

## Hormonal Regulation of Total Antioxidant Capacity in Seminal Plasma

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**ABSTRACT:** Infertility is associated with oxidative stress, normally counterbalanced by different antioxidant systems. In order to explore the hormonal control of seminal plasma total antioxidant capacity (TAC) we evaluated TAC and hormone patterns in a group of unselected infertile patients and control subjects. One hundred and ten infertile patients (divided into 3 groups: inflammation, varicocele, and other etiologies) and 31 fertile men were examined, evaluating blood serum gonadotropins, testosterone, estradiol, free tri-iodothyronine, free tetraiodothyronine (FT4), thyrotropin, prolactin (PRL), seminal parameters, and TAC. TAC was measured using the H<sub>2</sub>O<sub>2</sub>-metmyoglobin system, which generates the spectroscopically detectable radical cation of the chromogenous compound 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate). The "lag time" of its appearance is proportional to the antioxidant activity. Lag phase

was significantly higher in varicocele vs controls, whereas it was lower in patients with inflammation vs varicocele or other kinds of infertility. The correlation analysis between hormones and seminal parameters showed an inverse correlation between PRL and sperm motility, and a direct correlation of TAC with PRL and FT4, but not with gonadotropins or gonadal steroids. Our data suggest that systemic hormones may play a role in regulating seminal antioxidant capacity. This is interesting also because some hormones, such as thyroid and pituitary hormones, are not usually tested in the first-level evaluation of male patients with fertility problems.

Key words: Male infertility, thyroid hormones, prolactin, antioxidant capacity, oxidative stress.

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Oxidative stress has been associated with male infertility, though low levels of reactive oxygen species (ROS) are involved in physiological processes, such as capacitation and acrosomal reaction (Aitken and Krausz, 2001; Mancini et al, 2006). Seminal plasma is well endowed with an antioxidant buffer capacity, thanks to which it protects spermatozoa against ROS-induced damage implicated in sperm dysfunction. This protective system includes chain-breaking antioxidants, molecules that neutralize the oxidant radicals and stop the propagation of free-radical chain reactions.

Some studies have shown that infertile men have an impaired seminal plasma nonenzymatic antioxidant capacity (also called total antioxidant capacity [TAC]), suggesting that a decreased TAC may play a pathogenetic role in male infertility (Lewis et al, 1995). Different urogenital diseases and damage factors, such as varicocele, inflammation, and smoking, are well known to influence the balance between radical production and

antioxidant defense (Hendin et al, 1999; Pasqualotto et al, 2000; Saleh et al, 2002b).

However, it is not fully understood whether TAC is also under a systemic control, in particular by the endocrine system. We previously demonstrated that TAC is negatively correlated with follicle-stimulating hormone (FSH) levels in patients with varicocele, suggesting a role of systemic hormones in the regulation of seminal antioxidants (Meucci et al, 2003). Preliminary data also indicate a possible influence on these antioxidants by thyroid hormones (Mancini et al, 2005).

In order to verify whether a systemic endocrine control is superimposed over the local regulation of the seminal antioxidant defense system, TAC and its association with seminal parameters and serum hormone levels were evaluated in a group of infertile male patients and fertile normal subjects.

### Materials and Methods

#### Experimental Subjects

From our male infertility clinic, 110 patients, aged 26–41 years, affected with primary infertility (defined as the inability to conceive after 2 years of exposure to the risk of pregnancy),

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and 31 fertile control subjects, aged 21–43 years, were enrolled. Thirty-four patients were affected with prostatovesiculitis (inflammation), 30 with varicocele, 46 with other etiologies (29 with idiopathic oligozoospermia, 5 with past cryptorchidism, and 12 with secondary hypogonadism caused by transphenoidal operation or empty sella).

The research was approved by our Institutional Board and all subjects participated in this study after giving informed consent according to the guidelines of the Declaration of Helsinki.

None of the patients' partners had any diagnosed anatomic or physiological alterations causing infertility.

Diagnosis of varicocele was confirmed by the Doppler technique (Hirsh et al, 1980) and varicoceles were graded according to the Dubin and Amelar (1970) classification: grade 0 ( $n = 3$ ), subclinical varicocele; grade 1 ( $n = 14$ ), a distinct dilatation of the internal spermatic veins palpable during a Valsalva maneuver when upright; grade 2 ( $n = 11$ ), a palpable vein when upright with no Valsalva maneuver; grade 3 ( $n = 2$ ), a vein both palpable and visible through the scrotal skin when upright, with no Valsalva maneuver. All varicocele patients were affected by an isolated left form.

Patients with postoperative hypopituitarism were studied at least 1 month after hormone replacement therapy restored the physiological hormone levels.

### *Semen Analysis*

In all patients a standard semen analysis was performed, assessing the ejaculate volume and pH, sperm count, and percentages of sperm motility and morphology, according to World Health Organization (1999) classification. Each semen specimen was analyzed at 1 hour from collection.

Liquefied semen samples were centrifuged at  $700 \times g$  for 10 minutes in order to obtain the seminal plasma. Seminal plasma was divided in 0.5-mL aliquots, which were immediately frozen at  $-80^{\circ}\text{C}$  until TAC was assayed within 5 months. Repeated assays on a reference seminal plasma showed that sample storage under these conditions did not significantly influence the evaluated parameter.

TAC was measured using the  $\text{H}_2\text{O}_2$ -metmyoglobin system as source of radicals. They interact with the chromogenous compound 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) generating its radical cationic form ( $\text{ABTS}^+$ ), which can be spectroscopically and kinetically detected. This colorimetric assay was compared with the enhanced chemiluminescence one, which is the most commonly used method for measuring TAC in seminal plasma (Said et al, 2003). The colorimetric assay was found to be a reliable and accurate method, simpler and cheaper than the chemiluminescence one. Antioxidants induce a "lag time" in accumulation and appearance of  $\text{ABTS}^+$  that is proportional to the antioxidant concentration itself, so that TAC can be expressed as lag phase. The possible release of intracellular antioxidants from broken cells was preliminarily excluded by measuring the enzyme lactate dehydrogenase in seminal plasma specimens (Mancini et al, 1994).

### *Hormone Assays*

Free tri-iodothyronine (FT3), free tetraiodothyronine (FT4), thyrotropin (TSH), FSH, luteinizing hormone (LH), prolactin

(PRL), testosterone (T), and estradiol ( $\text{E}_2$ ) concentrations were determined at 8:00 AM in fasting basal blood serum samples. All hormones were measured by a radioimmunoassay, except LH and FSH, which were determined by an immunoradiometric assay (Radim, Pomezia, Italy). Intra-assay and interassay coefficients of variation were, respectively, 3.8% and 3.9% for FT3, 4.1% and 4.9% for FT4, 4.5% and 3.4% for TSH, 6.9% and 8.4% for FSH, 5.6% and 9.1% for LH, 2.1% and 3.1% for PRL, 6.1% and 9.3% for T, and 2.3% and 3.5% for  $\text{E}_2$ .

Normal values of the studied hormones were 2.3–4.2 pg/mL for FT3, 9.5–15.5 pg/mL for FT4, 0.35–2.80  $\mu\text{UI}/\text{mL}$  for TSH, 2.5–11 mUI/mL for FSH, 2.5–10 mUI/mL for LH, 3.5–15.5 ng/mL for PRL, 3.5–10 ng/mL for T, and 10–35 pg/mL for  $\text{E}_2$ .

### *Statistical Analysis*

Distribution of the data was evaluated by the Kolmogorov-Smirnov test. Because data were not normally distributed, a Mann-Whitney test for comparison among groups, a median test, and an analysis of Spearman rank correlation coefficient were also carried out.

A logistic regression was also performed testing hormone concentrations, lag time, and sperm density, motility, and morphology. The dependent variable was a dichotomous one (to be infertile or not, the latter as reference group). A stepwise approach (backward elimination) was applied. The goodness of fit of the model was assessed by the Hosmer and Lemeshow test.

We fixed the statistical significance level at  $P \leq .05$ . Analysis was performed using SPSS 12.0 software for Windows.

## **Results**

Seminal parameters of patients and controls are reported in Table 1. Patients were divided into 3 nosological groups: 1) inflammation, 2) varicocele, and 3) other kinds of infertility. All patient groups presented sperm density and percentages of forward progressive motility and normal morphology cells significantly lower than controls. As expected, lag phase was significantly lower in the inflammation group vs the other 2 groups, but not vs controls. However, it was significantly higher in varicocele vs controls. No difference was found among the different stages of varicocele in any parameters.

Hormone values in our patients and controls are reported in Table 2. No differences were discovered among groups, except for TSH, which, however, was always within the normal range.

The correlation analysis between hormones and seminal parameters showed an inverse correlation of PRL with both sperm density and motility, and of both LH and FSH with sperm density.  $\text{E}_2$  and sperm motility showed a nearly significant inverse correlation. The correlation analysis between lag and hormones showed a direct correlation with PRL and FT4, but not with

Table 1. Descriptive statistics of the seminal parameters, lag included, of the 3 groups of patients and controls and Mann-Whitney Test<sup>a</sup>

	Cell Density, 10 <sup>6</sup> /mL		Forward Progressive Motility, %		Normal Forms, %		Lag Phase, s	
	Mean ± SEM	Median (IR)	Mean ± SEM	Median (IR)	Mean ± SEM	Median (IR)	Mean ± SEM	Median (IR)
Inflammation	31.46 ± 6.07	15.50 (52.75–8.25)	20.34 ± 3.01	14.50 (28.50–7.87)	33.41 ± 3.35	36.50 (47.00–16.75)	108.82 ± 12.21	90.00 (120.00–70.00)
<i>P</i> <sup>b</sup>	.117		.467		.241		<.001	
<i>P</i> <sup>c</sup>	.700		.097		.837		.002	
<i>P</i> <sup>d</sup>	<.001		<.001		<.001		.438	
Varicocele	45.14 ± 7.47	33.00 (61.00–7.95)	23.48 ± 3.41	22.50 (30.50–4.00)	40.40 ± 3.37	38.00 (48.50–30.00)	150.27 ± 9.60	150.00 (180.00–100.00)
<i>P</i> <sup>b</sup>	.067		.037		.138		.486	
<i>P</i> <sup>c</sup>	.006		<.001		.002		<.001	
Other etiology	32.37 ± 5.91	16.50 (45.00–1.75)	14.17 ± 2.07	9.50 (24.25–2.75)	31.63 ± 2.80	37.50 (44.00–19.50)	143.93 ± 10.22	125.00 (170.00–101.25)
<i>P</i> <sup>d</sup>	<.001		<.001		<.001		.005	
Controls	72.64 ± 7.47	68.00 (98.50–38.00)	47.03 ± 3.29	52.00 (60.00–35.00)	55.00 ± 3.31	52.00 (70.00–37.00)	101.00 ± 5.45	100.00 (125.00–80.00)

Abbreviation: IR, interquartile range.

<sup>a</sup> Significant *P* values in bold.

<sup>b</sup> Vs varicocele.

<sup>c</sup> Vs others.

<sup>d</sup> Vs controls.

Table 2. Descriptive statistics and median tests of the hormone panel of the 3 groups of patients and controls

	Inflammation		Varicocele		Other Etiology		Controls	
	Mean ± SEM	Median (IR)	Mean ± SEM	Median (IR)	Mean ± SEM	Median (IR)	Mean ± SEM	Median (IR)
T (ng/mL)	4.18 ± 0.26	3.91 (5.34–3.23)	5.36 ± 0.59	4.62 (7.09–3.75)	4.68 ± 0.79	3.76 (5.70–2.79)	6.14 ± 0.72	5.87 (8.43–4.05)
<i>P</i>								
E <sub>2</sub> (pg/mL)	27.79 ± 3.40	24.00 (33.00–19.66)	29.21 ± 6.42	26.00 (33.50–13.90)	29.38 ± 2.18	29.00 (33.20–23.75)	42.18 ± 8.70	26.00 (76.00–21.00)
<i>P</i>								
FSH (mIU/mL)	6.08 ± 1.05	4.20 (7.50–2.60)	4.71 ± 0.80	3.90 (6.41–2.45)	7.08 ± 1.02	5.20 (8.52–2.70)	4.09 ± 0.89	3.25 (6.83–0.88)
<i>P</i>								
LH (mIU/mL)	4.18 ± 0.60	3.00 (5.47–1.96)	4.30 ± 0.59	3.90 (5.60–2.50)	4.14 ± 0.59	3.19 (5.92–1.90)	3.39 ± 0.68	2.90 (5.15–1.60)
<i>P</i>								
PRL (ng/mL)	8.52 ± 0.76	7.60 (10.20–5.13)	7.07 ± 0.69	6.50 (8.75–5.70)	9.25 ± 1.01	9.08 (10.52–5.85)	9.93 ± 1.17	10.40 (13.98–5.85)
<i>P</i>								
FT3 (pg/mL)	3.50 ± 0.11	3.40 (3.85–3.10)	4.80 ± 0.70	3.60 (4.42–3.10)	3.81 ± 0.39	3.50 (4.02–2.85)	2.89 ± 0.19	2.80 (3.60–2.22)
<i>P</i>								
FT4 (pg/mL)	10.38 ± 0.69	11.70 (121.45–10.00)	11.78 ± 0.69	12.45 (14.17–11.50)	13.47 ± 1.51	12.60 (13.42–11.47)	11.85 ± 1.29	12.90 (14.15–11.40)
<i>P</i>								
TSH (μIU/mL)	2.27 ± 0.44	1.60 (2.19–1.21)	1.54 ± 0.11	1.37 (1.85–1.19)	1.87 ± 0.18	1.75 (2.42–0.93)	2.03 ± 1.07	0.88 (1.39–0.22)
<i>P</i>								

Abbreviations: E<sub>2</sub>, estradiol; FSH, follicle-stimulating hormone; FT3, free triiodothyronine; FT4, free tetraiodothyronine; IR, interquartile range; LH, luteinizing hormone; PRL, prolactin; T, testosterone; TSH, thyrotropin.

Table 3. Spearman correlation coefficients<sup>a</sup>

		Cell Density	Forward Progressive Motility	Normal Forms	Lag Phase
T	<i>r<sub>s</sub></i>	0.07	0.04	0.09	-0.04
	<i>P</i>	.556	.706	.457	.757
E <sub>2</sub>	<i>r<sub>s</sub></i>	0.68	-0.23	0.00	-0.01
	<i>P</i>	.565	.054	.970	.936
FSH	<i>r<sub>s</sub></i>	-0.31	0.02	-0.11	0.06
	<i>P</i>	<b>.007</b>	.895	.337	.604
LH	<i>r<sub>s</sub></i>	-0.31	-0.03	-0.12	0.12
	<i>P</i>	<b>.007</b>	.791	.292	.301
PRL	<i>r<sub>s</sub></i>	-0.27	-0.28	-0.19	0.39
	<i>P</i>	<b>.018</b>	<b>.017</b>	.101	<b>.001</b>
FT3	<i>r<sub>s</sub></i>	-0.08	-0.04	-0.15	0.12
	<i>P</i>	.396	.708	.118	.219
FT4	<i>r<sub>s</sub></i>	-0.08	0.04	-0.12	0.28
	<i>P</i>	.422	.714	.214	<b>.004</b>
TSH	<i>r<sub>s</sub></i>	-0.11	0.00	-0.08	0.12
	<i>P</i>	.278	.999	.433	.200

Abbreviations: E<sub>2</sub>, estradiol; FSH, follicle-stimulating hormone; FT3, free tri-iodothyronine; FT4, free tetraiodothyronine; LH, luteinizing hormone; PRL, prolactin; T, testosterone; TSH, thyrotropin.

<sup>a</sup> Significant *P* values in bold.

gonadotropins or gonadal steroids (Table 3). A graphical representation of the significant correlations is presented in the Figure.

The multivariate regression model (logistic regression) found the following variables significantly associated with infertility: forward progressive motility (odds ratio [OR] 0.91; 95% confidence interval [CI], 0.88–0.95; *P* < .001) and lag phase (OR 1.02; IC 95%: 1.00–1.03; *P* = .041). The results of univariate and multivariate analysis are shown in Table 4.

## Discussion

The role of antioxidants as protective for sperm survival and function is well known. Nevertheless, there is no definite knowledge on the role of systemic hormones in the regulation of seminal antioxidants. We had previously shown a significant inverse correlation between FSH and TAC in varicocele patients (Meucci et al, 2003). In this paper we focused our attention on various hormones, besides gonadotropins, in an unselected population of infertile male patients with physiological hormone levels. We did not study parameters such as nuclear DNA damage and polymorphism of the mitochondrial DNA polymerase gamma gene (*POLG*), recently associated with unexplained infertility (Saleh et al, 2002a; Jensen et al, 2004), because our main objective was just to study biochemical alterations linked to endocrine control.

We did not find correlations between lag phase and gonadotropins or sexual hormones, even if some correlations were discovered between these hormones and

seminal parameters, probably because of a direct effect not mediated by the antioxidant systems. The inverse correlation between gonadotropins and sperm density in a physiological hormone range might express a compensatory feedback mechanism. As far as T was concerned, we recently showed that male hypogonadism is accompanied by low TAC levels in blood serum, which are corrected by T administration (Mancini et al, 2008). However, this study concerned blood, not seminal fluid, and pathological hormone values. At a physiological level, no correlation with the seminal plasma TAC was detectable in this study, possibly suggesting that a threshold effect could be present. On the contrary, a significant correlation was found between PRL and FT4 on the one hand and lag on the other.

The physiological role of PRL in male fertility is not yet well known, whereas a detrimental effect of hyperprolactinemia is clear (Luciano 1999; Aleem et al, 2005). Hyperprolactinemia affects seminal fluid through different mechanisms, causing spermiogenic arrest and impairing sperm motility, with cytological findings of sperm cells similar to those of prepubertal testis (Segal et al, 1979). But contrasting results concern the effects of pharmacological treatment of hyperprolactinemia. The lack of significant changes in seminal fluid parameters during bromocriptine therapy was reported (Thorner et al, 1974). Another study found a significant increase in the number, total and rapid-progressive motility, and normal morphology of spermatozoa after 6 months of treatment with both cabergoline and bromocriptine (De Rosa et al, 1998). PRL receptors are present in all stages of the cycle of the seminiferous epithelium, Leydig cells, and Sertoli cells in male rats (Hondo et al, 1995). PRL acts synergistically with LH and T and regulates the conversion of T precursors (Bartke, 1971; Hafiez et al, 1972). A more relevant datum for our study is the positive effect of PRL on the epididymis, which in turn plays a role in the defense against oxidative stress (Robaire and Hermo, 1998). The epididymis is the place of synthesis of carnitine, the positive effects of which have been also shown in pharmacological studies (Lenzi et al, 2004). Nonetheless, a prolonged time spent in this conduct has been related to infertility (Johnson and Varver, 1988). Specific PRL-binding proteins (receptors) have been found in homogenized epididymides of rats (Aragona and Friesen, 1973) and rabbits (Orgebin-Crist and Djiane, 1979). In this site PRL receptors had a concentration higher than in any other tissue of the male reproductive tract in rabbits, but among the lowest in rats. The possible coregulation of the release of gonadotropins and PRL, the presence of the above mentioned receptors, and the proposed role of PRL in ion transport (Shiu and Friesen, 1980) seem to suggest a major role of

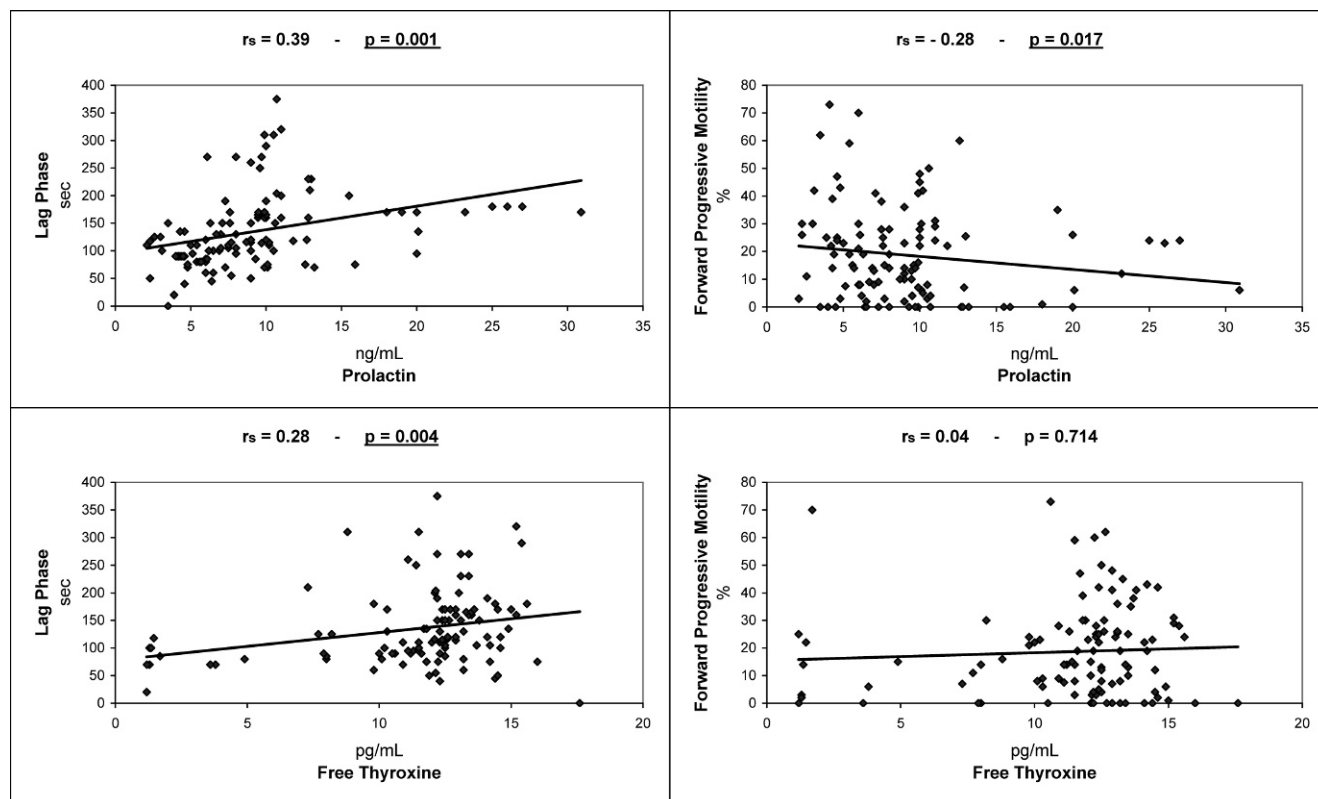


Figure. Graphical representation (scattered plot) of linear regression analysis between free tetraiodothyronine (FT4) or prolactin (PRL) and forward progressive motility or lag phase.

the hormone in the control of some epididymal physiological functions (Robaire and Hermo, 1998).

An interesting new finding of the present paper is the inverse correlation between PRL and sperm motility,

Table 4. Results of univariate and multivariate analysis<sup>a</sup>

	OR Crude (95% CI)	OR Adjusted (95% CI) <sup>b</sup>
Cell density	<b>0.98 (0.97–0.99)</b>	
Forward progressive motility	<b>0.93 (0.90–0.95)</b>	<b>0.91 (0.88–0.95)</b>
Normal forms	<b>0.94 (0.91–0.97)</b>	
Lag phase	<b>1.01 (1.00–1.02)</b>	<b>1.02 (1.00–1.03)</b>
T	0.91 (0.78–1.05)	
E <sub>2</sub>	0.97 (0.95–1.00)	
FSH	1.14 (0.95–1.36)	
LH	1.11 (0.89–1.39)	
PRL	0.95 (0.85–1.06)	
FT3	<b>2.76 (1.24–6.15)</b>	
FT4	1.01 (0.92–1.10)	
TSH	0.97 (0.75–1.26)	

Abbreviations: CI, confidence interval; E<sub>2</sub>, estradiol; FSH, follicle-stimulating hormone; FT3, free tri-iodothyronine; FT4, free tetraiodothyronine; LH, luteinizing hormone; OR, odds ratio; PRL, prolactin; T, testosterone; TSH, thyrotropin.

<sup>a</sup> Significant results in bold.

<sup>b</sup> Adjusted according to multivariable model.

which might seem contradictory, because PRL correlates positively with lag phase. We can hypothesize that an increase in antioxidant systems is compensatory to the increase in oxidative stress, in accordance with the idea formulated in other conditions (Elsayed, 2001; Comhair and Erzurum, 2002). Another hypothesis, not excluding the former, is that augmented lag can express a reduced utilization of the antioxidants, as better discussed below. In fact, this datum is also consistent with the finding of high lag values in patients with varicocele or other kinds of infertility.

Regarding thyroid hormones, previous studies have shown that both hyperthyroidism and hypothyroidism are associated with enhanced oxidative stress involving enzymatic and nonenzymatic antioxidants (Resch et al, 2002). Besides, some complications of hyperthyroidism are caused just by the oxidative stress in target tissues (Asayama and Kato, 1990). Thyroid hormones per se can act as oxidants and produce DNA damage (contrasted by catalase), probably through the phenolic group, similar to that of steroidal estrogens (Dobrzynska et al, 2004). Many other mechanisms, reviewed by Venditti and Di Meo (2006), can be involved: enhanced nitric oxide synthase gene expression with nitric oxide overproduction; activation of hepatic

nuclear factor kappa B and following increase of cytokines stimulating ROS generation; uncoupling mechanisms involving uncoupling protein (UCP)-2 and UCP-3, regulated by thyroid hormones; increased turnover of mitochondrial proteins; mitoptosis, regulated by peroxisome proliferator-activated receptor gamma coactivator-1, which is up-regulated by T3 administration. Thyroid hormones influence lipid composition of rat tissues (Hoch, 1988) and therefore the susceptibility to oxidative stress. At a systemic level, also in humans, hyperthyroidism has been associated with reduced circulating levels of alpha-tocopherol (Ademoglu et al, 1998; Bianchi et al, 1990) and coenzyme Q<sub>10</sub> (Bianchi et al, 1990; Mancini et al, 1991).

However, there is a specificity in tissue response, and discrepant effects of T3 and T4 are possible. In rat liver, T3-induced hyperthyroidism was found to be associated with altered lipid-peroxidation indices, including elevated levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (Fernandez et al, 1985; Venditti et al, 1997, 1999; Huh et al, 1998). On the contrary, no change in TBARS was found in homogenized livers from rats made hyperthyroid by administration of T4 over a 4-week period (Asayama et al, 1987). As regards testis, no significant change (TBARS or hydroperoxides) was observed in lipid peroxidation of hyperthyroid adult rats, but hyperthyroidism promoted protein oxidation rate as indicated by an enhanced content of protein-bound carbonyls (Choudhury et al, 2003). In conclusion, we should emphasize the fact of a tissue-linked variability in the effects of hyperthyroidism on the activity of antioxidant enzymes (Mn-superoxide dismutase [SOD] or Cu,Zn-SOD, catalase, glutathione-peroxidase) with differential effects of the 2 thyroid hormones (Venditti and Di Meo, 2006).

All the referred studies did not explore physiological hormone levels, but pharmacological manipulations of the thyroid status. Therefore, our results become even more meaningful by showing a correlation between FT4 and lag in this context.

Varicocele represents a different particular model when considering TAC modulation. In previous studies we showed a significant inverse correlation between FSH levels and seminal plasma TAC in varicocele patients (Meucci et al, 2003; Mancini et al, 2007). Furthermore, varicocele exhibited higher lag values, although it is well known to be a condition of increased oxidative stress. This feature was confirmed in the present study and accounted for the weak but significant OR between lag phase and infertility. A possible reason is an ineffective utilization of the antioxidant systems, though a compensatory increase in their level, in varicocele.

In conclusion, even if our results are based on statistical inferences, systemic hormones seem to pro-

duce a modulatory effect on the local regulation of the balance between oxidant species and antioxidant systems, which is obviously affected by urogenital diseases. In fact, our data indicate that systemic hormones can have a complex integrated action in regulating seminal antioxidant capacity, including hormones, such as thyroid and pituitary hormones, which are not usually tested in the first-level evaluation of an infertile male patient.

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