Reproductive BioMedicine Online (2013) **26**, 345–352

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ARTICLE

Elevated blood plasma antioxidant status is favourable for achieving IVF/ICSI pregnancy

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Abstract The aim of the study was to determine the roles of intrafollicular and systemic oxidative stress and antioxidant response in ovarian stimulation and intracytoplasmic sperm injection (ICSI) outcomes. For this purpose, 102 ICSI patients undergoing controlled ovarian stimulation were enrolled and samples were collected on the day of follicle puncture. Total peroxide (TPX) concentrations and total antioxidant response (TAR) were measured in follicular fluid and blood plasma, and an oxidative stress index (OSI) was calculated based on these two parameters. Urinary concentrations of 8-iso-prostaglandin F_{2a} (F_2IsoP) were measured. Elevated intrafollicular oxidative stress was positively correlated with ovarian stimulation outcome: less FSH per retrieved oocyte was used, more oocytes were collected and higher serum oestradiol concentrations were measured in patients with higher follicular OSI. However, high urinary F_2IsoP related to lower embryo quality and F_2IsoP was also elevated in smoking patients. Patients with endometriosis had lower follicular antioxidant status. Most importantly, higher systemic blood TAR was significantly favourable for achieving clinical pregnancy ($P = 0.03$). In conclusion, the findings suggest clear associations between oxidative stress, antioxidant status and several aspects of ovarian stimulation and IVF/ICSI outcome, including pregnancy rate.

KEYWORDS: antioxidative activity, female infertility, follicular fluid, IVF, ovarian stimulation, oxidative stress

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2012.12.012

Introduction

Ovarian stimulation followed by IVF creates stress in the female body. In combination with genetic predisposition and life-style habits, the outcome of IVF may be significantly altered by stress and by the natural defence mechanisms necessary for coping with it (Ruder et al., 2008, 2009).

Several endogenously synthesized reactive oxygen species (ROS) play important roles in the cellular signalling events leading to cell growth, differentiation, migration, mitosis and other processes (Droge, 2002; Duleba et al., 2004; Valko et al., 2007). Total antioxidant status regulates the concentrations of different ROS to maintain optimal physiologically low concentrations, as their excess can lead to high-grade oxidative stress, causing aberrations in DNA, proteins and lipids, and in the long term are deleterious to the cell or tissue (Valko et al., 2007). A stressful imbalance in a tissue may be caused by the malfunctioning of endogenous pro- and antioxidants as well as by various exogenous factors: smoking, extreme body mass index (BMI), nutrient intake and other environmental and life-style-related features influencing the level of oxidative stress (Ruder et al., 2008). It is proposed, however, that the expression of antioxidant system components in complex organisms varies between tissues, and responses to environmental oxidative stress factors may be tissue- and organ-specific (Limon-Pacheco and Gonsebatt, 2009). Therefore, in order to acquire more information about the oxidative stress and antioxidant balance, it is necessary to test simultaneously systemic and local oxidative stress and total antioxidant status.

A positive outcome in an IVF procedure requires the successful occurrence of several events: folliculogenesis, oocyte maturation, fertilization, embryo implantation and normal development. Hence, both systemic (measured in blood or urine) and intrafollicular oxidative stress and total antioxidant status are potential indicators of the outcome of IVF. Several groups have described single enzymic and non-enzymic components of total antioxidant status in relation to natural folliculogenesis or the outcome of IVF. The most abundantly studied of these are superoxide dismutase (Suzuki et al., 1999; Sugino et al., 2000; Matos et al., 2009; Pasqualotto et al., 2009), catalase (Behl and Pandey, 2002; Pasqualotto et al., 2009), glutathione peroxidase (Paszkowski et al., 1995), thioredoxin (Lambrinoudaki et al., 2009) and ascorbic acid (Paszkowski and Clarke, 1999), among others. Estimation of oxidative DNA damage in granulosa cells or lipid peroxidation products in follicular fluid are alternative methods for studying the end results of chain reactions producing ROS (Seino et al., 2002; Gupta et al., 2008; Fujimoto et al., 2011). However, in order to obtain a broader overview of the redox state of a tissue or organism, collective markers for oxidative stress and total antioxidant status should be assessed.

The goal of the current study was to describe the significance of oxidative stress and total antioxidant status in the oocyte maturation environment as well as systemically in women undergoing intracytoplasmic sperm injection (ICSI) and correlate the results with the outcome of ovarian follicle stimulation, clinical pregnancy rate, aetiology of infertility and smoking habits. For this purpose, this study collected blood plasma, urine samples and follicular fluid (FF) from 102 ovarian stimulation patients assigned to ICSI. Total peroxide (TPX) concentration as a marker of oxidative stress, and total antioxidant status measured via the total antioxidant response (TAR) method, was determined in FF and blood plasma by means of previously established methods (Erel, 2004; Kosecik et al., 2005). An oxidative stress index (OSI) was calculated from TPX and TAR data, reflecting the overall balance of oxidative stress and total antioxidant status in the sample. Similar methods have been previously used in association studies, carried out to determine the levels of oxidative stress in relation to various disease states (Harma and Erel, 2005; Devi et al., 2008). The content of 8-iso-prostaglandin F_{2a} (F_2IsoP), an endproduct of lipid peroxidation and a widely accepted additional marker of systemic oxidative stress (Minuz et al., 2006), was measured in the urine samples.

Materials and methods

Patients and IVF procedure

A total of 102 women, aged 23—41 years (mean ± SD 32.9 ± 4.2 years), BMI 18.0—36.3 kg/m^2 (23.6 ± 4.05 kg/m^2) undergoing consecutive ICSI and embryo transfer at Nova Vita Clinic (Tallinn, Estonia) were enrolled. All patients had been unable to conceive naturally for at least 1 year before entering the study. The reasons for the couples' infertility were male factor infertility (n = 45), polycystic ovary syndrome (n = 8), tubal occlusion (n = 29) and endometriosis (n = 16). Two patients were single women and for two couples the aetiology of infertility remained unknown. Fifteen women were regular smokers during ovarian stimulation.

ICSI was chosen for oocyte fertilization in all cases, because of male factor infertility or oocyte fertilization failure during previous cycles of conventional IVF. The study was approved by the Ethics Committee of the University of Tartu (approval reference number 160/9, dated 21 May 2007) and informed consent was obtained from all participants.

Ovarian hormonal stimulation was conducted according to the gonadotrophin-releasing hormone antagonist (Cetrotide; Merck Serono, Geneva, Switzerland) protocol with the administration of recombinant FSH (Gonal-F; Merck Serono, or Puregon; Schering-Plough, Kenilworth, NJ, USA). On average, 2072.2 ± 701.8 IU FSH was used during the 9.5 ± 0.8 days of ovarian stimulation. All patients underwent ovarian retrieval 36 h after human chorionic gonadotrophin administration (Ovitrelle; Merck Serono) with the puncture of follicles $\geq$15 mm in diameter. The total number of oocytes retrieved from each patient was 10.5 ± 6.6, of which 8.8 ± 5.5 were considered mature, as the polar body was clearly visible in microscopic inspection. ICSI was used to fertilize the oocytes 4—6 h after ovarian retrieval with a success rate of 69.3%. Oocytes from four patients failed to fertilize. Embryos were graded according to their morphological appearance 48 h after fertilization. An embryo with at least four equally sized blastomeres and $\leq$20% of acellular fragments was considered to be of good quality on day 2 and the percentage of good-quality embryos on day 2 was calculated for all patients, irrespective of the

day of embryo transfer. Up to three (1.9 ± 0.5) day 2 (n = 94) or day 3 (n = 4) embryos were transferred, resulting in a clinical pregnancy rate of 27.5% (n = 28) per ovarian retrieval and 28.6% per embryo transfer, eight (28.6%) of these being twin pregnancies. The overall implantation rate was 18.6 ± 32.9%. All embryos of good quality that were not transferred (1.9 ± 2.3 embryos per couple) were cryopreserved by slow freezing and preserved in liquid nitrogen. All procedures from ovarian retrieval to embryo transfer and freezing were performed using solutions and culture media from Origio (Måløv, Denmark), according to the manufacturer's instructions. Incubation of oocytes and embryos was performed in humidified conditions with 5.5% CO_2 at 37°C.

Sample collection

FF was collected during oocyte retrieval by follicle aspiration under ultrasonographic control and frozen immediately at −80°C. Only material from the first aspirated follicle (diameter 21.1 ± 3.0 mm) was used in order to avoid contamination with blood. No contaminated samples were used for further analysis. Total protein concentrations were determined by the Lowry method (Lowry et al., 1951) and all measurements in FF were normalized according to protein concentration.

Two blood samples were collected on the day of ovarian retrieval. For oxidative stress marker determination, blood in lithium-heparin Vacuette tubes (Greiner Bio-One, Kremsmünster, Austria) was centrifuged at 1200*g* for 10 min, and extracted plasma was kept at −80°C until analysis. For hormone analyses, samples were collected without anticoagulants and immediately sent for commercial assay of oestradiol and progesterone (Quattromed, Tallinn, Estonia).

Urine samples were collected on the same day into sterile cups and 600 μl was immediately frozen at −80°C. The rest of the sample was directly sent for routine commercial assay of creatinine (Quattromed), necessary for standardizing other measurements according to urine dilution.

Total peroxide assay

TPX concentrations in plasma and FF samples were determined by ferrous oxidation using xylenol orange assay version 2 (FOX2; Miyazawa, 1989), with minor modifications (Kosecik et al., 2005). The FOX2 assay is based on oxidation of ferrous ion to ferric ion by various types of peroxides within the samples to produce a ferric–xylenol orange complex, the absorbance of which can be measured at 560 nm. The TPX content of the samples was determined using a solution of H_2O_2 as a standard. Measurements were performed on a spectrophotometer (Jenway 6405; Bibby Scientific, Staffordshire, UK). The results were expressed as micromoles of H_2O_2 per litre. Within- and between-assay coefficients of variation were 4.4% and 4.7%, respectively.

Total antioxidant response assay

The method has been previously described in detail (Erel, 2004). Estimation of TAR is based on the reaction of Fe^{2+}-*O*-dianisidine complex with hydrogen peroxide, producing hydroxyl radicals. Colourless *O*-dianisidine molecules are reduced by ROS to yellow-brown dianisidyl radicals that can be measured at 412 nm by spectrophotometry. Antioxidants in the sample suppress colour formation to a degree that is proportional to their concentrations. Suppression of colour formation is calibrated using Trolox, a derivative of the antioxidant vitamin E, which is widely used as a traditional standard in TAR assays. The results are expressed in terms of millimolar Trolox equivalent per litre. The coefficients of variation for within- and between assay precision were 2.4% and 2.9%.

Oxidative stress index calculation

The percentage ratio of TPX to TAR (TPX μmol H_2O_2 equivalent/l ÷ TAR μmol Trolox equivalent/l × 100) was used for the OSI, being widely used as a measure for characterization of systemic oxidative stress (Horoz et al., 2006).

8-iso-Prostaglandin F_{2a} assay

Urinary concentrations of F_2IsoP were analysed by a commercial competitive ELISA (BIOXYTECH 8-F_2IsoP Assay; Oxis Research, Portland, OR, USA) according to the manufacturer's instructions. A Sunrise microplate reader (Tecan Austria, Salzburg, Austria) was used for measuring signal intensity. The acquired results were divided by the measured values of creatinine. Accordingly, concentrations of F_2IsoP are presented as ng/mmol creatinine.

Statistics

All data analysis was performed using PASW Statistics 18 software (SPSS, Chicago, IL, USA). Data was transformed logarithmically, if necessary, to achieve normal distribution. Values of oxidative stress markers in FF and blood plasma were compared using Student's paired t-test preceded by Levene's test for equality of variances. Partial correlation was used for analysing linear data. FSH per oocyte, the number of retrieved oocytes and plasma oestradiol concentration were corrected for patient's age; FF oxidative stress markers were corrected for follicle diameter; and F_2IsoP was corrected for BMI. Between-groups data was analysed using either the Mann–Whitney *U*-test (for two groups) or Kruskal–Wallis one-way ANOVA (for more than two groups) and differences are presented as medians and 95% confidence intervals (CI). The general linear model was used to correct F_2IsoP concentration for BMI and to compare the results between patients with different smoking habits. The results are presented as estimated marginal means with 95% CI. Chi-squared or Fisher's Exact tests were used for comparing categorical data. $P < 0.05$ was considered statistically significant in all tests.

Results

Data concerning all measurements of oxidative stress markers are presented in **Table 1**. TAR was significantly lower ($P < 0.001$) and OSI was significantly higher ($P < 0.001$) in FF compared with blood plasma samples. TAR showed a statistically significant positive correlation between plasma and FF ($P < 0.037$).

Table 1 Oxidative stress marker concentrations in the body fluids of IVF patients.

	Blood plasma		*Follicular fluid*		*Urine*		*Paired t-test P-value*	*Correlation*	
	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD		R	P-*value*
TAR (mmol Trolox equivalent/l)	0.71	0.74 ± 0.20	0.67	0.65 ± 0.24	–	–	<0.001	0.212	0.037
TPX (μmol H_2O_2 equivalent/l)	10.50	11.95 ± 5.96	12.00	12.88 ± 5.51	–	–	NS	−0.132	NS
Oxidative stress index	1.49	1.69 ± 0.90	2.02	2.30 ± 1.30	–	–	<0.001	0.059	NS
F_2IsoP (ng/mmol creatinine)	–	–	–	–	82.94	93.06 ± 46.38	–	–	–

Student's paired t-test and Pearson's correlation analysis were performed on logarithmically transformed data. F_2IsoP = 8-iso-prostaglandin F_{2a}; NS = not statistically significant; OSI = oxidative stress index; TAR = total antioxidant response; TPX = total peroxide.

The oxidative stress markers were studied in regard to the outcome of the ovarian stimulation cycle during which the samples were collected (**Table 2**). As there was a significant correlation between FF TPX ($R = -0.25$; $P = 0.015$) and follicle diameter, further associations involving FF oxidative stress markers were adjusted for this parameter. More oocytes were retrieved from and higher oestradiol concentrations were measured in the sera of patients with elevated follicular oxidative stress as indicated by higher FF TPX concentrations and OSI values. Data analysis also revealed that lower amounts of FSH per oocyte were required for patients with higher FF OSI values. Higher concentrations of urinary F_2IsoP reflected IVF outcome as they were associated with a decreased percentage of good-quality embryos on day 2. In the correlation analysis, concentrations of F_2IsoP were corrected for BMI, because of a positive association between the two data pools ($R = 0.24$; $P = 0.016$). Embryo quality was assessed only for patients with more than three embryos ($n = 69$) in order to observe a clear tendency of a patient's embryo developmental potential. The measured oxidative stress markers did not significantly correlate with the patients' age, serum progesterone concentration or the percentage of mature oocytes retrieved.

The association between a positive IVF outcome and patients' oxidative stress marker concentrations was also studied. Twenty-eight women (27.5%) achieved clinical pregnancy per ovarian retrieval, determined by visualization of an embryonic sac at vaginal ultrasonography 4 weeks after embryo transfer. The concentration of plasma TAR was significantly elevated in pregnant women ($P = 0.030$,

Table 2 Correlation of oxidative stress markers with parameters of ovarian stimulation and outcome.

	FSH/oocyte[a]		*Retrieved oocytes*[a]		*Plasma oestradiol*[a]		*Good-quality embryo (%)*[b]	
	R	P	R	P	R	P	R	P
Plasma								
TAR	0.032	NS	0.000	NS	0.003	NS	−0.002	NS
TPX	0.039	NS	−0.097	NS	−0.040	NS	−0.112	NS
OSI	0.020	NS	−0.090	NS	−0.037	NS	−0.102	NS
Follicular fluid								
TAR[c]	0.122	NS	−0.183	NS	−0.087	NS	−0.014	NS
TPX[c]	−0.160	NS	0.215	0.038	0.214	0.040	−0.142	NS
OSI[c]	−0.225	0.031	0.320	0.002	0.232	0.025	−0.121	NS
Urine								
F_2IsoP[d]	−0.135	NS	0.126	NS	0.039	NS	−0.303	0.012

[a]Corrected for age.
[b]Embryos with ≥4 equally sized blastomeres and ≤20% of fragmentation on day 2 were considered of good quality. Only patients with more than three embryos were selected ($n = 69$).
[c]Corrected for follicle diameter.
[d]Corrected for body mass index.
F_2IsoP = 8-iso-prostaglandin F_{2a};
NS = not statistically significant;
OSI = oxidative stress index;
TAR = total antioxidant response;
TPX = total peroxide.

Table 3 Oxidative stress marker concentrations and characteristics of patients according to IVF pregnancy outcome.

	Not pregnant (n = 74)		*Pregnant* (n = 28)	
	Median	*Mean ± SD*	*Median*	*Mean ± SD*
Age (years)	33.50	33.14 ± 4.30	32.00	32.11 ± 3.70
BMI (kg/m^2)	22.70	23.97 ± 3.99	21.32	22.64 ± 4.15
Plasma				
TAR (mmol Trolox equivalent/l)[a]	0.68	0.72 ± 0.19	0.76	0.80 ± 0.21
TPX (μmol H_2O_2 equivalent/l)	10.57	12.04 ± 5.90	10.13	11.70 ± 6.23
OSI	1.59	1.76 ± 0.93	1.31	1.50 ± 0.82
Follicular fluid				
TAR (mmol Trolox equivalent/l)[a]	0.67	0.63 ± 0.23	0.69	0.66 ± 0.20
TPX (μmol H_2O_2 equivalent/l)	12.10	12.69 ± 5.41	12.71	12.80 ± 4.76
OSI	1.95	2.32 ± 1.24	1.87	2.19 ± 1.45
Urine F_2IsoP (ng/mmol creatinine)	87.06	94.91 ± 50.88	80.35	88.18 ± 31.86
Serum progesterone (nmol/l)	30.09	35.40 ± 22.17	30.70	33.51 ± 16.84
Serum oestradiol (nmol/l)	2.98	3.24 ± 1.87	3.66	5.89 ± 11.89
Total exogenous FSH (IU)	2025.00	2163.54 ± 757.71	1800.00	1912.96 ± 647.83
FSH/oocyte (IU)	207.95	389.25 ± 503.59	180.00	298.60 ± 386.93
No. of oocytes	9.00	10.55 ± 6.83	10.00	11.39 ± 6.27
Mature oocyte (%)	81.82	79.09 ± 18.47	80.00	81.06 ± 18.30
Fertilization (%)	55.56	54.61 ± 22.73	56.07	58.07 ± 20.04
Good-quality embryo (%)[b]	60.00	57.57 ± 26.25	60.00	63.52 ± 21.72
No. of transferred embryos	2.00	1.81 ± 0.66	2.00	1.93 ± 0.26

[a]Mann—Whitney *U*-test showed that plasma TAR was significantly different between groups ($P = 0.03$). No statistically significant differences were found for any of the other parameters.
[b]Only patients with more than three embryos were included ($n = 69$). F_2IsoP = 8-iso-prostaglandin F_{2a}; OSI = oxidative stress index; TAR = total antioxidant response; TPX = total peroxide.

Table 3). The women were divided into three groups according to embryo implantation rate: 0%, 50% or 100% of transferred embryos implanted successfully. The concentration of plasma TAR differed significantly between the groups (overall Kruskal—Wallis $P = 0.029$). Significantly lower plasma TAR was measured in the group with no embryo implantation (median plasma TAR 0.68, 95% CI 0.64—0.72 mmol/l) as compared with the group with 50% implantation rate (median plasma TAR 0.76, 95% CI 0.73—1.03 mmol/l; $P = 0.006$). Plasma TAR concentration was not affected by the number of implanted embryos once the pregnancy was achieved (plasma TAR 0.75, 95% CI 0.48—0.87 mmol/l in women with 100% implantation rate). Oxidative stress markers in FF and urine were comparable between groups according to pregnancy outcome, as were all other physiological parameters (**Table 3**), aetiology of infertility and smoking habits (data not shown).

Concentrations of oxidative stress markers were compared in 16 patients with endometriosis versus 49 patients without any determined gynaecological diagnosis. The control group consisted of couples with male factor infertility and unknown cause of infertility, as well as single women. Significant differences were observed at the follicular level, where lower FF TAR were measured in the endometriosis patients (median FF TAR 0.44, 95% CI 0.34—0.70 mmol/l in endometriosis patients versus 0.68, 95% CI 0.56—0.77 mmol/l in healthy women; $P = 0.016$), indicating a reduced antioxidant response in the follicular environment among these patients. No differences in oxidative stress markers were observed when comparing patients with other aetiologies of infertility (polycystic ovary syndrome or tubal factor infertility) versus controls. Importantly, stimulation efficiency (measured by the number of retrieved oocytes and the amount of FSH administered per retrieved oocyte) was not different between patient groups according to aetiology of infertility.

There were no significant differences between the proportions of smokers when groups were compared according to their clinical pregnancy outcome or the aetiology of infertility. However, when comparing current smokers ($n = 15$) and non-smokers ($n = 87$), a significant difference in concentrations of F_2IsoP was observed (BMI-adjusted estimated marginal mean 83.92, 95% CI 73.91—93.94 ng/mmol creatinine in non-smokers and 127.47, 95% CI 105.43—149.51 ng/mmol creatinine in smokers; $P = 0.001$).

Discussion

One of the main aims of this study was to compare the intrafollicular and systemic balance of the pro-oxidant/antioxidant system in ICSI patients. To achieve this goal, three markers reflecting oxidative stress and total antioxidant status were measured in blood plasma and FF (TPX and TAR) or urine (F_2IsoP). This study also calculated OSI that describes

the oxidative stress/total antioxidant status balance and shows higher values if the tissue is under elevated oxidative stress. Estimation of ovarian oxidative stress was carried out using FF from the patient's first follicle to avoid blood contamination. Thus this study did not aim to study the fate of individual oocytes and subsequent embryos, but rather ovarian physiology as a whole and differences between patient groups.

The results showed that TPX concentrations were independent as regards FF and plasma, while TAR correlated well between the two body fluids, the latter result having been shown before (Appasamy et al., 2008). The higher overall OSI in FF in comparison with plasma reflects active metabolism in the follicle and possibly the whole ovary as a result of multiple follicle stimulation. It is well known that oxidative metabolism is involved in every stage of ovarian follicular development as well as oocyte maturation (Krisher, 2004). Also, high ovarian ROS concentrations are a byproduct of steroidogenesis and are necessary for successful ovulation signalling in mammals (Fujii et al., 2005). In addition, LH has been shown to stimulate superoxide production in rat pre-ovulatory follicles (Kodaman and Behrman, 2001). An excess of intra-ovarian ROS scavengers significantly reduces the rate of ovulation in mice by interfering with LH-dependent pathways, leading to perturbations in cumulus cell expansion, a decrease in progesterone synthesis and LH-dependent expression of ovulation-specific genes (Shkolnik et al., 2011). However, an upper limit at which ROS in FF commences oocyte damage, leading to reduced embryo quality, has been proposed (Jana et al., 2010). The present data, which correlate retrieved oocyte number to elevated FF TPX and OSI, support the idea that growth of a higher number of mature follicles is associated with ROS production. The positive correlation between FF OSI and serum oestradiol concentrations was expected, as maturing follicles are the primary source of oestradiol in IVF patients. To what extent elevated oxidative stress in the follicle could be a consequence of hormonal stimulation has not been extensively studied. It has been shown that ROS production by granulosa cells does not differ in stimulated versus non-stimulated patients (Jancar et al., 2008), but investigation of local and systemic oxidative stress markers in non-stimulated subjects remains to be carried out.

This study observed, as far as is known for the first time, that follicle stimulation sensitivity was elevated when higher FF OSI was calculated, as less FSH per retrieved oocyte was administered to these patients. This result was not significant when FF TAR and TPX were considered separately. It would be necessary to investigate further how ROS influence FSH signalling in recruited follicles to better explain this result. However, a favourable shift towards a moderate oxidative stress environment during oocyte maturation coincides well with data showing that cell-permeable antioxidants inhibit the resumption of oocyte meiosis in rats (Takami et al., 1999, 2000).

This study included urinary F_2IsoP as one of the parameters for systemic oxidative stress evaluation. Concentrations of urinary F_2IsoP were positively associated with BMI, which has been previously mentioned (Keaney et al., 2003; Mutlu-Turkoglu et al., 2003), while BMI has been related to reduced pregnancy outcome in IVF patients (Aurrekoetxea et al., 2010). In the current study, concentrations of F_2IsoP also presented the only systemic parameter that reflected an influence on in-vitro embryo development by being negatively correlated with the percentage of good-quality embryos. Previously, only FF lipid peroxidation concentrations have been correlated to IVF outcome, with conflicting results (Pasqualotto et al., 2004; Das et al., 2006; Oral et al., 2006). The current results support a link between lipid peroxidation products and embryo quality, although it is surprising that none of the FF markers showed a similar correlation. The mechanism of how systemic oxidative stress influences folliculogenesis can only be surmised. Several studies have demonstrated the negative impact of circulating F_2IsoP on angiogenesis via the activation of thromboxane receptor signalling and RhoA-mediated pathways leading to anti-angiogenic effects during chronic surplus of ROS (Benndorf et al., 2008; Sauer and Wartenberg, 2008). On the other hand, reduced peri-follicular angiogenesis and increased intrafollicular hypoxia cause chromosomal disturbances in the oocyte and lead to poor embryo quality (Chui et al., 1997; Van Blerkom et al., 1997; Monteleone et al., 2008). Analysis of the dynamics of folliculogenesis in women demonstrating dissimilar concentrations of urinary F_2IsoP would be useful in order to investigate the possible link between systemic lipid peroxidation products and follicular vascularization.

Concentrations of F_2IsoP also represented the only marker varying between smokers and non-smokers, as described elsewhere (Yanbaeva et al., 2007), but there was no significant difference in embryo quality between these groups. The information regarding the amount of cigarettes smoked was missing in this study's questionnaire and therefore this result needs further verification. However, this study did not observe whether smoking would influence other parameters in serum and follicular fluid.

Blood plasma TAR was elevated in women who achieved clinical pregnancy as a result of IVF. The increase was most markedly observed when embryo implantation rate increased from 0% to 50% (reflecting the implantation of one embryo) and did not change as more embryos implanted. These results suggest that a link exists between systemic antioxidant defence and endometrial receptivity. It has been shown that superoxide dismutase expression and activity increase in endometrial stromal cells upon decidualization (Sugino, 2007). Changes in endometrial total antioxidant status to balance the production of ROS may influence the systemic markers or there may be other, as-yet unknown, means by which elevated total antioxidant status is favourable to pregnancy achievement. A recent study revealed a correlation between systemic total antioxidant status and positive pregnancy outcome when excluding IVF patients with male factor infertility (Aurrekoetxea et al., 2010), suggesting that there is great variation in the influence of oxidative stress on implantation in different patient groups.

Very little information is available on FF markers of oxidative stress in endometriosis patients, as peritoneal fluid has been more extensively studied in relation to this aetiology of infertility, without finding any significant associations (Ruder et al., 2008). The current study observed that a reduced FF TAR described the patient group with endometriosis. A previous study revealed abundant oxidative

stress-related DNA damage in granulosa cells from endometriosis patients and that the degree of DNA damage in these cells correlated with reduced oocyte fertilization and embryo quality (Seino et al., 2002). It has also been suggested that increased total antioxidant status is necessary to obtain normal oocytes after ovarian stimulation in case of endometriosis (Bedaiwy et al., 2012). The current findings strengthen the hypothesis that normal oocyte development requires an optimal concentration of oxygen in the follicle, which may subsequently lead to oxidative stress, thus generating a need to be balanced with adequate antioxidant response in order to prevent ROS-induced abnormalities (Hu et al., 2001). In endometriosis patients, this response seems to be compromised and inadequate.

In conclusion, the primary results of this study demonstrate that achievement of clinical pregnancy is favoured by elevated systemic total antioxidant status and that follicular oxidative stress markers are positively correlated with ovarian stimulation efficiency in infertile patients. Although the small numbers of women in the groups with different aetiologies of infertility is a weakness of this investigation, nevertheless it is shown that reduced intrafollicular total antioxidant status is a characteristic of endometriosis patients. This work clearly reveals an association of both systemic and local oxidative stress and total antioxidant status markers with various aspects of IVF outcome.

Acknowledgements

This work was supported by the Estonian Ministry of Education and Science (core Grant no. SF0180044s09), Enterprise Estonia (Grant no. EU30020), target financing (no. 0180105s08) from the European Union through the European Regional Development Fund, the World Federation of Scientists. We thank our colleagues from the Nova Vita Clinic for their assistance in sample collection, Kaija Tammekivi for technical assistance, Dr Nick Bolton for revising the language, and all patient volunteers for their participation in the study.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 30 July 2012; refereed 22 November 2012; accepted 19 December 2012.