Cervico-vaginal secretion cytokine profile: A non-invasive approach to study the endometrial receptivity in IVF cycles

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Problem: Cytokines have a significant role in the process of embryo implantation, trophoblast growth, and differentiation by modulating the immune and endocrine system. The aim of this study was to investigate the profile of a large set of cytokines in the cervico-vaginal washing of women undergoing IVF, to explore the association of these proteins with a good receptive endometrium.

Method of study: A cohort of 155 women scheduled for IVF cycle was recruited. All patients were asymptomatic for genitourinary infections and had been screened for chlamydia, mycoplasma, and other bacterial infections. All IVF subjects were treated according to standard clinical and laboratory protocols. A panel of 48 immune factors was analyzed on cervico-vaginal washing, using magnetic bead-based multiplex immunoassays (Bio-Plex, BIO-RAD Laboratories, Milano, Italy).

Results: A total of 99 patients reached embryo transfer, of which 31 had a clinical pregnancy. A pattern of four pro-inflammatory immune molecules, IL-12p40, IFN-a, MIF, and MCP3 ($P < 0.001$), was found significantly up-regulated in the cervico-vaginal fluid of women with clinical pregnancy. A significantly increased expression of IL-9, Gro_{α}, and SDF-1_{α} ($P < 0.05$) was observed in the presence of endometriosis, while high levels of IL-13 and L-15 were associated with ovulatory infertility factor ($P < 0.05$).

Conclusion: In this pilot study, we demonstrated that the expression of specific cytokines in the cervico-vaginal washing on the day of oocyte retrieval might have a positive correlation with the potential clinical pregnancy. Therefore, cervico-vaginal secretion cytokine profiling might be a new, non-invasive approach to study the endometrial receptivity in IVF management.

KEYWORDS
cytokines, endometrium, implantation, infertility, IVF, pregnancy

1 | INTRODUCTION

Several assisted reproductive techniques (ART), including conventional in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI), are currently available for treatment of infertility. In most ART treatment cycles, one or more good-quality embryos are obtained. Nevertheless, in the last world report, the mean delivery rate per aspiration for fresh cycles reached approximately the 20%,[1] suggesting that a large percentage of good-quality embryos fail to implant. Successful embryo implantation and pregnancy require not only a good-quality embryo, but also a receptive endometrium and effective endometrial-embryonic interactions.[2] This embryo-uterine cross-talk seems to be regulated by several molecules leading to modulation of different cellular functions, such as maternal steroid

hormones, growth factors, cytokines, and chemokines.[3] Cytokines and chemokines have a significant role in the process of embryo implantation, endometrial development, trophoblast growth, and differentiation by modulating the immune and endocrine system. In particular, endometrial decidualization is promoted by cytokines influencing the processes of edema and angiogenesis, essential for endometrial tissue, recruitment of leukocytes, and production of molecules, such as immune mediators and prostaglandins, involved in maternal-embryo communication.[4]

Several studies have tried to identify the role of cytokines in implantation and clinical pregnancy in different biological samples such as blood,[5,6] follicular fluid,[7,8] endometrial secretions,[9] endometrial washing, and biopsy.[10]

In our study, we investigated the profile of a large set of cytokines, growth factors, and chemokines in the cervico-vaginal washing of women undergoing IVF. This study aimed to explore the relationship of these immune mediators with implantation of embryos and clinical pregnancy through the use of a non-invasive sampling approach.

2 | MATERIALS AND METHODS

2.1 | Study population

Women scheduled for IVF cycle at the Assisted Reproduction Unit of the Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, were recruited. All patients had been screened for chlamydia, mycoplasma, and other bacterial infections. Patient inclusion criteria were female aged between 18 and 43 years, suitability for IVF treatment with motile spermatozoa, no intracavitary pathology, such as polyps, submucous myomas, or intramural myomas without impression or deformation of the uterine cavity, negative screening for chlamydia, mycoplasma, and other bacterial infections. Exclusion criteria were concomitant symptoms or signs of genitourinary infections, chronic diseases requiring regular treatment, and use of donor oocytes.

The study was approved by the Institutional Review Board and the Independent Bioethics Committee of the Institute for Maternal and Child Health, "IRCCS—"Burlo Garofolo" of Trieste, Italy (RC 26/13), and the enrolled patients signed an informed consent form and the study was conducted in accordance with the Declaration of Helsinki.

2.2 | ART procedures

All IVF subjects were treated according to standard clinical and laboratory protocols as previously described.[11] The patients received a long gonadotrophin-releasing hormone (GnRH) analog protocol or a flexible GnRH antagonist protocol with oral contraceptive pre-treatment. Recombinant FSH (rec-FSH) 150-225 IU/d was administered when pituitary down-regulation was established or from Day 2 of the cycle, in GnRH agonist and antagonist protocol, respectively. The rec-FSH dose was adjusted and/or highly purified menotropin (hphMG) was added, after 4 days of stimulation, according to the ovarian response, as assessed by serum estradiol (E2) levels and ultrasound. Human chorionic gonadotropin (hCG) 5000 or 10 000 IU was given to induce final oocyte maturation when at least two leading follicles reached a mean diameter of 18 mm. Trans-vaginal ultrasound-guided oocyte retrieval was performed 36 hours later. Two or three embryos were transferred 3 days after oocyte pickup. Veeck's morphological grading system was adopted for day 3 embryo scoring.[12] For luteal phase support, intravaginal micronized progesterone 200 mg was given three times daily, starting on the day after oocyte retrieval. Serum hCG levels were measured 14 days after embryo transfer, and if positive, an ultrasound scan was scheduled 2 weeks later to assess the number and status of the implanted embryos. According to the ICMART glossary of ART terminology, a biochemical pregnancy was defined as a pregnancy diagnosed only by the detection of HCG in serum or urine and that did not develop into a clinical pregnancy. Clinical pregnancy was defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy.[13,14]

2.3 | Cytokines analysis

The analysis of 48 cytokines was carried out on cervico-vaginal fluid washing. Immediately before the oocyte retrieval, the vagina was irrigated with 50 mL of sterile water, and 3 mL of cervico-vaginal lavage fluid was collected for our investigations. Cytokine analysis was performed using magnetic bead-based multiplex immunoassays (Bio-Plex®; BIO-RAD Laboratories, Milano, Italy) using the Bio-Plex 200 reader (Luminex, Austin, TX, USA), as previously described.[15] In brief, 50 μL of cervico-vaginal washing and standards was added in duplicate to a 96-multiwell plate containing analyte beads. After incubation for 30 minutes at room temperature and washing, the antibody-biotin reporter was added and incubated for 10 minutes with streptavidin-phycoerythrin. The concentrations of the cytokines were determined by a digital processor managed data output, and Bio-Plex Manager® software presented data as median

TABLE 1 Data regarding patients after stimulation protocol

	Clinical pregnancy (n. 31)	No clinical pregnancy (n. 68)	*P* value
Mean age	33.7 ± 4.6 y	36.2 ± 4.3 y	0.07
Serum estradiol levels	1814 ± 633 pg/mL	1549 ± 781 pg/mL	0.190
Oocyte fertilization rate	69.1%	69.2%	0.912
Number of embryos transferred	2.0 ± 0.6	1.8 ± 0.7	0.281
Mean grade of embryos transferred	1.4 ± 0.5	1.9 ± 0.9	0.068

TABLE 2 Concentrations of cytokines and chemokines (pg/mL) in the vaginal washing, collected before oocyte retrieval, in women with or without a clinical pregnancy

Cytokines	Clinical pregnancy (n. 31)	No clinical pregnancy (n. 68)	*P* value	OR (CI)
IL-1α	17.29 (7.39-36.62)	10.83 (3.71-39.65)	0.725	0.99 (0.99-1.00)
IL-1β	2.21 (0.20-6.83)	2.21 (1.47-6.25)	0.961	1 (0.99-1)
IL-2	2.12 (1.17-3.25)	2.98 (2.21-3.90)	0.079	0.76 (0.57-1.03)
IL-2 ra	21.17 (13.44-29.19)	14.70 (11.57-23.85)	0.145	1.02 (0.99-1.06)
IL-3	43.39 (33.44-53.10)	40.09 (28.24-50.11)	0.532	1 (0.98-1.02)
IL-4	0.19 (0.13-0.43)	0.29 (0.11-0.48)	0.860	1.08 (0.42-2.76)
IL-5	nd	nd	nd	nd
IL-6	1.50 (0.58-2.77)	1.50 (0.58-1.5)	0.122	1.12 (0.96-1.29)
IL-7	0.60 (0.26-1.06)	1.06 (1.06-1.5)	0.643	0.92 (0.65-1.3)
IL-8	31.91 (10.26-196.6)	10.65 (2.98-101.9)	0.602	0.99 (0.99-1)
IL-9	2.01 (1.26-3.34)	2.99 (2.27-4.26)	0.049	0.74 (0.55-0.99)
IL-10	2.05 (1.21-4.38)	2.78 (2-5.14)	0.227	0.89 (0.75-1.06)
IL-12(p 40)*	268 (185.1-362.9)	118.3 (2.5-191.3)	0.000	1.01 (1-1.01)
IL-12(p 70)	2.37 (1.08-5.09)	4.26 (2.50-8.07)	0.167	0.94 (0.86-1.02)
IL-13	0.75 (0.49-0.97)	0.80 (0.52-0.80).	0.022	0.09 (0.01-0.7)
IL-15	1.34 (0.42-6.62)	5.54 (3.18-10.55)	0.006	0.86 (0.77-0.95)
IL-16	10.88 (5.79-15.26)	5.86 (4-10.43)	0.032	1.05 (1-1.11)
IL-17	2.57 (1.83-28)	17.53 (3.75-117.2)	0.007	0.98 (0.97-0.99)
IL-18	27.71 (9.08-101.9)	6.69 (3.4-20.99)	0.894	0.99 (0.99-1)
LIF	13.15 (10.56-16.95)	12.54 (11.04-16.53)	0.559	1.02 (0.95-1.09)
IFN-α2*	33.22 (9.98-36.97)	9.01 (0.29-10.89)	0.000	1.12 (1.07-1.17)
INF-γ	4.72 (2.09-11.89)	8.2 (2.35-14.78)	0.276	0.97 (0.92-1.02)
G-CSF	58.36 (12.10-166.7)	13.89 (6.69-51.93)	0.097	1 (0.99-1)
M-CSF	20.87 (11.46-32.80)	16.22 (10.84-26.30)	0.288	1 (0.99-1.01)
GM-CSF	44.83 (41.33-80.91)	87.59 (75.58-229.2)	0.002	0.97 (0.96-0.99)
TNF-α	1.84 (1.63-4.69)	1.84 (1.50-4.52)	0.600	0.97 (0.88-1.07)
TNF-β	1.16 (0.87-1.43)	1.16 (1.16-1.67)	0.088	0.55 (0.28-1.09)
SCF	7.22 (1.55-14.31)	1.50 (1.10-1.50)	0.001	1.13 (1.05-1.22)
MIF*	12 815 (49.48-25 549)	138.4 (7-531.8)	0.000	1 (1.0006-1.0017)
IL-1Ra	2826 (964.2-5563)	1582 (349.3-5935)	0.569	0.99 (0.99-1)
VEGF	7.41 (1.45-32.68)	25.74 (11.98-72.46)	0.016	0.98 (0.96-0.99)
Basic FGF	6.84 (5.76-23.45)	17.76 (6.17-33.36)	0.013	0.95 (0.92-0.99)
PDGF	2.26 (0.55-6.33)	3.77 (2.65-5.92)	0.195	1.10 (0.95-1.27)
HGF	13.05 (5.2-31.25)	28.51 (5.17-28.51)	0.994	0.99 (0.99-1)
NGF-β	0.65 (0.58-2.00)	0.65 (0.42-2)	0.337	1.74 (0.56-5.44)
SCGF-β	5.73 (5.73-113.2)	5.73 (5.73-5.73)	0.245	1 (0.99-1)
EOTAXIN	3.37 (1.78-8.21)	3.89 (1.82-7.82)	0.556	0.98 (0.95-1.02)
IP-10	48.35 (17.98-293.30)	40.09 (10.44-123.1)	0.137	1 (0.99-1)
MCP-1	2.53 (2.02-4.75)	3.06 (2.14-6.39)	0.247	0.96 (0.90-1.02)
MIP-1α	nd	nd	nd	nd
MIP-1β	1.47 (0.60-3.24)	1.53 (1.03-2.77)	0.958	0.99 (0.96-1.03)
RANTES	2.36 (1.41-4.91)	3.77 (2.36-4.44)	0.408	0.92 (0.75-1.11)
CTACK	3.63 (0.85-4.95)	0.85 (0.85-5.96)	0.761	1.02 (0.88-1.18)
Groα	354.5 (61.52-2906)	58.44 (21.68-247.6)	0.005	1 (1-1.07)

(Continues)

TABLE 2 (Continued)

Cytokines	Clinical pregnancy (n. 31)	No clinical pregnancy (n. 68)	*P* value	OR (CI)
MCP-3*	13.30 (2.89-33.25)	3.27 (1.77-7.38)	0.000	1.07 (1.03-1.12)
SDF-1α	88.06 (20.55-352.4)	28.30 (18.71-121.9)	0.018	1 (1.0005-1.006)
MIG	188.8 (53.37-790.2)	90.10 (19.29-186.9)	0.188	1 (0.99-1)
TRAIL	5.54 (3.78-8.99)	7.48 (5.75-9.55)	0.054	0.88 (0.77-1)

Values are expressed as medians and interquartile ranges. Odds ratios with confidence intervals and *P*-values are the result of bivariate logistic regressions. The significant results are marked with asterisks *$P < 0.001$.

fluorescence intensity (MFI) and as concentration (pg/mL; BIO-RAD Laboratories).

2.4 | Statistical analysis

Descriptive analyses were carried out, for continuous variables, using means and standard deviations (SDs) or medians and interquartile ranges (IQR) depending on the distribution of the data. Differences in the values were measured with Student's *t* tests or Mann-Whitney rank-sum tests. The association between pregnancy outcomes and the expression of the 48 cytokines was analyzed with bivariate logistic regressions. For logistic regressions, we applied the Bonferroni correction to the significance level, and considering the 48 multiple comparisons, we fixed it to $P < 0.001$. Data are presented as medians and IQRs. The association between infertility factors and concentration of cytokines was analyzed with logistic regressions, with statistical significance defined as $P < 0.05$. All analyses were carried out with Stata/IC 14.2 (StataCorp LLC, College Station, TX, USA).

3 | RESULTS

3.1 | ART outcomes

A total of 155 women were recruited. Fifty-six patients did not have embryo transfer because of a risk of ovarian hyperstimulation, no mature oocyte retrieval, fertilization, or cleavage failure. Ninety-nine patients (mean age: 36.6 ± 4.4 years) reached embryo transfer and were available for the analysis. The causes of infertility were distributed as follows: male factor infertility (41%, n = 41), tubal factor infertility (TFI; 8%, n = 8), ovulatory factor (20%, n = 20), endometriosis (14%, n = 14), and unexplained infertility (16%, n = 16). Fourteen out of 99 women who reached ET had a biochemical pregnancy, 54 women were negative to β-HCG test, and 31 had a clinical pregnancy.

For data analysis, the patients were divided into two groups: one composed of women with clinical pregnancies, and one group formed by women with a biochemical pregnancy or resulted negative to β-HCG test (no clinical pregnancies). Between the two groups of patients, there were no significant differences in the mean age, serum estradiol levels on the day of hCG administration, oocyte fertilization rate, number of embryos transferred, and mean grade of embryos transferred, as reported in Table 1.

3.2 | Cytokines profile

The concentration of 48 soluble immune proteins, including Th1/pro-inflammatory and anti-inflammatory cytokines, chemokines, and trophic factors, was measured in the cervico-vaginal washing of women subjected to IVF. The values measured as pg/mL, and the degree of statistical significance is reported in Table 2. A pattern of four pro-inflammatory immune molecules, composed of IL-12p40, IFN-α_2, MIF, and MCP3, was found to be significantly higher ($P < 0.001$) in the cervico-vaginal fluid of women with clinical pregnancies compared to women with no clinical pregnancies (Table 2; Figure 1). Also, we examined whether the cause of infertility, including endometriosis, tubal factor infertility (TFI), ovulatory factor, and male factor, could influence the cytokine expression of the cervico-vaginal microenvironment. Women with endometriosis presented significantly increased expression of the anti-inflammatory interleukin 9, and the trophic factors such as Gro$_\alpha$ and SDF-1$_\alpha$

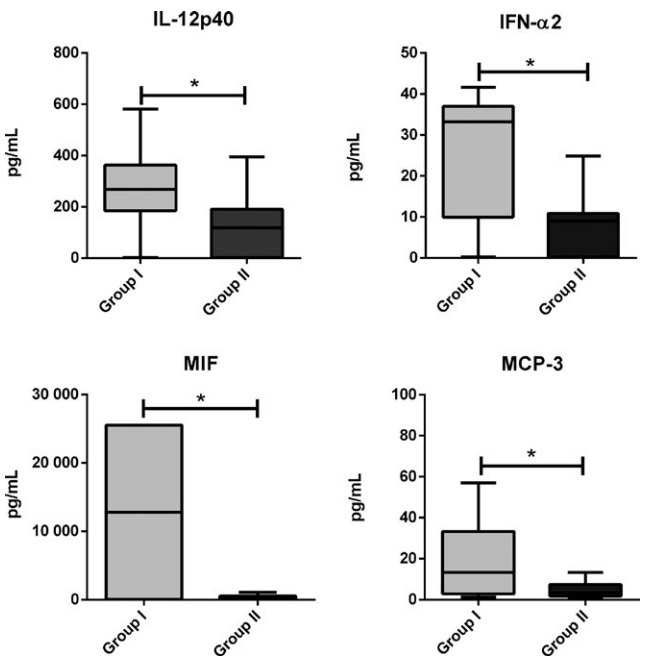

FIGURE 1 Expression of significant immune mediators in cervico-vaginal fluid of women undergoing IVF. The box plots show the expression levels (pg/mL) of the cytokines found to be statistically significant between women with clinical pregnancy (Group I) vs women with biochemical pregnancy or resulted negative β-HCG test (Group II). The significant results are marked with asterisks: *$P < 0.001$

TABLE 3 Expression of significant immune mediators in cervico-vaginal fluid from patients with and without endometriosis

Cytokines	Endometriosis present (n. 14)	Endometriosis absent (n. 85)	OR (CI)	*P* value
IL-9	4 (3.55-6.78)	1.4 (1.2-2.8)	1.31 (1.01-1.71)	0.040
Gro_{α}	407 (20.81-2198)	89.63 (33.11-497)	1 (1.00002-1.0004)	0.045
$SDF\text{-}1_{\alpha}$	144.2 (18.87-316.2)	50.1 (19.64-138.6)	1.002 (1.0005-1.005)	0.046

Values are expressed as medians and interquartile ranges. Odds ratios with confidence intervals and *P*-values are the result of bivariate logistic regressions.

($P < 0.05$; Table 3; Figure 2). Patients with an ovulatory infertility factor showed significantly higher levels of the interleukins, IL-13 and IL-15, and of the angiogenic factor VEGF ($P < 0.05$; Table 4; Figure 2). Particularly, the concentrations of IL-13 and IL-15 were found to be highly expressed in women with ovulation dysfunctions. Conversely, no significant differences in cytokine expression were associated with any other infertility factor.

4 | DISCUSSION

Cervico-vaginal washing contains a very wide range of cytokines, chemokines, and growth factors coming from different structures of the genital tract, in particular from vaginal transudate, cervical and endometrial secretions, and vaginal and cervical desquamated epithelial cells. These proteins, secreted partly by the vaginal epithelial and stromal cells and by cells of the immune system, and partly by the uterus, are involved in first host defense and the reproductive function.[16,17] This study associated the expression of a panel of 48 different biomarkers in the cervico-vaginal washing samples with clinically recognized pregnancies, identifying factors predictive of endometrial receptivity, embryo implantation, and IVF outcomes. The increase in the concentration of two pro-inflammatory cytokines, IL-12p40 and $IFN\text{-}_{\alpha 2}$, and of the trophic factors, MIF and MCP-3, was found to be significantly associated with good outcome ART. IL-12 is a Th1 cytokine and regulates cell-mediated immune responses. In particular, its p40 subunit is essential for recruitment and activation of inflammatory cell types.[18] Previous studies have been conducted, examining the concentration of IL-12p40 in the follicular fluid and in endometrial secretions, and have shown that IL-12 is associated with a negative effect on oocyte and implantation, and can have cytotoxic effects at high levels.[19,20] Conversely, recent studies found a positive association between the concentration level of IL-12 and the quality of oocytes and embryos.[21] According to these data, we suggest a possible dose-dependent role of IL-12p40 in the reproduction, probably exercising its deleterious effects only at higher concentrations. $IFN\text{-}_{\alpha 2}$ is a cytokine with anti-viral, antiproliferative, and immunoregulatory properties. A positive role for $IFN\text{-}_{\alpha 2}$ in reproduction and a positive correlation between the level of

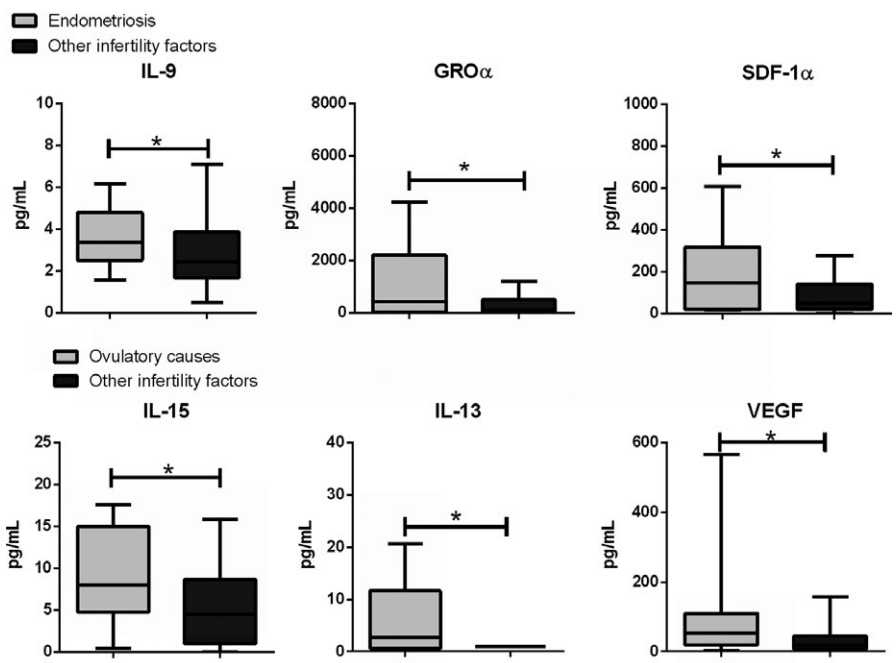

FIGURE 2 Expression of significant cervico-vaginal immune mediators associated with factors of infertility. The box plots show the expression of cytokine concentration found, in the cervico-vaginal fluid, associated with endometriosis and ovulatory factors of infertility. The significant results are marked with asterisks: $*P < 0.05$

TABLE 4 Expression of significant immune mediators in cervico-vaginal fluid from patients with and without ovulatory dysfunctions

Cytokines	Ovulatory dysfunctions present (n. 20)	Ovulatory dysfunctions absent (n. 79)	OR (CI)	*P* value
IL-15	8.2 (4.7-16.01)	4 (0.90-8.6)	1.11 (1.01-1.21)	0.019
IL-13	2.9 (1.6-12)	0.97 (0.97-0.97)	0.99 (0.97-1.02)	0.02
VEGF	53.48 (19.74-110.1)	18.64 (6.82-45)	1.01 (1.001-1.02)	0.026

Values are expressed as medians and interquartile ranges. Odds ratios with confidence intervals and *P*-values are the result of bivariate logistic regressions.

this cytokine and the quality of oocytes, in the absence of the infection, have already been shown.[22] Therefore, our data reinforce the hypothesis of a possible involvement of this cytokine in the good outcome of pregnancy. Several studies have demonstrated a possible contribution of the trophic factor MIF in the cytokine network for embryo development, and this protein may be involved in defending the oocytes and embryos from oxidative stress, favoring embryo implantation and in the first phases of embryogenesis.[23,24] Monocyte chemotactic protein-3 (MCP-3) is a pro-inflammatory chemokine that regulates macrophage function and attracts the monocytes in the site of inflammation,[25] To date, no studies have been conducted on the role of this chemokine in female infertility.

Endometriosis is a well-known cause of infertility, although the pathophysiological mechanism by which it causes infertility remains not fully ascertained. Endometriosis is characterized by the growth of ectopic lesions that stimulate infiltration of immune cells and the production of cytokines from endometriotic cells, inducing a progressive and chronic state of inflammation.[26,27] For this reason, in our study, we examined the possible association between the cytokine profile from cervico-vaginal washing and endometriosis as a cause of infertility. Interleukin 9, in combination with the immune factors, Gro_{α} and SDF-1_{α}, was found to be significantly overexpressed in the presence of endometriosis. IL-9 is a generally pleiotropic cytokine, with both pro-inflammatory and regulatory inflammatory functions, depending on the context.[28] It has been demonstrated that this cytokine is produced by Treg cells that might regulate the immune response, inducing an inflammatory tolerance state in the endometriotic tissue.[29,30] Regarding Gro_{α} and SDF-1_{α}, these trophic factors, as suggested by previous studies, might play an important role in the progression of endometriosis, in chronic inflammation and influence embryo implantation.[31-34]

It is known that the ovulatory dysfunction is a strong risk factor for pregnancy complications and the adverse pregnancy outcomes.[35,36]

In our study, a significant increase in the concentration levels of IL-15 and IL-13 was found associated with an ovulatory cause of infertility. Previously, it has been reported that IL-13 concentrations in follicular fluid (FF) were significantly higher in the patients with PCOS (polycystic ovary syndrome) than in the normally ovulating women.[37] No data are available for IL-15 levels in cervico-vaginal fluid or FF, although it has been observed that circulating levels in the blood of subjects with normal cycle and PCOS were not different.[38] High levels of IL-15 and IL-13 in the reproductive tract of women with implantation failure and recurrent spontaneous abortions have been observed.[39,40] Accordingly, in the present study, the concentration of these cytokines was found lower in the cervico-vaginal fluid of women with clinical pregnancy respect to women with no clinical pregnancy, but the differences were not significant (Table 2).

Experimental studies have shown that VEGF plays important roles during implantation.[41] In our population, VEGF expression was found down-regulated in the cervico-vaginal fluid of women with clinical pregnancy compared to women with no clinical pregnancy, but the differences in concentration were not statistically significant. Conversely, a significant association of high VEGF levels and ovulatory infertility factor was observed. This finding agrees with several studies that suggest an important role of VEGF in the pathophysiology of ovarian dysfunction such as the polycystic ovary syndrome.[42] However, our results on the association between cytokine profile and endometriosis or ovarian dysfunction are preliminary due to the small number of women with these pathologies.

In conclusion, in this pilot study, a wide range of cytokines was explored in the cervico-vaginal fluid of women undergoing IVF, with the aim to select some possible "fertility-related" proteins associated with the outcome of IVF procedure. Analysis of cervico-vaginal secretion cytokines is a new, non-disruptive, and non-invasive approach to study the endometrial receptivity in IVF management. We showed that the expression of specific cytokines and growth factors in the cervico-vaginal washing on the day of oocyte retrieval is significantly associated with the potential clinical pregnancy. IL-12p40, IFN-α_2, MIF, and MCP3 expression seems to be crucial for implantation and could be considered as potential surrogate clinical markers of endometrial receptivity. If confirmed in a larger cohort, the assessment of the concentration of these cytokines in the cervico-vaginal fluid, before the embryo transfer, might be useful to improve the efficacy of IVF in the identification of a "window of implantation," and the subsequent timing of embryo transfer, thus reducing the time and cost of the procedures.

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CONFLICT OF INTEREST

All authors disclose no potential sources of conflict of interest.

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