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Can the endometrioma be an obstacle to complete oocyte retrieval in IVF cycles? A retrospective study

G.M. BALDINI[1,2], A.S. LAGANÀ[3], A. MASTROROCCO[4], A. MALVASI[2], D. BALDINI[1], D. FERRI[1], R. SCIORIO[5], G. TROJANO[6]

[1]IVF Center, Momo Fertilife, Bisceglie, Italy
[2]Obstetrics and Gynecology Unit, Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro", Bari, Italy
[3]Unit of Obstetrics and Gynecology "Paolo Giacone" Hospital, Department of Health Promotion, Mother and Child Care , Internal Medicine and Medical Specialities (PROMISE), University of Palermo, Palermo, Italy
[4]Department of Biosciences, Biotechnologies and Environment, University of Bari "Aldo Moro", Bari, Italy
[5]Edinburgh Assisted Conception Programme, Royal Infirmary of Edinburgh, Edinburgh, UK
[6]Department of Maternal and Child Health, Madonna delle Grazie Hospital, Matera, Italy

Abstract. – OBJECTIVE: The study aimed to evaluate the utility and safety of ancillary maneuvers during oocyte retrieval for patients with endometrioma that makes ovum pick-up hard due to poor ovarian surgical accessibility.

PATIENTS AND METHODS: Cases of 251 women with ovarian endometriomas undergoing *in vitro* fertilization (IVF) in our infertility unit were retrospectively analyzed to evaluate the clinical IVF cycle outcomes after oocyte retrieval. Controls (n = 251) were age-matched women without endometriomas who underwent an uncomplicated oocyte retrieval.

RESULTS: No statistically significant differences were observed between groups except for the number of oocytes retrieved, which was higher in the control group than in the group of women with endometrioma. On the contrary, there were no differences between the experimental groups in the fertilization rate and number of embryos and neither were there in the pregnancy and live birth rate. Moreover, the surgical complications were infrequent and similar between the two analyzed groups. Accidental or voluntary endometrioma punctures were not accompanied by increases in the risk of a pelvic infection.

CONCLUSIONS: In conclusion, patients with endometrioma can undergo high-performance oocyte recovery procedures thanks to safe accessory maneuvers during the ovum pick-up.

Key Words:
In vitro fertilization/intracytoplasmic sperm injection, Endometrioma, Oocyte retrieval.

Introduction

Endometriosis is a chronic and common estrogen-dependent inflammatory gynecologic disease characterized by the presence and maintenance of endometrial-like tissue (glandular epithelium and stroma) in aberrant locations outside the uterus[1]. Recent epidemiological studies[2,3] have reported that it affects up to 50% of sub- and infertile women, although it can also be found in 10-15% of women of reproductive age. Endometriosis is a serious problem for women's health, and it can negatively affect the chances of pregnancy. More specifically, endometriosis is related to reduced ovarian reserve and worse fecundity rates due to altered molecular composition of follicular fluid with high concentrations of cytokines, leading to abnormal and reduced follicular and oocyte development and poor embryo quality[4].

In addition, endometriosis may cause an endometrioma, one or more cysts in the ovarian tissues, often diagnosed during infertility work-up in about 17-44% of women with endometriosis[5-7]. For the above reasons, most women with ovarian endometrioma-related infertility generally undergo an IVF treatment to achieve pregnancy.

Most publications[8-11] show that surgery does not increase the success rate of *in vitro* fertilization (IVF) and may even harm assisted reproductive techniques (ART) by leading to reduced ovarian response to controlled ovarian stimulation (COH).

In addition, several studies[12-15] have reported a reduction in anti-mullerian hormone (AMH) after endometriosis surgery, likely related to the removal of healthy parenchyma. Antral follicle counts appear to be relatively less affected[16,17]. Conversely, some studies[18-20] have shown recovery of ovarian reserve after endometriosis surgery, but

Corresponding Author: Domenico Baldini, MD; e-mail: info@momofertilife.it; dbaldini@libero.it

only up to one year, so spontaneous conception can be expected one year after surgery.

The presence of endometrioma, however, in many cases made the ultrasound monitoring of follicular growth difficult, the ovarian response to hyperstimulation inadequate and the ovum pickup more problematic since endometrioma may interfere with ovarian accessibility[21]. Therefore, an incomplete recovery of oocytes can occur, and the recovered oocytes may have low competence[21,22]. Moreover, the accidental rupture of the cyst can lead to a shedding of endometriotic material into the peritoneum with possibly consequent inflammatory diseases and pelvic sepsis[23]. On the other hand, there is evidence[24-26] that endometrioma did not significantly affect embryo development and pregnancy rates.

In cases where the presence of an endometrioma makes ovum pick-up hard due to poor ovarian surgical accessibility, some accessory maneuvers during the procedure can be helpful.

The purposes of this study were:

- Verify whether the use of ancillary maneuvers during oocyte retrieval can help reach the ovary and collect all the present oocytes.
- Evaluate if the accidental or volunteer rupture of the endometrioma followed by the total aspiration and washing of cysts may cause side effects.

Patients And Methods

Patients

From January 2012 to December 2023, 6,204 IVF cycles at the Momò Fertilife – Center for Reproductive Medicine in Bisceglie (Italy) were performed. Of these, 4,241 were fresh embryo transfers, and 2,143 were frozen embryo transfers. Of 4,241 cycles, 861 patients (20,7%) had a primary or secondary diagnosis of endometriosis. Patients were screened against their eligibility criteria. Exclusion criteria included previous pelvic and abdominal surgery and surgery to remove endometriosis, pelvic Inflammatory disease (PID) anamnesis, non-endometriosis ovarian cysts, body mass index (BMI) greater than 30, concomitant male infertility factor, adenomyosis, deep endometriosis.

Considering exclusion criteria, 616 cycles of 793 were selected. 251 patients of 616 (42%) had endometrioma diagnosis. 7 patients were lost to follow-up. The representativeness of the sample size was achieved with a 95% confidence interval and a margin of error of 5.2% for approximately 861 patients with endometriosis in the same study period. These data resulted in an optimal sample calculation of 251 cases.

The sampling was random stratified cluster type. Considering exclusion criteria, 3,363 of the remaining 4,241 fresh embryo transfer cycles were eligible as controls since those patients did not have an endometriosis diagnosis. From that group, 251 patients with similar social-demographical characteristics to the group of patients with endometrioma were selected as controls by using Meditex software version number 2.8.7.4 (available at: www.meditex-ivf.com) (Figure 1).

All the women underwent a baseline evaluation transvaginal ultrasound, antral follicles count (AFC), and blood tests, including hormone analysis [AMH, basal Follicle-stimulating hormone (FSH), and estradiol level] two months before starting the ART cycle date.

The diagnosis of presumed ovarian cysts was recorded at the baseline transvaginal ultrasound evaluation and examination on at least two menstrual cycles apart. Moreover, the presence of the ovarian cysts was confirmed during ovarian stimulation. Cystic mass of round shape, with a minimum diameter of 10 mm, thick walls, regular margins, low homogeneous exogenous fluid content with scattered internal echoes, and without papillary projections was defined as endometrioma[27]. Endometrioma diameter was calculated as the average of three perpendicular diameters.

Exclusion criteria were patients with one or more clinically atypical or suspected lesions (cysts whose ultrasound appearance was compatible but not distinctive of endometriosis) and/or with deep invasive endometriosis nodules.

All patients who underwent oocyte retrieval were monitored and managed according to standard procedures. The starting dose of gonadotrophin was determined based on maternal age, ovarian reserve (AMH and AFC) hormone tests and any outcomes of previous fertilization cycles. Thereafter, the patients underwent controlled ovarian stimulation (COS) using the gonadotrophin-releasing hormone (GnRH) antagonist protocol. The recombinant follicle-stimulating hormone (FSH; GONAL f, Merck Serono, Weiterstadt, Germany) was administered from the second day of menstruation; the starting dose was determined always using age, AMH, and AFC. Follicular maturation was monitored through serum estradiol (E2) and progesterone (P) levels and in parallel, also with transvaginal follicular ultrasound, respectively, on days 5, 7,

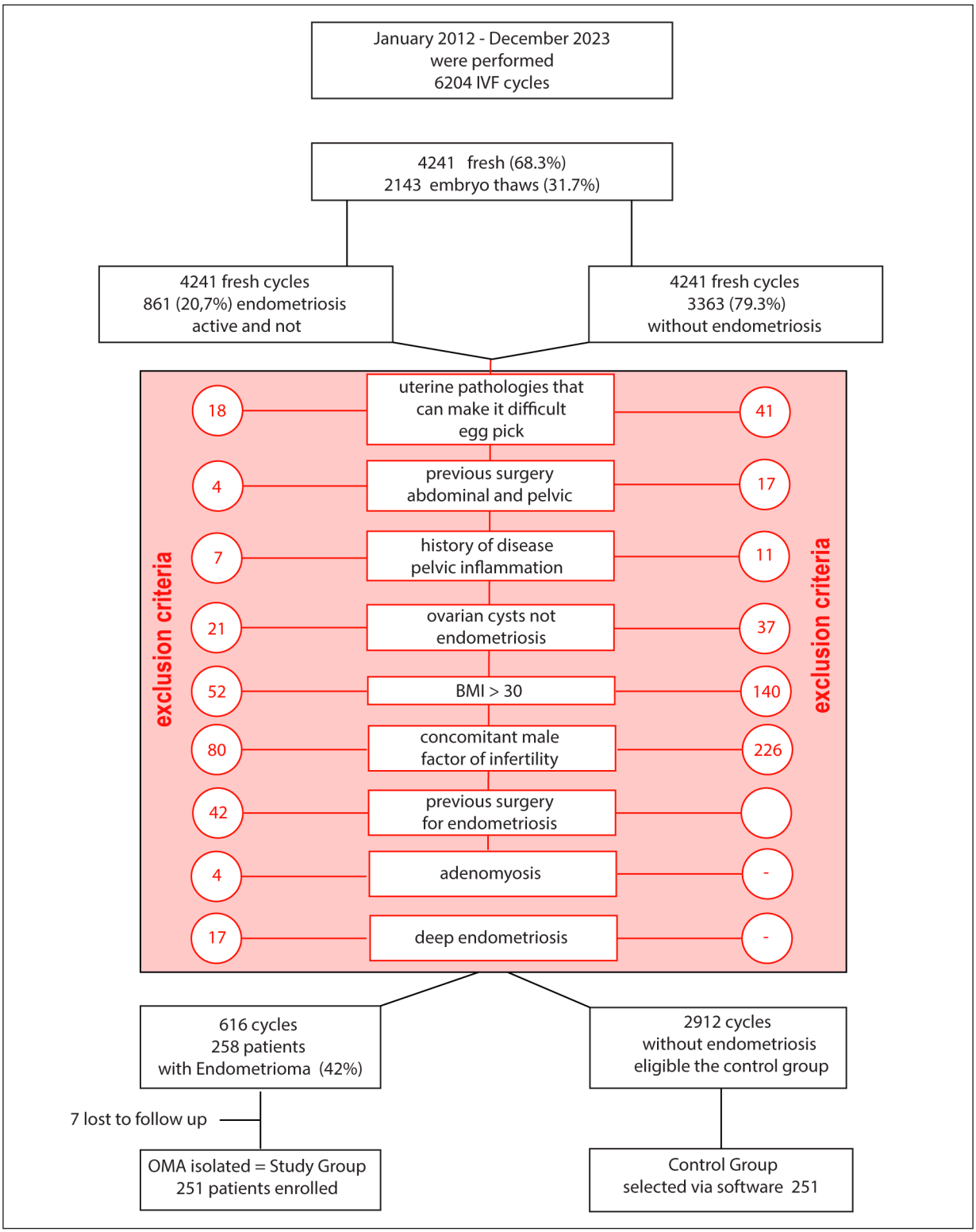

Figure 1. Study design. The flow diagram shows a selection of IVF/ICSI cycles.

10 and 12 from the start of therapy. When one or more follicles reached 13-14 mm size, 0.25 mg of Cetrotide (Merck Serono, Weiterstadt, Germany) was administered daily to avoid ovulation. To induce ovulation, the final dose of 10,000 IU of human Chorionic Gonadotropin (hCG; GONASI,

IBSA, Lugano, Switzerland) injection was administered subcutaneously when three or more follicles reached > 18 mm diameters and 34-37 hours later the pick-up takes.

All patients enrolled voluntarily in the study provided written informed consent after being well-informed of the aims and procedures of the study, and all the procedures were performed according to the Declaration of Helsinki relevant guidelines and regulations. Moreover, the patients enrolled in the study provided written consent to the use of their clinical data for research purposes. The Clinical Research Local Ethics Committee approved the present study (No. 7; registration date, 12/07/2018).

Oocyte Collection Procedures

Oocyte aspiration was performed with the administration of anesthetic drugs intravenously (15 mg Midazolam and 0.05 mg Fentanyl). Oocytes were collected by follicular puncture using a 17-gauge aspiration needle (COOK Medical, Bloomington, IN, USA) connected to a guide on the transvaginal ultrasound device (GE Voluson S8, GE Co Ltd, Boston, MA, USA).

IVF procedures were performed by three different doctors, and experts in *in vitro* fertilization. The capability of these doctors to be able to full oocyte recovery in 98% of cases and carry out additional maneuvers in case of difficulties was evaluated[28]. All these aspects were given a positive medical expertise evaluation.

Oocyte recovery was performed by using the standard procedure used in our clinic[29]. Briefly, this technique provides the minimal insertion of the oocyte aspiration needle into the ovaries, and all the follicles present were aspirated thanks to probe and needle maneuvers. Moreover, during the final stage of follicle aspiration, a needle spinning around its axis was performed. If the doctor could not aspirate all the follicles with a single insertion of the needle into the ovary, a new introduction of the needle into the ovary was made.

Ancillary maneuvers were carried out in the presence of endometriomas or misplaced ovaries. Ovary position was defined as dislocated about its normal anatomic position (each ovary is normally allocated laterally to the uterus in the ovarian fossa and behind and inside the external iliac vessels[30].

The maneuvers were:

- 1st Maneuver: bimanual abdominal pressure and vaginal probe pressure
- 2nd Maneuver: anti Trendelenburg position
- 3rd Maneuver: cervical clamp traction
- 4th Maneuver: trans-abdominal oocyte retrieval
- 5th Maneuver: trans-myometrial oocyte retrieval

In the event that the above-mentioned manipulations were not sufficient to perform the complete follicle aspiration, endometrioma was transfixed. Endometrioma transfixion consisted of the passage through the endometrioma, without aspirating, to reach the follicles behind the cysts, and the needle was washed with oocyte culture media. At the end of the follicle aspiration procedure with endometrioma transfixion, the cysts were completely aspirated and washed with a physiological solution. The cyst fluid was discarded.

Follicle retrieval was considered completed after the aspiration of all follicles with > 11-12 mm diameter size. In some cases, the complete aspiration could not be finalized due to the inability to achieve all the follicles, which could pose additional risks for the patient. In the case of accidental endometrioma aspiration reported by the doctor, it was confirmed by follicular fluid evaluation under a stereomicroscope with the presence of endometrioma content in aspirated follicular fluid: a cloudy, dark brown fluid with several fine black particles[31,32].

To assess ovum pick-up side effects and any risks of complications, the medical conditions (ultrasound and blood count) of the patients were evaluated immediately after 24 hours of the ovum pick-up. Oocytes retrieved from tubes contaminated with endometrioma contents and those not contaminated were treated similarly.

Insemination Procedure

Conventional IVF

The semen was prepared following the density gradient procedure. Then, the sperm sample was washed with a media for sperm handling (G-MOPS, Vitrolife, Gothenburg, Sweden) to remove any debris, non-motile sperm, and seminal fluid components that could potentially interfere with fertilization. Then, the sample underwent a swim-up for about 30/45 min in the incubator at 37°C. Subsequently, the supernatant was collected to prepare the IVF insemination by diluting it with sperm handling media (G-Mops, Vitrolife, Viby J, Denmark) until we reached the sperm concentration between 50.000 to 100.000 sperms per milliliter. Finally, the IVF insemination was placed in drops containing individual oocytes and left overnight.

ICSI (Intracytoplasmic Sperm Injection)

Cumulus-oocyte complexes were immediately collected, washed, and transferred in IVF medium (G IVF, Vitrolife, Sweden) for 3 hours at 37°C, 5% CO_2. Then, cumulus-oocyte complexes were exposed to a hyaluronidase solution (25 IU/ml) to remove the corona radiata. The cleaned oocytes were inspected and evaluated under the stereomicroscope (Nikon SMZ 1500, Tokyo, Japan) to select only metaphase II (MII) oocytes. Semen was collected after 3 to 4 days of sexual abstinence and prepared according to the method used in our center[33].

The ICSI procedure was performed at 37°C under an inverted microscope (Nikon Eclipse, TE 200, Tokyo, Japan) using a microinjection system with 400x magnification. After insemination, fertilized oocytes were cultured in Global medium (Cooper Surgical, Trumbull, CT, USA) for up to 6 days.

The obtained embryos were cultured in the Geri-time Lapse system (Genea Biomedx, Sydney, Australia) to check fertilization and cleavage rates.

Transfer Procedure

On the third and fifth days of culture, fresh embryos were transferred as single embryo transfer (SET). The best-quality embryos were transferred. In cases where fewer than three embryos were available, SET was performed after 48 hours after the fertilization procedure. The transfer was performed under ultrasound guidance (GE Voluson S8, GE Co Ltd., Boston, MA, USA) with the transabdominal probe. Pregnancy was confirmed by serum levels of beta-human chorionic gonadotrophin (beta-hCG) exceeding 15 mIu/mL, 9 days after transfer and intrauterine gestational sac identification via transvaginal ultrasonography.

The criteria for selecting embryo transfer on day 3 or day 5 were mainly based on the number of embryos obtained and the patient's age. In fact, when the number of embryos was less than 3 and the age was ≥ 40, the embryo transfer on day 3 was chosen over day 5.

Statistical Analysis

Patients' demographic characteristics were homogeneous using the Student's *t*-test (Table I). The data were analyzed using Prism 8 version 8.4.3 software (GraphPad Software, Boston, MA, USA). An accurate statistical assessment of the samples was obtained by analyzing general characteristics such as age, and clinical data of patients.

Data were presented as mean and standard deviation for continuous parametric variables or as percentages for categorical variables. Statistical evaluations of the clinic parameters were compared between groups using the Student's *t*-test or Chi-square test. Differences with p-value < 0.005 were considered statistically significant in the present study.

In detail, statistical evaluations regarding the characteristics of fertilization rate, number of viable embryos, fresh transfer performed at the cleavage stage, fresh transfer performed at the blastocyst stage, the pregnancy rate per retrieval, the live birth rate per retrieval, the number of retrieved oocytes, displaced ovaries, and the average number of entry needles were compared using the Chi-square test.

Accessory maneuvers to complete the pick-up, incomplete right pick-up, incomplete left pick-up, transfixion endometrioma, bleeding ovary, minimal vaginal bleeding, vaginal suture, homeostatic clamping, pelvic infection, normal check after 24

Table I. General and clinical characteristics of the two groups.

Characteristics	Endometrioma group (251)	Control group (251)	*p*-value
Age	36.1 ± 3.7	35.9 ± 3.6	0.471
BMI (kg/m^2)	25.3 ± 2.1	25.5 ± 2.4	0.135
Duration infertility (years)	1.9 ± 2.0	1.7 ± 2.1	0.126
Smokers (%)	28.7 ± 3,1	29.2 ± 2.7	0.178
Patients who have given birth (%)	7.4 ± 2,1	7.6 ± 2	0.189
AMH (ng/ml)	1.7 ± 1.4	1.9 ± 1.6	0.142
AFC	8.5 ± 5.1	9.4 ± 5.3	0.031
Total dose gonadotropin (UI)	2,275 ± 1,125	2,185 ± 1,205	0.362
Duration stimulation day	10 ± 3.1	9.7 ± 3.3	0.072
ICSI (%)	63	61	0.838
Classical IVF (%)	27	29	0.471

Student's *t*-test; p-value > 0.05 was considered not statistically significant. BMI = Body Mass Index; AMH = Anti Mullerian Hormone; AFC = Antral Follicle Count; ICSI = Intracytoplasmic Sperm Injection; IVF = *In vitro* Fertilization.

hours, abnormal check after 24 hours were compared using the Chi-square test.

The Chi-square test was also used to analyze the size of the endometrioma, complete recovery from both ovaries, and the number of transfixions according to the endometrioma location.

Results

The population consisted of 251 women with endometrioma and 251 women without endometrioma who underwent the ART cycle (control group) and showed comparable baseline characteristics (Table I). The two groups were evaluated as homogeneous and comparable.

Clinical characteristics about the number of oocytes retrieved, fertilization rate, number of viable embryos, percentage of fresh transfer at cleavage and blastocyst stage, pregnancy rate and live birth rate were reported in Table II. No statistically significant differences were observed between groups except for the number of oocytes retrieved, which was higher in the control group compared to the group of women with endometrioma (Chi-square test $p < 0.05$; Table II).

Table III reports data on the number of displaced ovaries, average number of entry needles, number of accessory maneuvers performed to complete the pick-up, number of rights and left pick-ups, and number of endometriomas transfixed only for the group of women with endometrioma. No differences were noticed between groups. The health assessment of the patients was reported in the same table. As reported, all values were comparable between the two groups. Moreover, the surgical complications were infrequent and similar both immediately and after 24 h between the two analyzed groups. No serious complications occurred in either group.

Table IV reported the correlations between the endometrioma location and the size of the endometrioma, complete recovery from both ovaries and the number of transfixions. The location of the endometrioma did not affect the parameters mentioned above.

Discussion

Endometriosis is a pathology that has a major impact on female health and reproductive capacity. The treatment of endometriosis for reproductive purposes has been the subject of numerous discussions in recent years. Recently, in patients with endometriosis, it may often be best to achieve pregnancy before surgery. Endometriosis hurts ovarian reserve and the outcome of IVF treatments[34,35]. Physiological ovulation in these patients, especially those operated for endometriomas, is also deficient[36]. In some cases, surgery for endometrioma results in a progressive loss of the follicular heritage, leading to atresia[37,38]. Other studies[21] have highlighted that even endometrioma at the time of oocyte retrieval constitutes a problem for carrying out the procedure and, in particular, for completing it. The results of the present study show that in patients with endometrioma, a complete ovum pick-up is possible. Only in a few cases was it not possible, and there were no statistically significant differences between the patients with endometrioma (5 cases) and the control group (2 patients). It is important to point out, however, that, in the present study, ovum pick-up procedures were performed by already experienced gynecologists in the field (98% success rate)

Table II. Descriptive data of reproductive clinical outcomes between the two groups.

Characteristics	Endometrioma group (251)	Control group (251)	*p*-value
Number oocytes retrieved	6 ± 2.1	7 ± 2.9	0.0001
Fertilization rate (%)	64	64.2	0.997
MII oocytes	4 ± 2.1	5 ± 3.9	0.051
Number of viable embryo	2.8 ± 1.2	3 ± 1.7	0.272
Top embryo quality (%)	31.4	33.1	0.239
Fresh transfer performed	101	108	0.673
At cleavage stage (%)	57	59	0.564
At blastocyst stage (%)	44	49	0.527
Pregnancy rate per retrieval (%)	33	36	0.686
Early miscarriage rate (%)	15.1	14.9	0.723
Live birth rate per retrieval (%)	26	28	0.754

Chi-square test; *p*-values < 0.05 were considered to be statically significant. MII= metaphase II.

and with confidence to carry out accessory maneuvers to complete difficult pick-up procedures. The other studies[22] that detected incomplete follicular aspiration probably gave this result because the egg collection had been entrusted to all the doctors in the team and not only to those who had achieved documented experience.

Moreover, contrary to how much was published from more papers[23], accidental or voluntary endometrioma punctures were not accompanied by increases in the risk of a pelvic infection. This finding can be explained by the fact that I) endometrioma punctures were accompanied by total aspiration and washing of the cysts as already described also by Somigliana et al[39]; II) moreover, the accessory maneuvers have prevented the loss of endocystic content in the patient's peritoneum responsible for the inflammatory and infectious process. In our study, there have been two cases of pelvic infections, but in the control group, so they were not related to endometrioma presence and/or transfixion. However, although it was a very wide sample group, to be sure of the validity of our conclusions, a larger sample should be treated and explored by a gynecologist with the same expertise and experience.

Another key aspect was the significant reduction in the oocyte recovery rate in the group of patients with endometrioma. It is argued that the difference is justified by the presence of the endometrioma.

On the contrary, there were no differences between the experimental groups in the fertilization rate and number of embryos, as well as in the pregnancy and live birth rates.

Moreover, the presence of endometrioma was not a discriminating factor of ovarian displacement, and the number of accesses and accessory maneuvers required to perform the sampling led us to believe that complete egg recovery in endometrioma patients is possible. Moreover, the position of the ovary involved did not affect the procedure.

Table III. Characteristics of oocyte retrieval in the group with and without endometrioma.

Characteristics	Endometrioma group (251)	Control group (251)	*p*-value
Displaced ovaries	33	38	0.997
Average number of entry needles	3	3	0.818
Accessory maneuvers to complete the pick-up	5	6	0.958
Incomplete right pick-up	3	2	0.459
Incomplete left pick-up	1	1	0.894
Trans abdominal retrieval	0	0	-
Trans myometrial retrieval	1	1	-
Trans-fixation endometrioma	14	0	-
Bleeding ovary	0	0	-
Vaginal suture	0	0	-
Hemostatic clamping	3	4	0.897
Hemoperitoneum	0	0	-
Pelvic infection	0	0	-
Normal check after 24 hours	251	251	-
Abnormal check after 24 hours	0	0	-
Pelvic infection after two months	0	0	-

Complications after egg retrieval. Chi-square test; *p*-values < 0.05 were considered to be statically significant.

Table IV. Topographic characteristics of the endometrioma group.

Side	Right ovary	Left ovary	Total	*p*-value
Endometrioma group (251)	117	134	251	
Size				
≤ 1 cm	72	86		0.758
1-3 cm	27	28		0.995
> 3 cm	18	20		0.192
Average diameter				
Total	117	134	251	

Chi-square test; *p*-values < 0.05 were considered to be statically significant.

One of this study's major limitations is its retrospective nature. Moreover, another important limit is operators' manual skills, which cannot be defined precisely for statistical purposes. Further studies are needed to clarify the impact of the endometrioma on the number of oocytes recovered, excluding endometriomas up to 3 cm that objectively rarely constitute an obstacle to ovum pick-up. In general, case studies should be increased to extract the most conclusions.

Conclusions

In conclusion, patients with endometrioma can undergo an IVF cycle and a complete oocyte recovery procedure. However, in some cases, endometrioma could be an obstacle to complete oocyte recovery, and accessory maneuvers during the ovum pick-up will be necessary to aspirate all the follicles. In cases where, despite the appropriate maneuvers, endometrioma prevented complete oocyte recovery, endometrioma transfixion could be practiced safely for patients.

Conflict of Interest
None.

Funding
None.

Ethics Approval
This study was regularly approved by the Local Ethical Board of the Momò Fertilife Institute (approval number 7 on 07/2018) according to the Declaration of Helsinki ethical principles.

Informed Consent
Written informed consent was obtained at the first clinical evaluation from all patients enrolled voluntarily in the study after being well-informed about the hypothetical risks of the procedure.

Data Availability
The datasets generated during and/or analyzed during the current study are not publicly available as they are not anonymous, but they are available from the corresponding author upon reasonable request.

Authors' Contributions
Conceptualization, G.M.B. and D.B.; methodology, D.B. and A.S.L. ; software, G.M.B. and D.B; formal analysis, and investigation, resources, R.S. and A.Mas.; data curation, A. Mal. ; writing G.T.; original draft preparation, writing – review and editing G.M.B., supervision D.B. All authors have read and agreed to the published version of the manuscript.

ORCID ID
Giorgio Maria Baldini: 0000-0002-5061-8007
Antonio Simone Laganà: 0000-0003-1543-2802
Antonela Mastrorocco: 0000-0001-7136-4029
Antonio Malvasi: 0000-0001-6940-7608
Baldini Domenico: 0000-0003-1433-504X
Daniele Ferri: 0000-0003-2921-5999
Romualdo Sciorio: 0000-0002-7698-8823
Giuseppe Trojano: 0000-0002-8737-9943.

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