AMERICAN JOURNAL OF HUMAN BIOLOGY 8:751–759 (1996)

Ovarian Function in the Latter Half of the Reproductive Lifespan

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ABSTRACT The relationship of age to four aspects of ovarian function was investigated: daily progesterone levels, pulsatile progesterone secretion, follicular and luteal estradiol levels, and preovulatory estradiol levels. Daily progesterone levels decrease after age 40. Pulsatile progesterone secretion remains approximately stable with age, though older women have somewhat higher late luteal activity. Daily follicular and luteal estradiol levels decrease with advancing age, but preovulatory peak estradiol remains stable. Some of these changes undoubtedly have negative effects on fecundity, such as lower follicular estradiol and average progesterone, via effects on endometrial development and support. But other changes identified, such as stability of preovulatory estradiol levels and thereby presumptive capacity to stimulate a luteinizing hormone (LH) surge despite lower follicular and luteal levels, as well as increased pulsatile progesterone secretion around the time of implantation, appear designed to conserve and maintain function. Thus, ovarian endocrine function over the course of reproductive life represents a process of change, but not one of generalized functional decline. Rather, aging with respect to ovarian endocrine function may proceed on a track, or on multiple tracks, which are largely separable from the continual depletion of oocyte stores which occurs over the lifetime.

The ovary is an organ with two related functions: manufacture, storage, and release of oocytes, and production of reproductive hormones, especially estradiol and progesterone. Oocytes with their enclosing follicles are formed during prenatal life, and a woman's supply of gametes is continuously, indeed exponentially (Block, 1952; Baker, 1963; Richardson et al., 1987), depleted over her lifetime, with ultimate exhaustion or near exhaustion leading to menopause. Ovarian endocrine function is closely tied to the cyclic, monthly process of follicular maturation and ovulation. Yet, as we will show here, hormone production as women age does not follow a trajectory paralleling that of declining gamete supply over the life course, which suggests that endocrine function is not linked to oocyte stores in a simple mechanistic fashion.

During the first portion, or follicular phase, of the menstrual cycle, developing follicles secrete increasing amounts of estradiol. After ovulation occurs at about midcycle, the remaining follicular cells form the corpus luteum, which secretes progesterone and estradiol during the latter portion, or luteal phase, of the cycle. Both estradiol and progesterone regulate their own production by exerting negative feedback on the stimulatory hormones of the hypothalamus and pituitary. Follicular estradiol promotes endometrial proliferation and affects the maturation and ultimate fertilizability of the oocyte (Yoshimura and Wallach, 1987; Kreiner et al., 1987; Maslar, 1988; Zelinski-Wooten et al., 1994). As titers become sufficiently high, estradiol ceases to suppress hypothalamic and pituitary action, and instead serves as a positive stimulus to the luteinizing hormone (LH) surge which in turn triggers ovulation (Knobil and Hotchkiss, 1988; Yen, 1991). The function of luteal estradiol is less well understood (Ghosh et al., 1994; Younis et al., 1994), although it acts synergistically

Received November 17, 1994; accepted January 27, 1996.

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with progesterone by promoting formation of progesterone receptors and may have additional effects on the maintenance of a functional endometrium (Fritz et al., 1987; Baulieu, 1990). Progesterone from the corpus luteum is the major hormone of the second half of the cycle. It serves to vascularize and maintain the integrity of the endometrium; progesterone is essential for the support of early pregnancy (Weitlauf, 1988). Luteal-phase progesterone is secreted in two modes by two different populations of luteal cells: tonically or continuously, and episodically or intermittently; episodic progesterone secretion is subject to stimulation by the LH, whereas tonic secretion is not (Filicori et al., 1984; Caruso et al., 1987; Veldhuis et al., 1988; Jones, 1990; Retamales et al., 1994).

If ovarian endocrine function need not change in tandem with oocyte stores, then how does it alter with advancing age? To what extent does it decline? By means of salivary steroid radioimmunoassay, age-related variation in four aspects of ovarian function was investigated: 1) daily progesterone levels, 2) pulsatile or episodic progesterone patterns, 3) follicular and luteal-phase estradiol levels, and 4) midcycle or preovulatory estradiol levels. Ovarian endocrine function changes as women get older, but only in some respects do the changes represent a decline in functional capacity; in other cases they do not. This paper reports on new findings in this area and reviews earlier ones with the overall aim of beginning to formulate a comprehensive picture of age-related change in ovarian endocrine function.

SUBJECTS AND METHODS

Details of subject selection and laboratory methods are reported elsewhere (Ellison et al., 1987; O'Rourke and Ellison, 1990, 1993; Lipson and Ellison, 1992; O'Rourke, 1992) and are only briefly summarized.

Subjects

All subjects were healthy, regularly-cycling women of stable weight who took no medications which could influence hormone levels and who engaged in vigorous exercise for less than 3 hours per week. Weight was between 80% and 125% ideal body weight (IBW; Metropolitan Life Insurance Company, 1983); average weight for all groups of women compared ranged between 93% and 100% IBW. Subjects were recruited from the Harvard University community and the Boston metropolitan area and were compensated for their participation. All studies were approved by the Harvard University Committee on the Use of Human Subjects in Research. The total number of subjects for the daily progesterone study was 124, 18–44 years; for pulsatile progesterone, 54, 18–48 years; for estradiol, 53, 25–48 years.

Collection and assay protocols

For studies of daily progesterone and estradiol, subjects collected saliva samples at home, using sugarless gum to stimulate salivation, into polystyrene tubes pretreated with sodium azide as a preservative. Samples were stored at ambient temperature during the month of collection. At the end of the month, samples were returned to the laboratory, where they were frozen at −20°C until assayed. For pulsatile progesterone studies, subjects came into the laboratory, where samples were collected every 15 minutes for 8 hours; all samples were collected during daytime hours. Samples were stored overnight at ambient temperature and frozen at −20°C the next day. All collection and storage protocols had been validated in earlier studies (Ellison, 1988; Lipson and Ellison, 1989).

Samples were extracted twice in diethyl ether and assayed for progesterone or estradiol using highly specific antisera (progesterone, no. 337; estradiol, no. 244; Dr. G. D. Niswender, Colorado State University; Korenman et al., 1974; Gibori et al., 1977) and a four-position tritiated competitor (Amersham-Searle, Arlington Heights, IL). Values for standard doses were fit to a log-logit curve according to the method of Rodbard (1978). Procedural losses were monitored by the addition of a small amount of tracer to each sample prior to extraction.

Pulsatile progesterone analysis

Progesterone peaks were identified using the PULSAR peak detection program of Merriam and Wachter (1982). This program first uses an iterative procedure to construct a series of baseline values over time, and then measures observed values against this baseline in units of an assay "noise factor." The noise factor was estimated from the variability of the standard curve, using an equation derived from a series of 40 standard curves, which took into account the fact that intraassay variation in observed values is a function

of dose. Single-point deviations were defined as peaks if they were elevated 1.96 SD above baseline, corresponding to the 0.025 level of significance (one tailed). For a series of points above baseline, less stringent criteria were applied (ranging from 1.65 SDs for two points in series to 0.68 SDs for five points), although in practice every peak identified contained at least one point exceeding 1.96 SDs.

An underlying assumption of analysis of pulsatile or episodic progesterone patterns is that these are related to functional changes in the corpus luteum as it grows, reaches maximum secretory capacity, and gradually degenerates. This assumption is borne out by investigations which show overall low pulsatile activity early in the luteal phase, increased activity around the central portion, and diminished activity as the next menses approach, at least for women in their 20s and early 30s (Filicori et al., 1984; Crowley et al., 1985; Soules et al., 1988; O'Rourke and Ellison, 1990).

In the present study, 15 subjects, 25–34 years, and 22 subjects, 35–48 years, also had daily progesterone profiles available for examination; pulsatile progesterone data presented here are based on these 37 subjects only. These daily profiles permitted determination of day of peak progesterone secretion for each woman; this marker was used in turn to estimate the functional stage of the corpus luteum. For these data, mid-luteal is defined as the period from day −2 to day +2 relative to day of peak secretion, and late luteal, as the period from day +3 to +7.

Several sample statistics and indices were defined and applied to each sample series:

1. Mean pulse frequency per hour: number of peaks/number of hours.
2. Mean peak amplitude (pmol/L): maximum deviation of each peak from baseline. Peaks in which the maximum deviation was adjacent to a break in the data stream (first or last point of the day, last point before or first point after a lunch break if one was taken) were not included in the calculation.
3. Mean progesterone (pmol/L): average of observed values over the sample series.
4. Observed baseline difference (pmol/L): Mean progesterone minus mean baseline (average of baseline values constructed by the PULSAR program). This represents a composite index of pulsatile activity.
5. Summed area under peaks: Calculated in arbitrary units by treating each peak as a triangle and summing the values for each sample series; incomplete peaks were included. It also represents a composite index of pulsatile activity.

Data analysis

Differences between groups were tested by 1- or 2-factor analysis of variance (ANOVA) or repeated-measures ANOVA. When appropriate, values were log-transformed prior to analysis to normalize distributions.

RESULTS

Daily progesterone levels

Women over 40 clearly have progesterone profiles which are suppressed relative to women in their late 20s or in their early or late 30s (Lipson and Ellison, 1992) (Fig. 1). Comparison of all groups by repeated-measures ANOVA using an autoregressive covariance structure indicates highly significant between-group differences ($P < 0.001$) (Lipson and Ellison, 1992). Other indices such as average luteal progesterone over the 16 days prior to menstrual onset and average mid-luteal progesterone (days −5 to −9 prior to menstrual onset) also showed significant differences between 40–44 and 25–29-year-old women (average luteal progesterone 205 ± 18 (SE) vs. 261 ± 14 pmol/L, $P < 0.05$; average mid-luteal progesterone 295 ± 26 vs. 380 ± 27 pmol/L, $P < 0.05$) (Lipson and Ellison, 1992).

Pulsatile progesterone

Table 1 shows the values for five statistics or indices in the mid- and late luteal phase for the two groups of women. Women 35–48 years have pulsatile profiles which are broadly comparable to those of women 25–34 years; these older women do not show any significant dimunition of capacity for pulsatile progesterone production. There are, however, some differences in the timing of maximal pulsatile activity between the two groups. In the mid-luteal, the older women have slightly, but not significantly, lower values, though the difference in average progesterone approaches significance ($P = 0.07$). In the late luteal, by contrast, the older women have significantly higher values for two indices: amplitude ($P = 0.02$) and summed area under peaks ($P = 0.05$).

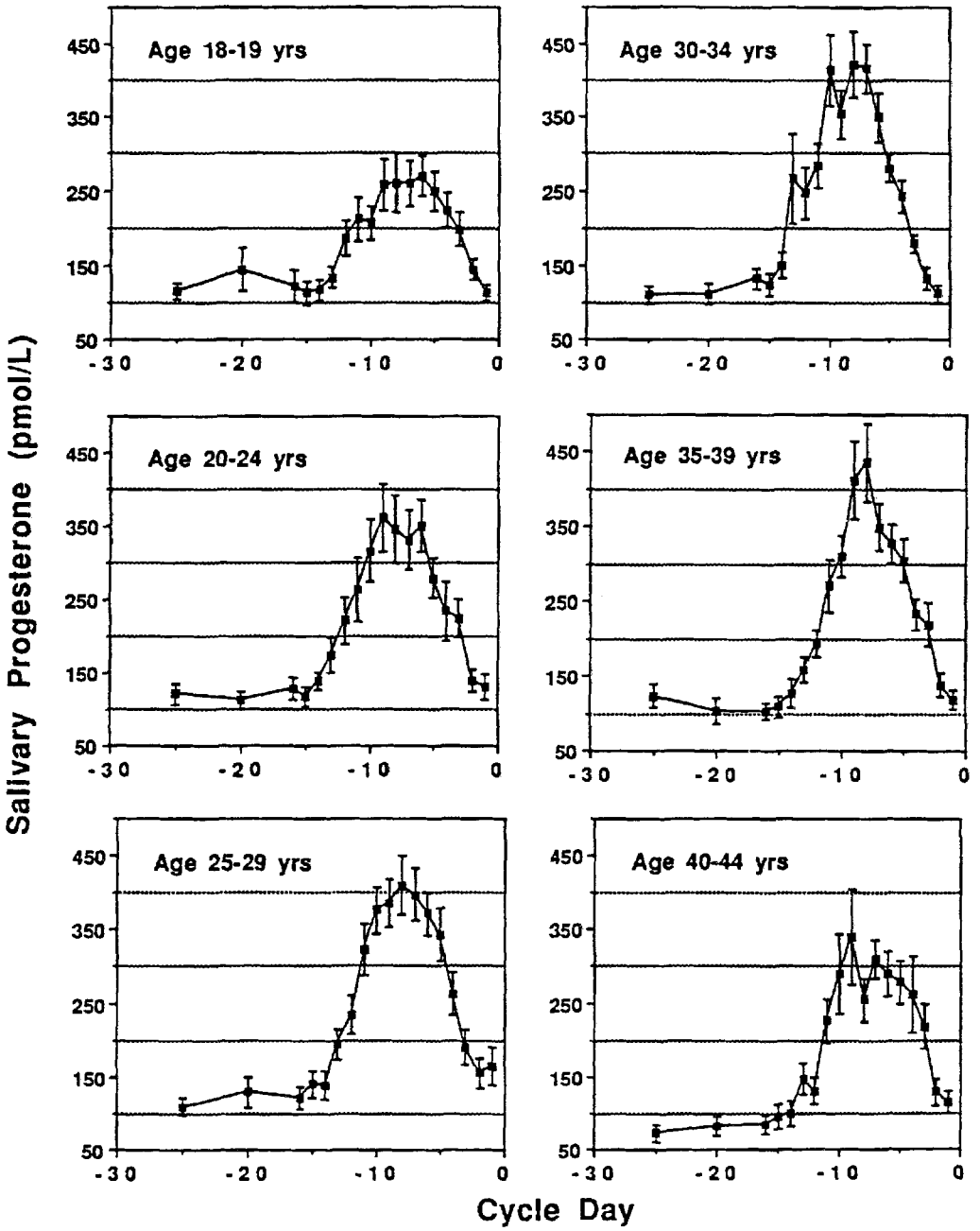

Fig. 1. Average profiles of salivary progesterone (±SE) for six age groups of women, aligned retrospectively on day of next menstrual onset. (Modified from Lipson and Ellison, 1992.)

TABLE 1. Arithmetic (SE) or geometric mean (range of mean ± 1 SE) for pulsatile progesterone sample statistics or indices, comparing women 25–34 and 35–48 years of age[1]

	Age 25–34		Age 35–48	
	ML	LL	ML	LL
FRQ	0.47 (0.11)	0.32 (0.04)	0.32 (0.06)	0.31 (0.07)
AMP	134 (118–152)	99 (86–114)	132 (112–156)	169 (147–193)
AREA	667 (435–1,023)	285 (198–411)	420 (299–590)	863 (600–1,242)
OBS-BSL	25.8 (7.0)	14.8 (6.1)	15.4 (5.0)	28.3 (9.9)
AVE P	381 (45)	256 (40)	285 (25)	273 (42)

[1]FRQ = frequency; AMP = amplitude; AREA = summed area under peaks; OBS-BSL = observed baseline difference; AVE P = mean progesterone; ML = mid-luteal; LL = late luteal.

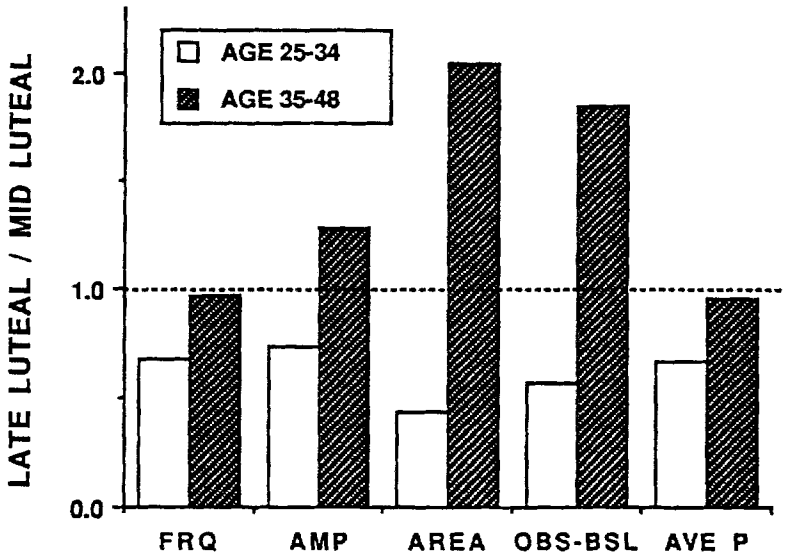

Fig. 2. Comparison of pulsatile progesterone sample statistics or indices between the mid- and late luteal phases within each of two age groups of women. For each statistic or index, the value shown is late luteal as a proportion of mid-luteal. See Table 1 for key.

This difference is more evident from comparison of phase differences within each age group of women. This is illustrated in Figure 2, which shows the late luteal value for each statistic or index as a proportion of the mid-luteal value. For women 25–34 years, all ratios are less than 1, indicating a lower level of pulsatile activity as the luteal phase approaches its end. For women 35–48 years, however, values in the late luteal are equivalent to, or higher than, mid-luteal values. Thus, the subjects do not show a uniform loss of capacity to secrete progesterone episodically, but rather concentrate secretion predominantly in the latter portion of the luteal phase, at which point average progesterone levels are about equal to those of the 25–34-year-old women. This further suggests that late luteal tonic secretion may be suppressed in older women because they appear to require greater pulsatile production, particularly peaks of higher amplitude, than younger women in order to achieve comparable average progesterone levels.

Follicular and luteal estradiol

Average daily estradiol profiles for women 25–48 years, aligned on day of peak estradiol level, are illustrated in Figure 3 (O'Rourke and Ellison, 1993). Data for women 35–39, 40–44, and 45–48 years are combined; average profiles for the three groups do not differ significantly. During both the follicular and luteal phases, women 25–29 years have the

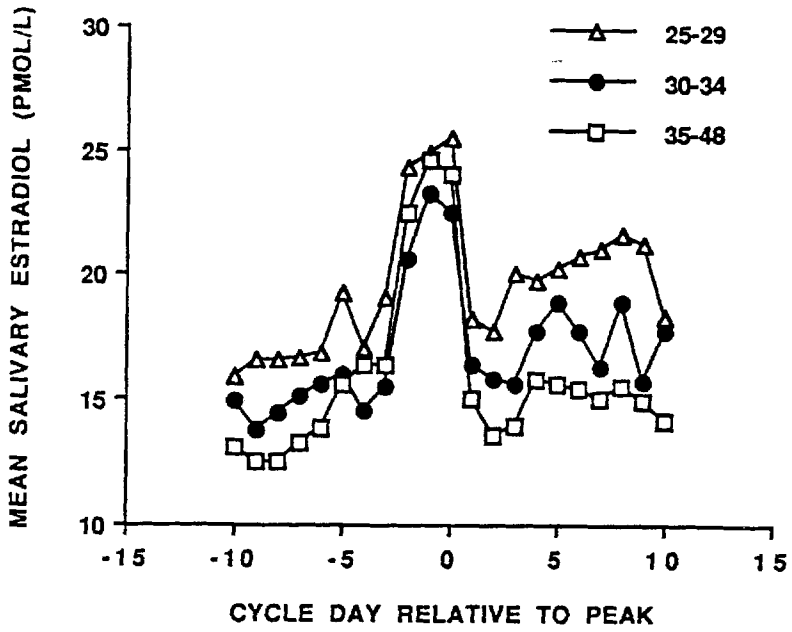

Fig. 3. Mean profiles of salivary estradiol for three age groups of women, aligned on day of estradiol peak. (Modified from O'Rourke and Ellison, 1993.)

highest estradiol levels; women in the early 30s are intermediate; and the lowest average levels are observed in women over 35 years (for interval days −10 to −5 relative to peak, means [SE] are 17.0 pmol/L [0.7] for 25–29 years; 15.0 [0.7] for 30–34 years; 13.5 [0.3] 35–48 years, $P = 0.04$; for interval days +1 to +10, respective means are 19.8 [0.6], 17.1 [0.5], 14.9 [0.3], $P = 0.01$) (O'Rourke and Ellison, 1993). Thus changes in average follicular and luteal estradiol appear to begin earlier, by the second half of the 30s, than changes in average luteal progesterone, which are not evident until after 40 years.

Preovulatory estradiol

Estradiol levels at the preovulatory peak, which show no difference by age, are also illustrated in Figure 3 (for interval days −4 to 0 relative to peak, age 25–29, means [SE] are 22.2 [1.2] for 25–29 years, 19.3 [1.0] for 30–34 years, 20.8 [0.6] for 35–48 years, $P = 0.55$) (O'Rourke and Ellison, 1993). Despite lower follicular baseline estradiol levels, older women achieve peak estradiol levels comparable to those of younger women in the same amount of time. Consequently, the rate of increase in production during the follicular phase must be more rapid in the older women.

DISCUSSION

To summarize the four aspects of ovarian function investigated: daily progesterone levels decrease after 40 years, but pulsatile progesterone secretion remains approximately stable, with some evidence for increased activity in the late luteal phase in older women; daily follicular and luteal estradiol levels also decrease with age, but midcycle peak estradiol levels do not change. Thus, in only some respects do the age-related changes represent functional decline. Equally notable is the extent to which some of these aspects of ovarian endocrine function are conserved into late reproductive life.

Considerable evidence, largely derived from studies of assisted reproduction, has established beyond question that fecundity decreases as women age (Albrecht et al., 1982; DeCherney and Berkowitz, 1982; Federation CECOS et al., 1982; Virro and Shewchuk, 1984; Padilla and Garcia, 1989; Edvinsson et al., 1990; Sauer et al., 1990; Levran et al., 1991; Navot et al., 1991; Pearlstone et al., 1992; FIVNAT, 1993; Meldrum, 1993). Changes in ovarian endocrine function documented here are consistent with observations of lower fecundity in older women. For example, lower follicular estra-

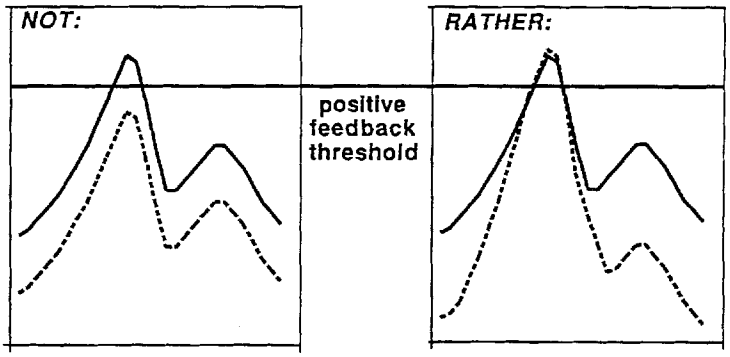

Fig. 4. Schematic representation of change in the shape of the estradiol profile with age, contrasting younger women (solid line) with older (dashed line). Average peak estradiol levels are stable with age despite differences in follicular and luteal levels.

diol may reduce fecundity by means of negative effects on endometrial proliferation and on the maturation and fertilizability of the oocyte (Yoshimura and Wallach, 1987; Kreiner et al., 1987; Maslar, 1988; Zelinski-Wooten et al., 1994). Recent clinical research supports the idea that a thinner endometrial layer is associated with lower pregnancy rates in assisted reproduction procedures (Shoham et al., 1991; Dickey et al., 1993; Abdalla et al., 1994). Similarly, lower progesterone may be associated with inadequate endometrial vascularization or support of early pregnancy (Lenton et al., 1982, 1988a). These effects in their turn could be expected to lead to longer interbirth intervals as women get older.

Lower follicular estradiol levels in older women may be related to the continuously decreasing size of the oocyte pool. As the total pool size declines, so should the size of the cohort of follicles that enters into the final stage of maturation each month (Koering, 1983; Gosden, 1985). Lower estradiol early in the follicular phase could, in part, reflect the smaller number of follicles contributing to total production. On the other hand, the predominant source of follicular estradiol is generally considered to be the dominant follicle, which becomes the ovulatory follicle (Hodgen, 1981; Findlay, 1986) so that the impact of follicular cohort size is uncertain.

In any case, what seems to occur is not a simple down-regulation of follicular estradiol production, which could have profound negative effects on fecundity if it prevented attainment of estradiol levels sufficient to trigger the positive feedback signal leading to the LH surge and ovulation, as illustrated schematically in Figure 4. The consistency of average peak estradiol levels across age groups suggests that this positive feedback threshold does not vary with age. The change in the shape of the estradiol curve may represent a positive adjustment or compensatory mechanism which tends to maintain fecundity, or slow its reduction, by sustaining the capacity to ovulate. The higher rate of increase in follicular estradiol production observed in older women may itself be driven by increased levels of a pituitary hormone, follicle-stimulating hormone (FSH). FSH promotes estradiol synthesis by stimulating production of enzymes necessary to convert androgens into estrogens (McNatty et al., 1979; Yen, 1990), and FSH levels are known to increase in women well before menopause (Gosden, 1985; Utian, 1987; Lenton et al., 1988b). FSH secretion and action are in turn regulated in negative feedback fashion by the hormone inhibin, produced in the follicle (Yen, 1991; Findlay, 1986). It has been suggested that reduced negative feedback secondary to a smaller cohort of maturing follicles is the cause of higher FSH in older women (Gosden, 1985). Quite recent evidence, however, indicates that reduction in the capacity of follicular cells to produce inhibin may be more important than cohort size (Pellicer et al., 1994).

The sources of lower luteal estradiol levels in older women have not been as well investigated, probably because the importance of luteal estradiol to implantation and maintenance of early pregnancy has not been firmly established (Nakamura et al., 1993; Ghosh et al., 1994; Lewin et al., 1994; Younis et al., 1994). One possibility is that older women

may ovulate from smaller follicles and develop smaller corpora lutea than younger women (Ahmed Ebbiary et al., 1994). But regardless of source, lower luteal estradiol levels may provide a mechanism whereby pulsatile progesterone secretion is maintained in older women in the second half of the luteal phase. Although both progesterone and estradiol serve to inhibit LH production, estradiol is the more potent suppressor (deZiegler et al., 1992). Low luteal estradiol, then, may allow LH levels to rise, which in their turn stimulate the luteal cells responsible for episodic progesterone production. Although the full functional capacity of the corpus luteum seems slower to develop in the older women that we studied, they do catch up to or even surpass younger women about mid-way through the luteal phase. Maintenance of adequate progesterone levels would be especially critical at this stage, since this is about the time that implantation begins.

While some of the changes in ovarian endocrine function in late reproductive life undoubtedly have negative effects on fecundity, others, including potential compensatory mechanisms, appear designed to conserve and maintain function. Thus, it is not accurate to characterize this process as a generalized functional decline. Rather, it may be the case that aging with respect to ovarian endocrine function proceeds on a track, or on multiple tracks, which are largely separable from the depletion of oocyte stores.

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