IUD-Induced Delay in Postcoital Rise in 20α-Hydroxypregn-4-en-3-one in the Female Rabbit (36321)

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The influence of an intrauterine contraceptive device (IUD) on the hypothalamo–hypophyseal–ovarian system has been studied in many species (1–5). From these studies, it was concluded that the systemic effects of an IUD, particularly on the central nervous system, are restricted. However, Janakiraman and Casida (6) have suggested that in rabbits, a reflex-ovulating species, the IUD causes a delay in postcoital ovulation. They made direct determinations of the pituitary LH content and suggested that the delay in ovulation is truly secondary to an alteration in the LH secretion pattern. On the other hand, Hilliard *et al.* (7) have clearly shown that postcoital pattern of secretion of pituitary LH has a close relationship with that of ovarian 20α-hydroxypregn-4-en-3-one (20α-OH-P) in this species.

The present study was designed to determine this ovarian progestin in the serum at various times following mating and to clarify the systemic effect of IUD in the rabbit.

Materials and Methods. New Zealand virgin female rabbits (8–12 months old) were used in this experiment. They were caged individually, fed rabbit chow and water *ad libitum*, and maintained under an artificial light regimen of 14 hr light (500–1900) and 10 hr darkness. They were divided into two groups. Animals from one group were anesthetized with pentobarbital (Nembutal 25 mg/kg); and silicone rubber tubing (10 cm long, 0.020 in. i.d.; 0.037 in. o.d.; Silastics, Dow Corning) was inserted in both horns of the uterus as intrauterine contraceptive devices (IUD). The upper end of each IUD was fixed to the antimesometrial wall close to the utero-tubal junction with silk suture and the lower free end of the tube was extended to just above the cervix. Animals from another group were left intact as control. After a short recovery period of 1 week to 10 days, these and control animals were mated with fertile males; and, beginning 1 hr after mating, about 10 ml of blood were collected in a few minutes from ear arteries of conscious animals every 2 hr. Serum 20α-OH-P was determined as follows.

The blood samples were allowed to clot, were centrifuged 30 min at 3000 rpm, and the serum was collected. Radiolabeled 20α-OH-P was added to all samples and was extracted with 4 vol of anhydrous ether. Ether extract was then evaporated almost to dryness; and the residue was spotted on a silica gel plate and chromatographed in hexane:distilled ethyl acetate (5:2) mixture. Ultraviolet absorption spots corresponding to appropriate steroid standards were eluted with chloroform:methanol (2:1) and dried under nitrogen. The residue was spotted again on an Eastman-Kodak silica plate and chromatographed with a mixture of methylene chloride:ether (6:2). This procedure is important to separate 20 α-OH-P clearly from progesterone and other analogous substances. The final residue was analyzed by gas–liquid chromatography employing a Packard Model 804-873 gas chromatograph equipped with a flame ionization detector, using the solid injection method. The column packing was GAC-CHROM Q (80/100 mesh) coated with 3% QF-1 as stationary phase. The amount of steroid in the sample was calculated from the peak heights of the sample and corrected for recovery by measuring radioactivity in the aliquots with a Packard Tri-Carb liquid-scintillation spectrometer (Model No. 3375).

TABLE I. Serum 20α-OH-Progesterone Concentration in Normal Rabbits During Preovulatory Periods.

Animal no.	Serum 20α-OH-progesterone (ng/100 ml of serum); hr pc: 1	3	5	7	9
M44	15,534[a]	6060	6140	2043	1110
M43	34,774[a]	14,655	4314	933	642
M61	5525[a]	3742	3006	524	ND[b]
M23	7950[a]	949	ND	ND	ND
M58	1506	6886[a]	469	275	ND
M59	8872	10,778[a]	6014	998	ND

[a] Highest value in each animal.
[b] Not detectable.

After blood collection, each rabbit was opened by ventrolateral incision under pentobarbital anesthesia and the number of ovarian stigmata counted at 12 and 24 hr postcoitum (pc).

Results. Table I shows serum 20α-OH-P concentration in normal intact rabbits after mating. The blood samples were collected every 2 hr beginning 1 hr after coitus. Although serum concentrations of 20α-OH-P during preovulatory periods showed much variation in each animal, the highest concentrations of this progestin, in our study, were detected in the first hour (4 animals) and third hour after mating (2 animals). The level of 20α-OH-P remained relatively high for 5 hr pc, then fell off rapidly to undetectable levels at 9 hr pc (4 animals).

In animals with IUD, 20α-OH-P concentration in the peripheral blood was changed as shown in Table II. In 2 animals out of 5, progesterone was nonmeasurable in peripheral blood at the first hour. The highest value of this steroid in the serum was found 3 hr pc (2 animals) and 5 hr pc (3 animals). Nine hours later, serum level of 20α-OH-P was detected in only one animal.

The number of ovarian stigmata in both groups was counted 12 and 24 hr after mating and these data are shown in Table III. There was no significant difference in the number of ovarian stigmata between the treated and untreated groups, indicating that IUD has no effect on the ovulation rate in the rabbit. There was a slight increase in the number of ovarian stigmata at 24 hr pc compared to those at 12 hr pc in the IUD group, but this is not significant because ovarian stigmata in the control group was also slightly increased.

Discussion. Many experimental data suggest that progesterone has a biphasic action—facilitatory and inhibitory—on ovulation (9–12). In 1961, Sawyer and Kawakami (13) demonstrated that progesterone administered to ovariectomized, estrogen-primed rabbits induced a biphasic change in the paradoxical sleep threshold of the hypothalamus. We also found that subcutaneous injection of 10 mg of progesterone altered the after-discharge threshold of the amygdaloid nucleus and the hippocampus of the limbic system biphasically in the ovariectomized estrogen-primed rabbits (14). Thus progesterone has an important role in the hypothalamo–hypophyseal–ovarian axis and can be a trigger of ovulation if it is administered at an appropriate time in animals.

20α-OH-P secretion in the rabbit is rapidly accelerated following coitus or after administration of exogenous gonadotropin; and this progestin acts as a positive feedback agent to prolong and heighten LH discharge in the mated rabbit (7). Since 20α-OH-P is a main progestin, its secretion pattern could be a good indicator of the hypothalamo–hypophyseal–ovarian relationship in the rabbit. These results obtained from our intact animals are in good agreement with those of Hilliard *et al.* (8), although they measured

TABLE II. Serum 20α-OH-Progesterone Concentration in IUD Rabbits.

Animal no.	Serum 20α-OH-progesterone (ng/100 ml of serum); hr pc: 1	3	5	7	9
M41	2303	5684[a]	1870	985	ND[b]
M39	9262	40,315[a]	38,040	14,774	5655
M13	ND	1367	3154[a]	727	ND
M30	ND	ND	1623[a]	ND	ND
M54	3602	6296	6609[a]	299	ND

[a] Highest value in each animal.
[b] Not detectable.

TABLE III. Changes in the Number of Ovarian Stigmata in Both Groups.

	Ovarian stigmata			
	12 hr pc		24 hr pc	
Animals	R	L	R	L
IUD (15)[a]	5.20 ± 2.39[b]	4.60 ± 1.14	5.60 ± 2.30	5.40 ± 0.89
Normal (6)	3.83 ± 1.72	3.83 ± 2.32	4.17 ± 1.47	4.50 ± 1.38

[a] No. of rats in parentheses.
[b] Mean ± SD.

ovarian venous progestin directly and the animals were anesthetized with pentobarbital. In our IUD animals, however, the highest value of 20α-OH-P was obtained at 3 and 5 hr pc calculated statistically. The time of attainment of the highest value in both groups was 1.67 ± 1.03 hr and 4.20 ± 1.10 hr pc, respectively, assuming that the peak of the actual secretory pattern coincides with that obtained in this study. Because of the 2-hr interval sampling, the highest serum 20α-OH-P concentrations may have been missed in some animals in both groups. Nonetheless, the difference in concentrations measured were significant at the level of $p < .005$, indicating that the presence of IUD caused a delay in the postcoital rise in 20α-OH-P in this species.

It may also be argued that the stress of frequent bleeding changed the secretion pattern of 20α-OH-P by the ovary. To minimize such stress, the blood was collected in a few minutes from ear arteries instead of the marginal ear vein every 2 hr and other artifacts such as anesthesia and laparotomy were avoided during blood collection.

There was no significant difference in the number of corpora lutea between control and IUD groups. Although Janakiramen and Casida (6) demonstrated a difference in the number of corpora lutea at 11 and 24 hr after mating in rabbits bearing a polyethylene plastic spiral, our data obtained from the rabbit with silastic tubing as IUD did not show this difference in number of corpora lutea at 12 and 24 hr after mating. These data suggest that the direct assay for serum LH and more frequent observation of ovarian stigmata, starting at least 8 hr after mating, are needed to demonstrate the mechanism of delay in ovulation in the rabbit with IUD.

Summary. Silicone rubber tubing (0.020 in. i.d.; 0.037 in. o.d.) was inserted in both horns of New Zealand white female rabbits (8–12 months old) as intrauterine contraceptive devices (IUD). After a short recovery period of 1 week to 10 days, these animals were mated with fertile males and about 10 ml of blood were collected from ear arteries every 2 hr beginning 1 hr postcoitum (pc). Serum 20α-hydroxypregn-4-en-3-one (20α-OH-P) was extracted with ether, isolated, and measured by thin-layer and gas–liquid chromatography. The concentrations of 20α-OH-P in serum from mated animals bearing IUD were compared with those from control animals without IUD. It was found that the highest peak of 20α-OH-P was detected at 1.67 ± 1.03 hr pc in intact animals. These values fell significantly by 3–5 hr and were lowest at 9 hr pc. In the IUD group the highest peak of 20α-OH-P occurred at 4.20 ± 1.10 hr pc and fell to lowest value at 9 hr. The difference of the values between these two groups is significant at $p < .005$. Although the number of stigmata on the ovary were not significantly different between these two groups, it is clear that the presence of IUD causes a delay in the postcoital rise in 20α-OH-P in this reflex-ovulating species.

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