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Effects of Superovulation and Early Embryonic Development by Pituitary Transplants

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뇌하수체 이식이 과배란 및 초기태아 발육에 미치는 영향

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초록 : 본 연구는 한개의 뇌하수체를 이식시켜 과배란된 미성숙 흰쥐에서 초기태아 발육과 착상효과를 관찰하기 위하여 시도되었다. 30일령 숫컷 흰쥐에서 뇌하수체를 제거하기 15일 전에 정소를 제거시켰으며 정소가 제거된 쥐에서 얻은 한개의 뇌하수체를 실험 시작일(임신 3일전 : D-2) 오전 7시에서 10시 사이에 28일령의 암컷 흰쥐의 우측 신장 피막 아래 이식시켰다. 대조군은 같은 날 오전 10시에 4 IU PMSG를 투여하였다.

실험에 사용된 쥐들은 난소 및 자궁 무게의 변화를 조사하기 위하여 임신 3일전, 2일전, 1일전, 임신 1일, 2일, 3일 및 5일에 희생시켰다. 또 다른 쥐들은 난 회수 및 난소를 관찰하기 위하여 임신 1일, 2일, 3일 및 5일에 희생시켰으며 임신 8일에는 착상 상태를 조사하였다. 임신 1일에는 질도말법에 의해 발정주기를 조사하였다.

본 실험에서 얻은 결과는 다음과 같다.

1. 뇌하수체를 이식시키거나 4 IU PMSG를 투여함으로써 발정 동기화를 이룰 수 있었다. 뇌하수체를 이식시킨 쥐와 대조군에서 임신 1일 전인 발정전기에 있는 흰쥐는 각각 64.7%, 71.3%이었다.

2. 뇌하수체 이식군 및 대조군의 교배율은 각각 75.0% 및 80.2%였으며 첫 배란은 각 처치후 3일 이내에 일어났다.

3. 과배란된 흰쥐에서 임신 1일에 황체화된 난포와 임신 황체수는 평균 46.1±2.9개였으며 임신 2일부터 그 수는 임신 1일 보다 많았다.

4. 과배란된 흰쥐에서 회수된 난의 수는 임신 1일과 2일에 각각 평균 46.1±2.9 및 49.8±4.2개였으며 대조군은 8.6±0.3 및 8.9±0.4개로 나타났다($p<0.001$).

5. 임신 3일부터 과배란된 흰쥐에서 난의 회수율은 임신 2일과 비교할 때 현저하게 감소되었으며 ($p<0.001$) 난의 발육은 지연되거나 퇴행되었다. 난 발육단계의 분포는 임신 3일과 5일에 특히 변이가 많았다.

6. 과배란된 흰쥐에서 임신 8일에 착상 부위수는 대조군에 비해 현저한 증가를 보였으며($p<0.001$), 18마리의 과배란된 흰쥐 중 특히 10마리에서는 28.1±0.7개의 착상부위가 확인되었다.

7. 과배란된 흰쥐의 난소 무게는 임신 1일전부터 임신 3일까지 계속 증가하였으며 대조군은 이 기간동안 큰 변화가 없었다.

Introduction

The induction of superovulation in immature rats by exogenous gonadotrophins has frequently been used as a model for studying the superovulatory mechanisms in adult rats.

Pituitary transplantations from male donors under the kidney capsule of immature female rats were also capable of advancing the day of vaginal opening, the first ovulation and puberty in immature female rats.[1,2,20,22,25] A marked increase in plasma follicle-stimulating hormone(FSH) was maintained at least 12 hrs after the transplantation, and these rats showed preovulatory surges of luteinizing hormone(LH) and FSH at 54 hrs after grafting.[22] But the transplantation of the cyclic female pituitary gland did not induce superovulation because of the insufficient contents of LH and FSH in the gland.[19]

In immature female rats received a pituitary gland, prolactin appears to advance the onset of puberty by acting on a central nervous system site and also by increasing the sensitivity of the ovary to gonadotrophins.[1,8,25]

Excessive dose of exogenous gonadotrophins to induce superovulation may be associated with the reduced fertility resulting from excessive follicular stimulation, which may cause loss of early embryos, abnormal development and degeneration of embryos, implantation failure, and polyploidy and chromosomal abnormalities in mice,[4,5,11,14] rats,[6,15,17,18,26] and rabbits.[10] The abnormal development and degeneration of early embryos in the superovulated rats treated with PMSG are resulted from the abnormal ovarian hormone levels, especially the hypersecretion of estrogen after the time of fertilization.[18] Gonadotropin release may be facilitated by the synergistic effect of estrogen and progesterone.[12] Likewise the superovulatory treatments alter the ovarian steroid hormonal *milieu* after ovulation resulting abnormal oviductal and uterine function.

It is important to establish whether the early embryos recovered from rats receiving a pituitary gland are capable of normal development and implantation in an appropriate genital tract. There appears to be no reports until the present time indicating the early embryonic development in the superovulated rats by transplantation of a pituitary gland.

Therefore the present study was undertaken to investigate the early embryonic development and implantation in immature female rats transplanted a pituitary gland into the subcapsular region of the kidney.

Materials and Methods

Animals: Sprague-Dawley rats, 28~30 days of age, were housed 3 or 4 per cage at the room temperature range of 20~24°C and 12 hrs light cycle and were fed on a pellet diet(Samyang Co.) and tap water *ad libitum*.

Induction of superovulation: Thirty-day-old male rats were performed orchidectomy under Ketamine hydrochloride(Yuhan Co.) anesthesia (50mg/kg, IM) and were adapted again to controlled conditions for 15 days. A pituitary gland removed from male rats 15 days after orchidectomy(at 45 days of age) was immediately transplanted into the subcapsular area of the right kidney of 28-day-old female rats. This day of the experiment was designated as Day -2. To induce normal ovulation and gestation, 4 IU PMSG (Intervet) was administered subcutaneously to the control rats at 1000 h on Day -2.[15,16] Vaginal smears were taken to determine the estrous cycle daily after vaginal opening and examined microscopically (AO, 100x magnification) between 1500 and 1600h on Day 0. The animals with opened vagina and at proestrus cycle were caged with fertile proven Sprague-Dawley males at 1730 h of Day 0.

Development of early embryos: At the following morning (Day 1) females were scored for the occurrence of mating between 0800 and 0900h. The mated rats were allotted at random to groups to be sacrificed on Days 1, 2, 3, 5 and 8. The ovaries were dissected from oviducts and adjacent adipose tissues, paired, blotted, weighed and examined with a stereoscopic microscope(AO) at 20x magnification for counting the number of follicles and corpora lutea of pregnancy. The ova in oviducts were collected in an egg bowl between 1500 and 1600h on Days 1, 2 and 3. Each uterus was flushed with 0.6ml of 0.9% NaCl

solution to recover the ova in uterus between 1500 and 1600 h on Days 3 and 5 and then blotted and weighed.

The ova were counted in a watch glass with 0.9% NaCl solution under a stereoscopic microscope (40x magnification). The ova were also examined under a binocular microscope (AO) at 100x magnification and under a phase contrast microscope (AO) at 200x magnification to differentiate normal ova from abnormal ones.

The other mated rats were sacrificed between 1500 and 1600h on Days 5 and 8 to ascertain the implantation sites. The number of implantation sites(blue bands and/or constriction rings) and corpora lutea of pregnancy were recorded. The data of the experiments were analyzed by student's t-test and the one-way analysis of variance.

Results

Estrous cycle and mating rate: In the rats bearing a pituitary gland from orchidectomized rats and the control rats receiving 4 IU PMSG on Day 0, 64.7% and 71.3% of the rats were in proestrus and 17.9% and 21.7% of the rats in estrus respectively at 1500~1600h on Day 0. Furthermore mating rate of Day 1 were 75.0% in the rats bearing a pituitary gland and 80.2% in the rats receiving 4 IU PMSG, respectively(Table 1).

Development and degeneration of early embryos: In the rats transplanted a pituitary gland from castrated males, the number of corpora lutea of pregnancy was increased significantly on Days 1, 2, 3 and 5

Table 1. Estrous Cycle On Day 0 and Mating Rate On Day 1 in the Rats Transplanted a Pituitary Gland and Administered 4 IU PMSG

Treatment	No. of rats	Estrous cycle on Day 0(%)				Mating rate(%) on Day 1
		Diestrus	Proestrus	Estrus	Metestrus	
PGT	207	17.3	64.7	17.9	0	75.0(123/164)
4 IU PMSG	129	7.0	71.3	21.9	0	80.2(77/96)

* Number in parenthesis: Number of mated rats/Number of female rats housed with male rats. PGT: Pituitary gland transplantation.

Table 2. Effects of Transplantation of a Pituitary Gland and Administration of 4 IU PMSG on Recovery Rate of Ova(mean±SEM)

	Day of pregnancy			
	1	2	3	5*
PGT				
No. of rats	12	12	17	12
No. of CLP+No. of LF[a)]	46.1±2.9[c)]	49.8±4.2[c)]	49.3±3.7	49.7±7.2
No. of ova recovered[b)]	44.8±3.8[d)]	49.3±4.4[d)]	39.1±4.4[d)e)]	29.0±3.6[e)]
Oviducts	44.8±3.8	49.3±4.4	38.4±4.3	2.6±2.6
Uteri	0	0	0.7±0.4	26.4±3.7
4 IU PMSG				
No. of rats	9	10	10	11
No. of CLP+No. of LF[a)]	8.6±0.3	8.9±0.4	8.4±0.4	8.9±0.4
No. of ova recovered[b)]	8.1±0.1	8.4±0.6	8.0±0.4	8.7±0.5
Oviducts	8.1±0.1	8.4±0.6	7.8±0.5	0.3±0.3
Uteri	0	0	0.2±0.2	8.4±0.5

*: Number of ova on Day 5=Number of flushed ova from reproductive tracts+Number of blue bands and/or constriction rings indicating implantation sites.
CLP: Corpora lutea of pregnancy. LF: Luteinized follicles. PGT: Pituitary gland transplantation.
a), b), c), d), e) : $p<0.001$. The same superscripts mean significant difference from each other.

Table 3. Development of Early Embryos in Immature Female Rats Transplanted a Pituitary Gland and Administered 4 IU PMSG

			Total No of ova[a]	1 cell[b]	2 cell[b]	3~4 cell[b]	8~32 cell[b]	Morula[b]	Blastocyst[b]	BB and /or CR[b]	Degeneration[b]
D_1	PGT	Oviducts	573(12)	512(95.3)	9(1.6)	6(1.1)	·	·	·	·	10(1.9)
	4 IU PMSG	Oviducts	73(9)	73(100)	·	·	·	·	·	·	·
D_2	PGT	Oviducts	591(12)	32(5.4)	502(84.9)	23(3.9)	·	·	·	·	34(5.8)
	4 IU PMSG	Oviducts	84(10)	9(10.7)	69(82.1)	3(3.6)	·	·	·	·	3(3.6)
D_3	PGT	Oviducts	665(17)	108(16.2)	235(35.3)	179(26.9)	29(4.4)	·	·	·	101(15.2)
		Uteri		·	2(0.3)	8(1.2)	·	·	·	·	3(0.5)
	4 IU PMSG	Oviducts	80(10)	6(7.5)	9(11.3)	60(75.0)	·	·	·	·	3(3.8)
		Uteri		·	2(2.5)	·	·	·	·	·	·
D_5	PGT	Oviducts	348(12)	17(4.9)	1(0.3)	·	·	·	·	·	13(3.7)
		Uteri		·	3(0.9)	10(2.9)	3(0.9)	31(8.9)	153(44.0)	13(3.7)	104(29.9)
	4 IU PMSG	Oviducts	96(11)	·	·	·	·	·	·	·	3(3.1)
		Uteri					1(1.0)	16(16.7)	59(61.5)	16(16.7)	1(1.0)

a) Number in parenthesis: Number of rats. b) Number in parenthesis: Percentages of cell stages.
BB: Blue band. CR: Constriction rings. PGT: Pituitary gland transplantation.

(mean range of 46.1~49.8, $p<0.001$) when compared with that of the rats received 4 IU PMSG (mean range of 8.4~8.9). The mean number of corpora lutea of pregnancy (46.1±2.9) in the superovulated rats on Day 1 was significantly lower than the mean numbers of corpora lutea (49.3~49.8) on the other days ($p<0.001$). The total number of ova recovered from oviduct (38.4±4.3) and uterine horn(0.7±0.4) was decreased remarkably($p<0.001$) on Day 3 when compared with that of on Day 2. The total number of ova recovered from oviduct(2.6±2.6) and uterine horn(26.4±3.7) on Day 5 was decreased greatly when compared with that of previous days ($p<0.001$) as shown in Table 2. The percentages of ova recovered in 1 cell stage on Day 1 were 95% in the rats transplanted a pituitary gland and 100% in the rats receiving 4 IU PMSG and those of in 2 cell stage on day 2 were 85% and 82% respectively, showing that the number of ova lost, degenerated or retarded up to this stage was small (Table 3). In the rats transplanted a pituitary gland the stages on Day 3 were variable compared with those of 4 IU PMSG-treated rats. About 16% of the ova recovered from superovulated animals were retarded or degenerated on Day 3. The ova and/or embryos recovered on Day 5 in the superovulated rats showed higher variability in their stages from 1 cell stage to blue band and/or constriction ring than in the rats received 4 IU PM SG. Especially the degeneration rate in the transplanted group (34%) was higher than 4 IU PMSG treated group (4%).

Implantation: As shown in Table 4, there was a significant increase ($p<0.001$) in the number of implantation sites per rat (18.1±4.0) compared with that of rats received 4 IU PMSG (8.5±3.2). The mean number of implantation sites on Day 8 (18.1±4.0) was significantly lower ($p<0.001$) than the mean number of ova and/or embryos recovered on Day 5 (29.0±3.6). However, 10 rats out of 18 superovulated rats particularly showed 25 to 32 implantation sites per rat and the mean of them (28.1±0.7, $n=10$) showed higher proportion ($p<0.001$) than the mean of implantation sites of 18 rats (18.1±4.0, $n=18$).

Changes of uterine and ovarian weights: The uterine weight per 100 g of body weight (113±3.1mg) at 12hrs after the transplantation increased nearly twofold (217±8.4mg) on Day 0. It was changed a little from Day 0 to Day 1 and followed by a decline. The uterine weight of control rats was remarkably

Table 4. Effects of Transplantation of a Pituitary Gland and Administration of 4 IU PMSG on Implantation on Day 8

Treatment	No. of rats	No. of CLP +No. of LF (mean±SEM)	No. of IM sites (mean±SEM)	No. of IM occurrences		No. of degeneration sites	Total No. of IM sites
				BB	CR		
PGT	18*	47.1±4.9[a)]	18.1±4.0[b)]	63	262	1	326
4 IU PMSG	11	8.9±0.3[a)]	8.5±3.2[b)]	25	66	2	93

*: Ten rats out of 18 rats had 28.1±0.7 implantation sites per rat.
BB: Blue band. CLP:Corpora lutea of pregnancy. CR: Constriction rings.
IM: Implantation. LF: Luteinized follicles. PGT: Pituitary gland transplantation.
a), b): $p<0.001$. The same superscripts mean significant difference from each other.

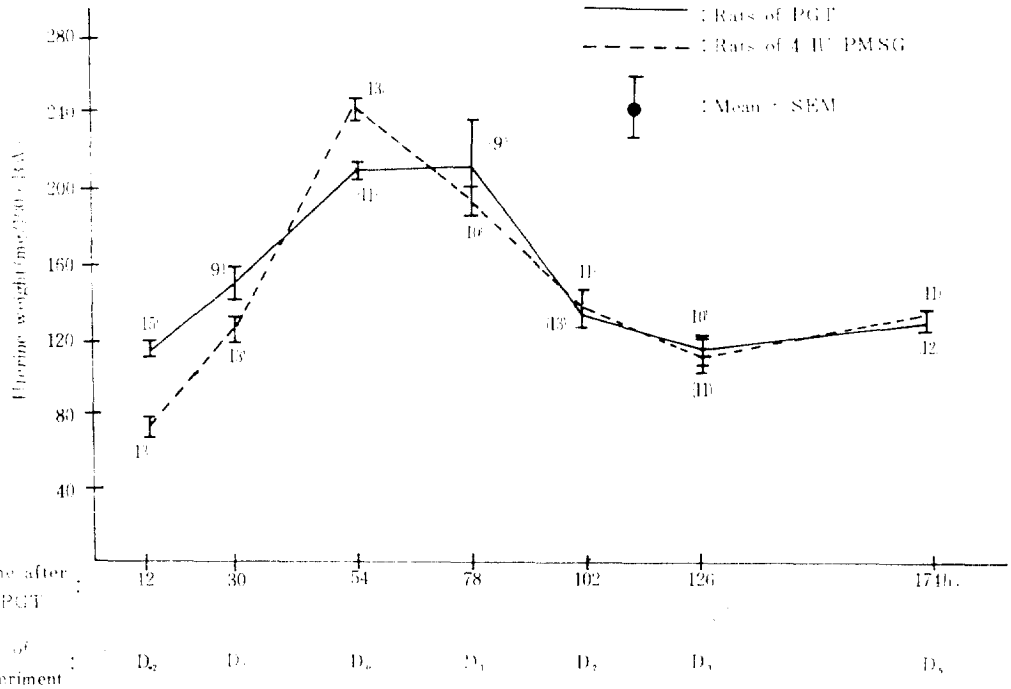

Fig 1. Changes of uterine weight in immature female rats transplanted a pituitary gland and administered 4 IU PMSG.
Number in parenthesis: Number of rats. PGT: Pituitary gland transplantation.

increased from 74mg on Day -2 to 243mg on Day 0, reaching a peak and then it was decreased linearly until Day 2. The pattern of variation between Days 2 and 5 was almost the same in both groups(Fig. 1). In the control rats the ovarian weight was changed within a limited level between Days -2 and 1 and after then there was a tendency to decrease slightly until Day 5. In the superovulated rats, the ovarian weight was about 40mg between 12 and 30 hrs after the transplantation, but the ovarian weight after Day -1 continued to increase, reaching a peak of 95mg on Day 3 and then tended to decline until Day 5(Fig. 2). There were significant differences ($p<0.001$) between two groups in all cases except Day -1 ($p<0.005$).

Discussion

The results demonstrated that the superovulation

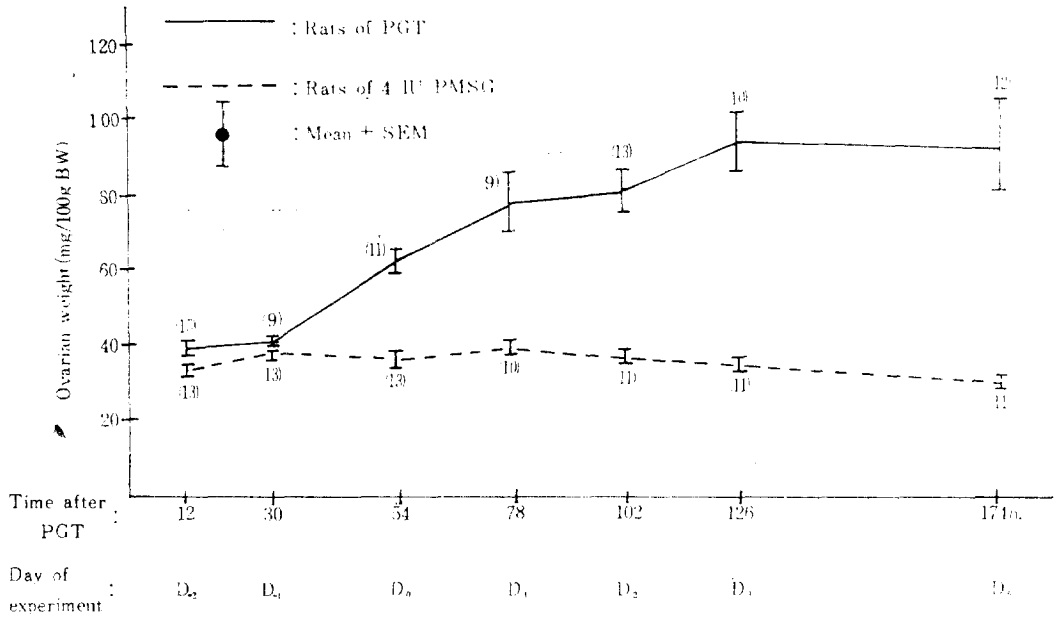

Fig. 2. Changes of ovarian weight in immature female rats transplanted a pituitary gland and administered 4 IU PMSG.
Number in parenthesis: Number of rats. PGT: Pituitary gland transplantation.

could be induced atleast within 3 days after the transplantation and did not disturb proestrus on Day 0 and estrus on Day 1.

Advis and Ojeda[23] reported that FSH, LH and prolactin were released spontaneously from the grafted pituitary gland of an adult male rat within 2 hrs after transplantation into female rat. Also they reported that gonadotrophin levels were not elevated by the pituitary gland of host, as a consequence of stress of the surgical procedure, but by the transplanted pituitary gland. It was confirmed by the fact that the sham-grafted animals with a piece of muscle did not show such an increase.

According to the report of Sameshima *et al*[19], the development of many follicles in the transplanted rats is mainly due to the gonadotrophins released by the grafted pituitary gland. The facts suggest that the large amounts of FSH and LH released from the pituitary gland transplanted under the kidney capsule of immature female rat could sufficiently stimulate the growth of many follicles for superovulation and result in releasing of estrogen and progesterone from the follicles.

Welschen and Rutte[24] showed that the absence of ovulation after treatment of PMSG alone in adult cyclic female rats was due to an inhibition of preovulatory LH surge, but the pituitary transplants had no inhibitory effect on preovulatory LH surge.[19] Thus, no additional HCG or LH injection was needed in this experiment to induce superovulation because of the large amounts of LH in the grafted pituitary gland.

In the present study most of the immature rats transplanted a pituitary gland or received 4 IU PMSG exhibited proestrus stage in about 54 hrs after each treatment and followed by estrus next day. This fact suggests that the synchronization of estrous cycle and the induction of ovulation are possible by either treatment. A retardation in growth and degeneration of the ova recovered in the superovulated rats were observed on Day 3. The recovery rate of ova was decreased drastically on Days 3 and 5. On Day 5 the number of degenerated cells in the superovulated rats increased higher(33.6%) than those in the control rats(4.1%). And the cells were developed up to blastocyst and blue band and/or constriction

ring were less in the superovulated rats(47.7%) than those in the control rats (78.2%). The number of premature ova arriving in the uteri on Day 5 showed fewer in superovulated rats (13.6%) than that of control (17.7%) with the exception of degenerated cells. Such premature exposure to uteri may be deleterious to early embryonic survival. The loss of viability may have resulted from the metabolic deprivation due to a decrease in the protein contents of uterine fluid and/or by changes in the protein structure.[3,21] These ova may be degenerated, but are usually promptly expelled from the uterus via the cervix.[6,9] Butcher and Pope[7] reported that the development of the rat embryos to blastocyst stage was retarded in animals which have been exposed to high serum estradiol for an extended preovulatory period. Miller and Armstrong[16] noted that there was the rapid loss of ova from the oviducts and uteri of superovulated rats treated with 40 IU PMSG which were probably caused by the high serum estradiol levels between Days 0 and 3. All the above studies suggest that the high levels of plasma estrogen in peri-ovulation and early pregnancy may provide both detrimental oviductal and uterine environments.

In the rats receiving 40 IU PMSG on Day 5, no ova and/or embryos were found in the uterus[26] and a few ova could be recovered.[13,14,16] But in this study the recovery rate of ova on Day 5 was considerably higher in the superovulated rats transplanted a pituitary gland than that of 40 IU PMSG treated rats. Likewise, these rats showed also a very high implantation rate (8.1±4.0) on Day 8 when compared with the complete infertility in the rats treated with 40 IU PMSG.[26] Especially 10 rats, out of 18 rats used to this experiment showed 25 to 32 implantation sites per rat.

Conclusions

The present study was undertaken to investigate the development of early embryos, the implantation rate in immature rats superovulated by transplantation of a pituitary gland.

Thirty-day-old male rats were performed orchidectomy 15 days before removal of each pituitary gland. A pituitary gland was transplanted under the right kidney capsule of 28-day-old female rat between 0700 and 1000h on the starting day of experiment which was designated as Day -2. Control rats were administered 4 IU PMSG at 1000 on day -2. Some of rats used to experiment were sacrificed to examine the changes of ovarian and uterine weights on Days -2, -1, 0, 1, 2, 3 and 5. Other rats were sacrificed to recover the ova and observe the ovaries on Days 1, 2, 3 and 5. The implantation sites were counted on Day 8. The results were summarized as follows.

1. The synchronization of estrous cycle could be achieved by transplantation of a pituitary gland or administration of 4 IU PMSG. On Day 0, 64.7% of the rats bearing a grafted pituitary gland and 71.3% of the rats receiving 4 IU PMSG were in the stage of proestrus.

2. The mating rate of immature rats receiving a pituitary gland and 4 IU PMSG were 75.0 and 80.2 %, respectively. The rats showed the first ovulation within 3 days by either treatments.

3. The number of luteinized follicles and corpora lutea of pregnancy in the superovulated rats was 46.1 ±2.9 on Day 1 and from Day 2 onwards the number was higher (49.3~49.8) than that of Day 1($p<0.001$).

4. The mean number of the recovered ova in the superovulated rats was 46.1±2.9 and 49.8±4.2 on Days 1 and 2, respectively. The number in control rats was 8.6±0.3 and 8.9±0.4, respectively.

5. From Day 3 onwards the recovery rate of ova in the superovulated rats was decreased remarkably ($p<0.001$) when compared with those of Day 2. The development of ova was retarded or degenerated gradually. The distribution of their developmental stages was variable especially on Days 3 and 5.

6. The number of implantation sites per rat (18.1 ±4.0) on Day 8 in the superovulated rats was increased significantly when compared with control rats. Particularly ten rats out of 18 superovulated rats showed 28.1±0.7 implantation sites per rat.

7. The ovarian weights of superovulated rats were increased continuously from Day -1 to 3, but those of control rats did not show significant change during this interval.

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