THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (January–February 2014) 64(1):47–52
DOI 10.1007/s13224-013-0451-x

ORIGINAL ARTICLE

Serum Levels of Anti-histone and Anti-double-Strand DNA Antibodies Before and After Laparoscopic Ovarian Drilling in Women with Polycystic Ovarian Syndrome

Samsami Dehaghani Alamtaj · Razmjoei Parisa · Parsanezhad Mohammad Ebrahim

Received: 30 December 2011 / Accepted: 14 June 2012 / Published online: 25 September 2013

Abstract

Objectives To determine the serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies after laparoscopic ovarian electrocauterization in patients with polycystic ovarian syndrome (PCOS).

Methods Our study was performed on 35 patients with PCOS resistant to medical therapy, 35 patients with unexplained infertility, and 35 healthy fertile individuals. Patients with PCOS underwent laparoscopic electrocauterization while those with unexplained infertility underwent diagnostic laparoscopy. Serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies were measured at baseline and 1 month after operation and were compared between groups.

Result Baseline characteristics were similar between groups. Patients with PCOS had significantly higher levels of anti-dsDNA compared to unexplained infertility ($p < 0.001$) and control groups ($p = 0.001$). Anti-histone antibodies were higher in PCOS group compared to control group ($p = 0.001$). In those patients suffering from PCOS, anti-histone antibody increased significantly 1 month after ovarian electrocauterization ($p = 0.017$). Similarly, serum levels of anti-nucleosome antibodies increased significantly 1 month after operation ($p < 0.001$).

Conclusion Laparoscopic ovarian electrocauterization in patients with PCOS results in increased levels of anti-histone and anti-nucleosome antibodies. Anti-dsDNA, anti-histone, and anti-nucleosome antibodies also increase after diagnostic laparoscopy in those with unexplained infertility. Patients with PCOS have higher levels of anti-dsDNA and anti-histone antibodies compared to those with unexplained infertility and healthy fertile subjects.

Keywords: Polycystic ovarian syndrome (PCOS) · Laparoscopic ovarian electrocauterization · Anti-dsDNA antibody · Anti-histone antibody · Anti-nucleosome antibody

Introduction

Polycystic ovarian syndrome (PCOS) is a form of functional ovarian hyperandrogenism which affects approximately 5–10 % of women of reproductive age [1]. It is the most common cause of female infertility and is characterized by ovarian hyperandrogenism and chronic anovulation [2]. Insulin resistance with compensatory hyperinsulinemia appears to be the most universal feature of the polycystic ovarian disease and has a pathophysiologic role in the hyperandrogenism of the disorder [2]. Nearly 20 % of obese women with PCOS have an impaired glucose tolerance test or diabetes [1].

Samsami D. A., Professor · Razmjoei P., Resident · Parsanezhad M. E., Professor
Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, P.O. Box 71345-1798, Shiraz, Iran

Samsami D. A. (✉), Professor
Department of Obstetrics and Gynecology, Shahid Faghihi Hospital, Zand Avenue, P.O. Box 7134-3119, Shiraz, Iran
e-mail: samsamia@sums.ac.ir

One of the most problems with these patients is anovulation and infertility being a dilemma to gynecologists. Current approach to infertility in patients with PCOS is initiating ovulation induction by medical therapy. Clomiphen citrate is the main choice for ovulation induction in patients with PCOS. About 10–15 % of the patients are found to be resistant to clomiphen citrate treatment and even increasing doses of clomiphen will not result in induced ovulation in these patients. Several other treatment options including gonadotropins with or without GnRH therapy [3] or insulin-sensitizing agents including metformin [4] have been shown to be effective in treatment of patients with clomiphen-resistant PCOS.

For those who are irresponsive to these medical therapies, surgical procedures are considered in order to improve the ovulation dysfunction. Wedge resection of the ovaries was the first surgical procedure which was previously performed in those with PCOS suffering from chronic anovulation. However, it was associated with several complications including peri-ovarian adhesions as well as premature ovarian failure (POF). In the recent decade wedge resection has been replaced by many laparoscopic procedures including biopsy, cauterization, multielectrocoagulation, laser, etc., which are less invasive and associated with less complications. Common complications of these procedures include periadnexal adhesions and ovarian destruction leading to ovarian failure. Several pathogenesis mechanisms have been identified for reduced ovarian reserve in those undergoing ovarian cauterization or drilling [5, 6]. One theory is the exposure of occult ovarian antigens to immune system leading to autoimmune reactions toward ovaries. Following ovarian cauterization, ovaries develop inflammation and ischemic changes which result in tissue rearrangements. This causes the ovarian antigens which have been hidden to be exposed to the immune system. It is also postulated that inflammation causes alterations of normal antigens to abnormal ones which stimulate immune system to produce anti-ovarian antibodies. In this regards Alborzi et al. [7] examined this hypothesis by measuring anti-ovarian antibodies (AOA) in clomiphen-resistant PCOS patients before and after ovarian elecrocauterization. However, they found that this laparoscopic procedure does not result in increased levels of AOA.

In addition, it has been shown that patients with PCOS have susceptibility to autoimmune processes [8]. Some recent studies have shown that patients with PCOS have significantly higher levels of autoantibodies including anti-histone and anti-dsDNA suggestive of autoimmune process in PCOS [8, 9]. It has been also shown that PCOS is associated with some other autoimmune disorders including autoimmune thyroiditis [10]. Thus an autoimmune basis of the disease should be taken into consideration. As data regarding this issue are scarce and the role of autoimmune process following laparoscopic ovarian cauterization is yet to be identified, we performed this study in order to determine the serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies after laparoscopic ovarian electrocauterization in patients with PCOS.

Material and Method

Study Population

This was a randomized clinical trial being performed in Zeinabieh hospital, a tertiary healthcare center affiliated with Shiraz University of Medical Sciences, during a 12-months period from February 2010 to February 2011. We performed this study in order to investigate the effects of ovarian electrocauterization on serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies in PCOS patients resistant to medical therapy. The study protocol was approved by the institutional review board (IRB) of Shiraz University of Medical Sciences and the approval of the Ethics Committee was achieved before the beginning of the study. All patients gave their informed written consent.

We included 35 patients diagnosed to have PCOS suffering from infertility who referred to infertility clinics of Zeinabieh hospital for medical assistant. PCOS was defined according to Rotterdam European Society for Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) PCOS consensus workshop. All patients had at least two of the three following criteria: (I) chronic anovulation, (II) clinical and/or biochemical evidence of androgen excess, and (III) polycystic-appearing ovaries on transvaginal ultrasound. None of the subjects had taken any medication that could influence carbohydrate metabolism for 2 months before the onset of the study, including oral contraceptives. All the patients had received metformin (1,500 mg/day for 3 months), clomiphen citrate (150 mg/day from the fifth to ninth day of each cycle for five cycles), and dexamethasone (0.5 mg/day for 1 month) without any response.

Diabetes was excluded with a fasting glucose of $<$120 mg/dL. Patients with Cushing's syndrome, hyperprolactinemia ($>$25 ng/dL), diabetes mellitus, thyroid disease adrenal hyperplasia, and androgen-secreting tumors or other endocrinopathies have been excluded from the study. Patients with adrenal hyperplasia were excluded by ACTH-stimulated 17-hydroxyprogesterone levels less than 10 ng/mL, and ACTH-stimulated 11-deoxycortisol levels less than 21 ng/mL [threefold the 95th percentile of a historical control group of 60 healthy women controls]. All the patients had LH/FSH ratio $>$2.5. Those subjects who

had kidney or liver diseases and those who were smokers or had breast cancer were also excluded from the study. None of the participants received oral contraceptives, steroid hormones, or any medications that interfere with lipid metabolism, ovarian and pituitary and hypothalamic function, or insulin sensitivity in the last 3 months before study. All patients followed almost the same exercise and diet protocols during the study period. All subjects were non-smokers and had usual physical activity, and none drank alcoholic beverages. None of the patients with PCOS had history of previous abdominal surgeries.

We also included 35 patients aging 21–38 years suffering from unexplained infertility who referred to Zeinabieh infertility clinics as control group. Infertility was defined as 1 year of unprotected intercourse without conception. Semen analysis (for the partners); hormonal assay including prolactin, thyroid stimulating hormone (TSH), prolactin (to rule out hypophyseal adenomas), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (to rule out ovarian dysfunction such as premature ovarian failure); and hysterosalpingogram (HSG) were performed for all the patients in order to find the etiology of the infertility. All women had normal plasma concentrations of LH, FSH, and progesterone; normal tests of renal and hepatic function; normal complete blood counts; normal HSG and negative pregnancy tests. We excluded, those who had hirsutism, autoimmune disorders, endocrinopathies, cirrhosis, and other chronic liver diseases and those who have been diagnosed to have endometriosis. None of these participants had history of previous abdominal surgeries.

We also included 35 fertile healthy women aging 21–38 years who had at least one pregnancy with no history of pregnancy loss and whose last child was delivered a maximum of 1 year before the present study. All these healthy controls had normal hormonal assay including prolactin, TSH, FSH, and LH. None of these subjects had history of previous abdominal surgeries and all of them had given birth to their children by normal vaginal delivery.

Study Protocol

All patients were visited by a physician before the study and underwent a complete history and physical examination and the findings were recorded in a standard questionnaire. On the first day of a spontaneous or progesterone-induced menstrual cycle, after an overnight fast, blood samples were drawn to determine serum FSH, LH, total testosterone (T), dehydroepiandrosterone sulfate (DHEAS), prolactin (PRL), fasting blood sugar (FBS), and fasting insulin. Moreover, oral glucose tolerance test was performed by Auto-analyzer method. Serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies were also measured as baseline.

In all the patients who had PCOS resistance to medical therapy, laparoscopic ovarian electrocauterization was performed. Three puncture techniques were used for laparoscopic surgery. The pelvic and abdomen structures were evaluated. The insulated cautery needle (no. 18) was then introduced through the third puncture site. The needle was inserted against the ovarian surface with gentle pressure until penetration of the ovarian capsule was achieved. Approximately six to eight cautery points were applied to each ovary. A cutting current of 40 W was used for cauterization. The duration of cauterization was 3 s. When necessary, hemostasis was achieved with bipolar forceps applied on the ovarian parenchyma. Cooling of the ovary was performed by irrigation at the end of the cauterization to minimize the risk of adhesion.

In those with unexplained infertility, diagnostic laparoscopy was performed. Two puncture was used for laparoscopy. Laparoscope was inserted from the subumblical incision while supra-pubic incision was used for inserting grasping forceps. All the pelvic organs were evaluated thoroughly.

Patients were followed for 1 month after operation then serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies were measured again 1 month after laparoscopic electrocauterization or diagnostic laparoscopy.

All the hormonal assays were performed in Endocrinology Research center of Nemazee hospital. Serum FSH (FSH-IRMA, KIP0264, BIOSOURCE, Nivelles, Belgium), LH (LH-IRMA CT, REF KP7CT, RADIM, Rome, Italy), DHEAS (DHEA-S-IRMA, KIP0481; BIOSOURCE), and PRL (PRL-IRMA, KIP1406, BIOSOURCE, Nivelles, Belgium) were measured with immunoradiometric assay. Serum levels were measured with radioimmunoassay (ESTO-RIA-CT, KIP1709, BIOSOURCE, Nivelles, Belgium). All immunology measurements were performed in Shiraz Institute for Cancer Research. Serum levels of anti-dsDNA (dsDNA-G, REF 3142, Nivelles, Belgium), anti-histone (Histone-C, REF 3150, Nivelles, Belgium), and anti-nucleosome (Nucleo-h, REF 3130, Nivelles, Belgium) antibodies were measured by enzyme-linked immunosorbent assay (ELISA). The intra-assay and inter-assay coefficients of variation were <6 % for all assays performed.

Statistical Analysis

Based on 90 % power to detect significant differences between corresponding variables ($p = 0.05$, 2-sided), 30 patients were required in each group. To compensate for possible nonevaluable data, we enrolled 35 participants in each group. The statistical software package SPSS for Windows, version 16.0 (SPSS, Chicago, IL, USA) was

used for data analysis. The paired t test was used to compare results within groups, the independent t test to compare results between the groups, and the χ^2 test to compare proportions. Data were reported as mean ± SD. $p < 0.05$ was considered significant.

Results

Overall we included 35 patients with PCOS resistant to medical therapy, 35 patients with unexplained infertility, and 35 healthy fertile individuals. The mean age of the patients with PCOS, unexplained infertility, and healthy individuals was 30.1 ± 2.3, 30.8 ± 2.1, and 29.9 ± 3.1 years, respectively ($p = 0.233$). Patients with PCOS had significantly higher levels of baseline anti-dsDNA antibody compared to unexplained infertility ($p < 0.001$) and control groups ($p = 0.029$). In the same way, serum levels of anti-histone antibodies were higher in PCOS group compared to unexplained infertility group ($p = 0.035$). Patients with unexplained infertility had significantly lower levels of anti-nucleosome antibody compared to those with PCOS ($p < 0.001$) and healthy subjects ($p < 0.001$). Patients with PCOS had significantly higher positive and equivocal results for anti-dsDNA ($p = 0.004$) compared to other groups (Table 1). However, there was not any significant difference between three study groups regarding the positive results for anti-histone antibodies ($p = 0.051$) (Table 1). All patients and controls were negative for anti-nucleosome antibodies.

Patients with PCOS had significantly higher postoperation serum level of anti-dsDNA antibody compared to unexplained infertility ($p < 0.001$) and control groups ($p = 0.001$). The serum level of postoperation anti-histone antibody were higher in patient with PCOS compared to control group ($p = 0.001$). In the case of postoperation anti-nucleosome antibody, patients with PCOS had significantly higher levels of this antibody compared to unexplained infertility ($p < 0.001$) and control groups ($p = 0.001$). There was not any significant difference between unexplained infertility and healthy subjects regarding postoperation serum levels of these antibodies. Patients with PCOS had significantly higher positive and equivocal results for anti-dsDNA ($p < 0.001$), anti-histone ($p = 0.001$), and anti-nucleosome ($p = 0.001$) antibodies. Table 2 demonstrates the postoperation results of anti-dsDNA, anti-histone, and anti-nucleosome antibodies in the three studied groups.

In those patients suffering from PCOS, serum levels of anti-histone antibody increased significantly 1 month after ovarian electrocauterization ($p = 0.017$). In the same way, serum levels of anti-nucleosome antibodies increased significantly 1 month after operation ($p < 0.001$) (Table 3). In those patients suffering from unexplained infertility, serum levels of anti-dsDNA increased significantly after operation ($p = 0.005$). Serum levels of anti-histone antibody increased significantly 1 month after ovarian electrocauterization ($p < 0.001$). In the same way serum levels of anti-nucleosome antibodies increased significantly 1 month after operation ($p < 0.001$) (Table 4).

Table 1 Baseline anti-dsDNA and anti-histone serum antibody levels detected in PCOS, unexplained infertility, and control groups

	Group								
	PCOS, *N* (%)			Unexplained infertility, *N* (%)			Control, *N* (%)		
	Pos.	Neg.	Equi.	Pos.	Neg.	Equi.	Pos.	Neg.	Equi.
Anti-dsDNA	8 (22.9)	21 (60)	6 (17.1)	0	33 (94.3)	2 (5.7)	2 (5.7)	30 (85.7)	3 (8.6)
Anti-Histone	4 (11.4)	24 (68.6)	7 (20)	0	31 (88.6)	4 (11.4)	1 (2.9)	32 (91.4)	2 (5.7)

Pos positive, *Neg* negative, *Equi* equivocal

Table 2 Postoperation anti-dsDNA, anti-histone, and anti-nucleosome serum antibody levels detected in PCOS, unexplained infertility, and control groups

	Group								
	PCOS, *N* (%)			Unexplained infertility, *N* (%)			Control, *N* (%)		
	Pos.	Neg.	Equi.	Pos.	Neg.	Equi.	Pos.	Neg.	Equi.
Anti-dsDNA	13 (37.1)	11 (31.4)	11 (31.4)	1 (2.9)	28 (80)	6 (17.1)	2 (5.7)	30 (85.7)	3 (8.6)
Anti-histone	10 (28.6)	24 (68.6)	1 (2.9)	2 (5.7)	26 (74.3)	7 (20.0)	1 (2.9)	32 (91.4)	2 (5.7)
Anti-nucleosome	5 (14.3)	26 (74.3)	4 (11.4)	0	35 (100)	0	0	35 (100)	0

Pos positive, *Neg* negative, *Equi* equivocal

Table 3 Serum levels of autoantibodies before and after electrocauterization in patients with PCOS

	Before	After	*p* value
Anti-dsDNA (μ/mL)	18.1 ± 12.6	19.71 ± 15.6	0.465
Anti-Histone (μ/mL)	8.74 ± 5.7	12.1 ± 8.3	0.017
Anti-Nucleosome (μ/mL)	2.28 ± 1.6	7.54 ± 6.52	<0.001

Table 4 Serum levels of autoantibodies before and after diagnostic laparoscopy in patients with unexplained infertility

	Before	After	*p* value
Anti-dsDNA (μ/mL)	5.33 ± 2.7	8.3 ± 5.8	0.005
Anti-histone (μ/mL)	5.91 ± 3.7	9.1 ± 4.7	<0.001
Anti-nucleosome (μ/mL)	0.15 ± 0.06	2.11 ± 0.97	<0.001

Discussion

In this study we tried to determine the effects of laparoscopic electrocauterization on serum levels of autoantibodies including anti-dsDNA, anti-histone, and anti-nucleosome antibodies in patients with PCOS compared to those with unexplained infertility and healthy subjects. We also tried to examine the role of these autoantibodies in pathogenesis of PCOS and unexplained infertility. We found that patients with PCOS have significantly higher levels of anti-dsDNA and anti-histone antibodies compared to those with unexplained infertility and healthy fertile subjects. These findings are consistent with previous studies [8, 9] suggestive of an autoimmune etiology in pathogenesis of PCOS. We also found that laparoscopic ovarian electrocauterization results in increased levels of anti-dsDNA and anti-histone antibodies in patients with PCOS. The interesting finding of our study was that diagnostic laparoscopy resulted in increased serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies in patients with unexplained infertility. This may be due to electrocauterization of posterior and anterior wall as well as posterior cul-de-sac and uterosacral endometriosis in those with unexplained infertility. Adhesion release in those who had tubo-ovarian adhesions may also result in increased serum levels of these autoantibodies after laparoscopy. In future studies, those patients with unexplained infertility should be included as control group that do not undergo any manipulation. This will help to exclude the adhesion release and endometriosis electrocauterization as confounding factors.

In 1935, when there was no knowledge of pituitary–ovarian axis, PCOS was described by Stein and Leventhal [11]. They found that wedge resection of the ovaries increases the ovulation and pregnancy rate in patients with PCOS. In the era there was not any medical treatment for PCOS patients because of lack of ovulation induction drugs, anti-androgenic and insulin-sensitizing agents. Thus ovarian wedge resection remained the mainstay of PCOS treatment for the next 40 years. As ovarian wedge resection accompanied many complications including tubo-ovarian adhesions, it was abandoned till 1984 when laparoscopic method was introduced [12]. Laparoscopic electrocautery resulted in 90 % increase in ovulation rate and 70 % increase in pregnancy rate which were favorable results.

The mechanism of ovulation induction following electrocauterization is unclear. However, several studies have demonstrated its efficacy in treatment of infertility in PCOS [7, 13]. Peritubal adhesion leading to infertility is the most important complication of this procedure being studied to a great extend [5, 6]. Several studies have shown that reducing the number of holes per each ovary will result in decreased risk of consequent adhesion formation. Jamal [14] showed that electrocauterization of only one ovary will result in decreased risk of subsequent adhesion formation with achieving favorable result [14]. However, it is postulated that decreaseing the number of holes will result in decreased efficacy of the procedure.

Reduced ovarian reserve and premature ovarian failure (POF) is another complication of laparoscopic ovarian electrocauterization. Several pathogenesis mechanisms have been identified for reduced ovarian reserve in those undergoing ovarian cauterization or drilling [5, 6]. One theory is the exposure of occult ovarian antigens to immune system leading to autoimmune reactions toward ovaries. Following ovarian cauterization, ovaries develop inflammation and ischemic changes which result in tissue rearrangements. This causes the ovarian antigens which has been occult to be exposed to the immune system. It is also postulated that inflammation causes alterations of normal antigens to abnormal ones which stimulate immune system to produce anti-ovarian antibodies (AOA). In this regards Alborzi et al. [7] examined this hypothesis by measuring AOA in clomiphen-resistant PCOS patients before and after ovarian elecrocauterization. They included 64 infertile patients with clomiphen citrate-resistant PCOS and 50 healthy fertile subjects as control group. Those 64 patients with PCOS underwent laparoscopic ovarian electrocauterization. Serum levels of AOA (IgG, IgM, and IgA) were measured before and after operation. Although these antibodies appeared in the sera of the patients after operation, their quantitative value was not significant. Thus they concluded that this laparoscopic procedure does not result in increased levels of AOA [7].

Several lines of evidence indicate that autoimmunity is responsible for several ovarian disorders including POF, endometriosis, unexplained infertility, and PCOS [8, 15]. Detection of AOA in these patients is suggestive of an autoimmune process in pathogenesis of the disease. It has

been shown that aggressive procedures including IVF puncture, radiotherapy, chemotherapy, and surgeries result in exposition of ovarian antigens to the immune system [6, 13]. On this basis we hypothesized that laparoscopic ovarian electrocauterization results in exposition of ovarian antigens and consequently serum levels of autoantibodies increase after the procedure. The results of this study confirm the hypothesis because serum levels of anti-histone and anti-nucleosome antibodies increased significantly 1 month after laparoscopic ovarian electrocauterization. However, serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies increased significantly 1 month after diagnostic laparoscopy.

In addition, it has been shown that patients with PCOS have susceptibility to autoimmune processes [8]. Some recent studies have shown that patients with PCOS have significantly higher levels of autoantibodies including anti-histone and anti-dsDNA suggestive of autoimmune process in PCOS [8, 9]. It has been also shown that PCOS is associated with some other autoimmune disorders including autoimmune thyroiditis [10]. Thus an autoimmune basis of the disease should be taken into consideration. As data regarding this issue are scarce and the role of autoimmune process in complications of laparoscopic ovarian cauterization is yet to be identified.

In conclusion, laparoscopic ovarian electrocauterization in patients with PCOS results in increased levels of anti-histone and anti-nucleosome antibodies. Serum levels of anti-dsDNA, anti-histone, and anti-nucleosome antibodies also increases after diagnostic laparoscopy in those with unexplained infertility. Patients with PCOS have higher levels of anti-dsDNA and anti-histone antibodies compared to those with unexplained infertility and healthy fertile subjects.

Acknowledgment This work financially was supported by Shiraz University of Medical Sciences and in part by Shiraz Institute for Cancer Research.

Conflict of interest Authors declare no conflict of interest.

References

1. Parsanezhad ME, Alborzi S, Zarei A, et al. Insulin resistance in clomiphene responders and non-responders with polycystic ovarian disease and therapeutic effects of metformin. Int J Gynaecol Obstet. 2001;75(1):43–50.
2. Spiroff L, Fritz M. Clinical gynecology endocrinology and infertility. 7th ed. Philadelphia: Williams & Wilkins; 2005. p. 465–92.
3. Cheng J, Lv J, Li CY, et al. Clinical outcomes of ovulation induction with metformin, clomiphene citrate and human menopausal gonadotrophin in polycystic ovary syndrome. J Int Med Res. 2010;38(4):1250–8.
4. Kazerooni T, Ghaffarpasand F, Kazerooni Y, et al. Short-term metformin treatment for clomiphene citrate-resistant women with polycystic ovary syndrome. Int J Gynaecol Obstet. 2009;107(1):50–3.
5. Taskin O, Sadik S, Onoglu A, et al. Adhesion formation after micro laparoscopic and laparoscopic ovarian coagulation for polycystic ovary disease. J Am Assoc Gynecol Laparosc. 1992;6:159–63.
6. Chapron C, Pierre F, Querleu D, et al. Complications of laparoscopy in gynecology. Gynecol Obstet Fertil. 2001;29:605–12.
7. Alborzi S, Tavazoo F, Dehaghani AS, et al. Determination of antiovarian antibodies after laparoscopic ovarian electrocauterization in patients with polycystic ovary syndrome. Fertil Steril. 2009;91(4):1159–63.
8. Petríková J, Lazúrová I, Yehuda S, et al. Polycystic ovary syndrome and autoimmunity. Eur J Intern Med. 2010;21(5):369–71.
9. Hefler-Frischmuth K, Walch K, Huebl W, et al. Serologic markers of autoimmunity in women with polycystic ovary syndrome. Fertil Steril. 2010;93(7):2291–4.
10. Janssen OE, Mehlmauer N, Hahn S, et al. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. Eur J Endocrinol. 2004;150(3):363–9.
11. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29(35):181–5.
12. Gjoannaess H. Polycystic ovarian syndrome treated by ovarian electrocautery through the laparoscope. Fertil Steril. 1984; 41(1):20–5.
13. Kaminski P, Gajewska M, Wielgos M, et al. The usefulness of laparoscopy and hysteroscopy in the diagnosis and treatment of infertility. Neuro Endocrinol Lett. 2006;27:813–7.
14. Jamal HS. Bilateral or unilateral KTP laser ovarian drilling in polycystic ovarian disease. Ann Saudi Med. 1992;20:165–7.
15. Forges T, Monnier-Barbarino P, Faure CG, et al. Autoimmunity and antigenic targets in ovarian pathology. Hum Reprod. 2004; 10:163–75.