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Local Ovarian Effects of an Intrauterine Device in Intact and Unilaterally Ovariectomized Guinea Pigs.* (31600)

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Intrauterine foreign bodies produce shortened estrous cycles in cattle, guinea pigs and sheep. In cattle(1) and in sheep(2) a plastic coil in one uterine horn results in shorter estrous cycles when the coil is in the horn adjacent to the ovary containing the corpus luteum (CL) than when the coil is opposite to the CL. The present experiments were designed to study the local utero-ovarian influences of an intrauterine device (IUD) on the CL and on the ovulation rate in guinea pigs.

Methods. A total of 107 virgin guinea pigs weighing 400 to 600 g was used in 3 separate experiments. The animals were examined approximately every 12 hours and the estrous

cycle length was determined by means of the vaginal smear technique. The day of estrus was designated day 1 of the estrous cycle. In all 3 studies the IUD consisted of a 7.0 mm length of 3.3 mm diameter plastic tubing.† For surgery the guinea pigs were anesthetized with an intraperitoneal injection of pentobarbital sodium, supplemented when necessary with the application of ether. The ovaries and uterine horns were exposed by means of 2 high lumbar, transverse abdominal incisions, one on each side of the body. The IUD was inserted through a small longitudinal incision in the caudal portion of the uterine horn and was pushed forward with a probe to a point approximately 10 mm from the cranial end of the horn. The device was sutured in place and the uterine incision was closed with 5-0 silk suture. The side of insertion of the IUD (right or left horn) was assigned alternately for successive guinea pigs within each treatment group at the time of surgery.

Experiment 1 was conducted during June and July, 1965. It was designed to determine: a) if an IUD, inserted in one uterine

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† Intramedic Polyethylene Tubing, PE280, Clay-Adams Inc., New York.

horn of the guinea pig would result in smaller CL in the adjacent ovary than in the opposite ovary and b) if grafting the horn containing the IUD to the opposite horn would result in luteal interference in the opposite ovary. Nineteen guinea pigs were laparotomized on day 2 of an estrous cycle and animals that had newly forming CL in only one ovary were discarded. The remaining animals were assigned alternately to one of two treatment groups: 1) IUD inserted into one uterine horn and 2) IUD inserted into one uterine horn and that horn then grafted to the opposite horn. Elimination of unilaterally ovulating animals plus one that died during the experimental period resulted in 6 and 7 guinea pigs in groups 1 and 2, respectively. The uterine horns were prepared for grafting by scarifying the medial aspect of the cranial 10 mm of each horn. The horn containing the IUD was freed by severing the oviduct at the tubo-uterine junction and the scarified areas of the 2 uterine horns were placed in apposition and sutured together with 5-0 silk suture. In both groups of guinea pigs the horn that did not receive an IUD was incised and the incision closed with suture. The CL were marked with India ink at the time of surgery to aid later identification. The animals were killed on day 10 of the operative cycle (8 days after surgery) and the CL were dissected from the ovarian stroma under a dissecting microscope. The mean CL weights were analysed statistically by analysis of variance and a multiple range test. When more than one CL was present in an ovary the average CL weight was used.

Experiment 2 was conducted during December, 1965. It was designed to determine if shorter estrous cycles occur in unilaterally ovariectomized guinea pigs when an IUD is in the uterine horn adjacent to the retained ovary than when an IUD is opposite to the retained ovary. Twenty-eight animals were assigned randomly to one of two treatment groups: 1) one ovary removed, IUD adjacent to the retained ovary and 2) one ovary removed, IUD opposite to the retained ovary. The IUD was inserted and the assigned ovary was removed on day 3 of an estrous cycle. Elimination of unilaterally ovulating guinea

pigs resulted in 11 and 10 animals in groups 1 and 2, respectively. The cycle lengths were analysed by analysis of variance.

Experiment 3 was conducted from March to June, 1966. Preliminary studies had suggested that: a) in ovarian-intact guinea pigs with CL in each ovary shortened cycles may occur when an IUD is present in only one horn, but the effect may be less pronounced than when an IUD is present in each horn; b) the cycle-shortening effect of an IUD may be less pronounced during an operative cycle (IUD inserted on day 3) than during a subsequent cycle. Experiment 3 was designed to test these possibilities and to confirm the results of Experiment 2. In addition the effects of an IUD on the ovulation rate were studied.

Sixty guinea pigs were assigned randomly to 5 treatment groups: 1) ovaries intact, sham-operated; 2) ovaries intact, IUD in only one uterine horn; 3) ovaries intact, one IUD in each uterine horn; 4) one ovary removed, IUD adjacent to retained ovary; 5) one ovary removed, IUD opposite to retained ovary. All guinea pigs were laparotomized on day 3 of an estrous cycle (operative estrous cycle), an IUD was inserted (Groups 2, 3, 4, 5) and a unilateral ovariectomy was performed (Groups 4, 5). The guinea pigs were relaparotomized on day 3 of the following cycle (subsequent cycle) and the position of the IUD was confirmed and the location (right or left ovary) of newly forming CL was recorded. Animals in all groups that did not have newly forming CL in both ovaries at the time of the first laparotomy and all ovarian-intact animals (Groups 1, 2, 3) that did not have newly forming CL in both ovaries at the time of the second laparotomy were not used. The elimination of unilaterally ovulating animals as well as 5 animals that died during the experimental period resulted in 7, 7, 7, 10 and 9 guinea pigs, respectively, for the 5 treatment groups. The cycle lengths were analysed statistically by analysis of variance and by a multiple range test of treatment means.

Results and discussion. The side of insertion of the IUD (right or left horn) did not appear to influence the results in any

TABLE I. Effects of an Intrauterine Device (IUD) on Weight of Corpora Lutea (CL) in Intact Guinea Pigs and in Guinea Pigs with the Uterine Horns Grafted Together (Exp 1).

Uterine horns	No. animals	Treatment of individual uterine horns*	Mean CL wt in adjacent ovary (mg)†
Normal	6	Sham-operated	2.91
		IUD inserted	1.86
Grafted together	7	Sham-operated	2.18
		IUD inserted	2.27

* IUD inserted and grafting performed on day 2 of an estrous cycle and animals killed on day 10.

† Probability values for differences in CL weights: 2.91 vs 1.86 ($P < 0.05$); 2.91 vs 2.18 ($P < 0.05$); 2.91 vs 2.27 ($P < 0.06$); 1.86 vs 2.18 (n.s.); 1.86 vs 2.27 (n.s.); 2.18 vs 2.27 (n.s.).

of the experiments and therefore the data for right and left sides were combined.

Experiment 1. The results are summarized in Table I. In the 6 guinea pigs without a uterine graft the CL were smaller ($P < 0.05$) when they were in an ovary adjacent to the horn containing the IUD than when they were adjacent to the sham-operated horn (1.86 vs 2.91—Table I). Since this experiment was conducted, a preliminary study has been reported which indicates that a glass bead inserted into one uterine horn of the guinea pig results in smaller CL volume in the adjacent ovary than in the opposite ovary(3). This is consistent with the present experiment. A similar unilateral effect of an intrauterine plastic coil on CL weight in sheep has been reported(2). In the 7 guinea pigs with an IUD in only one horn, but with the two uterine horns grafted together the CL in both ovaries were reduced in weight (2.18 and 2.27 are significantly smaller than 2.91, but are not different from 1.86—Table I). It should be noted, however, that a group of control guinea pigs with fused uterine horns, but without the presence of an IUD in either horn, was not incorporated into this study.

Experiment 2. The results are summarized in Table II. Shorter estrous cycles occurred in the unilaterally ovariectomized guinea pigs where the retained ovary (and therefore the CL) was adjacent to the horn containing the IUD, than where the retained ovary was opposite to the IUD. These results are consistent with reports of unilateral effects of intrauterine plastic coils in cattle and sheep

where cycle length was also used as the end-point.

Experiment 3. The results are summarized in Table III. There was a significant difference ($P < 0.01$) in mean estrous cycle lengths among the 5 treatment groups. The cycles were shorter ($P < 0.05$) in: a) intact guinea pigs with an IUD in each uterine horn and b) unilaterally ovariectomized guinea pigs with an IUD in the horn adjacent to the retained ovary than in: a) intact guinea pigs that were sham-operated, b) intact guinea pigs with an IUD in only one horn or c) unilaterally ovariectomized guinea pigs with an IUD opposite to the retained ovary. Shortened cycles did not occur in ovarian-intact guinea pigs with CL in each ovary and with an IUD in only one horn, since their cycle length was not significantly different from those of the sham-operated controls. No evidence was obtained that the IUD had a more pronounced effect on shortening the estrous cycle during the subsequent cycles than during the operative cycles. Over all treatment groups, the subsequent cycles actually were longer ($P < 0.01$) than the operative cycles, but no explanation is being offered for this occurrence. There was no significant interaction between treatment groups and cycle groups (operative and subsequent cycles) suggesting the main treatment effects were apparent in either cycle group.

No evidence was obtained that the presence of an IUD influenced the rate of ovulation as indicated by a comparison of the average number of newly forming CL present at surgery and at relaparotomy. The newly forming CL present at the time of surgery (avg no., 3.1) resulted from ovulations which occurred prior to IUD insertion. The CL

TABLE II. Effect of Intrauterine Device (IUD) on Estrous Cycle Length in Unilaterally Ovariectomized Guinea Pigs (Exp 2).

Treatment	No. animals	Length of estrous cycle (days)	
		Mean*	Range
IUD in horn adjacent to retained ovary	11	14.7	14-16
IUD in horn opposite to retained ovary	10	15.6	15-17

* $P < 0.025$.

TABLE III. Effect of an Intrauterine Device (IUD) on the Estrous Cycle Length of Intact and Unilaterally Ovariectomized Guinea Pigs (Exp 3).

Item	Both ovaries intact			One ovary removed	
	No IUD (sham-operated)	IUD in only one horn	IUD in both horns	IUD in horn adjacent to retained ovary	IUD in horn opposite to retained ovary
No. guinea pigs	7	7	7	10	9
Operative* cycle length (days)	16.1	15.5	14.3	13.0	16.1
Subsequent* cycle length (days)	16.2	16.6	14.6	14.8	17.2
Both operative and subsequent cycle length (days)	16.2†	16.1†	14.4‡	13.9‡	16.7‡

* Mean squares for the 5 treatment groups, 25.10 ($P < 0.01$); operative cycle vs subsequent cycle, 18.00 ($P < 0.01$); interaction, 1.92; error for treatment, 3.46; error for cycle and for interaction, 2.41.
 †, ‡ Means bearing different reference marks are significantly different from each other ($P < 0.05$).

present at the time of laparotomy during the subsequent cycle (avg no., 2.9) resulted from ovulations which occurred in the presence of an IUD. There was no significant difference in CL numbers among the 5 treatment groups or between the operative and subsequent cycles, nor was there any interaction between treatment and cycle. Removal of one ovary (Groups 4, 5) during the operative cycle did not change significantly the number of ovulations per animal during the subsequent cycle (3.0 vs 2.7, respectively).

Summary. The effects of an intrauterine device (IUD) on the corpora lutea, estrous cycle length and ovulation rate were studied in guinea pigs. Insertion of an IUD on day 2 of an estrous cycle resulted in smaller corpora lutea in the ovary adjacent to the

IUD than in the opposite ovary. The average estrous cycle length was shortened in ovarian-intact guinea pigs with an IUD in each uterine horn and in unilaterally ovariectomized guinea pigs with the IUD adjacent to the retained ovary. Estrous cycles were not shortened in ovarian-intact guinea pigs with an IUD in only one horn or in unilaterally ovariectomized guinea pigs with the IUD opposite to the retained ovary. No evidence was obtained that the IUD influenced the rate of ovulation.

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New Metabolizable Immunologic Adjuvant for Human Use.

4. Development of Highly Purified Influenza Virus Vaccine in Adjuvant 65. (31601)

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Previous reports(1-4) from this laboratory described the development and clinical evaluation of a new immunologic adjuvant, called adjuvant 65, which was applied to influenza virus vaccine. The adjuvant vaccine consisted of a water-in-oil emulsion of the aqueous vaccine in highly refined peanut oil using

Arlacel A (mannide monooleate) as emulsifier and aluminum monostearate as stabilizer. A detailed analytic review of adjuvants and adjuvant activity has been recorded(5) elsewhere. Further studies of influenza-adjuvant vaccine led to the development of a highly purified aqueous influenza virus vaccine which