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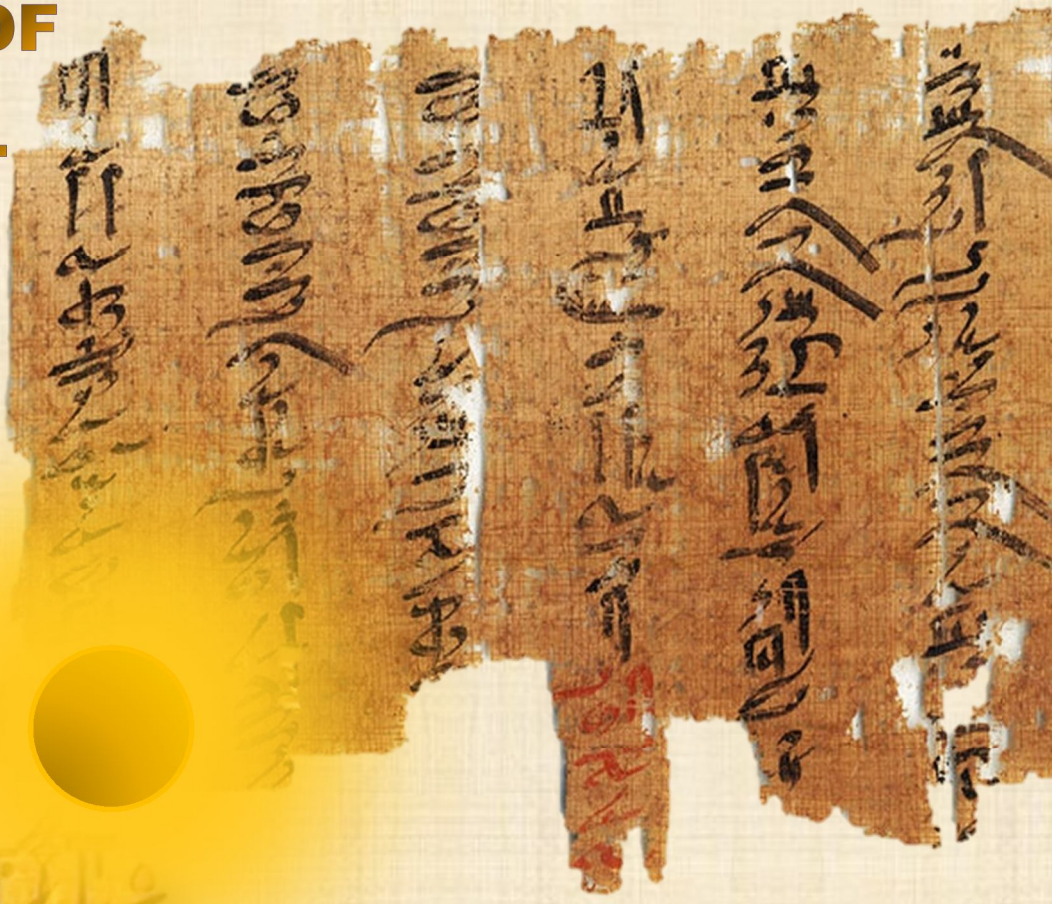

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Original article

Ovarian Reserve after Laparoscopic Ovarian Cystectomy in Infertile Patients

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ABSTRACT

Background: Ovarian reserve is the key target of infertility treatment; its accurate evaluation is extremely important for outcomes prediction of in-vitro fertilization [IVF]. Ovarian cysts act as a common gynecological site that occurred in 6.6% of patients between twenty-five and forty years old. When its surgical removal is indicated, stripping the ovarian cyst wall by laparoscopic approach is the technique of choice.

Aim of the Work: The present study was designed to evaluate ovarian reserve after laparoscopic ovarian cystectomy by using basal Follicular Stimulating hormone [FSH], Anti-Mullerian hormone [AMH], and Antral follicle count [AFC] as a marker for evaluation.

Patients and Methods: This was an observational study which was conducted on infertile patients after laparoscopic ovarian cystectomy from the Obstetrics and Gynecology department Al-Azhar University hospital [New Damietta] from October 2018 to last of November 2019.

Results: AFC had been increased significantly post-cystectomy when compared to corresponding preoperative values [17.8±2.24 vs 5.57±3.16 respectively]. On the other side, both FSH and AMH significantly decreased after cystectomy when compared to preoperative values. These results are constant regardless of the type of the cyst, the surgical procedure, or its polarity.

Conclusion: The FSH and AFC levels increased after laparoscopic cystectomy for benign ovarian cysts, indicating significant ovarian damage post operatively.

Keywords: Follicle Stimulating Hormone; Luteinizing Hormone; Antral Follicle Count; Ovarian Cystectomy; Ovarian Reserve

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* Main subject and any subcategories have been classified according to research topic.

INTRODUCTION

Ovarian reserve is a term used to assess the ability of the ovary to provide the fertilizer for a safe and productive pregnancy. Basal follicles stimulating hormones, anti-Mullerian hormones, inhibin-B, base estradiol, clomiphene citrate test, ovarian ova reserve test, ovarian vascularity, ultrasound parameters such as follicles counts, ovarian volume and ovaries may be evaluated in ovarian reserves[1].

Ovarian cyst is a growing concern in women both of reproductive age and those undergoing infertility research. Surgical care of laparoscopic cystectomy is also the procedure to reduce possible complications such as ruptures or malignancy while maximizing fertility preservation. The cyst's effect on folliculogenesis depends on the existence, dimensions and number of cysts[2].

Endometrioma is a type of ovarian growth of endometrial tissue. This affects women during their pregnancy and can cause chronic menstrual pelvic pain. The endometrioma is lined with endometrium tissue that produces the internal fluid from the discharge of active implants into the cyst through the accumulation of menstrual debris. Repeating inflammation allows cells to proliferate in a cytokine cascade and in reactive oxygen species[3]. The most common surgical technique is laparoscopic cyst excision including stripping of the cyst wall; this is preferred than ablation or drilling of the cyst. Excision of the cyst wall has several advantages, such as better success in relieving pain symptoms, a lower recurrence rate and an increased chance of spontaneous pregnancy[4].

The anti-Mullerian hormone [AMH] belongs to the β-class of growth factor transforming and falls from primary granulosa cells to small antral follicles, and is associated with the small antral follicles. The anti-Mullerian hormone is the only menstrual cycle independent ovarian marker that does not need oral contraceptive or gonadotropin releasing hormone [GnRH] agonists [5]. The AMH acts as an ovarian reserve marker, provide many advantages over other markers; it is stable during the menstrual cycle, and is fairly autonomous from the use of hormone therapy[6]. AMH level decrease after surgical cyst excision[7].

Determination of ovarian reserve after surgical interventions for ovaries are of utmost importance. A condition that is not adequately investigated.

AIM OF THE WORK

The aim of our study was to evaluate ovarian reserve after laparoscopic ovarian cystectomy using AMH, basal follicular stimulating hormone [FSH] and AFC as a marker for evaluation.

PATIENTS AND METHODS

This was an observational study conducted at Al-Azhar University [New Damietta] from October 2018 until November 2019, by the participation of infertile patients after laparoscopic ovarian cystectomy. After interviews and laboratory tests, eligible patients had given written consent. In addition, the study protocol had been approved by the local Institutional Review Board [IRB] [ADIM-IRB 15032019]. An administration consent was also obtained. In addition, patient confidentiality and privacy have been respected.

The inclusion criteria were: [age 20-35 years, patients after laparoscopic ovarian cystectomy and infertile lady [primary or secondary]. Otherwise, exclusion criteria were patients < 20 years and > 35 years, patients with history of previous ovarian surgery, history of chemotherapy or radiotherapy, history of smoking, family history of early meno-pause and history suggestive of any endocrinal disease.

Patients eligible for the study were submitted to the following: full history, complete examination including general, abdominal, pelvic clinical and ultrasound examination. Ultrasound scan was performed using a Voluson 730 Pro machine [GE, Milan, Italy] equipped with a multifrequency volume endovaginal probe. Antral follicles count had been completed by 2D transvaginal ultrasound, as described before[8]. The AMH was estimated by the enzyme-enhanced, two-site immunoassay AMH Gen II ELISA[9]. An immunocometrical test [FSH IRMA, Laboratory of Diagnostics, TX, USA], was used to measure serum FSH[10].

Laparoscopic ovarian cystectomy: Patient was anesthetized and put in the lithotomy position, the abdomen and vagina were sterilized and draped thoroughly. The umbilicus introduced 10 mM laparoscope, Veress needle, whereas the three 10 mm accessory trocars were placed sub-umbilically 5 cm from the superior iliac spine to introduce the

auxiliary instruments after initial diagnostic assessment of pelvis and abdomen and normal saline washing. Different techniques of ovarian cystectomy were recorded either stripping, aspiration and excision or ovarian cystectomy. Also, the usage of electric energy either mono- or bi-polar. Then and after 5 months, AFC, basal FSH and AMH were assayed again. For histo-pathological examination, the samples excised from the patient were used, which were preserved in a formalin solution of 10%. Tissue samples were taken using paraffin blocks, various sections were obtained and hematoxylin & eosin [Hx and E], stained and examined under a light microscope.

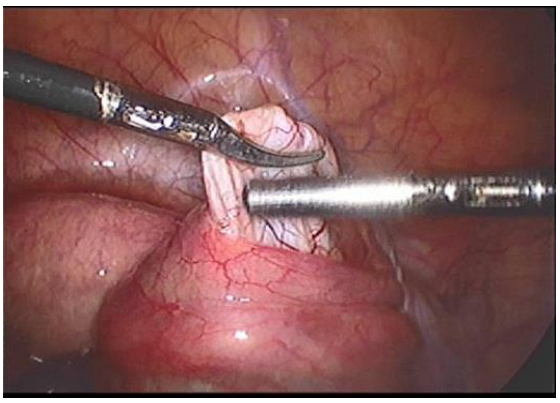

Figure [1]: Functional cyst

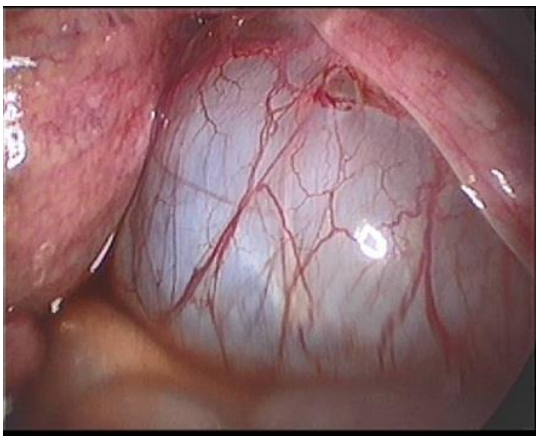

Figure [2]: Removal of cyst wall

Figure [3]: Dissection cyst wall by bipolar

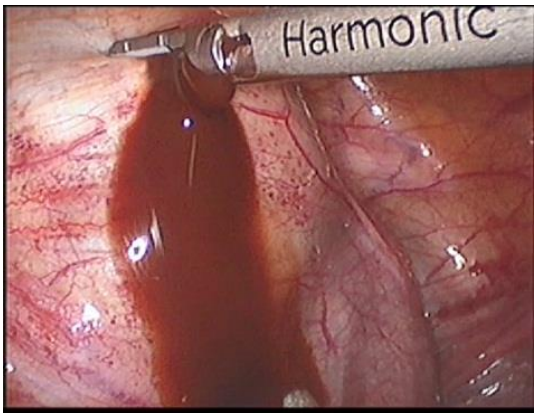

Figure [4]: Dissection cyst wall by harmonic

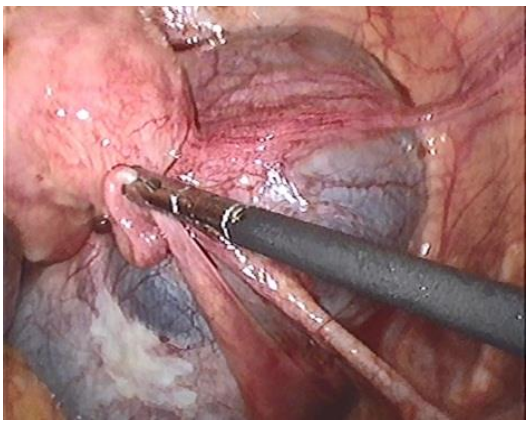

Figure [5]: Drilling ovarian cyst by unipolar

Statistical analysis: The data collected were organized, tabulated and analyzed statistically using IBM-compatible IBM-enabled version 19 [SPSS Inc., Chicago, USA] statistical package for social sciences [SPSS]. Frequency and percentage distributions have been calculated for qualitative data. Minimum and maximum are calculated for quantitative data, mean, standard deviation [SD]. The independent samples [t] test was used for comparison between two groups. All tests have been judged to have p-value < 0.05. All tests were considered to have a p value of > 0.05.

RESULTS

Table [1] presented the demographic data of the studied patients. Most of our patients had primary infertility [64%]. The mean infertility duration was 2.22±1.48 years. There were 9 [18%] simple cysts with focal inflammation of the fibrous wall and sloughed lining, 11 [22%] endometriotic cysts, 12 [24%] benign serous cyst, 11 [22%] hemorrhagic retention cyst with fibrolytic wall and 7 [14%] cystic teratomata. AFC increased significantly after surgery than before surgery, and associated with significant

reduction of FSH and AMH after surgery **[Table 2].** In addition, total antral follicle count was lower among patients before surgery than post ovarian cystectomy and associated with significant increase of AMH and FSH in patient before surgery than post-ovarian cystectomy **[Table 3].**

There were 13 cases [26%] with unipolar, 21 [42%] with bipolar and 16 [32%] with harmonic laparoscopic ovarian cystectomy. AFC increased significantly in post unipolar, bipolar and harmonic laparoscopic ovarian cystectomy when compared with values before surgery and associated with significant reduction of AMH and FSH after surgery when compared to corresponding preoperative values **[Table 4].** AFC increased significantly in post-laparoscopic excision ovarian cystectomy when compared with corresponding preoperative values [5.96±0.67 Vs 8.53±1.68], FSH was higher among patients before surgery [10.81±0.71] than post ovarian cystectomy group [6.84±0.73] and AMH decreased after laparoscopy when compared with values before it [1.56±0.14 ng/ml Vs 0.71±0.15 ng/ml]. AFC increased significantly in women post laparoscopic stripping ovarian cystectomy when compared with pre-surgery [6.26±1.27 Vs 8.77±0.59], FSH was higher among patients before surgery [10.8±0.46] than post intervention [6.46±0.58] and AMH decreased after operation when compared with before [1.6±0.26 ng/ml Vs 0.79±0.09 ng/ml] with statistical significant differences **[Table 5].**

Table [1]: Demographic data of the studied groups

Variable	Cases [n= 50]
Age [years]	24.49 ± 4.2
BMI [Kg/m²]	25.09 ± 2.74
Parity	1.06 ± 0.8
Period of infertility [years]	2.22 ± 1.48

Table [2]: Comparison between AFC, FSH and AMH regarding histo-pathological diagnosis of the studied cases.

	AFC		FSH		AMH	
	Pre	Post	Pre	Post	Pre	Post
Simple cyst [9]	4.93±0.75	8.5±2.3*	10.83±0.49	7.27±0.5*	1.63±0.29	0.83±0.06*
Endometriotic cyst [11]	6.3±1.5	8.8±0.55*	10.27±0.81	7.17±0.67*	1.6±0.26	0.83±0.06*
Benign serous cyst [12]	6.47±1.54	8.6±0.52*	10.77±0.84	6.76±1.23*	1.7±0.17	0.83±0.06*
Hemmorgic cyst [11]	6.67±1.01	9.0±0.71*	10.07±0.64	6.57±0.57*	1.5±0.17	0.77±0.11*
Cystic teratomata [7]	5.6±0.01	8.7±0.57*	11.15±0.21	6.0±0.56*	1.4±0.56	0.7±0.14*

Table [3]: Antral follicle count among studied groups

	Pre	Post-cystectomy	*t*	P
AFC	5.57±3.16	17.8±2.24	17.25	<0.001*
FSH [U/L]	9.74±1.14	6.43±0.83	17.25	<0.001*
AMH [ng/ml]	1.78±0.14	0.61±0.25	7.06	<0.001*

Table [4]: AFC, FSH and AMH among studied groups as regard usage of unipolar, bipolar and harmonic laparoscopic

		Pre	Post-cystectomy	t	P
Unipolar [13]	**AFC**	3.96±2.91	15.84±3.17	6.17	<0.001*
	FSH [U/L]	9.36±1.06	6.62±0.29	5.57	<0.001*
	AMH [ng/ml]	1.45±0.52	0.52±0.06	3.05	0.01*
Bipolar [21]	**AFC**	6.57±3.44	17.71±0.78	10.47	<0.001*
	FSH [U/L]	10.01±1.3	6.2±0.88	8.02	<0.001*
	AMH [ng/ml]	2.21±1.44	0.56±0.3	3.69	0.001*
Harmonic **[16]**	**AFC**	4.17±2.85	19.98±0.51	14.43	<0.001*
	FSH [U/L]	8.97±0.89	5.8±0.7	7.41	<0.001*
	AMH [ng/ml]	1.6±0.58	0.68±0.17	3.99	0.002*

Table [5]: AFC, FSH and AMH among studied groups as regard usage of laparoscopic excision and stripping

		Pre	Post-cystectomy	*t*	P
Excision [26]	**AFC**	5.96±0.67	8.53±1.68	3.43	0.002
	FSH [U/L]	10.81±0.71	6.84±0.73	12.037	<0.001*
	AMH [ng/ml]	1.56±0.14	0.71±0.15	12.86	<0.001*
Stripping [24]	**AFC**	6.26±1.27	8.77±0.59	3.23	0.003
	FSH [U/L]	10.8±0.46	6.46±0.58	18.44	<0.001*
	AMH [ng/ml]	1.6±0.26	0.79±0.09	9.26	<0.001*

DISCUSSION

Laparoscopic ovarian cystectomy is often considered as the preferred approach for the treatment of benign ovarian cysts. In comparison to laparoscopy, a quicker recovery, better and closer vision, shorter hospital stays and lower possible adhesion formation are associated with laparoscopy[11].

The technique used for surgery is therefore essential for the saving of ovarian tissues and residual ovarian function after opera-treated treatment; it is important to consider benign ovarian cystics such as ovarian endometrioma[12]. AMH and AFCs were often regarded as biomarkers inter-changeable for ovarian reserve detections[13].

AMH is made of pre-antral and early antral cells, it controls oocyte recruitment and folliculo-genesis[14].

Its serum measurement is applied to a variety of clinical applications based mainly on its ability to reflect the number of antral and pre-antral in ovaries[15]. After its introduction in endocrinology more than a decade ago, AMH has quickly become the best available marker on the ovarian reserves[16].

AFC has been the subject of increasing interest during the last decade from the improvement of ovarian ultrasonography[17].

There has been considerable research interest in the impact of endometriomas on the ovarian reserve per se and their operative removal[18]. Thus, the aim of this work is to evaluate ovarian reserve after laparoscopic ovarian cystectomy using AMH, basal FSH and AFC as a marker for evaluation.

In the present study, AFC increased significantly in patient after ovarian cystectomy. This results agreed with **Pados et al.**[19] who reported significant increase of AFC six month after laparoscopy.

After cystectomy, AFC was found to be significantly increased. It had been speculated that it can lead to a lack of account of preoperative AFC beyond the ovarian tumor or to an ovarian parenchyma reactivation after operation[20]. The AFC was largely used in studies to estimate the ovarian reserve of women under surgery.

Biacchiardi et al.[21] assessed AFC among 43 women with endometrioma before surgery, they reported that the AFC in the affected ovary was significantly lower than in the healthy ovary [3.3±3.2 Vs 8.4±6.0, respectively]. In addition, **Uncu et al.**[22] reported significantly lower endometrioma AFCs than controls women.

In contrast to our results, **Ercan et al.**[23] assessed AFC for 36 women with unilateral endometrioma in comparison with the contralateral ovary before surgery. The difference between the endometrioma and the contra-sided ovaries was not statistically significant. [4.5±2 Vs 5.2±3.5].

In addition, the combined analysis of pre-operative AFC shows that, for the ovary, the mean of AFC is below the contralateral but not statistically significant [p=0.20][24]. The impact of sample size,

the different age groups and the severity of the disease could lead to this variation[13].

The impact of the surgery for an ovarian endometrioma on the AFC assessed ovarian reservation was examined and meta-analyzed systemically. Of the 24 detailed studies considered, 13 were taken into account, including a total of 597 patients, for both data extraction and meta-analysis. This study showed that after operation, the AFC of the ovary operated did not change significantly. In addition, operated ovary demonstrated significantly lower AFC than the contralateral ovary, although, as previously mentioned, it was already before the operation. This difference was present. The results concluded that endometrioma surgery does not significantly affect the AFC assessed ovarian reserve[24].

In the present work, basal Follicular stimulating hormone [FSH] results agree with **Bulletti et al.[25] and Zaitoun et al.[26]** that showed significant increase in follicle stimulating hormone level for benign ovarian cysts after laparoscopic cystectomy.

The ovarian reserve after surgery is related with tumor recurrence when the ovarian tumor is reported with reproductive age group. The laparoscopic cystectomy is considered as a first line treatment in specific endometriomas by stripping technique. The removal of ovarian tissue is led by the removal of any benign cyst inadvertently; however, the impact is more substantial in endometriomas. It has been observed that due to the presence of a pseudocapsule, this difference was observed in an endometrioma as compared to a real capsule in a non-endometriotic cyst, which already had an independent tissue plane allowing the dissection from tissue easily[27].

Van der Steeg et al.[28] found that serum FSH levels declined significantly 3 months post-operatively in ovarian endometriomy patients compared to non-endo-metriomal cysts.

Celik et al.[29] reported that FSH serum levels are not different than preoperative levels at 6 months after surgery.

As regard to serum AMH, current results are consistent with **Celik et al.[29]** who measured the serum AMH until 6 month after cystectomy and noticed it gradually declined.

Chang et al.[30] reported that the serum AMH declined gradually after cystectomy after one month and that 65% preoperative level recovered 3 months later.

The impact of surgery on ovarian reserves for endometrioma was examined and meta-analyzed in 2012 based on serum AMH. Due to the hetero-geneous designs and the variables reported. **Somigliana et al.[31]** had included 11 articles. After surgery, nine papers showed a decline in AMH levels. There were 21 studies, 8 for meta-analysis and 29 for inclusion in systems evaluation, identified in a further study. Pooled analysis of 237 patients showed a substantial decrease, although heterogeneous, in serum AMH levels after ovarian cystectomy [1.13ng/ml]. The heterogeneity was decreased in the sensitivity analysis of the baseline AMH level < 3.1 ng/mL, yet the AMH concentration [1.52ng/mL] was significantly reduced post-operatively[7].

The mean AMH level reported by **Shebl et al.[32]** was considerably lower than in the control group [2.75±2.0 ng / ml Vs 3.46±2.30 Ng / ml, p<0.001].

In addition, 195 patients were involved in a prospective case-control trial: 130 fertile patients and 65 patients, diagnosed with laparoscopy and histology, with stage III and IV endometriosis. AMH serum levels were measured in both groups. Cases had statistically significantly lower mean AMH serum levels [0.97±0.59 ng/ml] than control [1.72±0.63 ng/ml] [p = 0.001][33].

Additionally, 102 women with endometrioma versus 102 body mass index [BMI] matched controls have also participated in another case – control retrospective study. In Endometriotic cases, serum

AMH and AMH median multiples [AMH-MoM] were lower, but statistically this wasn't significant. Women with stage IV endometriosis also had lower AMH and AMH-MoM serum than controls[34].

The prospective study in Turkey showed that, in baseline, women with infertility had considerably lower levels of AMH than the controls [2.81±2.15 ng / ml vs 4.20±1.26 ng/ml] to evaluate their ovarian reservations before [cases versus controls] and after surgery [cases at baseline versus 1- and 6-month follows][22]. The lower sample size and the high values of AMH among both cases and controls should be considered.

In another prospective research 40 women with endometriomas [as study group] diagnosed as infertility, 36 women with infertility factor tubes [as the control group 1], and 22 women with other benign ovarian cysts [as the control group 2] were also reported. The impact on ovarian reserves of endometrium and laparoscopic cystectomy was assessed by serum AMH levels. Prior to the operation, the group with endometrioma had markedly reduced levels of A MHOs [1.53±1.37 ng/ml] as compared with other ovaries with ovaries [2.20±1.23ng/ml] and tubes [2.82±1.74ng/ml] infertility[35].

It has to be noted that the results of AMH show great variation of its values among different studies, confirming the continuous debate about accurate laboratory analysis[13].

Another point of interest, AMH was further reduced after surgery which supports our results as regard the exaggerated effect of endometrioma on AFC rather than AMH. AMH impedes the initiation of original ovarian reserve follicle growth[36].

It reduces follicle sensitivity to the FSH, thus inhibiting FSH-induced growth and can contribute to the recruitment of antral follicle cohorts[37].

Various potential mechanisms underlying ovarian reserve reductions can be assumed to be ovarian cystectomy[38]. First, the damage can occur before surgery, i.e. the cyst itself can adversely affect the surrounding tissue. Second, the ovary tissue with follicles is unintentionally removed from a considerable amount of the tissue and therefore the ovarian reserve is reduced[39].

The elimination of any benign cyst inadvertently results in ovarian tissue deletion, but the effect in endometriomas is more significant[12].

Muzii et al.[40] suggested that this difference was caused by an endometriome pseudocapsule versus an actual capsule within a non-endometrial cyst that had a tissue plane that made tissue dissecting easy from the tissue.

The level of AMH in serum had significantly dropped 3 months postoperatively in patients with ovarian endodometriomas compared with the non-endometriomic cyst. Electric surgery can damage the clotting of the no stasis directly. Furthermore, the loss of healthy ovary follicles may be due to damages to the ovarian vasculature and/or inflammation-mediated injuries[32].

Conclusion: The FSH and AFC level increased after laparoscopic cystectomy for benign ovarian cysts, indicating significant ovarian damage post operatively. FSH, LH and AMH are significantly decreased after ovarian cystectomy in the next 5 months post operative, we recommend for further tests after 1 year post operative to see the final effect on the biochemical markers.

Limitations of this study include the small number of cases that meet the inclusion criteria.

Financial and Non-Financial Relationships and Activities of Interest:

None

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