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Review article

Sperm proteome and reproductive technologies in mammals

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ABSTRACT

Sperm is highly differentiated cell that can be easily obtained and purified. Mature sperm is considered to be transcriptionally and translationally silent and incapable of protein synthesis. Recently, a large number of proteins have been identified in sperm from different species by using the proteomic approaches. Clinically, sperm proteins can be used as markers for male infertility due to different protein profiles identified in sperm from fertile and infertile male animals. Recent evidences have shown that the conditions of sperm preservation *in vitro* can also change the sperm protein profiles. This paper reviews the recent scientific publications available to address sperm proteome and their relationship with sperm cryopreservation, capacitation, fertilization, and separation of X and Y sperm. Future directions in the application of sperm proteomics to develop or optimize reproductive technologies in mammals are also discussed.

Contents

1. Introduction

The essential role of male animals in reproduction is to transmit the paternal genome to the next generation. In the testis, there are a large number of thin and coiled seminiferous tubules providing the environment for sperm cell reproduction. Spermatogenesis is regulated by different pathways including endocrine, paracrine, and autocrine (Ramaswamy and Weinbauer, 2015; Cheng et al., 2010). Two major types of somatic cells (Sertoli cells and Leydig cells) and different types of sperm cells are localized in seminiferous tubules. Follicle stimulating hormone and luteinizing hormone secreted by pituitary gland control the proliferation and secretion of Sertoli cells and Ley-

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dig cells through the specific receptors expressed in these cells. Sertoli cells guarantee the effective spermatogenesis *via* providing the essential nutrients. However, Leydig cells mainly synthesize the testosterone to regulate animal sexual behavior and spermatogenesis. Highly complex cellular changes occur during spermatogenesis, including the proliferative phase, the meiotic phase, and the differentiation phase. Firstly, primordial germ cells are differentiated into spermatogonia, followed by the production of primary spermatocytes, secondary spermatocytes, spermatids, and finally motile sperm cells (Ramm et al., 2014). During the final step of the formation of round spermatids, most of the cytoplasm is removed, and the flagella and the acrosomal vesicle are assembled. Meanwhile, the histones are replaced by the highly positively charged protamines which enhance the extensive remodeling of chromatin (Zalenskaya et al., 2000). The final maturation of spermatozoa occurs in the epididymis where chromatin is further compacted through interacting with protamines. Although mature sperm is highly differentiated cell and its transcription and translation are considered to be silent, different molecules have been identified, including mRNAs (Li and Zhou, 2012), non-coding RNAs (Curry et al., 2009) and proteins (Hoalland and Ohlendieck, 2014). The present review focuses on the most relevant results available concerning the analysis of the sperm proteins using the different proteomic methodologies in different animal models. Furthermore, we discuss the relationship of sperm protein profile with reproductive technologies in mammals.

2. Proteomic profiling of sperm cells

Spermatogenesis is a precisely regulated biological process leading to the production of spermatozoa. To better understand the process of spermatogenesis, we firstly must identify the genes involved and explore what roles they play. To date, a number of genes have been considered to be important in regulating spermatogenesis and sperm functions by gene modified animal models (James and Wilkinson, 2005). Mature sperm is a unique type of highly differentiated cells, its transcription and translation is silent, indicating that transcriptional microarray and genomic studies are less informative in this context (Ward and Zalensky, 1996). It is difficult to culture sperm for long time and to study gene function in regulating sperm function *in vitro*. Some evidences have shown that proteins localized in sperm membrane may be responsible for sperm functions (Li et al., 2010, 2011a,b). Highly sensitive LC–MS/MS technology and sequence database analysis has been used to identify 1030 proteins in ejaculated equine spermatozoa, Gene ontology analysis showed that these proteins are involved in metabolism, antioxidant defences, and mitochondrial functions (Swegen et al., 2015). Emerging proteomic technologies can provide a powerful tool to study protein roles in sperm functions.

2.1. Separation of sperm proteins

Friedrich Miescher in 1874 firstly isolated basic component from the sperm of the salmon, and found that the protamine was the most abundant sperm nuclear protein. In addition to the nuclear proteins, the sperm cell have a large amount of tail proteins such as tubulin, acrosomal proteins such as acrosin, mitochondrial proteins, nuclear matrix and cytoskelctal proteins (du Plessis et al., 2011). Before isolation of sperm proteins with different methods, purification of motile sperm from semen sample is also an essential step. The fresh semen consists of various cellular components, for example, blood cells, and epithelial cells. Cryopreservation semen contains egg yolk and milk proteins (Amirat et al., 2004). During sperm proteomic analysis, contamination of other proteins will affect the accuracy. To remove or minimize this type of contamination, sperm always is purified through density gradient centrifugation or through swim-up method (Sakkas et al., 2000). Specific primers for leukocytes (for example CD 45) are used to further confirm the contamination of non-sperm cells (Lambard et al., 2004).

Once the sperm cells have been purified, the sperm proteins will be analyzed by methods based on electrophoretic or chromatographic characterization of proteins followed by the protein sequencing methods. Mostly, proteins were initially separated by using two-dimensional gel electrophoresis (2DE), an important approach used to identify many proteins from different types of cells (Rabilloud, 2002). The 2DE techniques coupled to the use of western blotting analysis lead to substantial advances in the study of sperm composition and function. The methods based on 2D separation of proteins have advantages: they can be operated by most laboratories and the data obtained from 2D map can be easily understood (Rabilloud, 2002). However, compared with the method relying on separation of peptides using liquid chromatography (LC), the throughput capability of the 2DE approach is lower (Hu et al., 2005). For example, 2DE approaches can identify 10–200 proteins per study, whereas LC–MS/MS approaches can easily identify thousands of different proteins in a relatively short time. A number of studies have clearly demonstrated that different approaches reveal unique results, and hence other accurate approaches should be used to obtain reliable results. For example, the standard methods for extracting proteins from cells are usually different and will result in failure of getting all proteins. In particular multiprotein complexes can only be solubilized with aggressive procedures (Tan et al., 2008). A study using different methods to isolate proteins of human cilia presented different results (Ostrowski et al., 2002). Analysis by two-dimensional (2-D) PAGE with trypsin digestion liquid chromatography/tandem mass spectrometry (LC/MS/MS) sequencing obtained 38 potential ciliary proteins. To identify ciliary components not resolved by 2-D PAGE, axonemal proteins were separated on a one-dimensional gel. The gel lane was divided into 45 individual slices, each of which was analyzed by LC/MS/MS. This procedure got an additional 110 proteins. Finally, preparations of isolated axonemes were digested with Lys-C, and the resulting peptides were analyzed directly by LC/MS/MS or by multidimensional LC/MS/MS, leading to the identification of a further 66 proteins (Ostrowski et al., 2002).