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Fluid Retention by the Rabbit Oviduct (40947)

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Abstract. Factors affecting occlusion of the isthmic portion of the oviduct in Dutch-belted rabbits were evaluated by measuring the amount of fluid that accumulated in oviducts that were ligated only at the ovarian end. Very little fluid accumulated in the oviducts of estrous rabbits. Induction of ovulation with human chorionic gonadotropin (hCG) or treatment with Depo-estradiol cypionate (ECP) caused a significant increase in the accumulation of tubal fluid at 48 hr. Indomethacin inhibited fluid retention in ECP-treated rabbits, but had no effect in rabbits injected with hCG. Progesterone antagonized the effect of hCG, but not that of ECP. The effect of progesterone in hCG-treated rabbits could not be attributed to a decrease in fluid secretion rate since the amount of fluid in oviducts that were ligated at both ends was similar in rabbits treated with hCG or hCG plus progesterone. In contrast to the results at 48 hr, indomethacin had no effect on fluid accumulation at 72 hr in ECP-treated rabbits. However, indomethacin did reduce the amount of fluid that accumulated in the oviducts of rabbits injected with both hCG and ECP. Prostaglandin (PG) E_1, PGE_2, and $PGF_{2\alpha}$ had no effect on fluid retention. These data indicate that an interaction exists between the occlusive effects of hCG and ECP. At 48 hr the effect of hCG seems not to be mediated by PGs, but the effect of ECP is PG related. However, at 72 hr the effect of ECP cannot be blocked by inhibiting PG synthesis, but the occlusive effect of hCG plus ECP may be mediated by PGs.

Fluid is retained in rabbit oviducts ligated only at the ovarian end (1–3). Occlusion of the isthmic portion of the oviduct is apparently very effective since fluid accumulates in sufficient volume to cause distention of the thinner-walled ampulla. The amount of fluid accumulated in the oviduct decreases at a time corresponding to that when ova normally pass from the oviduct into the uterus. Bellve and McDonald (4) studied the directional flow of sheep oviduct fluid. During the first few days of the estrous cycle fluid passed out of the ovarian end of the oviduct; but on Day 4 of the cycle, when ova are being transported into the uterus, the majority of fluid passed through the tubo-uterine junction. The mechanism that controls occlusion of the proximal oviduct in the rabbit, so that fluid is retained in ligated oviducts and ova are retained under normal conditions, is not known. However, ovarian steroids, adrenergic innervation, and prostaglandins have been implicated in this mechanism.

The experiments reported here were designed to study factors that affect occlusion of the oviduct and cause retention of oviductal fluid in the rabbit. The mechanism that controls fluid retention may be the same as, or similar to, the mechanism controlling retention of ova in the oviducts.

Materials and methods. Dutch-belted rabbits that had been individually caged for at least 3 weeks were used. Three or four rabbits were assigned to each treatment group. The numbers in parentheses on the figures are the number of oviducts included in each group. Animals were anesthetized with sodium pentobarbital supplemented with ether as needed. Through a midventral laparotomy the oviducts were ligated either at the fimbriated end or at both ends with a single 2-O silk suture. Both ovaries were observed to be certain that large follicles were present and that no recent ovulatory stigmata or corpora lutea were present.

Immediately following surgery the animals were injected intravenously with saline or 75 IU human chorionic gonadotropin (hCG) to induce ovulation. The time of saline or hCG administration is referred to as 0 hr. Groups of rabbits also received the following treatments: indomethacin, 5 mg/kg subcutaneously (sc) at −2, 0, +2, +8, and +24 hr; Depo-estradiol cypionate (ECP), 250 μg intramuscularly (im) at 0 hr;

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progesterone, 1.5 mg im at −48, −24, and 0 hr; prostaglandin (PG) E_1, PGE_2, or $PGF_{2\alpha}$, 2.5 mg/kg sc at +45 hr. Additional animals received combinations of these treatments as indicated under Results.

The tubo-uterine junctions were ligated with 2-O silk suture when the animals were sacrificed at either +48 or +72 hr. The oviducts were excised, carefully trimmed free of fat and mesosalpinx, and weighed. The oviducts were then cut open longitudinally, the retained fluid was expelled, and the oviducts were blotted on absorbent paper. The oviducts were reweighed, and the weight of retained fluid was calculated by difference.

Treatment effects were tested using a rank transformation according to the method of Kruskal and Wallis as reported by Hollander and Wolfe (5).

Results. Mean oviduct fluid weights for animals sacrificed at 48 hr after saline or hCG administration are shown in Fig. 1. Very little fluid accumulated in ligated oviducts of estrous rabbits injected only with saline. Induction of ovulation with hCG caused a significant ($P < 0.03$) increase in the amount of fluid retained in the oviducts. The administration of ECP to estrous rabbits that had been injected with saline also caused an increase in tubal fluid; the amount of oviductal fluid in these animals was significantly ($P < 0.03$) greater than that in rabbits injected only with saline, but was not different ($P > 0.05$) from that in rabbits injected with hCG. Progesterone caused a significant ($P < 0.03$) decrease in the amount of tubal fluid in hCG-treated rabbits, but had no effect ($P > 0.05$) in saline-treated rabbits or in rabbits treated with saline plus ECP. The administration of ECP to rabbits injected with hCG caused an increase in tubal fluid but the amount of fluid was not statistically ($P > .0.05$) greater than that in animals injected only with hCG. Indomethacin blocked the increase in tubal fluid caused by ECP in saline-injected rabbits ($P < 0.03$), but did not block the increase in tubal fluid caused by hCG administration ($P > 0.05$).

It is known that estrogen causes an increase and that progesterone causes a decrease in the volume of fluid secreted by the rabbit oviduct (6). Therefore, oviducts were ligated at both the ovarian and uterine ends in additional rabbits to determine if some of the treatment effects shown in Fig. 1 were merely due to differences in the amount of fluid that was secreted. These data are shown in Fig. 2. The amounts of fluid in the oviducts of rabbits treated with hCG, saline plus ECP, or saline plus ECP plus progesterone were similar ($P > 0.05$) in animals in which the oviducts had been ligated at both ends. The amount of fluid in animals treated with hCG plus progesterone was greater than that in animals treated with saline plus ECP ($P < 0.02$), but was not different from the amount of oviductal fluid in the other two groups ($P > 0.05$).

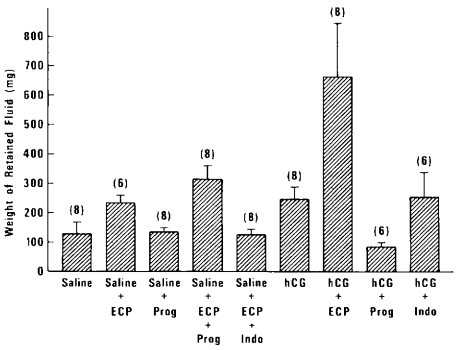

FIG. 1. Weight (mean ± SEM) of retained fluid at 48 hr after intravenous injection of saline or hCG in rabbit oviducts ligated only at the ovarian ends. The number of oviducts in each group is shown in parentheses.

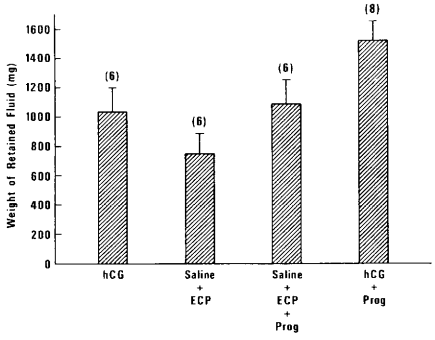

FIG. 2. Weight (mean ± SEM) of retained fluid at 48 hr after an intravenous injection of saline or hCG in rabbit oviducts ligated at both the ovarian and uterine ends. The number of oviducts in each group is shown in parentheses.

When animals were sacrificed at 72 hr (Fig. 3) there was no longer a significant ($P > 0.05$) difference in the amount of retained fluid in the oviducts of rabbits treated with saline or hCG. However, the amount of tubal fluid in these animals was significantly ($P < 0.03$) less than that in animals treated with saline plus ECP, saline plus ECP plus indomethacin, hCG plus ECP, or hCG plus ECP plus indomethacin. These latter two groups were different from each other ($P < 0.01$) and were greater than the groups treated with saline plus ECP or saline plus ECP plus indomethacin ($P < 0.01$).

Prostaglandins did not have a statistically significant ($P > 0.05$) effect on the amount of fluid that was retained in the oviducts of rabbits injected with hCG and sacrificed at 48 hr. The amount of fluid retained in these animals was: hCG, 246 ± 44 mg (mean ± SEM); hCG plus PGE_1, 208 ± 29 mg; hCG plus PGE_2, 455 ± 101 mg; hCG plus $PGF_{2\alpha}$, 503 ± 151 mg.

Discussion. The present data confirm earlier reports (1–3) that fluid is retained until approximately 72 hr after hCG administration in oviducts ligated only at the ovarian end. This corresponds to the time when ova are moving from the oviducts into the uterus under normal circumstances. In addition, these experiments have demonstrated that fluid does not accumulate to an appreciable extent in the oviducts of estrous rabbits. This is consistent with the observation that ova transferred to the oviducts of estrous rabbits are not retained, but are lost rapidly from the oviducts (7, 8). These data support the contention that ova are retained in the oviduct for approximately 60 hr after ovulation because of constriction of the proximal region of the tube. This constriction is efficient enough not only to retain ova, but also to cause fluid accumulation to the extent that the ampullary portion of the oviduct becomes greatly distended.

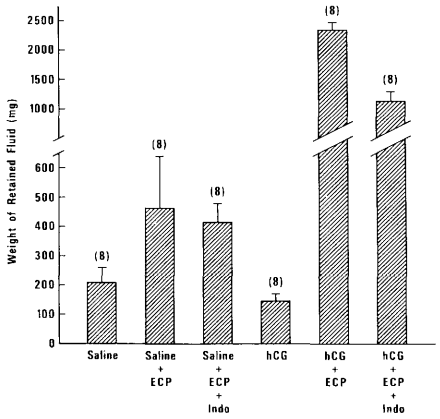

FIG. 3. Weight (mean ± SEM) of retained fluid at 72 hr after an intravenous injection of saline or hCG in rabbit oviducts ligated only at the ovarian ends. The number of oviducts in each group is shown in parentheses.

The mechanism responsible for occlusion of the proximal oviduct after ovulation is not clear. Experimental evidence has implicated the adrenergic nervous system (9), ovarian steroids (7, 8, 10, 11), and prostaglandins (12). The present data demonstrated that an injection of ECP caused an amount of fluid accumulation similar to that caused by the induction of ovulation with hCG. The increase in fluid accumulation in rabbits treated with ECP could not be attributed to a greater rate of fluid secretion than occurred in rabbits injected with hCG (Fig. 2), but was more likely due to an effect on occlusion of the proximal oviduct. A surge of estradiol secretion occurs approximately 6 hr after hCG administration in the rabbit (13). This estradiol surge may be, at least partially, responsible for occlusion of the proximal oviduct. The luminal diameter of the rabbit oviductal isthmus decreases following hCG-induced ovulation, and exogenous estradiol enhances this constriction (14).

It was suggested that the effect of estradiol may be mediated through an increase in PG synthesis by oviductal tissue (12). In the present experiments a PG synthesis inhibitor, indomethacin, had no effect on fluid accumulation at 48 hr in rabbits injected with hCG; but did inhibit fluid accumulation in rabbits treated with saline plus ECP. However, at 72 hr the amount of tubal fluid was the same in animals injected with saline plus ECP with or without indomethacin treatment. In contrast, indomethacin did inhibit fluid accumulation at 72 hr in rabbits treated with both hCG and ECP. These data indicate that a com-

plex interaction exists between the occlusive effects of hCG and estrogen. At 48 hr the effect of hCG seems not to be mediated by PGs, but the effect of ECP is PG related. However, at 72 hr the effect of ECP is not mediated by PGs; but the interactive occlusive effect of hCG plus ECP is related to PGs.

The total amount of fluid accumulated in the oviducts at 72 hr in rabbits treated with hCG plus ECP also indicates an interactive, synergistic effect of these agents. The amount of oviductal fluid in animals treated with both hCG and ECP was approximately fourfold greater than the sum of the fluid accumulated in rabbits treated with only saline plus ECP or only hCG. While a synergistic effect of hCG and ECP was apparent at 48 hr, it was not as profound as that at 72 hr. These data indicate that the occlusive properties of hCG and ECP, and thus fluid accumulation, are mediated by separate mechanisms but are interactive. This conclusion is substantiated by the observation that indomethacin blocked the effect of ECP but not that of hCG at 48 hr, and had no effect on ECP but blocked the combined effect of hCG plus ECP at 72 hr.

Progesterone and ECP treatments similar to those used here are known to accelerate and retard, respectively, the rate of ovum transport in the rabbit (15). Therefore, there is a good correlation between the effects of these steroids on ovum retention and fluid retention. This was not the case with regard to the effects of the PGs. It is known that PGE_1 and $PGF_{2\alpha}$ but not PGE_2 accelerate ovum transport in rabbits (12). None of these PGs had an effect on fluid retention. The precise reason for this disparity, an effect on ovum retention but not on fluid retention, is not known. One possible explanation is that the effects of PGs on ovum transport are due to the induction of peristaltic-type contractions on the oviduct which do not affect the occlusion of the tube that is responsible for fluid retention.

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