Compensatory Increase in Ovarian Aromatase in Older Regularly Cycling Women

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Context: Serum estradiol (E2) levels are preserved in older reproductive-aged women with regular menstrual cycles despite declining ovarian function.

Objective: To determine whether increased granulosa cell aromatase expression and activity account for preservation of E2 levels in older, regularly cycling women.

Design: Daily blood sampling and dominant follicle aspirations at an academic medical center during a natural menstrual cycle.

Subjects: Healthy, regularly cycling older (36–45 yrs; n=13) and younger (22–34 yrs; n=14) women.

Main Outcome Measures: Hormone levels were measured in peripheral blood and follicular fluid aspirates and granulosa cell *CYP19A1* (aromatase) and *FSH-R* mRNA expression was determined.

Results: Older women had higher FSH levels than younger women during the early follicular phase with similar E2 but lower inhibin B and AMH levels. Late follicular phase serum E2 was also similar in the two groups. Follicular fluid E2 (O = 960.0 [IQR 765.0–1419.0]; Y = 994.5 [647.3–1426.5] ng/ml, p=1.0), E1 (O = 39.6 [29.5–54.1]; Y = 28.8 [22.5–42.1] ng/ml, p=0.3), and the E2/T ratio (O = 109.0±41.9; Y = 83.0±18.6, p=0.5) were preserved in older women. Granulosa cell *CYP19A1* expression was increased 3-fold in older compared with younger women ($p < 0.001$) with no difference in *FSH-R* expression.

Conclusions: Ovarian aromatase expression increases with age in regularly cycling women. Thus, upregulation of aromatase activity appears to compensate for the known age-related decrease in granulosa cell number in the dominant follicle to maintain ovarian estrogen production in older pre-menopausal women.

The dramatic acceleration in the depletion of ovarian follicles that occurs at approximately 35 years of age (1–3) manifests clinically as a decrease in antral follicle count (AFC) on ultrasound, a fall in serum inhibin B (INH-B) and antimullerian hormone (AMH), and a rise in early follicular phase (EFP) FSH levels in response to decreased negative feedback by INH-B (4). Despite anatomic and physiological evidence for a decline in follicle number in older, regularly cycling women, there is a surprising preservation of ovarian steroidogenesis with age. Several large epidemiologic studies (5–8) in women followed across the menopausal transition have demonstrated that there is a precipitous decline in INH-B and AMH accompanied by a rise in FSH approximately 7 years before the final menstrual period, whereas estradiol (E2) remains relatively stable until 2 years before the final menstrual period. These findings are consistent with the results of many smaller, detailed physiologic studies showing that follic-

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ular phase E2 levels are higher or the same in older (> 35 years) compared with younger regularly cycling women (9–20).

There are several physiological mechanisms that could potentially account for preserved or higher E2 secretion with aging. Preservation of E2 would occur in the setting of increased follicle number or granulosa cell (GC) number. However, it is widely known that follicle number decreases with age based on both anatomic studies (1, 3) and ultrasound determination of AFC (21), and it has now been shown that the number of GCs within each follicle also declines with age (22, 23). While maturation of more than a single dominant follicle occurs more commonly in older reproductive aged women (12), we have reported that follicular phase E2 levels are similar in older and younger regularly cycling women even in the presence of a single dominant follicle of similar size (24). A second possibility is increased availability of aromatase substrates (androstenedione [AD] and testosterone [T]). However, there is no evidence of increased androgen substrate as a function of aging and, in fact, late follicular phase AD levels are lower in older compared with younger regularly cycling women (24). A third possibility is that aromatase activity is increased with ovarian aging. The higher ratio of estrone (E1) to AD across the cycle in older compared with younger regularly cycling women provides support for the hypothesis that increased aromatase activity accounts for the preservation of serum E2 levels with aging (24).

To test the hypothesis that increased aromatase activity in the dominant follicle maintains serum E2 levels with aging in regularly cycling women, we measured GC aromatase expression and reproductive hormones in follicular fluid (FF) aspirates from the dominant follicle in a spontaneous menstrual cycle in older and younger women. The current studies show that GC aromatase expression is higher in older compared with younger women, suggesting that an upregulation of aromatase with aging is responsible for the preservation of both serum and FF E2 levels in older reproductive-aged women.

Materials and Methods

Subjects

Healthy older (36–45 years; n = 13) and younger (22–34 years; n = 14) regularly cycling Caucasian women were studied. All subjects had a history of regular menstrual cycles (25–35 days) with an ovulatory cycle preceding participation, confirmed by a luteal phase progesterone (P) > 3 ng/ml (9.5 nmol/l). Subjects had no clinical evidence of androgen excess, were euthyroid, had normal prolactin levels, did not exercise excessively [ran < 20 miles or equivalent/wk (25)], were nonsmokers, and were not taking any medications or overthe-counter supplements that interact with the reproductive axis. In addition, subjects had a normal hemoglobin, platelet count, prothrombin time, and partial thromboplastin time (PTT), and a transvaginal ultrasound documenting normal ovarian position. African-American women were specifically excluded as we have previously demonstrated higher serum E2 and ovarian aromatase activity in African-American compared with Caucasian women (26).

The study was approved by the Human Research Committee of the Massachusetts General Hospital, and signed informed consent was obtained from each subject.

Experimental Protocol

Phenotyping studies

Subjects underwent daily blood sampling from the first day of menses until the day of follicle aspiration for measurement of LH, FSH, estradiol (E2), estrone (E1), and androstenedione (AD). Inhibin B (INH-B) and antimullerian hormone (AMH) were measured on cycle day 3 and on the day of aspiration, and inhibin A (INH-A), progesterone (P) and testosterone (T) were measured on the day of aspiration. Blood samples were drawn within a two hour window in the late afternoon to coincide with timing of follicle aspirations. Transvaginal ultrasounds were performed every 2 to 3 days from cycle day 7 onward to monitor follicle growth.

Follicle Aspirations

The dominant follicle was aspirated when it reached a diameter of ≥ 13 mm (calculated as the mean of follicle length and width). Aspirations were performed before the LH surge due to the rapid downregulation of aromatase after ovulation (27, 28). Aspirations were performed in the in vitro fertilization (IVF) suite of the Brigham and Women's Hospital using standard oocyte retrieval techniques, as previously described (29). The largest follicle was identified by transvaginal ultrasound, aspirated into a sterile needle and tubing, and flushed with 2 mL of DMEM. FF samples were centrifuged (3,000 RPM x 15 minutes) and the supernatants recovered and stored at −80 C for subsequent hormone analyses. Pellets were separated, mixed with 250 ul Trizol, and frozen at −80 C until mRNA extraction.

RNA extraction, cDNA synthesis, and Quantitative Real-Time PCR

Total RNA was extracted from GCs using the RNeasy Mini Kit (QIAGEN, Valencia, CA). Reverse transcription was performed with the Affinity Script QPCR cDNA Synthesis Kit (Agilent Technologies, La Jolla, CA) using oligo(dT) primers. Quantitative real-time PCR was performed for the expression of aromatase (*CYP19A1*) with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as an endogenous control using Fast SYBR Green MasterMix (Applied Biosystems). Primers were designed for the aromatase splice variant found in human ovaries (30), while previously published and validated primers were selected for *GAPDH* expression. Both primer pairs were validated to have 90%–100% efficiency in standard curve reactions (31). Primer sequences were as follows: *CYP19A1* forward 5′-GCA ACA GGA GCT ATA GAT GAA C-3′, *CYP19A1* reverse 5′-AGG CAC GAT GCT GGT GAT G-3′, *GAPDH* forward 5′-ACC CAC TCC TCC ACC TTT G-3′, *GAPDH* reverse 5′-CTC TTG TGC TCT TGC TGG G-3′. FSH receptor (*FSH-R*) expression was assessed using the Taqman gene expression assays (Life

Technologies, Grand Island, NY) using probes for *FSH-R* (Hs00174865 m1) and beta actin (*ACTB*) which served as an endogenous control (Hs01060665 g1). All reactions were run with an Applied Biosystems StepOnePlus Real-Time PCR system (Life Technologies, Grand Island, NY). Relative quantification was determined using the $2^{-\Delta\Delta C}{}_T$ method (32) and melt curves were examined to verify amplification of pure mRNA samples. PCR amplification was successful in 12 samples from the older women and in 11 samples from the younger women for *CYP19A1* expression, and in 10 samples from older women and 9 samples from younger women for *FSH-R* expression (3 samples were removed in the latter experiment because of lack of amplification of *FSH-R*).

Hormone Assays

Hormone and peptide measurements were performed in serum and FF using the same assay with the exception of E2. FF measurements were adjusted for the DMEM volume used during aspiration. Serum E2 was measured using the AxSYM immunoassay (Abbott Laboratories, Abbott Park, IL), as previously described (33). The functional sensitivity of the assay is 20 pg/ml (73.4 pmol/l), and the interassay CV was 6.5%–10% within the range of reported samples. Cross-reactivity with E1 was 0.1%. FF E2 was measured using the Architect immunoassay (Abbott Laboratories), which has a functional sensitivity of 25 pg/ml (91.8 pmol/l) and an interassay CV of 10%–15%. Cross-reactivity with E1 was 0.07%. The Architect E2 assay was standardized and calibrated against liquid chromatography/tandem mass spectrometry (LC/MS) (34, 35). E1 was measured using an RIA (Diagnostic Systems Laboratories, Webster, TX) with a functional sensitivity of 12 pg/mL and an interassay CV of 4.1% within the range of reported samples (36).

P was measured using enzyme amplified chemiluminescence (Immulite 1000; Siemens, Los Angeles, CA) which has a sensitivity of 0.2 ng/ml (0.6 nmol/l) and interassay CV of 10.6%–14%. AD was measured by RIA (Diagnostic Product Corps [DPC], Los Angeles, CA) (37) which has an assay sensitivity of 0.04 ng/ml (1.4 pmol/l) and a CV of 6%–8%. T was measured using the Coat-A-Count RIA kit (DPC) with an interassay CV of $< 10\%$ and a functional sensitivity of 4 ng/dl (138.7 pmol/l) (38). The T assay was standardized and calibrated against LC/MS (34, 35). LH and FSH were measured using a 2-site monoclonal nonisotopic system (AxSYM; Abbott Laboratories) as previously described (39) with assay sensitivities of 0.3 and 0.7 IU/L for LH and FSH, respectively and CVs of $< 4\%$. LH and FSH levels are expressed in international units per liter (IU/L) as equivalents of the Second International Pituitary Reference Preparation (second IPRP) 80/552 for LH and the second IPRP 78/549 for FSH.

INH-A was measured by ELISA (Serotec, Oxford, England) (40) using a lyophilized human FF calibrator standardized as equivalents of the World Health Organization recombinant human (rh) INH-A preparation 91/624. The interassay CV was $<$ 10% and the functional sensitivity was 8 pg/mL. INH-B was measured by ELISA (Serotec) (41), with a CV of 15%–18% and a limit of detection of 15 pg/ml. AMH was measured using a 2-site ELISA (Diagnostic Systems Laboratory, Beckman-Coulter, Webster, TX) which has a detection limit of 0.02 ng/ml (0.14 pmol/l) and an interassay CV of $< 11\%$ for quality control (QC) serum containing 2.65 ng/ml (18.9 pmol/l).

Data analysis

Data that was not normally distributed was log-transformed for analysis. E1/AD and E2/T were calculated as indices of aromatase activity and E1/E2 as an index of type 1 17β-hydroxysteroid dehydrogenase (17βHSD) activity. The inflection point for the midfollicular rise in serum E2 was defined as the day when E2 exceeded the mean of all preceding cycle days by at least 50% and demonstrated a sustained rise, as previously described (13). The slope of the E2 rise was calculated from the day of the rise to the day of peak E2. All FF hormone levels were compared between older and younger women using ANCOVA, controlling for follicle size. Daily serum hormone levels were compared between older and younger women using repeated measures ANOVA with Student-Newman-Keuls post hoc testing. Messenger RNA levels of *CYP19A1* and *FSH-R* were determined using the $2^{-\Delta\Delta C}{}_T$ method to calculate relative quantification (RQ) and to correct for expression of endogenous controls and were compared between groups using unpaired t-tests. Data are expressed as the mean $\pm$ SEM unless otherwise indicated, and a P value ≤ 0.05 was considered statistically significant.

Results

Thirteen older (39.7 $\pm$ 1.0 years) and 14 younger (26.4 $\pm$ 1.0 years) Caucasian women with regular menstrual cycles were studied. Subjects were of normal weight (BMI: O 24.5 $\pm$ 0.6 vs. Y 22.7 $\pm$ 0.6 kg/m^2; $P = .1$) with no difference between the two groups.

Serum Reproductive Hormones and Peptides Prior to Follicle Aspiration

E2 levels were not different between groups (**Figure 1**) across the follicular phase, nor were levels of E1 different between groups. FSH levels were higher in the older women during the EFP (cycle days 1–5), but decreased to normal during the midfollicular phase. INH-B was lower in the EFP, consistent with our previous studies (42). LH levels were not different between the two groups in the EFP, while AD levels were lower in older vs younger women, (**Figure 1**), as seen in previous studies (24). Cycle day 3 AMH levels were lower in older compared with younger women (O vs Y: 1.0 $\pm$ 0.3 vs. 2.6 $\pm$ 0.4 ng/ml, $P <$ .01).

A sustained rise in E2 occurred 3 days earlier in older women compared with younger women (O vs. Y: cycle day 6.6 $\pm$ 0.7 vs. 9.9 $\pm$ 0.6; $P = .03$), consistent with previous studies (13) (**Figure 2**), but there was no difference in the slope of the increase in E2 (O vs. Y: 22.0 $\pm$ 2.8 vs. 23.8 $\pm$ 5.1 pg/ml/d; $P = .8$).

Follicle Dynamics

Serial ultrasounds before aspiration demonstrated that each subject grew a single dominant follicle ($\geq$ 10 mm). Older women grew a dominant follicle faster than younger

women (O vs Y: cycle day 4.6 ± 0.1 vs. 8.9 ± 1.1, $P = .04$), but the subsequent growth rate of the dominant follicle until aspiration was the same (O vs Y: 1.2 ± 0.2 vs. 1.5 ± 0.2 mm/d, $P = .3$). As aromatase expression and E2 synthesis commence at a follicle diameter of 7 mm (43), we also counted the number of nondominant follicles ≥ 7 mm in both ovaries and found no difference between younger and older women (O vs Y: 2.5 ± 0.5 vs. 2.8 ± 0.5 follicles, $P = .7$). Aspirations were performed on cycle day 11.5 ± 0.4 in older women and cycle day 13.1 ± 0.7 ($P = .07$), with no difference in the size of the dominant follicle between the two groups (O vs. Y: 16.6 ± 0.6 vs. 17.1 ± 0.6 mm, $P = .6$) at the time of aspiration.

Serum Reproductive Hormones and Peptides on the Day of Aspiration. On the day of follicle aspiration, there were no differences in serum FSH (O vs Y: 4.4 ± 0.1 vs. 4.4 ± 0.1 IU/L), E2, AD or E1, but serum T was lower in the older women (Table 1). Older women also had higher E2/T and E1/AD ratios, consistent with increased aromatase activity. Similar to cycle day 3, older women had lower AMH levels than younger women on the day of aspiration. Re-

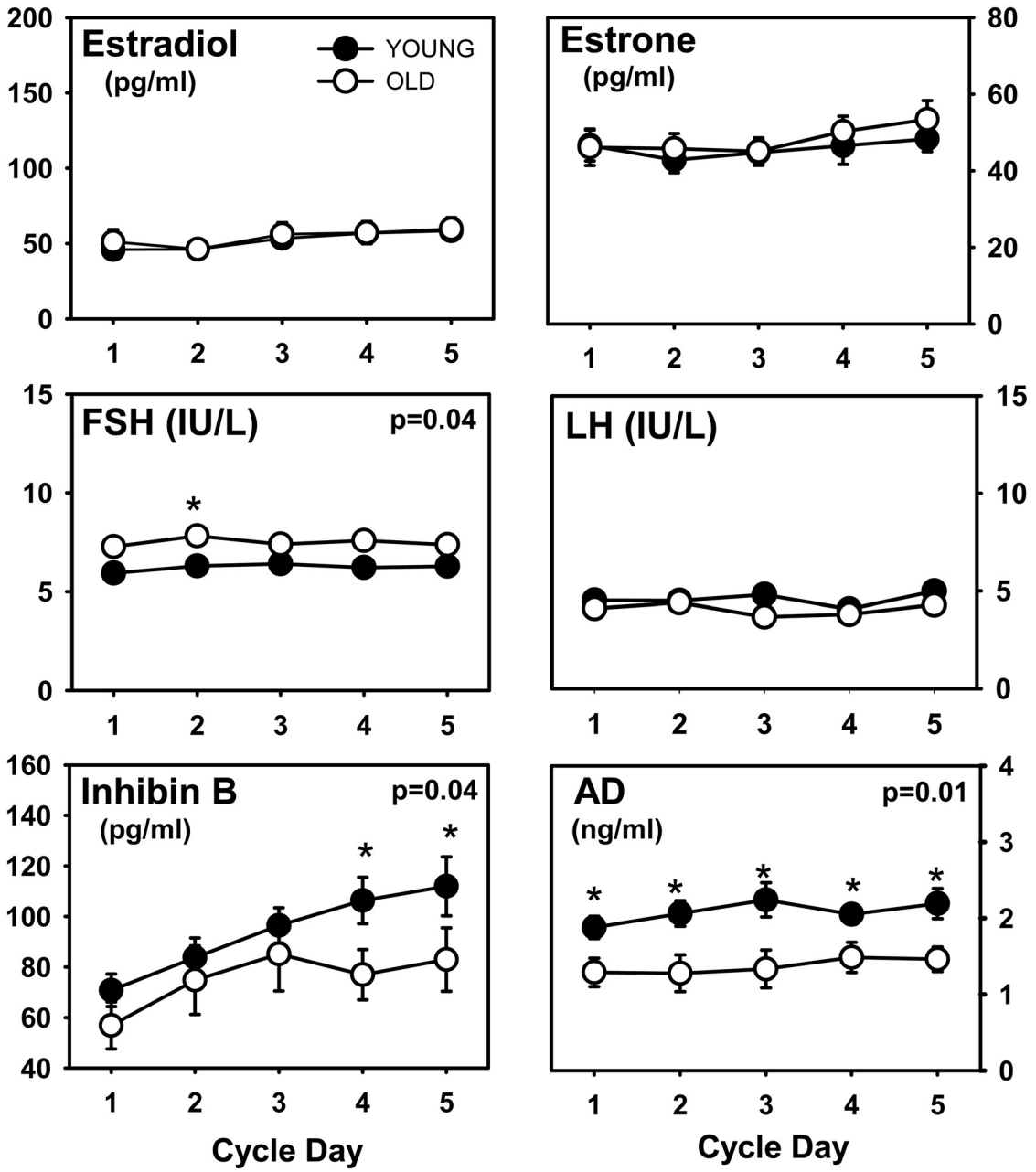

Figure 1. Older women had higher FSH ($P = .04$), lower inhibin B ($P = .04$), and lower androstenedione ($P = .01$) levels during the early follicular phase compared with younger women with preservation of estradiol and estrone secretion. There was no difference in LH levels between older and younger women. Levels were compared using repeated measures 2-way ANOVA. *$P < .05$ on post hoc testing.

Table 1. Effects of age on serum and follicular fluid hormone and peptide levels in regularly cycling women on the day of follicle aspiration (older women: cycle day 11.5 ± 0.4, younger women: cycle day 13.1 ± 0.7). Values are reported in pg/ml for estradiol, estrone, inhibin A and B; ng/ml for androstenedione, progesterone, and AMH; and ng/dl for testosterone. To convert serum values to SI units, multiple by 3.67 for estradiol and estrone (pmol/liter), 35 for androstenedione and testosterone (pmol/liter), 3.2 for progesterone (nmol/liter), 1 for inhibin A and B (ng/liter), and 7 for AMH (pmol/liter). All follicular fluid values are reported in ng/ml. P-values were unchanged after adjustment for dominant follicle size.

	Serum (Day of Aspiration)			Follicular Fluid		
	Older	**Younger**	p-value	**Older**	**Younger**	p-value
Follicle Size				16.6 ± 0.6 mm	17.1 ± 0.6 mm	0.6
Estradiol (E2)	145.4 ± 13.3 pg/ml	163.9 ± 21.2 pg/ml	0.5	960.0 (765.0–1419.0) ng/ml	994.5 (647.3–1426.5)* ng/ml	1.0
Androstenedione (AD)	2.0 ± 0.2 ng/ml	2.2 ± 0.2 ng/ml	0.7	331.0 (240.0–601.9) ng/ml	504.7 (151.8–647.5)* ng/ml	1.0
Estrone (E1)	101.5 ± 8.6 pg/ml	88.6 ± 8.5 pg/ml	0.3	39.6 (29.5–54.1) ng/ml	28.8 (22.5–42.1)* ng/ml	0.3
Testosterone (T)	20.0 (17.0–27.0) ng/dl	28.0 (23.3–32.8)* ng/dl	**0.05**[a]	20.0 ± 5.0 ng/ml	19.2 ± 2.7 ng/ml	0.7
E2 to T ratio	7.6 (6.3–8.4)	5.3 (3.9–7.3)*	**0.04**[a]	109.0 ± 41.9	83.0 ± 18.6	0.5
E1 to AD ratio	64.5 ± 7.0	44.4 ± 4.8	**0.03**[a]	0.2 ± 0.07	0.2 ± 0.04	0.4
E1 to E2 ratio	0.7 ± 0.05	0.6 ± 0.1	0.5	0.05 ± 0.004	0.04 ± 0.008	0.5
Inhibin A	27.5 ± 3.9 pg/ml	19.8 ± 2.6 pg/ml	0.1	21.5 ± 2.3 ng/ml	21.7 ± 2.7 ng/ml	1.0
Inhibin B	54.2 ± 8.9 pg/ml	59.8 ± 5.3 pg/ml	0.6	77.9 (62.0–88.0) ng/ml	70.2 (53.0–83.6)* ng/ml	0.7
Progesterone	0.5 ± 0.05 ng/ml	0.4 ± 0.05 ng/ml	0.2	546.0 (346.0–771.0) ng/ml	506.0 (273.5–916)* ng/ml	0.9
Anti-mullerian hormone	1.1 ± 0.3 ng/ml	3.1 ± 0.5 ng/ml	**0.003**[a]	4.6 (1.5–11.6) ng/ml	3.8 (3.2–5.9)* ng/ml	0.6

*Values are reported as median (interquartile range) due to non-normality.

[a]Statistically significant difference between older and younger women.

sults were unchanged after adjusting for dominant follicle size at aspiration.

Follicular Fluid and GC Analyses. Consistent with the hypothesis that the ovary is the source of increased aromatase activity in older women, GC aromatase expression, measured by quantitative RT-PCR, was 3-fold higher in older compared with younger women ($P < .006$, **Figure 3A**). There was no difference in *FSH-R* expression between the two groups (**Figure 3B**). FF estrogens (E2, E1), androgens (T, AD), aromatase (E2/T, E1/AD) and type 1 17ß-HSD (E1/E2) product to substrate ratios, and P levels were preserved in older women (Table 1). FF AMH levels were also similar in older and younger women (Table 1). AMH is predominantly expressed in secondary, preantral,

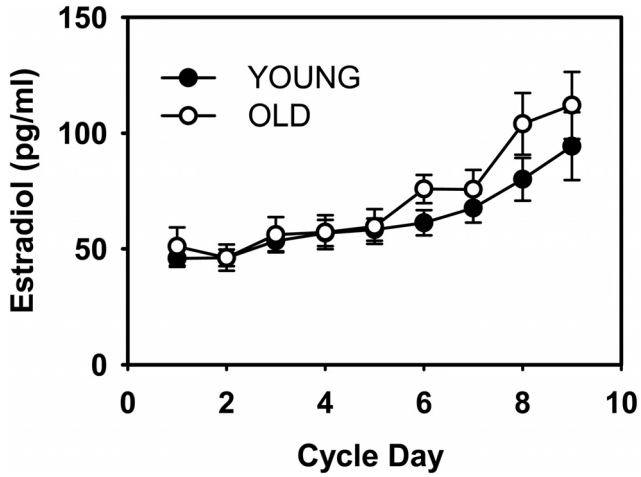

Figure 2. There was no difference in serum estradiol levels in older and younger women from cycle day 1 until the day of the first follicle aspiration procedure (cycle day 9), however, older women demonstrated an earlier rise in estradiol consistent with accelerated folliculogenesis.

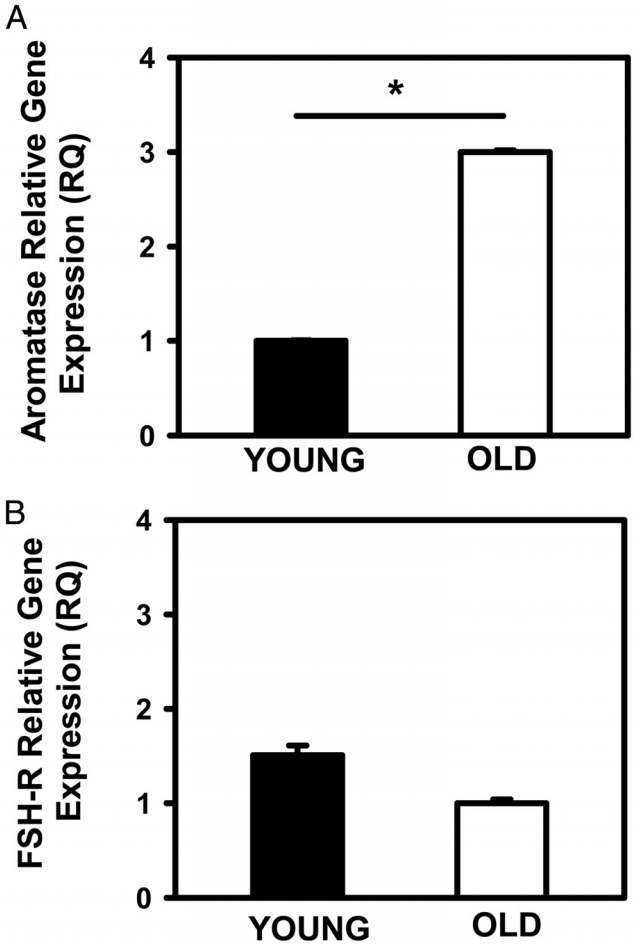

Figure 3. Mean (SEM) relative quantification (RQ) of A. aromatase gene expression (Young, n = 11; Old, n = 12) and B. *FSH-R* gene expression (Young, n = 9; Old, n = 10) in aspirated granulosa cells, demonstrating a 3-fold higher expression of aromatase in older compared with younger women with no difference in *FSH-R* expression. *, $P < 0.006$

and small antral follicles (44). Lower levels of AMH in serum in older women on the day of aspiration in the absence of a difference in FF AMH is therefore consistent with the known decrease in AFC in older women (1–3) rather than to decreased AMH production by the dominant follicle.

Discussion

Previous studies have clearly demonstrated that follicular phase serum E2 is paradoxically preserved or increased in older compared with younger regularly cycling women at a time when other biomarkers of ovarian reserve (INH-B, AMH, and AFC) show a marked decline. Preservation of estrogen with reproductive aging is accompanied by a decline in serum androgens and the increased ratio of estrogens to androgens is consistent with increased aromatase activity. While adrenal androgen production declines with age in men and women (45), the ovary is the predominant source of estrogen in premenopausal women, leading us to hypothesize that the selective preservation of E2 synthesis in older premenopausal women is due to increased ovarian aromatase activity. Results of the current study which demonstrated greater expression of aromatase in GCs isolated from dominant follicles in older women and preservation of serum and FF E2, E1, and FF aromatase product to substrate ratios (FF E2/T, E1/T) in older women, support this hypothesis.

While a large number of studies have examined changes in sex steroid levels in serum as a function of age in premenopausal women (6;9–20;46), no previous studies have measured sex steroids in FF or measured GC aromatase expression as a function of age in this demographic during naturally occurring cycles. Sex steroids and aromatase transcripts have been measured in FF sampled after the administration of hCG (in IVF cycles) in older and younger women (47–49). The inconsistent results from these studies likely represent the use of suboptimal methods to investigate the impact of aging on aromatase activity, as hCG, like the LH surge, induces a rapid decrease in aromatase transcription and transcript stability (27, 28).

Estradiol production by the ovary is a function not only of steroidogenic enzyme activity but also of androgenic substrate availability and follicle and GC number. There was no evidence of increased androgenic substrate in older women as a means to maintain E2 levels in the current studies; rather, older women had slightly lower serum T levels on the day of aspiration and FF T levels were similar between age groups. As older women are more likely to grow more than one dominant follicle (12), we confirmed the presence of a single dominant follicle by serial ultrasounds in all subjects. Studies in human GCs have demonstrated that aromatase transcription (43) and enzyme activity (50) first become detectable at a follicle size of 7–8 mm. Thus, it is theoretically possible that E2 production by a larger pool of nondominant follicles might compensate for compromised E2 production by the dominant follicle in older women. However, the dominant follicle produces approximately 8 times as much E2 as nondominant follicles aspirated from the same ovary (29), indicating that a sizeable increase in the pool of small follicles would be required to preserve E2. Furthermore, in the current studies we found no difference in the total number of nondominant follicles (>7 mm) between older and younger women.

An increase in GC number with aging might explain the maintenance of serum and FF E2 levels in older women. However, studies in women undergoing IVF cycles have demonstrated the opposite; older women (> 40 years) and women with decreased ovarian reserve (FSH > 10) have 33%–74% fewer luteinized GCs per follicle compared with younger women (< 35 years) and those with normal ovarian reserve (FSH < 6), respectively (22, 23). While we were unable to measure the total number of GCs per aspirated follicle in the current study, the above studies suggest that the preservation of E2 and E2/T in the older women in the current studies is achieved with significantly fewer GCs. While we did not separate cumulus from mural GCs, we can assume that the highly steroidogenic mural GCs (51) are the major contributor to increased aromatase expression in the current study. Aromatase activity is primarily regulated by changes at the level of transcription (52), and the current studies would thus predict that the greater number of aromatase transcripts in older women would be accompanied by increased aromatase activity. However, we cannot exclude the possibility of a discrepancy between transcript and protein levels due to the potential for accelerated mRNA decay with aging (reviewed in (53, 54).

Understanding the cellular mechanisms underlying the age-related increase in ovarian aromatase transcription will require further study, but several possibilities should be considered. FSH is the primary driver of ovarian aromatase expression (55). Demonstration of higher FSH levels in older compared with younger women in the EFP, but not later in the cycle, does not exclude the possibility that the age-related difference in aromatase activity in the dominant follicle is FSH-dependent. Exposure of GCs to higher FSH levels in the EFP may upregulate GC aromatase expression for the duration of dominant follicle maturation via epigenetic mechanisms. In vitro studies in GCs, for example, have demonstrated that FSH activates its target

genes in part through phosphorylation of histone H3 (56) and that there is an inverse correlation between the concentration of aromatase transcripts and methylation of the ovarian aromatase promoter (57). In previous studies in older and younger regularly cycling women, the estrogen response to exogenous FSH in the late follicular phase was maintained with aging when controlled for follicular number despite a presumed decrease in the number of GCs per follicle (24). Induction of epigenetic changes in aromatase in older women by higher endogenous EFP FSH may explain these findings. A second study that addressed the effect of age on the estrogen response to controlled FSH stimulation after pretreatment with a GnRH agonist concluded that the secretory capacity of the ovary is not impaired with aging and that increased FSH is therefore not necessary to maintain E2 secretion in older cycling women (58). However, even in this study, FSH levels remained higher in the older group, and thus these conclusions may be premature given our current understanding of epigenetic effects early in follicle development.

The current studies demonstrate that *FSH-R* expression in GCs of the dominant follicle does not increase with aging; however, it remains to be determined whether older women have greater *FSH-R* expression in the EFP when higher FSH levels would be expected to upregulate *FSH-R* expression (59). T, E2, and activin synergize with FSH downstream of the FSH-R to increase aromatase transcription in rat GCs (60, 61), while IGF-1 independently increases aromatase transcription in human luteinized GCs (62). In contrast, AMH (63, 64) and prolactin (65) inhibit FSH-induced aromatase transcription in GCs. In the current study, there were no differences in FF T, E2 or AMH levels between older and younger women. While all women had normal screening prolactin levels, prolactin was not measured in FF. FF activin and IGF-1 were not measured in the current study, however Klein et al reported lower IGF-1 and higher activin A levels in FF from luteinized GCs of older compared with younger, regularly cycling women (66). Reduced local IGF-1 action in older women would be expected to decrease rather than increase aromatase expression, and it is unlikely that activin plays a role in differential aromatase expression in the current studies as activin in human FF is completely bound and neutralized by follistatin (29).

In summary, ovarian steroidogenesis is selectively preserved with aging in regularly cycling women at a time when declining levels of AMH and early follicular phase INH-B indicate that the process of ovarian aging has already commenced. The current studies demonstrate that ovarian aromatase expression increases with age in regularly cycling women. We hypothesize that upregulation of GC aromatase activity in the face of the known decrease in GC number in the dominant follicle of older women accounts for the preservation of FF E2 levels with aging. Further studies of the epigenetic role of increased FSH early in follicle development on aromatase expression in the dominant follicle will provide important insights into the potential mechanisms responsible for aromatase upregulation in older women, as will studies of the synergistic and sustained role of lower levels of AMH.

Acknowledgments

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The authors have nothing to declare.

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Precis: *Enhanced ovarian aromatase expression in older compared with younger reproductive aged women may explain the selective preservation of serum estradiol levels with aging.*

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