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Human semen as an early, sensitive biomarker of highly polluted living environment in healthy men: A pilot biomonitoring study on trace elements in blood and semen and their relationship with sperm quality and RedOx status

Paolo Bergamo[a], Maria Grazia Volpe[a], Stefano Lorenzetti[b], Alberto Mantovani[b], Tiziana Notari[c], Ennio Cocca[d], Stefano Cerullo[a], Michele Di Stasio[a], Pellegrino Cerino[e], Luigi Montano[f,*]

[a] Institute of Food Sciences, National Research Council (CNR-ISA), via Roma 64, 83100 Avellino, Italy
[b] Istituto Superiore di Sanità (ISS), Dept. of Food Safety and Veterinary Public Health, viale Regina Elena 299, 00161 Rome, Italy
[c] Infertility Center of the "San Luca" Hospital, Local Health Authority (ASL) Salerno, via Francesco Cammarota, Vallo della Lucania (SA), Italy
[d] Institute of Biosciences and Bioresources, National Research Council (CNR-IBBR), Via Pietro Castellino 111, 80131 Naples, Italy
[e] Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM), via della Salute 2, 80055 Portici (NA), Italy
[f] Andrology Unit of the "San Francesco d'Assisi" Hospital, Local Health Authority (ASL) Salerno, EcoFoodFertility Project Coordination Unit, via M. Clemente, 84020 Oliveto Citra (SA), Italy

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ABSTRACT

The Campania region in Italy is facing an environmental crisis due to the illegal disposal of toxic waste. Herein, a pilot study (EcoFoodFertility initiative) was conducted to investigate the use of human semen as an early biomarker of pollution on 110 healthy males living in various areas of Campania with either high or low environmental impact. The semen from the "high impact" group showed higher zinc, copper, chromium and reduced iron levels, as well as reduced sperm motility and higher sperm DNA Fragmentation Index (DFI). Redox biomarkers (total antioxidant capacity, TAC, and glutathione, GSH) and the activity of antioxidant enzymes in semen were lower in the "high impact" group. The percentage of immotile spermatozoa showed a significant inverse correlation with TAC and GSH. Overall, several semen parameters (reduced sperm quality and antioxidant defenses, altered chemical element pattern), which were associated with residence in a high polluted environment, could be used in a further larger scale study, as early biomarkers of environmental pollution.

1. Introduction

During the last decades, the increasing occurrence of disorders of the male reproductive system in humans has raised attention about the possible environmental risk factors. In particular, the multifaceted toxicity of metals along with the widespread exposure of the general population pointed out to the potential association with the increased incidence of male infertility and the decline in human semen quality [1]. A major toxicity mechanism of many trace elements is their ability to produce high levels of reactive oxygen species (ROS), resulting in an unbalanced reduction-oxidation (RedOx) status affecting biological molecules, including lipid peroxidation [2]. Also an essential element like copper (Cu) at excessive exposure may increase oxidative damage with adverse effects on spermatogenesis and male fertility [3]. ROS are needed for the regulation of normal sperm physiology [4], but oxidative stress

Abbreviations: DFI, DNA fragmentation Index; G6PD, glucose 6-phosphate dehydrogenase; GCL, γ-Glutamate Cysteine Ligase; GPX, glutathione peroxidase; GSH, reduced glutathione; GSR, Glutathione-S reductase; GSSG, oxidized glutathione; GST, glutathione S-transferase; HIGH, high exposure; LOW, low exposure; Nrf2, nuclear factor erythroid-2-related factor 2; PC, protein bound carbonyls; PCA, Principal Component Analysis; ROS, reactive oxygen species; SH, total sulfhydryl groups; TAC, total antioxidant capacity.

* Corresponding author.
E-mail addresses: paolo.bergamo@isa.cnr.it (P. Bergamo), mgvolpe@isa.cnr.it (M.G. Volpe), stefano.lorenzetti@iss.it (S. Lorenzetti), alberto.mantovani@iss.it (A. Mantovani), tiziananotari7@gmail.com (T. Notari), ennio.cocca@ibbr.cnr.it (E. Cocca), dyer88@hotmail.it (S. Cerullo), michele.distasio@isa.cnr.it (M. Di Stasio), strategia@izsmportici.it (P. Cerino), l.montano@aslsalerno.it (L. Montano).

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conditions can adversely affect human sperm function and quality [5]. Consequently, an array of antioxidant molecules is present in seminal plasma, compensating for the deficiency in cytoplasmic defenses of sperm cells [6]. Among them the tripeptide glutathione (GSH) and thiols (SH) have ROS scavenging properties and are therefore suggested to be important in sperm function and fertilization besides playing a role in the defense against oxidative damage and toxicants. The nuclear factor erythroid-2-related factor 2 (Nrf2) is a master regulator of cellular defenses against oxidative stress and environmental toxicants [7] which activates the transcription of antioxidant and detoxifying enzymes (phase 2) (*e.g.,* Glutathione Peroxidase, GPX; Glutathione-S-transferase, GST; Glutathione-S-reductase, GSR, responsible for the recycling of oxidized glutathione, GSSG; Glucose-6-phosphate dehydrogenase, G6PD cofactor of GSR). Their reduced mRNA expression and/or efficiency have been associated to a declined fertility in humans [8,9].

Reduced fertility also correlates directly with sperm DNA damage [10]. In many epidemiological studies – including those investigating environmental exposures – sperm quality has been evaluated by using routine clinical parameters (seminal volume, pH, sperm concentration, motility, and morphology) [11], nevertheless evidence points out to the importance of sensitive molecular biomarkers such as the measurement of sperm DNA damage [10,12]. Moreover, the possible utilization of this indicator for male fertility diagnosis was recently suggested by the positive correlation between sperm DNA damage (TUNEL assay) and ROS yield [13].

During the past three decades, the northern part of the metropolitan area of Naples (the so-called "Land of fires" in popular media language) of Campania region (South-Western Italy) has been going through an environmental crisis due to both intense human activities (vehicle traffic, industry and agriculture) and illegal disposal of toxic waste [14]. As a result, there are serious concerns for the long-term effects on community health, including reproductive health, albeit a proper risk assessment has yet to be finalized. Interestingly, environmental bio-monitoring indicated that toxic elements (*e.g.*, Cr, Ni, Pb) feature prominently in the area [15,16] and other main contaminant (dioxins) were identified by Pacini and collaborators [17]. As a response to such serious concerns, the EcoFoodFertility initiative (http://www.ecofoodfertility.it/the-project.html), a multicenter, multidisciplinary research connecting human lifestyle and dietary habits to the environmental consequences of exposure to toxicants – has been recently launched with the aim of: (i) developing a better understanding of the environmental impact of toxicants on healthy humans, and (ii) use human seminal plasma as an early and sensitive biomarker of environmental exposures to pollutants as well as of the quality of living environment. Previous studies in the metropolitan area of Naples supported the relationship between lower sperm motility and high environmental exposures to traffic emissions or heavy metals [18,19]; Furthermore, preliminary EcoFoodFertility data indicated the impairment of several semen quality parameters, particularly, increased sperm DNA damage in clinically healthy male volunteers living in the "Land of Fires" [20,21].

The first aim of this pilot study was to investigate, which biological or chemical markers among those selected (semen quality, blood and seminal plasma levels of trace elements, RedOx status in blood and seminal plasma) allow to discriminate between healthy males living in geographical regions either at high or low environmental impact "(HIGH and LOW)".

The following analyses were carried out: (i) semen quality parameters, according to WHO criteria [11]; (ii) blood and seminal plasma content of 22 chemical elements potentially toxic and/or able to impact on semen quality – aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), cuprum (Cu), iron (Fe), lead (Pb), lithium (Li) magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), potassium (K), selenium (Se), sodium (Na), strontium (Sr) and zinc (Zn)]; (iii) RedOx status parameters in either (iii(a)) blood antioxidant measures (Total Antioxidant Capacity, TAC; total sulfhydryl groups, SH; antioxidant vitamins, vitamins A and E) or (iii(b)) seminal plasma antioxidant measures (TAC, SH, as well as GSH/GSSG ratio and Carbonylated Protein accumulation, PC; as indicators of oxidative stress extent).

The second objective of the study was to evaluate the link between sperm quality and/or trace element bioaccumulation with the efficiency of seminal antioxidant/detoxifying defenses and with noxious effects on sperm DNA. Therefore, the efficiency of Nrf2-activated defenses at both enzyme (Glutathione Reductase, GSR; Glutathione Peroxidase, GPX; Glutathione-S transferase, GST; Glucose-6-Phosphate Dehydrogenase, G6PD) and mRNA level (GCL, γ-Glutamate Cysteine Ligase; GSR, G6PD), and the DNA Fragmentation Index (DFI) were evaluated. Enzyme activities, mRNA levels and DFI levels were examined in two subsets (n = 20) of randomly selected subjects each in the HIGH or LOW group.

2. Material and methods

2.1. Recruitment

The study was carried out in accordance with the Code of Ethics of the World Medical Association [22] upon approval of the Ethical Committee of the Local Health Authority Campania Sud-Salerno. The recruitment was conducted between July and December 2015 in San Francesco d'Assisi Hospital (Oliveto Citra—Province of Salerno) and Medicina Futura center (Acerra—Province of Naples) and the participants were randomly selected within clinically healthy male volunteers in fertile age (20–40 years old). The HIGH group healthy males (n = 60, mean age 28 ± 5 years, BMI 24.5 ± 2.4) resided in 9 (Acerra, Caivano, Afragola, Casalnuovo, Pomigliano, Brusciano, Giugliano, Cardito and Marigliano; belonging to the Provinces of Naples and Caserta) out of 88 municipalities of the so-called "Land of fires": this area is officially recognized as a high environmental impact area on the basis of the Campania Region Environmental Protection Agency report (2008) [23], that identified the "Land of Fires" as the Campania area with the highest concentration of illegal disposal sites of toxic waste (Fig. 1). The LOW group healthy males (n = 50, mean age 28 ± 7 years, BMI 24.5 ± 2.4) resided in 7 municipalities (Oliveto Citra, Contursi Terme, San Gregorio Magno, Buccino, Ricigliano, Valva and Colliano) of a Southern Campania area, in Salerno province, known as "Alto-medio Sele": this area has a low environmental impact [23] and its economy based mainly on low-to-medium scale farming and without known illegal disposal of toxic wastes (Fig. 1). Enrolment criteria were as follows: residence for at least 10 years in the study area, no known chronic diseases (diabetes or other systemic diseases), no varicocele, no prostatitis and other factors that could affect semen quality (such as fever, medications, exposure to X rays etc.), no reported history of drug abuse and no known occupational exposures to toxic chemicals. Data were collected by questionnaire and physical examination, including the urogenital evaluation (testis volume and transrectal prostate evaluation). All recruited subjects got informed of the study objectives and gave their signed consent before sample collection. Upon enrolment, a code number (1,2,3... n) was assigned to each volunteer by the recruiting andrologist (the recruiter). Each code number was uploaded into a computer database along with personal and clinical information (e.g. age, BMI, area of residence). The examining andrologist (the evaluator, different from the recruiter) performed semen quality evaluation having access only to the code number assigned to each sample.