

## Effects of aging on menstrual cycle hormones and endometrial maturation\*†

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**Objective:** To investigate changes in menstrual cycle hormones and endometrial maturation that may contribute to the decline in fertility with aging.

**Design:** Prospective controlled clinical study.

**Setting:** Normal human volunteers in an academic research institution.

**Subjects:** Women with regular menstrual cycles.

**Interventions:** Thirty-two women, aged 20 to 30 or 40 to 50 years, had daily blood drawing starting on cycle day 6 to 10 and continuing until 2 days after the onset of next menses. In addition, 60 women, aged 20 to 30 or 40 to 50 years, had a total of 93 endometrial biopsies performed on day 7 to 9 after the LH surge.

**Main Outcome Measures:** Serum LH, FSH, E<sub>2</sub>, inhibin, P, and placental protein 14 (PP14) levels and histologic maturation of the endometrium.

**Results:** Serum FSH levels were increased whereas inhibin concentrations were reduced in the luteal-follicular transition of women >40 years. No other hormonal changes were seen in this population, including P and PP14 secretion. Disruption of endometrial maturation occurred at a similar frequency in both age groups.

**Conclusions:** Follicular recruitment, but not luteal function or endometrial maturation, is disturbed in cycling women >40 years and may contribute to the decline in fertility with aging. *Fertil Steril* 1995;64:492-9

**Key Words:** Luteinizing hormone, follicle-stimulating hormone, estradiol, progesterone, inhibin, placental protein 14, endometrial biopsy

Deferment of marriage and first birth until after the age of 35 years has become increasingly common in developed countries (1). Because the fertility rate

decreases in women after this age, more couples seek medical advice each year because of an inability to conceive (1, 2). Thus, it is of utmost importance to study the causes underlying the decline in fertility with aging. Both ovarian and uterine factors have been implicated in this process, although the contribution of each remains unsettled (1). The ovarian oocyte pool gradually is exhausted as women ap-

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proach menopause, resulting in a higher rate of anovulatory and thus sterile cycles (3). In addition, oocyte quality and embryo viability decline with aging and may be the major factors responsible for reduced fertility in cycling women >40 years (1). However, some authors have suggested that the development of the endometrium is frequently abnormal in this population, implying that endometrial receptivity to implantation also may deteriorate with advancing age (1, 4). This could be due to deficient P secretion by the corpus luteum or to inability of the endometrium to respond to P stimulation (4).

In the present study, we investigated changes in menstrual cycle hormones and endometrial maturation in normal women >40 years to gain insight into the causes underlying the decline in fertility with aging. To evaluate the effects of aging on ovarian and endometrial secretory function, we compared daily serum levels of LH, FSH, E<sub>2</sub>, and P, and also of the two more recently isolated glycoproteins, inhibin and placental protein 14 (PP14), in normal women aged 20 to 30 and 40 to 50 years. Inhibin is secreted by granulosa cells of the follicle and lutein-granulosa cells of the corpus luteum (5). Serum inhibin levels increase in the late follicular phase and again in the midluteal phase and thus may provide useful information about follicular and luteal function (6, 7). Placental protein 14 is a major product of the P-induced secretory endometrium that is released into both the uterine lumen and bloodstream (8). Serum PP14 concentrations rise in the second half of the luteal phase and peak at the onset of next menses, accompanying the normal differentiation of the endometrium (8, 9). Thus they may provide a measure of endometrial secretory function.

To investigate the effects of aging on endometrial maturation, we also examined the development of the endometrium at the time of nidation in normal women aged 20 to 30 and 40 to 50 years. Accordingly, an endometrial biopsy was performed in the midluteal phase to evaluate histologic abnormalities that potentially could interfere with endometrial receptivity to implantation.

## MATERIALS AND METHODS

The protocol was approved by the Clinical Research Subpanel of the National Institute of Child Health and Human Development. Informed consent was obtained from each subject.

All women participating in this protocol had regular menstrual cycles ≤ 36 days; this was documented by revising the dates of their menstrual periods and calculating the length of their cycles in the last 2 to 6 months before entering the study. None had a prior

history of infertility nor was trying to conceive at the time of the study. Likewise, none had used steroid contraceptives, intrauterine devices, or chronic medications in the previous 3 months. All subjects used barrier methods of contraception or abstained from sexual intercourse during the study. Pregnancy was excluded by a plasma β-hCG concentration ≤ 2.0 ng/mL (the threshold for the detection of pregnancy in this assay) at the beginning of the study cycles.

### Study I

Thirty-two healthy women with regular menstrual cycles of 23 to 34 days and weight between 87% and 130% of ideal value were recruited as consecutive subjects according to their age. Sixteen were aged 23 to 30 years (27.2 ± 2.4 years; mean ± SD) and weighed 47 to 95 kg (67.8 ± 13.6 kg). The remaining 16 subjects were aged 40 to 49 years (44.1 ± 2.8 years) and weighed 45 to 79 kg (60.8 ± 11.2 kg; *P* not significant compared with the younger group by unpaired two-tailed *t*-test). Nine and 11 women in the younger and older group, respectively, had been pregnant in the past; the remaining subjects had never attempted conception.

The study consisted of one menstrual cycle. Blood was drawn daily at 7:00 to 10:00 A.M. for serum measurements of LH, FSH, E<sub>2</sub>, inhibin, P, and PP14 starting on cycle day 6 to 10 and continuing until 2 days after the onset of next menses. Inhibin concentrations were not available in eight subjects aged 20 to 30 years, whereas LH levels were missing in one woman aged 40 to 50 years.

Serum levels of LH (10), FSH (10), and P (11) were measured by previously described RIA methods. Serum E<sub>2</sub> concentrations were determined by a commercial RIA kit (Diagnostic Products Corporation, Los Angeles, CA). Serum inhibin levels were measured in a heterologous double antibody RIA based on purified 31 K bovine follicular fluid (FF) inhibin (6, 7). The latter compound was radioiodinated with <sup>125</sup>I in the preparation of the tracer (6). It also was used to immunize rabbits to raise an inhibin antiserum (6). A serum pool obtained from women undergoing ovulation induction was calibrated against a partially purified human FF preparation and used as the standard in the inhibin assay (6, 7). Serum PP14 concentrations were determined in a double antibody RIA (9). A purified PP14 preparation obtained from human placenta (lot no. 120/135; Behringwerke AG, Marburg, Germany) was used as standard in the assay. Radioiodination of PP14 was carried out by lactoperoxidase, followed by purification of the tracer in Sephadex and concanavalin A-Sepharose columns (9). A rabbit anti-PP14 antibody

(lot no. 201ZA; Behringwerke AG) was used at a final dilution of 1:40,000. The sensitivities of the assays were 5.0 mIU/mL (5.0 IU/L) for LH, 3.0 mIU/mL (3.0 IU/L) for FSH, 20 pg/mL (74 pmol/L) for E<sub>2</sub>, 90 U/L for inhibin, 0.1 ng/mL (0.3 nmol/L) for P, and 7.0 ng/mL for PP14. Intra-assay and interassay coefficients of variation were  $\leq 12\%$  and  $15\%$ , respectively, for all hormones.

## Study II

Sixty healthy women with regular menstrual cycles of 22 to 36 days and weight between 87% and 130% of ideal value were recruited for various protocols conducted at the National Institutes of Health (Bethesda, MD) involving an endometrial biopsy. Part of these results have been published elsewhere (12). Several subjects volunteered for more than one clinical protocol at different times and therefore had two or three biopsies done within a period of 1 year. All women enrolled in study I also participated in study II in a different cycle so as to avoid any effect of the biopsy on P and PP14 secretion. Thirty-seven subjects in study II were aged 22 to 30 years ( $26.6 \pm 2.4$  years), weighed 47 to 95 kg ( $64.6 \pm 10.9$  kg), and had a total of 51 endometrial biopsies. The other 23 women were aged 40 to 48 years ( $44.0 \pm 2.6$  years), weighed 45 to 82 kg ( $61.3 \pm 11.0$  kg; *P* not significant compared with the younger group by unpaired two-tailed *t*-test), and underwent a total of 42 endometrial biopsies. Nineteen and 18 women in the younger and older groups, respectively, had been pregnant in the past; the remaining subjects had never attempted conception.

All endometrial specimens were obtained in spontaneous cycles, which included frequent blood drawing but no other intervention before the biopsy. Plasma LH levels were measured daily by a rapid RIA assay (Diagnostic Products Corporation), starting on cycle day 6 to 10 and continuing for a few days after the LH surge was detected. All biopsies were scheduled prospectively and performed 7 to 9 days after the LH peak to evaluate endometrial maturation at nidation. Specimens were obtained from the uterine fundus using the Pipelle (Prodimed, Neuilly-en-Thelle, France) as described in detail elsewhere (12). At the end of the study, all slides were reviewed independently by two pathologists (M.M. and C.A), who were unaware of the subject's age or the day of the cycle when the biopsy was obtained. Histologic dates were assigned according to the criteria of Noyes et al. (13), using a 2-day reading, e.g., secretory day 22 to 23 (12). An overall histologic date could not be assigned when the glandular and stromal parts of the specimen were at

different stages of endometrial maturation (dysynchronous pattern). Chronological dating of the biopsy was done prospectively by counting forward from the day of the LH surge (taken as cycle day 14) until the day of the biopsy (12). Endometrial maturation was evaluated by correlating the chronological date with the most advanced histologic date of the biopsy, in keeping with Noyes' recommendation that dating should rely primarily on the morphology of the most advanced portion or feature of the endometrium (13). The development of the endometrium was considered abnormal when the histologic dating lagged  $>2$  days behind the chronological dating of the biopsy (delayed pattern) or when the endometrial pattern was dysynchronous (4, 12).

## Analysis

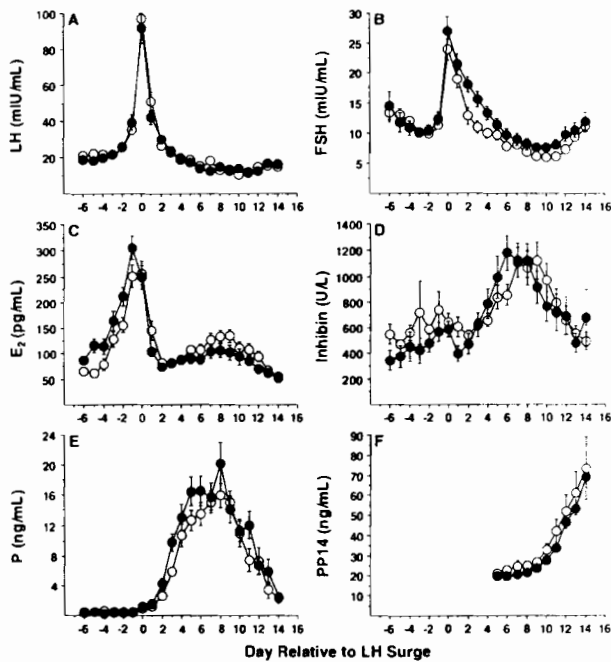
The follicular phase was defined as the days elapsed between the onset of menses until (and including) the day of the LH surge. The luteal phase was defined as the days after the LH surge until (but not including) the onset of next menses. Data were plotted according to the day of the LH surge (day 0) when evaluating the follicular and luteal phases and according to the 1st day of next menses (day 0) when analyzing the luteal-follicular transition.

Results are presented as means  $\pm$  SEM. The duration of the cycle was compared in women aged 20 to 30 and 40 to 50 years by two-tailed unpaired *t*-tests. The frequency of endometrial abnormalities in these two age groups was evaluated by  $\chi^2$  analysis. Hormonal profiles were examined after dividing the menstrual cycle into five intervals: late follicular phase (day -3 to 0 relative to the LH surge), early luteal phase (day 1 to 4 relative to the LH surge), midluteal phase (day 5 to 8 relative to the LH surge), late luteal phase (day 9 to 12 relative to the LH surge), and luteal-follicular transition (day -2 to 2 relative to the onset of menses). Serum concentrations for each hormone over a given interval were compared in younger and older women by two-factor analysis of variance with repeated measures. This test examined first the pattern of hormonal changes in the two age groups to determine whether hormonal profiles were parallel or divergent in a given interval. If they were parallel, the analysis proceeded to test for significant differences in daily hormonal levels within and between groups. Differences were considered significant at *P*  $> 0.05$ .

## RESULTS

### Cycle Length

There was no difference in cycle length in women aged 20 to 30 and 40 to 50 years who participated



**Figure 1** Serum levels of LH (A), FSH (B), E<sub>2</sub> (C), inhibin (D), P (E), and PP14 (F) in healthy cycling women aged 20 to 30 (○) or 40 to 50 years (●) plotted according to the day of the LH surge (day 0). Each point represents the mean  $\pm$  SEM of 16 measurements, except for inhibin in the younger group ( $n = 8$ ) and LH in the older group ( $n = 15$ ). Conversion factors to SI units, 1.00 (for LH and FSH), 3.671 (for E<sub>2</sub>), and 3.180 (for P).

in study I ( $27.6 \pm 0.5$  versus  $27.7 \pm 0.7$  days; mean  $\pm$  SEM;  $P = 0.880$ ). Likewise, the duration of the follicular phase ( $14.4 \pm 0.5$  versus  $14.8 \pm 0.8$  days;  $P = 0.639$ ) and of the luteal phase ( $13.2 \pm 0.3$  versus  $12.9 \pm 0.5$  days;  $P = 0.528$ ) was similar in these two groups.

### Follicular Phase

Serum LH levels increased, whereas FSH levels declined, in the late follicular phase of all women (Fig. 1A and B). Both hormones exhibited a sharp peak at midcycle, characterizing the preovulatory gonadotropin surge. Serum E<sub>2</sub> and, to a lesser degree, inhibin concentrations increased steadily in the late follicular phase, peaking 1 day before or on the day of the LH surge and declining thereafter (Fig. 1C and D).

Hormonal profiles were parallel in the late follicular phase of women aged 20 to 30 and 40 to 50 years except for E<sub>2</sub> ( $P = 0.024$ ). Thus, E<sub>2</sub> concentrations rose to an earlier peak in subjects  $>40$  years, dropping thereafter (Fig. 1C). Maximal preovulatory E<sub>2</sub> levels, however, were similar in younger and older women ( $291.4 \pm 23.4$  versus  $317.6 \pm 23.6$  pg/mL [ $1,069.7 \pm 85.9$  versus  $1,165.9 \pm 86.6$  pmol/L];  $P$

$= 0.436$  by two-tailed unpaired  $t$ -test). There were no differences in daily concentrations of LH ( $P = 0.400$ ), FSH ( $P = 0.959$ ), inhibin ( $P = 0.230$ ), and P ( $P = 0.791$ ) in the late follicular phase of both age groups.

### Luteal Phase

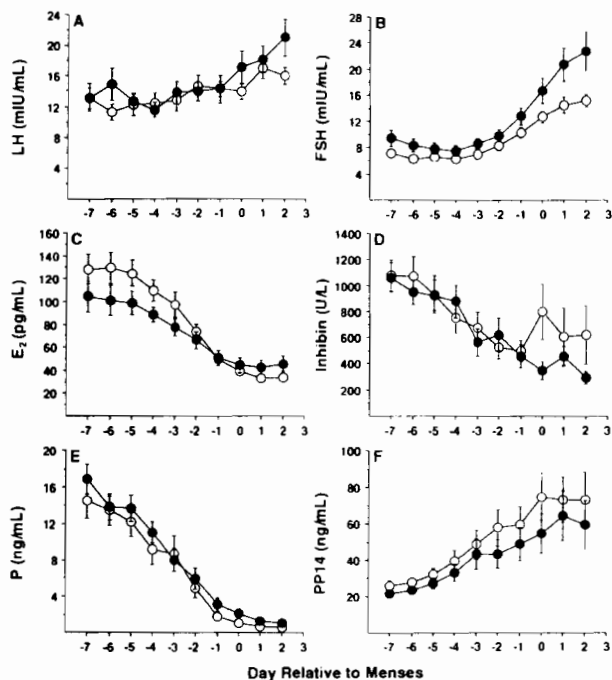
Serum LH and FSH levels declined throughout most of the luteal phase in both age groups, starting to rise only on day 12 after the LH surge (Fig. 1A and B). Serum E<sub>2</sub>, inhibin, and P concentrations increased steadily in the first half of the luteal phase, peaking on day 6 to 8 after the LH surge and declining thereafter (Fig. 1C to E). Serum PP14 levels began to rise only on day 9 after the LH surge, reaching maximal values at the end of the luteal phase (Fig. 1F).

Hormonal profiles were parallel throughout the postovulatory period of women aged 20 to 30 and 40 to 50 years except for E<sub>2</sub> ( $P = 0.024$ ) and inhibin ( $P = 0.017$ ) in the early luteal phase. Thus, E<sub>2</sub> concentrations seemed to drop more abruptly after ovulation (Fig. 1C), whereas inhibin levels appeared to rise at a faster rate in the first 4 days after the LH surge in subjects  $>40$  years (Fig. 1D). In this population, daily FSH concentrations also were higher in the early ( $P = 0.023$ ) and late luteal phases ( $P = 0.048$ ), whereas P levels were increased in the early luteal phase ( $P = 0.047$ ). There were no differences between groups in daily concentrations of E<sub>2</sub> ( $P = 0.092$  and  $0.241$ ), inhibin ( $P = 0.301$  and  $0.740$ ), P ( $P = 0.125$  and  $0.252$ ), and PP14 ( $P = 0.073$  and  $0.353$ ) in the midluteal and late luteal phases; in FSH levels in the midluteal phase ( $P = 0.119$ ); and in LH levels in the early, midluteal, and late luteal phases ( $P = 0.606$ ,  $0.234$ , and  $0.462$ ).

### Luteal-Follicular Transition

Serum LH and FSH levels started to rise on the last 2 days of the luteal phase and continued to increase after the onset of next menses in both age groups (Fig. 2A and B). Serum E<sub>2</sub>, P, and inhibin concentrations declined steeply during the second half of the luteal phase (Fig. 2C to E). After menstruation, E<sub>2</sub> and P tended to plateau, whereas inhibin rose sharply in the younger but not older group (Fig. 2C to E). Serum PP14 concentrations increased steadily throughout the latter part of the luteal phase, peaking on the 1st day of next menses or 1 day later (Fig. 2F).

Luteinizing hormone, E<sub>2</sub>, and inhibin exhibited different profiles ( $P = 0.017$ ,  $0.012$ , and  $0.017$ ) throughout the luteal-follicular transition of women aged 20 to 30 and 40 to 50 years. Thus, LH levels



**Figure 2** Serum levels of LH (A), FSH (B), E<sub>2</sub> (C), inhibin (D), P (E), and PP14 (F) in healthy cycling women aged 20 to 30 (○) or 40 to 50 years (●) plotted according to the 1st day of menses (day 0). Each point represents the mean ± SEM of 16 measurements, except for inhibin in the younger group (n = 8) and LH in the older group (n = 15). Conversion factors to SI units, 1.00 (for LH and FSH), 3.671 (for E<sub>2</sub>), and 3.180 (for P).

appeared to rise at a faster rate (Fig. 2A), E<sub>2</sub> concentrations decreased more slowly (Fig. 2C), and inhibin levels continued to decline (Fig. 2D) in subjects >40 years, particularly after the onset of menses. In contrast, there were no differences in the pattern or in daily concentrations of FSH ( $P = 0.068$ ), P ( $P = 0.205$ ), and PP14 ( $P = 0.362$ ) throughout the luteal-follicular transition of both age groups. However, if the very early follicular phase of the next cycle (i.e., day 0 to 2 relative to the onset of menses) was analyzed separately from the preceding luteal phase, FSH levels tended to be higher ( $P = 0.054$ ), whereas inhibin concentrations were lower ( $P = 0.018$ ) in women >40 years.

### Endometrial Maturation

Two pathologists (M.M. and C.A.) agreed on the pattern of endometrial maturation (normal, delayed, or dysynchronous) in 96% of biopsies. The mean difference between histologic and chronological dating of the biopsies was similar in women aged 20 to 30 and 40 to 50 years ( $0.16 \pm 0.26$  versus  $0.17 \pm 0.30$  days according to pathologist M.M.,  $P = 0.947$ ; or  $0.16 \pm 0.24$  versus  $0.29 \pm 0.29$  days according to pathologist C.A.,  $P = 0.718$  by unpaired two-tailed

*t*-test). Likewise, histologic abnormalities of the endometrium, including delayed or dysynchronous patterns, occurred at a similar frequency in these two groups ( $P > 0.05$ ; Table 1). No woman had more than one abnormal biopsy.

### DISCUSSION

The decline in fertility with aging has been ascribed to defective oocytes remaining in the ovaries in the latter part of reproductive life as well as to a decline in endometrial receptivity to implantation (1). Satisfactory pregnancy rates may be achieved in infertile women >40 years receiving oocytes from younger donors, suggesting that egg quality plays a key role in reduced fertility in the older population (14, 15). In oocyte donation programs, however, recipients usually are treated with supraphysiologic P doses, which could stimulate the differentiation of the endometrium and correct any age-related defects in endometrial development (1, 14, 15). Thus, it remains unclear whether endometrial maturation is often disrupted after age 40, leading perhaps to a hostile endometrium unable to sustain implantation of an embryo. To evaluate ovarian and endometrial factors that may contribute to the decline in fertility with aging, we characterized changes in female reproductive hormones, namely LH, FSH, E<sub>2</sub>, P, inhibin, and PP14, as well as defects in endometrial development in normal subjects with regular menstrual cycles aged 20 to 30 and 40 to 50 years. Our data suggest that cycling women >40 years have an abnormality in the cohort of follicles that are recruited in the beginning of the cycle. In contrast, we found no evidence of an increased rate of corpus luteum or endometrial insufficiency to support the hypothesis of implantation failure in this age group.

The duration of the follicular and luteal phases was not altered with aging in our study, suggesting

**Table 1** Frequency of Endometrial Abnormalities\*

Pathologist†	Age group	Endometrial maturation‡		
		Normal	Delayed	Dysynchronous
y				
M.M.	20 to 30	45	5	1
	40 to 50	37	4	1
C.A.	20 to 30	46	4	1
	40 to 50	38	3	1

\* Fifty-one and 42 biopsies were performed on luteal phase day 7 to 9 in women aged 20 to 30 and 40 to 50 years, respectively.

† Specimens were dated histologically by two pathologists (M.M. and C.A.) according to the criteria of Noyes et al. (13).

‡ There were no significant differences between groups.

that women >40 years recruited for this protocol had grossly normal functioning of their reproductive system. Others have reported changes in the length of the follicular phase in this age group, ranging from a shorter duration due to accelerated follicular maturation to a prolonged duration accompanying anovulatory cycles (16, 17). Perimenopausal women also may have shorter luteal phases associated with inadequate P secretion (16, 17).

Follicular recruitment usually begins during the late luteal phase after corpus luteum secretion of P, E<sub>2</sub>, and inhibin has started to decline (18). It is characterized and dependent mainly on rising FSH levels, which are required to stimulate a cohort of follicles to grow (18). In our study, a significant reduction in inhibin but not E<sub>2</sub> concentrations was seen in association with high-normal FSH levels in the luteal-follicular transition of women >40 years, especially in the very early follicular phase of the succeeding cycle. We cannot exclude, however, that our statistical analysis was somewhat biased by the fact that inhibin levels were not available in all of our younger subjects. In addition, comparison of FSH data in the late luteal phase and in the very early follicular phase of the next cycle yielded *P* values slightly lower (0.048) or slightly higher (0.054) than 0.05, an arbitrarily selected limit used in the present and in most other studies to define statistical significance. Thus, FSH levels tended to be higher but were not increased definitely in the luteal-follicular transition of women aged 40 to 50 years compared with the younger group. Possibly, if more subjects were included in these two populations, this difference would be more striking. In two other studies involving single or multiple blood sampling in the first half of the follicular phase but not in the preceding luteal phase, high FSH and low E<sub>2</sub> concentrations were seen in women aged 45 to 49 (19) or 40 to 48 years (20). Inhibin levels were reduced in the former population (19), whereas in the latter subjects they tended to be lower but were not decreased significantly compared with the control group (20).

Our present results suggest that inhibin levels are decreased in cycling women >40 years at the time of follicular recruitment, implying a primary abnormality in the cohort of follicles that begin to grow in the luteal-follicular transition. Lower inhibin concentrations result in less suppressible and thus rising FSH levels, which could then stimulate the ovaries and partially overcome the deficient inhibin secretion. Reduced inhibin concentrations may be related to the gradual decline in the number or quality of oocytes in the aging ovary (3). Because inhibin has been postulated as one of the ovarian signals

required for the initiation of folliculogenesis (18), decreased inhibin levels could be responsible for the disruption in follicular recruitment seen after age 40. It is also noteworthy that inhibin rather than E<sub>2</sub> concentrations declined first in this age group, suggesting that the former hormone may be a better marker of ovarian function than the latter towards the end of reproductive life. This view is supported by the findings of Hughes et al. (21), who showed an age-related reduction in inhibin but not E<sub>2</sub> response to ovarian hyperstimulation.

Follicular maturation is characterized by increasing E<sub>2</sub> and inhibin secretion by the dominant follicle that presumably suppresses FSH levels in the late follicular phase (6, 7). In our study, LH and FSH concentrations were normal, whereas E<sub>2</sub> and inhibin peaked at similar levels in the late follicular phase of women aged 40 to 50 years compared with the younger group. This suggests that follicular maturation and hormonal production by the dominant follicle were not altered by aging. However, others have reported normal or reduced E<sub>2</sub> levels in the preovulatory phase of women >40 years, in association with normal or elevated FSH concentrations and low-normal inhibin levels (16, 17, 20, 22).

The corpus luteum normally secretes E<sub>2</sub>, P, and inhibin in response to LH stimulation (23). Progesterone acts on the endometrium to induce its differentiation and synthesis of PP14, a major glycoprotein that may be involved in the immunomodulation of implantation and early pregnancy (8, 9). In our study, serum E<sub>2</sub>, P, and inhibin concentrations were either normal or increased throughout the luteal phase of women >40 years. Although these subjects had a slight trend toward decreased PP14 levels in the midluteal phase, no significant changes were seen at the end of the cycle, i.e., at the time of maximal PP14 secretion. Thus, our results suggest that luteal and endometrial secretory function do not decline significantly with aging. In other studies of women >40 years, P and E<sub>2</sub> levels were normal or reduced in the luteal phase, whereas those of inhibin were normal (16, 17, 20, 22). To our knowledge, there are no reports on PP14 concentrations in this population.

Disruption of endometrial maturation occurred at a similar frequency in women aged 20 to 30 and 40 to 50 years included in our study, suggesting that endometrial development and perhaps endometrial receptivity to implantation do not deteriorate with aging. Histologic data presented in this paper were pooled from various protocols involving normal volunteers conducted at the National Institutes of Health (Bethesda, MD). This group, albeit larger and somewhat different, included all subjects partic-

ipating in the first part of our study, i.e., in the evaluation of hormonal profiles of the menstrual cycle. All endometrial biopsies were performed and interpreted in a very controlled fashion. Specimens consistently were obtained 7 to 9 days after the LH surge to evaluate the development of the endometrium at the time of nidation. This timing of the biopsy also was chosen because histologic dating appears to be more precise when specimens are obtained in the midluteal phase as opposed to the late luteal phase, as done by other investigators (24). All specimens were read by only two pathologists, who agreed on the pattern of endometrial maturation in a high percentage (96%) of cases. Histologic dating of the endometrium was done by the traditional Noyes' criteria (13), a simple and frequently used method that has gained wide acceptance as the standard approach to evaluate the development of the endometrium (4). It is possible, however, that more sophisticated techniques such as quantitative morphometric analysis or scanning electron microscopy of the endometrium would have shown subtle changes in endometrial maturation that otherwise were not detected by Noyes' criteria (13).

Our data suggesting normal luteal and endometrial secretory function and normal endometrial maturation in cycling women >40 years indicate that implantation failure due to a hostile endometrium does not seem to play a significant role in the decline of fertility in this population. Our results contrast with those reported by Sterzik et al. (25), who performed endometrial biopsies in cycles stimulated for IVF and found that histologic abnormalities of the endometrium were significantly more common after age 35. In protocols used for oocyte retrieval, however, the endometrium is exposed to very high and perhaps deleterious steroid levels, which may explain the different results found in stimulated and spontaneous cycles such as the ones evaluated in the present study. In addition, Sterzik et al. (25) obtained endometrial tissue on day 2 after hCG-induced ovulation in infertile women and not at the time of nidation, as was done in normal subjects in our protocol.

Conflicting results seen in our and other studies of reproductive changes after age 40 may be due to sampling differences in the populations recruited by the various authors, including fertility status (16, 17, 20, 22). In normal women aged 40 to 50 years who menstruate regularly, our data suggest that a primary abnormality in follicular recruitment is the first step in the natural aging of the female reproductive system. At this stage, follicular maturation and ovarian hormonogenesis seem to progress normally after the dominant follicle is selected. Likewise, lu-

teal and endometrial secretory function and endometrial development appear unaffected in these subjects, arguing against an increased rate of corpus luteum and endometrial insufficiency in cycling women >40 years.

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