Systemic and follicular oxidative stress and antioxidant status in IVF patients

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INTRODUCTION

Several reactive oxygen species (ROS) play an important role in the cellular signaling events leading to cell growth, differentiation, migration, mitosis and other processes. Total antioxidant status (TAS) regulates the levels of different ROS to maintain their optimal physiological low level as their excess can lead to high-grade oxidative stress (OS), being in the long term deleterious for the cell or tissue. It has been proposed that in complex organisms the response to environmental OS factors may be tissue- and organ-specific. A positive outcome in *in vitro* fertilization (IVF) procedure requires the successful occurrence of several events in reproductive organs: from folliculogenesis, oocyte maturation and fertilization up to embryo implantation and normal development. Therefore, the levels of both systemic (measured in blood or urine) and intra-follicular OS and TAS are potential indicators for the outcome of IVF. In the current study, our goal was to describe the significance of OS and TAS in ovarian follicle, blood plasma and urine in IVF female patients and correlate the results with the outcome of ovarian follicle stimulation, clinical pregnancy rate and etiology of infertility.

MATERIALS AND METHODS

Blood plasma, urine samples and follicular fluid (FF) were collected from 102 IVF patients. Total peroxide concentration (TPX) as a marker of OS was determined by ferrous oxidation in xylenol orange assay. TAS was measured via the total antioxidant response (TAR) method based on the capacity of antioxidants in a sample to suppress Fe^{2+}-o-dianisidine reduction by H_2O_2. Both TPX and TAR were determined in FF and blood. The oxidative stress index (OSI) was calculated as the percentage ratio of TPX to TAR reflecting the overall balance of OS and TAS in the sample. The urinary content of 8-iso-prostaglandin F_{2a} (F_2IsoPs) was measured as a well recognized marker of systemic oxidative stress.

RESULTS

1. The values of TAR were significantly lower and those of OSI significantly higher in FF compared with blood plasma samples. Only TAR values showed statistically significant positive correlation between plasma and FF (Table 1).
2. Follicular TPX and OSI positively associated with blood estradiol on the day of follicle puncture and the number of oocytes obtained, while OSI negatively correlated with the dose of FSH used per oocyte retrieved. High urinary F_2IsoPs related to lower embryo quality (Table 2).
3. Some OS markers showed a tendency or significant difference in certain patient groups depending on IVF pregnancy outcome, etiology of infertility or smoking habits (Figure 1).

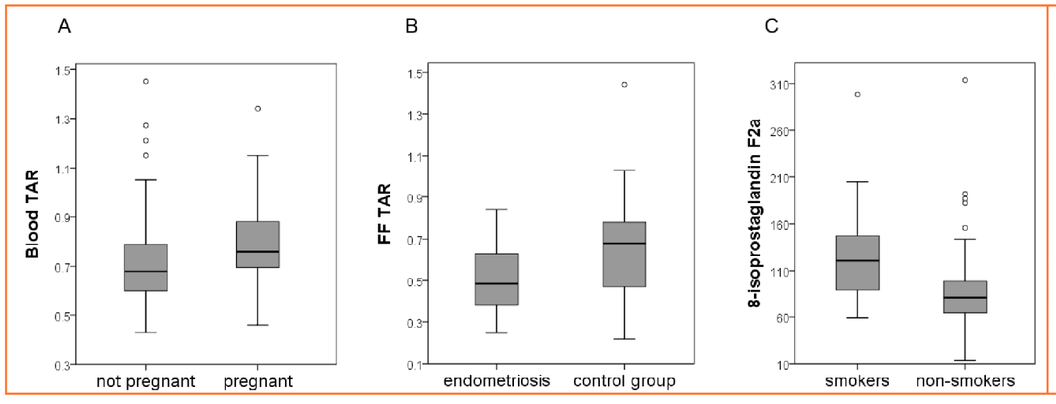

FIGURE 1.
OS markers in patient groups A: Blood TAR showed a statistical tendency to be elevated in pregnant patients (p=0.052). B: FF TAR was significantly lower in endometriosis patients (p=0.022).
C: Concentration of urinary F_2IsoPs was higher in patients who were regularly smoking during the stimulation period (p=0.001).

TABLE 1: Levels of TAR, TPX and OSI and their correlation between FF and blood plasma samples of IVF patients. Statistically significant differences are depicted in red (p<0.05).

	Plasma			Follicular fluid			Paired t-test p-value[a]	r[a]	Correlation p-value[a]
	M	median	SD	M	median	SD			
TAR (mmol Trolox equivalent/L)	0.74	0.71	0.20	0.64	0.68	0.22	0.000	0.245	0.016
TPX (μM)	11.95	10.50	5.96	12.72	12.10	5.20	0.129	-0.073	0.476
OSI	1.69	1.49	0.90	2.28	1.91	1.30	0.000	0.096	0.347

[a] Statistical analysis was performed on log-transformed data.
M = arithmetic mean
SD = standard deviation
r = Pearson's correlation coefficient

TABLE 2: Correlation between OS markers and IVF outcome. Statistically significant results are noted in red (p<0.05).

	FSH/oocyte		Serum estradiol		Oocyte number		Good quality embryos % (n>3)	
	r	p	r	p	r	p	r	p
plasma TAR	0.08	0.460	-0.01	0.916	-0.04	0.670	0.03	0.795
plasma TPX	0.00	0.982	0.00	0.984	-0.06	0.586	0.11	0.367
plasma OSI	-0.04	0.724	0.00	0.973	-0.03	0.775	0.08	0.527
FF TAR	0.13	0.225	-0.06	0.541	-0.19	0.060	0.04	0.737
FF TPX	-0.15	0.140	0.21	0.040	0.23	0.024	-0.06	0.664
FF OSI	-0.21	0.046	0.23	0.022	0.32	0.002	-0.07	0.595
Isoprostane[a]	-0.01	0.933	-0.16	0.217	0.06	0.636	-0.31	0.013

[a] Partial correlation adjusted to BMI was used.
r = Pearson's correlation coefficient

CONCLUSIONS

We clearly present for the first time that follicular OS markers correlate to stimulation efficiency and that the redox environment is completely different between blood plasma and follicular fluid in IVF patients. We could demonstrate that reduced intra-follicular TAS is a characteristic of endometriosis patients and systemic oxidative stress is elevated in smoking women. As an important result, we showed that stronger antioxidant status is favorable for the achievement of clinical pregnancy.