

ESTIMATION OF ANTIOVARIAN ANTIBODY LEVELS BEFORE AND AFTER LAPAROSCOPIC OVARIAN ELECTROCAUTERIZATION IN PATIENTS WITH POLYCYSTIC OVARIAN DISEASE

By

**Haithem Abd El-Rahim Fahmi Weshahy, Yehia Abd El-Salam Wafa,
Emad Abd El-Rahman El-Tmamy and Salah El-Beltagy***

Departments of Obstetrics & Gynecology and Clinical Pathology*, Faculty of Medicine
Al-Azhar University

***Corresponding author:** Haithem Abd El-Rahim Fahmi,

E-mail: dr.haithem_weshahy@gmail.com

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common and perplexing endocrine disorder of women in their reproductive years, with a prevalence of up to 10%. Clinical expression of the syndrome varies but commonly includes menstrual cycle disturbance, hyperandrogenism, insulin resistance and obesity. PCOS is not only the most common endocrine disorder in reproductive age women, but also a predominant cause of anovulatory infertility.

Objective: To determine the laparoscopic electrocauterization in patients with PCOS on development of a humoral immunity and production of antiovarian antibodies (AOAs).

Patients and Methods: This prospective cross-sectional study was conducted on 54 reproductive age women (18-35 y of age) with clomiphene citrate-resistant (≥ 150 mg/d). PCOS were enrolled in as study group, and 26 healthy women of reproductive age (<35 years) had no evidence of autoimmune disease or fertility problems, as a control group. During period from 2017 to 2020 at Al-Hussein University Hospital, Al-Azhar University. Blood samples were taken before and about 30-40 days after laparoscopic ovarian electrocauterization.

Results: There were 61.1% (33 women out of 54) women ovulated after laparoscopic ovarian drilling (LOD). Before LOD, there were 20.4% (11 women out of 54) women showed regular menstrual pattern and 79.6% (43 out of 54) women complained of irregular menstrual pattern. After LOD, there were 45(83.3%) women restore regular cycles, and 9 (16.7%) still complaining of irregular cycle. This difference was found to be statistically significance difference between before and after LOD ($p=0.001$) in regularity of the cycles. Antiovarian antibodies assessment of the studied group showed difference before and after LOD and this difference was found to be statistically significant. There was significant relationship between cycle regularity and LH value before and after LOD, and there was significant difference between LH: FSH ratio before and after LOD. The compression between antiovarian antibodies (AOAs) before LOD in study group according luteinising hormone (LH): follicle stimulating hormone (FSH) ratio. There was no significant difference between AOA before LOD in study group according to LH:FSH ratio.

Conclusion: Antiovarian antibodies assessment of the studied group (patients with clomiphene citrate-resistant (≥ 150 mg/d) PCOS) showed difference before and after LOD, and this difference was found to be statistically significant.

Keywords: Antiovarian Antibody, Laparoscopic Ovarian Electrocauterization, Polycystic Ovarian Disease.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction that is frequently associated with the systemic condition of insulin resistance. Its cardinal features are hyperandrogenism and polycystic ovary morphology. Its clinical manifestations can include infertility, menstrual irregularity or absence, signs of androgen excess and obesity. Although PCOS is the most common endocrine disturbance to affect women of reproductive age, its definition has been controversial and aspects of its pathophysiology and natural history remain unclear. Therapy is aimed at amelioration of symptoms and a variety of interventions have been proposed, ranging from modifications in lifestyle, to medical therapy or ovarian surgery (*Hart et al., 2012*).

According to the 2010 Rotterdam criteria, the diagnosis of PCOS requires at least two of the three following features: oligo- or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries on ultrasonography (*ESHRE and Group, 2010*).

Clomiphene citrate (CC), a selective estrogen receptor modulator, still remains the first line of treatment for ovulation induction (OI) in PCOS patients (*Vause et al., 2010*). CC-resistance refers to the failure to ovulate with 150 mg of CC for at least 3 cycles, while CC-failure is defined as failure to conceive with CC despite successful regular ovulation for 6-9 cycles (*Amer et al., 2012*).

Laparoscopic ovarian drilling (LOD) has evolved into a safe and effective surgical option for CC-resistant PCOS cases. It is as effective as gonadotropins in

terms of clinical pregnancy rates and live birth rates with the obvious advantages of spontaneous mono-ovulation thereby minimizing the need for intensive monitoring and eliminating the risks of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies (*Amer et al., 2012* and *Hashim et al., 2013*). However, there are concerns regarding the long-term effects on ovarian function, especially iatrogenic adhesions and decreased ovarian reserve, which may potentially jeopardize future fertility. Hence, this procedure should be employed rationally in selected CC-resistant cases for the sole purpose of correction of anovulatory infertility (*Mitra et al., 2015*).

The human ovary can be the target of an autoimmune attack in various circumstances, including several organ-specific or systemic autoimmune diseases. Clinically, the ensuing ovarian dysfunction often results in premature ovarian failure (POF), but other pathologies involving the ovaries, such as unexplained infertility, polycystic ovary syndrome (PCOS) and endometriosis have been associated with antiovarian autoimmunity (*Luborsky, 2010*).

Surgical-related injury may be represented by the development of an autoimmune process. The ovarian trauma could induce the release of significant amounts of ovarian antigens of the internal ovarian layers that are not usually encountered by the immune system and potentially able to elicit the production of antiovarian antibodies (*Forges et al., 2011*). This risk has strong implications for the development of a premature ovarian failure (POF) and a worse reproductive performance. The presence

of ovarian autoantibodies was evaluated by two different approaches—an indirect immunofluorescence test and an ELISA assay (*Chiodo et al., 2011*).

This study was undertaken to determine the laparoscopic electrocauterization in patients with PCOS results in development of a humoral immunity and production of AOA.

PATIENTS AND METHODS

This prospective cross-sectional study was conducted on 54 reproductive age women (18-35 y of age) with clomiphene citrate–resistance (≥ 150 mg/d) PCOS, and 26 healthy women of reproductive age (<35 years) had no evidence of autoimmune disease or fertility problems, as a control group, during the period from 2017 to 2020 at Al-Hussein University Hospital. Blood sample were taken before and about 30-40 days after laparoscopic ovarian electrocauterization:

Group 1 (study group): (Clomiphene resistant patients are those who did not ovulate in response to doses of CC up to 150 mg for 3 successive cycles) PCOS, and Group 2 (control group): had no evidence of autoimmune disease or fertility problems.

Inclusion criteria for study group: All women age ≤ 35 years, primary infertility secondary to anovulation, as indicated by oligomenorrhea or amenorrhea, and clinical or biochemical evidence of PCO and excess androgen, and the patients had not received oral contraceptive pills or progestins for at least three months before surgery.

Polycystic ovary syndrome was diagnosed on the basis of the following criteria (ESHRE/ASRM, Rotterdam

consensus workshop group, 2010) (2 out of 3): Menstrual disturbances (oligo or amenorrhea), clinical and/or biochemical features hyperandrogenism, and typical ultrasonographic findings of polycystic ovaries.

Exclusion criteria for study group: History of previous abdominopelvic operation or with evidence of previous autoimmune and neoplastic disorders were excluded from the study, history of ovarian operations including ovarian drilling, all women had no other infertility related factors, and received any medications at the time of blood sampling.

Inclusion criteria for the control group: Age: ≤ 35 years, had at least one normal vaginal delivery, and A history of regular menstruation.

Exclusion criteria for the control group: Used an intrauterine device or other hormonal methods as contraception, received any medications at the time of blood sampling, and history of previous abdominopelvic operation or with evidence of previous autoimmune and neoplastic disorders.

All patients were subjected to:

- 1. History:** A Full medical and surgical history was taken, and each was also asked about previous investigations done for infertility and previous treatment given for induction of ovulation.
- 2. Examination:** General examination was done to exclude any endocrinological abnormalities, body Mass Index (BMI). Abdominal examination was done to exclude any abdominal or pelvi-abdominal masses,

and pelvic examination was done to exclude any pathology.

3. Investigation:

- Baseline vaginal U/S scan between day 3-5 of cycle in studies cases to exclude any organic pelvic pathology, and confirm the presence of polycystic ovaries with more than 10 cystic 2-8 mm in diameter, scattered either around or through an echo-dense, thickened central stoma in study group.
- Investigation previously done by the patients included hormonal profile on day 2-5 of the cycle: FSH and LH.
- Assessment of anti-ovarian antibody: The anti-ovarian antibody by ELISA was assessed in serum of patient before and about 30–40 days after laparoscopic ovarian drilling.

Principles of the assayed method for detection of AOAs:

The presence of ovarian autoantibodies was evaluated by ELISA quantitative determination of anti-ovary antibodies in human serum from DRG Diagnostics (DRG Instruments GmbH, Marburg, Germany).

Follow up of those all women included in this study was done through repeated transvaginal ultrasonic and hormonal

profile. Repeated transvaginal ultrasonic folliculometry which started at day 9 of the cycle and every other day according to the follicular size. Good response is achieved when at least one mature follicle reaches 18-20 mm in diameter. If there was no follicular response till the 20th day of the cycle, or if the size of the follicle becomes > 24 mm, this hormonal profile on day 2-5 of the cycle: FSH, LH, before and after laparoscopic ovarian drilling was done.

Statistical Analysis:

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test (χ^2) was used to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean \pm SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). For non-parametric data; Mann-Whitney U test was used to compare between the two group. P value < 0.05 was considered significant.

RESULTS

The mean age ± SD of patients enrolled in this study was 27.65 ± 3.86 years (range, 20–35 y). Women in the control group (normal healthy and fertile women) had the same age range (20–35 y) mean age 26.85 ±3.2 y), with no significant statistical difference. The mean BMI (Kg/m2) of the patient was 30.8 ± 2.5 Kg/m2 (range 27-36 Kg/m2). Women in control group, the mean BMI (Kg/m2) was 28.6 ± 2.3 Kg/m2 (range 24-32 Kg/m2). The mean duration of infertility

in the patient group was 3.5±1.3 years (range, 2–6 y).

FSH mean value for study group before LOP was 49±2.44 mIU/ml and for control group was 5.5 ± 0.155 mIU/ml. LH mean value for study group before LOP was 8.41±2.66 mIU/ml and for control group was 5.6 ± 0.15mIU/ml. LH: FSH ratio mean for study group before LOP was 1.74±0.84 and for control group was 1.03 ± 0.002 (Table 1).

Table (1): Description of age, BMI, quantitative variable (FSH, LH, LH: FSH ratio) according among study group before laparoscopic ovarian drilling (LOD) and control group

Parameters	Study group(n=56)		Control group (n=26)	
	Range	Mean±SD	Range	Mean±SD
Age	20-35	27.65 ± 3.86	20-35	26.85 ±3.2 y
BMI	27-36	30.8 ± 2.5	24-32	28.6 ± 2.3
Duration of infertility	2–6	3.5±1.3		
FSH (mIU/ml)	2.1-11.7	49±2.44	2.5 -10.4	5.5 ± 0.155
LH (mIU/ml)	3.1-14.5	8.41±2.66	2.4 -9.6	5.6 ± 0.15
LH: FSH ratio	0.8- 4.9	1.74±0.84	10.6-1.1	1.03 ± 0.002

There were 23 women 42.6% positive for AOA and 31 women 57.4% negative for AOA in 54 women of the study group. There was 2 women (7.7%) positive for AOA, and 24 women (92.3%) negative for

AOA in 26 women of the control group. There was a significance difference between AOA value before and after LOD (p<0.001) (Table 2).

Table (2): Compression between case before LOD and control in AOA

Groups	Cases (n= 54)		Control (n=26)		Total	P. value
	No.	%	No.	%		
Positive AOA						
Positive	23	42.6	2	7.7	25	<0.001
Negative	31	57.4	24	92.3	55	
Total	54	100.0	26	100.	70	

Before LOD, there was 31 (57.4%) women with negative AOA and 23 (42.6%) women with positive AOA. After LOD, there was 14(74.4%) women with

negative AOA and 40 (74.1%) with positive AOA. There was significant difference between AOA before and after LOD ($P < 0.001$) (Table 3).

Table (3): Description of patients' AOA before and after LOD

Cases \ Patients	Before (n= 54)		After (n=54)		P. value
	No.	%	No.	%	
Positive	23	42.6	40	74.1	<0.001
Negative	31	57.4	14	25.9	

FSH mean value before LOD was 5.49 ± 2.44 . FSH mean value after LOD was 5.6 ± 1.61 . There was no significance difference between FSH value before and after LOD ($p = 0.213$). LH mean value before LOD was 8.41 ± 2.66 . LH mean value after LOD was 4.8 ± 1.48 . There was significance difference between FSH value before and after LOD ($p < 0.001$).

LH: FSH mean value before LOD was 1.74 ± 0.84 . LH: FSH mean value after LOD was 0.87 ± 0.93 . There was significance difference between LH: FSH ratio before and after LOD ($p < 0.001$). AOA mean value before LOD was 1.25 ± 0.89 . AOA mean value after LOD was 1.95 ± 0.94 . There was a significance difference between AOA value before and after LOD ($p < 0.001$) (Table 4).

Table (4): Compression between of FSH, LH, LH: FSH ratio and AOA before and after LOD

Parameters \ Patients	Before LOD	After LOD	P. value
	Mean \pm SD	Mean \pm SD	
FSH	5.49 ± 2.44	5.6 ± 1.61	0.213
LH	8.41 ± 2.66	4.8 ± 1.48	<0.001
AOA	1.25 ± 0.89	1.95 ± 0.94	<0.001
LH:FSH	1.74 ± 0.84	0.87 ± 0.93	<0.001

There was no significant different between AOA before and after LOD in

study group according to LH: FSH ratio (Table 5).

Table (5): Compression between AOA before and after LOD in cases according LH: FSH ratio

Parameters \ Cases	Positive (n=23)	Negative (n=31)	P. value
	Before: LH: FSH ratio	1.6 ± 0.4	
After: LH:FSH ratio	0.88 ± 0.2	0.85 ± 0.16	0.551

Before LOD, there was 11 (20.4%) women with regular menstrual pattern and 43(79.6%) women complained of irregular menstrual pattern. After LOD, there was 45(83.3%) women restored

regular cycles, and 9 (16.7%) still complaining of irregular cycle. There was significant difference between before and after LOD (p=0.001) in regularity of the cycles (Table 6).

Table (6): Compression between Regular cycle and Irregular cycle in case

Parameters \ Patients	Before		After		P. value
	NO.	%	NO.	%	
Regular cycle	11	20.40%	45	83.30%	<0.001
Irregular cycle	43	79.60%	9	16.7	
Total	54				

There was 33 (61.1%) out of 54 women ovulated after LOD. There was

21(38.9%) out of 54 women showed no ovulation after LOD (Table 7).

Table (7): Description of patients' ovulation pattern after laparoscopy

	Frequency	Percentage
Ovulation	33	61.1%
No ovulation	21	38.9%
Total	54	100%

There was significant relationship between cycle regularity and AOA ratio before and after LOD (Table 8).

Table (8): Comparison between cases according to cycle regularity before and after LOD regarding AOA

Cases \ AOA	Before	After	P. value
	Mean±SD	Mean±SD	
Regular cycle	0.69±0.10	2.2±0.85	<0.001
Irregular cycle	1.39±0.94	0.73±0.13	<0.001
P. value	<0.001	<0.001	

DISCUSSION

In this study, we have two groups with mean age 27.65±3.86 years, control group mean age 26.85 ±3.2 years, mean BMI = 31.74 kg/m² and mean duration of infertility 3.5±1.3 years. Also, group included 79.6% women with history of oligomenorrhea and 13% women with amenorrhea and 7.4% women with irregular bleeding and 61.1% women with hirsutism and 42.6% women with acne and 11.1% women with galactorrhea.

Women included in the group were assessed by serum sample for measurement of hormonal profile on day 2-5 of the cycle: FSH, LH before laparoscopic ovarian drilling and serum sample for assessment the Antiovarian antibodies (AOAb), before laparoscopic ovarian drilling. Then follow up about one month after laparoscopic ovarian drilling by also measurement of hormonal Profile on day-2 5 of the cycle: FSH, LH and assessment the Antiovarian antibodies

(AOAb), and they followed up for resumption of normal regular menstrual pattern, ovulation rate and Spontaneous pregnancy for 6 months.

During the period of follow up 83.3% experienced regular pattern of menstruation following laparoscopic ovarian drilling whereas 16.7% women still suffering from oligomenorrhea.

This result was slightly less than the results of *Api et al. (2010)* who reported that 93.3% of the patients with polycystic ovary syndrome used to have regular pattern of menstruation for many months or until they became pregnant following laparoscopic ovarian drilling. However, the result of current study was higher than the results reported by *Zahiri Sorouri et al. (2015)* where only 71.1% who reported that had spontaneous menstruation within 6 weeks after ovarian drilling in clomiphene citrate resistance polycystic ovary syndrome patients.

During follow up, spontaneous ovulation after laparoscopic ovarian drilling occurred in 61%. This result is slightly less than the results of *Kriplani et al. (2010)*, *Su et al. (2011)* and *Zahiri Sorouri et al. (2015)* who reported spontaneous ovulation in 64.4%, 81.8% and 83.3% of patients after laparoscopic ovarian drilling respectively.

This result was higher than that of *de Groot et al. (2011)* who found that 68% - 61% of women had normal ovulation following laparoscopic ovarian drilling. This result was in agreement with *Campo (2010)* and *Felemban et al. (2010)* who reported spontaneous ovulation in 78.8% and 73.2% of patients after laparoscopic ovarian drilling respectively.

Although the mechanism of ovulation is uncertain in ovarian cauterization, an increasing number of studies have reported the effectiveness of this procedure. For this reason, some authorities advocate a strategy of minimizing the number of holes in each ovary, with the intention of reducing the periovarian adhesion, and they have even suggested cauterization of only one ovary (*Kaminski et al., 2012*). It appeared that reduction of the ovarian surface injury affects the efficacy of the procedure. However, for resistant patients with PCOS, laparoscopic technique is the treatment of choice and has gained increasing acceptance among gynecological surgeons.

In our study, every effort was made to avoid damage to the ovarian cortex. The punctures were placed evenly around the ovary taking care to avoid the hilum, thereby averting bleeding and avoiding the risk of compromised ovarian blood supply.

During follow up, spontaneous pregnancy after laparoscopic ovarian drilling occurred in 64.8%. This result was slightly less than the results of *Ott et al. (2011)* in 80.6% of patients after laparoscopic ovarian drilling.

This result was an agreement with *Felemban et al. (2010)* and *Kaur et al. (2013)* who reported spontaneous ovulation in 58% and 47.3% of patients after laparoscopic ovarian drilling respectively.

Follow up of hormonal profile before laparoscopic ovarian drilling (LOD) Follicle Stimulating Hormone (FSH) mean = 5.49 ± 2.44 and Lutenising Hormone (LH) mean = 8.1 ± 2.66 LH/FSH

ratio mean= 1.74 ± 0.84 . Hormonal profile after laparoscopic ovarian drilling FSH mean = 5.6 ± 1.61 and LH mean = 4.8 ± 1.48 . LH/FSH ratio mean= 1.87 ± 0.93 . This was in agreement with report of *Li et al. (2010)* who reported that pre-treatment LH levels did not seem to influence the ovulation. However, once ovulation is achieved, LH levels appear to have a significant impact on pregnancy rate. LOD responders with higher LH levels have a significantly higher chance of conception than those with lower LH levels.

LH levels or a lower LH/FSH ratio were more likely to continue to benefit from the treatment for a longer period compared with those who had higher pre-treatment LH or LH/FSH ratio who were more likely to experience a recurrence of their anovulatory status after several months of treatment. A possible explanation for this is that the higher LH or LH/FSH ratio may be indicative of greater severity of the condition with a higher chance of early recurrence of the anovulatory status. However, these results were interpreted with caution as women who resumed a regular menstrual pattern after LOD were lost to follow up 1 year of the operation (*Imani et al., 2010*).

In this study there was no significance relation between antiovarian antibodies and variables and was no significance relation between antiovarian antibodies and FSH, LH, LH: FSH ration before and after LOD.

The search for antiovarian antibodies has been undertaken in numerous studies, but their results still remain conflicting, partly because of differences in the methods used for their detection.

Nevertheless, the localization of these antibodies by indirect immunofluorescence initially enabled the identification of their targets at the cellular level, whereas in more recent biochemical approaches, some of these targets were further characterized at the molecular level (*Forges et al., 2011*).

CONCLUSION

Antiovarian antibodies assessment of the studied group (patients with clomiphene citrate-resistant (≥ 150 mg/d) PCOS) showed difference before and after LOD and this difference was found to be statistically significant.

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تحديد الأجسام المضادة للمبيض ما قبل وما بعد الكي الإلكتروني للمبيض بواسطة المنظار لمرضى تكيس المبيض

هيثم عبد الرحيم فهمي وشاحي، يحيى عبدالسلام وفا، عماد عبدالرحمن التمامي،

صلاح البلتاجي*

قسمي أمراض النساء والتوليد، والباثولوجيا الاكلينيكية*، كلية الطب جامعة الأزهر

E-mail: dr.haithem_weshahy@gmail.com

خلفية البحث: تعد متلازمة تكيس المبايض هي خلل وظيفي للمبيض مصحوب في الأغلب باعتلال باجهزة الجسم بسبب مقاومة الانسولين. ومن سماته الأساسية فرط الأندروجين ومورفولوجيا المبيض المتعدد التكيسات. كما تكون مصحوب بمظاهره الاكلينيكية في صورة العقم، وعدم إنتظام الدورة الشهرية أو غيابها، وعلامات زيادة الأندروجين والسمنة. وتعد متلازمة تكيس المبايض واحدة من أكثر إضطرابات الغدد الصماء شيوعاً لدى النساء في سن الإنجاب وتؤثر على حوالي 10% من النساء في جميع أنحاء العالم.

الهدف من البحث: تحديد تأثير الكي الكهربائي بالمنظار لدى مرضى متلازمة تكيس المبايض علي تطوير حصانة خلطية وإنتاج اجسام مضاد للمبيض.

المریضات وطرق البحث: أجريت هذه الدراسة المقطعية المستعرضة على 54 سيدة في سن الإنجاب (18-35 سنة) وتعانين من متلازمة تكيس المبايض مقاومة لعقار سترات الكلوميدين. كمجموعة دراسة، مع 26 سيدة يتمتعن بصحة جيدة في سن الإنجاب (أقل من 35 عامًا) لم يكن لديهن دليل على أمراض المناعة الذاتية أو مشاكل الخصوبة كمجموعة ضابطة. وذلك خلال الفترة من 2017 إلى 2020 في مستشفى الحسين الجامعي بجامعة الأزهر. وقد تم أخذ عينة الدم قبل وبعد حوالي 30-40 يومًا من الكي الكهربائي بالمبيض بالمنظار.

نتائج البحث: كان هناك 61.1% (33 امرأة من أصل 54) امرأة تم التبويض بعد حفر المبيض بالمنظار. قبل حفر المبيض بالمنظار، كان هناك 20.4% (11 امرأة من 54) امرأة أظهرن نمط حيض منتظم، 79.6% (43 من 54) نساء يشتكين من

عدم انتظام الدورة الشهرية. بعد اللد، كان هناك 45 (83.3%) من النساء يعيدن دورات منتظمة و 9 (16.7%) ما زلن يشكين من الدورة غير المنتظمة. وكان هذا الإختلاف فرق ذو دلالة إحصائية بين قبل وبعد حفر المبيض بالمنظار في إنتظام الدورات. وقد أظهر تقييم الأجسام المضادة للبيض للمجموعة المدروسة فرقاً قبل وبعد حفر المبيض بالمنظار، وأن هذا الاختلاف ذو دلالة إحصائية. وكانت هناك علاقة معنوية بين انتظام الدورة وقيمة الهرمون اللوتيني قبل وبعد حفر المبيض بالمنظار، وكان هناك فرق معنوي بين الهرمون اللوتيني ونسبة الهرمون المنبه للجريب قبل وبعد حفر المبيض بالمنظار، ولم يكن هناك فرق معنوي بين الأجسام المضادة للبيضات قبل حفر المبيض بالمنظار في مجموعة الدراسة وفقاً للهرمون اللوتيني ونسبة الهرمون المنبه للجريب.

الاستنتاج: تحسن معدل إنتظام الدورة الشهرية تلقائياً وارتفاع معدلات حدوث تبويض، إن معدل حدوث الحمل لدى السيدات اللآتى تم عمل لهن تنقيب حرارى للمبيضين عن طريق منظار البطن قد وصل إلى 64.8% خلال فترة المتابعة. وإذداد معدل نسبة الأجسام المضادة للمبايض قبل وبعد التنقيب الحرارى للمبايض.

الكلمات الدالة: الأجسام المضادة للمبيض، الكي الإلكتروني للمبيض بالمنظار، مرض تكيس المبيض.