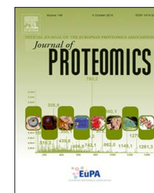
Journal of Proteomics 174 (2018) 85–100

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot



Soluble protein fraction of human seminal plasma

Laura Bianchi[a,*], Chiara Carnemolla[a], Viola Viviani[a], Claudia Landi[a], Valentina Pavone[b], Alice Luddi[c], Paola Piomboni[c], Luca Bini[a]

[a] Functional Proteomics Laboratory, Department of Life Sciences, Siena University, Via Aldo Moro 2, 53100 Siena, Italy
[b] Department of Life Sciences, Siena University, Via Aldo Moro, 53100 Siena, Italy
[c] Department of Molecular and Developmental Medicine, University of Siena, Viale Bracci 14, 53100 Siena, Italy

ARTICLE INFO

Keywords:
Extracellular vesicle
Prostate-specific antigen (PSA)
Proteomics
Protein species
Nervous system
In vitro fertilization

ABSTRACT

Human seminal plasma (SP) is a complex fluid where sperm cells are bathed. Until recently, SP was simply retained a spermatozoa transport medium with nourishing functions. Growing evidences are nowadays recognizing it as a main actor "on the stage" of reproduction: SP orchestrates the synchronized cascade of events that make spermatozoa able to fertilize and modulates male and female reproductive fitness in physiological and in pathological states.

Despite all SP studies have focused on "whole" SP or on its extracellular vesicles, to obtain an in-depth functional comprehension of SP, also the merely vesicle-free SP (vf-SP) soluble fraction deserves consideration. Here we present the first vf-SP functional proteomic study applying 2-DE, MALDI-TOF MS, and cluster and pathway analyses.

Our work evidenced the occurrence of few unique proteins in vf-SP and an unexpected high heterogeneity of their corresponding protein species, conceivably derived by massive co- and/or post-translational modification events. Generated nets and clusters revealed tight functional correlations among identified proteins as well as their involvement in key functions for spermatozoa support and fertilization. Moreover, since SP is released by urogenital tissues and glands, our work may pave the way to the evaluation of vf-SP expression-profiles in diagnosing pathological processes in the secreting tissues.

Biological significance: Seminal plasma vesicles are emerging as rich reservoirs of biomarkers for male infertility and urogenital disorders. Our proteomic approach to human SP diverges from the general trend in SP characterization, investigating not sperm extracellular vesicles but rather the vesicle-depleted SP fraction. Here we discussed how also SP soluble fraction may be considered actively involved in spermatozoa maturation, and we suggested that vf-SP protein profile may offer a precious overview on spermatogenesis, epididymal maturation, ancillary gland functionality, and sperm quality, thus highlighting its potentiality as a biomarker source in man health and reproduction.

The present work provided the first 2-DE reference map of the so far underestimated vf-SP and an innovative and comprehensive functional-overview of its proteins. Obtained data may concur to clarify biochemical and molecular processes that the *in fieri* and the ejaculated semen undergoes as well as how semen properties can affect fertility or may reflect genital disorders.

This paper is dedicated to the memory of Dr Riccardo Focarelli, associated professor in Comparative Anatomy and Glycobiology at the Dpt. of Life Sciences, Siena University, to acknowledge his fundamental contribution to the design of this study and in recognition of his distinguished research legacy.

1. Introduction

The ejaculated human semen can be described as a heterogeneous biological fluid in which spermatozoa constantly swim. In assisted reproduction technology (ART), semen is fractionated, after liquefaction and spermatozoa parameters evaluation, into two major components. These are the cellular fraction, which mainly corresponds, in physiological conditions, to spermatozoa, and the acellular one that is known as seminal plasma (SP).

In ART, seminal plasma is routinely discarded not only for the obvious necessity to concentrate gametes but also for its supposed deleterious effect in spermatozoa fertility maintenance during

* Corresponding author at: Department of Life Sciences, University of Siena, Via A. Moro 2, 53100 Siena, Italy.
E-mail address: bianchi12@unisi.it (L. Bianchi).

https://doi.org/10.1016/j.jprot.2017.12.015
Received 2 October 2017; Received in revised form 4 December 2017; Accepted 21 December 2017
Available online 27 December 2017
1874-3919/ © 2018 Elsevier B.V. All rights reserved.

cryopreservation. As a consequence, SP is the most abundant male "waste product" in assisted reproduction and, as for the female follicular fluid, it is in the spotlight as a precious sample to noninvasively investigate functional dynamics and biochemical properties of human sperm.

Seminal plasma represents about the 90% of the ejaculated volume and it comprises a highly concentrated protein-rich fluid and numerous and different extracellular vesicles (EVs), which are heterogeneous for dimensions, origin, and for "molecular cargo" [1].

Several secreting epithelia and glands differentially concur to the production of seminal plasma. While testes and epididymes supply solely 2–5% of SP, male accessory sex glands, under a strict regulation of neuroendocrine system, contribute to a greater extent to SP production [2–4]. In particular, vesicular glands and prostate provide the main contribution releasing about 65% and 25% of SP, respectively [5].

For a long time, seminal plasma was regarded as a quite irrelevant medium with not properly defined spermatozoa-supporting attitude. But, since the discovery of the above mentioned extracellular vesicles [6–9], plasma was widely reconsidered. Growing evidences have actually attested its involvement in sperm viability and prefertilizing activities, in orchestrating the profound remodelling of spermatozoa during maturation and acquisition of fertilizing capacity, and in male–female seminal fluid signalling for activation of female responses, which facilitate conception and sustain progression to pregnancy [10–13]. SP has been hence proposed as a promising source of biomarkers for reproduction disorders, contraception, and, according to its own nature, for diagnosis, prognosis and prevention of urogenital pathologies (*e.g.* varicocele, prostatitis, prostatic hyperplasia or carcinoma) [14,15,5].

To the best of our knowledge, almost all papers concerning human seminal plasma investigated vesicular fraction(s). Only a few of them analyzed not-processed "whole" seminal plasma, which corresponds to liquefied semen merely deprived of spermatozoa. Vesicles are retained to act pivotal roles in spermatozoa functional biology and to offer precious material for urogenital disease assessment. As above stated, vesicle-free seminal plasma (vf-SP) proteins are instead often relegated to minor and neglected roles in sperm physiology. In addition, SP proteins are usually considered, accounting for about 85% of semen proteins [16], undesirable and far too abundant contaminants in spermatozoa and vesicle purification.

Beginning with the pioneer work of Fung et al. in 2004 [17] and getting to the recent SP protein redundancy overcoming by Gilany et al. [18], proteomic approaches to the characterization of human whole-SP allowed the overall identification of 2168 different non-redundant SP proteins, with intracellular factors representing the highest percentage [5,19–22]. Despite a plausible physiological presence in SP of intracellular proteins derived from massive secretion events and shredding of urogenital epithelial cells, EVs may be considered, along with lysed spermatozoa, the chief source of those cellular components in seminal plasma [19,23]. Even though they account for only 3% of total seminal proteins [16], EVs are retained selectively enriched in specific proteins that may result detectable contaminants of the seminal plasma soluble fraction. Independently from their occurrence in semen, many cellular proteins are more profusely studied and described than the merely secreted semen plasma proteins. As a consequence, SP properties are *in bona fide* shadowed by biomolecular roles of vesicular/cellular proteins. Actually, simultaneous functional processing of whole-SP proteins may *de facto* diverts attention from seminal plasma real roles to the well-known cellular multitasking proteins and to their related networks. In addition, to worse this bioinformatic bias, a number of secretory plasma proteins have been poorly studied or they have not been functionally characterized in semen microenvironment. Indeed, the protein pattern of the SP soluble fraction and its molecular functions, as well as their implications in reproduction and in male patho-physiological states, may be exactly defined only reducing SP contamination by vesicular/cellular proteins. Being it impossible to avoid protein contamination from lysed sperm cells, EV removal, which is feasible even with minimal sample handling, may be worthwhile in minimizing cellular protein concentration in SP.

Here, we present the first functional proteomic overview on SP soluble fraction depleted of EVs. Proteomic data were obtained by 2-DE and MALDI-TOF MS. Then they were processed by bioinformatic resources for cluster and pathway analyses. Vf-SP resulted to include a very limited set of highly abundant unique-proteins that conceivably underwent massive co- and/or post-translational modification events. Owing to the complementarity of gel and MS based approaches, previous off-gel MS studies [18,19,21] did not describe or properly cover the complex protein heterogeneity that our performed 2-DE analysis depicted in SP. The obtained vf-SP overview suggested a fine modulation of SP properties *via* differential processing of its soluble protein fraction.

Based on GO molecular function and biological process ontologies, identified proteins emerged to predominantly have catalytic and binding activity, with principal involvement in immune response, apoptotic process and central nervous system development and morpho-functional maintenance. The original and comprehensive functional overview we obtained on vf-SP proteins not only confirmed vf-SP as a catalytic milieu of proteases and corresponding inhibitors, active in semen coagulation and liquefaction, but our approach also depicted complex pathways that are involved in modulating female immune deviation, redox balancing, and lipid metabolism and transport. Finally, to further stress fundamental roles that the soluble fraction of SP plays in sperm functionality and viability, several of the vf-SP proteins we identified have been previously proved to be the main differentially occurring proteins in whole-SP samples from men with aberrant semen parameters, despite the presence in those SP specimens of EVs [24]. In fact, some vf-SP proteins are supposed, and partially proved, to timely and spatially concur to modulate structural and functional properties of spermatozoa during sperm passage throughout male and female genital tracts.

In light of our analyses, the idea that vf-SP proteins merely generate a fluid matrix for spermatozoa maintenance is an "outdated concept" to be definitely abandoned. Vf-SP proteomic study are hence expected to improve biochemical and molecular knowledge about the *in fieri* semen and about the implication of semen properties in fertility and genital physiology.

2. Materials and methods

2.1. Semen sample collection

Two semen donors were recruited at the Centre for Diagnosis and Treatment of Couple Sterility, Institute of Obstetrics and Gynaecology, "Le Scotte" General Hospital in Siena. Donors were normozoospermic healthy subjects with proven fertility, fathering at least one child, naturally conceived. They were 25 and 30 years old non-smokers and non-drinkers with healthy habits. Sperm samples were collected after at least 36 h of sexual abstinence and following the World Health Organization (WHO) guidelines [25]. The use of human semen samples for the purposes of this research was secured by Institutional ethical approval and donors signed informed consent. After ejaculate collection and its liquefaction, samples were scored as normal, according to the WHO criteria [25], by assessing sperm morphology, sperm count and motility.

2.2. Vesicle-free seminal plasma sample recovery

Freshly-ejaculated liquefied semen samples were centrifuged at $800 \times g$ for 10 min at 4 °C to separate spermatozoa from seminal plasma (SP).

Recovered sperm cells were thoroughly washed in phosphate-buffered saline (PBS: 150 mM NaCl, 50 mM KH_2PO_4, pH 7.4), pelleted at

Download English Version:

https://daneshyari.com/en/article/7633702

Download Persian Version:

https://daneshyari.com/article/7633702

Daneshyari.com