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Ovarian stimulation outcome in infertile women with endometriosis undergoing IVF

Wyniki stymulacji jajników u niepłodnych kobiet z endometriozą poddawanych IVF

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Abstract

Objectives: *The aim of our study was to assess the influence of ovarian endometriosis on the outcome of controlled ovarian stimulation (COS) in IVF patients with normal functional ovarian reserve.*

Material and methods: *This was a retrospective case-control study of patients undergoing IVF/ICSI treatment between January 2013 and September 2014, aged ⩽40 years and a good ovarian reserve, characterized by antral follicle count ⩾7, anti-mullerian hormone levels ⩾0.8 ng/ml and day 3 serum FSH values ⩽12mUI/ml. The study group (1) consisted of 28 patients with ovarian endometriosis. The control group (2) included 57 patients with laparoscopically diagnosed tubal-factor infertility, without endometriosis. These groups were analyzed for the number of stimulation days, the total amount of gonadotropins used for COS, number of total or M2 oocytes and ovarian sensitivity index (OSI).*

Results: *The mean stimulation days, in groups 1 and 2, was 9.2±1.5 and 9.3±1.6, respectively. The mean number of retrieved oocytes in groups 1 and 2 was 10.5±4.7 and 9.0±4.5, respectively. The average number of metaphase II oocytes, in groups 1 and 2, was 8.8±4.71 and 8.2±4.1, respectively. The average of the total amount of gonadotropins used for stimulation, and the OSI were similar for both groups. We found no statistically significant differences in terms of the number of stimulation days, number of oocytes retrieved, total dose of FSH used and the OSI, between the two groups.*

Conclusions: *The simple presence of ovarian endometriosis does not seem to affect the outcome of the exogenous gonadotropin stimulation, when the ovarian functional reserve is not significantly impaired.*

Key words: **endometriosis** / **ovarian reserve** / **IVF** /

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Streszczenie

Cel pracy: *Celem naszego badania była ocena wpływu endometriozy jajnikowej na wyniki kontrolowanej stymulacji jajników (COS) u pacjentek poddawanych zapłodnieniu pozaustrojowemu z prawidłową rezerwą jajnikową.*

Materiał i metoda: *badanie retrospektywne z grupą kontrolną przeprowadzone wśród pacjentek poddawanych IVF/ICSI pomiędzy styczniem 2013 a wrześniem 2014, w wieku ⩽40 lat i z prawidłową rezerwą jajnikową, ocenioną jako liczba pęcherzyków antralnych ⩾7, poziom hormonu anty-mullerowskiego⩾0,8 ng/ml i poziom surowiczego FSH w 3 dniu cyklu ⩽12mUI/ml. Grupa badana (1) składała się z 28 kobiet z endometriozą jajnikową. Do grupy kontrolnej (2) włączono 57 pacjentek z laparoskopowo potwierdzonym czynnikiem jajowodowym niepłodności, bez endometriozy. Obie grupy przeanalizowano pod względem liczby dni stymulacji, całkowitej ilości zużytych gonadotropin do COS, liczby wszystkich oocytów i M2 oocytów i indeksu wrażliwości jajników (OSI).*

Wyniki: *średnia liczba dni stymulacji w grupie 1 I 2 wynosiła odpowiednio 9.2±1.5 i 9.3±1.6. Średnia liczba uzyskanych oocytów w grupie 1 i 2 wynosiła odpowiednio 10.5±4.7 i 9.0±4.5. Średnia liczba oocytów w metafazie II w grupie 1 i 2 wynosiła odpowiednio 8.8±4.71 i 8.2±4.1. Średnia ilość gonadotropin zużytych do stymulacji i indeks wrażliwości jajników były podobne w obu grupach. Nie znaleźliśmy istotnych statystycznie różnic w odniesieniu do liczby dni stymulacji, liczby uzyskanych oocytów, całkowitej dawki FSH i OSI pomiędzy obiema grupami.*

Wnioski: *Obecność endometriozy jajnikowej wydaje się nie wpływać na wyniki stymulacji egzogennymi gonadotropinami, gdy funkcjonalna rezerwa jajnikowa nie jest istotnie upośledzona.*

Słowa kluczowe: **endomtrioza** / **rezerwa jajnikowa** / **IVF** /

Endometriosis is a common gynecological disorder, affecting approximately 10% of women in the reproductive age [1]. It is an important cause of infertility and a reason for a growing number of IVF treatments. Over the years, the prevalence of endometriosis has dramatically increased, to 25%–50% in infertile women. In fact, 30–50% of women with endometriosis are infertile [1,2].

IVF studies have suggested that women with advanced endometriosis have poor ovarian reserve, low oocyte and embryo quality, and poor implantation [3]. The definite cause-effect relationship is still controversial and the mechanisms by which endometriosis affects female fertility remain to be fully understood. They might include abnormal folliculogenesis, elevated oxidative stress, immune alterations, modified hormonal milieu in the follicular and peritoneal environment, and reduced endometrial receptivity [4].

Objectives

Endometriosis is associated with impaired IVF outcomes [5]. We wondered whether endometriosis affected the outcomes of controlled ovarian stimulation (COS) through various changes in ovarian physiology or whether it was associated with another mechanism such as diminished ovarian reserve. The aim of the study was to investigate the impact of endometriosis on some IVF outcomes in patients with normal functional ovarian reserve.

Material and methods

It was a retrospective, non-interventional case-control study of patients undergoing IVF/ICSI treatment between January 2013 and September 2014. Local Ethics Committee approved of the study. All data were retrospectively collected to evaluate the impact of endometriosis on the treatment outcome.

Within the study period, we selected patients, aged ≤40 years, at the onset of the controlled ovarian hyperstimulation (COH) cycle and with good ovarian reserve, proven by an antral follicle count (AFC) of ≥7, anti-Mullerian hormone (AMH) serum levels of ≥0.8 ng/ml, and day 3 serum FSH values of ≤12mUI/ml. We excluded patients with technical difficulties impairing follicle aspiration of the ovaries at oocyte retrieval or those who previously had one of the ovaries surgically removed. AMH levels were assessed <6 months prior to the IVF procedure.

AFC and basal FSH levels were documented on the day the COS was started for the patients with the antagonist protocol. For the down-regulation protocol, AFC and basal FSH were measured on days 2-3 of the previous cycle. AFC was estimated as the total number of follicles with the diameter of <10 mm, as described previously [6].

The study group consisted of women diagnosed with ovarian endometriosis, either during previous surgery with histopathological confirmation or by the presence of an ultrasound image highly suggestive of ovarian endometrioma, with a mean diameter of ≤40 mm. Ultrasound diagnosis of ovarian endometrioma was based on the visualization of round-shaped, homogenous hypoechoic mass of low-levels echoes within the ovary, as described previously [7]. Voluson V730 Expert (GE Healthcare, Milwaukee, WI) was used. The endometrioma was measured in three dimensions, and the average diameter was calculated.

The control group included women who underwent IVF treatment for a laparoscopically diagnosed tubal factor, but without any evidence of pelvic endometriosis.

IVF procedure

On the first day of COS, the procedure was initiated if there was no follicle of ≥10 mm in diameter at transvaginal ultrasound and a) if estradiol levels were <50 pg/mL for the antagonist protocol or b) if estradiol levels were <15 pg/ml and endometrial thickness ≤4 mm for the long protocol.

COS was achieved by one of the two different regimens: a) long down-regulation protocol using GnRH agonist triptorelin (Gonapeptyl®; Ferring) or b) short protocols using 0.25 mg GnRH antagonists (Cetrotide®; Merck Orgalutran® Serono or ®; Organon). Ovarian stimulations were conducted with daily subcutaneous injections of rFSH (Follitopin alfa Gonal-f®, Merck-Serono), at appropriate doses (100-350 IU) estimated according to the age, AFC and AMH levels of the woman.

Ovarian response to gonadotropins was monitored by transvaginal ultrasound plus serum estradiol measurement every other day from stimulation day 6. Final follicular maturation was triggered when at least one follicle was ≥18 mm, with appropriate serum estradiol levels, using recombinant human chorionic gonadotropin (hCG, Ovitrelle®, Merck Serono), 0.25 μg, single subcutaneous injection. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h after triggering.

AMH and FSH measurements

AMH was assayed using an ELISA kit (AMH Gen II, Beckman Coulter, #A73818, Brea, CA, USA), I accordance with the manufacturer's protocol. The detection limit of AMH was 0.01 ng/mL. Concentrations of FSH were measured by commercial electrochemiluminescence immunoassay (ECLIA) kit (Elecsys FSH kit, Roche Diagnostics, Mannheim, Germany).

Data collection

We followed patient demographics, stimulation protocol, total doses of gonadotropins used for stimulation, number of stimulation days, number of total oocytes and mature (metaphase II) oocytes retrieved. The 'ovarian sensitivity index' was defined as the ratio between the total dose of rFSH (IU) used in COS and the number of the retrieved oocytes.

Statistical analysis

Statistical analyses were performed with SPSS program, version 21.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were compared using the Student's test. The *p*-value of <0.05 was considered as statistically significant.

Results

The study population included a total of 85 patients, 28 with endometriosis and 57 controls. The baseline data and the IVF/ICSI cycle outcomes for women undergoing assisted reproduction with or without endometriosis are presented in Table 1.

There were no statistically significant differences between the baseline data from the study groups regarding age, AFC, AMH, and basal FSH levels. Endometriosis patients had significantly lower BMI as compared to controls.

We found no statistically significant differences between the two groups in terms of the number of stimulation days, total amount of gonadotropins used for COS, number of total or M2 oocytes, and the ovarian sensitivity index (OSI).

Discussion

Impaired fertility in endometriosis patients, both with regard to spontaneous conception as well as IVF success rates, is a widely accepted fact but the full pathogenic mechanisms are far from acknowledged [3]. The suggested mechanisms associated with poor outcome include lower oocyte number and embryo quality [8, 9], as well as decreased implantation rates resulting from diminished endometrial receptivity.

Our study evaluated the impact of endometriosis on IVF/ICSI outcomes in patients with normal functional ovarian reserve. We found no statistically significant differences between endometriosis patients and controls in terms of the number of stimulation days, total amount of gonadotropins used for COS, number of oocytes, and the ovarian sensitivity index (OSI).

OSI [10] shows an effective ovarian response to FSH stimulation, independently of the total amount of the administered FSH. Patients with more abundant ovarian follicular reserve tend to display higher ovarian sensitivity to exogenous FSH, whereas women with a low ovarian reserve usually have lower ovarian sensitivity. Some authors consider OSI to be a better parameter for evaluating the ovarian response [11, 12].

Dong et al. [13], reported prolonged ovarian stimulation in endometriosis patients, associated with the need for a higher gonadotropin dosage, for all stages of endometriosis. However, they observed similar pregnancy rates for the affected patients and controls, except for patients with stage III-IV endometriosis. Additionally, in a recent study on patients with diminished ovarian reserve, Roustan et al. [14], noted a significantly longer ovarian stimulation and higher gonadotropin dosage for the endometrioma-treated group.

Our study ascertained a similar number of stimulation days for both, endometriosis and control groups, taking into consideration only patients with normal ovarian reserve. Recent findings support our results as various authors demonstrate lack of statistically significant correlations between AFC and the stage of endometriosis [15]. Moreover, the number of the follicles is not reduced after surgical treatment of an endometrioma [16]. Variations of the stimulation length might result from heterogeneity of protocols (e.g. step-up, step-down protocols, gonadotropin administration on trigger day) and other independent factors, e.g. individual follicular phase duration. The need for individualized stimulation protocols leads to massive data heterogeneity and raises significant difficulties in designing studies with reproducible conditions, resulting in a large number of studies with contradictory results within the field of assisted reproduction.

Several authors report higher gonadotropin doses in patients with endometriosis undergoing COS for IVF as compared to endometriosis-free groups [17, 18]. Data are consistent only for patients with advanced (III-IV, ASRM classification) stage of endometriosis and consecutive diminished ovarian reserve.

In their study on two normal responder groups, one with ovarian endometriomas and the other with simple ovarian cysts, Kumbak et al., showed higher gonadotropin doses in the endometriosis group. Their findings suggest that the adverse effect of endometriosis is the result of the disease itself, not the presence of a cystic mass [19]. One possible explanation could be a more important impact of the biochemical changes in the follicular microenvironment [20] in advanced stages of endometriosis.

In our study, we selected patients with normal ovarian reserve, what could be the main reason behind the need for the same gonadotropin doses.

Studies assessing the number and quality of the retrieved oocytes from patients undergoing IVF procedures in the presence

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Table I. Baseline data and IVF/ICSI cycle characteristics for women with or without endometriosis undergoing assisted reproduction.

	Endometriosis (n=28)	Control (n=57)	p
Age (years)	34.00±3.06	32.72 ±3.56	.107
BMI (kg/m^2)	21.59±1.41	22.75±2.65	.033
AMH (ng/ml)	2.17±1.06	2.81±1.90	.099
AFC (n)	12.54±5.26	13.39±5.86	.517
Day-3 FSH (mIU/ml)	6.45±1.96	7.01±2.18	.262
Stimulation days (n)	9.21±1.50	9.37±1.58	.668
Retrieved oocytes (n)	10.50±4.74	9.02±4.53	.166
M2 oocytes (n)	8.79±4.65	8.23±4.06	.572
Total FSH dose (IU)	2442.86±1048.51	1989.04±932.88	.058
OSI (IU FSH / oocyte)	285.97±178.72	295±230.80	.854

Data are presented as mean ± standard deviation. BMI: body mass index; AMH: anti-Mullerian hormone; AFC: antral follicle count; FSH: follicle-stimulating hormone; OSI: ovarian sensitivity index.

of endometriosis show contradictory results, when compared with patients without endometriosis.

A recent study by Filippi et al., shows no significant differences in oocyte developmental competence in the presence of ovarian endometriomas in women undergoing IVF [21]. Other studies conclude that patients with endometriosis have an impaired follicular milieu and poor oocyte quality [20].

Several authors report a lower oocyte yield from patients with endometriosis as compared to endometriosis-free groups [17, 18, 22], both in moderate-severe endometriosis group only [22], as well as patients with advanced (III-IV, ASRM classification) stage endometriosis and consecutive diminished ovarian reserve [17,18]. Moreover, endometriosis affects the oocyte number irrespectively of the presence of an ovarian endometrioma [23], but these studies lack information about technical difficulties in transvaginal oocyte retrieval, which might occur due to pelvic anatomy changes associated with advanced stage endometriosis.

Our findings, which are consistent with recent reports in the literature, found no statistically significant differences between the number of total or M2 oocytes in the endometriosis and control groups. Likewise, a study comparing the number of the retrieved oocytes from an intact gonad and from an ovary containing an endometrioma (in IVF patients with unilateral ovarian endometriosis) compared with those of patients with no endometriosis found no statistically significant differences between the groups [24]. Also, Kiran et al., found a similar number of the retrieved oocytes and metaphase II oocytes in both, endometrioma and unexplained infertility groups [25].

Quantitative and qualitative assessment of the retrieved oocytes in ART patients with endometriosis is important for the outcome prognosis. Data about embryo quality and implantation rates are better predictors of the success rate, but other important factors (male, endometrial) are also involved. Heterogeneity of infertility causes is a major drawback in designing studies with a high relevance in ART/IVF. Also, financial and ethical limitations need to be taken into consideration in large prospective randomized trials, which might have a better clinical relevance due to their sample size.

The retrospective case-control design constitutes a major limitation of our study. However, randomized controlled trials present a challenge in this case. Although the study sample is relatively small, our study provides statistically significant evidence that the primary outcomes of COS are no different in endometriosis patients than controls, if they have a similar ovarian reserve.

Another limitation of our study is the fact that patients from the endometriosis group had a statistically lower BMI than the control group. However, both groups included patients with normal weight, so it is relatively unlikely that this difference had any influence on gonadotropin requirement and the number of the retrieved oocytes. Studies reporting significant weight-dependent differences in gonadotropin doses are conclusive only for overweight (>24.9 kg /m^2) [26] and obese (BMI>29 kg/m^2) women [27, 28], but even in these cases the influence of the weight is controversial [29], for both types of major stimulation protocols used [30]. BMI seems to have a reduced influence on basal FSH levels, gonadotropin dosage, as well as oocyte number and quality [31-33]. Also, the amount of hCG required as the trigger is not significantly different with respect of BMI, even for obese patients [34].

Conclusions

The mere presence of endometriosis appears to have no effect on the ovarian response to exogenous gonadotropin stimulation when the ovarian reserve is not significantly impaired. In our study, the outcome of COS, in terms of quantitative (total number of retrieved oocytes) as well as qualitative (M2 oocytes) assessment, was similar for both investigated groups.

Authors' contribution:

1. Alexandru Luca – concept, data analysis, article draft and revision.
2. Dragos Nemescu – article draft, corresponding author.
3. Maria Butnaru –data acquisition.
4. Andreea Butnariu – data acquisition.
5. Mircea Onofriescu – revised article critically.

Authors' statement

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