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 (β_{FSH} = -0.59, P = 0.001 and β_{Oestradiol} = -0.019, P = 0.029) and MOD (β_{FSH} = -0.52, P = 0.003 and β_{Oestradiol} = -0.021, P = 0.017) and had a borderline negative significant correlation with ovarian length (β_{FSH} = -0.49, P = 0.077 and β_{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 preliminary 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 contro-
trolled 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 
avery at all ages (Broekmans et al., 2006; 
Coccia and Rizzello, 2008). A precise and 
valid measure of the quantitative OR cons-
isists 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 in-
fertility work-up are proxy variables for oo-
cyte quality which are currently applied 
(Broekmans et al., 2006). According to the 
ESHRE working group following a consen-
sus, poor ovarian responders (POR) are 
specified when at least two of the follow-
ings 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 al., 2000; Rou-
de bush et al., 2008). Day-3 FSH and oes-
tradiol are both measured typically (Sharara 
and McClamrock, 1999; Toner, 2003; Barn-
hart 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 ovar-
ian response to stimulative treatments - has 
been investigated in a few studies (Fratt-
tarelli et al., 2000; 2002; Bowen et al., 
2007). A study carried out by Frattarelli et 
al. (2000) on 278 infertile female candi-
dates of IVF showed that a significant asso-
ciation was found between MOD (which 
calculated as the sum of ovarian length and 
avarian width divided by two) and age, se-
rum FSH and FSH:LH ratio after some co-
founder factors such as smoking and body 
mass index (BMI) were controlled. In a re-
cent study by Bowen et al. (2007) on 69 re-
productive-age patients, a significant nega-
tive correlation was found between all ovar-
ian 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 associa-
tion 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. Like-
wise, we aimed to show whether ovarian di-
mensions can be a good or reliable predic-
tor for ovarian reserve.

METHODS

Participants

The study population consisted of 85 in-
fertile 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 pre-
vious unilateral oophorectomy, were ex-
cluded. 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 \( N = \left( \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(1-r^2)} \right) \). 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 ± 6.1 years. Table 1 shows the subjects’ descriptive information.
The mean serum FSH and oestradiol levels were 7.3 ± 3.5 ml U/ml and 72.4 ± 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 ± 8.5 mm and 29 ± 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 linear 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 ± 6.1 (19-43)    |
| BMI (kg/m$^2$)                   | 23.8 ± 3.1 (18-33)     |
| FSH (mIU/ml)                     | 7.3 ± 3.5 (2-18/7)     |
| Oestradiol (pg/ml)*              | 72.4 ± 66.9 (12-463)   |
| Ovarian length (mm)*             | 45 ± 8.5 (29-69)       |
| Ovarian width (mm)*              | 29 ± 5.6 (9-42)        |
| Overall Ovarian Diameter (mm)*   | 37 ± 6 (20-54)         |
| Infertility reasons (No., %)**   |                        |
| 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 ± 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

|                | Univariate model |                  | Multivariate model ¥ |
|----------------|------------------|------------------|-----------------------|
|                | β crude          | P value          | R²                    | β Adjusted         | P value | R²  |
| Width          |                  |                  |                       |                     |
| 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. R² 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 (R²) 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). R² 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. Ultrasoundography 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
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.
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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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