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Correlation between the Serum Luteinizing Hormone to Follicle-stimulating Hormone Ratio and the Anti-Müllerian Hormone Levels in Normo-ovulatory Women

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Serum anti-Müllerian hormone (AMH) levels are regarded as an age-specific marker for predicting the ovarian reserve in women of reproductive age. Some studies have shown that the luteinizing hormone (LH)/follicle stimulating hormone (FSH) ratio can be used as a predictor of ovarian reserve. The purpose of this study was to assess the variation of LH/FSH ratio with aging and to evaluate the correlation between serum LH/FSH ratio and AMH levels as a predictor of the ovarian reserve in normo-ovulatory women. We retrospectively analyzed the day 3 serum hormone levels in 1,251 patients (age range: 20-50 yr) between January 2010 and January 2011. We divided the patients into 6 groups according to their age. Relation between serum AMH level and LH/FSH ratio was analyzed statistically. The serum AMH level was inversely correlated with age (r = -0.400, P < 0.001). A significant negative correlation was found between serum LH/FSH ratio and age (r = -0.213, P < 0.001). There was a significant partial correlation between serum LH/FSH ratio and AMH level when adjusted by age (r = 0.348, P < 0.001). The LH/FSH ratio could be considered as a useful marker for the ovarian reserve and could be applied to the clinical evaluation with AMH.

Keywords: Anti-Müllerian Hormone; LH/FSH Ratio; Regular Menstruation; Ovarian Reserve

INTRODUCTION

An evaluation of the ovarian reserve is essential for the infertility work up and treatment. Advanced female age is associated with diminished ovarian reserve, but the age by itself does not accurately predict the ovarian reserve (1). The commonly used tests for ovarian reserve are basal follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), inhibin B levels and antral follicle count (AFC) by ultrasonography. However, these values are influenced by the menstrual cycle, i.e., intra- and inter-cycle variation and month-by-month variation and therefore are limited usable for the prediction of poor and high responders (2). Ultrasonographic markers, such as AFC and ovarian volumes have also been shown to be affected by observer variation (3-6). Moreover, serologic test and ultrasonographic evaluation should be measured in the early menstrual period (3-6).

The serum anti-Müllerian hormone (AMH) has recently emerged as a novel clinical marker of ovarian reserve. AMH is expressed in the growing pre-antral or small antral follicles in the ovary and reflects the recruited ovarian follicular pool. The advantages of AMH test are its little inter- and intra-cycle variability and gradually decreased value according to the increasing age. Serum AMH levels decrease steadily with ageing and are undetectable after menopause. Yoo et al. reported the age-specific reference values of AMH for Korean women with normal menstruation patterns between 20 and 50 yr of age (7). Some studies presented that the serum AMH levels are age-specific and readily available clinical marker of the ovarian reserve. Therefore, AMH is regarded as a useful ovarian reserve test nowadays (7-12).

Despite the several limitations of conventional tests, physicians usually check the serum basal FSH, LH, E2, and AMH for the evaluation of infertility at the same time during menstruation. Recently, some researchers concerned over the serum LH/FSH ratio in case of polycystic ovary syndrome (PCOS) patients (13). They showed that patients with polycystic ovaries have statistically significant higher LH levels and LH/FSH ratios than patients with normal ovaries (13). And some studies suggested that the LH/FSH ratio could be used as a marker of fertility or ovarian reserve (14-19). However, only serum FSH and AMH levels have been still focused and regarded as useful markers to predict the ovarian reserve in women of reproductive age. As the serum FSH levels are slightly elevated in the late period of reproduction and in poor responders, we could not precisely predict the decreased ovarian reserve in young women through

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serum FSH only. When we would evaluate the change of serum LH/FSH ratio, could we have more information? In theory, the LH/FSH ratio may decrease as the ovarian reserve begins to fall off, because FSH is known to increase more significantly than LH as the ovarian reserve declines. Therefore, we focused on the variation of LH/FSH ratio as a predictor of ovarian reserve.

The purpose of the present study was to evaluate age-related reference values for the LH/FSH ratio in normo-ovulatory women of reproductive age and was to assess the correlation between serum LH/FSH ratio and AMH levels. For these, we analyzed the change of LH/FSH ratio across a Korean female population of different ages with regular menstrual cycle.

MATERIALS AND METHODS

Study design

We retrospectively analyzed day 3 LH, FSH, AMH levels in 1,251 patients (age range 20-50 yr) between January 2010 and January 2011 in Dongguk University Ilsan Hospital and Cheil General Hospital. This study population included a total of 1,251 women with regular menstrual cycles (interval: 21-45 days) undergoing in vitro fertilization (IVF). Exclusion criteria were as follows: 1) history of uterus or adnexal surgery; 2) polycystic ovarian syndrome (PCOS); 3) body mass index (BMI) ≥ 30 kg/m^2; 4) serum prolactin levels > 50 ng/mL; 5) irregular menstruation (interval: less than 21 days, more than 45 days); 6) other endocrine diseases (thyroid disease, diabetes mellitus, Cushing's syndrome). Because serum AMH level is age-specific marker for the ovarian reserve, the patients were classified into 6 groups according to their age (Group 1 = under 31 yr [n = 394]; Group 2 = 32-34 yr [n = 301]; Group 3 = 35-37 yr [n = 259]; Group 4 = 38-40 yr [n = 173]; Group 5 = 41-43 yr [n = 85]; Group 6 = over 44 yr [n = 39]).

Measurements

Blood samples for AMH, LH, FSH, TSH and prolactin were obtained by venipuncture on day 2-3 of the menstrual period. The serum AMH levels were measured by enzyme immunoassay using an AMH/MIS EIA kit, two–immunological step sandwich type assay (Immunotech version; Beckman Coulter, Marseille, France).

Statistical analysis

All data analyses were performed using the Statistical Package for the Social Sciences software (version 19.0, IBM SPSS, Armonk, NY, USA). Comparisons of AMH, LH/FSH ratio, BMI, menstruation interval, TSH and prolactin between age groups were performed using one-way analysis of variance (ANOVA). The partial correlation between AMH and LH/FSH ratio was analyzed after adjustment of age. Variables were presented as mean ± S.D. $P < 0.05$ was considered statistically significant.

Ethics statement

The review board for human research of Dongguk University Ilsan Hospital approved this study (IRB No. 2013-116). The data used for this study do not include any identifiable personal information. As such, informed consent was waived by the board.

RESULTS

The mean level for serum FSH was 8.87 ± 5.95 mIU/mL, for LH/FSH ratio 0.79 ± 0.51 and for AMH 3.53 ± 3.02 ng/mL. The basal characteristics and the mean levels of serum AMH, LH/FSH ratio according to the age groups are presented in Table 1. BMI and the basal FSH levels were significantly increased according to age, however, the serum LH levels are increased at the late reproductive age group (≥ 44 yr) only. Menstrual interval, the serum AMH levels and the LH/FSH ratio were significantly decreased in patients with advanced age.

Fig. 1 shows the distribution of AMH, FSH, and LH/FSH ratio according to age. A significant negative correlation was demonstrated between the serum LH/FSH ratio and age (r = -0.213,

Table 1. Basal characteristics of patients according to age groups

Parameters (n = 1,251)	Age (yr)					
	Group1	Group 2	Group 3	Group 4	Group 5	Group 6
Range of age (yr)	≤ 31	32-34	35-37	38-40	41-43	≥ 44
No.	394	301	259	173	85	39
BMI (kg/m^2)	20.2 ± 2.4	20.5 ± 2.7	21.0 ± 2.5*	21.1 ± 2.6*	21.4 ± 2.6*	22.4 ± 3.2*,†,‡
Mense interval (d)	29.7 ± 3.0	29.2 ± 2.5	28.9 ± 2.8*	28.4 ± 2.2*,†	28.0 ± 2.2*,†	28.0 ± 2.0*
AMH (ng/mL)	4.7 ± 3.4	4.1 ± 3.1*	3.2 ± 2.4*,†	2.0 ± 1.8*,†,‡	1.4 ± 1.2*,†,‡	0.8 ± 0.8*,†,‡
Basal FSH (mIU/mL)	7.7 ± 6.2	7.8 ± 2.9	8.9 ± 4.7	10.2 ± 5.6*,†	12.0 ± 8.7*,†,‡	16.0 ± 11.6*,†,‡,§,¶
Basal LH (mIU/mL)	6.3 ± 3.4	5.8 ± 2.7	5.8 ± 2.8	6.5 ± 7.5	6.4 ± 4.6	9.2 ± 9.1*
LH/FSH ratio	0.95 ± 0.60	0.81 ± 0.44	0.74 ± 0.36*	0.68 ± 0.62*	0.58 ± 0.24*,†	0.62 ± 0.52*
TSH (μU/mL)	2.3 ± 1.3	2.2 ± 1.3	2.4 ± 1.4	2.3 ± 1.2	2.2 ± 1.4	2.4 ± 1.7
Prolactin (ng/mL)	14.8 ± 7.2	13.9 ± 7.0	14.5 ± 6.9	14.8 ± 8.1	12.5 ± 6.4	13.0 ± 8.5

The values are expressed as mean ± S.D. *,†,‡,§,¶One-way analysis of variance (ANOVA). *$P < 0.05$ compared to group 1; †$P < 0.05$ compared to group 2; ‡$P < 0.05$ compared to group 3; §$P < 0.05$ compared to group 4; ¶$P < 0.05$ compared to group 5. BMI, body mass index; AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; TSH, thyroid stimulating hormone.

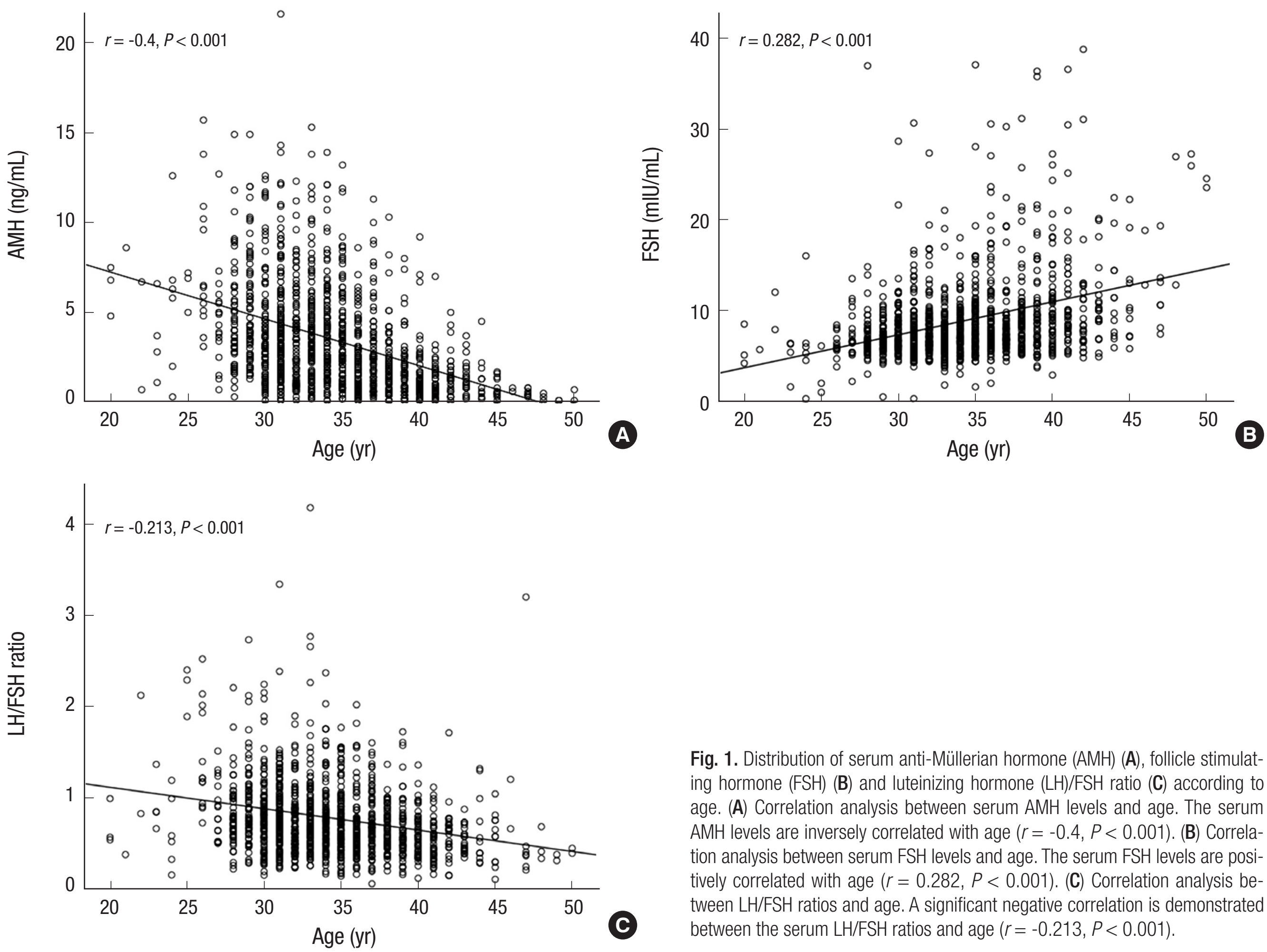

Fig. 1. Distribution of serum anti-Müllerian hormone (AMH) (**A**), follicle stimulating hormone (FSH) (**B**) and luteinizing hormone (LH)/FSH ratio (**C**) according to age. (**A**) Correlation analysis between serum AMH levels and age. The serum AMH levels are inversely correlated with age (r = -0.4, P < 0.001). (**B**) Correlation analysis between serum FSH levels and age. The serum FSH levels are positively correlated with age (r = 0.282, P < 0.001). (**C**) Correlation analysis between LH/FSH ratios and age. A significant negative correlation is demonstrated between the serum LH/FSH ratios and age (r = -0.213, P < 0.001).

P < 0.001). The age was also correlated with the serum AMH level (r = -0.4, P < 0.001) and serum FSH (r = 0.282, P < 0.001). Other variables, such as serum LH (r = 0.064, P = 0.024), BMI (r = 0.198, P < 0.001) and menstrual interval (r = -0.182, P < 0.001) showed weak correlation with age also (data not shown).

In accordance to our hypothesis, there was a significant partial correlation between the serum LH/FSH ratio and the AMH when adjusted by age (r = 0.348, P < 0.001).

DISCUSSION

FSH and LH levels are commonly used for the prediction of the ovarian reserve in the early follicular phase even though they have a lower predictive value. Recently, there was a report that elevated day 3 FSH/LH ratio ≥ 2 is associated with higher rates of cancellation in In Vitro Fertilization (IVF)-embryo transfer cycles (14) and some studies have shown that the day 3 FSH to LH or LH to FSH ratio can be used as a predictor of the ovarian reserve (14-19). The day 3 LH/FSH ratio is relatively easy to obtain and shows more predictive power as the day 3 FSH level alone. As the serum FSH increases early in reproductive aging and a rise in LH is observed at a later stage, the decreased basal LH/FSH ratio might be a sign of diminished ovarian reserve even with normal basal FSH. As the LH/FSH ratio may decrease as the ovarian reserve declines, the value of LH/FSH ratio could play a significant role in determining the appropriate status of ovarian reserve.

In our data, the LH/FSH ratio was steadily and predictably decreased with age. A significant negative correlation was demonstrated between serum LH/FSH ratio and age (r = -0.213, P < 0.001), implying that the LH/FSH ratio is related to age. The present study also illustrates that powerful marker of ovarian reserve, namely AMH levels are falling with age. As assumed, a significant partial correlation adjusted by age was observed between AMH and LH/FSH ratio (r = 0.348, P < 0.001).

Fig. 1 shows that the serum AMH levels and the LH/FSH ratio were inversely correlated with age. In Table 1, the LH/FSH was slightly elevated in groups 5 and 6 of late reproductive age contrary to the decreasing tendency. A possible explanation might be that the LH levels generally increase later than FSH, especially in peri-menopause woman where ovary ceases to function and the ratio increases therefore.

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The LH/FSH ratio is commonly used in the assessment of PCOS although its sufficient utility has not been proven in this setting yet. However, the ratio has been little investigated as a marker of fertility or ovarian reserve. Recently, the LH/FSH ratio was evaluated in the field of infertility, in which the LH/FSH ratio showed the highest correlation with clinical pregnancy over other measures of ovarian reserve (14). And another study reported that a high FSH/LH ratio may be used as an early biomarker of poor ovarian response (15). Seckin et al. suggested that an elevated day 3 FSH/LH ratio is useful in the prediction of the IVF outcome in older women, but does not seem to be an accurate predictor in younger women (16). But they did not show a detail analysis according to age. Kim et al. suggested that the best prediction of reproductive age could be obtained by using the combination of FSH and LH on day 1 of the menstrual cycle (17). Sixty six women were divided into two groups, the younger (20-25 yr) and the older (40-45 yr) group and were simply compared. However, we divided our patients into six groups on the basis of their ages to present the age-specific level and compared LH/FSH ratio with AMH levels to confirm the predictability of this ratio. To the best of our knowledge, this is the first report on the correlation between the serum LH/FSH ratio and AMH levels.

There are few studies only about the LH/FSH ratio and no age-related reference values for LH/FSH ratio were provided based upon a large population. In a previous study, 178 patients were reviewed to assess the values of AMH and day 3 FSH/LH ratio (18). It was reported a higher pregnancy rate could be expected if the day 3 FSH/LH ratio < 2 and the AMH ≥ 5. However, they did not show direct age-related values for the LH/FSH ratio and did not analyze the relation between LH/FSH ratio and AMH. The strength of the present study is that we compared the LH/FSH ratio with AMH values in a larger sample size than previous studies. The limitation of our study is that we just evaluated the correlation between LH/FSH ratio and age. Further investigations combined with the present correlation results are needed on the direct ovarian response to a controlled ovarian stimulation according to the LH/FSH ratio. This is necessary to estimate the value of markers of exact ovarian reserve.

The clinical usefulness of AMH has been confirmed by numerous studies. In this study, we found that the clinically valuable meaning of basal LH/FSH ratio and this ratio as well as AMH could be considered as useful marker for ovarian reserve. As the day 3 serum FSH and LH concentrations are already usually available markers, their ratio could be used without additional effort as an important predictor for the compromised ovarian reserve. The LH/FSH ratio instead of serum AMH could be an age-related reference value for the ovarian reserve in women of reproductive age and also could be applied to the clinical evaluation for the infertility work up. Most of all, we can predict the ovarian reserve using commonly checked tests with a higher accuracy in women of reproductive age. Especially in infertile patients, the age-specific value of LH/FSH ratio may have a role in determining the appropriate stimulation protocol and assist in patient counseling. The LH/FSH ratio may allow physicians to stimulate patients in a more individualized treatment and will maximize the IVF outcome. Although further active and prospective studies are necessary to confirm such a role of LH/ FSH ratio in a clinical setting, our results will be helpful to evaluate the ovarian reserve of women.

In summary, there was a significant negative correlation between the serum LH/FSH ratio and age and a significant partial correlation was found between the serum LH/FSH ratio and AMH level. This suggests that the LH/FSH ratio could be considered as a useful marker for the ovarian reserve and could be applied to the clinical evaluation with AMH.

DISCLOSURE

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conception and coordination of the study: Lee JE, Yoon SH, Min EG. Design of ethical issues: Lee JE, Yoon SH, Min EG. Acquisition of data: Lee JE, Yoon SH, Kim HY, Min EG. Data review: Lee JE, Yoon SH, Kim HY, Min EG. Statistical analysis: Lee JE, Yoon SH, Kim HY. Manuscript preparation: Lee JE, Yoon SH, Kim HY, Min EG. Manuscript approval: Lee JE, Yoon SH, Kim HY, Min EG.

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