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ORIGINAL ARTICLE *Infertility*

Do endometriomas induce an inflammatory reaction in nearby follicles?

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STUDY QUESTION: Do endometriomas induce an inflammatory reaction with increased cytokine concentrations in nearby follicles and thereby affect follicular development during controlled ovarian stimulation for *in vitro* fertilization (IVF)?

SUMMARY ANSWER: With most endometriomas, there is no evidence of increased cytokine concentrations in the ipsilateral leading follicle. Infrequently, the concentration of inflammatory cytokines is increased in the follicular fluid (FF) and associated with diminished ovarian response.

WHAT IS KNOWN ALREADY: The link between peritoneal endometriosis, inflammation and infertility is well established; however, the association between intraovarian inflammation and endometrioma is unknown.

STUDY DESIGN, SIZE, DURATION: This prospective cohort study included 117 infertile women undergoing IVF in a tertiary infertility clinic at Oslo University Hospital Rikshospitalet, Norway, during the period May 2009 to September 2011.

PARTICIPANTS, SETTING, METHODS: There were 47 patients with unilateral endometrioma and 17 patients with bilateral endometrioma, while the 53 control patients had unexplained or male factor infertility. Concentrations of IL-1β, IL-6, IL-8, IL-10, IL-12 and TNF-α were measured in serum and in the fluid of the largest pre-ovulatory follicles from each ovary of each participant.

MAIN RESULTS AND THE ROLE OF CHANCE: Cytokine levels in the follicular fluid from the two ovaries in women with unilateral endometriomas were comparable, and were not significantly altered compared with that of control groups with male factor infertility, unexplained infertility or bilateral endometriomas. Compared with serum levels, the follicular fluid levels of IL-8 and IL-6 were higher, suggesting a local production or recruitment. The follicular fluid IL-8 level varied considerably and showed an inverse relationship with IL-12, IL-10 and TNF-∝, suggesting a complex interaction between various immune cells. A small group of patients ($n = 3$) had increased levels of all follicular fluid cytokines combined with moderately to slightly elevated serum levels and these patients had a significantly lower ovarian response.

LIMITATIONS, REASONS FOR CAUTION: For ethical reasons, the endometriomas were diagnosed indirectly by ultrasound rather than by histology.

WIDER IMPLICATIONS OF THE FINDINGS: This paper reveals that endometriomas seldom induce inflammation in nearby follicles during IVF; therefore, routine cystectomy prior to IVF may not be necessary. Cytokine levels in the follicular fluid, nonetheless, show distinctive patterns and increased levels may be linked to reduced ovarian response independent of the cause of infertility.

STUDY FUNDING/COMPETING INTERESTS: The project was funded by the Norwegian Resource Centre for Women's Health, Oslo University Hospital. The authors have no conflicts of interests.

Key words: endometrioma / cytokines / inflammation / *in vitro* fertilization / IL-8

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Introduction

Endometriosis is defined as the presence of endometrial-like tissue outside of the uterus that induces a chronic inflammatory reaction (Kennedy *et al.*, 2005), implying that inflammation is an important part of the disease. Endometriosis is more frequent amongst infertile women, and an inflammatory state can impact fertility in many ways (Hull, 1992; D' Hooghe *et al.*, 2003).

Women with peritoneal endometriosis have an increased volume of peritoneal fluid with elevated levels of macrophages and various proinflammatory cytokines in comparison with fertile controls (Syrop and Halme, 1987; Hill *et al.*, 1988; Harada *et al.*, 2001; Lebovic *et al.*, 2001). These women have reduced spontaneous pregnancy rates and surgical removal of peritoneal endometriosis improves the spontaneous fecundity and success rates of artificial insemination and *in vitro* fertilization (IVF) (Marcoux *et al.*, 1997; Werbrouck *et al.*, 2006; Opoien *et al.*, 2011).

Whether intraovarian endometriosis, or endometriomas, also generates an unfavorable fluid environment as observed with peritoneal endometriosis, is a subject of debate. This matter is especially relevant in IVF, where the gametes and embryos are never in direct contact with the hostile milieu in the peritoneal fluid, but the developing oocytes would be in contact with the affected follicular fluid. It is also relevant for the ongoing discussion on the role of surgery in the management of endometriomas prior to IVF.

In order to study a possible association between endometriomas and signs of intrafollicular inflammation in follicles close to the endometrioma, we investigated the presence and levels of cytokines in follicular fluid derived from women with unilateral and bilateral endometriomas and from infertile women without endometriosis but treated for male factor infertility or unexplained infertility. We also assessed whether various cytokine patterns could be related to ovarian responsiveness or IVF cycle outcome.

Methods

This was a prospective cohort study of infertile woman undergoing IVF or intracytoplasmic sperm injection (ICSI) at the Section for Reproductive Medicine, Oslo University Hospital Rikshospitalet, during the period May 2009 to September 2011.

The study group consisted of women with uni- or bilateral ovarian endometriomas, and in women with unilateral lesions, findings in the unaffected contralateral ovary were used for comparison. In women with endometriosis, concomitant infertility like male factor might also be present. Infertile women with unexplained and male factor infertility, in whom laparoscopy and ultrasound scans had excluded the presence of endometriosis, were selected as controls.

Prior to IVF, all couples underwent a routine infertility workup including semen analysis, hormone analyses (AMH, FSH, LH, estrogen, progesterone, prolactin, TSH, T4 and anti-TPO), pelvic ultrasound scans and laparoscopy to assess tubal patency and to investigate if endometriosis was present. The endometrioma diagnosis was established after repeated gray-scale ultrasound evaluations confirming an ovarian cyst with diffuse low-level internal echoes during diagnostics and stimulation. The sizes of the cysts were recorded at the time of oocyte retrieval (OR).

Ovarian stimulation was individualized, and patients received either a luteal phase down-regulation GnRH agonist protocol or a GnRH antagonist protocol. Notably, no differences have been found in follicular fluid concentration of the selected cytokines between the two protocols (Ficicioglu *et al.*, 2010). During the GnRH agonist protocol, women received FSH Gonal F, (Merck Serono, Germany), Puregon (Organon, The Netherlands) or HMG (Menopur, Ferring, Switzerland) after adequate down-regulation with Synarela (Pfizer, USA) was achieved (serum estradiol concentration <0.2 nmol/l). None of the women received prolonged down-regulation protocols. In the GnRH antagonist protocol, women received Orgalutran (Organon), when the leading follicle was 14–15 mm. With both protocols the starting FSH dose was, in most cases, 150 IU per day for patients aged 35 years or younger and 225 IU for those older than 35 years. Final follicular maturation was induced with 6500 IU hCG (Ovitrelle, Merck Serono) when three or more follicles were $\geq$18 mm in diameter in the agonist protocol or 17 mm in the antagonist protocol. OR was performed 34–36 h later. In order to avoid contamination of the follicular fluid samples by blood or fluid of other follicles, follicular fluid was collected with a new aspiration needle each time the first and largest (also termed leading) ovarian follicle was punctured and with a new or flushed aspiration needle each time the first ovarian follicle of the opposite ovary was punctured. Patients who needed to have their endometrioma punctured in order to access follicles received prophylactic antibiotics (100–200 mg for 5 days of Doxylin, Actavis, Iceland).

In order to measure cytokine levels in the peripheral circulation, a blood sample was taken from the antecubital vein in each woman within an hour prior to follicle aspiration.

The protocols for IVF and embryo culture have previously been described (Bjercke *et al.*, 2010).

Laboratory procedures

The three test samples (fluid from the leading follicle of each ovary and serum) of each patient were centrifuged at 3000*g* for 20 min, and supernatants were frozen at -80°C for later assays.

Collected follicular fluid and serum samples were assayed using Bio-Plex Pro Human Cytokine Assay *(Bio-Rad Laboratories, Oslo, Norway)*. The custom-designed 6-plex kit included reagents to detect human cytokines IL-1β, IL-6, IL-8, IL-10, IL-12 (p70) and TNF-α. The test was performed according to the manufacturer's instructions and has previously been described (Polec *et al.*, 2011). Briefly, capture beads (25 µl pr well) were added to pre-wetted filter plates, then standards and test samples were transferred to respective sample wells (25 µl pr test) in duplicates. A mixture of detection antibodies was added (25 µl pr test) as the first detection step and streptavidin-PE (Invitrogen) was added (25 µl pr test) as the second detection step. Following each of the aforementioned steps, the test samples were incubated for 30 min at room temperature on a vibrating platform shielded from light after each step.

Instrument settings, signal acquisition, data analysis and calculations of cytokine concentrations were performed using the Bio-Plex Manager software. The lower limits of quantification (LLOQ) for this essay were as follows: 3.2 pg/ml for IL-1β, 2.3 pg/ml for IL-6, 1.9 pg/ml for IL-8, 2.2 pg/ml for IL-10, 3.3 pg/ml for IL-12 and 5.8 pg/ml for TNF-α, whereas the limits of detection were 0.6 pg/ml for IL-1β, 2.6 pg/ml for IL-6, 1.0 pg/ml for IL-8, 0.3 pg/ml for IL-10, 3.5 pg/ml for IL-12 and 6.0 pg/ml for TNF-α. Cytokine concentrations calculated by the Bio-Plex Manager software were used in subsequent analyses even if these were below the manufacturer's limits.

Statistics

Continuous data with a normal distribution are shown as mean $\pm$ SD, otherwise as median (range) values. Comparisons were done using one-way analyses of variance (ANOVA), with Bonferroni post hoc adjustments, or the χ^2-test (SPSS version 18 (SPSS Inc., USA). $P < 0.05$ was considered statistically significant.

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Cluster analysis was used to explore serum and follicular fluid cytokine concentrations patterns and to identify subgroups of patients with related patterns. First, serum and follicular fluid concentrations of the six assayed cytokines were normalized within patients with the scale function of R (www.r-project.org). The kmeans function was then applied with an increasing number of clusters in order to assess the optimal number of subgroups ($n = 4$). Finally, Ward hierarchical clustering was performed with four clusters to classify patients with similar serum and follicular fluid cytokine patterns.

Ethics

Written and informed consent were obtained from the participants. The study was approved by the Regional Ethics Committee (Ref No. S-05058).

Results

A total of 125 patients were invited to participate in the study. Two women declined, and six were excluded because their ovarian cysts were found not to be endometriomas on ultrasound control. Follicular fluid (FF) and peripheral blood samples were collected from 117 women undergoing IVF with or without ICSI.

Demographics and treatment data are shown in Table I. There was no difference in the distribution of age, BMI, primary infertility rate or number of cycles performed. ICSI was more often performed in male factor infertility, but also in the two endometriosis groups combined compared with unexplained infertility ($P < 0.05$). Patients with bilateral endometriomas required significantly increased total doses of FSH during stimulation compared with non-endometrioma groups. There were 3 (21.4%) patients with bilateral endometriomas and 13 (27.7%) patients with unilateral endometriomas who had a history of former ovarian surgery. At OR, the mean endometrioma size was 27 mm (SD 11). All endometrioma patients had significantly fewer oocytes retrieved, but the diploid fertilization rate and quality of the transferred embryos were comparable with that of patients with unexplained and male factor infertility. Implantation and pregnancy rates were not significantly different between groups.

A possible association between endometrioma and local intrafollicular inflammation was examined by comparing cytokine concentrations in the follicular fluid from the two ovaries across infertility diagnoses (Table II). The concentrations of IL-6 and IL-8 in follicular fluid were comparable in the affected and non-affected ovary of women with unilateral endometriomas, in women with bilateral endometriomas and in infertile women without endometriosis. TNF-α was detected in follicular fluid derived from women with bilateral endometriomas only. Follicular fluid concentrations of IL-1β, IL-10 and IL-12 were below the lower limit of quantification.

Comparing cytokine concentrations of parallel follicular fluid and serum samples may reveal whether the source of cytokines was

Table I Baseline characteristics and assisted reproduction treatment among women with endometrioma, unexplained infertility and male factor infertility.

	Unilateral endometrioma	Bilateral endometrioma	Unexplained infertility	Male factor infertility
Number of patients	47	17	28	25
Age (years)	34.8 ± 3.45	31.9 ± 4.79	33.6 ± 3.38	33.2 ± 5.2
BMI (kg/m^2)	23.1 ± 3.9	22.9 ± 3.6	23.5 ± 4.0	24.1 ± 3.77
Primary infertility(%)	60	76	61	76
Median number of treatment cycles (range)	2 (1–3)	1 (1–3)	1 (1–3)	1 (1–3)
Proportion of IVF treatments (of IVF + ICSI) (%)	57[a]	65[a]	79[b]	16
Total FSH dose (IU)	2352 ± 883	2829 ± 1035[c,d]	1816 ± 614	1957 ± 766
Number of collected oocytes	7.2 ± 3.7[d,e]	5.9 ± 3.0[d,e]	11.6 ± 4.97	10.6 ± 6.86
Number of diploid zygotes	3.9 ± 2.7	2.7 ± 1.8	5.3 ± 4.8	4.6 ± 4.5
Diploid fertilization rate	0.53	0.45	0.45	0.43
Number of blastomers of the transferred embryo with the highest quality	4 (2–5)	4 (2–6)	4 (2–8)	4 (2–7)
Quality score of the transferred embryo with the highest quality	2.1 (2.0–3.1)	2.1 (1.0–3.2)	2.1 (1.0–3.2)	2.1 (2.0–3.2)
Number of transferred embryos	1.0 (1–2)	1.0 (1–2)	1.0 (1–2)	1.0 (1–2)
Implantation rate (%)	23.6 (13/55)	20.0 (4/20)	41.3 (12/29)	30.0 (9/30)
Pregnancy rate per oocyte retrieval (%)	27.6 (13/47)	23.5 (4/17)	42.8 (12/28)	36.0 (9/25)
Number of treatments without fertilization or embryo development	5	2	4	4
Pregnancy rate per embryo transfer (%)	31.0 (13/42)	26.7 (4/15)	50.0 (12/24)	42.9 (9/21)

[a]$P = 0.01$ to male factor.
[b]$P < 0.001$ to male factor.
[c]$P < 0.01$ to male factor.
[d]$P < 0.001$ to unexplained infertility.
[e]$P < 0.05$ to male factor.

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Table II Median and range of cytokine concentrations in serum and follicular fluid at oocyte retrieval in women with endometrioma, unexplained infertility and male factor infertility.

Cytokine	Unilateral endometrioma ($n = 47$)			Bilateral endometrioma ($n = 17$)		Unexplained infertility ($n = 28$)		Male factor infertility ($n = 25$)	
	Serum (pg/ml)	Follicular fluid unaffected side (pg/ml)	Follicular fluid affected side (pg/ml)	Serum (pg/ml)	Follicular fluid (pg/ml)	Serum (pg/ml)	Follicular fluid (pg/ml)	Serum (pg/ml)	Follicular fluid (pg/ml)
IL-1β	0.070 (0–1050)	0.15 (0–1.5)	0.075 (0–4.6)	0.13 (0–0.58)	0.075 (0–1.5)	0.14 (0.10–0.47)	0.16 (0.01–0.64)	0.010 (0–1.1)	0.01 (0–11)
IL6	0.69 (0–2711)	3.0 (0.20–262)	3.0 (0.30–47)	0.73 (0.04–988)	4.5 (0.70–711)	1.2 (0.22–4000)	4.2 (0.40–19)	0.64 (0–3700)	2.7 (0.60–9.3)
IL8	0.88 (0–1407)	89 (19–760)	70 (1.5–409)	0.16 (0–13)	62 (4.7–499)	1.4 (0–3.8)	103 (1.8–488)	0.39 (0–3557)	93 (6.2–529)
IL-10	0.44 (0–2344)	0.41 (0–2.8)	0.40 (0–2.9)	0.28 (0–921)	0.30 (0–2.8)	0.41 (0–6.9)	0.54 (0.06–2.0)	0.17 (0–1900)	0.29 (0–7.9)
IL-12	1.1 (0–4638)	0.48 (0–9)	0.48 (0.10–2.9)	0.84 (0–2564)	0.64 (0.01–11)	1.1 (0.22–3.5)	0.64 (0–2.4)	0.61 (0–11)	0.42 (0.01–13)
TNF-α	4.5 (0–139)	4.7 (0.01–48)	3.8 (0–21)	3.4 (0–4888)	6.3 (0–180)	3.1 (0.91–24526)	5.6 (0.16–27)	2.1 (0–13223)	4.0 (0–21)

intraovarian or systemic. However, serum concentrations of cytokines were below the limit of quantification for 113 of 117 patients, precluding this analysis.

Cluster analysis of serum and follicular fluid cytokine concentrations identified four subgroups with distinct concentration profiles (Fig. 1). These clusters were unrelated to the infertility diagnoses ($P = 0.14$). In general, the concentration of all cytokines in serum was comparable and low, while in follicular fluid the concentration of IL-8 varied considerably and the following patterns appeared: increased follicular fluid concentrations of IL-8 were associated with reduced concentrations of IL-10 and IL-12 (cluster 2, $n = 17$), and reduced follicular fluid concentrations of IL-8 were associated with increased concentrations of either IL-10, IL-12 or TNF-α (some patients of cluster 1, $n = 93$) (Fig. 2). Additionally, there were two minor groups with either increased serum cytokine levels in combination with moderate to low levels in follicular fluid (cluster 3, $n = 4$) or moderately elevated serum levels in combination with consistently increased follicular fluid levels (cluster 4, $n = 3$) (Fig. 2). The latter group consisted of one patient with male factor infertility and two patients with endometriomas, none with any co-morbidity.

To reveal the clinical importance of these cytokine profiles, we compared the profile groups for body mass index, serum concentration of AMH, age, daily FSH dose, number of oocytes collected, number of diploid zygotes, number of blastomeres and score of the top quality embryo (Fig. 3). Notably, women in cluster 4, with a cytokine profile characterized by high follicular fluid levels and moderate to slightly elevated serum levels of IL-1β, IL-8, IL-10 and TNF-α, had significantly fewer oocytes extracted ($P < 0.05$). The oocyte count in this group was significantly lower also after adjusting for age and the daily FSH dose by regression analysis ($P < 0.05$).

Discussion

Endometriosis is characterized by a state of chronic inflammation at the site of disease. Indeed, the concentrations of activated macrophages and certain cytokines are elevated in the peritoneal fluid of women with peritoneal endometriosis. In this study we examined whether intraovarian endometriosis is associated with a local inflammatory reaction and whether inflammation affects follicular development and oocyte quality.

We found no difference in serum levels of several cytokines when comparing patients with and without endometriosis, indicating that ovarian endometriosis does not induce a systemic inflammatory response. Furthermore, in most patients, the cytokine levels in the leading follicle adjacent to endometriomas, whether uni- or bilateral, did not show alterations indicating inflammation. However, independently of the infertility diagnosis, we observed some distinctive cytokine patterns in follicular fluid. IL-8, which is mainly synthesized by granulosa cells and acts as chemoattractant and neutrophil activator, seems to vary inversely with IL-10, IL-12 and TNF-α, indicating that immune cells may interact with granulosa cell function. In a subgroup of patients with high follicular fluid cytokine levels (IL8, IL-1β, IL-10, IL-12 and TNF-α), an inferior ovarian response during IVF was observed, which was independent of clinical background and stimulation data.

Almost every reproductive step is thought to be affected by endometriosis (Fernandez-Shaw *et al.*, 1993; Lessey, 2002; Kissler *et al.*,

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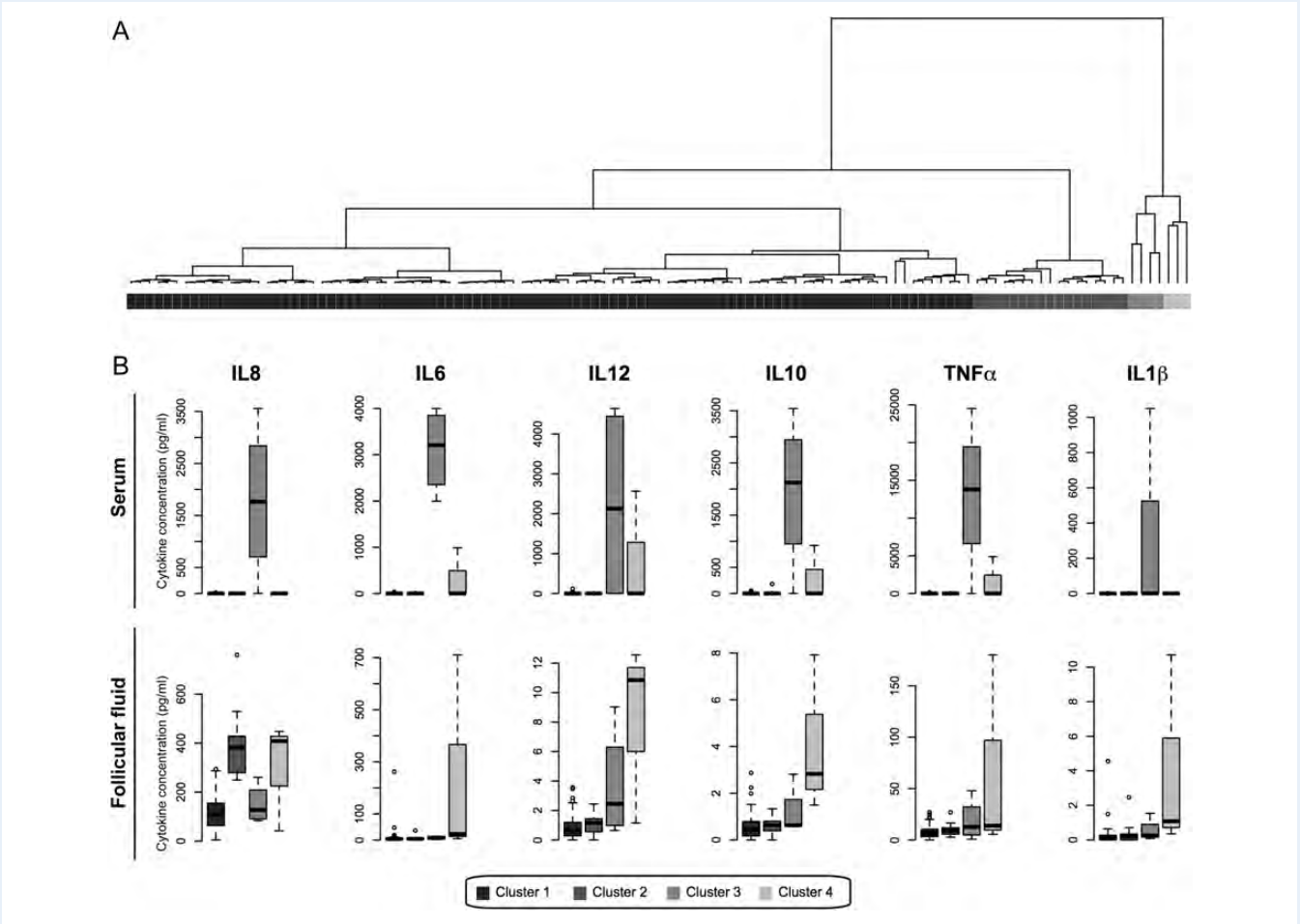

Figure 1 Subgroups of women undergoing assisted reproduction treatment according to concentration profiles of IL-8, IL-6, IL-12, IL-10, TNF-α and IL-1β in serum and follicular fluid. (**A**) Cluster analysis identifies four subgroups, marked with shades of gray, which share related concentration profiles (cluster 1 = 93 patients, cluster 2 = 17 patients, cluster 3 = 4 patients, cluster 4 = 3 patients). Length of dendrogram branches indicate relatedness. (**B**) Concentration of IL-8, IL-6, IL-12, IL-10, TNF-α and IL-1β in serum and follicular fluid for subgroups identified by cluster analysis. Boxplots indicate median, 75% percentiles and range concentrations.

2006; Tomassetti *et al.*, 2006; Bulun, 2009). Oocyte donation programs showed reduced pregnancy rates when the oocytes came from donors with endometriosis; however, normal pregnancy rates were observed when solely the recipients had endometriosis (Simon *et al.*, 1994). When oocytes from a healthy donor were split in the same cycle and sibling oocytes were given to recipients with and without endometriosis, the pregnancy rates were similar (Diaz *et al.*, 2000). These studies from oocyte donation programs indicate that in patients with endometriosis, a low success rate with IVF is caused by poor oocyte and embryo quality rather than a low receptive endometrium (Halis and Arici, 2004). Increased apoptosis, alterations in the cell cycle and higher incidence of oxidative stress have all been observed in granulosa cells derived from women with all ASRM stages of endometriosis, including endometriomas, when compared with the granulosa cells of women with other causes of infertility (Saito *et al.*, 2002). Endometriosis, therefore, seems to have a detrimental effect on oocyte quality.

Nonetheless, it is uncertain whether endometriomas, like peritoneal endometriotic lesions, represent an active inflammatory state or are residues of past inflammatory reactions. Our findings of low follicular fluid cytokines together with comparable fertilization rates, implantation rates and pregnancy rates in various study groups indicate that endometriomas, in most patients, do not induce an unfavorable inflammatory environment compromising surrounding follicles.

The inverse relationship between the IL-8 produced by granulosa cells and the anti-inflammatory IL-10 and proinflammatory cytokines IL-1β, IL-12 and TNF-α may represent a normal pre-ovulatory, intrafollicular status. Just prior to ovulation there is a massive influx of granulocytes in and around the follicle, and this is believed to play a vital role in a timely follicular rupture. The influx is believed to be promoted by the chemoattractant properties of granulosa cell-derived IL-8 (Zeineh *et al.*, 2003; Polec *et al.*, 2009). Concentrations of IL-8 in the follicular fluid have been observed to increase substantially following hCG administration for final follicle maturation and a 14-fold gradient between serum and follicular fluid has been shown (Arici *et al.*, 1996), consistent our results. IL-8 secretion by granulosa cells is also enhanced by modulators such as the otherwise proinflammatory TNF-α (Zeineh *et al.*, 2003).

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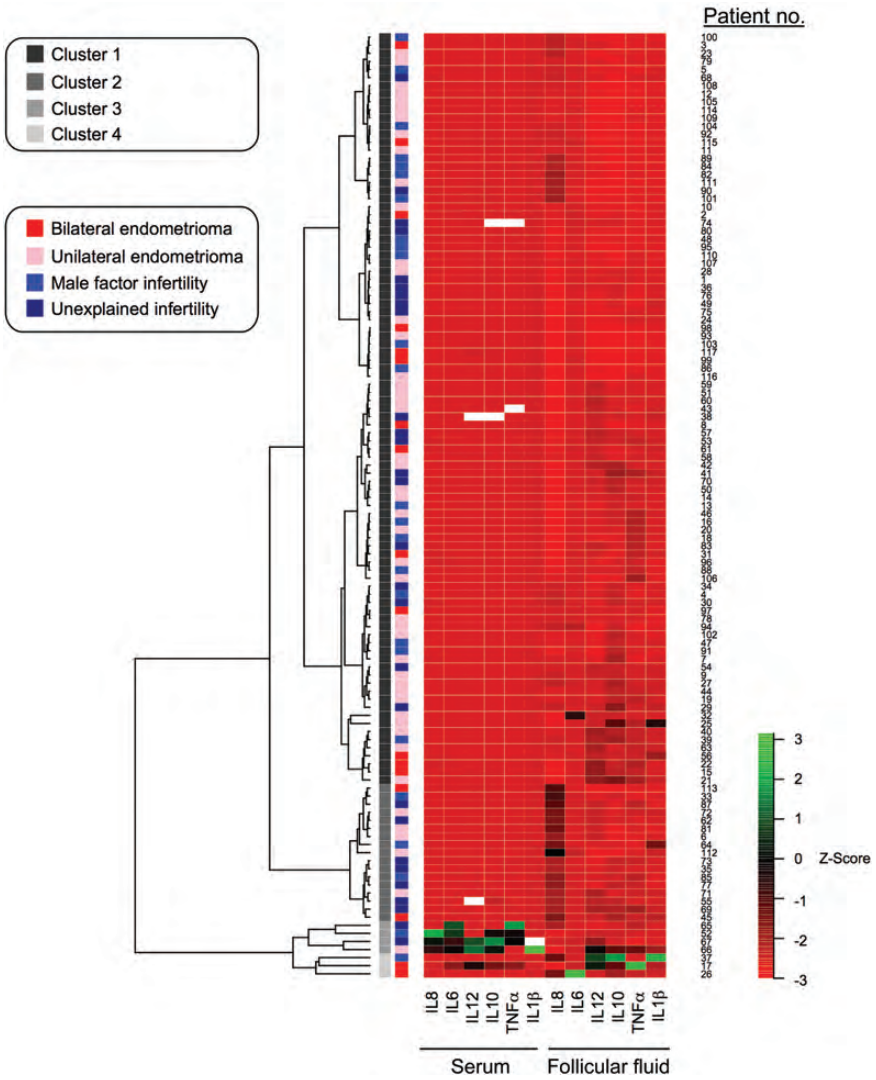

Figure 2 Distribution and individual variability of serum and follicular fluid concentrations of IL-8, IL-6, IL-12, IL-10, TNF-α and IL-1β in women undergoing assisted reproduction treatment. Patients were grouped according to concentration profiles with cluster analysis as indicated by the dendrogram (*left*). Concentrations for individual patients are shown by color-coded map, where green reflects high levels and red reflects low levels normalized to mean levels of the total sample (*right*).

We characterized a subgroup of patients with high follicular fluid cytokine concentrations, but normal levels in the peripheral circulation. These patients had an inferior ovarian response, which could be secondary to a local intraovarian inflammation. IL-1β is a key inflammatory mediator that is usually found in very low concentrations in follicular fluid prior to ovulation (Buscher *et al.*, 1999; Sarapik *et al.*, 2012); however, in this subgroup the follicular fluid concentration of IL-1β was high. IL-12 is a proinflammatory cytokine known to be cytotoxic in high concentrations and with putative dose-dependent detrimental effects on oocyte quality (Gazvani *et al.*, 2000; Bedaiwy *et al.*, 2007; Ledee *et al.*, 2008; Sarapik *et al.*, 2010, 2012). Our finding of increased IL-12 levels in follicular fluid of this small group of patients with inferior ovarian response is consistent with this.

A pattern of high local follicular fluid cytokines levels caused by an inflammatory reaction is what we expected to find among women with endometriomas. However, our cytokine results did not support local inflammation as a cause for adverse follicular development in endometrioma patients. Our endometrioma study group did show a reduced ovarian response during IVF, in terms of significantly higher FSH doses required and fewer oocytes extracted, as recently supported by others (Benaglia *et al.*, 2012), but the diploid fertilization rate and quality of the transferred embryos were similar to the non-endometriosis control groups. Local ovarian inflammation may yet be important in relation to subfertility and IVF, but may not be specifically related to endometriomas.

Our results may have consequences for the management of endometriomas prior to IVF. ESHRE recommends ovarian cystectomy if the endometrioma is $\geq$4 cm (Kennedy *et al.*, 2005). However, a meta-analysis concluded that the odds of a clinical pregnancy after IVF-ET are not affected significantly in patients with ovarian

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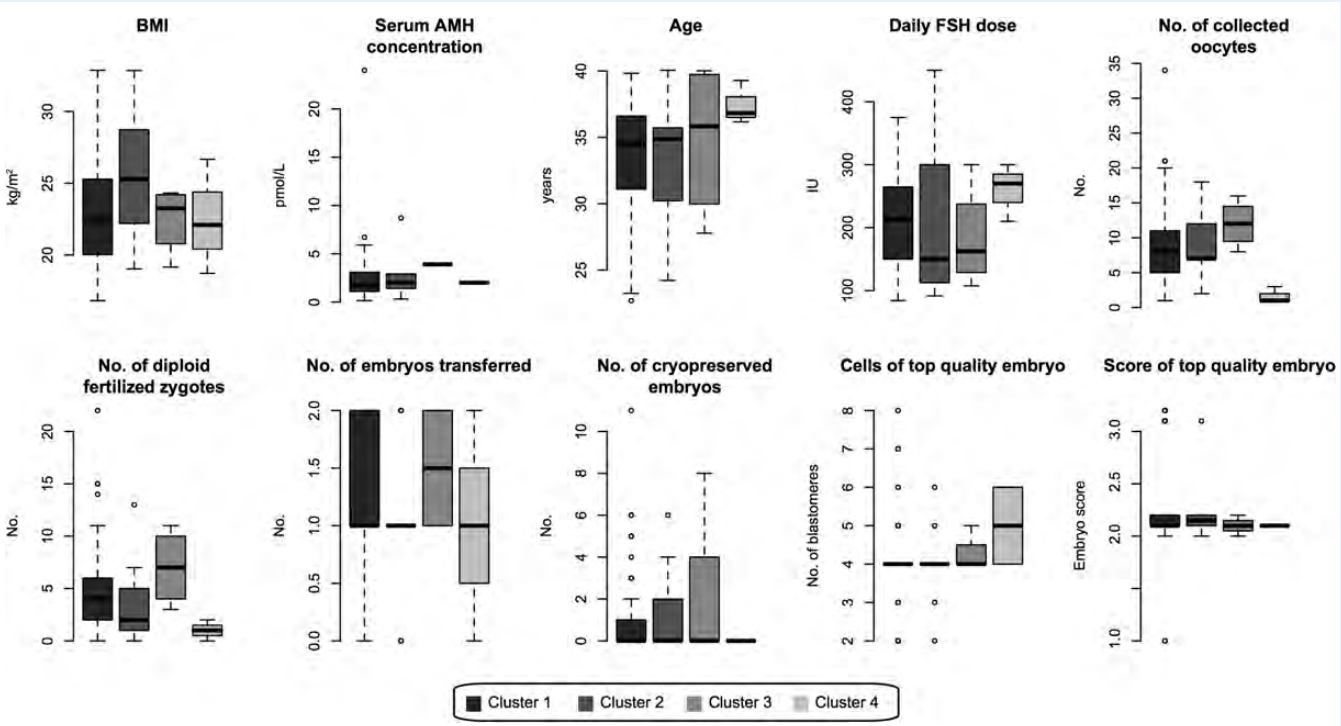

Figure 3 Baseline characteristics and assisted reproduction treatment in women grouped according to relatedness of serum and follicular fluid concentrations of IL-8, IL-6, IL-12, IL-10, TNF-α and IL-1β. Subgroups were identified by cluster analysis and are indicated by shades of gray corresponding to notations in Figs. 1 and 2.

endometriomas compared with controls (OR 1.07, $P = 0.79$) (Gupta *et al.*, 2006). Furthermore, a randomized clinical trial concluded that even with decreased ovarian responsiveness, the fertilization and pregnancy rates are not altered when comparing endometrioma patients with or without ovarian surgery before IVF-ET (Demirol *et al.*, 2006). In a previous study from our group, we found an inferior pregnancy rate in a group of endometrioma patients in whom 78% were operated prior to IVF (Opoien *et al.*, 2012). Our findings, therefore, challenge ESHRE's recommendation that endometriomas should be removed. Two recent meta-analyses have shown reduced post surgical AMH (Raffi *et al.*, 2012; Somigliana *et al.*, 2012), indicating potential surgical damage to remaining ovarian tissue, loss of follicles, diminished ovarian reserve and inferior responsiveness to stimulation. On the other hand, biopsies of the ovarian cortex surrounding endometriomas show a reduced number of follicles (Maneschi *et al.*, 1993), so we can also speculate that mechanical stretching and reduced vascularity to some extent harm the tissue. Still, ovarian surgery without a proper indication may increase the costs of treatment, prolong time to pregnancy, cause lower success rate and expose the patient to risks of surgical complications.

The strengths of this study are its prospective nature and the fact that follicular fluid taken from the ovaries of women with unilateral endometriomas was compared with the findings on the unaffected side, which excluded other potential influencing factors. The weaknesses are the interpretation of low cytokine concentrations around the limit of detection, such as for TNF-α, that the clusters of patients with high intrafollicular cytokine levels were small and that the endometrioma diagnoses had to be made indirectly by ultrasound. On the other hand, high sensitivities and specificities of transvaginal ultrasound for detection of endometriomas have been reported to be 84–100 and 90–100%, respectively (Mais *et al.*, 1993; Patel *et al.*, 1999; Eskenazi *et al.*, 2001). The most common differential diagnoses of endometrioma are hemorrhagic cysts or bleeding into corpus luteum cysts, which more frequently present with acute symptoms and have additional sonographic appearances of fibrinous strands and retracting clots. Furthermore, in most cases they spontaneously resolve over time, as opposed to endometrimas that will not resolve if left untreated.

Conclusion

We found that local intrafollicular increases in proinflammatory cytokines may be associated with an inferior ovarian response, but we were unable to find a link between this observation and the presence of ovarian endometriomas. The findings strengthen the argument against surgical removal of endometriomas prior IVF.

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Authors' roles

H.K.O.: Designing the study, collection, analysis and interpretation of data, drafting the article and final approval. P.F.: Substantial contributions to design; analysis and interpretation of data, drafting the article, revising it critically for important intellectual content and final approval of the version to be published. A.P.: Collection, analysis and interpretation of data. Co-writing the article, revising it critically and giving final approval of the version to be published. M.S.: Collection and interpretation of data. Revising it critically for important content and giving final approval of the version to be published. T.Å.: Contributions to design, revising the article critically and final approval of the version to be published. T.T.: Substantial contributions to design, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content and final approval of the version to be published.

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Conflict of interest

None declared.

References

Arici A, Oral E, Bukulmez O, Buradagunta S, Engin O, Olive DL. Interleukin-8 expression and modulation in human preovulatory follicles and ovarian cells. *Endocrinology* 1996;**137**:3762–3769.

Bedaiwy M, Shahin AY, AbulHassan AM, Goldberg JM, Sharma RK, Agarwal A, Falcone T. Differential expression of follicular fluid cytokines: relationship to subsequent pregnancy in IVF cycles. *Reprod Biomed Online* 2007;**15**:321–325.

Benaglia L, Bermejo A, Somigliana E, Scarduelli C, Ragni G, Fedele L, Garcia-Velasco JA. Pregnancy outcome in women with endometriomas achieving pregnancy through IVF. *Hum Reprod* 2012;**27**:1663–1667.

Bjercke S, Tanbo T, Abyholm T, Omland A, Opoien HK, Fedorcsak P. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing their first treatment cycle of IVF or ICSI. *Acta Obstet Gynecol Scand* 2010;**89**:1053–1060.

Bulun SE. Endometriosis. *N Engl J Med* 2009;**360**:268–279.

Buscher U, Chen FC, Kentenich H, Schmiady H. Cytokines in the follicular fluid of stimulated and non-stimulated human ovaries; is ovulation a suppressed inflammatory reaction? *Hum Reprod* 1999;**14**:162–166.

D'Hooghe TM, Debrock S, Hill JA, Meuleman C. Endometriosis and subfertility: is the relationship resolved? *Semin Reprod Med* 2003;**21**:243–254.

Demirol A, Guven S, Baykal C, Gurgan T. Effect of endometrioma cystectomy on IVF outcome: a prospective randomized study. *Reprod Biomed Online* 2006;**12**:639–643.

Diaz I, Navarro J, Blasco L, Simon C, Pellicer A, Remohi J. Impact of stage III-IV endometriosis on recipients of sibling oocytes: matched case-control study. *Fertil Steril* 2000;**74**:31–34.

Eskenazi B, Warner M, Bonsignore L, Olive D, Samuels S, Vercellini P. Validation study of nonsurgical diagnosis of endometriosis. *Fertil Steril* 2001;**76**:929–935.

Fernandez-Shaw S, Hicks BR, Yudkin PL, Kennedy S, Barlow DH, Starkey PM. Anti-endometrial and anti-endothelial auto-antibodies in women with endometriosis. *Hum Reprod* 1993;**8**:310–315.

Ficicioglu C, Kumbak B, Akcin O, Attar R, Yildirim G, Yesildaglar N. Comparison of follicular fluid and serum cytokine concentrations in women undergoing assisted reproductive treatment with GnRH agonist long and antagonist protocols. *Gynecol Endocrinol* 2010;**26**:181–186.

Gazvani MR, Bates M, Vince G, Christmas S, Lewis-Jones DI, Kingsland C. Follicular fluid concentrations of interleukin-12 and interleukin-8 in IVF cycles. *Fertil Steril* 2000;**74**:953–958.

Gupta S, Agarwal A, Agarwal R, Loret de Mola JR. Impact of ovarian endometrioma on assisted reproduction outcomes. *Reprod Biomed Online* 2006;**13**:349–360.

Halis G, Arici A. Endometriosis and inflammation in infertility. *Ann N Y Acad Sci,* 2004;**1034**:300–315.

Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. *Fertil Steril* 2001;**76**:1–10.

Hill JA, Faris HM, Schiff I, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. *Fertil Steril* 1988;**50**:216–222.

Hull MG. Infertility treatment: relative effectiveness of conventional and assisted conception methods. *Hum Reprod* 1992;**7**:785–796.

Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, Hummelshoj L, Prentice A, Saridogan E. ESHRE guideline for the diagnosis and treatment of endometriosis. *Hum Reprod* 2005;**20**:2698–2704.

Kissler S, Hamscho N, Zangos S, Wiegratz I, Schlichter S, Menzel C, Doebert N, Gruenwald F, Vogl TJ, Gaetje R *et al.* Uterotubal transport disorder in adenomyosis and endometriosis—a cause for infertility. *BJOG* 2006;**113**:902–908.

Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. *Fertil Steril* 2001;**75**:1–10.

Ledee N, Lombroso R, Lombardelli L, Selva J, Dubanchet S, Chaouat G, Frankenne F, Foidart JM, Maggi E, Romagnani S *et al.* Cytokines and chemokines in follicular fluids and potential of the corresponding embryo: the role of granulocyte colony-stimulating factor. *Hum Reprod* 2008;**23**:2001–2009.

Lessey BA. Implantation defects in infertile women with endometriosis. *Ann N Y Acad Sci* 2002;**955**:265–280.

Mais V, Guerriero S, Ajossa S, Angiolucci M, Paoletti AM, Melis GB. The efficiency of transvaginal ultrasonography in the diagnosis of endometrioma. *Fertil Steril* 1993;**60**:776–780.

Maneschi F, Marasa L, Incandela S, Mazzarese M, Zupi E. Ovarian cortex surrounding benign neoplasms: a histologic study. *Am J Obstet Gynecol* 1993;**169**:388–393.

Marcoux S, Maheux R, Berube S. Laparoscopic surgery in infertile women with minimal or mild endometriosis. Canadian Collaborative Group on Endometriosis. *N Engl J Med* 1997;**337**:217–222.

Opoien HK, Fedorcsak P, Byholm T, Tanbo T. Complete surgical removal of minimal and mild endometriosis improves outcome of subsequent IVF/ICSI treatment. *Reprod Biomed Online* 2011;**23**:389–395.

Opoien HK, Fedorcsak P, Omland AK, Abyholm T, Bjercke S, Ertzeid G, Oldereid N, Mellembakken JR, Tanbo T. In vitro fertilization is a successful treatment in endometriosis-associated infertility. *Fertil Steril* 2012;**97**:912–918.

Patel MD, Feldstein VA, Chen DC, Lipson SD, Filly RA. Endometriomas: diagnostic performance of US. *Radiology* 1999;**210**:739–745.

Polec A, Tanbo T, Fedorcsak P. Cellular interaction regulates interleukin-8 secretion by granulosa-lutein cells and monocytes/macrophages. *Am J Reprod Immunol* 2009;**61**:85–94.

Polec A, Raki M, Abyholm T, Tanbo TG, Fedorcsak P. Interaction between granulosa-lutein cells and monocytes regulates secretion of angiogenic factors in vitro. *Hum Reprod* 2011;**26**:2819–2829.

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Raffi F, Metwally M, Amer S. The impact of excision of ovarian endometrioma on ovarian reserve: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2012;**97**:3146–3154.

Saito H, Seino T, Kaneko T, Nakahara K, Toya M, Kurachi H. Endometriosis and oocyte quality. *Gynecol Obstet Invest* 2002;**53**(Suppl 1):46–51.

Sarapik A, Haller-Kikkatalo K, Utt M, Teesalu K, Salumets A, Uibo R. Serum anti-endometrial antibodies in infertile women—potential risk factor for implantation failure. *Am J Reprod Immunol* 2010;**63**:349–357.

Sarapik A, Velthut A, Haller-Kikkatalo K, Faure GC, Bene MC, de Carvalho BM, Massin F, Uibo R, Salumets A. Follicular proinflammatory cytokines and chemokines as markers of IVF success. *Clin Dev Immunol* 2012;**2012**:606459.

Simon C, Gutierrez A, Vidal A, de los Santos MJ, Tarin JJ, Remohi J, Pellicer A. Outcome of patients with endometriosis in assisted reproduction: results from in-vitro fertilization and oocyte donation. *Hum Reprod* 1994;**9**:725–729.

Somigliana E, Berlanda N, Benaglia L, Vigano P, Vercellini P, Fedele L. Surgical excision of endometriomas and ovarian reserve: a systematic review on serum antimullerian hormone level modifications. *Fertil Steril* 2012;**98**:1531–1538.

Syrop CH, Halme J. Cyclic changes of peritoneal fluid parameters in normal and infertile patients. *Obstet Gynecol* 1987;**69**:416–418.

Tomassetti C, Meuleman C, Pexsters A, Mihalyi A, Kyama C, Simsa P, D'Hooghe TM. Endometriosis, recurrent miscarriage and implantation failure: is there an immunological link? *Reprod Biomed Online* 2006;**13**:58–64.

Werbrouck E, Spiessens C, Meuleman C, D'Hooghe T. No difference in cycle pregnancy rate and in cumulative live-birth rate between women with surgically treated minimal to mild endometriosis and women with unexplained infertility after controlled ovarian hyperstimulation and intrauterine insemination. *Fertil Steril* 2006;**86**:566–571.

Zeineh K, Kawano Y, Fukuda J, Nasu K, Narahara H, Miyakawa I. Possible modulators of IL-8 and GRO-alpha production by granulosa cells. *Am J Reprod Immunol* 2003;**50**:98–103.

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