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# EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ ON EGG RECOVERY FROM THE VAGINA AND EGG TRANSPORT IN SUPEROVULATED RABBITS

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## Introduction

When 5 mg/kg of body weight of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) was injected subcutaneously in rabbits, 21 to 27 hours after mating, high egg recovery rates (62.6 to 82.7%) were attained by multiple vaginal washings from 1 to 36 hours after injection.<sup>15)</sup> In superovulated rabbits approximate 44% of eggs ovulated were recovered by multiple vaginal washings from 24 to 192 hours after mating. The total number of eggs recovered from the vagina per doe was 36.6 in the study.<sup>20)</sup> Accordingly, it was speculated that an administration with prostaglandin  $F_{2\alpha}$  to the superovulated doe might have a more powerful effect on the egg recovery from the vagina compared with each treatment alone.

## Materials and Methods

Fifty-five Japanese adult female white rabbits used in Experiments I and II, were superovulated by intramuscular injection of 200 IU PMSG (Serotropin, Teikoku-zōki, Japan), followed by mating with intravenous injection of 100 IU HCG (Primogonyl, Schering) 80 hours later.

In Experiment I, 30 superovulated does were divided into 6 groups depending on the time and dose of  $PGF_{2\alpha}$  administration (Table 1). The vagina of these does was washed 1, 2, 3, 6, 9, 12, 24, 36 and 48 hours after  $PGF_{2\alpha}$  administration and eggs in the fluid recovered from the vagina were located using dissecting microscope according the method reported previously.<sup>16)</sup> The animals were laparotomized 9 days after mating, and the numbers of corpora lutea and sites of implantation were recorded.

In Experiment II, 25 superovulated does were received 5 mg/kg of PGF<sub>2α</sub> 24 hours after mating, and were separated into 5 groups depending on the time of sacrifice by an overdose of sodium pentobarbital (Somnopenyl) 1, 3, 6, 12 or 24 hours after PGF<sub>2α</sub> treatment. The vagina of these does was washed 1 to 7 times before sacrificing (see schedule in Table 2). Immediately after the final vaginal washing, the does were sacrificed to investigate the distribution of eggs in the oviduct and uterine horn. The abdominal wall was opened immediately after killing, most of the digestive tracts were removed quickly, and the whole reproductive organ was frozen *in situ* by pouring liquid nitrogen into the abdominal cavity, as described by HOWE<sup>10</sup>. After thawing, the oviduct and the uterine horn with the cervix were excised *en bloc*, trimmed, and cleared in benzyl benzoate according to the clearing technique by ORSINI<sup>11</sup>. Eggs in the cleared oviduct, uterus and cervix were located under a dissecting microscope with transmitted light. Locations of eggs in the oviducts or uteri were expressed by the percentage of distance travelled from the ostium of oviduct or from the uterotubal junction (Fig. 3). Corpora lutea were counted after removing the reproductive tract.

## Results

### Experiment I

Numbers and developmental stages of eggs recovered from the vagina are illustrated in Figs. 1 and 2 according to experimental groups and hours after PGF<sub>2α</sub> administration. The earliest eggs were recovered from the vagina one hour after treatment in most of the groups, similar to the previous experiment of PGF<sub>2α</sub> administration on normally ovulated does<sup>10</sup>. Recovery rate of vaginal eggs was higher in the group of 36 hours administration, compared with the groups of 20 and 24 hours administration (Table 1). In the groups of 20 and 24 hours administration, recovery rates of eggs were lower in cases of 2 mg/kg of PGF<sub>2α</sub> administration than in cases of 5 mg/kg, and most of the eggs were recovered after 12 hours following administration. In groups of 5 mg/kg administration 20 or 24 hours after mating, however, the recovery rates reached about 30% in both groups and numbers of eggs recovered per doe were about 11 for the group of 20 hours administration and 16 for the group of 24 hours administration. In the group of 36 hours administration, the numbers of eggs recovered per doe were 24 for the group of 2 mg/kg administration and 15 for the group of 5 mg/kg. No significant difference between recovery rates of eggs for the group of 2 mg/kg administration (39%) and that for 5 mg/kg (35%) was noted as those in groups of 20 or 24 hours administration.

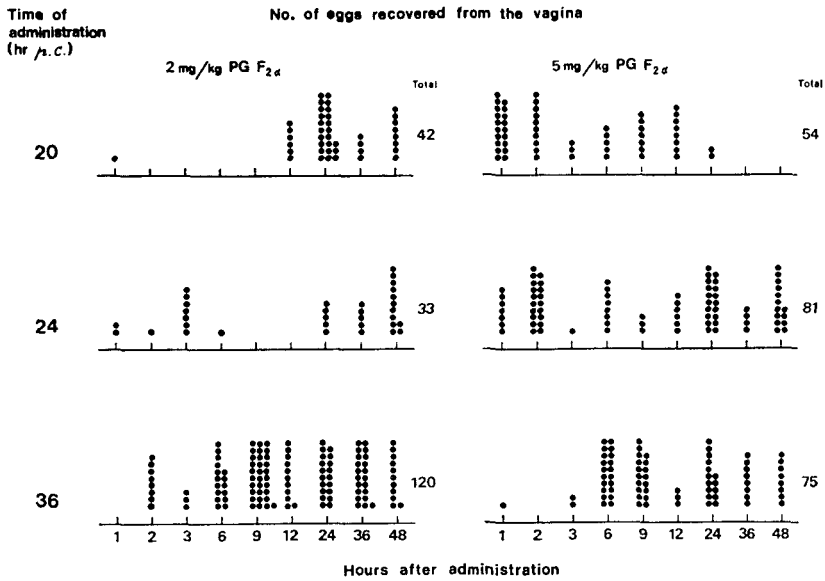


Fig. 1. Number of eggs recovered from the vagina of superovulated rabbits each hour after administration of  $PGF_{2\alpha}$ .

Morphology of eggs	Time of vaginal washing after $PGF_{2\alpha}$ administration (hr)									Total no. of eggs	
	1	2	3	6	9	12	24	36	48		
Undivided	●●●●●●●● ○○○○○○○○	●●●●●●●● ○○○○○○○○	●● ○○	●● ○○	●●●● ○○○○	●●●● ○○○○	●●● ○○○	●●●●●●●●●● ○○○○○○○○○○	●●●●●●●● ○○○○○○○○○○	●● ○○	91
2-cell	○	●●●●●●●● ○○○○○○○○	●●	●● ○○	●●	●					25
4-cell	▲	○	○ ▲▲	○○○	○○○	○○○○○○○○					24
8-cell		●●●●●●●● ▲▲▲▲▲▲	▲	●●●●●●●● ▲▲▲▲▲▲	●●●●●	●● ○○ ▲	●●●●●●●● ○○○○○○○○	●●			56
16-cell			▲▲	●●●●●●	●●●●●●●● ▲▲▲▲▲▲	●●●●●●●● ▲▲▲▲▲▲	●●●● ○○○ ▲▲▲	●			57
32-cell						▲	●●●●●●●● ▲▲▲▲▲▲	○○○	●● ○		22
Morula							▲	○○ ●●●●●●●●	●●●●●●●● ○○○○○○○○○○	●●●● ○○○○○○○○○○	29
Blastocyst									▲▲▲▲		5
Degenerated and/or fragmented		●	○	● ○○	●●●● ○○○○	● ○○	●●●●●●●● ▲▲▲▲	● ○○	●● ○○○○○○○○○○	●● ○○○○○○○○○○	81
Empty zona pellucida	●	○	○				●●●●●●●● ○○○○○○○○○○				15

Fig. 2. Developmental stages and numbers of eggs recovered from the vagina of superovulated does following  $PGF_{2\alpha}$  administration 20 hours after mating (●), 24 hours after mating (○) and 36 hours after mating (▲).

TABLE 1. Dosage of PGF<sub>2α</sub> and vaginal egg recovery in superovulated rabbits

Time of administration (hr after mating)	Dose of PGF <sub>2α</sub> (mg/kg)	No. of does	Time of vaginal washing (hr after mating)		No. of corpora lutea	No. of eggs recovered	Recovery rate (%)
			The first—	The last	Mean (range)	Mean (range)	Mean (range)
20	2	5	21—	68	53.6 (32-78)	8.4 (0-35)	14.5 (0-61.4)
	5	5			42.6 (26-47)	10.8 (0-25)	29.5 (0-58.1)
24	2	5	25—	72	82.0 (43-100)*	6.6 (1-15)	10.8 (0.5-28.6)*
	5	5			51.8 (40-71)	16.2 (8-25)	29.9 (17.5-47.1)
36	2	5	37—	84	65.0 (34-100)*	24.0 (5-56)	39.0 (14.7-70.6)*
	5	5			40.8 (18-39)	15.0** (5-35)	34.5 (17.0-55.6)

\* Two does in the 24 hour administration group and one doe in the 36 hour administration group had over a hundred corpora lutea and their number of corpora lutea were designated as 100 for calculation, because of the difficulty in counting.

\*\* Because one doe in this group urinated habitually at each vaginal washing, 4 times out of 9 vaginal washings were useless.

## Experiment II

The number of eggs recovered from the vagina per hour before sacrificing is shown in Table 2. Lower recovery rate of eggs from the vagina in groups of 12 or 24 hours after treatment was noticed when compared with that in group of 5 mg/kg administration 24 hours after mating in Experiment I. Changes in the distribution pattern of eggs located in the reproductive tract are illustrated in Fig. 3 in relation to time in hours after PGF<sub>2α</sub> administration, and the number of eggs found in the oviduct and uterus is shown in Table 3. If eggs found in the uterine horn or cervix and/or in the vagina are left out of consideration, the distribution of eggs of the does one hour after administration had a mode near the ampullary-isthmic junction of the oviduct, and the pattern of distribution of eggs was very similar to that in normally ovulated does 24 hours after mating,<sup>18</sup> in spite of the wide distribution after PGF<sub>2α</sub> administration. At 24 hours after administration (48 hours after mating) most of the eggs were distributed in the proximal half of isthmus and the mode of the distribution was in segment 7 of the isthmus. Such a distribution pattern was very similar to that in the normally ovulated does 66 hours after mating.<sup>18</sup> Relatively few eggs were found in the uterine horn and cervix, although many eggs were recovered from the vagina.

Fourteen eggs (9 eggs in 2-cell stage and 5 eggs in 4-cell stage) recovered

TABLE 2. Time of acrificing of superovulated rabbits treated with PGF<sub>2α</sub> 24 hours after mating and vaginal recovery of eggs from the does before sacrificing

Time of sacrificing after treatment (hr)	No. of does	No. of eggs recovered from the vagina at each hour after administration of PGF <sub>2α</sub>							Total no. of eggs recovered	Recovery rate* (%)
		1	2	3	6	9	12	24		
1	5	4							4	1.7
3	5	6	19	2					27	11.5
6	5	10	14	1	2				27	9.2
12	5	21	7	8	2	4	4		46	19.6
24	5	0	1	0	1	2	2	16	22	10.4

\* The recovery rate against the number of ovulation points or corpora lutea.

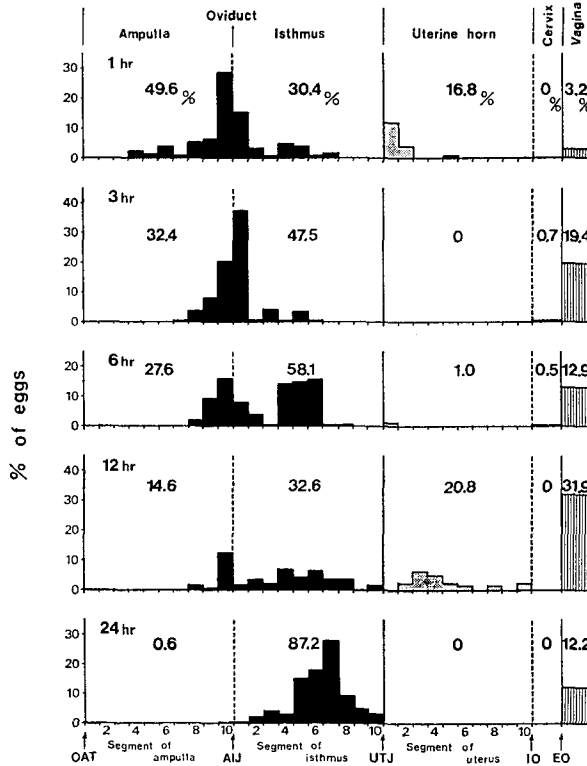


Fig. 3. Changes of distribution of eggs in relation to time in hours after treatment of PGF<sub>2α</sub> 24 hours after mating in the reproductive tracts of superovulated does. OAT, ostium abdominal tubae; AIJ, ampullary-isthmic junction; UTJ, uterotubal junction; IO, internal os; EO, external os.

TABLE 3. Numbers of eggs detected from the reproductive tracts of superovulated does treated with PGF<sub>2α</sub> 24 hours after mating

Hours after treatment	No. of corpora lutea	No. of eggs detected in					Total	Detection rate* (%)
		Ampulla	Isthmus	Uterine horn	Cervix	Vagina		
1	229	62	38	21	0	4	125	54.6
3	235	45	66	0	1	27	135	59.1
6	295	58	122	2	1	27	210	71.2
12	235	21	47	30	0	46	144	61.3
24	212	1	157	0	0	22	180	84.9

\* Total no. of eggs/no. of corpora lutea.

from the vagina 2 and 6 hours after PGF<sub>2α</sub> treatment in group 24 hours after mating were transferred into oviducts of a synchronized pseudopregnant doe, additionally. Two sites of implantation were observed by laparotomy 9 days after mating, and a normal young was obtained.

### Discussion

Calculation of the previous data<sup>20</sup> showed that rate of eggs recovered from the vagina of superovulated does from 24 to 78 hours after mating was about 38%. This figure was equivalent to the egg recovery rate in group of 36 hours administration of PGF<sub>2α</sub> in Experiment I of the present study. However, the egg recovery rates (about 30%) in groups of 20 or 24 hours administration of 5 mg/kg were somewhat low in comparison with that obtained during 24 to 78 hours after mating in superovulated does in the previous report.<sup>20</sup> The results imply that as far as concerned the number of eggs recovered from the vagina, PGF<sub>2α</sub> treatment did not act effectively to expel the eggs into the vagina. However, the time of beginning of egg recovery from the vagina could be greatly advanced at 20 and some hours after mating by the administration of PGF<sub>2α</sub>. It was, also, pointed out that in the group of 36 hours administration (Experiment I) no effective action of PGF<sub>2α</sub> was recognizable, because the egg recovery rate from the vagina became lower in 5 mg/kg group than in 2 mg/kg. It is considered, therefore, that the administration of PGF<sub>2α</sub> to the superovulated does is effective on the egg recovery from the vagina during the early hours after mating such as 20 or 24 hours after mating, but the effect of PGF<sub>2α</sub> becomes lesser until 36 hours after mating. Because the administration of 5 mg/kg of PGF<sub>2α</sub>

in normally ovulated does was very effective on the egg recovery from the vagina until 60 hours after mating,<sup>10)</sup> effective period of  $\text{PGF}_{2\alpha}$  on the egg recovery may be shortened by treatments of superovulation.

Although there are many studies on the levels of progestational steroids in blood during pseudopregnancy and/or pregnancy in the rabbit<sup>1-4,6-9,14,17)</sup>, there are only a few investigations on the hormonal fluctuations in blood of superovulated does.<sup>5,12)</sup> POLIDORO and BLACK<sup>12)</sup> showed no significant differences in levels of progesterone in peripheral plasma between normally ovulated and superovulated does in blood at 10, 20 and 30 days after mating. However, recently our data<sup>19)</sup> showed that the progesterone level in peripheral blood serum was elevated sharply within several hours after treatment of superovulation. The elevation in progesterone level may lead to scanty response of  $\text{PGF}_{2\alpha}$  on the reproductive tract, because progesterone inhibits the myometrial response to  $\text{PGF}_{2\alpha}$  in the rabbit.<sup>13)</sup>

The pattern of egg distribution in the oviduct of superovulated does after one hour administration of 5 mg/kg of  $\text{PGF}_{2\alpha}$  in Experiment II, was similar to that in superovulated does with no  $\text{PGF}_{2\alpha}$  treatment at 24 hours after mating.<sup>20)</sup> Pattern of egg distribution in the oviduct at 48 hours after mating in superovulated does did not differ regardless of the treatment with  $\text{PGF}_{2\alpha}$ , although a relatively large number of eggs appeared in the uterine horn, cervix and vagina. In the previous paper<sup>20)</sup> the authors suggested that the egg transport through the uterine horn in the rabbit is biphasic; a quick transport and a slow transport. This phenomenon may be applicable to the eggs in the oviduct of superovulated does, and the eggs transported quickly through the oviduct may be expelled in the vaginal lumen immediately after entrance to the uterine horn.

The rate of the total number of eggs which were found in the cleared specimen and recovered from the vagina against the number of corpora lutea (Table 3) was somewhat low in comparison with that of a previous study<sup>20)</sup> with no treatment of  $\text{PGF}_{2\alpha}$ . The trend was marked especially in does which were sacrificed in the early hours after  $\text{PGF}_{2\alpha}$  administration, and the rate seemed to recover to normal levels until 24 hours after treatment (48 hours after mating). The reason for this remains unknown.

The eggs recovered from the vagina of superovulated does in Experiment I were classified into three groups according to time of  $\text{PGF}_{2\alpha}$  administration (20, 24 and 36 hours after mating). It appeared that cleavage of the eggs was not disturbed by  $\text{PGF}_{2\alpha}$  administration in superovulated does. However, several empty zona pellucidae were recovered up till 48 hours after mating in the 20 and 24 hour administration groups, in spite of recovering after



144 hours post mating in superovulated does in the previous study.<sup>20</sup> No empty zonae pellucidae were recovered in the 36 hour administration group. This may indicate that the administration of PGF<sub>2α</sub> to the superovulated does at 20 or 24 hours after mating is effective on the contractility of the oviduct and/or uterus, although the administration of PGF<sub>2α</sub> at 36 hours after mating is ineffective.

### Summary

Fifty-five superovulated and mated rabbits were dealt with in two experiments. In Experiment I, 30 does received 2 or 5 mg/kg of PGF<sub>2α</sub> at 20, 24 or 36 hours after mating, respectively, and their vaginal lumina were washed to recover eggs 9 times during 1 to 48 hours after PGF<sub>2α</sub> administration. Although the recovery rate of the eggs was the highest in the 36 hour administration group (39.0% for group of 2 mg/kg administration and 34.5% for 5 mg/kg), the rate did not differ from that in superovulated does with no treatment of PGF<sub>2α</sub>. By PGF<sub>2α</sub> administration, however, initial time of egg recovery from the vagina was greatly advanced in the early hours after mating.

In Experiment II, 25 superovulated does receiving 5 mg/kg of PGF<sub>2α</sub> at 24 hours after mating were sacrificed from 1 to 24 hours after PGF<sub>2α</sub> treatment, and their reproductive tracts were cleared to locate eggs. Before sacrificing vaginal washings were made 1 to 8 times. No changes in the distribution pattern of eggs in the oviduct at 48 hours after mating in superovulated does were found regardless of the treatment of PGF<sub>2α</sub>.

The results suggested that an administration of PGF<sub>2α</sub> to the superovulated does 20 or 24 hours after mating is effective to recover eggs from the vagina and the administration of PGF<sub>2α</sub> 36 hours after mating is ineffective.

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