Human Reproduction Vol.16, No.10 pp. 2073–2078, 2001

# Follicular fluid concentration of leukaemia inhibitory factor is decreased among women with polycystic ovarian syndrome during assisted reproduction cycles

**N.Lédée-Bataille[1,5], G.Laprée-Delage[2], J.L.Taupin[3], S.Dubanchet[2], J.Taieb[4], J.F.Moreau[3] and G.Chaouat[2]**

[1]Service de Gynécologie-Obstétrique et Médecine de la Reproduction, Hôpital Antoine Béclère, [2]INSERM U131, [3]CNRS, UMR 5540, Bordeaux, [4]Service de Biochimie, Hôpital Antoine Béclère, Clamart, France

[5]To whom correspondence should be addressed at: Service de Gynécologie-Obstétrique et Médecine de la Reproduction, Hôpital Antoine Béclère, 157 Rue de la Porte de Trivaux, 92140 Clamart, France. E-mail: Ledeenathalie@aol.com

**BACKGROUND: The possibility that a specific cytokine profile could be detected in the ovaries of patients with polycystic ovarian syndrome (PCOS) was investigated. METHOD: Enzyme-linked immunosorbent assay (ELISA) or bioassays were used to assess the concentrations of leukaemia inhibitory factor (LIF), tumour necrosis factor, interleukin 11, gamma interferon, progesterone and oestradiol in follicular fluids from preovulatory follicles collected after ovarian stimulation from 15 PCOS patients, 15 infertile control patients with regular cycles, and 8 oocyte donors. RESULTS: LIF and progesterone concentrations were significantly lower in the follicular fluid of PCOS patients (LIF median: 265 pg/ml) compared with controls (LIF median: 816 pg/ml); LIF and progesterone follicular fluid concentrations were correlated ($r = 0.720$, $P = 0.0001$). The LH/FSH ratio was negatively correlated with LIF concentrations ($r = -0.714$, $P = 0.0075$). Although the PCOS and control patients did not differ significantly in age, ovarian reserve or IVF indication, the implantation rate was significantly lower among the women with PCOS (IR = 9 versus 21%, $P = < 0.01$). CONCLUSION: The specific cytokine profile of the PCOS patients is probably related to the lower implantation rate since follicular fluid LIF appears to function as an embryotrophic agent.**

*Key words:* cytokines/follicular fluid/leukaemia inhibitory factor/polycystic ovarian syndrome

## Introduction

Follicular growth and maturation is a complex process regulated by autocrine and paracrine factors. Follicular fluids provide the environment in which oocyte maturation occurs. Follicular fluids may therefore affect fertilization and early embryonic development (Brannström and Norman, 1993, Vinatier and Monnier, 1993; Adashi, 1994). For this reason, several authors have postulated that cytokines are involved in ovarian function (Deshpande, 2000). Some cytokines have been detected in human follicular fluids and even in human embryo culture medium (probably because it contains residual follicular fluids) (Austgulen, 1995).

Four cytokines were the focus of this study: leukaemia inhibitory factor (LIF), interleukin-11 (IL-11), tumour necrosis factor (TNF), and gamma interferon (IFNγ). The first two are already known to be key determinants of successful implantation and the latter two are responsible at high doses for implantation failure and early pregnancy loss in mice and humans.

LIF is the first cytokine found to be mandatory for implantation in mice (Stewart *et al.*, 1992). Indeed, LIF–/– transgenic mice obtained by gene knockout are fertile (the number of LIF–/– embryos is normal), but neither LIF–/– nor LIF+/+ embryos implant in LIF–/– foster mothers. Both types of embryo do implant in LIF+ foster mothers, and the implantation blockade observed in LIF– mice can be partly corrected by an infusion of recombinant LIF in the peritoneal cavity. The presence of LIF in the follicular environment has been described by several authors (Arici *et al.*, 1997; Coskun *et al.,*1998; Jean *et al.*, 1999; Ozornek *et al.*, 1999), but its role is not yet clear: it cannot be essential because LIF-deficient mice usually ovulate. Nonetheless, the concentration of LIF in human follicular fluid rises around the time of ovulation (Arici *et al.*, 1997). In-vitro LIF improves the development of murine blastocysts (Mitchell *et al.*, 1994; Kauma and Matt, 1995) and increases the rate of hatching in both murine and ovine species (Lavranos *et al.,*1995; Tsai *et al.*, 1999). LIF also decreases the rate of embryo degeneration and increases the pregnancy rate in ovine experiments (Fry *et al.*, 1992). In humans, follicular fluid LIF has been described as a marker of oocyte quality (Jean *et al.,* 1997) and correlated with embryo quality (Arici *et al.*, 1997). Follicular fluid LIF in humans has also been reported to enhance the rate of blastocyst formation (Dunglison *et al.*, 1996), although this

© European Society of Human Reproduction and Embryology

finding has not been confirmed (Jurisicova *et al.*, 1995) and thus remains controversial. Ovarian granulosa, stromal cells and macrophages all express LIF mRNA and actively secrete the protein (Loukides *et al.*, 1990).

IL-11, reported to be present in preovulatory follicular fluids, belongs to a cytokine subfamily that shares a common signal transducing receptor, gp130, with LIF. Although its paracrine and/or autocrine role in human ovarian follicular function is not yet known, IL-11 is strongly suspected to be an immunomodulator that may down-regulate the expression of various pro-inflammatory cytokines. Reports of high IL-11 concentrations in the follicular fluids of atretic follicles raise the issue of this protein's involvement in the process of atresia (Branisteanu *et al.*, 1997).

TNF is a cytokine with an essential role in folliculogenesis and ovarian maturation (Wang *et al.*, 1992). It is a potent modulator of ovarian function, affecting steroidogenesis of both granulosa and interstitial thecal cells (Spaczynski *et al.,* 1999). TNF has been shown to play an important role in oestradiol secretion (Wang *et al.*, 1992, Cianci *et al.*, 1996) as well as in the induction and demise of the corpus luteum (Wang *et al.*, 1992). The administration of dexamethasone to patients with polycystic ovarian disease has been shown to decrease follicular fluid TNFα concentrations (Zolti *et al.,* 1992).

The pro-inflammatory cytokine IFNγ has been found in follicular fluids, while other pro-inflammatory cytokines are either not detectable or detected in insignificant amounts (Srivastava *et al.*, 1996). This suggests that it has a specific role, perhaps the prevention of infection. In mice, some gene knockout experiments have shown that neither IFNγ nor its receptor is essential for fertility and maintenance of pregnancy (Dalton *et al.*, 1993; Huang *et al.*, 1993), but other studies report the contrary (Ashkar and Croy, 1999; Croy *et al.*, 2000). The reason for such discrepancies between various strains of knockout mice is still unresolved.

We postulated that the cytokine profiles of women who spontaneously ovulate (control and donor patients) should differ from those of women with polycystic ovarian syndrome (PCOS) who have difficulty ovulating without stimulation. Nonetheless, ovarian stimulation of these patients usually leads to abnormal results, such as a premature ovarian response to LH by granulosa cells, albeit healthy (Almahbobi *et al.,* 1996; Willis *et al.*, 1998), and a tendency to ovarian hyperstimulation. Accordingly, patterns of cytokine expression in follicular fluids were investigated that might be related specifically to PCOS and that might lead to a better understanding of this syndrome, which has not yet been completely characterized.

## Materials and methods

### *Patient characteristics*

This study enrolled 38 patients between November 1998 and November 1999. All patients provided an informed consent, and this investigation was approved by our Institutional Review Board. Human follicular fluids were collected from preovulatory follicles of patients (aged 26–41 years) undergoing IVF and embryo transfer at the Antoine Béclère Hospital, Clamart, France.

Diagnosis of PCOS ($n = 15$) was based on oligo- or anovulatory cycles associated with oligo-amenorrhea or amenorrhea, LH/FSH >2, or on ovarian ultrasonography findings of increased stromal and ovarian volume and many (>10) tiny (<8 mm) cysts studded along the periphery of the ovary (Dewailly *et al.*, 1992; Dewailly, 1997). Patients were selected if they exhibited all the criteria for the diagnosis of PCOS. Eight patients had hyperandrogenism and seven did not. IVF indications in the PCOS group were male factors ($n = 8$), tubal disease ($n = 3$) or idiopathic infertility ($n = 4$). The women in the control group ($n = 15$) and the oocyte donors ($n = 8$) had regular cycles, an LH/FSH ratio <1, and <10 follicles at the beginning of the cycle, with normal stromal volume. The patients in the control group were selected according to the inclusion criteria during the same period. In the control group, the IVF indications were male factors ($n = 7$), tubal disease ($n = 3$), or idiopathic infertility ($n = 5$).

### *Protocol for controlled ovarian stimulation*

All patients received a standard gonadotrophin-releasing hormone (GnRH) agonist regimen that began on day 21 of a spontaneous menstrual cycle. Leuprolide acetate (1 mg per day s.c. Lucrin; Takeda Pharmaceuticals, Paris, France) was administered for 10–14 days until complete pituitary desensitization was documented. Stimulation was initiated once sonographic evidence indicated no ovarian follicular activity and an endometrial lining <5 mm thick and serum oestradiol measured <50 pg/ml. Recombinant gonadotrophin (rFSH) (Puregon; Organon Pharmaceuticals, Saint Denis, France) therapy then began at 225 IU/day for control and oocyte-donor groups and 150 IU/day for the PCOS group for the first five days of controlled ovarian stimulation. Further rFSH doses were determined according to the standard criterion of follicular maturation, assessed by ultrasound and serum oestradiol measurements. Similarly, HCG was administered (10 000 IU i.m.'endo' chorionic gonadotrophin; Organon Pharmaceuticals) when at least three follicles exceeded 17 mm in diameter and when the oestrogen concentration per mature follicle (diameter >17 mm) was >300 pg/ml. 36 h after HCG administration, oocytes were retrieved by needle aspiration, with transvaginal ultrasound guidance and routine intravenous sedation. After the cumulus–oocyte complexes were removed, the preovulatory follicular fluid were pooled for each patient and then centrifuged at 600 *g* for 10 min. The cell-free supernatants were then divided into aliquots and stored at –80°C until assay.

### *Cytokine assays*

The enzyme-linked immunosorbent assay (ELISA) used to quantify LIF has been described previously (Taupin *et al.*, 1997). The two monoclonal antibodies it uses are known not to interfere with the ligand receptor binding; therefore the test is not affected by the presence of soluble receptors in the samples. The detection threshold was calculated by adding 2 standard deviations to the mean of 6 blank wells and never exceeded 25 pg/ml. Results are expressed in pg/ml. Intra-assay coefficients of variation were respectively 4.45, 3.55 and 4.59 for LIF concentration of 80, 400 and 1000 pg/ml. Inter-assay coefficients of variation were respectively 4.26, 3.42 and 1.02 for LIF concentration of 80, 400 and 1000 pg/ml.

TNF was evaluated by the standard cytotoxicity bioassay, with the L929 murine fibroblast cell line (Wang *et al.*, 1985; Hogan and Vogel, 1988). This assay, while it does not distinguish between the alpha and beta isoforms, measures all—and only—the bioactive TNF present in the samples (Wang *et al.*, 1985). Murine and human TNF lyse equally well with the L929 murine fibroblast cell. Results are expressed in units, each corresponding to the TNF concentration required for half maximal lysis.

**Table I.** Patient characteristics

| | PCOS | Control | *P* |
|---|---|---|---|
| Age (years) | 34 (28–41) | 34 (28–41) | NS |
| Duration of infertility (years) | 5 (2–12) | 4 (2–12) | NS |
| FSH at day 3 (mIU/ml) | 3.90 (1.70–6.90) | 4.20 (3.20–7.90) | NS |
| LH/FSH | 1.65 (0.47–4.05) | 0.63 (0.44–0.90) | 0.0002 |
| Oestradiol at day 3 (pg/ml) | 25 (10–87) | 24 (15–62) | NS |
| Gonadotrophins (IU) | 1038 (750–2600) | 2363 (1350–3600) | $< 0.0001$ |
| Number of oocytes | 8 (2–19) | 9 (4–15) | NS |
| Number of embryos | 4 (0–13) | 4 (1–11) | NS |
| Fertilization rate (%) | 60 (25–100) | 60 (25–92) | NS |
| Implantation rate (%) | 9 | 21 | $< 0.01$ |

Values are reported as medians with the range in parentheses. The fertilization and implantation rates are percentages. The LH/FSH ratio, the number of gonadotrophin units administered for ovarian stimulation and the implantation rate were significantly different in the PCOS and control groups ($P < 0.05$).
PCOS = polycystic ovarian syndrome.

IL-11 concentrations were measured with the Quantikine kit (R&D, Abingdon, UK); its sensitivity, calculated as the 95% coefficient of variation of repeated blank measurements, was 8 pg/ml. Intra- and inter-assay coefficient of variation values were 2.4 and 1.8% respectively. For IFNγ we used the commercial ELISA kit produced by Coulter-Immunotech, Marseille, France. Its sensitivity was 0.08 IU/ml . Intra- and inter-assay coefficient of variation values were 4.7 and 7.8% respectively.

### *Oestradiol and progesterone assays*

Oestradiol and progesterone concentrations in serum were determined on the day of triggering, and in follicular fluid, on the day of oocyte collection. Hormone measurements were performed with an automated chemiluminescence system (ACS-180; Bayer Diagnosis, USA). Sensitivities were 30 pg/ml (conversion factor to SI units, 3.67) for oestradiol and 0.1 ng/ml (conversion factor to SI units, 3.18) for progesterone. Intra- and inter-assay coefficient of variation values were respectively, 5 and 7% for oestradiol and 3.5 and 7% for progesterone. Before testing, follicular fluid was diluted with a specific diluent at 1/100 for oestradiol and 1/10 for progesterone.

### *Evaluation of embryo quality*

The quality of embryos was evaluated by their morphological aspect, the regularity of the embryo cells and the percentage of fragmentation.

### *Statistical analysis*

The normal distribution of the follicular fluid cytokine concentrations could not be confirmed. Therefore, results were expressed as median values. The nonparametric Mann–Whitney *U*-test was used to compare groups. We used the Spearman's rank test for correlation analysis of the control group, the PCOS group and the pooled group of all infertile patients (control and PCOS). Embryo quality was compared with the $\chi^2$ test. The statistical assessment used StatView software (Abacus Concepts, Inc., Berkeley, CA, USA). A $P$ value $< 0.05$ was considered significant.

## Results

### *Patients, controlled ovarian stimulation and embryology data*

Table I summarizes the patients' clinical features. The PCOS and control groups did not differ significantly as to mean (± SEM) age, duration of infertility, indication for IVF, serum FSH and oestradiol on the third day of the menstrual cycle, or number of oocytes and embryos collected. Stimulation of PCOS patients used significantly fewer units of gonadotrophin ($P < 0.0001$), in accordance with their ovarian profiles.

### *Cytokine concentrations in the follicular fluid*

All follicular fluid samples contained LIF. In the control group, the LIF concentrations ranged from 403 to 2894 pg/ml with a median value of 816 pg/ml, not significantly different from the donor group (range: 553–1230 ; median: 749 pg/ml). These values were significantly higher than those in the PCOS group ($P < 0.0001$), where LIF ranged from 78 to 594 pg/ml, with a median at 265 pg/ml (Table II). In the PCOS group, median LIF values did not differ significantly according to hyperandrogenism.

TNF was detected in all samples of follicular fluid except for a single case in the PCOS group (Table II). The PCOS and control groups did not differ significantly for TNF concentrations, but significant differences were observed for the donors, compared with both the control ($P = 0.0225$) and PCOS patients ($P = 0.0340$).

IL-11 and IFNγ, on the other hand, were less commonly found in follicular fluid samples. IL-11 was found in 50% of donor follicular fluid samples, 29% of control samples and only 21% of PCOS patients. Although the donor median differed, there were no significant statistical differences between the 3 groups (Table II).

IFNγ was detected in 50% of control follicular fluid samples, 66% of PCOS patients, and 75% of donors. Again, the differences were not significant (Table II).

### *Oestradiol and progesterone in follicular fluids*

The follicular fluid oestradiol concentrations did not differ significantly, with medians of $655\times10^3$ pg/ml for controls, $753\times10^3$ pg/ml for donors, and $526\times10^3$ pg/ml for PCOS patients (Table II). follicular fluid progesterone concentrations were significantly lower among the PCOS group than among the control ($P = 0.0046$) or donor ($P = 0.0072$) groups.

### *Correlation between cytokines and hormones*

Follicular fluid concentrations of LIF and progesterone were correlated in the control group ($r = 0.550$, $P = 0.0396$) and

**Table II.** Cytokine and steroid concentrations in follicular fluids

| | PCOS ($n$ = 15) | Controls ($n$ = 15) | Donors ($n$ = 8) |
|---|---|---|---|
| LIF pg/ml[a] | 265 (78–594) | 816 (403–2894) | 749 (553–1230) |
| TNF IU/ml[b] | 12.60 (0–44.26) | 16.55 (9.41–38.35) | 29.19 (17.76–101.98) |
| IL-11 pg/ml | 0 (0–187) | 0 (0–79) | 22 (0–113) |
| IFNγ IU/ml | 2.64 (0–8.41) | 0.09 (0–10.16) | 0.84 (0–1.83) |
| Oestradiol (pg/ml $\times 10^3$) | 526 (281–1522) | 655 (228–1184) | 753 (297–2461) |
| Progesterone (ng/ml)[c] | 7377 (2585–22 314) | 13 396 (6330–70 660) | 17 972 (9978–20 000) |

[a]$P < 0.0001$ for PCOS versus controls and for PCOS versus donors.
[b]$P = 0.034$ for PCOS versus donors.
[c]$P = 0.0046$ for PCOS versus controls and $P = 0.0072$ for PCOS versus donors.
Values are reported as medians with the range in parentheses. Cytokine and steroid concentrations were measured in the follicular fluid of women undergoing ovarian stimulation for infertility or for oocyte donation. PCOS = polycystic ovarian syndrome.

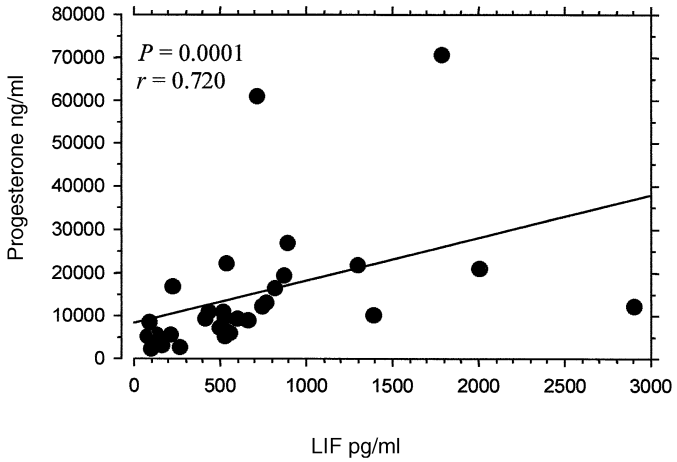


**Figure 1.** Correlation between follicular fluid progesterone (Pg) concentrations and follicular fluid leukaemia inhibitory factor (LIF) concentrations. LIF concentrations were measured in the follicular fluids of women undergoing ovarian stimulation for infertility ($n$ = 30, pooling patients with polycystic ovarian syndrome with control patients) and plotted against the median follicular fluid progesterone concentration for each woman.

the infertile group (controls and PCOS) ($r$ = 0.720, $P$ = 0.0001) (Figure 1). LIF and TNF concentrations were not correlated, nor were those of LIF or TNF correlated with follicular fluid oestradiol.

The LH/FSH ratio was negatively correlated with the LIF concentration in the PCOS group ($r$ = –0.714, $P$ = 0.0075) and in the infertile group (control and PCOS) ($r$ = –0.716, $P$ = 0.0001). In the PCOS group, IFNγ concentrations were also negatively correlated with follicular fluid oestradiol ($r$ = –0.673, $P$ = 0.0257).

### *IVF results*

Embryo quality was similar in the control and PCOS groups, but the implantation rate was significantly lower in PCOS patients (9 versus 21% in the control group, $P < 0.01$).

## Discussion

A growing body of evidence points to an interaction between the immune and reproductive systems. Cytokines and growth factors are the candidates most likely to participate in such an interaction. Nevertheless, whether high follicular fluid cytokine concentrations have any significance for ovarian function must be assessed.

In this study the highest cytokine concentrations were found in the donor follicular fluid and the lowest in the PCOS follicular fluid. TNF has usually been described as deleterious in follicular fluid (Yan *et al.*, 1993), but in studies that used an ELISA assay specific for TNFα which is unable to measure bioactive TNF. The current investigation used a bioassay that is unable to distinguish between the α and β forms of TNF but measures all the bioactive TNF (Wang *et al.*, 1985; Hogan and Vogel, 1988).

LIF concentrations <15 pg/ml have usually been reported in pooled follicular fluids isolated from preovulatory follicles and collected in conditions like ours (Arici *et al.*, 1997; Coskun *et al.*, 1998). The results of the current study differ dramatically from those reported previously. This discrepancy may be explained by the fact that the ELISA assay used measured all LIF fractions, whether or not they were bound to soluble receptors. The specificity of our LIF ELISA assay made possible a 100-fold increase in follicular fluid LIF detection. Such differences in the specificity of LIF ELISA tests have been reported previously (Taupin *et al;* 1997; Jean *et al.,* 1999).

A previous report found follicular fluid LIF to be correlated with follicular fluid oestradiol (Arici *et al.,* 1997). The strong correlation between follicular fluid LIF and progesterone found in this study was more in agreement with that reported by Ozornek *et al.* (Ozornek *et al.,* 1999).

Except in cases of severe inflammation, LIF is reported to be absent from circulating blood due to its short half-life in the vascular compartment (Hilton *et al.*, 1991). Therefore, blood contamination is very unlikely to be responsible for the high follicular fluid LIF concentrations. The precise sites that produce and secrete LIF and TNF have not yet been determined. Candidate sources include granulosa cells, resident ovarian cells or leukocytes present in follicular fluid. Resident macrophages and monocytes make up 5–15% of human follicular tissue cells. The lymphocyte concentration and T-cell subpopulations in follicular fluid change significantly during oocyte maturation (Loukides *et al.*, 1990). Although all these cells may contribute to the regulation of the cytokines tested here, the best candidate is the granulosa cell, which expresses *LIF*

mRNA at the preovulatory follicle stage (Arici *et al.*, 1997). Indirect support for this proposition may be found in the difference in the LIF detected in control and PCOS follicular fluid and the correlation between LIF and progesterone. Indeed, PCO granulosa cells have an abnormal capacity to synthesize progesterone *in vivo* and *in vitro* (Doldi *et al.,* 1998). Recent data suggest that follicular fluids may play an important role in the endocrine balance of PCOS by its effect on the relation between theca and granulosa cells. An abnormal interaction between PCO granulosa cells and their own follicular fluids may be implicated in the altered steroidal response of the granulosa (Andreani *et al.*, 1996). The strong negative correlation between the LH/FSH ratio, one of the criteria by which PCOS is defined (Tarlatzis *et al.,* 1995), and the follicular fluid LIF concentration may thus be a key factor in this abnormal interaction.

Recombinant human LIF has already been tested in murine IVF. Its addition to mouse embryo culture medium enhances blastocyst development (Kauma and Matt, 1995; Tsai *et al.*, 1999), significantly increases the number of embryos that hatch and improves embryo survival *in vivo* (Fry *et al.*, 1992; Lavranos *et al.*,1995).

The effects of adding recombinant human LIF to culture medium for human blastocysts is still a matter of debate. It has been found that adding LIF to the medium enhances in-vitro human blastocyst formation (Dunglison *et al.*, 1996), while others have not found such an effect (Jurisicova *et al.*, 1995). Nonetheless, follicular fluid LIF concentration has been found to be correlated with embryo quality in an oocyte donor group; and a similar correlation has been reported between LIF concentration and oocyte quality (Arici *et al.,* 1997). LIF receptors have been found in human embryos (Charnock-Jones *et al.,* 1994; Sharkey *et al.*, 1995; Van Eijk *et al.*, 1996; Chen *et al.,* 1999): this suggests that LIF may affect embryo development by acting as an 'embryotrophin' before implantation and as a factor required for embryo implantation.

In this study lower concentrations of LIF follicular fluid were found in the PCOS group. We therefore speculate that the lower implantation rate in this group might be due to the LIF concentrations.

## Acknowledgements

This work was supported by INSERM, CEFIPRA, la Fondation fertilité stérilité and la Ligue de Lutte contre le Cancer de la Gironde. N.L. and G.L-D. contributed equally to this work.

## References

Adashi, E.Y. (1994) Growth factors and ovarian function: the IGF-I paradigm. *Horm. Res*., **42**, 44–48.

Almahbobi, G., Anderiesz, C., Hutchinson, P. *et al.* (1996) Functional integrity of granulosa cells from polycystic ovaries. *Clin. Endocrinol.*, **44**, 571–580.

Andreani, C.L., Pierro, E., Lazzarin, N. *et al.* (1996) Effect of follicular fluid on granulosa luteal cells from polycystic ovary. *Hum. Reprod.*, **11**, 2107–2113.

Arici, A., Oral, E., Bahtiyar, O. *et al.* (1997) Leukaemia inhibitory factor expression in human follicular fluid and ovarian cells. *Hum. Reprod.*, **12**, 1233–1239.

Ashkar, A.A. and Croy, B.A. (1999) Interferon-gamma contributes to the normalcy of murine pregnancy. *Biol. Reprod.*, **61**, 493–502.

Austgulen, R., Arntzen, K.J., Vatten, L.J. *et al.* (1995) Detection of cytokines (interleukin-1, interleukin-6, transforming growth factor-beta) and soluble tumour necrosis factor receptors in embryo culture fluids during in-vitro fertilization. *Hum. Reprod.*, **10**, 171–176.

Branisteanu, I., Pijnenborg, R., Spiessens, C. *et al.* (1997) Detection of immunoreactive interleukin-11 in human follicular fluid: correlations with ovarian steroid, insulin-like growth factor I concentrations, and follicular maturity. *Fertil. Steril.*, **67**, 1054–1058.

Brannström, M. and Norman, R.J. (1993) Involvement of leukocytes and cytokines in the ovulatory process and corpus luteum function. *Hum. Reprod.*, **8**, 1762–1775.

Charnock-Jones, D.S., Sharkey, A.M., Fenwick, P. and Smith, S.K. (1994) Leukaemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. *J. Reprod. Fertil.*, **101**, 421–426.

Chen, H.F., Shew, J.Y., Ho, H.N. *et al.* (1999) Expression of leukaemia inhibitory factor and its receptor in preimplantation embryos. *Fertil. Steril.*, **72**, 713–719.

Cianci, A., Calogero, A.E., Palumbo, M.A. *et al.* (1996) Relationship between tumour necrosis factor alpha and sex steroid concentrations in the follicular fluid of women with immunological infertility. *Hum. Reprod.*, **11**, 265–268.

Coskun, S., Uzumcu, M., Jaroudi, K. *et al.* (1998) Presence of leukaemia inhibitory factor and interleukin-12 in human follicular fluid during follicular growth. *Am. J. Reprod. Immunol*., **40**, 13–18.

Croy, B.A., Ashkar, A.A., Minhas, K. and Greenwood, J.D. (2000) Can murine uterine natural killer cells give insights into the pathogenesis of preeclampsia? *J. Soc. Gynecol. Invest*., **7**, 12–20.

Dalton, D.K., Pitts-Meek, S., Keshav, S. *et al.* (1993) Multiple defects of immune cell function in mice with disrupted interferon-gamma genes [see comments]. *Science*, **259**, 1739–1742.

Deshpande, R.R., Chang, M.Y., Chapman, J.C. and Michael, S.D. (2000) Alteration of cytokine production in follicular cystic ovaries induced in mice by neonatal estradiol injection. *Am. J. Reprod. Immunol.*; **44**, 80–88.

Dewailly, D. (1997) Definition and significance of polycystic ovaries. *Baillieres Clin. Obstet. Gynaecol*., **11**, 349–368.

Dewailly, D., Cortet-Rudelli, C., Nobels, F. *et al.* (1992) Insulin resistance and polycystic ovary syndrome. *Ann. Endocrinol.*, **53**, 1–7.

Doldi, N., Gessi, A., Destefani, A. *et al.* (1998) Polycystic ovary syndrome: anomalies in progesterone production. *Hum. Reprod.*, **13**, 290–293.

Dunglison, G.F., Barlow, D.H. and Sargent, I.L. (1996) Leukaemia inhibitory factor significantly enhances the blastocyst formation rates of human embryos cultured in serum-free medium. *Hum. Reprod.*, **11**, 191–196.

Fry, R.C., Batt, P.A., Fairclough, R.J. and Parr, R.A. (1992) Human leukaemia inhibitory factor improves the viability of cultured ovine embryos. *Biol. Reprod.*, **46**, 470–474.

Hilton, D.J., Nicola, A., Waring, P.M. and Melcalf, D. (1991) Clearance and fate of leukaemia-inhibitory factor (LIF) after injection into mice. *J. Cell, Physio*., **148**, 430–439.

Hogan, M.M. and Vogel, S.N. (1988) Production of tumor necrosis factor by rIFN-gamma-primed C3H/HeJ (Lpsd) macrophages requires the presence of lipid A-associated proteins. *J. Immunol.*, **141**, 4196–4202.

Huang, S., Hendriks, W., Althage, A. *et al.* (1993) Immune response in mice that lack the interferon-gamma receptor. *Science*, **259**, 1742–1745.

Jean, M., Mirallie, S., Barriere, P. *et al.* (1999) Leukaemia inhibitory factor expression in human follicular fluid. *Hum. Reprod.*, **14**, 571.

Jurisicova, A., Ben-Chetrit, A., Varmuza, S.L. and Casper, R.F. (1995) Recombinant human leukaemia inhibitory factor does not enhance in-vitro human blastocyst formation. *Fertil. Steril.*, **64**, 999–1002.

Kauma, S.W. and Matt, D.W. (1995) Coculture cells that express leukaemia inhibitory factor (LIF) enhance mouse blastocyst development *in vitro*. *J. Assist. Reprod. Genet*., **12**, 153–156.

Lavranos, T.C., Rathjen, P.D. and Seamark, R.F. (1995) Trophic effects of myeloid leukaemia inhibitory factor (LIF) on mouse embryos. *J. Reprod. Fertil.*, **105**, 331–338.

Loukides, J.A., Loy, R.A., Edwards, R. *et al.* (1990) Human follicular fluids contain tissue macrophages. *J. Clin. Endocrinol. Metab.*, **71**, 1363–1367.

Mitchell, M.H.S.R., Hodgen, G.D. and Oehninger, S. (1994) Enhancement of in-vitro murine embryo development by recombinant leukaemia inhibitory factor. *J. Soc. Gynecol. Invest*., **1**, 215–219.

Ozornek, M.H., Bielfeld, P., Krussel, J.S. *et al.* (1999) Epidermal growth factor and leukaemia inhibitory factor levels in follicular fluid. Association with in-vitro fertilization outcome. *J. Reprod. Med*., **44**, 367–369.

Sharkey, A.M., Dellow, K., Blayney, M. *et al.* (1995) Stage-specific expression of cytokine and receptor messenger ribonucleic acids in human preimplantation embryos. *Biol. Reprod.*, **53**, 974–981.

Spaczynski, R.Z., Arici, A. and Duleba, A.J. (1999) Tumor necrosis factor-alpha stimulates proliferation of rat ovarian theca-interstitial cells. *Biol. Reprod.*, **61**, 993–998.

Srivastava, M.D., Lippes, J. and Srivastava, B.I. (1996) Cytokines of the human reproductive tract. *Am. J. Reprod. Immunol*., **36**, 157–166.

Stewart, C.L., Kaspar, P., Brunet, L.J. *et al.* (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature*, **359**, 76–79.

Tarlatzis, B.C., Grimbizis, G., Pournaropoulos, F. *et al.* (1995) The prognostic value of basal luteinizing hormone:follicle-stimulating hormone ratio in the treatment of patients with polycystic ovarian syndrome by assisted reproduction techniques. *Hum. Reprod.*, **10**, 2545–2549.

Taupin, J.L., Gualde, N. and Moreau, J.F. (1997) A monoclonal antibody based elisa for quantitation of human leukaemia inhibitory factor. *Cytokine*, **9**, 112–118.

Tsai, H.D., Chang, C.C., Hsieh, Y.Y. *et al.* (1999) Recombinant human leukaemia inhibitory factor enhances the development of preimplantation mouse embryo *in vitro. Fertil. Steril.*, **71**, 722–725.

Van Eijk, M.J., Mandelbaum, J., Salat-Baroux, J. *et al.* (1996) Expression of leukaemia inhibitory factor receptor subunits LIFR beta and gp130 in human oocytes and preimplantation embryos. *Mol. Hum. Reprod.*, **2**, 355–360.

Vinatier, D. and Monnier, J.C. (1993) Immunological recognition in pregnancy: physiology. *J. Gynecol. Obstet. Biol. Reprod.*, **22**, 709–721.

Wang, A.M., Creasey, A.A., Ladner, M.B. *et al.* (1985) Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science*, **228**, 149–154.

Wang, L.J., Brannstrom, M., Robertson, S.A. and Norman, R.J. (1992) Tumor necrosis factor alpha in the human ovary: presence in follicular fluid and effects on cell proliferation and prostaglandin production. *Fertil. Steril.*, **58**, 934–940.

Willis, D.S., Watson, H., Mason, H.D. *et al.* (1998) Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. *J. Clin. Endocrinol. Metab.*, **83**, 3984–3991.

Yan, Z., Hunter, V., Weed, J. *et al.* (1993) Tumor necrosis factor-alpha alters steroidogenesis and stimulates proliferation of human ovarian granulosal cells *in vitro. Fertil. Steril.*, **59**, 332–338.

Zolti, M., Bider, D., Seidman, D.S. *et al.* (1992) Cytokine levels in follicular fluid of polycystic ovaries in patients treated with dexamethasone. *Fertil. Steril.*, **57**, 501–504.

*Received on February 14, 2001; accepted on June 26, 2001*