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ARTICLE

Influence of ovarian stimulation for IVF/ICSI on the antioxidant defence system and relationship to outcome

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Abstract Ovarian stimulation is used with IVF/intracytoplasmic sperm injection (ICSI) cycles to obtain multiple oocytes and improve pregnancy rates; however, it also induces perturbation in the oxidant–antioxidant balance leading to oxidation stress. The present study monitored the plasma antioxidant status in women undergoing a long agonist protocol of ovarian stimulation at three different time points: at baseline (T0), after pituitary suppression (T1) and on the day of oocyte retrieval (T2). The antioxidant composition of follicular fluid samples collected on T2 was also evaluated. Significant decreases ($P < 0.05$) of plasma vitamin C, vitamin E and carotenoids were found between T1 and T2 but not between T0 and T1. At T2, high plasma vitamin E was associated with high numbers of total and mature oocytes retrieved per patient, which, in turn, were favourable for achieving pregnancy. Accordingly, women who became pregnant presented higher vitamin E concentrations both in plasma and FF than those who did not. In conclusion, this study confirmed the occurrence of significant modifications of the plasma antioxidant profile during ovarian stimulation with gonadotrophins; at the same time, it was found that both systemic and follicular antioxidant status may be related to IVF/ICSI outcome.

KEYWORDS: antioxidant profile, assisted reproduction, follicular fluid, ovarian stimulation, oxidation stress, pregnancy

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Introduction

Oxidation stress occurs when the production of reactive oxygen species (ROS) exceeds the antioxidant-scavenging capacity of a cell or tissue. The link between oxidation stress and male infertility has received substantial scientific support (Agarwal et al., 2008a; Gharagozloo and Aitken, 2011; Lanzafame et al., 2009); indeed, excessive ROS production negatively impacts sperm quality and function due to the induction of detrimental chemical and structural modifications to sperm DNA and membrane lipids (Benedetti et al., 2012; Khosrowbeygi and Zarghami, 2007; Shiva et al., 2011). The involvement of free radicals in female infertility is less well known and controversial. Evidence has suggested that ROS play an essential physiological role in oocyte maturation, formation of the corpus luteum and luteolysis (Agarwal et al., 2012; Ruder et al., 2009; Sugino, 2005). However, debate continues as to whether oocyte potential is adversely influenced by oxidation stress within the follicle. Several studies have focused on the microenvironment surrounding the oocyte and the ROS and antioxidants found in the follicular fluid (FF) (Appasamy et al., 2008; Fujimoto et al., 2011; Oyawoye et al., 2003; Pasqualotto et al., 2004); indeed, an imbalance between ROS and the antioxidant defence system in the FF could be responsible for abnormal oocyte development, causing damage to the DNA, cytoskeleton and cell membrane, which would result in lower egg quality (Paine et al., 2013).

The impact of follicular oxidation stress on oocyte maturation, fertilization and embryo implantation has gained particular attention in relation to IVF/intracytoplasmic sperm injection (ICSI) outcome (Agarwal et al., 2008b; Bedaiwy et al., 2012; Pasqualotto et al., 2004). In fact, despite advances in assisted reproduction treatment, poor oocyte quality remains a subtle problem for female infertility, and the investigation of factors that affect IVF/ICSI outcome may help to improve success rates.

Although oxidation stress has been suggested as one of the most important factors that negatively affect assisted reproduction outcome (Agarwal et al., 2012), to date this matter has still received little attention in women undergoing ovarian stimulation in preparation to IVF/ICSI. Gonadotrophin stimulation may have direct impact on oxidation stress markers; indeed, IVF cycles have been associated with the production of ROS and perturbation in the oxidant–antioxidant balance, leading to reduced protection against oxidation (Aurrekoetxea et al., 2010). Recently, the gonadotrophin-releasing hormone (GnRH) antagonist protocol has also been related with increased oxidation stress (Celik et al., 2012).

The present study of women undergoing a long agonist protocol of ovarian stimulation in preparation to IVF/ICSI monitored the plasma antioxidant concentrations at three different experimental time points (baseline, after pituitary suppression by GnRH agonists and the day of oocyte retrieval after ovarian stimulation by gonadotrophins), corresponding to key steps of the IVF/ICSI pharmacological treatment. This study also evaluated the antioxidant composition of FF collected on the day of oocyte retrieval and investigated the relationship between FF antioxidant profile, plasma antioxidant profile and IVF/ICSI outcome.

Materials and methods

Subjects

The study included 25 women (mean age 33 ± 4 years) admitted at the Physiopathology of Reproduction Unit (Cervesi Hospital, Cattolica, RN, Italy) for fertility treatment with ovarian stimulation for IVF/ICSI. Aetiology of infertility was attributed to male factors ($n = 9$), female factors (tubal factor $n = 7$, endometriosis $n = 2$) or unexplained ($n = 7$). Criteria for participating in the study included no vitamin supplementation, no cardiovascular medical history, no hypertensive disorders and no metabolic disease. The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans following local Ethics Committee approval (prot. 2733/2013, approved 5 June 2013).

Ovarian stimulation protocol

Patients were submitted to a long agonist protocol of ovarian stimulation. Women received an intramuscular injection of leuprolide acetate at a dose of 3.75 mg (Enantone; Takeda Italia Farmaceutici, Roma) starting in the mid-luteal phase of the previous cycle. After suppression of pituitary, recombinant FSH (Gonal-F;Serono, Switzerland) was commenced at a dose of 225–450 IU/day according to age, ovarian reserve and previous induction cycles. When there was consistent rise in serum oestradiol concentration and the presence of two or more follicles 16–18 mm in diameter, 10,000 IU/ml human chorionic gonadotrophin (HCG; Gonasi; IBSA Farmaceutici Italia, Lodi, Italy) was administered subcutaneously 36 h before transvaginal oocyte retrieval. IVF or ICSI was performed according to the aetiology of infertility. Embryo transfer was performed transcervically 2–5 days after oocyte retrieval. Pregnancies were initially diagnosed by serum HCG and then confirmed as clinical pregnancies by ultrasound visualization of gestational sac with heartbeat.

Sample collection

After obtaining informed consent, fasting blood samples were taken in heparinized tubes from each subject at the following experimental time points: T0, before the intramuscular injection of the agonist leuprolide acetate (baseline); T1, after pituitary suppression and immediately before FSH administration; and T2; the day of oocyte retrieval before anaesthesia induction.

At T2, an average of three FF samples was also collected from each patient. Fluids were aspirated individually with a separate needle avoiding blood contamination, for a total of 73 aspirates. Among them, 54 derived from follicles containing metaphase-II mature oocytes, seven from follicles containing metaphase-I immature oocytes and 12 from follicles containing no oocytes.

Blood and FF were processed by centrifugation (1500*g* for 10 min at 4°C), and plasma and FF aliquots were stored at −80°C until assay.

Biochemical analyses

In plasma and FF, vitamin C (ascorbic acid), vitamin E (alpha tocopherol), vitamin A (retinol) and carotenoids (lutein, lycopene and β-carotene) were monitored. Measurements in FF were normalized according to protein concentration.

Vitamin C

Plasma and FF vitamin C concentrations were evaluated by HPLC (Jasco Corporation, Tokyo, Japan) at 265 nm as previously reported (Colagar and Marzony, 2009; Kand'àr and Zàkovà, 2008). Briefly, samples were diluted in cold methanol, vortexed and centrifuged at 10,000*g* for 10 min at 4°C. Supernatants were injected into an Alltima C18 column (4.6 × 250 mm, 5 μm, from Alltech, Milan, Italy) and eluted at 0.5 ml/min with a mobile phase consisting of 5% methanol in 25 mmol/l NaH_2PO_4 (pH 4.8). Methanol was pure HPLC-grade (VWR International, Milan, Italy) and NaH_2PO_4 was obtained from Sigma–Aldrich (Milan, Italy).

Vitamins A and E and carotenoids

Plasma and FF concentrations of lipid-soluble antioxidants were measured by HPLC following sample deproteinization with ethanol and extraction with hexane (Aebischer et al., 1999). After centrifugation at 10,000*g* for 10 min at 4°C, the organic layer was removed and evaporated; the residue was dissolved in the eluent phase and injected into the HPLC system (Alltima C18 column). The eluent phase was acetonitrile/tetrahydrofuran/methanol (68:22:7, v/v) adjusted to 100 v/v with 1% ammonium acetate (Sigma–Aldrich); the flow rate was 1.5 ml/min. UV and fluorescent detectors were programmed according to absorption, excitation and emission wavelengths of each molecule. All solvents were pure HPLC-grade (VWR International).

Protein concentration

Protein concentration in FF aspirates was determined according to the method of Bradford (1976) at 595 nm.

Statistics and data processing

Descriptive analysis included mean ± standard deviation (SD). Differences in the plasma antioxidant profile between the three experimental time points (T0 versus T1 versus T2) were assessed by ANOVA for repeated measures using t-test for paired data as the post test for multiple comparisons, corrected according to Bonferroni's criterion. Differences in the follicular antioxidant profile between the three groups of patients on the basis of the aetiology of infertility (male versus female versus idiopathic) were assessed by one-way ANOVA using Tukey's as the post test for multiple comparisons. Differences between pregnant and nonpregnant groups were analysed using t-test for unpaired data. Probability values <0.05 were accepted. Normality of the distribution was evaluated by the Kolmogorov–Smirnov test. Correlations were calculated using linear regression analysis. Statistics were obtained using WinSTAT (R. Fitch Software).

Results

Two out of 25 patients did not undergo IVF/ICSI after oocyte retrieval due to ovarian hyperstimulation syndrome.

IVF/ICSI outcome

Table 1 shows the characteristics and outcomes of the 23 women included according to cycle outcome. The numbers of total and mature oocytes retrieved per patient were significantly higher (both $P = 0.002$) in women who achieved pregnancy than in those who did not. Taking into account the aetiology of infertility, the best pregnancy rate was observed in women with male factor infertility, while female factors appeared to negatively affect pregnancy.

Plasma antioxidant profile

Table 2 shows the plasma antioxidant profiles at T0, T1 and T2. Significant differences ($P = 0.043$ for ascorbic acid, $P = 0.0003$ for α-tocopherol, $P = 0.018$ for lycopene and $P = 0.0004$ for β-carotene) were found among the three experimental time points: indeed, while no relevant modifications were detected between T0 and T1 antioxidant concentrations, significant decreases were observed at T2 as compared with T1.

Follicular fluid antioxidant profile

Table 3 shows the antioxidant profile of FF collected from follicles containing metaphase-II oocytes. Significant differences ($P = 0.0001$ for ascorbic acid, $P = 0.0007$ for α-tocopherol and $P = 0.017$ for β-carotene) were observed on the basis of the aetiology of infertility; in fact, ascorbic acid, α-tocopherol and β-carotene concentrations were significantly lower in women with infertility due to female or unexplained factors than in those with male infertility (comparable to healthy control subjects).

FF from follicles containing metaphase-I oocytes ($n = 7$) or no oocytes ($n = 12$) were also collected on the day of oocyte retrieval to investigate possible differences in the antioxidant profile. However, the reduced sample size of these two groups did not allow any statistically valid comparison and no significant differences were revealed in the antioxidant profile as compared with FF from follicles containing metaphase-II oocytes ($n = 54$).

Plasma antioxidant profile versus follicular fluid antioxidant profile

Significant positive correlations were found between plasma and follicular antioxidant concentrations at T2, particularly for ascorbic acid, lutein and β-carotene ($R = 0.883$ and $P < 0.0001$ for ascorbic acid; $R = 0.319$ and $P = 0.019$ for α-tocopherol; $R = 0.588$ and $P < 0.0001$ for retinol; $R = 0.823$ and $P < 0.0001$ for lutein; $R = 0.329$ and $P = 0.019$ for lycopene; $R = 0.826$ and $P < 0.0001$ for β-carotene; Table 4). In addition, evaluating the proportions of each antioxidant in plasma and FF revealed highly significant decreases of all lipid-soluble antioxidants in FF compared with plasma ($P < 0.001$), while a significant increase of vitamin C was found ($P < 0.001$) (Table 5).

Plasma and follicular antioxidant profiles in relation to cycle outcome

Relating the plasma antioxidant profile at T2 to cycle outcome, this study found positive correlations between

Table 1 Characteristics and IVF/ICSI outcomes of women who became pregnant and those who did not.

Parameter	*Total (n = 23)*	*Pregnant (n = 8)*	*Not pregnant (n = 15)*	P-*value*
Infertility factor				
Male	30.4	50.0	20.0	–
Female	39.2	12.5	53.0	
Unknown	30.4	37.5	27.0	
Age (years)	33.3 ± 3.9	33.4 ± 3.4	33.3 ± 4.1	NS
Total oocytes retrieved per patient	10.3 ± 4.5	14.0 ± 2.7	8.3 ± 3.9	0.002
Mature oocytes retrieved per patient	8.8 ± 3.8	11.9 ± 2.0	7.2 ± 3.3	0.002
Oocytes fertilized per patient	6.6 ± 3.00	7.6 ± 3.0	6.0 ± 2.7	NS
Embryos transferred per patient	2.2 ± 0.6	2.1 ± 0.6	2.2 ± 0.5	NS

Values are % or mean ± SD. *P*-values comparing pregnant versus nonpregnant women calculated using t-test for unpaired data.

Table 2 Plasma antioxidant profile at baseline, after pituitary suppression and on day of oocyte retrieval.

Antioxidant (μmol/l)	*T0 (n = 23)*	*T1 (n = 23)*	*T2 (n = 23)*	P-*value*
Ascorbic acid	43.49 ± 4.57	41.94 ± 5.84	36.03 ± 5.98[a]	0.043
α-Tocopherol	17.58 ± 1.22	18.17 ± 0.63	15.42 ± 0.5[a]	0.0003
Retinol	1.41 ± 0.1	1.38 ± 0.05	1.36 ± 0.04	NS
Lutein	0.29 ± 0.05	0.27 ± 0.02	0.24 ± 0.02	NS
Lycopene	0.69 ± 0.07	0.54 ± 0.03	0.46 ± 0.04[a]	0.018
β-Carotene	0.57 ± 0.08	0.48 ± 0.04	0.44 ± 0.04[a]	0.0004

P-values calculated using ANOVA for repeated measures.
NS = not statistically significant; T0 = baseline; T1 = after pituitary suppression; T2 = day of oocyte retrieval.
[a]*P*-value < 0.05 versus T1; t-test for paired data used for post-ANOVA multiple comparisons, corrected according to Bonferroni's criterion.

Table 3 Antioxidant concentrations in follicular fluid from follicles containing metaphase-II oocytes according to aetiology of infertility.

Antioxidant (nmol/g protein)	*Total (n = 54)*	*Male (n = 17)*	*Female (n = 22)*	*Unexplained (n = 15)*	P-*value*
Ascorbic acid	1313 ± 349	1727 ± 297	1406 ± 279[a]	1078 ± 115[a]	0.0001
α-Tocopherol	99.1 ± 24.9	116.8 ± 15.0	92.3 ± 26.8[a]	88.1 ± 21.0[a]	0.0007
Retinol	30.3 ± 8.1	32.9 ± 5.2	28.4 ± 9.7	29.9 ± 8.2	NS
Lutein	2.72 ± 0.93	2.72 ± 0.60	2.73 ± 0.93	2.68 ± 1.21	NS
Lycopene	2.04 ± 0.82	2.15 ± 0.70	1.98 ± 0.93	1.99 ± 0.80	NS
β-Carotene	1.16 ± 0.69	1.55 ± 0.86	1.06 ± 0.66[a]	0.88 ± 0.26[a]	0.017

P-values calculated using one-way ANOVA.
NS = not statistically significant.
[a]$P < 0.05$ versus male infertility (Tukey test used for post-ANOVA multiple comparisons).

α-tocopherol concentration and the number of oocytes retrieved per patient ($R = 0.509$, $P = 0.018$), as well as α-tocopherol concentration and the number of mature oocytes ($R = 0.451$, $P = 0.040$).

By comparing the plasma and follicular antioxidant profiles at T2 in women who achieved pregnancy and those who did not (Table 6), significant differences ($P < 0.05$) were observed as regards the plasma and follicular concentrations of α-tocopherol and β-carotene; indeed, both antioxidants were higher in women who became pregnant. The same trend was observed for ascorbic acid concentration, but the difference was not significant. FF retinol concentration was significantly lower in women who became pregnant than in those who did not ($P < 0.05$).

Discussion

Ovarian stimulation is frequently used with IVF and ICSI cycles to obtain multiple oocytes and to improve pregnancy

Table 4 Positive correlations between plasma and follicular antioxidant concentrations (evaluated at T2).

Antioxidant	R	P-*value*
Ascorbic acid	0.883	<0.0001
α-Tocopherol	0.319	0.019
Retinol	0.588	<0.0001
Lutein	0.823	<0.0001
Lycopene	0.329	0.019
β-Carotene	0.826	<0.0001

Table 5 Distribution of antioxidants in plasma and follicular fluid on day of retrieval.

Antioxidant	*Plasma* (n = *23*)	*Follicular fluid* (n = *54*)	P-*value*
Ascorbic acid	62.0 ± 14.5	91.6 ± 2.7	<0.001
α-Tocopherol	32.6 ± 13.0	6.3 ± 2.2	<0.001
Retinol	2.87 ± 1.09	1.78 ± 0.57	<0.001
Lutein	0.60 ± 0.26	0.22 ± 0.06	<0.001
Lycopene	1.17 ± 0.45	0.13 ± 0.06	<0.001
β-Carotene	0.83 ± 0.40	0.07 ± 0.05	<0.001

P-values calculated using t-test for unpaired data.

rates. However, it has been demonstrated that ovarian stimulation also induces ROS production and perturbation in the oxidant–antioxidant balance, leading to oxidation stress, one of the most important factors that negatively affect assisted reproduction outcome (Agarwal et al., 2008b). Accordingly, previous studies have shown that serum was less protected from oxidation after an IVF cycle, showing a lower resistance to in-vitro oxidation, reduced total antioxidant capacity and decreased concentrations of hydrophilic antioxidants and vitamin E (Aurrekoetxea et al., 2010). At the same time, the GnRH antagonist protocol has been associated with increased oxidation stress as compared with the agonist protocol (Celik et al., 2012), but the authors concluded that the relation of GnRH analogues with oxidation stress and its implication in follicular growth needed to be addressed in further studies.

The present study focused on modifications of the plasma antioxidant profile during a standard agonist long protocol of ovarian stimulation in preparation for IVF/ICSI. Antioxidant concentrations were monitored at three experimental time points: at baseline before the beginning of the pharmacological treatment in preparation for assisted reproduction treatment (T0); after pituitary suppression by a GnRH agonist and immediately before the ovarian stimulation by FSH (T1); and on the day of oocyte retrieval. As far as is known, this is one of the first reports monitoring step by step the antioxidant profile in women undergoing IVF/ICSI; indeed, most studies have carried out analysis on oxidation stress parameters in plasma samples collected only on the day of oocyte retrieval (Appasamy et al., 2008; Celik et al., 2012; Velthut et al., 2013).

From these data, there is no significant modification of the basal antioxidant profile after administration of the agonist leuprolide acetate; indeed, at T1, women presented mean plasma antioxidant concentrations comparable to those at T0. At T2, there were significant decreases in ascorbic acid, α-tocopherol, lycopene and β-carotene.

The differences between T1 and T2 may be due in part to the differences between luteal and follicular phases; in fact, it has been demonstrated that serum antioxidant concentrations may be significantly lower during the follicular phase as compared with the luteal phase (Palan et al., 2006). However, by comparing serum antioxidant concentrations after gonadotrophin stimulation in women undergoing IVF with those found during the follicular phase of the natural cycle, Aurrekoetxea et al. (2010) demonstrated that gonadotrophin stimulation produced a perturbation in the oxidant–antioxidant balance leading to decreased plasma concentrations of hydrophilic antioxidants and vitamin E and to lower serum protection against oxidation. As a consequence, the decreases in plasma antioxidant concentrations seen between T1 and T2 were not simply due to the follicular phase, but also to gonadotrophin administration.

This study also evaluated the antioxidant composition of FF collected on the day of oocyte retrieval. On the basis of the aetiology of infertility, women with male factor infertility, which can be considered as healthy control subjects, presented the best follicular antioxidant profile in comparison to those with female or unexplained infertility, confirming the presence of oxidation stress and reduced antioxidant capacity in FF from women with reproductive

Table 6 Plasma and follicular antioxidant profiles on day of retrieval in women who became pregnant and those who did not.

Antioxidant	*Plasma (μmol/l)*		*Follicular fluid (nmol/g protein)*	
	Pregnant (n = *8*)	*Not pregnant* (n = *15*)	*Pregnant* (n = *8*)	*Not pregnant* (n = *15*)
Ascorbic acid	45.23 ± 6.71	38.41 ± 5.64	1307 ± 382	1112 ± 188
α-Tocopherol	20.05 ± 2.76	16.98 ± 2.96[a]	109.68 ± 21.41	92.98 ± 22.30[a]
Retinol	1.37 ± 0.16	1.43 ± 0.22	27.06 ± 5.02	32.56 ± 7.73[a]
Lutein	0.28 ± 0.13	0.24 ± 0.10	2.52 ± 0.73	2.79 ± 1.01
Lycopene	0.50 ± 0.15	0.56 ± 0.17	1.85 ± 0.72	2.04 ± 0.76
β-Carotene	0.66 ± 0.18	0.39 ± 0.13[a]	1.47 ± 0.96	1.00 ± 0.52[a]

[a] $P < 0.05$ versus pregnant (t-test for unpaired data).

diseases (Prieto et al., 2012; Velthut et al., 2013). The present study also found that follicular antioxidant concentrations were strongly correlated with corresponding plasma concentrations. These findings are in accord with previous observations indicating a positive correlation between plasma and FF total antioxidant capacity (Appasamy et al., 2008; Velthut et al., 2013), thus confirming that systemic oxidation profile could influence follicular oxidation profile.

In addition, evaluating the distribution of each antioxidant in plasma and FF revealed a significant decrease of all lipid-soluble antioxidants in FF compared with plasma, while a significant increase of water-soluble vitamin C was found. To interpret possible specific differences between plasma and FF, the transfer of these components from plasma across the blood–follicle barrier to the follicle should be taken into account. It has been demonstrated that, among lipoproteins, very-low density and low-density lipoproteins are mostly excluded from the transfer and that high-density lipoproteins are predominant in FF (Browne et al., 2008); consequently, only carotenoids and α-tocopherol associated with the high-density lipoproteins fraction are recovered from FF (Schweigert et al., 2003). The intrafollicular concentrations of some of the investigated components may also be affected by other means, such as local conversion of carotenoids with provitamin A activity (β-carotene) into vitamin A (retinol) or other selective metabolic mechanisms within the follicle itself. The high content of vitamin C in FF in the present study confirms that it is an important antioxidant system within follicles (Paszkowski and Clarke, 1999); accordingly, it has been demonstrated that the lack of vitamin C results in ovarian atrophy and extensive follicular atresia, supporting the important role that it plays against oxidation stress (Gupta et al., 2009).

Taking into account possible relationships between the antioxidant profile and IVF/ICSI outcome, this study observed that women with higher plasma concentrations of vitamin E also presented a higher number of total oocytes as well as mature oocytes retrieved per patient. These two parameters, in turn, were strictly related to pregnancy; in fact, the numbers of total and mature oocytes retrieved per patient were significantly higher in women who became pregnant than in those who did not. In this context, Aurrekoetxea et al. (2010) also found that the total number of oocytes retrieved per patient was higher in conception cycles.

Notably, comparing the plasma and follicular antioxidant profiles in pregnant and nonpregnant women revealed that the plasma and follicular concentrations of α-tocopherol as well as β-carotene were higher in women who achieved pregnancy. Together, these findings suggest a clear association between the antioxidant status and several aspects of ovarian stimulation and IVF/ICSI outcome, including pregnancy rate. In accordance with this study, previous reports have shown that follicular total antioxidant capacity is positively correlated with pregnancy rate (Bedaiwy et al., 2012; Pasqualotto et al., 2004); at the same time, a very recent study demonstrated that elevated blood plasma antioxidant status was favourable for achieving clinical pregnancy (Velthut et al., 2013). Interestingly, it has been shown that multivitamin and mineral supplementation modulates oxidation stress and antioxidant vitamin concentrations in serum and FF of women undergoing IVF (Ozkaya and Nazıroğlu, 2011; Özkaya et al., 2010), suggesting that antioxidant administration throughout ovarian induction may be a useful tool to counteract vitamin depletion by gonadotrophins, possibly favouring the achievement of clinical pregnancy. However, further controlled clinical trials are needed to confirm this hypothesis.

In conclusion, the biochemical evaluations carried out in the present study provided the following evidence: (i) in women undergoing a long agonist protocol of ovarian stimulation, there are reduced plasma antioxidant concentrations after gonadotrophin administration but not after pituitary suppression; (ii) high plasma concentration of vitamin E are associated with higher numbers of total and mature oocytes retrieved per patient, which, in turn, are favourable for achieving IVF/ICSI pregnancy; accordingly, women who became pregnant presented higher plasma concentrations of vitamin E than those who did not; and (iii) plasma and follicular antioxidant concentrations are strongly correlated; as a consequence, women who achieved pregnancy presented higher vitamin E concentrations in FF than those who did not. In short, both systemic and local antioxidant status appears to be in strict relation with assisted reproduction outcome.

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