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Issue Article

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THE ASSOCIATION BETWEEN FOLLICULAR ASPIRATION FLUID CYTOKINES AND RESULTS OF IN VITRO FERTILIZATION PROCEDURE

***Abstract*: *The pupose of the study was** to investigate the association between the levels of cytokines in follicular aspiration fluid (FAF) and the outcomes of in vitro fertilization (IVF) procedure.*

***Material and Methods:** 131 patients were included in the study. They were grouped based on the outcome or parameters of the IVF procedure (successful or not), pregnancy status and female infertility. Cytokines (IL-1β, TNF-a, IFN-γ, IL-4, IL-5, IL6, and IL-7) were measured by ELISA in the follicular aspiration fluid obtained during the process of oocyte pick-up (OPU).*

***Results:** Among the infertility factor the most common was person factors 45.0% (n=59). The ovarian factor was revealed in 26 (19.8%) of patients, tube factor – in 13 (9.9%), unexplained reason – in 19 (14.5%), gender selection – in 4 (3.1%) and infertility depends on both sides - in 10 (7.6%) of women. According to the results of hysterosalpingogram, tubes were open in 118 (90.1%) of patients, were closed in 13 (9.9%) of all women, involved in the study. The pregnancy outcomes were as following: no transfer –in 7 (5.3%), no pregnancy – in 60 (45.8%), presence of pregnancy – in 56 (42.7%), menstrual cycle cancelled – in 8 (6.1%%) of patients. IL-1β levels were statistically significant different in the follicular aspiration fluid of patients with pregnancy and without pregnancy (p=0.039).*

***Conclusion:** There is statistically significant association between IL-1β in follicular aspiration fluid and successful pregnancy in IVF procedure.*

***Key words**: cytokines, IL-1β, pregnancy, in vitro fertilization, follicular aspiration fluid.*
***Language**: English*
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Introduction

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Assisted reproductive technology (ART) includes fertility treatment in which either eggs or embryos are handled outside a female's body to promote successful pregnancies and healthy offspring [1]. As of 2020**,** an estimated 8 million children had been conceived by ART [2].

Current ART procedures encompass in vitro fertilization with or without intracytoplasmic sperm injection. The success of an IVF procedure and the results depend on various factors, of which normal folliculogenesis is considered to be crucial [1].

A normal balance of different parts of the immune system is necessary for the proper development of follicles. Cytokines are key modulators of the immune system and also contribute to regulation of the ovarian cycle [3]. Correlations between embryo quality and concentrations of specific cytokines in culture media of human embryos have been investigated for many years [4].

The dysregulation of immune cells and cytokine profiles in the follicular fluid may play an important role in the competence of the oocyte and the development of the embryo, but the mechanism remains largely unknown [5].

Cytokine support of embryonic development includes promotion of implantation and protection of blastomeres from cell stress and apoptosis [4]. Cytokines promote proper oocyte maturation, timely rupture of follicles, and neoangiogenesis, indirectly contributing to the supply of oxygen, nutrients, and substrates for subsequent steroidogenesis [6]. Regarding folliculogenesis, cytokines regulate cell proliferation or differentiation, follicle survival or atresia, and oocyte maturation [7]. According to some sources, among the cytokines, elevation of CCL15, CCL27, and CXCL-12 in culture media were significantly associated with in the top-quality embryo in IVF procedure. These results suggested that specific cytokines measured in human embryo culture media can be used to predict embryo quality and IVF outcomes [4].

The central role of cytokines suggests that any modulation during follicle development and oocyte maturation may have a significant impact on the development of physiological conditions for fertilisation. Pro-inflammatory cytokines are crucial in the maturation process of the ovarian follicle, in addition to the process of embryo implantation. Immune imbalances have a negative impact on the prognosis of the effectiveness of IVF and possibly on natural fertilization [8].

The analysis of the cytokine and hormonal profile of follicular fluid in natural and stimulated physiological cycles is crucial in the assessment of its role in follicle development [9].

Despite many recent publications, knowledge of the immunology of follicular fluid in the context of reproductive system pathology is superficial, and further research is needed to better understand the role of individual cytokines in reproductive pathology. In the future, this knowledge may allow for the individual qualification of patients for individual methods of infertility treatment, taking into account the patient's immune profile. Thus, studying the role of various cytokines in the procedure of in vitro fertilization may ultimately help in establishing an individual approach to the fertility treatment.

The purpose of the study was to investigate the association between cytokines in follicular aspiration fluid (FAF) and in vitro fertilization outcomes in women undergoing in vitro fertilization procedure.

Materials and methods

The study involved 131 women who were treated with the IVF procedure in the period from 2020 to 2022 in the reproduction department of the Caspian International Hospital. Patients were grouped and analyzed based on following parameters from IVF procedure outcomes: IVF outcome (positive or negative); pregnancy (positive or negative); infertility factors. In addition to general clinical studies, hysterosalpingogram was performed to determine the condition of tubes.

IVF was performed according to standard clinical procedures.

Cytokines were measured in the serum and follicular aspiration fluid obtained during the process of OPU. All samples were taken at the day of OPU from 84 patients. Samples were centrifuged at 1,000xg for 15 minutes and then stored at -800C until used. Standard ELISA kits (Thermofisher) were used to measure IL-1β, TNF-a, IFN-y, IL-4, IL-5, IL-6, and IL-7 using STAT FAX 303 PLUS instrument in Caspian international Hospital Laboratory.

Statistical Analysis was performed by NCSS (Number Cruncher Statistical System) program. Descriptive statistical methods (mean, standard deviation, frequency, percentage) were used while evaluating the study data. The conformity of the quantitative data to the normal distribution was tested with the Shapiro-Wilk test and graphical examinations. A Mann-Whitney U test was used for comparisons between two groups of non-normally distributed quantitative variables. Statistical significance was accepted as $p<0.05$.

Results

The results of studying the anamnesis of the examined women are presented in Table 1

Table 1. The anamnestic data of women involved in study

Age	Mean±SD	31.02±6.01
	19-35 years	103 (78.6%)
	36-50 years	28 (21.4%)
Pregnancy history	Not present	104 (79.4)
	Present	27 (20.6%)
Number of pregnancy (n=27)	Mean±SD	1.89±1.31
	1 time	13 (48.1%)
	≥2 times	14 (51.9%)
Birth (n=27)	Not present	19 (70.4%)
	Present	8 (29.6%)

Number of births (n=8)	Mean±SD	1.50±0.76
	1 birth	5 (62.5%)
	≥2 births	3 (37.5%)
Abortion (n=27)	Not present	10 (37.0%)
	Present	17 (63.0%)
Number of abortions (n=17)	Mean±SD	1.59±0.94
	1 time	11 (64.7%)
	≥2 times	6 (35.3%)
Medical abortion (n=27)	Not present	26 (96.3%)
	Present	1 (3.7%)
Number of medical abortions (n=1)	5 times	1 (100.0%)
Ectopic pregnancy (n=27)	Not present	20 (74.1%)
	Present	7 (25.9%)

According to the results obtained, among the infertility factor the most common was person factors 45.0% (n=59). The ovarian factor was revealed in 26 (19.8%) of patients, tube factor – in 13 (9.9%), unexplained reason – in 19 (14.5%), gender selection – in 4 (3.1%) and infertility depends on both sides - in 10 (7.6%) of women. The hysterosalpingography revealed that tubes were open in 118 (90.1%) of patients, were closed in 13 (9.9%) of all women, involved in the study. The outcomes were as following: no pregnancy – in 60 (45.8%), presence of pregnancy – in 56 (42.7%), menstrual cycle cancelled – in 8 (6.1%), no transfer –in 7 (5.3%) of patients.

Some cytokines' levels were different in the follicular aspiration fluid of patients with IVF successful results and without them. Despite the levels of IL-1β and IL-6 in women with IVF (+) was lower than in patients with IVF (-), (1009.07±1597.7 vs 1381.18±2474.95, respectively for IL-1β and 69.36±134.91 vs 119.63±240.59, respectively for IL-6), the differences were not statistically significant (p>0.05). The other cytokines had practically the same levels in compared groups (p>0.05).

Table 2. The levels of cytokines in follicular aspiration fluid based IVF success status

Parameters		Groups: IVF not successful (-) **(n=8)**	Groups: IVF successful (+) **(n=76)**	***P***
IL-1β pg/ml	Mean±SD	1381.18±2474.95	1009.07±1597.7	***[a]0,851***
TNF-α pg/ml	Mean±SD	95.1±109.11	102.64±251.45	***[a]0,453***
IFN-γ pg/ml	Mean±SD	75.49±136.83	75.49±136.83	***[a]0,325***
IL-4 pg/ml	Mean±SD	153.49±292.7	128.1±231.87	***[a]0,424***
IL-5 pg/ml	Mean±SD	145.13±272.32	115.67±216.25	***[a]0,295***
IL-6 pg/ml	Mean±SD	119.63±240.59	69.36±134.91	***[a]0,994***
IL-7 pg/ml	Mean±SD	192.76±359.57	150.7±291.35	***[a]0,502***

[a]Mann Whitney's U Test / Values are for pg/ml.

The patients' markers were analyzed based the pregnancy status. IL-1β level was different in the follicular aspiration fluid of patients with pregnancy and without pregnancy (p=0.039).

The cytokines' levels were analyzed based on the infertility status in patients' follicular aspiration liquid (infertility (+), n=32; infertility (-), n=50), there was no significant difference in these markers in patients based on infertility status (p>0.05).

We studied the relationship between various infertility factors and cytokines in FAF. Our patients were grouped based on infertility factors. One group

(n=28) included patients with factors such as tubes over reserve and gender selection. The other group (n=48) included patients with factors such as male factor, azoospermia, and unexplained cause. The levels of cytokines in the patient follicular aspiration fluid were not significantly different (p>0.05).

Discussion

Our study revealed that the concentrations of cytokines in FAF did not demonstrate significant differences (only IL-1β, depends on pregnancy status; p=0.039).

However, there are some articles with different results.

So, Lédée et al., by assessment the concentrations of 28 cytokines and chemokines in FAF collected from 132 women subjected to IVF-ICSI, found that a significantly higher concentration of IL-2 and INF-ɣ was present in the FAF of embryos that underwent early cleavage [10].

Very interesting study was published by Sarapik et al., who presented an analysis of pro- (IL-1β, IL-6, IL-18, IFN-γ, IFN-α, TNF-α, IL-12, and IL-23) and anti-inflammatory cytokines (G-CSF), in addition to which selected chemokines (MIP-1α, MIP-1β, MCP-1, RANTES, and IL-8) in FAF were collected from women undergoing IVF. The authors revealed that lower levels of IL-β and INF-α were correlated with tubal infertility. In our study we did not find statistically significant differences depends on infertility factors [3].

In our patients with IVF negative results we also observed a reduced level of IL-6 in FAF. Among cytokines, such as IL-4, IL-6 and IL-10 which are primarily secreted by Th2 cells, IL-4 and IL-10 can cooperate with Treg cells in the regulation of immune balance to ensure a conducive environment for embryo implantation and maintenance of pregnancy. Zhang Y, et al indicated the changes of IL-4 and IL-10 and their ratio in patients with recurrent implantation failure [11]. But we did not revealed differences of IL-4 levels in FAF among women observed.

The same results demonstrated IL-5 concentration in FAF – there were no significant difference. It is interesting that Alhilali MJS, et al (2019) in their study found out higher levels of IL-5 in follicular fluid as a negative predictor of the intracytoplasmic sperm injection outcome [12].

Conclusions

In women undergoing IVF procedure the levels of IL-1β in follicular aspiration fluid statistically significant (p=0.039) differ in groups based pregnancy status (present or not present). There are no any association between cytokines levels in FAF depend on IVF success and infertility factors.

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