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Original Article

Laparoscopic Ovarian Drilling Improve Endometrial Receptivity by Increasing Production of Endometrial Metabolites

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ABSTRACT

Objective: Laparoscopic ovarian drilling normalizes ovulation by reducing cortical thickening, lowering androgen production, and regulating luteinizing hormone pulse frequency. On the other hand, the effect of laparoscopic ovarian drilling on the endometrium is unknown. This study was planned to investigate the changes in the functional markers of choline, creatine, lactate, and lipid metabolites of the endometrium before and after laparoscopic ovarian drilling in women with polycystic ovary syndrome.

Methods: Twenty women diagnosed with clomiphene-resistant polycystic ovary syndrome who did not ovulate successfully despite the administration of clomiphene citrate or aromatase inhibitor were included in the study. Patients were offered Assisted Reproductive Technology (ART) or laparoscopic ovarian drilling options. Patients who accepted laparoscopic ovarian drilling formed the study group. Endometrial MR spectroscopy was applied to the participants in the mid-luteal phase before laparoscopic ovarian drilling. Choline, creatine, lactate, and lipid metabolites of all patients were measured and denominated parts per million. The second MR spectroscopy was performed 2 months after the first MR spectroscopy, and the changes in endometrial metabolites after laparoscopic ovarian drilling were recorded. Twenty patients who did not have clinical and laboratory findings of polycystic ovary syndrome and were matched for age and body mass index were accepted as the control group. The patients in this group were selected from fertile women with at least 2 children. MR spectroscopy was performed in the mid-luteal phase in fertile women. The obtained results were compared within and between groups and the possible effects of laparoscopic ovarian drilling on metabolite synthesis were tried to be determined.

Results: During MR spectroscopy examination main endometrial metabolites choline, creatine, lactate, and lipid were detected in the polycystic ovary syndrome group. The most prominent metabolite peak before and after laparoscopic ovarian drilling was recorded as choline and creatine. There was a significant increase in choline and creatine peaks after laparoscopic ovarian drilling compared to the values before laparoscopic ovarian drilling. There was no significant increase in lactate and lipid signals before and after laparoscopic ovarian drilling. The choline and creatine metabolite levels of the women with polycystic ovary syndrome before laparoscopic ovarian drilling were significantly lower than those of the fertile women. The choline and creatine metabolite levels of the women with polycystic ovary syndrome after m laparoscopic ovarian drilling were similar to those of the fertile women. There was no significant difference between lactate and lipid signals before and after laparoscopic ovarian drilling.

Conclusion: Laparoscopic ovarian drilling improves polycystic ovary syndrome-related subfertility by increasing endometrial choline and creatine metabolite levels to those of fertile women.

Keywords: Laparoscopic ovarian drilling, polycystic ovary syndrome, endometrium, metabolite, subfertility

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine problem encountered in infertility practice and is one of the most difficult causes of subfertility for clinicians to treat. Although oligo-anovulation is accepted as the main culprit as a cause of subfertility, it is known that the endometrium of PCOS patients varies considerably compared to fertile patients.[1] In PCOS endometrium, both receptivity genes, steroid receptor expressions, and glucose transport are impaired compared to fertile cases.[2,3] All these reasons may lead to inadequate decidualization and inadequate implantation in PCOS patients. The nearly double increase in obstetric complications in PCOS is another proof that the endometrial environment is defective.[4] While rising insulin and insulin resistance increase luteinizing hormone (LH)-mediated androgen release, decrease hepatic synthesis of sex hormone-binding globulin.[5] Increased androgen

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levels and hyperinsulinemia contribute to subfertility by impairing IGFBP-1 synthesis in the endometrial stroma.[6] All these data are proof that not only the ovulatory process but also the endometrial environment is defective in PCOS.

Lifestyle change and weight loss are the main treatment approaches in subfertile PCOS patients.[1,5,6] Although clomiphene citrate is the first-line treatment in the medical treatment of subfertility, it is very difficult to get an ovulatory response to Clomiphene Citrate (CC) in most of the cases. In CC-resistant PCOS patients, adding low-dose gonadotropin to the treatment or a combination with insulin-sensitizing treatments can be tried in the second step. If there is no response to all these approaches, treatment with aromatase inhibitors is tried. After this stage, patients are either referred to ART, or ovarian drilling is recommended.[7]

Laparoscopic ovarian drilling (LOD) provides an ovulatory process equivalent to medical therapy in patients with clomiphene-resistant PCOS. In addition, it increases the pregnancy rates[7-9] by causing an improvement in the hormonal parameters and insulin resistance of PCOS. There is only one study investigating the effects of LOD on the endometrium.[7] The main reason for the low number of studies is the need for biopsy, which is an invasive procedure, for endometrial evaluation. MR spectroscopy is a non-invasive method used in the evaluation of the endometrium and provides clues about the function of the endometrium. It provides clear information about the current state of endometrial cells according to the metabolite density obtained.[10,11] Choline (Cho), creatine (Cr), lactate, and lipid are the main metabolites detected in the spectroscopy panel of healthy endometrial cells.[10,11] When reviewing the literature the effect of LOD on endometrial Cho, Cr, lactate, and lipid metabolites has not been studied yet. We, therefore, attempted to investigate whether LOD alters the expression levels of basic functional metabolites in the endometrium of infertile women with clomiphene-resistant PCOS.

METHODS

The participants of this case-controlled study consisted of 40 patients, 20 of whom were PCOS and 20 were fertile. Twenty women diagnosed with clomiphene-resistant polycystic ovary syndrome who did not ovulate successfully despite the administration of clomiphene citrate or aromatase inhibitor were included in the study.

Main Points

- Laparoscopic ovarian drilling normalizes ovulation by reducing cortical thickening, lowering androgen production, and regulating luteinizing hormone pulse frequency.
- Laparoscopic ovarian drilling improves polycystic ovary syndrome-related subfertility by increasing endometrial choline and creatine metabolite levels to those of fertile women.
- Mid-luteal phase MR spectroscopy can be used for the evaluation of endometrium metabolite levels.

Patient Selection

The participants of this case-controlled study consisted of 40 patients, 20 of whom were PCOS and 20 were fertile. Twenty women diagnosed with clomiphene-resistant polycystic ovary syndrome who did not ovulate successfully despite the administration of clomiphene citrate or aromatase inhibitor were included in the study. Polycystic ovary syndrome was defined according to revised Rotterdam criteria, which require 2 of the following 3 manifestations: (i) oligo-anovulation (oligomenorrhoea or amenorrhea); (ii) high concentrations of androgen in the bloodstream and/or clinical signs of hyperandrogenism; and (iii) polycystic ovaries shown by ultrasonography (more than 12 follicles measuring 2-9 mm on at least one ovary). When CC treatment fails, defined as failure to ovulate after 6 months of treatment at an appropriate dose, the patient is regarded as resistant to CC. Induction with letrozole was planned as second-line treatment in PCOS patients for whom the diagnosis of CC resistance was certain. Women with PCOS were offered ART or LOD options. Patients who accepted LOD formed the study group. Endometrial MR spectroscopy (MRS) was applied to the participants in the mid-luteal phase before LOD. Twenty patients who did not have clinical and laboratory findings of PCOS and matched for age and body mass index (BMI) were accepted as the control group. The patients in this group were selected from fertile women with at least 2 children. MR spectroscopy was performed in the mid-luteal phase in fertile women. The obtained results were compared within and between groups and the possible effects of LOD on metabolite synthesis were tried to be determined.

Women with PCOS were subjected to progesterone-induced withdrawal bleeding to determine their secretory phases. Preoperative blood samples were taken from PCOS and control subjects for complete hormonal assays and insulin analysis. Insulin resistance was calculated using the homeostasis model assessment-insulin resistance index (HOMA-IR). Hormonal parameters were re-evaluated after the second MRS. Patients who used hormonal medication in the last 6 months or had a history of ovarian surgery and patients diagnosed with hydrosalpinx were not included in the study. Those with endocrine disease, especially diabetes and hypo/hyperthyroidism, were not included in the study. Women taking antiandrogens, antidiabetics, and lipid-lowering drugs were excluded. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee of Vocational School of Beykent University (Date: May 21, 2020, Decision no: 2020/66).

Drilling Procedure

In the PCOS group, LOD was performed in the mid-luteal phase, which was calculated according to progesterone withdrawal bleeding. Laparoscopic ovarian drilling was performed with a monopolar hook and 5 perforations of 2-3 mm deep in the capsule of per ovary bilaterally. Thermal dose adjusted according to ovarian volume (60 J/cm^3 of ovarian tissue). Since there was no difference between performing 5 or 10 punctures (450-750 J) on the ovarian cortex in terms of clinical and reproductive results, each ovary was exposed to 5 punctures.[7] Electrocautery was used due to its ease of application and access to necessary equipment.

Endometrium Spectroscopy

Before ovarian drilling of the endometrium, MR spectroscopy was performed using MR imaging. T1-weighted images (500/20) and T2-WI (1600/80) with 4-mm thick sections were obtained in the axial and coronal planes. Both single and multi-voxel point-resolved spectroscopy sequences with short and long TEs were used. The metabolite ratios of the peaks were determined using Magnetic Resonance User Interface software. Although their intensities were different, the main metabolites detected before or after LOD were the same. The endometrium was first visualized using magnetic resonance imaging before the voxel was placed. Due to the importance of the voxel location on the appropriate endometrial area, the volume of interest was placed to the center of the endometrium. Choline, Cr, lactate, and lipid metabolites of all patients were measured and denominated parts per million. The second MRS was performed 2 months after the first MRS, and the changes in endometrial metabolites after LOD were recorded.[10,11]

Statistical Analysis

The data was analyzed with the Statistical Package for Social Sciences software 21.0 (IBM SPSS Corp.; Armonk, NY, USA) Normality of data was examined by the Shapiro–Wilk test. Pearson's Chisquare was used to test categorical variables. Continuous variables were analysed with Mann–Whitney U-test. Data are presented as mean ± SD. A p value <.05 was accepted statistically significant.

RESULTS

Patients in the PCOS and control groups were found to be similar in terms of mean age and BMI values. Total testosterone, LH, and insulin levels measured before LOD were significantly higher in the PCOS group compared to the control group (Table 1). Similarly, HOMA-IR values of women with PCOS before LOD were significantly higher than the control group. There was a significant decrease in serum testosterone, LH and insulin levels, and HOMA-IR value after LOD (Table 2).

Table 1. Demographic and Hormonal Characteristics of PCOS and Fertile Groups

	PCOS (n=20)	Fertile Control (n=20)	*P*
Age (years)	27.5 ± 0.13	28.6 ± 1.44	.65
BMI (kg/m^2)	24.7 ± 1.43	24.9 ± 2.33	.08
Infertility duration (years)	3.44 ± 2.03	NA	NA
Endometrial thickness (mm)	8.66 ± 2.05	9.12 ± 0.22	.86
Testosterone (ng/dL)	84.6 ± 4.14	44.5 ± 5.11	.001*
LH (mIU/mL)	11.3 ± 1.20	5.43 ± 2.11	.01*
FSH (mIU/mL)	5.08 ± 1.01	4.46 ± 1.43	.32
Insulin (mU/L)	11.4 ± 1.44	6.19 ± 2.04	.03*
HOMA–IR	3.78 ± 1.50	1.56 ± 0.66	.02*

Data are presented as means ± SD.
*$P < .05$.
BMI, body mass index; FSH, follicle–stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; HOMA–IR, homeostasis model assessment–insulin resistance index; NA, not applicable; SD, standard deviation.

Table 2. Comparison of Endometrial Metabolites and Hormonal Values Before and After Ovarian Drilling

	Before Ovarian Drilling	After Ovarian Drilling	Control	*P**
Choline	1.65 ± 0.33	2.67 ± 1.03	2.87 ± 1.09	.02
Creatine	1.10 ± 0.11	1.85 ± 1.77	1.99 ± 0.33	.03
Lactate	1.02 ± 0.02	0.98 ± 0.08	0.95 ± 0.03	.08
Lipid	0.89 ± 0.30	0.80 ± 0.32	0.81 ± 0.05	.60
Total testosterone (ng/dL)	84.6 ± 4.14	66.5 ± 5.44	44.5 ± 5.11	.01
HOMA–IR	3.78 ± 1.50	2.32 ± 1.03	1.56 ± 0.66	.03
Fasting insulin (mU/mL)	11.4 ± 1.44	8.02 ± 2.54	6.19 ± 2.04	.001
LH (mIU/mL)	11.3 ± 1.20	7.34 ± 2.05	5.43 ± 2.11	.02

Data are presented as means ± SD.
**P* values compared before and after drilling.
BMI, body mass index; FSH, follicle–stimulating hormone; LH, luteinizing hormone; HOMA–IR, homeostasis model assessment–insulin resistance index; SD, standard deviation.

During MRS examination main endometrial metabolites Cho, Cr, lactate, and lipid were detected in the PCOS group (Table 2). The most prominent metabolite peak before and after LOD was recorded as Cho and Cr. There was a significant increase in Cho and Cr peaks after LOD compared to the values before LOD. There was no significant alteration in lactate and lipid signals after LOD. The Cho and Cr metabolite levels of the women with PCOS before LOD were significantly lower than those of the fertile women. The Cho and Cr metabolite levels of the women with PCOS after LOD were similar to those of the fertile women. There was no significant difference between lactate and lipid signals before and after LOD. The decrease in insulin and testosterone levels after LOD was correlated with an increase in Cho and Cr signals. However, the current correlation did not reach statistical significance. No significant correlation was found between the other hormonal and demographic characteristics of the patients in the PCOS group and their endometrial metabolite levels.

DISCUSSION

In subfertile PCOS patients, the following treatment protocols should be tried before going to ART, and if no success is achieved, a higher-level treatment should be started; (i) lifestyle change and weight loss, (ii) ovulation induction with clomiphene citrate, (iii) low-dose gonadotropin addition to treatment in CC-resistant cases or combination with insulin-sensitizing drugs,

(iv) aromatase inhibitors or LOD, and (v) ART.[1,5-7,12] Since most researchers attribute infertility due to PCOS only to the ovulatory factor, treatment approaches have also been directed toward it. However, in the last decade, it has been reported that the endometrium of PCOS patients is defective, both morphologically and molecularly, compared to fertile controls.[2-4] It has been suggested that increased androgen and insulin levels impair decidualization in stromal cells via mitogen-activated protein kinase (MAPK) pathways and Insulin Like Growth Factor Binding Protein 1 (IGFBP-1) synthesis and cause subfertility.[6,13] It has also been reported that the synthesis of homeobox 10, the basic gene of endometrial receptivity, is lower in PCOS patients compared to healthy controls.[7,14] It has been suggested by another researcher that the reason for the decrease in the homeobox gene in PCOS patients may be increased testosterone levels.[14] However, due to the invasive nature of endometrial sampling, adequate receptivity studies could not be performed in subfertile PCOS patients. Moreover, the variability of inter and intra-observer variations of transcriptomics tests has limited their routine use.[10]

MR spectroscopy is a non-invasive imaging method that can detect the life activities of cells in living tissues at the physiological or pathological level. It has been reported that this method can be used for diagnostic or screening purposes in many tissues, especially the brain, endometrium, myometrium, and ovaries.[10,11] In the presence of normal cellular functions, the metabolites that dominate the spectrum are Cho and Cr, indicating the cell's membrane integrity and energy balance, respectively. In case of deviation from normal cellular functions, the metabolites that dominate the spectrum are lactate and lipid. In the light of these data, we non-invasively tested the change of endometrial metabolites in endometrial MR spectroscopy performed before and after ovarian drilling in subfertile PCOS patients. Laparoscopic ovarian drilling is a minimally invasive method used in subfertile women with PCOS who are resistant to clomiphene citrate administration. When LOD is applied in patients with BMI >30 and LH >10 IU/L, it improves both ovarian morphology and endocrine picture.[5,6] We performed endometrial spectroscopy before LOD in patients in the PCOS group and repeated the measurement 2 months later. We found that endometrial Cho and Cr metabolites increased significantly in control measurements. Choline and Cr levels measured before LOD were significantly lower than infertile patients. After LOD, Cho and Cr levels increased and reached the levels of fertile cases. The increase in Cho and Cr levels after LOD may have developed due to the decrease in hormonal parameters and insulin resistance. However, a positive but insignificant correlation was found between the increase in Cho and Cr and the levels of insulin and testosterone. A significant correlation may not have been found due to the low number of cases.

On the other hand, there was no change in lactate and lipid levels, which are pathological endometrial metabolites, after LOD. Lactate is an indicator of anaerobic glycolysis and its levels increase in case of hypoxia. Since lactate levels do not change in subfertile PCOS cases, it suggests that there is no pathology in endometrial oxygenation.[10,11] Similarly, the lipid metabolite is an indicator of membrane integrity and its levels did not change after LOD. This finding is important evidence that endometrial integrity is preserved in PCOS patients.

Our study showed that LOD significantly increased the levels of endometrial metabolites Cho and Cr in subfertile PCOS patients, but did not affect the levels of pathological metabolites lactate and lipid. The increase in Cho and Cr levels may have a positive effect on endometrial receptivity. However, studies comparing metabolite levels and receptivity genes are needed to make a clear comment on this issue. Despite the small number of cases, this study showed for the first time non-invasively that LOD affects the endometrium. Thanks to spectroscopy, we can have preliminary information about endometrial receptivity without biopsy.

CONCLUSIONS

In addition to ovulatory dysfunction and hyperandrogenemia, changes in endometrial metabolites occur in subfertile PCOS patients. These changes can be detected non-invasively by spectroscopy and receptivity can be increased by performing interventions for the endometrium. The fact that spectroscopy provides preliminary information about the endometrium without the need for a biopsy may enable this method to be used in the future to calculate the receptivity timing and to determine the transfer day.

Ethics Committee Approval: Ethics committee approval was received from the Vocational School of Beykent University (Date: May 21, 2020, Decision no: 2020/66).

Informed Consent: Participants were selected from patients with consent for laparoscopy

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