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Seasonal pattern on semen characteristics and correlation with testosterone levels in farm-raised ostrich (*Struthio camelus*)

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Abstract
Correlation between semen characteristics and testosterone levels in ostrich were studied for a period of 12 months during the year 2015. The mean volume of semen per ejaculate at winter, summer, southwest monsoon and northeast monsoon were observed to be 1.21, 1.33, 1.52 and 1.40 ml, respectively. Significantly ($P \leq 0.01$) higher mass activity score was observed in semen collected in summer (4.66) and winter (4.57), followed by southwest (3.93) and northeast monsoon (3.85). Semen collected during summer season had significantly ($P \leq 0.01$) higher per cent motility (85.25), followed by winter (83.68), southwest monsoon (74.83) and northeast monsoon (73.48) and this trend was similar to mass activity. The seasonal pattern on spermatozoa concentration ($x10^9$/ml) was observed that the concentration increased from northeast monsoon season (2.57) and reached peak during winter season (3.20) then gradually declined during summer (2.88) and southwest monsoon (2.27). The mean values of live spermatozoa (%) were 88.17 in winter, 90.20 in summer, 82.59 in southwest and 81.00 in northeast monsoon season. The blood plasma and seminal plasma testosterone levels showed highly significant differences ($P<0.01$) among seasons. The blood plasma testosterone levels were found to increase from northeast monsoon and maintaining peak during winter and summer thereafter, the level gradually reduced during southwest monsoon. A similar trend was also observed for seminal plasma testosterone suggesting that semen characteristics and testosterone levels are seasonally influenced in farmed-raised ostriches under tropical conditions.

Keywords: Semen characteristics, Testosterone, 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. Ostrich are considered to be seasonal breeders, although they may also breed all year round (Degen *et al*., 1994) [5]. Unpredictable egg production, unstable fertility, poor hatchability and poor chick survival are some of the major constraints in viable ostrich farming. Seasonal effects on testosterone and semen characteristics in ostrich have not been well documented and could provide information that is needed to understand the underlying endocrine mechanisms associated with testicular function. Such information may be useful in developing breeding strategies in ostrich reproduction. Hence, this study was carried out to assess the effect of season on semen characteristics and correlation with testosterone levels in ostriches under tropical conditions.

Materials and Methods
Experimental Location
This experiment was carried out at Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University, Kattupakkam, Kanchipuram district in Tamil Nadu, India during 2015. The station is situated approximately at 12.5ºN latitude and 80º to 81ºE longitudes and at the altitude of 48 meter above mean sea level. Being nearer to East coast of India it enjoys a tropical maritime monsoon climate. This station gets most of its seasonal rainfall from the northeast monsoon i.e. during October to December (Indian Metrological Department, Pune).

Selection of ostrich and semen collection method
Nine adult male ostrich aged four to nine years were selected based on their phenotypic characters and mating behavior; and housed in trios i.e. one male with two females providing a floor space of 1500 m^2 per trio. All the birds were housed under standard management conditions.

Selected male ostrich were trained for semen collection by teaser method as recommended by earlier authors (Malecki and Martin 2005; Rybnik *et al*., 2008) [10, 13].

Evaluation of semen

The semen collected from a total of nine individual male birds were evaluated for volume, pH, mass activity, motility, concentration, live and abnormal spermatozoa. The volume of the semen was measured directly by observing the marking on the collection tube with 0.1ml accuracy. The mass activity of the spermatozoa was estimated based on the wave motion. The activity was ranked on a 0 (no motility) to 5 scale (vigorus progressive movement, rapid edges) (Allen and Champion, 1955) [2]. The motility was assessed and expressed in percentage (Parker *et al*., 1942) [11]. The concentration of spermatozoa in fresh undiluted semen was determined by using a "NEUBAUER" type hemocytometer and the final concentration of spermatozoa expressed as millions ($\times 10^9$) per ml (Allen and Champion, 1955) [2]. The viability of spermatozoa was determined by Eosin-Nigrosin staining procedure (Bakst and Cecil 1997) [3] and abnormal spermatozoa were expressed in percentage (Alkan *et al*., 2002) [1]. The semen collected during a period of 12 months consequently were analysed to know the seasonal effects. The seasons were categorized as winter (January to February), summer (March to May), southwest (June to September) and northeast (October to December) monsoon. (*Indian Metrological Department, Pune, India*).

Estimation of testosterone

In this experiment, testosterone was assessed from the above nine birds for a period of 12 months. Blood sample was collected from wing vein using heparinized vial and the vial was centrifuged (2500 rpm) for 15 min and the resulting plasma was collected and stored at -80°C until assayed. The quantitative determination of total testosterone both in plasma of peripheral blood and seminal plasma of male ostrich were carried out by competitive immunoassay by using Testosterone ELISA kit (Enzo Life Sciences Inc, Farmingdale, NY 11735) as per the test procedure (Catalog #: ADI-900-065, Enzo Life Sciences Inc, Farmingdale, NY 11735)

Statistical Analyses

The effect of various sources of variation on seminal atributes and testosterone levels were analysed by One-way ANOVA as per the procedure of Duncan's multiple comparison test (Duncan, 1955) [6]. Proportion data were converted to Arcsine values before ANOVA. A value of $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 20.0 (*SPSS Inc., Illinois, USA)*.

Results

Seasonal pattern on semen characteristics

Effect of season on semen characteristics of ostrich are presented in Table 1.

Table 1: Effect of season on semen characteristics (mean ± SE) of ostrich.

3	Semen volume (ml)	pH	Mass activity Score (1-5)	Sperm motility (%)	Sperm conc. ($\times 10^9$/ml)	Live sperms (%)	Abnormal spermatozoa (%)		
							Head	Tail	Total
Winter (Jan-Feb) (n= 38)	$1.21^{b}\pm0.10$	$7.17^{a}\pm 0.05$	$4.57^{a}\pm0.10$	$83.68^{a}\pm1.71$	$3.20^{a}\pm0.11$	$88.17^{a}\pm1.57$	$6.63^{ab}\pm0.32$	$7.87^{a}\pm 0.38$	$14.50^{ab}\pm0.36$
Summer (March-May) (n= 59)	$1.33^{ab}\pm0.10$	$7.19^{a}\pm 0.04$	$4.66^{a}\pm0.08$	$85.25^{a}\pm1.37$	$2.88^{b}\pm0.09$	$90.20^{a}\pm1.26$	$6.01^{a}\pm0.26$	$7.37^{a}\pm 0.21$	$13.38^{a}\pm0.53$
Southwest monsoon (June-Sep) (n= 59)	$1.52^{a}\pm0.08$	$7.35^{b}\pm 0.04$	$3.93^{b}\pm0.08$	$74.83^{b}\pm1.36$	$2.27^{d}\pm0.09$	$82.59^{b}\pm1.25$	$7.10^{b}\pm0.26$	$8.23^{a}\pm 0.31$	$15.34^{b}\pm0.52$
Northeast monsoon (Oct-Dec) (n= 46)	$1.40^{ab}\pm0.10$	$7.39^{b}\pm 0.05$	$3.85^{b}\pm0.09$	$73.48^{b}\pm1.86$	$2.57^{c}\pm0.10$	$81.00^{b}\pm1.63$	$8.54^{c}\pm0.39$	$9.81^{b}\pm 0.35$	$18.35^{c}\pm0.60$
Overall mean (n=202)	1.38±0.05	7.28± 0.02	4.26±0.04	79.21±0.85	2.69±0.05	85.71±0.73	7.02±0.16	8.27± 0.18	15.30±0.31
F value	2.218	5.164	23.220	16.350	16.480	9.261	14.293	9.785	13.571
Significance	*	**	**	**	**	**	**	**	**

n=No. of ejaculate/season; Means bearing different superscripts within the same column differ significantly; ** ($P \leq 0.01$); * ($P \leq 0.05$).

The mean volume of semen per ejaculate at winter, summer, southwest monsoon and northeast monsoon were observed to be 1.21 ± 0.10, 1.33 ± 0.10, 1.52±0.08 and 1.40 ± 0.10 ml, respectively. Significantly ($P \leq 0.01$) higher mass activity score was observed in semen collected in summer (4.66 ± 0.08) and winter (4.57 ± 0.10), followed by southwest (3.93 ± 0.08) and northeast monsoon (3.85 ± 0.09), which indicate the effect of season on mass activity. In contrast Rybnik *et al.* (2008) [13] observed no significant variation in spermatozoa motility among different season. Semen collected during summer season had significantly ($P \leq 0.01$) higher per cent motility (85.25 ± 1.37), followed by winter (83.68±1.71), southwest monsoon (74.83 ± 1.36) and northeast monsoon (73.48 ± 1.86) and this trend was similar to mass activity. In this study, the spermatozoa concentration ($x10^9$/ml) increased during northeast monsoon (2.57 ± 0.10) and reached the peak during winter season (3.20 ± 0.11), then gradually declined during summer (2.88 ± 0.09) and southwest monsoon seasons (2.27 ± 0.09), which concurs with the findings (Rybnik *et al.,* 2008) [13] in ostrich. The mean values of per cent live spermatozoa were 88.17 ± 1.57 in winter, 90.20 ± 1.26 in summer, 82.59 ± 1.25 in southwest and 81.00 ± 1.63 in northeast monsoon season, indicating significant ($P \leq 0.01$) differences among seasons. Percentage of live spermatozoa observed in this study was almost similar with the earlier findings (Rybnik *et al.,* 2008) [13] in ostrich. This pattern revealed that the winter and summer seasons are the main breeding season and southwest and northeast monsoon seasons are the off-season in ostrich breeding. The semen collected during summer season had significantly ($P \leq 0.01$) lesser (13.38 %) abnormal spermatozoa followed by winter (14.50 %), southwest (15.34 %) and northeast monsoon (18.35 ± %) seasons. Similarly, Bertschinger *et al.* (1992) [4], Irons *et al.* (1996) [9] and Rozenboim *et al.* (2003) [12] in ostrich observed more abnormal spermatozoa at the beginning and end of breeding season than mid season.

Season pattern on blood plasma and seminal plasma testosterone levels

Blood plasma and seminal plasma testosterone levels in ostrich as influenced by different seasons are presented in Table 2.

Table 2: Effect of season on testosterone levels (mean ± SE) in ostrich.

Season	Blood plasma testosterone (ng/ml)	Seminal plasma testosterone (ng/ml)
Winter (Jan-Feb)(n=18)	$11.04^{a} \pm 0.93$	$8.85^{a} \pm 0.90$
Summer (March-May)(n=27)	$9.10^{b} \pm 0.65$	$7.95^{a} \pm 0.91$
Southwest monsoon(June-Sep)(n=36)	$8.31^{b} \pm 0.34$	$6.00^{b} \pm 0.19$
Northeast monsoon (Oct-Dec)(n=27)	$9.55^{ab} \pm 0.39$	$8.12^{a} \pm 0.31$
Overall mean(n=108)	9.17 ± 0.28	7.26 ± 0.31
F value	3.919	4.173
Significance	** (P≤0.01).	

Figure in parenthesis indicate number of observation/season; Means bearing different superscripts within the same column differ significantly.

Seasonal influence on blood plasma and seminal plasma testosterone of ostrich are highly significant (P≤0.01). Both blood plasma and seminal testosterone levels escalated to increase from northeast monsoon (9.55 ng/ml and 8.12 ng/ml) and maintained peak during winter (11.04 ng/ml and 8.85 ng/ml) and summer (9.10 and 7.95 ng/ml), thereafter the level gradually reduced during southwest monsoon (8.31 ng/ml and 6.00 ng/ml). This pattern is coinciding with observations of Walsangkar (2010) [15] in ostrich who found, increasing level of blood and seminal plasma testosterone during early (3.5 ng/ml and 0.45 ng/ml) and peak seasons (14.89 ng/ml and 1.7 ng/ml). Further, the present finding was also in agreement with earlier reports of Sundaresan (2014) [14] in emu, who observed increasing level of serum testosterone level from early to peak seasons (2.47 to 11.69 ng/ml) and thereafter the level reduced (7.43 ng/ml) at the end of the breeding season. However, comparatively higher levels of blood testosterone during off-season and breeding season were also observed by Bertschinger *et al*. (1992) [4] in ostrich (19.81 and 144.90 ng/ml, respectively) and Goes *et al.* (2010) [7] in captive rhea (5.57 and 53.28 ng/ml, respectively).

Correlation coefficient of testosterone levels with semen characteristics

Correlation coefficients of blood plasma and seminal plasma testosterone levels with semen characteristics of ostrich are presented in Table 3.

Table 3: Correlation coefficient of testosterone levels with semen characteristics of ostrich.

Parameters	Semen volume	Sperm motility	Sperm concentration	Live sperm	Head abnormality	Tail abnormality	Total abnormality	Blood plasma testosterone	Seminal plasma testosterone
Blood plasma testosterone (n=96)	0.024	0.478**	0.455**	0.398*	-0.071	-0.167	-0.142	1.000	0.799**
Seminal plasma testosterone (n=66)	0.109	0.560**	0.486**	0.447*	-0.179	-0.136	-0.173	0.799**	1.000

** Highly significant (P≤0.01) * Significant (P≤0.05)

Highly significant (P<0.01) positive association among blood plasma and seminal plasma testosterone with semen characteristics were revealed. Reproductive hormones play an important role in ostrich breeding. The vital quality of semen, especially spermatozoa motility, concentration and live spermatozoa had very strong significant (P<0.01) positive correlations with blood plasma and seminal plasma testosterone which have also been found in other species. This is evident from the earlier findings of Bertschinger *et al.* (1992) [4] who observed increased density of spermatozoa with elevated level of testosterone concentration in ostrich. This correlation may be explained by the fact that spermatozoa production depends on testosterone levels and stimulate spermatogenesis as reported by Hafez and Hafez (2004) [8]. The correlation between semen volume and testosterone may be due to enlargement of testes size, increase in parenchymal volume and number of Leydig cells, which is responsible for testosterone production, would lead to higher spermatozoa production (Goes *et al.,* 2010) [7]. Though not significant (P>0.05), the per cent head, tail and total abnormal spermatozoa showed negative correlations with both blood plasma and seminal plasma testosterone.

Conclusion

Based on this study it is concluded that a raise and fall in semen quality attributes was noticed from winter and summer to southwest and northeast monsoon seasons for all the semen characteristics except abnormal spermatozoa. Further, the effect of season on blood plasma and seminal plasma testosterone showed significant (P≤0.01) difference and the level increased from northeast monsoon and maintained peak during winter and summer thereafter, the level gradually reduced during southwest monsoon clearly indicated that the ostrich is a seasonal breeder and the semen characteristics and testosterone levels are differed among seasons. Similarly, relationship between semen characteristics and testosterone level gives an insight to the physiological signaling in ostrich reproduction which will be useful in designing better breeding programme in ostrich.

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