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Effect of month and season on female reproductive hormones in farmed ostriches (*Struthio camelus*)

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Abstract
Assessment of reproductive hormones levels *viz.*, estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) during breeding and non-breeding seasons were studied for a period of 12 months in four to 12 year-old female ostrich. The mean blood plasma estrogen, FSH and LH in female ostrich were 6.99 pg/ml, 0.48 ng/ml and 2.23 ng/ml, respectively. The effect of the month and season on estrogen, FSH and LH level showed significant ($P \leq 0.01$) difference. The estrogen (pg/ml), FSH (ng/ml) and LH (ng/ml) levels were found to increase during the northeast monsoon (7.28 ± 0.21, 0.55 ± 0.02 and 2.17 ± 0.10, respectively) and reached peak during winter (8.84 ± 0.26, 0.58 ± 0.02 and 2.52 ± 0.11, respectively), followed by summer (7.02 ± 0.25, 0.44±0.02 and 2.48 ± 0.08, respectively) and then maintained at baseline level during southwest monsoon (5.82 ± 0.16, 0.40 ± 0.01 and 1.94 ± 0.04, respectively).

Keywords: Reproductive hormone, seasonal effect, female ostrich

1. 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 is very adaptable and can be farmed in almost all climatic conditions, but are ideally suited to dry and arid climate. Ostrich is considered to be seasonal breeders, although they may also breed all year round [5]. During the breeding season, male and female ostrich are reproductively active for six to eight months of the year [10]. Reproductive wastage makes the production performance of ostrich bird highly inefficient; this is due to low egg fertility and hatchability. Many farms attempt to solve problems in production efficiency by changing their management practices or by applying the latest research outcomes, but progress is very slow and often geographically isolated [8]. Not much work has been done on the female reproductive hormone i.e. Estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in ostrich; hence the present work becomes a pioneering study in India. With these backgrounds, the present investigation has been taken up to assess the above reproductive hormone levels during breeding and non-breeding seasons in the tropical climate of India.

2. Materials and Methods
2.1 Experimental locations
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 meters above mean sea level. Being nearer to East coast of India it enjoys a tropical maritime monsoon climate. During this study period, the average maximum and minimum temperature, relative humidity and the annual rainfall were recorded as 34.2 ºC, 23.2 ºC, 87.3 per cent and 1391 mm, respectively. This station gets most of its seasonal rainfall from the northeast monsoon i.e. during October to December. 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).

2.2 Selection of Ostrich

Nine female ostrich aged four to 12 years were selected based on their reproductive characters (fertility above 20% and egg production of between 30 to 40 eggs per year) 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 managed conditions.

2.3 Experimental design, blood collection and estimation of reproductive hormones

Hormone profiles like estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in female ostrich were assessed 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.

2.4 Estimation of estrogen, FSH and LH

The quantitative determination of estradiol-17 β in blood plasma of female ostrich was carried out by enzyme linked immunosorbent assay (ELISA) based on the principle of competitive binding by using estradiol 17 β ELISA kit (IBL International GMBH, Germany). Test procedure was carried out as per the Catalog #: RE52041, IBL International GMBH, Germany. The quantitative determination of FSH in blood plasma of female ostrich birds was carried out by sandwich principle using an FSH ELISA kit (IBL International GMBH, Germany). Test procedure was carried out as per the Catalog #: RE 52121, IBL International GMBH, Germany. The quantitative determination of LH in blood plasma of female ostrich was carried out by typical two-step capture or sandwich principle using a LH ELISA kit (LDN Labor Diagnostika North GmbH & Co.KG). Test procedure was carried out as per the Catalog#: FR E-2600, LDN Labor Diagnostika North GmbH & Co.KG, AM Eichenhain, Nordhorn.

2.5 Statistical Analysis

The effect of various sources of variation in reproductive hormone levels was analysed by One-way ANOVA as per the procedure of Duncan's multiple comparison test (Duncan, 1955) [6]. A value of $P<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Illinois, USA).

3. Results and discussion

3.1 Reproductive hormones in ostrich

Blood plasma estrogen, FSH and LH levels of ostrich are presented in Table 1. The overall mean values of blood plasma estrogen, FSH and LH of a female ostrich were 6.99±0.17 pg/ml, 0.48 ± 0.01 ng/ml and 2.23 ± 0.04 ng/ml, respectively.

Table 1: Blood plasma estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (mean ± SE) in ostrich

Bird No.	Reproductive hormone		
	Estrogen (pg/ml)	FSH (ng/ml)	LH (ng/ml)
01 (n=12)	7.01 ± 0.34	0.45 ± 0.03	2.12 ± 0.05
02 (n=12)	7.81 ± 0.63	0.47 ± 0.03	2.34 ± 0.21
03 (n=12)	6.47 ± 0.52	0.46 ± 0.02	2.27 ± 0.12
04 (n=12)	7.07 ± 0.57	0.47 ± 0.03	1.99 ± 0.16
05 (n=12)	7.40 ± 0.50	0.43 ± 0.03	2.12 ± 0.12
06 (n=12)	6.37 ± 0.43	0.54 ± 0.04	2.35 ± 0.15
07 (n=12)	7.41 ± 0.41	0.48 ± 0.02	2.15 ± 0.09
08 (n=12)	6.55 ± 0.69	0.48 ± 0.04	2.29 ± 0.16
09 (n=12)	6.80 ± 0.37	0.51 ± 0.04	2.43 ± 0.13
Over all mean (n=108)	6.99 ± 0.17	0.48 ± 0.01	2.23 ± 0.04
F value	0.928	0.894	1.030
Significance	($P>0.05$)		

n=No. of observation/bird

The mean concentration of blood plasma estrogen in female ostrich observed in this study was 6.99 pg/ml, which is comparable to the earlier reports [3] in ostrich, who have observed 17.9, 28.3 and 7.0, (pg/ml) during 24 h and 6 h before, and 24 h after ovulation, respectively. The wide variations in concentration of estrogen were also observed in ostrich (10-70pg/ml) [5] and in emu (3.81 to 4.41 ng/ml) [11]. The mean concentration of blood plasma FSH observed in female ostrich was 0.48 ng/ml. The mean concentration of blood plasma LH observed in female ostrich was 2.23 ng/ml which, concurs with evidence of 1.9, 2.7 and 1.7 ng/ml during the periods of 24 h and 6 h before, and 24 h after ovulation in ostrich [3, 5]; and in emu (0.5 to 2.5 ng/ml) [2]. In our study the FSH concentration does not fluctuate as much as that of estrogen and LH, this is because of the added regulatory feedback mechanism of inhibin within the regulatory pathways for FSH secretion. The increasing or decreasing levels of estrogen, FSH and LH reported in the literature could be ascribed to the difference in breed, strain, age, geographical location, nutrition and analysing methods.

3.2 Effect of month on reproductive hormones

Blood plasma estrogen, FSH and LH in ostrich as influenced by different months are presented in Table 2.

Table 2: Effect of month on blood plasma estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels (mean ± SE) in ostrich

Month (2015)	Reproductive hormone		
	Estrogen (pg/ml)	FSH (ng/ml)	LH (ng/ml)
January (n=9)	$9.14^{a} \pm 0.28$	$0.61^{a} \pm 0.03$	$2.66^{a} \pm 0.18$
February (n=9)	$8.53^{ab} \pm 0.44$	$0.55^{abc} \pm 0.02$	$2.37^{abc} \pm 0.13$
March (n=9)	$7.84^{abc} \pm 0.48$	$0.45^{bcde} \pm 0.03$	$2.54^{ab} \pm 0.16$
April (n=9)	$7.06^{bcd} \pm 0.42$	$0.52^{abcd} \pm 0.02$	$2.44^{abc} \pm 0.12$
May (n=9)	$6.16^{cd} \pm 0.21$	$0.34^{e} \pm 0.01$	$2.47^{abc} \pm 0.16$
June (n=9)	$6.07^{cd} \pm 0.15$	$0.34^{e} \pm 0.01$	$2.10^{abc} \pm 0.05$
July (n=9)	$6.19^{cd} \pm 0.17$	$0.43^{cde} \pm 0.02$	$1.87^{c} \pm 0.13$
August (n=9)	$5.66^{d} \pm 0.40$	$0.41^{de} \pm 0.01$	$1.93^{bc} \pm 0.08$
September (n=9)	$5.37^{d} \pm 0.45$	$0.45^{bcde} \pm 0.03$	$1.88^{bc} \pm 0.07$
October (n=9)	$5.52^{d} \pm 0.68$	$0.51^{abcd} \pm 0.03$	$1.93^{bc} \pm 0.11$
November (n=9)	$7.11^{bcd} \pm 0.47$	$0.59^{a} \pm 0.03$	$2.25^{abc} \pm 0.25$
December (n=9)	$9.22^{a} \pm 0.27$	$0.55^{ab} \pm 0.04$	$2.32^{abc} \pm 0.15$
Overall mean (n=108)	6.99 ± 0.17	0.48 ± 0.01	2.23 ± 0.05
F value	12.295	11.311	3.991
Significance	** ($P \leq 0.01$)		

n=No. of observation/month; Means bearing different superscripts within the same column differ significantly.

Effect of months on blood plasma estrogen, FSH and LH levels in ostrich are significant ($P<0.01$). The blood plasma estrogen started to increase during November (7.11 pg/ml) and maintained the maximum level between December and April (8.35 pg/ml) then reduced to baseline level between May and October (5.82 pg/ml), which is in agreement with reports [5] who have observed maximum level during May (70 pg/ml) and baseline value between October and December (10 pg/ml). Similar trend was also observed by [12] in captive rhea, who observed maximum level (5.5 pg/ml) between November and December and baseline level between April and February (3.5 pg/ml). The concentration of FSH started to increase from October (0.51 ng/ml) and reached peak during January (0.61 ng/ml) then gradually reduced and maintained a baseline level between May and September (0.39 ng/ml). Similarly, maximum level of LH was maintained between November and June (2.39 ng/ml) and minimum level between July and October (1.90 ng/ml), which is similar to the findings [5] in ostrich who have observed maximum level between February and March (4.0 ng/ml) and minimum baseline level between October to December (2.0 ng/ml).

In this study the maximum level of estrogen concentration observed between December and March months indicates the major reproductive activity existed during this period. This may be due to that estradiol is involved in the reproductive tract development and mainly produced by the thecal internal cells of matured follicle and peaks during the egg laying cycle [4]. Similarly, LH in female birds is mainly involved in maturation of the follicle and in ovulation and stimulates ovary steroidogenesis [7, 9]. In this study, baseline level of LH observed between July and November months indicate the poor ovary steroidogenesis during these periods.

3.3 Effect of season on reproductive hormones

Blood plasma estrogen, FSH and LH in ostrich as influenced by different seasons are presented in Table 3.

Table 3: Effect of season on blood plasma estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels (mean ± SE) in ostrich.

Season	Reproductive hormone		
	Estrogen (pg/ml)	FSH (ng/ml)	LH (ng/ml)
Winter (Jan-Feb)(n=18)	$8.84^{a} \pm 0.26$	$0.58^{a} \pm 0.02$	$2.52^{a} \pm 0.11$
Summer (March-May) (n=27)	$7.02^{b} \pm 0.25$	$0.44^{b} \pm 0.02$	$2.48^{a} \pm 0.08$
Southwest monsoon (June-Sep)(n=36)	$5.82^{c} \pm 0.16$	$0.40^{b} \pm 0.01$	$1.94^{b} \pm 0.04$
Northeast monsoon (Oct-Dec)(n=27)	$7.28^{b} \pm 0.21$	$0.55^{a} \pm 0.02$	$2.17^{b} \pm 0.10$
Overall mean (n=108)	6.99 ± 0.17	0.48 ± 0.01	2.23 ± 0.05
F value	17.960	23.474	12.166
Significance	** ($P \leq 0.01$)		

n=No. of observation/season; Means bearing different superscripts within the same column differ significantly.

The estrogen (pg/ml), FSH (ng/ml) and LH (ng/ml) started to increase during the northeast monsoon (7.28, 0.55 and 2.17, respectively) and reached maximum during winter (8.84, 0.58 and 2.52, respectively), followed by summer (7.02, 0.44 and 2.48, respectively) then reduced to baseline level during southwest monsoon (5.82, 0.40 and 1.94, respectively). Similarly, in emu observed the highest level of LH during winter months (4 to 5 ng/ml), plateau in the spring and baseline level at the end of breeding season [1]. Results of this study proved that the changes in the hormone levels are seasonality dependent in ostrich.

4. Conclusion

From the study, it is evident that the female reproductive hormone viz., estrogen, FSH and LH levels in ostrich along started to increase during northeast monsoon and reached peak during winter and maintaining the maximum level during summer then gradually decrease towards southwest monsoon provide indication that mechanism of initiating and terminating the hormone level in ostrich are seasonal dependent, so called winter-summer breeders, although they also breed all round the year.

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