Oocytes retrieval difficulties in women with ovarian endometriomas

Running title: Oocyte retrieval in women with endometriomas

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Abstract

There is growing consensus that women with small ovarian endometriomas should not be operated prior to IVF because of the risk of damage to the ovarian reserve. However, conservative management is not without risks. In this study, we investigated one of the potential drawbacks associated to the presence of endometriomas, i.e. the technical difficulties that physicians may encounter during oocytes retrieval. To this aim, we prospectively recruited women undergoing IVF and compared technical difficulties between women with (n=56) and without (n=227) endometriomas. In exposed women, the cyst had to be transfixed in 8 cases (14%, 95%CI: 7-25%) and accidental contamination of the follicular fluid with the endometrioma content was recorded in 9 women (16%, 95%CI: 8-27%). Moreover, follicular aspiration was more frequently incomplete (OR=3.6, 95%CI: 1.4-9.6). In contrast, the retrieval was not deemed more technically difficult by engaged physicians and the rate of oocytes retrieved per developed follicle did not differ. No pelvic infections neither cyst ruptures were recorded (0%, 95%CI: 0-5%). In conclusion, oocytes retrieval in women with ovarian endometriomas is more problematic but the magnitude of these increased difficulties is modest. Overall, the presence of these cysts does not appear to represent a clinically significant obstacle.

Key message: Oocytes retrieval in women with ovarian endometriomas is more challenging However, these difficulties are only fairly enhanced and their clinical relevance is doubtful.

Key words: endometrioma / oocytes retrieval / complication

Introduction

Classical surgical management of endometriotic ovarian cysts using the laparoscopic stripping technique has been questioned because of the possible damage to the ovarian reserve (Somigliana *et al.*, 2015). The rate of spontaneous ovulation is lower in operated ovaries, serum levels of anti-mullerian hormone (AMH) shrink after surgery and responsiveness to ovarian hyper-stimulation is halved (Somigliana *et al.*, 2015). Even if data on this burning topic is not fully consistent (for instance, evidence obtained with the use of Antral Follicle Count failed to document a significant damage) (Muzzii *et al.*, 2014), conservative management has grown in recent years and there is now an increasing agreement that small endometriomas should not be removed before IVF (Practice Committee of ASRM, 2012; Dunselmann *et al.*, 2014; Vercellini *et al.*, 2016). The conservative approach is also facilitated by the high accuracy of the non-invasive diagnosis of ovarian endometriomas using transvaginal sonography (Exacoustos *et al.*, 2014; Guerriero *et al.*, 2016).

However, conservative management is not without potential drawbacks and risks (Somigliana *et al.*, 2015). The presence of endometriomas may interfere with ovarian responsiveness to hyperstimulation (Somigliana *et al.*, 2015) and with oocytes competence (Sanchez *et al.*, 2017), it may be associated with higher risk of pelvic infections (Somigliana *et al.*, 2015), pregnancy outcome may be affected (Fernando *et al.*, 2009) and there is the hazard of missing occult malignancies or causing later-in-life cancer development (Kobayashi *et al.*, 2007). The overall magnitude of these risks is considered modest and does not justify systematic surgery prior to IVF (Somigliana *et al.*, 2015). On the other hand, it has to be recognized that evidence to support this conclusion is generally weak. In this study, we aimed at specifically investigating a neglected but potentially relevant aspect of this argument, i.e. the technical difficulties that physicians may encounter during oocytes retrieval in women with ovarian endometriomas. The impact of these cysts on IVF outcome has been extensively studied in the past (Hamdan *et al.*, 2015) but, to our

knowledge, there is no previous studies specifically designed to evaluate the additional technical difficulties.

Materials and Methods

Women undergoing oocytes retrieval aimed at IVF-ICSI between March 2015 and December 2015 at the Infertility Unit of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico were prospectively considered for study entry. Eligibility for study entry was assessed at the time of oocytes retrieval. Women were selected as exposed if they had an ultrasound diagnosis of one or more presumed ovarian endometriomas. Previous surgery for endometriosis was not an exclusion criterion for the exposed group. Non-exposed subjects were women without a history of surgery for endometriosis and without ultrasound evidence of endometriomas (diagnostic laparoscopy to rule out endometriosis is not part of the infertility diagnostic work-up of our Unit). Women without endometriomas who were diagnosed deep invasive endometriotic nodules were also excluded from this group. In fact, the non-exposed group consisted of women with tubal factor, unexplained infertility and male infertility. Women with an ultrasound diagnosis of diffuse severe adenomyosis, large fibroids (mean diameter > 3cm), multiple fibroids and non-endometriotic ovarian cysts were excluded from both study groups. Women could be included in the study only once. The study was approved by the local Institutional Review Board and all women gave a written informed consent to participate.

In our Unit, all women scheduled for IVF performed a general assessment (transvaginal ultrasound and blood tests) the month preceding the attempt. The presence of ovarian cysts was systematically recorded at this baseline evaluation and their presence confirmed during the stimulation. The diagnosis of presumed endometriomas was made before initiating the treatment cycle. Specifically, endometrioma was defined as a round-shaped cystic masses with a minimum diameter of 10 mm,

with thick walls, regular margins, homogeneous low echogenic fluid content with scattered internal echoes, and without papillary projections (Exacoustos *et al.*, 2014). Lesions had to be documented at least twice at least two months apart. Doubtful cases were excluded. Women carrying endometriomas larger than 4 cm were counseled about the opportunity to undergo surgery before IVF: removal was however not mandatory and women refusing the intervention could be included in this study.

The regimen of hyper-stimulation used and the dose of gonadotropins were determined on an individual basis according to data from previous IVF cycles, age, day 3 serum FSH, serum AMH and Antral Follicle Count (AFC). During the stimulation, women were monitored and managed according to a standardized clinical protocol as reported elsewhere (Benaglia *et al.*, 2014b). Briefly, they underwent serial transvaginal ultrasounds and, when three or more leading follicles with a mean diameter >18 mm were visualized, human chorionic gonadotropin (hCG) was administered subcutaneously. Oocyte retrieval was performed transvaginally 36 hours later. Even if the benefits of the prophylaxis with antibiotics remains debated (Bhandari *et al.*, 2015; Kaye *et al.*, 2017), the policy of our unit was to systematically administer Ceftriaxone 2 g intravenously (Clarithromycin 500 mg for women who were allergic) at the time of the retrieval.

The anesthetic technique included the intra-venous administration of midazolam 15 mg and fentanyl 0.05 mg associated to a paracervical block with 10 ml of 2% mepivacaine. Oocytes retrieval was done using a single lumen 17 gauge needle and flushing was not performed. Several follicles could be aspirated in each test tube. In women with endometriomas, all efforts were made to avoid the puncture of the cysts because this might facilitate infection, cyst rupture or follicular fluid contamination (Somigliana *et al.*, 2015). However, endometriomas could be transfixed (but not aspirated) to reach follicles behind the cysts if deemed clinically relevant (insufficient number of oocytes retrieved). Transfixion was defined as the voluntary decision to pass through the endometrioma with the needle (without aspirating) in order to aspirate follicles located behind the

cysts and that could not be safely reached in other manners. The accidental aspiration of an endometrioma was defined as the presence of endometrioma content in the aspirated follicular fluid. It was suspected by the physician based on the macroscopic contamination of the follicular fluid and confirmed by the biologist who described the presence of chocolate-like fluid at stereo-microscopic evaluation. More specifically, when the physicians suspected the contamination, aspiration was interrupted and the biologist was requested to check the tube content to confirm or to exclude the diagnosis. The biological diagnosis was based on the observation of a fluid with turbid dark-brown appearance containing many fine black particles (Khamsy *et al*., 2001; Benaglia *et al*., 2014b). If contamination was confirmed, the needle was flushed with oocytes culture media and then the follicular aspiration was resumed and completed. Eight different physicians with a long-lasting expertise in IVF performed all the procedures. The retrieval was transiently interrupted after the aspiration of the first ovary to allow the biologists to count the number of oocytes retrieved separately for the two gonads.

After the end of the retrieval, the physician performing the procedure was requested to fill a specific chart inquiring on the following; 1) completeness of the aspiration (complete versus incomplete), 2) technical difficulties encountered, 3) location of the ovaries, 4) need to transfix the endometrioma and 5) accidental contamination of the follicular fluid. Specifically, aspiration was defined as complete if all follicles with a mean diameter ≥11 mm were aspirated. Otherwise, it was defined as incomplete. Incompleteness was a conscious decision of the physicians based on the possible additional risks associated to reach distant follicles, in particular if requiring transfixion of the endometrioma. Technical difficulties encountered were subjectively classified by the physician using a 5-points Likert scale (very easy, easy, medium difficulty, difficult and very difficult). This variable was then dichotomized into difficult (difficult and very difficult) and not difficult (very easy, easy, medium difficulty) for the analysis. The location of the ovaries (normally located or dislocated) was defined based on their normal anatomical location site, i.e. lateral to uterus in the

ovarian fossa, behind and inside the external iliac vessels (Mihu *et al*., 2011). Ovaries that did not satisfy these anatomical relations were defined as dislocated. All this information was collected separately for the two ovaries.

Women were requested to evaluate pain at the time of oocytes retrieval and two hours later. This symptom was assessed using an 11-points numeric rating scale (NRS), with 0 indicating the absence of pain and 10 indicating pain that is as bad as it can be. This data was used as continuous variable. Nurses had to report on the need to use pain killers or ice bag. Finally, women were contacted by phone two to three weeks later to investigate clinical complications occurring after hospital discharge. They were asked about the occurrence of fever and pain and the need to assume pain-killers or other medical treatments. They were also asked whether they had to refer to any emergency department and how they were managed.

Data were analyzed using the Statistical Package for the Social Sciences software 18.0 (Chicago, IL, USA). P values below 0.05 were considered statistically significant. Two main analyses were performed. Firstly, we compared exposed to non-exposed women. We then performed secondary analyses comparing women with larger and smaller endometriomas (divided the group of exposed women into two groups based on the median dimension of the cyst) and comparing those who were or were not previously operated for endometriosis. Finally, we selected the subgroup of women with unilateral endometriomas and performed paired comparisons between the affected and contralateral unaffected gonads. The main variables investigated included: the proportion of difficult retrievals, the proportion of women with dislocated ovaries, the ratio of oocytes retrieved per developed follicles and the proportion of incomplete aspiration. The Fisher's Exact test, the unpaired Student's *t*-test and the Mann-Whitney nonparametric test were used for unpaired comparisons. The McNemar test and the paired Wilcoxon nonparametric test were used for paired analyses. A binomial distribution model was used to calculate the 95% Confidence Interval (95%CI) of the most relevant proportions. We calculated the sample size setting type I and II errors at 0.05 and 0.20, respectively, considering a rate of difficult retrieval in unexposed women of 5%,

hypothesizing a ratio of exposed to non-exposed of about 1:4 and claiming as clinically relevant showing a more than four-fold increased risk (>20%) in women with endometriomas. On these bases, the required sample size consisted in 54 exposed and 216 non-exposed women.

Results

We ultimately recruited 56 women with endometriomas. The flow chart of the study is shown in Figure 1. The most relevant clinical features of the disease are shown in Table 1. The range of the diameter of the endometriomas varied between 10 and 45 mm. The diameter exceeded 40 mm in three women (5%). Two-hundred twenty-seven women were recruited as non-exposed subjects. The comparison of the baseline clinical characteristics of the two groups is illustrated in Table 2. Antral Follicular Count (AFC) but not AMH was significantly higher in non-exposed women. The remaining characteristics were similar in the two study groups. Considering the indications to IVF, a concomitant male factor of infertility was documented in 21 (37%) women with endometriomas. Indications to the procedure in the non-exposed group were as follows: male factor (n=104, 46%), tubal factor (n=26, 11%), unexplained infertility (n=81, 36%) and mixed factor (n=16, 7%).

In women with endometriomas, the cyst had to be transfixed during oocytes retrieval in 8 cases (14%, 95%CI: 7-25%). Accidental contamination of the follicular fluid with the endometrioma content was recorded in 9 women (16%, 95%CI: 8-27%). Both conditions (transfixion and contamination) occurred in 6 women (11%, 95%CI: 4-20%). The main characteristics of the oocytes retrievals in exposed and non-exposed women are shown in Table 3. The number of developed follicles and the number of oocytes retrieved were significantly higher in non-exposed compared with exposed patients. However, the rate of oocytes retrieved per developed follicles did not differ. Moreover, no differences between the study groups emerged for the proportion of women with dislocated gonads and for the rate of clinically difficult aspiration. In contrast, follicular

aspiration was more frequently incomplete in affected women. The Odds Ratio (OR) was 3.6 (95%CI: 1.4-9.6).

Data on pain and complications after oocyte retrieval are reported in Table 4. The number of hospital accesses after discharge was more common in women with endometriomas. Four exposed women (7%) referred to the emergency department of our hospital for pain complaints, of whom one was ultimately admitted to the gynaecological ward. This woman was discharged the following day without a diagnosis of infection. No pelvic infections neither cyst ruptures were recorded (0%, 95%CI: 0-5%). Among non-exposed women, four (2%) referred to the emergency department for pain complaints and none was admitted in the gynaecological ward.

Finally, we performed three subgroups analyses. Firstly, we divided exposed in women into two groups according to the median of the diameter of the endometriomas (27 mm). The main results of this analysis are shown in Table 5. No statistically significant difference emerged even if the frequency of transfixion and accidental contamination may be higher in the group of women carrying the largest cysts. Secondly, we compared women who did (n=27) and did not (n=29) undergo previous surgery for endometriosis (Table 6). Accidental contamination with the endometrioma content and transfixion of the cysts were more common in unoperated women (p=0.03 and 0.052, respectively). The other studied outcomes did not differ (Table 6). Finally, we performed a paired *per ovary* comparison in the subgroup of women with unilateral endometriomas (n=44). Results are shown in Table 7. A statistically significant difference was documented for the rate of oocytes retrieved per developed follicles. Specifically, the median (IQR) rate was 50% (33-75%) and 75% (50-100%) in the affected and contralateral unaffected ovary respectively (p=0.04). Conversely, no differences emerged for the proportions of dislocated ovary, incomplete aspiration and difficult retrieval.

Discussion

Oocytes retrieval is more complicated in women with ovarian endometriomas. In our study, the frequency of a non-complete follicular aspiration was more than three-folds higher in affected women. On the other hand, we failed to observe significant differences for other relevant variables such as in particular the rate of difficult retrievals, the proportion of women with dislocated ovaries and the ratio between oocytes retrieved and developed follicles. Moreover, we did not find any difference in technical difficulty at the time of oocyte retrieval according to the dimension of the cysts.

The higher frequency of incomplete aspiration in the absence of increased technical difficulties may suggest that physicians are not challenged by the specific condition but, conversely, that they consciously have a cautious attitude. Some follicles were presumably not punctured with the aim of avoiding the transfixion of the endometriomas. This clinical conduct is mainly guided by the remote and unproven hypothesis that transfixion may facilitate infection, cyst rupture or harm to the collected oocytes (Somigliana *et al.*, 2015). Noteworthy, despite this cautious approach, endometriomas had to be transfixed in 14% of exposed women and accidental contamination of the follicular fluid with the endometrioma content occurred in 16% of cases and no infections neither cyst ruptures occurred. The frequencies of transfixion or contamination tended to be higher in women with larger cysts. In general, the lack of any clinically relevant complications such as ovarian abscess or cyst rupture questions against the cautious attitude used in our Unit. On the other hand, it has to be pointed out that these complications may be demanding and even low frequencies may be of clinical relevance. Our sample size is actually insufficient to drawn definitive conclusions on this point, the upper limit of the 95%CI of the frequency of these complications being 5% (low but not nil). In this context, it has also to be underlined that previous studies reported a lower frequency of follicular fluid contamination compared to our present data (Benaglia *et al.*, 2008; Benaglia *et al.*, 2014b). A recent review of the literature estimated this risk at 5% (95%CI: 3-

7%) (Somigliana *et al*., 2015) while we observed a rate of 16%. This inconsistency may be due to the study design. Previous estimations were based on retrospective studies (contamination was presumably under-reported) whereas our study has a prospective design.

A point of interest here is the potential clinical relevance of follicular fluid contamination. Previous evidence on this issue is contrasting (Somigliana *et al*., 2015). An experimental study in mice reported detrimental effects that could be observed at the blastocyst stage of development (Piromlertamorn *et al*., 2013). Data in human is based on small series and is inevitably exposed to confounders. Khamsi *et al*. (2001) failed to detect any effect on the chances of pregnancy whereas Suwajanakorn *et al*. (2001) and Benaglia *et al*. (2014b) reported lower pregnancy rates. In our present study, we failed to observe significant difference between contaminated and non-contaminated cases in terms of embryological development and pregnancy rate (data not shown) but the sample size (9 women) is too low for meaningful conclusions.

Our primary analysis does not consent to rule out a detrimental role of endometriosis in general rather than of ovarian endometriomas. For this reason, we performed a subgroup analysis in women with unilateral cysts that actually confirmed the independent role of endometriomas. Indeed, we observed a significantly lower ratio between oocytes retrieved and developed follicles in the affected gonads. On the other hand, we failed to show a different proportion of incomplete follicular aspiration. We interpreted this contrasting data as a type II error. The sample size was actually insufficient to draw robust conclusions for the second outcome.

Some limitations of our study deserve to be commented.

Firstly, since this is the first study specifically designed to investigate endometrioma-related impact on the oocytes retrieval procedure, future evidence from independent groups is warranted. Confirmation is always mandatory in Science but this aspect is even more relevant here considering that several studied outcomes were inherently subjective and may thus be biased. They actually

reflect the clinical concerns of the involved physicians and in particular their fear to cause complications. In this regard, it is important to point out that the incompleteness of the retrieval is mainly based on individual physician preference. In fact, we did not establish criteria *a priori* to interrupt the aspiration. This, however, would have been a complex task because the decision comes from the combination of several intricate aspects including the number of oocytes already collected, the number of oocytes that the transfixion is expected to provide, the position of the ovary, the possible technical difficulties if a urgent surgery will be required because of a complication and, last but not least, the willingness of the patient to face additional significant risks. We performed an analysis aimed at evaluate whether the rate of incomplete aspiration varied according to the physician involved (data not shown) but the total number of events (n=18) and the total number of physicians involved (n=8) impeded meaningful conclusion. If possible, future studies should consider alternative study designs to overcome this limitation.

Secondly, we cannot exclude that some women in the non-exposed group had some forms of endometriosis that was missed at transvaginal sonography. Even if ultrasound is highly accurate for the diagnosis of advanced endometriosis, it is less reliable for some forms of the disease such as adhesions, superficial peritoneal lesions and some deep peritoneal lesions (Guerriero *et al*., 2016; Turocy and Benacerraf, 2017). Diagnostic laparoscopy is the gold-standard for the diagnosis of the disease but it is not routinely recommended (Practice Committee of the ASRM, 2012; Dunselman *et al*., 2014) and it is not part of the fertility work-up in our clinic. However, we did not deem this confounder of relevance. We aimed at studying the independent impact of ovarian endometriomas on oocytes retrieval difficulties, not of endometriosis in general. The presence of some missed cases of non-ovarian endometriosis in the group of non-exposed women is unlikely to influence our conclusions. In fact, at the time of study design, we even considered to exclusively recruit women with endometriosis and to compare those with and without endometriomas. This study design would have consented to better disentangle the independent effects of ovarian endometriomas.

However, this option did not also consent to overcome all possible confounders (history of surgery and phenotypes differ) and it was abandoned also because a much longer recruitment period would have been necessary to reach the same statistical power. Moreover, in order to exclude the confounding effect of the different diagnoses of non-exposed women, we performed subgroups analyses according to the diagnostic group but failed to detect significant differences in the outcomes (data not shown).

Thirdly, physicians were not blinded to the condition of the patients. In particular, the subjective judgment on the difficulty of the oocytes retrieval could be exposed to this confounder. For this reason, we dichotomized the variable at the time of the analysis. We deemed this potential confounder less relevant if we exclusively focussed on the rate of most demanding procedures. Noteworthy, the assessment of the location of the ovary and the completeness of the aspiration might somehow also be exposed to this confounder. On the other hand, the ratio between oocytes retrieved and the developed follicles was less vulnerable.

Fourthly, a type II error cannot be excluded for some of the investigated items. This may be particularly true for the subgroup analysis according to the dimension of the endometrioma (Table 5). In this regard, it is however important to point out that we designed a pragmatic study. We were actually not interested in mild differences between women with and without endometriomas (that presumably do exist) but, instead, to clinically relevant differences that could lead to re-discuss the potential role of surgery before IVF.

Fifthly, the discrepancy between the results observed for the rate of incomplete aspiration (statistically significant) and the ratio between oocytes retrieved and developed follicles (no significant differences) is surprising. This is particularly troubling if we consider that the opposite occurred in the subgroup analysis per ovary in women with unilateral endometriomas. A type I and II errors might have a role. In addition, one may speculate that physicians performed the retrieval with enhanced care for the follicles that could be safely reached in order to avoid puncture of those

at higher risk of complications. Finally, when aspiration was incomplete, the number of non-punctured follicles was presumably low.

Finally, one may also argue that we may have mis-classified some exposed women because we relied on the ultrasound diagnosis of endometriomas without surgical confirmation. We deemed this possible criticism poorly relevant. In fact, even if surgical diagnosis remains the gold standard, the ultrasound diagnosis of ovarian endometriomas is now considered very accurate. According to a recent Cochrane meta-analysis, transvaginal ultrasound for ovarian endometriomas had a sensitivity of 93% (95%CI: 87-99%) and a specificity of 96% (95%CI: 92-99%) (Nisenblat *et al*., 2016). In fact, the authors of this meta-analysis concluded that transvaginal ultrasound "*approaches the criteria for replacement*" (i.e. for definitively substituting laparoscopy with ultrasound). Moreover, we included only women with a definite sonographic diagnosis: doubtful cases were excluded.

From a clinical point of view, the findings emerging from our analysis deserve some comments. Even if the study was not designed to investigate the possible beneficial effect of surgery, the magnitude of the observed differences is modest and thus cannot be used to justify surgical removal of the endometriomas prior to IVF. Indeed, the difference in the proportion of incomplete aspirations is considerable (14% in exposed and 4% in non-exposed women) but the clinical relevance of this difference is debatable. Unfortunately, we did not record the precise number of non-punctured follicles but the observation that the number of retrieved oocytes per developed follicles is not significantly affected suggests that their number was very low. Of note, we failed to observe significant differences in terms of pregnancy rate between women with and without complete aspiration (data not shown). In addition, we failed to record clinically relevant complications such as in particular pelvic abscesses development or cyst rupture, two possible complications of oocytes retrieval in women with endometriomas (Somigliana *et al*., 2015). In fact, it has to be underlined that the stringent statement to avoid endometrioma puncture to prevent infection, rupture or damage to the collected oocytes is based on commonsense and lacks solid

evidence (Garcia-Velasco and Somigliana, 2009). On the other hand, we observed a trend towards a higher risk of hospital referral in the two weeks after the retrieval among exposed women (7% versus 2%, p= 0.052). However, none of these women was diagnosed with infection and all promptly recovered. We speculate that this increased referral may be consequent to the underlying condition (endometriosis) that favors pain rather than the mere presence of endometriomas. To note, surgery cannot be expected to improve the number of oocytes retrieved. Indeed, even if one may properly assume that the absence of the endometrioma would reduce the frequency of incomplete aspiration, the possible damage of surgery on the ovarian reserve could shrink the total number of developed follicles (Raffi *et al.*, 2012; Somigliana *et al.*, 2015). In other words, one may expect after surgery to aspirate all follicles but also to have less follicles to aspirate. On the other hand, it is intriguing to note that we observed a lower frequency of accidental contamination and transfixion of the cysts in women who were previously operated for endometriosis compared to those who were not (Table 6). The two groups, however, presumably differ in several characteristics and drawing inferences on the possible beneficial effects of surgery based on these findings is unjustified. Finally, our study was not powered to assess the impact of these difficulties on pregnancy rate. The live birth rate per oocytes retrieval did not differ between the two groups (24% and 25% in women with and without endometriomas) but definitive conclusions could not be drawn. To note, even if not markedly high, the chances of live birth in our study are in line with those reported in the European Consortium (European IVF-monitoring Consortium, 2017).

In conclusion, oocytes retrieval in women with ovarian endometriomas is more challenging because of the generally accepted (but not demonstrated) assumption that puncture of these cyst should be avoided. The magnitude of these increased difficulties is however modest. Overall, the presence of these cysts does not appear to represent a clinically significant obstacle.

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References

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 26, 1270-1283.

Bhandari H, Agrawal R, Weissman A, Shoham G, Leong M, Shoham Z, 2015. Minimizing the Risk of Infection and Bleeding at Trans-Vaginal Ultrasound-Guided Ovum Pick-up: Results of a Prospective Web-Based World-Wide Survey. J Obstet Gynaecol India. 65, 389-395.

Benaglia L, Cardellicchio L, Leonardi M, Faulisi S, Vercellini P, Paffoni A, Somigliana E, Fedele L, 2014a. Asymptomatic adenomyosis and embryo implantation in IVF cycles. Reprod Biomed Online. 29, 606-611.

Benaglia L, Cardellicchio L, Guarneri C, Paffoni A, Restelli L, Somigliana E, Fedele L, 2014b. IVF outcome in women with accidental contamination of follicular fluid with endometrioma content. Eur J Obstet Gynecol Reprod Biol. 181, 130-134.

Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, Heikinheimo O, Horne AW, Kiesel L, Nap A, Prentice A, Saridogan E, Soriano D, Nelen W, 2014. European Society of Human Reproduction and Embryology.. ESHRE guideline: management of women with endometriosis. Hum Reprod. 29, 400-412.

European IVF-monitoring Consortium (EIM); European Society of Human Reproduction and Embryology (ESHRE), Calhaz-Jorge C, De Geyter C, Kupka MS, de Mouzon J, Erb K, Mocanu E, Motrenko T, Scaravelli G, Wyns C, Goossens V, 2017. Assisted reproductive technology in Europe, 2013: results generated from European registers by ESHRE. Hum Reprod. 32, 1957-1973.

Exacoustos C, Manganaro L, Zupi E, 2014. Imaging for the evaluation of endometriosis and adenomyosis. Best Pract Res Clin Obstet Gynaecol. 28, 655-681.

Fernando S, Breheny S, Jaques AM,Halliday JL, Baker G, Healy D, 2009. Preterm birth, ovarian endometriomata, and assisted reproduction technologies. Fertil Steril. 91, 325–330.

Garcia-Velasco JA, Somigliana E, 2009. Management of endometriomas in women requiring IVF: to touch or not to touch. Hum Reprod. 24, 496-501

Guerriero S, Condous G, van den Bosch T, Valentin L, Leone FP, Van Schoubroeck D, Exacoustos C, Installé AJ, Martins WP, Abrao MS, Hudelist G, Bazot M, Alcazar JL, Gonçalves MO, Pascual MA, Ajossa S, Savelli L, Dunham R, Reid S, Menakaya U, Bourne T, Ferrero S, Leon M, Bignardi T, Holland T, Jurkovic D, Benacerraf B, Osuga Y, Somigliana E, Timmerman D, 2016. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International Deep Endometriosis Analysis (IDEA) group. Ultrasound Obstet Gynecol. 48, 318-332.

Hamdan M, Dunselman G, Li TC, Cheong Y, 2015. The impact of endometrioma on IVF/ICSI outcomes: a systematic review and meta-analysis. Hum Reprod Update. 21, 809-825.

Khamsi F, Yavas Y, Lacanna IC, Roberge S, Endman M, Wong JC, 2001. Exposure of human oocytes to endometrioma fluid does not alter fertilization or early embryo development. J Assist Reprod Genet. 18, 6-9.

Kaye L, Bartels C, Bartolucci A, Engmann L, Nulsen J, Benadiva C, 2017. Old habits die hard: retrospective analysis of outcomes with use of corticosteroids and antibiotics before embryo transfer. Fertil Steril. 107, 1336-1340.

Kobayashi H, Sumimoto K, Moniwa N, Imai M, Takakura K, Kuromaki T, Morioka E, Arisawa K, Terao T. Risk of developing ovarian cancer among women with ovarian endometrioma: a cohort study in Shizuoka, Japan. Int J Gynecol Cancer 2007;17:37–43.

Mihu D and Mihu CM, 2011. Ultrasonography of the uterus and ovaries. Medical Ultrasonography 2011, Vol. 13, no. 3, 249-252.

Muzii L, Di Tucci C, Di Feliciantonio M, Marchetti C, Perniola G, Panici PB, 2014. The effect of surgery for endometrioma on ovarian reserve evaluated by antral follicle count: a systematic review and meta-analysis. Hum Reprod. 29, 2190-2198.

Nisenblat V, Bossuyt PM, Farquhar C, Johnson N, Hull ML. 2016 Imaging modalities for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev. Feb 26;2:CD009591.

Piromlertamorn W, Saeng-Anan U, Vutyavanich T. 2013 Effects of ovarian endometriotic fluid exposure on fertilization rate of mouse oocytes and subsequent embryo development. Reprod Biol Endocrinol. 11, 4.

Practice Committee of the American Society for Reproductive Medicine, 2012. Endometriosis and infertility: a committee opinion. Fertil Steril. 98, 591-598.

Raffi F, Metwally M, Amer S, 2012. The impact of excision of ovarian endometrioma on ovarian

Sanchez AM, Viganò P, Somigliana E, Panina-Bordignon P, Vercellini P, Candiani M. 2014 The distinguishing cellular and molecular features of the endometriotic ovarian cyst: from pathophysiology to the potential endometrioma-mediated damage to the ovary. Hum Reprod Update. 20, 217-30.

Sanchez AM, Vanni VS, Bartiromo L, Papaleo E, Zilberberg E, Candiani M, Orvieto R, Viganò P, 2017. Is the oocyte quality affected by endometriosis? A review of the literature. J Ovarian Res. 10, 43..

Somigliana E, Benaglia L, Paffoni A, Busnelli A, Vigano P, Vercellini P, 2015. Risks of conservative management in women with ovarian endometriomas undergoing IVF. Hum Reprod Update. 21, 486-99.

Suwajanakorn S, Pruksananonda K, Sereepapong W, Ahnonkitpanit V, Chompurat D, Boonkasemsanti W, Virutamasen P. 2001 Effects of contaminated endometriotic contents on quality of oocytes. J Med Assoc Thai. 84, Suppl 1:S371-6.

Turocy JM, Benacerraf BR. Transvaginal sonography in the diagnosis of deep infiltrating endometriosis: A review. J Clin Ultrasound. 2017 Apr 17. [Epub ahead of print]

Vercellini P, Buggio L, Berlanda N, Barbara G, Somigliana E, Bosari S, 2016 Estrogen-progestins and progestins for the management of endometriosis. Fertil Steril. 106, 1552-1571.

Figure legend

Figure 1: Flow chart of the study.

Table 1. Characteristics of women with endometriomas (n=56)

Characteristics	Number (%) or mean ± SD or [range]
Endometrioma location	
Right ovary	12 (22%)
Left ovary	32 (56%)
Bilateral	12 (22%)
Number of endometriomas	
Right ovary	
1	20 (83%)
≥2	4 (17%)
Left ovary	
1	39 (89%)
≥2	5 (11%)
Endometrioma mean diameter (mm) [a]	
Right ovary	26.3 ± 8.1 [12-40]
Left ovary	26.3 ± 8.2 [10-45]
Deep peritoneal endometriotic lesions	9 (16%)
Previous surgery for endometriosis	27 (48%)
Endometrioma excision	
Unilateral	13 (23%)
Bilateral	8 (14%)
Deep endometriosis surgery	3 (5%)

[a] If more than one endometrioma per ovary was documented, the diameter of the largest one was considered

Table 2. Baseline characteristics of exposed and non-exposed women.

Characteristics	Exposed (n=56)	Non-exposed (n=227)	p value
Age (years)	35.8 ± 4.0	36.1 ± 4.2	0.64
BMI (Kg/m^2)	21.6 ± 2.4	21.9 ± 3.4	0.39
Duration of infertility (years)	2.3 ± 1.8	2.8 ± 2.3	0.17
Previous deliveries	4 (7%)	20 (9%)	0.46
Day 3 serum FSH (IU/ml)	7.5 ± 3.3	7.1 ± 2.9	0.36
AMH (ng/ml)	1.8 ± 1.2	2.0 ± 1.4	0.32
Antral Follicle Count (AFC)	9.5 ± 6.3	12.5 ± 8.1	0.01
Protocol of ovarian stimulation			0.83
Long protocol	8 (14%)	36 (16%)	
GnRH antagonists	36 (64%)	150 (66%)	
Short protocol	12 (22%)	41 (18%)	
Total dose of gonadotropins (IU)	2,351 ± 1,012	2,245 ± 1,075	0.51
Duration of stimulation (days)	8.3 ± 2.3	8.4 ± 2.4	0.75
Number of oocytes retrieved	4 (2-8)	6 (3-11)	0.03
Number of oocytes used	4 (2-7)	4 (2-8)	0.50
No suitable oocytes obtained	4 (7%)	19 (8%)	1.00
Technique used [a]			0.035
Classical IVF	25 (48%)	68 (33%)	
ICSI	27 (52%)	140 (67%)	
Fertilization rate [a]	61% (45-77%)	60% (38-80%)	0.53
Number of cleavage stage embryos [a]	2 (1-4)	2 (1-5)	0.11
No viable embryos obtained [a]	4 (8%)	14 (7%)	0.76
Fresh transfer performed [b]			1.00
at cleavage stage	46 (96%)	187 (96%)	
at blastocyst stage	2 (4%)	7 (4%)	
Women performing frozen embryos transfer [b]	7 (13%)	19 (8%)	0.31
Implantated embryos / embryos transferred	18 / 77 (23%)	72 / 312 (23%)	0.96
Pregnancy per retrieval [c]	18 (32%)	69 (30%)	0.80
~~Cumulative~~ Live births per retrieval [c]	14 (25%)	55 (24%)	1.00

Data are expressed as number (%), median (Interquartile Range) or mean ± SD and compared using Fisher Exact test, Mann-Whitney test or unpaired Student *t*-test

[a] Refers to women retrieving at least one suitable oocyte.

[b] Refers to women who had at least one viable embryo

[c] Pregnancies obtained with both fresh and frozen embryo transfers were considered

Table 3. Oocytes retrieval in exposed and non-exposed population.

Characteristics	Exposed (n=56)	Non-exposed (n=227)	p
Dislocated ovaries [a]	6 (13%)	30 (13%)	0.82
Number of developped follicles >11 mm	7 (5-12)	10 (5-14)	0.07
Number of oocytes retrieved	4 (2-8)	6 (3-11)	0.03
Oocytes retrieved / developed follicles	61% (40-80%)	67% (50-87%)	0.15
Incomplete aspiration	8 (14%)	10 (4%)	0.01
Difficult oocyte retrieval [b]	5 (9%)	8 (4%)	0.14

Data is reported as number (%) or median (interquartile range)

[a] At least one dislocated ovary

[b] Retrieval was considered difficult if physicians reported a difficult/very difficult retrieval for at least of the two ovaries.

Table 4. Symptoms during and after oocytes retrieval in exposed and non-exposed women

Characteristics	Exposed (n=56)	Non-exposed (n=227)	p
During hospitalization			
Pain during oocyte retrieval (NRS)	2.5 ± 1.4	2.8 ± 1.4	0.23
Pain two hours after oocyte retrieval	1.3 ± 1.0	1.4 ± 1.2	0.41
Pain therapy			
Ice pack	12 (21%)	49 (22%)	0.98
Painkillers	2 (4%)	28 (12%)	0.09
After hospital discharge			
Fever	0 (0%)	0 (0%)	1.00
Pain			0.40
No	22 (39%)	76 (33%)	
Yes (painkillers not assumed)	21 (38%)	108 (48%)	
Yes (painkillers assumed)	13 (23%)	43 (19%)	
Hospital referral	4 (7%)	4 (2%)	0.052

NRS: Numeric Rating Scale.

Table 5. Subgroup analysis according to the diameter of the endometrioma

Characteristics	≤ 27 mm [a] (n=29)	> 27 mm [a] (n=27)	p
Transfixion of the endometrioma	2 (7%)	6 (22%)	0.14
Accidental contamination with endometrioma content	2 (7%)	7 (26%)	0.07
Dislocated ovaries [b]	4 (14%)	2 (7%)	0.67
Number of developped follicles >11 mm	7 (5-12)	8 (5-13)	0.74
Number of oocytes retrieved	4 (2-8)	5 (3-8)	0.74
Oocytes retrieved / developed follicles	67% (38-80%)	60% (46-85%)	0.81
Incomplete aspiration	5 (17%)	3 (11%)	0.71
Difficult oocyte retrieval [c]	2 (7%)	3 (11%)	0.66

Data is reported as number (%) or median (interquartile range)

[a] A cut-off of 27 mm was chosen based on the median of the distribution of the diameters. If more than one endometrioma was present, the diameter of the largest one was used for categorization.

[b] At least one dislocated ovary

[c] Retrieval was considered difficult if physicians reported a difficult/very difficult retrieval for at least one of the two ovaries.

Table 6. Subgroup analysis according to previous surgery for endometriosis.

Characteristics	Prior surgery (n=27)	No prior surgery (n=29)	p
Transfixion of the endometrioma	1 (4%)	7 (24%)	0.052
Accidental contamination with endometrioma content	1 (4%)	8 (28%)	0.03
Dislocated ovaries [a]	2 (7%)	4 (14%)	0.44
Number of developed follicles >11 mm	7 (5-9)	9 (5-13)	0.18
Number of oocytes retrieved	4 (2-6)	5 (2.5-9.5)	0.78
Oocytes retrieved / developed follicles	63% (40-80%)	60% (39-82%)	1.00
Incomplete aspiration	3 (11%)	5 (17%)	0.51
Difficult oocyte retrieval [b]	1 (4%)	4 (14%)	0.19

Data is reported as number (%) or median (interquartile range)

[a] At least one dislocated ovary

[b] Retrieval was considered difficult if physicians reported a difficult/very difficult retrieval for at least of the two ovaries.

Table 7. Intrapatient comparison of the oocytes retrieval in women with unilateral endometriomas (n=44)

Characteristics	Affected ovary	Intact ovary	p
Dislocated ovary	4 (9%)	1 (2%)	0.25
Number of developped follicles >11 mm	4 (3-6)	4 (2-6)	0.91
Number of oocytes retrieved	2 (1-4)	3 (1-4)	0.18
Oocytes retrieved / developed follicles	50% (33-75%)	75% (50-100%)	0.04
Incomplete aspiration	3 (7%)	1 (2%)	0.62
Difficult oocyte retrieval	3 (7%)	2 (5%)	1.00

Data is reported as number (%) or median (interquartile range)