Journal of Applied Molecular Biology (JAMB)
Molecular biology Research & Studies institute , Assuit University, Egypt
ISSN 2974 – 4008
Volume 3 (1): January 2025

https://jamb.journals.ekb.eg

Relation of Interleukin-6 level in the follicular fluid on intracytoplasmic sperm injection outcome

Shymaa F. A. Abdellatif[1*], Asmaa A. A. Hussein [1,2], Ahmed Momen Kamel[1,3], Ahmed A. Youssef[1]

[1]Department of Molecular Biology, Molecular Biology Research & Studies Institute, Assiut University, Egypt
[2]Department of zoo noses, Faculty of Veterinary Medicine, Assiut University, Egypt
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Egypt.

[*]Corresponding Author: shoshet.agaa@gmail.com

ARTICLE INFO

Article History:
Received:02-10-2024
Accepted: 24-12-2024
Online: 27-01-2025

Keywords:
Interleukin-6 (IL-6), follicular fluid (FF), intracytoplasmic sperm injection (ICSI)

ABSTRACT

This study aims to use follicular fluid interleukin-6 (FF IL-6) levels as a biomarker for the prediction of intracytoplasmic sperm injection (ICSI) outcomes. A total of 82 couples receiving ICSI at the Assiut Centre for IVF and ICSI at El-Hyat Hospital, Assiut, Egypt, were included in this study between August 2021 and December 2022. The participants were between the ages of 20 and 40, with body mass index (BMI) ranging from 19 to 35 kg/m2. They received a typical antagonist regimen (Cetrorelix) and recombinant follicle-stimulating hormone (rFSH) until at least three follicles reached 18 mm. Ovum pick-up occurred approximately 36–37 hours after the introduction of human chorionic gonadotropin (HCG). Follicular fluid (FF) samples were collected, subjected to centrifugation, and stored for further analysis. The oocytes obtained were 12.67 ± 6.33 oocytes per patient, with a 78.12 ± 15.06% maturity rate. Pregnancy rate was 44%. FF IL-6 levels exhibiting a favourable correlation with the amount of blastocysts produced on the fifth day ($r = 0.318$, $p = 0.013$). FF IL-6 levels were significantly elevated in patients who successfully achieved pregnancy compared to those who did not ($p < 0.00001$). FF IL-6 levels affected the percentage of embryo fragmentation ($p < 0.001$) but not symmetry or cell number at the cleavage stage. The study suggests that FF IL-6 levels might be a potential biomarker for predicting ICSI success in patients with male factor and unexplained infertility. Higher levels of IL-6 are associated with increasing blastocyst formation, higher pregnancy rates, and less embryo fragmentation; however, more research will be needed.

1. INTRODUCTION

The FF surrounding the oocyte constitutes a dynamic biomolecular microenvironment, offering critical insights into oocyte maturation and differentiation by reflecting its metabolic demands and signaling pathways [1]. The composition of the follicular fluid including cytokine levels can reflect the local microenviroment and potential influence oocyte quality and subsequent embryo development.

Cytokines play a crucial role in controlling ovarian functions, specifically in the processes of follicle development and release of eggs. They help create a favorable environment for the selection and growth of follicles [2]. Interleukin-6 (IL-6), as a A pleiotropic cytokine has various actions on different cells, here are some key cellular effects of IL-6:

1. Inflammatory Response: It is a major mediator that stimulates the production of acute-phase proteins which are important for the body's response to infection, injury, or inflammation.
2. Regulation of Immune Response: It enhances the process of distinguishing and stimulating immune cells, such as T cells, B cells, and macrophages.
3. Responsible for regulating Hematopoiesis (the process of blood cell formation) [3].
4. Metabolic Regulation: Including glucose and lipid metabolism. It can induce insulin resistance in certain tissues and promote lipolysis
5. Cell Growth and Differentiation in various cell types: Including tissue regeneration, repair processes, besides the development and progression of certain diseases, including cancer [4].
6. Neurological Effects: It has been implicated in the central nervous system, where it can influence neuronal function and neuroinflammation [5].

Overall, IL-6 has diverse effects on various cell types and physiological processes. It present in the FF, arises potential influence on oocyte quality [6]. Several independent studies have confirmed the IL-6 of FF presence in human [7, 8]. The disparity in IL-6 levels between FF and serum suggests a specific and potentially active contribution of this cytokine to follicular processes and oocyte development [9].

A Bayesian network was utilized to investigate the correlation between the cytokine network in human follicular fluid and mature oocytes at the protein level. The results suggest that IL-6 was situated in a pivotal position within the network [10].

Clinical investigations have revealed a positive association between elevated IL-6 levels and compromised embryo quality. Additionally, these studies suggest a reduction in pregnancy rates among patients with higher IL-6 concentrations [11]. Conversely, studies have reported a beneficial role of elevated IL-6 levels in the FF during oocyte maturation suggesting a potential association between high IL-6 concentrations and enhanced clinical pregnancy rates and embryo implantation, counterpoint to the observations linking IL-6 with compromised embryo quality [12].

Controlled ovarian stimulation (COS) using gonadotrophins induces dynamic alterations in the IL-6 landscape within the follicular micoenviroment, inducing a notable elevation

compared to natural cycles in some studies [13]. These dynamic changes in FF present a potential avenue for predicting ICSI outcomes directly, through parameters like oocyte maturity and fertilization rate, or indirectly, by influencing embryo quality, pregnancy rate, and implantation rate [14]. Prior investigations did not take into account the influence of these elements. Also prior studies that assessed the relationship between biomarkers and their capacity to forecast the result of ICSI either employed single FF samples [15, 16, 17, 18, 19, 20] or combined FF samples [21, 22, 23, 24]. Pooled FF can provide a realistic representation of the dynamic micro-environment in which oocytes are produced [20]. Moreover, the process of single follicle aspiration poses challenges for both the patient and the physician due to the need for many vaginal punctures, hence elevating the risk of vaginal hemorrhage [2].

Our study was conducted to examine the associations between IL-6 levels in the pooled FF and outcomes in laboratory indexes utilized for assessing the embryos and biochemical pregnancy.

2. MATERIALS AND METHODS

This study was cross sectional prospective study enrolled 82 participants undergoing ovarian stimulation and ICSI at the Assiut Center for IVF and ICSI at El-Hyat Hospital, Assiut, Egypt, between August 2021 and December 2022. Participants received a typical Cetrorelix antagonist regimen (Cetrotide®, Merck Serono, France; 0.25 mg) and rFSH (Gonapure, Minapharm, Egypt) until at least three follicles reached 18 mm. Oocyte retrieval occurred 36-37 hours post HCG administration (choriomon, IBSA, Netherlands; 10000 IU). Individual pools of FF of all follicles more than 16 mm in diameter were collected via transvaginal ultrasound-guided follicle aspiration, centrifuged at 2000 revolutions per minute (rpm) for 10 minutes, and stored at -80°C. To ensure sample integrity, only FF devoid of flushing solution was collected.

2.1. Study protocol

2.1.1. Hormonal profile assessment:

Detection of the hormonal profile including anti-Müllerian hormone (AMH), Follicle-stimulating hormone (FSH) levels for all participants in day 2 or 3 of their last menstrual cycle. Estradiol (E2) level also was assessed in the blood on the day of HCG administration.

2.1.2. Inclusion criteria:

Normal responder Participants were aged 20-40 years with BMIs 19-35 kg/m2 and ICSI is necessary in cases of unexplained infertility or mild male factor infertility (MFI).

2.1.3. Exclusion criteria:

Poor responders were aged > 40 years, women with ovarian hyper stimulation syndrome (OHSS) and infertility due to severe male factor.

2.1.4. Oocytes assessment:

Within two hours after oocytes collection, cumulus cell denudation of the oocyte-cumulus complexes was performed enzymatically using hyalourinidase (SAGE, 80 U/ml, 30 Second). The quality of oocytes was directly evaluated based on the criteria

established by The Istanbul consensus workshop on embryo assessment [25], within 20-30 minutes of collection. Oocytes that displayed an elongated sun-flare corona radiata were categorized as high-quality.

Microscopic evaluation of oocyte nuclear morphology classified them as necrotic, germinal vesicle (GV) stage, prophase I, or metaphase II (MII) as shown in Figure 1. Only MII oocytes, indicative of meiotic maturity, were selected for insemination. The remaining oocytes, including GV and necrotic ones, were excluded. The proportions of each oocyte stage were determined for each patient by dividing the respective oocyte count by the total retrieved oocyte count, then multiplying by 100.

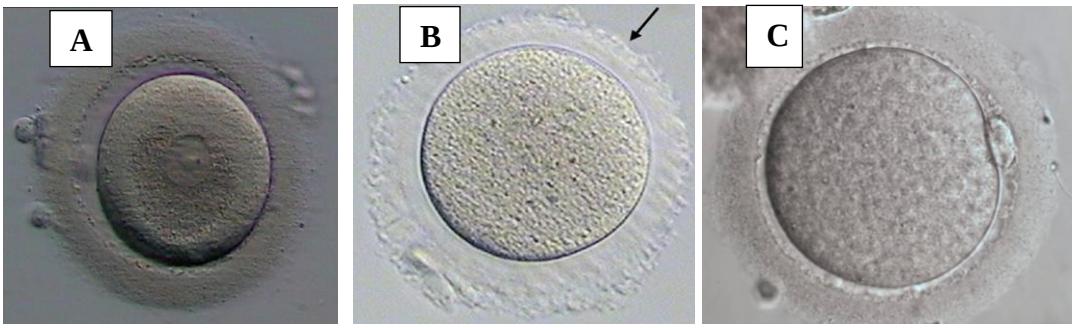

Figure 1. Showing different stages of nuclear maturation of the oocytes (A: GV , B: MI, C: MII)

2.2. ICSI outcome

Approximately 16-18 hours after ICSI, oocyte fertilization assessment was conducted. The presence of two pronuclei (2PN) under microscopic observation served as a confirmation of successful fertilization and zygote formation. Furthermore, the fertilization rate (FR) was calculated by dividing the number of two pronuclei (2PN) by the total number of MII oocytes and expressing it as a percentage. This computation was performed by dividing the number of 2PN oocytes by the total number of MII oocytes and then multiplying the result by 100.

On the third day after ICSI, embryos were graded and assessed. The generated embryos were scored as follows:

1. Fragmentation grade: fragmentation is the lost cytoplasmic content of the dividing cells of the embryos that can be classified into 4 grades as followsIV: Less than 10% fragmentation, III: 10 to 20% fragmentation, II: 20 to 50% fragmentation, I: More than 50% fragmentation.
2. Blastomere symmetry: the blastomere diameter difference that has 2 grades: (1):≤ 25%, (0): > 25%.
3. Number of cells (n).

Two embryos were chosen for transfer on day 5 to the uterus under vaginal ultrasound guidance and the number and rate of blastocyst formation on day 5 were determined for all embryos that continued to day 5 for transfer or vitrification. A positive HCG level (>5 IU/mL) in blood 14 days after intrauterine embryo transfer was assessed as an indicator for biochemical pregnancy.

2.3 Quantification of IL-6 in FF

IL-6 ELISA kits (BT LAB cat. No E0090Hu) were used. The procedure was performed in compliance with the kit's instructions.

2.3.1. Ethics approval and consent to participate:

The study protocol underwent rigorous ethical review and received approval from the Research Ethics Committee (REC) of the Molecular Biology Research and Studies Institute (MBRSI) at Assiut University, Egypt. This ensured adherence to the highest ethical standards throughout the research process, safeguarding the rights and well-being of all participants.

2.3.2. Informed Consent: All participants provided written informed consent for the release of anonymised data and study outcomes.

2.4. Statistical analysis:

Data entry and analysis were performed using SPSS version 22 (Statistical Package for Social Science). Independent samples t-test was used to compare quantitative variables between groups in case of parametric data. Mann-Whitney test was used to compare quantitative variables between groups. Spearman correlation was done to measure correlation between quantitative variables in case of non-parametric data. P- value< 0.05 was considered to be statistically significant.

3 . RESULTS

The 82 patients in this study were all normal responders who were having ICSI due to male factor and infertility that could not be explained. Table 1 displays the individuals' baseline characteristics. The mean values for age, BMI, infertility duration and antral follicle count (AFC) were 28.95 ± 4.87 years, 26.35 ± 2.81 Kg/m2, 6.12 ± 3.58 years and 9.33 ± 4.06 and mean values of their hormonal profile including AMH, FSH and TSH were, 1.97 ± 0.75 ng/ml and 4.97 ± 2.09 mIU/ml, respectively.

Table 1. The baseline characters and hormonal profile of the participants in this study

Baseline data	Mean ± SD	Median (Range)
Age (years)	28.95 ± 4.87	30.0 (20.0-39.0)
BMI (Kg/m^2)	26.35 ± 2.81	26.7 (19.3-33.0)
Infertility duration (years)	6.12 ± 3.58	6.0 (1.0-16.0)
AFC	9.33 ± 4.06	8.0 (7.0-25.0)
AMH (ng/ml)	1.97 ± 0.75	1.8 (1.0-3.1)
FSH (mIU/ml)	4.97 ± 2.09	4.9 (1.1-10.9)

BMI: Body mass index, AFC: Antral follicle count, AMH: Anti-Müllerian hormone, FSH: follicle stimulating hormone.

Mean values for GnRH used, E2 level in the blood on the day of HCG administration., duration of stimulation, oocytes retrieved, maturity rate and IL-6 concentration were 270.73 ± 57.44 IU, 1897.56 ± 975.19 pg/ml, 8.85 ± 0.94 days, 12.67± 6.33 and 78.12 ±

15.06 % and 450.23 ± 242.55 ng/l respectively as presented in Table 2. The pregnancy rate was 44%.

Table 2. Gn used, participant data during induction and after ovum pick-up.

Parameters	Mean ± SD	Median (Range)
GnRH (IU)	270.73 ± 57.44	300.0 (150.0-375.0)
Serum E2 on HCG day (pg/ml)	1897.56 ± 975.19	1919.0 (701.0-4014.0)
Duration of stimulation (days)	8.85 ± 0.94	9.0 (8.0-12.0)
Expected oocyte (n)	12.29 ± 5.34	12.0 (5.0-25.0)
Retrieved oocytes (n)	12.67 ± 6.33	11.5 (3.0-25.0)
MII oocytes (n)	10.18 ± 5.95	9.0 (2.0-25.0)
Maturity rate (%)	78.12 ± 15.06	80.0 (28.6-100.0)
IL-6 Concentration (ng/L)	450.23 ± 242.55	334.0 (158.1-935.3)

GnRH: gonadotropin releasing hormone, E2: estradiol, HCG: human chrionic gonadotropin, MII: metaphase II, IL-6: interleukin-6.

An evident and meaningful association was found between FF and IL-6 levels and the quantity of blastocysts produced on the fifth day of culture ($r = 0.328$, $p = 0.003$*). In order to examine the possible impact of COH on FF IL-6 levels, the correlation between FF IL-6 levels and different COH parameters was evaluated on the day of oocyte extraction Table 3. There were no notable associations discovered between FF IL-6 levels and age ($r = 0.020$, $p = 0.858$), BMI ($r = -0.036$, $p = 0.746$), infertility duration ($r = -0.001$, $p = 0.991$), AFC ($r = 0.100$, $p = 0.370$), AMH ($r = 0.115$, $p = 0.303$), serum E2 on the day of HCG administration ($r = 0.024$, $p = 0.834$), number of retrieved oocytes ($r = 0.126$, $p = 0.260$), number of mature MII oocytes ($r = 0.165$, $p = 0.137$), number of embryos ($r = 0.209$, $p = 0.060$), type of GnRH used ($r = -0.052$, $p = 0.645$), or duration of COH stimulation ($r = -0.047$, $p = 0.673$).

All 82 patients underwent embryo transfer. As indicated in Table 4, A substantial disparity in FF IL-6 levels was detected between the pregnant and non-pregnancy cohorts ($p < 0.00001$), having elevated levels in the pregnant cohort. Importantly, there were no substantial disparities seen across the groups in terms of their initial features and hormone profiles (Table 4), including age ($p = 0.643$), BMI ($p = 0.252$), infertility duration ($p = 0.989$), AFC ($p = 0.273$), AMH ($p = 0.120$), FSH ($p = 0.795$). Table 5 There is no notable distinction observed between the pregnant and non-pregnant groups in terms of the type of GnRH utilized ($p = 0.784$), duration of stimulation ($p = 0.353$) and serum E2 levels on the HCG day ($p = 0.701$). Furthermore, according to laboratory data, no significant differences were found in the total number of retrieved oocytes ($p = 0.386$) or the number of mature oocytes (MII) ($p = 0.327$) between both groups.

Table 3. The relationship between important parameters in controlled ovarian hyperstimulation and FF IL-6 on the day of oocyte retrieval.

Parameters	IL-6 Concentration (ng/L)	
	r-value	P-value
Age (years)	0.020	0.858
BMI (Kg/m^2)	-0.036	0.746
Infertility duration (years)	-0.001	0.991
AFC (n)	0.100	0.370
AMH (ng/ml)	0.115	0.303
FSH (mIU/ml)	-0.143	0.200
Serum E2 on HCG day (pg/ml)	0.024	0.834
GnRH	-0.052	0.645
Duration of stimulation (days)	-0.047	0.673
Retrieved oocytes	0.126	0.260
MII oocytes (n)	0.165	0.137
MII rate %	0.135	0.227
Immature (n)	-0.024	0.832
Embryo number (n)	0.209	0.060
Blastocyst Number (n)	0.328	0.003*

BMI: body mass index, AFC: antral follicle count, AMH,anti-müllerian hormone, FSH, follicule stimulating hormone, E2: estradiol, HCG: human chrionic gonadotropin, GnRH: gonadotropin releasing hormone, MII: metaphase II, *.Correlation is significant at the 0.05 level (2-tailed).

Table 4. Differences between pregnant and non- pregnant groups according to patient characteristics and hormonal profile.

Parameters data	Pregnant (n= 36)	Not pregnant (n= 46)	P-value
Age: (years)			
Mean ± SD	28.67 ± 4.76	29.17±4.99	0.643
Range	22.0-39.0	20.0-39.0	
BMI: (Kg/m2)			
Mean ± SD	25.95±2.72	26.66±2.86	0.252
Range	19.3-33.0	19.9-32.9	
Infertility duration: (years)			
Mean ± SD	5.89 ±3.24	6.17 ± 3.76	0.989

Median (Range)	6.0 (1.0-12.0)	6.0 (1.0-16.0)	
AFC:			
Mean ± SD	9.89 ± 4.46	8.89 ± 3.72	0.273
Median (Range)	8.5 (7.0-25.0)	7.0 (7.0-25.0)	
AMH: (ng/ml)			
Mean ± SD	2.12 ± 0.77	1.86 ± 0.74	0.120
Median (Range)	2.1 (1.1-3.1)	1.62 (1.0-3.4)	
FSH: (mIU/ml)			
Mean ± SD	4.09 ± 2.18	5.02 ± 2.05	0.795
Median (Range)	4.6 (1.59-10.0)	5.0 (1.1-10.9)	

BMI: body mass index, AFC: antral follicle count, AMH: anti-müllerian hormone, FSH: follicule stimulating hormone,**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

Table 5. Differences in stimulation protocol and lab parameters between pregnant and non-pregnant groups

Parameters data	Pregnant (n= 36)	Not pregnant (n= 46)	P-value
GnRH:			
Mean ± SD	268.75 ± 54.89	272.28 ± 59.91	0.784
Median (Range)	262.5 (150.0-375.0)	300.0 (150.0-375.0)	
Serum E2 on HCG day: (pg/ml)			
Mean ± SD	1944.69 ± 937.75	1860.67 ± 1012.26	0.701
Median (Range)	2095.5 (812.0-3917.0)	1865.5 (701.0-4014.0)	
Duration of stimulation:(days)			
Mean ± SD	8.75 ± 0.65	8.93 ± 1.12	0.353
Median (Range)	9.0 (8.0-10.0)	9.0 (8.0-12.0)	
Retrieved oocytes:			
Mean ± SD	13.36 ± 5.93	12.13 ± 6.64	0.386
Median (Range)	13.5 (4.0-24.0)	10.0 (3.0-25.0)	
MII oocytes:			
Mean ± SD	10.92 ± 5.68	9.61 ± 6.16	0.327
Median (Range)	10.0 (3.0-23.0)	9.0 (2.0-25.0)	
IL-6 concentration (ng/l)			

Mean ± SD	681.53 ± 182.2	269.22 ± 61.51	0.000*
Median (Range)	760.6 (224.7-935.3)	273.1 (158.1-393.7)	

GnRH: gonadotropin releasing hormone, E2: estradiol, HCG: human chrionic gonadotropin, MII: metaphase II, IL-6: interleukin-6. **. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).

The relation between IL-6 and Embryo grading

IL-6 can effect on the percentage of fragmentation in embryos and its level ($p<0.001$), however it does not effect on the symmetry and number of cells in all embryos at cleavage stage as shown in Figure 2. Table 6 demonstrated a statistically noteworthy distinction according to the number of blastocyst formed in day 5 and thus in their rate of formation (P= 0.045, P= 0.000** respectively).

Table 6. Differences in blastocyst formed and blastulation rate between pregnant and non-pregnant groups

Parameters	Pregnant (n= 36)	Not-pregnant (n= 46)	P-value
Blastocyst No.:			
Mean ± SD	5.72 ± 4.73	3.59 ± 4.71	0.045*
Median (Range)	4.0 (0.0-20.0)	2.0 (0.0-21.0)	
Blastulation rate (%):			
Mean ± SD	60.28 ± 22.99	34.87 ± 24.50	0.000**
Median (Range)	60.8 (0.00-100)	36.4 (0.00-91.30)	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

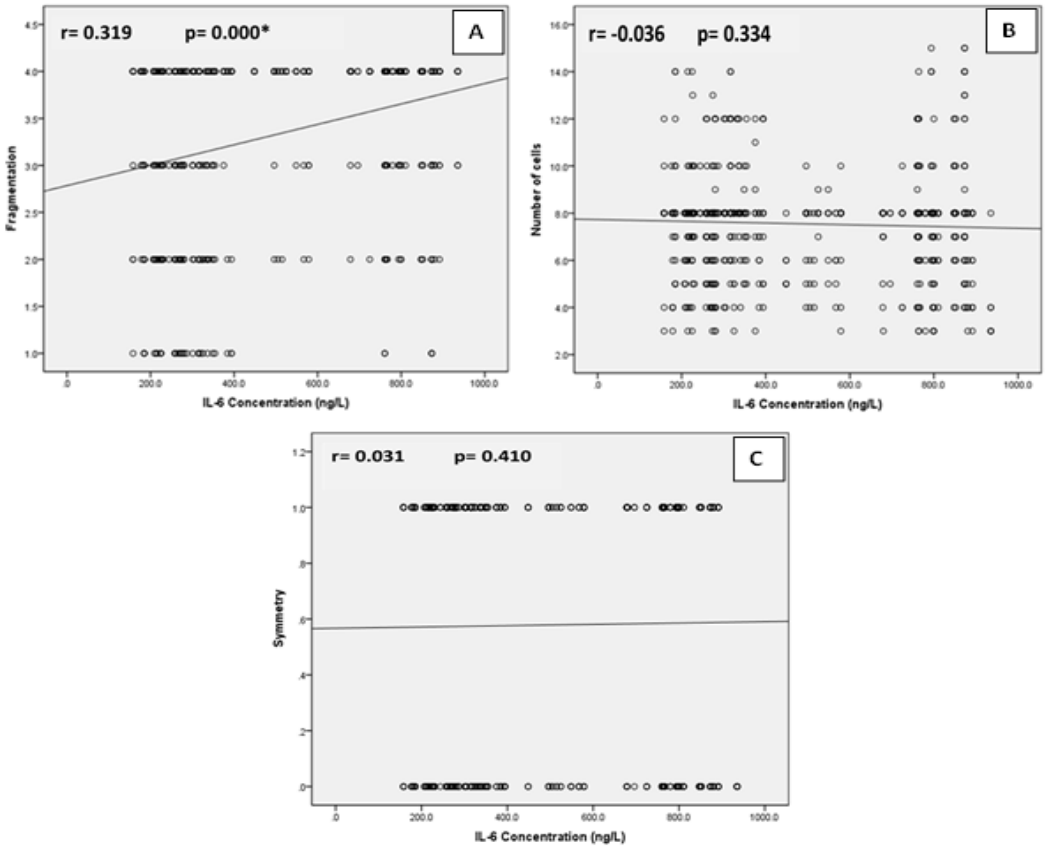

Figure 2: Showing the relation between IL-6 concentration and day 3 embryo fragmentations (A), cell numbers (B) and cell symmetry (C).

4. DISCUSSION

IL-6, is a cytokine with multicellular functions. It contributes to progress and maturation of follicles. It is one of several mediators produced in the process of incomplete abrogation of the HCG which induced ovulation rate. Gonadal cells (GCs) produce IL-6 in reaction to HCG during the process of ovulation [8]. IL-6 concentrations were enhanced in preovulatory FF, supporting its participation in ovulation [26] and inducing cumulus-oocyte complex. Furthermore, IL-6 cooperates to stimulate the formation of the corpus luteum [27].

This study aimed to examine the correlation between the concentration of IL-6 in the follicular fluid following COH by antagonist protocol and the outcome of ICSI; with regarding to implantation potential and biochemical pregnancy success. In agreement with a previous finding by [5]. The occurrence of the biochemical pregnancy and the IL-6 concentration are positively correlated because the epithelial and stromal endometrial cells release a variety of interleukin classes during implantation, including IL-6. These interleukins form a complex network that promotes trophoblastic invasion, decidua formation, and embryo implantation, regulates the activity of regulatory T and B cells, suppresses the release of antifetal antibodies, and coordinates the maturation and proliferation of uterine natural killer cells [28].

In order to investigate the function of FF IL-6 in the development of embryos, we analyzed the relationship between FF IL-6 and three important indicators that indicate the capacity for embryonic development: cell quantity, blastomere symmetry, and embryo fragmentation. Our findings show that FF IL-6 had no effect on embryo division rate or blastomere symmetry on day three. Nevertheless, elevated levels of FF IL-6 were linked to a significant reduction in embryo fragmentation which aligns with the discovery made by [5].

One of the most important markers of its developmental potential is embryo fragmentation. Apoptotic blastomeres may be the source of the fragmented cells, which indicate compromised cellular integrity and are intimately linked to the arrest of embryonic development [29, 30]. Research conducted in vitro has repeatedly shown that adding recombinant IL-6 to growth medium dramatically lowers the percentage of apoptotic cells [31]. Our data supports earlier findings that indicate positive embryonic development is associated with higher levels of FF IL-6. The potential beneficial role of IL-6 in this specific scenario was supported by the substantial negative connection observed between increased FF IL-6 levels and embryo fragmentation, which was accompanied by a discernible increase in clinical pregnancy rates.

According to Yang et al.[5], IL-6 can effect on the percentage of fragmentation in embryos and its level was higher in pregnant than non- pregnant women undergoing ICSI and had long agonist protocol and Our research showed the same outcomes when we used an antagonist protocol for induction.

We evaluate the relationship between FF IL-6 concentrations and the other ICSI outcome including oocytes and embryo grading and blastocyst formation and we analyzed the blastulation rate in pregnant and non-pregnant women and our result ensured that increased levels of IL-6 increased the blastocyst formation rate significantly which agree with a previous study by Kelley and Gardner [32]. They found that, IL-6 enhanced the growth and blastocyst formation of the separately cultured mouse embryo.

The relationship between specific gonadotropin (Gn) regimens (antagonist) and FF IL-6 concentrations was analyzed. In agreement with a previous finding by [5] that used agonist protocol, There is no substantial distinction in the total quantity of Gn used, the length of stimulation, and FF IL-6 levels when comparing the usage of agonist protocol with antagonist protocol. Consequently, the utilization of Gn did not impact the final IL-6 concentration in the follicular fluid. Furthermore, the levels of IL-6 did not show any correlation with the estradiol level on the day of HCG administration, which aligns with the discovery made by Wu et al. [12].

Building upon the promising work of Stojanovic Gavrilovic et al. [30], our study further supports the potential of FF IL-6 levels as a predictive marker for successful Assisted reproductive technology (ART) outcomes.

CONCLUSION

This Study demonstrates that increased levels of IL-6 in the fluid around the ovarian follicles can reduce the breakdown of embryos and improve the likelihood of successful pregnancies and FF IL-6 levels may be used as a potential biomarker for predicting ICSI success patients with unexplained or mild male factor infertility. Higher IL-6 levels were

associated with less embryo fragmentation and increased blastocyst formation, higher pregnancy rates. However, further research is needed to confirm these findings and determine their clinical utility in practical use because the study's sample size is tiny. Moreover, blood contamination during sample can make FF's accurate IL-6.

REFERENCES

1. Rodgers, R. J., Irving-Rodgers, H. F., 2010. Formation of the ovarian follicular antrum and follicular fluid. Biology of Reproduction, (82), 1021-1029.
2. Alhilali, M. J. S., Parham, A., Attaranzadeh, A., Amirian, M., Azizzadeh, M., 2019. IL-5 in follicular fluid as a negative predictor of the intracytoplasmic sperm injection outcome. Cytokine, (113), 265-271.
3. Li, B., Jones, L. L., Geiger, T. L., 2018. IL-6 promotes T cell proliferation and expansion under inflammatory conditions in association with low-level RORγt expression. The Journal of Immunology, (201), 2934-2946.
4. Garbers, C., Heink, S., Korn, T., Rose-John, S., 2018. Interleukin-6: designing specific therapeutics for a complex cytokine. Nature reviews Drug discovery (17), 395-412.
5. Yang, J., Yang, X., Yang, H., Bai, Y., Zha, H., Jiang, F., Meng, Y., 2020. Interleuki n 6 in follicular fluid reduces embryo fragmentation and improves the clinical pregnancy rate. Journal of Assisted Reproduction and Genetics, (37), 1171-1176.
6. Stojanovic Gavrilovic, A. Z., Cekovic, J. M., Parandilovic, A. Z., Nikolov, A. B., Sazdanovic, P. S., Velickovic, A. M., Andjelkovic, M. V., Sorak, M. P., 2022. IL-6 of follicular fluid and outcome of in vitro fertilization. Medicine, (101), e29624.
7. Buyalos, R. P., Watson, J. M.a., Martinez-Maza, O., 1992. Detection of interleukin-6 in human follicular fluid**Presented at the 46th Annual Meeting of The American Fertility Society, Washington, D.C., October 15 to 18, 1990.††Supported by a grant from the California Institute for Cancer Research, Los Angeles, California, and by grants CA 01588 from the National Institutes of Health and CA 09120 from the National Institutes of Health Tumor Immunology Institutional Training, Bethesda, Maryland. Fertility and sterility, (57), 1230-1234.
8. Machelon, V., Emilie, D., Lefevre, A., Nome, F., Durand-Gasselin, I., Testart, J., 1994. Interleukin-6 biosynthesis in human preovulatory follicles: some of its potential roles at ovulation. The Journal of Clinical Endocrinology & Metabolism, (79), 633-642.
9. Kollmann, Z., Schneider, S., Fux, M., Bersinger, N. A., von Wolff, M., 2017. Gonadotrophin stimulation in IVF alters the immune cell profile in follicular fluid and the cytokine concentrations in follicular fluid and serum. Human Reproduction, (32), 820-831
10. Field, S. L., Dasgupta, T., Cummings, M., Orsi, N. M., 2014. Cytokines in ovarian folliculogenesis, oocyte maturation and luteinisation. Molecular Reproduction and Development, (81), 284-314.
11. Semeniuk, L. M., Likhachov, V. K., Yuzvenko, T. Y., Dobrovolska, L. M., Makarov, O. G., 2018. Risk markers of reproductive loss in women with hyperandrogenism. Wiadomosci Lekarskie (Warsaw, Poland: 1960), (71), 1550-1553.
12. Wu, G., Bersinger, N. A., Mueller, M., von Wolff, M., 2017. Intrafollicular inflammatory cytokines but not steroid hormone concentrations are increased in

naturally matured follicles of women with proven endometriosis. Journal of Assisted Reproduction and Genetics, (34), 357-364.

13. Geva, E., Lessing, J. B., Lerner-Geva, L., Azem, F., Yovel, I., Amit, A., 1997. Elevated levels of interleukin-6 in the follicular fluid at the time of oocyte retrieval for in vitro fertilization may predict the development of early-form ovarian hyperstimulation syndrome. Fertility and sterility, (68), 133-137.
14. Singh, A. K., Dutta, M., Chattopadhyay, R., Chakravarty, B., Chaudhury, K., 2016. Intrafollicular interleukin-8, interleukin-12, and adrenomedullin are the promising prognostic markers of oocyte and embryo quality in women with endometriosis. Journal of Assisted Reproduction and Genetics, (33), 1363-1372.
15. Baskind, N. E., Orsi, N. M., Sharma, V., 2014b. Impact of exogenous gonadotropin stimulation on circulatory and follicular fluid cytokine profiles. International Journal of Reproductive Medicine, 2014.
16. de los Santos, M. J., García-Láez, V., Beltrán-Torregrosa, D., Horcajadas, J. A., Martínez-Conejero, J. A., Esteban, F. J., Pellicer, A., Labarta, E., 2012. Hormonal and molecular characterization of follicular fluid, cumulus cells and oocytes from pre-ovulatory follicles in stimulated and unstimulated cycles. Human Reproduction, (27), 1596-1605.
17. Lédée, N., Frydman, R., Osipova, A., Taieb, J., Gallot, V., Lombardelli, L., Logiodice, F., Petitbarat, M., Fanchin, R., Chaouat, G., 2011. Levels of follicular G-CSF and interleukin-15 appear as noninvasive biomarkers of subsequent successful birth in modified natural in vitro fertilization/intracytoplasmic sperm injection cycles. Fertility and sterility, (95), 94-98.
18. Lédée, N., Gridelet, V., Ravet, S., Jouan, C., Gaspard, O., Wenders, F., Thonon, F., Hincourt, N., Dubois, M., Foidart, J. M., 2013. Impact of follicular G-CSF quantification on subsequent embryo transfer decisions: a proof of concept study. Human Reproduction (28), 406-413.
19. Ledee, N., Petitbarat, M., Frydman, R., Rahmati, M., Wainer, B., Dubanchet, S., Foidart, J., Munaut, C., Piccinni, M., Chaouat, G., 2010. Peri-conceptual cytokine environment and consequences for human pregnancy. Journal of Reproductive Immunology, (1), 19.
20. Southcombe, J. H., Ledee, N., d'Hauterive, S. P., Turner, K., Child, T., Snider, J. V., Redman, C.W., Sargent, I. L., Granne, I., 2013. Detection of soluble ST2 in human follicular fluid and luteinized granulosa cells. PloS one (8), e74385.
21. Altun, T., Jindal, S., Greenseid, K., Shu, J., Pal, L., 2011. Low follicular fluid IL-6 levels in IVF patients are associated with increased likelihood of clinical pregnancy. Journal of Assisted Reproduction and Genetics, (28), 245-251.
22. Asimakopoulos, B., Demirel, C., Felberbaum, R., Waczek, S., Nikolettos, N., Koester, F., Al-Hasani, S., Diedrich, K., 2010. Concentrations of inflammatory cytokines and the outcome in ICSI cycles. in vivo, (24), 495-500
23. Cataldi, T., Cordeiro, F. B., da Costa, L. D. V. T., Pilau, E. J., Ferreira, C. R., Gozzo, F. C., Eberlin, M. N., Bertolla, R. P., Cedenho, A. P., Lo Turco, E. G., 2013. Lipid profiling of follicular fluid from women undergoing IVF: young poor ovarian responders versus normal responders. Human fertility, (16), 269-277.
24. Mehta, B. N., Chimote, M. N., Chimote, N. N., Nath, N. M., Chimote, N. M., 2013. Follicular-fluid anti-Mullerian hormone (FF AMH) is a plausible biochemical

indicator of functional viability of oocyte in conventional in vitro fertilization (IVF) cycles. Journal of human reproductive sciences, (6), 99.

25. Balaban, B., Brison, D., Calderon, G., Catt, J., Conaghan, J., Cowan, L., Ebner, T., Gardner, D., Hardarson, T., Lundin, K., 2011. Alpha scientists in reproductive medicine and ESHRE special interest group of embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction, (26), 1270-1283.
26. Büscher, U., Chen, F. C., Kentenich, H., &, Schmiady, H., 1999. Cytokines in the follicular fluid of stimulated and non-stimulated human ovaries; is ovulation a suppressed inflammatory reaction? Human reproduction (Oxford, England), (14), 162–166.
27. Baskind, N. E., Orsi, N. M., Sharma, V., 2014a. Follicular-phase ovarian follicular fluid and plasma cytokine profiling of natural cycle in vitro fertilization patients. Fertility and sterility, (102), 410-418.
28. Pantos, K., Grigoriadis, S., Maziotis, E., Pistola, K., Xystra, P., Pantou, A., Kokkali, G., Pappas, A., Lambropoulou, M., Sfakianoudis, K., 2022. The role of interleukins in recurrent implantation failure: a comprehensive review of the literature. International journal of molecular sciences, (23), 2198.
29. Chi, H. J., Koo, J. J., Choi, S. Y., Jeong, H. J., Roh, S. I., 2011. Fragmentation of embryos is associated with both necrosis and apoptosis. Fertility and sterility, (96), 187-192.
30. Stensen, M. H., Tanbo, T. G., Storeng, R., Åbyholm, T., Fedorcsak, P., 2015. Fragmentation of human cleavage-stage embryos is related to the progression through meiotic and mitotic cell cycles. Fertility and sterility, (103), 374-381.e374.
31. Shen, X. H., Han, Y. J., Zhang, D. X., Cui, X. S., Kim, N. H., 2009. A link between the interleukin-6/Stat3 anti-apoptotic pathway and microRNA-21 in preimplantation mouse embryos. Molecular Reproduction and Development, (76), 854-862.
32. Kelley, R. L., Gardner, D. K., 2017. Addition of interleukin-6 to mouse embryo culture increases blastocyst cell number and influences the inner cell mass to trophectoderm ratio. Clinical and Experimental Reproductive Medicine, (44), 119.