

# Impact of Laparoscopic Ovarian Drilling on Anti-Müllerian Hormone Levels and Ovarian Stromal Blood Flow Using 2D Power Doppler in Women with Anovulatory Polycystic Ovary Syndrome

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## ABSTRACT

**Aim of the work:** this study aimed to evaluate the effect of laparoscopic ovarian drilling (LOD) on plasma levels of anti-Müllerian hormone (AMH) and ovarian stromal blood flow changes, by using 2D power Doppler ultrasonography, in polycystic ovary syndrome (PCOS), previously described as being clomiphene citrate resistant and to evaluate the value of these parameters in predicting the clinical outcome of this line of treatment. **Patients and methods:** this prospective controlled was conducted in Al-Hussien University Hospital, Al-Azhar University and Police Authority Hospitals. This study was included twenty-three anovulatory clomiphene citrate (CC)-resistant women with PCOS and 20 fertile women as a control group. Laparoscopic ovarian drilling was done. Serum levels of hormonal profile were measured (AMH, LH, FSH, LH/FSH ratio and total testosterone), ovarian stromal blood flow Doppler indices (RI and PI) and occurrence of ovulation or pregnancy. **Result:** in this study we reported our findings regarding the effects of LOD on AMH, hormonal profile and ovarian stromal blood flow in women with PCOS with Clomiphene resistance. These results suggested that the measurement of AMH, LH, LH/FSH ratio, total testosterone, ovarian volume and ovarian stromal blood flow by color Doppler were in discrimination of PCOS from potentially normal women. The data in our study also suggested that there were no significant differences as regard AMH, hormonal profile except total testosterone, ultrasound ovarian findings and ovarian stromal blood flow before and after LOD, but there were significant differences as regard total testosterone before and after LOD. Also, there was good predictive value for AMH after LOD for ovulation and clinical pregnancy. **Conclusion:** measuring AMH for women with anovulatory PCOS undergoing LOD may provide a useful tool in evaluating the outcome of LOD, but ovarian stromal blood flow 2D Doppler indices did not show significant changes predicting ovulation or pregnancy rate after LOD.

**Keywords:** polycystic ovary syndrome, laparoscopic ovarian drilling, ovulation, anti-Mullerian hormone, ovarian stromal blood flow.

## INTRODUCTION

Anti-Mullerian Hormone (AMH) also known as Mullerian Inhibiting Substance (MIS), is a member of the transforming growth factor  $\beta$  family of growth and differentiation factors. In the ovary, AMH has an inhibitory effect on primordial follicle recruitment as well as on the responsiveness of growing follicles to Follicle-Stimulating Hormone (FSH). The ovary-specific expression pattern in granulosa cells of growing non-selected follicles makes AMH an ideal marker for the size of the ovarian follicle pool<sup>(1)</sup>. AMH serum levels were shown to be highly correlated with the number of antral follicles before treatment and number of oocytes retrieved upon ovarian stimulation<sup>(2)</sup> and had a better predictive value than serum levels of FSH, inhibin B and E<sub>2</sub>, and that the predictive values for AMH and antral follicle count were almost identical. Similarly, cycle day-5 AMH levels were better markers of ovarian responsiveness than inhibin B levels<sup>(3)</sup>. Polycystic ovary syndrome (PCOS) is a common endocrine disturbance in women of reproductive

age and the most common cause of anovulatory infertility, accounting for more than 70% of all cases<sup>(4)</sup> and affecting 5%–10% of females of reproductive age<sup>(5)</sup>.

The diagnostic criteria of polycystic ovary syndrome according to the Rotterdam concession, 2003 included any 2 of the following three parameters: 1) oligo- and/or anovulation; 2) clinical and/or biochemical signs of hyper-androgenism and 3) polycystic ovaries by ultrasound and also with exclusion of other etiologies of hyper-androgenism (Congenital adrenal hyperplasia, androgen secreting tumors and Cushing's syndrome)<sup>(6)</sup>.

There had been much interest regarding the potential role of two dimensional (2D) transvaginal color Doppler ultrasound in assessing the intra-ovarian blood flow in women with PCOS. Those women with PCOS had a significant difference in intra-ovarian hemodynamics when compared to women having normal ovaries. The intra-ovarian blood flow was assessed by studying blood vessels in the ovarian stroma (Small arteries

in the ovarian stroma not close to the surface of the ovary or near the wall of a follicle) <sup>(7)</sup>. The ovarian stromal blood flow differences were likely to be due to a primary disorder within the polycystic ovary, or vice-versa <sup>(8)</sup>. These women with PCOS when compared to normal women without PCOS had an increased ovarian stromal blood flow velocity in the early follicular phase of the menstrual cycle <sup>(9)</sup>. This increase in the ovarian stromal blood flow velocity had also been observed after pituitary suppression and after controlled super-ovulation in females undergoing in-vitro fertilization <sup>(10)</sup>.

Laparoscopic ovarian drilling (LOD) represented an effective treatment for CC-resistant patients and possesses numerous advantages over gonadotrophin therapy such as mono-ovulation, no risk of ovarian hyper stimulation syndrome, no increase in the incidence of multiple pregnancy, less cost and does not require intensive monitoring. In those patients, LOD results in ovulation in about 80% of patients and pregnancy in about 50%-60% of patients <sup>(11- 13)</sup>. This study aimed to evaluate the effect of laparoscopic ovarian drilling (LOD) on plasma levels of anti-Müllerian hormone (AMH) and ovarian stromal blood flow changes, by using 2D power Doppler ultrasonography, in polycystic ovary syndrome (PCOS), previously described as being clomiphene citrate resistant and to evaluate the value of these parameters in predicting the clinical outcome of this line of treatment.

## PATIENTS AND METHODS

This prospective controlled clinical study was carried out in the Department of Obstetrics and Gynecology, Faculty of Medicine, Al-Azhar University (Al-Hossien University Hospital) and Police Authority Hospitals in the period between November 2015 to August 2017. This study included 23 infertile anovulatory women with CC-resistant PCOS who underwent LOD (PCOS group) and 20 healthy fertile women with a regular menstrual cycle and normal ovaries (By ultrasound examination). **Inclusion criteria were:** 1- Patients' ages 18 to 35 years.

2- All patients were infertile (primary or secondary infertility) and were diagnosed as PCOS according to Rotterdam criteria (two criteria were sufficient for diagnosis of PCOS: i) oligo-and/or an-ovulation; which was manifested clinically by secondary amenorrhoea or oligomenorrhoea, ii) hyperandrogenism (clinical and/or biochemical); (hirsutism and/or elevated serum level of total testosterone), iii) polycystic ovaries (should be present in all cases) by ultrasonography (each ovary contained 12 or more

follicles measured 2–9 mm and/ or ovarian volume more than 10 ml) and previously documented anovulation by transvaginal ultrasound follicular monitoring, while taking incremental doses of clomiphene citrate (clomiphene citrate resistant). 3- History of Clomiphene citrate resistance which was defined as failure to ovulate after CC administration up to a daily dose of 150 mg from cycle days 2–6 for at least three consecutive cycles. 4- Body mass index (BMI):from25-30 i.e. over weight. **Exclusion criteria were:** 1- Women with single ovary; previous ovarian cystectomy. 2- Any organicpelvic diseases at laparoscopy or diseases potentially affecting the ovarianenvironment and/or function (including endometriosis and leiomyomas). 3- Current or previous use of metformin. 4- Tubal or male factor infertility investigated with hysterosalpingography and standard semen analysis. 5- Associated medical conditions e.g.thyroid disease,hyperprolactinaemia, diabetes, cardiac disease, renal disease... etc.

## Ethical considerations

The study protocol was approved by the local Ethical Committee of Faculty of Medicine, Al Azhar University. An informed written consent was taken from all patients and their husbands before starting the study and every patient had the right to leave the study at any time.

## Methods

A full detailed history was taken and systematic examination was done for all patients. Trans-abdominal and/or trans-vaginal ultrasound was done to exclude patients with ovarian masses or pelvi-abdominal masses. Other investigations were done to fulfill the inclusion and exclusion criteria (Serum prolactin level, free T3, free T4, TSH).

Hormonal profile as regard serum levels of AMH, LH, FSH and total testosterone were measured in the early follicular phase (days 2-4 of spontaneous cycle in oligomenorrhic patients). To start the study in amenorrhic patients (after exclusion of pregnancy) they received progesterone (Oral Norethisterone acetate 10 mg daily for 5 days) to induce withdrawal bleeding and hormonal profile was measured in days 2-4 of this withdrawal bleeding. Hormonal assay was done for all patients in the Laboratory of Police Hospitals. Assays for AMH were performed by an automated chemiluminescent immunoassay (Architect analyzer, Abbott Diagnostics, USA). The sensitivity of the assay was 99.69% and the specificity is 99.6%. Assays for LH, FSH and T were performed by an automated microparticle

enzyme immunoassay (Abbott AxSYM analyser; Abbott Diagnostics).

Ultrasound examinations were performed using trans-vaginal 7.5-MHz power Doppler ultrasound (Philips Clear Vue 350 ultrasound system, USA). While, the patients at lithotomy position after they had evacuated their urinary bladder and on the same days of the hormonal assay, baseline 2D TVS was used to examine the uterus for any abnormality and measuring the uterine size and endometrial thickness and then to identify PCO criteria in both ovaries and ovarian volume was measured using ellipsoid prolate formulae (length X width X height X 0.523, which was calculated automatically by the software of the ultrasound machine) then color Doppler ultrasound scanning was performed to assess the ovarian stromal blood flow.

Both right and left ovaries were observed and analyzed in each woman using color and power Doppler flow ultrasonography. By means of color and power Doppler flow imaging, color signals were searched for in the ovarian stroma away from ovarian surface and away from the wall of the follicles. By placing the color Doppler gate over the ovarian stroma, areas of maximum color intensity, representing the greatest Doppler frequency shifts, were visualized, then selected for pulsed Doppler examination, pulsatility index (PI) and resistance index (RI) were calculated in each selected Doppler wave. Both right and left ovaries were observed and analyzed in each patient, revealing no statistical significance in Doppler parameters of ovarian stromal arteries. Therefore, the mean value for all ovarian blood flow parameters was calculated and used in the statistical analysis.

Laparoscopic ovarian drilling was done by a specially designed monopolar electrocautery probe was used to penetrate the ovarian capsule at 4 points <sup>(14)</sup> (regardless of the size of the ovary), with the aid of a short burst of monopolar diathermy. The probe (which had a distal stainless steel needle measuring 10 mm in length and 2 mm in diameter) was applied to the surface of the ovary at a right angle to avoid slippage and to minimize surface damage.

A monopolar coagulating current at a 40 W power setting was used. The needle was pushed through the ovarian capsule for about 4 mm depth into the ovarian tissue and electricity was activated for 4 seconds <sup>(15)</sup>. The ovary was then cooled using 200 ml crystalloid solution before releasing the ligament.

## Follow up

Hormonal assay (AMH - FSH - LH - total testosterone) was performed in the early follicular phase (days 2-4 of the menstrual cycle) of the first post-operative spontaneous menstruation (which occurred within 6 – 8 weeks after the operation). In non-menstruating patients, hormonal assay (FSH - LH - total testosterone) was performed by the end of the 8 weeks. Blood flow assessment (PI - RI) was performed in the early follicular phase (days 2-4 of the menstrual cycle) of the first post-operative spontaneous menstruation (which occurred within 8 weeks after the operation). In non-menstruating patients, the blood flow assessment was performed by the end of the 8 weeks. In menstruating patients, this cycle was monitored to assess hormonal profile, ovarian stromal blood flow Doppler parameters and finally to detect ovulation. Ovulation was assessed by serial trans-vaginal ultrasound until visualization of pre-ovulatory follicle of at least 18 mm <sup>(16)</sup>. Ovulation was confirmed by seeing follicle collapse on subsequent trans-vaginal ultrasound, appearance of fluid in the Cul-de-sac and elevated mid-luteal serum progesterone level > 5 ng/ml <sup>(7)</sup>. Ovulating group was informed to report the occurrence of natural conception for 6 months after LOD. Patients who did not menstruate (pregnancy should be excluded at first) or did not ovulate within 8 weeks after drilling as evidenced by poor or no follicular growth by serial transvaginal ultrasound folliculometry, and low mid-luteal serum progesterone level < 5 ng/ml were referred to be re-evaluated.

## Statistical analysis

Data were analyzed using Stata® version 14.2 (StataCorp LLC, College Station, TX, USA) and MedCalc© version 14.8 (MedCalc© Software bvba, Ostend, Belgium).

Numerical variables were presented as mean and standard deviation (SD) and intergroup differences between PCOS group and the control group were compared using the independent-samples t test. Paired numerical data in the study group before and after LOD were compared using the paired t test. Correlations were tested using the Pearson product-moment correlation.

Receiver-operating characteristic (ROC) curve analysis was used to examine the predictive value of ovarian volume, AMH or ovarian Doppler indices. P-value <0.05 was considered statistically significant. The statistical results were as the following:

**Table 1: receiver-operating characteristic (ROC) curve analysis for discrimination between PCOS patients and controls using total (Sum) ovarian volume, ovarian RI, ovarian PI, AMH or testosterone**

ROC parameter	Predictor				
	Total ovarian volume	Average ovarian RI	Average ovarian PI	AMH	Testosterone
AUC	0.889	0.865	0.891	1.000	0.987
SE	0.050	0.055	0.058	0.000	0.012
95% CI	0.756 to 0.964	0.726 to 0.950	0.759 to 0.966	0.918 to 1.000	0.894 to 1.000
z statistic	7.835	6.700	6.788	NA	42.394
p-value	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Youden index	0.650	0.600	0.757	1.000	0.913
Cut-off criterion	>19.1 ml	≤0.86	≤2.93	>3.97 ng/ml	>2.23 nmol/l
Sensitivity	100%	100%	95.7%	100%	91.3%
Specificity	65%	60%	80%	100%	100%

AUC, area under the ROC curve; SE, standard error; 95% CI, 95% confidence interval.

\*DeLong method.

**Table 2: comparison of clinical, power Doppler and hormonal measures before and after LOD in patients with PCOS**

Variable	Before LOD		After LOD		p-value*
	Mean	SD	Mean	SD	
BMI (kg/m <sup>2</sup> )	27.3	1.4	27.4	1.4	0.803
Right ovarian volume (ml)	11.8	2.2	12.0	2.5	0.834
Left ovarian volume (ml)	12.0	2.4	13.7	3.1	0.061
Average ovarian volume (ml)	11.9	1.4	12.8	1.7	0.053
Total (Sum) ovarian volume (ml)	23.8	2.8	25.7	3.5	0.053
AFC	52	12	35	17	<b>0.001</b>
Right ovarian RI	0.81	0.07	0.83	0.07	0.561
Left ovarian RI	0.76	0.07	0.82	0.07	<b>0.013</b>
Average ovarian RI	0.79	0.05	0.82	0.05	<b>0.039</b>
Right ovarian PI	2.45	0.62	3.03	0.69	<b>0.010</b>
Left ovarian PI	2.12	0.62	3.00	0.56	<b>0.000</b>
Average ovarian PI	2.28	0.40	3.01	0.49	<b>&lt;0.001</b>
AMH (ng/ml)	6.2	1.5	6.6	1.5	0.483
FSH (IU/l)	4.4	2.4	4.2	2.1	0.776
LH (IU/l)	12.7	3.2	11.6	2.8	0.273
LH/FSH ratio	3.5	1.5	3.7	2.6	0.702
Testosterone (nmol/l)	3.4	1.0	2.5	0.7	<b>0.003</b>

Data are mean and standard deviation (SD).

\*Paired t test.

**Table 3: receiver-operating characteristic (ROC) curve analysis for prediction of ovulation or clinical pregnancy**

ROC curve parameter	Outcome	Ovulation		Clinical pregnancy
	Predictor	BMI before LOD	AMH after LOD	AMH after LOD
AUC		0.795	0.866	0.794
SE		0.109	0.109	0.113
95% CI		0.576 to 0.933	0.660 to 0.971	0.576 to 0.932
z statistic		2.692	3.366	2.610
p-value*		<b>0.007</b>	<b>0.001</b>	<b>0.009</b>
Youden index J		0.482	0.714	0.598
Cut-off criterion		≤26.72 kg/m <sup>2</sup>	≤7.97 ng/ml	≤5.86 ng/ml
Sensitivity		62.5%	100%	83.3%
Specificity		85.7%	71.4%	76.5%

AUC, area under the ROC curve; SE, standard error; 95% CI, 95% confidence interval.

\*DeLong method.

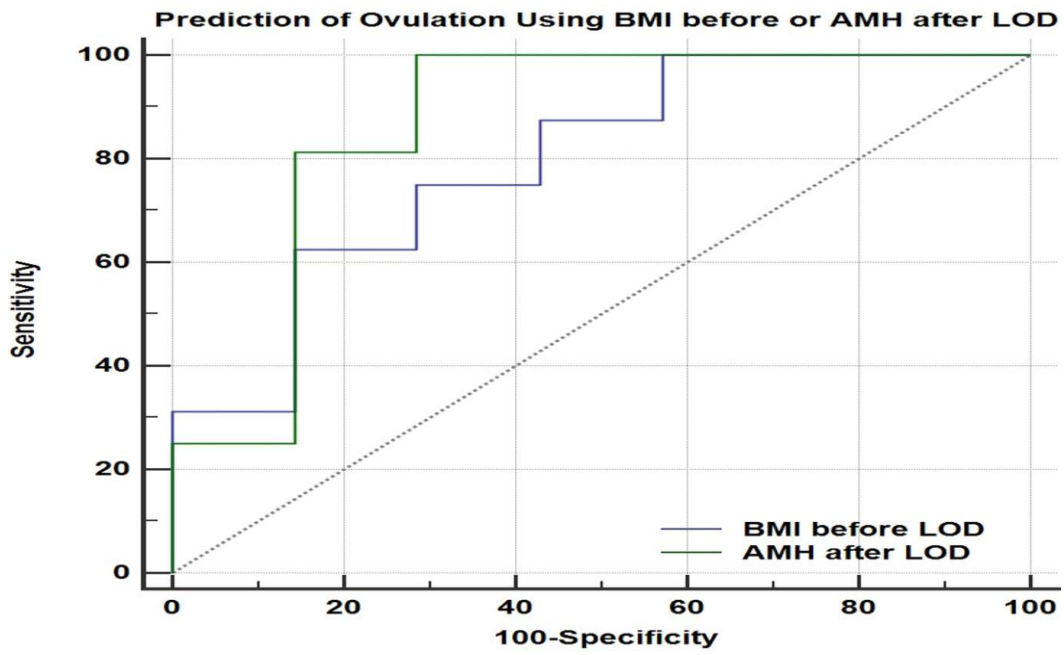


Figure 1: receiver-operating characteristic (ROC) curves for prediction of ovulation using BMI before or AMH level after LOD.

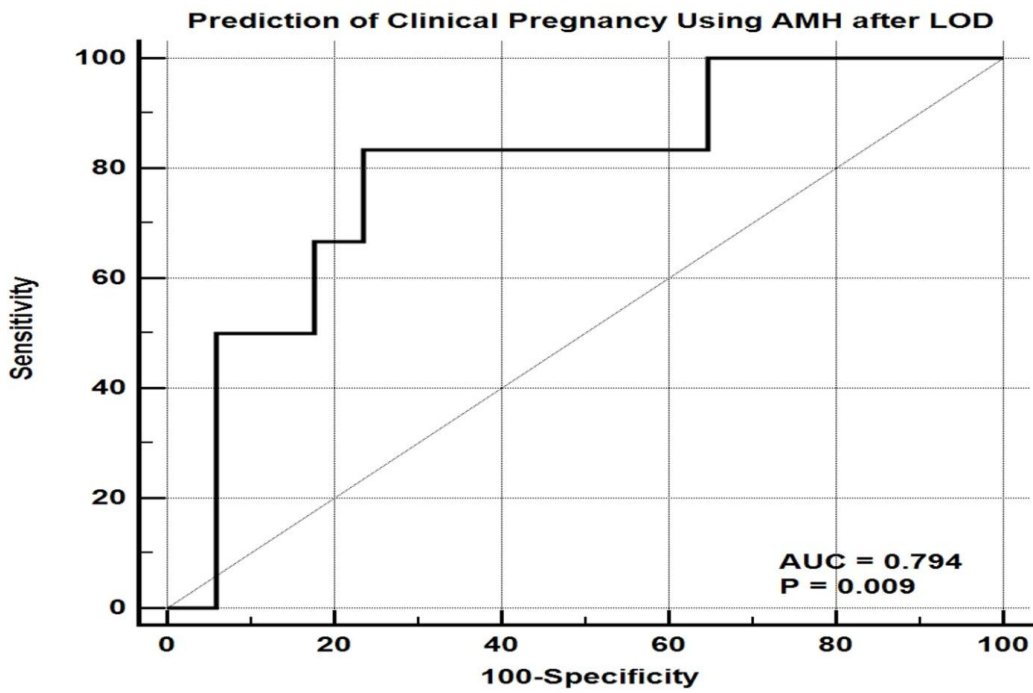
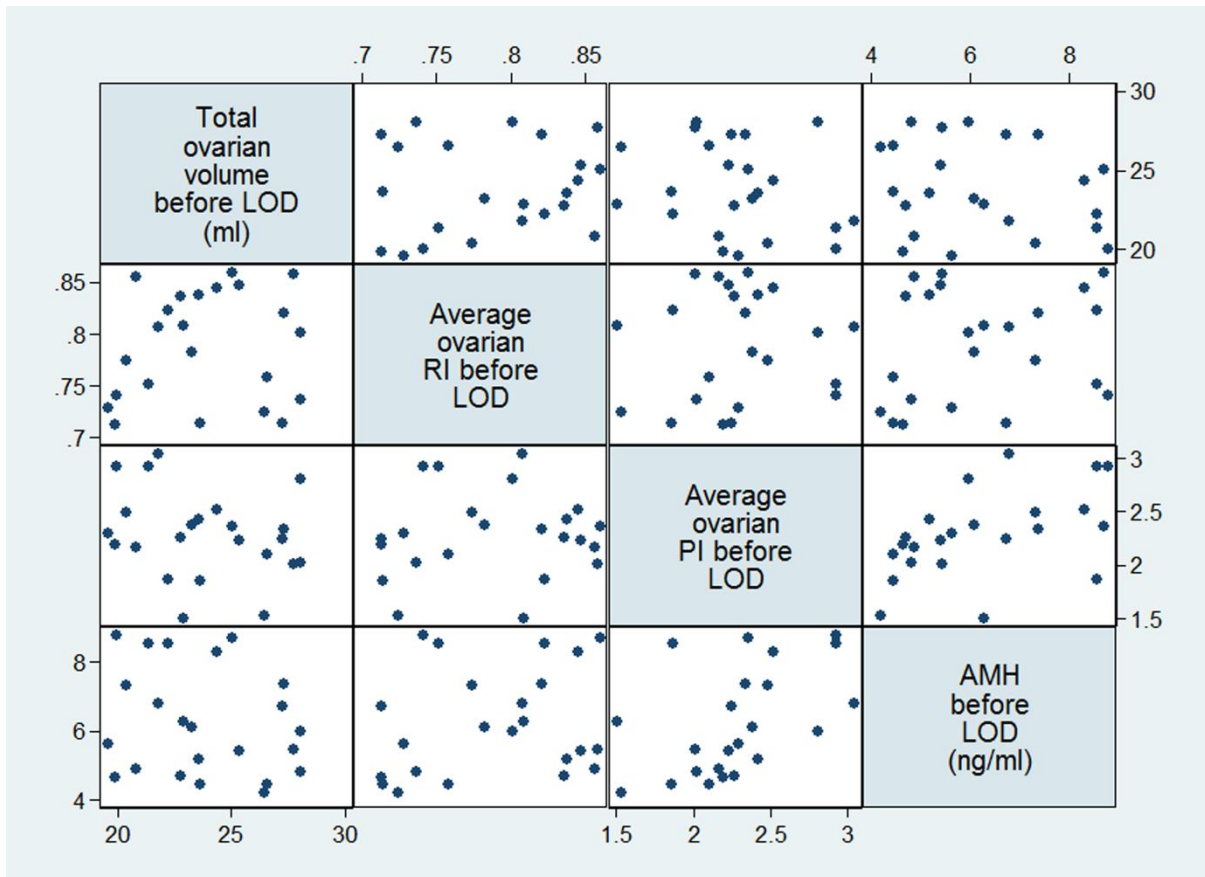
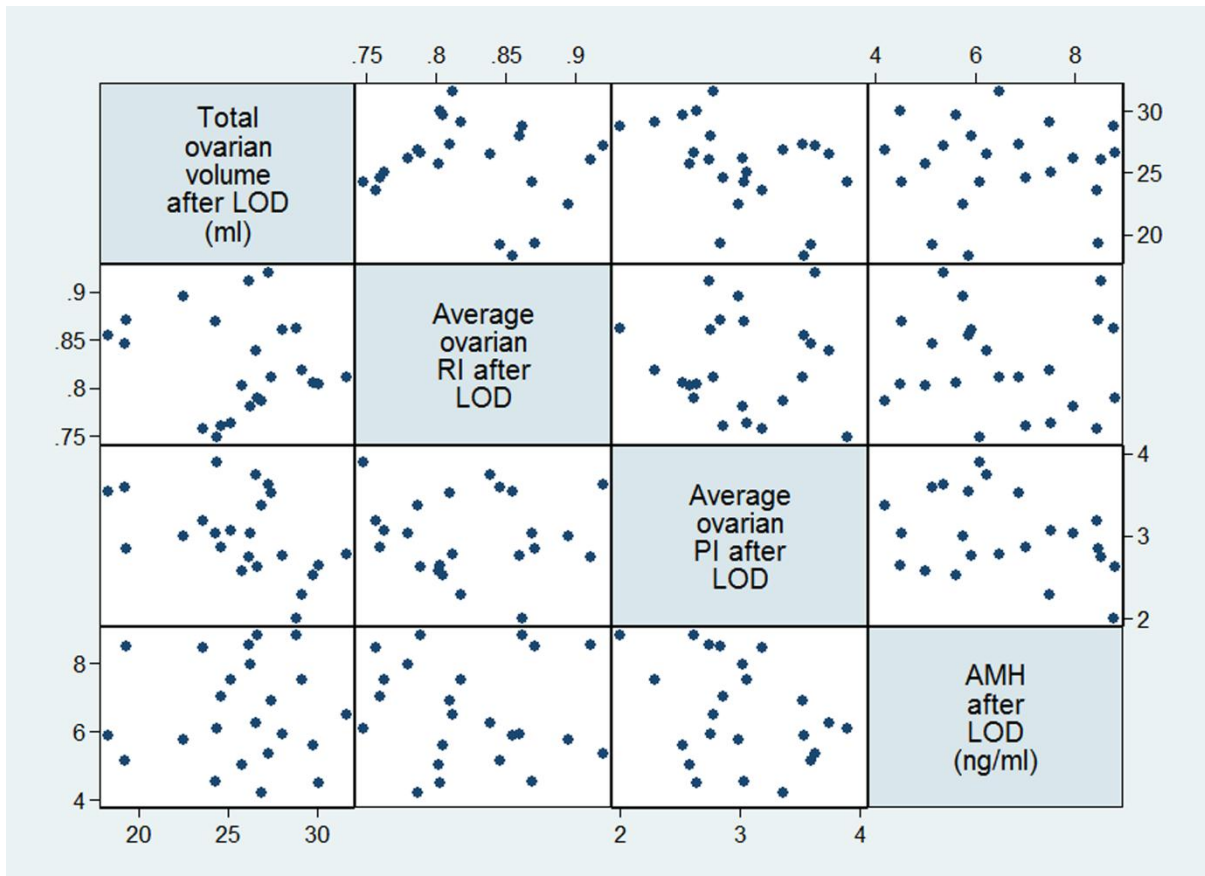


Figure 2: receiver-operating characteristic (ROC) curve for prediction of clinical pregnancy using AMH level after LOD.



**Figure 3:** scatter plot matrix showing the correlations among total ovarian volume, AMH and ovarian Doppler indices before LOD



**Figure 4:** scatter plot matrix showing the correlations among total ovarian volume, AMH and ovarian Doppler indices after LOD.

## DISCUSSION

In our study, we found that age of menarche did not significantly affect results of our study, but BMI showed a statistical significant difference before LOD between patients with ovulation and patients without ovulation (P value 0.024), but did not affect the pregnancy rates. Also, the results of receiver-operating characteristic (ROC) curve analysis for prediction of ovulation showed that BMI before LOD had good value for prediction of ovulation (AUC = 0.795). The best cut-off was a BMI of  $\leq 26.72$  kg/m<sup>2</sup> (Sensitivity = 62.5%, specificity = 85.7%)

In study of **Amer *et al.*** it was found that ovulation and pregnancy rates were significantly decreased in patients with increasing BMI ( $> 35$  kg / m<sup>2</sup>). Multiple logistic regression analysis showed that BMI was one of the most important independent predictor of ovulation after LOD. The presence or absence of acne and menstrual cycle pattern did not seem to predict the outcome of LOD in the same study. Proper identification of predictors of success can help in selection of ideal cases for LOD and ideal cases for gonadotrophin therapy<sup>(17)</sup>.

The hormonal pattern of the studied group was consistent with the diagnosis of PCOS. AMH, LH levels, LH: FSH ratio and total testosterone were elevated. There was a significant difference between PCOS group and the control group as regard AMH (mean values: 6.2 +/- 1.5 and 2.9 +/- 0.6, respectively; P. value  $< 0.001$ ), LH (mean values: 12.7 +/- 3.2 and 5.5 +/- 3.6, respectively with P. value  $< 0.001$ ), LH/FSH ratio (mean values: 3.47 +/- 1.48 and 1.91 +/- 1.74, respectively with P value 0.003) and Testosterone (mean values: 3.4 +/- 1.0 and 1.5 +/- 0.5, respectively with P value  $< 0.001$ ). AMH and testosterone had excellent diagnostic value for PCOS (AUCs = 1.0 and 0.987, respectively). The best cut-offs were an AMH level of  $> 3.97$  ng/ml (sensitivity = 100%, specificity = 100%) and a testosterone level of  $> 2.23$  nmol/l (sensitivity = 91.3%, specificity = 60%). But, there was a non-significant difference between PCOS group and the control group as regard FSH (P value 0.800).

PCOS was characterized by an increase in follicle number that had been shown to occur at the earliest stages. The ability of AMH to alter early follicle growth made it a candidate for causing this change characteristic of PCOS, which in turn increased AMH secretion. However, a study demonstrated that the increase in AMH concentration was largely due to the increase in production of AMH by each follicle and not just a consequence of an increase in follicle number<sup>(18)</sup>.

AMH has been shown to be two- three-folds higher in serum from women with PCOS than in women with normal ovaries. Similarly, concentrations of AMH were found to be five times higher in follicular fluid from unstimulated follicles from women with anovulatory PCOS compared to women who were ovulatory. Serum concentration of AMH correlated with the severity of symptoms, with the ovulatory group having lower concentrations than those who were equally hyper-androgenic but anovulatory<sup>(19)</sup>. In our study there was a statistical significant difference as regard total testosterone before and after LOD in the studied group with mean values 3.4 +/- 1 and 2.5 +/- 0.7, respectively (Pvalue = 0.003). There was no statistical significant difference as regard AMH (6.2 +/- 1.5 before and 6.6 +/- 1.5 after LOD with Pvalue = 0.483). In addition, there was no significant difference before and after LOD as regard LH (mean values were 12.7 +/- 3.2 and 11.6 +/- 2.8, respectively with P.value = 0.273), LH/FSH ratio (Pvalue = 0.702) and FSH levels (mean values were 4.4 +/- 2.4 before and 4.2 +/- 2.1 after LOD with Pvalue = 0.776).

The same was detected in a study done by **Elmashed *et al.***<sup>(20)</sup>, they realized that LH decreased insignificantly from 11.7±1.3 before LOD to 10.8± 1.8 after LOD. FSH decreased insignificantly from 4.2± 1.3 before LOD to 4.1±1.4 after LOD. Total testosterone decreased significantly from 4.2± 0.4 nmol/L before LOD to 2.6±0.6 nmol/L after LOD.

In a study carried out by **Samy *et al.***<sup>(21)</sup> LH decreased significantly from (12.57±4.28 before LOD to 9.35±3.12 after LOD and total testosterone decreased significantly from 2.79±1.6 to 1.98±1.13. As regard FSH, it was decreased insignificantly from 6.34±2.83 before LOD to 6.33±2.44 after LOD.

In another study reported by **Parsanezhad *et al.***<sup>(7)</sup> within 6 to 10 weeks after LOD in ovulating group, LH decreased significantly from 16.86±4.53 before LOD to 11.76±4.82 after LOD (Pvalue = 0.001) and total testosterone decreased significantly from 1.18±0.32 to 0.72 ± 0.28 (P value = 0.001). As regard FSH, it increased significantly from 6.42 ± 1.85 before LOD to 7.55± 1.98 after LOD (P value = 0.03).

In a study carried out by **Abou Sekkein *et al.***<sup>(22)</sup> LH decreased significantly from 12.96 ± 2.1 before LOD to 10.62 ± 1.8 after LOD. FSH decreased insignificantly from 5.8 ± 0.4 before LOD to 5.3 ± 0.51 after LOD. In a study carried out by **Safdarian *et al.***<sup>(23)</sup> LH decreased significantly from 13.23 ± 0.56 before LOD to 8.61 ± 0.62 after LOD. As regard FSH, it

increased insignificantly from  $6.62 \pm 0.41$  before LOD to  $8.70 \pm 0.52$  after LOD. Another study was reported by **Onofriescu et al.** <sup>(24)</sup> within 6 weeks after LOD. In their study, LH decreased significantly from  $5.62 \pm 0.33$  before LOD to  $4.47 \pm 0.3$  after LOD and total testosterone decreased significantly from  $0.73 \pm 0.16$  to  $0.66 \pm 0.11$ . As regard FSH, it increased significantly from  $3.95 \pm 0.21$  before LOD to  $4.64 \pm 0.21$  after LOD.

The mechanism of action of LOD is the destruction of the androgen-producing stroma in the ovary resulting in decrease in the circulating androgens and also decrease in the circulating estrone (E1) due to decreased peripheral aromatization of androgens. This E1 fall resulted in decreased positive feedback on LH and decreased negative feedback on FSH at the level of the pituitary, so LH decreases and FSH increases resulting in follicular development. Another theory is the production of non-steroidal factors due to ovarian injury resulting in restoration of the normal ovarian-pituitary relationship. The last theory is that injury to the ovarian tissue results in production of certain growth factors (such as insulin like growth factor-I), which increase the sensitivity of the ovary to the circulating gonadotrophins resulting in normal follicular growth <sup>(14)</sup>.

Similar results to our study was reported by **Tulandi et al.** <sup>(25)</sup>, they did not find a significant difference in the hormonal profile before and after LOD. A few studies reported no change in LH level weeks to months after surgery <sup>(26,27)</sup>. Also a few studies reported no significant change in testosterone concentration after ovarian surgery <sup>(28,29)</sup>. The cause for different results mostly was the use of different techniques for LOD which was associated with less thermal injury (Less number of punctures- less duration of application- less amount of electric current).

In our study, 30.4% of PCO group fail to respond to LOD. It may be due to the amount of electric current which was not sufficient to produce an effect in those patients. But a study revealed that LOD increased the endogenous FSH and only a minimal amount of thermal energy was required. Also unilateral ovarian drilling is sufficient to produce ovulation in the responders. Another possible explanation may be an inherent resistance of the ovary to the effects of drilling. Another cause may be hyper-prolactaemia observed in some patients after LOD. It was important to monitor the patients for prolactin levels after LOD. The drawback with LOD was to quantify the dose of diathermy to a particular patient. It was difficult to decide the dose for a

particular patient without knowing the dose response. There is a need to optimize the dose of thermal energy in LOD in response to ovarian size <sup>(30)</sup>. In our study, we did not determine the amount of thermal energy according to the ovarian volume or size but the amount of thermal energy was fixed in all patients regardless the ovarian size.

The aetiology of high ovarian stromal blood flow in PCOS patients was not clear up till now. Serum estradiol (E2) may a role in regulation of uterine and ovarian blood flow <sup>(31)</sup>. **Greenblatt and Casper** <sup>(32)</sup> proved in their study that E2 level decreased the first day after drilling, reaching the lowest level by day 4 after operation and begin to rise after that. So there was a vague relation between serum E2 levels and ovarian blood flow changes after LOD. Ovarian stimulation with gonadotrophins was followed by significant reduction in vascular impedance to blood flow in the ovarian artery <sup>(33)</sup> and in arteries around the follicles, in correlation with an increase in the number of follicles and serum E2 concentration <sup>(34)</sup>. Considering these observations and the data reported by **Schurz et al.** <sup>(35)</sup> it seemed that factors other than E2 was the etiology of high ovarian stromal blood flow in PCOS patients.

In our study, there was significant difference between PCOS group and control group as regard AFC (mean values were  $52 \pm 12$  and  $17 \pm 6$ , respectively with P value  $<0.001$ ). Average ovarian volume was  $11.9 \pm 1.4$  and  $9.7 \pm 1.2$ , respectively with P value  $<0.001$ ) and sum ovarian volume was  $23.8 \pm 2.8$  and  $19.4 \pm 2.3$ , respectively with P value  $<0.001$ ). There was a significant difference between PCOS group and the control group as regard Doppler indices presented in average ovarian RI was  $0.79 \pm 0.05$  and  $0.87 \pm 0.05$ , respectively with P value  $<0.001$ ) and average ovarian PI was  $2.28 \pm 0.40$  and  $3.32 \pm 0.65$  with P value  $<0.001$ ).

The results of receiver-operating characteristic (ROC) curve analysis for discrimination between PCOS patients and controls using total ovarian volume, ovarian RI and ovarian PI showed that total (Sum) ovarian volume, average ovarian RI and average ovarian PI had good value for discrimination between PCO patients and controls (AUCs = 0.889, 0.865 and 0.891, respectively). The best cut-offs were a total ovarian volume of  $>19.1$  ml (sensitivity = 100%, specificity = 65%), an average ovarian RI of  $\leq 0.86$  (sensitivity = 100%, specificity = 60%) and an average ovarian PI of  $\leq 2.93$  (sensitivity = 95.7%, specificity = 80%). Ultrasound and Doppler indices in our study before and after LOD in patients with PCOS had shown that there was a statistical significant difference as regard AFC before and after LOD in



the study group was 52 +/- 12 and 35 +/- 17 with P value 0.001). A statistical significant difference before and after LOD as regard Doppler Indices in the form of verage Ovarian RI (0.79 +/- 0.05 and 0.82 +/- 0.05, respectively with P. value 0.039) and Average Ovarian PI (2.28 +/- 0.40 and 3.01 +/- 0.49, respectively with Pvalue <0.001). There was no statistical significant difference as regard sum ovarian volume before and after LOD was 23.8 +/- 2.8 and 25.7 +/- 0.35, respectively with P value 0.053) .

As regard Doppler indices, this was the same as reported by **Parsanezhad *et al.***<sup>(7)</sup> 6 - 10 weeks after LOD. In their study in ovulating group, PI increased significantly from 0.98 + 0.36 before LOD to 1.78 + 0.72 after LOD (P value = 0.001). As regard RI, it increased significantly from 0.55 + 0.16 before LOD to 0.71+ 0.19 after LOD (P value = 0.001).

In a study carried out by **Abou Sekkein *et al.***<sup>(22)</sup>, they reported that PI increased insignificantly from 0.85 + 0.11 before LOD to 0.9 + 0.14 after LOD. In a study by carried out by **Safdarian *et al.***<sup>(23)</sup> they reported that PI increased significantly from 2.01 ± 0.64 before LOD to 2.89 ± 0.57 after LOD. As regard RI, it increased significantly from 0.76 ± 0.11 before LOD to 0.84 ± 0.08 after LOD.

**Vizer and co-workers**<sup>(18)</sup> reported that ovarian stromal blood flow increased after LOD, laparoscopic ovarian electrocautery was applied on both ovaries (40W monopolar current) and 15–20 cauterization points were performed at a depth of 5–7 mm and three-dimensional (3D) sonography was used to assess the intraovarian blood flow. There were no studies dealing with the literature having the same results of **Visser *et al.***<sup>(1)</sup>.

**El Behery *et al.***<sup>(36)</sup> used three-dimensional (3D) sonography to assess the intraovarian blood flow after LOD in patients with PCOS, they concluded that the Doppler indices of ovarian stromal blood flow were significantly higher in the PCOS group than in the control group and the ovarian stromal blood flow Doppler indices were significantly reduced in the PCOS group after LOD<sup>(36)</sup>.

Ovarian stroma is the source of blood supply to the small preantral follicles. Follicular blood flow was increased with growth of primary follicles. Laparoscopic ovarian drilling decreased the number of small and intermediate follicles that usually seen in PCOS and it had the same effect on ovarian stromal tissue and the contained blood vessels<sup>(37)</sup>. Regarding these effects, we can hypothesize that the decline in ovarian stromal

blood flow velocity could be the result of the direct electrical and/or thermal effects of LOD.

Considering the increased ovarian stromal blood flow velocity in PCOS and its possible effects on ovarian steroidogenesis, there might be a possible beneficial effect of diminished ovarian stromal blood flow velocity on ovarian steroidogenesis in PCOS<sup>(5)</sup>.

In our study, in the patients before LOD, there was moderate positive correlation between AMH and average ovarian PI (Pearson r 0.497 and P value 0.016). While, AMH showed moderate negative correlation with BMI (Pearson r -0.449 and Pvalue 0.032).In the patients after LOD, there was moderate negative correlation between sum ovarian volume and average ovarian PI (Pearson r -0.451 and Pvalue 0.031). While, AMH showed moderate positive correlation with the age at the time of the study (Pearson r 0.489 and P value 0.018), Age at Menarche (Pearson r 0.581 and P value 0.004) and LH levels (Pearson r 0.457 and P value 0.022). On the other hand, we did not find any correlation between other parameters in our study. In a study carried out by **Parsanezhad *et al.***<sup>(7)</sup> they found a significant negative correlation between LH and PI (r = 0.43, P = 0.001), testosterone and PI (r = 0.40, P = 0.003), testosterone and RI (r = 0.30, P = 0.043), LH/FSH ratio and PI (r = 0.53, P < 0.001) and RI (r = 0.43, P = 0.001).While, correlation was useful in discovering possible connections between variables, it did not prove or disprove any cause-and-effect (causal) relationships between them. A very rapid response had been reported following LOD, with ovulation occurring within 2- 4 weeks and menses within 4-6 weeks in the responders. Restoration of regular ovulatory cycles occurred in about two thirds of cases<sup>(14)</sup>. In our study, menses and ovulation occurred in 16 patients (69.6% of cases) during the follow up period (8 weeks) and 7 patients (30.4% of cases) did not ovulate during the same period and we found that there was a statistical significant difference as regard BMI before LOD between patients with ovulation and patients without ovulation (P value 0.024); also there was a statistical significant difference as regard AMH After LOD between patients with ovulation and patients without ovulation (P value 0.002). Consequently, BMI before LOD and AMH after LOD had good value for prediction of ovulation (AUC = 0.795 and 0.866, respectively). The best cut-offs were a BMI of ≤26.72 kg/m<sup>2</sup> (sensitivity = 62.5%, specificity = 85.7%) and an AMH level of ≤7.97 ng/ml (sensitivity = 100%, specificity = 71.4%) .

This ovulation rate was less than that reported by **Parsanezhad *et al.*** (73.1%)<sup>(7)</sup> and more than reported by **Abou Sekkein *et al.*** (69.23%)<sup>(22)</sup> and **Amer *et al.*** (67%)<sup>(38)</sup>. The cause for this difference in ovulation rate may be due to different techniques of drilling (monopolar or bipolar type of diathermy, laser or diathermy, duration of electric current application, different number, diameter and depth of punctures and different amount of thermal energy). Importance of AMH in prediction of ovulation after different types of treatment of anovulation make it was possible to hypothesize that normal levels of AMH were necessary to achieve optimal ovarian responsiveness to ovulation induction. Both low and high levels of AMH seem to be detrimental to ovarian responsiveness to stimulation<sup>(38)</sup>. As regard occurrence of pregnancy in patients with ovulation there were 6 cases (26% of the study group; 37.5 % of ovulation group) showed clinical pregnancy during 6 months of follow up after LOD and there was only a statistical significant difference as regard AMH After LOD between patients who got pregnant and patients without (P value 0.040). AMH after LOD had good value for prediction of clinical pregnancy (AUC = 0.794). The best cut-off was an AMH level of  $\leq 5.86$  ng/ml (sensitivity = 83.3%, specificity = 76.5%). In this study, we reported our findings regarding the effects of LOD on AMH, hormonal profile and ovarian stromal blood flow in women with PCOS with Clomiphene resistance. These results suggested that the measurement of AMH, LH, LH/FSH ratio, total testosterone, ovarian volume and ovarian stromal blood flow by colored Doppler might be of value in discrimination of PCOS from potentially normal women.

Data of our study also suggested that there were no significant differences as regard AMH, hormonal profile except total testosterone, ultrasound ovarian findings and ovarian stromal blood flow before and after LOD, but there were significant differences as regard total testosterone before and after LOD. Also, there was good predictive value for AMH after LOD for ovulation and clinical pregnancy.

However, we believed that further research on a larger sample size was needed to determine whether an interaction occurs between LOD, ovarian stromal blood flow, AMH and ovarian steroidogenesis or other surgical methods can be used.

Comparing LOD for PCOS patients with other surgical interventions like ovarian wedge resection which was done formerly, we found that reports had confirmed the benefits of the procedure of wedge resection, with varying rates

of success in resumption of ovulatory cycles and pregnancy rates. However, It was clear, that the procedure was often associated with the development of periadnexal adhesions obviating the beneficial effects of surgery<sup>(39)</sup>, but with modified ovarian wedge resection with anti-adhesion techniques that progressed sufficiently to be able to feel confident that a surgical procedure such as modified ovarian wedge resection could be performed without any great risk of forming adhesions.<sup>(40)</sup>

Laparoscopic ovarian wedge resection had also been reported in a series of 33 patients treated with laparoscopic ovarian wedge resection using harmonic scalpel, a 67% pregnancy rate was reported<sup>(41)</sup>.

**Yildirim *et al.*** performed ovarian wedge resection by laparotomy and reported a pregnancy rate of 90% in 2 years. They also stated that adhesions were found in five of 44 patients who underwent a laparoscopy or caesarean deliveries<sup>(42)</sup>.

## CONCLUSION

Finally, we can concluded that laparoscopic ovarian drilling was a good second choice to induce ovulation in patients with PCOS who were clomiphene citrate resistant. Measuring AMH in those patients may provide a useful tool in evaluating the outcome of LOD, but ovarian stromal blood flow 2D Doppler indices did not show significant changes predicting ovulation or pregnancy rate after LOD. However, after LOD and association with no ovulation, the patient was considered showing failure to the thermal effect of LOD and should be reevaluated for possibility of starting immediately other options for ovulation induction. Fortunately, the ovaries of patients who did not respond to LOD will become more sensitive to ovulation inducing drugs.

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