

The Gonadotropin Secretion Pattern in Normal Women of Advanced Reproductive Age in Relation to the Monotropic FSH Rise

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OBJECTIVE: *Women of advanced reproductive age are known to demonstrate subtle FSH elevations (monotropic FSH rise) while still retaining ovulatory function. The purpose of this study was to investigate the hypothesis that the physiologic basis for the monotropic FSH rise is an alteration in the secretion pattern of the GnRH pulse generator.*

METHODS: *The subjects were 11 normal women age 40–45 years who underwent 24 hours of frequent blood sampling in the follicular (EF) and/or midluteal (ML) phases of spontaneous menstrual cycles. The controls were 11 normal women age 20–25 years. The respective gonadotropin secretion patterns were analyzed for LH pulse frequency, mean LH and FSH levels, and LH pulse amplitude.*

RESULTS: *There were no differences between the groups for estradiol (E2) and progesterone when the respective cycle phases were compared. The 24-hour mean FSH level was significantly increased in the older women in both the EF and ML phases. There were no differences between the groups in either cycle phase for LH pulse frequency, LH pulse amplitude, and mean LH levels.*

CONCLUSION: *The results lend no support to the hypothesis that a slowing or other alteration of the GnRH pulse generator is the basis for the monotropic FSH rise in older ovulatory women. Other possibilities include the dynamics of E2 secretion or changes in FSH-modulating peptides (ie, inhibin) in these women. (J Soc Gynecol Invest 1996;3:27–32)*

KEY WORDS: Reproductive aging, monotropic FSH rise, LH secretion pattern, GnRH pulse generator.

Aging of the normal female reproductive system is not well understood. Cessation of ovarian function (menopause) occurs long before the failure of the other major organ systems. The first signs of reproductive aging in women generally precede the menopause by about a decade. Before the perimenopause, which is characterized by irregular menstrual cycles, older ovulatory women in their fifth decade demonstrate subtle signs of reproductive aging while maintaining their regular menstrual cyclicality. The first sign of reproductive aging that has been noted consistently is an isolated (monotropic) rise in serum FSH, which is first manifested in the early

follicular phase of ovulatory cycles.^{1–3} This FSH rise is temporally associated with the accelerated loss of primordial ovarian follicles that begins when a woman is about age 38.^{4–6} It is unclear whether the monotropic FSH elevation is the cause or effect of this accelerated follicular atresia in the latter reproductive years. Nevertheless, when a woman demonstrates a monotropic FSH rise, she is distinctly less fertile, although she will usually maintain regular ovulatory menstrual cycles for several more years before lapsing into the perimenopausal period.

A key element to an understanding of reproductive aging in women is the function of the hypothalamic-pituitary-ovarian (HPO) axis. The monotropic FSH rise represents a subtle change in HPO axis function. Whether the monotropic FSH rise occurs initially because there is reproductive aging of the brain (hypothalamus-pituitary) or secondary to reduced negative feedback ovarian hormones has yet to be determined.

As part of a series of studies on reproductive aging, we examined hypothalamic-pituitary function in older ovulatory reproductive-age women. We specifically focused on the hypothalamic GnRH pulse generator by examining pulsatile indices of pituitary LH secretion in the follicular and luteal phases

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in older ovulatory women. The peripheral LH secretion pattern as detected by frequent sampling of circulating LH levels is generally considered to accurately reflect the endogenous activity of the GnRH pulse generator.⁷ It has been determined that the GnRH pulse frequency is a major determinant of the relative circulating levels of LH and FSH.⁸ When the GnRH pulse frequency was modulated in hypothalamic-lesioned macaques, the serum levels of LH and FSH could be manipulated.⁸ A relatively slow GnRH pulse frequency was associated with an FSH rise and an LH decline that could be maintained as long as the frequency was sustained. The well-described slowing of the GnRH (LH) pulse frequency in the luteal phase of the normal human menstrual cycle is thought to be the basis for the intercycle FSH rise.^{7,9} In consideration of these facts, we hypothesized that the monotropic FSH rise in older ovulatory women occurred because of a slowing of the GnRH pulse generator that would be reflected in a decrease in the LH pulse frequency and perhaps other LH secretion characteristics. Although a change in function of the hypothalamus and pituitary in these older women would not necessarily prove that the FSH rise is a primary neuroendocrine change, a better understanding of how the monotropic FSH rise was maintained would aid our overall understanding of female reproductive aging.

MATERIALS AND METHODS

Subjects

Two groups of normal women were selected to participate in this study. The first group consisted of women age 40–45 years, and women age 20–25 years comprised the second group. The women in both groups were required to meet the following criteria to be considered normal for the purposes of this study: 1) have regular menstrual cycles every 25–35 days, 2) be of normal weight for height (body mass index 18–24 kg/m²),¹⁰ 3) be on no medications, 4) not have a medical history of past or current reproductive endocrine problems (eg, galactorrhea, hirsutism) or infertility, 5) not participate in aerobic exercise more than 5 hours/week, 6) consume a standard, balanced diet, 7) have a normal medical history and pass a complete physical examination, 8) demonstrate that her menstrual cycles were ovulatory by completing one or more biphasic basal body temperature graphs, and 9) have serum hormone levels within the following boundaries as drawn in the midluteal phase of an ovulatory cycle preceding the study cycle—prolactin less than 20 ng/mL, progesterone greater than 3 ng/mL, and testosterone less than 60 ng/mL.

Based on these criteria, 16 older women (group O) and 12 younger women (group Y) were selected to enter the study. All participants had daily venous blood samples drawn between 0800 and 1000 hours from cycle day 1 of the study cycle until cycle day 5 of their subsequent menstrual cycle. The samples were allowed to clot and the serum was separated, then frozen and stored at –20°C. These serum samples were subsequently analyzed for LH, FSH, estradiol (E2), and progesterone. In addition, each subject had a daily pelvic sonographic study to

determine follicle size and the dynamics of ovulation in her periovulatory cycle interval. The findings from this larger study are the subject of a separate report.¹¹

This report presents the findings from data generated during a frequent blood sampling episode in subgroups of older ($n = 11$; \bar{x} age 42.9 ± 0.5 years) and younger ($n = 11$; \bar{x} age 22.7 ± 0.6 years) women from the larger study. The frequent sampling occurred during an admission to the clinical research center in either early follicular (EF) or midluteal (ML) phases of participants' cycles. The EF phase was defined as the first 4 days of the study cycle; the ML phase was defined as cycle days +6 to +9 after the peak daily serum LH value (the LH surge = day 0). Of the 11 younger women (group Y) who volunteered for this study, there were six EF study periods and seven ML study periods in this group (some individuals were studied in both cycle phases). For the 11 older women (group O) who volunteered for this study, there were seven EF study periods and nine ML study periods in this group (some individuals were studied in both cycle phases). All volunteers provided informed consent. The study protocol was approved by the Human Subjects Review Board (University of Washington).

Sampling Procedures

During each admission to the Clinical Research Center, a subject had an indwelling intravenous line placed with a stop-cock arrangement to facilitate frequent blood sampling. After a rest period, a 3-mL blood sample was obtained every 20 minutes for 24 hours beginning at 0800 hours on the cycle day selected. During this time period each subject was at bed rest, ate three balanced meals, slept as desired, and was not allowed to consume caffeine or nicotine. Each blood sample was allowed to clot, and the serum was separated and frozen at –20°C until subsequent analysis for LH and FSH. An extra aliquot of blood was obtained at the beginning and end of each 24-hour study period and later analyzed for E2 and progesterone.

Hormone Assays

Serum hormone concentrations were determined in blood samples obtained on a daily basis and during the admission(s).

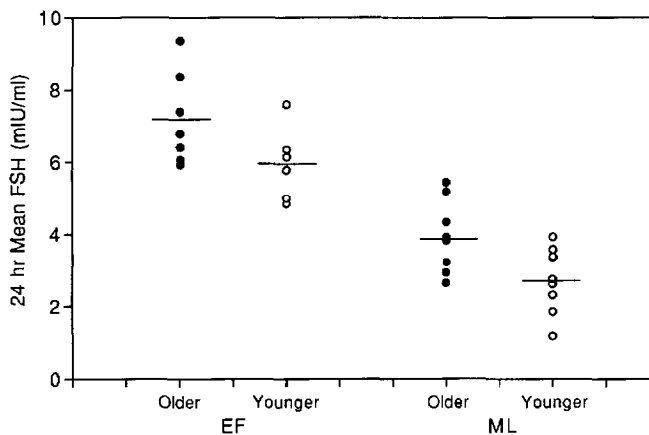


Figure 1. A mean 24-hour FSH level (the average of 73 samples) was calculated and plotted for each subject in the cycle phases studied. A mean of the means is indicated by the horizontal bar for each group. EF = early follicular phase; ML = midluteal phase.

Table 1. Study Participants: Menstrual Cycle Data

	Age* (y)	Mean cycle day (relative to LH surge = D0)	Mean cycle day (relative to last or next MP)	Estradiol* (pg/mL)	Progesterone* (ng/mL)	FSH D2-4 mean (mIU/mL)	FSH 24-h mean (mIU/mL)
Early follicular phase							
Older (n = 7)	42.6 ± 0.48	-10.0	3.1	56.6 ± 9.4	0.50 ± 0.09	7.9	7.2 ± 0.5
Younger (n = 6)	22.8 ± 0.87	-11.7	3.3	41.6 ± 6.9	0.87 ± 0.14	6.3	5.9 ± 0.4
P (between age groups)	<.01	.22	.67	.22	.06	.26	<.05
Midluteal phase							
Older (n = 9)	43.1 ± 0.59	8.1	-5.4	107 ± 17.7	7.8 ± 1.2	8.0	3.9 ± 0.3
Younger (n = 7)	22.6 ± 0.75	7.1	-5.6	117 ± 21.7	8.3 ± 1.4	6.0	2.8 ± 0.4
P (between age groups)	<.01	.17	.90	.33	.65	<.01	<.05

D = day; MP = menstrual period.
 * Mean ± standard error of the mean.

All the samples from an individual woman were analyzed in one hormone assay. Serum samples were analyzed in duplicate for LH and FSH by an immunoradiometric method (MAIA clone; Serono Laboratories, Geneva, Switzerland). The inter-assay variabilities for LH and FSH were 12 and 14%, respectively. The intra-assay variabilities were 5 and 9% for LH and FSH, respectively.

Serum progesterone concentrations were determined in duplicate by radioimmunoassay using reagents supplied by Diagnostic Systems Laboratories (Webster, TX). The antibody cross-reactivity is less than 5% with all other steroids. The inter-assay and intra-assay coefficients of variation were 13 and 11%, respectively.

The radioimmunoassay for serum E2 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 more than 1% with all other steroids. The inter-assay and intra-assay coefficients of variation were 16 and 7%, respectively.

Pulse Analysis

An adaptive-threshold method was used to determine the time and amplitude of hormone pulses.¹² A pulse was defined as an increase from local minimum to local maximum that was greater than a threshold value. The correct threshold was determined in an iterative manner.

Statistics

Results are presented as calculated mean values plus or minus standard error of the mean ($\bar{x} \pm SEM$). Student *t* tests (two-tailed) were performed to compare mean results between groups. Daily hormone levels across the menstrual cycle were compared between the two groups by analysis of variance with repeated measures. *P* < .05 was selected to indicate a significant difference.

RESULTS

To make meaningful comparisons of various indices of gonadotropin secretion between study groups, the groups had to have been studied during comparable cycle phases. Although

the volunteers were studied during preselected cycle phases (EF, ML), we could only determine whether the groups were comparable after completion of their menstrual cycles. The data in Table 1 indicate groups O and Y were studied in similar cycle phases, both in relation to the LH surge and menstrual period. In addition, the E2 and progesterone levels for each group were also quite similar for each cycle phase (Table 1).

The mean 24-hour FSH level were significantly different, with group O having higher levels than group Y in both cycle

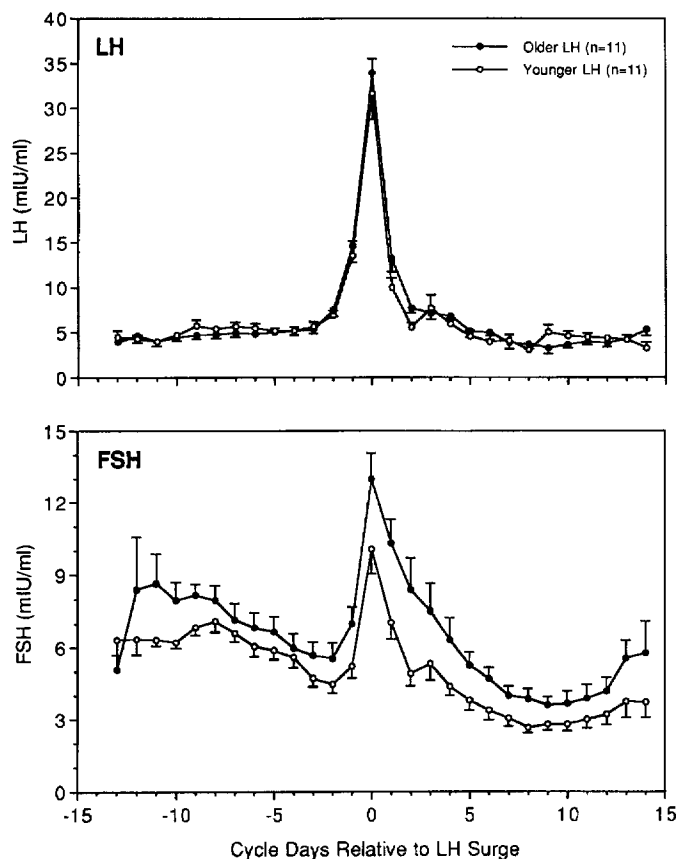


Figure 2. The daily ($\bar{x} \pm$ standard error of the mean) LH and FSH levels across the menstrual cycle for the 11 women in each of the study groups.

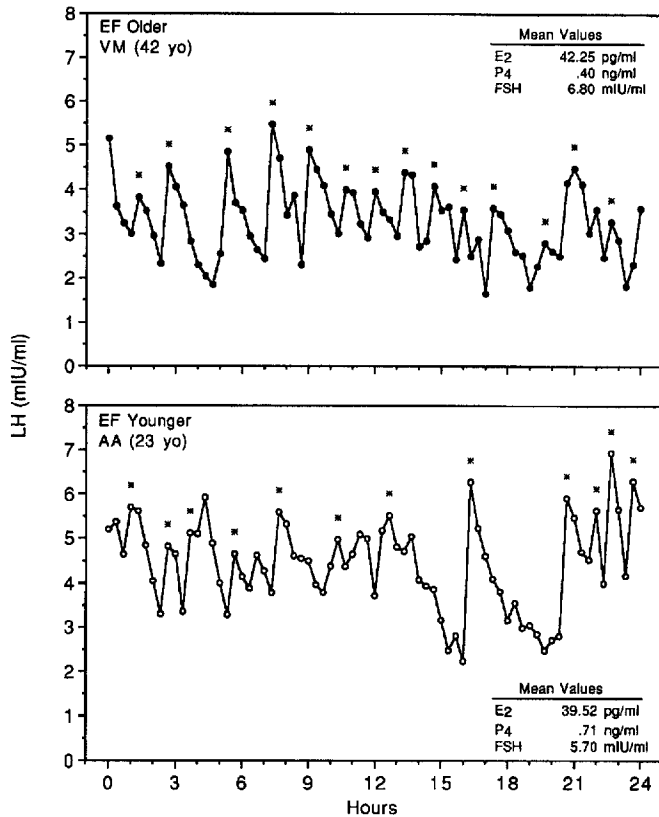


Figure 3. Early follicular (EF) phase. The LH secretion pattern over 24 hours is illustrated for representative women from the older and younger study groups. E₂ = estradiol; P₄ = progesterone. FSH level is the mean of 73 samples. *Secretory pulse.

phases (7.2 ± 0.48 versus 5.9 ± 0.41 mIU/mL, EF) and (3.9 ± 0.32 versus 2.8 ± 0.35 mIU/mL, ML) (Figure 1, Table 1), respectively. The daily LH and FSH levels across the menstrual cycle for the older and younger women in this study are presented in Figure 2. There was a significant ($P < .05$) elevation of FSH across the cycle in the older group. These elevated FSH values both across the menstrual cycle and for particular cycle phases in older women are in agreement with a general elevation of daily FSH levels throughout the menstrual cycle in the larger group of 40–45-year-old women in our companion study.¹¹ There were significant differences between mean FSH levels in the EF and ML cycle phases within both groups (Table 1), with EF mean FSH levels consistently higher than ML levels.

Figures 3 and 4 present LH pulse pattern graphs over 24 hours for EF and ML phases, respectively, for represented individuals from both groups. Despite elevations in FSH, the LH secretory data appear very similar between individuals in groups O and Y. In fact, there were no discernible differences in LH pulse frequency, LH pulse amplitude, and mean LH levels between the two groups in either cycle phase (Table 2).

Because FSH has been found to be chronologically first and most prominently elevated in the EF phase of the cycle in older ovulatory women, we were especially interested whether there was a relationship between the LH pulse frequency in the ML

phase and the FSH level in the EF phase. To this end, the ML pulse frequency was plotted against the EF mean FSH level for cycle days 2–4 for each individual who underwent a frequent sampling study in the ML phase (Figure 5). Although there was a trend toward a negative correlation between LH frequency and the day 2–4 FSH level, it was not significant ($r = 0.12$; $P > .1$).

DISCUSSION

Reproductive aging has special significance for the current clinical practice of reproductive medicine. In the United States, the relatively large group of women who were born in the decade after World War II (the “baby boomers”) are now 30–50 years of age. This generation of women has some unique demographic characteristics in relation to reproduction. Compared with earlier generations, as a group these women 1) are more educated (48% had earned college diplomas in a 1985 survey), 2) have had more access to effective contraception, and 3) marry at a relatively older age (24.3 years in 1986).^{13–15} All of these changes have led to a strong trend toward a delay in childbearing. The only group of women in the population experiencing an increase in the birth rate in 1988 were women age 30–39 years.¹³ Never before in history have so many women delayed procreation until their latter

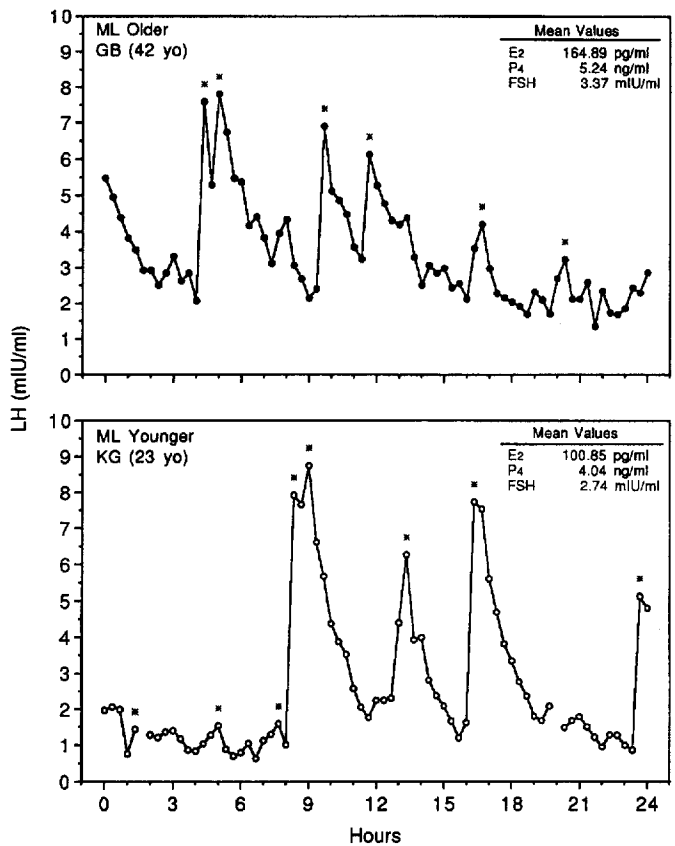


Figure 4. Midluteal (ML) phase. The LH secretion pattern over 24 hours is illustrated for representative women from the older and younger study groups. E₂ = estradiol; P₄ = progesterone. FSH level is the mean of 73 samples. *Secretory pulse.

Table 2. Gonadotropin Pulse Data

	LH pulse frequency (pulse/24 h)	LH pulse amplitude (mIU/mL)	LH 24 h mean (mIU/mL)
EF phase			
Older (<i>n</i> = 7)	14.1 ± 1.5	1.6 ± 0.2	3.7 ± 0.3
Younger (<i>n</i> = 6)	12.7 ± 1.1	2.0 ± 0.2	3.8 ± 0.3
<i>P</i> (between age groups)	.43	.17	.80
ML phase			
Older (<i>n</i> = 9)	6.6 ± 0.7	2.7 ± 0.2	2.7 ± 0.3
Younger (<i>n</i> = 7)	6.4 ± 1.2	3.5 ± 1.1	2.4 ± 0.3
<i>P</i> (between age groups)	.93	.46	.53
<i>P</i> (EF vs ML)			
Within older group	.001	.002	.010
Within younger group	.002	.210	.011

EF = early follicular; ML = midluteal.
Data are presented as mean ± standard error of the mean.

reproductive years. Women in their late 30s and early 40s are distinctly less fertile and less likely to conceive than younger women.^{16,17} Considering these trends and the current shift in age of those attempting to conceive, a better understanding of the earlier stages of reproductive aging is compelling.

Aging of any organ system is defined as a gradual loss of physiologic function due to the passage of time (natural) as opposed to a disease process. The HPO axis is the primary component of the primate female reproductive system that demonstrates loss of function with age. The first reported sign of a change in HPO function in older yet ovulatory women is a monotropic rise in serum FSH levels.¹⁻³ This FSH rise may be the cause of accelerated depletion of ovarian follicles and the primary change of reproductive aging or it may simply be an indirect sign of other primary causes of reproductive senescence. An understanding of how the monotropic FSH rise is maintained could provide important clues as to how it occurred. One potential mechanism that could effect a change in circulating FSH levels is hypothalamic GnRH secretion as reflected in the pulsatile pattern of LH secretion. This study examined the LH secretion pattern in older ovulatory women in two phases of their menstrual cycles.

The subjects of this study were not a random cross section of older or younger women. The age span of 20–25 years was selected for the controls to ensure ample ovarian follicular reserve while being a sufficient number of years beyond puberty to have matured their HPO axes. Both subjects and controls were highly selected for their normal status both in terms of reproduction and general health. The subjects themselves were age 40–45 years, a time span wherein a woman can lose her ovarian function completely (menopause) and still be normal.¹⁸ We purposely chose older women who were relatively young in terms of reproductive function by selecting those who had retained ovulatory function. Most women past age 40 who become anovulatory and have irregular menstrual cycles are further advanced in terms of reproductive aging and closer to the menopause. It seemed more relevant to study older ovulatory women at an earlier stage of reproductive aging in order to examine the aging process before it was obscured by secondary changes. This ovulatory group is also more relevant in terms of clinical medicine. The infertility associated with

reproductive aging is more advanced in the anovulatory patient and less likely to respond to any therapeutic measures.

Follicle-stimulating hormone levels were found to be significantly elevated in the EF phase in the older group, as has been reported previously. Although the FSH levels were the highest in this cycle phase in our subjects, they demonstrated elevated FSH levels throughout their entire cycle compared with controls. If these FSH changes were due to differences in activity of the GnRH pulse generator, we would have expected to have seen a slowing of the LH pulse frequency or some other changes in either the EF or ML phase. However, the LH pulse indices were quite similar between the groups in both cycle phases. Although there was a trend toward a significant correlation between a slowing of the LH pulse frequency in the ML phase and FSH levels in the EF phase (Figure 5), the phase where we hypothesized it most likely would be found, it was not impressive.

Altogether, the findings in this study lend little support to the hypothesis that a change in the GnRH secretion pattern is the basis for the monotropic FSH rise of older ovulatory women. Several facets of the study might have hindered our

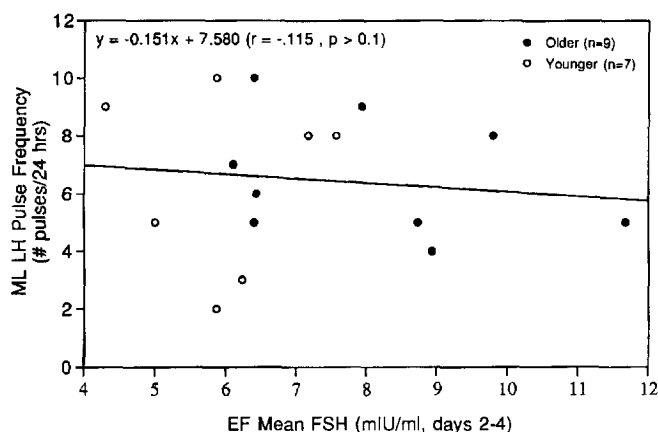


Figure 5. The mean FSH level from days 2–4 of the menstrual cycle as calculated from daily levels for each younger and older subject is plotted versus their respective LH pulse frequency in the midluteal (ML) phase. This graph is restricted to those subjects who had ML studies. An equation was calculated for a line that best fits the data points ($r = -0.115$; $P > .1$).

ability to uncover subtle differences in GnRH secretion between the two groups. Although the number of subjects in each group was relatively large for frequent sample (intensive) studies, the addition of more volunteers might have uncovered small but real differences. A protocol that called for more frequent samples than every 20 minutes also might have uncovered some differences. However, a 20-minute sample interval is a common interval for 24-hour studies and has been shown capable of detecting subtle changes in GnRH activity.¹⁹ An examination of additional phases of the menstrual cycle, such as the late follicular or late luteal phases, also could have led to positive findings. Although these aspects of study design might have obscured real differences, the fact that the findings were so close between the groups leads us to doubt that further investigation of this hypothesis would be fruitful.

If changes in GnRH secretion are not the explanation for the monotropic FSH rise, then it is necessary to invoke other hypotheses. Alternative explanations could include intrinsic changes in relative LH and FSH secretion by the pituitary gonadotrophs. Such intrinsic changes could be secondary to aging of the pituitary itself (eg, change in sensitivity to feedback) or modulated by hormones known to effect FSH secretion by the gonadotrophs. Estradiol is known to have both positive and negative feedback effects on FSH secretion. In older ovulatory women, the peak and preovulatory levels of E2 are not different when compared to controls.¹¹ However, the older women have a shortened follicular phase, indicating that the changes in E2 occur in an accelerated manner when compared with younger subjects.¹¹ Perhaps the dynamics of E2 change could potentiate FSH secretion in older women. Other hormones that could modulate FSH secretion at the pituitary level are circulating levels of inhibin and/or activin. Relative changes in inhibin or activin within the pituitary could also affect these FSH changes by a paracrine effect as well. We were not able to demonstrate any differences in circulating inhibin levels between groups O and Y across the menstrual cycle in our companion study.¹¹ We did detect subtle differences in the response of the anterior pituitary to a GnRH challenge between these two groups.²⁰ Altogether, a clear physiologic mechanism to explain the monotropic FSH rise has not yet been demonstrated.

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