PHYSIOLOGY

Cytokines in the Human Ovary: Presence in Follicular Fluid and Correlation with Leukotriene B₄

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Purpose: This study was undertaken to correlate the follicular levels of interleukin (IL)-1 α , IL-2, tumor necrosis factor- α (TNF- α), and leukotriene (LT) B₄ with oocyte maturity, fertilization, and achievement of pregnancy.

Methods: The material was obtained from 22 women undergoing IVF, 8 of whom became pregnant and 14 of whom did not.

Results: All of the studied cytokines and LT B₄ were found in follicular fluids, but there were no significant differences according to oocyte maturity, fertilization, embryo quality, and achievement of pregnancy. On the other hand, a significant positive correlation was found between IL-1 α and TNF- α , IL-1 α , and LT B₄ as well as between TNF- α and LT B₄ in follicular fluids with subsequently fertilized oocytes.

Conclusions: It seems that IL-1 α , TNF- α and LT B₄ may take part in the process of follicle wall degradation, and their follicular correlations may suggest more optimal follicular and oocyte development and maturation.

KEY WORDS: interleukin-1 α ; interleukin-2; tumor necrosis factor- α ; leukotriene B₄; human follicular fluid.

INTRODUCTION

Currently, there is growing interest in the interaction between the endocrine and the immune systems, especially in the ovary. Cytokines are proteinaceous mediators of the immune system and important intercellular regulators in a wide range of physiological and pathophysiological events (1). Moreover, recent studies have demonstrated an active role for cytokines in the process leading to ovulation, as well as in fertilization and early embryonic cleavage (2,3). These substances are produced locally in the ovaries and exert their action on various cell types (4). In addition, cytokine production and function appear to be modulated by gonadal steroids and pituitary hormones (2,4).

On the other hand, it has been suggested that cyclooxygenase products of arachidonic acid, e.g., prostaglandins (PGs) of the E and F series, may have a physiological role in follicular rupture (5). Furthermore, their production seems to be regulated by some cytokines, i.e., interleukin (IL)-1 and tumor necrosis factor- α (TNF- α), because they can stimulate monocytes to produce PGs (6,7).

Nevertheless, limited data are available regarding the involvement of the arachidonic acid products derived from the lipoxygenase pathway, e.g., leukotriene (LT) B₄, in ovarian physiology and the ovulatory process (5,8). This led us to measure IL-1 α , IL-2, TNF- α , and LT B₄ levels in human periovulatory follicular fluids (FFs) to study their relationship with oocyte maturity and fertilization, as well as with the achievement of pregnancy after in vitro fertilization (IVF).

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MATERIALS AND METHODS

For this study, 69 blood-free FF samples were obtained from 22 women undergoing IVF due to tubal-factor and idiopathic infertility. Eight of 22 women became pregnant after the treatment, whereas 14 did not. Patient age ranged between 26 and 41 years (mean, 32.1 ± 4.0 years). All women who had participated in this study were in good general health, and no couples with male infertility were included. All semen samples were examined thoroughly according to World Health Organization criteria and were within the normal range, to eliminate the influence of sperm or non-ovarian factors on the IVF outcome. The 69 FF samples were divided into groups according to the parameters studied.

In all women, buserelin (Suprefact; Hoechst, Frankfurt am Main, Germany) was given intranasally at a dose of 1 mg/day, starting on day 1 of the cycle. Ovarian stimulation was initiated after complete pituitary desensitization [estradiol (E_2) < 40 pg/ml], with daily administration of 150 IU of human menopausal gonadotropin (hMG) [Humegon (Organon, Oss, The Netherlands) or Pergonal (Serono, Aubonne, Switzerland)]. Follicular development was assessed in all patients by daily morning serum E_2 determinations (Leeco Diagnostics, Detroit, MI) and ovarian ultrasonography (Kretz, Combison 320; Kretztechnik, Austria). The criteria for human chorionic gonadotropin (hCG) [Pregnyl (Organon) or Profasi (Serono)] administration, oocyte retrieval and maturity evaluation, sperm preparation, oocyte insemination, and embryo transfer have been described elsewhere (9–11).

A total of 113 FF samples was obtained by transvaginal aspiration and immediately centrifuged at 1000g for 10 min to remove all cells. FF samples were not diluted with culture medium. The supernatants were frozen at -30° until assay. Forty-four FF samples that contained blood, that did not contain an oocyte, or that contained more than a single oocyte were excluded from the study. Those 44 FF samples came from both pregnant and nonpregnant women.

Measurements of IL-1 α , IL-2, TNF- α , and LT B $_4$ were performed using an enzyme-linked immunosor-

bent assay (ELISA) technique. ELISA kits from RD Systems (Minneapolis, MN) were used for cytokine determinations, whereas ELISA kits from Advanced Magnetics Inc. (MA) were used for LT B $_4$ measurements. The sensitivity of the method was 0.3 pg/ml for IL-1 α , 6 pg/ml for IL-2, and 1.5 pg/ml for TNF- α , with intraassay variations of 7.1, 6, and 8.3%, respectively, and interassay variations of 8.3, 8.2, and 9.3%, respectively.

For LT B $_4$, the sensitivity of the method was 8.7 pg/ml and there was very little cross-reactivity (e.g., less than 1% for PGs). The intra- and interassay variations for LT B $_4$ were 6.4 and 7.3%, respectively.

Follicular fluid levels of IL-1 α , IL-2, and TNF- α were correlated with LT B $_4$ levels and all of them with oocyte maturity and fertilization, types of embryos, and implantation after embryo transfer.

Statistics

Data are presented as mean $\pm$ standard deviation. Student's *t* test and linear regression analysis were used for statistical analysis of the results as appropriate. A *P* value of < 0.05 was considered statistically significant.

RESULTS

We were able to detect IL-1 α , IL-2, TNF- α and LT B $_4$ in all FFs obtained after ovarian stimulation, at concentrations that ranged from 26.5 to 74.7, 7.1 to 82.3, 20.0 to 87.4, and 1224 to 3843 pg/ml, respectively.

The immunoreactive levels of IL-1 α , IL-2, TNF- α and LT B $_4$ detected in FFs that were grouped according to oocyte maturity, fertilization, embryo quality, and achievement of pregnancy are shown in Tables I, II, III, and IV, respectively. No significant differences in FF levels of the studied cytokines and LT B $_4$ were found between these groups (*P* = NS). On the other hand, there were considerable variations in IL-1 α (Fig. 1), IL-2, TNF- α and LT B $_4$ (Fig. 2) concentrations in FFs obtained from the same patient, suggesting

Table I. Concentrations of IL-1 α , IL-2, TNF- α , and LT B $_4$ in FFs from Mature and Immature Oocytes*

Oocytes	IL-1 α (pg/ml)	IL-2 (pg/ml)	TNF- α (pg/ml)	LT B $_4$ (pg/ml)
Mature (<i>n</i> = 31)	41.8 $\pm$ 8.6	22.0 $\pm$ 15.8	41.5 $\pm$ 16.0	2358.2 $\pm$ 666.9
Immature (<i>n</i> = 38)	42.0 $\pm$ 9.5	27.9 $\pm$ 18.5	35.7 $\pm$ 10.3	2332.4 $\pm$ 685.6

* *P* = NS.

Table II. Concentrations of IL-1 α , IL-2, TNF- α , and LT B₄ in FFs from Fertilized and Nonfertilized Oocytes*

Oocytes	IL-1 α (pg/ml)	IL-2 (pg/ml)	TNF- α (pg/ml)	LT B ₄ (pg/ml)
Fertilized ($n = 32$)	43.6 $\pm$ 9.7	23.6 $\pm$ 17.3	40.7 $\pm$ 15.9	2410.0 $\pm$ 567.4
Nonfertilized ($n = 37$)	40.6 $\pm$ 8.4	26.7 $\pm$ 17.7	36.2 $\pm$ 10.5	2281.6 $\pm$ 749.7

* $P = \text{NS}$.**Table III.** Concentrations of IL-1 α , IL-2, TNF- α , and LT B₄ in FFs According to the Subsequent Types of Embryos*

Embryos	IL-1 α (pg/ml)	IL-2 (pg/ml)	TNF- α (pg/ml)	LT B ₄ (pg/ml)
Types 1 and 2 ($n = 22$)	42.8 $\pm$ 9.6	23.8 $\pm$ 18.0	39.5 $\pm$ 1.42	2381.9 $\pm$ 601.3
Type 3 ($n = 7$)	46.0 $\pm$ 7.1	22.7 $\pm$ 19.2	39.0 $\pm$ 15.4	2524.4 $\pm$ 610.7

* $P = \text{NS}$.

intraovarian asynchrony between the developing follicles.

A significant positive correlation was found between IL-1 α and TNF- α levels in FFs containing mature oocytes ($r = 0.57$, $P < 0.001$), fertilized oocytes ($r = 0.59$, $P < 0.001$) (Fig. 3), and good-quality embryos ($r = 0.58$, $P < 0.005$), as well as in FFs from both pregnant ($r = 0.40$, $P < 0.05$) and nonpregnant ($r = 0.43$, $P < 0.005$) women. On the other hand, linear regression analysis between IL-1 α and TNF- α FF levels showed no significant correlation in FF with immature oocytes ($r = 0.25$, $P = 0.13$), unfertilized oocytes ($r = 0.05$, $P = 0.75$), or poor-quality embryos ($r = 0.47$, $P = 0.28$). Concerning IL-2, no significant correlation could be demonstrated between this cytokine and IL-1 α , TNF- α , or LT B₄, in any group of FF studied.

A significant positive correlation was also found between IL-1 α and LT B₄ levels in FFs from subsequently fertilized oocytes ($r = 0.63$, $P < 0.001$) (Fig. 4) and from good-scoring embryos ($r = 0.64$, $P < 0.002$), whereas no correlation was observed in FFs from mature ($r = 0.29$, $P = 0.11$) or immature ($r = 0.24$, $P = 0.15$) oocytes, from unfertilized oocytes ($r = 0.08$, $P = 0.95$), or from women who achieved (r

$= 0.27$, $P = 0.17$) or did not achieve ($r = 0.29$, $P = 0.06$) a pregnancy. In addition, TNF- α and LT B₄ levels correlated significantly in FFs from fertilized oocytes ($r = 0.37$, $P < 0.05$) but not in FFs from mature ($r = 0.27$, $P = 0.14$) or immature ($r = 0.23$, $P = 0.16$) oocytes, from unfertilized oocytes ($r = 0.17$, $P = 0.3$), from good-quality ($r = 0.37$, $P = 0.09$) as well as bad-quality ($r = 0.64$, $P = 0.11$) embryos, or from pregnant ($r = 0.36$, $P = 0.06$) or nonpregnant ($r = 0.26$, $P = 0.09$) women.

DISCUSSION

Our data confirm the presence of the cytokines IL-1 α , IL-2, and TNF- α , as well as of LT B₄, which is a product of arachidonic acid via the lipoxygenase metabolic pathway, in human preovulatory FFs following ovarian stimulation with GnRHa/hMG. Our results are in agreement with those of previous studies that also identified the existence of IL-1 α , IL-2, and TNF- α in human FF (7, 12). Some differences observed in the follicular concentrations may be due to the different stimulation protocols used by various centers or to the

Table IV. Concentrations of IL-1 α , IL-2, TNF- α , and LT B₄ in FFs from Pregnant ($n = 8$) and Nonpregnant ($n = 14$) Women*

FF	IL-1 α (pg/ml)	IL-2 (pg/ml)	TNF- α (pg/ml)	LT B ₄ (pg/ml)
Pregnant ($n = 27$)	44.4 $\pm$ 9.6	26.9 $\pm$ 19.0	37.2 $\pm$ 13.0	2380.8 $\pm$ 656.1
Nonpregnant ($n = 42$)	40.4 $\pm$ 8.5	24.2 $\pm$ 16.5	39.1 $\pm$ 13.7	2291.9 $\pm$ 679.9

* $P = \text{NS}$.

Fig. 1. Concentrations of IL-1 α in individual FF samples from pregnant and nonpregnant patients.

Fig. 2. Concentrations of LT B₄ in individual FF samples from pregnant and nonpregnant patients.

different assays used for measurements of the immunoreactive cytokines.

Cytokines are products of the immune cells, especially of monocytes, as well as of other types of cells, e.g., mesenchymal cells (3). Granulosa cells of the ovary have been shown to be a source for TNF- α and possibly for IL-1 as well as a target for these cytokines (4). During the past years, there has been growing evidence indicating that cytokines, particularly IL-1, IL-2, and TNF, are involved in the ovulatory process. The action of IL-1 and TNF- α is associated with several events including increased membrane fluidity and permeability (4) and prostaglandin production (13, 14). Furthermore, they may play a role in cellular proliferation within the follicle (15), because IL-1 and TNF- α were shown to increase during the follicular phase, reaching their maximal levels around ovulation (16).

On the other hand, the data concerning the role of eicosanoids and, particularly, of leukotrienes in human ovarian physiology are much more limited. Earlier studies have identified lipoxygenase activity (6) and measurable leukotriene levels within the preovulatory dominant follicle, in unstimulated women undergoing sterilization (17), suggesting the possible involvement

of lipoxygenase products of arachidonic acid in the mechanism of ovulation. In the present study, we have demonstrated that in preovulatory FFs obtained from stimulated women undergoing IVF, there are significant concentrations of LT B₄, a leukotriene which has

Fig. 3. Correlation between the level of IL-1 α and that of TNF- α in FFs with subsequently fertilized oocytes.

Fig. 4. Correlation between the level of IL-1 α and that of LT B₄ in FFs with subsequently fertilized oocytes.

a degranulating effect and increases vascular permeability *in vivo* (18). The high levels of LT B₄ that we found in FFs may be due to the administration of hCG to all patients during ovarian stimulation, because in earlier studies, it has been demonstrated that lipoxygenase activity in proestrous rat ovaries and preovulatory follicles is enhanced both by endogenous LH and by exogenous hCG (5).

The origin of LT B₄ in FF is not fully elucidated. It has been shown that human granulosa cells release LT B₄ *in vitro*, an action that is induced by LT B₄ itself (19). Other potential cellular sources of LT B₄ in FF include cells of the myeloid lineage, e.g., polymorphonuclear leukocytes or monocytes/macrophages, which have been also identified in human FF (20). Thus, LT B₄ may be produced locally and/or transferred from the serum into the follicle.

No significant differences in FF levels of the studied cytokines and LT B₄ were identified according to oocyte maturity, fertilization, embryo quality, and achievement of pregnancy, which is in accordance with previous studies (21,22) that reported similar results concerning IL-1 and TNF FF levels. Moreover, there were some variations of IL-1 α , IL-2, TNF- α , and LT B₄ in different FFs from the same patient, possibly reflecting the intraovarian asynchrony observed during ovarian stimulation for IVF (23). Hence, the follicular levels of these substances cannot be used as markers of IVF outcome. A significant positive correlation was found between FF IL-1 α and TNF- α . This observation, together with the known property of IL-1 to stimulate macrophages to produce both IL-1 and TNF (24), may suggest that FF IL-1 α supports the production of and

increase in TNF- α before ovulation, contributing to the rupture of the follicular wall. Conversely, no correlation was found between FF IL-2 and the other substances studied. It seems, therefore, that follicular IL-1 α , TNF- α , and LT B₄ have no involvement in IL-2 production, which is secreted by different cell types (activated T lymphocytes), but it may act synergistically with them in the process of ovulation.

The significant positive correlation between FF IL-1 α and LT B₄ as well as between TNF- α and LT B₄ observed in our study may indicate that the production of LT B₄ is partially induced by those cytokines. On the other hand, leukotrienes were reported to exert a stimulatory effect on IL-1 synthesis (25). Therefore, the interaction among IL-1 α , TNF- α and LT B₄ seems to be complex and the regulatory roles of cytokines in their own secretion, as well as their involvement in the regulation of LT B₄, support the existence of a cytokine-leukotriene network. Taking into account the degranulating effect of LT B₄ (17), it seems reasonable to assume that IL-1 α , TNF- α and LT B₄ may play a crucial role in the follicle degradation and rupture processes in humans.

It is noteworthy that all the above correlations were observed in FF with subsequently fertilized oocytes. However, we were not able to demonstrate the aforementioned correlations in FF from mature oocytes (although there was a tendency), possibly due to the difficulty in assessing oocyte maturity accurately (23). Hence, the presence of a positive correlation among FF IL-1 α , TNF- α , and LT B₄ may suggest more optimal follicular and oocyte development in those women.

In conclusion, IL-1 α , TNF- α and LT B₄ may act as parts of a concordant network within the follicular microenvironment, with possible action in the process of follicular maturation and follicle wall degradation. On the other hand, much remains to be learned about the participation of cytokines and leukotrienes in these mechanisms, and further research should elucidate the degree to which these substances contribute to this process.

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REFERENCES

1. Oppenheim JJ, Ruscetti FW, Faltynek C: Cytokines. *In* Basic and Clinical Immunology, DP Stites, AI Terr (eds). East Norwalk, CT, Appleton and Lange, 1991, 7th ed, p 78

2. Simon C, Polan ML: Cytokines and reproduction. *West J Med* 1994;160:425-429
3. Zolti M, Ben-Rafael Z, Meirom R, Shemesh M, Bider D, Mashiach S, Apte RN: Cytokine involvement in oocytes and early embryos. *Fertil Steril* 1991;56:256-272
4. Tabibzadeh S: Cytokines and the hypothalamic-pituitary-ovarian-endometrial axis. *Hum Reprod* 1994;9:947-967
5. Feldman E, Haberman S, Abisogun AO, Reich R, Levran D, Maschiach S, Zuckermann H, Rudak E, Dor J, Tsafiriri A: Arachidonic acid metabolism in human granulosa cells: Evidence for cyclooxygenase and lipoxygenase activity in vitro. *Hum Reprod* 1986;1:353-356
6. Watanabe H, Nagai K, Yamaguchi M, Ikenoue T, Mori N: Concentration of interleukin-1 β correlates with prostaglandin E₂ and F₂ α in human preovulatory follicular fluid. *Hum Reprod* 1994;9:9-12
7. Wang LJ, Brännström M, Robertson SA, Norman RJ: Tumor necrosis factor α in the human ovary: Presence in follicular fluid and effects on cell proliferation and prostaglandin production. *Fertil Steril* 1992;58:934-940
8. Lipner H: Mechanisms of mammalian ovulation. In *The Physiology of Reproduction*, E Knobil, J Neil (eds). New York, Raven Press, 1988, p 447
9. Tarlatzis B, Pados G, Bontis J, Lagos S, Grimbizis G, Spanos E, Mantalenakis S: Ovarian stimulation with buserelin/HMG/HCG: Prospective randomized study of short versus long protocol. *Hum Reprod* 1993;8:807-812
10. Tarlatzis BC, DeCherney AH: Semen preparation for IVF. In *Foundations of In Vitro Fertilization*, CM Fredericks, J Paulson, AH DeCherney (eds). Washington, DC, Hemisphere, 1987, p 60
11. Pados G, Tarlatzis BC, Bontis J, Lagos S, Papadimas J, Spanos S, Mantalenakis S: Evaluation of different ovarian stimulation protocols for in vitro fertilization. *Gynecol Endocrinol* 1995;9:103-112
12. Wang LJ, Norman RJ: Concentrations of immunoreactive interleukin-1 and interleukin-2 in human preovulatory follicular fluid. *Hum Reprod* 1992;7:147-150
13. Godfrey RW, Johnson WJ, Hoffstein ST: Recombinant tumor necrosis factor and interleukin-1 both stimulate human synovial cell arachidonic acid release and phospholipid metabolism. *Biochem Biophys Res Commun* 1987;142:235-241
14. Kokia E, Hurwitz A, Ricciarelli E, Tedeschi C, Resnick CE, Mitchell MD, Adashi EY: Interleukin-1 stimulates ovarian prostaglandin bio-synthesis: Evidence for heterologous contact independent cell-cell interaction. *Endocrinology* 1992;130:3095-3097
15. Wang LJ, Robertson S, Seamark RF, Norman RJ: Lymphokines, including interleukin-2, alter gonadotropin-stimulated progesterone production and proliferation of human granulosa-luteal cells in vitro. *J Clin Endocrinol Metab* 1991;72:824-831
16. Zolti M, Bider D, Seidman DS, Maschiach S, Ben-Rafael Z: Cytokine levels in follicular fluid of polycystic ovaries in patients treated with dexamethasone. *Fertil Steril* 1992;57:501-504
17. Priddy AR, Killick SR, Elstein M, Morris J, Sullivan M, Patel L, Elder MG: Ovarian follicular fluid eicosanoid concentrations during the pre-ovulatory period in humans. *Prostaglandins* 1989;38:197-202
18. Knapp HR: Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor. *N Engl J Med* 1990;323:1745-1748
19. Rabe T, Przylipek A, Grunwald K, Parta S, Przylipek M, Runnebaum B: Action of leukotriene B₄ in human granulosa cells in vitro. *Mol Hum Reprod* 1995;1:1881-1884
20. Loukides JA, Loy RA, Edwards R, Honig J, Visintin I, Polan ML: Human follicular fluids contain tissue macrophages. *J Clin Endocrinol Metab* 1990;71:1363-1367
21. Barak V, Mordel N, Horzer H, Zajicek G, Treves AJ, Laufer N: The correlation of interleukin-1 and tumour necrosis factor to oestradiol, progesterone and testosterone levels in periovulatory follicular fluid of in vitro fertilization patients. *Hum Reprod* 1992;7:462-464
22. Huyser C, Fourie FLR, Bosmans E, Levay PF: Interleukin-1 β , interleukin-6, and growth hormone levels in human follicular fluid. *J Assist Reprod Genet* 1994;11:193-202
23. DeCherney AH, Tarlatzis BC, Laufer N: Follicular development: Lessons learned from human in vitro fertilization. *Am J Obstet Gynecol* 1985;153:911-923
24. Atkins RC: Interleukin-1 in crescentic glomerulonephritis. *Kidney Int* 1995;48:576-586
25. Oppenheim JJ, Ruscetti FW, Faltynek C: Cytokines. In *Basic and Clinical Immunology*, DP Stites, AI Terr (eds). East Norwalk, CT, Appleton and Lange, 1991, 7th ed, p 78