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Intraperitoneal Insemination of the Guinea Pig with Synchronized Estrus Induced by Progesterone Implant

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Abstract: Female guinea pigs with synchronized ovulation by means of implantation of progesterone-filled tubing (P-tube) followed by a progesterone injection, were inseminated by intraperitoneal injection with sperm suspension. First, to obtain the optimum conditions for insemination, the females were inseminated singly over the range of $1-10 \times 10^7$ spermatozoa before and after the synchronized ovulation. The incidence of conception and implantation was 100% in the females given more than 5×10^7 /animal at 9:00 h on the 5th day after removal of the P-tube. Second, the reproductive ability of the inseminated females under this optimal condition was observed throughout the pregnancy to delivery. Inseminated females had a mean $\pm$ S.D. gestation period of 68.7 ± 0.5 days, a litter size of 2.8 ± 0.6 pups and body weight of 110 ± 14 g. These data were comparable to those of naturally-mated females. Our findings suggest that the artificial insemination by intraperitoneal injection in combination with the synchronized estrus technique is very useful for production control in a small colony of guinea pigs.

Key words: artificial insemination, guinea pig, synchronized ovulation

There are two typical techniques for artificial insemination in guinea pigs: intravaginal [3] and intraperitoneal [10] administrations of sperm. Artificial insemination by the latter intraperitoneal injection was developed by using an anatomical characteristic: in the ovarian bursa there is an opening in the ventral portion of the bursa in guinea pigs. For this reason, sperm cells injected intraperitoneally can pass through the opening in the bursa and into the oviduct. It is reported that intraperitoneal injection has been shown

to be a simple method of insemination which produces a high conception rate in guinea pigs, but the intraperitoneal injection method of insemination in this species cannot be claimed to have practical applications, because it is necessary 1) to obtain spermatozoa from the epididymis surgically, and this involves killing males; and 2) to maintain a large colony to collect many females at the same estrous stage. It has therefore not been a useful tool for the reproduction of guinea pigs [9].

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We have previously demonstrated that long-term implantation of progesterone-filled tubing (P-tube), done at any estrous stage of female guinea pigs, induces the synchronized ovulation within 5–6 days after removal of the P-tube [7]; and additional progesterone injection at 13:00 h on the 4th day after the removal of the P-tube concentrates the ovulation time more closely at a fixed time [13].

It may be considered that this synchronizing technique is a useful method to supplement the practical application of artificial insemination by intraperitoneal injection. In this study, we tried to investigate the optimum conditions for artificial insemination by intraperitoneal injection in guinea pigs.

The adult female guinea pigs (Crj: Hartley, weight 500–900 g) used in the present study were purchased from Charles River Japan, Inc. Adult male guinea pigs of the Hartley strain (weight 575–925 g), which were maintained as a closed population in our laboratory, were used for sperm collection and mating. The animals were kept under controlled light (12 hr light, 12 hr dark; light on at 07:00) and temperature (at $24 \pm 2^\circ\text{C}$). They were provided with commercial pellets (GC4, Oriental Yeast Co., Inc.) and water *ad libitum*. The animals used in the present studies were reared, treated, and killed in accordance with the provisions for animal welfare in The Institute of Environmental Toxicology which followed the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science [6].

Synchronization of the estrous cycle in guinea pigs was induced by implantation of the P-tube as described previously [7, 13]. Females received a subcutaneous implant (Silastic tubing, 1 cm long, 0.4 cm i.d.) for 2 weeks. Additionally, 0.5 mg progesterone/0.1 ml corn oil/animal was administered subcutaneously at 13:00 h on the 4th day after the removal of the P-tube, in order to synchronize the ovulation time more closely at a fixed time.

Spermatozoa taken from the ductus deferens and the caudal epididymis of freshly killed males were prepared and used for insemination in accordance to the methods of Rowlands [10] and Blandau and Young [3]. That is, the ductus deferens and caudal epididymis were removed and placed in Baker's solution [1] of the following composition: glucose 3.0 g; $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 0.298 g; NaCl 0.2 g; KH_2PO_4 0.03 g; distilled water

100 ml. The contents of the ductus deferens of each animal were squeezed out with forceps into a Baker's solution. The caudal epididymis was cut with scissors until a thick and milky suspension of spermatozoa was obtained. These sperm suspensions from the caudal epididymis and the ductus deferens were mixed in one tube/one male. Preparation of sperm suspensions was conducted within 30 min. The sperm concentration was measured with a Thoma type haemocytometer and adjusted to about 1×10^8 sperm cells/ml with Baker's solution. The mean ($\pm$ S.D.) number of spermatozoa collected from one male was $82.3 (\pm 12.3) \times 10^7$.

Female guinea pigs with synchronized estrus were injected intraperitoneally with sperm suspension according to the method of Rowlands [10] at a volume of 0.5–1 ml/animal. The sperm suspension was taken up into a sterilized syringe with a 23 gauge needle and injected immediately into the abdominal cavity of the female. The animal was inclined head downwards and the insemination conducted from a mid-ventral point about 2 cm in front of the teats.

Experiment 1: The following 7 groups were subjected to examination of the conception rate. Females in 4 groups received the artificial insemination at 09:00 h and 17:00 h on the 4th or 5th day after removal of the P-tube at a concentration of 5×10^7 sperm cells/animal; and females in the another 3 groups received artificial insemination at 09:00 h on the 5th day at different concentrations of 1, 3, and 10×10^7 sperm cells/animal. The time schedule of the artificial insemination is shown in Fig. 1. Treated females were killed 10 days after insemination. In addition, 6 intact females were mated naturally with adult males and killed 10 days after copulation as the control group. The numbers of implanted conceptuses in these animals were recorded.

Experiment 2: Rates of pregnancy and delivery by artificial insemination and natural mating were examined. One group received the artificial insemination at 09:00 h on the 5th day after removal of the P-tube (Day 0 of pregnancy). Females in the other group were mated naturally with adult males. The day on which sperm were found in the vaginal smear and/or vaginal plug was designated as Day 0 of pregnancy. The duration of pregnancy, number of pups and body weights of pups were recorded.

Statistical evaluations of incidence in the control and inseminated groups were carried out with the Chi-square

Fig. 1. Time schedule of artificial insemination.

test. The Mann-whitney U test was employed to assess statistical differences in the duration of pregnancy, number of pups and body weights of pups in the inseminated and mated animals.

To obtain optimum conditions for the concentration of sperm inseminated and/or the timing of estrus inseminated by intraperitoneal injection, the following experiment (shown in Table 1) was carried out. First, a constant number of sperm (5×10^7 sperm cells/animal) were injected into female guinea pigs with synchronized estrus at different timing of estrus, before or after ovulation. The conception rates in the females given sperm cells at 09:00 h and 17:00 h on the 4th day after removal of the P-tube were 0% (0/6) and 50% (3/6), respectively. Then the conception rate reached 100% in the females given at 09:00 h on the 5th day, which was the same as that of the control group, and had decreased at 17:00 h on the 5th day (3/6, 50%). Implantation rates showed the same tendency as conception rates. The conception rates and implantation rates at 09:00, 17:00 on Day 4 and 17:00 on Day 5 were significantly ($P < 0.01$ or $P < 0.05$) lower than those in the control group. Second, females were inseminated at different concentrations of 1, 3, and 10×10^7 sperm cells at 09:00 h on the 5th day. The conception rates in the females inseminated with 1×10^7 and 3×10^7 were 14% (1/7) and 71% (10/14); and the implantation rates in these animals were 5 and 55%, respectively. Insemination with more than 5×10^7 at 09:00 h on the 5th day resulted in 100% conception and implantation rates. The conception rate of the group inseminated with 1×10^7 and the implantation rates of the groups inseminated with 1 and 3×10^7 were significantly ($P < 0.01$) lower than those in the control group.

Our previous report showed that the females given the same progesterone regimen as described in this study exhibited ovulation from 05:00 h to 09:00 h on the 5th day after removal of the P-tube [7]. The result of this experiment clearly shows that the optimal time for insemination is 09:00 h on the 5th day (when all ova have been ovulated), and the minimal number of spermatozoa required to evoke maximal fertility is 5×10^7 .

In experiment 2, females with synchronized estrus were inseminated with 5×10^7 sperm cells at 09:00 on the 5th day. On the other hand, intact females were mated as the natural mating control. The mean ($\pm$ S.D.) duration of pregnancy, number of pups and body weights of pups in artificially inseminated females (Table 2), 68.7 ± 0.6 days, 2.8 ± 0.6 days and 110 ± 14 g, were comparable to those of naturally mated females. There was no significant difference between the control and inseminated groups. In a previous paper, guinea pigs with synchronized estrous cycles treated with long-term implantation of progesterone were mated with adult males and delivered normally [14]. The gestation period, litter size and body weights of pups obtained from the above study were similar to those inseminated intraperitoneally in this study. These findings suggest that the reproductive ability of females artificially inseminated would not differ from the ability of naturally mated females.

The sperm of guinea pigs can be obtained a) by using an electro-ejaculation technique [4, 5], b) by flushing the genital tract of mated females [8], and/or c) by squeezing the caudal epididymis [3] and the ductus deferens [10]. Although sperm collection was performed many times in one male by methods a) and b), difficulty would seem to arise because semen solidifies very

Table 1. Effect of timing of insemination and number of sperm on the fertility of guinea pigs with synchronized estrus

Time of insemination (after removal of P. tube)	No. of sperm cells injected ($\times 10^7/\text{Anim.}$)	No. of pregnant animals/No. of animals inseminated	Conception rate ^{a)} (%)	No. of embryos implanted/No. of corpora lutea	Estimated implantation rate ^{b)} (%)
Day 4 9:00	5	0/6	0**	0/3, 0/4, 0/3, 0/3 0/3, 0/3	0**
Day 4 17:00	5	3/6	50*	1/3, 2/4, 0/3, 2/4 0/3, 0/3	25**
Day 5 9:00	1	1/7	14**	0/3, 0/3, 1/3, 0/2 0/3, 0/3, 0/2	5**
	3	10/14	71	4/4, 4/4, 3/3, 0/3, 2/3 1/3, 1/4, 0/3, 3/3, 2/3 2/3, 0/3, 2/2, 0/3	55**
	5	8/8	100	4/4, 3/3, 3/3, 3/3 3/3, 3/3, 3/3, 3/3	100
	10	4/4	100	3/3, 3/3, 2/2, 3/3	100
Day 5 17:00	5	3/6	50*	0/4, 4/4, 1/3, 0/4 0/4, 1/3	27**
Natural mating of intact animals		6/6	100	3/4, 3/3, 3/3, 4/4 4/4, 3/4	91

^{a)}: No. of pregnant animals/No. of animals inseminated $\times 100$ ^{b)}: No. of embryos implanted / No. of corpora lutea $\times 100$ * $P < 0.05$, ** $P < 0.01$ VS Natural mating of intact animals**Table 2.** Comparison of reproduction following intraperitoneal insemination in guinea pigs with synchronized estrous and natural mating in intact guinea pigs

Treatment	Mating method	No. of dam	Duration of the gestation (day) Mean $\pm$ S.D.	No. of pups delivered	Litter size Mean $\pm$ S.D.	Mortality of pups ^{a)} (%)	Sexual rate ^{b)} (%)	B.W. ^{c)} of live pups (g) Mean $\pm$ S.D.
Synchro- nization	AI	10	68.7 $\pm$ 0.5	28	2.8 $\pm$ 0.6	2/28 (7)	19/28 (68)	110 $\pm$ 14
Intact	Natural mating	12	69.1 $\pm$ 1.2	33	2.8 $\pm$ 1.0	2/33 (6)	19/33 (58)	105 $\pm$ 18

^{a)}: No. of dead pups/No. of pups delivered.^{b)}: No. of male pups/No. of pups delivered.^{c)}: Body weight.AI: Artificial insemination by intraperitoneal injection of 5×10^7 sperm.

rapidly and becomes unmanageable. For this reason, the sperm collected by these methods would not be satisfactory in a program of artificial insemination. It is therefore considered that collection of sperm by c) method might be suitable for artificial insemination in guinea pigs. In this study, approximately 8×10^8 sperm

cells were able to be collected from one adult male. When we apply our insemination method to guinea pigs with synchronized estrus, ideally sixteen females can be inseminated with sperm from one male.

Concerning to sperm-capacitation, Yanagimachi and Chang [15] first reported that mammalian spermatozoa

taken from the caudal epididymis can be capacitated *in vitro*. Toyoda *et al.* [12] also reported successful *in vitro* fertilization of mouse eggs with a simple "chemically defined" medium. In guinea pigs, Barros [2] found that the majority of guinea pig spermatozoa undergo the acrosome reaction in 1 to 4 hr when they are placed in a simple salt solution containing some energy sources. These experiments of successful *in vitro* fertilization surely indicate successful *in vitro* capacitation of spermatozoa taken from the caudal epididymis. And Rawlands [10] reported that proestrous intraperitoneal insemination of spermatozoa from the caudal epididymis was as successful as natural mating estrus when the insemination was carried out at 0 to 16 hr after ovulation. Taken together with these facts and our successful findings on artificial insemination at the time of ovulation, the spermatozoa of the caudal epididymis seem to be capacitated by passing through the abdominal cavity.

As mentioned above, since spermatozoa were already capacitated in the oviduct, some candidates for factors affecting the conception rate will be nominated from both sides: spermatozoa and ovulated ova. Sperm reaching the oviduct retain their fertilizing ability for about 22 hr in guinea pigs [11]. The aging of sperm cells was considered to occur gradually. As for the aging effect of ova, Blandau and Young [3] showed that the aging effect was first noted 8 hr after ovulation and normal embryo development was usually noted only in ova fertilized within 20 hr of ovulation in guinea pigs. The environmental condition at fertilization also is one of the factors affecting the conception rate. In general, freshly ejaculated sperm reach the ampullar region to meet ovulated ova, and a very high proportion of ovulated ova undergo fertilization under natural mating conditions. Nevertheless, our observation of previous unpublished data showed that ovulated ova located in the ampullar region at 09:00 on the 5th day were transported to the isthmus region at 17:00 on the 5th day after P-tube removal. In the present study low conception rates were noted when the animals were inseminated about half and 1 day before ovulation or about 8 hr after ovulation. It is considered that some factors, for example, the initial aging effects of spermatozoa and/or of ova, some difficulties in fertilization in the isthmus portion and/or the ability for embryo development, lead to a low conception rate. These

possible causes of low conception rates should be clarified in future experiments.

It is difficult to conduct artificial insemination in a large colony of guinea pigs at a scheduled time, because females have a long estrous cycle of about 16 days and/or semen for insemination is not available from living males. In order to solve this problem, in our present study, first, we prepared the females with synchronized ovulation by implanting a P-tube for 2 weeks followed by 0.5 mg progesterone injection; and second, we used a technique of artificial insemination by intraperitoneal injection of a suspension of sperm which were obtained from single sacrificed males. These inseminated females became pregnant and delivered as well as naturally mated females.

The results of this study suggest that artificial insemination would be a good method of reproduction even in guinea pigs, and the technique used in this study should be useful for scheduled programs of experiments in embryology, teratology, endocrinology and some other fields in this species.

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