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Associations of the levels of adipokines and cytokines in individual follicles with in vitro fertilization outcomes in women with different ovarian reserves

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Abstract

Background To a large extent, the ovarian reserve determines a woman's reproductive potential. The etiological and pathological mechanisms of diminished ovarian reserve (DOR) remain unclear, and no reliable treatment is currently available for DOR. Adipokines and cytokines in follicular fluid (FF) play pivotal roles in follicular development and maturation. The concentrations of adipokines and cytokines in FF from individual follicles of women with DOR undergoing in vitro fertilization (IVF) were studied. In particular, we investigated the associations between the levels of adipokines and cytokines in individual FFs from women with different ovarian reserves and between the follicular levels of adipokines and cytokines and IVF outcomes in individual follicles.

Methods A total of 115 women who underwent IVF were recruited. Patients diagnosed with DOR, defined as a basal antral follicle count < 5 or an anti-Mullerian hormone concentration < 1.1 ng/mL, were assigned to the DOR group, while patients with a normal ovarian reserve (NOR) were assigned to the NOR group. FF was sampled from the first follicle with a diameter of approximately 18–20 mm from each patient, and the IVF outcome of the oocyte from the corresponding follicle was tracked. The levels of 5 adipokines (including visfatin-1, monocyte chemoattractant protein-1 [MCP-1], resistin, leptin, and chemerin) and 3 cytokines (including interleukin [IL]-6, IL-12p70, and tumor necrosis factor [TNF]-α) in FF were determined by Luminex technology.

Results The follicular levels of TNF-α, IL-6, visafatin, MCP-1, IL-12, and chemerin were significantly lower in women with NOR than in those with DOR. The follicular level of IL-6 was negatively correlated with the quality of embryos according to the binary logistic regression analysis, while the follicular levels of adipokines and other cytokines did not correlate with IVF outcomes regardless of the woman's ovarian reserve.

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Conclusions Our study demonstrated that the levels of adipokines and cytokines in individual follicles in women with DOR were different from those in women with NOR, indicating that increased intrafollicular inflammation might be related to DOR. Moreover, a high follicular level of IL-6 might negatively impact embryo quality.

Keywords Diminished ovarian reserve, Follicular fluid, Adipokines, Cytokines, In vitro fertilization

Introduction

One of the most common challenges in current clinical reproductive medicine is how to treat patients with diminished ovarian reserve (DOR), which is characterized by increased levels of follicle stimulating hormone, low antral follicle counts (AFCs), and decreased anti-Mullerian hormone (AMH) levels, affecting approximately 6–30% of women worldwide [1–3]. The most common risk factor for DOR is advanced age, but the specific etiology of DOR remains unclear [4, 5].

The pathological mechanism of DOR has recently been hypothesized to be associated with the dynamic environment of follicular fluid (FF), which is composed of immune cells, and various factors, including hormones, adipokines, and cytokines [6–9]. Cytokines are important intercellular regulators of large-scale physiological and pathophysiological events, including oocyte maturation, folliculogenesis, and ovulation, indicating that their concentration in the FF affects oocyte viability and developmental potential [10–12].

Recently, increasing attention has been given to the role of adipokines in maintaining ovarian function and promoting ovarian follicle development. Different adipokines, such as leptin, adiponectin, chemerin, resistin, monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), and visfatin, are involved in FF [13]. Adipokines are widely expressed in the ovaries and have been shown to affect different cells of the ovaries, including oocytes, theca, and granulosa cells, by regulating cell proliferation, cell cycle progression, and apoptosis [13, 14]. Moreover, adipokines play a crucial role in regulating the microenvironment that supports follicle maturation by controlling ovarian steroidogenesis and improving folliculogenesis and ovulation [15–18]. Therefore, evaluating the concentrations of cytokines and adipokines in FF is highly important for studying the role of these factors during the development of follicles and exploring the potential physiological mechanism of DOR [19].

Preliminary studies have indicated that adipokines and cytokines in FF might be associated with oocyte and embryo development, ovarian reserve, and the clinical pregnancy rate [20–23]. However, the specific roles of these factors in follicle maturation remain unclear [13, 24]. While most studies have evaluated the concentrations of adipokines and cytokines in pooled FFs [6, 24, 25], the levels of adipokines and cytokines in individual follicles are strongly correlated with the quality of the oocytes and embryos derived from these corresponding follicles; therefore, evaluating their roles in follicle maturation and embryo development is important .

Our study investigated the associations between the levels of adipokines and cytokines in individual FFs of women with different ovarian reserves and between the follicular levels of adipokines and cytokines and in vitro fertilization (IVF) outcomes in individual follicles.

Methods and materials

Study design and participants

We conducted a prospective study at the Reproductive Medicine Center at Shunde Hospital of Southern Medical University (The First People's Hospital of Shunde) from May 2022 to July 2023. All patients under 40 years of age who participated in an IVF program during this period were included. The exclusion criteria were as follows: polycystic ovary syndrome (PCOS), endometriosis, diabetes history, and IVF failure. Male patients with severe conditions, such as non-ejaculation, azoospermia, and a sperm morphology less than 1%, were excluded from the study. Only one cycle from each patient was included in this study.

Patients were divided into two groups according to their own ovarian reserve. Patients diagnosed with DOR, defined as a basal AFC < 5 or AMH < 1.1 ng/mL, were assigned to the DOR group, while patients with a normal ovarian reserve (NOR) were assigned to the NOR group.

Ovarian stimulation and IVF protocols

Controlled ovarian stimulation was performed with the gonadotrophin releasing hormone (GnRH) antagonist protocol. Ovarian stimulation was initiated on days 2 to 4 of withdrawal bleeding with recombinant follicle-stimulating hormone (FSH) (Gonal F, Merck Serono, Italy). Cetrorelix (Cetrotide, Merck Serono, Germany) of 0.25 mg was initiated daily when one of the following criteria was reached: (i) dominant follicle measuring ≥ 14 mm, (ii) serum E2 ≥ 600 pg/mL, or (iii) serum luteinizing hormone (LH) level ≥ 10 IU/l until and including the day of trigger [26]. Oocyte retrieval was performed 35–36 h after triggering with hCG, GnRH agonist, or combined hCG and GnRH agonist by experienced physicians. At the end of oocyte

retrieval, the sperm were diluted to 0.3 × 106/mL with G-IVF PLUS (10136, Vitrolife, Sweden). Then, 100 μl droplets of the sperm suspension were placed into a 60 mm petri dish (3652, Falcon, USA) covered with liquid oil and equilibrated in the incubators. After 3–4 h of preincubation in G-IVF medium, each oocyte was transferred to a microdroplet containing motile sperm. Oocytes and sperm were incubated for 16–20 h until fertilization. The morphology of day 3 embryos was assessed according to the Istanbul consensus criteria [27]. Embryos with 7–9 cells, equal size, regular shape, and < 20% fragmentation were considered good quality embryos.

FF collection and measurement

FFs were sampled from the first follicle, which had a diameter of approximately 18–20 mm, from each patient. During aspiration, mixing of fluids from different follicles and flushing with medium were avoided. The FF of the first follicle was aspirated separately and examined to detect and remove the oocyte corona cumulus complex. Then, the corresponding oocytes were isolated for evaluation and culture. Only the FF without obvious blood contamination was stored. FF was centrifuged at 2000 rpm for 10 min, and the FF supernatants were collected and stored at − 80 ℃ until analysis. The FF and the corresponding oocyte from the individual follicle shared the same code.

The concentrations of the 5 adipokines and 3 cytokines were measured in each FF sample with Luminex technology. The concentrations of 5 adipokines and 3 cytokines involved in different cell signaling pathways, namely, basic visfatin-1, MCP-1, resistin, leptin, chemerin, interleukin (IL)-6, IL-12p70, and tumor necrosis factor (TNF)-α, were determined in the FF by multiplex fluorescence bead-based technology (Luminex X-200, USA) by using a commercial Luminex screening assay kit (human magnetic Luminex 8-Plex Assay; R&D Systems, Minneapolis, USA). The FF samples were diluted twofold before incubation with specific antibody-coated fluorescent beads according to the manufacturer's recommendations. Fifty microliters of each individually diluted FF sample was added to 50 μL of antibody-conjugated beads directed against the adipokines/cytokines listed above in a 96-well filter plate (R&D Systems). After a 30-minute incubation, the plate was washed, and 25 μL of biotinylated anti-cytokine antibody solution was added to each well before another 30-minute incubation. The plate was then washed, and 50 μL of streptavidin-conjugated phycoerithrin was added to each well. After a final wash, each well was resuspended in 125 μL of assay buffer (R&D Systems) and analyzed by a Luminex X-200.

Hormone measurement

This study involved biochemical measurements in the Department of Clinical Laboratory of the Shunde Hospital of Southern Medical University (The First People's Hospital of Shunde). Automated chemiluminescent immunoassay kits (DxI 800 Access Immunoassay System, Beckman Coulter Inc., USA) were used to measure FSH, LH, estradiol (E2), progesterone (P), prolactin (PRL), and testosterone (TT). The intra- and interassay coefficients of variation of the different parameters were < 4.3% and 5.6% for FSH, < 5.4% and 6.4% for LH, < 7.7% and 4.7% for E2, < 9.19% and 9.57% for P, < 3.93% and 7.08% for TT, and < 1.61% and 6.92% for PRL, respectively. AMH was measured using a chemiluminescent immunoassay diagnostic kit (Guangzhou Kangrun Biotech Co., Ltd., China). The intra- and interassay coefficients of variation were < 8.0% and 15.0%, respectively.

Sample size

The prespecified sample size was calculated according to the data of our preliminary experiment. Based on our calculations, 116 patients (58 patients in each group) were sufficient to detect differences, with a 5% type I error and a power of 80% in a two-sided test.

Statistical methods

All the statistical analyses were performed using SPSS version 26.0 software (SPSS Inc., Chicago, USA). The data are presented as the means ± standard deviations for continuous variables and as frequencies with proportions for categorical variables. Differences in continuous variables between two groups were analyzed using the Mann – Whitney U test. For categorical variables, Pearson's chi-square test was used. Binary logistic regression analysis was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) of fertilization and embryo development outcomes for the concentrations of adipokines and cytokines in different groups after adjusting for age, body mass index (BMI), and infertility type. All P values were two-sided, and $P < 0.05$ was considered to indicate statistical significance.

Results

The patients' demographic and baseline characteristics are shown in Table 1. Higher concentrations of FSH and lower concentrations of AFC and AMH were detected in women with DOR (mean ± SD of 10.46 ± 4.60 mIU/mL, 3.36 ± 2.02, and 0.53 ± 0.25 ng/mL, respectively; $P < 0.001$). Moreover, the proportion of patients with primary infertility in the NOR group was significantly greater than that in the DOR group (53.4% vs. 28.6%, respectively, $p = 0.010$). No significant

Table 1 Demographic and clinical characteristics

	DOR group	NOR group	*P* value
No. of cycles	42	73	
Age at OPU (years)	33.29 ± 4.11	32.10 ± 4.21	0.216
Duration of infertility (years)	3.81 ± 3.07	3.30 ± 2.95	0.419
Primary infertility	12(28.6%)	39(53.4%)	**0.010***
Body mass index (kg/m^2)	21.07 ± 2.20	21.59 ± 2.69	0.288
AMH(ng/mL)	0.53 ± 0.25	3.37 ± 1.87	**< 0.001***
FSH(mIU/mL)	10.46 ± 4.60	7.46 ± 2.23	**< 0.001***
AFC	3.36 ± 2.02	13.12 ± 7.18	**< 0.001***

*$P < 0.05$ are statistically significant difference. Date expressed as mean ± standard deviation

Table 2 Concentrations of Adipokines and cytokines in FF

	DOR group	NOR group	*P* value
No. of cycles	42	73	
TNF-α(pg/ml)	3.71 ± 2.29	2.79 ± 0.87	**0.001***
IL-6(pg/ml)	3.77 ± 2.47	2.81 ± 0.94	**0.001***
Visfatin1(pg/ml)	4210.87 ± 2650.43	3368.38 ± 1319.68	**0.025***
MCP-1(pg/ml)	32.06 ± 16.28	25.11 ± 8.85	**0.008***
Resistin(pg/ml)	669.68 ± 416.31	885.55 ± 627.59	0.056
IL-12(pg/ml)	46.84 ± 34.80	34.00 ± 12.82	**0.002***
Leptin(pg/ml)	1386.79 ± 1110.02	1395.42 ± 779.41	0.419
Chemerin(pg/ml)	1647.03 ± 965.00	1310.75 ± 353.84	**0.003***

*$P < 0.05$ are statistically significant difference. Date expressed as mean ± standard deviation

differences in age at oocyte pick-up (OPU), infertility duration, or BMI were detected between women in the NOR group and those in the DOR group.

The concentrations of adipokines and cytokines in the first FF of patients with NOR and DOR are presented in Table 2. The follicular levels of TNF-α, IL-6, visafatin, MCP-1, IL-12, and chemerin were significantly lower in women with NOR than in those with DOR. No significant differences were detected between women with NOR and those with DOR with regard to resistin or leptin.

Furthermore, we investigated the relationship between the levels of follicular adipokines and cytokines and the IVF outcomes of oocytes from the corresponding follicles. However, none of the differences in the levels of adipokines or cytokines were statistically significant when comparing successful fertilization with failure or abnormal fertilization in the DOR group, NOR group, or all participants (Table 3).

The relationships between the levels of adipokines and cytokines in FF and embryo development outcomes are shown in Table 4. The level of resistin was significantly lower in the good-quality embryo group than in the poor-quality embryo group for all participants. However, after subgroup analysis, no statistically significant difference was observed. Other FF adipokine and cytokine levels were similar across the different groups.

Table 3 Relationships between the concentrations of Adipokines and cytokines in FF and the fertilization outcomes of the corresponding oocytes

	All			DOR group			NOR group		
	Successful fertilization (2PN)	Failure or abnormal fertilization	*P* value	Successful fertilization (2PN)	Failure or abnormal fertilization	*P* value	Successful fertilization (2PN)	Failure or abnormal fertilization	*P* value
No. of cycles	87	28		32	10		55	18	
TNF-α(pg/ml)	3.19 ± 1.78	2.93 ± 0.82	0.835	3.84 ± 2.56	3.29 ± 0.99	0.723	2.81 ± 0.94	2.73 ± 0.65	0.878
IL-6(pg/ml)	3.24 ± 1.91	2.93 ± 0.91	0.701	3.97 ± 2.76	3.12 ± 1.00	0.275	2.81 ± 0.97	2.83 ± 0.86	0.682
Visfatin1(pg/ml)	3763.50 ± 2136.28	3404.42 ± 1172.57	0.674	4421.36 ± 2944.67	3537.30 ± 1213.70	0.443	3380.75 ± 1372.80	3330.59 ± 1178.00	0.878
MCP-1(pg/ml)	28.15 ± 13.61	26.07 ± 8.10	0.850	33.14 ± 17.98	28.60 ± 8.75	0.679	25.25 ± 9.28	24.66 ± 7.60	0.949
Resistin(pg/ml)	730.30 ± 439.70	1044.13 ± 816.36	0.118	631.78 ± 296.20	790.96 ± 682.55	0.850	787.62 ± 498.44	1184.79 ± 867.85	0.060
IL-12(pg/ml)	39.62 ± 26.77	35.80 ± 12.10	0.799	49.05 ± 39.05	39.76 ± 13.86	0.616	34.13 ± 13.51	33.59 ± 10.79	0.908
Leptin(pg/ml)	1367.85 ± 811.27	1468.14 ± 1177.63	0.979	1207.93 ± 787.99	1959.14 ± 1731.09	0.140	1460.89 ± 817.16	1195.37 ± 628.90	0.266
Chemerin(pg/ml)	1447.90 ± 738.62	1389.04 ± 345.21	0.794	1706.37 ± 1084.50	1457.13 ± 372.16	0.616	1297.51 ± 362.00	1351.20 ± 334.21	0.539

Table 4 Association between the concentrations of Adipokines and cytokines in FF and Good-Quality embryos

	All			DOR group			NOR group		
	Good quality embryo	Poor quality embryo	*P* value	Good quality embryo	Poor quality embryo	*P* value	Good quality embryo	Poor quality embryo	*P* value
No. of cycles	54	50		19	19		35	31	
TNF-α(pg/ml)	2.88 ± 0.90	3.43 ± 2.18	0.202	3.26 ± 1.04	4.25 ± 3.18	0.389	2.67 ± 0.74	2.92 ± 1.02	0.365
IL-6(pg/ml)	2.88 ± 1.03	3.53 ± 2.31	0.056	3.45 ± 1.30	4.26 ± 3.40	0.589	2.57 ± 0.68	3.07 ± 1.12	0.052
Visfatin1(pg/ml)	3424.10 ± 1493.66	4054.81 ± 2441.59	0.092	3859.06 ± 1815.89	4769.51 ± 3448.57	0.314	3187.98 ± 1252.58	3616.77 ± 1442.06	0.175
MCP-1(pg/ml)	25.64 ± 10.15	30.24 ± 15.17	0.080	28.80 ± 13.03	35.66 ± 20.03	0.175	23.92 ± 7.88	26.92 ± 10.25	0.307
Resistin(pg/ml)	645.12 ± 336.89	960.71 ± 690.83	**0.017***	575.92 ± 295.61	809.13 ± 494.82	0.125	682.68 ± 355.71	1053.68 ± 355.71	0.052
IL-12(pg/ml)	35.18 ± 12.73	42.98 ± 33.24	0.229	40.42 ± 15.06	55.03 ± 48.64	0.328	32.33 ± 10.42	35.60 ± 15.38	0.468
Leptin(pg/ml)	1452.90 ± 902.72	1389.16 ± 981.22	0.701	1180.17 ± 894.54	1640.48 ± 1341.58	0.133	1600.96 ± 884.59	1235.12 ± 654.33	0.089
Chemerin(pg/ml)	1328.98 ± 384.09	1562.52 ± 905.01	0.180	1446.79 ± 490.14	1895.19 ± 1324.17	0.184	1265.03 ± 301.35	1358.63 ± 415.52	0.763

*$P < 0.05$ are statistically significant difference

We performed binary logistic regression analysis to further identify the associations of fertilization and embryo development outcomes with the concentrations of adipokines and cytokines in FF (Table 5). The level of IL-6 was negatively correlated with the quality of the embryos in the NOR group (OR = 0.4480, 95% CI 0.247 to 0.935), but the levels of other FF adipokines and cytokines were similar across the different groups.

Discussion

Our study demonstrated that the levels of adipokines and cytokines in the FFs of women with DOR were different from those in the FFs of women with NOR, indicating that increased intrafollicular inflammation might be related to DOR. Moreover, we investigated the relationship between the follicular levels of adipokines and cytokines and IVF outcomes in individual follicles. These results enrich our understanding of the role of adipokines and cytokines in FF and the potential pathological mechanism of DOR.

Our study revealed that the concentrations of visfatin, MCP-1, chemerin, TNF-α, IL-6, and IL-12 in FFs were greater in the DOR group than in the NOR group. TNF-α, IL-6, and IL-12 are widely known proinflammatory cytokines [18], and some studies came to the same conclusion as our findings showing increased IL-6 and TNF-α levels in the FF of DOR patients [7, 28]. Visfatin and chemerin are proinflammatory adipokines with immunological activity, while MCP-1 can recruit and activate monocytes and macrophages [13]. Multiple studies have suggested that the FF levels of chemerin and MCP-1 are involved in the inflammatory milieu associated with oocyte maturation, resulting in decreased oocyte yield and pregnancy rate [25, 29–31]. Given the evidence mentioned above, our results indicated that there might be greater intrafollicular inflammation in women with DOR than in women with NOR.

Our binary logistic regression analysis revealed that the concentrations of IL-6 were negatively correlated with the quality of the embryos, which was consistent with previous studies demonstrating that FF IL-6 levels are negatively associated with the clinical pregnancy rate [32, 33]. We also found that the concentration of resistin was significantly greater in the poor-quality embryo group than in the good-quality embryo group for all patients. However, after subgroup analysis and binary logistic regression analysis, we found that the differences were not statistically significant. Previous studies have shown that several factors, such as TNF-α, IL-6, vascular endothelial growth factor, and leptin, are associated with oocyte maturation and embryo development by improving folliculogenesis and ovulation [13, 15, 17, 21, 24, 34, 35]. Resistin and

Table 5 Binary logistic regression analysis for outcomes

	All				DOR group				NOR group			
	Successfully fertilization		Good quality embryo		Successfully fertilization		Good quality embryo		Successfully fertilization		Good quality embryo	
	OR(95%CI)	*P* value	OR(95%CI)	*P* value	OR(95%CI)	*P* value	OR(95%CI)	*P* value	OR(95%CI)	*P* value	OR(95%CI)	*P* value
TNF-α(pg/ml)	1.171(0.793,1.727)	0.427	0.729(0.497,1.070)	0.106	1.157(0.693,1.929)	0.577	0.723(0.415,1.257)	0.250	1.206(0.634,2.295)	0.568	0.687(0.372,1.268)	0.230
IL-6(pg/ml)	1.146(0.794,1.653)	0.467	0.702(0.479,1.028)	0.069	1.292(0.668,2.496)	0.447	0.846(0.595,1.202)	0.351	1.029(0.577,1.834)	0.922	0.480(0.247,0.935)	**0.031***
Visfatin1(pg/ml)	1.000(1.000,1.000)	0.404	1.000(1.000,1.000)	0.098	1.000(1.000,1.001)	0.499	1.000(1.000,1.000)	0.323	1.000(1.000,1.001)	0.584	1.000(0.999,1.000)	0.173
MCP-1(pg/ml)	1.012(0.972,1.055)	0.557	0.965(0.930,1.001)	0.057	1.025(0.957,1.098)	0.482	0.973(0.927,1.021)	0.271	1.009(0.947,1.075)	0.776	0.954(0.900,1.012)	0.115
Resistin(pg/ml)	0.999(0.998,1.000)	**0.015***	0.999(0.998,1.000)	**0.008***	0.999(0.997,1.001)	0.350	0.998(0.996,1.000)	0.121	0.999(0.998,1.000)	**0.037***	0.999(0.998,1.000)	**0.019***
IL-12(pg/ml)	1.011(0.984,1.039)	0.437	0.980(0.954,1.006)	0.127	1.012(0.973,1.052)	0.556	0.978(0.939,1.018)	0.268	1.011(0.968,1.056)	0.629	0.978(0.938,1.020)	0.306
Leptin(pg/ml)	1.000(0.999,1.000)	0.308	1.000(1.000,1.001)	0.827	0.999(0.999,1.000)	0.144	1.000(0.999,1.000)	0.272	1.000(0.999,1.001)	0.463	1.001(1.000,1.001)	0.091
Chemerin(pg/ml)	1.000(0.999,1.001)	0.653	0.999(0.998,1.000)	0.091	1.000(0.999,1.002)	0.525	0.999(0.998,1.001)	0.227	1.000(0.998,1.002)	0.957	0.999(0.998,1.001)	0.242

*$P<0.05$ are statistically significant difference

visfatin are involved in the regulation of ovarian function because they can affect follicle proliferation and steroidogenesis, subsequently correlating with oocyte yield [13, 36–40]. However, studies have reached the opposite conclusion: MCP-1, resistin, and chemerin are negatively associated with oocyte yield, embryo implantation rate, and pregnancy rate and seem to be correlated with several diseases, such as endometriosis and PCOS, through their involvement in the inflammatory milieu [25, 29–31, 41, 42]. Therefore, the exact roles of adipokines and cytokines in FF remain unclear.

Based on our findings, we hypothesized that higher concentrations of proinflammatory factors are associated with DOR. However, our results failed to show that adipokines and other cytokines in FF were related to fertilization and embryonic development outcomes. It may be due to the young age of our participants, as age is still the most pivotal factor in reflecting oocyte health and determining the success rates of fertility treatments [43–46]. Future studies concerning this relationship are needed to reveal the role of follicular adipokines and cytokines in oocyte and embryonic development.

Our study had several strengths that should be taken into consideration. The first strength is that our study is the first to comprehensively examine the levels of adipokines included in FF and to study the relationships between the concentrations of these factors and the fertilization and embryo development outcomes of the oocyte from the corresponding follicle, which deepens our understanding of the role of adipokines in FF. Second, compared with previously published studies, we collected FF from individual follicles, and we traced the fertilization and subsequent embryo development of the oocyte from the corresponding follicle, which enables a broad assessment of the relationship between ovarian reserve, adipokines, and cytokines in FF and oocyte development. Given this limitation, the sample size of our study was relatively small due to the difficulty of collecting samples because we wanted to correlate each follicle with the fertilization and embryo development outcomes of the oocyte. Another potential limitation is that we lacked data on patient IVF outcomes during pregnancy, and further studies are needed.

Conclusions

Our results suggest that increased intrafollicular inflammation is related to DOR and that IL-6 may be negatively correlated with the quality of embryos. Our study provides insight into the role of follicular adipokines and cytokines in ovarian functions. Furthermore, this study provides additional biochemical information and a research basis for the exploration

of the pathogenesis of DOR. To the best of our knowledge, this is the first study exploring the relationships between the concentrations of adipokines and cytokines in FF and the fertilization and embryo outcomes of oocytes from corresponding follicles. This correlation remains to be further studied to document its significance in follicle and oocyte maturation.

Abbreviations

DOR	Diminished ovarian reserve
AFC	Antral follicle count
AMH	Anti-Mullerian hormone
FF	Follicular fluid
MCP-1	Monocyte chemoattractant protein-1
PAI-1	Plasminogen activator inhibitor-1
IVF	In vitro fertilization
GnRH	Gonadotrophin releasing hormone
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
IL	Interleukin
TNF-α	Tumor necrosis factor-α
E2	Estradiol
P	Progesterone
PRL	Prolactin
TT	Testosterone
OR	Odds ratio
CI	Confidence interval
OPU	Oocyte pick-up
BMI	Body mass index
PCOS	Polycystic ovary syndrome

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Author contributions
Xuelan Li and Xin Chen: design of the study, analysis and interpretation of data, and revise the article. Xinning Li: design of the study, analysis and interpretation of data. Chujun Li: acquisition and analysis of data, drafting the article. Jie Yang, Min Li and Xianli Zhou: revise the article, analysis and interpretation of data. Ziyang Su and Yuting Zhang: collection of data and samples. All authors approved the final version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The approval for this study was obtained from the Ethical Committee of Shunde Hospital, Southern Medical University (20210703). All the patients signed their written informed consent. All of the research processes in this study are in accordance with the Helsinki Declaration.

Consent for publication
Written informed consent for publication was obtained from the patients.

Competing interests
The authors declare no competing interests.

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