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ORIGINAL ARTICLE.

Changes in Ovarian Reserve Parameters after Myomectomy in Women at Reproductive Age at Zagazig Univeristy .

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ABSTRACT

Background: Ovarian reserve is defined as the existent quantitative and qualitative supply of follicles which are found in the ovaries which can potentially develop into mature follicles and determines a woman's reproductive potential. This study aimed to evaluate the effect of open myomectomy on ovarian reserve in females in reproductive age.

Methods: We used a sample size of 30 women in childbearing period with age ranging from 18 to 40 years old with negative pregnancy test, not using Gonadotrophine-Releasing Hormone Agonists in the past 3 months and without surgical history of ovarian operation. From all ovarian reserve parameters, we use antral follicle count (AFC) and anti-mullerian hormone (AMH) to determine this effect due to their high specificity and sensitivity. The 2 parameters were measured again 6 weeks postoperative with the same method before the operation.

Results: From the results we obtained, we found that P- value of AMH was 0.713 and of AFC, 0.252.

Conclusion: We concluded that there was no significant effect of open myomectomy on ovarian reserve parameters.

Key words: Ovarian reserve, uterine fibroids, open myomectomy, AMH and AFC.

INTRODUCTION

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

The commonly employed tests of ovarian reserve can be divided into static markers (estradiol (E2), FSH, inhibin-B and Anti-Mullerian Hormone (AMH)), dynamic markers (tests of stimulation with clomiphene citrate, gonadotrophins and gonadotrophin releasing hormone analogue) and ultrasonographic markers (antral follicle count (AFC), ovarian volume and ovarian bloodflow) **[2]**.

Anti-mullerian Homone is a glycoprotein hormone known as Müllerian inhibiting substance is one of the best markers of ovarian reserve **[3]**. AMH is thought to be expressed by the granulosa cells of pre-antral and small antral follicles measuring 6.0 mm or less in diameter. The biological role of AMH is still unclear, but rodent data suggest that it acts as a modulator of follicle recruitment and ovarian steroidogenesis **[4]**.

Transvaginal ultrasonographic assessment of the ovaries for ovarian volume, AFC and stromal blood flow has been described in relation to the assessment of ovarian reserve, 2D ultrasound is widely used for this application.Ultrasonographic assessment of the total number of antral follicles measuring 2-10 mm is generally considered a reliable determinant of ovarian reserve **[5]**.

Uterine fibroids are the most common benign genital tract tumours and the most frequently seen benign disorder of the uterus in women of reproductive age **[6]**.

When contemplating the best course of treatment for uterine fibroids in reproductive-aged women, it is important to consider the potential impact of each of the procedures on ovarian reserve, because ovarian dysfunction can lead to accelerated onset of menopause and diminished fertility **[7]**.

This study aimed to evaluate the effect of open myomectomy on ovarian reserve in females in reproductive age.

AIM OF WORK

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

MATERIAL AND METHODS

Ethical clearance:

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Patients:

30 female patient between age from 18 to 40 years with fibroid uterus, not using Gonadotrophine-Releasing Hormone Agonists in the past 3 months, with negative pregnancy test and with no ovarian surgery (at the same operation or in a past surgery) were scheduled for open myomectomy.

ELISA kit:

FSHMIS/AMH enzyme-linked immune-sorbent assay kit (Diagnostic Systems Laboratories, Zagazig University, Egypt).

Experimental design:

30 patients included in this study, were subjected to full history taking, full general examination including Body Mass Index (BMI), abdominal examination for scar of a past pelvic surgery and palpation of myoma if can be felt abdominally and Ultrasonographic imaging of cases. All patients were examined with a 4.3-7.5 MHz RIC 2D endovaginal multi frequency probe on a Voluson® p6 730Pro (General Electric Medical Systems Kretztechnic GmbH & Co. OHG 2003, Austria) ultrasound device at Zagazig Univerisity hospitals.

We used ultrasound to assess 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]**, diagnosis of myoma with detection of its dimension, site, number, stage and differential diagnosis with other pelvic masses and in the post-operative follow up.

Collection of serum samples

Within 30 minutes of collection of blood samples, they were centrifuged for 20 min at 4°C and 4000 rpm to separate the serum. The serum sample was frozen at -20°C and stored for subsequent analysis of AMH.

Surgical treatment

Abdominal myomectomy.

- **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 checkupand correction of anemia in anemic patients by blood transfusion, extensive bowel preparation and finally an informed cosent.

- **Operative technique: [9]**

A low transverse abdominal (Pfannestiel) skin incision was made almost 3 cm above the pubic symphysis. The subcutaneous and the abdominal fascia were also opened by transverse incision, while the abdominal muscle and the peritoneum longitudinally. Longitudinal uterine incision (cold knife) of anterior or fundal wall, depending on the myomas location detected by uterine palpation, was the incision of preference to reduce the postoperative adhesions.

Furthermore, the uterine incision was made as midline as possible to avoid potential damages of the saplings' tubes. Consequently, myomectomy is practically always feasible and relatively easy for all fibroids no matter their size, number or location. The number of serosal incisions must be as low as possible, trying to extract all accessible fibroids through the same incision. Finally, we close the myometrial defect in a hemostatic manner that obliterates dead space, but maintains the anatomical normality as much as possible. If the endometrial cavity is opened, we repair it with small-caliber, interrupted sutures inverting the endometrium. Myometrial closure is accomplished in one or two layers using interrupted absorbable sutures (1-0 caliber vicryl). The serosa must not be resected even if there is an apparent excess of it.

- **Follow up:**

All patients were observed after the operation in regard to oral feeding which began within 6 hours associated with early ampulation and leg rising, hospital stay which lasted for 2-3 days ended by drain removal and patients allowed to be discharged.

Patients were examined 6 weeks post-operative to assess success of the operation by detecting any residual myomas, assessment of AFC in both ovaries to compare it with the pre-operative one and taking blood samples 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. Independent-samples t-test of significance was used when comparing between two means of normally distributed data. A one-way analysis of variance (ANOVA) was used when comparing between more than two means if data is normally distributed. Pearson's coefficients were calculated to assess relationship between study parameters, (+) sign indicate direct correlation & (-) sign indicate inverse correlation, also values near to 1 indicate strong correlation & values near zero indicate weak correlation. Paired-samples t-test compares two means t hat are from the same individual, object, or related units. The two means typically represent two different times (e.g., pre-test and post-test with an intervention between the two-time points). The P-value $\leq$0.05 was considered significant, P-value $\leq$0.001 was considered as highly significant and P-value >0.05 was considered insignificant.

RESULTS

Table S1 is showing presentation of cases according to age and BMI. We have 30 cases with mean age 29.6, two of them have BMI <18.5 (6.7%), 15 cases (50%) with BMI 18.5-24.5, 11 case (36.6%) with BMI 25-34.9 and only 2 cases representing 6.7 % of cases with BMI ≥ 35. Table 1 is showing 10 cases present with uterine bleeding with percentage (33.3%), 15 cases (50 %) present with infertility factor and 5 cases (16.7 %) present with lower abdominal pain. Table 2 is 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 S2 is showing correlation between age and AMH 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.

Table S3 is showing correlation between AFC and age before and after the operation. It shows the same correlation as in AMH with no significant changes after the operation. Table S4 is showing correlation analysis between AFC and AMH before and after the operation. There was positive correlation between AMH and AFC with no significant changes after the operation. Table S5 is comparing values of AMH and AFC before and after the operation with results showing that the changes are completely insignificant. Tables S6 and 3 are showing the effect of site of myoma on AMH and AFC. This was repeated postoperative with no significant changes in the results after the operation. Tables 4 and 5 are showing the effect of number of myoma on AMH and AFC. This was repeated post-operative with no significant changes in the results after the operation. Tables 6 and S7 are 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.

Table (1): Clinical data of the study population.

Clinical data	All patients
Count (%)	30 (100%)
Complaint	
Uterine bleeding	10 (33.3%)
Infertility factor	15 (50%)
Lower abdominal pain	5 (16.7%)
Number of myoma	
Solitary fibroid	18 (60%)
Multiple fibroids	12 (40%)
Site of myoma	
Subserous	4 (13.3%)
Intramural	14 (46.7%)
Submucous	8 (26.7%)
Others	4 (13.3%)

Table (2): 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)

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

Table (3): Comparison between the sites of myoma regarding the AFC.

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 (4): Comparison between the studied numbers of myoma regarding the AMH.

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)		

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

Table (5): Comparison between the studied numbers of myoma regarding the AFC.

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 (6): Comparison between BMI subgroups regarding the AMH.

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)		

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

Table (S1): Demographic data of the study population.

Demographic data	All patients
Count (%)	30 (100%)
Age (years)	
Mean ± SD	29.9 ± 6.6
Median (IQR)	28.5 (18 – 39)
BMI (kg/m^2)	
< 18.5	2 (6.7%)
18.5 – 24.9	15 (50%)
25 – 34.9	11 (36.6%)
≥ 35	2 (6.7%)

Table (S2): Correlation analysis between AMH and age.

Variable	AMH before (ng/ml)		AMH after (ng/ml)	
	R	P	r	P
Age (years)	-0.754	<0.001	-0.578	0.001

Table (S3): Correlation analysis between, AFC and age before and after the operation.

Variable	AFC before		AFC after	
	R	P	r	P
Age (years)	-0.523	0.063	-0.088	0.644

Table (S4): Correlation analysis between, AFC and AMH.

Variable	AMH before (ng/ml)		AMH after (ng/ml)	
	R	P	r	P
AFC before	-0.466	0.069	0.320	0.085
AFC after	0.066	0.730	0.091	0.632

Table (S5): Comparison between the AMH and AFC before and after myomectomy (n=30).

Laboratory data	Before	After	Test	P-value (Sig.)
AMH (ng/ml)				
Mean ± SD	1.57 ± 0.88	1.60 ± 0.66	-0.371 ₽	0.713(NS)
AFC				
Mean ± SD	7.6 ± 1.8	7.9 ± 1.9	-1.170 ₽	0.252(NS)

₽ Paired samples t-test.
p< 0.05 is significant.
Sig.: significance.

Table (S6): Comparison between the sites of myoma regarding the AMH.

AMH (ng/ml)	Before	After	Test	P-value (Sig.)
	Mean ± SD	Mean ± SD		
Subserous (n=4)	1.19 ± 0.96	1.61 ± 0.65	-1.256 ₽	0.298(NS)
Intramural (n=14)	1.45 ± 0.59	1.50 ± 0.52	-0.529 ₽	0.605(NS)
Submucous (n=8)	1.71 ± 1.03	1.93 ± 0.76	-1.649 ₽	0.143(NS)
Others (n=4)	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)		

Table (S7): Comparison between BMI subgroups regarding the AFC.

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.

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 30 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 (AMH...p-value 0.713) (AFC…p- value 0.252).

These results are in partial agreement with another study done by **Migahed et al. [8]** 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.

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

Wang et al. [11] 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 hysterectomy group, serum AMH level was found to be reduced 2 days after operation and remained at the lower level 3 months after operation. In the myomectomy group, serum AMH level was also significantly reduced 2 days after operation but was comparable to the pre-operative level 3 months after operation **[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.

Göksedef et al. [13] made a similar study 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 number of early antral follicles and serum AMH levels was significantly stronger than age and serum levels of FSH. The count of the number of antral follicles by ultrasonography is the best predictor for the quantitative aspect of ovarian reserve. There is no consensus on identification of the antral follicles; however several evidence based studies suggested to select the follicles as antral follicles based on a diameter of measurement (2 to 10 mm). It has been reported that human antral follicles measuring <6 mm express the greatest amount of AMH, and that its level declines when antral follicles increase in size.

They observed that serum AMH levels are strongly related to early AFC, with a significance that was remarkably stronger than age, serum levels of inhibin B, E2, FSH and LH. 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 **[14]**.

Barbakadze et al. [15] also confirmed these results in their study. Not only **Barbakads et al. [15]** who confirmed this, but also **Gada et al. [16]** said the same results.

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.

Our results were consistent with the results of a similar study by **LaMarca et al. [17]** who 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 said that AMH was undetectable at birth and increased to the ages of 2–4 years, remaining stable thereafter until adulthood.

Kelsey et al. [18] 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.

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

Bozdag et al. [19] 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.

Tehraninezhad et al. [20] 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. 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 **[20]**.

Loy et al. [21] 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.

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. [22] worked on a similar study 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.

According to **Simoes-pereira et al. [23]** BMI does not seem to affect AMH levels.

Malhotra et al. [24] 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.

When the association between AMH serum level and BMI was analyzed according to age group, they found that in patients with age group ≤ 30 years old and 30–35 years old AMH and BMI were positively correlated and the association was maintained after adjustment for age . In turn, in patients over 35 years of age, AMH and BMI were not correlated 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 **[25]**.

Unlike the all previous studies which were in agreement with our results, **Bernardi et al. [26]** 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 **[26]**.

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. [27]** who gave results about the effect of sites of myomas on ovarian reserve and it was in agreement with our results.

Another study declared the possible effect of both site and number of myomas on ovarian reserve with result showing no significant difference **[28]**.

CONCLUSION

In this study we assessed the changes in ovarian reserve parameters after open myomectomy in order to evaluate the impact of myomectomy on ovarian reserve. We used AMH and AFC to assess this relationship.We noticed that there were no significant changes after the operation and the impact was not significant (AMH P- value…..0.713)(AFC P-value…..0.252).

Conflict of interest

The authors declare that there is no conflict of interest.

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