

## Experimental Induction of Superfecundation in the Dog

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In the dog, mating results in fertilization when it occurs during a period from the beginning of the onset of sexual receptivity to 108 hr after ovulation, which is about 7 days [3]. Canine oocytes are still in the stage of metaphase I by the time of ovulation, or 48-72 hr after the acceptance of the male. These oocytes grow into the secondary stage (metaphase II) 48 to 72 hr after ovulation [4]. Superfecundation thus may occur when the bitch is subjected to mating to several males during the same estrous period. There have been no reports concerning superfecundation in dogs so far. The present experiment was carried out to establish a time schedule of mating for the induction of superfecundation.

Eleven beagles and seven mongrels were used once or twice, for a total of 21 cases of pregnancy being obtained. Two beagle and two mongrel males were used for mating. They had semen of normal quality, having the experience in mating.

In bitches, the time of ovulation was estimated by the grade of maturity of the ovarian follicle in the same manner as already reported [2, 3]. Laparotomy was performed on each bitch under general anesthesia with Ketamine hydrochloride 48 or 60 hr after the start of estrus.

The time of ovulation was estimated by direct observation of the ovaries by the use of laparotomy. The condition of the ovary in a period of ovulation was as follows: At 24 hr before ovulation follicles protruded about 1mm from the ovarian surface, the ovarian bursa containing a small amount of transparent fluid. At 12 hr before ovulation. Follicles protruded about 2mm from the ovarian surface, the ovarian bursa containing 2 to 3 ml of transparent fluid. After that a small amount of hemorrhage was observed at the ovulatory point and the ovarian bursa was filled with pink to red fluid. These conditions served as our judging that the dogs were in a period of ovulation.

At 12 hr postovulation, the ovulatory point and the adjacent area were red in color. A small

amount of hemorrhage was still observed as the ovulatory point was touched through the mesosalpinx. There was a small amount of transparent fluid in the ovarian bursa.

The mating schedule for experimental dogs is shown in Fig. 1.

The first mating was conducted 36 or 48 hr after the start of estrus. The second ones were done during a period from 24 to 96 hr after the first, resulting in 12, 24, 36, 48, 60, 72 or 84 hr after ovulation. The blood of those pups was examined by the D blood group system [1] to identify their father. If two female dogs were both ready for breeding on the same day, they were scheduled to mate with different blood type male dogs. Four bitches were bred in this manner and hair coat and body size of pups were used to differentiate sires. All the bitches used in the present experiment had blood type D<sub>2</sub>.

In case the ovulatory process had been completed by the time of laparotomy, the number of ovulations can be determined. If a bitch was in the pre-ovulatory stage at the time, another laparotomy was performed during pregnancy or after parturition, using the number of corpora lutea for estimating the number of ovulations. The number of pups were recorded at the time of parturition.

Twelve dogs underwent laparotomy 48 hr after the onset of estrus; 4 dogs were 12 hr pre-ovulation, 7 dogs in the ovulation period and 1 dog 12 hr postovulation. Nine dogs also underwent laparotomy 60 hr after the onset of estrus; 2 dogs were 12 hr pre-ovulation, 3 dogs in the

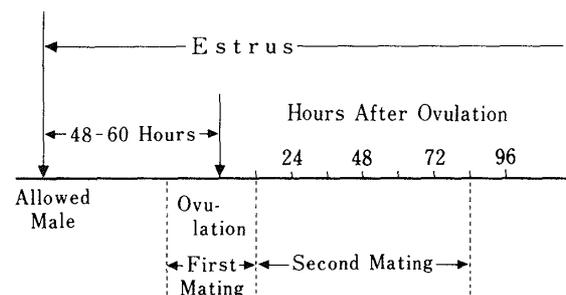


Fig. 1. Mating Schedule for Experimental Dogs.

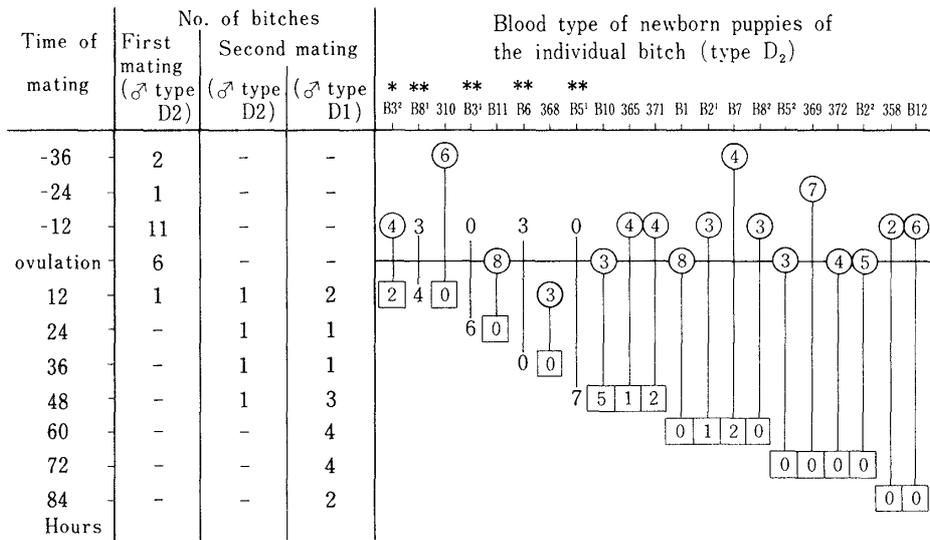


Fig. 2. Experimental induction of superfecundation \* B indicates beagle. \*\* The sire was identified by judgment of hair coat and body size. The circle (○) indicates blood type D<sub>2</sub> and the square (□) indicates blood type D<sub>1</sub> D<sub>2</sub>. Number in the circle or square indicates number of puppies.

Table 1. Number of corpora lutea and newborn pups

Bitch No.	No. of corpora lutea	No. of newborn pups
B <sub>3</sub> <sup>2</sup>	7	6
B <sub>8</sub> <sup>1</sup>	7	7
310	7	6
B <sub>3</sub> <sup>1</sup>	8	6
B <sub>11</sub>	10	8
B <sub>6</sub>	8	3
368	4	4
B <sub>5</sub> <sup>1</sup>	7	7
B <sub>10</sub>	8	8
365	5	5
373	8	6
B <sub>1</sub>	8	8
B <sub>2</sub> <sup>1</sup>	6	4
B <sub>7</sub>	7	6
B <sub>8</sub> <sup>2</sup>	8	3
B <sub>5</sub> <sup>2</sup>	6	3
369	7	7
372	4	4
B <sub>2</sub> <sup>2</sup>	5	5
358	7	2
B <sub>12</sub>	10	7
Total	147	115
m±S.D.	7.0±1.7	5.5±1.8

ovulation period and 4 dogs 12 hr postovulation.

Time intervals between the first mating and the time of ovulation varied from 36 hr before ovulation to 12 hr after ovulation, as shown in Fig. 2.

Table 1 shows the numbers of corpora lutea and newborn pups in the 21 experimental bitches. The mean numbers of corpora lutea and newborn pups per animal were 7.0±1.7 (S.D.) and 5.5±1.8, respectively. The mean rate of fertilization was 78.2% in all 21 bitches, among which nine bitches showing 100%.

Fig. 1 shows the results of experimental induction of superfecundation.

The second mating was conducted on eleven bitches at 12, 24, 36 or 48 hr after ovulation. Superfecundation occurred in five, all of which were born prematurely. Four bitches were subjected to the second mating at 60 hr after ovulation, and superfecundation occurred in two, both of which were also premature. No superfecundation occurred in six bitches bred for the second time at 72 or 84 hr after ovulation, all of which being pregnant by the first mating.

The data in Table 2 indicate that a litter balanced evenly between 2 sires can occur if the inseminations occur by 48 hr postovulation and preferably earlier. In this study, 10 pups were obtained from the first mating and 11 pups from the second mating in the three pregnancies in

Table 2. Contribution of first or second mating on litter composition

Bitch No.	No. of pups		Relationship of 2 <sup>nd</sup> mating to ovulation (time in hr)
	1 <sup>st</sup> mating	2 <sup>nd</sup> mating	
B3	4	2	+12
B8	3	4	+12
B10	3	5	+48
365	4	1	+48
371	4	2	+48
B2	3	1	+60
B7	4	2	+60

which the second mating was done 12, 12 and 48 hr after ovulation. These results reaffirm the previous findings of the senior author concerning the time of formation of the first polar body, i.e. between 48 and 60 hr post ovulation [4]. Because formation of the first polar body must occur before fertilization takes place, it is clear that it had not yet occurred in 2 dogs at 60 hr post ovulation (B2<sup>1</sup> and B7) when insemination at this time was able to result in the fertilization of oocytes. It is also clear that formation of the first polar body had occurred prior to 72 hr post ovulation when none of the second inseminations done at this time produced pups. Because ovulation is usually completed within a few hours in the dog [2], there to be appear some variation in

the time of the formation of the first polar body. The results from animal B10 (3 pups from the first insemination and 5 pups from the second) suggest the first polar bodies had not been formed as yet prior to the second insemination at 48 hr. However, in two other animals inseminated for the second time at 48 hr (365 and 371), more pups were produced from the first insemination [8] than the second [3]. This suggests that some polar bodies had been shed by 48 hr. Again, the fact that there is not a clear shift in the data collected at 48 and 60 hr (second insemination) suggests that there might be a significant variation (perhaps 12 hr) in the time when the first polar body is formed, even within an animal.

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#### 要 約

犬における実験的同期複妊娠について(短報): 筒井敏彦・江島博康(日本獣医畜産大学, 獣医臨床繁殖学教室) — 雌犬21頭に異なる雄犬と2回交配させ、子犬の雄親を判定することによって同期複妊娠の成立状況を検討した。開腹手術によって観察した卵胞の成熟状態から排卵時期を推定して、排卵36時間前から排卵84時間の間に24~96時間の間隔で2回交配させ、娩出された子犬の血液型および体型で雄親を判定したところ、2回目の交配を排卵後60時間までに行なった場合に同期複妊娠が成立することがわかった。