JOURNAL OF PROTEOMICS XX (2014) XXX–XXX

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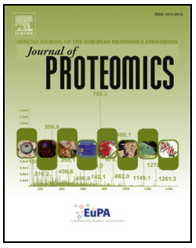

Qualitative and quantitative peptidomic and proteomic approaches to phenotyping chicken semen

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ARTICLE INFO

Article history:
Received 24 February 2014
Accepted 17 July 2014

Keywords:
Chicken (*Gallus gallus*)
Semen
Fertility
Proteomic
ICM-MS
Top-down MS

ABSTRACT

Understanding of the avian male gamete biology is essential to improve the conservation of genetic resources and performance in farming. In this study, the chicken semen peptidome/proteome and the molecular phenotype related to sperm quality were investigated. Spermatozoa (SPZ) and corresponding seminal plasma (SP) from 11 males with different fertilizing capacity were analyzed using three quantitative strategies (fluid and intact cells MALDI-MS, SDS-PAGE combined to LC–MS/MS with spectral counting and XIC methods). Individual MALDI profiling in combination with top-down MS allowed to characterize specific profiles per male and to identify 16 biomolecules (e.g.VMO1, AvBD10 and AvBD9 including polymorphism).

Qualitative analysis identified 1165 proteins mainly involved in oxidoreduction mechanisms, energy processes, proteolysis and protein localization. Comparative analyses between the most and the least fertile males were performed. The enzymes involved in energy metabolism, respiratory chain or oxido-reduction activity were over-represented in SPZ of the most fertile males. The SP of the most and the least fertile males differed also on many proteins (e.g. ACE, AvBD10 and AvBD9, NEL precursor, acrosin). Thus proteomic is a "phenomic molecular tool" that may help to discriminate avian males on their reproductive capacity. The data have been deposited with ProteomeXchange (identifier PXD000287).

Biological significance
This peptidomic and proteomic study i) characterized for the first time the semen protein composition of the main domestic avian species (*Gallus gallus*) by analysis of ejaculated

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http://dx.doi.org/10.1016/j.jprot.2014.07.024
1874-3919/

spermatozoa and corresponding seminal plasma; ii) established a characteristic molecular phenotype distinguishing semen and males at an individual level; and iii) proposedthe first evidence of biomarkers related to fertility.

1. Introduction

Birds are oviparian species with internal fertilization that displays many original features such as long in vivo sperm storage and polyspermic fertilization. These specific features are of great importance in the management of chicken reproduction (*Gallus gallus*), bearing in mind that the chicken constitutes a main source of animal protein for human food [1,2]. Differences in semen composition may affect sperm fertilizing ability in both birds and mammals. Various molecular studies have been undertaken in birds to improve understanding of semen biology and its consequences for fertility [1–3]. However, greater understanding of semen composition is still needed in order to improve avian reproduction, farming output and the preservation of diversity of genetic resources. Proteomic and peptidomic approaches [4,5] offer new insights to evaluate the molecular phenotype of semen, and these were explored in the present study on chicken.

Many in vitro sperm quality tests have been developed in order to correlate semen quality observed at ejaculation and individual male fertilizing ability in the chicken. Most of these tests (viability, motility and mobility, acrosome reaction, in vitro egg penetration) [6] are derived from tests developed in other internal fertilization species; mainly domestic mammals and humans. As in these other species, none of the tests show clear correlation with male fertility. The key test to evaluate the fertility of male birds is therefore still to measure the fertilization rate of eggs laid during one to three weeks after insemination of a defined number of hens with a standardized number of sperm originating from individual males. However, this is a very time and money consuming method that cannot be widely used for high-throughput screening.

Moreover, semen quality includes the complex relationship between sperm cells and seminal plasma. Seminal plasma is a complex fluid that results from a combination of secretions from the testes, epididymis and other deferent duct secretions such as accessory sex glands in mammals. It has been demonstrated to contain a wide protein and peptide profile which affects sperm maturation and fertilizing ability in mammals [7,8]. Very early results from our laboratory showed the existence of key seminal proteins and lipoproteins in the chicken [9,10] and the effects of specific seminal plasma fractions on sperm fertilizing ability [11]. Seventeen seminal proteins were subsequently identified [12]. However, the nature and the quantity of the majority of the seminal biomolecules remain to be identified. While the application of mass spectrometry-based proteomics could greatly extend our knowledge of semen composition in avian models, only two proteomic investigations have been reported recently in the chicken [12,13]. A comparative analysis of differential sperm motility, and a qualitative study to assess a number of seminal plasma protein were performed using 2D gel electrophoresis and tandem mass spectrometry, respectively. Both allowed identification of a limited number of proteins while proteomic studies conducted on mammals have shown that numerous proteins are present in sperm and seminal plasma [14–17], some of which have been demonstrated to be involved in the maturation or the fertilizing ability of male gametes [4,18–20].

The protein and peptide inventory of specific sperm and seminal plasma fractions have thus never been described in chicken semen. Moreover, differential and quantitative peptidomic and proteomic analyses have never been performed to distinguish avian semen in terms of their fertilization capacity, although semen composition at the level of sperm and/or seminal plasma may be of crucial importance in male gamete quality. We hypothesized that proteomic studies would provide new tools to phenotype individual male chickens on their semen composition in relation to semen quality.

The aim of the study presented here was therefore i) to define the semen protein composition of the main domestic avian species (*G. gallus*) by analysis of ejaculated spermatozoa and the corresponding seminal plasma; ii) to establish a characteristic molecular phenotype distinguishing semen and males at the individual level; and iii) to propose an initial approach to characterize biomarkers for fertile and infertile males.

In order to characterize, identify and/or quantify a wide range of proteoforms and peptidoforms, we used several complementary techniques based on MALDI MS profiling of whole cells (Intact Cells MALDI-TOF Mass Spectrometry) and fluids, followed by targeted top-down high resolution mass spectrometry and bottom-up proteomic approaches. In addition, from all the mass spectrometry-based quantitative technology approaches currently available with or without labeling [21,22], we chose three complementary label-free methods based on MALDI-TOF profiling or bottom-up MS (from protein extracts) using GeLC–MS/MS (protein samples included in polyacrylamide gel after fractionation or not by SDS-PAGE are analyzed by nanoLC–MS/MS after in-gel digestion [23]) combined with the spectral counting (SC) [24–26] or eXtracted Ion Chromatogram (XIC) [27–33] peptide pattern quantitative methods.

2. Materials and methods

2.1. Animals

The males and females used were adult chickens of a standardized genetic origin [34] housed in the INRA PEAT experimental unit (Nouzilly, France) under a 14L:10D photoperiod and fed a standard diet of 12.5 MJ/day, supplemented with calcium for the females.

2.2. Semen collection

Semen was routinely collected twice a week by massage [35]. Sperm concentrations were immediately determined by light absorption of semen with a photometer (Jeanway 6051 colorimeter, Jeanway LTD, Dunmon, UK) at a wavelength of 640 nm

Please cite this article as: Labas V, et al, Qualitative and quantitative peptidomic and proteomic approaches to phenotyping chicken semen, J Prot (2014), http://dx.doi.org/10.1016/j.jprot.2014.07.024