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The effect of individual oocyte matched follicular fluid oxidant, antioxidant status, and pro- and anti-inflammatory cytokines on IVF outcomes of patients with diminished ovarian reserve

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Abstract

Oocyte matched follicular fluid oxidant, antioxidant status, and pro- and anti-inflammatory cytokine levels were assessed to reveal a possible effect of local-intrafollicular levels of these markers on the individual oocyte with its quality, ability to achieve fertilization, further embryo development, and pregnancy. A cross-sectional study of infertile women with diminished ovarian reserve undergoing antagonist protocol in vitro fertilization (IVF); in the form of ICSI, and fresh single embryo transfer were included. When follicular fluid was collected, each ovarian follicle was aspirated independently, and each follicular fluid was collected into a separate test tube to match it with a single cumulus-oocyte complex obtained from the same follicle. Oocyte matched follicular fluid samples and blood specimens were taken from the participants. Relationships of total antioxidant status, total oxidant status, oxidative stress index, total thiol, interleukin (IL)-6, IL-8, and IL-10 levels of each follicle with oocyte grade, grade of transferred embryos, and pregnancy rate of a given follicle were assessed. A total of 23 infertile women with diminished ovarian reserve and 79 individual follicles of these women were assessed. Serum total oxidant status level of metaphase II (MII) group was significantly lower than non-MII group ($P < .001$). Follicular fluid IL-6 level of MII group was significantly lower than non-MII group ($P = .005$). Follicular fluid IL-8 value was significantly low with positive pregnancy results ($P < .001$). Serum oxidative stress status and follicular fluid pro-inflammatory cytokines were associated with IVF outcomes. This unique study might guide IVF practice with the aim of developing and establishing more effective therapeutic strategies and choosing embryos with more potential for success.

Abbreviations: DOR = diminished ovarian reserve, IL = interleukin, IVF = in vitro fertilization, MI = metaphase I, MII = metaphase II, OSI = oxidative stress index, ROS = reactive oxygen species, TAS = total antioxidant status, TOS = total oxidant status.

Keywords: diminished ovarian reserve, follicular fluid, IL-10, IL-6, IL-8, TAS, TOS, total thiol

1. Introduction

Follicular fluid provides an important microenvironment for the development of oocytes and it is easily available during oocyte pick-up. The intrafollicular concentrations of various hormones and mediators are essential for successful oocyte growth, maturation, fertilization and pregnancy rate.[1,2] Since, excessive reactive oxygen species (ROS) levels in ovarian follicle may cause negative effects on the aforementioned processes, oxidative stress has been stated as an etiopathogenetic factor in female infertility.[3,4]

ROS may induce an inflammatory response accompanied by the releasing of pro-inflammatory cytokines.[5] Interleukin (IL)-6 as an inflammatory cytokine decreases aromatase activity within follicles and leads to reduction in intrafollicular estradiol concentration, fertility and fertilizing capacity.[5] IL-8 is mainly produced in monocytes and macrophages. IL-8 contributes to the modulation of the inflammatory response.[5,6] IL-10 is an anti-inflammatory cytokine and downregulates expression of pro-inflammatory cytokines. Increased production of IL-6, IL-8 and decreased production of IL-10 may lead to an imbalance between pro- and anti-inflammatory cytokines which results in altered steroidogenesis, delayed follicular maturation and ovarian dysfunction.[5,6]

There are many oxidant and antioxidant molecules establishing the oxidative stress state in the organism, but measuring

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their levels separately is both costly and difficult. Practically, total oxidant status (TOS) and total antioxidant status (TAS) are measured[7,8] and the oxidative stress index (OSI), that reflects the degree of oxidative stress, is calculated by proportioning TOS to TAS.[9] Antioxidant defense systems such as thiol also play a protective role in cells and tissues against the harmful effects of ROS via different mechanisms such as thiol-disulfide redox buffer, radical quenchers and chelators of metal ions.[10]

Oxidative stress and inflammation in follicular fluid surrounding the oocyte has been linked to female infertility.[3,4] Accordingly, in this study we herein examined oocyte matched follicular fluid oxidant, antioxidant status and pro- and anti-inflammatory cytokine levels to reveal a possible effect of local-intrafollicular levels of these markers on the individual oocyte. With the aim of developing and establishing therapeutic strategies the individual oocyte's quality and ability to achieve fertilization, further embryo development and pregnancy was also assessed. To the best of our knowledge, this is the first study investigating the direct correlation of the "local intrafollicular oxidative stress" and "inflammatory markers" of a given individual follicle with in vitro fertilization (IVF) outcome of the same follicle.

2. Methods

We conducted a cross-sectional study of infertile women with diminished ovarian reserve from October 2020 to March 2021 in the IVF Unit of Istanbul Research and Education Hospital. The study was approved by the ethics committee of the hospital (2550/2020), and all patients provided informed consent. We included patients between 23 to 45 years old, unable to conceive naturally for at least 1 year who had an infertility etiology of diminished ovarian reserve (DOR) and were undergoing their first or second cycle of ovarian stimulation antagonist protocol IVF, in the form of ICSI, and fresh single embryo transfer. DOR was defined as the presence of at least 2 of the following features: Age ≥ 40 years, or any other poor ovarian response risk factor; An earlier prior poor ovarian response that is, a history of cycle cancelation or 3 ≥ oocytes at retrieval after conventional gonadotropin stimulation; and An abnormal ovarian reserve test (low antral follicle count or low anti-Müllerian hormone.[11]

The exclusion criteria were moderate or severe male factor (based on semen quality on the day of oocyte retrieval), presence of hydrosalpinx or unexplained infertility. Patients with endocrine disorders (hyperprolactinemia, diabetes mellitus, thyroid dysfunction, Cushing syndrome, and congenital adrenal hyperplasia), diseases of the immune system, hematologic malignancies, ovarian tumors, uterine abnormality or patients who were smokers were also excluded.

For controlled ovarian stimulation, we utilized a GnRH-antagonist protocol. The GnRH-antagonist protocol was started on the third day of the menstrual cycle with either hMG or rFSH (Menogon, Ferring, Istanbul, Turkey or Gonal F 75 IU ampules; Serono, Istanbul, Turkey). Cetrotide (Cetrorelix acetate, Merck Serono, Istanbul, Turkey) was administered when the leading follicle reached 12 to 14 mm in size. When the dominant follicle reached ≥ 18 mm in the presence of at least 2 16 mm follicles, Ovitrelle 250 micrograms (choriogonadotropin-alfa, Merck Serono, Istanbul, Turkey) was ordered to trigger ovulation.

Oocyte retrieval was performed 35 to 36 hours after hCG injection, using a 17-gauge needle under transvaginal ultrasound guidance. When follicular fluid was collected, each ovarian follicle was aspirated independently, and each individual follicular fluid was collected into a separate test tube to match it with a single cumulus-oocyte complex obtained from the same follicle. After puncture of the first follicle, the needle was removed and flushed, and then the air was aspirated until the tubing was empty. The remaining follicles were aspirated 1 by 1 by with the same technique and all tubes were numbered. Following cumulus cell separation around the oocyte, the nuclear state of the denuded oocytes was then determined. The oocytes were graded into the following 3 classes: Metaphase I – absence of the first polar body; metaphase II (MII) – presence of the first polar body; and germinal vesicle breakdown – presence of a clearly defined germinal vesicle containing the typical prominent nucleolus and degenerated oocytes.[12] Oocyte quality of each tube was noted as MI, MII, germinal vesicle and empty follicles. ICSI was done after 2 hours of incubation. The oocytes were checked 16 to 18 hours following injection to determine the presence of pronuclei. At 24 hours after oocyte retrieval, patients began receiving luteal phase supplementation consisting of intramuscular progesteron (50 mg/mL once in a day, progestan, Koçak, Istanbul, Turkey). Embryos were selected for fresh transfer on Day 2 to 3 or Day-4 and single embryo transfer was performed.

Cleavage-stage embryo scoring system consensus opinion states that an optimal Day-2 embryo would have 4 equally sized mononucleated blastomeres with <10% fragmentation.[13] An optimal Day-3 embryo would have 8 equally sized mononucleated blastomeres, with <10% fragmentation.[12] An optimal embryo at Day-4 would be compacted or compacting, and have entered into a fourth round of cleavage. Compaction should include virtually all the embryo volume. Consensus scoring system rates cleavage-stage embryos and Day-4 embryos as good, fair, poor (Grade 1, 2, 3 respectively).[13]

Oocyte matched follicular fluid samples were centrifuged at 10,000*g* for 10 minutes, and the supernatants were aliquoted and stored at –80°C for subsequent analysis. Blood specimens of all patients were drawn at the oocyte retrieval day. IL-6, IL-8 and IL-10 concentrations were measured by the sandwich enzyme immunoassay ELISA method based on a human monoclonal antibody (Rel Assay Diagnostics, Turkey) according to the manufacturer's instructions. TAS and TOS levels were measured spectrophotomerically according to Erel method.[7,8] Additionally, the OSI value of each participant was calculated by proportioning TOS to TAS.[14,15] Thiol level was measured with the spectrophotometric method defined by Erel & Neselioglu.[14,15]

Age, body mass index, gravida, parity, duration of infertility, day 3 hormone profile, and number of retrieved oocytes were noted. Possible relationship of TAS, TOS, OSI, total thiol, IL-6, IL-8, and IL-10 levels of each follicle with oocyte grade, grade of embryos transferred, and pregnancy rate of a given follicle were assessed.

2.1. Statistical analysis

Statistical analysis was performed with the SPSS version 17.0 program. The conformity of the variables to the normal distribution was examined by the Kolmogorov–Smirnov test. Mean, standard deviation, and median values were used when presenting descriptive analyses. Categorical variables were compared with the Pearson chi-square test. The Mann–Whitney *U* test was used when evaluating non-normally distributed (nonparametric) variables between 2 groups, and the Kruskal–Wallis test was used when evaluating between more than 2 groups. $P < .05$ were considered as statistically significant results. The power analysis was carried out with the G Power 3.1.9.7 (Franz Faul, Germany) program. It was assumed that the effect size would be d: 1.532. In the calculation made with the determined effect size and 5% margin of error, the power of the study was found to be 85.29%. The sample size could not be calculated because there was no reference study. In the power analysis, it was seen that the number of samples studied was sufficient.

3. Results

A total of 23 infertile women with DOR were identified in this study according to the inclusion and exclusion

criteria. Altogether, 79 follicles of those women were assessed. Demographic features, biochemical values and distribution of oocyte grade, embryo grade, and B-hcg results were given in Table 1. Follicular fluid TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 levels were compared between MII and non-MII groups. The follicular fluid IL-6 level of MII group was significantly lower than non-MII group ($P = .005$). Serum levels of TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 of MII and non-MII groups were compared and serum TOS level of MII group was significantly lower than non-MII group ($P < .001$) (Table 2).

TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 levels of follicles were compared according to embryo grades. Serum levels of TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 were also compared according to embryo grades. There was no statistically significant difference among the groups ($P > .05$) (Table 3). TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 levels of follicles resulted in positive B-hcg were compared with those with negative B-hcg results. Accordingly, follicular fluid IL-8 value was significantly low with positive pregnancy results. When serum values were compared according to pregnancy outcome, no statistically significant difference was found among the groups ($P > .05$) (Table 4).

The cutoff value of follicular fluid IL-6 and serum TOS level that could predict MII oocyte and follicular fluid IL-8 level that could predict B-hcg positivity was calculated by ROC analysis. When the cutoff value was <48 pg/mL for follicular fluid IL-6, 47.06% sensitivity, 78.57% specificity, 80% PPD, and 44.90% NPD were obtained. When the cutoff value was <1.23 pg/mL for serum TOS, 96.08% sensitivity, 72.22% specificity, 90.74% PPD, and 86.67% NPD were obtained. When the cutoff value was <88.5 pg/mL for follicular fluid IL-8, 100% sensitivity, 78.57% specificity, 57.14% PPD, and 100% NPD were obtained (Table 5).

4. Discussion

Our present study showed that serum oxidative stress status and follicular fluid pro-inflammatory cytokines were associated with IVF outcomes. Low serum TOS and low follicular fluid IL-6 levels were found to be associated with more mature oocyte retrieval and low follicular fluid IL-8 level was found to be associated significantly with positive pregnancy results. Neither serum nor follicular fluid TAS levels were found to be associated with IVF results including oocyte maturity, embryo grade, and positive pregnancy results. However low serum TOS levels were found to be associated with more mature oocyte retrieval. We interpreted these findings such as that; the follicular fluid may act as a mirror of what happens at the molecular level in the ovary. The intrafollicular hormonal environment of the developing oocyte is the first critical indicator of the ability of an individual oocyte to be fertilized and develop into an embryo.[1,2] Therefore, in the present study, we matched each follicular fluid content with the given oocyte from the same follicle.

DOR and poor ovarian response are bothersome issues in the field of IVF practice. Since DOR often results in diminished oocyte quantity, decreased oocyte quality and reduced fecundability, DOR patients usually have a higher rate of IVF failure.[15] Different interventions have been tried on patients with poor ovarian response, such as different stimulation protocols and adjuvant therapies to improve rates of ovarian response and pregnancy. Unfortunately, none of these regimes have shown to be superior over others.[16] Since decreased oocyte capacity is believed to be partly attributed to oxidative stress, antioxidants in repairing oxidative damage to the ovary by decreasing the accumulation of ROS has been extensively studied.[17] In the present study; the study population was consisted of infertile women with DOR. The reason we chose purely women with DOR was both the homogenization of the study group and the reevaluation of previous data stating oxidative stress affects IVF outcomes.[3,4]

TAS in follicular fluid has been previously studied in the literature. Jana et al showed that significantly decreased TAS in follicular fluid was correlated with poor oocyte and embryo quality and a low fertilization rate.[18] Oyawoye et al[19] demonstrated that higher follicular fluid TAS level improves fertilization in women undergoing IVF. No correlations were observed between TAS and IVF outcomes in another study.[20] Conflictingly, TAS showed a positive correlation with embryo quality in IVF in a more recent study.[21] Pasqualotto et al[21] stated that patients who did not become pregnant had significantly lower levels of TAS than those who became pregnant. The uniqueness of our study, which differentiates it from previous studies that were conducted is that we matched and labeled individual oocytes and not the pooled follicular fluid.

Follicular fluid/serum inflammatory markers and oxidative stress markers were assessed in a wide variety of patient groups undergoing IVF.[22,23] Singh et al stated that intrafollicular interleukin-8, interleukin-6 were the promising prognostic markers of oocyte and embryo quality in women with endometriosis.[24] In another study endometriosis patients had higher serum levels of the inflammatory molecules IL-6 and IL-8 and decreased concentrations of the anti-inflammatory IL-10. Serum IL-8 and follicular fluid IL-10 demonstrated great correlation with positive IVF outcome.[25] In a separate study,[26] the study group was women with PCOS. The researchers examined follicular fluid of women with PCOS and showed elevated concentration of TOS, IL-6, and IL-8 compared to controls. Levels of TAS, thiol groups, and IL-10 were lower in the PCOS group compared to controls. Similarly, the results of our study showed that follicular fluid pro-inflammatory cytokines were associated with IVF outcomes. Low follicular fluid IL-6 levels were found to be associated with more mature oocyte retrieval and follicular fluid IL-8 level was found significantly low with positive pregnancy results. The cutoff value that follicular fluid IL-6 and serum TOS level could predict MII oocyte and follicular fluid IL-8 level could predict B-hcg positivity was calculated. When the cutoff value was <48 pg/mL for follicular fluid IL-6, 47.06% sensitivity, 78.57% specificity, 80% PPD, and 44.90% NPD

Table 1

Demographic features, biochemical values and distribution of oocyte grade, embryo grade, and B-hcg results.

	Mean ± SD
Age (yr)	33.19 ± 0.49
BMI (kg/m^2)	21.41 ± 2.17
Gravida	0.35 ± 0.85
Parity	0.10 ± 0.40
Abortus	0.22 ± 0.63
Duration of infertility (yr)	5.35 ± 3.82
AMH (ng/mL)	0.87 ± 0.67
FSH (mIU/mL)	9.78 ± 2.97
Duration of stimulation (d)	10.29 ± 1.66
	n (%)
Oocyte grade	
MII	51 (64.56)
Non-MII	28 (35.44)
Embryo grade	
1 (Good)	5 (21.74)
2 (Fair)	7 (30.43)
3 (Poor)	11 (47.83)
B-hcg	
–	19 (82.61)
+	4 (17.39)

AMH = anti-Mullerian hormone, BMI = body mass index, FSH = follicle-stimulating hormone, MII = metaphase II, n = number.

Table 2

Comparison of follicular fluid and serum TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 levels of MII and non-MII groups.

	Oocyte grade				
	MII		Non-MII		
	Mean ± SD	Median	Mean ± SD	Median	P value
TAS*	1.08 ± 0.10	1.08	1.08 ± 0.13	1.10	.906
TOS*	7.07 ± 2.20	6.77	6.88 ± 2.91	6.81	.492
OSI*	0.66 ± 0.23	0.60	0.67 ± 0.39	0.58	.412
TTL*	591.29 ± 654.56	491.00	457.25 ± 88.96	441.00	.078
IL-6*	50.98 ± 12.69	49.53	57.03 ± 8.51	56.86	**.005**
IL-8*	92.15 ± 9.44	89.82	90.52 ± 10.38	91.20	.641
IL-10*	14.56 ± 1.88	14.11	14.57 ± 1.39	14.52	.439
TAS †	7.07 ± 2.20	6.77	9.80 ± 6.18	7.59	.057
TOS †	1.08 ± 0.10	1.08	1.43 ± 0.55	1.31	**<.001**
OSI †	0.66 ± 0.23	0.60	0.70 ± 0.44	0.56	.393
TTL †	591.29 ± 654.56	491.00	570.44 ± 130.43	587.50	.053
IL-6 †	*14.56 ± 1.88*	14.11	15.29 ± 2.05	14.83	.136
IL-8 †	92.15 ± 9.44	89.82	91.63 ± 8.96	92.26	.946
IL-10†	50.98 ± 12.69	49.53	56.65 ± 13.59	52.69	.153

Mann–Whitney *U* test.

IL = interleukin (pg/mL), MII = metaphase II, OSI = oxidative stress index, TAS = total antioxidant status (mmol/L), TOS = total oxidant status (μmol/L), TTL = total thiol.

* Follicular fluid.

† Serum.

Table 3

Comparison of follicular fluid and serum TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 levels and embryo grade.

	Grade 1		Grade 2		Grade 3		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	*P* value
TAS*	1.10 ± 0.13	1.04	1.09 ± 0.06	1.07	1.10 ± 0.11	1.12	.711
TOS*	7.71 ± 1.96	7.27	7.18 ± 1.92	6.77	6.50 ± 1.36	6.26	.563
OSI*	0.72 ± 0.23	0.68	0.66 ± 0.18	0.58	0.59 ± 0.12	0.63	.707
TTL*	1437.00 ± 2057.04	588.00	448.43 ± 80.36	470.00	548.44 ± 139.18	564.00	.492
IL-6*	13.47 ± 0.70	13.72	15.43 ± 1.60	14.97	14.36 ± 0.98	14.61	.144
IL-8*	83.30 ± 2.91	83.74	99.50 ± 12.12	103.38	94.52 ± 8.65	93.00	.076
IL-10*	45.51 ± 6.74	46.32	51.93 ± 8.13	54.11	48.97 ± 6.90	44.96	.570
TAS†	1.30 ± 0.10	1.30	1.68 ± 0.95	1.33	1.35 ± 0.07	1.36	.211
TOS†	11.37 ± 8.22	7.97	8.98 ± 7.42	7.62	9.08 ± 4.78	7.18	.905
OSI†	0.91 ± 0.73	0.59	0.51 ± 0.24	0.55	0.67 ± 0.36	0.55	.435
TTL†	639.50 ± 85.53	642.00	599.50 ± 132.96	593.50	537.17 ± 127.86	525.50	.381
IL-6†	16.02 ± 2.44	15.78	15.31 ± 1.34	15.11	14.82 ± 2.75	14.58	.918
IL-8†	91.99 ± 12.29	94.22	91.30 ± 5.78	92.26	90.70 ± 10.93	91.94	.983
IL-10†	68.00 ± 15.42	65.05	51.96 ± 10.40	49.62	51.06 ± 5.82	49.42	.250

Kruskal–Wallis test.

IL = interleukin (pg/mL), OSI = oxidative stress index, TAS = total antioxidant status(mmol/L), TOS = total oxidant status (μmol/L), TTL = total thiol.

* Follicular fluid.

† Serum.

were obtained. When the cutoff value was <1.23 pg/mL for serum TOS, 96.08% sensitivity, 72.22% specificity, 90.74% PPD, and 86.67% NPD were obtained. When the cutoff value was <88.5 pg/mL for follicular fluid IL-8, 100% sensitivity, 78.57% specificity, 57.14% PPD, and 100% NPD were obtained.

This research has the following strengths: First, we assessed a homogenous group of infertile patients with DOR. Second, the studies up to date; measured similar markers from only 1 follicle or pooled follicular fluid from several pooled follicles[19,21,27,28] without allowing for the ability to link the level of individual follicular oxidant, antioxidant status and pro- and anti-inflammatory cytokines with the fate of the oocyte derived from the same follicle. To the best of our knowledge, this is the first study allowing the direct correlation of the local intrafollicular oxidant, antioxidant status and pro- and anti-inflammatory cytokines of a given follicle with the quality of the oocyte collected from the same follicle and the ability of the same oocyte to undergo fertilization and pregnancy. Another strength of the study is that we also studied the serum levels of these markers. Our research obviously has limitations. The small number and scope of the research population is mainly related to our strict inclusion and exclusion criteria. It is necessary to further expand the research to include a broader patient group in the future.

Table 4

Comparison of follicular fluid and serum TAS, TOS, OSI, TTL, IL-6, IL-8, and IL-10 levels and pregnancy results.

	B-hcg				
	–		+		
	Mean ± SD	Median	Mean ± SD	Median	*P* value
TAS*	1.09 ± 0.09	1.08	1.13 ± 0.13	1.09	.871
TOS*	6.80 ± 1.28	6.77	8.22 ± 2.71	7.98	.417
OSI*	0.63 ± 0.13	0.63	0.75 ± 0.30	0.73	.516
TTL*	503.74 ± 117.54	483.00	1658.25 ± 2305.03	570.50	.465
IL-6*	14.58 ± 1.51	14.61	13.63 ± 0.79	13.97	.239
IL-8*	95.42 ± 10.21	94.93	84.11 ± 2.17	83.74	**.039**
IL-10*	48.45 ± 8.40	48.26	51.92 ± 5.01	51.82	.441
TAS†	1.45 ± 0.63	1.31	1.35 ± 0.10	1.36	.366
TOS†	10.94 ± 6.43	7.59	5.83 ± 3.22	6.96	.243
OSI†	0.78 ± 0.46	0.59	0.42 ± 0.22	0.51	.110
TTL†	552.86 ± 137.36	587.50	632.00 ± 90.23	627.00	.395
IL-6†	15.01 ± 2.05	14.61	16.25 ± 2.03	16.09	.242
IL-8†	91.17 ± 8.41	90.88	93.26 ± 12.01	96.76	.595
IL-10†	54.65 ± 14.43	49.42	63.65 ± 7.75	65.05	.056

Mann–Whitney *U* test

IL = interleukin (pg/mL), OSI = oxidative stress index, TAS = total antioxidant status(mmol/L), TOS = total oxidant status (μmol/L), TTL = total thiol.

* Follicular fluid.

† Serum.

Table 5

Cutoff value for follicular fluid IL-6, serum TOS, and follicular fluid IL-8.

				95% confidence interval						
	AUC	Std. error	*P* value	Lower limit	Upper limit	Cutoff	Sensitivity	Specificity	PPV	NPV
IL-6*	0.693	0.058	**.005**	0.578	0.807	<48	47.06%	78.57%	80%	44.90%
TOS†	0.928	0.033	**.001**	0.863	0.992	<1.23	96.08%	72.22%	90.74%	86.67%
IL-8*	0.920	0.066	**.039**	0.789	1000	<88.5	100%	78.57%	57.14%	100%

ROC analysis.

AUC = area under curve, IL = interleukin (pg/mL), NPV = negative predictive value, PPV = positive predictive value, TOS = total oxidant status (μmol/L).

* Follicular fluid.

† Serum.

5. Conclusion

For clinical scope, many IVF centers worldwide select embryos according to their morphological features and development rate determined by light microscopy. However, taking into account serum oxidative stress status and follicular fluid pro-inflammatory cytokines could improve IVF outcomes. This preliminary study might guide IVF practice with the aim of developing and establishing more effective therapeutic strategies and choosing embryos with more potential for success.

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