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PROTEOMICS OF THE SPERMATOZOON

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ABSTRACT

The study of the sperm proteins is crucial for understanding its normal function and alterations in infertile patients. The sperm is a highly specialized cell with a very large flagella, with little cytoplasm and a highly condensed nucleus. The most abundant proteins in the nucleus of mammalian sperm are the protamines. The main functions of the protamines are the condensation of the DNA, possibly contributing to the generation of a more hydrodynamic sperm head and to the protection of the genetic message. However, in addition to protamines, about 5.0-15.0% of the paternal genome is also complexed with histones and histone variants. It has also demonstrated a differential distribution of genes in regions associated with histone and protamine-associated regions, suggesting a potential epigenetic relevance in embryonic development. More recently, detailed lists of proteins have been described corresponding to the different compartments of the sperm cell thanks to the application of recent proteomic techniques based on mass spectrometry (MS). Differential proteomics is also being applied to identify the presence of protein abnormalities found in infertile patients.

Keywords: Proteomics, Proteome, Sperm chromatin, Epigenetics, Infertile

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The Nucleohistone-Nucleoprotamine Transition and Organization of the DNA in the Sperm Nucleus. Spermatogenesis involves radical changes in chromatin structure to give rise to the mature sperm [1,2]. The nucleosome structure present in spermatogonia, spermatocytes and round spermatids, is disassembled in spermiogenesis and is temporarily replaced by transition proteins and finally by protamines [1-4].

While most of the genome in the sperm cell (about 85.0-95.0%) is tightly packaged by protamines in the form of toroidal structures, it is also important to note that about 5.0-15.0% of sperm DNA is organized by histone proteins, many of which are sperm-specific variants [3-5]. The distribution of genes in genomic regions organized by protamine and the genomic regions organized by histones is not random. Recent studies based on analysis of the paternal genome associated with each of these domains using microarrays, have led to the basic conclusion that the regions associated

with the nucleohistone are associated with gene regulatory regions [6]. In another recent study [7], based on massive genome sequencing, it was found that nucleosomes associated regions are significantly enriched in genes important for development, including imprinted genes, microRNAs, Hox genes, promoters and transcription developmental genes and signaling factors. It has also been shown that histone modifications (H3K4me2, H3K27me3) are reached at certain loci associated with developmental genes, and promoters associated with developmental genes are hypomethylated in the sperm, but are methylated during maturation [7,8].

In addition to these epigenetic marks determined by the differential distribution of genes in the domains associated with the nucleohistone and nucleoprotamine, other types of epigenetic information are potentially transmitted by the sperm nucleus to the oocyte. One of the best known is contrasted DNA methylation. More recently, the identification of RNAs present in the sperm and the demonstration of oocyte transfer, opens the possibility of their role in fertilization. Another potential source of epigenetic information could be the presence of other proteins in the sperm nucleus, in addition to histones and protamines [9,10].

More recently, proteomic analysis of proteins identified in mature sperm has provided some unexpected results. For example, transcription factors, DNA binding proteins and proteins involved in the metabolism of the chromatin in cells that are transcriptionally inactive [9,10]. The catalogs for the proteomes of human sperm are available [9,11,12]. Most notable is the presence of proteins such as histone acetyltransferase and deacetylase, histone methyltransferase, DNA methyltransferase, topoisomerase, helicase, transcription factors, zinc finguers, homeobox proteins, cromodominio proteins, centrosomal proteins, and telomerase in cells that are transcriptionally inert and have at least 85.0% of their DNA packaged by protamines [9]. A crucial question is whether these transcription factors and proteins newly identified in the cores, represent remnants of the process of spermatogenesis or are making some regions of the paternal genome and have an epigenetic basis [9,13].

Abnormalities in the content of protamine in subfertile patients have already been described over 20 years ago [14]. Subsequently, other studies confirmed the link between abnormal protamine content and alterations in sperm parameters in infertile patients [15,16]. One of the potential causes of abnormal protamine ratio (P1/P2) can be found in abnormal processing of protamine 2 and increased protamine precursors in a subset of patients [15,17]. The results of the content of protamines and histones have been correlated with alterations in the integrity of DNA and the results of assisted reproduction [18].

Identification of Sperm Proteins and Their Alterations in the Spermatozoa Through Proteomic Techniques. Essentially, two different alternatives can be used to study the sperm proteome through mass spectrometry (MS) (Figure 1): ***1)*** two dimensional (2D) separation of the proteins followed by their identification by Matrix-assisted laser desorption/ionization (MALDI)-MS or liquid chro-matography-tandem MS (LC-MS/MS), and ***2)*** the initial digestion of proteins to generate peptides, followed by separation and LC-MS/MS analysis [9]. The first alternative generally involves the separation of proteins using isoelectric focusing and is followed by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) for separating proteins in a second dimension based on their molecular weight. This alternative has been widely used in the past to identify many proteins present in the sperm cell [11,19]. Of the two alternatives, the initial generation of peptides and analysis by LC-MS/MS is of much higher throughput. For example, through 2D and MALDI-TOF (time of flight) or LC-MS/ MS, it has been possible to identify some hundreds of proteins [11,20], whereas the generation of peptides followed by LC-MS/MS allows the identification of up to about 1000 different proteins [9,12].

In addition to the generation of catalogs of proteins, proteomics has also been applied to the identification of the presence of anomalies in infertile patients. There are several strategies to analyze the differential protein content in two or more different samples. One method is 2D-DIGE (differential in-gel electrophoresis) and is based on the differential identification of fluorochrome-labeled proteins extracted from the control (for example, labeled green) and experimental cells (for example, labeled red). This is followed by mixing of the proteins and their separation in the same 2D system, followed by detection that can detect increased or decreased pro-

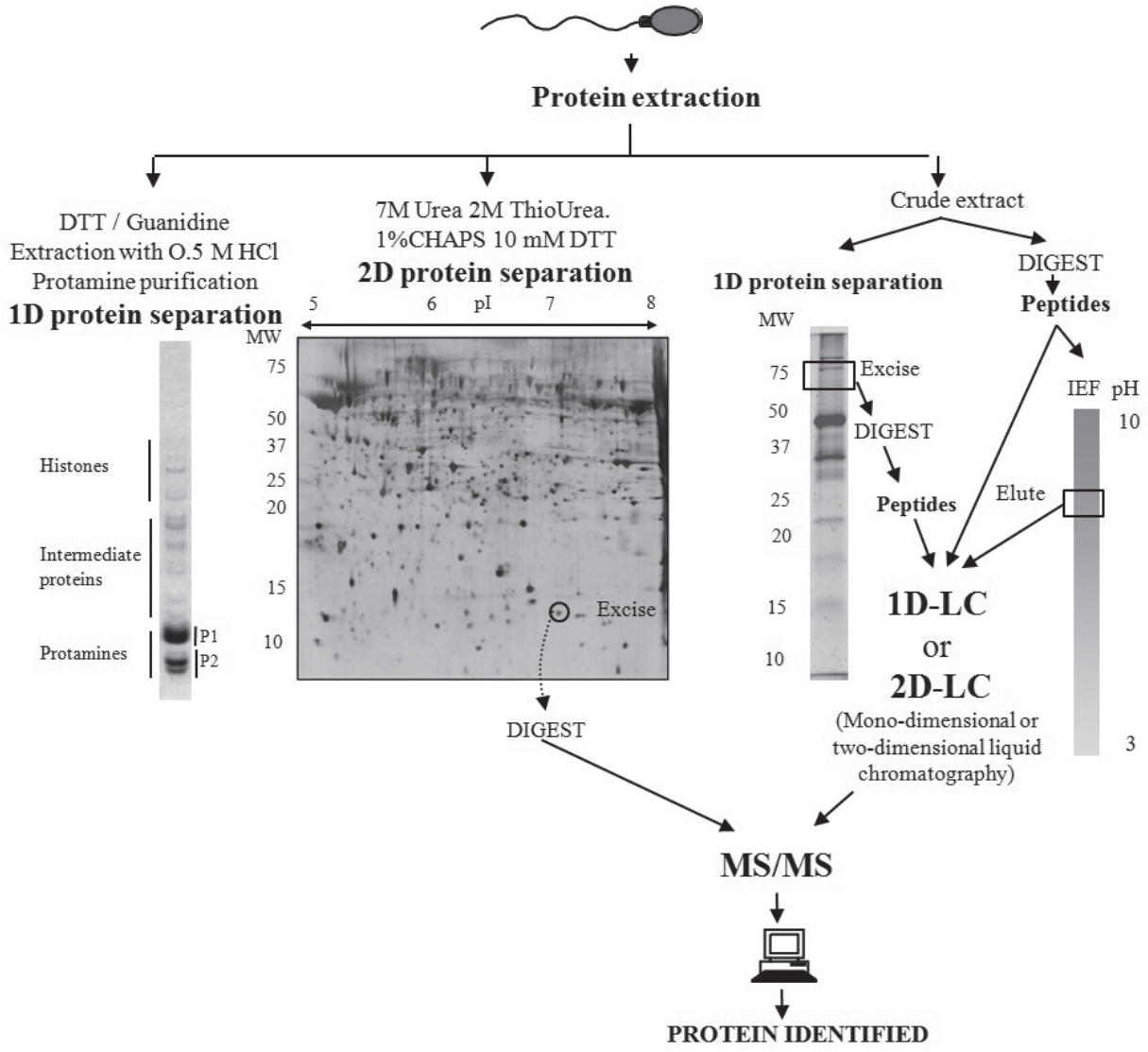

Figure 1. Strategies available for studying the sperm cell proteome. Typical extraction from sperm protamines consisting of reducing the disulfide bridges of protamines with DTT/guanidine hydrochloride, followed by extraction with 0.5 M HCl, precipitation and purification of the proteins and their separation by PAGE acid (left). With this strategy it is possible to identify the protamine 1 (P1) and a set of bands corresponding to the family of protamine 2 (P2). To analyze the total proteome it is possible to use 2D electrophoresis of the proteins. The identification of proteins is then performed *via* MS. A more robust strategy involves the initial generation of peptides followed by their separation by liquid chromatography and identification using MS (right). Based on [9] with modifications.

teins, observing the deviation of the fluorescence to one of the fluorochromes [9,12]. Another alternative is the quantification and comparison of the relative abundance of the different proteins in separate gels. Newer strategies are being developed based on non radioactive isotopic labeling of the test samples and control [9,12].

The first description of the potential of 2D proteomic analysis in the study of defects in sperm was performed in a patient with repeated failure of *in vitro* fertilization techniques [20]. The proteome of this patient showed 20 differences compared with controls, and identified several proteins differentials. It was later applied to the identification of the differential proteins in astenozoospermic patients, oligozoospermic patients, and patients with alterations in the content of protamines or the integrity of DNA [19].

The application of proteomics techniques in andrology and reproductive biology is in its infancy

but the data available to date indicate their enormous potential. It is foreseeable that in the future it will allow the molecular dissection of the various causes of male infertility, allowing both the identification of the pathophysiologic mechanisms involved and its application to the diagnosis, prognosis, and development of new therapeutic strategies.

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