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Relationship between Follicle-Stimulating Hormone Levels at the Beginning of the Human Menstrual Cycle, Length of the Follicular Phase and Excreted Estrogens: The FREEDOM Study

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Although reproductive aging has been separately related to elevated FSH and shorter follicular phase (FP), the direct association between both parameters has not been investigated. Also, the exact effects of increased FSH on estrogen production are yet to be established.

A large database of daily urinary concentrations of FSH, LH, and estrone 3-glucuronide (E1G) from 37 regularly menstruating women (median 11 cycles per patient) was used. Initial FSH levels (iFSH) were estimated as the mean value of d 1–5. The day of E1G take-off (ETO) was determined by an algorithm, and accordingly, the FP was divided into early (d 1 to ETO) and late (ETO+1 to LH peak). FP maximum and integrated E1G were calculated.

Subjects were distributed according to their mean iFSH into three categories (≤5, >5 to 10, and >10 IU/liter). There was a gradual decrease in FP length with increasing category (15.2 ± 3.8, 14.1 ± 3.6, and 13 ± 2.6 d, respectively; $P < 0.0001$). A similar effect occurred in early FP (7.5 ± 4, 6.4 ± 3.7, and 5.4 ± 2.7; $P < 0.0001$); in contrast, late FP was unaffected (7.7 ± 2.1, 7.7 ± 2.1, and 7.6 ± 2.4; $P = 0.86$). No consistent increase in E1G was found with advancing iFSH category; however, women with mean initial LH higher than 6 IU/liter had significantly elevated maximum ($P < 0.0001$) and integrated ($P = 0.002$) E1G.

FP length decreases in parallel with increasing iFSH, with a selective effect on the early FP. Increased FSH does not affect E1G; however, elevated initial LH level was related to higher E1G. (*J Clin Endocrinol Metab* 89: 3270–3275, 2004)

INCREASED FSH AT the beginning of the menstrual cycle is an established early sign of reproductive aging in the woman (1–5). Throughout the reproductive life, there is a steady reduction in the number of newly recruited ovarian follicles. Because these follicles produce inhibin B, a dimeric glycoprotein that inhibits FSH secretion by the anterior pituitary (6–12), a diminution in the number of recruited follicles results in raised FSH. Because the number of these follicles reflects ovarian reserve, FSH is an indirect indicator of reproductive age. Indeed, d 3 FSH measurement is currently part of the routine in the clinical management of infertility (13, 14).

Another early feature of reproductive aging is a reduction in the length of the cycle (15–17), which is due to the shortening of the follicular phase (FP) (4, 18, 19). During the FP, there is a process of follicle recruitment, growth, and selection as well as synthesis of estrogens. Because FSH plays a central role in this process by stimulating the granulosa cells (20), a rise in FSH might affect FP length, possibly by accelerating follicular development (4). Nevertheless, although there are several studies proposing a relationship between increased FSH as well as reduced FP length with advancing age, a direct association between increasing FSH and shortening FP has not been shown.

The FP can be divided into two distinct stages according to the onset of estrogen take-off (ETO): early FP (from d 1 to ETO) and late FP (from the day after ETO to the day of LH peak). At the early FP, follicles are recruited, and begin to grow, with very low steroidogenic activity. At the late FP, follicles (mainly the dominant one) start to produce estrogens increasingly. A reduction in FP length could in principle affect both the early and late stages. However, if FSH is involved in this reduction, because levels are higher during the early FP, this stage should be more affected than the late FP.

The second stage of the FP is characterized by the production of estrogens. Research on estrogen variation with reproductive aging has produced diverse results (1, 2, 4). Those studies, however, are based exclusively on chronological age. Because hormonal parameters appear to be better indicators of reproductive aging, examining estrogen changes in relation to increasing FSH might be more appropriate.

In this study we investigated the relationship between initial levels of FSH in the menstrual cycle and length of the FP. To examine it in further detail, we divided the FP into early and late, according to the onset of estrone 3-glucuronide (E1G) rise. In addition, we investigated possible changes in E1G production during the FP in relation to increasing FSH. As the source of information, we used a large database of daily urinary hormonal profiles over several menstrual cycles from women with menstrual regularity.

Abbreviations: E1G, Estrone 3-glucuronide; ETO, E1G take-off; FP, follicular phase; iFSH, initial FSH level; iLH, initial LH level; S, surge size.

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Subjects and Methods

Subjects

This is part of a large study on changes in reproductive hormones during aging (FREEDOM study: Fertility Recognition Enabling Early Detection Of Menopause), which started in 1998 (Miro, F., S. Parker, L. Aspinall, J. Coley, P. Perry, J. Ellis, manuscript submitted). The study involved, initially, a total of 112 women of different reproductive ages, from women with normal regular cycles to postmenopausal ones. Each woman collected daily urine samples for a variable period of time (6–18 months) and kept daily records of days of menses, medication taken, and other relevant information. The use of exogenous hormones or any medication known to interfere with the secretion of the hormonal markers investigated was an exclusion criterion. In this part of the study, we regarded only those women with regular cycles.

Data from 37 healthy women with regular menstrual cycles (mean length 24–33 d) and no abnormal bleeding were considered. The ages of the volunteers ranged from 30 to 52 yr (median value 44) with a median body mass index of 23.

Hormonal analyses

Urine samples (first morning void) were collected in universal specimen bottles containing sodium azide (0.1% volume) as preservative. Volunteers were asked to keep the samples refrigerated until delivered to the laboratory (on a weekly basis). On arrival, the specimens were aliquoted and stored at 4 C until the analyses were performed.

The samples were analyzed for LH, E1G, and pregnanediol 3-glucuronide by immunoassay, using AutoDelfia (Perkin-Elmer Life Sciences, Cambridge, UK) following in-house established and validated protocols, as previously described (20). FSH was measured using a similar procedure as used for LH. The antibody reagents used in the FSH assay were biotin-labeled anti-αFSH (clone 4882) together with strepavidin-coated microtitration strips and europium-labeled anti-βFSH (clone 5948). The sensitivity of the FSH assay was 0.17 IU/liter, with intraassay coefficients of variation of 5.1, 2.4, and 1.8% for FSH concentrations of 1.8, 8.2, and 42.9 IU/liter, respectively. The interassay coefficients of variation at the same FSH concentrations were 4.0, 3.4, and 2.4%. The FSH assay had no cross-reactivity to LH, human chorionic gonadotrophin, and TSH (<0.1%).

Data preparation and analyses

The concentrations of FSH, LH, and E1G were adjusted to compensate for urine volume fluctuations as previously described (Miro, F., J. Coley, M. Gani, P. Perry, D. Talbot, L. Aspinall, manuscript submitted). Briefly, a smooth curve was fitted to ln(pregnanediol 3-glucuronide) and the residuals were used to adjust the concentrations of the other hormones. This was achieved by using the SAS/IML Splinec routine with a smoothing parameter of 10 (23) (Miro, F., J. Coley, M. Gani, P. Perry, D. Talbot, L. Aspinall, manuscript submitted). FSH and LH results are expressed as international units per liter and E1G as nanograms per milliliter.

Parameters, algorithms, and statistics

Initial levels of FSH and LH in the follicular phase (iFSH, iLH) were estimated as the mean concentration of FSH or LH over the first 5 d of the cycle, starting from first day of bleeding.

The onset of ETO in the FP was determined by an algorithm that retrospectively detects the first day of a sustained increase in E1G concentration. The algorithm was applied to smoothed-adjusted values of E1G throughout the menstrual cycle and works by measuring the surge size (S) for each day in the cycle having a positive slope, excluding the last 10 d of the cycle (Fig. 1). Thus, S is based on the change in E1G slope relative to the current E1G level:

S = {Right slope − Left slope*}/current [E1G]**

*, If the left slope is negative, then it is substituted by 0. **, If the E1G concentration is less 4 ng/ml, then it is substituted by 4, where right slope = the slope of the line joining the current day with the day R days later and left slope = the slope of the line joining the current day with the day L days earlier.

The value for L is fixed in the model as 2. However, R depends on the overall length of the cycle. For cycles 30 d or longer, R = 3, and it increases at a rate of 1 for each 5-d increase in cycle length up to 10, for cycle lengths longer than 60 d. This allows for longer cycles tending to have longer sustained E1G rises. Dividing the difference in the slopes by the current E1G level gives greater weight to changes in the slopes at low E1G concentrations. Setting the minimum E1G level to 4 ng/ml avoids undue sensitivity of the algorithm at very low concentrations.

The day of ETO is defined as the first day in the cycle for which $S > 0.25$ or $S > 0.65 \times$ maximum(S). The latter condition provides adaptation to the cycle, ensuring that S is always defined.

FP of the cycle is defined from d 1 of cycle to the day of LH peak and is divided according to the onset of ETO, into:

Early FP from d 1 of the cycle until the ETO day.

Late FP from the day after ETO to the day of the LH peak (Fig. 2).

Maximum E1G value during the FP is defined as the maximum concentration of E1G during the FP in smooth profiles. This day must be no more than 2 d from the LH peak day.

The integrated E1G during the FP was calculated as the sum of adjusted E1G concentration in the smooth profiles, over the period from the day of E1G take-off and up to the day of maximum E1G. From this value the minimum E1G for each volunteer was subtracted to correct for the baseline E1G in each subject (Fig. 2).

Differences between categories were determined using one-way ANOVA. Results are expressed as mean ± SD.

Results

Incomplete cycles were excluded from the study. Equally, there were eight cycles with very high basal levels of E1G (*i.e.* ≥ 20 ng/ml). Most of these cycles were unusually short in length (mean 19 d long), although there were two cycles longer than normal (41 and 44 d). Because initial FSH levels were considerably lower in these cycles, compared with the

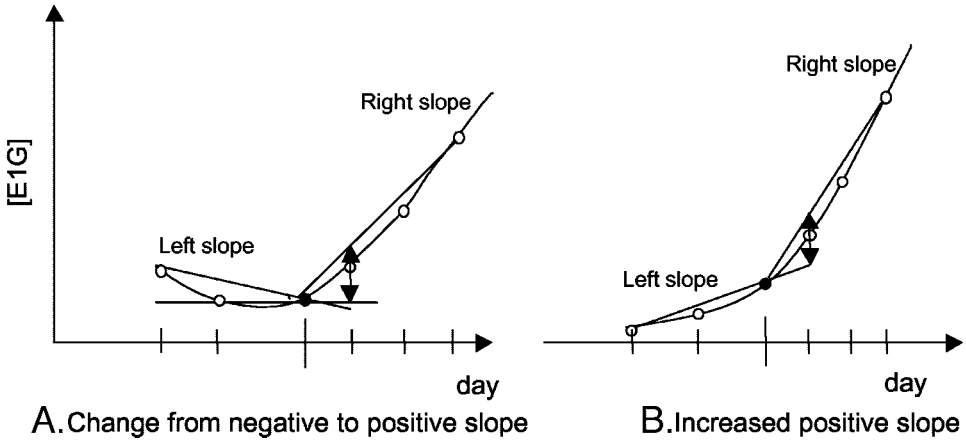

FIG. 1. Algorithm aimed to determine onset of ETO. The algorithm detects the first sustained increase in E1G by estimating the magnitude of S in E1G for each day in the cycle. The *filled circles* correspond to the current E1G level, and the *double-headed arrow* illustrates the value of right slope/left slope. In these plots, L = 2 d and R = 3 d; thus, S depends on the current levels of E1G and the values 2 d earlier and 3 d later. If the left slope is negative, it is substituted by zero, corresponding to a *horizontal line*.

rest of the cycles within the same woman, it was judged that the elevated levels of estrogen affected FSH production and thus were excluded from the analyses. Overall, 386 cycles were analyzed (median 11 per volunteer).

Variation in total FP length in relation to iFSH levels

To determine the precise relationship between FSH and FP length, we distributed the subjects into three categories according to their mean iFSH levels. Three categories allows a robust enough statistical analysis while providing a perception of gradual variation. The groups were established taking the 33.3 and 66.6 centiles of the distribution as guidance, which were 5.1 and 10.4, respectively.

Low: iFSH 5 IU/liter or less (mean 3.6, SD 1.5), n = 12 cases, 125 cycles. Overall, the mean number of cycles per subject with iFSH less than 5 IU/liter was 83% (SD 13.7), with 0% above 10 IU/liter.

Medium: iFSH more than 5, 10 IU/liter or less (mean 7.2, SD 3.6), n = 15 cases, 153 cycles. Overall, the mean number of cycles per subject with iFSH more than 5, 10 IU/liter or less, was 64% (SD 20), with 22% (SD 15) below, and 14% (SD 9.5) above 5 IU/liter.

High: iFSH more than 10 IU/liter (mean 11.4, SD 4.9), n = 10 cases, 108 cycles. Overall, the mean number of cycles per subject with iFSH more than 10 IU/liter was 60% (SD 15), with 37% (SD 15) more than 5, 10 IU/liter or less, and 3% (SD 5.6) less than 5 IU/liter.

The mean and range of age for each category was: 41 yr for low (30–52); 44.5 for medium (39–50); and 44 for high (30–52). These three categories correspond to three stages in reproductive aging (1, 2a, and 2b) used in a parallel study (Miro, F., S. Parker, L. Aspinall, J. Coley, P. Perry, J. Ellis, manuscript submitted).

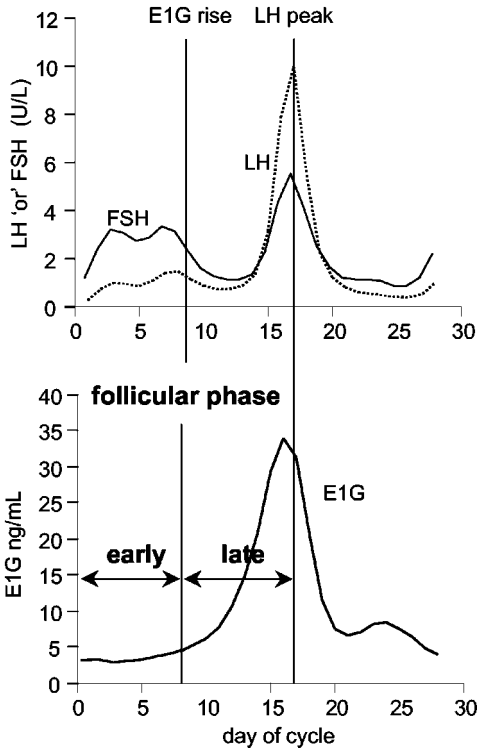

FIG. 2. Application of the algorithm to detect the day of ETO in a real cycle. The *upper panel* shows FSH and LH concentrations, the *lower* E1G. ETO divides the FP into early (from d 1 to ETO) and late (from the first day after ETO to the day of LH peak).

Variation in the length of early and late FP in relation to iFSH

The length of the FP was compared among the three resulting categories of subjects with respect to overall FP, early FP, and late FP. With regard to overall FP length, we found a progressive reduction in the length from the low FSH category to high (Fig. 3) of 15.2 ± 3.8, 14.1 ± 3.6, and 13.0 ± 2.6 d, respectively ($P < 0.0001$).

A preliminary comparison between the SD of the early and late FPs for each individual revealed a much higher degree of variability in the early stage of the FP, as determined by the sign test ($P < 0.0001$). This suggests that changes in the overall length of the FP are more likely to affect the early stage.

To investigate this further, we determined the distribution of the length of the early and late FP in relation to each category (Fig. 3). The mean length of the early FP showed a similar trend to that of the total FP length, with lengths of 7.5 ± 4.0, 6.4 ± 3.7, and 5.4 ± 2.7 d ($P < 0.0001$), respectively, for low to high FSH. In contrast, there was no difference in the length of the late FP among the three categories, *i.e.* 7.7 ± 2.14, 7.7 ± 2.1, and 7.6 ± 2.4 d ($P = 0.86$).

Variation in FP estrogen production in relationship to iFSH

To investigate the relationship between iFSH and E1G production, we compared the maximum FP values of E1G as

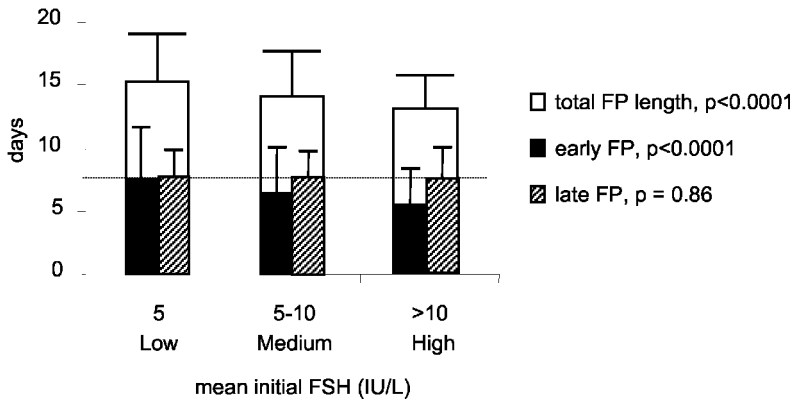

FIG. 3. Changes in the length of the FP with increasing iFSH. Volunteers were distributed according to their mean iFSH into three categories as shown, and the mean length of the FP, early FP (up to ETO), and late FP (from ETO to LH peak) for each category was compared. *Error bars* indicate SD. Comparisons were made using one-way ANOVA.

well as integrated E1G among the three categories. As shown in Table 1, there was no consistent tendency toward increasing E1G levels in parallel with FSH. Women with medium initial levels of FSH had higher levels of E1G; however, there was no statistical significance in this difference ($P = 0.072$). Integrated and maximum E1G was very similar in the groups of low and high FSH, whereas the medium FSH group had slightly higher values ($P = 0.021$).

Although not as pronounced as FSH, there is a parallel rise in LH at the beginning of the cycle. Because estrogen production involves the action of both FSH and LH, there is a possibility that the LH rise might be implicated in increased production of estrogen. When the volunteers were distributed into three categories according to iLH levels, namely less than 3 IU/liter, 3–6 IU/liter, and more than 6 IU/liter, it was found that although the first two categories had similar E1G levels, the third category had substantially higher maximum as well as integrated E1G (Table 1).

Discussion

Reproductive aging in the woman is associated with increased FSH at the beginning of the cycle (1–5), and shorter FP (4, 18, 19). Such parallelism, together with the fact that FSH is the major director of follicular development, suggests a direct association between increasing FSH and reduced FP length; however, this association has not been investigated to date. We have addressed the issue using a large database on daily urinary hormonal concentrations of reproductive hormones (37 women, 386 cycles). Our results indicate the occurrence of a gradual reduction in the length of the FP in parallel with increasing FSH.

To better understand our finding, we examined the FP length in further detail. Because the day of ETO provides a suitable reference of the emergence of the dominant follicle (24, 25), the FP was divided into early (before) and late (after ETO). Although a reduction in FP length could in principle involve both stages, we found that this FSH-related shortening selectively affects the early FP, with no apparent effect on the late FP.

Two different explanations for the age-related reduction in follicular phase have been proposed: accelerated follicular growth (26) and earlier start of follicular growth (27). At the present, the former explanation has received more experimental support, and our results are consistent with it. Some researchers have found evidence of faster follicular development (4, 26), and even earlier achievement of dominance (28), in women of advanced reproductive age. Moreover, these authors too have found indications that the shortening of the follicular phase affects selectively events occurring early in the follicular phase (28).

Our results here, based on a large number of cases, agree with the concept that the cause of the reduction in the length of the follicular phase involved in reproductive aging is accelerated follicular growth (4, 26) and that this affects selectively early events in the FP (28). In addition, our approach detects a gradual relationship between increasing levels of FSH and reduction in FP length. Our approach too, differs in fundamental ways from that used before. We use urine instead of blood as the analytical medium, our reference for reproductive aging is the level of FSH instead of age, and our analysis of the substages of the FP is based on the onset of ETO instead of the FSH peak that follows it.

The concept of accelerated follicular growth receives further support from the finding that granulosa cells in the growing follicles from women exposed to ovarian hyperstimulation with exogenous FSH present increased mitotic index, compared with spontaneous cycles (29). Consistently, too, granulosa cells from women over 40 yr of age (which usually present elevated FSH) also have higher mitotic index, compared with younger women (30).

The steadiness in the length of the late FP found was somehow expected because FSH levels decline as estrogens rise (31). Nevertheless, early exposure to FSH might affect later aspects of follicular development, perhaps by increasing cellular numbers or a more advanced degree of cell differentiation. This, however, appears not to be the case.

Considerable evidence has shown a gradual decline in natural fertility throughout life, with a pronounced decline starting early in the fourth decade (32). This is well reflected by results from artificial insemination (33, 34). Statistical research indicates the existence of a 6-d fertile window in the cycle, during which nearly all pregnancies occur (35–37). This fertile window contains the day of ovulation and the 5 d before. Because ovulation occurs approximately 24 h after the LH peak, the fertile window thus corresponds mostly with the late FP in our study. A recent ample investigation concluded that although fertility declines with age, there is no evidence of reduction in the length of the fertile window (38). This is consistent with constancy in the late FP.

It remains to be elucidated whether the effects of higher FSH on the early events of the FP have an impact on fecundity. Inhibin B is a glycoprotein with suppressive effects on FSH secretion. Because inhibin B is produced by the newly recruited follicles from the beginning of the cycle (6–12), it is conceivable that keeping circulating levels of FSH relatively low might be desirable to ensure favorable conditions

TABLE 1. Estrogen production during the follicular phase: relationship with initial levels of FSH and LH

	E1G (ng/ml)	Mean initial levels			*P*
FSH (IU/liter)		Low (≤5)	Medium (>5 to 10)	High (>10)	
	Integrated	146 ± 84	168 ± 109	147 ± 60	0.072
	Maximum	41.6 ± 20	48.2 ± 25	42.5 ± 17	0.021
LH (IU/liter)		Low (≤3)	Medium (>3 to 6)	High (>6)	
	Integrated	149 ± 71	145 ± 89	187 ± 114	0.002
	Maximum	41 ± 16	42 ± 22	54.8 ± 26	<0.0001

Data show mean ± SD.

for follicular development. Some authors have suggested that the resulting increase in FSH might be a compensatory mechanism to maintain normal levels of estrogens (39). Further research is required to establish this point, and there might be important implications for the treatment of natural decline in fertility with increasing age.

Because, in addition to follicular growth, FSH stimulates estrogen production in granulosa cells, we investigated changes in estrogen production related to increased FSH. We found no consistent elevation in E1G (integrated or maximum) with increasing FSH.

Because ovarian estrogen synthesis involves two different cell types, stimulated by two gonadotropins, FSH and LH (40–43), we considered a possible relationship between increasing iLH and E1G. Although we did not see a gradual rise in E1G with increasing LH, women with the highest iLH had higher values of E1G. These findings are difficult to interpret because early in the FP, granulosa cells have very little, if any, responsiveness to LH (44–46). However, a comparison between iLH with those on d 7–9 (data not shown) reveals a strong correlation ($R^2 = 0.54$, $P < 0.0001$). Interestingly, in ovarian hyperstimulation, increasing the LH dose during mid-FP, with a fixed FSH dose, results in a significant increase in plasma estradiol (47–49).

Research on variation in estrogens with age has yielded conflicting results. Initial work found a decrease in estradiol (1) or excreted estrogens (50) throughout the cycle with increasing age. Other studies, although, did not find such decrease (51, 52), and indeed some even found increased circulating FP estradiol with increasing age, concomitant with a rise in FSH and LH (2). More recent studies have produced results in one or the other direction. One reported a brief decrease in preovulatory estradiol in women 36–38 yr old, followed by an increase thereafter (53). A comparison between women 40–45 and 20–25 yr old found higher serum FSH, shorter FPs, and elevated estradiol in the former (4). Interestingly, those older women had an earlier onset of ETO. Finally, there are reports of mild, although not significant, increase in FP estradiol with age (49, 54).

There is a high degree of correlation between plasma estradiol and urinary estrogens throughout the cycle, in particular E1G (55–57), and there are a few studies on age-related changes in urinary estrogens. One such study reported a substantial increase in urinary estrone during the FP in older women, who also had higher FSH (55). Other researchers, however, found an increase in excreted estradiol, estrone, and estriol with increasing age, although significant differences were found only for estradiol (58).

In summary, these results support the concept that the FP of the menstrual cycle shortens concomitantly with increasing iFSH levels. The relation between increasing FSH and reduction in FP length appears to be gradual and affects selective events occurring prior to the rise in estrogens. Also, the rise in FSH does not seem to have a significant impact on estrogen synthesis in the late FP. The finding of increased FP estrogens with relatively elevated iLH levels might explain some discrepancies reported in the literature regarding increased estrogens and aging and requires further research.

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References

1. **Sherman BM, West JA, Korenman SG** 1976 The menopausal transition: analysis of LH, FSH, estradiol and progesterone concentrations during menstrual cycles of older women. J Clin Endocrinol Metab 42:629–636
2. **Reyes FI, Winter JS, Faiman C** 1977 Pituitary-ovarian relationships preceding the menopause. I. A cross-sectional study of serum follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol and progesterone level. Am J Obstet Gynecol 129:557–564
3. **Ahmed Ebbiary NA, Lenton EA, Cooke ID** 1994 Hypothalamic-pituitary ageing: progressive increase in FSH and LH concentrations throughout the reproductive life in regularly menstruating women. Clin Endocrinol (Oxf) 41:199–206
4. **Klein NA, Battaglia DE, Miller PB, Branigan EF, Giudice LC, Soules MR** 1996 Reproductive aging: accelerated ovarian follicular development associated with a monotropic follicle stimulating hormone rise in normal older women. J Clin Endocrinol Metab 81:1038–1045
5. **Oosterhuis GJE, Vermes I, Michgelsen HWB, Schoemaker J, Lambalk CB** 2002 Follicle stimulating hormone measured in unextracted urine throughout the menstrual cycle correlates with age and ovarian reserve. Hum Reprod 17:641–646
6. **Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, McNeilly AS** 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 81:1401–1405
7. **Roberts VJ, Barth S, el-Roeiy A, Yen SS** 1993 Expression of inhibin/activin subunits and follistatin messenger ribonucleic acids and proteins in ovarian follicles and the corpus luteum during the human menstrual cycle. J Clin Endocrinol Metab 77:1402–1410
8. **Klein NA, Illingworth PJ, Groome NP, McNeilly AS, Battaglia DE, Soules MR** 1996 Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: a study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. J Clin Endocrinol Metab 81:2742–2745
9. **Hayes FJ, Hall JE, Boepple PA, Crowley Jr WF** 1998 Differential control of gonadotropin secretion in the human: endocrine role of inhibin. J Clin Endocrinol Metab 83:1835–1841
10. **Welt CK, McNicholl DJ, Taylor AE, Hall JE** 1999 Female reproductive aging is marked by decreased secretion of dimeric inhibin. J Clin Endocrinol Metab 84:105–111
11. **Burger HG, Dudley EC, Hopper JL, Groome N, Guthrie JR, Green A, Dennerstein L** 1999 Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. J Clin Endocrinol Metab 84:4025–4030
12. **Burger HG, Groome NP, Robertson DM** 1998 Both Inhibin A and B respond to exogenous follicle-stimulating hormone in the follicular phase of the human menstrual cycle. J Clin Endocrinol Metab 83:4167–4169
13. **Toner JP, Philput CB, Jones GS, Muasher SJ** 1991 Basal follicle-stimulating hormone level is a better predictor of *in vitro* fertilization performance than age. Fertil Steril 55:784–791
14. **Hédon B, Dechaud H, Galtier-Dereure F, Guigue N** 1999 Comment évaluer la réserve ovarienne en 1999? Contracept Fertil Sex 27:412–416
15. **Collet ME, Wertenberger GE, Fiske VM** 1954 The effect of age upon the pattern of the menstrual cycle. Fertil Steril 5:437–448
16. **Treloar A, Boynton R, Behn B, Brown B** 1967 Variation of the human menstrual cycle through reproductive life. Int J Fertil 12:77–126
17. **Vollman RF** 1977 The length of the menstrual cycle. In: Vollman RF, ed. The menstrual cycle. Philadelphia: WB Saunders; 19–72
18. **Sherman BM, Korenman SG** 1975 Hormonal characteristics of the human menstrual cycle throughout reproductive life. J Clin Invest 55:699–706
19. **Lenton EA, Landgren BM, Sexton L, Harper R** 1984 Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronological age. Br J Obstet Gynaecol 91:681–684
20. **McGee EA, Hsueh AJW** 2000 Initial and cyclic recruitment of ovarian follicles. Endocr Rev 21:200–214
21. Deleted in proof
22. Deleted in proof
23. **Littell RC, Milliken GA, Stroup WW, Wolfinger RD** 1996 SAS System for mixed models. Cary, NC: SAS Institute Inc.
24. **Fauser BCJM, van Heusden AM** 1997 Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocr Rev 18:71–106

25. **van Santbrink EJ, Hop WC, van Dessel TJ, de Jong FH, Fauser BC** 1995 Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. Fertil Steril 64:37–43
26. **Klein NA, Battaglia DE, Miller PB, Branigan EF, Giudice LC, Soules MR** 1996 Ovarian follicular development and the follicular fluid hormones and growth factors in normal women of advanced reproductive age. J Clin Endocrinol Metab 81:1946–1951
27. **van Zonneveld P, Scheffer GJ, Broekmans FJM, Blankenstein MA, de Jong FH, Looman CWN, Habbema JDF, te Velde ER** 2003 Do cycle disturbances explain the age-related decline of female fertility? Cycle characteristics of women aged over 40 years compared with a reference population of young women. Hum Reprod 18:495–501
28. **Klein NA, Harper AJ, Houmard BS, Sluss PM, Soules MR** 2002 Is the short follicular phase in the older women secondary to advanced or accelerated dominant follicle development? J Clin Endocrinol Metab 87:5746–5750
29. **Gougeon A, Testart J** 1990 Influence of human menopausal gonadotropin on the recruitment of human ovarian follicles. Fertil Steril 54:848–852
30. **Gougeon A** 1998 Ovarian follicular growth in humans: ovarian ageing and population of growing follicles. Maturitas 30:137–141
31. **Brown JB** 1978 Pituitary control of ovarian function. Concepts derived from gonadotrophin therapy. Aust NZ J Obstet Gynaecol 18:47–54
32. **Wood JW** 1989 Fecundity and natural fertility in humans. Oxford Rev Reprod Biol 11:61–109
33. **Schwartz D, Mayaux MJ** 1982 Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. Federation CECOS. N Engl J Med 306:404–406
34. **Van Noord-Zaadstra BM, Looman CW, Alsbach H, Habbema JD, te Velde ER, Karbaat J** 1991 Delaying childbearing: effect of age on fecundity and outcome of pregnancy. Br Med J 302:1361–1365
35. **Wilcox AJ, Weinberg CR, Baird DD** 1998 Post-ovulatory ageing of the human oocyte and embryo failure. Hum Reprod 13:394–397
36. **Dunson DB, Baird DD, Wilcox AJ, Weinberg CR** 1999 Day-specific probabilities of clinical pregnancy based on two studies with imperfect measures of ovulation. Hum Reprod 14:1835–1839
37. **World Health Organization** 1984 A prospective multicentre trial of the ovulation method of natural family planning. III. Characteristics of the menstrual cycle and of the fertile phase. Fertil Steril 40:773–778
38. **Dunson DB, Colombo B, Baird DD** 2002 Changes with age in the level and duration of fertility in the menstrual cycle. Hum Reprod 17:1399–1403
39. **Burger HG, Dudley EC, Robertson DM, Dennerstein L** 2002 Hormonal changes in the menopausal transition. Recent Prog Horm Res 57:257–275
40. **Fevold HL** 1941 Synergism of the follicle-stimulating and luteinizing hormones in producing estrogen secretion. Endocrinology 28:33–36
41. **Bogovich K, Richards JS** 1982 Androgen biosynthesis in developing ovarian follicles: evidence that luteinizing hormone regulates theca 17 α-hydroxylase and C17–20 lyase activity. Endocrinology 111:1201–1208
42. **Hillier SG, Whitelaw PF, Smyth CD** 1994 Follicular oestrogen synthesis: "the two-cell, two-gonadotrophin" model revisited. Mol Cell Endocrinol 100:51–54
43. **Erickson GF, Wang C, Hsueh AJW** 1979 FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium. Nature 279:336–338
44. **Minegishi T, Tano M, Abe Y, Nakamura K, Ibuki Y, Miyamoto K** 1997 Expression of luteinizing hormone human chorionic gonadotrophin (LH/hCG) receptor mRNA in the human ovary. Mol Hum Reprod 3:101–107
45. **Takao Y, Honda T, Ueda M, Hattori N, Yamada S, Maeda M, Fujiwara H, Mori T, Wimalasena J** 1997 Immunohistochemical localization of the LH/hCG receptor in human ovary: hCG enhances cell surface expression of LH/hCG receptor on luteinizing granulosa cells *in vitro*. Mol Hum Reprod 3:569–578
46. **Adonakis G, Deshpande N, Yates RWS, Fleming R** 1998 Luteinizing hormone increases estradiol secretion but has no effect on progesterone concentrations in the late follicular phase of *in vitro* fertilization cycles in women treated with gonadotropin-releasing hormone agonist and follicle-stimulating hormone. Fertil Steril 69:450–453
47. **Fleming R, Rehka P, Deshpande N, Jamieson ME, Yates RWS, Lyall H** 2000 Suppression of LH during ovarian stimulation: effects differ in cycles stimulated with purified with purified urinary FSH and recombinant FSH. Hum Reprod 15:1440–1445
48. **Burgues S** 2001 The effectiveness and safety of recombinant human LH to support follicular development induced by recombinant human FSH in WHO group I anovulation: evidence from a multicentre study in Spain. Hum Reprod 16:2525–2532
49. **Filicori M, Cognigni GE, Taraborrelli S, Spettoli D, Ciampaglia W, de Fatis CT, Pocognoli P, Cantelli B, Boschi S** 2001 Luteinizing hormone activity in menotropins optimizes folliculogenesis and treatment in controlled ovarian stimulation. J Clin Endocrinol Metab 86:337–343
50. **Pincus G, Romanoff LP, Carlo J** 1954 The excretion of urinary steroids by men and women of various ages. J Gerontol 9:113–132
51. **England PC, Skinner LG, Cottrell KM, Sellwood RA** 1974 Serum oestradiol-17β in normal women. Br J Cancer 29:462–469
52. **Zumoff B, Fishman J, Cassouto J, Gallagher TF, Hellman L** 1968 Influence of age and sex on normal estradiol metabolism. J Clin Endocrinol Metab 28:937–941
53. **Lee SJ, Lenton EA, Sexton L, Cooke ID** 1988 The effect of age on the cyclical patterns of plasma LH, FSH, estradiol and progesterone in women with regular menstrual cycles. Hum Reprod 3:851–855
54. **Reame NE, Wyman TL, Phillips DJ, de Kretser DM, Padmanabhan V** 1998 Net increase in stimulatory input resulting from a decrease in inhibin B and an increase in activin A may contribute in part to the rise in follicular phase follicle-stimulating hormone of aging cycling women. J Clin Endocrinol Metab 83:3302–3307
55. **Santoro N, Rosenberg Brown J, Adel T, Skurnick JH** 1996 Characterization of reproductive hormonal dynamics in the perimenopause. J Clin Endocrinol Metab 81:1495–1501
56. **Wilcox AJ, Baird DD, Weinberg CR, Armstrong EG, Musey PI, Wehmann RE, Canfield RE** 1987 The use of biochemical assays in epidemiological studies of reproduction. Environ Health Perspect 75:29–35
57. **Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL** 1991 Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. Clin Chem 37:838–844
58. **Musey VC, Collins DC, Musey PI, Martino-Saltzman D, Preedy JRK** 1987 Age-related changes in the female hormonal environment during reproductive life. Am J Obstet Gynecol 157:312–317

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