Glutathione peroxidase activity in seminal plasma and its relationship to classical sperm parameters and in vitro fertilization-intracytoplasmic sperm injection outcome

Lorena Crisol, B.S.,[a,b] Roberto Matorras, M.D., Ph.D.,[a] Fermín Aspichueta, B.S.,[a,b] Antonia Expósito, Ph.D.,[a] Maria Luisa Hernández, Ph.D.,[b] Maria Begoña Ruiz-Larrea, Ph.D.,[b] Rosario Mendoza, B.S.,[a] and José Ignacio Ruiz-Sanz, Ph.D.[b]

[a] Human Reproduction Unit, Department of Obstetrics and Gynecology, Hospital de Cruces, University of the Basque Country, and [b] Department of Human Physiology, University of the Basque Country, Vizcaya, Spain

Objective: To relate the glutathione peroxidase (GPX) activity level in human seminal plasma with standard semen parameters and spermatozoa fertilization potential in terms of fertilization and pregnancy rates in an IVF program.
Design: Prospective study.
Setting: Human Reproduction Unit at Cruces Hospital (Vizcaya, Spain).
Patient(s): Three hundred consecutive males from infertile couples participating in the IVF program.
Intervention(s): None.
Main Outcome Measure(s): Analysis of GPX activity in seminal plasma by spectrophotometry.
Result(s): GPX activity in seminal plasma was significantly lower in patients with abnormal sperm as assessed by 1999 and 2010 World Health Organization (WHO) criteria, compared with normozoospermic individuals. There was a more significant decrease in those samples with severe sperm pathologies. GPX values were significantly lower in samples with severe asthenozoospermia, oligozoospermia, and teratozoospermia compared with normal samples. However, there was no correlation between GPX activity in seminal plasma in IVF patients and fertilization rates or pregnancy outcome.
Conclusion(s): Although seminal plasma GPX activity was related to semen quality according to WHO parameters, such an association was not found with IVF–intracytoplasmic sperm injection (ICSI) outcome, presumably because of the well-known ability of IVF-ICSI procedures to overcome sperm deficiencies in the fertilization process. (Fertil Steril® 2012;97:852–7. ©2012 by American Society for Reproductive Medicine.)
Key Words: Glutathione peroxidase (GPX), semen parameters, in vitro fertilization, fertilization rate, pregnancy rate

During cellular metabolism, active forms of oxygen metabolites and peroxidized molecules, called reactive oxygen species (ROS), are generated. These molecules are strong oxidants that may cause severe cell injury, leading to the free-radical cell destruction (1). Some evidence has shown the pivotal role of ROS in sperm dysfunction and male factor infertility, causing lipid peroxidation as well as DNA and protein injury (2–4). However, besides their noxious effects, a small, controlled amount of ROS is responsible for the physiological control of certain processes in sperm such as maturation and capacitation, acrosome reaction, and oocyte binding (5). It is therefore necessary to be a balance between ROS production and elimination around spermatozoa. Something similar has been recently described in women undergoing IVF, suggesting a role for ROS in conception (6).

Oxidative damage in the male reproductive tract is controlled by various antioxidant molecules and enzymatic systems within spermatozoa seminal plasma and secreted along the entire reproductive tract. One of the most important systems governing the elimination of ROS is the glutathione peroxidase-glutathione reductase system. Glutathione peroxidase (GPX) catalyses the reduction of organic and inorganic hydroperoxides using reduced glutathione as an electron donor. The GPX family is constituted by several proteins classified according to their sequence, substrate specificity, and subcellular localization (7, 8).

Received September 27, 2011; revised and accepted January 11, 2012; published online January 31, 2012.
L.C. has nothing to disclose. R.M. has nothing to disclose. F.A. has nothing to disclose. A.E. has nothing to disclose. M.L.H. has nothing to disclose. M.B.R.-L. has nothing to disclose. R.M. has nothing to disclose. J.I.R.-S. has nothing to disclose.
Reprint requests: Lorena Crisol, B.S., Hospital de Cruces, Plaza de Cruces s/n 48903, Cruces, Barakaldo, Spain (E-mail: lorena.crisolortiz@osakidetza.net).

Fertility and Sterility® Vol. 97, No. 4, April 2012 0015-0282/$36.00

doi:10.1016/j.fertnstert.2012.01.097

Several studies have demonstrated the importance of the GPX family during spermatogenesis and its relationship with male factor fertility (9–13). Likewise, in vitro experiments have shown the pivotal role of GPX activity against lipid peroxidation in human spermatozoa (14).

The World Health Organization (WHO) standard for semen analysis does not provide complete diagnostic information and is inadequate to determine the underlying cause of infertility, so many men with normal WHO parameters will actually be infertile. Owing to the involvement of ROS in sperm pathologies and the value of seminal plasma enzymatic ROS scavengers, GPX activity in seminal plasma could be a useful biomarker for male factor infertility. The present study was designed to establish whether there is an association between GPX activity in seminal plasma and WHO semen parameters or the outcomes of IVF in terms of fertilization and pregnancy rates.

MATERIALS AND METHODS

Seminal Samples

Semen samples were obtained from 300 consecutive males from infertile couples participating in the IVF/intracytoplasmic sperm injection (ICSI) program of the Human Reproduction Unit at Cruces Hospital (Vizcaya, Spain) for either male or female factor infertility. The inclusion criteria were duration of infertility of at least 12 months and female age less than 40, as well as the use of the patient's own oocytes in fresh embryo cycles. Azoospermic men were excluded from the study.

The study was approved by the Institutional Review Board of Cruces Hospital, and all participants gave written informed consent for the research.

Before the implementation of the assisted reproduction technique, semen samples were collected in sterile containers by masturbation after 3–5 days of sexual abstinence. After liquefaction of the semen at room temperature (22°C) for 30 minutes, the samples were assessed in accordance with WHO guidelines (15). The variables taken into consideration were ejaculate volume (in milliliters), sperm concentration ($n \times 10^6$/mL), progressive motility (a+b, %), and morphology (normal forms, %). Sperm morphology was studied using Kruger's strict criteria (16). Subsequently, the samples were also classified according to 2010 WHO semen parameters (normozoospermia: $\geq$15/million/mL, $\geq$32% progressive motility, and $\geq$4% normal forms) (17). All the sperm samples were analyzed by the same biologist (L.C.).

An aliquot of each sample was collected to obtain sperm-free seminal plasma by centrifugation at 400 *g* for 10 minutes and then frozen at −80°C until enzyme analysis.

The results of the sperm swim-up procedure (sperm concentration and motility) were also recorded for the study.

IVF Cycle Management

Our IVF management has been described elsewhere (18). Briefly, it consists of down-regulation with a GnRH analogue, triptorelin acetate (Decapeptyl, Ipsen) on a long protocol, ovarian stimulation with recombinant FSH (Gonal F, Merck Serono), and highly purified urinary menopausal gonadotropins (Menopur, Ferring), with ovulation being triggered with 250 μg Ovitrelle (Serono). Transvaginal ultrasound-guided oocyte retrieval was scheduled 36 hours after hCG injection. The luteal phase was supplemented with micronized progesterone (Utrogestan, Laboratorios Seid), vaginally 200 mg/12 hours.

Concerning the choice of insemination technique, ICSI was performed in cases with [1] less than 5 million motile sperm recovered after swim-up, [2] low rates of fertilization (<30%) in a previous IVF cycle, and/or [3] previous IUI failures. In the remaining cases, conventional IVF was used. In borderline cases with 10 or more oocytes, both IVF and ICSI were carried out. Overall, ICSI was performed in 181 cases (60.3%), IVF in 71 (23.6%), and IVF-ICSI in 48 cases (16%).

Since GPX analysis was performed after the IVF procedure, the selection of insemination technique was not influenced by the GPX results.

The fertilization rate (FR) was defined as the number of fertilized oocytes with two well formed male and female pronuclei/total oocytes inseminated. Pregnancy was defined as the visualization of the gestational sac by vaginal ultrasound 3–4 weeks after ET.

GPX Activity Measurement

GPX activity in seminal plasma was determined spectrophotometrically by an adaptation to microreader well plates of the method described by Flohe and Gunzler (19). Briefly, each well contained 170 μL of the reaction mixture consisting of phosphate buffer 50 mM, pH = 7, GSH 0.45 mM, NADPH 0.2 mM, sodium azide 1.5 mM, and 0.45 units of glutathione reductase (Sigma) and 30 μL of thawed sample. The reaction started by the addition of 25 μL of 0.72 mM cumene hydroperoxide. The absorbance change was continuously registered at 340 nm and 30°C for 15 minutes. GPX catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, oxidized glutathione is immediately converted into the reduced form with concomitant oxidation of NADPH to $NADP^+$. This oxidation process is associated with a decrease in absorbance at 340 nm, allowing GPX activity to be monitored spectrophotometrically.

GPX activity was expressed as mU/mL seminal plasma. One enzymatic unit was defined as 1 μmol of oxidized NADPH per minute at 30°C by the glutathione reductase-linked kinetic.

Statistical Analysis

Data were analysed using the SPSS 18.0 statistical package. Analysis of variance, Student's *t*-tests, and Pearson correlations were used to evaluate the relationships between GPX activity and semen parameters and in a second step to determine whether GPX activity was associated with pregnancy and FR. Significance was defined as $P<.05$.

For the analysis of the relationship between GPX and sperm parameters, sample size was calculated as follows: accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, assuming a GPX mean value of 20 mU/mL and an SD of 5 (based on our previous unpublished experience) in the normal population, 99 subjects are necessary in the study group and 99 in the control group to recognize as statistically