Original Research

Seasonal Variations in Seminal Plasma Protein of Ostrich (*Struthio camelus*)

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Abstract

This study was designed to evaluate the influence of season on seminal plasma protein expression of ostrich semen. The ostrich seminal plasma (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) were observed in the present study. Months and season had significant influence on the molecular weight of ostrich seminal plasma protein. The southwest monsoon had significant effects on molecular weight, when compared with other seasons. From the study, it was concluded that seminal plasma contains specific proteins in particular season, which may be associated with some of the semen characteristics, and these proteins could be used as markers of the semen quality of ostrich.

Key words: Ostrich, Seminal Plasma Protein, Seasonal Variations

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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. Further, composition of seminal plasma has a great influence on the biological quality of the ostrich semen. Seminal plasma (SP) is known to play

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an important role in fertilization. Proteins are one of the major component inseminal plasma 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 designed to evaluate the influence of season on seminal plasma protein expression of ostrich semen.

Commented [R1]: Component in seminal plasma

Material 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, India. This experiment was designed to analyse the protein profile of seminal plasma in nine ostrich. The protein profile of seminal plasma for a period of 12 months was analysed to know the seasonal effects. Nine male ostrich were selectedfor 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 120 seminal plasma samples 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 NCBI reference sequences in GenBank to find out the proteins.

Commented [R2]: delete

Statistical Methods

The effect of various sources of variation on continuous traits were analysed by One-way ANOVA as per the procedure of Duncan's multiple comparison test (Duncan 1955). A value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Protein Profile of Ostrich Seminal Plasma

The protein bands in ostrich seminal plasma (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.

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Molecular Weight of Different Protein Bands of Ostrich 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.

Effect of Age on Molecular Weight of Different Protein Bands of Ostrich Seminal Plasma

The male age had no significant difference on molecular weight expression in seminal plasma protein of ostrich. The average molecular weight of different protein bands varied from 23.46 kDa to 97.64 kDa.

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. Month had significant influence on the molecular weight of ostrich seminal plasma protein. The identified five major protein bands had the maximum range of molecular weight between May and September months and the remaining months show no variation.

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

Months (2015)	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)	$93.93^{cde} \pm 2.01$	$85.24^{bc} \pm 3.25$	62.9 ± 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	**	**

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

Molecular weight of different protein bands of ostrich seminal plasma as influenced by different seasons are presented in Table 2.

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

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] ± 1.66	83.75[b] ± 2.52	67.73[ab] ± 2.32	32.29[c] ± 1.30	23.31[b] ± 0.70
Summer (March-May) (n=30)	95.36[b] ± 1.70	85.03[b] ± 1.89	67.48[ab] ± 1.96	36.98[ab] ± 1.28	20.77[c] ± 0.59
Southwest monsoon (June-Sep) (n=39)	104.73[a] ± 1.43	93.33[a] ± 1.06	72.25[a] ± 2.06	40.90[a] ± 1.37	27.27[a] ± 0.77
Northeast monsoon (Oct-Dec) (n=32)	93.73[b] ± 1.19	85.58[b] ± 1.94	64.68[b] ± 1.83	30.83[c] ± 1.18	21.42[b] ± 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	6.69	2.719	12.529	18.62
Significance	**	**	*	**	**

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).

Conclusion

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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