Interleukin in endometriosis-associated infertility-pelvic pain: systematic review and meta-analysis

Helena Malvezzi, Camila Hernandez, Carla A Piccinato and Sérgio Podgaec

Hospital Israelita Albert Einstein, São Paulo, São Paulo, Brazil

Correspondence should be addressed to H Malvezzi; Email: helenamalvezzi@gmail.com

Abstract

The objective is to study the significance of altered interleukin levels in endometriosis-related infertility or pelvic pain. The present systematic review and meta-analysis includes a discussion on the roles of interleukin in the physiopathology of endometriosis-associated infertility and/or pelvic pain. We included all studies in which interleukins in peritoneal fluid, follicular fluid or serum from patients were measured and that correlated the findings with either peritoneal or deep endometriosis-associated infertility or pelvic pain. For the meta-analysis, we selected studies on the following cytokines: interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8). Endometriosis is a chronic inflammatory disease. Inflammatory processes clearly participate in the etiology of endometriosis. Cytokines are mediators of inflammation, and increase in their concentration in plasma or other body fluids signals the presence and extent of tissue lesions. A number of studies have reported on the association between higher cytokine levels and progression or maintenance of endometriosis and coexisting infertility or pelvic pain. The results of the analyses support that an association exists between elevated serum IL-6 and/or IL-8 concentrations and the occurrence of endometriosis-associated infertility. Such association was not found for endometriosis-associated pain. In spite of accumulated evidence on the association of pro-inflammatory cytokines and endometriosis, it still is not clear if and how these mediators participate in the physiopathology of endometriosis-associated infertility or pelvic pain, in part due to poor quality of the evidence established in the vast majority of interleukins and challenges in endometriosis research reproducibility. In summary, the results of the analyses support that an association exists between elevated serum IL-6 and/or IL-8 concentrations and the occurrence of endometriosis-associated infertility.

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Introduction

Endometriosis is a chronic inflammatory disease characterized by the presence of endometrial tissue outside of the uterine cavity, mainly attached to the peritoneum, pelvic organs or ligaments (Vercellini *et al.* 2014). The prevalence of endometriosis is assumed to be 10% of women in reproductive age (Kvaskoff *et al.* 2015). However, due to difficulties in understanding the diversity of the lesion's phenotypes, clinical symptoms and, in image diagnosis, the actual disease prevalence is unknown. The diagnostic difficulties frequently make endometriosis treatment a challenge. Nonetheless, untreated endometriosis can cause low quality of life, depression, inability to work, sexual dysfunction, infertility or diverse pain symptoms, including dysmenorrhea, deep dyspareunia and chronic pelvic pain (Carvalho *et al.* 2013). Although the factors determining the most common symptoms, such as infertility and pelvic pain, which affect respectively 40% (Eisenberg *et al.* 2018) and 50% (Andres *et al.* 2014) of the patients have not been all identified, it is clear that inflammatory processes participate in the etiology of endometriosis (Jiang *et al.* 2016).

Relative to the causes of endometriosis-associated infertility, inflammatory mediators found in peritoneal and follicular fluids can act on cell proliferation, differentiation and blastocyst invasion. Among the described noxious effects are diminished oocyte quality (Da Broi & Navarro 2016, Malvezzi *et al.* 2018); sperm/oocyte interaction disorders (Hsu *et al.* 2015); embryo quality and its implantation and development (Stilley *et al.* 2012). Any of these effects leads to an altered reproductive condition (Haydardedeoglu & Zeyneloglu 2015). Pain symptoms are also aggravated by the local inflammatory response secondary to the activation of immune responses (Arnold *et al.* 2013).

Cells involved in the immune response such as monocytes, macrophages, natural killer cells, T and B cells, regulatory T cells, as well as secreted cytokines and chemokines, participate in inflammation. Their role in endometriosis has been extensively studied, as cell numbers and cytokine concentrations were higher or altered in the peritoneal fluid of the endometriosis patients (Nesbitt-Hawes & Ledger 2004, Podgaec *et al.* 2012, Gupta *et al.* 2016, Nisenblat *et al.* 2016).

Those cells, and also the ectopic endometrial implants and mesothelial cells of the peritoneum,

synthesize and secrete cytokines, many of which were found to be related to endometriosis. Interleukin-1 (IL-1), a stimulant of prostaglandins' synthesis (important pain and fever mediators), is increased in peritoneal fluid of endometriosis patients (Akoum *et al.* 2008). Interleukin-6 (IL-6) is a pleiotropic cytokine also secreted by macrophages that promotes endometrial cell proliferation and angiogenesis in endometriosis (Symons *et al.* 2018). Interleukin-8 (IL-8) is a potent angiogenic, pro-inflammatory and cellular proliferation cytokine, which was also found in the peritoneal fluid and facilitates endometrial cell adhesion (Sikora *et al.* 2017). Interleukin-10 may contribute to the altered immune regulation observed in endometriosis by limiting and terminating local inflammation and also leading to the removal of apoptotic cells which will reduce the local inflammatory response (Suen *et al.* 2014). It was reported to be elevated in serum from endometriosis stage III and IV patients (Suen *et al.* 2014).

Other cytokines such as IL-2, IL-4, IL-11, IL-12, IL-13, IL-15, IL-17, IL-18, IL-23 and IL-33 have also been associated with endometriosis. IL-4, IL-12, IL-15, IL-18, IL-23 and IL-33 were found to be elevated in endometriosis in the majority of studies, whereas IL-2 and IL-17 were downregulated or were not different from controls (McLaren *et al.* 1997, Furuya *et al.* 2003, Arici *et al.* 2003a,b, Zhang *et al.* 2005, Mikolajczyk *et al.* 2006, Fairbanks *et al.* 2009, Andreoli *et al.* 2011, Santulli *et al.* 2012).

Considering the controversial amount of information provided, the objective of this systematic review was to conduct a critical survey of the published papers reporting on interleukins quantified in serum, peritoneal fluid (PF) or follicular fluid (FF) in endometriosis-associated infertility and/or endometriosis-associated pelvic pain and to perform a meta-analysis of the papers whose data fulfilled the requirements for such analysis.

Methods

This study was designed according to the PRISMA statement for systematic reviews (Supplementary Fig. 1, see section on [supplementary data](#) given at the end of this article), and it was carried out at Hospital Israelita Albert Einstein in São Paulo, Brazil. The focal point keywords were 'interleukins', 'endometriosis', 'pelvic pain' and 'infertility', but we also included different keywords for a more complete combination of results. The search method was applied to PubMed, Medline, EMBASE, Scopus and Lilacs databases (the last search was performed on 1 June, 2018). The search builders are shown in Supplementary Fig. 2.

For meta-analysis, we selected studies that had well-described result data and only articles that had results expressed by measures of central tendency, variability and *P* values or, at least, by measures of central tendency and *P* values clearly depicted in figures, graphs or in text. Meta-analysis was carried out in the following conditions: (a) a minimum of two studies with information on the outcome of interest and effect

size in the same direction; (b) a minimum of three studies, regardless of the direction of effect estimation (Valentine *et al.* 2010). Random effect models were considered and effect sizes (standardized mean differences) were estimated with a 95% confidence interval. Absence of difference between groups was determined when *P* values were greater than 0.05 or when the 95% confidence interval contained zero. The I² index and Cochran Q test were used to measure the heterogeneity of the different studies. After reviewing all selected papers, the ones that evaluated IL-1 and/or IL-6 and/or IL-8 in relation to endometriosis presented enough data for meta-analysis.

The heat map (Fig. 1) represents a compilation of results from all published articles stipulated by values ranging from -1 to 1 for every article result according to the interleukin evaluated. To each article a score was given as follows: one (1), when the IL concentrations were significantly higher ($P \leq 0.05$) in the group of endometriosis patients compared to the control group; zero (0) when the difference in IL concentrations between these groups was not statistically significant ($P > 0.05$); minus one (-1) when IL concentrations were significantly higher ($P \leq 0.05$) in the control group compared to endometriosis patients. Colors were chosen considering the sum of the results; vivid red for higher score, which denotes more articles reporting significantly increased IL concentrations in endometriosis fluids compared to control patients and blue for articles reporting significantly lower IL concentrations in endometriosis patients versus controls.

Quality evaluation of included studies was performed by the methodological index for non-randomized studies – MINORS – (Fig. 2) and followed the literature scope to characterize the risk of bias in the compilation of articles selected for this review (Slim *et al.* 2003).

MINORS is a verified scoring tool for non-randomized studies and includes a 12-item assessment. The score of each item ranges from -1 (not reported), 0 (reported but inadequate) to 1 (reported and adequate); a question mark (?) is used when evaluation was not possible.

Selection and exclusion criteria

All studies that associated IL with endometriosis-associated infertility or pelvic pain were included. No data limits were imposed. Two reviewers performed eligibility assessment independently in an unblinded standardized manner. Disagreement on study inclusion was solved by consensus. A systematic review was performed analyzing the number of patients involved in the study, type of study, results and conclusions of each study, which interleukins were evaluated, association with infertility and/or pelvic pain. To be included in the systematic review, the study had to present data on interleukin concentrations in serum, PF or FF samples from endometriosis patients and had to compare the findings with the occurrence of peritoneal or deep endometriosis-associated infertility or pelvic pain.

Results

A total of 426 articles were retrieved through database searching: 172 from PubMed/Medline, 206 from EMBASE, 48 from Scopus and 0 from Lilacs. From

A				B		
	PF	FF	S		PF	S
IL-1	3	2	2	IL-1	-1	nm
IL-2	-1	1	1	IL-2	1	nm
IL-4	-1	1	1	IL-4	1	nm
IL-6	0	3	2	IL-6	2	nm
IL-8	2	1	1	IL-8	0	0
IL-10	0	1	1	IL-18	2	nm
IL-12	0	1	2	IL-33	1	nm
IL-15	1	0	nm			
IL-17	0	nm	0			
IL-18	2	0	0			
IL-23	1	nm	0			

Figure 1 Values range from vivid red for higher number of articles demonstrating significant increased interleukin (IL) concentration in endometriosis fluids compared to control patients to blue for significant decreased IL concentration in endometriosis. This table is a compilation of articles results, where to each article a score was given, being 1 for positive significance, 0 for non-significant and -1 for negative significance in IL concentration in endometriosis patients compared to control. For a complete description of results see Tables 1, 2, 3, 4, 5 and 6. (A) 3.1. Interleukins and infertility in endometriosis patients: IL-1: PF=3 studies >; 4 studies ns; FF=2 studies >; 2 studies ns; S=2 studies >; 5 studies ns. IL-2: PF=1 studies <; 1 study ns; FF=1 study >; S=study 1>. IL-4: PF=1 study<; 1ns; FF=1>;S=1 study >. IL-6: PF=2 studies>; 2<; 3 studies ns; FF=3 studies >; 3 studies ns; S=3 studies >; 1 study <; 1 study ns; IL-8: PF=2 studies >; 1 study ns; FF=1 study >; 2 studies ns; S=1 study >; 2 studies ns. IL-10: PF=3 studies ns; FF=1 study >; S=1 study >; 1 study ns. IL-12: PF=3 studies ns; FF=1 study >; S=2 studies >. IL-15: PF=1 study >; FF=1 study ns. IL-17: PF=2 studies ns; S=1 study ns. IL-18: PF=2 studies >; 1 study ns; FF=1 study ns; S=3 studies ns. IL-23: PF=1 study >; S=1 study ns. (B) Interleukins associated with pelvic pain in endometriosis patients: IL-1β: PF=<; 1 study ns. IL-2: PF=1 study >. IL-4: PF=1 study >. IL-6: PF=2 studies >; 1 study ns. IL-8: PF=1 study ns; S=1 study ns. IL-18: PF=2 studies >. IL-33: PF=1 study >. PF=peritoneal fluid; FF=follicular fluid; S=serum; >=higher IL concentration in endometriosis compared to control; ns=non-significant IL difference between endometriosis and control; <lower IL concentration in endometriosis compared to control; nm=not measured.

this list, all duplicates were removed resulting in 218 articles. After eliminating reviews and articles written in languages other than English or Portuguese, the final list consisted of 167 articles. Abstracts and references of all studies identified by the search strategy were examined and 67 relevant papers were read in full. All papers excluded from the review and the respective reasons for it appear in Supplementary Table 1. Finally, as shown in Supplementary Fig. 1, 26 studies fulfilled the selection criteria and were included in the systematic review. For meta-analysis, eight studies were selected; in fact, when considering different interleukins and fluids, the number of studies with available data ranges between 2 and 4. The heat map, quality score and baseline characteristics of included studies are shown in Figs 1 and 2 and Tables 1, 2, 3, 4, 5 and 6.

Interleukins and infertility in endometriosis patients

Interleukin-1 (IL-1, IL-1α and IL-1β)

IL-1, which includes the related cytokines IL-1α and IL-1β, exerts strong pro-inflammatory activities. In endometriosis, increased IL-1 levels have been associated with major symptoms; however, the association between IL-1 levels in serum, FF and PF and endometriosis-associated infertility is not evident (Table 1). For instance, five out of ten eligible articles belonging to our systematic review found no significant differences between FF, PF and serum IL-1 concentrations in endometriosis infertile

patients compared to the respective control groups (Ueki *et al.* 1994, Pellicer *et al.* 1998, Oku *et al.* 2004, Skrzypczak *et al.* 2005, Wunder *et al.* 2006).

Regarding PF IL-1, meta-analysis information was available in two studies: one reported higher values for the endometriosis-associated infertility group (SMD 2.48, 95% CI 1.81–3.14), while in the other, there was no difference between groups (SMD, -0.06; 95% CI, -0.63 to 0.52). Since there are only two non-concordant effects studies, the estimate combined effect was not determined. The I^2 value was 96.88% (95% CI 84.32 to >99.9%), indicating a high degree of heterogeneity. Moreover, one study quantified PF IL-1 in endometriosis but IL-1 was undetected in control patients (Kondera-Anasz *et al.* 2005). Additionally, in PF of infertile women with endometriosis, IL-1 was quantified as significantly higher than control patients (Akoum *et al.* 2008, Michaud *et al.* 2011).

An interesting result compared different stages of endometriosis. On one hand, PF IL-1 was higher in early stages of the disease (Akoum *et al.* 2008), whereas on the other hand, advanced stages presented higher PF and serum IL-1 (Kondera-Anasz *et al.* 2005).

IL-1 information on FF was available for three studies. The SMD between women with endometriosis-associated infertility and controls was estimated at 2.18 (95% CI -0.04 to 4.40, P 0.054) showing that there was not sufficient evidence that FF IL-1 values were increased among women with endometriosis and were

	A clearly stated aim	Inclusion of consecutive patients	Prospective collection of data	Endpoints appropriate to the aim of the study	Unbiased assessment of the study endpoint	Follow-up period appropriate to the aim of the study	Loss to follow up less than 5%	Prospective calculation of the study size	An adequate control group	Contemporary groups	Baseline equivalence of groups	Adequate statistical analyses
Altered expression of	1	1	1	1	-1	?	-1	-1	?	1	-1	1
Changes of cytokines	0	1	1	0	-1	?	1	-1	?	1	-1	1
Concentrations of int	1	1	1	1	-1	?	1	-1	?	1	-1	1
Decreased	1	1	1	0	-1	?	1	-1	?	1	-1	1
Detection of	1	1	1	0	-1	?	1	-1	?	1	-1	1
Imbalance in the	1	1	1	1	-1	?	1	-1	?	1	1	1
Immunological and	1	1	1	0	-1	?	1	-1	?	-1	-1	1
Increased ENA-78 in	1	1	1	1	-1	?	1	-1	?	1	-1	1
Increased interleukin-	1	1	1	1	-1	?	1	-1	?	1	1	1
Increased levels of	1	1	1	1	-1	?	1	-1	?	1	-1	1
Interleukin-6 and	1	1	1	1	-1	?	1	-1	?	1	-1	1
Interleukin-8 serum	1	1	1	1	-1	?	1	-1	?	1	0	1
Intrafollicular	1	1	1	1	1	?	1	1	?	1	1	1
Intrafollicular	1	1	1	1	-1	?	1	-1	?	1	-1	1
Macrophage	0	1	1	0	-1	?	1	-1	?	1	-1	1
Serum and peritoneal	1	1	1	1	-1	?	1	-1	?	1	-1	1
Peritoneal and serum	1	1	1	1	-1	?	1	-1	?	1	-1	1
Peritoneal fluid	1	1	1	1	-1	?	1	-1	?	1	-1	1
Peritoneal fluid	1	1	1	1	-1	?	1	-1	?	1	-1	1
Peritoneal	1	1	1	1	-1	?	1	-1	?	1	1	1
Peritoneal fluid	1	1	1	1	-1	?	1	-1	?	1	-1	1
Selected cytokines	1	1	1	1	-1	?	1	-1	?	1	1	1
Serum and peritoneal	1	1	1	1	-1	?	1	-1	?	1	1	1
T helper (Th)1, Th2,	1	1	1	1	-1	?	1	-1	?	1	-1	1
The follicular and	1	1	1	1	-1	?	1	-1	?	1	-1	1
Role of IL-18 in	1	1	1	1	-1	?	1	-1	?	1	-1	1

Figure 2 The quality scores of included studies by methodological index for non-randomized studies. The scored -1 represents: not reported, 0: reported but inadequate; 1: reported and adequate and ?: not evaluated.

also infertile. The I^2 and Q test values were 98.61% (95% CI 94.82 to >99.9%) and less than 0.0001 respectively, indicating a high degree of heterogeneity. Higher FF IL-1 concentrations were described in endometriosis patients compared to FF from non-endometriotic infertile patients (Singh *et al.* 2016, Wu *et al.* 2017).

Serum IL-1 information was available for three studies (SMD -0.48, 95% CI -3.92 to 2.96; $P=0.783$) showing that there is not sufficient evidence that serum IL-1 values are different between women with and without endometriosis. The I^2 value and P value for the Q were 98.91% (95% CI 95.98 to >99.9%) and less than 0.0001 respectively, indicating a high degree of heterogeneity.

Interleukins IL-2, IL-12, IL-4, IL-10

Interleukins IL-2 and IL-12 are closely related to T helper 1 (Th1) activation, whereas IL-4 and IL-10 are related to T helper 2 (Th2) activation during immunological responses. Th1-type responses are generally considered as pro-inflammatory in which the cytokines IL-2 and

IFN- γ are synthesized by Th1 lymphocytes and IL-12 mainly by dendritic cells. Th2-type responses are considered as anti-inflammatory; Th2 lymphocytes synthesize IL-4 and IL-10, but several other cells also secrete IL-10, a cytokine associated to a regulatory phenotype. Both types of T cell responses are activated and regulated during the immune responses (Raphael *et al.* 2015). Four studies reported on IL-2, IL-12, IL-4 or IL-10 concentrations in endometriosis-associated infertility patients; however, the results are diverse and we emphasize that different corporal fluids were used in these studies (Table 2) (Furuya *et al.* 2003, Andreoli *et al.* 2011, Drosdzol-Cop *et al.* 2012, Singh *et al.* 2016).

Whereas Singh *et al.* (2016) reported statistically higher IL-2, IL-12, IL-4 or IL-10 concentrations in both FF and serum of endometriosis patients compared to controls undergoing *in vitro* fertilization procedures, the study by Furuya *et al.* (2003) did not find differences in PF dosages of the same cytokines between infertile endometriosis patients and controls. Corroborating Furuya *et al.* (2003) data, other researchers found no

Table 1 Interleukin-1 distribution among endometriosis-associated infertility and control patients.

Reference	E (n)	C (n)	C type	Type of fluid	E	C	P value	Additional information
Kondera-Anasz <i>et al.</i> (2005)	49	20	Diagnostic for infertility	PF	>	<	<0.0001	not detected in control
	49	20	Diagnostic for infertility	S	>	<	<0.0001	not detected in control
Michaud <i>et al.</i> (2011)	70	76	Tubal ligation or reanastomosis	PF	>	<	<0.05	
Akoum <i>et al.</i> (2008)	154	147	Tubal ligation or reanastomosis	PF	>	<	<0.05	Initial stages are related to infertility
Wunder <i>et al.</i> (2006)	47	279	Assisted reproduction without endometriosis	FF		ns	0.1	Type of infertility not specified
Wu <i>et al.</i> (2017)	17	17	Tubal, male, myoma or idiopathic infertility	FF	>	<	<0.001	
Singh <i>et al.</i> (2016)	200	140	Tubal infertility	FF	>	<	<0.05	
	200	140	Tubal infertility	S	>	<	<0.05	
Ueki <i>et al.</i> (1994)	7	9	Fertile with myoma or ovarian cyst	PF		ns	α NA	
Skrzypczak <i>et al.</i> (2005)	28	20	Diagnostic for pain	PF		ns	α NA	
	20	10	Diagnostic for pain	PF		ns	α NA	
Pellicer <i>et al.</i> (1998)	8	7	Diagnostic for infertility	S		ns	>0.05	
	12	11	Assisted reproduction without endometriosis	FF		ns	>0.05	
	8	7	Diagnostic for infertility	S		ns	>0.05	Natural cycle
	12	11	Assisted reproduction without endometriosis	S		ns	>0.05	Before hcg
	12	11	Assisted reproduction without endometriosis	S		ns	>0.05	After hcg
Oku <i>et al.</i> (2004)	39	19	Pelvic disease	PF		ns	>0.05	
	39	19	Pelvic disease	S		ns	>0.05	

α NA, α error not available; C, control; E, endometriosis; FF, follicular fluid; ND, not detected; ns, non-significant; PF, peritoneal fluid; S, serum.

difference in PF and serum levels of IL-10 or IL-12 also between infertile endometriosis patients and controls (Andreoli *et al.* 2011). On the other hand, Drosdzol-Cop *et al.* (2012) reported higher IL-4 and low IL-2 levels in the serum and PF from endometriosis patients suggesting activation of a Th2 response (Drosdzol-Cop *et al.* 2012).

Interleukin-6

Despite being the most investigated interleukin in endometriosis, its relation with endometriosis-associated infertility is still unclear. IL-6 is a multifunctional cytokine involved in numerous immunological processes such as regulation of the immune response, inflammation,

Table 2 Interleukin-2, -4, -10 and -12 distribution among endometriosis-associated infertility and control patients.

Author, year	E (n)	C (n)	C type	Type of fluid	IL	E	C	P value
Furuya <i>et al.</i> (2003)	14	3	Pelvic inflammatory disease	PF	IL-2	<	>	<0.05
	14	3	Pelvic inflammatory disease	PF	IL-4	<	>	<0.05
	14	3	Pelvic inflammatory disease	PF	IL-10		ns	α NA
	14	3	Pelvic inflammatory disease	PF	IL-12		ns	α NA
	14	12	Benign ovarian cyst	PF	IL-2		ns	α NA
	14	12	Benign ovarian cyst	PF	IL-4		ns	α NA
	14	12	Benign ovarian cyst	PF	IL-10		ns	α NA
	14	12	Benign ovarian cyst	PF	IL-12		ns	α NA
Singh <i>et al.</i> (2016)	200	140	Tubal infertility	FF	IL-2	>	<	<0.05
	200	140	Tubal infertility	FF	IL-4	>	<	<0.05
	200	140	Tubal infertility	FF	IL-10	>	<	<0.05
	200	140	Tubal infertility	FF	IL-12	>	<	<0.05
	200	140	Tubal infertility	S	IL-2	>	<	<0.05
	200	140	Tubal infertility	S	IL-4	>	<	<0.05
	200	140	Tubal infertility	S	IL-10	>	<	<0.05
	200	140	Tubal infertility	S	IL-12	>	<	<0.05
Andreoli <i>et al.</i> (2011)	40	40	Tubal ligation	PF	IL-10		ns	0.209
	40	40	Tubal ligation	PF	IL-12		ns	0.344
	40	40	Tubal ligation	S	IL-10		ns	0.604
	40	40	Tubal ligation	S	IL-12		ns	0.203

α NA, α error not available; C, control; E, endometriosis; FF, follicular fluid; IL, interleukin; ND, not detected; ns, non-significant; PF, peritoneal fluid; S, serum.

Table 3 Interleukin-6 distribution among endometriosis-associated infertility and control patients.

Author, year	E (n)	C (n)	C type	Type of fluid	E	C	P value	Additional information
Liu <i>et al.</i> (2000)	14	11	Diagnostic laparoscopy for infertility	PF	>	<	<0.01	
Buyalos <i>et al.</i> (1992)	10	10	Tubal infertility	FF	Ns		α NA	
	10	10	Antisperm antibody	FF	Ns		α NA	
Furuya <i>et al.</i> (2003)	14	3	Pelvic inflammatory disease	PF	<	>	<0.01	
	14	12	Benign ovarian cyst	PF	>	<	<0.05	
Wunder <i>et al.</i> (2006)	47	279	Assisted reproduction without endometriosis	FF	ns		0.34	
Harada <i>et al.</i> (1997)	19	19	Diagnostic laparoscopy for infertility	PF	<	>	<0.05	
Wu <i>et al.</i> (2017)	17	17	Tubal, male, myoma or idiopathic infertility	FF	>	<	0.01	
Singh <i>et al.</i> (2016)	200	140	Tubal infertility	FF	>	<	<0.05	
	200	140	Tubal infertility	S	>	<	<0.05	
Ueki <i>et al.</i> (1994)	7	9	Fertile with myoma or ovarian cyst	PF	ns		α NA	
Skrzypczak <i>et al.</i> (2005)	28	20	Infertile without endometriosis	PF	ns		α NA	Infertile endometriosis
	20	10	Fertile	PF	ns		α NA	Fertile endometriosis
Pellicer <i>et al.</i> (1998)	8	7	Diagnostic laparoscopy for infertility	S	<	>	<0.05	
	12	11	Assisted reproduction without endometriosis	FF	>	<	<0.05	
	8	7	Diagnostic laparoscopy for infertility	S	>	<	<0.05	Natural cycle
	12	11	Assisted reproduction without endometriosis	S	>	<	<0.05	Before hcg
	12	11	Assisted reproduction without endometriosis	S	ns		α NA	After hcg

α NA, α error not available; C, control; E, endometriosis; FF, follicular fluid; ND, not detected; ns, non-significant; PF, peritoneal fluid; S, serum.

angiogenesis and pregnancy outcome. Several groups (Table 3) studied its production and association with infertility. Five out of nine studies failed to find any difference in PF IL-6 levels between endometriosis-associated infertility patients and controls (Ueki *et al.* 1994, Furuya *et al.* 2003), FF (Buyalos *et al.* 1992, Wunder *et al.* 2006) and also in serum (Drosdzol-Cop & Skrzypulec-Plinta 2012). In four studies higher IL-6 concentrations in infertile endometriosis compared with non-endometriosis patients were found in the PF (Harada *et al.* 1997, Liu *et al.* 2000) and FF (Singh *et al.* 2016, Wu *et al.* 2017). One particular study (Pellicer *et al.* 1998) compared within the endometriosis

patients group, those undergoing natural cycle with those with stimulated cycles, and also with controls (tubal factor controls). The authors found that in natural cycling women, serum IL-6 was higher in endometriosis compared to controls; during stimulated cycles, serum IL-6 decreased in endometriosis patients and increased just after human chorionic hormone administration; FF IL-6 was also higher in endometriosis. Other authors reported that when fertile women with or without endometriosis were compared with infertile endometriosis patients, IL-6 concentrations were significantly higher in the fertile group ($P<0.05$) (Skrzypczak *et al.* 2005).

Table 4 Interleukin-8 distribution among endometriosis-associated infertility and control patients.

Author, year	E (n)	C (n)	C type	Type of fluid	E	C	P value	Additional information
Wunder <i>et al.</i> (2006)	47	279	Assisted reproduction without endometriosis	FF	ns		0.63	
Wu <i>et al.</i> (2017)	17	17	Tubal, male, myoma or idiopathic infertility	FF	ns		α NA	
	17	17	Tubal, male, myoma or idiopathic infertility	S	ns		α NA	
Singh <i>et al.</i> (2016)	200	140	Tubal infertility	FF	>	<	<0.01	
	200	140	Tubal infertility	S	>	<	<0.01	
Gazvani <i>et al.</i> (1998)	25	22	Diagnostic laparoscopy for pain or infertility	PF	>	<	0.02	
	25	22	Diagnostic laparoscopy for pain or infertility	S	ns		0.27	
Skrzypczak <i>et al.</i> (2005)	28	20	Infertile without endometriosis	PF	>	<	<0.01	Infertile endometriosis
	20	10	Fertile	PF	ns		α NA	Fertile endometriosis

α NA, α error not available; C, control; E, endometriosis; FF, follicular fluid; ns, non-significant; PF, peritoneal fluid; S, serum.

Table 5 Interleukin-15, -17, -18 and -23 distribution among endometriosis-associated infertility and control patients.

Author, year	E (n)	C (n)	C type	Type of fluid	IL	E	C	P value
Arici <i>et al.</i> (2003a)	50	18	Tubal ligation	PF	IL-18	>	<	0.005
	47	279	Assisted reproduction without endometriosis	FF	IL-15	ns		0.78
Arici <i>et al.</i> (2003b)	55	18	Tubal ligation	PF	IL-15	>	<	<0.001
Velasco <i>et al.</i> (2010)	84	24	Ovarian cyst	OMA	IL-17	ND		
Wu <i>et al.</i> (2017)	17	17	Tubal, male, myoma or idiopathic infertility	FF	IL-18	ns		α NA
	17	17	Tubal, male, myoma or idiopathic infertility	S	IL-18	ns		α NA
Glitz <i>et al.</i> (2009)	34	22	Tubal ligation	PF	IL-18	ns		>0.05
	34	22	Tubal ligation	S	IL-18	ns		>0.05
Zhang <i>et al.</i> (2005)	36	26	Tubal ligation	PF	IL-17	ns		>0.05
Andreoli <i>et al.</i> (2011)	40	40	Tubal ligation	PF	IL-17	ns		0.445
	40	40	Tubal ligation	PF	IL-23	>	<	0.006
	40	40	Tubal ligation	S	IL-17	ns		0.325
	40	40	Tubal ligation	S	IL-23	ns		0.209
Oku <i>et al.</i> (2004)	39	19	Pelvic disease	PF	IL-18	>	<	<0.019
	39	19	Pelvic disease	S	IL-18	ns		0.945

α NA, α error not available; C, control; E, endometriosis; FF, follicular fluid; IL, interleukin; ND, not detected; ns, non-significant; PF, peritoneal fluid; S, serum.

IL-6 information in FF was available for meta-analysis in four studies (SMD 0.82, 95% CI -0.60 to 2.25, $P=0.258$). The conclusion is that no sufficient evidence exists that FF IL-6 values are different between women with and without endometriosis. The I^2 and Q test values were 97.42% (95% CI of 91.45–99.82%) and less than 0.0001 respectively, indicating a high degree of heterogeneity, so that the estimation of effect size is inconclusive.

In the PF two studies were available; one had higher values for the endometriosis group (SMD 6.35, 95% CI: 4.42–8.28) and, in the other, no differences between groups were described (SMD 0.00, 95% CI: -0.57 to 0.57). Due to the non-concordant effect studies, the combined effect was not estimated. The I^2 value was 97.4% (95% CI of 86.9 to >99.7%), indicating a high degree of heterogeneity.

On the other hand, two studies were available for serum IL-6, and both reported higher values in the endometriosis group (SMD 3.92, 95% CI 3.56–4.28, $P<0.001$). In conclusion, even though coming from only two studies, there was sufficient evidence that serum IL-6 values are increased among women with endometriosis-associated infertility. The I^2 value was 0.0% (95% CI of 0.0–96.1%), indicating mild degree or absence of heterogeneity.

Interleukin-8

IL-8 is secreted by leukocytes, macrophages and endothelial cells stimulated, for instance, by IL-1 or TNF- α . It is a pro-inflammatory cytokine known to be a potent regulator of angiogenesis and a growth promoting factor (Long *et al.* 2016). Both properties may be important to

Table 6 Interleukin distribution among endometriosis-associated pain and control patients.

Author, year	E (n)	C (n)	C type	Type of fluid	IL	E	C	P value	Additional information
Arici <i>et al.</i> (2003a)	50	18	Tubal ligation	PF	IL-18	>	<	0.005	No dispersion values available
Michaud <i>et al.</i> (2011)	70	76	Tubal ligation or reanastomosis	PF	IL-1 β	$P<0.05$		<0.05	
Velasco <i>et al.</i> (2010)	84	24	Ovarian cyst	PF	IL-6	>	<	<0.01	
	84	24	Ovarian cyst	PF	IL-8	ns	α NA		
Manero & Alcazar (2010)	26	25	Diagnostic laparoscopy for pain	S	IL-8	ns	α NA		
Santulli <i>et al.</i> (2012)	44	36	Diagnostic laparoscopy for pain	PF	IL-33	>	<	0.013	
Rapkin <i>et al.</i> (2000)	29	23	Pelvic adhesions or no visible alteration	PF	IL-6	ns	α NA		Comparison between pain and no pain among groups
Drosdzol-Cop & Skrzypulec-Plinta (2012)	33	11	Diagnostic laparoscopy for pain	PF	IL-6	>	<	0.0002	
Drosdzol-Cop <i>et al.</i> (2012)	33	17	Diagnostic laparoscopy for pain	PF	IL-2	<	>	0.01	
	33	17	Diagnostic laparoscopy for pain	PF	IL-4	>	<	0.03	
Oku <i>et al.</i> (2004)	39	19	Pelvic disease	PF	IL-1 β	ns		>0.05	
	39	19	Pelvic disease	PF	IL-18	>	<	<0.019	

α NA, α error not available; C, control; E, endometriosis; FF, follicular fluid; IL, interleukin; ND, not detected; ns, non-significant; PF, peritoneal fluid; S, serum.

endometriosis as they could possibly contribute to the inflammatory pathogenesis and infertility associated with the disease. The relationship between IL-8 and infertility was reported in five published papers (Table 4) and all the studies that evaluated IL-8 in the PF found higher concentrations of this cytokine in endometriosis patients compared to control patients. Especially when comparing infertile women with and without endometriosis, IL-8 levels were higher in the endometriosis group (Gazvani *et al.* 1998, Skrzypczak *et al.* 2005).

The differences found for IL-8 PF levels, however, were not consistent among the studies that reported on FF and IL-8. Two groups found no statistically significant differences in IL-8 levels in FF (Wunder *et al.* 2006, Wu *et al.* 2017) and one study reported higher serum and FF IL-8 levels in infertile endometriosis patients compared to control patients enrolled in *in vitro* fertilization protocols (Singh *et al.* 2016).

Follicular and PF IL-8 data information was available in two studies. One of these studies reported higher values for the endometriosis group in the FF and in PF (SMD 9.27; 95% CI 9.54–10.0 for FF; 5.51, 95% CI 4.26–6.76 for PF) (Gazvani *et al.* 1998, Singh *et al.* 2016). The other study did not find any difference in IL-8 concentrations between endometriosis and control groups (SMD of 0.07, 95% CI –0.24 to 0.38 for FF; 0.51, 95% CI –0.07 to 1.10 for PF) (Skrzypczak *et al.* 2005, Wunder *et al.* 2006). Therefore, no combined effect was estimated for FF and PF IL-8. The I^2 value was 99.8% (95% CI 99.0 to >99.9%) and 98.0% (95% CI 90.0 to >99.8%) respectively, indicating a high degree of heterogeneity. For serum IL-8 both studies observed higher values in the endometriosis-associated infertility group (SMD 4.01, 95% CI 3.45–4.33, $P < 0.001$). In conclusion, there is sufficient analytical evidence that serum IL-8 values are increased in women with endometriosis-associated infertility. The I^2 value was 18.4% (95% CI of 0.0 to >99%), indicating a small degree of heterogeneity.

Interleukin-15, -17, -18 and -23

Endometriosis is widely known as an inflammatory condition, and IL-15, IL-17, IL-18 and IL-23 are pro-inflammatory cytokines that have been investigated in endometriosis (Table 5) (Musso *et al.* 1998, Oku *et al.* 2004, Lubberts 2015). Data on IL-23 appear in just one study and no difference was seen in serum and PF between endometriosis and control patients and no association of IL-23 was found with infertility (Andreoli *et al.* 2011). IL-23 is known to stimulate a Th17 response, but like IL-23, no difference in concentrations of IL-17 in PF or serum were found between endometriosis patients and controls (Zhang *et al.* 2005, Andreoli *et al.* 2011) and, in one study, neither was even detected in serum or PF (Velasco *et al.* 2010). Nevertheless, Zhang and collaborators reported significant difference in IL-17 levels between minimal/mild endometriosis versus

moderate/severe stages of the disease and higher IL-17 concentrations in infertile versus fertile endometriosis patients (Zhang *et al.* 2005).

Lymphocytes and macrophages can secrete IL-15 and IL-18. PF of endometriosis patients contains higher numbers of macrophages (Santanam *et al.* 2002). Still, it is interesting to notice that the studies on IL-15 and IL-18 have conflicting results (Arici *et al.* 2003a,b, Oku *et al.* 2004, Wunder *et al.* 2006, Glitz *et al.* 2009, Wu *et al.* 2017).

While two groups found increased concentrations of IL-18 in PF of infertile endometriosis patients compared to controls, there was no association with infertility (Arici *et al.* 2003a,b, Oku *et al.* 2004). Another study found no difference in FF IL-18 between endometriosis and non-endometriosis patients (Wu *et al.* 2017) and still another research group did not find significant differences in PF IL-18 concentrations between the two groups of patients (Glitz *et al.* 2009).

With respect to IL-15, in one study, IL-15 was not detected either in FF or in serum from infertile women with endometriosis (Wu *et al.* 2017). In another study, no difference was observed in PF IL-15 (Wunder *et al.* 2006), and in a third study, PF IL-15 levels in endometriosis patients were higher compared to PF from controls. In this last study, PF IL-15 concentrations were found to be significantly different between minimal/mild endometriosis versus moderate/severe endometriosis. Similar differential result for PF IL-15 was also reported between deep endometriosis in comparison to endometrioma (Arici *et al.* 2003a,b).

Interleukins and pelvic pain in endometriosis patients

The association between IL-1, IL-2, IL-4, IL-6, IL-8, IL-18 or IL-33 and endometriosis-related pelvic pain was investigated by different research groups (Table 6) (Rapkin *et al.* 2000, Arici *et al.* 2003a,b, Oku *et al.* 2004, Manero & Alcazar 2010, Velasco *et al.* 2010, Michaud *et al.* 2011, Drosdzol-Cop & Skrzypulec-Plinta 2012, Drosdzol-Cop *et al.* 2012, Malhotra *et al.* 2012, Santulli *et al.* 2012).

The majority of these studies indicate an association between endometriosis-associated pain with cytokine concentration by reporting increased interleukin levels in PF from endometriosis patients compared to control participants. Only two of these studies report similar significant associations for serum cytokine levels in patients compared to controls (Drosdzol-Cop *et al.* 2012, Santulli *et al.* 2012).

Peritoneal fluid IL-1 and IL-18, IL-4 from PF and serum, and IL-33 from serum were augmented in all mentioned studies (Arici *et al.* 2003a,b, Oku *et al.* 2004, Michaud *et al.* 2011, Drosdzol-Cop *et al.* 2012, Santulli *et al.* 2012). However, positive correlation with dysmenorrhea ($r = 0.270$, $P < 0.05$) and pelvic pain ($r = 0.247$, $P < 0.05$) in endometriosis patients could only

be established for IL-6 in one study (Velasco *et al.* 2010), and for IL-33 and dysmenorrhea also in just one study ($r=0.174$; $P=0.028$) (Santulli *et al.* 2012).

Despite possibly being the most frequently studied interleukins in endometriosis, investigations on IL-6 and IL-8 led to inconsistent results. Some authors found significantly higher concentrations in endometriosis patients (Manero & Alcazar 2010, Velasco *et al.* 2010, Drosdzol-Cop & Skrzypulec-Plinta 2012), whereas others reported no difference in patients versus controls regardless of the evaluated fluid (Rapkin *et al.* 2000, Manero & Alcazar 2010). The heat maps of endometriosis-associated pain IL can be seen in Fig. 1B.

Discussion

The present review and meta-analysis support the association between elevated serum pro-inflammatory interleukins, IL-6 and IL-8, and endometriosis-associated infertility. Similar studies failed to show the association of higher IL-6 and IL-8 levels with endometriosis-associated pain. In addition, the analysis of the included papers did not provide consistent results relative to differences in follicular or PF concentrations of any of the investigated cytokines (IL-6 and IL-8 included).

IL-1 studies, taken together, did not supply sufficient evidence of differences in concentration between endometriosis and control patients in the three fluids (serum, FF and PF).

Despite the small meta-analysis results, in accordance with other reviews (Harada *et al.* 2001, Arici 2002) our present analysis observed higher interleukin concentrations in infertile endometriosis patients compared to control participants, regardless of the inclusion criteria chosen for the control group (Fig. 1A and Tables 1, 2, 3, 4 and 5). The results for IL-1, IL-8, IL-10, IL-12, IL-15 and IL-23 concentrations in PF, FF and serum are either non-significant or higher in endometriosis. Regarding IL-2, IL-4 and IL-6 results from the analyzed studies are more diverse and in some of the studies, the IL concentrations reflect the menstrual cycle phase (Liu *et al.* 2000) or stage dependent (Skrzypczak *et al.* 2005).

The MINORS gives us the idea of how endometriosis research has been developed and, in that way, how challenging can its reproducibility and the robustness of its results given by poor quality of the evidence established be. Almost all selected papers (96%) have bias in the assessment of study endpoints and have not calculated the study sample size prior to its beginning or, at least, it was not described. Another important point is the assessment of equivalence of groups and type of lesion, since the eligibility criteria for the control group and the presence, local and type of endometriosis described were very different among studies. Different hypotheses and endpoints require comparison with adequate control groups and, therefore, different results are expected. Some studies compared infertile

endometriosis groups with fertile control groups, while others compared infertile endometriosis with infertile control or infertile versus fertile endometriosis.

When looking at the heat map (Fig. 1A), it is noticeable that the majority of positive results for higher concentration of ILs were found in the serum or FF from infertile endometriosis patients. On the other hand, results for PF are diverse, which could be due to patient or control group selection or to the cytokine-quantifying method (Fig. 2).

Apart from the results of the meta-analysis, the systematic review emphasizes an important fact: interleukin concentrations vary among infertile endometriosis patients depending on the menstrual cycle phase and endometriosis grade, and differences between studies depend on the chosen control group. Concerning endometriosis-associated pelvic pain, the results appear to be less conflicting. While looking at the heat map (Fig. 1A), the impression is that higher than control, IL concentrations tend to be reported more frequently for endometriosis patients in the existing studies. However, as the number of analyzable studies is still small, this assumption may be precocious. There is need for more studies on interleukin and chronic pelvic pain.

Since endometriosis is an inflammatory disease, the existing evidence suggests that infertility and pelvic pain associated with endometriosis result from the local peritoneal inflammatory reaction caused by the ectopic endometrial implants (Miller *et al.* 2017). After attachment to the peritoneum, the viability of the endometriotic focus may be maintained by local angiogenesis and secretion of growth factors and cytokines, mainly by peritoneal neutrophils and macrophages (Izumi *et al.* 2018). It is also known that macrophages and natural killer (NK) cells of patients with endometriosis may not eliminate all endometrial cells in the peritoneal cavity, which leads to aberrant cytokine secretion and inflammation (Izumi *et al.* 2018).

The presence of ectopic endometrium as well as cytokines and chemokines possibly recruit more cells (B and T lymphocytes, macrophages, NK cells, neutrophils), a cause of more inflammation. Cytokines are secreted by all these cell types and they activate the synthesis of other cytokines, inflammatory mediators such as prostaglandins and leukotrienes and chemokines. Chemokines are a family of secreted proteins that are chemotactic to a great variety of cells whose receptors are expressed upon stimulation on virtually all cell types. Interleukins and other cytokines either stimulate or downregulate cell proliferation and the synthesis of other cytokines and mediators in target cells, depending on the cytokine and target cell. Examples are the activation of macrophages by interferon- γ to a microbicidal state, and the stimulation of IL-2 and IFN- γ synthesis and consequent Th1 proliferation by IL-12 synthesized by dendritic cells. Macrophages and epithelial cells release IL-8 a potent chemokine for neutrophils. IL-6 is rapidly

produced by many cell types in response to injury and also by activated macrophages. It is considered a pro-inflammatory cytokine implicated in the maintenance of inflammation and activation of Th17 lymphocytes, active in multiple chronic inflammatory diseases. Altogether, these complex interactions and inflammation can affect fertility and cause significant abdominal pain to patients with endometriosis.

To our knowledge, this systematic review and meta-analysis is the first one compiling all cytokines or interleukins ever evaluated in endometriosis patient fluids in relation to two main symptoms, infertility and pelvic pain. Our survey may have been affected by intrinsic study biases, as all evidence came from observational studies, which are at higher risk of bias than randomized, controlled trials. Therefore, the quality of the evidence may be reduced because the studies present several limitations, as for instance the way results and data information were presented in the papers (Fig. 2). Also, the IL-6 meta-analysis results, even though it showed higher expression in serum from endometriosis group, should be interpreted with caution, since it is derived from only two studies, with mild-degree heterogeneity and, concrete assumption and association made from these results are subjected to miscalculation.

Some points have to be addressed in future research. Among the first things that should be debated is endometriosis classification and the type of lesions that have to be considered and specified in the studies. Some authors use the term *early stages*, referring to stages I and II and *advanced stages* for stages III and IV; others classify endometriosis patients by the presence of peritoneal and endometriotic ovarian cysts. In addition, there is reference to superficial and deep nodules of the disease. According to the latest World Endometriosis Society consensus on the classification of endometriosis published in the beginning of 2017 (Johnson *et al.* 2017), endometriosis final classification include stage I (minimal), II (mild), III (moderate) and IV (severe). To conform to this classification, a series of grades that include observation of peritoneal and ovary lesions should be considered. The location of the lesion and its type should be described, and also whether it is superficial or deep and other individualities. Therefore, when comparing groups of patients, all these characteristics should be addressed and described in the article, especially when the comparison is among endometriosis groups.

Furthermore, the choice of an ideal control group to be compared with endometriosis will mostly depend on the hypothesis and the expected endpoint that is under consideration. Since endometriosis is a multifactorial disease and each stage or type of lesion is sometimes considered a different disease (Nisolle & Donnez 1997), it is important to clarify what type of disease the researches are working on, describe each patient (comorbidities, use of hormone, therapies) and the selection criteria of all groups. Nevertheless, the

establishment of a constant control group used in the studies or, at least, more homogeneous groups would be an important but quite hard purpose to accomplish. As observed, the control groups present in the studies considered in this review are widely different and possibly may have influenced the results. Examples of conditions of patients who were in the control groups are the following: pelvic inflammatory disease; pelvic disease; benign ovarian cyst; idiopathic infertility; tubal infertility; diagnostic laparoscopy for infertility; antisperm antibody investigation; assisted reproduction without endometriosis; uterine fibroids; leiomyoma; tubal ligation or reanastomosis.

Since the approach to the abovementioned studies are related to inflammatory conditions, the selection of control groups solely by absence of endometriotic lesions as the only inclusion criterion, disregarding other inflammatory diseases, may be the major factor for the non-significant, hard to interpret, findings of most studies. Crohn's diseases, for example, as well as other inflammatory diseases, are known to have cytokines involved in their pathogenesis (Guan & Zhang 2017). Even stressed people can have in their body fluids deregulated cytokine levels (Clark *et al.* 2019). Because of the involvement of these molecules in a number of other pathological conditions, patients included in the control groups should be better analyzed and considered prior to their participation in endometriosis-related studies. The task is not simple since many of these other inflammatory diseases, as well as endometriosis, cannot be checked by a precise non-invasive diagnostic method.

Although the available evidence points to increased cytokine concentrations in endometriosis patients, it is still not clear if and how cytokines, especially interleukins, participate in the physiopathology of endometriosis-associated infertility and pelvic pain. Different fluids of the same patient may present different profiles of endometriosis-related molecules. In addition, the choice of an adequate control group is essential. Considering the potential use of cytokines as a future diagnostic or biomarker tool in endometriosis or for endometriosis-related infertility or pelvic pain, it is mandatory that investigators consider the correct choice of control groups, the endometriosis grade and the type of observed lesions and other inflammatory diseases that may obscure the results.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/REP-18-0618>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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