Alteration in the intrafollicular thiol–redox system in infertile women with endometriosis

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Abstract

The aim of this study was to compare intrafollicular biomarkers of thiol–redox system and chronic inflammation in infertile patients with and without endometriosis, and examine correlations between biomarkers and IVF outcomes. The study included 65 patients receiving IVF: 31 patients with endometriosis vs 34 patients without endometriosis. Follicular fluid (FF) was obtained from a single-dominant follicle during oocyte retrieval and stored at −70 °C. Malondialdehyde, superoxide dismutase, glutathione (GSH), glutathione peroxidase 3 (GPX3), thioredoxin (TRX), TRX-binding protein 2 (TBP2), and peroxiredoxin-4 levels were measured in the FF samples by ELISAs as biomarkers of oxidative stress. The inflammatory cytokines interleukin 1 beta (IL1β), IL6, IL8, and tumor-necrosis factor alpha (TNFα) were also measured by ELISAs. GSH levels were significantly lower in the endometriosis group compared with the controls. TBP2 levels were significantly higher in the endometriosis group. IL6, IL8, and TNFα levels were significantly higher in the endometriosis group. The levels of all of the inflammatory cytokines positively correlated with the levels of TRX. GSH levels positively correlated with the number of high-quality embryos. GPX3 and TRX levels negatively correlated with the percentage of mature oocytes. TNFα levels negatively correlated with the cumulative embryo score per embryo. Logistic regression analysis revealed that the number of high-quality embryos was an independent factor predicting clinical pregnancy. In conclusion, there may be an imbalance in the thiol–redox system and increased levels of inflammatory cytokines in the intrafollicular microenvironment of infertile patients with endometriosis, which may affect the qualities of the oocyte and embryo.


Introduction

Endometriosis is characterized by the presence of endometrial tissue outside of the uterine cavity and is associated with pain and infertility (Bulun 2009). Approximately 35–50% of women with infertility have endometriosis (Giudice & Kao 2004). Similarly, about 30–50% of patients with endometriosis have impaired fertility (Bulletti et al. 2010). Endometriosis is also associated with a reduced rate of pregnancy after IVF, which may be due to the poor qualities of oocytes and embryos (Garrido et al. 2000).

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 (Gupta et al. 2008). Abnormal folliculogenesis, elevated oxidative stress, altered immune function, changes in the hormonal milieu, or decreased endometrial receptivity may also contribute to reduced fertility (Harkki et al. 2010).

Oxidative stress develops due to an imbalance between generation of reactive oxygen species (ROS) and the scavenging capacity of antioxidants in the reproductive tract (Augoulea et al. 2009, Carvalho et al. 2012). 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 ovarioly dysfunction, poor oocyte quality, reduced fertilization, low-grade embryos, and reduced implantation (Garrido et al. 2003). Changes in the kinetics of granulosa cell cycle may also impair follicular growth and oocyte maturation in patients with endometriosis (Saito et al. 2002). 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. The thiol–redox system is crucial for 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 (Biswas et al. 2006). 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 (Chaiswing & Oberley 2010). 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 H2O2 (Chaiswing & Oberley 2010). 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 (Nordberg & Arner 2001). TRX is considered to be a good marker of oxidative stress. TRX-binding protein 2 (TBP2), which is also known as TRX-interacting protein, vitamin D3-upregulated protein, or TRX-interacting protein, regulates the expression and function of TRX (Nishiyama et al. 1999, Junn et al. 2000). 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 (Burton & Jauniaux 2011). 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 (Maulik & Das 2008). The PRX family of antioxidant proteins has been recently discovered and is ubiquitously synthesized and abundantly expressed in various organisms (Rhee et al. 2005). The PRX family includes at least six distinct mammalian PRX genes. The functional activities of PRX proteins depend on reduced forms of TRX and/or GSH (Kang et al. 2005, Rhee et al. 2005). In contrast to the intracellular localization of other family members, PRX4 is the only known secretory form (Jin et al. 1997, Giguere et al. 2007). PRX4 protects against oxidative damage by scavenging ROS in the extracellular space (Jin et al. 1997, Matsumoto et al. 1999).

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 AP1, p53, and nuclear factor (NF)-kappa B (Agarwal et al. 2012). Many pro-inflammatory cytokines increase the levels of ROS, which induces oxidative modification of cellular macromolecules through a process called oxidative stress (Schleicher & Friess 2007). The levels of inflammatory cytokines, such as interleukin 6 (IL6), IL1β, and tumor necrosis factor alpha (TNFα), are increased in the follicular fluid (FF) of patients with endometriosis (Pellicer et al. 1998, Garrido et al. 2000, Wunder et al. 2006). These inflammatory cytokines can activate apoptosis.

There have been several studies focused on ROS and antioxidants in the intrafollicular microenvironment (Oyawoye et al. 2003, Agarwal et al. 2005, Prieto et al. 2012, Singh et al. 2013). However, there have been few reports on the thiol–redox system in the intrafollicular microenvironment of infertile patients with endometriosis (Ebisch et al. 2006, Singh et al. 2013). In addition, although both oxidative stress and chronic inflammation are recognized features of endometriosis, there are few reports investigating the 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.

**Subjects and methods**

**Patients and controls**

Among candidates undergoing controlled ovarian stimulation (COS) for IVF and embryo transfer (IVF-ET) from June 2010 to April 2013, only those aged 30–39 years with a BMI of 16–29 kg/m² were included in this study. The following exclusion criteria were adopted: polycystic ovary syndrome, cycles with dominant FF contaminated blood during oocyte retrieval, cycles with dominant FF not yielding oocytes, and the presence of only one ovary. 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. Ultrasound scanning revealed recurrent ovarian endometrioma in 14 patients and six patients had not. Eleven patients were diagnosed with ovarian endometrioma 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.

This study was approved by the institutional review board of the Severance Hospital. Informed consent was obtained from each subject.
Protocols for COS
In the gonadotropin-releasing hormone agonist (GNRHa) long protocol, administration of the GNRH agonist triptorelin (Decapeptyl, Ferring, Malmö, Sweden) was initiated at 0.1 mg/day in the mid-luteal phase of the previous cycle. After pituitary release of GNRH was downregulated, the triptorelin dose was reduced to 0.05 mg/day and recombinant follicle-stimulating hormone (FSH) (Gonal-F, Serono) and/or human menopausal gonadotropin (hMG; IVF-M, LG Life Science, Seoul, Korea) were administered. The 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) was administrated daily at a dose of 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 17 mm. In both protocols, urinary human chionic gonadotropin (hCG) (10 000 IU, IVF-C, LG Life Science) or recombinant hCG (250 μg, Ovidrel, Serono) was administered 35 h before transvaginal oocyte retrieval. Up to four embryos were transferred 2 or 3 days after oocyte retrieval. The embryos were graded according to their morphologies and cleavage rates. On the day of embryo transfer, the grade of each embryo transferred was multiplied by the number of blastomeres to produce a score for each embryo, and the summation of the scores obtained for all the embryos transferred was defined as the cumulative embryo score (CES; Steer et al. 1992). 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, Paris, France). Progesterone support was initiated on the day of oocyte retrieval for 14 days and was continued for another 6–8 weeks if a pregnancy was achieved. A clinical pregnancy was identified 4–5 weeks after oocyte retrieval by the presence of an intrauterine gestational sac and a pulsating fetal heartbeat.

FF collection and laboratory assay
FF was obtained from a single-dominant follicle which had the largest diameter during oocyte retrieval. FF was centrifuged at 250 g for 15 min to separate cellular content and debris. The FF supernatant was transferred to sterile polypropylene tubes and stored at −80°C until assayed. FF samples that were contaminated with blood were excluded. Superoxide dismutase (SOD), GSH, GPX3, TRX, TBP2, and PRX4 (Wuhan EIAAB Science Co., Ltd, Wuhan, China for SOD, GPX3, TRX, TBP2, and PRX4; USCN Life Science, Inc., Wuhan, China for GSH) were measured by ELISAs as antioxidant biomarkers. In addition, malondialdehyde (MDA), an indicator of lipid peroxidation, was also measured by ELISA (Wuhan EIAAB Science Co., Ltd). The inflammatory cytokines IL1β, IL6, IL8, and TNFα (R&D Systems, MN, USA) were also measured by ELISAs. The intra- and inter-assay coefficients of variation were <10% in all of the assays.

Statistical analysis
The sample size was calculated to compare differences between TRX and TBP2 levels in the FF samples. As there has been no report on the intrafollicular levels of these markers, serum levels of these markers from our previous report were used to perform power analysis (Seo et al. 2010). 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 if a 30% difference in mean value was significant.

Data were analyzed with SPSS version 18.0 (SPSS, Inc.). The results were compared between the two groups and statistically analyzed using a Student’s t-test or χ² test. When normality of distribution was assessed by means of the test of Shapiro–Wilks, skewed variables were analyzed by Mann–Whitney U test. Pearson’s bivariate correlation coefficient analysis was performed to detect correlations. Multivariate logistic regression analysis was performed to identify factors predicting a clinical pregnancy. The model included potential confounders that were found to be P<0.1 in univariate analysis and those that were generally considered to be clinically significant among the various cycle parameters and biomarkers that were assayed. P<0.05 was considered statistically significant.

Results
Clinical characteristics of study subjects
There were no significant differences in age, duration of infertility, and basal serum FSH levels. 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/m², 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 1).

Outcomes of COS 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 with those in the control group. Serum E₂ levels (1585.8±1162.0 vs 3104.97±2041.8 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 with those in the control group. Other outcomes, such as number of embryos transferred, CESs
FF concentrations of inflammatory cytokines and oxidative stress biomarkers

The levels of IL6, IL8, and TNFz were significantly higher in the FF samples from the endometriosis group compared with 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). IL1β levels were higher in the FF samples from the endometriosis group compared with those from the control group, but the difference was not significant (Fig. 1).

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 TB2 were significantly higher in the FF samples from the endometriosis group compared with those of the control group (219.97 ± 507.23 vs 3.27 ± 6.14 ng/ml, P = 0.042). MDA, SOD, GPX3, TRX, and PRX4 levels were not significantly different between groups (Fig. 1).

Relationship between inflammatory cytokines and biomarkers of oxidative stress in FF

FF levels of IL6, IL8, and TNFz 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). Although it was not statistically significant, there was also a positive correlation between FF levels of IL1β and TRX (r = 0.248, P = 0.058) (Table 2).

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

FF 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 CES per embryo. The levels of GPX3 and TRX in the FF samples negatively correlated with the percentage of mature oocytes (r = 0.275, P = 0.046; r = 0.398, P = 0.004 respectively). TRX levels negatively correlated with the CES per embryo with a borderline significance (r = −0.261, P = 0.062). The levels of TNFz 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 3).

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 chronic inflammation in FF and IVF outcomes.

Discussion

In this study, there were significant differences in the FF levels of GSH and TB2 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 significantly and positively correlated with the TRX concentrations in the FF. Furthermore, the levels of inflammatory cytokines were found to be significantly elevated in the FF samples obtained from patients with endometriosis. In addition, TRX concentrations in the FF samples 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 GSH depletion (Gardiner et al. 1998). Glutathione plays important roles during maturation and post-fertilization processes in bovine oocytes (Hashimoto et al. 2000). The secreted GSH protects oocytes against excessive levels of ROS during ovulation, helping to ensure successful fertilization. In our study, the levels of GSH in the FF samples from the endometriosis group were significantly lower than those from the control group. In addition, GSH levels were shown to be 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 (Ebisch et al. 2006). However, because GSH levels were measured from pooled samples of FF from different follicles, this previous study may not accurately reflect the microenvironment of the dominant follicle. This study showed that GPX3 concentrations in FF samples were negatively correlated with oocyte maturity. GPX3 may indicate a hypoxic environment. Microarray analysis previously revealed that GPX3 gene

Table 2 Pearson’s correlation coefficients between inflammatory cytokines and biomarkers of oxidative stress in the FF of study subjects.

<table>
<thead>
<tr>
<th></th>
<th>IL1β</th>
<th>IL6</th>
<th>IL8</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>−0.143</td>
<td>0.232</td>
<td>−0.111</td>
<td>0.352</td>
</tr>
<tr>
<td>SOD</td>
<td>−0.156</td>
<td>0.188</td>
<td>−0.053</td>
<td>0.658</td>
</tr>
<tr>
<td>GSH</td>
<td>−0.137</td>
<td>0.245</td>
<td>−0.143</td>
<td>0.223</td>
</tr>
<tr>
<td>GPX3</td>
<td>−0.041</td>
<td>0.755</td>
<td>−0.058</td>
<td>0.659</td>
</tr>
<tr>
<td>TRX</td>
<td>0.248</td>
<td>0.058</td>
<td>0.280</td>
<td>0.032</td>
</tr>
<tr>
<td>TBP2</td>
<td>−0.097</td>
<td>0.488</td>
<td>−0.037</td>
<td>0.791</td>
</tr>
<tr>
<td>PRX4</td>
<td>0.034</td>
<td>0.774</td>
<td>0.024</td>
<td>0.838</td>
</tr>
</tbody>
</table>

FF, follicular fluid; IL, interleukin; TNF, tumor necrosis factor; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; GPX, glutathione peroxidase; TRX, thioredoxin; TBP, TRX-binding protein; PRX, peroxiredoxin.
expression was reduced in cumulus cells from oocytes that did not yield early-cleavage embryos (van Montfoort et al. 2008). Hypoxia produces ROS, which cause lipid peroxidation, enzymatic inactivation, and cell damage, resulting in apoptosis (Buttke & Sandstrom 1994) of cumulus cells and oocytes (Tatemoto et al. 2000). Hypoxia (Van Blerkom et al. 1997) and elevated concentrations of ROS in FF are negatively associated with embryonic development and pregnancy outcome (Pasqualotto et al. 2004, Das et al. 2006) and associated with a significantly higher incidence of aneuploidy and spindle defects in oocytes (Van Blerkom et al. 1997). Among women who are receiving IVF, the mean GPX activity is greater in follicles that yield oocytes that are subsequently fertilized compared with that in follicles with non-fertilized oocytes (Paszkowski et al. 1995). However, this study did not show a correlation between levels of GPX3 in FF samples and fertilization rate.

Endometriosis is associated with inflammatory changes in the FF. This study showed that the levels of IL6, IL8, and TNFα were significantly higher in the FF of women with endometriosis compared with those in the controls, which was consistent with previous studies (Pellicer et al. 1998, Garrido et al. 2000, Wunder et al. 2006). The pro-inflammatory intrafollicular microenvironment in women with endometriosis may influence the quality of oocytes and embryos. In this study, the levels of TNFα in FF samples negatively correlated with the quality of embryos. TNFα in FF has been proposed to be related with oocyte quality and IVF outcomes (Lee et al. 2000), which is consistent with our results. Previous work suggested that elevated levels of IL6 in FF may be detrimental to implantation (Altun et al. 2011). However, we did not observe this relationship in our study. Consistent with other reports (Gazvani et al. 2000, Hammadeh et al. 2002, 2003), we did not find a correlation between IL8 concentrations and IVF outcomes.

Although TRX levels in FF did not differ between groups, TBP2 levels were significantly higher in the FF samples from the endometriosis group compared with those from the control group. The levels of TRX and TBP2 in serum and peritoneal fluid samples from patients with endometriosis were previously reported to be similar to controls (Lambrinoudaki et al. 2009, Seo et al. 2010). These findings may be due to differences between the systemic environment and the local intrafollicular environment. TBP2 was originally identified as a negative regulator of TRX and acts as a suppressor of cell growth and regulator of lipid/glucose metabolism (Watanabe et al. 2010). In addition, TBP2 may enhance the atherosclerotic process by increasing vascular inflammation (World et al. 2006). Our study showed that the levels of TRX in FF were positively correlated with the levels of IL1β, IL6, IL8, and TNFα in FF. 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

### Table 3
Pearson’s correlation coefficients between biomarkers of oxidative stress and chronic inflammation in FF and IVF outcomes.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of mature oocytes/no. of total oocytes (%)</th>
<th>Fertilization rate (%)</th>
<th>CES/the no. of embryos transferred</th>
<th>No. of high-quality embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>MDA</td>
<td>−0.069</td>
<td>0.592</td>
<td>−0.001</td>
<td>0.991</td>
</tr>
<tr>
<td>SOD</td>
<td>−0.164</td>
<td>0.199</td>
<td>0.048</td>
<td>0.715</td>
</tr>
<tr>
<td>GSH</td>
<td>0.111</td>
<td>0.381</td>
<td>0.040</td>
<td>0.760</td>
</tr>
<tr>
<td>GPX3</td>
<td>−0.275</td>
<td>0.046</td>
<td>−0.198</td>
<td>0.164</td>
</tr>
<tr>
<td>TRX</td>
<td>−0.398</td>
<td>0.004</td>
<td>0.019</td>
<td>0.896</td>
</tr>
<tr>
<td>TBP2</td>
<td>0.018</td>
<td>0.905</td>
<td>0.072</td>
<td>0.640</td>
</tr>
<tr>
<td>PRX4</td>
<td>0.045</td>
<td>0.726</td>
<td>0.068</td>
<td>0.601</td>
</tr>
<tr>
<td>IL1β</td>
<td>0.114</td>
<td>0.372</td>
<td>0.042</td>
<td>0.748</td>
</tr>
<tr>
<td>IL6</td>
<td>−0.013</td>
<td>0.920</td>
<td>0.072</td>
<td>0.578</td>
</tr>
<tr>
<td>IL8</td>
<td>0.057</td>
<td>0.657</td>
<td>0.006</td>
<td>0.961</td>
</tr>
<tr>
<td>TNFα</td>
<td>−0.028</td>
<td>0.826</td>
<td>0.013</td>
<td>0.921</td>
</tr>
</tbody>
</table>

FF, follicular fluid; IL, interleukin; TNF, tumor necrosis factor; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; GPX, glutathione peroxidase; TRX, thioredoxin; TBP, TRX-binding protein; PRX, peroxiredoxin.

### Table 4
Logistic regression analysis of predictive factors for a clinical pregnancy.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Basal serum FSH</td>
<td>0.867 (0.739–1.017)</td>
<td>0.080</td>
</tr>
<tr>
<td>Total antral follicle count</td>
<td>1.091 (0.993–1.200)</td>
<td>0.069</td>
</tr>
<tr>
<td>Serum E2 levels on hCG day</td>
<td>1.000 (1.000–1.001)</td>
<td>0.033</td>
</tr>
<tr>
<td>No. of high-quality embryos</td>
<td>1.983 (1.168–3.365)</td>
<td>0.011</td>
</tr>
<tr>
<td>FF GSH</td>
<td>1.089 (1.002–1.184)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

FSH, follicle-stimulating hormone; E2, estradiol; hCG, human chorionic gonadotropin; FF, follicular fluid; GSH, glutathione.
embryo quality, and TNFα 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.

Although GSH, GPX3, TRX, and TNFα levels were significantly correlated with IVF outcomes, multivariate logistic regression analysis revealed that the number of high-quality embryos was significantly and independently correlated with 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. Firstly, 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 (Garcia-Velasco et al. 2010). Secondly, although we are concerned about microenvironment of dominant follicle, which is most likely to contain the mature oocyte, results of FF samples from a single-dominant follicle may not reflect the other follicles in the ovary. In this study, thirdly, there was a significant difference in ovarian reserve and the number of oocytes retrieved between patients with and without endometriosis. These differences may be due to the surgery and/or endometriosis itself in patients with endometriosis. However, markers of ovarian reserve and the number of oocytes retrieved were not correlated with biomarkers of oxidative stress and chronic inflammation. Therefore, differences in ovarian reserve may not affect the results of this study.

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.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


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