-tations	B/A ^{b)}	
136	223	0.5	4	91.0	4.0	L R	2 4	♂ 	1 0	1/2
163	218	1.0	4	98.8	3.5	L R	3 4	♂ 	3 2	5/3
163	221	0.4	4	96.1	8.0	L R	2 4	♂ 	2 2	4/2
170	218	1.5	4	94.9	3.7	L R	2 4	♂ 	3 3	6/2
171	223	0.6	4	86.2	5.7	L R	3 4	♂ 	1 2	3/3
173	223	0.5	4	91.0	4.0	L R	4 4	♂ 	3 2	5/4
175	223	0.5	3	74.0	4.4	L R	3 7	♂ 	4 5	9/3
175	221	0.3	4	92.8	8.3	L R	7 2	 ♂	2 2	4/2
Mean		0.7	3.9	90.8	5.2	7.4 ^{d)}				
SE		0.1	0.1	2.7	0.7	0.5				

a) Grade of sperm motility: 1=<25%, 2=25–50%, 3=50–75%, 4=75–100%.

b) A: No. of ovulations in ovary of inseminated side, B: No. of implantations of both ut. horns.

c) □: No. of ovulations in ovary of inseminated side<No. of implantations of both ut. horns.

d) Left + right.

the means of ovulation number in each experimental group was 7.3, 7.4, 7.5, 7.0, respectively. Conception rates were each 7/7 (100%), 8/8 (100%), 10/11 (90.9%), 2/7 (28.6%), respectively.

Cases in which the number of puppies exceeded the ovulation number of the inseminated side were each 5/7 (71.4%) in A, 6/8 (75.0%) in B, 0/10 in C, 0/2 in D, respectively. This fact makes it clear that in both groups A and B, sperms migrated from the inseminated side into the opposite side. The implantation rate toward bilateral ovulation rate in both these groups were

12.5–100% with a mean of 60.5% in A, and 16.7–100% with a mean of 61.8% in B. The implantation rate toward ovulation rate of the inseminated side in group C was 66.7–100% with a mean of 82.5%.

Conception of intrauterine insemination in an ipsilateral ovariectomized bitch: Semen judged to have fertilized ova ovulated from the inseminated side alone was 10×10^6 sperms/0.1 ml. Thus, this semen was inseminated during optimum mating time into the ovariectomized uterine horn of a bitch, which had ovariectomy performed unilaterally beforehand. Results are shown in

Table 3. Conception by using 10×10^6 sperms/0.1 ml into unilateral uterine horn

Bitch No.	Semen Quality					Findings on Laparotomy			
	Male No.	2nd Fraction Volume (ml)	Mo-a) tility Status	Vi-ability (%)	Count $\times 10^8$ /ml	No. of Ovulations	Inseminated Ut. Horn	No. of Implantations	B/A ^{b)}
124	195	0.7	4	90.0	9.0	L 4 R 7	♂	1 2	3/4
126	195	0.7	4	90.0	9.0	L 6 R 3	♂	1 2	3/3
136	218	1.3	4	95.0	3.0	L 6 R 0	♂	2 2	4/6
139	221	1.8	4	97.9	1.6	L 2 R 6	♂	4 2	6/6
159	218	0.5	4	98.5	5.8	L 4 R 3	♂	0 0	—
173	221	1.0	4	91.6	2.7	L 6 R 3	♂	0 2	2/3
173	218	1.5	4	95.5	1.9	L 3 R 4	♂	1 2	3/4
175	221	0.7	4	91.6	5.0	L 5 R 3	♂	1 1	2/3
179	185	0.6	4	95.8	4.0	L 4 R 2	♂	1 2	3/4
179	195	0.7	3	85.0	8.0	L 2 R 3	♂	1 2	3/3
179	218	1.2	4	97.0	4.0	L 4 R 2	♂	2 2	4/4
Mean		1.0	3.9	93.4	4.9	7.5 ^{c)}			
SE		0.1	0.1	1.3	0.8	0.5			

a) Grade of sperm motility: 1=<25%, 2=25–50%, 3=50–75%, 4=75–100%.

b) A: No. of ovulations in ovary of inseminated side, B: No. of implantations of both ut. horns.

c) Left + right.

Table 5, i. e., the mean value for semen quality of the 2nd F used in this experiment was 0.8 ml in volume, 3.8 in motility, 92.9% in viability and 4.6×10^8 sperms/ml in number. Also, ovulation number was 7–10 with a mean of 8.5. Conception rates for these were 1/11 (9.1%), and number of puppies was 1.

DISCUSSION

A high conception rate was obtained in this experiment by intrauterine insemination of 10×10^6 sperms/0.1 ml or more fresh semen. Furthermore, it was clear that 10×10^6 sperms/0.1 ml was appropriate for fertilizing the ova ovulated of the inseminated side alone. It was also clear that insemination of 20×10^6 sperms/0.1 ml caused sperms migration to the opposite uterine horn, fertilizing those ova also. But

doubling the number of sperms in the unilateral insemination did not increase the implantation rate. This is thought to be due to the fact that factors such as uterine movements prevent migration of sperms inseminated into the opposite horn. Therefore, inseminating over 10×10^6 sperms/0.1 ml semen into each uterine horn was thought to be effective for obtaining high implantation rates.

The authors have previously reported 2×10^8 sperms/1–3 ml fresh semen would be necessary to obtain high conception and fertilization rates by intravaginal insemination [27]. This is 10 times the amount of semen used for intrauterine insemination.

In this experiment, there was no difficulty found with the capacity of the uterine horn in parous beagles to inseminate 0.1 or 0.2 ml of semen into the upper point of the uterine horn.

Table 4. Conception by using $3-5 \times 10^6$ sperms/0.1 ml into unilateral uterine horn

Bitch No.	Semen Quality					Findings on Laparotomy			
	Male No.	2nd Fraction Volume (ml)	Mo- ^{a)} tility Status	Vi-ability (%)	Count $\times 10^8$ /ml	No. of Ovulations	Inseminated Ut. Horn	No. of Implantations	B/A ^{b)}
156	195	1.5	4	92.2	3.0	L 5 R 4	♂ (3×10^6)	0	—
173	300 195	0.4 0.8	4 4	90.0 95.7	3.0 1.1	L 5 R 3	♂ (3×10^6) ♂ (3×10^6)	0 0	—
163	213	1.0	4	90.1	3.9	L 0 R 7	♂ (5×10^6)	0	—
163	213 300	1.0 0.6	4 4	95.6 89.0	2.8 7.6	L 1 R 4	♂ (5×10^6) ♂ (5×10^6)	0 0	—
176	300 195	0.5 0.4	4 4	89.2 90.4	1.9 5.0	L 3 R 2	♂ (5×10^6) ♂ (5×10^6)	0 0	—
135	213	1.0	4	92.5	3.1	L 8 R 0	♂ (5×10^6)	3 1	4/8
183	218	1.3	4	93.5	2.8	L 3 R 4	♂ (5×10^6) ♂ (5×10^6)	2 2	2/3 2/4
Mean		0.9	4	91.8	3.4	7.0 ^{c)}			
SE		0.1	0	0.7	0.5	0.5			

a) Grade of sperm motility: 1=<25%, 2=25–50%, 3=50–75%, 4=75–100%.

b) A: No. of ovulations in ovary of inseminated side, B: No. of implantations of both ut. horns.

c) Left + right.

Table 5. Conception in a bitch unilaterally ovariectomized and using 10×10^6 sperms/0.1 ml into uterine horn of extracted side

Bitch No.	Semen Quality					Findings on Laparotomy	
	Male No.	2nd Fraction Volume (ml)	Mo- ^{a)} tility Status	Vi-ability (%)	Count $\times 10^8$ /ml	No. of Ovulations (Side)	No. of Implantations (Side)
124	218	0.8	4	97.0	3.0	9 (L)	0
124	218	0.4	4	87.0	5.7	9 (L)	0
126	218	0.9	4	97.0	4.3	9 (R)	1 (R)
126	221	1.0	4	96.4	4.0	9 (R)	0
135	221	1.8	3	80.0	1.9	9 (L)	0
141	185	0.5	4	97.1	3.5	10 (L)	0
146	218	0.8	4	95.8	4.9	7 (R)	0
156	185	0.8	3	82.0	2.8	7 (R)	0
170	218	0.7	4	95.8	7.5	8 (R)	0
178	226	0.6	4	97.6	9.0	9 (L)	0
180	185	1.0	4	96.5	3.8	8 (L)	0
Mean		0.8	3.8	92.9	4.6	8.5	
SE		0.1	0.1	2.0	0.6	0.3	

a) Grade of sperm motility: 1= $<25\%$, 2=25–50%, 3=50–75%, 4=75–100%.

b) No. of ovulations from one remaining ovary.

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要 約

犬の片側子宮内授精における精子数と着床率との関係：筒井敏彦・清水敏光・尾原規子・柴 祐子・廣中俊郎・織間博光・小笠 晃（日本獣医畜産大学獣医臨床繁殖学教室）——犬の子宮内授精において受胎に要する精子数を知る目的で実験を行った。試験は $40 \times 10^6/0.2\text{ml}$, $20 \times 10^6/0.1\text{ml}$, $10 \times 10^6/0.1\text{ml}$, $3-5 \times 10^6/0.1\text{ml}$ のグループで行った。授精は適期に開腹手術を行い、片側の子宮角先端部に行った。受胎成績はそれぞれ7/7, 8/8, 10/11, 2/7であった。これらのうち授精側排卵数を着床数が上まわった例は、それぞれ5/7, 6/8, 0/10, 0/2であった。このことから 20×10^6 以上では、精子は対側の卵とも受精することが分った。そこで 10×10^6 を片側卵巣摘出犬の摘出側子宮角に授精したところ、11頭中1頭が受胎したが、子犬は1匹であった。本報の成績では、授精した側の卵のみを受精させるには $10 \times 10^6/0.1\text{ml}$ の精液による子宮内授精が最適であった。