Induction of Ovulation in Adult Rats with Follicle Stimulating Hormone (34319)

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The generally accepted role of follicle stimulating hormone (FSH) is one in which this gonadotropin acts to stimulate development of the follicle preparing it for ovulation when acted upon by luteinizing hormone (LH). There are reports, however, employing hypophysectomized immature rats (1, 2) or immature rats primed with pregnant mare serum (PMS) (3, 4) suggesting that FSH may play an active role in the ovulatory process itself. Additional studies in adult animals related to the release of FSH on the day of proestrus have been reported for the rat (5, 6) and hamster (7). Declines in pituitary content of FSH have been associated with estrus and ovulation in sheep (8), cattle (9), and swine (10). These studies in adult animals (5–10) further support the concept that FSH participates in the ovulatory process itself.

There are no studies which demonstrate unequivocally that FSH, by itself, in the *adult animal* is capable of inducing ovulation. Studies in *immature animals* where FSH is implicated in the ovulatory process (1–4) are difficult to interpret since LH is administered on the same day of treatment with FSH, or high doses of FSH with the inherent danger of LH contamination, are used to effect ovulation. Furthermore, it is known that immature rats of the ages employed in these studies (3, 4) ovulate in response to PMS treatment alone as long as the pituitary is present, a response which has been related to endogenous release of LH. The present paper presents data illustrating that FSH, by itself, is capable of inducing ovulation in adult rats prevented from ovulating with chlorpromazine (CPZ).

Materials and Methods. Adult nonparous rats of Wistar origin (Royal Hart Farms), weighing between 185–275 g, were used in experiments designed to show the ovulatory effects of FSH. Females were housed as previously described, and only females with 4-day estrous cycles were used in these studies (11).

Ovulation was prevented by subcutaneous injection of 1 mg of CPZ between the hours of 11:30 a.m. and 12:00 p.m. on the day of proestrus. This dose of CPZ was selected since it has been shown to block the neurogenic mechanism effecting the release of the ovulatory hormone(s) during the "critical period" (2–4 p.m.) in proestrus females (12). Females so treated were always quiet at the time of injection of the various gonadotropins some 3–4 hr later. They would respond to handling indicating that CNS depression, if any, was minimal.

The gonadotropins (NIHLH-S11, NIH FSH-S6, NIHFSH-P1), contained in .1 ml of saline, were administered to females during the "critical period" as a single injection into the saphenous vein. The rats were anesthetized lightly with ether during injection of the various gonadotropins.

All rats were sacrificed the day following administration of gonadotropins and the ovaries, oviducts, and tip end of the uterine horns were removed and examined with a dissecting microscope (14× magnifications) for evidence of ovulation. In addition, all oviducts were flushed with physiological saline and the flushings were examined (60×) for presence of ova in tightly packed cumulus balls as the index of recent ovulation.

The LH activity of NIHFSH-S6 (100 μg/female) was determined in 40 immature rats (Royal Hart Farms, Wistar) using the ovarian ascorbic acid depletion assay (OAAD)

TABLE I. Incidence of Ovulation in CPZ Blocked Rats Treated with NIHLH-S11, NIHFSH-S6, or NIHFSH-P1.

Gonadotropin administered	Dose (μg/♀)	No. of ♀♀ ovulating/ ♀♀ on test	% ♀♀ ovulating	Total no. of ova recovered	Mean no. of ova/ovulating ♀	ED_{50}[a] (μg/♀)
CPZ	—	3/87	4	—	—	
NIHLH-S11	1.4	33/40	83	321	9.8	
	1.2	24/40	60	234	9.8	1.19 ± 0.03
	1.0	4/40	10	31	7.8	
NIHFSH-S6	30.0	10/10	100	124	12.4	
	10.0	35/40	87	357	10.2	
	6.0	10/40	25	39	3.9	7.02 ± 0.45
	3.0	2/40[b]	5	23	11.5	
	1.0	0/20	0	—	—	
NIHFSH-P1	20.0	22/28	79	219	10.0	
	10.0	8/28	28	32	4.0	13.46 ± 1.19
	5.0	0/28	0	—	—	

[a] 95% confidence limits.

[b] It was felt that these two females would have ovulated even though the gonadotropin had not been administered. Evidence that the animals were not blocked is obtained when one considers the rather large number of ova recovered from these two females as compared to the small number of ova recovered from the 10 females ovulating at the next higher dose. Also, approximately 4% of the females treated with CPZ at 1 mg will ovulate.

described by Parlow (13). NIHLH-S11 (0.6, 1.2, and 2.4 μg/rat) was used as the reference standard. The method of measuring ascorbic acid was that of Mindlin and Butler (14).

The dose of gonadotropin necessary to cause ovulation in 50% of the treated females was calculated according to the method of Berkson (15). The data obtained in the OAAD assay were analyzed by the method of covariance (16), as applied by Sakiz and Guillemin (17).

Results. Doses of NIHLH-S11 varying from 1.0 to 1.4 μg were capable of inducing ovulation in adult rats blocked with CPZ (Table I). The rather small confidence limits associated with the calculated ED_{50} indicates the high degree of repeatability of results from one test to another. Also, doses of 3–30 μg of NIHFSH-S6 caused ovulation in blocked rats. NIH-FSH-P1 caused ovulation at 10 and 20 μg, but was ineffective at 5 μg. The calculated ED_{50} of NIHFSH-P1 (13.46 μg/rat) was approximately twice that of NIHFSH-S6 (7.02 μg/rat).

A significant (p.01) depletion of ovarian ascorbic acid was obtained when immature rats were treated with 0.6, 1.2, or 2.4 μg of NIHLH-S11 (Table II). NIHFSH-S6 at a dose of 100 μg, did not significantly deplete ovarian ascorbic acid when compared with the saline-treated control group.

Discussion. Everett (18) reported that a dose of 2 μg of LH/100 g of body weight injected subcutaneously caused ovulation in pentobarbital-blocked rats. Investigators using immature rats (3,4) or hypophysectomized immature rats (2) report that 4–20 μg of LH were necessary to induce ovulation. The results in the present studies with CPZ blocked rats are in essential agreement with these reports.

The data obtained in these studies show for the first time that FSH by itself was able to induce ovulation in *adult rats*. It has been reported that NIHFSH-S6 contains 4.5 μg of LH in 1000 μg of FSH, and that NIHFSH-P1 contains 7.5 μg of LH in a similar quantity of FSH. Therefore, it seems reasonable to suggest that if the ovulation obtained in these studies was the result of contaminating LH one would have expected

TABLE II. The Effect of Varying Doses of Gonadotropins on Ovarian Ascorbic Acid Depletion.

Treatment group	No. of ♀ ♀	Dose (μg/♀)	Corrected means (OAA in μg)	% Depletion compared to control group
Control	8	—	90.0	—
NIHLH-S11	8	0.6	57.9[a]	36
	8	1.2	55.8[a]	38
	8	2.4	44.1[a]	51
NIHFSH-S6	8	100.0	79.0[b]	12

[a] Values significantly (p .01) lower than control values.
[b] Nonsignificant.

that the ED_{50} for porcine FSH to be lower than that of ovine FSH. Also, the maximum amount of LH that would have been administered as a contaminant of NIHFSH-S6 at the highest dose (30 μg) would have been the equivalent of 0.135 μg and for the highest dose of NIHFSH-P1 (20 μg) 0.150 μg of LH; amounts of LH well below the minimally effective dose of 1.0 μg of NIHLH-S11. Thus, the doses of gonadotropins argue against the possibility that ovulation was the result of contaminating LH.

Furthermore, had the resulting ovulation been ascribed to LH one would have expected a much steeper dose–response curve for the FSH than was obtained. Additional evidence that the effects of FSH were not due to LH contamination was obtained in the OAAD assay. In this study 100 μg of NIHFSH-S6 did not produce depletion of ovarian ascorbic acid, whereas a dose of NIHLH-S11 as low as 0.6 μg significantly (p.01) depleted ovarian ascorbic acid when compared with control values. Thus, in this test system, commonly used as a sensitive assay for LH, NIHFSH-S6 at 100 μg (a dose roughly 15 times greater than the calculated ED_{50} of 7.02 μg) was unable to elicit a significant response. Studies are currently in progress utilizing rats hypophysectomized at proestrus in order to more fully explore the ovulatory effects of FSH. The observation that FSH by itself is able to induce ovulation of course does not negate its role in follicular development, but points out that it is involved in ovulation as well.

Summary. Data are presented demonstrating that in adult rats in which the release of LH had been blocked, FSH will cause ovulation. Thus, while FSH plays a primary role in the development of follicles, the data also support the view that it can, by itself, produce release of ova from graafian follicles. In this sense it should be considered as an intimate part of the ovulatory hormone complex.

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