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# Pre and Post Operative Evaluation of Ovarian Reserve using Anti-mullerian Hormone level and Antral follicle count in Patients with Ovarian Endometrioma

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***Abstract***
*Endometriosis is a common finding in women with infertility with suggested negative effect on the ovarian reserve. 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. The classic ovarian reserve markers as early follicular phase serum follicle stimulating hormone (FSH), inhibin B, and estradiol (E2) levels showed limited clinical usefulness. On the other hand, ultrasonographic antral follicle count (AFC) assessment has been shown to be good predictor of ovarian reserve and response. AMH is reliably used for the prediction of ovarian response, being a marker that can estimate the quantity and activity of follicles in early stages of maturation. The AFC and AMH are the most significant predictors of poor response to ovarian stimulation during ART. Therapeutic approach for women with ovarian endometrioma may vary according to the age of women, size of the cyst, symptoms, and desire for future conception. Laparoscopic cystectomy for endometrioma is common and seems to be feasible in terms of postoperative fecundability and recurrence rate compared with that of fenestration and coagulation of the cyst wall. However, the safety of this technique with respect to residual ovarian damage has been questioned. Pre-and post-operative evaluation of ovarian reserve in endometriotic patients by using AMH and AFC. This is a prospective study comparing ovarian reserve following surgical treatment of endometriomas by either open cystectomy or through laparoscopy. This study was conducted on 80 infertile patients' due to ovarian endometriosis aging from 20-35 years old. It was performed at Al-Azhar university hospital, New Damietta. All patients are either subjected to laparoscopic or open cystectomy. Antral follicle count is measured by transvaginal ultrasonography in the early follicular phase by taking the mean of two perpendicular measurements. Also, serum Anti-müllerian hormone was measured in the same day. Pre- and post-operative evaluation after 2weeks of procedure was done using the same previous parameters. Present study revealed that AMH decreased significantly after surgery ($p < 0.001$). There was no significant difference between laparoscopic and open surgery groups ($p = 0.648$). Antral follicle count increased significantly after surgery ($p < 0.001$). However, it was significantly higher post-operatively in the laparoscopic group ($p < 0.001$). Although laparoscopic treatment of ovarian endometriomas carries some advantages compared to open laparotomy dealing with such a problem, both techniques possess the risk of decreasing ovarian reserve. The two techniques have a rather good result concerning pregnancy rate, however long-term effects of depleting ovarian reserve need a longer follow up trials.*

***Keywords:*** *Endometriosis,Ovarian endometrioma, laparoscopic surgery, Ovarian reserve*

## Introduction

Endometriosis is defined as the presence of endometrial-like tissue (glands or stroma) outside the uterus, which induces a chronic inflammatory reaction (Kennedy S. et al., 2005). The exact prevalence of endometriosis is unknown but estimated to range from 2 to 10% in women of childbearing age. Its prevalence rises up to 50% in women with infertility (D'Hooge et al., 2003). In women with endometriosis, there is a reduced monthly fecundity rate (2–10%) compared with fertile couples (15–20%). Although endometriosis impairs fertility, it does not usually completely prevent conception (The Practice Committee of the American Society for Reproductive Medicine,2004). The correlation between endometriosis and infertility is well documented, with monthly fecundity reported as 0.02 to 0.1, compared with couples without endometriosis of 0.07 to 0.2 per month (Rodriguez-Escudero et al., 1988). Despite this reduction in fecundity, a causal relationship has not been proven with theories including distorted anatomy, failure of implantation, hormonal imbalances, and peritoneal dysfunction (Giudice L. C 2010). Laparoscopic excision of endometriosis has been shown to significantly reduce pain and improve quality of life however the effect on fertility is still debated (Meuleman C et al., 2014). Surgery's aim is to

remove macroscopic endometriosis implants and restore normal pelvic anatomy. However, surgery may not be able to completely restore pelvic anatomy or to stop inflammatory process. Hence, it is important to weigh up benefits versus harm of surgical procedure. Laparoscopy is preferred to laparotomy because of advantages of minimal tissue damage, of magnification, of faster recovery, and shorter hospital stay (Royal College of Obstetricians and Gynecologists, 2006).

The ESHRE guideline for the management of women with endometriosis, recommended that clinicians should counsel infertile women with endometrioma regarding the risks of reduced ovarian function after surgery and the possible loss of the ovary. The decision to proceed with surgery should be considered carefully if woman has had previous ovarian surgery (Dunselman GA, 2014).
There are two types of tests of ovarian reserve: static and dynamic. Static tests assess specific parameters relating to ovarian reserve at a single point in time and involve both ultrasound and biochemical parameters. Dynamic tests assess ovarian response to exogenous stimulation. Usually this involves measurement of hormonal concentrations in a serum sample before and after stimulating the ovaries using FSH, clomiphene citrate (CC) or a gonadotrophin-releasing hormone (GnRH) agonist (Broekmans et al., 2006). It is widely accepted that the reduction of AMH levels in serum is the first indication of a decline in the follicular reserve of the ovaries. AMH concentration remains stable throughout the menstrual cycle. Recent data, however, have shown that there are fluctuations throughout the cycle (with lower levels during the early secretory phase) or even between consecutive cycles.7 Nevertheless, these fluctuations are not considered clinically significant to recommend the measurement of AMH (Streuli I, etal., 2009).Anti-Müllerian hormone (AMH) is a homodimeric glycoprotein, a member of the transforming growth factor-β superfamily (Panidis D, et al., 2010).AMH levels accurately reflect the ovarian follicular reserve and could, therefore, be considered as an extremely sensitive marker of ovarian aging and a valuable tool in the diagnosis and the recognition of recurrence of granulosa cell tumors. Furthermore, AMH evaluation is of clinical importance in predicting the success of in vitro fertilization (La Marca A et al., 2010). The number of the small AFs is related to the size of the primordial follicle pool. With the decrease in the number of the AFs with age AMH production appears to diminish and become undetectable at and after menopause. AMH levels strongly correlate with basal antral follicle count (AFC) measured by transvaginal ultrasonography. Unlike other biochemical markers, it can be measured on any day of the cycle and does not exhibit intercycle variability (Broer et al., 2011).

AMH and AFC are currently the two most commonly used methods to assess ovarian functional reserve (La Marca et al., 2010). However, pre - and post- operative evaluation for cases with ovarian endometrioma is still matter or research.

**Materials and methods**

*Patients and methods*

The study included 80 infertile patients due to ovarian endometriosis aging from 20-35 years old. It was performed at Al-Azhar university hospital, New Damietta, Obstetrics and Gynecology Department.

*Inclusion criteria include,*

Infertile patients (primary infertility) excluding male factor; Patients with regular menstrual cycle; Age: 20-35 years; BMI: 22-37 $kg/m^2$; Endometrioma volume: 2.1-3 $cm^3$

*Exclusion criteria include,*

Female patient who do not reach menarche or reach menopause or women with premature ovarian failure. Also previous ovarian operation is excluded.

After selection, counseling, explaining the procedure to all participants, and obtaining a written consent to participate in the study and the following were taken:

1- Full history taking: included age, parity, history of infertility and its type, duration of infertility and any other medical or surgical history.
2-Complete clinical examination: included general and full gynecological examination.
3- Investigations:
a- Ultrasonography: ultrasound scan was performed using Voluson 730 Pro machine (GE, Milan, Italy) equipped with a multifrequency volume endovaginal probe. Ovarian endometrioma was diagnosed if the typical pattern of *''a circular-shaped homogeneous hypoechoic mass of low-levels echoes''* was detected on B-mode ultrasonography. The endometrioma volume, antral follicle counts (AFC) was assessed bilaterally. The endometrioma volumes were calculated by the formula: height × length × width × 0.5233 and expressed in $cm^3$. Antral follicle count is measured by transvaginal ultrasonography in the early follicular phase by taking the mean of two perpendicular measurements.
b- Serum Anti-müllerian hormone: it was measured on 3rd day of menstrual cycle. The AMH is measured using immunoassay.

Patients are subjected to either laparoscopic or open cystectomy. Patients were then divided into two main groups viz.,
** Group A: patients undergoing open cystectomy using mono- polar diathermy.
** Group B: patients undergoing laparoscopic excision of the endometriomas using mono-polar diathermy.

Follow up of the patients was done after 2 weeks of operative procedure with the same parameters used before surgical management.

*Statistical analysis of data:* the collected data were organized, tabulated and statistically analyzed using SPSS, version 16, running on IBM compatible computer.

**Results**

This study was conducted at Al-Azhar university hospital, New Damietta, on 80 Infertile women (primary infertility) with ovarian endometriosis aging from 20-35 years old. Patients are subjected to either laparoscopic or open cystectomy. Patients were then divided into two equal groups, 40 patients in each. The mean age of studied women was 27.8±4.4 years, ranging from 20 to 35 years as shown in table (1)

**Table 1:** Age distribution of the studied group

| | Mean±SD | Median (Range) |
|---|---|---|
| **Age (Years)** | 27.8±4.4 | 28 (20-35) |

Endometriomas involved both ovaries in 16 patients (20%). The right ovary was affected in 35 cases and the left in 29 cases (Table 2). The mean value of the largest diameter of the affected ovary was 7.5±2.2 cm.

**Table 2:** Characteristics of the affected ovaries in the whole studied group

| Site | Number | % |
|---|---|---|
| **Right ovary** | 35 | 43.8 |
| **Left ovary** | 29 | 36.6 |
| **Bilateral** | 16 | 20.0 |
| **Largest Diameter (mean±SD, cm)** | 7.5±2.2 | |

**Table 3:** Anti-mullerian hormone (AMH) and antral follicle count (AFC) before and after operation.

| | | Mean±SD | Median (Range) | p value |
|---|---|---|---|---|
| **AMH** | **Before surgery** | 9.0±4.4 | 9.1 (0.9-23.7) | < 0.001 |
| | **After Surgery** | 2.9±1.8 | 2.5 (0.1-11.3) | |
| **AFC** | **Before surgery** | 2.8±1.2 | 3.0 (1.0-5.0) | < 0.001 |
| | **After Surgery** | 7.1±1.9 | 7.0 (3.0-12.0) | |

*AMH decreased significantly after surgery (p < 0.001). Total antral follicle count increased significantly after surgery (p < 0.001).*

**Table 4:** Correlation coefficient and significant of correlation of AMH and TAFC with age, duration of infertility and ovarian size in the whole studied group

| | | AMH | AFC |
|---|---|---|---|
| **Age** | Correlation coefficient | 0.074 | -0.457 |
| | P vale | 0.514 | <0.001 |
| **Size** | Correlation coefficient | 0.102 | -0.340 |
| | P vale | 0.367 | 0.002 |
| **AFC** | Correlation coefficient | -0.014 | |
| | P vale | 0.904 | |

Concerning levels before surgery, there was a good negative correlation between age and total antral follicular count (r = -0.457, p < 0.001). Also, there was a weak negative correlation between size of the affected ovary and TAFC (r = -0.340, p = 0.002). Meanwhile the level of AMH was not correlated to age, ovarian size or TAFC (Table 4).

**Discussion**

The potential deleterious effects to ovarian reserve associated with excision of endometriotic ovarian cysts did not pay much attention. There are at least 2 reasons to explain this. First, evaluation of ovarian reserve remains an elusive task of reproductive medicine. Because ovarian function cannot be measured directly, ovarian response to gonadotrophin hyper-stimulation is currently considered the most appropriate surrogate measurement for ovarian reserve. The use of serum dosages (follicle- stimulating hormone [FSH], inhibin B, 17β-estradiol, FSH/luteinizing hormone ratio, anti-müllerian hormone) and/or ultrasound variables (ovarian volume, antral follicle count, ovarian stromal blood flow) may be of help but are still considered less informative (Bukman&Heineman, 2001). Second, in most cases, only 1 gonad is involved (Vercellini et al., 1998; Al-Fozan&Tulandi, 2003). The contra-lateral intact ovary

does usually supply to the reduced function of the affected gonad (Lass, 1999). This study compared ovarian reserve following surgical treatment of endometriomas by either open surgery or laparoscopic surgery endometrioma. The study included 80 patients suffering from primary infertility with ovarian endometrioma. The 80 patients were divided into 2 equal groups, 40 patients in each. Forty patients underwent open cystectomy. The other forty patients underwent laparoscopic excision of the endometriomas with mono-polar diathermy. All patients were subjected measurement of anti-mullerian hormone (AMH) and antral follicle count (AFC) before and after surgery. AMH decreased significantly after surgery ($p < 0.001$). There was no significant difference between laparoscopic and open surgery groups ($p = 0.648$). Antral follicle count increased significantly after surgery ($p < 0.001$). However, it was significantly higher post-operatively in the laparoscopic group ($p < 0.001$). A recent study demonstrated that ovarian endometrioma perse is associated with reduced ovarian reserve, and laparoscopic cystectomy can further have exerted significant damage on ovarian reserve. Like our findings, authors found that serum AMH level in patients with previous cystectomy was significantly lower than patients with endometrioma without previous cystectomy (Hwu et al., 2011).

Contrary to our results, Hwu et al. (2011) reported that bilateral endometriomas had a more profound impact on serum AMH levels than unilateral endometriomas, regardless of either surgical cystectomy or conservative management. A recent meta-analysis of 8 studies involving 237 patients showed a statistically significant decrease in serum AMH concentration after ovarian cystectomy. These results suggested a negative impact of excision of endometriomas on ovarian reserve (Raffi et al., 2012). The rate of decline of the serum AMH level was significantly higher in the bilateral group than the unilateral group in another study (Hirokawa et al., 2011). In agreement with our results, Lee et al. (2011) concluded that laparoscopic ovarian cystectomy for endometrioma decreases serum AMH levels immediately after surgery. Serial measurements found that significant drop of serum AMH remained for up to 3 months. They reported similar patterns of change after cystectomy or oophorectomy. Ercan et al. (2011) compared the operated and non-operated ovaries after laparoscopic stripping in 36 patients to evaluate the ovarian reserve based on ovarian volumes, antral follicle counts, stromal blood flows and AMH levels. All patients had unilateral ovarian endometrioma. In agreement with our results, mean antral follicle counts (AFC) of the operated side ovaries were significantly lower on the second postoperative day and in the third month. They also reported a non- significant decrease in AMH levels in the third month after surgery. They concluded that endometrioma surgery techniques are important in preserving normal functioning ovaries and further studies are necessary for optimizing these surgical approaches. Ragni et al. (2005) previously concluded that laparoscopic excision of endometriomas has a quantitative rather than qualitative damage to ovarian reserve. They prospectively evaluated ovarian response to hyper-stimulation in women selected for IVF/ICSI who previously underwent laparoscopic enucleation of a unilateral endometrioma. They confirmed the evidence that laparoscopic cystectomy for endometrioma is associated with a reduced responsiveness to ovarian hyper-stimulation. However, fertilization rate and rate of high- quality embryos were not affected by previous surgery. These authors also documented a reduction in number of dominant follicles, oocytes retrieved, and embryos obtained of about 50%. A successful operation means not only removal of ovarian pathology but also maintenance of ovarian function (Yu et al., 2010). In the current study, we achieved an overall success rate of 48.8%. The success rate was higher in the laparoscopic group (57.5%) compared to open surgery group (40.0%), however the difference was not significant ($p = 0.117$). Hart et al. (2008) reported good evidence that excisional surgery for endometrioma provides for a more favorable outcome than drainage and ablation regarding the recurrence of the endometrioma, recurrence of pain symptoms, and subsequent spontaneous pregnancy. Consequently, this approach should be the favored surgical approach.

In a prospective randomized study to compare laparoscopic versus open surgery for the treatment of endometriomas revealed short term benefits of laparoscopy over laparotomy including less postoperative pain, less need for analgesics, shorter hospital stay and shorter period before resuming full normal activities (Mais et al. 1996). However, Chapron et al. (2002) commented that laparotomy might be indicated in cases of severe endometriosis associated with dense extensive adhesions (Crosignani & Vercellini, 1996). Milingos et al. (1999) also compared endoscopic versus laparotomy for the treatment of ovarian endometrioma. The pregnancy rates, monthly fecundity and cyst recurrence rates were comparable. The advantages of laparoscopy over laparotomy were also suggested by several studies (Adamson et al., 1992; Bateman et al., 1994; Catalona et al., 1996; Crosignani et et al., 1996; Busacca et al. 1999; Sawada et al., 1999).

**Conclusion**

Endometrioma perse may contribute to decreased ovarian reserve. A considerable body of molecular, histological and morphological evidence suggests that in cases of endometrioma, normal ovarian cortical tissue adjacent to the cyst is replaced by fibrosis leading to reduce follicular density and eventually decreased ovarian reserve. Stretching of the tissue nearby the cyst however has no role in declined ovarian reserve. The condition is known to leave a significant negative effect on ovarian reserve. Both superficial and deep infiltrating endometriosis decrease the ovarian reserve, ovulation rate and response to ovulation induction. As already pointed out, AFC and AMH are the most reliable markers for ovarian reserve and given the unique characteristics of AMH in progressive disease like endometriosis, its assay can potentially predict the ovarian reserve decrement. Laparoscopy is the preferred technique for diagnosis since endometriosis is located primarily on the pelvic organs. The optimal timing of laparoscopy at a specific time in the menstrual cycle is unclear, but it should not be performed during or within three months of hormonal treatment to avoid under-diagnosis. Ideally, if surgery is performed for diagnosis, consent is obtained for surgical resection/ablation of endometriosis at the same time. Although laparoscopic treatment of ovarian endometriomas carries some advantages compared to open laparotomy dealing

with such a problem, both techniques possess the risk of decreasing ovarian reserve. However long-term effects of depleting ovarian reserve need a longer follow up trials.

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