

Full Length Research Paper

Interleukin 6, interleukin 1 β , estradiol and testosterone concentrations in serum and follicular fluid of females with stimulated and non-stimulated ovaries

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The ovarian physiology is regulated by some complex factors such as hormones and growth factors. The hormones and growth factors are synthesized by follicular and ovarian cells during follicular maturation stage and cytokines are synthesized by the immune system. Interaction between immune and endocrine systems modulates ovarian function through the secretion of regulatory soluble factors, especially cytokines. There is a close contact between corona-cumulus-oocyte complex with follicular fluid which affects the quality and degree of oocyte maturation. The objectives of this study were to determine the levels of interleukin-6 (IL-6), interleukin 1 β , estradiol and testosterone concentration in serum and follicular fluid of women with stimulated and non-stimulated ovaries. A total of 41 women, 27 in stimulated and 14 non-stimulated cycles, undergoing intra cytoplasmic sperm injection (ICSI) treatment were included in this study. Follicular fluid and serum samples from all cases were collected at the time of oocyte retrieval and concentration of testosterone, estradiol, interleukin 6 and interleukin 1 β were measured. The results show that serum concentration of testosterone was significantly higher in non stimulated cases. Also, the serum and follicular fluid concentrations of interleukin 6 and interleukin 1 β in stimulated cases were significantly higher than non stimulated group. There was an important negative correlation between the level of testosterone in patient's serum and IL-6 and IL-1 β levels of follicular fluid. In conclusion, according to these results, it seems that the levels of testosterone and IL-6 and IL-1 β in patient's serum and follicular fluid are a good factor for prediction of maturity of oocytes.

Key words: Oocyte maturation, follicular fluid, interleukin 6, interleukin 1 β , testosterone, estradiol.

INTRODUCTION

A complex of hormones, growth factors and cytokines regulates the ovarian function. The interaction between immune and endocrine systems is triggered by the action of immune cells within the ovary. This interaction modu-

lates ovarian function through the secretion of regulatory soluble factors, especially cytokines (Adashi, 1992; Zolti et al, 1991; Bonetti et al., 2010).

The immune cells have been suggested to play an important role in ovarian physiology. They are a source of cytokines and growth factors within the ovary at various stages of follicular development (Brannstrom and Norman, 1993). In the pre-ovulatory stage, several kinds of leukocytes and cytokines seem to act synergistically to promote rupture of the follicle and change the ruptured follicle into a corpus luteum (Brannstrom and Norman, 1993; Ziltener et al., 1993; Hammadeh et al., 2002). Also, they mediate ovarian functions specifically during the

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Abbreviations: IL-6, Interleukin-6; 1 β , interleukin 1 β ; ICSI, intra cytoplasmic sperm injection; ART, assisted reproductive technology.

process of ovulation (Adashi, 1992; Barak et al., 1992; Hurwitz et al., 1991; Machelon et al., 1995).

In addition, macrophage secretes factors which play important roles in granulosa cell proliferation and the regulation of follicular steroidogenesis and follicular rupture (Cohen et al., 1996).

The hormones synthesized by granulosa cells during follicular maturation stage accumulate in follicular fluid. It is believed that there is a close contact between corona-cumulus-oocyte complex with follicular fluid and an association exists between the hormonal content of follicular fluid and the quality and degree of oocyte maturation. Therefore, the hormonal content of follicular fluid is presumed to be related to fertilization, embryo development and implantation rate (Costa et al., 2004; Fishel et al., 1983; Pellicer et al., 1998).

In the last decade, for ovulation induction, gonadotropin-releasing hormone (GnRH) agonists have been routinely used in assisted reproductive technology (ART). *In vivo* and *in vitro* studies have shown that GnRH reduces steroidogenesis granulosa cells and reduce steroid levels in follicular fluid (Dor et al., 2000; Costa et al., 2004). On the other hand, the use of GnRH agonists during ovulation induction has significantly improved the results of ART cycles, causing the relationship between steroid levels and oocyte maturity to be questioned. Recently, some studies were carried out to determine whether progesterone, estradiol and cytokines levels in serum and follicular fluid or their ratio may be used as parameters to discriminate between ovulatory (mature) oocytes and non-ovulatory (immature) oocytes. However, progesterone, estradiol and testosterone levels in follicular fluid are significantly modified during the preovulatory period, especially after the luteinizing hormone (LH) surge (Murdoch and Dunn, 1982; Hammadeh et al., 2002; Costa et al., 2004; Bonetti et al., 2010). However, the results were contradictory to that of Peek et al. (1986) and Wrambsy et al. (1981). The ability to determine the condition of oocytes maturation during ovulation induction cycles is the ambition of many ART clinics. If this can be successfully achieved, it would aid women at risk of premature ovarian failure, and possibly result in women no longer requiring an expensive drug regime and monitoring programme, which they currently have to undergo (Thomas et al., 2003).

The objectives of this study were to determine the levels of interleukin-6 (IL-6), interleukin 1 β , estradiol and testosterone concentration in serum and follicular fluid of women with stimulated and non-stimulated ovaries.

MATERIALS AND METHODS

The local ethical committee approved the study and written informed consents were obtained from all participants according to the criteria of the Hamadan University of medical sciences.

A total of 41 women, 27 in stimulated and 14 non-stimulated cycles, undergoing ICSI treatment in the Fatemeh Infertility Research Center were included in this study. The age of all the

patients was under 40 years and male factor was the main indication for ICSI treatment. In the stimulated cases, all patients underwent controlled ovarian hyperstimulation after they had been treated with GnRH agonist (Superfact, Hoechst, Germany), for pituitary down-regulation as described earlier (Farimani et al., 2006). Controlled ovarian hyperstimulation was induced with recombinant FSH (Gonal-F, Serono, Swiss). Administration started at a daily dose of 150 to 225 IU, depending on age. The dose of the gonadotropin was changed according to the follicular growth. When more than 2 follicles bigger than or equal to 18 mm were seen, HCG (Pregnyle, Organon, Germany) 10000 IU were injected to induce final oocyte maturation and 36 h later, the mature oocytes were retrieved.

In the non-stimulated cases, to initiate the treatment cycle, the patient with amenorrhea received vaginal 300 mg of progesterone once daily for 10 days to induce withdrawal bleeding. Between days 2 and 4 of menstrual bleeding, small ovarian follicles were monitored by transvaginal ultrasonography, confirming that there was no dominant follicle and endometrial thickness was 8 mm. Transvaginal ultrasound scans were repeated on day 8 of the cycle or on the day of HCG administration (Chian et al., 2000; Son et al., 2006) when endometrial thickness reached at least 6 mm.

The patient was given 10000 IU of HCG (Pregnyle, Organon, Germany) and 36 h later, the immature oocytes were retrieved (Chian et al., 2000). Immature oocytes were aspirated with 17.5 gauge needles (MDT, Netherlands) under the guidance of transvaginal ultrasonography. A portable aspiration pump was used with a pressure between 160 and 180 mmHg. The aspirates were collected in tubes with pre-warmed heparinized saline, immature oocytes were retrieved and follicular fluid was collected.

Follicular fluid samples from 27 stimulated and 14 non-stimulated cases were collected at the time of oocyte retrieval and immediately centrifuged (10 min by 300 g) to remove granulosa cells and clarified supernatants. Then, the supernatants were collected and stored in aliquots at -70°C until the hormones and cytokines concentration were determined. Only follicular fluid free of blood was used to determine cytokine and hormone determination.

A peripheral blood sample was obtained from all the cases on the day of oocyte retrieval. The serum were separated and stored at -70°C for further analysis.

Testosterone and estradiol assay

Serum and follicular fluid testosterone and estradiol were measured using a quantitative competitive immuno-assay kit (DRG instruments company, Germany). Testosterone and estradiol were analyzed in duplicate for each serum samples.

The level of testosterone is expressed as ng/ml, the level of estradiol is expressed as pg/ml.

Interleukin 6 and interleukin 1 β assay

Both serum and follicular fluid Interleukin 6 and interleukin 1 β were measured using enzyme linked immunosorbent assay for quantitative detection of human interleukin 6 and interleukin 1 β (Bender Med Systems, Austria) (IL-6 BMS 213/2 and IL-1 β BMS 224/2). The level of interleukin 6 and interleukin 1 β is expressed as pg/ml.

Statistical analysis

Statistical analysis was performed using the SPSS software package, version 16. Student t-test was used for analysis. The

Table 1. The levels of testosterone, estradiol, interleukin 6 and interleukin 1 β in the serum of stimulated and non-stimulated cases.

Parameter	Stimulated (mean \pm SEM)	Non-stimulated (mean \pm SEM)	P- value
Testosterone (ng/ml)	7.90 \pm 1.04	13.93 \pm 1.37	<0.001
Estradiol (pg/ml)	1518.2 \pm 5.64	1513.8 \pm 9.27	<0.672
Interleukin 6 (pg/ml)	1.80 \pm 0.26	0.21 \pm 0.05	0.000
Interleukin 1 β (pg/ml)	3.20 \pm 0.43	0.75 \pm 0.19	0.000

Table 2. The levels of testosterone, estradiol, interleukin 6 and interleukin 1 β in the follicular fluid of stimulated and non-stimulated cases.

Parameter	Stimulated (mean \pm SEM)	Non-Stimulated (mean \pm SEM)	P- value
Testosterone (ng/ml)	2.24 \pm 0.19	3.43 \pm 0.36	<0.003
Estradiol (pg/ml)	1207 \pm 70.65	1102.4 \pm 129.42	<0.443
Interleukin 6 (pg/ml)	9.08 \pm 0.58	2.32 \pm 0.7	0.000
Interleukin 1 β (pg/ml)	3.16 \pm 0.4	0.82 \pm 0.21	0.000

results were reported as mean \pm SEM. The linear dependence was measured using Pearson's correlation coefficient. A value of $P < 0.05$ was considered significant.

RESULTS

The study population consisted of 41 patient serum and follicular fluid, 27 patients with stimulated and 14 patient non-stimulated ovaries. The mean \pm SEM and differences of testosterone, estradiol and cytokines in the serum and follicular fluids of both groups are shown in Tables 1 and 2.

As shown in these tables, the level of testosterone in serum and follicular fluid of non stimulated group were significantly higher than that of stimulated ($P < 0.001$ and $P < 0.003$, respectively). However, there were no significant differences between the levels of estradiol in serum and follicular fluid of two groups. In comparing the concentration of cytokines, significant difference was shown between the groups. As shown in Tables 1 and 2, the levels of interleukin 6 and interleukin 1 β in serum and follicular fluid of stimulated cases were significantly higher than non-stimulated group ($P < 0.000$).

On the other hand, the statistical analysis of our results showed that the serum concentration of testosterone was negatively correlated with the concentration of IL-6 in

follicular fluid ($P < 0.006$, $r = -0.425$) (Figure 1). The same result was shown on the correlation between the serum concentration of testosterone and level of interleukin 1 β in follicular fluid ($P < 0.006$, $r = -0.468$) (Figure 2) but there were no correlation between serum concentration of estradiol and level of interleukin 6 and interleukin 1 β in follicular fluid.

DISCUSSION

The statistical analysis of our results showed that serum concentration of testosterone in non stimulated cases was significantly higher than that of stimulated patients. On the other hand, there was an important negative correlation between the level of testosterone in patient's serum and IL-6 and IL-1 β levels of follicular fluid, but there is no correlation between the level of estradiol in patient serum and IL-6 and IL-1 β levels of follicular fluid. According to our results, it seems that the levels of testosterone in patient serum are negatively correlated with the maturity of oocytes. However, we could not find a cut off point in the level of serum testosterone to predict the maturity of oocytes.

Several studies have shown that mature oocytes with a higher potential for fertilization are also associated with

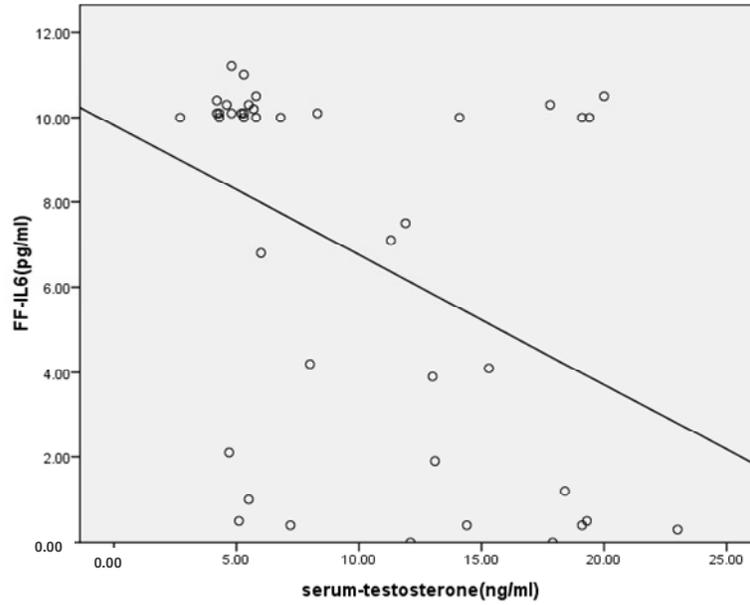


Figure 1. Negative correlation between the serum concentration of testosterone and level of interleukin 6 in follicular fluid (FF) of study population (41 cases), ($P < 0.006$, $r = -0.425$)

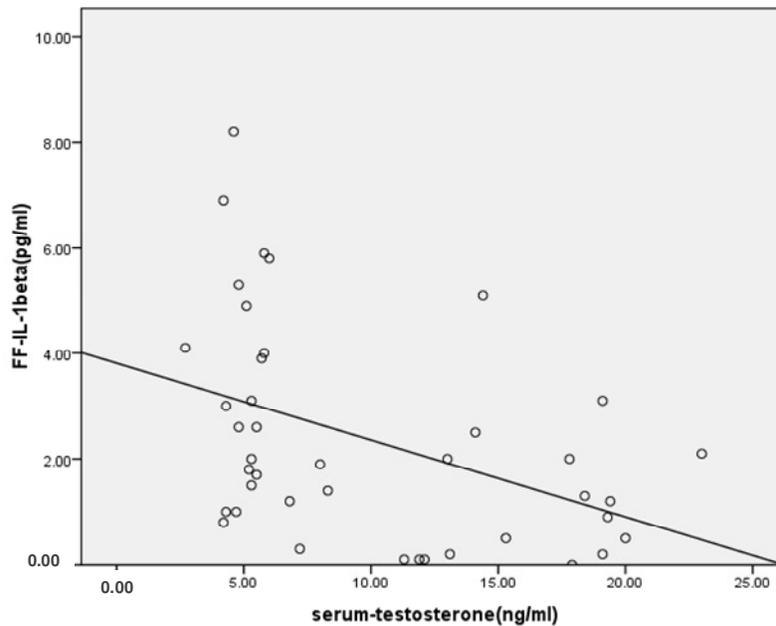


Figure 2. Negative correlation between the serum concentration of testosterone and level of interleukin 1 β in follicular fluid (FF) of study population (41 cases), ($P < 0.006$, $r = -0.468$).

lower testosterone levels in follicular fluid at the time of follicular rupture (Brailly et al., 1981; Vanluchene et al., 1991; Costa et al., 2004). Our results are in agreement with these data. The association between oocyte maturity and low testosterone concentration in follicular fluid of

stimulated cases is usually associated with immature oocyte with low count of granulosa cells. Estradiol levels were not different between mature and immature oocytes. The reduction of estradiol levels in preovulatory follicles seems to result from a modification of the

steroidogenic patterns of granulosa cells. Oocyte maturation is accompanied by a significant reduction in estradiol and testosterone levels and a positive association between them. Indeed, this could not be different, since androgens and testosterone in particular are immediate precursors of estradiol. Thus, if a reduction in testosterone production by thecal cells is observed, this fact definitely contributes to the reduction of estrogen synthesis observed in parallel (Costa et al., 2004).

Previous reports have indicated that IL-6 and IL-1 β have an important role in ovarian steroidogenesis, while it is known that these cytokines are produced by ovarian granulosa cells of preovulatory follicles (Salamonsen et al., 2007; Trundley et al., 2004), being a main mediator of inflammatory responses during ovulation (Buscher et al., 1999).

In conclusion, it seems that lower levels of cytokines in follicular fluid of immature oocytes indicate their role in follicular growth and oocyte maturation. For the time being, we are unable to provide a cut off point in levels of IL-6 and IL-1 β in serum and follicular fluid to predict the maturity of oocytes and future studies are needed to find these cut of points.

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REFERENCES

- Adashi E (1992). The potential relevance of cytokines to ovarian physiology. *J Steroid Biochem* . 43:439-444.
- Barak V, Mordel N, Holzer H, Zajicek G, Treves AJ, Laufer N (1992). The correlation of interleukin-1 and tumor necrosis factor to oestradiol, progesterone, and testosterone levels in preovulatory follicular fluid of *in vitro* fertilization patients. *Hum Reprod* . 7:462-464.
- Bonetti TC, Salomao R, Braunialti M, Braga DP, Borges E Jr, Silva ID (2010). Cytokine and hormonal profile in serum samples of patients undergoing controlled ovarian stimulation: interleukin-1 β predicts ongoing pregnancy. *Human Reprod* . 25(8):2101-6.
- Brailly S, Gougeon A, Milgrom E, Bomsel-Helmreich O, Papiernik E (1981). Androgens and progestins in the human ovarian follicles: differences in the evolution of preovulation, healthy nonovulatory, and atretic follicles. *J. Clin. Endocrinol. Metabolism* . 53: 128-134.
- Brannstrom M and Norman RJ (1993). Involvement of leukocytes and cytokines in the ovulatory process and corpus luteum function. *Hum Reprod* . 8:1762-1775.
- Buscher U, Chen FC, Kantenich H, Schmiady H (1999). Cytokines in the follicular fluid of stimulated and non-stimulated human ovaries; is ovulation a suppressed inflammatory reaction? *Hum Reprod* . 14(1):162-6.
- Chian RC, Buckett WM, Tulandi T, Tan SL (2000). Prospective randomized study of human chorionic *gonadotrophin* priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum Reprod* . 15:165-170.
- Cohen T, Nahari D, Cerem LW, Neufeld G, Levi BZ (1996). Interleukin 6 induces the expression of vascular endothelial growth factor. *J Biol. Chem* . 271:736-741.
- Costa LO, Mendes MC, Ferriani RA, Moura MD, Reis EM, Silva De Sa MF (2004). Estradiol and testosterone concentrations in follicular fluids as criteria to discriminate between mature and immature oocytes. *Braz. J Med. Biol. Res. Nov*;73(11):1747-1755.
- Dor J, Bider D, Shulman A, Levron JL, Shine S, Mashiach S, Rabinovici J (2000). Effects of gonadotrophin-releasing hormone agonists on human ovarian steroid secretion *in vivo* and *in vitro*. Results of a prospective, randomized *in vitro* fertilization study. *Human Reprod* . 15: 1225-1230.
- Farimani M, Amiri I, Hoseini S (2006). Day 3 inhibin-B level is not predictive of ovarian assisted reproductive technologies outcome. *IJRM* 4(1):1-5.
- Fishel SB, Edwards RG, Walters DE (1983). Follicular steroid as a prognosticator of successful fertilization of human oocytes *in vitro*. *J. Endocrinol* . 99: 335-344.
- Hammadeh ME, Ertan AK, Georg MT, Rosenbaum P, Schmidt W (2002). Relationship between ovarian stimulation regimen and interleukin level in pre-ovulatory follicular fluid and their effect on ICSI outcome. *AJRI* . 48:255-261.
- Hurwitz A, Ricciarelli E, Botero L, Rohan RM, Hernandez ER, Adashi EY (1991). Endocrine and autocrine mediated regulation of rat ovarian (theca interstitial) interleukin 1 β gene expression: Gonadotropin-dependent preovulatory acquisition. *Endocrinol* . 129:3427-3429.
- Machelon V, Nome F, Durand-Gasselin I, Emilie D (1995). Macrophage and granulosa interleukin-1 β mRNA in human ovulatory follicles. *Hum. Reprod* . Aug;10:2198-2203.
- Murdoch WJ, Dunn TG (1982). Alterations in follicular steroid hormones during the preovulatory period in the ewe. *Biol. Reprod* . 27: 300-307.
- Peek JC, Ghoy VJ, Watkins WB, Graham FM (1986). Levels of oxytocin-like activity and progesterone in follicular fluid from *in vitro* fertilization cycles. *J. In Vitro Fertilization and Embryo Transfer* . 3: 157-165.
- Pellicer A, Valbuena D, Bauset C, Albert C, Bonilla-Musoles F, Remohi J, Simon C (1998). The follicular endocrine environment in stimulated cycles of women with endometriosis: steroid levels and embryo quality. *Fertility and Sterility* . 69: 1135-1141.
- Salamonsen LA, Hannan NJ, Dimitriadis E (2007). Cytokines and chemokines during human embryo implantation: roles in implantation and early placentation. *Semin Reprod Med* . 25:437-444.
- Son WY, Yoon SH, Lim JH (2006). Effect of gonadotrophin priming on *in-vitro* maturation of oocytes collected from women at risk of OHSS. *RBM Online* . 13:340-348.
- Thomas FH, Walters KA, Telfer EE (2003). How to make a good oocyte: an update on *in-vitro* models to study follicle regulation. *Hum Reprod Update* . 9(6): 541-555.
- Trundley A, Moffett A (2004). Human uterine leukocytes and pregnancy. *Tissue Antigens* . 63:1-12.
- Vanluchene E, Hinting A, Dhont M, De Sutter P, Van Maele G, Vandekerckhove D (1991). Follicular fluid steroid levels in relation to oocyte maturity and *in vitro* fertilization. *J. Steroid Biochem. Mol. Biol* . 38: 83-87.
- Wramsby H, Kullander S, Liedholm P, Rannevik G, Sundstrom P, Thorell J (1981). The success rate of *in vitro* fertilization of human oocytes in relation to the concentrations of different hormones in follicular fluid and peripheral plasma. *Fertility and Sterility* . 36: 448-454.
- Ziltener HJ, Maines-Bandiera S, Schrader JW, Auersperg N (1993). Secretion of bioactive interleukin-1, interleukin-6, and colony-stimulating factors by human ovarian surface epithelium. *Biol. Reprod* . 49: 635-641.
- Zolti M, BenRafael Z, Meirom R, Shemesh M, Bider D, Mashiach S, Apte RN (1991). Cytokines involvement in oocytes and early embryos. *Fertil Steril* . 56: 265-272.