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Correlations between concentrations of interleukin-12 and interleukin-13 and lymphocyte subsets in the follicular fluid of women with and without polycystic ovary syndrome

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Objective: To investigate a possible correlation between interleukin-12 (IL-12) and IL-13 levels and lymphocyte subsets in the preovulatory follicles of patients with and without polycystic ovarian syndrome (PCOS).

Design: Controlled clinical study.

Setting: University hospital.

Patient(s): Seventy-eight infertile women undergoing IVF-embryo transfer.

Intervention(s): The subjects underwent blood sampling, ovum retrieval, and embryo transfer.

Main Outcome Measure(s): Follicular fluid levels of T, androstenedione (A); IL-12, IL-13, activated T cells, T helper, and T-suppressor lymphocytes.

Result(s): The level of IL-12 detected in follicular fluid (FF) was significantly lower in patients with PCOS than in normally ovulating women (mean: 1.47 ± 0.3 pg/mL vs. 2.25 ± 0.7 pg/mL, respectively); in contrast, FF IL-13 concentrations were significantly higher in the patients with PCOS than in the normally ovulating women (mean: 32.5 ± 3.7 pg/mL vs. 19.6 ± 2.5 pg/mL, respectively), as was the total number of activated T lymphocytes (11.5% ± 1.5% vs. 4.8% ± 0.4%). A significant correlation was observed between FF activated T-cell concentrations and FF IL-12, IL-13, T, and A levels. No significant differences were observed when these data were compared with embryological parameters.

Conclusion(s): The present study shows significant differences in the correlation between FF IL-12 and IL-13 levels and T lymphocyte numbers in the subset of patients with PCOS as compared to normally ovulating women. (Fertil Steril® 2003;79:1365–72. ©2003 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovarian syndrome, immunity, cytokines, IVF-embryo transfer, pregnancy rate

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Polycystic ovary syndrome (PCOS) is a common disorder of premenopausal women characterized by clinical and biochemical features (1, 2). The current recommended diagnostic criteria for PCOS are hyperandrogenism and ovulatory dysfunction with the exclusion of specific disorders such as congenital adrenal hyperplasia (1). Polycystic ovaries display increased ovarian stroma and eight or more subcapsular follicular cysts ≤10 mm in diameter (2), as detected by ultrasound diagnosis; they are consistent with, but not essential for, the diagnosis of the syndrome (1–3). Although the etiology of PCOS still remains unclear, it is considered to be a familial condition in which a genetic disorder of androgen biosynthesis may be associated with other genetic factors such as insulin resistance (4, 5), dysregulation of cytochrome P450c17 (6, 7), and polymorphism of the steroid metabolism gene CYP17 (8). Moreover, there are findings in the literature showing that the heterogeneous nature of PCOS could be explained by the interaction of this genetic disorder with environmental, endocrine, and paracrine/autocrine factors (9, 10).

Recently, conflicting evidence of possible associations of ovarian antibodies with PCOS has been reported (11, 12). The presence of significant amounts of cytokine-target immunocompetent cells such as monocyte/macrophages, B cells and T cells (9, 13), as well as active cellular interactions among T lymphocyte subpopulations, have been described in human preovulatory follicles (13–15). These cells seem to exert an effect in preventing the development of autoimmune diseases in the ovary (16), in the diagnosis of idiopathic infertility (17), and also in the immunoregulation of hormones involved in folliculogenesis (16). Investigators have demonstrated that some interleukins (IL), namely IL-1, IL-2, IL-6, IL-8, IL-11, and leukemia inhibitory factor produced by immunocompetent cells in vitro, inhibit steroidogenesis and are, in turn, inhibited by the granulosa luteal cells (16–21). Moreover, IL-1, IL-2, IL-6, and other cytokines have been shown to play an important role in ovulation and corpus luteum function (17, 18, 21). In particular, Macciò and colleagues (22) showed that the immunosuppressive activity of the follicular fluid (FF) is mediated by the specific inhibition of IL-1 and IL-2 production through the decreased expression of the IL-2 receptor p55 subunit. The elevated levels of CD11b+, CD16+, and CD4+ helper lymphocytes in FF observed by the same researchers suggest that they could be involved in other immune processes (22).

More recently, two interleukins, IL-12 and IL-13, have attracted the attention of scientists from different research fields for their paradigmatic and opposing properties in reproductive physiology. Interleukin-12 is a heterodimeric cytokine composed of a 35-kD chain (p35) and a 40-kD (p40) chain, produced by phagocytes and B lymphocytes (23). It is characterized by two primary properties: one has the capacity to enhance pro-inflammatory cytokine synthesis by activated macrophages and monocytes in different tissues (24); the other takes part in the activation of NK and cytotoxic T lymphocytes, in the differentiation of CD4+ and CD8+ lymphocytes and in the enhancement of interferon-γ production (25). Interleukin-13, in contrast, is a cytokine with pleotropic functions, most of which are shared with IL-4 (26). It inhibits macrophagic and lymphocyte activation, thereby stopping interferon (INF) activator factors; it increases the production of adhesion molecules on the endothelium (VCAM-1) (26) and, finally, it down-regulates prostaglandin (PG) and E_2 production (27). Women affected by endometriosis have a lower concentration of IL-13 in their peritoneal fluid than do patients without endometriosis (28).

With these experimental findings in mind, the aims of the present study were: [1] to investigate possible relationships between IL-12 and IL-13 levels in FF and between IL-12/IL-13 levels and FF lymphocyte subsets in patients undergoing assisted reproductive technologies (ART); [2] to compare these findings with biological parameters observed during ART cycles; and [3] to evaluate possible differences between patients undergoing ART for idiopathic infertility and those affected by suspected immune-linked pathologies, such as PCOS (9, 11–15).

MATERIALS AND METHODS

Patient Characteristics

Seventy-eight infertile women undergoing IVF-embryo transfer or intracytoplasmic sperm injection (ICSI) were enrolled in the study (age range: 25–37 years). The ICSI was performed exclusively for severe oligoasthenoteratozoospermia (Kruger criteria: concentration $<5 \times 10^6$; progressive motility <20%; normal morphology <20%). Thirty-eight patients enrolled in the study were normally ovulating women (group A), whereas 40 patients were affected by PCOS (group B).

Before admission to the study every woman underwent clinical and psychological examination and transvaginal ultrasonography. Moreover, hormonal evaluation included [1] measurement of baseline levels of E_2, androstenedione (A), P, T, and cortisol; [2] an LH pulsatility study as previously described (29); [3] assessment of LH release after GnRH stimulation (30); and [4] plasma androgen levels after dexametasone suppression (30). The criteria followed for inclusion in group A were absence of endocrinological disorders of the pituitary or ovary, such as hyperprolactinemia, hypogonadotropic hypogonadism, premature ovarian failure and premature menopause, or of abnormal adrenal or thyroid function.

The criteria for PCOS presented at the 1990 National Institutes of Health–National Institute of Child Health and Human Development conference on PCOS and at the 1995 Serono Symposium on PCOS were followed for the enrolment of patients in group B.

Briefly, PCOS was diagnosed by presence of amenorrhea or oligomenorrhea and hirsutism as assessed by the Ferriman-Gallwey score (31) with chronic ovulatory dysfunction (menstrual cycle >45 days in length, or luteal phase P level <2 ng/mL), plasma androgen values at the upper limit or above the normal range (T level: 0.17–0.58 ng/mL; A level: 0.57–1.7 ng/mL), and the presence at ultrasound diagnosis of bilaterally enlarged or normal-sized ovaries with 8–10 subcortical microcysts (<5 mm in diameter). A normal LH:FSH ratio was not considered an exclusion criterion (32). The body mass index (BMI) was also calculated for each woman enrolled in the study. The mean (SD) BMI of patients was 29.21 ± 5.3 kg/m^2, whereas the mean BMI of controls was 23.18 ± 5.71 kg/m^2 ($P<.05$). Obesity was defined as having a BMI of >25 kg/m^2.

The ART was used in patients without PCOS because of tubal obstruction in 15 women, oligoasthenoteratozoospermia in 20, and idiopathic infertility in 3. In the latter three patients the difficulty in becoming pregnant was probably related to a female age >37 years, despite the fact that serum FSH levels and ovarian antibodies concentrations (33) were

in the normal range. No subject from either of the two groups had undergone pharmacological treatment for infertility in the 6 months before the study, whereas for 45 of them this was their first attempt at ART. Implantation was accepted where the serum hCG was >100 IU/L 18 days after embryo transfer. The study protocol was approved by the Institutional Review Board of the University of Modena. All biological analyses were carried out by the same investigators (I.C. and M.S.), who were not told to which group each patient belonged.

Induction of Superovulation

To permit a statistical comparison between the two groups of subjects, all patients underwent the same standard protocol of superovulation using purified FSH and hCG after ovarian desensitization by a GnRH-analogue (GnRH-a). Patients were given GnRH-a (Enantone 3.75, Takeda, Milano, Italy) starting on days 15–20 of the menstrual cycle. Fourteen to 20 days were needed for complete ovarian suppression, as assessed by serum E_2 concentrations (<50 pg/mL; conversion factor to SI unit, 3.671) and by ovarian ultrasonography (no follicles >10 mm in the healthy controls). When the suppression criteria were satisfied, purified FSH (75 IU FSH, Metrodin HP, Serono, Rome, Italy) treatment was started. The patients received purified FSH (2 ampules, 150 IU/day) from day 1–5 of the menstrual cycle. From day 6 of the menstrual cycle, the ovarian response was monitored daily by transvaginal ultrasonography and serum E_2 assays. The dosage of purified FSH was adjusted for each woman according to the ovarian response, as judged by the serum E_2 level and follicular growth. When one or more follicles >17 mm in diameter and serum E_2 levels of 200 pg/mL per follicle >15 mm in diameter were obtained, 10,000 IU IM hCG was administered. Thirty-four to 36 hours after hCG administration, oocytes were retrieved by ultrasound-guided transvaginal aspiration. The retrieved oocytes were classified as mature (preovulatory), intermediate, immature, or atretic on the basis of their morphology and the appearance of the oocyte–cumulus–corona complex, according to the criteria of Garcia et al. (34). Sperm processing, insemination or ICSI, and embryo transfer were performed using standard techniques (35). Up to three embryos were transferred 48 hours after oocyte retrieval and the remaining embryos, if present, were cryopreserved. All patients undergoing embryo transfer received IM supplemental P (50 mg/day for 18 days; Prontogest, AMSA, Milano, Italy). Eighteen days after embryo transfer, a blood sample was obtained for β-hCG assay. Pregnancy was confirmed ultrasonically 4 weeks after oocyte retrieval.

Preparation of Samples

Follicular fluids were collected from the 78 women undergoing IVF-embryo transfer or ICSI treatment as described above. The volumes of FF obtained from follicles >18 mm in diameter were a mean of 4.1 ± 0.4 mL and 3.7 ± 0.3 mL per follicle in groups A and B, respectively. Only samples of FF not contaminated by visible blood or aspiration buffer, and stored in sterile conditions, were used in the subsequent assessments.

Cell Isolation

After centrifugation at 1,200 rpm for 10 minutes, the cells were separated from the supernatant and then resuspended in 2 mL of RPMI-1640 medium (ICN, Milano, Italy) supplemented with 10% (v/v) heat-inactivated fetal calf serum (ICN), 2% L-glutamine, and 1% penicillin-streptomycin (36). Means of $1.8 \pm 0.4 \times 10^6$ and $1.7 \pm 0.2 \times 10^6$ cells per follicle were recovered for groups A and B, respectively, as assessed by hemocytometric cell counting.

Antibody Labeling and Flow Cytometry

The surface antigens of follicular leukocytes were analyzed with a panel of monoclonal antibodies (mAbs) directly conjugated with different fluorochromes (four-color direct immunofluorescence MultiTEST CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC, Becton Dickinson Italia, Milan, Italy) (37). In particular, mAbs, directly conjugated with different fluorochromes, were used to analyze the surface antigens of follicular lymphocytes: anti-CD45 peridinin chlorophyll protein (PerCP [pan leukocyte]), anti-CD3 fluorescein isothiocyanate (FITC [pan T cell]), anti-CD8 phycoerythrin (PE [suppressor-cytotoxic T cell]), anti-CD4 allophycocyanine (APC [helper-inducer T cell]). A standard technique was used to identify the labeling of cells with this panel of mAbs (37).

Briefly, mAbs were incubated with 5×10^5 cells for 30 minutes at 4°C, washed twice, and fixed in 0.1% *p*-formaldehyde (ICN). The immunofluorescence reactivity of 10^4 cells/sample was then analyzed by cytofluorometry using an argon laser operating at an excitation wavelength of 488 nm with an intensity of 200 mW (Facstar, Becton Dickinson Italia). An isotype-matched, nonreactive, directly conjugated mAb was used to determine background fluorescence, which was <2% in all analyses performed. Non-CD45 cells were gated out of the analysis. Morphological confirmation was finally carried out by cytocentrifuging on glass slides and by Wright-Giemsa staining of the cells analyzed by cytofluorimetry. The lymphocyte content, evaluated under the optical microscope (×400), was constant in all the samples analyzed.

Flow Cytometric Analysis

As reported, FF leukocytes were directly conjugated and labeled with mAbs. To ensure that the combination of forward and side scatter used for the analysis did not exclude a significant proportion of leukocytes, a pan leukocyte mAb (anti-CD45) was used for labeling FF cells and back-gating was performed (38).

Wright-Giemsa staining was subsequently used to identify the sorted cells. The CD45- cells were primarily granulosa cells, whereas the CD45+ cells were mostly leukocytes (data not shown). The mean percentage of CD45+ cells

(>74% in each group of subjects) was measured to evaluate the proportion of leukocytes in each sample. The target antigen expression by labeling cells was then evaluated using FITC-, PE-, and APC-conjugated mAbs. Thereafter, we were able to measure the percentage of the different T lymphocytes present in the FF samples. All antigens analyzed were measured according to the number of CD45+ cells. Activated T lymphocytes (HLA-DR+ T lymphocytes) were detected using a two-color direct immunofluorescence reagent (Simultest CD3/Anti-HLA-DR; Becton Dickinson) with a gate based on lymphocyte scatter parameters (39).

Enzyme-Linked Immunosorbent Assays

Interleukins were evaluated in a total number of 100 follicles from each group of patients (control group, mean: 2.6 follicles per patient; PCOS group, mean: 2.5 follicles per patient). Both FF IL-12 and IL-13 concentrations were estimated using ELISA, performed with commercially available immunoassay kits (IL-12, Biosource International, Camarillo, CA; IL-13, Endogen, Woburn, MA). In the present study we followed the protocol previously described by Naz and Evans (40) for IL-12 detection and the manufacturer's instructions without significant variations for IL-13 analysis. Because the levels of IL-12 and IL-13 were directly proportional to the intensity (absorbence) of the resultant color of the reaction mixture (read at 450 nm), two Scatchard-plot curves were compared with the known standards to estimate the IL-12 and IL-13 concentrations present in each sample.

Inter- and intra-assay variability was 6.8% and 7.9% for IL-12 and 6.7% and 7.8% for IL-13, respectively. Fifty microliters of pools of three samples per patient was used for analysis. Samples were run in duplicate on two different days, and the mean value, expressed as picograms per milliliter of FF, was calculated. The control specimens were obtained from egg donors not enrolled in the study but respecting all the clinical and biological parameters of group A. The ELISA kits used were highly specific and ultrasensitive; they did not cross-react with any other human IL or with any other cytokine tested and their lower limits of detection (sensitivity) were <1 pg/mL for IL-12 and <7 pg/mL for IL-13.

Steroid Hormone Assays

As reported for ILs, steroids were evaluated in a total number of 100 follicles from the PCOS group and in 100 from the normally ovulating women group. Estradiol, A, and T were measured by RIA using tritiated tracers and charcoal separation (materials purchased from Radim, Rome, Italy). Intra- and interassay variation coefficients were 5.8% and 6.4%, respectively (41).

Comparison and Statistical Analysis

Immunological data comprise the FF counts of white blood cells (WBCs), total B and T cells, activated and nonactivated helper and suppressor T lymphocytes. Embryological parameters comprise the total number of ovarian follicles, the total number of mature, immature, degenerate, inseminated, and fertilized oocytes, and, finally, the total number of transferred embryos.

An unpaired Student's *t* test was used for comparison between groups (means ± SD). The linear regression analysis was performed between IL-12, IL-13, T cells, and FF hormone levels, as well as between these parameters and embryological or clinical ART variables. Statistical differences were considered significant for values of $P<.05$.

RESULTS

The two groups of patients analyzed were similar with regard to education level, age, years of infertility, and parity. On the day of oocyte retrieval, significantly lower FF IL-12 levels were detected in patients with PCOS than in the control group (mean: 1.47 ± 0.3 pg/mL vs. 2.25 ± 0.7 pg/mL, respectively; $P<.05$), whereas FF IL-13 concentrations were significantly higher in the former group than in the latter (mean: 32.5 ± 3.7 pg/mL vs. 19.6 ± 2.5 pg/mL, respectively; $P<.01$) (Fig. 1). Moreover, on the day of ovum pick-up, a higher number of activated T lymphocytes (mean: 11.5% ± 1.5% vs. 4.8% ± 0.4%, respectively; $P<.001$) was observed in group A than in group B (Fig. 2A). On average, the percentage of FF T helper lymphocytes (CD4+) was significantly higher in group A than in group B (48.2% ± 5.1% vs. 42.4% ± 4.8%; $P<.05$), whereas FF T suppressor lymphocytes (CD8+) were slightly but significantly higher in group B than in group A (36.2% ± 3.4% vs. 31.1% ± 3%; $P<.05$) (Fig. 2B). These changes determined an increase in the IL-13/IL-12 ratio and T suppressor/T helper ratio in the PCOS group (1.57 vs. 1.20 and 1.61 vs. 1.24, respectively; $P<.05$), but not in the control group.

Concerning the sex steroid hormone levels, FF T and A concentrations on the day of ovum pick-up were significantly higher in group B than in group A (T = 10.2 ± 1.2 ng/mL vs. 3.6 ± 0.7 ng/mL, $P<.01$; A = 47.1 ± 4.6 ng/mL vs. 20.3 ± 2.8 ng/mL, $P<.01$). Patients with PCOS showed an inverse correlation between FF androgen levels and concentrations of activated T cells or IL-12 (T and A vs. activated T cells: $r = -0.43$ and $r = -0.45$, respectively, $P<.01$; T and A vs. IL-12: $r = -0.41$ and $r = -0.4$, $P<.01$); on the other hand, in the same group of women, the FF androgen levels and FF IL-13 concentrations were positively correlated (T and A vs. IL-13: $r = 0.73$ and $r = 0.7$, $P<.01$). In addition, we observed that both groups A and B were characterized on the one hand by an inverse correlation between FF activated T cell number and IL-13 levels ($r = -0.51$ and $r = -0.48$, respectively, $P<.01$), and on the other hand by a positive correlation between FF activated T cell number and IL-12 concentrations ($r = 0.63$ and $r = 0.67$, respectively, $P<.01$) (Fig. 3).

No significant difference or correlation was detected when FF IL-12 and FF IL-13 and T cell levels were com-

FIGURE 1

Interleukin (IL)-12 and IL-13 levels in follicular fluid (FF). Values are reported as mean ± SD. *$P<.05$; **$P<.01$. FF IL-12 levels were significantly lower in patients with polycystic ovarian syndrome (PCOS) than in normally ovulating women (NOW) (mean: 1.47 ± 0.3 pg/mL vs. 2.25 ± 0.7 pg/mL, respectively; $P<.05$); in contrast, FF IL-13 concentrations were significantly higher in the PCOS than in the NOW group (mean: 32.5 ± 3.7 pg/mL vs. 19.6 ± 2.5 pg/mL, respectively; $P<.01$).

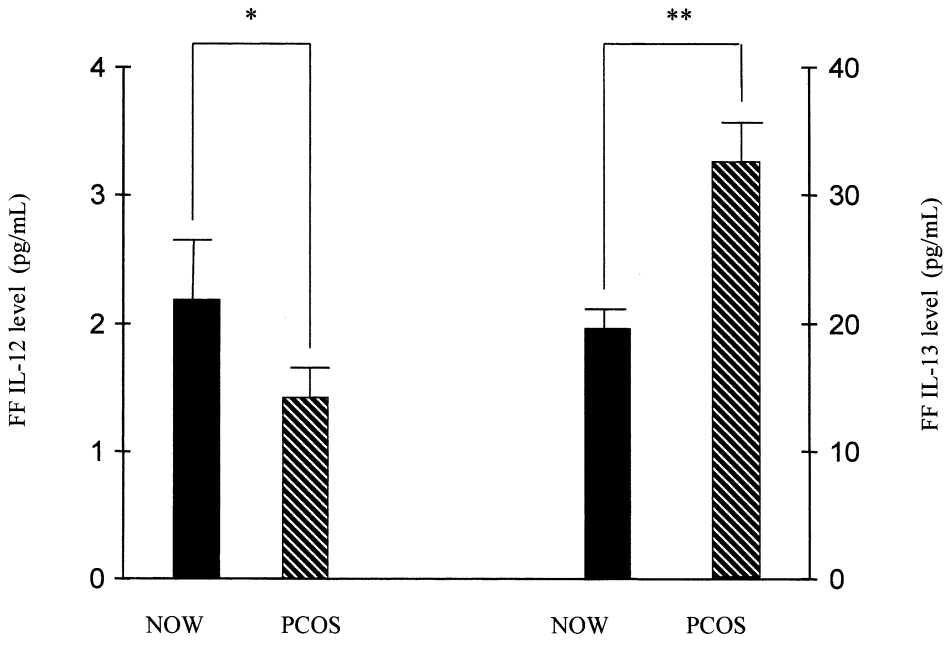

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FIGURE 2

(**A**) Activated T-lymphocyte levels in follicular fluid (FF). Values are expressed as percent of cell concentration (mean ± SD). ***$P<.001$. The total number of activated T lymphocytes detected in FF of normally ovulating women (NOW) was significantly higher than that observed in women with polycystic ovarian syndrome (PCOS) (11.5% ± 1.5% vs. 4.8% ± 0.4%; $P<0.001$). (**B**) Follicular fluid T-helper and T-suppressor cell levels. Values are expressed as percent of cell concentration. *$P<.05$. On average, the percentage of FF T-helper lymphocytes was significantly higher in NOW than in in women with PCOS (48.2% ± 5.1% vs. 42.4% ± 4.8%; $P<.05$, panel **A**), whereas FF T suppressor lymphocytes were significantly higher in women with PCOS than in NOW (36.2% ± 3.4% vs. 31.1% ± 3%; $P<.05$, panel **B**).

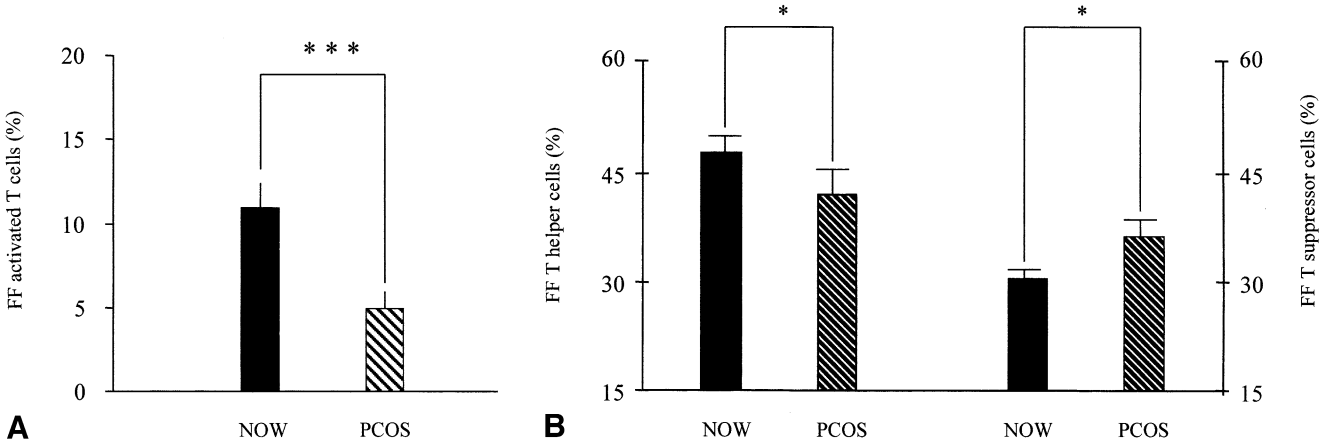

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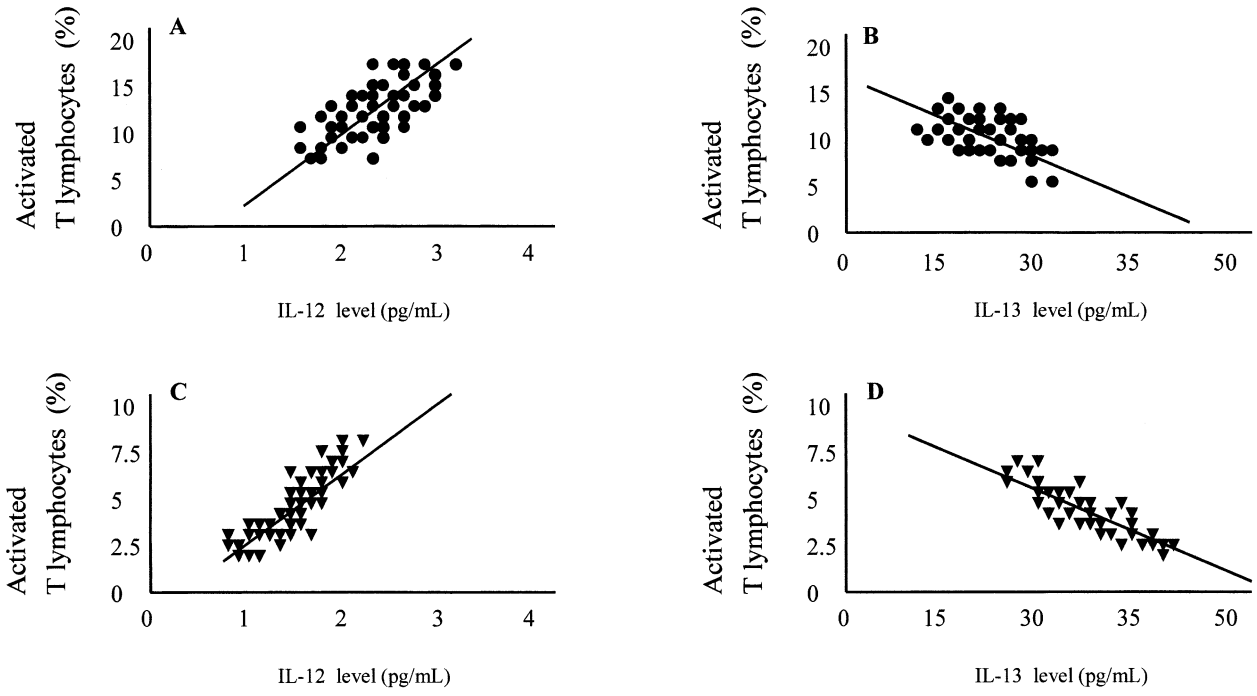
FIGURE 3

Correlations between follicular fluid (FF) activated T lymphocyte cells (ATC) amounts and FF interleukin (IL)-12 and IL-13 levels in the normally ovulating women (NOW) (dots) and in patients with PCOS (triangles). Values of the ATC are percent of cell concentration. A positive correlation between FF ATC and IL-12 concentrations ($r = 0.63$ and $r = 0.67$ for NOW and PCOS, respectively; $P<.01$; **A** and **B**) and, in contrast, an inverse correlation between FF ATC amounts and IL-13 levels ($r = -0.51$ and $r = -0.48$ for NOW and PCOS, respectively; $P<.01$; **C** and **D**).



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pared with other clinical or epidemiological parameters or embryological variables, such as the age of the patient, total FSH IU used, number of ovarian follicles obtained, characteristics of the oocytes, oocyte fertilization rate, and pregnancy rate (Table 1).

DISCUSSION

In the present study, we observed a significant decrease in IL-12 levels and a significant increase in IL-13 concentrations in the FF of patients with PCOS with respect to normally ovulating women on the day of ovum pick-up. In addition, we observed that the total number of FF-activated T lymphocytes as well as the FF T helper/T suppressor ratio were significantly higher in group A than in group B. Finally, both group A and group B displayed a positive correlation between FF activated T cells and FF IL-12 levels and, in contrast, an inverse correlation between FF activated T cells and FF IL-13 concentrations.

TABLE 1

Patient demographics, FF interleukin levels, and FF lymphocyte concentrations.

Variable	NOW	PCOS
Mean (± SD) age (y)	31.5 ± 0.8	32.1 ± 0.3
Mean (± SD) of uFSH (IU)	1,552.7 ± 98.6	1,517.8 ± 101.78
Mean (± SD) number of oocytes recovered	8.3 ± 0.5	7.8 ± 0.8
Mean (± SD) percentage fertilization rate	71.2 ± 10	67.5 ± 7.8
Mean (± SD) number of transferred embryos	2.8 ± 0.6	3.1 ± 0.3
Mean (± SD) percentage pregnancy rate	36.8 ± 4.1	33.9 ± 3.8

Note: P is not significant. NOW = normally ovulating women.

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In the past several reports have supported a role for an IL-regulated network in follicologenesis (16–21), but the clinical involvement of IL-12 and IL-13 in the reproductive system has only been shown more recently. Somigliana and colleagues (24) demonstrated that IL-12 promotes NK cell-mediated responses toward endometrial antigens, giving rise to an enhanced prevention of ectopic endometrial implantation in vitro; Naz and Evans (40) observed a significant correlation between the IL-12 concentrations and the morphology of the human sperm. Finally, IL-12 has been detected in human FF (42). Coskun and colleagues (42) in fact measured lower FF IL-12 levels in preovulatory than in immature follicles, whereas Gazvani et al. (43) demonstrated a marked association between the presence of IL-12 in the FF and the incidence of fertilization failure. Neither of these investigators observed any significant correlation between IL-12 concentrations and the cycle outcome.

The possible role of IL-13 in human reproduction is still largely unknown. It is weakly expressed in first-trimester human chorionic villi (44), but has not yet been detected in amniotic fluid (45). Significantly reduced amounts have been observed in the peritoneal fluid of patients with endometriosis, but no cycle-dependent variations in the immunolocalization of IL-13 have been observed (28). This suggests that peritoneal fluid IL-13 levels are not regulated by ovarian steroids.

Our findings suggest that IL-12 and IL-13, as previously demonstrated for other cytokines such as IL-1, IL-2, and IL-8 (16), may work in an interactive manner that involves cross-talk through a network of processes to regulate female fertility in a negative or positive way. The findings reported in the present study, concerning IL-12, IL-13, and T cells, appear to be independent events, because, on the one hand, inverse correlations were observed between FF androgen, activated T cells, and IL-12 concentrations; whereas, on the other hand, positive correlations were demonstrated between FF androgen, activated T cells, and FF IL-13 levels.

Our data permit speculation about the possibility of a simultaneous decrease in the IL-12/IL-13 and T helper/T suppressor ratios in the FF of subjects with PCOS, playing a role in the structural changes that are now considered a necessary step for preantral follicle rupture (16, 19). In other words, the abnormal levels of T cells and ILs observed could alter the mechanisms that normally cause the release of proteolytic enzymes, such as collagenase and elastase, which digest extracellular matrix proteins and thereby cause follicular rupture and ovulation.

Regarding a possible correlation between FF IL-12 levels and clinical parameters, our results do not confirm the data of Gazvani et al (43). In fact, we were unable to find a significant correlation either between fertilization rate and FF IL-12 concentrations or between FF lymphocyte subsets or IL-13 levels and any clinical or biological parameters analyzed in both groups of patients (Table 1). Because both studies used standard techniques and protocols to perform IVF-embryo transfer, this discrepancy may be due to technical variations in cytokine measurement due, for example, to the different sensitivity of the ELISA system used. Moreover, we did not pool the FFs, but tested each FF separately. Further studies are required to clarify this point.

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