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# Association between the seminal plasma proteome and sperm functional traits

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**Objective:** To analyze the seminal plasma proteome and biological functions associated with sperm functional alterations.
**Design:** Cross-sectional study.
**Setting:** University andrology and research laboratories.
**Patient(s):** A total of 156 normozoospermic men.
**Intervention(s):** Sperm mitochondrial activity, acrosome integrity, and DNA fragmentation were evaluated in a semen aliquot. Remaining semen was centrifuged, and seminal plasma was utilized for proteomic analysis (liquid chromatography–tandem mass spectrometry). Patients were divided into percentiles (15%) to form the following groups: substudy 1, high (control, n = 26) and low (study, n = 23) sperm mitochondrial activity; substudy 2, high (control, n = 23) and low (study, n = 22) sperm acrosome integrity; and substudy 3, low (control, n = 22) and high (study, n = 22) sperm DNA fragmentation. Groups were compared using univariate and multivariate analyses. Differentially expressed proteins were used for functional enrichment analysis.
**Main Outcome Measure(s):** Seminal plasma proteome and postgenomic pathways are associated with several sperm functional traits.
**Result(s):** In total, 506, 493, and 474 proteins were observed in substudies 1, 2, and 3, respectively. Enriched functions in substudy 1 were intramolecular oxidoreductase activity, aminoglycans catabolism, endopeptidases inhibition, lysosomes, and acute-phase response (study group). In substudy 2, main enriched functions were phospholipase inhibition, arachidonic acid metabolism, exocytosis, regulation of acute inflammation, response to hydrogen peroxide, and lysosomal transport (study group). In substudy 3, enriched functions were prostaglandin biosynthesis and fatty acid binding (study group). We proposed eight, six, and eight seminal biomarkers for substudies 1, 2, and 3, respectively.
**Conclusion(s):** Seminal plasma proteome reflects sperm mitochondrial activity reduction, acrosome damage, and DNA fragmentation, with several postgenomic functions related to these alterations. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
**Key Words:** Acrosome, DNA damage, mitochondria, proteomics, semen

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Sperm functional alterations, referring to any alteration in sperm that may affect essential functions, such as motility or fertilization ability, are recognized as one of the major cellular mechanisms underlying male infertility and can be observed in at least 25% of infertile men (1–3), such as men with varicocele (4–7), obesity (8), or smoking habits (9). Therefore, the evaluation of sperm function may provide further information regarding male fertility potential (10, 11). However, its relevance as an infertility diagnostic method is still the subject of debate (12, 13). Thus, new studies, mainly focusing on the molecular mechanisms involved with sperm functional alterations (14), could provide novel methods for sperm quality evaluation that might be clinically relevant (15). These studies

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are even more necessary in cases of idiopathic infertility, which account for approximately 25% of infertile couples and are possibly caused by sperm functional alterations (16).

In this context, studying the seminal plasma proteome could allow the discovery of protein biomarkers of sperm functional alterations that, after further studies, may provide a kit for an easy and noninvasive diagnostic tool of sperm functional alterations. This analysis, coupled with semen analysis, would provide a more complete diagnosis of male infertility. Given that this diagnostic method would rely on protein quantification, as it is already used for diagnosis of other diseases, such as prostatic cancer (prostate-specific antigen quantification), this would be feasible and cost-effective in the laboratory setting, quickly providing the detection of sperm functional alterations.

Accordingly, in a recently published study (17), we have already demonstrated that the seminal plasma proteome is associated with semen oxidative stress, and suggested a seminal biomarker (Mucin-5B). Although the sperm could be directly evaluated, most men who seek infertility treatment have low sperm concentration, which impairs the use of sperm as a source for protein evaluation in the clinical setting, especially if the semen sample will be further used for IVF or intracytoplasmic sperm injection. On the other hand, seminal plasma is often obtained in larger amounts, and its proteome might reflect sperm functional alterations for several reasons. First, approximately 10% of seminal plasma proteins are of testicular or epididymal origin (18). Studies have shown that seminal plasma epididymal proteins play a major role in sperm quality (19) and that proteins associated with testicular function may be observed in seminal plasma (20–22). Seminal plasma proteome alterations have already been associated with several infertility factors, such as nonobstructive azoospermia (23, 24), obstructive azoospermia (25), asthenozoospermia (19), varicocele (26), and vasectomy (18, 23), as well as with sperm functional alterations, such as sperm DNA fragmentation (22). Therefore, the seminal plasma proteome directly reflects spermatogenesis and epididymal maturation status. Additionally, proteins from altered sperm present throughout the male reproductive tract can compose the seminal plasma (27–29), demonstrating that the seminal plasma proteome also reflects sperm integrity. Last, seminal plasma proteins can regulate sperm function (20), which is corroborated by human and bovine studies showing that the addition of seminal plasma of high motility to low-motility sperm may improve its function (30–32). Thus, studying the seminal plasma proteome might provide some important insights regarding sperm function.

Among the main physiologic features necessary for sperm function, we highlight [1] the presence of active mitochondria, which is important for an adequate motility and hypermotility pattern and, consequently, for sperm transit through the female reproductive tract and fertilization ability (33), [2] an intact acrosome structure, essential for a correct acrosome reaction and, thus, for sperm penetration into the zona pellucida (34), and [3] DNA integrity, necessary for production of a viable embryo without genomic alterations, that can pursue a correct development, culminating in the birth of a healthy baby (35). Therefore, these sperm functions are required for male fertility, and understanding the effects of alterations on these features on the seminal plasma proteome would be of great interest.

Thus, we hypothesized that the seminal plasma proteomic profile could reflect alterations to sperm mitochondrial activity, acrosome integrity, and sperm DNA fragmentation, owing to direct alterations on spermatogenesis or epididymal maturation, and to sperm damage. To test this hypothesis, we performed a shotgun proteomic analysis of seminal plasma associated with these alterations. This untargeted approach provides a screening of seminal plasma proteome, which is the first step toward biomarker discovery (36). With our results, we were able to identify the differential protein expression related to each sperm functional alteration and, therefore, the seminal biomarkers of these alterations. Hence, after biomarker validation, these may be used as diagnostic methods of sperm functional alteration in the clinical setting. Moreover, through functional enrichment analysis we have identified the altered seminal plasma biological functions related to sperm functional quality, providing further insight into the mechanisms associated with these alterations.

## MATERIALS AND METHODS

### Study Design

This study received institutional review board approval from the São Paulo Federal University (Brazil) Research Ethics Committee. All included subjects provided informed, written consent.

A cross-sectional study was used including the patients referred to the Andrology Laboratory of the Human Reproduction Section from São Paulo Federal University. Inclusion factors were men aged 20–50 years, presenting sperm concentration $>15 \times 10^6$/mL. Exclusion factors were ejaculate volume $<1.5$ mL, progressive motility $<32\%$, morphology $<4\%$, or leukocyte concentration $\geq 1 \times 10^6$/mL. Only normozoospermic men were included in this study, because studies have already demonstrated that asthenozoospermic and teratozoospermic men present differences in their seminal plasma proteome (19, 37). Therefore, we tried to isolate our study factors (alterations in sperm mitochondrial activity, acrosome damage, and DNA fragmentation), excluding the bias that the inclusion of men with seminal alterations would add to our study, to detect the proteome changes indeed associated with sperm functional alterations.

Semen was collected at the Andrology Laboratory between July 2012 and November 2013, by masturbation after 2–5 days of ejaculatory abstinence. In total, 233 samples were collected, of which 77 were excluded owing to semen analysis alterations. Therefore, a total of 156 normozoospermic patients were included in this study. This cohort was previously used in another published study from our group (17).

After semen liquefaction, an aliquot was used for semen analysis, performed according to the World Health Organization (2010) criteria (38). Another aliquot was used for evaluation of sperm mitochondrial activity, acrosome integrity, and DNA fragmentation. Sperm functional analyses were performed in individual samples. The remaining semen volume