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# ARTIFICIAL INSEMINATION OF EMU SEMEN DILUTED WITH IMV AND MODIFIED BELTSVILLE POULTRY SEMEN EXTENDERS (MBPSE)

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***Abstract***

*A study was conducted for insemination of emu semen diluted with IMV poultry semen extender (IMV) and Modified Beltsville Poultry Semen Extender (MBPSE). Ten adult male and fifteen female emu birds aged 3 to 4 years were selected and housed individually in emu unit, University Research Farm, TANUVAS, Chennai – 600051. The male birds were selected based on their readiness in accepting human beings without fear. All the birds were housed properly under standard managemental condition. An Isocaloric and Isonitrogenous standard emu breeder ration was fed to birds and portable drinking water were made available ad libitum. The selected male emus were trained for semen collection by non-teaser method. After collection, the raw semen was diluted with IMV and MBPSE semen extenders at 1:3, 1:4 & 1:5 ratio and stored at $4^oC$ and evaluated all the seminal attribute in 1, 3 and 6 hours interval. The semen diluted 1:3 & 1:4 ratio stored for 1 hour showed better seminal characteristics and chosen for insemination. The female birds were restrained by casting on the floor; the artificial insemination was carried out with diluted semen. The female started laying eggs three to four day after insemination. All the eggs were set for incubation. A significant ($P \leq 0.01$) difference was observed in fertility of female emu birds inseminated with extended semen and the IMV showed better fertility and hatchability than MBPSE and the embryonic mortality did not showed any difference. It is concluded that the emu semen can be stored for a short period of 1 to 3 hour at 1:3 & 1:4 ratio of LSE in $4^oC$ with better seminal attributes for further processing like artificial insemination and cryopreservation.*

***Key words**: Emu, semen extender, artificial insemination*

## I. Introduction

Emu (*Dromaius novaehollandiae)* is a flightless, monogamous bird and is the second largest bird belonging to ratite family. Emu farming is gaining popularity in many parts of India for its skin, fat, feathers, meat and eggs to produce valuable products such as leather and oil. This species is well-suited for intensive rearing, adapts relatively easily to cold and hot environments, and has a high rate of reproduction (Malecki *et al.*, 2002; Sales, 2007). In natural mating, emu farmer has to keep equal number of breeder male and female, maintain surplus breeder males to achieve optimum fertility level, thus rise in cost of production. The monogamous innate behaviour of emu is

a major constraint for their genetic improvement. Apart from that, emu is a seasonal bird, breed between September to March months and it is not easy to transport for natural mating to other farms resulting in inbreeding and hence, the germplasm of superior birds cannot be disseminated. The alternate choice is Artificial Insemination (AI), hence, this study was conducted to know the fertility performance of emu inseminated with diluted semen.

## II. Materials and Methods

### Selection and training of male emu birds

Ten adult male emu birds aged 3 to 4 years were selected and housed individually in a 10' x 50' pen constructed in parallel rows at emu unit, University Research Farm, TANUVAS, Chennai, Tamilnadu, India. The male birds were selected based on their readiness in accepting human beings without fear. All the birds were housed properly under standard managemental condition. Standard emu breeder ration and portable drinking water was made available *ad libitum*.The selected male emus were trained for semen collection by non-teaser method (Malecki *et al*. 1997 and Malecki and Martin 2005).

### Dilution and evaluation of semen

Immediately after collection, the semen was kept in a water bath at 20ºC and then it was diluted with two semen extenders namely IMV avian semen extender (IMV) and Modified Beltsville Poultry Semen Extender-Table 1 (MBPSE) (Kumararaj and Omprakash, 1996) at 1:3, 1:4 & 1:5 ratio and stored at 4$^{o}$C for 1, 3 & 6 hours. The IMV avian diluent obtained from IMV Technology, ZI n$^{o}$ 1 Est, 61300 L'Aigle, France. The pH and osmolarity of the extenders were measured and adjusted according to standard levels using DALAL pH meter and OSMOMAT 030 cryoscopic osmo-meter.

Then the stored semen was evaluated for macroscopical seminal attributes namely volume, colour, consistency and pH and microscopical attributes namely mass activity, per cent motility, concentration, per cent live and abnormal spermatozoa as per the standard procedures. The semen diluted in 1:3 & 1:4 ratio in both extenders stored for 1&3 hours showed better seminal characteristics and chosen for insemination.

**Table 1. Modified Beltsville Poultry Semen Extender (MBPSE), (Kumararaj and Omprakash, 1996)**

| Component | g/100 ml |
|---|---|
| Sodium glutamate (Monohydrate) | 0.870 |
| Fructose | 0.500 |
| TRIS Buffer [(hydroxyl methyl) amino methane] | 0.180 |
| Potassium citrate (monohydrate) | 0.070 |

| | |
|---|---|
| Potassium di phosphate (Trihydrate) | 1.270 |
| Potassium monophosphate | 0.060 |
| Sodium acetate (Anhydrous) | 0.430 |
| Magnesium chloride (Octahydrate) | 0.030 |
| Deionozed triple distilled water | 100 ml |
| pH | 7.5 |
| Osmolarity (mos/kg $H_2O$) | 340 |

**Artificial insemination**

Artificial Insemination was carried out in female emu by using extended semen of selected treatment of combinations. Female emu was restrained and then the bird was averted and cast on its back by pulling the two legs in upward direction. After restraining the bird for artificial insemination, the inseminator knelt behind the female bird and inserted his left hand index finger in to the cloaca by slowly rotated his finger in clockwise to stimulate and relax the vaginal musculature (Plate 1 and 2). A glass speculum was then inserted into the cloaca and brought close to the vagina. The extended semen sample with minimum 400 million spermatozoa were taken in 1 ml serological plastic pipette. Through speculum this pipette was inserted in to the vagina until resistance was felt (approximately 1-2 cm in depth). Then the semen was released from the serological plastic pipette. The female emus were inseminated the day after every oviposition to maximize the duration of their fertile period (Malecki *et al.* 2002).

Plate: 1

Semen collection in male emu bird by artificial cloaca

Plate: 2

Insemination of diluted semen through pipette in female emu bird

**Fertility parameters**

The eggs were collected immediately after laying and fumigated with potassium premanganate method at 4X concentration. Then eggs were cleaned with disinfectants and stored between 60 to 68$^{o}$F with the relative humidity of 65-70 per cent for 3 days and

set in incubator with the constant temperature of 97$^{o}$F with the relative humidity of 45-50 per cent for 52 days. The eggs were turned once in an hour by automatic turner for 90$^{o}$ angle up to 48 days. Then the eggs were transferred to hatcher at 49$^{th}$ day for hatching. After hatching the remaining unhatched eggs were opened to record the infertile and embryonic mortalities. Fertility was calculated after deducting infertilities from the total number of eggs. Hatchability on total eggs set and on fertile eggs set was calculated. Mid and late embryonic mortalities were calculated based on fertile eggs set. All the fertility parameters were expressed in percentage. All the data recorded in this study were analysed as per Snedecor and Cochran (1994).

## III. Results and Discussion

The effect of AI by two semen extenders namely IMV and MBPSE are presented in Table 2. Highly significant results were obtained ($P \leq 0.01$) in fertility, and total and fertile hatchability, embryonic mortality showed significant ($P \leq 0.05$) difference.

Earlier fertility works on using different extended emu semen was not traceable. However, Malecki *et al.* (1995) obtained 80 per cent fertility with insemination of 0.5 ml raw semen on alternate days with a total of 2.5 ml. In this present study, only 0.2 ml of raw semen equivalent in an extended form was used for liquid semen insemination for female with a frequency of once in 3 days. Thus the total spermatozoa count per week amounts to 800 million spermatozoa, which is far less than the insemination dose of Malecki *et al.* (1995).

This evidently proved that the advantage of using semen extenders where in the semen of elite male can be inseminated to more number of females with moderate fertility. The fertility observed in this study is in accordance with Dzama (2009) and Rizzi *et al.* (2002) in ostrich in natural mating. However, Majewska (2001) in emu, and Cloete *et al.* (1998) in ostrich observed more than 80 per cent fertility under farm conditions. However, Agab *et al.* (2004) recorded 65 to 75 per cent fertility in natural mating.

The total and fertile hatchability had shown significant difference among selected treatment groups in liquid semen insemination with maximum values in IMV semen extender than MBPSE extended semen. The earlier research on influence of different

semen extender on total and fertile hatchability was not traceable. However, Malecki *et al.* (1995) observed 70 per cent fertile hatchability with 2.5ml of raw semen insemination compared to 0.4 ml per week in this study. Deeming (1996) observed comparable total and fertile hatchability in ostrich as observed in this study. Dzama (2009) observed that the per cent hatchability in wild ostrich ranged from 27 to 67 per cent, which is in agreement with present study. However, Agab *et al.* (2004) recorded more than 50 per cent hatchability in emu under natural mating.

The early embryonic mortality pattern observed after liquid insemination with selected treatment group showed no significant difference among extenders, however the late and total embryonic mortality showed significant ($P \leq 0.05$) difference. The value obtained in this study coincides with the findings of Agab *et al.* (2004) in emu have observed higher embryonic mortality than this study. However, Szczerbinska *et al.* (2004) observed less embryonic mortality under non comparable condition. However, Majewska (2001) in emu birds did not agree with this result.

## IV. Summary

The results of above study clearly indicated that the emu semen can be diluted with semen extenders and stored for short period at refrigeration temperature ($4^{o}C$) without affecting the seminal attributes. Emu females can be inseminated with diluted semen for getting fertile eggs and chicks by artificial incubation. The optimum fertility and hatchability can be obtained in liquid semen inseminations by using semen extended with IMV diluted at 1:3 ratio, stored for 3 hours in $4^{o}C$.

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**Table: 2 Fertility parameters of female emu artificially inseminated with extended semen (Mean±SE)**

| Sl. No. | Extended semen | Fertility** % | Total Hatchability * % | Fertile Hatchability * % | Embryonic Mortality % | | |
|---|---|---|---|---|---|---|---|
| | | | | | Mid NS | Late * | Total * |
| 1. | IMV(n=50eggs) | $60.79^{a}$±5.02 | $19.25^{a}$±2.76 | $30.43^{a}$±2.67 | 16.26±2.73 | $25.27^{a}$ ±4.03 | $41.53^{a}$ ±3.21 |
| 2. | MBPSE(n=40eggs) | $42.50^{b}$±4.27 | $10.00^{b}$±3.15 | $23.53^{c}$b6.71 | 17.90±3.11 | $14.70^{b}$ ±3.70 | $32.60^{b}$ ±2.27 |
| | **Overall mean** | **53.90±3.34** | **14.65±3.18** | **26.88±3.49** | **17.09±2.01** | **19.98±3.49** | **37.24±2.81** |

Mean with different super scripts in the same columns differ significantly, ** Highly significant (P<0.01), *Significant (P<0.05), NS Not significant (P>0.05).

**Extended semen**

1. IMV-Semen extended with IMV at 1:3 dilution ratio, stored at 6 hours in $4^{o}$ C.
2. MBPSE-Semen extended with MBPSE at 1:3 dilution ratio, stored at 6 hours in $4^{o}$ C.

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