

The anterior pituitary response to a gonadotropin-releasing hormone challenge test in normal older reproductive-age women*†

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Objective: To assess the pituitary responsiveness to GnRH stimulation of premenopausal women relative to age.

Design: Older and younger reproductive-age women underwent the GnRH stimulation test in the early follicular phase of the menstrual cycle.

Setting: Female subjects in an academic research environment.

Patients: Women aged 21 to 44 years consisting of normal volunteers and infertility patients.

Interventions: Gonadotropin-releasing hormone was administered intravenously between days 2 and 4 of the menstrual cycle. Blood samples were collected from -20 minutes before to 120 minutes after administration.

Main Outcome Measure: Luteinizing hormone, FSH, inhibin, and E₂ levels.

Results: No significant difference in baseline values existed between older and younger women with regard to LH, inhibin, and E₂, but basal FSH levels were higher in older women. A significantly diminished percent of LH and percent FSH change above baseline occurred 30 minutes after GnRH administration in the older women compared with younger women. No change in inhibin or E₂ levels could be detected during the sampling period.

Conclusions: The present study demonstrates marked attenuation of the acute pituitary LH response (sensitivity) to GnRH stimulation in older women when compared with a younger cohort. *Fertil Steril* 1996;65:539-44

Key Words: Reproductive aging, GnRH stimulation test, gonadotropins

Peak efficiency of the female reproductive system occurs at approximately 24 years. Thereafter, there is a steady decrease in fertility. The decline of reproductive capacity in normal cycling women during the fourth and fifth decades has been well documented (1, 2). This age-related decline in fecundity is thought to result from diminishing numbers of ovar-

ian follicles with an associated increase in ovarian resistance to gonadotropins. The concept of an age-related decline in ovarian reserve appears to be well supported in the literature (3, 4). Despite the popularity of the theory that premenopausal changes in reproductive function (e.g., fertility) are caused by an antecedent decrease in ovarian reserve, the effect of aging on the hypothalamic-pituitary axis in reproductive-age women remains uncertain.

The earliest identifiable endocrinologic phenomenon that occurs with aging in women is an FSH elevation unaccompanied by an identifiable rise of LH, otherwise known as the monotropic FSH rise (5-8). Transplant studies in rodents implicate the neuroendocrine unit as the primary site of both the monotropic FSH rise and reproductive aging (9). Despite an elevated FSH level, reduction of ovarian steroid output in ovulatory reproductive-age women does not occur. This implies that, as women age, the alterations in the normal negative feedback relation-

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ship between the ovary and the hypothalamic-pituitary (neuroendocrine) axis is altered (6).

Older yet ovulatory women can be characterized in part by an increased ovarian resistance to exogenous gonadotropins (2). Older infertile women require larger amounts of hMG and produce fewer numbers of follicles in clinical trials of controlled ovarian hyperstimulation (COH) (10). In the clinical setting, regardless of the therapy used, a clear and significant decrease in successful pregnancy rates occurs in infertile women greater than age 40 years when compared with younger women (11).

If the monotropic FSH rise is the initial change seen in older reproductive-age women, we sought to define a group of women who are relatively older and less fertile whose relatively advanced reproductive age could be "uncovered" by a classic endocrine stimulation test. To define more critically possible changes in the neuroendocrine axis associated with the monotropic FSH rise, we conducted a prospective study using GnRH stimulation to compare pituitary responsiveness between older and younger reproductive-age women. In a separate cohort of the infertility patients, response to hMG therapy for COH was compared with their prior response to GnRH stimulation.

MATERIALS AND METHODS

Subjects

A group of 47 women between the ages of 38 and 45 years comprised of 10 normal female volunteers and 37 infertility patients were the subjects for this study. Seventeen women between the ages of 21 and 25 years, comprised of 10 normal female volunteers and 7 women participating as oocyte donors in the University of Washington donor oocyte program, were studied as the younger control group. The study was approved by the Human Subjects Review Committee of the University of Washington. Informed consent was obtained from each woman. All control subjects were within $\pm 10\%$ of ideal body weight* (Metropolitan Life tables, 1980) and had regular menstrual cycles (25 to 35 days). All control subjects were required to demonstrate normal biphasic BBT charts, normal serum PRL (< 20 ng/mL; conversion factor to SI unit, 1.00), P (> 12 ng/mL; conversion factor to SI unit, 3.180), and T (< 60 ng/dL; conversion factor to SI unit, 3.467) in the midluteal phase of a cycle before participation. These women were taking no medication and had not received hormone therapy within the previous 2 months. Of 37 infertility patients studied, 22 pa-

tients underwent subsequent COH in the Fertility and Endocrine Center at the University of Washington School of Medicine.

Protocol

Each subject was admitted to the University of Washington Clinical Research Center during the early follicular phase of a spontaneous menstrual cycle (cycle days 1 to 4) for a period of approximately 3 hours. They were placed in a semirecumbent position for the duration of the study. Six blood samples were taken over the course of each admission. The first and second samples were drawn at -15 and -1 minute, respectively, before administration of GnRH. At time 0, $25 \mu\text{g}$ IV GnRH (Lutrapulse; Ferring Laboratories, Suffern, NY) was administered. Subsequent venous blood samples were taken at 30, 60, 90, and 120 minutes after the injection. All blood samples were allowed to clot and were centrifuged. Serum for hormone measurements was removed and frozen at -4°C until analysis.

Hormone Assays

Serum concentrations of FSH, LH, and E_2 were determined in all blood samples. Serum levels of inhibin were determined at baseline, $+60$, and $+120$ minutes only. All serum samples from an individual woman were analyzed in duplicate in one hormone assay to minimize effects of interassay variability. Luteinizing hormone and FSH assays were determined using DELFIA LH and FSHspec (Wallace, Inc., Gathersburg, MD), which are solid-phase two-site fluorometric (Delfia) assays in which two monoclonal antibodies are directed against two separate antigenic determinants on the human gonadotropin molecules. The FSH assay antibody cross-reacts $< 1\%$ with LH. The LH assay antibody cross-reacts $< 1\%$ with FSH. The LH intra-assay and interassay coefficients of variation were 2.8% and 4.7%, respectively. The intra-assay and interassay coefficients of variation for the FSH assay were 2.3% and 4.6%, respectively.

The RIA for serum E_2 was performed in duplicate using reagents supplied by ICN Biomedicals, Inc. (Costa Mesa, CA). The antibody cross-reacts 20% with estrone, 1.5% with estriol, and $< 1\%$ with all other steroids. The interassay and intra-assay coefficients of variation were 18% and 9%, respectively.

Serum inhibin was measured in a heterologous double-antibody RIA based on purified 31-kd bovine follicular fluid inhibin as described previously (12). The interassay and intra-assay coefficients of variation were 13.9% and 6.8%, respectively.

* Bulletin of the Metropolitan Insurance Company 1980; 40:1.

Controlled Ovarian Hyperstimulation

Twenty-two of the older infertility patients attempted 40 cycles of ovarian hyperstimulation with hMG as part of their infertility treatment. The remainder of the 37 infertility patients elected not to undergo a COH cycle, choosing to discontinue infertility treatment or a treatment that did not include COH. Patients received 300 to 450 IU hMG IM daily beginning day 3 of the cycle (with or without pituitary down-regulation using leuprolide acetate beginning in the midluteal phase of the preceding cycle). Initial gonadotropin doses were individualized according to patients' age, weight, and previous ovarian response to stimulation. Ovarian response was monitored with serial transvaginal ultrasound and serum E_2 levels beginning after 5 days of gonadotropins. Human chorionic gonadotropin (10,000 IU) was administered IM once the lead follicle(s) was ≥ 16 mm in mean diameter. Cycles were evaluated based on total dose of gonadotropin required, number of follicles > 10 mm, and serum E_2 level on the day of hCG.

Statistical Analysis

Statistical analysis was performed using parametric testing. All basal levels represent the mean of -15- and -1-minute sample measurements. Basal levels and responses to GnRH stimulation of serum LH, FSH, E_2 , and inhibin were compared between the groups using Student's unpaired *t*-tests. The absolute gonadotropin response (Δ LH or Δ FSH) was determined as the difference in gonadotropin levels between the mean of the preceding unstimulated baseline and stimulated level at each time point measured. Results are expressed as means \pm SEM.

RESULTS

Basal Endocrine Characteristics

Mean basal serum FSH in the older reproductive-age group was significantly higher (6.95 ± 0.59 mIU/mL; conversion factor to SI unit, 1.00) compared with the younger women (4.9 ± 0.33 mIU/mL; $P < 0.05$). Conversely, there was no significant difference in mean basal serum LH levels between the older and younger groups (4.29 ± 0.33 versus 3.7 ± 0.28 mIU/mL, respectively; $P = 0.34$; conversion factor to SI unit, 1.00). The mean basal serum E_2 level was higher in the older group (77.11 ± 9.45 pg/mL) compared with the younger group (55.75 ± 4.83 pg/mL) but the difference was not statistically significant ($P = 0.17$). The mean basal level of immunoreactive inhibin in the older group (443.29 ± 248.43 mIU/mL) was not significantly different from the younger group (241.89 ± 81.1 mIU/mL; $P = 0.63$).

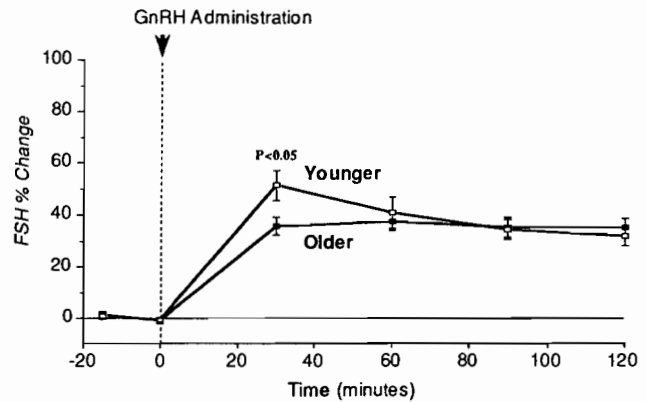


Figure 1 Mean \pm SEM FSH percent change above baseline for older and younger women. Statistical significance was achieved only at the time point +30 minutes ($P < 0.05$).

Endocrine Dynamics After GnRH Stimulation

In both age groups, prompt and sustained increases in gonadotropin levels were achieved in response to GnRH stimulation. Maximal gonadotropin response to GnRH stimulation occurred at +30 minutes in all subjects studied. In the older cohort, the mean serum FSH level achieved at +30 minutes was 9.54 ± 0.87 mIU/mL, corresponding to a percent change from baseline of $37.45\% \pm 3.64\%$. The mean serum FSH level 30 minutes after stimulation in the younger group was 7.49 ± 0.60 mIU/mL, with a corresponding percent change from baseline of $51.2\% \pm 5.88\%$, which was significantly greater than the older group ($P < 0.05$) (Fig. 1). Despite the significant difference in percent change from baseline of FSH, absolute FSH rise above baseline at +30 minutes was similar between older and younger groups (2.55 ± 0.35 versus 2.57 ± 0.35 mIU/mL, respectively; $P = 0.97$; Fig. 2). Neither FSH percent change from baseline nor absolute FSH rise above baseline were significantly different between the two groups at +60, +90, and +120 minutes after stimulation (Figs. 1 and 2).

The mean serum LH level achieved at +30 minutes for the older group was 14.16 ± 1.05 compared with 16.85 ± 1.83 mIU/mL in the younger group. The percent change from baseline of LH at +30 minutes after stimulation for the younger group ($369.29\% \pm 46.85\%$) was significantly higher than for the older group ($251.44\% \pm 19.60\%$; $P < 0.05$; Fig. 3). Unlike the FSH response, the absolute LH rise above baseline at +30 minutes was greater in the younger group compared with the older group, but this difference did not reach statistical significance (13.11 ± 1.70 versus 9.88 ± 0.86 mIU/mL, respectively; $P = 0.07$; Fig. 2). Comparison of the absolute LH rise above baseline at +30 minutes between older control subjects and older infertility pa-

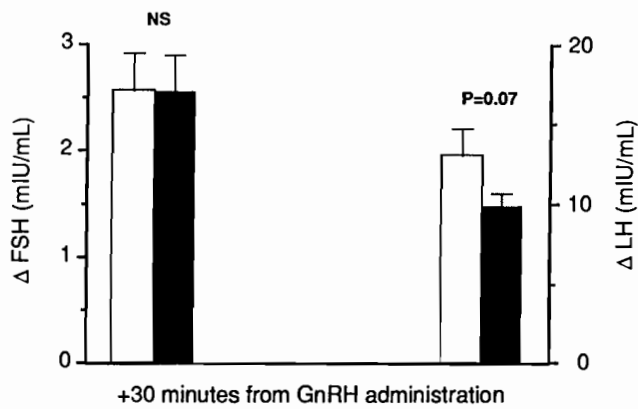


Figure 2 Δ FSH and Δ LH values for older and younger women at +30 minutes after GnRH administration. The Δ LH value at +30 minutes after GnRH stimulation approached statistical significance ($P = 0.06$). □, younger; ■, older.

tients revealed no difference (11.49 ± 2.15 versus 9.44 ± 0.93 mIU/mL, respectively; $P = 0.33$). Similar to the FSH response, no difference in percent change above baseline or absolute LH rise could be detected between the two groups at +60, +90, and +120 minutes after GnRH stimulation (Fig. 3).

Serum E_2 levels remained constant in each group throughout the duration of sampling in both age groups. Older women had a higher level of E_2 at all time points; however, with the exception of basal E_2 , none of these differences were statistically significant. Likewise, serum inhibin levels did not vary dramatically after GnRH stimulation. At +60 minutes after GnRH administration, the serum inhibin levels for the older and younger groups were 461.79 ± 236.72 and 209.762 ± 65.85 mIU/mL, respectively; $P = 0.52$. At +120 minutes after stimulation, serum levels of inhibin were 457.5 ± 257.76 and 235.78 ± 76.91 mIU/mL in older and younger groups, respectively ($P = 0.61$).

Controlled Ovarian Hyperstimulation

Patients who underwent gonadotropin stimulation ($n = 22$) were classified as either poor or normal responders according to their response. A poor responder was defined as a patient with one or more cycles cancelled because of inadequate ovarian response (serum E_2 level < 100 pg/mL after ≥ 5 days of gonadotropins) or with peak serum E_2 level < 500 pg/mL and five or fewer follicles > 10 mm on the day of hCG administration. Normal responders completed all attempted cycles and had serum E_2 levels > 500 pg/mL on the day of hCG with more than five follicles exceeding a mean diameter of 10 mm.

There were no differences in age between poor and normal responders (39.8 ± 0.7 versus 40.1 ± 0.4

years, respectively; $P = 0.7$). Compared with normal responders, poor responders required higher doses of gonadotropins ($4,111 \pm 405$ versus $2,362 \pm 165$ IU; $P < 0.01$) and had fewer follicles > 10 mm (2.9 ± 0.5 versus 6.8 ± 0.5 ; $P < 0.01$) and lower serum E_2 (475 ± 60 versus 868 ± 86 pg/mL; $P < 0.01$) on the day of hCG. Baseline FSH and response to 25 μ g of GnRH are shown in Table 1. Whereas there was a trend toward higher baseline FSH and lower percent change of both FSH and LH in the poor response group, none of these differences were significant. There were no differences in absolute change in FSH or LH between the two groups. Therefore, the GnRH stimulation test was not helpful in identifying patients who subsequently experienced a poor ovarian response to exogenous gonadotropins.

DISCUSSION

Our understanding of the mechanisms leading to the diminished capability of older reproductive-age women to achieve a successful pregnancy remains poor. It has been postulated that diminished ovarian reserve represents a primary reason for a decreased ability to conceive in the later stages of a woman's reproductive life. Evidence of an age-related decrease in fertility has been provided woman who undergo donor insemination. These patients in whom male fertility and coital frequency has been controlled nevertheless experience a decreased pregnancy rate with advancing age (13). It also is well established that a monotropic rise in basal FSH occurs during the early follicular phase of ovulatory cycles as the first sign of reproductive aging (8, 14). Even though it has not been established rigorously that diminished ovarian reserve is responsible for basal FSH elevation, many clinicians have assumed this relationship to be the case.

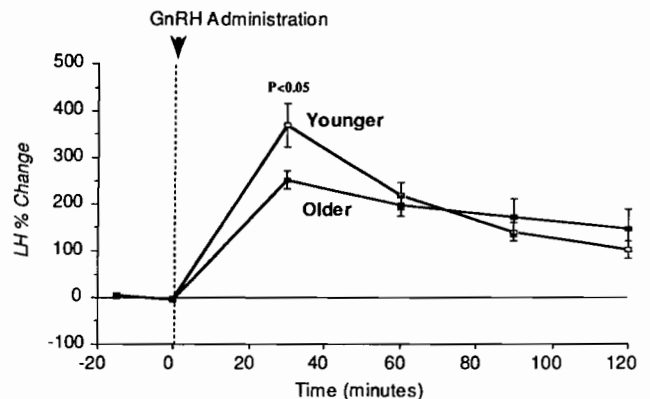


Figure 3 Mean \pm SEM LH percent change above baseline for older and younger women with statistical significance was achieved at the +30-minute time point ($P < 0.05$).

Table 1 Parameters of GnRH Stimulation Testing in Older Women Undergoing COH*

	Baseline FSH	FSH percent change (+30 minutes)	LH percent change (+30 minutes)	Δ FSH	Δ LH
	<i>mIU/mL</i>			<i>mIU/mL</i>	
Poor responders (n = 9)	6.9 \pm 0.6	32.0 \pm 5.6	216.0 \pm 45.0	2.1 \pm 0.4	7.5 \pm 1.2
Normal responders (n = 13)	5.8 \pm 0.4	37.9 \pm 7.0	305.0 \pm 50.0	2.1 \pm 0.3	9.8 \pm 1.3
P value	0.12	0.49	0.10	0.91	0.13

* Values are means \pm SEM. Conversion factors to SI units are as follows: LH and FSH, 1.00.

The elevation in basal FSH (reproductive aging) seen in older women likely represents an event influenced by both intrinsic ovarian and neuroendocrine changes. Ovarian transplantation experiments in aging female rats provide evidence for age-related changes at the level of the hypothalamic-pituitary axis independent of diminished ovarian reserve (9, 15). In the present study, the diminished LH response to GnRH stimulation in our older cohort of subjects would suggest a differential effect of aging on the regulation of gonadotropin secretion. This observation is in contrast to evidence provided by Muasher et al. (16) demonstrating no difference in the magnitude of increase in serum LH among groups of infertile premenopausal women with varying levels of basal serum FSH and LH. Our findings in this study suggest that intrinsic alterations within the human pituitary occur with aging that may influence directly the pituitary responsiveness to negative feedback. Evidence in favor of intrinsic changes in pituitary responsiveness with aging was published by Mobbs et al. (17). They demonstrated a reduction in the amplitude of the LH surge with aging by inducing the LH surge in ovariectomized mice treated with equivalent implant doses of E₂, suggesting an intrinsic decreased pituitary responsiveness to proestrus estrogen positive feedback. Alternatively, a second explanation would be that the changes in pituitary responsiveness seen are related directly to increased negative feedback by elevated basal E₂ levels in older women. However, previous studies have demonstrated that the overall E₂ production across the menstrual cycle is similar in older and younger reproductive-age women (6).

It has been observed that increased ovarian inhibin secretion potentiates negative feedback regulation of FSH secretion during the estrous cycle in rats (18). Pal et al. (19) found significantly higher levels of FSH in 14-month-old mice compared with 6-month-old mice despite similar levels of plasma α -inhibin, suggesting age-related alterations in pituitary responsiveness to inhibin. We did not find any differences in basal or stimulated serum inhibin levels between the older and younger groups of women; however, the variability of serum inhibin levels

found in our study for both younger and older groups of women was substantial. This variability in part may be due to the polyclonal nonspecific nature of the inhibin assay used for this study. Together with our observation of diminished pituitary LH responsiveness, these data confirm the role of age-related changes resulting in decreased pituitary responsiveness independent of ovarian inhibin secretion. Given the evidence for primary neuroendocrine changes in the pituitary gland, questions arise with regard to the influence of the aging process on the hypothalamic neuroendocrine unit that is related so intimately to the physiology of pituitary gonadotrophs.

With regard to IVF-ET success, previous studies have demonstrated a strong inverse correlation between pregnancy rates and basal FSH independent of age (20, 21). Several investigators have attempted to use the clomiphene citrate (CC) challenge test to discriminate further those older patients with diminished ovarian reserve despite normal basal FSH levels (22–24). Tanbo et al. (22) found an 85% predictive value of an abnormal CC challenge test for cycle cancellation due to poor ovarian responsiveness along with a 100% predictive value for failed conception. Similarly, Loumaye et al. (23) found the predictive value of an abnormal CC challenge test for failing to conceive to be 100% (23). Winslow et al. (25) demonstrated a GnRH analog stimulation test to be a sensitive predictor of stimulation response in the flare-up IVF cycle. In contrast to either of these provocative tests, we did not find any correlation between the GnRH-stimulated response of gonadotropins and the clinical response to exogenous hMG. Although the clinical portion of our study is limited by sample size, there appears to be no clear advantage to provocative GnRH stimulation testing over basal FSH level in identifying poor responders to subsequent hMG stimulation.

In summary, the significant changes in LH responsiveness to GnRH stimulation without alteration of the levels of ovarian feedback hormones, E₂ and inhibin, would imply the change in gonadotropin secretion to be due to a primary hypothalamic-pituitary aging phenomenon. Our findings support the hy-

pothesis that the alterations in gonadotropin secretion seen with reproductive aging in women are at least in part determined by diminished pituitary responsiveness. Although the GnRH stimulation test does not appear to be a more useful clinical tool than basal FSH to predict accurately the clinical responsiveness to hMG for COH in the older population of reproductive-age women, it does offer insight into the physiologic changes occurring in the reproductive neuroendocrine unit with age.

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