Assessment of ovarian reserve in patients with ovarian endometriosis following laparoscopic enucleation of a cyst accompanied by CO\textsubscript{2} laser ablation or electroablation

Introduction: Endometriosis affects about 5-15% of women in the reproductive period. One of the most important complications of pelvic endometriosis is infertility.

Objective: The assessment of ovarian endometriosis therapy with the use of combined laparoscopic techniques (cyst enucleation with electroablation/CO\textsubscript{2} laser ablation) and their influence on the preservation of the ovarian reserve.

Materials and Method: Fifty-eight patients aged 19-40 with diagnosed ovarian endometriosis underwent pre-surgical assessment of their ovarian reserve consisting of antral follicle count (AFC), basal ovarian volume (BOV) and FSH level. Twenty-four patients underwent laparoscopic enucleation of the cyst with CO\textsubscript{2} laser ablation and thirty-four patients had enucleation with successive electroablation. The ovarian reserve of the patients was reassessed during follow-up assessments three and six months after surgery.

Results: In neither group was there a statistically significant decrease of AFC after three or six months. Additionally, in the group that underwent enucleation with electroablation, a significant drop in FSH level was observed after three months. This group was characterized by a statistically significant decrease in BOV at the three- and six-month follow-up assessments. 17% patients had ovarian relapses visible in the ultrasound scan at the six month follow-up assessment. In the group of patients who underwent enucleation with CO\textsubscript{2} laser ablation, BOV had not changed significantly, while a high rate of ovarian relapses (39%) was observed.

Conclusions: The lack of significant changes in AFC level after the surgeries suggests an initial positive evaluation of both techniques in the context of ovarian reserve preservation. The detrimental BOV decrease after enucleation with electroablation Wstęp: Endometriozę dotyka około 5-15% kobiet w wieku rozrodczym. Jednym z najważniejszych powikłań występowania endometriozy miednicy mniejszej jest nieплодność.

Cel: Ocena leczenia endometriozy jajnika z wykorzystaniem łączonych technik laparoskopowych (wyluszczenie torbieli z elekta ablacją/laserem CO\textsubscript{2}) i ich wpływ na zachowanie rezerwy jajnikowej.

Materiał i metody: 58 pacjentek w wieku 19-40 lat z rozpoznaną endometriozą jajnika poddano ocenie rezerwy jajnikowej obejmującej określenie liczby pęcherzyków antralnych w jajnikach, pomiar całkowitej objętości jajników oraz oznaczenie poziomu FSH w surowicy krwi. 24 chore poddano następnie laparoskopowemu wyluszczeniu torbieli w połączeniu z ablacją laserem CO\textsubscript{2} 34 pacjentek wykonano wyluszczenie torbieli z nastepową elektroablacją. Rezerwa jajnikowa pacjentek została poddana ponownej ocenie po trzech i sześciu miesiącach od wykonania zabiegu.

Wyniki: W żadnej z grup nie zaobserwowano istotnie statystycznego spadku ilości pęcherzyków antralnych po trzech i sześciu miesiącach od wykonania zabiegu. Dodatkowo, w grupie po przebytym wyluszczeniu torbieli z nastepową elektroablacją zaobserwowano znacznym spadek poziomu FSH po trzech miesiącach od zabiegu. Grupa ta charakteryzowała się również istotnie statystycznie spadkiem całkowitej objętości jajników po trzech i sześciu miesiącach od zabiegu. U 17% pacjentek z tej grupy zaobserwowano nawroty choroby widoczne w USG po sześciu miesiącach obserwacji. W grupie pacjentek po wyluszczeniu torbieli z nastepowym uzyciem lasera CO\textsubscript{2} caolkwita objętość jajników nie zmieniła się znacząco, natomiast zaobserwowano wysoki odsetek nawrotów ultrasonograficznych endometriozy (39%).

Wniosek: Brak istotnych zmian w ilości pęcherzyków antralnych po operacji sugeruje wstępną pozytywną
and high relapse rate after enucleation after CO₂ laser ablation shows that further research is needed in order to optimize the laparoscopic techniques of endometriosis therapy.

Introduction
Endometriosis is an estrogen-dependent chronic disease with ectopic endometrial cells with glands and stroma outside the uterine cavity. The disease occurs in 5-15% of women with no clinical symptoms, and in 30-50% of cases it causes pain symptoms in the pelvic region [1]. Endometriosis can be divided into superficial, ovarian and deep infiltrating endometriosis (DIE) [2]. One of the most frequent causes of endometriosis is infertility. The presence of the foci of ectopic tissue in the pelvic region leads to the creation of a chronic inflammatory microenvironment, which affects both the gamete mobility and the implantation potential of the embryo. Apart from the adverse impact on hormonal and biochemical homeostasis, the structural and functional damage to the Fallopian tubes due to the formation of adhesions has an important role in the preservation of fertility [3]. One factor that is extremely important for the reproductive potential of women is the destruction of the ovarian cortex by the ovarian endometriosis, which leads to a decreased ovarian reserve [4]. Currently used techniques of ovarian reserve evaluation include: antral follicle count (AFC) and the assessment of basal ovarian volumes (BOV) during an ultrasound scan, as well as the measurement of the blood concentrations of follicle stimulating hormone (FSH), Anti-Müllerian Hormone (AMH) and inhibin B. The FSH assay plays an important role in patients below 35 years old with anovulatory cycles and patients with diagnosed endometriosis who are prepared for fertilization with assisted reproductive techniques (ART) [5,6]. A high number of antral follicles in the ovary (AFC) increases the chance for live births and for pregnancy in general [7,8]. In recent findings in the literature, the prognostic value of AFC is comparable to that of biochemical and hormonal parameters in patients being prepared for in vitro fertilization (IVF) [9,10].

With age, the ovarian reserve decreases, which is visible mainly in the drop in AFC, increase in FSH level and lowered serum concentration of AMH. The quickest decrease of the ovarian reserve can be observed after the age of 35 [11]. The treatment of endometriosis consists of both surgical and pharmacological therapy. The golden standard in the diagnosis and surgical treatment of this disease is laparoscopy [12]. Currently, one of the more efficient methods is the surgical excision of the endometrial foci and enucleation of cysts, known as excisional cystectomy (EC) [13]. This technique leads to the best therapeutic effect seen as a high rate of spontaneous pregnancies and lower relapse rate in comparison to other methods [14]. At the same time, there are papers suggesting that cyst fenestration and electroablation of pseudocapsule also has a high efficiency, comparable to EC, as the rate of positive response to hormonal stimulation is high [15,16]. However, in other research it was observed that, in comparison to surgical enucleation, this method entails a high relapse rate within six months from the procedure, as much as 10-20%, as well as a lower rate of pregnancies achieved in the post-surgical period [17-19]. The majority of the studies agree that surgical enucleation of the ovarian lesions causes damage to the ovarian parenchyma and induces local fibrosis of the healthy tissue, which results in a decrease in the ovarian reserve [20-22]. In the postsurgical period, the AFC and BOV parameters decrease, the level of FSH rises and the serum concentration of AMH falls [4,22-24]. Decreased fertility is observable both in spontaneous and ART-stimulated cycles. The relatively new technique of CO₂ laser ablation enables high precision of movement and control over tissue penetration depth, which results in smaller mechanical and thermal damage to the ovarian parenchyma [25]. As a result of the procedure, the number of antral follicles does not decrease and the normal ovarian volume can be maintained [4]. This fact is of crucial importance for the procreative plans of the patients as it gives more opportunities of achieving pregnancy in a natural way or with the use of ART. Long-term observations indicate that the chances for a positive therapeutic effect, i.e. a pregnancy, is statistically greater in those patients having the CO₂ laser treatment [26]. However, some sources report high relapse rates after the use of this surgical technique [27,28].

The aim of this paper is to analyze the efficiency of two laparoscopic methods of treatment of ovarian endometriosis: excisional cystectomy with CO₂ laser ablation and excisional cystectomy with electroablation, in terms of the preservation of the ovarian reserve.

Material and methods
Study design
The one-center, prospective, randomized study was carried out between February 2012 and January 2014. All patients scheduled for surgical treatment of ovarian endometriosis at the Gynecology and Oncology Clinic of the Jagiellonian University Medical College Krakow, Poland, were invited to participate in the study. The study was approved by the Ethical Committee of the Jagiellonian University Medical College (decision no. KBET/14/B/2012). All patients signed the informed consent form for participation in the study. The study was performed in accordance with Good Clinical Practice and confidentiality rules. The exclusion criteria included: laparotomy, inflammation in the pelvic area or neoplasm in the medical history, use of contraceptive drugs and pregnancy.

The information about the exclusion criteria was obtained by general and gynecological interview. Of the group initially invited to the study (n=110), seventy patients (n=70) were finally included in the study following verification of all the criteria. All the patients were given codes generated randomly by a computer system. On the basis of the randomization, the patients were divided into two groups. One half of the patients formed group A, undergoing excisional cystectomy (EC) with CO₂ laser ablation, and the other half formed group B, treated with EC combined with electroablation. Out of the seventy patients included in the study, sixty five (n=65) (92.8%) expressed their informed consent to participate in the study. Additionally, seven patients (10%) from both groups withdrew their consent for participation in the study just before the surgical procedure. The final total for the study was fifty-eight participants (82.9%) aged from 19 to 40 (24 patients in group A and 34 patients in group B).

Method
Before surgery, each patient underwent a transvaginal ultrasound with assessment of the ovarian reserve (AFC and BOV) and measurement of the endometrial cyst volume. Furthermore, between the first and the third day of the menstrual cycle, the serum concentration of FSH was assayed. All these procedures were performed again three and six months after the operation, together with an assessment of the relapse of the disease by ultrasound scan. All patients who became pregnant during the project were excluded from further participation in the study. The ultrasound device was used with a transvaginal volumetric scanner (2D/3D/4D 5-9 MHz Voluson E6). The assessment of AFC and BOV was performed with the 3D SonoAVC (Automated Volume Calculation) technology of GE Healthcare Voluson E6 BT12 and software 4D view 10.5. The progress of endometriosis was determined during laparoscopy with the revised Classification of Endometriosis of the American Society for Reproductive Medicine (ASRM) [29]. The procedure involved the removal of endometrial cysts in one or both ovaries. The ovarian lesions were 50-60% enucleated and then the pseudocapsule treated with electroablation or CO₂ laser ablation. Ablative techniques were also used for hemostasis. In order to confirm the diagnosis of ovarian endometriosis, the procedure included a sample for histopathological examination. The surgery was performed with an AcuPulse CO₂ laser (Lumienie, Israel) in the impulse mode, at a power of 10-20W and frequency of 0.01 Hz. The laser was connected to the laparoscope (Storz, Germany) and an Aida registering machine (Storz, Germany). The main spectra lines of the lasers were in the
wavelength range between 9.4 μm and 10.6 μm, with emitted power up to 1013 W in the impulse mode. The electroablation of the endometrial foci used an electrosurgical device by ERBE Elektromedizin GmbH with a bipolar 60-70 W ending.

Statistical analysis
The statistical analysis was conducted using Statistica 10 (StatSoft Inc.) software. The data were presented as averages with standard deviation (x±SD) or percentage values. Normal distribution was determined with the use of the Shapiro-Wilk test. The AFC and BOV differences between the groups were analyzed with the Mann-Whitney U test. The variance homogeneity was tested with the Levene U test. The variance homogeneity was tested with the Student’s t test. The statistically significant value was assumed as \( p < 0.05 \).

Results
Assessment of the endometriosis stage and the surgical procedures
After the intraoperative assessment of the stage of endometriosis according to the rASRM scale, ten patients (17%) belonged to stage IV, forty-one patients (71%) belonged to stage III, four patients (7%) belonged to stage II and three patients (5%) to stage I. The characteristic of the groups before and after surgery are presented in Table I.

In group A (EC/CO laser ablation) there were thirteen procedures on the right ovary (54.2%), six procedures on the left ovary (25%) and five bilateral procedures (21%). In group B (EC/electroablation) there were thirteen procedures on the right ovary (38%) and eighteen procedures on the left ovary (52%). In the latter group, endometriosis was removed from both ovaries in three patients (8.8%).

Results of the 3-month follow-up assessment
A total of fifty-seven patients (98%) took the three-month follow-up assessment: twenty four from group A (100%) and thirty three from group B (97%). In this period, one patient (3%) from group B had become spontaneously pregnant. The ultrasound scan showed relapses of ovarian endometriosis in seven patients from group A (29%) and eighteen procedures on the left ovary (38%) and five bilateral procedures (21%).

Results of the 6-month follow-up assessment
A total of fifty-two patients (89.6%) took the six-month follow-up assessment: twenty three patients from group A (100%) and thirty from group B (97%). In this period, one patient (3%) from group B had become spontaneously pregnant. The ultrasound scan showed relapses of ovarian endometriosis in twenty three patients from group A (95.8%) and eighteen procedures on the left ovary (38%), with emitted power up to 1013 W in the impulse mode. The electroablation of the endometrial foci used an electrosurgical device by ERBE Elektromedizin GmbH with a bipolar 60-70 W ending.

The analysis showed a significant drop in FSH level at six months in the group treated with EC/electroablation (\( p=0.023 \)) while in the group treated with EC/CO laser ablation no significant changes in the FSH level were observed in this period. At six months after the surgery, there were no further changes in FSH level in either group.

BOV analysis
The analysis showed that the Basal Ovarian Volume (BOV) decreased in group B both at the follow-up assessments at three months (\( p=0.002 \)) and six months (\( p=0.001 \)) from surgery. It was also observed that the decrease was the greatest on the second follow-up assessment. No differences in the BOV were observed in group A. The analysis

<table>
<thead>
<tr>
<th>Data</th>
<th>Group A (±SD)</th>
<th>Group B (±SD)</th>
</tr>
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<tbody>
<tr>
<td>Endometriosis progress (rASRM)</td>
<td>2.95 ± 0.7</td>
<td>3.08 ± 0.58</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.97 ± 5.1</td>
<td>31.70 ± 7.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.57 ± 2.65</td>
<td>21.17 ± 2.36</td>
</tr>
<tr>
<td>Right ovary volume (cm³)</td>
<td>13.01 ± 6.96</td>
<td>10.40 ± 9.12</td>
</tr>
<tr>
<td>Left ovary volume (cm³)</td>
<td>12.40 ± 10.10</td>
<td>9.59 ± 4.47</td>
</tr>
<tr>
<td>AFC right ovary</td>
<td>9.44 ± 5.4</td>
<td>10.69 ± 6.86</td>
</tr>
<tr>
<td>AFC left ovary</td>
<td>10.40 ± 7.12</td>
<td>11.75 ± 7.59</td>
</tr>
<tr>
<td>AFC left ovary</td>
<td>10.40 ± 7.12</td>
<td>11.75 ± 7.59</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>7.87 ± 4.67</td>
<td>6.15 ± 2.5</td>
</tr>
<tr>
<td>Right ovary cyst volume before surgery (cm³)</td>
<td>9.7 ± 16.15</td>
<td>10.8 ± 9.2</td>
</tr>
<tr>
<td>Left ovary cyst volume before surgery (cm³)</td>
<td>12.33 ± 8.46</td>
<td>11.79 ± 5.45</td>
</tr>
</tbody>
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Figure 1
BOV [cm³] changes on follow-up visits after 3 and 6 months
Zmiany objętości jajnika [cm³] po 3 i 6 miesiącach obserwacji.

Table II
Relapse rate and cyst volume in the groups at the three- and six-month follow-up assessments.
Odsetek nawrotów oraz objętość ewentualnych torbieli w obu grupach po 3 i 6 miesiącach obserwacji.

<table>
<thead>
<tr>
<th>Relapses</th>
<th>CO₂ laser ablation (group A)</th>
<th>Electroablation (group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of relapses</td>
<td>29%</td>
<td>39%</td>
</tr>
<tr>
<td>Relapse volume (cm³)</td>
<td>4.67 ± 6.21 SD</td>
<td>3.76 ± 3.77 SD</td>
</tr>
</tbody>
</table>
of BOV changes is presented in Figure 1.

AFC analysis
The study did not reveal any significant changes in AFC values at three and six months after the surgery in either group and regardless of the operated ovary (right vs. left ovary).

Discussion
Summary of main findings
The study formed an analysis of the impact of the laparoscopic removal of ovarian endometriosis on the preservation of the ovarian reserve, comparing two combined laparoscopic techniques: EC/electroablation and EC/CO2 laser ablation. Neither group showed any statistically significant decrease of AFC at three or six months. Additionally, in the group treated with EC/electroablation, a significant decrease in FSH level and BOV was observed three and six months after the surgery. Among the patients from this group, five (15.2%) became spontaneously pregnant and six (17%) had relapses of the disease visible in the ultrasound image after six months. On the other hand, the group treated with EC/CO2 laser ablation showed no changes in the BOV at three or six months; however, the relapse rate was high (39%). At the six-month follow-up assessment, no spontaneous or stimulated pregnancy was confirmed in this group.

Comparison with other studies
The majority of the studies on the preservation of the ovarian reserve in the surgical treatment of endometriosis focus mainly on the comparison of the therapeutic effects of the chosen laparoscopic techniques, such as excisional cystectomy (EC) and fenestration with electrical or laser vaporization of the pseudocapsule, and the results varied. Frequently, a statistically significant, high reduction of BOV, lowered AFC and increase in FSH were observed after laparoscopic excisional cystectomy [23,24,30-32]. The results indicate that the changes were more intense than in the case of ablative techniques. However, it was emphasized that excisional cystectomy is associated with higher pregnancy rate and lower endometriosis relapse rate [14,19,31]. This is of crucial importance for the treatment of patients in the reproductive period, especially those women undergoing preparation for the in vitro fertilization procedure (IVF).

In this study, beneficial results were obtained for both types of procedures, especially concerning AFC, currently considered the most important prognostic factor for the preservation of ovarian reserve [33]. The positive influence of CO2 laser ablation on AFC was confirmed in retrospective studies by Shimizu et al. and Wyns et al. [26,34]. The studies consisted of an analysis of the number of pregnancies from natural and/or stimulated cycles in patients with endometriosis who had undergone laser treatment. Apart from that, the lack of any negative influence of electroablation on AFC was proved in the studies by Aborzi et al. and the recent study by Donnez et al., analyzing the therapeutic effect of combined laparoscopic techniques of EC and CO2 laser ablation [15,35]. The analysis conducted by Donnez et al. revealed a minor influence of such treatment on the ovarian cortex, which resulted in the preservation of AFC and maintenance of BOV comparable to that in healthy women. The latest metaanalysis by Muzzi et al. suggests that laparoscopic excisional cystectomy has no negative influence on the preservation of AFC [36]. Furthermore, the prospective study by Uncu et al. also did not prove any changes of AFC within six months of laparoscopic excisional cystectomy in the treatment of ovarian endometriosis [37]. The decrease in FSH level observed in our study three months after the surgery in those patients who underwent EC/electroablation is an advantageous factor for further assessment of combined laparoscopic techniques for the preservation of ovarian reserve. In our study groups, the obtained pregnancy rates of 15.2% spontaneous pregnancies after EC/electroablation and no pregnancies after EC/laser ablation are substantially lower than in studies by other authors. For comparison, the pregnancy rates after laparoscopic treatment of ovarian endometriosis in the studies by Moschini et al. and Gandhi et al. were 22% and 20%, respectively [38,39]. It must be remembered, though, that in the mentioned studies the follow-up period lasted two years after the surgery. In our study, in the group of patients after EC/CO2 laser ablation, a high rate (39%) was observed of relapses visible in the ultrasound scan, which is comparable to the results of the metaanalyses by Vercellini et al. with a relapse rate of 18.4%, and the final results by Carmen et al. of 37% [18,27]. According to Lass et al., the BOV value is a significant prognostic factor, especially for the prediction of response to the stimulation of ovulation with gonadotropins [40]. In our study, the reduction of BOV three and six months after the surgery in patients who underwent EC/electroablation is a disadvantageous factor for this type of method. Similar results were obtained by Roman et al. who analyzed the efficiency of EC/electroablation and minimally invasive ablative techniques in the treatment of ovarian endometriosis [41]. In the study by Roman et al., BOV reduction was observed in 31% of patients and the decrease in AFC. The importance of BOV reduction for the preservation of the ovarian reserve needs to be considered. It is worth mentioning that in the metaanalysis conducted by Hendrix et al., AFC correlates better than BOV with the response to stimulation in the patients undergoing IVF [42].

Interpretation of the results
The results allow us to look positively at the usefulness of the combined laparoscopic technique in the treatment of ovarian endometriosis with the preservation of the ovarian reserve. The resignation from ablative techniques caused by a too high relapse and low pregnancy rate makes these methods characterized with a limited scope of damage to the ovarian parenchyma should bring desired effects - a lower relapse rate and preservation of the ovarian reserve. Taking into consideration the aspect of the ovarian reserve, an important element of the surgical therapy would be simultaneous radical removal of the cyst with the pseudocapsule in a manner as non-invasive as possible while preserving the ovarian cortex. Unfortunately, it is often physically impossible to remove the whole cyst surgically without damaging the ovarian structures. In order to avoid this, we should first consider CO2 laser ablation as a method that is more precise with lower penetration scope, which results in less damage to the healthy tissue in comparison to electroablation. It seems that the potential advantages of CO2 laser ablation are much less than expected. The small difference in AFC between the two combined techniques, simultaneous higher relapse rate and lower pregnancy rate within six months in six months after EC/CO2 laser ablation means that EC/electroablation is the more advantageous technique. It may be assumed that one possible reason for such a high relapse rate visible in the ultrasound scan after EC/CO2 laser ablation is imperfect surgical technique, the “human factor”, which is difficult to eliminate. According to Muzzi [43,44], the thickness of endometrial capsule ranges from 1.5 to 1.9 mm. The ablative mode of the laser applied in the study (pulsation mode, power 10-20W) destroys about 0.1 mm of tissue, which may be insufficient for the removal of the cyst remnants after enucleation. This may be the cause of relapses. The implementation of a mode with deeper penetration of the lesion and maintenance of the precision in removing only the lesions without penetrating into the healthy ovarian tissue could improve the therapeutic effect of CO2 laser ablation. It must be remembered, though, that the increased scope of impact (penetration depth) of the laser beam may deteriorate the results as far as the ovarian reserve (AFC, FSH, BOV) is concerned.

Conclusions
A combined method of using classical laparoscopic surgical removal with CO2 laser ablation or electroablation for the treatment of ovarian endometriosis was found to be effective in the preservation of AFC. This gives an initial positive evaluation of both techniques in the context of ovarian reserve preservation.

The current study found that in the short term observation period, excisional cystectomy (EC) with electroablation was effective in the treatment of ovarian endometriosis, especially due to the significant reduction of FSH. A lower rate of relapses, identified...
in follow up ultrasound scans, and higher pregnancy rate were also found. This has also been confirmed by other studies.

The treatment of ovarian endometrioses with excisional cystectomy (EC) and CO2 laser ablation was found to preserve BOV levels. Further research on larger numbers of patients with possible modification of the surgical technique and longer follow-up period may give improved answers to the question concerning the justification of combined laparoscopic techniques in the treatment of ovarian endometriosis in patients of reproductive age.

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12. Donnez J, Nisolle M, Gillet N, Smets M, Bassil S, Casanas-Roux F. Ovarian cystectomy versus CO2 laser ablation was found to preserve BOV levels. Further research on larger numbers of patients with possible modification of the surgical technique and longer follow-up period may give improved answers to the question concerning the justification of combined laparoscopic techniques in the treatment of ovarian endometriosis in patients of reproductive age.

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