Ovarian follicular concentration of IL-12, IL-15, IL-18 and p40 subunit of IL-12 and IL-23

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BACKGROUND: The aim of the study was to determine the presence of interleukin (IL)-12, IL-15, IL-18 and p40 subunit of IL-12/IL-23 in follicular fluid from spontaneous cycles and the relation between the concentration of selected cytokines and IVF–embryo transfer outcome. METHODS: IVF–embryo transfer and enzyme immunoassay (EIA) (R&D Systems, Minneapolis, MN, USA and MBL, Nagoya, Japan) were used. RESULTS: Follicular fluid of women included in the IVF–embryo transfer procedure contained common p40 subunit of IL-12/IL-23 (median 70.1 pg/ml), IL-15 (median 1.3 pg/ml) and IL-18 (median 38.2 pg/ml). There was a significant negative correlation between follicular fluid concentrations of IL-15 and IL-18 (R = –0.392, P = 0.003). Significantly higher concentrations of common p40 subunit of IL-12/IL-23 (median 79.8 pg/ml) were found in the follicular fluid taken from follicles containing oocytes, when compared with those without an oocyte (median 44.5 pg/ml, P = 0.006). Patients who achieved clinical pregnancy had significantly decreased concentration of IL-15 (median 0.8 pg/ml) compared with patients without successful IVF–embryo transfer outcome (median 1.4 pg/ml, P = 0.047). CONCLUSION: Follicular fluid collected from spontaneous cycles contains detectable levels of p40 subunit of IL-12/IL-23, IL-15 and IL-18. Increased concentrations of p40 subunit of IL-12/IL-23 in follicles containing oocytes suggest an important role of this cytokine in reproduction. Possible negative value of IL-15 as a predictor of IVF–embryo transfer success remains to be determined.

Key words: cytokines/follicular fluid/IL-12/IL-15/IL-18/subunit p40 IL-12 and IL-23

Introduction

Follicular fluid contains a variety of autocrine and paracrine factors responsible for the regulation of oocyte development, folliculogenesis and ovulation. The complex composition of the regulatory factors (hormones, cytokines and growth factors) and their concentration in the follicular fluid has an influence, either direct or indirect, on oocyte viability and development potential (Mendoza et al., 1999, 2002).

The influence of the immune system on various aspects of reproduction, especially on the reproductive outcome, has been extensively studied. There is growing evidence that the interactions between the immune and endocrine systems can influence ovarian function. Consequently, the composition of regulatory factors in the follicular fluid is indirectly linked to fertilization and early embryonic development (Mendoza et al., 2002; Hammadah et al., 2003, 2004).

Resident and infiltrating leukocytes are the principal sources of cytokine synthesis into follicular fluid and surrounding tissue. Additionally, ovarian somatic cells, including luteal, stromal, thecal and granulosa cells, are an important cellular source of cytokines as well. Cytokines play an important role in the regulation of ovarian function, gonadal steroid secretion, corpus luteum function, embryo development and implantation.

Many studies demonstrated the presence of various cytokines [interleukins (ILs), interferons (IFNs), chemokines, tumour necrosis factors (TNFs), colony-stimulating factors (CSFs) and growth factors] in follicular fluid (Ledur et al., 1995; Aziz et al., 1999; Whiteside, 2002; Židovec et al., 2004). However, due to heterogeneous and sometimes conflicting results of studies using commercially available enzyme-linked immunosorbent assays (ELISAs) by different manufacturers, the true predictive value of cytokines for IVF–embryo transfer outcome is unclear (Vujisić and Židovec, 2004). To our knowledge, there is no experimental evidence of the presence of IL-3, IL-4, IL-5, IL-7, IL-9, IL-14, IL-15, IL-16, IL-17, IL-19 and IL-29 in follicular fluid.

This study focused on the four cytokines: IL-12, IL-15, IL-18 and p40 subunit of IL-12/IL-23.

IL-12 is a pleiotropic type 1 cytokine that mediates several biological activities on human T cells and natural killer (NK) cells, including induction of IFN-γ production, enhancement of
cell-mediated cytotoxicity and comitogenic effects on resting T cells (Langrish et al., 2004). This proinflammatory heterodimeric cytokine shares important biological features with recently characterized cytokines IL-23 and IL-27.

IL-23 is a heterodimeric cytokine composed of a unique p19 subunit and a common p40 subunit shared with IL-12 (Langrish et al., 2004). This cytokine is important for the recruitment and activation of a whole range of inflammatory cells that are required for the induction of chronic inflammation and granuloma formation. Recent studies by Bettelli and Kuchroo (2005) noticed the importance of IL-23 for the proliferation of autoreactive pathogenic Th17 cells.

IL-15 is an IL-2-related cytokine, which induces T-cell proliferation and is an important regulator of NK cell functions (Fehniger et al., 1999; Fehniger and Caligiuri, 2001). IL-15 is structurally related to IL-2, IL-3, IL-6, IL-7, CSFs and growth hormone.

IL-18 is a member of the IL-1 family of cytokines with proinflammatory and tumour-suppressive properties (Lauwersy et al., 1999, 2000). Its ability to augment the synthesis of IFN-γ and regulate NK activity makes this cytokine an important immunomodulatory molecule. High levels of IL-18 can, in humans, be detected in acute and chronic viral or bacterial infections.

Earlier investigations on the cytokine composition of follicular fluid were on the basis of the analysis of stimulated cycles. Under those conditions, follicular fluid was analysed after exposure to exogenous hormones, which changed the natural composition of soluble factors in the follicle. Namely, the ability of hormones to influence the ovarian synthesis of various inflammatory mediators, including cytokines, has been already recognized. To avoid the influence of high doses of exogenous hormones on cytokine composition in follicular fluid, our study enrolled patients with spontaneous menstrual cycles.

This study was undertaken to determine whether human follicular fluid from spontaneous cycles contains IL-12, IL-15, IL-18 and p40 subunit of IL-12/IL-23 and to determine the relation between the concentration of selected cytokines and relevant reproductive parameters. To our knowledge, follicular fluid concentrations of IL-15 and p40 subunit of IL-12/IL-23 have not been previously investigated.

Materials and methods

Patients’ characteristics

This study included 40 married couples who entered the IVF/embryo transfer programme at the Department of Obstetrics and Gynecology, “Sveti Duh” Hospital, Zagreb, Croatia. The median age of female patients was 35 years (range 27–42 years).

Infertility in the 37 female patients of our study group was caused by tubal factor in 10 (tubal occlusion n = 3 and absent of tubae after ectopic pregnancies n = 7), male factor (n = 4), unexplained infertility (n = 5) and mixed cause of infertility (both female and male, n = 18). The three patients with endometriosis were analysed separately.

The results of routine haematological, serological, microbiological and molecular tests (differential blood count, sedimentation, routine serological assays for blood donors, presence of anaerobic and aerobic bacteria, human papillomaviruses and Chlamydia trachomatis in cervical swabs) did not reveal acute or chronic infections in our patients.

The basal serum level (on the third day of the menstrual cycle) of FSH, LH, estradiol (E2) and prolactin was measured by the commercially available MEIA (IMx; Abbott Diagnostic, Abbott Park, IL, USA).

LH surge was detected by Ovunos Premium immunochromatographic urinary test (Biognost doo, Zagreb, Croatia), according to manufacturer’s instruction.

Written consent for the use of follicular fluid samples obtained during oocyte recovery was provided by all patients. The ethics committee of the hospital approved this study.

Sample collection

Follicular fluid was taken after oocyte isolation and centrifuged at 600 g for 10 min at room temperature. Supernatant was stored at –80°C until cytokine quantification by enzymelinked immunosorbent assays (EILAs).

IVF–embryo transfer procedure

Forty patients with spontaneous menstrual cycles underwent the transvaginal ultrasound follow-up of follicular development from day 8. When a follicle reached 18 mm in diameter with the appropriate result of LH urinary test, ovulation was triggered by administration of 5000 IU hCG (Primogonyl, Schering AG, Berlin, Deutschland).

After recovery, oocytes were washed free from the follicular fluid. Maturity of the oocyte was assessed after mechanical dissection of cumulus oophorus and corona radiate. Oocytes were pre-incubated for 4 h in IVF medium with 5.0 mg/ml of human serum albumin (HSA) (SAGE In Vitro Fertilization, Trumbull, CT, USA) at 37°C in 5.8% CO2 and humidified air before insemination. Semen samples were washed by centrifugation at 300 g with Quinn’s Sperm Washing Medium (SAGE In Vitro Fertilization) and subsequently processed by swim-up method in the IVF medium with 5.0 mg/ml of HSA (SAGE In Vitro Fertilization). Each oocyte was inseminated with 40–80 × 105 motile sperm. Fertilization was monitored for about 20 h after insemination, and zygotes were placed into the IVF cleavage medium with 5.0 mg/ml of HSA (SAGE In Vitro Fertilization). If oocytes did not contain pronuclea, we checked fertilization once more after another 24 h. Embryos were graded (I–IV) on the transfer day according to their morphology by determining the number of blastomeres and the relative proportion of embryo volume occupied by anucleate cell fragments. Grade I embryos were the best and were defined as round and well-shaped blastomeres without fragments. Embryos with 10–20% fragmentation, with 20–30% fragmentation and with >30% fragmentation were referred to as grade II, III and IV, respectively. After evaluation, the embryos were placed in IVF blastocyst medium with 5.0 mg/ml of HSA (SAGE In Vitro Fertilization). Embryos were transferred after ultrasound evaluation of uterus and ovaries. Luteal phase support was accomplished with micronized progesterone (Utrogestan; Laboratories Piette International S.A., Brussels, Belgium) 600 mg/day starting from the day after oocyte retrieval.

Cytokine assays

Quantification of cytokines in follicular fluid was performed at the Division of Cellular Immunology, University Hospital for Infectious Diseases “Dr Fran Mihaljević”, Zagreb, Croatia, according to manufacturer’s instructions. Follicular fluid concentrations of IL-12, p40 subunit of IL-12 and IL-15 were determined using Quantikine human IL-12 Immunoassay, Quantikine human IL-12 p40 Immunoassay and Quantikine human IL-15 Immunoassay, respectively (R&D Systems, Minneapolis, MN, USA). IL-18 in the follicular fluid was quantified using Human IL-18 ELISA Kit (MBL, Nagoya, Japan). The minimum detectable doses of commercially available EILAs for IL-12, p40 subunit of IL-12/IL-23 and IL-15 were typically less than 5, 15 and 2 pg/ml, respectively. Although the minimum detectable dose for IL-15 was typically <2 pg/ml, there is no threshold value (the test is matrix dependent). For IL-18, minimum sensitivity was 12.5 pg/ml.
**Statistical analysis**

Statistical analysis was performed using Statistica, version 7.1 (StatSoft, Tulsa, OK, USA). The difference in immunologic parameters between individual independent groups was determined by Mann–Whitney U-test, and \( P < 0.05 \) was considered to be significant. Results of the cytokine quantification are presented as median and inter-quartile range (IQR).

**Results**

**Oocytes, embryos and pregnancy rate**

Of 40 follicular aspirations in the spontaneous cycles, we detected and isolated oocytes from follicular fluid in 28 cases. The general observations on oocytes and embryos were as follows: of 28 oocytes, 22 reached meiotic maturity (metaphase II), 4 were immature and 2 were degenerative. IVF resulted in the development of 18 two-pronucleated zygotes, and the fertilization rate was 81.81%. As for embryo morphology, four cleaving embryos were grade I, eight were grade II, five were grade III and one was grade IV at the same time of observation. Pregnancy rate per embryo transfer was 27.8%.

**Cytokines in the follicular fluid**

Follicular fluid of the 37 women included in the IVF–embryo transfer procedure contained common p40 subunit of IL-12/IL-23 (median 70.1 pg/ml), IL-15 (median 1.3 pg/ml) and IL-18 (median 38.2 pg/ml) (Table I). IL-12 was undetectable in the follicular fluid samples. There was a significant negative correlation between follicular fluid concentrations of IL-15 and IL-18 (\( R = -0.392, \ P = 0.003 \)) (Figure 1). Correlations between the concentrations of common p40 subunit of IL-12/IL-23 and IL-15 or IL-18 were not statistically significant.

Follicular fluid concentrations of selected cytokines were evaluated considering the presence of endometriosis in three patients. Concentrations of p40 subunit of IL-12/IL-23 in the patients with endometriosis were 82.2, 63.8 and 192.3 pg/ml; concentrations of IL-15 were 6.8, 16.1 and 18.8 pg/ml and concentrations of IL-18 were 28.7, 21.6 and 17.7 pg/ml. Owing to the small number of patients in the endometriotic group, the statistical analysis was not performed.

**Basal serum levels of FSH, LH, E2 and prolactin regarding oocyte presence**

Median basal serum level of FSH in patients with oocytes retrieved was 5.9 IU/l (range 4.1–7.39), whereas in the patients without oocytes, it was 5.03 IU/l (4.3–6.4). Median basal serum level of LH in the patients with oocytes was 3.8 IU/l (range 2.6–7.5), whereas in the patients without oocytes, it was 3.8 IU/l (range 2.1–5.9). Median basal serum level of E2 in the patients with oocytes was 157 pg/ml (range 83.1–278), whereas in the patients without oocytes, it was 153.7 pg/ml (range 112–270). Median basal serum level of prolactin in the patients with oocytes was 351 mIU/l (range 135–617), whereas in the patients without oocytes, it was 311 mIU/l (range 163–655). Serum concentrations of analysed hormones in the patients with and without oocytes were not significantly different.

**Follicular fluid cytokines and presence of oocyte**

Follicular fluid concentrations of selected cytokines were also evaluated considering the presence of an oocyte in the follicle (Table II). Significantly higher concentration of common p40 subunit of IL-12/IL-23 (median 79.8 pg/ml) was found in follicular fluid taken from follicles containing oocytes, compared with those without an oocyte (median 44.5 pg/ml, \( P = 0.006 \)). Concentration of IL-15 in follicular fluid taken from follicles containing an oocyte (median 1.3 pg/ml) and those without an oocyte (median 1.5 pg/ml) was not significantly different (\( P = 0.777 \)). Similarly, IL-18 concentration in the follicles that

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**Table I. Concentration of cytokines in follicular fluid of women undergoing IVF–embryo transfer (spontaneous cycles)**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cytokine concentration in follicular fluid (pg/ml) [median (inter-quartile range)]</th>
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<tbody>
<tr>
<td>IL-12</td>
<td>Not detectable</td>
</tr>
<tr>
<td>p40 subunit of IL-12 and IL-23</td>
<td>70.1 (44.6–91.4)</td>
</tr>
<tr>
<td>IL-15</td>
<td>1.3 (0.72–1.95)</td>
</tr>
<tr>
<td>IL-18</td>
<td>38.2 (25.1–50.3)</td>
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IL, interleukin.

**Table II. Cytokine concentration in follicular fluid with regard to the presence of oocyte**

<table>
<thead>
<tr>
<th>Presence of oocyte</th>
<th>Concentration of cytokines (pg/ml) [median (inter-quartile range)]</th>
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<tbody>
<tr>
<td></td>
<td>Common p40 subunit of IL-12 and IL-23</td>
</tr>
<tr>
<td>Yes (n = 28)</td>
<td>79.8* (58.3–113.3)</td>
</tr>
<tr>
<td>No (n = 9)</td>
<td>44.5 (28.6–57.9)</td>
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IL, interleukin.

*Statistically significant difference (\( P = 0.006 \)). Mann–Whitney \( U \)-test differences between the groups for IL-15 and IL-18 were not statistically significant.

Figure 1. Correlation analysis for p40 subunit of interleukin (IL)-12/IL-23, IL-15 and IL-18 in follicular fluid of women undergoing IVF (significant negative correlation between IL-15 and IL-18, \( R = -0.392; P < 0.05 \), Spearman’s rank order correlation).
contained (median 38.2 pg/ml) and those that did not contain (median 38.2 pg/ml) an oocyte was not significantly different ($P = 0.571$). We failed to observe significant correlations between the concentration of cytokines (p40 subunit of IL-12/IL-23, IL-15 and IL-18) in the follicular fluid and the serum hormone levels (FSH, LH, E$_2$ and prolactin).

**Follicular fluid cytokines and oocyte quality**

A possible association between the concentration of the selected cytokines and oocyte quality was also investigated. Median concentrations of p40 subunit of IL-12/IL-23 in follicular fluid of mature, immature and atretic oocytes were 86.4 pg/ml (IQR 62.5–113.6), 51 pg/ml (IQR 39.1–62.8) and 105.8 pg/ml (IQR 76.2–135.4), respectively.

Medians concentrations of IL-15 in follicular fluid of follicles with mature, immature and atretic oocytes were 1.3 pg/ml (IQR 0.7–2.2), 1.0 pg/ml (IQR 0.7–1.6) and 1.5 pg/ml (IQR 1.0–2.0), respectively.

Medians concentrations of IL-18 in follicular fluid of follicles with mature, immature and atretic oocytes were 38.2 pg/ml (IQR 26.0–55.5), 35.9 pg/ml (IQR 23.4–53.8) and 37.4 pg/ml (IQR 18.2–56.7), respectively.

Statistical analysis was not performed because of the small number of patients with immature and atretic oocytes.

**Follicular fluid cytokines and fertilization**

Concentration of p40 subunit of IL12/IL-23 in the follicular fluid of oocytes with subsequent successful fertilization (median 88.8 pg/ml and IQR 64.3–113.6 pg/ml) was higher compared with those that failed to fertilize (median 64.5 pg/ml and IQR 54.9–76.2 pg/ml), but the difference did not reach statistical significance ($P = 0.136$). Similarly, concentrations of IL-15 (median 1.3 pg/ml and IQR 0.7–1.8 pg/ml) and IL-18 (median 39.1 pg/ml and IQR 24.8–50.8 pg/ml) in the follicular fluid of oocytes with successful fertilization were not significantly different compared with follicular fluid of oocytes that failed to fertilize (for IL-15, median was 1.3 pg/ml and IQR 0.7–1.4 pg/ml; for IL-18, median was 38.5 pg/ml and IQR 26.9–48.5 pg/ml and the differences between groups were $P = 0.837$ and $P = 0.471$, respectively).

**Follicular fluid cytokines and embryo quality**

We compared follicular fluid concentrations of cytokines regarding the good-quality (grades I and II) and poor-quality (grades III and IV) embryos. Concentrations of p40 subunit of IL-12/IL-23 in follicular fluid of good-quality (median 91.4 pg/ml and IQR 40.7–124.7 pg/ml) or of poor-quality (median 86.4 pg/ml and IQR 78.1–105.9 pg/ml) embryos were not significantly different ($P = 0.964$). Similarly, concentrations of IL-15 in follicular fluid of good-quality (median 1.0 pg/ml and IQR 0.7–1.4 pg/ml) and poor-quality (median 1.8 pg/ml and IQR 1.3–3.6 pg/ml) embryos were not significantly different ($P = 0.094$). Concentration of IL-18 in the follicular fluid of good-quality embryos (median 42.3 pg/ml and IQR 34.2–59.2 pg/ml) was higher compared with poor-quality ones (median 26.0 pg/ml and IQR 22.1–50.3 pg/ml), but the difference did not reach statistical significance ($P = 0.342$).

**Follicular fluid cytokines and IVF–embryo transfer outcome**

Concentration of follicular fluid cytokines was also evaluated with regard to IVF–embryo transfer outcome (Table III). Patients who achieved clinical pregnancy had significantly decreased ($P = 0.047$) concentrations of IL-15 (median 0.8 pg/ml) compared with patients without successful IVF–embryo transfer outcome (median 1.4 pg/ml). Concentrations of common p40 subunit of IL-12/IL-23 (median 112.9 pg/ml) and IL-18 (42.3 pg/ml) in the follicular fluid of patients with successful IVF–embryo transfer outcome were higher compared with patients who failed to do so (medians 78.1 and 36.6 pg/ml, respectively), but the difference did not reach statistical significance ($P = 0.126$).

**Discussion**

Our study showed the presence of p40 subunit of IL-12/IL-23, IL-15 and IL-18 in follicular fluid collected in spontaneous menstrual cycles. Follicular fluid IL-15 and IL-18 concentrations in follicular fluid were negatively correlated. Significantly higher concentrations of p40 subunit of IL-12/IL-23 were found in follicular fluid taken from follicles containing an oocyte compared with empty ones. Additionally, significantly increased concentrations of IL-15 were found in follicular fluid from women who failed to achieve clinical pregnancy.

We failed to show detectable levels of IL-12 by using a commercially available EIA with a minimum detectable dose <5 pg/ml. Srivastava et al. (1996) also failed to detect IL-12 in follicular fluid as well as in human oviductal fluid. To our knowledge, three recent studies investigated IL-12 in follicular fluid. Coskun et al. (1998) analysed the kinetics of ovarian IL-12 synthesis during follicular maturation. They detected higher concentrations of IL-12 in immature follicles (10.9 ± 5.0 pg/ml) compared with pre-ovulatory follicles (1.3 ± 0.4 pg/ml). Whereas Coskun et al. (1998) showed a negative correlation between oocyte maturity and IL-12, Gazvani et al. (2000) showed a negative correlation between fertilization rate and IL-12 and suggested this cytokine as a predictor of negative outcome of IVF–embryo transfer procedure. Gallinelli et al. (2003) detected significantly lower IL-12 concentrations in follicular fluid from patients with polycystic ovary syndrome compared with controls (mean 1.47 ± 0.3 versus 2.25 ± 0.7 pg/ml, respectively; $P < 0.5$) on the day of oocyte retrieval.

**Table III.** Cytokine concentration in follicular fluid from women undergoing IVF–embryo transfer (spontaneous cycles) regarding pregnancy

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Concentration of cytokines (pg/ml) [median (inter-quartile range)]</th>
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<tbody>
<tr>
<td></td>
<td>Common p40 subunit of IL-12/IL-23</td>
</tr>
<tr>
<td>Yes ($n = 5$)</td>
<td>112.9 (105.9–113.6)</td>
</tr>
<tr>
<td>No ($n = 13$)</td>
<td>78.1 (59.1–91.2)</td>
</tr>
</tbody>
</table>

*Statistically significant difference ($P = 0.047$). Mann–Whitney U-test differences between groups in cytokine concentration for p40 subunit and IL-18 were not statistically significant. [source: https://academic.oup.com/humrep/article-abstract/21/10/2650/2914244?abstract_only=true&download_selected=true]
Concentrations of IL-12 detected in pre-ovulatory follicles by Coskun et al. (1998) and Gallinelli et al. (2003) were lower than the minimum detectable dose of our selected EIA. On the basis of fact that we decided not to extrapolate the results of our assay below the value recommended by the manufacturer, a direct comparison of our results with the mentioned studies is not possible. Moreover, in the studies mentioned above, follicular fluid was collected from hormonally stimulated cycles. Considering the ability of hormones to induce the synthesis of various inflammatory mediators (Licht et al., 2001; Orvieto, 2004; Huck et al., 2005), we propose that the absence of hormonal stimulation in our patients explains the existing difference in the results between ours and other studies.

Although we failed to detect IL-12 in follicular fluid of our patients, we found high concentrations of a common p40 subunit of IL-12/IL-23. To our knowledge, this is the first experimental evidence of the presence of p40 subunit of IL-12/IL-23 in follicular fluid. Moreover, increased concentrations of p40 subunit of IL-12/IL-23 were found in the fluid from follicles, which contained an oocyte compared with empty ones. This finding suggests that p40 subunit of IL-12/IL-23 could be involved in follicular maturation.

Additionally, we showed significantly decreased concentration of IL-15 in follicular fluid from women who achieved clinical pregnancy. IL-15 and IL-18 in follicular fluid were negatively correlated. The only data available in the literature on the correlation between IL-15 and IL-18 in reproduction was a study by Ledee-Bataille et al. (2005). The authors investigated the expression of cytokines in the endometrium of infertile women and found a positive correlation between IL-15 and IL-18 on mRNA level. A possible reason why we observed a negative correlation and they observed a positive correlation between IL-15 and IL-18 is probably that because the cytokine analysis was performed at different phases of the menstrual cycle (endometrium was investigated in mid-luteal phase, and follicular fluid was obtained during the late follicular phase). The expression of cytokines in human uterine mucosa corresponds to the fluctuation of uterine NK cells, and its production is hormonally controlled, especially by progesterone (Ledee-Bataille et al., 2004). These findings confirmed results from. It seems that the local hormonal milieu influences cytokine expression.

Gutman et al. (2004) provided the first experimental evidence of the presence of IL-18 in pre-ovulatory follicular fluid of 30 patients undergoing IVF–embryo transfer (in stimulated cycles). Concentration of IL-18 in follicular fluid collected from stimulated cycles reported in their study (228.9 ± 208 pg/ml) was higher compared with IL-18 levels in follicular fluid collected from spontaneous cycles, as reported in our study. Much higher quantities of IL-18 in follicular fluid collected from stimulated cycles, compared with spontaneous ones, clearly supports the concept of exogenous hormones as powerful modifiers of cytokine synthesis in follicular fluid. The influence of hormonal stimulation on IL-18 synthesis is also supported by significantly increased IL-18 levels at the day of ovum retrieval in patients with ovarian hyperstimulation syndrome (OHSS) compared with patients without OHSS (Gutman et al., 2004).

The importance of IL-18 in reproduction is supported by two studies by Wilson et al. (2004a,b). The authors showed reduced concentrations of IL-18 in the serum of non-pregnant women who had a history of at least three previous miscarriages. These results were significantly decreased compared with non-pregnant women without such a history (Wilson et al., 2004a). Moreover, women with a history of recurrent miscarriages who became pregnant and miscarried again had significantly higher IL-18 levels when not pregnant than those women whose next pregnancies were successful. Furthermore, Wilson et al. (2004b) showed that increased levels of IL-18 were able to discriminate between pregnancies that continued and those that ended in miscarriage.

In our study, which was conducted on patients with a natural cycle, we could not detect a relation between basal serum concentration of FSH, LH, E2 and prolactin on the one hand and local IL concentration in relation to oocyte presence on the other. Earlier studies by Reljic and Vlaisavljevic (1999) on patients in natural IVF/ICSI cycles showed that there was no difference in mean E2 levels between the groups with successful and unsuccessful oocyte retrieval. The further step of investigation should be analysis of the E2 as well as the progesterone concentration at the local level and putting it into the relation with IL concentrations and oocyte development potential.

Elevated p40 subunit of IL-12/IL-23 and IL-15 concentrations were present in the patients with endometriosis. The pathogenesis of this enigmatic disorder still remains controversial despite extensive research. Ulukus and Arici (2005) proposed that there is substantial evidence to support that alterations in both cell-mediated and humoral immunities contribute to the pathogenesis of endometriosis. Moreover, the increased levels of several cytokines and growth factors which are secreted by both immune and endometrial cells seem to promote implantation and growth of ectopic endometrium by inducing proliferation and angiogenesis. This is a special field of infertility and needs special further attention at the local level.

In conclusion, human follicular fluid from women undergoing IVF–embryo transfer collected from spontaneous cycles contains p40 subunit of IL-12/IL-23, IL-15 and IL-18. Increased concentrations of p40 subunit of IL-12/IL-23 in follicles containing oocytes suggest an important role of this cytokine in reproduction. The possible negative value of IL-15 as a predictor of IVF–embryo transfer success remains to be determined.

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Follicular fluid cytokines


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