

**ASSESSMENT OF REPRODUCTIVE EFFICIENCY IN
OSTRICH (*Struthio camelus*)**

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CERTIFICATE

This is to certify that the thesis entitled "ASSESSMENT OF REPRODUCTIVE EFFICIENCY IN OSTRICH (*Struthio camelus*)" submitted in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY in POULTRY SCIENCE** to the **TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY, CHENNAI - 600 051**, is a record of bonafide research work carried out by **C. PANDIAN**, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journals or magazine.

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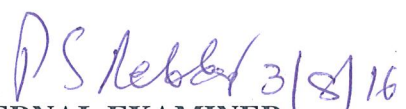

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ABSTRACT

ASSESSMENT OF REPRODUCTIVE EFFICIENCY IN OSTRICH (*Struthio camelus*)

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A study was conducted to evaluate the semen characteristics and biometry of spermatozoa, reproductive hormonal levels during breeding and non-breeding seasons and their effects on the follicular development, protein profile of semen, sperm-egg interaction assay and standardization of artificial insemination technique in ostrich. Ten adult male and twenty female ostrich aged between three to ten years were selected based on their phenotypic characters and mating behaviour. All the birds were maintained under standard feeding and management conditions.

Totally five experiments were conducted. In experiment-1, evaluation of semen characteristics and biometry of spermatozoa in ostrich were studied. The male ostrich were trained for semen collection by teaser method using artificial cloaca. The overall mean values for frequency of semen collection, semen

volume, pH, mass activity, per cent motility, concentration (10^9 per ml), percent live and total abnormal spermatozoa of ostrich were 17.36 days, 1.38 ml, 7.28, 4.26, 79.21, 2.69, 85.71 and 15.30, respectively. Age, month and season had a highly significant ($P \leq 0.01$) influence on seminal attributes. In morphometric analyses of ostrich spermatozoa, the acrosome and nucleus mean length was $1.22 \mu\text{m}$ and $14.14 \mu\text{m}$, giving a mean total head length of $15.36 \mu\text{m}$. The mean length of mid-piece, principal piece and end piece were $3.04 \mu\text{m}$, $54.82 \mu\text{m}$ and $2.99 \mu\text{m}$, respectively. The mean total tail length was $60.86 \mu\text{m}$ and the mean total spermatozoa length was $76.22 \mu\text{m}$. The mean nucleus, mid-piece and principal piece width were $1.05 \mu\text{m}$, $0.63 \mu\text{m}$ and $0.39 \mu\text{m}$, respectively.

In experiment-2, assessment of reproductive hormonal levels during breeding and non-breeding seasons and their effects on the follicular development were studied. Highly significant difference ($P \leq 0.01$) was observed in blood plasma (9.17 ng/ml) and seminal plasma testosterone level (7.26 ng/ml) between individual male ostrich. Effect of month and season showed highly significant ($P \leq 0.01$) influence on blood plasma testosterone. The blood plasma testosterone levels were found to increase from northeast monsoon (9.55 ng/ml) and maintained peak during winter (11.04 ng/ml) and summer (9.10 ng/ml), thereafter, the level gradually reduced during southwest monsoon (8.31 ng/ml). Similar trend was also observed for seminal plasma testosterone. There was no significant difference observed in reproductive hormones namely, blood plasma estrogen, FSH and LH between individual female and the overall mean values were 6.99 pg/ml , 0.48 ng/ml and 2.23 ng/ml , respectively. However, blood plasma estrogen, FSH, LH and ultrasonography features of ovarian follicle were significantly ($P \leq 0.01$) influenced by months and seasons. The estrogen, FSH and LH levels were found to increase during the northeast monsoon and reached peak during winter followed by summer and then maintained baseline level during southwest monsoon. Similarly, the highest number of total follicles were observed during winter (6.39), followed by summer (3.70) and southwest monsoon (3.03) then gradually decreased towards northeast monsoon (0.74).

In experiment-3, protein profiles of ostrich seminal plasma were studied. Five major protein bands in ostrich seminal plasma (OSP) were identified by MALDI MS proteomics analysis and the molecular weight of each protein band was predicted by using SDS-PAGE. The identified five major protein bands in ostrich seminal plasma were GATA Zinc finger domain containing protein 1 (OSP-I and II), low quality protein: E3 ubiquitin protein ligase RNF 216 (OSP-III), mitotic spindle assembly checkpoint protein MAD1 (OSP IV) and dual specificity phosphatase DUPD1 (OSP-V). All the five major expressed protein bands had a molecular weight ranging from 21.66 kDa to 102.34 kDa.

In experiment-4, sperm-egg interaction assay was carried out by Inner Perivitelline layer (IPVL) and Outer Perivitelline layer (OPVL) technique. Initial number of IPVL-sperm holes after last mating and rate of sperm holes decline pattern was demonstrated using a simple regression and the relationship was expressed as $Y = -2.85x + 24.60$ ($R^2=0.987$). This study revealed that the number of IPVL-sperm holes declined at the rate of $2.76/\text{mm}^2$ per day and expected to have 1.97 IPVL-sperm holes/ mm^2 on 8th day in the GD after last mating. Similarly, initial number of OPVL-sperm and rate of sperm decline pattern was also demonstrated using a simple regression and the relationship is expressed as $Y = -8.237x + 75.67$ ($R^2=0.932$). From the equation it is arrived that the OPVL-sperm declined at the rate of $7.99/\text{mm}^2$ per day and expected to have 2.31 OPVL-sperm per mm^2 on 9th day in the GD after last mating.

In experiment-5, artificial insemination was standardized and it was clearly evident that as the inseminated group had shown significantly higher values of per cent fertility (23.20), total hatchability (12.80) than naturally mated (9.60 and 5.08 per cent, respectively) ostrich hens.

Key Words: Ostrich semen characteristics, reproductive hormone level, seminal plasma protein, sperm-egg interaction assay and artificial insemination.