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Is endometrioma-associated damage to ovarian reserve progressive? Insights from IVF cycles.

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Abstract

Objective: The relation between endometriomas and damage to ovarian reserve remains controversial. In this study, we hypothesized that this damage may not be present at the time of endometrioma formation but may conversely gradually develop over time.

Study design: To investigate the possibility of a time-related detrimental effect of endometriomas on ovarian reserve, we retrospectively selected 29 women with unilateral cysts who underwent at least two IVF cycles at least 6 months apart and evaluated ovarian responsiveness over time. Women were excluded if they conceived, developed new endometriomas or necessitated new medical or surgical therapies for endometriosis during the interval between the two cycles,

Results: The mean ± SD of the diameter of the endometriomas was 26 ± 8 mm. Most women (*n*=25) had only one endometrioma. In the first cycle, the number of developing follicles in the affected and contralateral intact gonads was 4.9 ± 2.5 and 5.9 ± 2.4, respectively (p=0.10). In the second cycle, it was 5.0 ± 2.9 and 6.0 ± 2.8, respectively (p=0.13). The median (Interquartile Range) proportion of follicles developing in the affected ovaries in the first and second cycles was 44% (31-58%) and 44% (35-55%), respectively (p=0.97). Subgroup analyses according to the duration of the time interval between the two assessments, the dimension of the endometriomas and the history of previous surgery for endometriosis did not show subgroups at significant risk of time-related damage.

Conclusions: We failed to observe an endometrioma-related reduction of ovarian responsiveness with time. However, evidence from larger series obtained in women carrying larger cysts and enrolled for longer time period of time are required for a definitive conclusion.

Key words: endometrioma / ovarian reserve / IVF

Introduction

Endometrioma-related damage to ovarian reserve is a debated and unsolved question (1-4). On one hand, there is cumulative biological and histological evidence suggesting a detrimental effect of these cysts on the surrounding ovarian tissue. Endometriomas contain a plethora of factors such as free iron, reactive oxygen species (ROS), proteolytic enzymes and inflammatory molecules that can diffuse through the cyst wall and damage the adjacent pool of primordial follicles (5,6). Moreover, the presence of these cysts causes a persistent and prolonged stretching to the ovarian cortex that can also disrupt ovarian function and further contribute to ovarian injury (5). Recent histological findings also documented a significant loss of primordial follicles in the ovarian cortex of affected gonads (7,8).

On the other hand, available clinical evidence is less worrying (4). Initial studies showed that ovulation could be partly impaired in affected gonads (9,10) but a recent large cohort study did not confirm detrimental effects (11). Serum AMH is not significantly reduced in women with unoperated ovarian endometriomas, with the possible exception of bilateral cases (12-14). Finally, in women with unilateral endometriomas undergoing *in vitro* fertilization (IVF), ovarian responsiveness to hyper-stimulation did not markedly differ between the affected and intact gonads in women carrying small unilateral endometriomas (4). A reduction in ovarian responsiveness could be observed only in women with larger cysts (15) and in those with bilateral cysts (16,17). In this regard, it has also to be highlighted that, even if robust evidence is lacking, one cannot exclude a concomitant detrimental effect of endometriosis in general on ovarian reserve, in particular for advanced cases. Inflammations and adhesions might indeed perturb ovarian function on their own (18).

In this study, we hypothesized a possible effect of time on the endometrioma-related damage to ovarian reserve. This injury may actually be a time-related event that takes place progressively. In other words, the damage may not be present at the time of endometrioma formation but may

conversely gradually develop over time. Differences in time passed since their development may affect the magnitude of the damage and may at least in part also explain the discrepancies emerging from previous evidence.

To investigate the possibility of a time-related detrimental effect of endometriomas on ovarian reserve, we retrospectively selected women with unilateral cysts who underwent at least two IVF cycles at least 6 months apart and evaluated ovarian responsiveness over time. The contralateral intact gonads were used as controls to protect the findings from the independent deleterious effect of time per se on the ovarian reserve and the confounding effect of the regimen of hyper-stimulation used.

Materials and Methods

This is a retrospective study performed at the Infertility Unit of the Department of Obstetrics and Gynecology of the Fondazione Ca' Granda, Ospedale Maggiore Policlinico, Milan Italy. Clinical charts from women who underwent IVF cycles between January 2005 and June 2013 were reviewed. Inclusion criteria were as follows: 1) sonographic diagnosis of at least one unilateral ovarian endometrioma at the time of the first cycle (women with bilateral endometriomas and those with non-endometriotic ovarian cysts were conversely excluded) ; 2) age $\leq$ 42 years at the time of the second cycle; 3) development of at least one follicle > 10 mm per ovary in the first cycle; 4) two IVF cycles reaching hCG administration at a time interval $\geq$ 6 months. Patients with a past history of ovarian surgery for endometriotic and non-endometriotic cysts could be included regardless of the site of the recurrence (both women with ipsilateral and contralateral recurrences could be included). Exclusion criteria were aimed at excluding new conditions occurring in the interval between the two cycles that may independently affect ovarian responsiveness, i.e. new endometriomas, pregnancy, surgery and new hormonal therapies (19-21). Specifically, exclusion criteria were as follows: 1) development of one or more new endometriomas between the two

studied cycles; 2) pregnancy during the interval between the two studied cycles; 3) surgery between the two studied cycles; 4) need to initiate, discontinue or modify the hormonal therapy (progestins or estroprogestins) between the two studied cycles. Noteworthy, according to the policy of our unit, women who were receiving hormonal treatments (progestins or estro-progestins) prior to start an IVF cycle, re-started assumption if pregnancy did not occur. Women adhering to this policy were not excluded. If more than two cycles satisfied the selection criteria, the first and last cycles were chosen for the comparison. Women could be included only once. A specific informed consent was not obtained since this is a retrospective study. However, all women referring to our unit were routinely requested to provide an informed consent for their clinical data to be used for researches purposes and those denying this consensus were excluded. The local institutional review board approved the study.

The primary outcome of the study was evaluating whether the relative contribution of the affected gonad to the overall ovarian responsiveness (i.e. the total number of developed follicles in the two ovaries) changed over time.

The diagnosis of endometrioma was performed by transvaginal ultrasound and had to be documented on at least two occasions at least two menstrual cycles apart. More specifically, ovarian endometrioma was defined as a round-shaped cystic mass with a minimum diameter of 10 mm, with thick walls, regular margins, homogeneous low echogenic fluid content with scattered internal echoes and without papillary proliferations (22). Women with doubtful lesions, i.e. cysts whose sonographic appearance was compatible but not distinctive of endometriosis were excluded. The diameter of the endometriomas was calculated as the mean of three perpendicular diameters. All sonographic evaluations were performed by physicians with long-standing experience in reproductive medicine.

IVF cycles were managed in a standardized manner as previously described (17). Oocyte retrieval was performed transvaginally 36 hours after hCG injection. Embryo transfer was performed 48-72 hours after oocyte collection or, in properly selected subjects, at the stage of blastocyst. Clinical

pregnancy was defined as the ultrasonographic demonstration of an intrauterine gestational sac 4 weeks after embryo transfer.

Data analysis was carried out with the Statistics Package for Social Sciences (SPSS 18.0, Chicago, IL, USA). Data is reported as number (%), mean ± SD or median (interquartile range: IQR). Data was compared using the paired or unpaired Student *t*-test or the paired or unpaired Wilcoxon non-parametric test or the Fisher Exact test, as appropriate. P-values below 0.05 were considered statistically significant. The main study outcome was the total number of follicles with a mean diameter ≥ 11 mm at the time of hCG administration. The analyses were performed as follows. Firstly, we did a paired analysis of the follicles in the affected-unaffected gonad in the first and second included cycles (using the paired Student *t*-test). Then, we calculated the proportion of follicles developing in the affected gonad (P) according to the following formula:

P = 100 * N. follicles in the affected gonad / (N. follicles in the affected gonad + N. follicles in the contralateral intact gonad)

Finally, we evaluated whether this proportion varied over time. To this aim, we compared this proportion in the first and second cycle using the paired Wilcoxon non-parametric test.

Results

Twenty-nine women were ultimately recruited. The majority (*n*=16) did not undergo previous surgery for endometriosis. Of the remaining 13 women, 11 were operated once while the other two were operated twice. Operated women were staged as Stage I-II, III and IV in one, six and six cases, respectively. Eleven women (38%) were previously operated for ovarian endometriomas (bilateral in one case). In five of the 10 women who underwent unilateral excision of endometriomas, recurrence was on the same side. Endometriomas were located in the right and left ovaries in 11 and 18 cases, respectively. Most women (*n*=25) had only one endometrioma. Multiple endometriomas were observed in the remaining four (two cysts in three cases and four cysts in one case).

Basal general characteristics of the studied population are shown in Table 1. The mean ± SD and the median (IQR) time between the two selected IVF cycles were 13 ± 9 and 11 (8-14) months, respectively. Overall, the time range varied between 6 and 54 months. The mean diameter of the endometriotic cysts in the first and second IVF cycles was 26 ± 8 mm and 26 ± 11 mm, respectively (p=0.64). The IVF cycles characteristics of the first and second IVF cycles are shown in Table 2. Eight women (28%) became pregnant after the second cycle.

Ovarian responsiveness in the affected and contralateral intact ovaries at the first and second IVF cycles are illustrated in Figure 1. Specifically, in the first cycle, the number of developing follicles was 4.9 ± 2.5 and 5.9 ± 2.4, respectively (p=0.10). In the second cycle, it was 5.0 ± 2.9 and 6.0 ± 2.8, respectively (p=0.13). The median (IQR) proportion of follicles developing in the affected ovaries in the first and second cycles was 44% (31-58%) and 44% (35-55%), respectively (p=0.97).

Finally, we performed subgroup analyses according to the duration of the interval between the two IVF cycles, the history of surgery for endometriosis and the diameter of the endometriomas. Results are shown in Table 3. No significant findings were observed.

Comment

The contribution of an ovary with an endometrioma to the whole ovarian response to hyper-stimulation did not change over time. The proportion of follicles developing in the affected ovaries in the first and second cycles was very similar. Moreover, subgroup analyses according to the duration of the interval between the cycles, the dimension of the endometriomas and the surgical history did not consent to identify a group at significant risk of damage. Overall, our results do not support the hypothesis that endometrioma-related damage to ovarian reserve takes place gradually over time.

Our study aimed at shedding some light on the paradox of the relation between endometriomas and ovarian reserve, i.e. the contrast between the worrying biological evidence and the reassuring

clinical data. Indeed, the possibility of a detrimental effect over time would have provided a possible explanation. Endometrioma would display a toxic effect on the surrounding ovarian tissue but this would require time to take place. The presumably short period of time between the formation of these cysts and the evaluation of the functional damage would have explained the scanty clinical impact observed in most cases. However, our findings tend to rule out this possibility.

Given that endometriomas contain a plethora of toxic molecules that may be harmful to the surrounding ovarian tissue, our negative findings deserve some explanations. It may be speculated that some compensatory effects may play a critical role. The 1-2 mm thickness of the fibrotic cyst wall may represent a valid barrier against the diffusion of toxic molecules, in particular those with a larger molecular weight (5). However, ROS and ions such as iron may still diffuse. There is consistent biological evidence demonstrating this phenomenon (5). For instance, 8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA damage, is 10 fold higher in the ovarian tissue surrounding endometriomas compared to other benign cysts (23).

On the other hand, there is also evidence demonstrating that this event can trigger compensatory mechanisms. ROS may induce reactive tissue fibrosis resulting in an increase in the thickness of the cyst wall and, thus, a consequent reduction of their diffusion from the endometrioma content (24). Interestingly, it has also been shown that the increased iron concentration in follicles developing close to ovarian endometriomas (25) is accompanied by a concomitant local increase in ferritine, a molecule that is known to effectively sequester free iron (25,26). Decreasing the labile iron pool would prevent the cascade of events responsible for ROS formation.

At least in part, the differences between biological and clinical evidence emerging from the literature may also be consequent to methodological or interpretation issues. Firstly, the magnitude of biological differences may not be equivalent to clinical differences. In other words, statistically significant biological differences may not translate into clinically significant differences. Secondly, biological evidence generally originates from specimen obtained at the time of surgery whereas

functional clinical studies are acquired from non-operated cysts (4). The lesions may differ. Operated endometriomas are generally larger and are more commonly associated with pelvic pain, thus indirectly suggesting a different inflammatory environment. Of utmost relevance here is the recent demonstration that ovarian responsiveness to hyper-stimulation is hampered when exclusively focussing on large endometriomas (≥5 cm) (15).

Finally, the stretching effect is a neglected but potentially important confounder, in particular for histological studies. In this regard, it has to be highlighted that, for mere geometrical reasons, an endometrioma with a diameter of 4 cm is expected to cause a 12 folds distension of the ovarian cortex. This increases to 27 folds for cysts of 6 cm. By definition, a similar reduction in the density of primordial follicles would be expected even in the absence of any damage.

Some limitations of our study should be acknowledged. Firstly, we lack a definite histological diagnosis of endometriosis since women were not operated. However, this limitation is unlikely to play a relevant confounding effect. The accuracy of the sonographic diagnosis of endometriosis is well established. According to a recent Cochrane meta-analysis, transvaginal ultrasound for ovarian endometriomas has a sensitivity of 93% (95%CI: 87-99%) and a specificity of 96% (95%CI: 92-99%) (27). 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). Finally, it is worth mentioning that this limitation is a common and accepted drawback of most available studies on the functional impact of ovarian endometriomas (4).

Secondly, our population was highly selected and does not reflect the characteristics of all women with endometriomas. Included women were infertile and scheduled for IVF, dimension of the cysts was generally small (the mean diameter was below 3 cm) and women requiring surgery or a new hormonal treatment after the first cycle were excluded. We thus presumably selected women at better prognosis. Further evidence on the potential detrimental role of time using different study designs and selection criteria is thus warranted for a definitive conclusion. On the other hand, the rigid inclusion of women with unilateral lesions and the exclusion of women achieving pregnancy,

developing new endometriomas or undergoing new treatments for endometriosis protect our analyses from other confounders. Interestingly, in line with a previous study of our group (28), dimension of the endometriomas did not change over time thus further supporting the view that IVF does not affect the size of these lesions. In this regard, it has however to be noted that we did not exclude women resuming medical hormonal therapy after the failed attempt and we cannot rule out some beneficial effects of medical therapy on the dimension of the cysts (that could have compensated the impact of hyper-stimulation).

Thirdly, the sample size is undersized to perform robust subgroup analyses. A larger sample size would have allowed us to draw reliable information on the presence of detrimental effect in subgroups of women with peculiar clinical characteristics. Of particular relevance here is the length of the inter-cycle interval. We performed a subgroup analyses for cases with an interval above 12 months and still failed to show any difference, but the sample size was too modest (n=14) for robust conclusions. Unfortunately, the rigid criteria used for inclusion hampered the recruitment of a significantly larger sample size. Moreover, the specific context (women repeating IVF cycles) did not consent to select women with long intervals between the assessments: alternative study designs are required to investigate the impact of longer period of time. In this regard, it is also worth mentioning that we lack the data on the time between the origin of the endometrioma and the first IVF cycle. The availability of this information and the evaluation of its impact could have been of interest. This variable is however not routinely recorded in clinical practice and prospective studies are required to overcome this limitation. Finally, it has also to be recognized that more robust subgroup analyses on the dimension of the cysts (including in particular only cysts with a mean diameter $\geq$ 5 cm) and on the role of previous surgery would have also been of great interest considering the recent evidence on the relevance of these two factors in the determinism of the damage (15,29)

Fourthly, even if ovarian responsiveness to hyper-stimulation is considered a valid surrogate measurement of the ovarian reserve, it remains an indirect finding. We cannot exclude that studying

differences in ovarian responsiveness is an instrument that is too inaccurate and raw to capture detrimental effects over such short period of time. Moreover, data on the oocytes rather than on the number of follicles would have provided interesting information on the quality of the ovarian function. Unfortunately, our retrospective study design did not consent us to obtain this data. Further studies using different study designs and focussing on biological outcomes are thus also warranted.

In conclusion, we failed to observe an endometrioma-related gradual damage to ovarian reserve with time. However, further evidence using larger series, longer follow-up and different study designs is required prior to draw definite conclusions. Studies specifically focussing on women with larger endometriomas would be particularly important.

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Figure legend

Figure 1: Ovarian responsiveness in affected (blu columns) and contralateral intact (red columns) gonads according to study cycle.

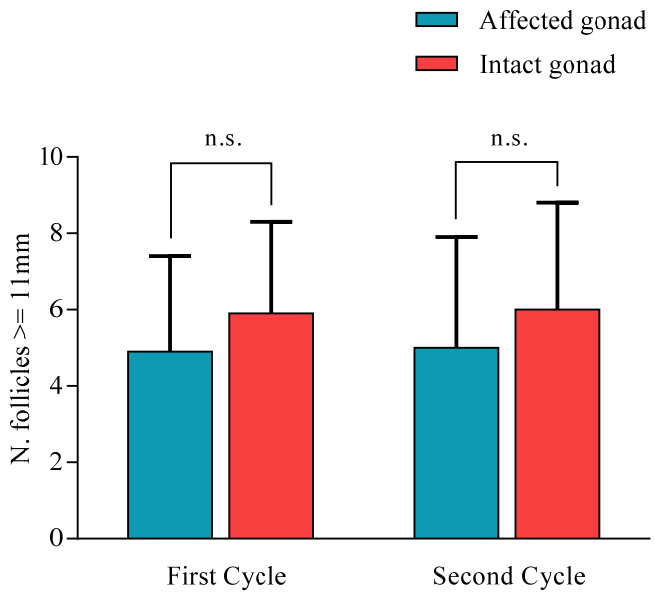

Table 1. Baseline characteristics of the studied population (n=29).

Characteristics	Number (%), Mean ± SD or Median (IQR)
Age (years)	36.1 ± 4.0
BMI (Kg/m^2)	22.3 ± 3.8
Previous pregnancies	4 (14%)
Smoking	3 (10%)
Duration of infertility (years)	3.2 ± 1.2
Concomitant male factor of infertility	13 (45%)
Previous IVF cycles	4 (14%)
Day 3 serum FSH (IU/ml)	8.4 ± 3.7
AMH (ng/ml)	2.7 (0.7-5.7)
AFC (both ovaries)	10 (6-14)
CA-125 (IU/ml)	52 (25-93)
Medical treatment prior to enter the cycle [a]	
Oral contraceptives	6 (21%)
Progestins	5 (17%)

IQR: Interquartile Range

[a] All women resumed the same therapy after the first failed cycle

Table 2. IVF outcome in the two studied cycles (n=29).

Characteristics	First cycle	Second cycle	p
Stimulation protocol			0.26
Long protocol	12 (41%)	9 (31%)	
GnRH antagonist	9 (31%)	6 (21%)	
Other protocol	8 (28%)	14 (48%)	
Total dose of administered FSH (IU)	2,947 ± 1,372	3,363 ± 1,641	0.15
Duration of stimulation (days)	10.2 ± 2.0	10.1 ± 3.1	0.94
Total number of follicles ≥ 11 mm	10.9 ± 3.8	10.9 ± 4.5	0.96
Total number of oocyte retrieved	6.8 ± 4.5	6.2 ± 4.1	0.51
Technique used [a]			
Classical IVF	20 (69%)	18 (69%)	
ICSI	9 (31%)	8 (31%)	
Total number of embryos obtained	2 (1-3)	2 (1-4)	0.04

Data is reported as mean ± SD, number (%) or median (Interquartile range), as appropriate.

[a] Total number of cycles is only 26 in the second cycle because three women failed to retrieve competent oocytes.

Table 3. Subgroup analyses according to the duration of the interval, the history of surgery for endometriosis and the dimension of the endometriomas.

Subgroup	N.	First cycle	Second cycle	p
Duration of the interval				
< 12 months	15	43% (29-53%)	44% (38-50%)	0.39
≥ 12 months	14	50% (33-64%)	47% (19-60%)	0.43
Previous sugey for endometriosis				
No	16	38% (27-57%)	43% (33-50%)	0.76
Yes	13	50% (35-58%)	55% (41-62%)	0.70
Diameter of the endometriomas [a]				
≤ 25 mm	15	38% (29-53%)	44% (33-50%)	0.69
> 25 mm	14	50% (36-60%)	52% (33-61%)	0.78

The percentages refer to the relative contribution (%) of the affected ovary to the overall number of developed follicles.

Data is reported as percentage (Interquartile range of the percentage). Comparisons were made using the non parametric Wilcoxon test for paired data.

[a] If more than one endometrioma was present, the data refers to the largest one.