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# Inhibin: Differences in Bioactivity within Human Follicular Fluid in the Follicular and Luteal Stages of the Menstrual Cycle[1] (40768)

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Recently, the presence of a nonsteroidal substance (inhibin) has been demonstrated in porcine (1), bovine (2), and human (3) follicular fluid. This ovarian substance exerts an inhibitory effect on pituitary follicle-stimulating hormone (FSH) secretion and may play a role in the regulation of ovulation in female mammals. A similar material isolated from hamster ovaries can suppress the release of FSH that occurs in female hamsters immediately after ovariectomy or in an *in vitro* pituitary cell culture system after luteinizing hormone-releasing hormone treatment (4). This substance exerts only a slight inhibitory effect on luteinizing hormone (LH) secretion. Because one of us (S. C. C.) has determined that inhibin is present within steroid-free extracts of hamster ovaries at a specific stage (proestrus) of the estrous cycle (5), we performed experiments to determine (a) whether inhibin is present within human follicular fluid (HFF) and if so, (b) whether this material is present within ovarian follicular fluid at specific times during the menstrual cycle. The knowledge of relative changes in levels of inhibin present within HFF during the menstrual cycle may allow investigators to postulate its role in the regulation of ovulatory cyclicity in the human female.

*Materials and methods.* The HFF was obtained from women at the time of hysterectomy and/or ovariectomy. Proliferative phase FF was obtained from patients undergoing tubal ligations for contraceptive purposes or who were undergoing tuboplasty. The luteal phase specimens were obtained from portions of the routine surgical specimen obtained at oophorectomy performed for endometrial carcinoma. Immediately before surgical intervention, a peripheral blood sample was obtained. The HFF was treated twice with charcoal (50 mg Norit/ml) for removal of estradiol ($E_2$) and progesterone (P). The charcoal-treated HFF and peripheral serum samples were analyzed for $E_2$ and P by the appropriate radioimmunoassay (6, 7). Residual steroid levels in HFF samples so treated were found to be at the sensitivity of the assays ($E_2 < 10$ pg/ml; $P < 1$ ng/ml).

*Determination of inhibin content in HFF samples.* The presence of inhibin within HFF samples was determined by two methods. The first was an *in vivo* model that employed ovariectomized rhesus monkeys. Each monkey had been fitted previously with an indwelling stainless-steel cannula aimed at the anterior pituitary gland (8). After six hourly preinfusion blood samples (2 ml each) 25 $\mu$l of steroid-free HFF collected from one of five women was infused through the catheter into the anterior pituitary gland. The infusion time was always less than 1 min. Thereafter, a 2-ml blood collection was obtained at hourly intervals for 18 hr. Serum concentrations of LH and FSH were determined by the appropriate radioimmunoassay (6) and expressed in terms of the standard LER-1909-2. HFF samples that induced a decline in serum FSH but not LH concentrations were classified as inhibin containing.

The second method was an *in vitro* hamster pituitary cell culture system. Female hamsters that had been ovariectomized for

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at least 1 month were treated for 3 days with 100 μg of estradiol benzoate and 5 mg of progesterone (sc). On the fourth day, animals were decapitated and anterior pituitary cells were enzymatically dispersed as described previously (4, 10). After 4 days of growth in Dulbecco's modified Eagle's medium plus horse serum (3%) and fetal calf's serum (FCS;15%), each culture dish (500,000 cells/dish) was washed three times with serumless medium. Each dish was then resuspended in serumless medium that contained 10 μl of the HFF sample to be tested or 25 μl of steroid-free fetal calf's serum as a control. This substance was used as a control due to its lack of detectable inhibin content in our bioassay. All cultures were allowed to incubate overnight as preliminary studies had demonstrated a highly significant effect of inhibin upon gonadotropin release at that time. The next day, each dish was washed with medium three times and resuspended in serumless medium, medium plus luteinizing hormone-releasing hormone (LHRH; $10^{-8}$ *M*) plus 25 μl of steroid-free FCS, or medium plus LHRH plus 10 μl of HFF. Each HFF sample was tested in duplicate cultures. All dishes were again incubated for 4 hr at 37°C. The medium was removed, centrifuged (2,200*g* for 1 hr), and stored frozen. The concentrations of LH and FSH were determined within the medium from each culture dish by the appropriate radioimmunoassay (4, 5). The criterion for the presence of inhibin in HFF was an observed increase in medium concentrations of LH in the absence of a measurable concomitant increase in FSH concentrations after treatment of the pituitary cells with LHRH. If present within HFF, inhibin was expected to decrease the LHRH-induced increase in FSH secretion without affecting the LHRH-induced increase in LH concentrations in the medium. The concentrations of LH and FSH within tissue culture medium are expressed as a percentage of the LHRH- and FCS-treated controls. The effects of HFF treatment were determined by the one-way analysis of variance (ANOVA; *in vivo* tests) or the Student's *t* test (*in vitro* tests).

*Results.* As shown in Table 1, the HFF from each patient was classified according to the serum levels of estradiol and progesterone present at the time of collection. None of the HFF donors were undergoing surgery for ovarian diseases. Of the 11 samples, the first 6 were judged to have been collected from women during the follicular phase of the menstrual cycle. The remaining 5 samples were collected during the luteal phase.

The results of the infusion of 25 μl of HFF collected from patients WE, MO, and SU into ovariectomized rhesus monkeys are shown in Fig. 1. Only the HFF collected from patient SU exerted an inhibitory ($P < 0.01$) effect on serum FSH but not LH levels. Serum FSH concentrations decreased from a preinjection level of 25.9 μg/ml to 8.0 μg/ml at the end of the blood

TABLE I. SERUM CONCENTRATIONS OF ESTRADIOL AND PROGESTERONE OF WOMEN AT THE TIME OF FOLLICULAR FLUID COLLECTION

| Patient | Age | Estradiol (pg/ml) | Progesterone (ng/ml) | Estimated stage of cycle |
|---|---|---|---|---|
| FU | 29 | 115 | 1.3 | Early follicular |
| BO | 30 | 124 | 0.79 | Early follicular |
| PI | 39 | 376 | 1.36 | Follicular |
| RO | 32 | 198 | 1.98 | Follicular |
| CA | 24 | 287 | 0.91 | Follicular |
| SU | 30 | 581 | 2.6 | Late follicular |
| MO[a] | 40 | 587 | 31.6 | Luteal |
| WE[a] | 32 | 395 | 11.4 | Luteal |
| JA | 40 | 79 | 6.44 | Luteal |
| MC | 45 | 39 | 2.55 | Late luteal |
| SM[a] | 27 | 16 | 3.64 | Late luteal |

[a] A corpus luteum was observed when follicular fluid was collected.

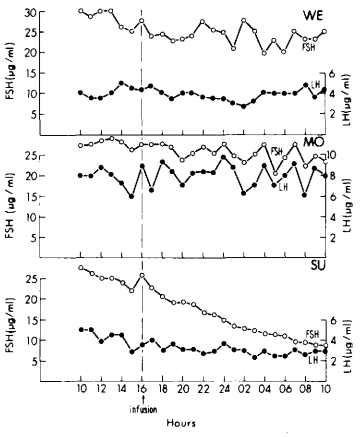


FIG. 1. Serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) at hourly intervals in three ovariectomized rhesus monkeys before and after the infusion (at 1600 hr) of 25 μl of human follicular fluid (HFF). The initials of the HFF donor in each trial are given in the upper right corner of each panel.

collection period, a 70% reduction. Infusion of HFF collected from patients MO and WE did not exert an effect on the serum levels of either gonadotropin. In two trials not shown, HFF collected from patients FU and PI during the follicular phase elicited a decrement in serum FSH but not LH levels following the infusion. The reduction in circulating FSH levels after the infusion of HFF collected from these two patients was approximately 30% of preinfusion levels.

When 10 μl of steroid-free HFF collected from each woman described in Table 1 was tested individually in the *in vitro* pituitary cell culture system, no inhibitory effect on the LHRH-induced LH release was observed. The concentrations of LH within the media of each test dish was similar to those of LHRH-treated controls, and elevated ($P < 0.01$) above the values for control dishes not treated with LHRH (Fig. 2). Pituitary cell cultures treated with LHRH and 10 μl of HFF collected from women judged to be in the follicular phase of the menstrual cycle exerted an inhibitory effect on the LHRH-induced FSH secretion compared with HFF collected during the luteal phase (Fig. 3). Medium FSH concentrations from cultures incubated with follicular phase HFF were 50–65% of that found in control LHRH-treated medium and similar to FSH concentrations in cultures not stimulated with LHRH (medium only). However, the HFF collected from patient BO during the early follicular phase did not contain inhibin activity. The HFF collected from women during the luteal phase of the menstrual cycle exerted no effect on the LHRH-induced FSH release. Pituitary cells that received 10 μl of HFF from women in

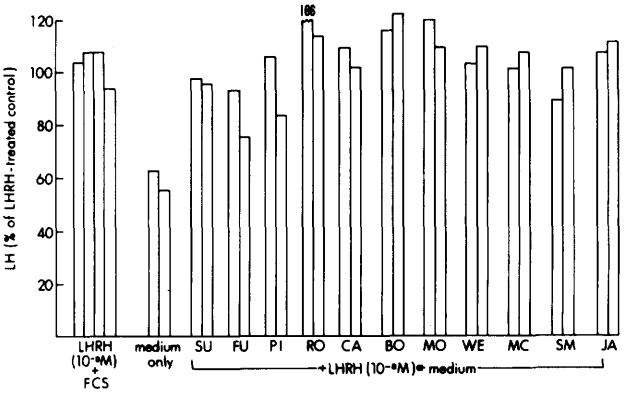


FIG. 2. The failure of 10 μl of human follicular fluid (HFF) collected from 11 women at different stages of the menstrual cycle to affect the release of luteinizing hormone (LH) stimulated by luteinizing hormone-releasing hormone (LHRH) in a hamster pituitary cell culture system. Duplicate trials were performed with each HFF sample; the LHRH-treated control test was run four times in the presence of 25 μl of steroid-free fetal calf's serum (FCS). The LH concentrations are expressed as percentages of the average LH concentration in the LHRH-treated controls (LHRH plus FCS).

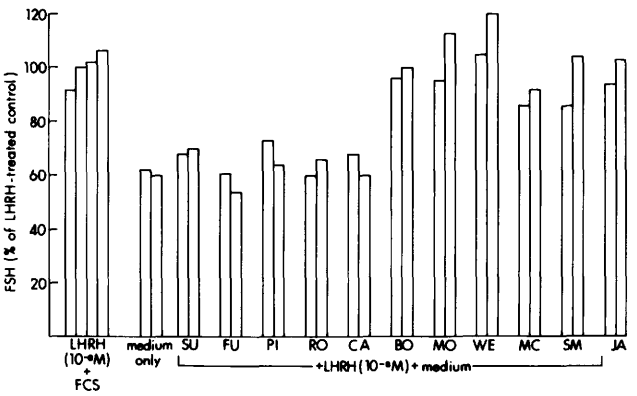


FIG. 3. The ability of 10 $\mu$l of human follicular fluid (HFF) collected from patients SU, FU, PI, RO, and CA to suppress the release of follicle-stimulating hormone (FSH) induced by luteinizing hormone-releasing hormone (LHRH). The HFF collected from the other patients exerted no inhibitory effect on FSH release vs fetal calf serum (FCS)-treated controls. The FSH concentrations are expressed as percentages of the average FSH concentration of the LHRH-treated controls (LHRH plus FCS).

the follicular phase of the menstrual cycle released significantly less ($P < 0.01$) FSH after LHRH treatment than cells receiving luteal HFF.

*Discussion.* To our knowledge, this study is the first that demonstrates the presence of inhibin within HFF at only certain stages of the menstrual cycle. However, until inhibin concentrations within ovarian venous blood have been determined throughout the menstrual cycle, these results must be viewed with caution. Follicle size and ovarian blood supply change throughout the cycle and may exert an effect upon inhibin within HFF. These results support previous findings that inhibin is present within the fluid from human Graafian follicles (3, 9) and that greater amounts of inhibin are present in fluid collected from large-diameter bovine follicles than in fluid from smaller ones (11). They also support the previous observation that this FSH-inhibiting material was detectable only in hamster ovaries collected immediately before ovulation (5). Inhibin may be present within HFF at other times of the cycle but in amounts below the sensitivity of our bioassay. These results suggest that inhibin may exert an influence on FSH secretion at specific stages of the mammalian reproductive cycle. The lack of inhibin activity in the HFF of patient BO during the early follicular phase suggests that this material may not exert an effect on pituitary FSH secretion at that time. Since serum estradiol levels are also low during this period, the lack of steroidal and nonsteroidal negative feedback may provide the proper hormonal milieu for the rise in serum FSH observed in female rhesus monkeys (6) and humans (12) during the early follicular period. That this substance affects the secretion of FSH from primate and rodent anterior pituitary cells suggests a pituitary site of action. This substance may be present in many mammalian species; studies with human, bovine, porcine, and hamster inhibin support this hypothesis.

The physiological role of this substance in women and female animals has yet to be determined. However, its ability to affect the secretion of FSH, without altering LH, may shed light on how just one releasing hromone (LHRH) can regulate the secretion of both gonadotropins.

*Summary.* The presence of a nonsteroidal substance (inhibin) within human follicular fluid (HFF) with the ability to suppress pituitary follicle-stimulating hormone (FSH) but not luteinizing hormone (LH) secretion was determined by two methods. The first method consisted of infusion of HFF directly into the anterior pituitary gland of ovariectomized rhesus monkeys. The second employed a pituitary cell culture system. Release of LH and FSH was stimulated in HFF-treated cells by the addition of luteinizing hormone-releasing

hormone (LHRH) to the tissue culture medium. In both methods, to determine the presence of inhibin, we used the criterion of a decreased release of FSH into the medium or serum that was not accompanied by an concomitant decrement in LH concentrations. Both methods detected the presence of inhibin within HFF from women during the follicular but not the luteal phase of the menstrual cycle. This finding suggests that this substance may play a role in the regulation of FSH secretion at a specific stage of the reproductive cycle in women.

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