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Induction of Ovulation in the Hypophysectomized Proestrous Hamster by Purified FSH or LH (40992)[1]

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Abstract. Hamsters hypophysectomized at 1300 hr of proestrus were immediately injected ip with various doses of highly purified ovine FSH or LH. The FSH preparation was more potent than LH in inducing ovulation. The results cannot be attributed to significant LH contamination in the injected FSH. Despite the ability of FSH to induce ovulation, the resultant corpora lutea did not secrete progesterone on the morning of estrus.

The ability of exogenous FSH to act as an ovulating hormone has been demonstrated in a variety of animal models (see (1, 2) for Ref.). One such model consists of hamsters hypophysectomized on the afternoon of proestrus—before the preovulatory surges of LH and FSH—and injected immediately with ovine FSH or LH, provided by NIH (1). The results showed that 10 μg FSH was capable of inducing ovulation in 80% of the acutely hypophysectomized hamsters but the next morning the newly formed corpora lutea did not secrete progesterone (1). The minimal effective doses of FSH contained the equivalent of 100 ng of NIH–LH–S1 which was far below the dose of 2.5 μg LH needed to induce ovulation (1).

The availability of highly purified and potent ovine FSH (3, 4) and LH (5) prompted the present study which utilized the hypophysectomized proestrous hamster as the animal model.

Materials and methods. Golden hamsters (*Mesocricetus auratus*) were maintained on a 14-hr light:10-hr dark schedule with the lights on from 0500 to 1900 hr (CST). At least three consecutive 4-day cycles were monitored before the animals were used. The postovulatory discharge on Day 1 of the cycle (=estrus) was used to determine the stage of the cycle; Day 4 corresponds to proestrus.

The hamsters were hypophysectomized at 1300 hr of proestrus by a parapharyngeal approach while under Nembutal anesthesia. The animals were then immediately given a single intraperitoneal injection of various doses of purified ovine FSH and LH, prepared by H. Papkoff (see below). The animals were decapitated the next morning (estrus) at 0900 hr and trunk blood was saved for radioimmunoassay (RIA) of progesterone. At necropsy the sella turcica was examined with a dissecting microscope and animals with fragments of pituitary were discarded from the study. The ovaries were examined for newly formed corpora lutea and the oviducts flushed to recover recently ovulated ova which were still embedded in granulosa cells; the granulosa cells were dispersed with hyaluronidase (Wydase, Wyeth) and the eggs counted at 25× magnification.

Hormones. The ovine LH (G3-222B) was estimated to be 2.75 × NIH–LH–S1 by the ovarian ascorbic acid depletion assay and 2.69 × LH–S12 by RIA. The FSH activity was 0.0144 × NIH–FSH–S11 by RIA. The ovine FSH preparation (G4-150C) was 54 × NIH–FSH–S1 by the Steelman–Pohley assay. It contained minimal LH activity: less than 0.02 × NIH–LH–S1, as determined by the ovarian ascorbic acid depletion assay as well as by the rat Leydig cell assay (6). The hormones were administered ip in 0.1 ml of physiological saline.

RIA of progesterone. Serum progesterone was determined by a modification (7) of the RIA method of Thorneycroft (8) utilizing an antiserum provided by Dr. Ian

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Thorneycroft [11α-succinylprogesterone bovine serum albumin (1HT-R-11-1516-1)].

Results. Injection of 2.5 or 5.0 μg FSH was effective in inducing ovulation in almost all animals and with the same number of ovulations as in sham-hypophysectomized hamsters (Table I). Even 1 μg FSH induced ovulation in 40% of the animals whereas 1 μg LH was wholly ineffective. Even though 2.5–5.0 μg of FSH induced ovulation and there were new corpora lutea present the next morning, serum levels of progesterone did not differ from the values in untreated hypophysectomized hamsters (Table I). In contrast, the corpora lutea induced by 2.5 μg LH were associated with serum progesterone in the range of the sham-hypophysectomized control group.

In the next experiment, various amounts of subliminal FSH and LH were combined to determine whether synergism could be achieved. The hormones were administered as separate ip injections. As shown in Table II, 1 μg FSH plus 1.5 μg LH resulted in all animals ovulating but the other combinations were not much more effective than 1 μg FSH alone.

Discussion. Using these purified preparations, FSH was a better ovulating hormone than LH (Table I). The minimal effective dose of FSH was 2.5 μg which contained at most the equivalent of 50 ng LH (see Materials and methods). Since even 1 μg of LH was unable to induce ovulation, the ovulating ability of the FSH preparation cannot be attributed to significant LH contamination and therefore represents an inherent property of FSH. Moreover, attempts to combine low doses of FSH and LH indicates little evidence of synergism until LH was increased to values very close to the amount of LH required alone to produce 100% ovulation (Tables I and II).

The minimal effective dose of FSH needed to induce ovulation was 2.5 μg (Table I) compared to 10 μg of ovine FSH–NIH in a previous study (1). Since the present FSH preparation was 54× as potent as NIH–FSH–1, we initially thought that a dose considerably below 2.5 μg might be effective. However, the potency of 54× NIH–FSH is based on the results of the Steelman–Pohley assay which involves the *chronic* administration of FSH over a 3-day period. The present animal model tests the *acute* effects of FSH. The different lengths of time involved in arriving at the respective endpoints most likely accounts for the lowered potency of the purified FSH in this investigation.

In confirmation of previous results (1), the corpora lutea induced by FSH are not secreting progesterone on the morning of their formation, as evidenced by the serum levels of immunoassayable progesterone (Table I). A recent study has shown, however, that the FSH induced corpora lutea on the morning of ovulation (i.e., estrus), despite low serum levels of progesterone, are capable *in vitro* of producing substantial amounts of the hormone; in fact these corpora lutea produce approximately twice as much progesterone as corpora lutea induced by LH (9). By Day 2 of the "artificial" cycle, serum levels of progesterone

TABLE I. THE OVULATORY ACTIVITY OF FSH OR LH INJECTED IP INTO HYPOPHYSECTOMIZED (H) PROESTROUS HAMSTERS

Treatment after H̄ at 1300-hr proestrus	No. ovulating at estrus / Total no. animals	Mean number of ovulations ± SEM	Serum progesterone at estrus (ng/ml ± SEM)
Sham	8/8	10.9 ± .76 (8)	6.27 ± 0.45 (5)
Saline	0/5	—	1.19 ± 0.86 (5)
5 μg FSH	5/6	12.0 ± .63 (5)	1.04 ± 0.58 (5)
2.5 μg FSH	10/10	10.9 ± .56 (10)	1.27 ± 0.33 (5)
1.0 μg FSH	4/10	10.0 ± 2.3 (4)	1.09 ± 0.81 (5)
0.5 μg FSH	0/3	—	—[a]
2.5 μg LH	6/6	10.8 ± .74 (6)	4.88 ± 1.3 (5)
1.0 μg LH	0/8	—	1.79 ± 1.0 (5)

[a] Not determined.

TABLE II. THE OVULATORY ACTIVITY OF COMBINED FSH AND LH IN THE HYPOPHYSECTOMIZED ($\bar{H}$) PROESTROUS HAMSTER

Treatment after $\bar{H}$ at 1300-hr proestrus	No. ovulating at estrus / Total No. animals	Mean number ovulations ± SEM	Serum progesterone at estrus (ng/ml ± SEM)
1 μg FSH + 0.1 ml saline	3/8	7.7 ± 3.2 (3)	0.27 ± 0.07 (5)
1 μg FSH + 0.1 μg LH	2/8	7.5 (2)	0.33 ± .20 (5)
1 μg FSH + 1.0 μg LH	5/8	10.6 ± 1.6 (5)	0.72 ± 0.8 (5)
1 μg FSH + 1.5 μg LH	8/8	9.4 ± 1.3 (8)	2.26 ± .64 (5)

are similar whether FSH or LH was used as the ovulating hormone. Hence, the initial retardation in steroidogenesis by the corpora lutea induced by FSH is eventually overcome.

A necessary caveat is that although these experiments and others demonstrate inherent ovulating activity in the FSH molecule this is not meant to detract from the normal situation *in vivo* in which LH probably plays a predominant role as *the* ovulating hormone (for references see (10)).

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