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Research Article

Ovarian Reserve after Abdominal Myomectomy in Women at Childbearing Period -

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ABSTRACT

Introduction: Ovarian reserve is defined as the existent quantitative and qualitative follicular supply found in the ovaries which may turn into mature follicles and assigns a woman's reproductive potential. The commonly appointed tests of ovarian reserve can be divided into static markers (FSH, estradiol, inhibin-B and [AMH] Anti-Mullerian Hormone), dynamic markers (clomiphene citrate, gonadotrophins and Gonadotrophin Releasing Hormone [GnRh] analogue stimulation tests) and ultrasonographic markers (Antral Follicle Count [AFC], ovarian volume and ovarian blood flow). Leiomyomas are the most common genital tract tumors of benign nature and the most frequent benign uterine disorder in women of reproductive period.

Aim of study: The study evaluated the effect of open myomectomy on ovarian reserve. From all ovarian reserve parameters, we use AFC and AMH to determine this effect due to their high specificity and sensitivity.

Material and method: The study was prospective cohort study done at Armed Forces Hospitals of South Region. Gazan KSA, from May 2018 to June 2019 included 90 women in childbearing period with age ranging from 18 to 40 years old with negative pregnancy test and without surgical history of ovarian operation. AFC is determined by counting the number of follicles measuring (2-8 mm) by TVS one day before the operation. While AMH is measured using blood sample to be determined by ELISA technique. The 2 parameters were measured again 6 weeks postoperative with the same method before the operation to evaluate them as markers of ovarian reserve. The data we obtained was summarized, presented in tables and charts and analyzed in detailed methods by computerized system.

Result: "There were no significant changes in AMH and AFC after the operation (p = 0.713 for AMH) (p = 0.252 for AFC). There was positive correlation between AMH and AFC, which held true to both before and after the operation."

Conclusion: From the results we obtained, we found that there was no significant effect of open myomectomy on ovarian reserve parameters.

Keywords: Ovarian reserve; Myomectomy; Reproductive age

INTRODUCTION

Ovarian reserve is a term that used to determine the capacity of the ovary to provide eggs that are capable of fertilization resulting in a healthy and successful pregnancy [1]. It is also defined as the existent qualitative and quantitative follicular supply found in the ovaries that can potentially produce mature follicles which assigns a woman's reproductive potential.

The commonly appointed tests of ovarian reserve can be divided into static markers (FSH, E2, inhibin-B and AMH), dynamic markers (stimulation tests with clomiphene citrate, gonadotrophins and GnRh analogue) and ultrasonographic markers (AFC, ovarian volume and ovarian blood flow) [2].

AMH is considered to be released from granulosa cells of antral and pre-antral follicles measuring ≤ 6 mm. The effect of AMH is still biologically unclear, but experimental data suggest that it modulates ovarian steroidogenesis and follicular recruitment [3]. AMH is gonadotropin independent, so it remains relatively constant within and between the menstrual cycles and it is considered a promising screening test [4].

TVS assessment of the ovarian reserve is conducted by AFC of those measuring 2-10 mm, ovarian volume, and stromal blood flow, 2D U/S is widely used for this. U/S assessment of the total AFC is generally considered a reliable determinant of ovarian reserve [5].

Leiomyomas are the most common female genital tract neoplasm of benign nature and the most frequent benign uterine disorder in women of reproductive period [6]. It is important to consider the potential impact of each of the procedures of uterine fibroids management on the ovarian reserve, as possible resulted ovarian dysfunction can lead to accelerated menopause onset and diminished fertility [7] as decrease of the ovarian blood flow during the surgery" may account for possible ovarian reserve-decrement.

AIM OF THE WORK

The present study was conducted to evaluate ovarian reserve parameters changes after myomectomy especially in women seeking for fertility.

METHODOLOGY

This was prospective cohort study done at Armed Forces Hospitals of south Region, Gazan KSA. The sample size was 90 patients using open Epi, CI 0.5 % and power of test 80%.

Inclusion criteria: All women were scheduled for open myomectomy with age from 18 to 40 years.

Exclusion criteria:

1- Incapable of informed consent.

2- Women over 40 years.

3- Use of Gonadotropin Releasing Hormone Agonists, in the past 3 months.

4- Positive pregnancy test.

5- Ovarian surgery (at the same time).

All patients were subjected to the following:

i. Full history taking

Including (age, sex, smoking, parity, menstruation, contraception, pelvic surgery).

ii. Full general examination: - including Body Mass Index (BMI).

iii. Abdominal examination: -

For scar of a past pelvic surgery and palpation of myoma if can be felt abdominally.

iv. Ultrasonographic imaging of cases: -

The study included 30 women. All patients were examined with a 4.3-7.5 MHz RIC 2D endovaginal multi frequency probe on a Voluson® p6 730 Pro (General Electric Medical Systems Kretztechnic

GmbH & Co. OHG 2003, Austria) ultrasound device MEDISON ultrasound device at Zagazig University hospitals.

The following were assessed: -

1- AFC by counting number of follicles in both ovaries, this was done 1 day before and 6 weeks after the operation on day 2 of follicular phase [8].

2- Diagnosis of myoma with detection of its dimension, site, number, stage and differential diagnosis with other pelvic masses.

3- Post-operative follow up.

v. Hormonal Assay:

Collected blood samples should be processed within 30 minutes, centrifugation to separate the serum for 20 min at 4000 rpm and 4°C. The samples of serum was kept at -20°C for subsequent AMH and FSH analysis using enzyme-linked immune-sorbent assay kit (Diagnostic Systems Laboratories, Zagazig University, Egypt). The lowest limit of detection was 0.006 ng/mL and the intra and inter-assay coefficients of variation below 5% and 8% respectively [8].

- Preoperative preparation of cases:

All patients were admitted to the hospital several days before the operation for full history taking, clinical examination, basic investigation, pre-anesthetic checkup and correction of anemia in anemic patients by blood transfusion, extensive bowel preparation and finally an informed consent [9].

- Open abdominal myomectomy was performed

- Follow up:

1- Patients were examined 6 weeks post-operative to assess the following: -

a. Follow up and assessment of success of the operation by detecting any residual myomas.

b. Assessment of AFC in both ovaries to compare it with the pre-operative one.

c. Taking blood sample to measure post-operative value of AMH.

STATISTICAL ANALYSIS

Data were analyzed using Statistical Program for Social Science (SPSS) version 25.0 for windows (SPSS Inc., Chicago, IL, USA).

Quantitative data were expressed as mean ± Standard Deviation (SD). Median and Inter-Quartile Range (IQR) were also calculated for quantitative data. Qualitative data were expressed as frequency and percentage.

RESULTS

This was prospective cohort study done at Armed Forces Hospitals of South Region. Gazan KSA. The sample size was 90 patients using open Epi, CI 0.5% and power of test 80%.

Table 1 is showing presentation of cases according to age and BMI. We have 90 cases with mean age 29.6, six of them have BMI < 18.5 (6.7%), 45 cases (50%) with BMI 18.5-24.5, 33 case (36.6%) with BMI 25-34.9 and only 6 cases representing 6.7% of cases with BMI ≥ 35.

Table 1: Demographic data of the study population.

Demographic data	All patients
Count (%)	90 (100%)
Age (years)	
Mean ± SD	29.9 ± 6.6
Median (IQR)	28.5 (24-37)
BMI (kg/m²)	
< 18.5	6 (6.7%)
18.5 - 24.9	45 (50%)
25 - 34.9	33 (36.6%)
≥ 35	6 (6.7%)

In the table 2 Clinical data of the study population including complaint, number and type of myomas 30 cases present with uterine bleeding with percentage (33.3%), 45 cases (50%) present with infertility factor and 15 cases (16.7%) present with lower abdominal pain. According to the number 54 (60%) patients had solitary fibroid and 36 (40%) had multiple fibroids and according the site of tumors, 12 of them were subserous (13.3%), 42 intramural (46.7%), 24 sub mucosal (26.7%) and 12 cases had tumors in other classification like cases with multiple myomas with different types or mixed types (intramural sub mucosal - intramural subserous - hybrid myomas)

Table 3 showing changes in AMH and AFC before and after myomectomy with Mean value of AMH before operation 1.57 ± 0.88 and after operation becomes 1.60 ± 0.66. According to AFC, the mean value before the operation was 7.6 ± 1.8 and after the operation becomes 7.9 ± 1.9.

Table 4 showing correlation between age, AMH and AFC before and after operation. AMH levels reached their peaks at the age 24.5, with maximum values between 18 to 30 years and begin to decrease after the age of 31 year with no significant changes after the operation. AFC shows the same correlation as in AMH with no significant changes after the operation

Table 5 showing the effect of site of myomas on AMH and AFC. This was repeated postoperative with no significant changes in the results after the operation

Table 2: Clinical data of the study population including complaint, number and type of myomas.

Clinical data	All patients
Count (%)	90 (100%)
Complaint	
Uterine bleeding	30 (33.3%)
Infertility factor	45 (50%)
Lower abdominal pain	15 (16.7%)
Number of myomas	
Solitary fibroid	54 (60%)
Multiple fibroids	36 (40%)
Site of myomas	
Subserous	12 (13.3%)
Intramural	42 (46.7%)
Submucous	24 (26.7%)
Others	12 (13.3%)

Table 3: Laboratory data of the study population (*n* = 30).

Laboratory data	Before	After
AMH (ng/ml)		
Mean ± SD	1.57 ± 0.88	1.60 ± 0.66
Median (IQR)	1.55 (0.88-2.14)	1.43 (1.18-1.98)
AFC		
Mean ± SD	7.6 ± 1.8	7.9 ± 1.9
Median (IQR)	7.5 (6-9)	8 (6.75-9)

Table 4: Correlation between age vs. postoperative AMH or AFC.

Variable	AMH before (ng/ml)		AMH after (ng/ml)	
	R	*p*	R	*p*
Age (years)	-0.754	< 0.001	-0.578	0.001
Variable	**AFC before**		**AFC after**	
	R	*p*	R	*p*
Age (years)	-0.523	0.063	-0.088	0.644

Table 5: Comparison between the sites of myoma regarding the AMH and AFC.

AMH (ng/ml)	Before	After	Test	*p*-value (Sig.)
	Mean ± SD	Mean ± SD		
Subserous (*n* = 12)	1.19 ± 0.96	1.61 ± 0.65	-1.256 ₱	0.298 (NS)
Intramural (*n* = 42)	1.45 ± 0.59	1.50 ± 0.52	-0.529 ₱	0.605 (NS)
Submucous (*n* = 24)	1.71 ± 1.03	1.93 ± 0.76	-1.649 ₱	0.143 (NS)
Others (*n* = 12)	2.07 ± 1.41	1.31 ± 0.89	2.651 ₱	0.077 (NS)
Test	0.810 ₳	1.026 ₳		
***p*-value (Sig.)**	0.500 (NS)	0.397 (NS)		
AFC	**Before**	**After**	**Test**	***p*-value (Sig.)**
	Mean ± SD	**Mean ± SD**		
Subserous (*n* = 4)	7.8 ± 1.7	9.8 ± 1.3	-2.828 ₱	0.066 (NS)
Intramural (*n* = 14)	7.3 ± 1.9	7.2 ± 2.2	0.221 ₱	0.828 (NS)
Submucous (*n* = 8)	7.5 ± 1.9	8.4 ± 1.1	-1.433 ₱	0.195 (NS)
Others (*n* = 4)	8.5 ± 1.7	7.5 ± 1.0	2.449 ₱	0.092 (NS)
Test	0.445 ₳	1.026 ₳		
***p*-value (Sig.)**	0.723 (NS)	0.397 (NS)		

₳ One-way Anova test.
₱ Paired samples t-test.
$p < 0.05$ is significant.
Sig.: Significance.

Table 6 are showing the effect of number of myomas on AMH and AFC. This was repeated post-operative with no significant changes in the results after the operation.

Table 7 demonstrating the effect of BMI on AMH and AFC with results confirming that BMI has no effect on ovarian reserve and any associated infertility condition mostly related to other factors like endometrial factors or poor development of ova. The same study was repeated post-operative with no significant changes in the results.

DISCUSSION

Open myomectomy still one of the most surgical options in treating uterine fibroids. This study was made to determine possible effect of this procedure on ovarian reserve. AMH and AFC are the most accurate tests to evaluate ovarian reserve.

We made a study on 90 women to assess the possible effect of abdominal myomectomy on ovarian reserve by evaluating changes in AMH and AFC after the operation. Values of AMH and AFC were assessed 1 day before the operation to be compared with the new values of the same 2 parameters 6 week after the operation in day 2 or 3 of menstrual cycle.

Our results found that, there were no significant changes in values of AMH and AFC after the operation ($p = 0.713$ for AMH) ($p = 0.252$ for AFC).

These results are in partial agreement with another study done by Migahed, et al. in 2014 to evaluate the efficacy of open myomectomy on AMH. AMH was measured one day before, one day after and 6 weeks after the operation with results showing no significant changes in AMH levels after the operation except only minimal decrease in its levels one day postoperative [8].

Browne, et al. had made a similar study in 2008 and found that the difference was not statistically significant.

The same previous study determined the possible effect of open myomectomy on FSH as a marker of ovarian reserve and also gave

Table 6: Comparison between the studied numbers of myoma regarding the AMH and AFC.

AMH (ng/ml)	Before	After	Test	*p*-value (Sig.)
	Mean ± SD	Mean ± SD		
Solitary fibroid (*n* = 18)	1.39 ± 0.88	1.34 ± 0.69	-1.337 ₱	0.062 (NS)
Multiple fibroids (*n* = 12)	1.82 ± 0.87	1.54 ± 0.63	1.911 ₱	0.082 (NS)
Test	-1.126*	0.378*		
***p*-value (Sig.)**	0.146 (NS)	0.594 (NS)		
AFC	**Before**	**After**	**Test**	***p*-value (Sig.)**
	Mean ± SD	**Mean ± SD**		
Solitary fibroid (*n* = 18)	7.1 ± 1.6	7.16 ± 1.5	-1.150 ₱	0.061 (NS)
Multiple fibroids (*n* = 12)	7.3 ± 2.0	7.8 ± 2.1	1.293 ₱	0.139 (NS)
Test	-1.261*	-0.177*		
***p*-value (Sig.)**	0.052 (NS)	0.676 (NS)		

*Independent samples Student's t-test.
₱ Paired-samples t-test.
$p < 0.05$ is significant.
Sig.: significance.

Table 7: Comparison between BMI subgroups regarding the AMH and AFC.

AMH (ng/ml)	Before	After	Test	*p*-value (Sig.)
	Mean ± SD	Mean ± SD		
BMI (< 18.5) (*n* = 2)	1.91 ± 0.72	1.19 ± 0.31	2.483 ₱	0.244 (NS)
BMI (18.5-24.9) (*n* = 15)	1.88 ± 0.98	1.90 ± 0.74	-0.162 ₱	0.873 (NS)
BMI (25-34.9) (*n* = 11)	1.14 ± 0.62	1.34 ± 0.39	-1.670 ₱	0.126 (NS)
BMI (≥ 35) (*n* = 2)	1.22 ± 0.96	1.20 ± 0.71	0.111 ₱	0.930 (NS)
Test	1.815 ₳	2.476 ₳		
***p*-value (Sig.)**	0.169 (NS)	0.084 (NS)		
AFC	**Before**	**After**	**Test**	***p*-value (Sig.)**
	Mean ± SD	**Mean ± SD**		
BMI (< 18.5) (*n* = 2)	8.0 ± 2.8	8.0 ± 0.00	< 0.001 ₱	1.000 (NS)
BMI (18.5-24.9) (*n* = 15)	7.9 ± 1.5	7.8 ± 1.6	0.202 ₱	0.843 (NS)
BMI (25-34.9) (*n* = 11)	7.2 ± 2.3	7.9 ± 2.3	-1.437 ₱	0.181 (NS)
BMI (≥ 35) (*n* = 2)	7.0 ± 1.4	8.5 ± 3.5	-1.000 ₱	0.500 (NS)
Test	0.371 ₳	0.077 ₳		
***p*-value (Sig.)**	0.775 (NS)	0.972 (NS)		

₳ One-way Anova test.
₱ Paired samples t-test.
$p < 0.05$ is significant.
Sig.: significance.

results in agreement with ours in concern the effect on ovarian reserve [10].

Wang, et al. also gave results which agreed with our results when they compared between open myomectomy and hysterectomy in regard to their effect on ovarian reserve.

In the group of hysterectomy, serum level of AMH was found to be decreased two days after operation and remained at the low level for three months postoperative. In the group of myomectomy, serum AMH level was also significantly reduced two days after operation but was comparable to the pre-operative level three months postoperative [11].

Another study comparing myomectomy and hysterectomy in regard to the effect on ovarian reserve gave results in partial agreement with the previous study and our study. The trial found that the AMH fallen in both groups but with some recovery in the hysterectomy group. There was no information on AMH levels following myomectomy [12].

We also tried to evaluate AMH and AFC as markers of ovarian reserve. We studied the relationship or the correlation between values of AMH and AFC. From our data and after detailed statistical analysis we found positive correlation between AMH and AFC before and after the operation.

Pina, et al. made a similar study in 2010 to detect correlation between AMH and AFC. Their results said that age, AMH and FSH were significantly correlated with the number of early antral follicles on cycle day 3.

The correlation between AFC and serum AMH levels was significantly stronger than age and serum levels of FSH.

The AFC by TVS is the best predictor for the quantitative aspect of ovarian reserve. Several evidence based studies suggested to select the follicles as antral follicles depending on a measured diameter (2-10 mm). It has been reported that antral follicles measuring < 6 mm express the greatest amount of AMH, and its level declines when antral follicles increase in size.

Serum AMH levels are significantly related to early AFC and remarkably stronger than age, FSH, LH, inhibin B and E2. Similar results were found by the previous published studies about the relationship between AMH and antral follicle count and the coefficients of correlation were reported [13].

Barbakadze, et al. also confirmed these results in their study 2015. Not only Barbakads, et al. who confirmed this.

Assessment of other biological parameters was presented in our study. The correlation between the age and the AMH was one of these, with results showing that the age was negatively correlated with the AMH. The AMH levels reached their peaks at the age of 25 years, with maximum values between 18 to 30 years and began to decrease after the age of 31 years. The same correlation was done post-operative with no significant changes in the results [14].

Our results were consistent with the results of a similar study by La Marca, et al. in 2010 that declared that age and AMH are in negative correlation with each other's and in a partial agreement with us in concern of the variation of AMH levels along age groups. They reported that AMH was undetectable at birth, then increased to the ages of 2-4 years and remained stable thereafter until adulthood [15].

Kelsey, et al. worked on the same point and gave results which were in complete agreement with our results in concern the correlation between age and AMH and the peak age of AMH was 24.5 years [16] .

Choi, et al. were some of those who were interested in studying this point and gave results also were in agreement with our results about the negative correlation between the age and the AMH [17].

On the other hand a similar relationship between AFC and age was found which confirmed positive correlation between AFC and AMH.

Bozdag, et al. was in agreement with our results and declared that the mean number of antral follicles count was found to be significantly in negative relationship with age [18].

Tehraninezhad, et al. made analysis to ovarian reserve markers including AMH, FSH and AFC in different age strata and found that AMH and AFC decreased with age while FSH increased [19].

Their results confirmed the idea of our study in using AMH and AFC as the main markers in studying and evaluation of ovarian reserve. Their study said that AMH is superior to FSH in studying ovarian reserve and a combination of AMH and AFC is more superior to both [19].

Loy, et al. also made an age related nomograms for AFC and AMH in sub-fertile Chinese women and found that there were a decline in AFC and AMH over age [20].

Our study tried to find the possible relationship between BMI and ovarian reserve which showed that BMI had no effect on ovarian reserve and any associated infertility condition usually related to another factors like endometrial factors or even improper maturation of oocytes. The same analysis was done post-operative and also there were no significant changes in these results.

Heidar, et al. worked on a similar study in 2018 with more concentration on this point and their results were in agreement with ours, declaring that there were no significant difference in serum AMH values between normal, overweight and obese females [21].

According to Simoes-pereira, et al. BMI does not seem to affect AMH levels [22].

Malhotra, et al. evaluated the relationship between AFC and BMI in 183 infertile women and the result had no relationship between AFC and BMI.

In another more detailed study to found the relationship between BMI and AMH, the results were that BMI was not significantly correlated with AMH serum level [23].

Association between AMH serum level and BMI was analyzed according to age groups. Positive correlated was found between AMH and BMI in patients of age group ≤ 30 years old and those in group 30-35 years old and the association was maintained after adjustment for age. But in patients > 35 years old, no correlation found in bivariate analysis or after adjustment for age in a multivariate linear regression model. However, the age of the patients was negatively associated with serum AMH level in all age groups after adjustment for BMI and this is the same we have in our study [24].

Unlike the all previous studies which were in agreement with our results, Bernardi, et al. found results not compatible with ours.

They found that AMH is inversely associated with BMI and there was significant association between AMH and multiple markers of obesity including current BMI, BMI in late teen years and Leptin [25].

In the last, we studied the possible effect of sites and types of myomas on ovarian reserve and we found that there was no significant effect of sites and types of myomas on ovarian reserve.

The previous studies that explain this possible effect were rare. Only Mara, et al. who gave results about the effect of sites of myomas on ovarian reserve and it was in agreement with our results [26].

Another study declared the possible effect of both site and number of myomas on ovarian reserve with result showing no significant difference [27].

CONCLUSION

There were no significant changes in ovarian reserve after the operation and the impact was not significant (AMH $p = 0.713$) (AFC $p = 0.252$). Other issues like number and types of myomas were assessed separately and also had no significant effect on ovarian reserve. This study also confirmed the power of AMH and AFC measurement as parameters of ovarian reserve and the good correlation between them. Further studies are recommended to evaluate the effect of other treatment modalities of fibroid on ovarian reserve with larger sample size.

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