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Seminal Plasma Proteomics and Its Molecular Changes Associated with Seasonality in Male Ostrich

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Abstract

The proteins 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). Seasons had significant influence on the molecular weight of seminal plasma protein. The southwest monsoon had significant effects on molecular weight, when compared with other seasons. 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.

Key words: Ostrich, Seminal plasma protein, Seasonality.

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 and its seasonal changes that can serve as potential biomarkers for male infertility.

Material and methods

This experiment was carried out at Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu. This experiment was designed to analyze 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).This experiment was designed to analyze the protein profile of seminal plasma in nine individual birds for a period of 12 months to know the seasonal effects.

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 analyzed 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 Gen Bank to find out the proteins. The data were analyzed by One-way ANOVA as per the procedure of Duncan's multiple comparison test (Duncan, 1955)

Result and discussion

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). 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 seminal plasma

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) were observed in the present study. 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. 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).

Effect of month on molecular weight of different protein bands of ostrich seminal plasma

Molecular weight of different protein bands of ostrich seminal plasma as influenced by different months are presented in Table 1. Molecular weight of different protein bands in ostrich seminal plasma differed significantly among months. The molecular weight of OSP-I, OSP-II, OSP-IV and OSP-V showed highly significant differences ($P \leq 0.01$), while OSP-III showed no significant difference.

Table 1. Effect of month on molecular weight (mean ± SE) (kDa) of different protein bands of ostrich seminal plasma(n=120)

Months	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)	OSP-V (Dual specificity phosphatase DUPD1)
January (n=11)	$92.45^{de} \pm 2.66$	$81.95^{bc} \pm 3.80$	64.31 ± 2.80	$30.23^{def} \pm 0.89$	$21.94^{cde} \pm 0.64$
February (n=8)	$94.38^{cde} \pm 1.62$	$86.23^{bc} \pm 2.98$	72.43 ± 3.48	$35.11^{bcdef} \pm 2.63$	$25.18^{bc} \pm 1.16$
March (n=7)	$96.18^{bcde} \pm 0.86$	$86.03^{bc} \pm 2.90$	70.67 ± 4.15	$27.92^{ef} \pm 0.69$	$21.26^{cde} \pm 0.86$
April (n=15)	$90.07^{e} \pm 2.14$	$79.64^{c} \pm 2.47$	61.37 ± 2.31	$40.44^{abc} \pm 1.56$	$18.58^{e} \pm 0.41$
May (n=8)	$104.56^{abc} \pm 2.76$	$94.27^{ab} \pm 2.56$	76.16 ± 2.25	$38.43^{abcd} \pm 1.48$	$24.44^{bc} \pm 0.98$
June (n=7)	$114.90^{a} \pm 3.32$	$98.16^{a} \pm 1.79$	69.69 ± 2.88	$49.02^{a} \pm 2.54$	$32.29^{a} \pm 2.01$
July (n=9)	$107.02^{ab} \pm 1.73$	$94.04^{ab} \pm 1.34$	75.93 ± 1.03	$42.07^{ab} \pm 1.90$	$29.30^{ab} \pm 1.37$
August (n=10)	$102.07^{bcd} \pm 2.26$	$92.57^{abc} \pm 1.73$	72.16 ± 2.27	$38.74^{abcd} \pm 3.22$	$24.89^{bc} \pm 1.06$
September (n=13)	$99.71^{bcde} \pm 2.17$	$90.82^{abc} \pm 2.37$	71.34 ± 1.72	$37.37^{bcde} \pm 1.63$	$24.99^{bc} \pm 0.96$
October (n=8)	$91.95^{de} \pm 1.83$	$83.75^{bc} \pm 3.54$	66.62 ± 3.15	$35.25^{bcdef} \pm 2.67$	$24.07^{cd} \pm 1.94$
November (n=13)	$93.93^{cde} \pm 2.01$	$85.24^{bc} \pm 3.25$	62.90 ± 3.08	$30.93^{cdef} \pm 1.91$	$21.67^{cde} \pm 1.03$
December (n=11)	$94.78^{cde} \pm 2.22$	$87.30^{bc} \pm 3.49$	65.38 ± 3.35	$27.49^{f} \pm 1.17$	$19.18^{de} \pm 0.79$
Overall mean (n=120)	97.64 ± 0.87	87.67 ± 0.94	68.36 ± 0.96	35.87 ± 0.76	23.46 ± 0.44
F value	9.277	3.635	2.118	8.768	12.603
Significance	**	**	NS	**	**

n=No. of observation/month; Means bearing different superscripts within the same column differ significantly; **Highly significant (P≤0.01), NS- Not significant.

Effect of season on molecular weight of different protein bands of ostrich seminal plasma

Molecular weight of different protein bands of ostrich seminal plasma as influenced by different seasons are presented in Table 2. Molecular weight of ostrich seminal plasma proteins bands differed significantly among different seasons. Molecular weight of OSP-I, OSP-II, OSP-IV and OSP-V showed highly significant difference ($P \leq 0.01$), while OSP-III showed significant difference ($P \leq 0.05$) among seasons. OSP-I had a maximum molecular weight during southwest monsoon (104.73 kDa). However, there was no significant difference on the molecular weight among summer (95.36 kDa), northeast monsoon (93.73 kDa) and winter (93.26 kDa) seasons. Similarly, OSP-II had maximum molecular weight during southwest monsoon (93.33 kDa), followed by northeast monsoon (85.58 kDa), summer (85.03 kDa) and winter (83.75 kDa) seasons. OSP-III had maximum molecular weight during southwest monsoon (72.25 kDa). Summer (67.48 kDa) and winter (67.73 kDa) seasons had no significant difference on molecular weight of OSP-III, and very low expression of the protein was observed during northeast monsoon (64.68 kDa). OSP-IV had maximum molecular weight during southwest monsoon (40.90 kDa), followed by summer (36.98 kDa), winter (32.29 kDa) and northeast monsoons (30.83 kDa). OSP-V had maximum molecular weight during southwest monsoon (27.27 kDa). Further, there was no significant difference observed among summer (20.77 kDa), winter (23.31 kDa) and northeast monsoon (21.42 kDa) seasons. The results revealed that seasons had significant influence on the molecular weight of seminal plasma protein. The southwest monsoon had significant effects on molecular weight, when compared with other seasons. Nandre *et al.* (2013) identified four commonly expressed protein spots in buffalo bull (B6, B7, B9 and B10) during winter and summer season and four differently (B37W, B48W, B59W and B61W) expressed protein spots in winter season, with range of molecular weight approximately 14 kDa to 120 kDa, where in the range of 14 kDa to 63 kDa expressed during winter season, while, 66 kDa to 120 kDa expressed during summer season. The reason behind the expression of these proteins in this range of molecular weight is unknown. However, higher range of molecular weight observed in this study between May and September months (i.e. end of summer to southwest monsoon) may be associated with linkage of subunits or enzyme with major seminal plasma protein during this period to protect the spermatozoa from heat stress which are in agreement with findings of Thurston *et al*. (1993), Moura *et al.* (2006) and Nandre *et al.* (2013).

Table 2. Effect of season on molecular weight (mean ± SE) (kDa) of different protein bands of ostrich seminal plasma (n=120)

Season	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)	OSP-V (Dual specificity phosphatase DUPD1)
Winter (Jan-Feb) (n=19)	$93.26^{b} \pm 1.66$	$83.75^{b} \pm 2.52$	$67.73^{ab} \pm 2.32$	$32.29^{c} \pm 1.30$	$23.31^{b} \pm 0.70$
Summer (March-May) (n=30)	$95.36^{b} \pm 1.70$	$85.03^{b} \pm 1.89$	$67.48^{ab} \pm 1.96$	$36.98^{ab} \pm 1.28$	$20.77^{c} \pm 0.59$
Southwest monsoon (June-Sep) (n=39)	$104.73^{a} \pm 1.43$	$93.33^{a} \pm 1.06$	$72.25^{a} \pm 2.06$	$40.90^{a} \pm 1.37$	$27.27^{a} \pm 0.77$
Northeast monsoon (Oct-Dec) (n=32)	$93.73^{b} \pm 1.19$	$85.58^{b} \pm 1.94$	$64.68^{b} \pm 1.83$	$30.83^{c} \pm 1.18$	$21.42^{b} \pm 0.75$

Overall mean (n=120)	97.64 ± 0.87	87.67 ± 0.94	68.36 ± 0.96	35.87 ± 0.76	23.46 ± 0.44
F value	15.387	6.690	2.719	12.529	18.620
Significance	**	**	*	**	**

n=No. of observation/season; Means bearing different superscripts within the same column differ significantly; **Highly significant (P≤ 0.01);* Significant (P≤ 0.05).

Conclusion

This present study concluded that major protein bands of seminal plasma had the maximum range of molecular weight during May and September and the remaining months showed no variation. Similarly, the southwest monsoon had higher molecular weight expression, when compared to other seasons. The 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.

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