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Evaluation of Ovarian Reserve after Laparoscopic Surgery in Patients with Polycystic Ovary Syndrome

Laya Farzadi[1], Mohammad Nouri[2*], Morteza Ghojazadeh[3], Mona Mohiti[3], Esmat Aghadavod[2,4*]

[1]*Women Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
[4]*Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*

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ABSTRACT

Introduction: Ovarian reserve depends on the size and quality of oocytes stored in ovary. Aging and some diseases reduce ovarian reserve, leading to decreased reproductive performance. Laparoscopic surgery is used to treat infertility in women with PCOS. The purpose of this study was measurement of ovarian reserve in patients with PCOS before and after laparoscopic surgery. ***Methods:*** In this study thirty infertile patients with PCOS that had not responded to initial treatment with drug were selected. We assessed the serum levels of anti-mullerian hormone, testosterone, luteinizing hormone (LH), and the number of antral follicles before laparoscopic surgery and 1 week, 3 and 6 months after the surgery. ***Results:*** The average amount of anti-mullerian hormone serum levels was 8.4±4.7 before laparoscopic surgery and 7.5±4.5, 7±4.5, and 7.7±4.4 ng/ml one week, 3 and 6 months after surgery, respectively. Serum levels of LH was 13.6±6.7 and 12.7±11.1 IU /ml (P=0.87) before surgery and 6 months after laparoscopy, respectively. Mean serum testosterone levels were 0.9±0.8, 0.8±0.9 ng/ml (P=0.86) before and after surgery. Annual reproductive rate was 26.7 percent in this population. ***Conclusion:*** Laparoscopic ovarian puncture didn't change anti-mullerian hormone serum, testosterone and LH in women with PCOS and hence has no adverse effects on the ovarian reserve.

Introduction

Ovarian reserve is related to the size, number, and quality of oocytes within follicles. The ovarian reserve is the reproductive ability of ovary that shows number of follicles in it. Aging decreases ovarian reserve and subsequently reproductive ability of women (Hansen *et al* 2008). Markers which are related to the ovarian reserve include age, sonographic variables (such as ovarian volume), Antral Follicle Count (AFC), ovarian stromal blood flow, hormonal parameters (for instance Follicle-Stimulating Hormone (FSH), estradiol (E_2), Luteinizing Hormone (LH), Anti-Mullerian Hormone (AMH)), inhibin B levels and the FSH/LH ratio (Sowers *et al* 2010).To assess ovarian reserve, Gleicher *et al* (2010) measured serum level of FSH, Inhibin B and E2 in the follicular phase. One of the best ovarian reserve determining factors is antral follicle count which is assessed by transvaginal ultrasound tests during the follicular phase. Within the last years, serum AMH measurement has been introduced as one of the best and easiest markers of ovarian reserve (Maheshwari *et al* 2009). AMH or Mullerian Inhibiting Substance (MIS) is a member of transforming growth factor ß (TGF-ß). It is a dimeric glycoprotein which is produced by granulosa cells from birth up to the menopause that controls primary follicles' formation by inhibiting follicular recruitment (Rey *et al* 2003). *In vivo* and *in vitro* studies have showed that AMH inhibits primordial follicles' employment and decreases growing follicles (Dumesic *et al* 2008). Therefore, AMH has an important role in ovarian folliculogenesis and its serum is related to reflective antral follicle numbers. Its principal function is the inhibition of primordial follicle growth organization that is important in dominant follicle selection (Barad *et al* 2009).

Polycystic Ovary Syndrome (PCOS) is an endocrine disorder that is common in 6-8 percent of women of reproductive age. It is the major cause of ovulation-related infertilities. This disease is characterized by ovulation disorders like oligomenorrhea and amenorrhea; in fact there are immature follicles that are unable to ovulate (Dumesic *et al* 2008).

***Corresponding authors:** Mohammad Nouri (PhD), Tel: +98-411-3364666, Fax: +98-411-3373959, Email: nourimd@yahoo.com
Esmat Aghadavod (PhD), Email: aghadavod_m@yahoo.com

Serum AMH evaluation is a practical marker in infertility as it assesses ovarian reserve and recognizes women poor fertility (Daniel *et al* 2009).

Studies show that ageing leads to a decline in female reproductive function due to the reduction in the ovarian follicle pool and in quality of the oocytes (Ficiciog *et al* 2006). Also serum circulating AMH is reduced at the third day of menstrual cycles in healthy women and after ovariectomy. AMH is also a marker of ovarian reserve in patients undergoing Assisted Reproductive Technology (ART) (Chie *et al* 2008).

In recent years AMH has been used as a probable predictor of ovarian response to stimulation during I*n Vitro* Fertilization (IVF) and may be applied as a method for determining ovarian supply (Mashiach *et al* 2010).

Laparoscopic Ovarian Diathermy (LOD) is widely used to induce ovulation in PCOS patients. However thirty percent of patients do not respond to laparoscopic treatment due to an unknown reason. LOD destroys parts of the ovaries and this surgery is not commonly used, however it can be used as an alternative option for infertile women who are still not ovulating in terms of lack of response to the drug (Fernandez *et al* 2011).

Laparoscopy can lead to injuries in ovarian tissue and induce the reduction of ovarian reserve. The extent of damage that affects ovarian reserve correlates with the content of ovarian tissue removed during surgery and the damage to the ovarian vascular system during laparoscopy (Fernandez *et al* 2011). Evaluation of AMH serum levels can be used as a predictor of laparoscopic possible damage to the ovarian reserve. On the other hand, serum AMH levels disappear in cycle bleeding and the whole cycle has minimum intra-cycle changes (Iwase *et al* 2010).

Since previous studies have shown that changes in serum AMH concentrations caused by laparoscopy can be used as an effective and appropriate factor within the ovary in the assessment of damage levels to ovarian tissue (Iwase *et al* 2010), the purpose of this study was to determine the changes in ovarian reserve by the comparison of serum level of AMH before and after laparoscopic surgery and to check whether laparoscopic surgery can probably reduce ovarian reserve.

Materials and methods

Patients

In this cross-sectional study, we selected thirty patients aged from 19 to 35 with infertility problems, PCOS and resistant to treatment with the drug clomiphene citrate. The number of patients and treatments was based on previous studies (Iwase *et al* 2010). These patients were selected based on the table of random numbers.

Setting

The study was performed in the Alzahra educational and medical center in Tabriz, Iran. The Study began in April 2010 and continued for 15 months.

Inclusion criteria

Patients were diagnosed according to the Rotterdam criteria as ovulatory disturbance, hyperandrogenism and presence more than 12 follicles 2- to 9-mm range in each ovary with ultrasound examination by female doctors.

Procedures

Before surgery, about 5cc blood sample was taken from each patient and maintained in tubes containing cloth activator material (serum separation, Deltalab Rubi, SPAIN). The samples were centrifuged with 3000 rpm and the serum was collected at 2ml microtubes and stored at –20° C freezer until subsequent analysis.

During the laparoscopy, the patients were punctured from 6 to 7 points in their ovary with monopolar cautery hook. Serum testosterone, LH, anti-mullerian hormone levels and antral follicles were measured in all patients. AMH was measured using a commercially available enzyme immunoassay kit (ELAab & USCNLIFE, Wuhan ELAab Science Co.Ltd). The lowest detection limit of this assay is 0.053 ng/ml. According to instructions provided in the analysis kit, testosterone levels as well as LH for all the patients was measured using an Enzyme Immunoassay method (EIA, Cayman chemical, 582701). After LOD, the level of serum anti-mullerian hormone was measured in one week, three and six months after that. Serum testosterone and LH were measured six months after surgery. Furthermore, we studied the annual pregnancy rate among these individuals.

Statistical analysis

Analysis was performed using SPSS version 13. Data were expressed as mean±SD or counts and percentages. Differences between groups were assessed by using independent samples' T-test. To evaluate the correlation between quantitative variables, Pearson correlation coefficient (r) was determined. In all cases, $P \leq 0.05$ was considered as significant.

Results

Table 1 indicates the information about age, BMI, duration of use of clomiphene citrate and the number of basic follicles in the subjects. As shown in Table 2, AMH concentrations did not differ significantly before and after surgery. After treatment, annual fertility rate was reported 26.7% (8 cases) among patients. There was a significant positive correlation between base serum anti-mullerian hormone and the number of antral follicles ($r=0.685$, $P \leq 0.05$).

Table 1. Information related to age, BMI, treatment duration with clomiphene citrate and number of basic follicles in patients

Variable	Mean±SD
Age (N=30)	28.4±2.3
BMI	29.1±1.5
Treatment duration with clomiphene citrate (period)	03.9±2.3
number of basic follicles	10.9±2.1

Table 2. Serum AMH, LH, testosterone levels pre- and post-surgery

Variable (ng/ml)	Before Surgery	After 1 week	After 3 months	After 6 months	*P* value
AMH	08.4±4.7	07.5±4.5	07.7±04.5	07.7±04.5	0.70
LH	13.1±6.7	-	-	12.7±11.1	0.87
Testosterone	00.9±0.8	-	-	00.8±00.9	0.86

Discussion

Studies showed that serum AMH levels in women with advancing age decrease and also confirmed that AMH has been one of the best markers of ovarian reserve (Maheswari *et al* 2009). IVF treatment according to the relationship between the number of follicles in the ovary and serum AMH levels demonstrated that AMH is a good predictor to evaluate the ovarian reserve. Therefore, serum AMH levels are affected by reduced number of antral follicle or ovarian tissue injures (Fernandez *et al* 2011).

The question is whether laparoscopy affected ovarian tissue and reduced ovarian reserve. Weerakiet *et al* (2007) investigated the changes in anti-mullerian hormone serum levels before and after LOD. They found that the average serum level of anti mullerian hormone in 21 patients with PCOS was 4.6 ng/ml three days after LOD which was not statistically significant. In our study, average anti-mullerian hormone levels before laparoscopy, 1 week, 3 months and 6 months after laparoscopy did not change significantly. Although one week after laparoscopy serum levels declined, this change was not statistically significant.

On the other hand, previous studies showed that women with PCOS have 2 to 3 times increased level of the serum AMH concentration which was related to increment in the number of small follicles (Dumesic *et al* 2008). According to Elmashad's research, significant decrease in serum AMH levels and AFC were detected at the 1st, 3rd, and 6th cycles after laparoscopy (Elmashad *et al* 2011). This could be explained by possible damage to the ovarian blood vessels and ovarian parenchyma after bipolar electrocoagulation during laparoscopy. The amount of ovarian tissue which is removed during laparoscopy affects AMH produced by antral follicle (Chang *et al* 2010). In this study, in women who experienced ovarian laparoscopy, serum AMH levels were reduced in the first week after surgery and AMH levels recovered 1 and 3 months after surgery to approximately 65% of the preoperative level. Studies indicate that laparoscopic ovarian puncture does not change durably anti-mullerian hormone serum levels in women with polycystic ovary and therefore has no adverse effects on the ovarian reserve (Amer *et al* 2009).

In fact, high levels of serum AMH in patients with PCOS reduce follicles' sensitivity to circulating FSH (La Marca *et al* 2005). Therefore, in these patients follicle selection are prevented and subsequently follicles in small antral phase are stopped. AMH also reduces aromatase activity, resulting in the reduction of the estradiol production from follicles (La Marca *et al* 2005). In patients treated with assisted reproductive technology (ART), AMH levels gradually reduced during taking FSH as a part of Controlled Ovarian Hyperstimulation (COH) (Iwase *et al* 2010).

In our study, slight decrease in AMH levels during FSH taking may be due to the negative effect of FSH on the secretion of AMH. Generally, serum AMH levels during COH have a positive correlation with the number of small follicles; although the serum AMH levels during the maturation of several small follicles decreased (Amer *et al* 2004). Therefore, based on previous studies it can be deduced that the LOD decreased slightly the serum AMH which is a significant factor in the selection of follicles, their maturation and length LOD operation, however AMH levels will not change noticeably; therefore, LOD has no negative effect on ovarian supply. These findings are completely comparable with the results of our research. In our study, there was a significant positive correlation between base serum anti-mullerian hormone and the number of antral. La Marca *et al* (2005) reported similar results with ours.

Amer *et al* examined 29 patients with PCOS after laparoscopic ovarian diathermy and showed that serum level of AMH was 6.1ng/ml which was reduced to 4.7 mg/ml after one week and remained at the same level. Reduction in the AMH levels maybe due to the use of bilateral diathermy technique (Amer *et al* 2004). Api (2009) showed that ovarian reserve of patients with PCOS does not change significantly after LOD; therefore, it can be stated that the possibility of traumatic AMH only shows that the patient's status is normal. Kandi and Salim examined ovarian reserve levels after treatment with clomiphene, unilateral LOD and bilateral LOD and showed that only after bilateral, LOD ovarian reserve reduced significantly.

Therefore, the likeliness of traumatic injury in this form of intervention is zero (Kandi *et al* 2005). Overall, it seems that although LOD leads to the decreased levels of AMH in patients with PCOS, these changes are not statistically significant and only indicate the patient's normality and has no negative impact on ovarian supply.

Due to the limited number of studies conducted in this area further researches need to be carried out in the future. Thereby, we suggest more controlled studies with greater sample size (an emphasis on the role of unilateral and bilateral LOD, separately).

Ethical issues

Before entering patients into the study, all patients signed written informed consent form. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran.

Conflict of interests

Authors declare no conflict of interests.

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