Evaluation of Ovarian Reserve in Infertile Women with Minimal/Mild Endometriosis

AHMED E.H. ELBOHOTY, M.D., M.R.C.O.G. *; AHMED A. THARWAT, M.D. *; IBRAHIM ZWIEL, M.D. *; MOHAMED I.M. AMER, M.D. *; and SANAA E. MOHAMED, M.D. **

The Departments of Obstetrics & Gynecology* and Biochemistry**, Faculty of Medicine, Ain Shams University

Abstract

**Aim:** To evaluate the ovarian reserve in infertile women with minimal to mild degrees of endometriosis in comparison to infertile women with tubal factor.

**Methods:** In a case-control study was run at Ain Shams University Maternity Hospital, 40 infertile patients, undergoing laparoscopy were divided into 2 groups Group A: Patients with minimal or mild endometriosis (Stage I or II) (n=20). Group B: Patients with tubal factor without endometriosis (n=20). The outcome Measures were serum FSH, E2, anti-Mullerian hormone (AMH), and antral follicular count (AFC).

**Results:** The number of antral follicles, follicles <8mm in diameter and the AMH level were significantly lower in endometriosis group in comparison to the control group. There was no statistically difference regarding E2 or FSH.

**Conclusions:** The ovarian reserve is decreased in minimal to mild endometriosis which is demonstrated by measuring AMH and AFC. This finding needs further investigation.

**Key Words:** FSH – E2 – AMH – AFC – Endometriosis – Infertility – Ovarian reserve.

Introduction

ENDOMETRIOSIS is a common finding in women with infertility [1] with suggested negative effect on the ovarian reserve [2,3]. Markers of ovarian reserve are increasingly used to aid management and counseling of infertile women, especially who are subjected to undergo assisted reproductive technology (ART). However, there is no universal agreement on the best marker for ovarian reserve in women with endometriosis [4]. The classic ovarian reserve markers as early follicular phase serum follicle stimulating hormone (FSH), inhibin B, and estradiol (E2) levels showed limited clinical usefulness [5,6]. On the other hand, pre-ART ultrasound follicular count (AFC) has been shown to be an excellent predictor of ovarian reserve and response in relation to other markers [7,8]. AMH is reliably used for the prediction of ovarian response, being a marker that can estimate the quantity and activity of retrievable follicles in early stages of maturation [9,10]. The AFC and AMH are the most significant predictors of poor response to ovarian stimulation during ART [11,12], with the latter proposed to be more superior and having the potential to be incorporated in work-up protocols with cutoffs of >3.75ng/mL and <1.0ng/mL having modest sensitivity and specificity in predicting the extremes of response [13].

A recent study suggests low ovarian reserve in patients with minimal/mild endometriosis [14]. The aim of the present study is to compare the ovarian reserve in infertile women with minimal to mild endometriosis and infertile women with tubal obstruction without endometriosis.

Material and Methods

This is a case-control cross-sectional study that was conducted in Ain Shams University Maternity Hospital in the period from April 2009 to March 2012. The population of this study comprised of 40 women considered for laparoscopy. Detailed medical history was obtained and all patients underwent complete clinical examination. Inclusion criteria included age 18-35 years, regular menses, normal serum prolactin, normal serum thyroid stimulating hormone (TSH), body mass index (BMI) <30kg/m², with both ovaries present and normal semen analysis of the husband. Exclusion criteria included patients with endocrine disorders, cases in which the cause for infertility was other than endometriosis (except for patients with tubal factor, in the control group) and patient...
with previous surgery for endometriosis. On cycle day 3, 10ml blood sample was withdrawn, centrifuged at 2,500rpm, with plasma separated and stored in Eppendorf tubes and frozen at –80ºC for later analysis of serum FSH (microplate immunoenzymatic assay; Accu Bind, USA), E2 (DRG Estradiol ELISA; DRG International, USA) and AMH (AMH Gen II ELISA; Beckman Coulter, USA). On the same day all patients underwent ultrasonographic transvaginal scanning (TVS) using 260 Corvus device (ESAOTE Pie Medical, Model No. 402171 Rev. B, Italy) to determine the ovarian antral follicular count (AFC) cohort by measuring number and diameter of all selectable follicles. After laparoscopy, those with normal pelvic findings were excluded; while those classified as minimal to mild (American Society for Reproductive Medicine I/II) 15 endometriosis (Group A, n=20), or tubal factor of infertility (Group B, n=20) were incorporated into the study.

A previous study reported a lowered mean level of AMH (1.26±0.7ng/ml) in patients with minimal/mild endometriosis compared to that of patients with tubal factor infertility (2.02±0.72ng/ml) [14]. Sample size was calculated with the program Stata 10.0 (Stata Corp LP, Texas, USA) based on the assumption of a type I error (α) of 0.05, a power (1 - β; type II error), and the required sample size for each group was proved to be 19 subjects at least. Based on this assumption, 20 cases were enrolled in each group.

Statistical analysis was performed with STATISTICA ver. 12.0 (Statsoft Inc., Tulsa, USA). Comparisons of data between groups were performed using Mann-Whitney U-test (for continuous data) and Spearman rank order correlation (for categorical data). Each variable is presented as number (percentage) or mean ±SD. Pearson’s correlation was utilized to analyze the correlations between variables. A p-value of below 0.05 was considered to be statistically significant.

**Results**

The patients’ characteristics in both groups were comparable with no statistical differences (Table 1). All studied subjects presented with serum FSH levels below 12IU/mL, and E2 below 81.5pg/ml (300pmol/ml). Markers for ovarian reserve in both groups showed that the endometriosis has a negative impact on most of them. The number of antral follicles (AFC) was comparable in both groups; however, the ratio of small follicles (<8mm in diameter)/AFC, and the AMH levels were significantly higher in tubal group; while the mean follicular diameter was significantly smaller. Categorical AMH level subgrouping showed it to be <1ng/ml in 35% of subjects with endometriosis compared to 5% of subjects with tubal disease, and 3.75ng/ml or more in 0% versus 20%; respectively (p<0.05) (Table 1). There were no significant statistical differences in serum E2 and FSH levels between the two groups. There were significant negative correlations between serum basal FSH and AMH (Fig. 1). Also, there was a negative correlation between AMH and mean diameter of antral follicles (n=40, r=-0.28, p=0.0795), approaching significance (p<0.08). Neither AMH nor FSH was found to be correlated to antral follicle count.

| Table (1): Demographic data and ovarian reserve markers in both groups. |
|---------------------------------|-----------------|-----------------|---|
|                                | Group A (Endometriosis) | Group B (Tubal factor) | **p** |
| **Age (years)**                | 29±4.69           | 29.2±2.97        | 0.935323 |
| **BMI (Kg/m^2)**              | 25.96±1.42        | 26.2±1.83        | 0.490335 |
| **Infertility duration (years)** | 3.2±1.56           | 4.05±2.78        | 0.570000 |
| **AFC**                        | 10.75±1.59        | 11.15±2.25       | 0.551776 |
| **Follicles <8mm/AFC Ratio**   | 0.752±0.106       | 0.848±0.079      | 0.002686 |
| **Follicles ≥8mm/AFC Ratio**   | 0.248±0.106       | 0.151±0.079      | 0.002049 |
| **Follicular Diameter (mm)**   | 6.44±0.21         | 5.9±0.3          | 0.000003 |
| **AMH (ng/ml):**               | 1.51±0.86         | 2.2±1.03         | 0.025640 |
| **Subgroups:**                 |                  |                  |    |
| Low <1                         | 7 (35)            | 1 (5)            | 0.00265 |
| Average 1<3.75                 | 13 (65)           | 15 (75)          |    |
| High ≥3.75                     | 0 (0)             | 4 (20)           |    |
| **FSH (miu/ml)**               | 8.54±1.63         | 7.6±2.08         | 0.126432 |
| **E2 (pmol/ml)**               | 122.85±58.44      | 143.65±65.25     | 0.244768 |

which shows also a negative correlation of AMH heterogeneous follicular cohort with larger mean follicular diameter in the minimal/mild endometriosis; however, this may not be evident with all ovarian reserve markers. The assessment of ovarian reserve remains a challenge. Among the different available markers of ovarian reserve, AFC and AMH have been shown to be excellent predictors of ovarian reserve and considered to be the only static markers that can potentially assess the follicular reserve as a whole, in quantity and quality [5,7-10,18,19]. Our data shows that both were significantly lower with significantly higher percentage of large follicles (>8mm in diameter) and larger mean follicular diameter in the minimal/mild endometriosis group. This agrees with the findings of a previous study [14]. The level of AMH expression in the granulosa cells was well demonstrated to be highest in secondary, preantral and small antral follicles (<4mm in diameter) and to decrease gradually till it disappears in larger follicles (8mm in diameter) [20]. That is why 8mm limit was chosen in the present study. Although the AFC was not decreased in endometriosis, the higher follicles >8mm/AFC ratio, and the lower ratio of smaller follicles (<8mm in diameter)/AFC along with the larger mean follicular diameter indicate a decrease in the relative number of smaller follicles. Other investigators have reported the presence of a more heterogeneous follicular cohort with larger mean follicular diameter in minimal/mild endometriosis compared with tubal factor infertility [14]. This concurs with the findings of the present study which shows also a negative correlation of AMH level and mean follicular diameter. The findings of the present study and others [14] might explain the decrease of the AMH level in endometriosis patients since it is produced mainly by small, early antral follicles.

It has been reported that E2 and FSH measurement have a limited role in assessing the ovarian reserve due to inter and intra-cyclic variability [19]. Basal FSH level is known to be modulated by the population of larger antral follicles [21]. The presence of significant negative correlation between FSH and AMH levels in the present study agrees with this, despite failure to show differences in basal levels of FSH in both groups. The presence of significantly lower basal AMH levels in patients with minimal or mild endometriosis compared to women with tubal factor infertility concurs with the findings of other two recent studies [14,22]. However, most other investigators included infertile patients with all stages of endometriosis. In one study, there were no differences in basal FSH and AMH between stages I/II and III/IV endometriosis; yet with significant elevation of basal FSH in whole endometriosis group compared to control [21]. Others found significant lower AMH and higher FSH in endometriosis compared to control, with the former being more correlated to the number of retrieved oocytes [23]. This discrepancy might reflect more sensitivity of AMH than FSH in assessing ovarian reserve. Women with minimal or mild endometriosis might respond adequately to exogenous FSH but may have impaired oocyte quality, reflected in lower fertilization rates [22]. Consistent with progressive loss of ovarian reserve in women with increasing stages of endometriosis, it has been reported that day 3 FSH levels were significantly elevated in women with stage III/IV endometriosis, and not in women with stage I/II endometriosis [17]. On the other hand, high cycle day 3 E2 is reported to be associated with higher cancellation rates [24,25], lower oocyte yield [25], and pregnancy rates [24]. Although the present study showed no significant difference in basal E2 levels in both study groups, the condition might vary in advanced (stage III/IV) endometriosis. Day 3 E2 was significantly elevated in women with stage III/IV endometriosis, and not in women with stage I/II endometriosis [17]. Other investigators failed to find differences in basal E2 between stages I/II and III/IV endometriosis [21].

The evident relatively smaller number of follicles (<8mm in diameter) in endometriosis group might represent an effect of endometriosis on the development of growing follicles; an effect even appearing in minimal to mild degrees of the disease. Autoimmunity might play role as several studies suggest the presence of correlation between anti-

![Correlation between serum basal FSH and AMH](image)

Fig. (1): Correlation between serum basal FSH and AMH (n=40, r=-0.3982, p=0.011).

**Discussion**

Endometriosis could affect the ovarian reserve and this has been previously assessed, however most of studies have included infertile women with all stages of endometriosis [16,17]. The overall findings of the present study showed that the ovarian reserve is decreased in minimal/mild endometriosis; however, this may not be evident with all ovarian reserve markers. The assessment of ovarian reserve remains a challenge. Among the different available markers of ovarian reserve, AFC and AMH have been shown to be excellent predictors of ovarian reserve and considered to be the only static markers that can potentially assess the follicular reserve as a whole, in quantity and quality [5,7-10,18,19]. Our data shows that both were significantly lower with significantly higher percentage of large follicles (>8mm in diameter) and larger mean follicular diameter in the minimal/mild endometriosis group. This agrees with the findings of a previous study [14]. The level of AMH expression in the granulosa cells was well demonstrated to be highest in secondary, preantral and small antral follicles (<4mm in diameter) and to decrease gradually till it disappears in larger follicles (8mm in diameter) [20]. That is why 8mm limit was chosen in the present study. Although the AFC was not decreased in endometriosis, the higher follicles >8mm/AFC ratio, and the lower ratio of smaller follicles (<8mm in diameter)/AFC along with the larger mean follicular diameter indicate a decrease in the relative number of smaller follicles. Other investigators have reported the presence of a more heterogeneous follicular cohort with larger mean follicular diameter in minimal/mild endometriosis compared with tubal factor infertility [14]. This concurs with the findings of the present study which shows also a negative correlation of AMH level and mean follicular diameter. The findings of the present study and others [14] might explain

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The evident relatively smaller number of follicles (<8mm in diameter) in endometriosis group might represent an effect of endometriosis on the development of growing follicles; an effect even appearing in minimal to mild degrees of the disease. Autoimmunity might play role as several studies suggest the presence of correlation between anti-
bodies to endometrial, ovarian and nuclear antigens, and endometriosis [26-28]. Another explanation is the apoptosis of granulosa cells which is caused by disturbance in oocyte growth and maturation and associated with decreased growth differentiation factor-9 (GDF-9) production [29]. Moreover, increased apoptosis with significantly higher incidence of apoptotic bodies in the granulose cells from women with endometriosis were found and it increased with the severity of the disease with reduced steroidogenesis [30].

In conclusion, patients with minimal/mild endometriosis present a decreased serum AMH level with heterogeneous follicular cohort entailing small relative number of small-sized follicles, denoting the low ovarian reserve. The other serum markers for ovarian reserve, including FSH and E2, seem to be less valuable.

Disclosure:
We declare that we have no conflict of interest.

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