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ORIGINAL ARTICLE

The Effect of Age on Ovarian Reserve Markers in Tehranian Women with Normal Fertility

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The main goal of this study was to evaluate the effect of age and body mass index (BMI) on ovarian reserve markers in fertile Iranian women.
Materials & methods: One hundred fifteen fertile women were included in this cross-sectional study conducted from May 2005 until December 2006 in the Arash Hospital, Tehran. A transvaginal ultrasound examination and blood test were carried out between the third and fifth day of their menstrual cycle (follicular phase). The ovarian volume was measured and total antral follicles (AFC) were counted. Hormonal markers such as FSH, LH and estradiol were measured.
Results: Age had positive correlation with FSH (r = 0.819, P <0.01) and FSH/LH (r = 0.452, P <0.01). Meanwhile age had negative correlation with total ovarian volume (r=-0.835) and AFC (r=-0.924) P value < 0.01. Age had no effect on LH and estradiol levels (P > 0.05). Comparison of ovarian reserve markers between the four age subgroups (25-30, 31-35, 36-40 and 41-45 years) showed the FSH level, total ovarian volume and AFC had significant differences between these groups, while LH and estradiol did not different between subgroups. Meanwhile BMI had moderate positive correlation with FSH (r = 0.35, P <0.01) and LH (r=0.30, P <0.01) and moderate negative correlation with estradiol (r=-0.20, P<0.05), total ovarian volume (r=-0.31) and AFC (r=-0.41) by P <0.01. In multivariate analysis after adjustment of age, BMI as an independent factor had no effect on ovarian reserve markers.
Conclusions: AFC had the best correlation with age, followed by ovarian volume, FSH level and FSH/LH, making it a better predictor of ovarian response than BMI.

Key Words: Age, Ovarian reserve marker, FSH/LH ratio, fertile women, AFC, Ovarian volume

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Introduction

Fertility is remarkably reduced with increasing age of women in both spontaneous conceptions[1] and assisted reproductive methods.[2] The period of optimal fertility lasts until the age of about 30 yr and decreases gradually thereafter.[3] Decrease in fertility is probably due to a decreasing number of primordial follicles after birth; by the age of 37-38 yr, the follicle pool is significantly decreased,[4] as is the quality and quantity of oocytes.[5]

On the other hand, today many women for various personal reasons, delay childbearing. An accurate assessment of reproductive age would be helpful in counselling these women about their fertility potential and perhaps in scheduling any pregnancies. A number of parameters known as ovarian reserve markers have been examined in many assisted reproduction technology (ART) programs to predict ovarian responses prior to ovarian stimulation with gonadotrophin.

Majority of ovarian reserve tests were performed in women with a higher reproductive age than fertile women, but had infertility problems; hence is inappropriate to generalize the data of infertile subjects to fertile ones.

Therefore in this study we evaluate the effect of age and BMI on some hormonal and ultrasound makers in fertile Iranian women, referred to Arash hospital.

Materials and Methods

In this cross-sectional study, all women attending the gynecology clinic of Arash hospital for Pap smear screening test from May 2005 until December 2006 were recruited if the following criteria were met: Age between 25-45 years old, history of spontaneous conception and at least one term pregnancy, regular cycles with cycle length of 21-35 days; exclusion criteria were history of ovarian surgery, poly cystic ovarian or existence of ovarian cyst ≥20 mm, history of infertility and taking of hormonal drugs within the previous 3 months. For the enrollled women, with the appropriate inclusion criteria, procedures were fully explained and informed consent were obtained from all participants; following this, subjects were requested to come to the clinic between the 3rd to the 5th day (follicular phase) of their menstrual cycle for a transvaginal ultrasound examination and blood test. We recorded the probable menstrual date of each participant, and subjects were followed up by telephone. To reduce the inter- and intraobserver variability, all ultrasound examination was carried with a dynamic sonography set, using a 6.5 MHz with MCX vaginal probe (Livingstone, Scotland, UK), by the same sonographist, using one protocol, in order to measure the length, height and width of each ovary in the sagittal and coronal planes. The ovarian volume was then obtained using a formula for the volume of an ellipsoid, i.e.

$\pi/6$ (length × height × width).

The number of antral follicles <20 mm in each ovary was counted, as well. Then, 5 ml of venous blood was taken for measurement of FSH, LH and estradiol. FSH and LH were measured by the Pishtaz kit (Iran) and estradiol was measured by DRG ELISA kit (Germany). All samples were obtained, centrifuged and frozen for subsequent analysis. Hormonal levels were measured by the same technician in the Arash hospital laboratory. The coefficients of variation for LH and FSH were both <8% and the coefficient of variation for estradiol was <6.5%.

To evaluate the normal distribution of variables, the Kolmogorov-Smirnov Z test was used. The concentrations of hormone levels were not normally distributed and were log-transformed. In order to evaluate the effect of age on ovarian reserves of women they were classified in four groups aged 25-30, 31-35, 36-40 and 41-45 years. Kruskal-Willis Test was used to compare variables. Pair wise comparison was conducted by Mann-Whitney Test. For analyzing correlation between variants, the Pearson Correlation test was used. In order to evaluate the effect of BMI on variables as independent factors, multivariate analysis was conducted. All statistical analyses were performed by SPSS software (Version 12.5). A P value less than 0.05 was considered to be statistically significant.

This study was approved by the ethics committee of Tehran university of Medical Sciences.

Results

A total of 115 women were included in this study, 36 in group 1, 20 in group 2, 36 in group 3 and 23 in group 4. Demographic data and markers of ovarian reserve are shown in Table 1. Table 2 shows age of the women to

Table 1. Demographic characteristics of participants and ovarian reserve markers

Variables	Mean±SD	Range
Age (years)	35.09 ± 6.43	25-45
BMI (kg/m^2)	24.28 ± 1.95	20.82-30.48
FSH (IU/L)	6.39 ± 1.93	3.7-11.0
LH (IU/L)	4.27 ± 1.17	1.6-7.0
Estradiol (pg/mL)	55.97 ± 18.97	20.0-100.0
Ovarian volume (cm^3)		
Right	4.09 ± 0.54	3.2- 5.0
Left	3.95 ± 0.60	3.0-5.0
Total	8.4 ± 1.09	6.2-9.8
Antral follicle count (AFC)		
Right	4.7 ± 1.20	3.0-7.0
Left	4.11 ± 1.24	2.0-7.0
Total	8.83 ± 2.22	5.0-13.0

BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone

have a strong positive correlation with FSH ($r=0.819$, $p<0.01$); age however had a strong negative correlation with total ovarian volume ($r=-0.835$, $p<0.01$) and antral follicle count (AFC) ($r=-0.924$, $p<0.01$) and had no significant correlation with LH and estradiol ($p>0.05$). On the other hand, Table 2 shows BMI had a moderate positive correlation with FSH ($r=0.352$, $p<0.01$) and LH ($r=0.300$, $p<0.01$) and a negative correlation with estradiol ($r=-0.201$, $p<0.05$), total ovarian volume ($r=-0.311$, $p<0.01$) and AFC ($r=-0.412$, $p<0.01$). The results demonstrate FSH/LH has significant correlations with age ($r=0.452$, $p<0.01$) but no so with in BMI (Table 2). Comparison of ovarian reserve markers between age subgroups (Table 3) showed FSH level, total ovarian volume and AFC had significant differences between the four groups, ($p<0.001$). But LH ($p=0.98$) and estradiol levels ($p=0.38$) did not differ significantly between groups. In pair-wise comparison, significant differences were observed in FSH level in all groups, except between second and third group ($p=0.08$). Total ovarian volume showed significant difference between the first and third group, but not between the third and fourth groups ($p=0.51$). Data showed AFC rate decreased with age; there were significant differences between groups, especially between the first and fourth group ($p<0.0001$).

In multivariate analysis after adjustment of age, BMI had no effect on ovarian reserves as an independent factor.

Table 2. Correlation coefficient between age and BMI with ovarian reserve markers

Ovarian reserve markers	Age	BMI
FSH level (IU/L)	0.819**	0.352**
LH level (IU/L)	0.076	0.300**
FSH/LH ratio	0.452**	0.18
Estradiol (pg/mL)	-0.032	-0.201*
Total ovarian volume (cm^3)	-0.835**	-0.311**
AFC	-0.924**	-0.412**

* P value less than 0.05
** P value less than 0.01

Table 3. Comparison of ovarian reserve markers in subgroup analyses of age

Ovarian reserve markers	Age group (yr)				P value
	25-30 (n=36)	31-35 (n=20)	36-40 (n=36)	41-45 (n=23)	
FSH (IU/L)	4.45±0.72*	6.10±0.75	6.93±1.54	8.80±1.20	< 0.001
LH (IU/L)	4.20 ± 1.20	4.34 ± 0.57	4.30 ± 1.27	4.29 ± 1.38	0.97
Estradiol (pg/mL)	56.89 ± 17.90	52.40 ± 10.89	59.56 ± 25.09	52.0 ±14.17	0.38
Total ovarian volume (cm3)	9.27 ± 0.70	8.44 ± 0.55	7.18 ± 0.38	7.10 ± 0.44	< 0.001
AFC	11.67 ± 0.68	8.80 ± 0.77	7.67 ± 0.68	6.22 ± 1.04	< 0.001

* Mean±SD

Discussion

Since human fertility is known to decline with increasing maternal age, after contraceptive-use patterns and behavioral factors are being taken into consideration. [6] Since age is only a rough estimate of ovarian reserve, many tests, such as FSH, LH, Inhibin, AFC and total ovarian volumes, have been develop to predict ovarian reserve more precisely. A combination of tests can provide more accurate information of ovarian reserve. [7] The AFC by ultrasound is promising and facilitates clinical use.

Our results clearly showed a strong negative correlation between age and AFC, indicating ovarian follicles decreased with increase in age, a finding in accordance with those of other studies.[8-11]In our study, AFC was seen to have the best predictive value. Many studies also confirm the importance of AFC in the prediction of ovarian response in infertile women but some of the studies were conducted in women with proven fertility. Reuss et al showed AFC decreased by 0.95 follicles per year of age, or 60% of 22 and 42 of 31 healthy Caucasian volunteers. [12] Another study in 162 fertile women also showed before the age of 37 years, AFC mean yearly decline of 4.8% compared with 11.7% thereafter. [8]

Total ovarian volume as another parameter has also been evaluated to predict ovarian responses during ART treatment and seems to be correlated with age.

Andolf et al showed the most important factor for ovarian size was age but they evaluated the women from a referral population rather than general population and about one third of the patients were postmenopausal. Meanwhile that study demonstrated ovarian size decreased with age in all women but had no relation to parity and day of menstrual cycle in the menstruating group of women over 40 years of age. [13]

Another study evaluated ovarian size in 1888 infertile women undergoing IVF and they found total ovarian volume did not change significantly across age groups (23-45 years). [14] However, the results of this study manifested significantly decreasing ovarian size by age, especially between age 25-30 year (youngest group) and 41-45 years (oldest group).

Based on the reports of another study, in postmenoposal women, one delivery enlarged the ovaries by 6%, two deliveries increased ovarian volume by approximately 12% and there was no effect of further deliveries. [15] But, in our study, all women had a history of at least one term pregnancy and we did not consider the effect of parity a limitation.

FSH levels in early follicular phase, taken prior to the treatment cycles, are widely used in many ART programs and one study showed it is better predictor of ovarian response than the age of the women. [16] There was a positive correlation of age with serum FSH level in our study, similar to results of another study.[11]

LH and Estradiol are two ovarian reserve markers which had no correlation with age, findings in agreement with those of another study conducted in fertile women. [17] These results may be due to the evaluation of fertile women in these two studies unlike the other

study, which was performed in infertile women. Another reason maybe the small sample size of this study, not enough to ascertain the association between LH and estradiol with age. Further studies with larger sample sizes are needed to evaluate this association.

On the other hand, FSH/LH ratio had moderate positive correlation with age. In one study, the best prediction of age differences was obtained by using the combination of FSH and LH.[18]

In multivariate analysis, after adjustment of age we found no association between BMI and ovarian reserve. Another study conducted on women in their later reproductive ages (40-52 years) also showed that ovarian volume, FSH and estradiol were not associated with BMI.[19]

In conclusion, we demonstrated that AFC had the best correlation with age, followed by total ovarian volume, basal FSH, FSH/LH and correlation of ovarian reserve markers with age is more significant; Hence, it is a better predictor of ovarian response than BMI.

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