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A Proteomic Approach to Identify Seminal Plasma Proteins in Ostrich (*Struthio camelus*)

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The protein bands in ostrich seminal plasma (OSP) were identified by using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS) proteomic analysis and compared with NCBI reference sequence. The results revealed that OSP had five major specific proteins bands namely, OSP-I (GATA Zinc finger domain containing protein 1), OSP-II (GATA Zinc finger domain containing protein 1), OSP- III (E3 ubiquitin protein ligase RNF 216, OSP-IV (Mitotic spindle assembly checkpoint protein MAD1) and OSP-V (Dual specificity phosphatase DUPD1). The estimated molecular weight of OSP-I (M_r94.51 to 102.34 kDa), OSP-II (M_r75.19 to 93.07 kDa), OSP-III (M_r59.58 to 72.76 kDa), OSP-IV (M_r30.71 to 40.90 kDa) and OSP-V (M_r21.66 to 26.26 kDa). The results of our study provide basic knowledge of the protein composition of ostrich seminal plasma highlighting important physiological pathways which may play crucial roles in the sperm environment after ejaculation. This knowledge can be the basis to further develop procedures improving the reproduction of farmed ostrich.

Introduction

Ratite species such as ostrich (*Struthio camelus*), emu (*Dromaius novaehollandiae)* and rhea (*Rhea americana*) are fundamentally attractive for farming to produce leather, meat, oil and feathers. Unpredictable egg production, unstable fertility, poor hatchability and poor chick survival are some of the major constraints in viable ostrich farming. To achieve rapid and sustained genetic improvement, ostrich farming needs to adopt advanced reproductive technological tools. In this composition of seminal plasma has a great influence on the biological quality of the ostrich semen. Seminal plasma (SP) is known to play an important role in fertilization. However, the variability found in its composition among species, males and even fractions of the same ejaculate has made difficult to completely understand its effect in sperm function. Proteins are one of the major seminal plasma components that modulate sperm functionality. Alterations at the molecular level in spermatozoa and seminal plasma can affect male fertility. There are also reports that seminal plasma proteins affect sperm motility (Yoshida *et al.*, 2008).

These proteins could either display negative (La Falci *et al.,* 2002) or positive effects on sperm motility (Qu *et al.,* 2007). Hence this study was carried out to determine seminal plasma protein expression of ostrich semen that can serve as potential biomarkers for male infertility.

Materials and Methods

This experiment was carried out at Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University, Kattupakkam, Kanchipuram, Tamil Nadu during 2014 - 2016. This experiment was designed to analyse the protein profile of seminal plasma in nine ostrich. Selected nine male ostrich were trained for semen collection by teaser method as recommended by earlier authors ***(***Rybnik *et al.,* 2007***).***

Seminal plasma was separated from the semen by centrifugation (2500 rpm for 15 min at 20°C) and stored at -80°C until assayed. A total of 126 seminal plasma sample (each 14 sample from nine male ostrich) were used for this study. The major proteins in seminal plasma were identified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) and the prominent protein bands were analysed by matrix assisted laser desorption ionization mass spectrometry (MALDI MS) to identify the protein**.** The MALDI MS results were compared with the available sequences in GenBank to find out the proteins.

Results and Discussion

Identification of seminal plasma protein in ostrich

Protein sequence results matches for each of the protein bands in ostrich seminal plasma are presented in table 1 and Plate 1.

Ostrich seminal plasma protein band I (OSP-I)

GATA Zinc finger domain containing protein 1 (*Struthio camelus australis*)

GATA zinc fingers are zinc-containing domains found in a number of transcription factors (erythroid specific transcription factor and nitrogen regulatory proteins). Some members of this class of zinc fingers specifically bind the DNA sequence (A/T) GATA (A/G) in the regulatory regions of genes giving rise to the name of the domain.

Ostrich seminal plasma protein band II (OSP-II)

GATA Zinc finger domain containing protein 1 (*Struthio camelus australis*)

The OSP-II protein band also matched with the GATA Zinc finger domain containing protein 1 (*Struthio camelus australis*).

Therefore, the following three assumptions were derived based on the results.

The OSP-II might be a subunit or isoform variant of protein band I (OSP-I).

OSP-II might be a degraded or cleaved product of OSP-I. OSP-I and OSP-II might be part of the so-called family protein.

Ostrich seminal plasma protein band III (OSP-III)

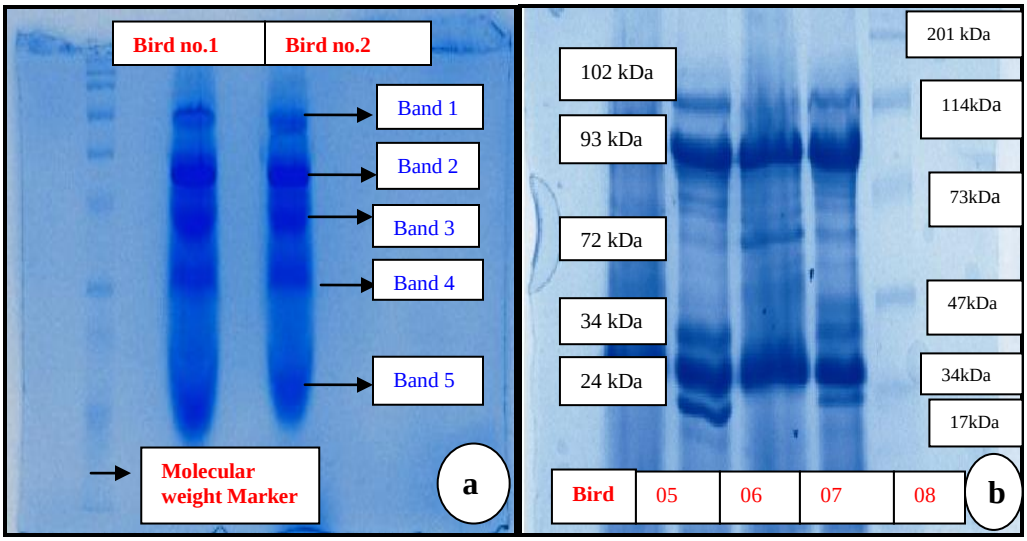
E3 ubiquitin protein ligase RNF 216 (*Struthio camelus australis*)

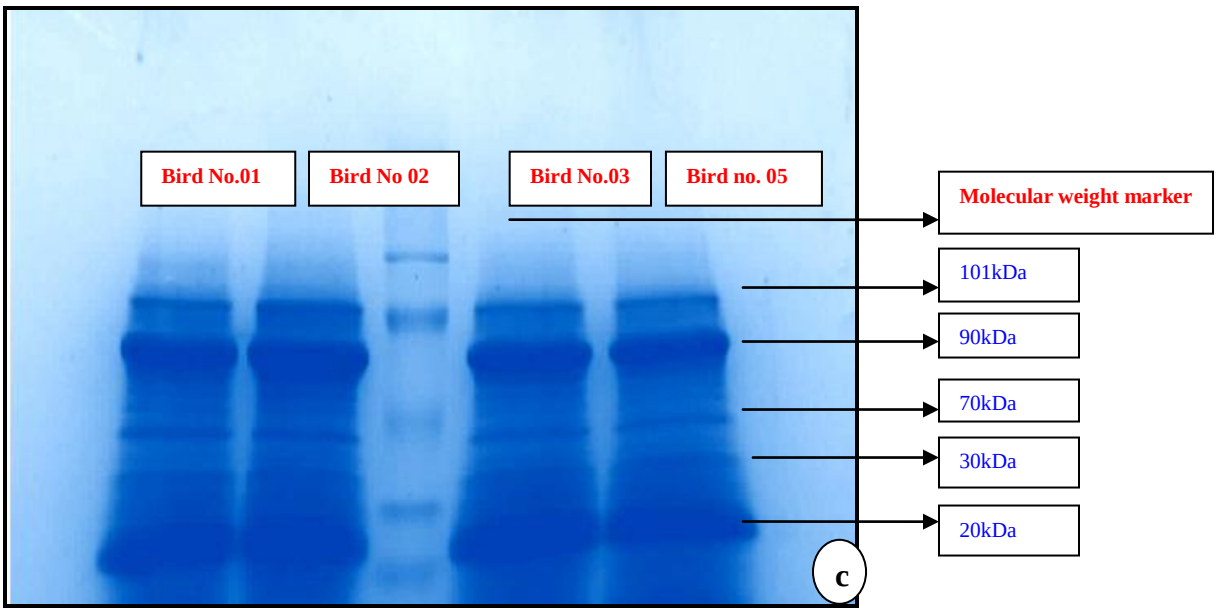
This was the 3rd major seminal plasma protein identified in the present study and the protein was reported to be involved in the capacitatition of spermatozoa which was located in acrosomal membrane.

Table.1 Major Protein bands identified in ostrich seminal plasma

Characteristics	**GATA Zinc finger domain containing protein 1** (*Struthio camelus australis*)	**GATA Zinc finger domain containing protein 1** (*Struthio camelus australis*)	**E3 ubiquitin protein ligase RNF 216** (*Struthio camelus australis*)	**Mitotic spindle assembly checkpoint protein MAD1** (*Struthio camelus australis*)	**Dual specificity phosphatase DUPD1** (*Struthio camelus australis*)
Protein band	**OSP-I**	**OSP-II**	**OSP-III**	**OSP-IV**	**OSP-V**
NCBI reference sequence	XP_009672619.1	XP_009672619.1	XP_00968359.1	XP_009688373.1	XP_009669492.1
Locus	XP_009672619	XP_009672619	XP_00968359	XP_009688373	XP_009669492
Amino acids	151 aa	151 aa	925 aa	717 aa	214 aa
Version	GI 697481422	GI 697481422	GI 697513075	GI 697438336	GI 697472637
Source/ organism	*Struthio camelus australis*	*Struthio camelus australis*	*Struthio camelus australis*	*Struthio camelus australis*	*Struthio camelus australis*
Isolate	BGI_N308	BGI_N308	BGI_N308	BGI_N308	BGI_N308
Sub species	*australis*	*australis*	*australis*	*australis*	*australis*
Gene prediction method	Gnomon, supported by mRNAand EST evidence	Gnomon, supported by mRNAand EST evidence	Gnomon, supported by EST evidence	Gnomon	Gnomon
Genomic sequence number	NW009271130.1	NW009271130.1	NW009272015.1	NW009270416.1	NW009270910.1
Documentation	NCBI'S Annotation process	NCBI'S Annotation process	NCBI'S Annotation process	NCBI'S Annotation process	NCBI'S Annotation process
Annotation status	Full annotation	Full annotation	Full annotation	Full annotation	Full annotation
Annotation pipeline	NCBI eukaryotic genome annotation	NCBI eukaryotic genome annotation	NCBI eukaryotic genome annotation	NCBI eukaryotic genome annotation	NCBI eukaryotic genome annotation
Completeness	Incomplete on the amino end	Incomplete on the amino end	Full length	Full length	Full length
Gene	GATAD1	GATAD1	RNF216	MAD1L1	DUPD1

Plate.1 SDS PAGE displaying the protein profile of seminal plasma in ostrich (a) & (b) Resolved protein bands (c) Molecular weight of different protein bands assessed through molecular weight marker





A large number of RING finger (RNF) proteins were present in eukaryotic cells and the majority of them are believed to act as E3 ubiquitin ligases. Pertinent to the search for new semen quality/fertility markers, ubiquitin is one of the secretory proteins in the epididymis and plays an important role in gametogenesis and fertilization.

Ostrich seminal plasma protein band IV (OSP-IV)

Mitotic spindle assembly checkpoint protein MAD1 (*Struthio camelus australis*)

This is the 4th major seminal plasma protein identified in the present study. This protein was mainly found in the secretion of epdidymis, seminal vesicle and testis.

Ostrich seminal plasma protein band V (OSP-V)

Dual specificity phosphatase DUPD1 (*Struthio camelus australis*)

This is the 5th major protein identified in the proteomic study of ostrich seminal plasma. Similar findings were observed by Thurston (1976) who identified six major protein fractions in turkey seminal plasma and observed that beta-3 fraction was the most prominent in turkey seminal plasma protein. Similarly, Thurston *et al.,* (1982) observed nine bands in guinea fowl seminal plasma and Marzoni *et al.,* (2013) detected a total of 83 protein spots in seminal plasma of chicken.

Molecular weight of different protein bands of ostrich seminal plasma

The molecular weight (kDa) of five major ostrich seminal plasma protein bands namely, OSP-I to OSP-V were estimated by using standard protein marker (Bio-Rad). The molecular weight of five ostrich seminal plasma protein bands (OSP I, II, III, IV and V) showed no significant difference among individual ostrich. All the 5 major expressed protein bands had a molecular weight ranging from 21.66 kDa to 102.34 kDa. The estimated molecular weight of ostrich seminal plasma protein *viz.,* OSP I (range between 94.51 kDa and 102.34 kDa), OSP II (75.19 kDa and 93.07 kDa), OSP III (59.58 kDa and 72.76 kDa), OSP IV (30.71 kDa and 40.90 kDa) and OSP V (21.66 kDa and 26.26 kDa) showed wide variations in expression. Thurston *et al.,* (1993) opined that the molecular weight of turkey seminal plasma proteins ranged from 25 to 80 kDa.

OSP-I and OSP-II identified in the present study are considered family of protein or isoform or identical subunits. Although the two proteins have different range of molecular weight, they are composed of same protein and thus may be isoenzymes, which is in agreement with the reports of Thurston *et al.,* (1993), who had found that, similar isoform for turkey seminal plasma enzyme. However, comprehensive proteomic study on ratites seminal plasma or even other poultry species has not been studied adequately for comparison. Ubiquitin (OSP-III) protein identified in this study is involved in the ubiquitin-proteosome pathway in gametogenesis and fertilization. Ubiquitination has been implicated in targeted proteolysis of histones and other proteins during spermatid elongation, in the degradation of the sperm mitochondria after fertilization, and in the sperm-zona penetration during fertilization as reported by Sutovsky *et al.,* (2004).

Mitotic spindle assembly checkpoint protein (MAD1) and Dual specificity phosphatase (DUPD1) protein were also identified in ostrich seminal plasma and many authors believe that this type of protein function to stabilize the spermatozoa against premature capacitation and spontaneous acrosome reaction.(Mortarino *et al.,* 1998; Moura *et al.,* 2006; Drabovich *et al.,* 2011; Milardi *et al.,* 2012).

This present study revealed, that proteomic analysis using SDS-PAGE reference map could represent a useful tool for the identification of still poorly understood nature and function of the ostrich seminal plasma proteins. Further,

correlation of seminal plasma protein with fertility parameters is the need of hour to identify potential marker for evaluation of fertility in male ostrich.

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