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Effect of Vaginally Administered 15[S]15-Methyl-PGF$_{2\alpha}$ on Egg Transport and Fertility in Rabbits (39262)

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Several investigators have demonstrated the effects of prostaglandins on egg transport in laboratory animals (1). The effects of PGs on egg transport vary among species, and at least in the rabbit seem to depend on the time of administration relative to the time of ovulation. Although the effect of vaginally administered PGs on oviductal motility has been reported previously (2), there are no published reports of the effects of vaginally administered PGs on egg transport.

The experiments reported here were designed to study the effects of 15[S]15-methyl-PGF$_{2\alpha}$ administered in vaginal suppositories on oviductal motility, egg transport, and fertility in rabbits.

Materials and methods. Dutch-belted and New Zealand rabbits were maintained on a standard rabbit ration and provided water *ad libitum*. The animals were caged individually for at least 3 weeks before being used in an experiment.

Vaginal suppositories were formulated to contain 1.0 mg of 15[S]15-methyl-PGF$_{2\alpha}$ in a lipid base. The suppositories, 8-mm in diameter and 19-mm long, were placed deep in the vagina with the aid of blunt forceps. Recording animals were treated with either placebo or drug-containing suppositories. Control animals in the egg transport and fertility experiments only had blunt forceps inserted in the vagina in the same manner as was used for suppository placement.

Muscular activity of the oviductal isthmus was monitored in unmated, estrous New Zealand rabbits. Motility was recorded on a Grass polygraph using pressure-sensitive silicone balloons implanted in the tubal lumen, as described previously (3). Recordings were made for 30 min before suppository treatment, and after treatment for at least 3 hr. Pretreatment and post-treatment amplitude and frequency of contractions were compared statistically using a paired Student's *t* test (4).

Vaginal suppositories were administered to Dutch-belted and New Zealand rabbits at 24 hr, at 18 and 24 hr, or at 24, 28, and 32 hr after an iv injection of 50 IU human chorionic gonadotropin (HCG, Nutritional Biochemicals Corp.). All animals were sacrificed at 48 hr after HCG to determine the location of eggs. An additional group of animals was treated with suppositories at 48 and 52 hr after HCG, and sacrificed at 64 hr after HCG. The tubal location of eggs was determined using the benzyl-benzoate clearing method described by Orsini (5) as modified by Longley and Black (6). Since the major intention of these experiments was to determine the treatment effect on tubal egg transport, no attempt was made to recover eggs from the uterus or vagina. Eggs that could not be found in the oviducts were considered to have been transported 100% of the oviductal length. The position of tubal eggs was expressed as a percentage of the total length of the oviduct. The percent egg recovery and mean distance traveled by the eggs in control and treated animals were compared using a nonparametric statistical test based on the Wilcoxon rank order statistic (4).

Six additional New Zealand rabbits were used to determine the effects of these suppositories on fertility. Suppositories were administered at 24, 28, and 32 hr after natural mating and the iv injection of 50 IU HCG. The animals were laparotomized on Day 12 of pregnancy (Day 0 is the day of mating), and the number of implantation sites was recorded. On Day 28 of pregnancy the animals were sacrificed and the numbers of viable fetuses, resorbing implantation sites, and corpora lutea were recorded. The control and treated groups were compared using Wilcoxon's test (4).

Results. The mean pretreatment and post-

treatment amplitudes and frequencies of contractions are shown in Table I. The amplitude of contractions began to increase within 15 to 30 min after the insertion of a 15[S]15-methyl-$PGF_{2\alpha}$ suppository, and the increased muscular activity persisted for an average of 2 hr. The maximal amplitude of contraction following suppository treatment was significantly greater than that before treatment ($P < 0.02$). In general, the amplitude of contractions following suppository treatment was three times greater than pretreatment levels. The decrease in the frequency of contractions following suppository treatment was statistically significant ($P < 0.04$).

The administration of placebo suppositories had no effect on oviductal motility in seven rabbits. The pretreatment amplitude of contractions (6.1 ± 1.9 mm Hg, mean ± SE) was not statistically ($P > 0.08$) different from the post-treatment amplitude (8.5 ± 3.0 mm Hg). Therefore, in subsequent experiments on egg transport and fertility the control animals only had blunt forceps inserted in the vagina.

The effects of postcoitally administered 15[S]15-methyl-$PGF_{2\alpha}$ on egg transport are shown in Table II. The number of eggs located in the oviduct was expressed as a percentage of the number of ovulation points counted on the ovaries. The distance traveled by the eggs was calculated by expressing the location of tubal eggs as a percentage of the total oviductal length, and expressing missing eggs as 100%. There was a statistically significant ($P < 0.025$) decrease in egg recovery from the oviducts in all three treatment groups in which the animals were sacrificed at 48 hr after HCG. However, there was a great deal of variation in ovum recovery in treated animals; recovery ranged from 0 to 100% in individual animals. Because the number of experimental animals is small, no statistical tests were performed on the data from animals sacrificed at 64 hr after HCG. It appears, however, that treatment with vaginal suppositories at 48 and 52 hr after HCG may be more effective in expelling eggs from the oviducts than treatments at earlier times; only 30.7% of the eggs were located in the oviducts as compared to an overall recovery of 51.7% in the three groups treated at earlier times. Treatment with vaginal suppositories at 18 and 24 hr, or at 24, 28, and 32 hr after HCG caused an increase in the median distance traveled by the eggs ($P < 0.05$); but the increase observed after treatment at 24 hr was not statistically significant. There was also no difference in the distance traveled between control animals and animals treated at 48 and 52 hr after HCG.

TABLE I. EFFECT OF 15[S]15-METHYL-$PGF_{2\alpha}$ VAGINAL SUPPOSITORIES ON THE AMPLITUDE AND FREQUENCY OF CONTRACTIONS OF THE RABBIT OVIDUCTAL ISTHMUS.[a]

Group (n = 11)	Amplitude (mm Hg)	Frequency (cont/min)
Pretreatment	6.6 ± 1.3[b]	9.8 ± 0.9
Posttreatment	27.1 ± 7.9[c]	8.8 ± 0.1[d]

[a] Vaginal suppositories contained 1.0 mg of 15[S]15-methyl-$PGF_{2\alpha}$ in a lipid base.

[b] Tabular values are means ± standard errors of the mean.

[c] Post-treatment significantly greater than pretreatment ($P < 0.02$).

[d] Post-treatment significantly less than pretreatment ($P < 0.04$).

The number of Day-12 implantation sites (Table III) was significantly reduced in the suppository treated animals ($P < 0.05$). The percentage of corpora lutea represented by Day-12 implants (51.1%) was similar to egg recovery rates in animals treated similarly (45.1%, Table II). Suppository treatment apparently had no detrimental effects on embryo development once implantation occurred. There was no difference ($P > 0.05$) in fetal survival from Day 12 to 28 of pregnancy. One control animal had two dead fetuses and one resorbing implantation site on Day 28, while two treated animals each contained one resorbing implantation site when sacrificed on Day 28.

Discussion. The pharmacological effects of natural PGs on oviductal motility and egg transport have been reviewed recently (1). Most reports described experiments in which PGs were administered sc. However, one report stated that solutions of crude PGs administered vaginally decreased activity of the rabbit oviduct (2). Vaginal suppositories containing 1.0 mg of 15[S]15-methyl-$PGF_{2\alpha}$ caused an increase in oviductal activity similar to that caused by the sc

TABLE II. EFFECTS OF 15[S]15-METHYL-$PGF_{2\alpha}$ VAGINAL SUPPOSITORIES ON TUBAL EGG TRANSPORT IN THE RABBIT.

Group	Treatment time[a]	Number of animals	Time of sacrifice[a]	Percentage egg recovery[b]	Mean distance traveled[c]
Control	–	10	48	99.0 ± 1.0[d]	76.0 ± 2.1[d]
	–	2	64	88.9 ± 11.1	94.2 ± 2.7
Treated	24	5	48	65.0 ± 14.0[e]	81.1 ± 5.9
	18, 24	6	48	43.7 ± 18.9[e]	90.2 ± 3.1[f]
	24, 28, 32	4	48	45.1 ± 15.6[e]	88.1 ± 4.3[f]
	48, 52	4	64	30.7 ± 14.5	94.7 ± 2.5

[a] Hours after HCG injection.
[b] Tubal eggs as a percentage of number of ovulation points.
[c] Location of tubal eggs expressed as a percentage of oviduct length, missing eggs considered 100%.
[d] Mean ± standard error of the mean.
[e] Significantly different from control ($P < 0.025$).
[f] Significantly different from control ($P < 0.05$).

injection of $PGF_{2\alpha}$ (3, 7). These vaginal suppositories did not cause an alteration of tubal motility that lasted longer than that following sc administration of 3 mg/kg of $PGF_{2\alpha}$. One of the limiting factors in producing a long-term effect on tubal motility may be the rate of vaginal absorption of the PG. It is unlikely that all of the PG analog contained in the suppositories was absorbed, and in some animals part of the suppository was expelled from the vagina.

The premature expulsion of eggs from the oviducts following treatment with 15[S]15-methyl-$PGF_{2\alpha}$ vaginal suppositories was similar to that following the sc administration of $PGF_{2\alpha}$ (7–10). Tubal egg recovery rates of 0 to 64% have been reported when $PGF_{2\alpha}$ was administered sc at a dose of approximately 3 mg/kg. The overall tubal recovery rate in animals treated with vaginal suppositories was 46%. In agreement with other reports (7, 9), treatment at later times after ovulation was apparently more effective in accelerating egg transport than was treatment soon after ovulation. The reason for this temporal difference is not easily explained, especially in view of the report that the effects on oviductal motility of 6 mg of $PGF_{2\alpha}$ sc were similar at 12 and 24 hr after mating (7). One possible explanation is that most of the eggs are still located in the ampulla soon after ovulation, and the strong, tonic contractions of the isthmus induced by PGFs may occlude the tubal lumen and prevent many of the eggs from passing through the isthmus into the uterus. At later times, when some of the eggs are already in the isthmus, the PGF-induced contractions may propel the eggs into the uterus.

TABLE III. EFFECT OF 15[S]15-METHYL-$PGF_{2\alpha}$ VAGINAL SUPPOSITORIES ON FERTILITY IN THE RABBIT.

	Control	Treated
Number of corpora lutea	8.5	10.3
Day-12 implants[a]	85.7%	51.1%[b]
Viable Day-28 fetuses	85.0%[c]	94.1%
	70.6%[d]	46.3%

[a] Day 12 implants as a percentage of number of corpora lutea.
[b] Significantly less than control ($P < 0.05$).
[c] Day 28 fetuses as a percentage of day 12 implants.
[d] Day 28 fetuses as a percentage of number of corpora lutea.

The reduction in fertility following suppository treatment is similar to the rate of expulsion of eggs from the oviduct following similar treatment. Chang (11) reported that only about 20% of 2-day embryos transferred from the oviducts to the uterus developed normally. The decrease in implantation rate observed in suppository treated animals is presumably due to accelerated passage of eggs through the oviducts. Those eggs arriving in the uterus prematurely following treatment probably do not implant and either degenerate or are expelled from the uterus, while those eggs whose tubal transport is not affected by the treatment implant after arriving in the uterus at the normal time. Since the number of Day-12 implants and Day-28 fetuses were not different in the treated animals, there is no evidence that the treatment imposed in these experiments had any latent effects on embryo development once implantation was

established. It has been reported that treatment with PGE_2 during the time of tubal transport reduces subsequent *in vitro* embryo development (12).

Summary. The effects of vaginal suppositories containing 1.0 mg of 15[S]15-methyl-$PGF_{2\alpha}$ on oviductal motility, egg transport, and fertility were determined in rabbits. Suppository treatment caused a significant increase ($P < 0.02$) in the amplitude of oviductal contractions, and a decrease in the frequency of contractions ($P < 0.04$). Altered oviductal motility persisted for an average of 2 hr after treatment. Treatment with 1, 2, or 3 suppositories at various times after ovulation caused a significant reduction in the number of eggs located in the oviducts ($P < 0.025$). There was, however, a great deal of variation in egg recovery in treated animals (range 0 to 100%). Treatment of mated rabbits during the time of tubal egg transport caused a significant reduction in the number of Day-12 implants ($P < 0.05$). The percentage of corpora lutea represented by Day-12 implants was similar to egg recovery rates in animals similarly treated. The treatment had no effect on fetal survival from Day 12 to 28 of pregnancy. The decrease in fertility caused by these vaginal suppositories is presumably due to the stimulatory effect on oviductal motility which accelerates tubal transport of the embryos into the uterus. Embryos that arrive in the uterus prematurely probably do not implant and degenerate or are expelled.

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