Biospectra : ISSN : 0973-7057, Vol. 13(2), Sept., 2018, pp. 77-80
An International Biannual Refereed Journal of Life Sciences

Animal Science

ISSN : 0973-7057

Int. Database Index: 663 www.mjl.clarivate.com

Studies on embryonic diapause as reproductive strategies in gravid female of garden lizard, *Calotes versicolor*

Pranav Kumar Pratap[a]*, Arun Kumar[a] & R.B. Jha[b]
[a]P.G. Department of Zoology, B.N. Mandal University, Madhepura, Bihar, India
[b]R.M. College, Saharsa, Bihar, India

Received : 19th June, 2018 ; Revised :14th August, 2018

Abstract: Embryonic dipause is one of the several exciting strategies in reproduction in female garden lizard, *Calotes versicolor*. The most important feature of this strategies is regulatory delay in the embryonic development of gravid female thereby preventing oviposition. The mechanism has been carefully studied by observing the time & body temperature dependent various stages of embryonic development in the test animal during the laboratory rearing of the lizard in 2015.

Key words : Body temperature, Embroyonic dipause, prolonged egg retention, *Calotes versicolor*

INTRODUCTION

Calotes versicolor, commonly called Indian garden lizard is famous for behavioural camouflaging by changing its body color with the environmental habitats. The internal biological constitution in terms of structure and function is also very interesting which make them a stout and strong reptile of the garden with no environmental threat.

These lizards are oviparous in reproductive behaviour and lay fertilized eggs after internal fertilisation for embryonic development outside the body of the mother. The present paper deals with the phenomenon of unique embryononic dipause as one of the reproductive strategies delaying the embryonic development in relation to the falling body temperature under the laboratory condition as well as environmental cooling and hibernation supported by the levels of hormonal plasma progesterone and luteal activity.[1,2,3]

*Corresponding author :
Phone : 09404308111
E-mail : pranabkpratap@gmail.com

As a matter of fact lizards are members of warm blooded homoeothermic vertebrates just evolved from the group of cold blooded poikilothermic[4,5] ancestors and maintaining the steady state of body temperature is another exciting biological program for them. Apart from this, the fluctuation in body temperature in terms of fall and rise may be either related with extreme environmental conditions or metabolic disorder are often encountered by the lizards which substantially affect other physiological activities including reproduction. Vis-à-vis the lizards have developed the phenomenon of embryonic diapause dealt in this paper as one of the reproductive strategies and extraordinary environmental adaptation for better survival.[6,7]

MATERIALS & METHODS

Lizards were collected by hand and noose from surroundings. Captured lizards were housed in terrariums. Suitable natural climatic condition was provided during experimental period. After hatching of eggs laid by lizards in captivity, our data collection process start-up. Suitable changes were arranged to study the mechanism of the

embryonic diapause. To validate data results may be analysed using bio statistical method.

For present study *C. Versicolor* collected from Madhepura district Bihar, India in May- June 2013. Six females in early gestation were identified by the presence of eggs that felt soft during palpation of abdomen and these were maintained in outdoor terrarium at P.G. Department of Zoology, B.N. Mandal University, Madhepura. Upon appearance of a single crease on the lateral side of the abdominal wall close to the hind limbs, the oviductal eggs in these lizards were deemed to have reached stage 27–28 embryos (Shanbhag *et al.*, 2003)[6]. The eggs with oviductal embryos at stage 27–28 were stripped from these lizards, since the oviposition in *C. versicolor* takes place at embryonic stage 27–28 (Muthukarruppan *et al.*, 1970)[7] and were incubated in the laboratory. Each gravid lizard yielded 20 ± 3 eggs (range 17–22, n = 6) and a total of 124 eggs were collected. The eggs were incubated in laboratory (at ambient temperature 27.71 ± 0.29 °C and moisture 40–45%) and hatchlings were obtained. Eggs were buried leaving roughly one-third of their top surface exposed so that they could be monitored without disturbance.

As soon as hatchlings emerged from the eggs (n = 114), they were placed in the glass terrarium (30×30×25 cm) with wire mesh at the top for ventilation. Up to 10 hatchlings were reared per terrarium and total 12 terrariums were used for rearing hatchlings. When lizards attained two months, they were maintained in 18 terrarium (90×90×60 cm) made up of wire mesh on all sides except bottom. The lizards (n = 107) were kept in 1:2 male/female ratio in captivity as the males are generally seen with more than one female in the wild during breeding season. The sex of the lizards was identified at hatching by protrusion of hemipenis (Harlow, 1996)[8]. Thus, each terrarium housed 6 individuals (2 males, 4 females) and was exposed to natural sunlight. When lizards grew six months old, they were maintained in outdoor terrarium of size 150×120×120 cm with wire mesh on top and two sides, and other two sides of transparent acrylic plates. They were provided with soil-sand mixed bed, large dried twigs for climbing, the potted plants, hiding places with broken earthen pots, and a water bowl. The newborn hatchlings were fed daily with termites and early instar larvae of various insects. Juveniles and sub- adults were fed with the small cockroaches and grasshoppers on alternate day. Adult lizards were likewise fed on alternate day with cockroaches, and grasshoppers and other insects caught in swapping of insect nets. A total of 122 hatchlings were used to study the growth patterns in this species. The snout-vent length (SVL), head length (HL), head width (HW), and head depth (HD) to the nearest mm were recorded at hatching. The hatchlings were marked by thermocol beads tied loosely on the posterior part of the trunk. Subsequently when hatchlings were one month old they were permanently marked by unique toe clipping. SVL and head size (HL, HW, and HD) of these lizards were recorded at monthly intervals.

To study embryonic diapause in *C. Versicolor* few containers were placed in a Percival environmental chamber at a constant 28°C; some eggs from each clutch were shifted to constant 25 and 30 °C temperature treatments after diapause had ended. These temperatures fall within the range of nest temperatures in nature for *C. versicolor*. Eggs were therefore incubated at 28 °C for about two months so that embryos would be at post-diapause stages when experiments were initiated. At the beginning of the experimental period, sub-sets of eggs from some clutches were assigned to two additional temperature treatments; one-third of each clutch was left at 28 °C, one-third was shifted to 25 °C, and one-third was shifted to 30 °C. This experimental protocol was used for three sets of observations. Moisture was not considered in the experimental design because the effects of temperature on development far outweigh the effects of moisture (Flatt *et al*., 2001[9]; Andrews, 2004)[4]. I determined the effect of incubation temperature on differentiation (stage) and growth (mass) of embryos. For these observations, eggs were assigned to the three treatments at 70 days of incubation. Eggs (n=56) from 12 clutches were sampled between 70 and 180 days. Embryos were removed from eggs and preserved in 70% ethyl alcohol. At the time that they were staged, embryos were blotted to remove surface alcohol and weighed on an electronic balance to the nearest 0.01mg. We determined the relationship between the area of the AV– YS–CAM and embryonic stage for eggs from three clutches. An egg from each clutch-treatment combination was randomly selected for sampling at 3–4 day intervals starting at 71 days when eggs were allocated among the three temperature treatments and ending when the AV–YS–CAM covered 100% of the shell. Eggs from an additional two

clutches incubated at 28 °C only were selected for sampling at 2–3 day intervals during the period 40–84 days to provide more information on AV–YS–CAM area and stage early in development. These latter two clutches were used for qualitative comparisons only.

Each egg sampled was weighed to the nearest 0.01 g. linear measurements of the major and minor axes of the egg and the AV–YS–CAM were made with dial callipers. Dimensions of the AV–YS–CAM for areas greater than 50% coverage of the egg were estimated from the a embryonic side of the egg by determining the area yet to be covered by the CAM and subtracting that area from the total surface area of the egg. Sampled eggs were preserved in 10% buffered formalin and staging was done at the end of the experiment. I determined the effect of temperature on growth of the YS– CAM for eggs from six clutches. Successive observations on individual eggs were made every 3–4 days from day 64 when embryos were shifted to the three experimental temperatures until the time when the YS–CAM covered 100% of the shell. At each sample date, eggs were weighed and the major and minor axes of eggs and their AV–YS–CAMs were measured as described in the previous paragraph. When measurements were completed, eggs were returned to the incubator. Because of embryo deaths in some clutch/ treatment combinations, analyses were conducted using the three of the six clutches with embryos in all three treatments. Conclusions were identical to those reported for the entire data set of 6 clutches.

During whole experimental process "Guidelines for care and use of animals in Scientific Research" were followed.

RESULTS & DISCUSSION

The lizard, *Calotes versicolor* can delays oviposition of oviductal eggs for as long as 6 months or more under unfavourable conditions. During this period of prolonged egg retention, the growth of oviductal embryos is arrested at stage 34. The present study shows for the first time among reptiles that the 'embryonic diapause' is manifested by the gravid females by lowering their body temperature (T_b) by 3-5°C during the period of egg retention by mechanism presently unknown **(Table-1).**

The T_bs of males, non-gravid females and females during normal gestation were comparable with each other and were very close to ambient temperature on any given day **(Table- 1).** A slight fall in T_b of gravid females was observed with advancement in the embryonic development i.e. after embryos had reached stage 27-28. However, a significant fall in the T_b was evident in the females with stage 34 embryos ($F(3.88)= 137.05$, $P<0.001$) in comparison with that of other groups. A fall of 3-5°C (mean 4.44 ± 0.2°C) in the T_b of females with prolonged egg retention was registered throughout the experiment. On one occasion the fall in T_b was as much as 6°C. Thus, in order to prolong egg retention, arrest of embryonic growth (diapause) is accomplished by lowering the body temperature (T_b).

TABLE -1

Variable	temperature Mean ±SE (^{0}C)
Ambient temperature	28.25±0.26
Females	
a. Non-gravid	27.64±0.30
b. Gravid:Normal gestation	27.01±0.31
Egg retained	23.93±0.20[a]
Males	27.80±0.26

[a]Significantly different from all other groups.

ACKNOWLEDGEMENT

I express my sincerioust thanks and gratitude to Professor & Head, Dept. of Zoology, Dr. Arun Kumar, B.N.Mandal University Madhepura, North Bihar for his immensed co-operation & motivation to complete this research paper.

REFERENCES

1. **Radder RS, Shanbhag BA, Saidapur SK. 1998.** Prolonged oviductal egg retention arrests embryonic growth at stage 34 in captive *Calotes versicolor*. *Herpetol Rev* **29:**217–218
2. **Radder RS, Shanbhag BA, Saidapur SK. 2002.** Influence of incubation temperature and substrate on eggs and embryos of the garden lizard, *Calotes versicolor* (Daud.). *Amphibia-Reptilia* **23:**71–82
3. **Shanbhag BA, Radder RS, Saidapur SK. 2001.** Plasma progesterone levels and luteal activity during gestation and prolonged oviductal egg retention in the tropical lizard, *Calotes versicolor*. *Gen Comp Endocrinol* **123:**73–79

4. **Andrews R. M. 2004.** Patterns of embryonic development. In Reptilian incubation environment, evolution and behaviour (ed. Deeming D. C., editor.), pp. 75–102 Nottingham, UK: Nottingham University Press

5. **Andrews R. M., Diaz P. C., Marco A., Portheault A. 2008.** Developmental arrest during embryonic development of the common chameleon (*Chamaeleo chamaeleon*) in Spain. Physiol. *Biochem. Zool.* **81:** 336–344.

6. **Shanbhag, B.A., Saidapur, S.K., Radder, R.S. 2003.** Lowering body temperature induces embryonic diapause during prolonged egg retention in the lizard, *Calotes versicolor*. *Naturwissenschaften.* **90:** 33–35.

7. **Muthukaruppan, V.R., Kanakambika, P., Manichavel, V., Veeraraghavan, K. 1970.** Analysis of the development of the Lizard, *Calotes versicolor.* I. A series of normal stages in the embryonic Development. *Indian J. Exp. Biol.* **37:** 300–301.

8. **Harlow, P.S. 1996.** A harmless technique for sexing hatchling lizards. *Herpetol. Rev.* **27:** 71–72.

9. **Flatt, T., Shine, R., Borges-Landaez, P.A., Downes, S.J., 2001.** Phenotypic variation in an oviparous montane lizard (Bassiana duperreyi): the effects of thermal and hydric incubation environments. *Biol. J. Linnean. Soc.* **74:**339–350.

***