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의학박사 학위논문

Alteration of the Intrafollicular Thiol-Redox System in Infertile Women with Endometriosis: Relationship with Oocyte and Embryo Quality

자궁내막증 환자의 난포액내 씨올-산화환원계의 변화와 난자 및 배아의 질과의 연관성에 관한 연구

2014년 2월

서울대학교 대학원

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ABSTRACT

INTRODUCTION: Oxidative stress and chronic inflammation play important roles in the pathophysiology of endometriosis. However, their roles in female infertility remain unclear. The thiol-redox system participates in a variety of diseases related to oxidative stress. Oxidative stress induces the expression of inflammatory cytokines, which further promote oxidative stress. The role of these systems in the intrafollicular microenvironment of infertile patients with endometriosis is unknown. The aim of this study was to compare biomarkers of the thiol-redox system and chronic inflammation in infertile patients with and without endometriosis who were receiving *in vitro* fertilization (IVF). We also examined correlations between biomarker concentrations and IVF outcome.

METHODS: Sixty-five patients receiving IVF were included in this prospective observational study. Patients were divided into two groups: 31 patients with endometriosis (endometriosis group) *vs.* 34 patients with unexplained infertility or infertility due to male or tubal factors (controls). Follicular fluid (FF) was obtained from a single dominant follicle during oocyte retrieval and stored at -70°C. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPX)-3, thioredoxin (TRX), TRX-binding protein (TBP)-2, and peroxiredoxin (PRX)-4 levels were measured in the FF samples with enzyme-linked immunosorbent assays (ELISAs) as biomarkers of oxidative stress. The inflammatory

cytokines interleukin (IL)-1β, IL-6, IL-8, and tumor-necrosis factor (TNF)-α were also measured by ELISAs. Expression levels were compared between groups and statistically analyzed with a Student's t-test or Mann-Whitney *U* test. Linear regression analysis was performed to correlate expression levels and IVF outcomes. Logistic regression analysis was performed to identify predictive factors for clinical pregnancy.

RESULTS: GSH levels were significantly lower in the FF samples from the endometriosis group compared to those from the control group (12.73±5.67 vs. 16.19±6.94 μg/mL, *P*=0.033). TBP-2 levels were significantly higher in the FF samples from the endometriosis group compared to those from the control group (219.97±507.23 vs. 3.27±6.14 ng/mL, *P*=0.042). MDA, SOD, GPX-3, TRX, and PRX-4 levels were not significantly different between the two groups. MDA levels negatively correlated with TRX levels in FF (*r*=-0.264, *P*=0.047) and positively correlated with TBP-2 levels (*r*=0.354, *P*=0.009). SOD levels negatively correlated with PRX-4 levels in FF (*r*=-0.247, *P*=0.035). IL-6, IL-8, and TNF-α levels were significantly higher in the endometriosis group compared to those in the control (16.97±29.62 vs. 4.11±2.89 pg/mL, *P*=0.022; 216.26±95.73 vs. 171.50±72.06 pg/mL, *P*=0.037; 0.93±1.01 vs. 0.43±0.33 pg/mL, *P=0.*036, respectively). IL-1β levels were higher in the endometriosis group compared to those in the control, but differences were not significant. There were significant positive correlations among the four inflammatory cytokines. The levels of all of the inflammatory cytokines positively correlated with the levels of TRX in the FF samples.

GSH levels were positively correlated with the number of high-quality embryos (r=0.299, P=0.024). GPX-3 and TRX levels were negatively correlated with the percentage of mature oocytes (r=0.275, P=0.046; r=0.398, P=0.004, respectively). TNF-α levels were negatively correlated with the cumulative embryo score per embryo (r=0.278, P=0.025). Logistic regression analysis revealed that the number of high-quality embryos was an independent factor predicting clinical pregnancy (OR 0.975, P=0.024).

CONCLUSIONS: These findings suggest that there may be an imbalance of the thiol-redox system and increased levels of inflammatory cytokines in the intrafollicular microenvironment of infertile patients with endometriosis who are receiving IVF. These factors may affect the qualities of the oocyte and embryo.

Keywords: endometriosis, *in vitro* fertilization (IVF), follicular fluid, thiol-redox system, inflammatory cytokines

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INTRODUCTION

Endometriosis is characterized by the presence of endometrial tissue outside of the uterine cavity and is associated with pain and infertility [1]. Approximately 35-50% of women with infertility have endometriosis [2]. Similarly, about 30-50% of patients with endometriosis have impaired fertility [3]. Endometriosis is also associated with a reduced rate of pregnancy after *in vitro* fertilization (IVF), which may be due to the poor qualities of oocytes and embryos [4].

The mechanisms of endometriosis-associated infertility are not fully understood. However, endometriosis may contribute to infertility by impairing ovarian and tubal function and reducing uterine receptivity [5]. Abnormal folliculogenesis, elevated oxidative stress, altered immune function, changes in the hormonal milieu, or decreased endometrial receptivity may also contribute to reduced fertility [6].

Oxidative stress develops due to an imbalance between the generation of reactive oxygen species (ROS) and the scavenging capacity of antioxidants in the reproductive tract [7,8]. An imbalance between ROS production and antioxidant activity causes cellular damage and dysfunction and may affect folliculogenesis. Altered folliculogenesis in patients with endometriosis may contribute to ovulatory dysfunction, poor oocyte quality, reduced fertilization, low-grade embryos, and reduced implantation [9]. Changes in granulosa cell

cycle kinetics may also impair follicular growth and oocyte maturation in patients with endometriosis [10]. Thus, oxidative stress contributes to the infertility associated with endometriosis.

Thiols are organic sulfur derivatives, identified by the presence of sulfhydryl residues (-SH) at their active site. Biological thiols include low-molecular-weight free thiols and protein thiols, the functional group of the amino acid cysteine. Thiol-redox system is crucial to the normal function of a specific protein and may affect a vast variety of functions including protein structure, protein–protein interactions, catalysis, electron transfer, ion channel modulation, phosphorylation-dependent signal transduction, post-translational protein modification and transcriptional activation[11]. The extracellular supply of thiols is critical for maintaining the redox state of the extracellular space or microenvironment. Cell-surface and extracellular thiols are important for many cellular functions, including ligand-receptor binding and signal transduction [12]. The thiol-redox system includes cysteine, glutathione (GSH), thioredoxin (TRX), glutaredoxin, and peroxiredoxin (PRX). GSH is found in various tissues and is expressed in oocytes and embryos. GSH is the substrate of glutathione peroxidase (GPX), which is the main antioxidant enzyme that protects cells from lipid hydroperoxides and H_2O_2 [12]. Thus, GSH represents the main non-enzymatic defense system against ROS. TRX is a redox-regulating antioxidant protein that prevents oxidative stress from damaging cells [13]. TRX is considered to be a good marker of oxidative stress. TRX-binding protein-2 (TBP-2), which is also known as TRX-

interacting protein, vitamin D3-up-regulated protein, or TRX-interacting protein, regulates the expression and function of TRX [14,15]. The TRX system regulates the functions of specific genes, coordinates various enzymatic activities, and plays roles in female reproduction and fetal development by regulating cell growth, differentiation, and death [16]. TRX is involved in multiple clinical conditions. TRX alterations have been implicated in cataract formation, ischemic heart disease, cancer, AIDS, rheumatoid arthritis, diabetic complications, and hepatic and renal diseases [17]. The PRX family of antioxidant proteins was recently discovered and is ubiquitously synthesized and abundantly expressed in various organisms [18]. The PRX family includes at least six distinct mammalian *PRX* genes. The functional activities of PRX proteins depend on reduced forms of thioredoxin and/or glutathione [18,19]. In contrast to the intracellular localization of other family members, PRX-4 is the only known secretory form [20,21]. PRX-4 protects against oxidative damage by scavenging ROS in the extracellular space [21,22].

Endometriosis is associated with inflammatory changes in the intrafollicular microenvironment. ROS-induced damage can occur through altered expression of cytokines or pro-inflammatory substrates via activation of the redox-sensitive transcription factors AP-1, p53, and nuclear factor (NF)-kappa B [23]. Many pro-inflammatory cytokines increase the levels of ROS, which induces oxidative modification of cellular macromolecules through a process called oxidative stress [24]. Levels of inflammatory

cytokines, such as interleukin (IL)–6, IL-1β, and tumor necrosis factor (TNF)-α, are increased in the follicular fluid (FF) of patients with endometriosis. These inflammatory cytokines can activate apoptosis [4,25,26].

There have been several studies focused on ROS and antioxidants in the intrafollicular microenvironment [27-29]. However, there have been few reports on the thiol-redox system in the intrafollicular microenvironment of infertile patients with endometriosis. Additionally, although both oxidative stress and chronic inflammation are recognized features of endometriosis, there are few reports investigating biomarkers of oxidative stress and chronic inflammation in the FF of patients with endometriosis.

The aim of this study was to compare thiol-redox and chronic inflammatory biomarker concentrations in FF samples obtained from infertile patients with and without endometriosis who were receiving IVF. We also investigated correlations between biomarker concentrations and IVF outcome.

Materials and Methods

Patients and controls

Sixty-five patients receiving IVF were included in this prospective observational study. The endometriosis group consisted of 31 patients with infertility due to endometriosis, which was diagnosed by ultrasound or laparoscopy. Among these patients, 20 patients were surgically treated for the endometriosis before the enrollment and 11 patients were diagnosed with endometriosis by ultrasound. Thirty-four patients with unexplained infertility (n=22) or infertility due to male (n=7) or tubal factors (n=5) served as controls. Although laparoscopy remains the gold standard for definitively diagnosing endometriosis, transvaginal ultrasound has a high specificity and sensitivity for diagnosing ovarian endometriomas [30,31]. Although other morphological features have been described, a typical endometrioma is diagnosed by transvaginal sonography as an adnexal mass with homogenous low-level echogenicity and an absence of specific neoplastic features.

This study was approved by the institutional review board of the Severance Hospital. Informed consent was obtained from each subject.

Protocols for controlled ovarian stimulation

In the gonadotropin-releasing hormone agonist (GnRHa) long protocol, administration of the GnRH agonist triptorelin (Decapeptyl, Ferring, Sweden)

was initiated at 0.1 mg/day in the mid-luteal phase of the previous cycle. After pituitary release of GnRH was down-regulated, the triptorelin dose was reduced to 0.05 mg/day and recombinant follicle-stimulating hormone (FSH) (Gonal-F, Serono, Switzerland) and/or human menopausal gonadotropin (hMG; IVF-M, LG Life Science, Korea) were administered. Doses were adjusted based on individual responses, until either the leading follicle reached a mean diameter of 18 mm, or two follicles or more reached diameters of 17 mm. In the GnRH antagonist (GnRHant) multiple-dose flexible protocol, recombinant FSH and/or hMG were administered on the third day of the menstrual cycle. The GnRH antagonist cetrorelix (Gonal-F, Serono, Switzerland) was administered daily at 0.25 mg, starting when the leading follicle reached a diameter of 14 mm until the leading follicle reached a mean diameter of 18 mm, or two follicles or more reached diameters of 17mm. In both protocols, urinary human chorionic gonadotropin (hCG) (10,000 IU, IVF-C, LG Life Science, Korea) or recombinant hCG (250 μg, Ovidrel, Serono) was administered 35 hours before transvaginal oocyte retrieval. Up to four embryos were transferred two or three days after oocyte retrieval. Embryos were graded according to their morphologies and cleavage rates. We defined high-quality embryos as those with morphologic grades of I/V or II/V and four or five blastomeres on day 2 and at least seven blastomeres on day 3 after fertilization. The luteal phase was supported with 50 mg progesterone in oil, 8% progesterone gel (Crinone, Serono) daily, or 800 mg micronized progesterone (Utrogestan, Laboratoires Besins International, France). Progesterone support was initiated on the day of oocyte

retrieval for 14 days and was continued for another 6 to 8 weeks if a pregnancy was achieved. A clinical pregnancy was identified four to five weeks after oocyte retrieval by the presence of an intrauterine gestational sac and a pulsating fetal heartbeat.

Follicular fluid collection and laboratory assay

Follicular fluid was obtained from a single dominant follicle, which had the largest diameter during oocyte retrieval. FF was centrifuged at 250×*g* for 15 minutes to separate cellular content and debris. The FF supernatant was transferred to sterile polypropylene tubes and stored at -70°C. FF samples that were contaminated with blood were excluded. Superoxide dismutase (SOD), GSH, GPX-3, TRX, TBP-2, and PRX-4 (Wuhan EIAAB Science Co., LTD., China for SOD, GPX-3, TRX, TBP-2, and PRX-4; USCN Life Science Inc., China for GSH) were measured by enzyme-linked immunosorbent assays (ELISAs) as antioxidant biomarkers. In addition, malondialdehyde (MDA) was also measured by ELISA (Wuhan EIAAB Science Co., LTD.) as an indicator of lipid peroxidation. The inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α (R&D systems, USA) were also measured by ELISAs. The intra- and inter-assay coefficients of variation were less than 10% in all of the assays.

Statistical analysis

The sample size was calculated to compare differences between TRX and TBP-2 levels in the FF samples. Power analysis showed that at least 31

patients were needed in each group to achieve 80% power at a 5% significance level with a two-sided equivalence test.

Data were analyzed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as numbers and percentages, and numerical data were expressed as means ± standard deviations. The results were compared between the two groups and statistically analyzed with a Student's *t*-test or chi-square test. Linear regression analysis was performed to detect correlations. Logistic regression analysis was performed to identify factors that were predictive for a clinical pregnancy among the various cycle parameters and biomarkers that were assayed. $P<0.05$ was considered statistically significant.

Results

Clinical characteristics in study subjects

There were no significant differences in age, duration of infertility, and basal serum FSH levels. Body mass index (BMI), serum anti-Müllerian hormone (AMH) levels, and total antral follicle counts were significantly lower in the endometriosis group than those in the control group (20.18±2.16 vs. 21.49±2.76 kg/m^2, *P*=0.039; 1.57±0.75 vs. 4.36±3.91 ng/mL, *P*=0.001; 6.90±3.66 vs. 12.39±6.10, *P*<0.001) (Table I).

Table I. Clinical characteristics of study subjects

	Endometriosis (n=31)	Controls (n=34)	*P* -value
Age (years)	34.71±3.22	35.50±3.57	0.354
Body mass index (kg/m^2)	20.18±2.16	21.49±2.76	0.039
Duration of infertility (mo)	37.42±27.25	40.50±22.68	0.627
Basal serum LH	5.62±2.20	4.68±1.27	0.095
Basal serum FSH (mIU/mL)	11.20±4.54	9.34±3.49	0.078
Serum AMH (ng/mL)	1.57±0.75	4.36±3.91	0.001
Total antral follicle count	6.90±3.66	12.39±6.10	<0.001
COS protocol used			0.084
GnRH agonist long protocol	19	13	
GnRH antagonist protocol	12	21	

LH, luteinizing hormone; FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; COS, controlled ovarian stimulation; GnRH, gonadotropin-releasing hormone

Mean±S.D.

Outcomes of controlled ovarian stimulation and IVF-ET

The duration of stimulation was significantly longer (11.19±3.01 vs. 9.11±1.47 days, *P*=0.001), and the total dose of gonadotropin (3796.77±1314.36 vs. 1984.03±1224.51 IU, *P*=0.001) was significantly higher in the endometriosis group compared to those in the control group. Serum E_2 levels (1,585.8±1,162.0 vs. 3104.97±2,041.8 pg/mL, *P*=0.001), the number of follicles ≥ 11 mm on the day of hCG administration (6.03±3.18 vs. 11.35±7.73, *P*=0.001), and the number of oocytes retrieved (5.71±4.58 vs. 12.12±8.00, *P*<0.001) were significantly lower in the endometriosis group compared to those in the control group. Other outcomes, such as number of embryos transferred, cumulative embryo scores per embryo, number of high-quality embryos, fertilization rate, implantation rate, and clinical pregnancy rate, did not differ between groups (Table II).

Table II. Outcomes of COS and IVF-ET between the two groups

	Endometriosis (n=31)	Controls (n=34)	*P*-value
Duration of COS (days)	11.19±3.01	9.11±1.47	0.001
Dose of gonadotropins used (IU)	3796.77±1314.36	1984.03±1224.51	0.001
Serum E_2 on hCG day (pg/mL)	1,585.8±1,162.0	3104.97±2,041.8	0.001
No. of follicles ≥ 11 mm on hCG day	6.03±3.18	11.35±7.73	0.001
Serum E_2 per follicle ≥ 11 mm on hCG day (pg/mL)	330.29±155.44	340.19±169.49	0.807
No. of oocytes retrieved	5.71±4.58	12.12±8.00	<0.001
No. of transferred embryos	2.42±1.07	2.50±0.93	0.771
Cumulative embryo scores per embryo transferred	24.23±14.69	29.64±7.66	0.073
No. of high-quality embryos	1.39±1.01	1.65±1.18	0.381
Fertilization rate (%)	107/177 (60.5)	232/412 (56.3)	0.365
Clinical pregnancy rate per cycle (%)	11/31 (35.5)	11/34 (32.4)	0.790
Implantation rate (%)	17/63 (26.98)	14/85 (16.47)	0.153
No. of frozen embryos	0.80±1.68	2.94±3.71	0.005

COS, controlled ovarian stimulation; IVF-ET, in vitro fertilization and embryo transfer

Mean±S.D.

Follicular fluid concentrations of oxidative stress biomarkers and their relationship

GSH levels were significantly lower in the FF samples from the endometriosis group than those in the control group (12.73±5.67 vs. 16.19±6.94 μg/mL, *P*=0.033). Similarly, the levels of TBP-2 were significantly higher in the FF samples from the endometriosis group compared to those of the control group (219.97±507.23 vs. 3.27±6.14 ng/mL, *P*=0.042). MDA, SOD, GPX-3, TRX, and PRX-4 levels were not significantly different between groups (Table III). MDA levels negatively correlated with TRX levels in the FF samples (*r*=-0.264, *P*=0.047) and positively correlated with TBP-2 levels (*r*=0.354, *P*=0.009). SOD levels negatively correlated with PRX-4 levels in the FF samples (*r*=-0.247, *P*=0.035) (Table IV).

Table III. Follicular fluid (FF) concentrations of oxidative stress biomarkers between the two groups

	Endometriosis (n=31)	Controls (n=34)	*P*-value
MDA (nmol/mL)	0.21±0.10	0.20±0.14	0.751
SOD (U/mL)	29.27±18.69	23.94±15.19	0.214
GSH (μg/mL)	12.73±5.67	16.19±6.94	**0.033**
GPX-3 (ng/mL)	0.38±0.26	0.39±0.20	0.826
TRX (ng/mL)	4.67±7.05	4.53±3.79	0.929
TBP-2 (ng/mL)	219.97±507.23	3.27±6.14	**0.042**
PRX-4 (pg/mL)	1058.75±496.64	1141.74±685.13	0.581

MDA, malondialdehyde; SOD, Superoxide dismutase; GSH, glutathione; GPX-3, Glutathione peroxidase-3; Thioredoxin, TRX; TBP, TRX-binding protein; PRX-4, Peroxiredoxin-4

Mean±S.D.

Table IV. Pearson's correlation coefficients between FF oxidative stress markers in study subjects

	MDA		SOD		GSH		GP-3		TRX		TBP-2		PRX-4	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
MDA			0.126	0.296	0.189	0.111	0.135	0.134	-0.264	0.047	0.354	0.009	-0.154	0.196
SOD	0.126	0.296			0.085	0.475	0.017	0.900	-0.112	0.399	0.161	0.253	-0.247	0.035
GSH	0.189	0.111	0.085	0.475			-0.153	0.240	-0.210	0.111	-0.013	0.926	-0.194	0.098
GPX-3	0.135	0.134	0.017	0.900	-0.153	0.240			0.072	0.623	0.049	0.746	-0.123	0.345
TRX	-0.264	0.047	-0.112	0.399	-0.210	0.111	0.072	0.623			0.015	0.927	0.120	0.367
TBP-2	0.354	0.009	0.161	0.253	-0.013	0.926	0.049	0.746	0.015	0.927			0.089	0.527
PRX-4	-0.154	0.196	-0.247	0.035	-0.194	0.098	-0.123	0.345	0.120	0.367	0.089	0.527		

FF, follicular fluid; MDA, malondialdehyde; SOD, Superoxide dismutase; GSH, glutathione; GPX-3, Glutathione peroxidase-3; Thioredoxin, TRX; TBP, TRX-binding protein; PRX-4, Peroxiredoxin-4

Follicular fluid concentrations of inflammatory cytokines and their relationship

IL-6, IL-8, and TNF-α levels were significantly higher in the FF samples from the endometriosis group compared to those from the control group (16.97±29.62 vs. 4.11±2.89 pg/mL, *P*=0.022; 216.26±95.73 vs. 171.50±72.06 pg/mL, *P*=0.037; 0.93±1.01 vs. 0.43±0.33 pg/mL, *P=0.*036, respectively). IL-1β levels were higher in the FF samples from the endometriosis group compared to those from the control group, but the difference was not significant (Table V). There were significant positive correlations among the inflammatory cytokines in the FF samples (Table VI).

Table V. Follicular fluid (FF) concentrations of inflammatory cytokines between the two groups

	Endometriosis (n=31)	Controls (n=34)	*P*-value
IL-1β (pg/mL)	1.65±3.38	0.84±3.67	0.140
IL-6 (pg/mL)	16.97±29.62	4.11±2.89	0.022
IL-8 (pg/mL)	216.26±95.73	171.50±72.06	0.037
TNF-α (pg/mL)	0.93±1.01	0.43±0.33	0.036

IL, interleukin; TNF, tumor necrosis factor

Mean±S.D.

Table VI. Pearson's correlation coefficients between inflammatory cytokines in the FF of study subjects

	IL-1β		IL-6		IL-8		TNF-α	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
IL-1β			0.488	<0.001	0.477	<0.001	0.434	0.001
IL-6	0.488	<0.001			0.962	<0.001	0.983	<0.001
IL-8	0.477	<0.001	0.962	<0.001			0.975	<0.001
TNF-α	0.434	0.001	0.983	<0.001	0.975	<0.001		

FF, follicular fluid; IL, interleukin; TNF, tumor necrosis factor

Relationship between inflammatory cytokines and biomarkers of oxidative stress in FF

Follicular fluid levels of IL-6, IL-8, and TNF- α were positively correlated with the FF levels of TRX (r=0.280, P=0.032; r=0.285, P=0.029; r=0.327, P=0.045, respectively). There was also a positive correlation between FF IL-1β and TRX levels with borderline significance (r=0.248, P=0.058) (Table VII).

Table VII. Pearson's correlation coefficients between inflammatory cytokines and biomarkers of oxidative stress in the FF of study subjects

	IL-1β		IL-6		IL-8		TNF-α	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
MDA	-0.143	0.232	-0.111	0.352	-0.166	0.167	-0.140	0.329
SOD	-0.156	0.188	-0.053	0.658	-0.115	0.336	-0.088	0.541
GSH	-0.137	0.245	-0.143	0.223	-0.164	0.166	-0.151	0.292
GPX-3	-0.041	0.755	-0.058	0.659	-0.023	0.864	-0.051	0.759
TRX	0.248	0.058	0.280	0.032	0.285	0.029	0.327	0.045
TBP-2	-0.097	0.488	-0.037	0.791	-0.009	0.950	-0.051	0.759
PRX-4	0.034	0.774	0.024	0.838	0.029	0.807	-0.040	0.781

FF, follicular fluid; IL, interleukin; TNF, tumor necrosis factor; MDA, malondialdehyde; SOD, Superoxide dismutase; GSH, glutathione; GPX-3, Glutathione peroxidase-3; Thioredoxin, TRX; TBP, TRX-binding protein; PRX-4, Peroxiredoxin-4

Relationship between biomarkers of oxidative stress and chronic inflammation in FF and IVF outcomes

Follicular fluid GSH levels were positively correlated with the number of high-quality embryos (r=0.299, P=0.024) but not with the percentage of mature oocytes, fertilization rate, or cumulative embryo score (CES) per embryo. The levels of GPX-3 and TRX in the FF samples were negatively correlated with the percentage of mature oocytes (r=0.275, P=0.046; r=0.398, P=0.004, respectively). TRX levels were negatively correlated with the CES per embryo with borderline significance (r=-0.261, P=0.062). The levels of TNF-α in the FF negatively correlated with the CES per embryo (r=0.278, P=0.025) and the number of high-quality embryos (r=-0.209, P=0.096). Other oxidative stress biomarkers and inflammatory cytokines did not correlate with cycle parameters (Table VIII). Similar results were shown in patients with endometriosis (Table IX), but not in the control group (Table X).

Table VIII. Pearson's correlation coefficients between biomarkers of oxidative stress and chronic inflammation in FF and IVF outcomes in all participants

	No. of Mature oocytes / No. of total oocytes (%)		Fertilization rate (%)		CES/the no. of embryos trasnferred		No. of high-quality embryos	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
MDA	-0.069	0.592	-0.001	0.991	-0.095	0.450	0.090	0.477
SOD	-0.164	0.199	0.048	0.715	-0.009	0.942	0.130	0.307
GSH	0.111	0.381	0.040	0.760	0.131	0.299	0.299	0.015
GPX-3	-0.275	0.046	-0.198	0.164	0.049	0.724	-0.159	0.250
TRX	-0.398	0.004	0.019	0.896	-0.261	0.062	-0.170	0.228
TBP-2	0.018	0.905	0.072	0.640	-0.179	0.224	-0.112	0.448
PRX-4	0.045	0.726	0.068	0.601	-0.018	0.886	0.030	0.812
IL-1β	0.114	0.372	0.042	0.748	-0.096	0.450	-0.026	0.838
IL-6	-0.013	0.920	0.072	0.578	-0.127	0.314	-0.029	0.821
IL-8	0.057	0.657	0.006	0.961	0.021	0.868	-0.037	0.771
TNF-α	-0.028	0.826	0.013	0.921	-0.278	0.025	-0.209	0.096

FF, follicular fluid; IL, interleukin; TNF, tumor necrosis factor; MDA, malondialdehyde; SOD, Superoxide dismutase; GSH, glutathione; GPX-3, Glutathione peroxidase-3; Thioredoxin, TRX; TBP, TRX-binding protein; PRX-4, Peroxiredoxin-4

Table IX. Pearson's correlation coefficients between biomarkers of oxidative stress and chronic inflammation in FF and IVF outcomes in patients with endometriosis

	No. of Mature oocytes / No. of total oocytes (%)		Fertilization rate (%)		CES/the no. of embryos trasnferred		No. of high-quality embryos	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
MDA	-0.081	0.547	-0.065	0.633	-0.048	0.707	0.105	0.454
SOD	-0.202	0.135	-0.095	0.486	-0.050	0.695	0.047	0.735
GSH	-0.128	0.343	0.014	0.921	0.129	0.314	0.237	0.090
GPX-3	-0.386	0.012	-0.172	0.278	0.007	0.963	-0.123	0.431
TRX	-0.095	0.540	0.000	1.000	-0.262	0.077	-0.127	0.431
TBP-2	-0.124	0.404	-0.163	0.286	-0.056	0.691	-0.172	0.261
PRX-4	0.133	0.325	0.286	0.037	-0.011	0.932	0.162	0.246
IL-1β	0.089	0.511	-0.052	0.705	-0.099	0.443	-0.087	0.532
IL-6	0.025	0.855	-0.052	0.855	-0.182	0.157	-0.027	0.844
IL-8	0.251	0.067	0.073	0.600	-0.026	0.844	-0.040	0.778
TNF-α	-0.043	0.754	-0.056	0.688	-0.220	0.095	-0.170	0.235

FF, follicular fluid; IL, interleukin; TNF, tumor necrosis factor; MDA, malondialdehyde; SOD, Superoxide dismutase; GSH, glutathione; GPX-3, Glutathione peroxidase-3; Thioredoxin, TRX; TBP, TRX-binding protein; PRX-4, Peroxiredoxin-4

Table X. Pearson's correlation coefficients between biomarkers of oxidative stress and chronic inflammation in FF and IVF outcomes in the control group

	No. of Mature oocytes / No. of total oocytes (%)		Fertilization rate (%)		CES/the no. of embryos trasnferred		No. of high-quality embryos	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
MDA	0.09	0.479	-0.073	0.555	0.076	0.544	0.062	0.642
SOD	0.153	0.225	0.262	0.034	0.051	0.685	0.23	0.084
GSH	0.313	0.012	0.085	0.485	-0.016	0.893	0.131	0.319
GPX-3	0.042	0.76	-0.04	0.764	0.041	0.763	-0.061	0.67
TRX	-0.219	0.113	0.011	0.937	-0.03	0.827	-0.146	0.315
TBP-2	0.115	0.472	-0.044	0.778	0.193	0.213	0.15	0.366
PRX-4	-0.009	0.94	-0.06	0.624	0.134	0.276	0.042	0.747
IL-1β	-0.012	0.925	-0.075	0.545	0.24	0.055	0.331	0.013
IL-6	-0.238	0.056	-0.363	0.003	-0.152	0.216	-0.01	0.939
IL-8	0.041	0.741	-0.007	0.953	0.048	0.698	0.093	0.48
TNF-α	0.067	0.604	-0.089	0.479	-0.091	0.477	-0.055	0.686

FF, follicular fluid; IL, interleukin; TNF, tumor necrosis factor; MDA, malondialdehyde; SOD, Superoxide dismutase; GSH, glutathione; GPX-3, Glutathione peroxidase-3; Thioredoxin, TRX; TBP, TRX-binding protein; PRX-4, Peroxiredoxin-4

Predictive factors for a clinical pregnancy

Univariate logistic regression analysis suggested that a successful clinical pregnancy was influenced by the serum E2 levels on the day of hCG administration, the number of high-quality embryos, and the level of GSH in the follicular fluid. Multivariate logistic regression analysis revealed that the number of high-quality embryos was an independent predictive factor for clinical pregnancy (OR 0.975, *P*=0.024) (Table XI).

Table XI. Logistic regression analysis of predictive factors for a clinical pregnancy

	Univariate		Multivariate	
Variables	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Basal serum FSH	0.867 (0.739-1.017)	0.080	-	-
Total AFC	1.091 (0.993-1.200)	0.069	-	-
Serum E_2 levels on hCG day	1.000 (1.000-1.001)	0.033	1.000 (1.000-1.001)	0.174
No. of high-quality embryos	1.983 (1.168-3.365)	0.011	1.904 (1.085-3.341)	0.025
FF GSH	1.089 (1.002-1.184)	0.044	1.044 (0.951-1.146)	0.364

FSH, follicle-stimulating hormone; AFC, antral follicle count ; E_2, estradiol; hCG, human chorionic gonadotropin; FF, follicular fluid; GSH, glutathione

Discussion

In the present study, there were significant differences in the FF levels of GSH and TBP-2 between the endometriosis and control groups. The FF GSH levels significantly and positively correlated with the quality of the embryo. The concentrations of inflammatory cytokines in the FF were significantly and positively correlated with the TRX concentrations in the follicular fluid. Furthermore, the levels of inflammatory cytokines were significantly elevated in the follicular fluid samples obtained from patients with endometriosis. In addition, TRX concentrations in the FF samples were negatively correlated with oocyte maturity and embryo quality. This is the first *in vivo* study to investigate the integrated effects of thiol-redox and inflammatory factors in the FF of women with endometriosis on IVF outcomes.

GSH has been shown to be present in secretions from the female reproductive tract. GSH protects pre-implantation embryos from the adverse effects of intracellular glutathione depletion [32]. Glutathione plays important roles during maturation and post-fertilization processes in bovine oocytes [33]. Secreted GSH protects oocytes against excessive levels of ROS during ovulation, helping to ensure successful fertilization. In our study, the levels of glutathione in the FF samples from the endometriosis group were significantly lower than those from the control group. In addition, glutathione levels were significantly and negatively correlated with the number of high-quality

embryos. Therefore, depletion of GSH from the FF may adversely affect the quality of embryos in women with endometriosis. In contrast, one study reported that there were no differences in GSH levels in the FF from women with endometriosis-related infertility and those from women with infertility due to tubal factors or unexplained infertility [34]. However, because GSH levels were measured from pooled samples of follicular fluid from different follicles, this previous study may not accurately reflect the microenvironment of the dominant follicle.

The present study showed that GPX-3 concentrations in FF samples were negatively correlated with oocyte maturity. GPX-3 may indicate a hypoxic environment. Microarray analysis previously revealed that *GPX-3* gene expression was reduced in cumulus cells from oocytes that did not yield early-cleavage embryos [35]. Hypoxia produces ROS, which cause lipid peroxidation, enzymatic inactivation, and cell damage, resulting in apoptosis [36] of cumulus cells and oocytes [37]. Hypoxia [38] and elevated concentrations of ROS in follicular fluid are negatively associated with embryonic development and pregnancy outcome [39,40] and associated with a significantly higher incidence of aneuploidy and spindle defects in oocytes [38]. Among women who are receiving IVF, the mean GPX activity is greater in follicles that yield oocytes that are subsequently fertilized compared to that in follicles with non-fertilized oocytes [41]. However, the present study did not show a correlation between levels of GPX-3 in FF samples and fertilization rate.

Endometriosis is associated with inflammatory changes in the follicular fluid. The present study showed that the levels of IL-6, IL-8, and TNF-α were significantly higher in the FF of women with endometriosis compared to those in the controls, which was consistent with previous studies [4,25,26]. The pro-inflammatory intrafollicular microenvironment in women with endometriosis may influence the qualities of oocytes and embryos. In the present study, the levels of TNF-α in FF samples negatively correlated with the quality of embryos. TNF-α in FF has been proposed to be related to oocyte quality and IVF outcomes [42], which is consistent with our results. Previous work suggested that elevated levels of IL-6 in FF may be detrimental to implantation [43]. However, we did not observe this relationship in our study. Consistent with other reports [44-46], we did not find a correlation between IL-8 concentrations and IVF outcomes.

Although TRX levels in FF did not differ between groups, TBP-2 levels were significantly higher in the FF samples from the endometriosis group compared to those from the control group. The levels of TRX and TBP-2 in serum and peritoneal fluid samples from patients with endometriosis were previously reported to be similar to controls [47,48]. These findings may be due to differences between the systemic environment and the local intrafollicular environment. TBP-2 was originally identified as a negative regulator of TRX and acts as a suppressor of cell growth and regulator of lipid/glucose metabolism [49]. In addition, TBP-2 may enhance the

atherosclerotic process by increasing vascular inflammation [50]. Our study showed that the levels of TRX in FF were positively correlated with the levels of IL-1β, IL-6, IL-8, and TNF-α in follicular fluid. MDA is a by-product of lipid peroxide decomposition and has been used to monitor the degree of peroxidative damage in cells. MDA positively correlated with TRX and negatively correlated with TBP-2 in the present study. Therefore, we hypothesize that oxidative damage activates the TRX system as a protective mechanism, which activates inflammatory cytokines in the intrafollicular microenvironment. In addition, TRX levels were negatively correlated with oocyte maturation and embryo quality, and TNF-α levels were negatively correlated with embryo quality. Thus, excessive oxidative stress and inflammatory changes in the intrafollicular microenvironment may affect the qualities of the oocyte and embryo.

Univariate logistic regression analysis revealed that the successful achievement of a clinical pregnancy was influenced by serum E_2 levels, the number of high-quality embryos, and glutathione levels in the FF. However, other biomarkers of oxidative stress and inflammation in the FF and other cycle characteristics did not affect whether a clinical pregnancy was achieved. However, multivariate logistic regression analysis identified only the number of high-quality embryos as an independent predictive factor for clinical pregnancy. Thus, the thiol-redox system and inflammatory cytokines might be involved in the achievement of a clinical pregnancy due to their impact on the qualities of the oocyte and embryo.

There are several limitations to our study. First, because diagnostic laparoscopy was not routinely performed in our hospital, we cannot rule out the existence of minimal and mild endometriosis in the control group. However, careful imaging has demonstrated to be sensitive enough to detect small cysts or firm adhesions [50]. Second, although we are concerned about microenvironment of dominant follicle which is most likely to contain a mature oocyte, results of FF samples from a single dominant follicle may not reflect the other follicles in the ovary. Third, since this study was performed in infertile patients receiving IVF treatment, there was no direct in vitro evidence on the effect of the thiol-redox system on the oocyte and embryo quality. Therefore, our results may not be a causal relationship. Fourth, since the two different protocols were performed for COS according to physician's preference, it may affect the qualities of oocyte and embryo. However, there was no significant difference in the distribution of the protocols used between the two groups. Additionally, there were no differences in the percentage of mature oocyte, fertililization rate, CES/embryos, and the number of high quality embryos.

In conclusion, alterations in the thiol-redox system and increased levels of inflammatory cytokines were found in the intrafollicular microenvironment of infertile patients with endometriosis who were receiving IVF. These changes may affect the qualities of the oocyte and embryo.

References

1. Bulun SE. Endometriosis. N Engl J Med. 2009;360:268-279.
2. Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364:1789-1799.
3. Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. J Assist Reprod Genet. 2010;27:441-447.
4. Garrido N, Navarro J, Remohi J, Simon C, Pellicer A. Follicular hormonal environment and embryo quality in women with endometriosis. Hum Reprod Update. 2000;6:67-74.
5. Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. Fertil Steril. 2008;90:247-257.
6. Harkki P, Tiitinen A, Ylikorkala O. Endometriosis and assisted reproduction techniques. Ann N Y Acad Sci. 2010;1205:207-213.
7. Augoulea A, Mastorakos G, Lambrinoudaki I, Christodoulakos G, Creatsas G. The role of the oxidative-stress in the endometriosis-related infertility. Gynecol Endocrinol. 2009;25:75-81.
8. Carvalho LF, Samadder AN, Agarwal A, Fernandes LF, Abrao MS. Oxidative stress biomarkers in patients with endometriosis: systematic review. Arch Gynecol Obstet. 2012;286:1033-1040.
9. Garrido N, Pellicer A, Remohi J, Simon C. Uterine and ovarian function in endometriosis. Semin Reprod Med. 2003;21:183-192.
10. Saito H, Seino T, Kaneko T, Nakahara K, Toya M, Kurachi H. Endometriosis and oocyte quality. Gynecol Obstet Invest. 2002;53

Suppl 1:46-51.

11. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol. 2006;71:551-564.

12. Chaiswing L, Oberley TD. Extracellular/microenvironmental redox state. Antioxid Redox Signal. 2010;13:449-465.

13. Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med. 2001;31:1287-1312.

14. Junn E, Han SH, Im JY, Yang Y, Cho EW, Um HD, Kim DK, et al. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. J Immunol. 2000;164:6287-6295.

15. Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, Nakamura H, Takagi Y, Sono H, Gon Y, Yodoi J. Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. J Biol Chem. 1999;274:21645-21650.

16. Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol. 2011;25:287-299.

17. Maulik N, Das DK. Emerging potential of thioredoxin and thioredoxin interacting proteins in various disease conditions. Biochim Biophys Acta. 2008;1780:1368-1382.

18. Rhee SG, Chae HZ, Kim K. Peroxiredoxins: a historical overview and

speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic Biol Med. 2005;38:1543-1552.

19. Kang SW, Rhee SG, Chang TS, Jeong W, Choi MH. 2-Cys peroxiredoxin function in intracellular signal transduction: therapeutic implications. Trends Mol Med. 2005;11:571-578.

20. Giguere P, Turcotte ME, Hamelin E, Parent A, Brisson J, Laroche G, Labrecque P, Dupuis G, Parent JL. Peroxiredoxin-4 interacts with and regulates the thromboxane A(2) receptor. FEBS Lett. 2007;581:3863-3868.

21. Jin DY, Chae HZ, Rhee SG, Jeang KT. Regulatory role for a novel human thioredoxin peroxidase in NF-kappaB activation. J Biol Chem. 1997;272:30952-30961.

22. Matsumoto A, Okado A, Fujii T, Fujii J, Egashira M, Niikawa N, Taniguchi N. Cloning of the peroxiredoxin gene family in rats and characterization of the fourth member. FEBS Lett. 1999;443:246-250.

23. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol. 2012;10:49.

24. Schleicher E, Friess U. Oxidative stress, AGE, and atherosclerosis. Kidney Int Suppl. 2007; doi:10.1038/sj.ki.5002382.S17-26.

25. Pellicer A, Albert C, Mercader A, Bonilla-Musoles F, Remohi J, Simon C. The follicular and endocrine environment in women with endometriosis: local and systemic cytokine production. Fertil Steril. 1998;70:425-431.

26. Wunder DM, Mueller MD, Birkhauser MH, Bersinger NA. Increased ENA-78 in the follicular fluid of patients with endometriosis. Acta Obstet Gynecol Scand. 2006;85:336-342.

27. Agarwal A, Gupta S, Sharma R. Oxidative stress and its implications in female infertility - a clinician's perspective. Reprod Biomed Online. 2005;11:641-650.

28. Oyawoye O, Abdel Gadir A, Garner A, Constantinovici N, Perrett C, Hardiman P. Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome. Hum Reprod. 2003;18:2270-2274.

29. Prieto L, Quesada JF, Cambero O, Pacheco A, Pellicer A, Codoceo R, Garcia-Velasco JA. Analysis of follicular fluid and serum markers of oxidative stress in women with infertility related to endometriosis. Fertil Steril. 2012;98:126-130.

30. Patel MD, Feldstein VA, Chen DC, Lipson SD, Filly RA. Endometriomas: diagnostic performance of US. Radiology. 1999;210:739-745.

31. Dogan MM, Ugur M, Soysal SK, Soysal ME, Ekici E, Gokmen O. Transvaginal sonographic diagnosis of ovarian endometrioma. Int J Gynaecol Obstet. 1996;52:145-149.

32. Gardiner CS, Salmen JJ, Brandt CJ, Stover SK. Glutathione is present in reproductive tract secretions and improves development of mouse embryos after chemically induced glutathione depletion. Biol Reprod. 1998;59:431-436.

33. Hashimoto S, Minami N, Yamada M, Imai H. Excessive concentration of glucose during in vitro maturation impairs the developmental competence of bovine oocytes after in vitro fertilization: relevance to intracellular reactive oxygen species and glutathione contents. Mol Reprod Dev. 2000;56:520-526.

34. Ebisch IM, Peters WH, Thomas CM, Wetzels AM, Peer PG, Steegers-Theunissen RP. Homocysteine, glutathione and related thiols affect fertility parameters in the (sub)fertile couple. Hum Reprod. 2006;21:1725-1733.

35. van Montfoort AP, Geraedts JP, Dumoulin JC, Stassen AP, Evers JL, Ayoubi TA. Differential gene expression in cumulus cells as a prognostic indicator of embryo viability: a microarray analysis. Mol Hum Reprod. 2008;14:157-168.

36. Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunol Today. 1994;15:7-10.

37. Tatemoto H, Sakurai N, Muto N. Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during In vitro maturation: role of cumulus cells. Biol Reprod. 2000;63:805-810.

38. Van Blerkom J, Antczak M, Schrader R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. Hum Reprod. 1997;12:1047-1055.

39. Pasqualotto EB, Agarwal A, Sharma RK, Izzo VM, Pinotti JA, Joshi

NJ, Rose BI. Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedures. Fertil Steril. 2004;81:973-976.

40. Das S, Chattopadhyay R, Ghosh S, Ghosh S, Goswami SK, Chakravarty BN, Chaudhury K. Reactive oxygen species level in follicular fluid--embryo quality marker in IVF? Hum Reprod. 2006;21:2403-2407.

41. Paszkowski T, Traub AI, Robinson SY, McMaster D. Selenium dependent glutathione peroxidase activity in human follicular fluid. Clin Chim Acta. 1995;236:173-180.

42. Lee KS, Joo BS, Na YJ, Yoon MS, Choi OH, Kim WW. Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. J Assist Reprod Genet. 2000;17:222-228.

43. Altun T, Jindal S, Greenseid K, Shu J, Pal L. Low follicular fluid IL-6 levels in IVF patients are associated with increased likelihood of clinical pregnancy. J Assist Reprod Genet. 2011;28:245-251.

44. Gazvani MR, Bates M, Vince G, Christmas S, Lewis-Jones DI, Kingsland C. Follicular fluid concentrations of interleukin-12 and interleukin-8 in IVF cycles. Fertil Steril. 2000;74:953-958.

45. Hammadeh ME, Fischer-Hammadeh C, Georg T, Rosenbaum P, Schmidt W. Comparison between cytokine concentration in follicular fluid of poor and high responder patients and their influence of ICSI-outcome. Am J Reprod Immunol. 2003;50:131-136.

46. Hammadeh ME, Ertan AK, Georg MT, Rosenbaum P, Schmidt W. Relationship between ovarian stimulation regimen and interleukin level in pre-ovulatory follicular fluid and their effect on ICSI outcome. Am J Reprod Immunol. 2002;48:255-261.

47. Seo SK, Yang HI, Lee KE, Kim HY, Cho S, Choi YS, Lee BS. The roles of thioredoxin and thioredoxin-binding protein-2 in endometriosis. Hum Reprod. 2010;25:1251-1258.

48. Lambrinoudaki IV, Augoulea A, Christodoulakos GE, Economou EV, Kaparos G, Kontoravdis A, Papadias C, Creatsas G. Measurable serum markers of oxidative stress response in women with endometriosis. Fertil Steril. 2009;91:46-50.

49. Watanabe R, Nakamura H, Masutani H, Yodoi J. Anti-oxidative, anti-cancer and anti-inflammatory actions by thioredoxin 1 and thioredoxin-binding protein-2. Pharmacol Ther. 2010;127:261-270.

50. World CJ, Yamawaki H, Berk BC. Thioredoxin in the cardiovascular system. J Mol Med (Berl). 2006;84:997-1003.

국문 초록

서론 : 산화 스트레스 및 만성 염증은 자궁내막증의 병태생리에서 중요한 역할을 하지만 여성 불임에서의 역할은 명확히 알려져 있지 않다. 씨올-산화환원계는 산화 스트레스와 관련된 다양한 질환에 관여하며 산화 스트레스는 염증성 시토카인의 발현을 유도하고 이는 다시 산화 스트레스를 더욱 촉진시킨다. 현재까지 자궁내막증을 가진 불임환자의 난포액내 미세환경에서 씨올-산화환원계 및 만성 염증의 역할은 명확히 알려져 있지 않다. 그러므로, 본 연구에서는 자궁내막증이 있는 불임환자와 자궁내막증이 없는 불임환자의 체외수정시술시 난포액내 씨올-산화환원계 및 만성염증의 생표지자들을 비교하고 생표지자들과 체외수정시술의 결과와의 연관성을 파악하고자 한다.

방법 : 65명의 체외수정시술 환자들이 본 전향적 관찰연구에 포함이 되었고 자궁내막증이 있는 불임환자 31명 (자궁내막증군)과 원인불명, 남성요인 및 난관요인의 불임 환자 34명(대조군)으로 구분하였다. 난포액은 난자채취 시에 하나의 우성난포로부터 채취하였고 영하 70℃에서 냉동보관 하였다. 산화 스트레스의 생표지자로 말론다이알데하이드 (malondialdehyde, MDA), 초과산화물디스뮤타아제 (superoxide dismutase, SOD), 글루타티온 (glutathione, GSH), 글루타티

온과산화효소 (glutathione peroxidase, GPX)-3, 씨오레독신 (thioredoxin, TRX), 씨오레독신 결합단백 (TRX binding protein, TBP)-2 및 페록시레독신 (peroxiredoxin, PRX)-4 농도를 염증성 시토카인으로 인터루킨 (interleukin, IL)-1β, IL-6, IL-8 및 종양괴사인자 (tumor necrosis factor, TNF)-α 농도를 효소결합면역흡착측정법 (enzyme-linked immunosorbent assay)으로 측정하였다. 양군 간의 생표지자 농도는 스투덴트 *t* 검정 또는 만-휘트니 *U* 검정을 통하여 분석하였고 각 생표지자 농도와 체외수정시술의 결과와의 관련성은 선형회귀분석을 통하여 분석하였다. 임상적 임신의 예측인자를 파악하기 위하여 로지스틱 회귀분석이 시행되었다.

결과 : 난포액내 GSH 농도는 대조군과 비교하여 자궁내막증군에서 유의하게 낮았다 (12.73±5.67 vs. 16.19±6.94 μg/mL, P=0.033). 난포액내 TBP-2 농도는 대조군과 비교하여 자궁내막증군에서 유의하게 높았다 (219.97±507.23 vs. 3.27±6.14 ng/mL, P=0.042). 난포액내 MDA, SOD, GPX-3, TRX, PRX-4 농도는 양 군간에 유의한 차이를 보이지 않았다. MDA 농도는 TRX 농도와 유의한 음의 상관관계를 보였고 (r=-0.264, P=0.047) TBP-2 농도와 유의한 양의 상관관계를 보였다 (r=0.354, P=0.009). SOD 농도는 PRX-4 농도와 유의한 음의 상관관계를 보였다 (r=-0.247, P=0.035). 난포액내 IL-6, IL-8, TNF-α 농도는 대조군과 비교하여 자궁내막증군에서 유의하게 높았다 (16.97±29.62 vs.

4.11±2.89 pg/mL, *P*=0.022; 216.26±95.73 vs. 171.50±72.06 pg/mL, *P*=0.037; 0.93±1.01 vs. 0.43±0.33 pg/mL, *P*=0.036, respectively). 난포액내 IL-1β 농도는 자궁내막증군에서 더 높았으나 통계적 유의성은 없었다. 각 시토카인들 간에 상호 유의한 양의 상관관계가 있었으며 모든 시토카인들이 TRX 농도와 양의 상관관계를 보였다. GSH 농도는 양질의 배아의 수와 양의 상관관계를 보였고 (*r*=0.299, *P*=0.024), GPX-3 및 TRX 농도는 채취된 난자들 중 성숙난자의 비율과 음의 상관관계를 보였다 (*r*=0.275, *P*=0.046; *r*=0.398, *P*=0.004, respectively). TNF-α 농도는 배아당 배아누적지수와 음의 상관관계를 보였다 (*r*=0.278, *P*=0.025). 로지스틱 회귀분석에서 임신을 예측하는 독립적인 인자는 양질의 배아의 수이었다 (OR 0.975, *P*=0.024).

결론 : 본 연구의 결과는 자궁내막증이 있는 불임환자의 체외수정시술시 난포액내 미세환경은 씨올-산화환원계의 불균형 및 염증성 씨토카인의 증가가 있으며 이러한 미세환경의 변화가 난자 및 배아의 질에 영향을 줄 수 있음을 제시한다.

주요어 : 자궁내막증, 체외수정시술, 난포액, 씨올-산화환원계, 염증성 시토카인

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