Investigation Study of Human Spermatozoa Proteome

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Abstract

The current study has been conducted on health human sperm to identify the different types of amino acids and entire proteins of sperm. Two methods have been applied to characterise of human sperm, firstly extract lysate of sperm, then digest and run it in mass spectrometry, secondly, total sperm lysate was run in SDS page gel and bands were stained with coomassie, then band was cut and digest to extract of whole amino acids and also was run in mass spectrometry. Result revealed that sperm have several proteins, which were involved with different binding proteins, soluble proteins, and glycoproteins, transmembrane proteins.

Current result was displayed that mass spectrometry can be used to recognise data of proteins of sperm and detected types of it. This technique was applied on whole lysate and gel bands of sperm and created database of sperm proteins which can be investigated and consider more interesting for researchers. This study is concluded that human sperm have important transmembrane, enzymes, glycosylated proteins, glycoproteins and uncharacterised proteins which might be answered many theoretical aspects of biological and biochemical sperm migrations before and after fertilization.

Keywords: *sperm, SDS page, mass spectrometry, glycoproteins, transmembrane, uncharacterised proteins.*

Introduction

The male reproductive is consisted of left and right testis, epididymis, vas deferens which is received products of accessory glands and joined into urethral part of urethra in the penis external male organ(1,2,3) .

Testes are surrounded by skin, then serous layer and tunica albuginea that extended into parenchyma of testis and divided it into incompletes lobules and constitutes many septa and mediastinum of testis (4). Internally, testis is consisted of coiled tubules is called convoluted seminiferous tubules that is contained the germinal epithelium of sperm and connected to epididymis via efferent ductuli (6).

In addition, it has special cells is called interstitial cells(leydig) that produce testosterone hormone, this hormone is responsible for development of sperm and secondary growth of male (7,8).

Sperms undergo different stages of development before maturing, and these stages continue after liberated sperm into lumen of seminiferous tubules (9,10,11).

Furthermore, sperm will undertake remodelling and biochemical changes in the epididymis, resulting acquired it the motility, and sperms become able to be a motile(12,13) also head of sperm would obtained specific β-defensin protein that support sperm in the motility, maturation and antimicrobial activity and it is essential of fertility(14,15,16). The sperm are surrounded by plasma membrane which constitutes with glycocalyx, and carbohydrates such as sialic acid which consist the major sugar of sperm (17,18) these carbohydrates would be mediated and reacted with the female reproductive tract during capacitation process of sperm and also during reservoir of sperm in uterine tube before fertilizations (19,20).

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Moreover, there are different putative recognition protein could play a role in sperm motility and fertilizations (21). For example beta-defensin family are expressed and coated sperm which might be protected it as antibacterial factor and might be involved in sperm capacitation to prepare sperm for fertility(14,22,23), more protein were identified on sperm included Siglecs family which could be mediated sperm activity before fertilizations and may be regulated the internal environment for sperm(17). Therefore, many recent studies are interested in characterization of proteins of sperm to investigate more about transmembrane proteins activity of sperm in relations with female reproductive tract. Proteomic studies were applied on whole lysate of sperm in different ways to detect the total proteins of it and understand it functions(24,25).

The overall aim of this study is to conduct a comprehensive characterisation of proteins of human sperm and created database of human sperm. Additionally, the behaviour proteomic analysis on whole lysates of sperm combine to electrophoresis of human sperm lysates to augment previous studies.

Material and Methods

Human Semen

Straws of semen from three donors obtained from Cryos Ltd. were used (numbers 5469-17, 5549-238 and 3825-40). Each straw contained approximately $1X10^8$ sperm in 500 µl of extender.

Gel formulation

Briefly, Bio-Rad protocol was used for making gel(26). The resolving gel (10% acrylamide/bisacrylamide). All solutions were mixed in order in a 15 ml Falcon tube, then mixed well after the TEMED was added and poured between the 0.75mm gap between the thick and thin glass plates, it was left for 25 minutes to solidify. The Stacking Gel (4% acrylamide/bisacrylamide) was made by mixing the reagents. A comb to create appropriate well volumes was inserted between the plates before pouring the stacking gel on top of the resolving gel.

Tissues lysis buffer

The Tissue Lysis buffer was made and before use one tablet of protease inhibitor (Roche cat no. 11836170001) was added per 10 ml of lysis buffer. It could then be stored at 4°C for up to two weeks.

Preparation of samples

Two different methods were used for preparation peptides acid of human sperm. These were applied on semen of bovine (27). Preparation of lysates of human had followed the protocol of(28). The samples were then ready for injection into the Mass spectral machine by Mass Spec machine (FT-ICR/Orbitrap) in the Conway institute. University College Dublin.

Micro BCA Assay Quantitation of Protein in human sperm

The Micro BCA Protein Assay Kit (Pierce product code #23235) was used to quantitate the protein in Human Sperm.

Coomassie Staining Solution

The whole lysate of human sperm was loaded on SDS-PAGE gels, and after migrated of sperm proteins, the gels were stained by Coomassie Blue Staining (Brilliant Blue). After that destain for Coomassie stain solution was used to remove the excess the stain. The banded of proteins were stained with blue and cut carefully as cubes and digest to extract amino acids (27)

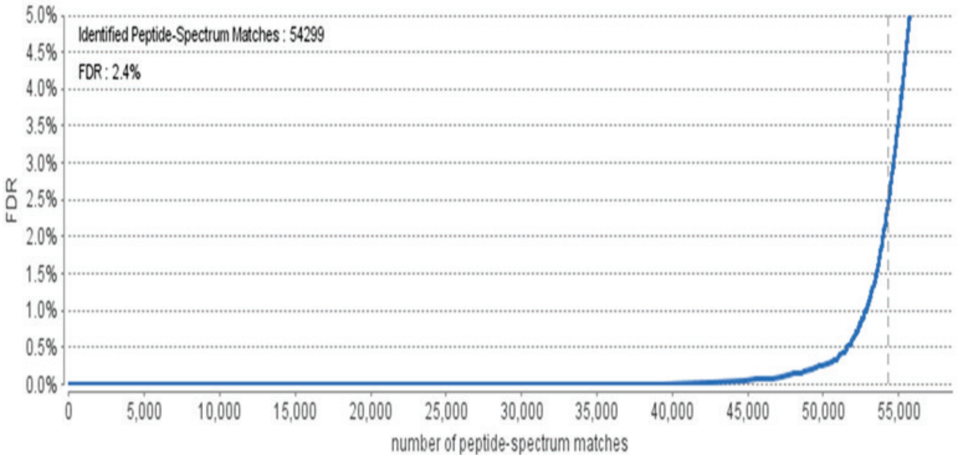

Figure (1): False discovery rate (FDR) curve. X axis is the number of peptide-spectrum matches (PSM) being kept. Y axis is the corresponding FDR.

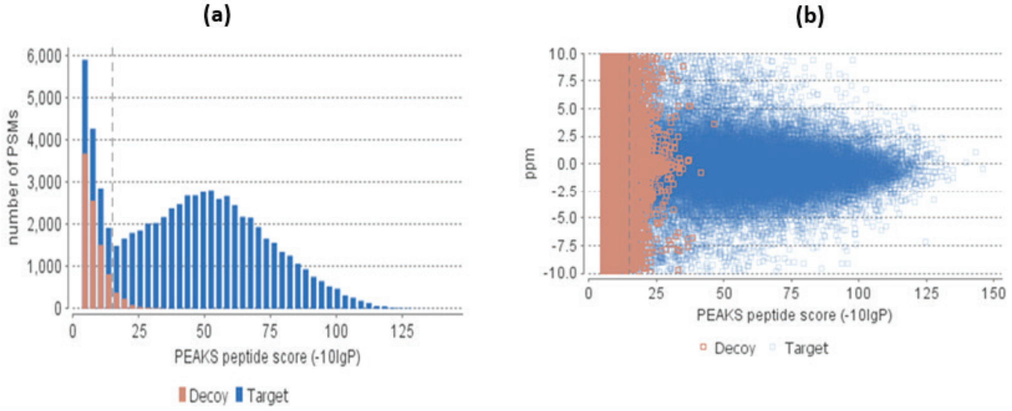

Figure (2): SM score distribution. (a) Distribution of PEAKS peptide score; (b) Scatterplot of PEAKS peptide score versus precursor mass error.

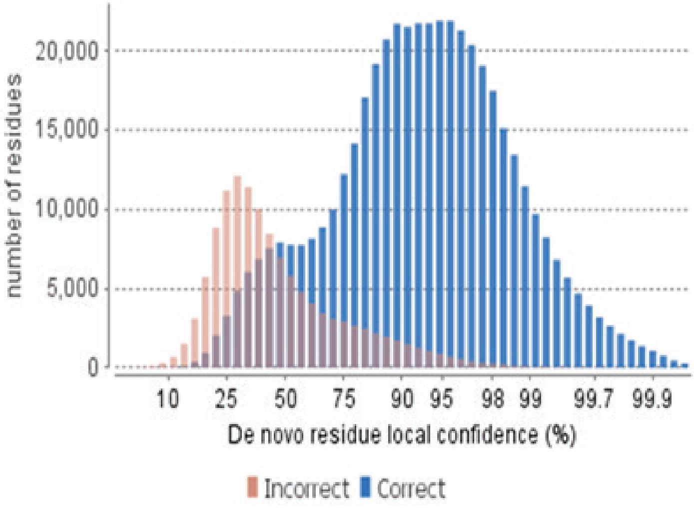

Figure (3): De novo result validation.

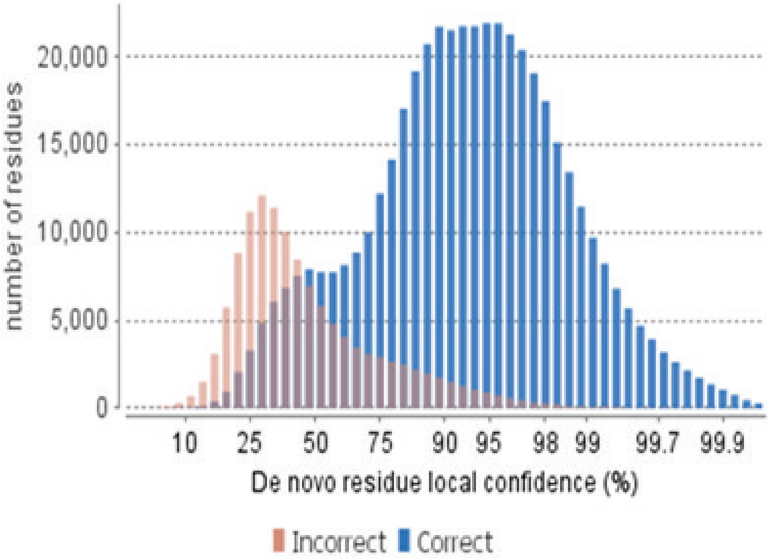

Figure (4): Precursor mass error of peptide-spectrum matches (PSM) in filtered result. Distribution of precursor mass error in ppm.

Table (1): Statistics of filtered result.

Peptide-Spectrum Matches	54301
Peptide Sequences	26781
Protein Groups	2994
Proteins	4576

Result and Discussion

Our results shown that sperm has many different proteins in sperm including those involved in membranes, glycoproteins, non-glycosylated proteins and different enzymes in human. Briefly, samples were prepared in two ways, the first was using cubes cut from 10% SDS polyacrylamide gels containing lysate of sperm samples, where proteins were trypsin digested in situ with Promega™ Trypsin Gold, Mass Spectrometry Grade product (Manufacturer: Promega™ V5280) then extracted from the gel for analysis. The peptides from the digest were resuspended in buffer and run in the Mass Spec machine (FT-ICR/Orbitrap)(28), the second method used whole lysates of sperm which were directly digested by trypsin (Manufacturer: Promega™ V5280), then run in the Mass Spec, with a minimum of three replicates being used for each sample. The data obtained was analysed by Peak7 studio software (29).

The results of both ways were detected different types and various proteins of sperm; however, the electrophoresis method was given better isolated and identifications of proteins. Also sliced of gel or band was more efficiency to recognise more proteins compare to the whole lysate. All biological replications of whole lysate and electrophoresis methods were combined and data was analysed. Proteomic of human sperm revealed that there are about 26781 peptide sequences, 2994 protein groups and 4576 proteins, Table (1). Figure

(1,2,3, and 4).

In this result, the major important proteins were selected which are included with constituents of human sperm as the following: Uncharacterized proteins: 44, Binding proteins: 187, Synthase proteins (enzyme): 68, Actinin: 9, Calcium Binding Proteins: 60, Transmembrane Proteins: 80, Mucin: 18, Galectin: 6, Beta-Defensin Protein: 8, Glycoproteins: 15, Myosin: 76, Actin: 13 (supplementary1).

Mass spectral analysis is one strategy that used in proteomic studies which identifies the relative abundance of amino acids and proteins by measuring deconvoluted overlapping peptide HPLC peaks to provide relative quantitation based on peak areas(30), It has been used to detect relative expression of proteins that characterise different diseases or to identify specific proteins in biological samples(31,32,33). These studies suggest that it is feasible to use mass spectrometry to identify proteins on human sperm. Several proteomic and transcriptomic studies of human and bovine sperm have already been conducted, and have identified different types of proteins which were displayed also different types of important proteins such as free proteins, transmembrane proteins, glycoproteins and enzymes, as well proteins related to immune system in human sperm(34,35,36),which were confirmed our finding in current study. Interestingly, our proteomic results provided evidence for identified many thousands of proteomic sperm with other studies that have been detected in proteomics studies, and could be used to diagnose the disease and fertility by identified normal proteins of sperm(37,38) . Our result identified from the analysis of the data from the mass spectral profiles that sperm surrounded by different types of transmembrane of protein which might be involve in glycosylation process of sperm during passage it via cervix and uterus and reservoir in uterine tube before meeting the ovum and fertilizations and are glycosylated and considered as glycoproteins(39 40,41), therefore It might be necessary to use a number of different proteomic methods to profile all of the proteins in human sperm, because mass spectrometry of glycoproteins is limited by technical issues due to the considerably larger differences and diverse chemical properties of individual glycoproteins(42, 43). There are many complicated reasons which can preclude accuracy in the analysis of glycoproteins in biological samples using mass spectroscopy especially in the case of proteins with low abundance (44). In conclusions, human sperm has large several proteins which can play a huge role in sperm motility, reservoir, internal regulation environment of spermatozoa, immune suppression of female reproductive tract and finally fertilisations.

Acknowledgement: We thank the laboratory staff in Conway institute of UCD for endless help in prepare the sample and created library and analysis for our data.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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