Peripheral estrogens in women with endometriosis undergoing IVF

Marco RESCHINI [1 *], Edgardo SOMIGLIANA [1,2], Andrea BUSNELLI [1,2],

Laura BENAGLIA [1], Paola VIGANO' [3], Paolo VERCELLINI [1,2]

[1] Obstet-Gynecol Dept, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

[2] Dept of Clinical Science and Community Health, University of Milan, Milan, Italy

[3] Division of Genetics and Cell Biology, Reproductive Sciences Laboratory, IRCCS Ospedale San Raffaele, Milan, Italy.

* To whom correspondence should be addressed

Marco RESCHINI

Infertility Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Via M. Fanti, 6 - 20122 - Milan - Italy

Tel: +39-02-55034304 - Fax: +39-02-55036581

E-mail: marco.reschini@policlinico.mi.it

Abstract

Background: A consistent body of *in vitro* evidence supports a detrimental effect of endometriosis on ovarian steroidogenesis, in particular the synthesis of estrogens. However, clinical evidence is scanty and methodologically weak. This study aimed at clarifying whether peripheral 17-β-estradiol during IVF are influenced by the presence of endometriosis.

Materials and methods: Women undergoing IVF were retrospectively reviewed. Cases were women with a diagnosis of endometriosis. Controls were matched to cases in a 1:1 ratio by study period, age, total number of developed follicles on the day of hCG administration, protocol of hyperstimulation, gonadotropin used and starting dose. The primary outcome was the ratio between serum levels of 17-β-estradiol and the total number of developed follicles.

Results: Fifty-three women with endometriosis and 53 controls were selected. The median ratio (Interquartile range) between serum 17-β-estradiol and the total number of developed follicles in the two groups was 207 (164 – 282) and 201 (144 – 268) pg/ml, respectively (p=0.46). Sensitivity analyses on the magnitude of the follicular response, the history of surgery for endometriomas and the presence of endometriomas did not show any subgroup at increased risk of peripheral estrogens impairment.

Conclusions: Endometriosis does not influence peripheral levels of 17-β-estradiol during IVF. Our findings argue against a biologically relevant effect of the disease on ovarian estrogen-synthesis.

Key words: endometriosis / estrogen / oocyte / in vitro fertilization

Introduction

Endometriosis is commonly associated to subfertility but the precise mechanisms interfering with natural conception remain debated.[1,2] Adhesions and pelvic inflammation are the most commonly advocated reasons. The former could perturb tubal motility while the latter could display direct detrimental effects on the released oocyte, the spermatozoa and the embryo.[1,2] On these bases, it is not surprising that surgery is commonly advocated to treat endometriosis-related infertility. Indeed, albeit demanding, a careful intervention that meticulously removes all lesions can turn off inflammation. It may also improve pelvic anatomy.[3,4] Alternatively or if surgery fails, endometriosis can be cured with *in vitro* fertilization (IVF), a procedure that is expected to overcome most of the postulated deleterious effects of the disease.[2,5-7].

However, several meta-analyses showed lower chances of pregnancy with IVF in women with endometriosis compared to other causes of infertility, in particular for advanced stages.[8-12] Other pathogenetic mechanisms that cannot be overcome by IVF should thus be considered. They include a less receptive endometrium or a deleterious effect during folliculogenesis.[13,14]

A growing and consistent body of evidence supports a negative influence of endometriosis on the growth, steroidogenesis and functionality of granulosa cells (GC).[15] In particular, four independent *in vitro* studies showed a reduction in the expression of P450 aromatase (the enzyme that converts androgens into estrogen) in affected women.[16-19] Moreover, estrogen concentrations in the culture media of granulosa cells and in the serum at the time of ovulation trigger are lower in affected women.[8,15,18] On the other hand, one cannot exclude biases, in particular one cannot exclude a confounding effect of the reduced ovarian reserve, a common situation in women with a history of surgery for endometriosis.[20] In other words, the altered estrogens synthesis and the reduced peripheral levels could be consequent to a previous quantitative damage rather than to a direct detrimental effect on the folliculogenesis.

In order to disentangle whether endometriosis is really associated with impaired and clinically relevant disruption of ovarian estrogen-synthesis, we designed a matched case-control study aimed at overcoming the spurious effects of potential confounders. Specifically, we matched cases and controls for the main variables that are known to influence peripheral levels of estrogens during IVF.

Materials and methods

Women undergoing IVF at the Infertility Unit of the Fondazione Ca' Granda Ospedale Maggiore Policlinico of Milan, Italy between December 2014 and January 2016 were retrospectively reviewed. Women were excluded if the cycle was aimed at fertility preservation or if the cycle was cancelled before the retrieval of the oocytes or if the ovaries could not be properly visualized at ultrasound because of severe anatomical distortions. Cases were women with a diagnosis of endometriosis. Controls were matched to cases in a 1:1 ratio by study period (the following patient satisfying the criteria for inclusion), age (± 1 year), total number of follicles with a mean diameter ≥ 11 mm on the day of human chorionic gonadotropin (hCG) administration (± 1), protocol of ovarian hyperstimulation (long protocol, flare-up or protocol with GnRH antagonists), specific gonadotropin used [recombinant Follicle Stimulating Hormone (FSH), urinary FSH or human Menopausal Gonadotropin (hMG)] and starting dose of gonadotropin used (± 50 IU). The study was approved by the local institutional review board. An informed consent was not required since this is a retrospective study. However, all women referring to our unit signed an informed consent for their data to be used for scientific purposes. Women who denied this consent were excluded.

Clinical information was retrieved from patients' charts. Women were diagnosed with endometriosis if they previously underwent surgery for the disease and/or had sonographic evidence of ovarian endometriomas or deep invasive lesions at the time of IVF initiation. The sonographic diagnosis of endometriosis was done according to previously described criteria.[21]

Ovarian hyper-stimulation was performed as reported in details elsewhere.[22,23] Briefly, the regimen of ovarian stimulation, the specific gonadotropins used and the starting dose were decided on an individual basis according to data from previous IVF cycles, age, day-3 serum FSH, serum anti-mullerian hormone (AMH) and antral follicle count (AFC). Ultralong protocol for IVF in women with endometriosis [24] was not considered during the study period. During stimulation, women underwent serial transvaginal ultrasounds and the dose of gonadotropins could be adjusted based on the ovarian response. When three or more leading follicles with a mean diameter greater than 18 mm were visualized, hCG was administered subcutaneously. Oocyte retrieval was carried out transvaginally 36 h later. If needed, assessment of serum 17-β-estradiol or progesterone could be requested during the stimulation. These assessments were mandatory on the day of hCG administration. Similarly, in that same day, all follicles with a mean diameter ≥ 11 mm were systematically recorded. Their dimension was obtained as the mean of three perpendicular diameters. All ultrasounds, including those aimed at monitoring follicular growth, were done by expert gynaecologists with at least five years experience in IVF and using a unique instrument (EUB 6000 HITACHI equipped with a 6 MHz curvilinear color Doppler probe).

Blood samples were collected and then centrifugated at room temperature at 800 g. Serum 17-β-estradiol concentrations was measured using electrochemiluminescence dosage technique (ECLIA) (Roche Diagnostics GmbH, Mannheim, Germany). The assay detection limit was 5 pg/mL. Intra- and inter-assay variation coefficient were < 8%. Besides the internal quality control checks performed daily by the institutional laboratory, the assays were calibrated whenever a new reactive batch was used or whenever an outcome outside the normal range was observed. The assay was used for the entire duration of the study and no corrective intervention had to be performed during the study period.

The primary outcome of the study was the ratio between serum levels of 17-β-estradiol and the total number of developed follicles with a mean diameter ≥ 11 mm on the day of hCG administration. Given the non-normal distribution of this variable, data was compared using the non-parametric

paired Wilcoxon test. Peripheral progesterone was compared in the same manner. The sample size (at least 50 women per group) was calculated setting type I and II errors at 0.05 and 0.20 and hypothesizing as clinically relevant demonstrating that serum estrogens were inferior in affected women in at least 70% of cases. Statistical analyses were conducted with SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) software version 23. Comparisons between the two groups for the baseline characteristics were done using paired analyses and, in particular, using the paired *t*-test, the non parametric Wilcoxon test and the Chi square test. A Spearman correlation was used to test the association between peripheral 17-β-estradiol level and the total number of follicles. The regressions lines between the two were drawn using the non-parametric regression analysis of Theil-Kendall. Sensitivity analyses for the primary outcome were primarily done for the magnitude of the ovarian response, for the history of surgery for endometriomas and for the presence of endometriomas at the time of the cycle.

Results

Fifty-three women with endometriosis were ultimately included. They were matched to 53 controls. Among cases, 25 women (47%) underwent at least one previous surgery for endometriosis, of whom 21 (84%) had to remove at least one ovarian endometrioma. At the time of the cycle, endometriosis could be demonstrated at ultrasound in 34 women (64%), of whom 31 had ovarian endometriomas (bilateral in 11 cases). In the control group, two women underwent ovarian surgery for non-endometriotic ovarian cysts.

Baseline characteristics of women with and without endometriosis are shown in Table 1. A statistically significant difference emerged only for duration of infertility. IVF cycle outcome in the two groups is shown in Table 2. None of the evaluated variables differ significantly.

Correlation between serum 17-β-estradiol and the total number of developed follicles resulted statistically significant in both women with and without endometriosis. Specifically, the Spearman

correlation coefficients were +0.60 ($p<0.001$) and +0.71 ($p<0.001$), respectively (Figure 1). The ratio between serum estrogens and the total number of developed follicles in the two groups was 207 (164 - 282) and 201 (144 - 268) pg/ml, respectively ($p=0.46$). The ratio between serum progesterone and the total number of developed follicles in the two groups was 84 (48 - 121) and 84 (49-142) pg/ml, respectively ($p=0.87$).

Sensitivity analyses on the ratio between serum 17-β-estradiol and the total number of developed follicles according to the magnitude of the follicular response, the history of surgery for endometriomas and for the presence of endometriomas did not identify any subgroup showing significant difference (Table 3). Other sensitivity analyses were done according to age, protocol of stimulation, gonadotropin prescribed, starting dose and total dose administered but none revealed any subgroup with estrogens production impairment (data not shown). All sensitivity analyses were repeated for the peripheral levels of progesterone but also failed to highlight any remarkable result (data not shown).

Discussion

Our study does not support a detrimental effect of endometriosis on the synthesis of ovarian estrogens. We actually failed to detect any difference in the peripheral levels of 17-β-estradiol at the time of IVF between women with and without the disease. This result was robust as no difference (neither trends) emerged also at sensitivity analyses. In particular, similar levels were observed regardless of the magnitude of the ovarian response, of a history of surgery for endometriomas or of the presence of these cysts at the time of the attempt. This latter finding is of particular relevance considering that endometriomas were claimed to be particularly detrimental to folliculogenesis.[25,26]

To our knowledge, this is the first study specifically designed to address this issue. Inconsistencies with previous clinical evidence in peripheral blood [8] should be explained with confounders in previous studies, in particular ovarian reserve-related confounders. To note, in the meta-analysis of

Barnhart *et al*., lower levels of peripheral estrogens were observed only in women with advanced stages, the same group of women who also retrieved a lower number of oocytes.[8] Inconsistencies with *in vitro* studies are more difficult to explain. In general, estimating clinical relevance of biological findings is challenging. In fact, our study tends to deny any relevant effect on the synthesis of estrogens but we cannot exclude that subtle local alterations may be of some biological relevance. Estrogens are both endocrine and paracrine agents and one may speculate that even a modest impairment of estrogen production (that cannot be revealed peripherally) may be somehow detrimental locally. In addition, one can argue that IVF does not reflect the situation occurring during natural cycle. Further evidence with different study designs is required to address these concerns. On the other hand, it is noteworthy that there is growing clinical findings on the use of aromatase inhibitors for ovarian hyperstimulation.[27] These agents interfere with estrogens production and shrink their peripheral levels, thus indirectly determining follicular growth through centrally governed feedback mechanisms. Letrozole has become the first line option for the treatment of anovulation in infertile anovulatory women [28] and is recommended as an adjuvant treatment during ovarian hyper-stimulation aimed at cryopreserve oocytes in women with estrogen-dependent cancers.[29] Albeit debated, some authors also advocate its systematic use for IVF.[30] Overall, the observation that good results can be achieved with the use of a drug interfering with estrogen-synthesis argues against a finely regulated role of local estrogens in folliculogenesis. It also argues against the above-mentioned hypothesis that a subtle disruption of ovarian production of estrogens that cannot be captured peripherally may be of some relevance in women with endometriosis.

The observation that endometriosis does not significantly affect estrogen-syntheses cannot be used to rule out a detrimental effect of the disease on folliculogenesis or oocyte quality. Indeed, other mechanisms can be detrimental. For instance, there is evidence that oocytes from affected women may have lower mitochondrial content, increased spindle abnormalities and hardening of the zona pellucida.[13] In general, even if our study provided negative findings, disentangling the possible

detrimental effects of endometriosis on folliculogenesis and oocyte quality is far from being clarified and remains crucial. Steroidogenesis is only one of the multiple phenomena occurring during folliculogenesis. To note, the origin and pathogenesis of endometriosis is complex and remains nebulous and debated. [31] A plethora of biological and molecular events is associated to the presence of the disease. In general, even if the *primum movens* of the disease is yet unclear, there is growing evidence that a complex network of immune cells, adhesion molecules, extracellular matrix metalloproteinases and pro-inflammatory cytokines activate/alter peritoneal microenvironment, creating the conditions for differentiation, adhesion, proliferation and survival of ectopic endometrial cells.[32-34] This same complex perturbation may interfere with other physiological functions of the reproductive system and in particular with folliculogenesis and oocytes competence. To note, investigating oocyte quality in women with endometriosis deserves utmost consideration because new insights may lead to modifications of clinical practice. In particular, if a clinically relevant damage will definitely emerge and will be shown to be progressive, fertility preservation in younger age or prompt surgery could be justified. To date, however, there is insufficient evidence to support this position.

Some strengths and limitations of our study deserve to be commented. Firstly, we did a very accurate matching. Even if this decision was taken to protect our findings from confounders one may have concerns about overmatching. In fact, we decided for a matched design because we were not fully confident on the capacity of mathematical modeling to properly control for confounders. Moreover, the effects of the variables used for matching on estrogen-synthesis are well-known and do not follow simple mathematical rules. Finally, even if in our unit there is not a peculiar policy for the management of IVF in women with endometriosis one cannot exclude that the presence of the disease may have influenced some clinical decisions that could ultimately influence the peripheral levels of estrogens. For instance, there is some mild evidence on the superiority of the long protocol regimen in women with endometriosis.[35] Secondly, our study design mainly rely on the accuracy of

the record of the follicular growth. One cannot exclude that the presence of endometriosis may have in some cases compromised ultrasound visibility. In addition, the decision to exclude cases with impaired visibility could be a source of bias (one cannot rule out that estrogen-synthesis could be impaired only in most severe cases with highly subverted local anatomy). On the other hand, it has to be underlined that all scans were done by expert gynecologists and that the number of women who were excluded because of difficulties in visualization was very low.

In conclusion, endometriosis does not influence peripheral levels of 17-β-estradiol during IVF. Our findings argue against a biologically relevant effect of the disease on estrogen-synthesis. Other mechanisms should be investigated to explain the possible deleterious impact of endometriosis on folliculogenesis and oocyte quality.

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Table 1. Baseline characteristics of women with and without endometriosis.

Characteristics	Endometriosis n=53	Controls n=53	p
Age (years)	35.7 ± 3.9	36.0 ± 3.2	0.45
BMI (Kg/m^2)	20.9 ± 2.4	21.8 ± 3.4	0.17
Duration of infertility (years)	2 (1 - 2.5)	2 (1.5 - 4)	0.002
Previous deliveries	3 (6%)	10 (19%)	0.07
Day 3 serum FSH (IU/ml)	7.6 (6.3 - 8.7)	7.6 (5.7 - 9.9)	0.55
AMH (ng/ml)	1.7 (0.7 - 2.8)	1.0 (0.5 - 2.6)	0.43
Antral Follicle Count (AFC)	8 (6 - 12.5)	9 (6 - 13)	0.25
Previous IVF cycles	22 (42%)	27 (51%)	0.44
Indication to IVF			< 0.001
Male factor	0 (0%)	23 (44%)	
Endometriosis	33 (62%)	0 (0%)	
Tubal factor (PID)	0 (0%)	8 (15%)	
Unexplained	0 (0%)	15 (28%)	
Mixed	20 (38%)	7 (13%)	

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

Data is compared using Chi square test, Fisher Exact test, Student *t*-test, Mann-Whitney test and Wilcoxon test as appropriate.

Table 2. Cycle outcome in women with and without endometriosis.

Characteristics	Endometriosis n=53	Controls n=53	p
Protocol of ovarian stimulation			1.00
Long protocol	8 (15%)	8 (15%)	
GnRH antagonists	34 (64%)	34 (64%)	
Flare up	11 (21%)	11 (21%)	
Gonaodotropin used			1.00
Recombinant FSH	8 (15%)	8 (15%)	
hMG	42 (79%)	42 (79%)	
Corrifollitropin	3 (6%)	3 (6%)	
Starting dose of gonadotropins (IU)	300 (200 - 400)	300 (200 - 400)	0.15
Total dose of gonadotropins (IU)	2,100 (1,775 - 3,000)	2,400 (1,525 - 3,000)	0.39
Duration of stimulation (days)	8.4 ± 2.2	8.3 ± 2.2	0.70
Total number of follicles ≥ 11 mm	8 (5 - 13)	7 (5 - 13)	0.60
Serum estrogens (pg/ml)	1,586 (1,146 - 2,787)	1,625 (1,060 - 2,322)	0.24
Serum estrogens ≥ 3,500 pg/ml	4 (8%)	2 (4%)	0.68
Serum progesterone (pg/ml)	641 (439 - 887)	599 (470 - 914)	0.54
Serum progesterone ≥ 1,500 pg/ml	3 (6%)	1 (2%)	0.62
Oocytes retrieval			
Total number of oocytes retrieved	4 (3 - 8)	5 (3 - 9)	0.11
Total number of suitable oocytes retrieved	4 (2 - 7)	4 (2 - 8)	0.56
No suitable oocyte retrieved	2 (4%)	2 (4%)	1.00
Embryo transfer [a]			
Fresh transfer	40 (76%)	41 (77%)	1.00
Not done for unavailability of embryos	4 (8%)	6 (12%)	0.74
Fresh transfer not done for other reasons	7 (14%)	4 (8%)	0.53
Frozen transfer(s)	16 (31%)	12 (24%)	0.51
Cumulative clinical pregnancy per retrieval	17 (32%)	18 (34%)	1.00
Cumulative live birth per retrieval	14 (26 %)	13 (25%)	1.00

Data is reported as number (%), mean ± SD or Median (interquartile range), as appropriate.
Data is compared using Chi square test, Fisher Exact test, Student *t*-test, Mann-Whitney test and Wilcoxon test as appropriate.

[a] Data refer to patients with suitable oocytes retrieved (51 case and 51 control subjects).

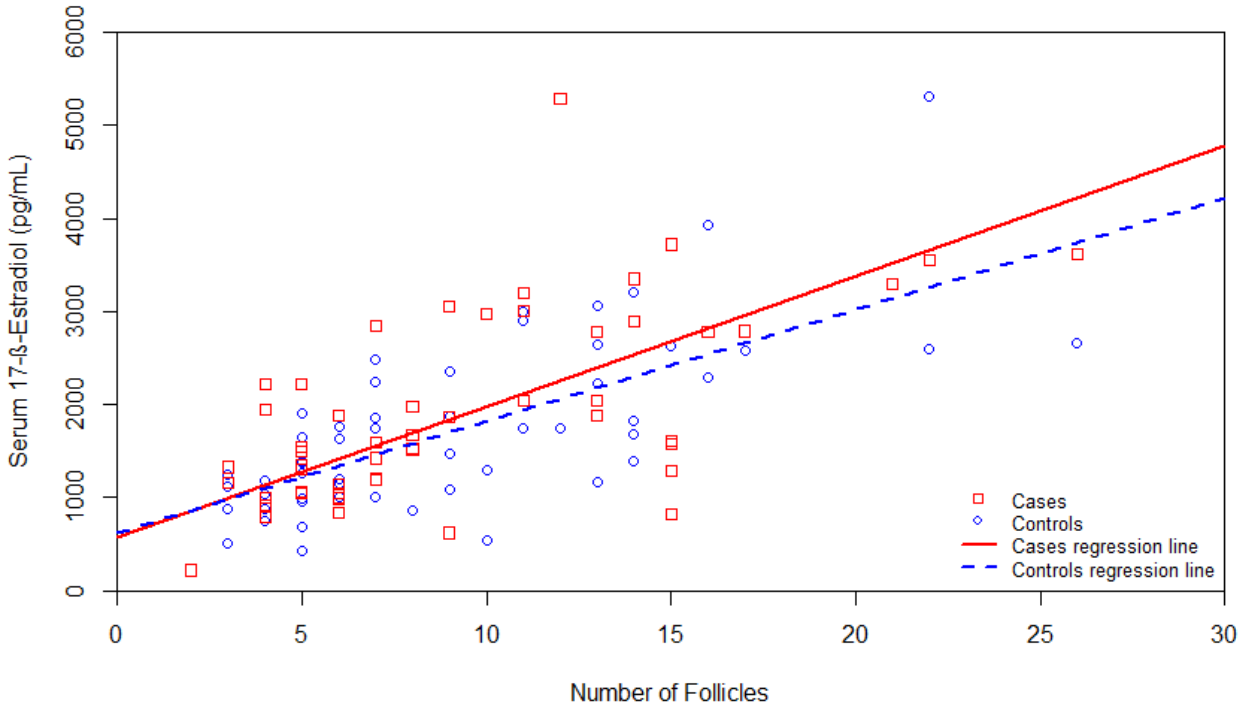

Figure 1: Correlation between the total number of developed follicles and serum 17-β-estradiol at the time of hCG administration. Women with and without endometriosis are represented in red and blue, respectively. The Spearman correlation coefficients were +0.60 ($p<0.001$) and +0.71 ($p<0.001$), respectively. Regressions lines were drawn using the non-parametric regression analysis of Theil-Kendall.