Seminal Fluid ROS-Buffering Capacity Relates to Sperm Parameters, Chromatin Integrity, and Embryo Developmental Competence

Laura Park, Queenie V. Neri, Zev Rosenwaks, and Gianpiero D. Palermo

Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, New York, USA

Abstract

Study question: We question whether seminal plasma's total antioxidant capacity (TAC) has any effect on spermatozoa parameters, predicts male genome integrity, or impacts the ability of the male gamete to participate in embryo development. Therefore we wonder whether TAC measurement may aid in the prognostication of male factor infertility.

Summary answer: TAC decreases by lengthening the abstinence period, but increases with sperm concentration, motility, and morphology. TAC protects the sperm's chromatin's, as inferred by mean sperm DFI. Most importantly, a compromised TAC in the inseminating specimen was accompanied by a lower chance of pregnancy.

What is known already: The use of seminal biomarkers to predict gamete health and embryo developmental competence is under investigation. During spermiogenesis and throughout the genital tract, reactive oxidative species (ROS) are generated from developing, maturing, and decaying spermatozoa. The natural buffering capacity of seminal plasma reduces the effect of oxidative insults and sustains spermatozoa motility, preserving their competence. Direct and indirect assays to measure ROS are currently being validated to screen male factor infertility.

Study design, size, duration: Prospectively during the last 5 months, we assessed TAC in ejaculates of 65 men. We plotted TAC against semen parameters and sperm DNA fragmentation index (DFI). For subjects that underwent IUI and ART with their female partners, the relationship between TAC and clinical outcome was investigated.

Participants/materials, setting, methods: TAC, in Trolox equivalents was assessed by a colormetric assay on an automated microplate reader (ΔOD_{405}). Direct ROS damage to sperm DNA was evaluated by TUNEL. For most of these men, their reproductive outcomes with IUI and ART were evaluated in relation to the TAC of the inseminating specimen.

Main results and the role of chance: In 65 men (39.7±8yrs), sperm concentration was 35.9±25x10^6/ml, motility 42.7±14%, and morphology 2.4±1%. The TAC for normozoospermic men (*n*=34) was 1965.9±212nmol/ml and for oligoasthenozoospermic (*n*=12), 1720.2±264.3nmol/ml (*P*=0.01). TAC inversely correlated to abstinence (*P*=0.01) while positively with semen concentration, motility, and morphology (*P*<0.05). In 13 subjects, a lower TAC was associated with compromised sperm DFI (*P*<0.001). In 22 couples (maternal age 35.5±5yrs) treated in 41 IUI cycles, clinical pregnancies of 17.1% (n=7) were accompanied by a TAC of 2001.0±133nmol/ml. This was higher than in couples whose treatments did not result in pregnancy (n=15; 1783.0±195.6nmol/ml) (*P*<0.01). Five couples (maternal age 37.7±5years) who failed IUI, along with 17 others with a suboptimal TAC (1,741.6±296nmol/ml) were treated in 32 ICSI cycles, resulting in a clinical pregnancy rate of 25.0%.

Limitations, reasons for caution: Even though the benefit of a higher antioxidant capacity exerted by the seminal plasma has an undisputed benefit on the health and performance of the male gamete, this biomarker is not yet validated as a valuable screening assay for male gamete performance.

Wider implications of the findings: The limitations of the semen analysis to screen for male factor infertility are apparent and additional biomarkers are needed to acquire further insight on spermatozoal function and its structural integrity. Seminal TAC, an indirect measurement of ROS, provides information on sperm DNA integrity and may help predict IUI outcomes. TAC level in the ejaculate improves male factor infertility screening and helps steer the infertile couple toward the proper insemination method.

Study funding/competing interest(s): Reproductive Medicine, Weill Cornell Medical College

Trial registration number: N/A

Background

Spermatozoa spontaneously produce a variety of reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide, and nitric oxide. Produced in small amounts, the ROS are functionally important in driving the tyrosine phosphorylation cascades associated with sperm capacitation. However, when the ROS production exceeds the seminal plasma's antioxidant defenses, the excessive oxidative environment may induce damage on the sperm plasma membrane and DNA strand breakage. This oxidative stress not only disrupts the fertilizing potential of human spermatozoa but also the ability to create normal healthy embryos.

We tested the feasibility of utilizing an inexpensive assay to measure the antioxidant capacity of seminal plasma in men screened for infertility at our Center and the significance of this assay as an add-on to a standard semen analysis on gathering information on spermatozoa integrity as well as their reproductive potential.

Methods

Patients undergoing male infertility screening were included in the study (IRB 1006101085). After complete liquefaction, semen parameters such as abstinence, volume, concentration, motility, and morphology were assessed to the 2010 WHO criteria. The specimens were then centrifuged at 3,000*g* for 10 minutes and the cell-void seminal plasma were aliquoted and cryopreserved at -80ºC until the time of TAC assay. To measure the effect of oxygen-free radicals on spermatozoal DNA integrity, DNA fragmentation was assessed.

Antioxidant assay: Total antioxidant capacity was assessed using a commercial kit (Antioxidant Assay Kit, Cayman Chemical). Seminal fluid was diluted 1:10 with assay buffer before assessment. After the preparation of the Trolox standards and reagents according to the manufacturer's instructions, 10µl of the Trolox standards and 10ul of the seminal plasma was loaded into each individual well in triplicate on a 96-well microplate. Then, 10µl metmyoglobin and 150µl chromogen was added to all Trolox and sample wells. To initiate the reaction, 40µl of hydrogen peroxide was quickly added to all wells and the microplate was incubated for 5 minutes at room temperature. The optical density was measured at ΔOD_{405} using a computerized automated microplate reader. Finally, the antioxidant concentration of the sample was calculated by substituting the average absorbance values for each sample into the equation obtained from the linear regression of the standard curve:

$$\text{Antioxidant}\left[\frac{\text{nmol}}{\text{ml}}\right] = \frac{\text{Average absorbance 405nm for unknown} - \text{Y intercept}}{\text{slope}} \times \text{dilution} \times 1000$$

A threshold of 1800nmol/ml TAC was adopted.

Sperm DNA fragmentation assessment: The integrity of the paternal genome was measured using terminal deoxynucleotidyl transferase dUTP NICK end labeling (TUNEL). Spermatozoa were smeared and fixed on a slide. Samples were then permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate and incubated with TdT and BrdUTP for the detection of DNA breaks. The detection of BrdU incorporation was achieved through an Alexa Fluor 488 dye-labeled anti-BrdU antibody (APO-BrdUTM TUNEL Assay Kit). At least 500 spermatozoa were counted and the proportion with fragmentation was calculated.

Reproductive potential: To measure the ability of spermatozoa to fertilize and participate in embryo development, we assessed the reproductive performance of couples undergoing IUI and ICSI in relation to TAC. Clinical pregnancies were considered positive when at least one fetal heartbeat was detected on their 7th week ultrasound.

Results

A total of 139 men whose average age, sperm characteristics, and average seminal TAC are shown below (Table 1). There was no significant relationship observed between TAC and abstinence period. However, when we plotted TAC against sperm concentration (*P* = 0.04), motility (*P* = 0.008), and morphology (*P* = 0.04), a positive correlation was identified between TAC and all three parameters (Figure 1). In addition, seminal TAC decreased with increasing male age (*P* = 0.01; Figure 2). While TAC for normozoospermic men (*n* = 46) averaged 1904.3 ± 228.6 nmol/ml, for the oligoasthenozoospermic counterpart (*n* = 21) TAC was only 1741.3 ± 273.1 nmol/ml (*P* = 0.02).

Table 1. Sperm characteristics and TAC concentration

Male Patients	139
Male Age (M yrs ± SD)	38.2 ± 7.5
Abstinence (M days ± SD)	3.9 ± 1.7
Seminal Fluid Volume (M ml ± SD)	3.1 ± 1.4
Sperm Concentration (M x10^6/ml ± SD)	25.8 ± 29.3
Motility (M% ± SD)	39.8 ± 14.4
Morphology (M% ± SD)	2.3 ± 1.0
Seminal TAC (M nmol/ml ± SD)	1829.7 ± 277.3

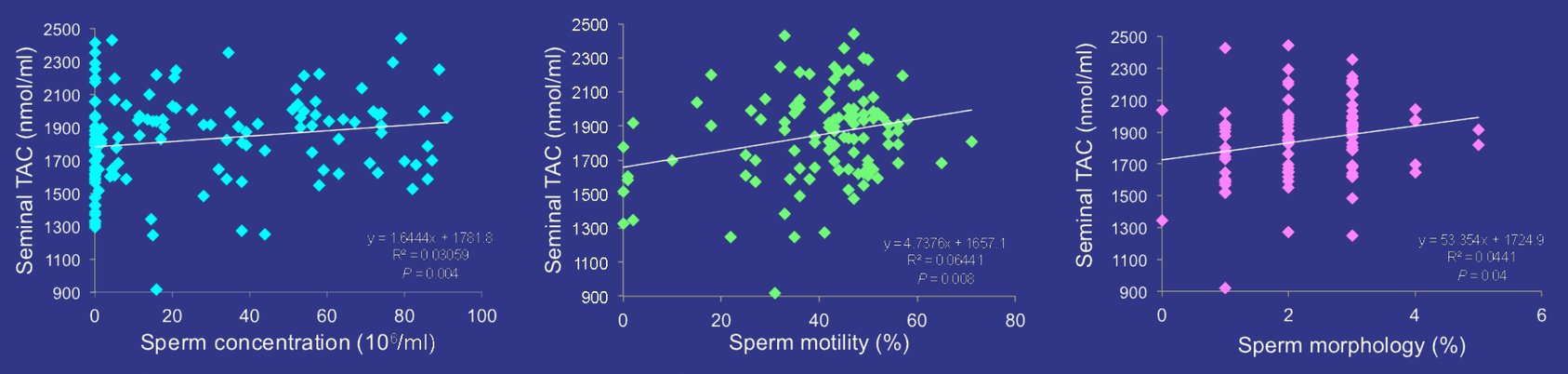

Figure 1. Semen characteristics according to seminal TAC concentration

Results (cont'd)

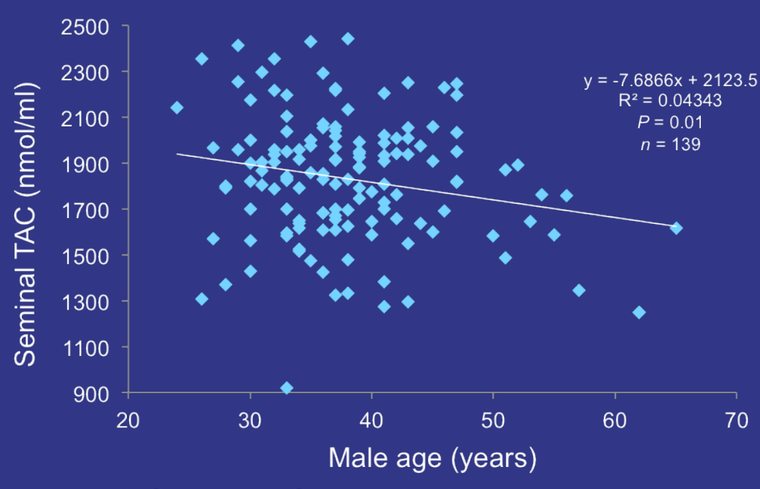

Figure 2. Seminal TAC and patient age

A group of 43 men were treated in 107 IUI cycles with their female partner, reporting a clinical pregnancy rate of 14.0% (Table 2).

Table 1. Sperm characteristics and TAC concentration

Patients	43
IUI Cycles	107
Male Age (M yrs ± SD)	37.4 ± 5.2
Female Age (M yrs ± SD)	35.8 ± 4.5
Sperm concentration (M x10^6/ml ± SD)	42.0 ± 25.8
Motility (M% ± SD)	42.5 ± 13.0
Total Motile Count (M x10^6 ± SD)	52.8 ± 40.9
Morphology (M% ± SD)	2.5 ± 1.1
Seminal TAC (M nmol/ml ± SD)	1859.7 ± 189.4

When we compared IUI clinical pregnancy outcome of these men in relation to their seminal TAC, patients that had a compromised TAC (*n* = 16) exhibited a significantly lower pregnancy rate than patients that had a TAC above the 1800nmol/ml threshold (*n* = 27) (Figure 3).

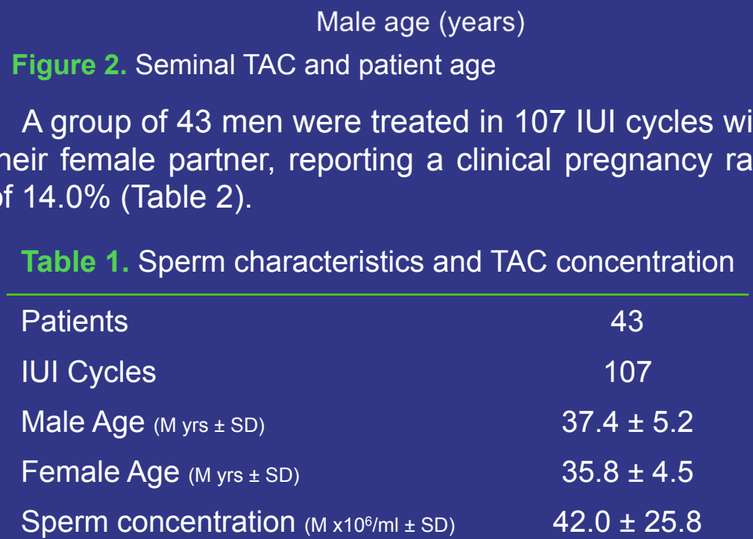

Figure 3. IUI pregnancy rates according to TAC level

In a subgroup of patients, a lower TAC was capable of predicting a higher sperm DFI (Figure 4).

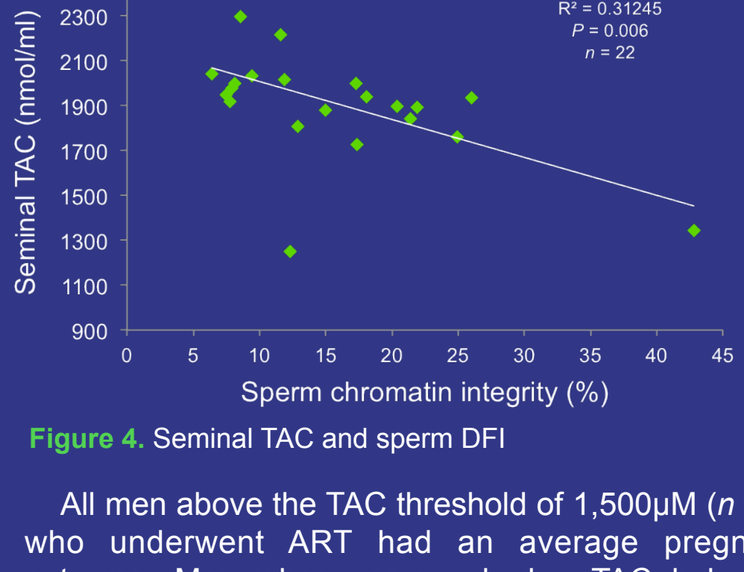

Figure 4. Seminal TAC and sperm DFI

All men above the TAC threshold of 1,500µM (*n* = 21) who underwent ART had an average pregnancy outcome. Men whose semen had a TAC below the threshold (*n* = 24) appear to repeatedly fail IUI cycles (3-5 cycles) and 5 of them were only able to achieve a pregnancy following ICSI.

Figure 5. Seminal TAC and ART outcome

Conclusions

The limited nature of a standard semen analysis to screen male patients is becoming more apparent. Supplemental assays are being implemented to gain information regarding spermatozoal function and structural integrity. In this work we saw a direct correlation between antioxidant capacity of seminal plasma and spermatozoa concentration and motility. Men with TAC below threshold presented with several IUI failure and particularly those with severely compromised TAC appear to attain a pregnancy only when the most invasive ART procedure is used. The implementation of this inexpensive and non-labor intensive assay may provide information on the environment and conditions of the male gamete and may serve as an add-on to a standard semen analysis.