

**Original article:**

**ASSOCIATION BETWEEN INDIVIDUAL OVARIAN DIMENSIONS  
WITH OVARIAN RESERVE INDICES**

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**ABSTRACT**

**Introduction:** In some young female candidates of assisted reproductive technology (ART), ovarian response to simulative treatments is less than what is expected. More precise assessment of oocyte quality and quantity through studying ovarian dimensions can be useful for determining the dose of ovarian stimulant drugs and for preventing ART cycles cancellation. The aim of the present study is to determine the association between ovarian dimensions and ovarian reserve (OR) indices and whether ovarian dimensions can predict ovarian reserve.

**Methods:** In this cross-sectional study, 85 infertile women were studied. In early follicular phase, ovarian diameters (including length and width of the ovaries) were measured using transvaginal ultrasonography. Mean ovarian diameters (MOD) were calculated according to average length and width of the ovaries. A serum sample was taken from all patients to measure the level of Follicular Stimulating Hormone (FSH) and oestradiol as OR indices.

**Results:** The results of univariate analysis showed that FSH and oestradiol had a negative significant association with width, length and MOD ( $P < 0.01$ ). The results of multivariate regression analysis showed that FSH and oestradiol had a negative significant association with width ( $\beta_{\text{FSH}} = -0.59, P = 0.001$  and  $\beta_{\text{Oestradiol}} = -0.019, P = 0.029$ ) and MOD ( $\beta_{\text{FSH}} = -0.52, P = 0.003$  and  $\beta_{\text{Oestradiol}} = -0.021, P = 0.017$ ) and had a borderline negative significant correlation with ovarian length ( $\beta_{\text{FSH}} = -0.49, P = 0.077$  and  $\beta_{\text{Oestradiol}} = 0.022, P = 0.08$ )

**Conclusions:** The results of this study revealed that despite a moderate correlation, ovarian diameters could be an applicable index for predicting OR. Using this method along with other methods may be useful in treatment with ovarian stimulants.

**Keywords:** Ovarian length, ovarian width, FSH, oestradiol, infertility

**INTRODUCTION**

About 10-15 % of reproductive-age couples suffer from infertility. Ovarian stimulation is one of the fundamental prelimi-

nary steps of assisted reproductive technology (ART) (Berek, 2007; Fritz and Speroff, 2011). The evaluation of ovarian reserve (OR) may help predict women who proba-

bly under-respond or over-respond to controlled ovarian hyperstimulation protocols in ART programs (Bukulmez and Arici, 2004; Vladimirov et al., 2005; Bancsi et al., 2002; Chang et al., 1998; La Marca et al., 2012).

OR is defined as the number and quality of the recruited follicles remained in the ovary at all ages (Broekmans et al., 2006; Coccia and Rizzello, 2008). A precise and valid measure of the quantitative OR consists of the enumeration of total follicles that existing both ovaries (Block, 1952). Pregnancy likelihood in infertility therapy such as intra-uterine insemination (IUI) and in-vitro fertilization (IVF), or in the follow-up of spouses within and then the initial infertility work-up are proxy variables for oocyte quality which are currently applied (Broekmans et al., 2006). According to the ESHRE working group following a consensus, poor ovarian responders (POR) are specified when at least two of the followings are met:

- 1) high maternal age or presence of other potential risk factors of POR,
- 2) previous history of POR,
- 3) Abnormal ovarian reserve test (ORT) (Ferraretti et al., 2011).

Follicular stimulating hormone(FSH) is the most commonly used test to assess OR. Third day of cycle is the best testing day because the expected low level of oestradiol affects FSH levels related to a negative feedback control (Perloe et la., 2000; Roubush et al., 2008). Day-3 FSH and oestradiol are both measured typically (Sharara and McClamrock, 1999; Toner, 2003; Barnhart and Osheroff, 1998). However, other blood tests like antimullerian hormone (AMH) and inhibin-B have become popular recently because they determine ovarian status more directly; day-3 FSH and oestradiol are indirect measurements (Dehghani-Firouzabadi et al., 2008; Jayaprakasan et al., 2010; Ebner et al., 2006; La Marca et al., 2007; Feyereisen et al., 2006; Visser et al., 2006).

Making use of ovarian dimensions - as criteria to evaluate OR and to predict ovarian response to stimulative treatments - has been investigated in a few studies (Frattarelli et al., 2000; 2002; Bowen et al., 2007). A study carried out by Frattarelli et al. (2000) on 278 infertile female candidates of IVF showed that a significant association was found between MOD (which calculated as the sum of ovarian length and ovarian width divided by two) and age, serum FSH and FSH:LH ratio after some co-founder factors such as smoking and body mass index (BMI) were controlled. In a recent study by Bowen et al. (2007) on 69 reproductive-age patients, a significant negative correlation was found between all ovarian diameters (including width, length and mean ovarian diameters) and serum FSH and age. Ovarian width was recognized as the strongest predictor of ovarian reserve but this study did not examine the association between ovarian dimensions and serum oestradiol (Bowen et al., 2007).

The present study aimed to determine the association between width, length and mean ovarian dimensions with OR indices including FSH and serum Oestradiol. Likewise, we aimed to show whether ovarian dimensions can be a good or reliable predictor for ovarian reserve.

## METHODS

### *Participants*

The study population consisted of 85 infertile women who referred to IVF unit of Mirza Koochak Khan Hospital (which is one of the teaching hospitals affiliated to Tehran University of Medical Sciences (TUMS)) from July 2009 to April 2010. These subjects were randomly selected to participate in this study. Women aged more than 44 and those who had a history of previous unilateral oophorectomy, were excluded. This study was approved by ethics committee of TUMS. A written informed consent was obtained from all patients who participated in the study.

### ***Ovarian reserve (OR) measurements***

Serum samples were taken in the first to third day of menstruation and FSH and serum oestradiol levels were measured [ELISA, Roche Elecsys1010, Roche Diagnostics, Indianapolis; Oestradiol II, minimal detection limit 5pg/ml, intra-assay CV 5.7 % and inter-assay CV 6.2 % and FSH, minimal detection limit 0.10 mIU/ml, intra-assay CV 1.8 % and inter-assay CV 5.3 %]. Serum sampling was done at the same day when ultrasonographic measurement was taken. The results were calculated with an automated method using a 4 PL (4 Parameter Logistics) curve fit.

### ***Ovarian dimensions measurements***

To avoid the reproducibility within and between observers, transvaginal ultrasonography was done only by one infertility fellowship. 7-MHz frequency of Medison ultrasound machine was used, and both length and width of every ovary were measured in millimeter in the biggest sagittal plan. The variable “ovarian length” was calculated as “the sum of right ovarian length and left ovarian length divided by two” and the variable “ovarian width” as “the sum of right ovarian width and left ovarian width divided by two”. Patients’ weight (kg) and height (m) were measured and their BMI was calculated as subjects’ weight (in kg) divided by height (in m) squared. All patients were asked verbally about their cigarette smoking. The cause of infertility was extracted from the patient’s recorded files.

### ***Sample size and statistical analysis***

The sample size was determined using correlation formula ( $N = ((Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (1-r^2))/(r^2)$ ). Where  $r$ , based on the data on Bowen et al. (2007) study, as the correlation between increasing levels of FSH and a significant decrease in the mean ovarian diameters, was equal to -0.3 and type I error ( $\alpha$ ) and the statistical power ( $1-\beta$ ) were 5 % and 80 %, respectively. Allowing for a 5 % dropout rate, the study required 85 subjects.

Data were presented as mean and standard deviation (SD) for continuous variables and relative and absolute frequencies for categorical variables. Pearson Correlation ( $r$ ) was used to determine the strength of the correlation between mean ovarian diameter and variables including FSH, oestradiol and age. To predict the ovarian reserve indices (FSH, oestradiol) using the ovarian dimensions parameters (length, width and mean ovarian dimensions), a univariate linear regression analysis was used. For each ovarian dimension, a linear regression was performed to fit concerning models and regression coefficients or Beta coefficients ( $\beta$ ) were reported. The interpretation of these beta coefficients is how much are the dependent variables (such as width, length and mean ovarian dimension here) expected to change (decrease or increase) for one-unit change in independent variables (such as FSH and oestradiol here). To control the effects of some potential confounder variables such as age, smoking and BMI, a multivariate linear regression analysis was used. To determine the accuracy of the regression models, coefficients of determination ( $R^2$ ) were calculated and reported. This value ( $R^2$ ) shows an insight into the “goodness of fit” of the statistical model, linear regression models in this study. This coefficient is valued between 0 and 1.  $R^2$  equal to 1 indicates that the models perfectly fit the data and  $R^2$  equal to 0 indicates that the fitted model does not explain variations at all. All statistical analysis was done using SPSS version 15 for windows software and STATA version 10.  $P < 0.05$  was considered statistically significant.

## **RESULTS**

85 patients were recruited in the study over the study period. The mean age of all subjects was  $32.2 \pm 6.1$  years. Table 1 shows the subjects’ descriptive information.

The mean serum FSH and oestradiol levels were  $7.3 \pm 3.5$  ml U/ml and  $72.4 \pm 66.9$  pg/ml, respectively. The most common causes of infertility were male-factor, tubal-factor, diminished OR and PCOD with frequency of 29.5, 16.5, 16.5 and 12.5 %, respectively. Table 1 lists other main causes regarded to the study population. The mean ovarian length and width were  $45 \pm 8.5$  mm and  $29 \pm 5.6$  mm, respectively. Nearly 5 % of patients were smokers.

**The association between ovarian width and OR indices**

Univariate linear regression analysis showed that FSH, oestradiol and age had a significant linear association with ovarian width. Beta coefficients of linear regression for these three variables were -0.75, -0.028 and -0.35mm, respectively ( $P < 0.05$ ). The highest level of coefficient of determination was 0.20 for FSH index (meaning, 20 % of

variations of ovarian width were explained by FSH).

After confounder factors including age, cigarette smoking and BMI were controlled, multivariate linear regression analysis revealed that FSH and oestradiol had a significant negative association with ovarian width. Coefficient of determination ( $R^2$ ) for FSH increased from 0.20 to 0.23 and for oestradiol from 0.10 to 0.16 (Table 2).

**The association between ovarian length and OR indices**

Results of univariate linear regression analysis showed that FSH, oestradiol and age had a significant negative association with ovarian length. Beta coefficients of linear regression for these three variables were -0.87, -0.035 and -0.65mm, respectively ( $P < 0.05$ ). The coefficient of determination for FSH was 0.12, (meaning, 12 % of variations of ovarian length were explained by FSH).

**Table 1:** Descriptive characteristics and main causes of infertility of study sample

Variables	Descriptive statistics
Age (years)	$32.38 \pm 6.1$ (19-43)
BMI ( $\text{kg}/\text{m}^2$ )	$23.8 \pm 3.1$ (18-33)
FSH (mIU/ml)	$7.3 \pm 3.5$ (2-18/7)
Oestradiol (pg/ml)*	$72.4 \pm 66.9$ (12-463)
Ovarian length (mm)*	$45 \pm 8.5$ (29-69)
Ovarian width(mm)*	$29 \pm 5.6$ (9-42)
Overall Ovarian Diameter (mm)*	$37 \pm 6$ (20-54)
<b>Infertility reasons (No., %)**</b>	
Diminished OR	14 (16.5)
Male factor	25 (29.5)
Tubal factor	14 (16.5)
PCOD	11 (12.5)
Unexplained	8 (9.5)
Endometriosis	8 (9.5)
Multifactorial	5 (5.9)

\*data are presented as: mean  $\pm$  SD (min-max), \*\* N (%)

**Table 2:** Univariate and multivariate linear regression of Follicular Stimulating Hormone (FSH) and oestradiol on ovarian length, ovarian width and overall ovarian mean

Width	Univariate model			Multivariate model *		
	$\beta$ crude	P value	R <sup>2</sup>	$\beta$ Adjusted	P value	R <sup>2</sup>
FSH	-0.75	< 0.001	0.20	-0.59	0.001	0.23
Oestradiol	-0.028	0.002	0.10	-0.019	0.029	0.16
Age	-0.35	< 0.001	0.15	-	-	-

Length						
FSH	-0.87	0.001	0.12	-0.46	0.077	0.23
Oestradiol	-0.038	0.005	0.089	-0.022	0.08	0.23
Age	-0.65	< 0.001	0.22	-	-	-

Overall Ovarian Mean						
FSH	-0.81	< 0.001	0.19	-0.52	0.003	0.32
Oestradiol	-0.033	0.001	0.13	-0.021	0.017	0.29
Age	-0.50	< 0.001	0.26	-	-	-

\*: adjusted for age, smoking status and BMI

Results of multivariate linear regression analysis showed that FSH and oestradiol had a borderline significant negative association with ovarian length. R<sup>2</sup> for FSH and oestradiol in multivariate analysis increased from 0.12 to 0.23 and from 0.08 to 0.23, respectively (Table 2).

#### ***The relationship between mean ovarian diameter and OR indices***

The Pearson correlation showed a significant negative correlation between mean ovarian diameter and FSH ( $r = -0.45$ ,  $P < 0.001$ ), oestradiol ( $r = -0.36$ ,  $P = 0.001$ ) and age ( $r = -0.52$ ,  $P < 0.001$ ) (Figure 1, 2, 3). According to results of the univariate linear regression analysis, FSH, oestradiol and age had a significant negative association with mean ovarian diameters (MOD). Beta coefficients of linear regression for these three variables were -0.81, -0.033 and -0.50 mm, respectively ( $P < 0.05$ ). The coefficient of determination (R<sup>2</sup>) for FSH, oestradiol and age was obtained as 0.19, 0.13 and 0.26, respectively.

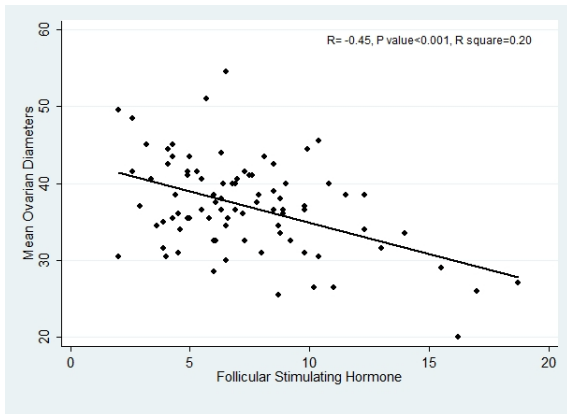
Results of multivariate linear regression analysis revealed that, FSH and oestradiol still had a significant reverse association

with ovarian length ( $P < 0.05$ ). R<sup>2</sup> for FSH and oestradiol increased from 0.19 to 0.32 and from 0.13 to 0.29, respectively (Table 2).

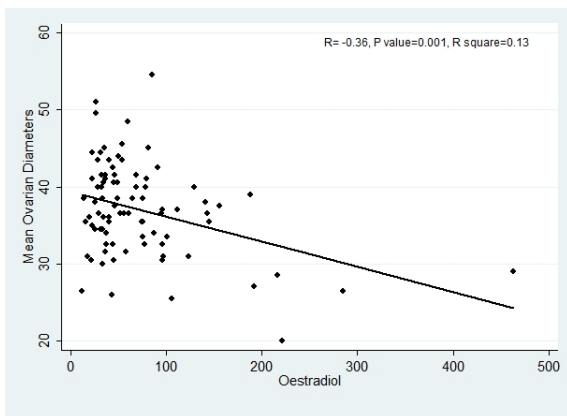
## **DISCUSSION**

Results showed that ovarian measurements had a strong and significant relationship with biological indices of ovarian reserve like FSH and serum oestradiol as well as patients' age. Results also revealed that by adjusting effects of some confounder variables like smoking, BMI and age, still a significant relationship can be observed between ovarian reserve and ovarian dimensions. The highest level of changes factor was related to FSH, that justified more than 20 % of changes of all three ovarian dimensions in the adjusted model.

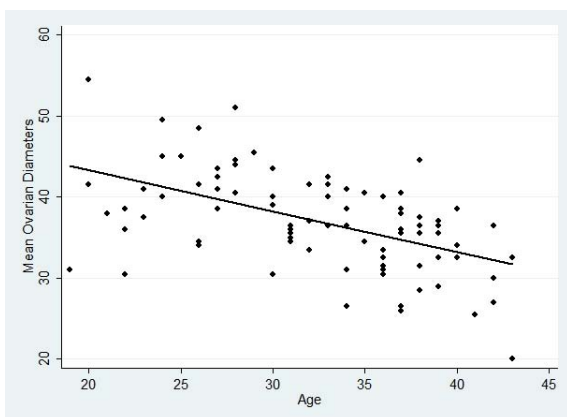
Several research has been carried out so far to discover a useful index for determining ovarian reserve. Ultrasonography can be a useful tool to determine ovarian performance. In a prospective study by Adibi et al. (2012) on fifty two women (aged 18-46) with regular cycles, antral follicles count (AFC) was considered a better



**Figure 1:** Pearson correlation (r) between mean ovarian diameters and Follicular Stimulating Hormone (R square: coefficients of determination)



**Figure 2:** Pearson correlation (r) between mean ovarian diameters and Oestradiol (R square: coefficients of determination)



**Figure 3:** Pearson correlation (r) between mean ovarian diameters and age (R square: coefficients of determination)

method for predicting ovarian response to ovulation induction protocol compared with ovarian volume and hormonal tests. In another recent study conducted by Kelsey et al. (2012) there was a strong and positive correlation ( $r = 0.89-0.99$  based on different statistical models) between ovarian volume and the number of non-growing follicles (NGFs) in the human ovary aged 25-51. According to these results, these researchers indicated that ovarian volume - as a surrogate measure - could be a useful indirect factor (index) for predicting human ovarian reserve for the individual woman (Kelsey et al., 2012). However, AFC is a subjective matter; in order for it to be a reliable marker of ovarian reserve, it needs a high-resolution machine and a report by the same observer. Furthermore, AFC & ovarian volume vary in different cycles. Thus, low AFC in a regularly-menstruating young woman with ovulation can not predict poor ovarian reserve (Roudebush et al., 2008).

Ovarian stromal blood flow velocity is one of the useful indices to predict ovarian responsiveness to stimulative treatments; however, results do not always correlate with advancing age. Likewise, it is not applicable in all infertility centers due to the need for doppler Ultrasonography (Roudebush et al., 2008).

In a recent study by Satwik et al. (2012), among FSH, AMH and age, AMH was the best factor to predict the overall ovarian response and poor response to ovulation induction. The outcome measure was the number of oocytes retrieved. Although 2 pmol/l seemed to be a reasonable cut-off of AMH levels for predicting poor response, there was an enormous overlap between average and poor responders in the AMH range of 2–10 pmol/l; however, it cannot be a definite predictor of non-responder status (Satwik et al., 2012).

In our study, ovarian dimensions were measured using transvaginal ultrasonography. Results showed that all parameters of ovarian dimensions including length, width and mean ovarian diameter were related to

ovarian reserve indices including serum FSH and age of patients. Our study was a controlled research concerning devastating variables including smoking status and BMI.

In a study carried out by Frattarelli et al. (2000) in 1999 on 60 patients, the relationship between mean ovarian diameter and mean ovarian volume was examined in infertile women who had undergone ART. Results showed that more than 90 % of correlation was observed between these two mentioned variables. In another study by Frattarelli et al. (2002), results of transvaginal Ultrasonography were studied on 278 patients in follicular phase. Ovarian size calculated as average length and width showed a significant relationship between age, serum FSH and ratio of FSH to LH. In this study, confounding factors like smoking and BMI were ignored.

In another research conducted by Bowen et al. in 2007, 69 patients were studied at their pregnancy ages. A significant correlation was found between all ovarian measurements and serum FSH and age; ovarian width was the strongest predictor of ovarian reserve. The relationship between ovarian dimensions and serum oestradiol wasn't examined in this study. In our extensive literature review, we couldn't find any studies that had compared or assessed the relationship between other markers of ovarian reserve such as AFC or AMH and ovarian dimensions.

In our study, the highest relationship with serum FSH was related to ovarian width and ovarian mean; the highest relationship with serum oestradiol and age was related to overall ovarian mean. The difference between results of our study and those of Bowen may be as a result of higher volume of study or racial differentiation in ovarian forms. In both studies, a significant relationship was observed between all ovarian dimensions and ovarian reserve indices including FSH and age.

### **Limitations**

The subjects of the study were selected using simple random sampling method which might influence the generalizability of the results of the present study. Likewise, our study was carried out only in one hospital; however, it was a referral hospital in our setting (Tehran city), but if we had selected more study population in various infertility centers, results might be more precise compared to the current findings. Another one is that at the time of the study we were not able to evaluate AMH of the patients. Although AMH (rather than FSH) is an acceptable measurement to obtain the ovarian reserve, we did not have the possibility to compare ovarian dimensions with the findings of AMH.

### **Recommendations**

Further studies are required for the proper interpretation of currently applied hormonal markers, ultrasound parameters, and hormone challenge tests. It seems reasonable to use a combination of these tests to improve their predictive value. This approach may reduce cancelled cycles, waste of resources, and emotional stress to the patient, and it may result in a reasonable pregnancy rate. Also, it is recommended to extend the study to evaluate the ovarian response to COH (e.g. number of co-dominant follicle or retrieved oocytes) which has been already started by the authors of this study

### **CONCLUSION**

Results of this research revealed that ovarian measurements, especially mean ovarian diameter, can be an acceptable index for predicting ovarian reserve; however, it needs more studies to confirm the results of our study and similar studies, and it needs to compare the results of this method with other alternative methods. This knowledge may allow physicians to evaluate and counsel patients immediately before ART stimulation and to optimize stimulation protocols.

## ACKNOWLEDGEMENTS

The authors would like to thank the respectable personnel of the IVF ward of the Mirza Koochak Khan Hospital for their great help in this study. We also thank all participants in this study and ethics committee of Tehran University of Medical Sciences.

## CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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