

ARTICLE

# Gonadotrophin dynamics during reproductive life

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## KEYWORDS

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## ABSTRACT

**Objective:** To evaluate the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in early follicular phase throughout the reproductive years. **Method:** FSH and LH concentrations were determined by radioimmunoassay (RIA). Linear and polynomial regressions were carried out considering basal FSH as the dependent and age as the independent variable. **Results:** FSH levels increased throughout the reproductive years ( $P<0.025$ ). A positive correlation between age and basal FSH levels was detected ( $P<0.05$ ). The Pearson squared coefficient of  $r^2=0.889$  was obtained. Using polynomial regression, the inclination of the parabole ( $Y=7.97-0.009x+0.057x^2$ ) was 0.359 and the generalized correlation coefficient was  $r=0.795$ . The goodness of fit analysis showed that the parabole may better represent the phenomenon ( $F=4.7$ ;  $P<0.05$ ). The LH levels remained constant, increasing only beyond 40 years of age. **Conclusion:** The FSH levels rose in a nonlinear way during the reproductive life and the LH concentrations increased discreetly only in patients over 40 years of age.

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## 1. Introduction

After the 20th week of intrauterine life, the number of follicles decreases gradually until menopause, when there will be only a few hundred of them. The speed of follicular attrition

is not the same at any time of life, occurring a new acceleration 7–10 years before menopause. In women at age of 50 years with regular menses, each ovary would still contain 2500–4000 follicles [1].

The complex functions of the hypothalamic–pituitary–ovarian axis result in variable levels of gonadotrophin, according to the phase of the menstrual cycle. Follicle-stimulating hormone (FSH) levels are related to the ovarian production

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of steroids and inhibin by a group of follicles that becomes sensitive to pituitary gonadotrophins in each cycle. The basal FSH levels, and in a lower scale of luteinizing hormone (LH), increase with age, reflecting a continuous decrease in ovarian reserve [2]. This fact is so relevant that an increase in FSH might mark the beginning of reproductive aging [3]. It is presumed that the monotropic FSH rise at the end of menopause indicates the beginning of accelerated follicular attrition. Thus, the serum FSH level is accepted as a sensitive biochemical marker of ovarian reserve [4]. In clinical setting, FSH is used to predict fertilization and pregnancy capability or indicate higher risk of recurrent fetal loss [5]. Even though FSH is employed as indicative of ovarian reserve, differences in the levels of this gonadotrophin in normal women at different ages should be established. Currently, there is no definition in which concentration FSH should be considered normal in fertile population at all ages. The proposal of this preliminary study was to verify the patterns of FSH and LH between the 3rd and 5th days of the menstrual cycle in unselected healthy women throughout the reproductive life.

## 2. Materials and methods

The study includes 246 healthy women, age range between 13 and 52 years. The subjects eligible for the study included women who reported still having regular periods between 26 and 34 days. Those who referred abnormal menstrual cycle, infertility, recurrent miscarriage, or have been on medication that could interfere with gonadotrophin levels in the last 3 months were excluded. Twenty milliliters of blood was drawn by venous puncture after fasting of 8–12 h, between the 3rd and 5th day of the menstrual cycle. Blood samples remained at room temperature for 60 min. After obtaining stable clot, the tubes were spun at 1500×g for 15 min, the supernatant was aspirated, transferred to a polypropylene tube, and stored at –20 °C. The study was approved by the Ethics Committee of the Julio Muller University Hospital and included only women who provided a written informed consent.

FSH concentrations were determined in duplicate by radioimmunoassay (RIA) calibrated against the Second International Reference Preparation (2nd IRP, 78/549) using reagents supplied by the Diagnostic Products Corporation (DPC, Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation in concentrations ranging from 1.6 to 4.6 mUI/ml were 3.4% and 6.2%,

respectively. According to the manufacturer the sensitivity of this assay is 0.06 mUI/ml. LH was also measured in duplicate by radioimmunoassay, calibrated against the First International Reference Preparation (1st IRP, 68/40), and supplied by the Diagnostic Production Corporation. The sensitivity of this assay is 0.15 mUI/ml and the intra-assay and inter-assay coefficients of variation were 2.9% and 7.6% in concentrations ranging from 2.1 to 9.3 mUI/ml.

FSH and LH concentrations are given as mean and standard deviation (SD). Comparison between the mean levels of gonadotrophin in individuals under 14 and over 50 was made by the one-sided *t*-test for nonpaired samples. Proportions between LH and FSH in subjects over 35 were compared by the *Z*-test. After 35 years of age, FSH and LH levels, distributed in 5-year age bands, were evaluated by the  $\chi^2_{TR}$  trend test. Correlation between the levels of FSH or LH and age was examined initially by linear regression. Correlation by polynomial regression ( $y=a+bx+cx^2$ ) was also estimated using a generalized correlation coefficient ( $r^2=\frac{\sum (y-\hat{y})^2}{\sum (y-y)^2}$ ) as the ratio between the explained and total variation. The assumption that the parabola offers a better representation of the phenomenon than the straight line, to analyze the goodness of fit, was verified by analysis of variance. The hypothesis that the parabola's inclination could show a better correlation between both tested variables was examined by Student's *t*-test. Values of  $P<0.05$  were considered significant.

## 3. Results

All women had serum FSH measured between the 3rd and 5th days of the menstrual cycle; in 168 of them, the levels of LH were also estimated. Means

**Table 1** Distribution of FSH and LH levels between the 3rd and 5th day of the menstrual cycle, according to age bands<sup>a</sup>

Age	FSH		LH	
	<i>n</i>	$\bar{x} \pm DP$	<i>n</i>	$\bar{x} \pm DP$
≤14	7	8.25±1.90	7	5.24±1.81
15–19	17	10.29±2.39	13	8.71±3.18
20–24	23	11.50±2.57	22	6.71±3.75
25–29	26	12.67±2.42	20	6.81±3.11
30–34	44	13.31±3.94	33	8.66±4.50
35–39	41	14.93±6.09	30	7.70±3.47
40–44	50	17.16±6.04	23	10.05±4.57
45–49	34	22.02±10.09	18	7.57±3.45
≥50	4	21.45±2.16	2	11.60±1.62
Total	246		168	

<sup>a</sup> Some women had only FSH measured.

**Table 2** Proportion of women beyond 35 years with high gonadotrophins, distributed in 5-year-old band\*

Age	FSH			LH		
	<15 mUI/ml, n (%)	>15 mUI/m, n (%)	Total n	<15 mUI/ml, n (%)	>15 mUI/m, n (%)	Total n
35–39	28 (68.3) <sup>a</sup>	13 (31.7)	41	29 (96.7)	1 (4.3)	30
40–44	19 (38.0)	31 (62.0)	50	23 (100.0)	0 (0.0)	23
45–49	09 (26.5)	25 (73.5)	34	17 (94.4)	1 (5.6)	18
≥50	00 (0.0)	04 (100.0)	04	00 (0.0)	2 (100.0)	2
Total	56 (43.4)	73 (56.6)	129	69 (94.5)	4 (5.5)	73

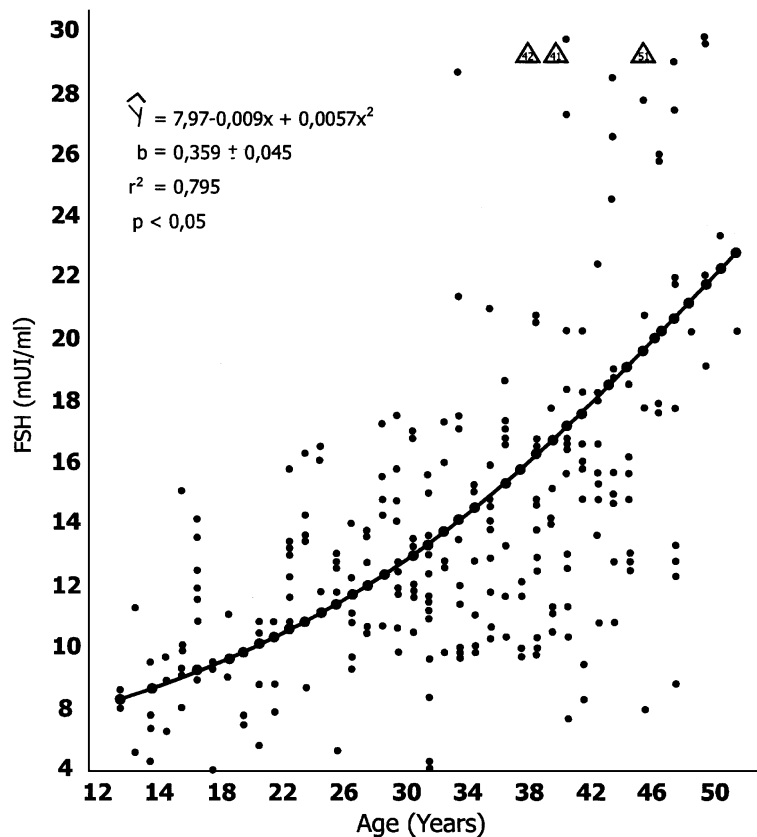
<sup>a</sup> Numbers among parentheses represent proportions for each group.

\*  $P < 0.05$  for proportions of women above 35 years, when LH and FSH >15 or <15 mUI/ml; Z-test.

and standard deviations of the gonadotrophin levels, distributed in 5-year age bands, are shown on Table 1. From the lower to the higher age bands, the mean levels of FSH increased from 8.2 to 22.0 mUI/ml, an increase of 2.6 times ( $P < 0.025$ ). The elevation of FSH levels was not constant during reproductive life; aging as a factor of faster FSH elevation was seen in women after 35–40 years. FSH levels higher than 15 mUI/ml were observed in 56.6% of 129 women over 35 years old (Table 2), and FSH levels  $\geq 10$  mUI/ml were found in 91% of them (data not shown).

A positive correlation between chronological age and the levels of FSH was detected. The increase in FSH was not a constant phenomenon, being accel-

erated after 35 years of age (Fig. 1). This positive correlation evaluated by polynomial regression was statistically significant ( $P < 0.05$ ) and the parabole's inclination was  $b = 0.359$ , IC 95% 0.315–0.404 ( $t_{39} = 5.26$ ;  $P < 0.05$ ). The generalized correlation coefficient, determined by the ratio between the explained and total variation, was  $r = 0.795$ . A Pearson squared coefficient  $r^2 = 0.889$  was obtained by simple linear regression, correlating age and FSH levels ( $F = 99.2$ ;  $P < 0.05$ ). In fact, goodness of fit analysis showed that the parabole better represents the phenomenon than the straight line ( $F = 4.7$ ;  $P < 0.05$ ). LH concentrations remained constant throughout the reproductive years, increasing from 5.2 mUI/ml in the 14-year-old band to 11.6



**Figure 1** Correlation between chronological age and serum levels of FSH. The symbol  $\Delta$  identifies possible outliers and their values are given inside.

mUI/ml in women over 50 (Table 1;  $P < 0.025$ ). LH levels  $\geq 15$  mUI/ml were detected in only 5.5% of women between the ages of 35 and 50 (Table 2). About 27.4% of those over 35 had LH levels higher than 10 mUI/ml. The levels of LH showed no correlation with age.

#### 4. Discussion

Depletion in ovarian follicular population occurs from the 20th week of the intrauterine life until menopause, either by atresia or ovulation. The speed of follicular loss, modulated by endocrine, paracrine, and intracrine mechanisms, is not the same at any stage of life. Later in life, the higher rate of follicular consumption has been associated with monotropic elevation of FSH [6]. In fact, increase in FSH, dissociated from LH, was noticed almost three decades ago [7]. Currently, FSH measurement in the early follicular phase has been used to estimate the ovarian reserve, especially in women over 35 years of age [8].

The present study showed that FSH levels increase very early, even in women under 20 years old. While the number of ovulatory cycles could not be ascertained, it should consider that the much lower incidence of ovulatory cycles in women under the age of 20 could have an important influence on the relative FSH concentrations in this group of women. When women under 15 were compared with those over 45, FSH levels were two to three times higher ( $8.2 \pm 1.9$  vs.  $22.0 \pm 10.0$  mUI/ml) in the last group. These findings are consistent to those reported by Hansen et al. [9] using an immunoradiometric assay calibrated against the same FSH standard, the 2nd International Reference Preparation 78/549, World Health Organization (WHO). Cramer et al. [10], using the same FSH assay employed by Hansen et al. [9], also calibrated against the WHO Standard Preparation 78/549, found mean FSH levels of 10 mUI/ml between the ages of 26 and 35. At this age range, the FSH levels were 29% higher with the radioimmunoassay employed in the current study. In the 40–45 age band, the mean FSH levels of 17 mUI/ml seen by us were comparable to those detected by Cramer et al. [10] who found FSH levels of 16 mUI/ml in women around 45 years old. Using RIA, the levels of FSH were again higher at this age in the present study; however, Seth et al. [11] showed that immunoradiometric assays for FSH give results on average 17% higher than radioimmunoassays.

FSH levels higher than 10 [12] or 15 mUI/ml [13] in the early follicular phase have been associated with poor reproductive results. Concerning these

studies, Pearlstone et al. used the Amerlex RIA standardized in terms of the WHO 2nd IRP (78/549) and Scott et al. employed the Leeco RIA also calibrated against the 2nd IRP WHO (78/549). In general, in several institutions, correlations between the different radioimmunoassays on FSH measurement have been high [14]. In the present study, 15% of women under age 35 (data not shown) and 56% of those over 35 had levels of FSH higher than 15 mUI/ml. In addition, a positive correlation between age and FSH levels was confirmed in this study ( $r^2 = 0.795$ ;  $P < 0.05$ ). The accelerated rise in FSH concentrations seen after 35–40 years of age seemed to be an exponential phenomenon. Although the polynomial regression had better adjustment than the straight line, this method may not be the best for graphical representation of the FSH increment yet. This study is limited to examining the dynamics of FSH in reproductive life and did not analyze the mechanisms involved in FSH elevation toward the end of reproductive stage. Also, it has the limitation of not having considered in details the menstrual cycle's characteristics and not excluding ovarian surgery or smoking, conditions potentially associated with rapid follicular consumption [15,16]. The LH did not increase significantly before the age of 40, and this observation is in agreement with previous report [17].

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