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Title
Impact of large ovarian endometriomas on the response to superovulation for in vitro fertilization: a retrospective study

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Condensation

Endometriomas (≥ 5 cm) at time of IVF significantly reduce the number of oocyte retrieved in the affected ovaries compared with the contralateral healthy ovaries.

Abstract

Objective: To assess the response to superovulation for in vitro fertilization (IVF) in patients with unilateral endometriomas with diameter ≥ 5 cm and in the contralateral healthy ovary.

Study design: This retrospective analysis of a prospectively collected database included patients who underwent superovulation for IVF/ICSI cycles and had unoperated single unilateral endometrioma with diameter ≥ 5 cm and healthy contralateral ovary. The primary outcome of the study was to compare the number of oocyte retrieved in the ovary with the endometrioma and the contralateral healthy ovary.

Results: The total number of follicles was lower in ovaries with endometriomas (2.6 ± 1.3) than in healthy ovaries (4.8 ± 2.0; p<0.001). The number of codominant follicles and the total number of oocytes retrieved were lower in ovaries with endometriomas (1.5 ± 0.9 and 2.0 ± 1.2) than in the contralateral ovaries (3.7 ± 1.5 and 4.2 ± 1.7; p<0.001, respectively). The number of oocytes retrieved suitable for fertilization was lower in ovaries with endometriomas (1.5±1.1) than in the healthy ovaries (3.3±1.5; p<0.001). The total number of oocytes retrieved and the number of oocytes retrieved suitable for fertilization were lower in ovaries with endometriomas respectively in 21 (80.8%) and in 20 (76.9%) cases. The decreased responsiveness to ovarian superovulation was confirmed considering women with ultrasonographic diagnosis of deep infiltrating endometriosis. 30.8% of patients had positive HCG; the pregnancy rate was 23.1%.

Conclusion: The presence of large endometriomas (≥ 5 cm) at time of IVF significantly decreases the number of oocyte retrieved compared with the contralateral healthy ovaries.
Keywords: Endometriomas; ICSI; in vitro fertilization; oocyte; pregnancy

Introduction
There is growing evidence that laparoscopic excision of ovarian endometriomas has a detrimental effect on ovarian reserve. Spontaneous ovulation occurs less frequently in the operated ovaries than in the contralateral healthy ovaries [1, 2] while it has similar frequency in healthy and unoperated ovaries with endometriomas [3]. Contradictory results have been reported on the effect of surgery for endometrioma on ovarian reserve. A systematic review and meta-analysis showed a significant decline in serum AMH concentrations after surgical treatment of endometriomas [4]. In contrast, another systematic review and meta-analysis showed that ovarian reserve evaluated by antral follicle count (AFC) is not decreased after surgical treatment of endometriomas [5]. However, in women undergoing in vitro fertilization (IVF) after surgical excision of unilateral endometrioma, the number of developing follicles and the number of oocytes retrieved in the operated ovary is significantly lower than in the contralateral ovary [6-11]. Furthermore, the outcome of IVF in women operated for bilateral endometriomas is significantly impaired [12-14]. A recent systematic review and meta-analysis showed that, in women who had surgical treatment in one ovary, a lower number of oocytes were retrieved compared with the contralateral normal ovary without endometrioma of the same patient [15]. Furthermore, among women with endometrioma, those who had surgical treatment before IVF/ICSI had a similar live birth rate, clinical pregnancy rate, miscarriage rate, number of oocyte retrieved and cancellation rate per cycle compared with those with untreated endometrioma; however, women with endometrioma who had surgical treatment had a lower AFC and required a higher dose of FSH [15].

Several previous studies showed that, in patients with unilateral endometriomas undergoing IVF, the affected and the healthy ovary produce a similar number of codominant follicles and oocytes [16-19]. In agreement with these observations, the guidelines of the European Society of Human
Reproduction and Embryology (ESHRE) suggest that patients with endometriomas larger than 3 cm should not undergo laparoscopic cystectomy before IVF in order to improve pregnancy rate [20]. However, the available studies comparing the response to IVF between ovaries with endometriomas and contralateral healthy ovaries included patients with mean diameter of the endometrioma ranging between 2 and 3 cm [16-19]. Therefore, we deem that it is important to further investigate the response to superovulation of ovaries with larger endometriomas.

The objective of the current study was to assess the response to superovulation for IVF in patients with unilateral endometriomas with diameter ≥ 5 cm and in the contralateral healthy ovary.

**Materials and methods**

This study was based on a retrospective analysis of a prospectively collected database of 26 patients with unoperated unilateral endometriomas who underwent their first IVF cycle at our center. In detail, the criteria for inclusion in the study were: women selected for IVF/ICSI cycles who underwent superovulation; age between 18 and 40 years; ultrasonographic diagnosis of single unilateral endometrioma with mean diameter ≥ 5 cm and healthy contralateral ovary; assessment of ovarian reserve (basal FSH and AMH) within 6 months before the IVF cycle; duration of infertility of at least one year.

Exclusion criteria for the study were as follows: basal FSH > 20 IU/l; previous ovarian surgery; previous surgery for non-ovarian endometriosis; atypical ultrasonographic characteristic of the endometrioma suspicious for malignancy; presence of ovarian cysts other than endometriomas; severe cause of male infertility (azoospermic cases requiring the use spermatozoa obtained from surgical procedures, those with a sperm concentration < 1 x 10^6 /ml, and those using frozen semen). The use of hormonal therapy prior to IVF was not an exclusion criterion for the study. Patients were included only for their first treatment cycle reaching oocyte retrieval.
Regional Ethic Committee approval was obtained. All the women gave a general consent for the use of their data for research purposes; no specific informed consent for the present analysis was obtained since this was a retrospective study.

All the scans were performed by the first author (S.F.) who is an expert ultrasonographer in the field of reproductive medicine and endometriosis (more than 5000 scans performed).

AFC was assessed by transvaginal ultrasonography between cycle day 3 and 5; the number of antral follicles measuring 2-10 mm in diameter in each ovary was recorded for each patient.

The diagnosis of endometriomas was based on transvaginal ultrasonography (TVS), which was performed by an experienced operator by using either a Voluson E6 or a Voluson S8 machine (GE Healthcare, Milwaukee, WI, USA) equipped with transvaginal probes. The ultrasonographic criteria for the diagnosis of endometrioma were: round cystic mass with thick walls, regular margins, homogeneous low echogenic fluid content with scattered internal echoes, and without papillary projections with no or poor vascularization of capsule [21]. The diagnosis of endometriomas was confirmed twice at least at two months interval in order to rule out hemorrhagic functional cysts.

The diameter and volume of the endometriomas were assessed during a baseline ultrasonography the month before the ovarian superovulation. The endometrioma was measured in three dimensions, and the average diameter was calculated. The volume of the endometrioma was estimated by using virtual organ computer-aided analysis (VOCAL, GE Healthcare, Milwaukee, WI, USA) as previously described [22]. Briefly, the VOCAL technique was used to obtain a sequence of 20 sections of each endometrioma around a fixed axis, each after 9° rotation from the previous section. The contour of each endometrioma was drawn manually using the roller ball cursor of the 3D-ultrasound machine to obtain a 3D volume measurement. The presence of deep infiltrating endometriosis (DIE) was investigated by ultrasonography and recorded. Briefly, rectovaginal endometriosis was diagnosed when a hypoechoic nodule was seen on TVS in the rectovaginal space below the line passing along the lower border of the posterior lip of the cervix (under the peritoneum) [23]. Rectal water-contrast TVS was performed when the presence of bowel infiltration
was suspected [24]. Bladder endometriosis appeared as a filling defect of the posterior wall with a variable protrusion into the lumen, with an iso/hypoechoic aspect sometimes with small transonic formations usually not vascularized [23].

During the IVF-ICSI cycle, patients were monitored and managed according to a standardized protocol. The regimen and the dose of gonadotropins were chosen for each patient on the basis of age, day-3 serum FSH, serum AMH and AFC. When planning the IVF, patients on long-term hormonal therapy were requested to temporarily interrupt the treatment. Day-3 serum FSH, serum AMH and AFC were assessed during the second menstrual cycle following the interruption of treatment which was restarted immediately after ovarian reserve assessment. During superovulation, the patients underwent serial ultrasound scans and serum estradiol measurements; the dose of FSH was adjusted according to the follicular response. An ultrasound scan was systematically performed on the day of hCG administration (when two or more leading follicles had mean diameter > 18 mm); during this scan, the number and size of all follicles with mean diameter > 11 mm and of codominant follicles (mean diameter > 15 mm) were recorded separately for the two ovaries. The diameter of the follicles was calculated as the mean of three perpendicular diameters. Oocyte retrieval was performed transvaginally under general anesthesia 36 hours after hCG administration. All follicles with a mean diameter > 10 mm were aspirated. Oocytes were considered suitable for fertilization if they were metaphase II oocytes (if ICSI was required) and type 1 cumulus-oocytes according to the European Society for Human Reproduction and Embryology Istanbul Consensus Conference [25] for IVF cycles. The endometriomas were transfixed only when this was required to reach the follicles. All study patients received antibiotics prophylactically at the time of oocyte retrieval (azithromycin 1 g orally or ceftriaxone 1 g intramuscularly). When the endometrioma was punctured the patients prophylactically received ceftriaxone 1 g intramuscularly for 4 days. The luteal phase was supported by vaginal administration of micronized progesterone (400 mg/day) from the day of ovarian puncture to the day of pregnancy test. Pregnancies were diagnosed by an increasing concentration of serum β-hCG,
which was tested 14 days after embryo transfer. Clinical pregnancies were confirmed by the presence of a gestational sac on transvaginal ultrasonography during the fifth week.

**Statistical analysis**

The primary outcome of the study was to compare the number of oocyte retrieved in the ovary with the endometrioma and the contralateral healthy ovary. The Mann-Whitney U-test was used to compare the differences between affected and contralateral healthy ovaries. The frequency of silent ovaries (absence of follicular growth) was compared between the two groups by the Fisher exact test. The null hypothesis stated that the number of codominant follicles did not differ between the ovary with the endometrioma and the contralateral healthy ovary. A p value < 0.05 was considered statistically significant. Data analysis was performed using the Statistics Package for Social Sciences (SPSS, version 20.0 Chicago, IL, USA).

**Results**

The baseline characteristics of the patients included in the study are shown in Table 1. There was no significant difference in the number of antral follicles in the ovary with the endometrioma and in the contralateral ovary (Table 2).

Eleven women (42.3%) were treated with a long protocol of stimulation (daily GnRH agonist started in the mid-luteal phase, two-three weeks before administering the gonadotropins). Fifteen women (57.7%) received gonadotropins from the third day of the cycle and then added daily GnRH antagonist to prevent spontaneous ovulation after the detection of a leading follicle with a mean diameter of 13-14 mm. The mean (± SD) total dose of recombinant FSH administered was 2780 (± 1053) IU; the duration of stimulation was 11.8 ± 2.0 days.

The total number of follicles was significantly lower in ovaries with endometriomas than in healthy ovaries (Table 2). The total number of follicles was lower in the ovaries with endometriomas in 19 cases (73.1%; 95% C.I., 52.2%-88.4%). When considering only the codominant follicles (diameter > 15 mm), their number was lower in ovaries with endometriomas than the contralateral ovary (Table 2). The number of codominant follicles was lower in ovaries with endometriomas in 22
cases (84.6%; 95% C.I., 65.1%-95.6%). Similarly, both the total number of oocytes retrieved and the number of oocytes retrieved suitable for fertilization was lower in ovaries with endometriomas than in the healthy ovary (Table 2). The total number of oocytes retrieved and the number of oocytes retrieved suitable for fertilization were lower in ovaries with endometriomas respectively in 21 (80.8%; 95% C.I., 60.6%-93.4%) and in 20 (76.9%; 95% C.I., 56.4%-91.0%) cases. Five endometriomas were punctured at the time of oocyte retrieval (in 19 ovaries the puncture of the endometrioma was avoided); no case of pelvic abscess was observed. There was an absence of follicular growth (silent ovaries) in 2 ovaries with endometriomas and in no healthy ovary (p=0.490).

A subanalysis was performed according to the presence of ultrasonographically diagnosed DIE. The decreased responsiveness to ovarian superovulation of ovaries with endometriomas was confirmed in women with ultrasonographic diagnosis of DIE (n = 19) but not in patients without DIE (n = 7) probably because of the small sample size of this group (Table 3).

No embryo was available for transfer in 2 patients (7.7%). In women undergoing embryo transfer, the mean (± SD) number of embryo transferred was 1.9 (± 0.7). Eight patients (30.8%) had a positive HCG; the pregnancy rate was 23.1% (6/26). The pregnancy rate was 20.0% (1/5) in patients who had the endometrioma punctured at the time of oocyte retrieval.

Following failure of the IVF cycle, 11 women (42.3%) underwent laparoscopic excision of endometriomas and the diagnosis was confirmed in all the cases.

Discussion

This study shows for the first time that ovaries with large endometrioma (diameter ≥ 5 cm) have a decreased responsiveness to superovulation compared with contralateral healthy ovaries. In fact, the affected ovaries produced a lower number of follicles, codominant follicles, oocyte retrieved and oocyte retrieved suitable for fertilization. This observation is in contrast with previous studies
showing that endometriomas with diameter of 2-3 cm do not negatively affect the response to ovarian superovulation [16-19].

It remains unclear why large endometriomas negatively affected ovarian response and the number of oocyte retrieved. Considering ovarian response, it is possible that blood flow is changed in the ovarian tissue stretched by the presence of large endometriomas. In these ovaries, a lower concentration of gonadotropin may reach the ovarian follicles. Concerning the number of oocyte retrieved, the presence of the endometrioma may be a mechanical obstacle to the pick up thus influencing the number of oocyte retrieved.

Previous studies showed that hormonal therapies (such as oral contraceptive pill, progestins and aromatase inhibitors) decrease the size of ovarian endometriotic cysts [22]. Future studies should evaluate whether the decrease of the size of endometriomas caused by hormonal therapies increases the response to superovulation. Alternatively, the aspiration of large endometriomas prior to IVF may decrease the mechanical compression of the cyst on the ovarian tissue and thus it may positively influence the response to superovulation.

This study has several limitations. Firstly, this was a retrospective study, despite it was based on a prospectively collected database. The retrospective study design did not allow providing data on the number and quality of the embryos obtained by affected and healthy ovaries. In fact, in clinical practice, the number of developing follicles and oocyte retrieved is recorded separately for the two ovaries, while data on the subsequent embryo development cannot be retrieved retrospectively because the oocytes are typically handled without recording the ovary of origin. Furthermore, the sample size of the study is quite small; however, this was a single center study and patients with large endometriomas often require surgery because of pain symptoms. In addition, we arbitrarily included in the study patients with endometriomas larger or equal than 5 cm. Considering potential limitations of the study, about 70% of the patients were using hormonal therapies before IVF and these therapies were interrupted immediately before ovarian superovulation; therefore, these patients were under hormonal therapy at the time of ultrasonographic assessment of the
characteristics of the endometriomas. It is well known that hormonal therapies decrease the size of endometriomas [22] and, therefore, it is likely that the baseline volume of the endometriomas was underestimated in some patients included in this study. However, this potential bias of the study may only have decreased the impact of the endometrioma on the responsiveness to superovulation.

In our institution, the decision for IVF or surgery is taken in agreement with patient’s priorities. Hence, if the woman’s priority is to become pregnant, IVF is proposed first, even in symptomatic patients and therefore the hormonal therapy is administered to control pain symptoms. In this study, the diagnosis of endometriomas was based on ultrasonography and the histological confirmation of the diagnosis was obtained only in the patients (42.3%) who did not conceive and underwent surgery after IVF. However, it is well known that endometriomas can be accurately diagnosed by ultrasonography [21, 26, 27]. Furthermore, the diagnosis of endometriomas was documented twice at least two months interval in order to rule out hemorrhagic cysts. Another limitation is that the reliability of AFC may be questioned due to the possible reduction of visualization of antral follicles that can be obscured by the endometrioma occupying a substantial portion of the ovary. In addition, the presence of the cyst also increases the distance between the ultrasound probe and the normal ovarian tissue worsening the resolution of the transvaginal ultrasound scan. However, in this study, the same experienced ultrasonographer performed all the scans, thus decreasing this potential bias. Finally, the last limitation of the current study is the lack of a control arm of patients without endometrioma, which prevents to evaluate if large endometriomas may affect the pregnancy rate.

A strength of this study is that the ovarian response was compared within the same patient between the healthy and the affected ovary thus controlling for potential confounding factors such as age of the patients, dose of gonadotropins administered, length of stimulation and presence of other forms of endometriosis (such as DIE).

A potential criticism to our study is that ovarian endometriomas might not be the unique form of endometriosis that can impair ovarian responsiveness to superovulation. Previous studies suggested that DIE has a negative impact on IVF outcome in patients with endometriomas [28]. About 70% of
the patients included in our study had ultrasonographic diagnosis of DIE and, in this population, the decreased responsiveness to ovarian superovulation of ovaries with endometriomas was confirmed. In contrast, only 7 patients with large endometriomas did not have ultrasonographic evidence of DIE; although in this population no statistically significant difference was observed between healthy and affected ovary, the analysis does not allow to draw any conclusion because it is limited by the very sample size. Furthermore, we are aware that other forms of the disease (such as superficial implants) may not be detected by ultrasonography and may potentially influence IVF outcome.

Conclusions

This study, although limited by the retrospective design and by the small sample size, suggests that the presence of large endometriomas (≥ 5 cm) at the time of IVF significantly decreases responsiveness to superovulation. If this observation is confirmed in prospective studies with larger sample size, hormonal treatment, aspiration of the cyst or surgery may be considered prior to IVF.

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References


### Tables

Table 1. Characteristics of the study population (n=26)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>34.2 ± 3.8</td>
</tr>
<tr>
<td>BMI (kg/m², mean ± SD)</td>
<td>22.1 ± 2.7</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td>5 (19.2)</td>
</tr>
<tr>
<td>Duration of infertility (months, mean ± SD)</td>
<td>30.5 ± 11.2</td>
</tr>
<tr>
<td>Concomitant male factor of infertility (n, %)</td>
<td>9 (34.6)</td>
</tr>
<tr>
<td>Side of the endometrioma (n, %)</td>
<td></td>
</tr>
<tr>
<td>- left</td>
<td>16 (61.5)</td>
</tr>
<tr>
<td>- right</td>
<td>10 (38.5)</td>
</tr>
<tr>
<td>Diameter of the endometrioma (cm, mean ± SD)</td>
<td>6.6 ± 0.9</td>
</tr>
<tr>
<td>Volume of the endometrioma (cm³, mean ± SD)</td>
<td>155.2 ± 62.7</td>
</tr>
<tr>
<td>Associated endometriotic lesions (n, %) *</td>
<td></td>
</tr>
<tr>
<td>- rectovaginal nodules</td>
<td>7 (26.9)</td>
</tr>
<tr>
<td>- uterosacral ligaments</td>
<td>14 (53.8)</td>
</tr>
<tr>
<td>- colorectal nodules</td>
<td>2 (7.7)</td>
</tr>
<tr>
<td>- bladder nodules</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>- vaginal nodule</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>Patients with deep endometriosis (n, %) *</td>
<td>19 (73.1)</td>
</tr>
<tr>
<td>CA 125 (IU/ml)</td>
<td>35.0 ± 11.2</td>
</tr>
<tr>
<td><strong>Hormonal therapy before entering the IVF cycle (n, %)</strong></td>
<td></td>
</tr>
<tr>
<td>- oral combined contraceptive</td>
<td>11 (42.3)</td>
</tr>
<tr>
<td>- vaginal ring</td>
<td>2 (7.7)</td>
</tr>
<tr>
<td>- progestin</td>
<td>9 (34.6)</td>
</tr>
<tr>
<td>Duration of hormonal therapy before entering the IVF cycle (months, mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>- oral combined contraceptive</td>
<td>5.7 ± 1.9</td>
</tr>
<tr>
<td>- vaginal ring</td>
<td>6.8 ± 1.7</td>
</tr>
<tr>
<td>- progestin</td>
<td>4.5 ± 2.1</td>
</tr>
<tr>
<td>Day-3 serum FSH (IU/ml, mean ± SD)</td>
<td>6.6 ± 2.0</td>
</tr>
<tr>
<td>Total antral follicle count (n, mean ± SD)</td>
<td>13.5 ± 4.0</td>
</tr>
<tr>
<td>AMH (ng/ml, mean ± SD)</td>
<td>2.7 ± 1.4</td>
</tr>
</tbody>
</table>

* diagnosed by ultrasonography
Table 2. Number of antral follicles, follicles and oocytes

<table>
<thead>
<tr>
<th></th>
<th>Ovary with endometrioma</th>
<th>Healthy ovary</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antral follicle count</td>
<td>6.7 ± 2.3</td>
<td>6.8 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Total follicles</td>
<td>2.6 ± 1.3</td>
<td>4.8 ± 2.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Codominant follicles</td>
<td>1.5 ± 0.9</td>
<td>3.7 ± 1.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>2.0 ± 1.2</td>
<td>4.2 ± 1.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Suitable oocytes retrieved</td>
<td>1.5 ± 1.1</td>
<td>3.3 ± 1.5</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Data are presented as number, mean ± SD. NS: not significant.

Table 3. Number of antral follicles, follicles and oocytes in women with and without deep endometriosis *

<table>
<thead>
<tr>
<th></th>
<th>Patients with deep endometriosis (n = 19)</th>
<th>Patients without deep endometriosis (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovary with endometrioma</td>
<td>Healthy ovary</td>
</tr>
<tr>
<td>Antral follicle count</td>
<td>6.8 ± 2.4</td>
<td>6.9 ± 2.2</td>
</tr>
<tr>
<td>Total follicles</td>
<td>2.5 ± 1.3</td>
<td>5.2 ± 1.9</td>
</tr>
<tr>
<td>Codominant follicles</td>
<td>1.5 ± 0.9</td>
<td>3.9 ± 1.2</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>1.9 ± 1.3</td>
<td>4.5 ± 1.7</td>
</tr>
<tr>
<td>Suitable oocytes retrieved</td>
<td>1.5 ± 1.1</td>
<td>3.5 ± 1.5</td>
</tr>
</tbody>
</table>

* diagnosed by transvaginal ultrasonography. NS: not significant.