https://doi.org/10.48047/AFJBS.6.15.2024.6091-6097

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Study of the ultrastructure of the ovarian follicles of *Mabuya multifasciata* along with its element composition.

Dr Moushumi Hazarika, D.K.Sharma

1 Former SRF, Gauhati University
2 Professor, USTM, Retired H.O.D of Gauhati University.
Corresponding author email : msmimsmi253@gmail.com

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.6091-6097

Abstract : Viviparous squamates are those who give birth to young ones instead of laying eggs. It includes lecithotrophic and placentotrophic. *Mabuya multifasciata* has allantoplacenta which provides most of the nutrients needed for embryonic development and they ovulate microlecithel eggs. So the genus *Mabuya* has a vast scope to study of the evolution of structures and their function leading to microlecithal egg as a transition to viviparity and placentotrophy. The present study was done during the period of 2011-2015 in Assam, India.

Key words : Vivarity, ultrastructure, ovary, *Mabuya multifasciata.*

Introduction :

All the known metazoans possesses yolked oocytes. Viviparous squamates include lecithotrophic species (the yolk is the principal source of nutrients for embryo development) and placentotrophic species (nutrients are provided through a placenta). Placentotrophic lizards of the genus *Mabuya* have an allantoplacenta which provides most of the nutrients needed for embryonic development and ovulate microlecithal eggs (Blackburn and Vitt, 2002). Therefore, the species of this genus represent potential models systems for the study of the evolution of structures and their function leading to microlecithal egg as a transition to viviparity and placentotrophy.

The present study deals with the ultrastructure of the ovary and certain element composition of it which was done during 2011-2015 in Assam,India.

1. Materials and methods :

2.1 **Certain elements in ovary**

Elemental estimation was carried out with the help of Atomic Absorption Spectrophotometer (Perkins Elmer 3110) at Sophisticated Analytical Instrumentation Facility (SAIF), North Eastern Hill University, Shillong, India.

2.2 Gonads and placenta (ultra structure)

The ovary, oviduct, placental tissue and extra embryonic membrane were fixed in 2.5% glutaraldehyde prepared in 0.1% N Sodiam Cacodylate buffer at Ph 7.2 to 7.4 at 4˚ C. Then it washed in buffer for overnight, post fixed in 1% buffered Osmium tetroxide for one hour and dehydrated through increasing concentration of acetone. The dehydrated samples were dried either in critical point drier (Samdri Pvt Tousimis) using acetone as the intermediate fluid and CO_2 as transitional fluid or by TMS drying technique (Dey et al.,1989). In this technique the dehydrated samples were dipped in tetra methyl silame (TMS) at 4^{o}C for 10 minutes. The samples then from TMS were placed in a glass slide and dried at room temperature. The samples were then secured horizontally to brass stub (10mm to 12 mm) with double coated adhesive tape connected via patch of silver paint to ensure change condition. A conductive coating was applied to the sample using TFC 1100 (Jeol) ion sputter coater. A relative low vacuum (10-3 tor) was established in the sputtering chamber, and gold was used as the target material. The preparations were examined with SEM, JSM-35 CF (Jeol) using the secondary electron transmission made at an accelerating voltage of 15 KV (SAIF NEHU, Shillong).

2.3 Sample preparation for FE-SEM/EDX analysis

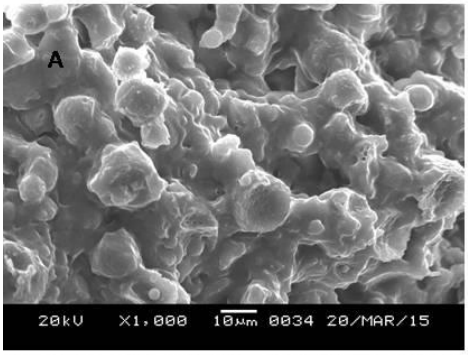
The sample preparation for biological specimen includes three major steps (i.e. fixation, dehydration and drying process). To fix the material it needs to wash first by using certain techniques to avoid damage of the fine structure of the samples due to surface tension and dehydrated as per procedure. After dehydration the samples were scan with SEM or FE-SEM,

the objects are first made conductive. It was done by coating them with extremely thin layer of gold (1.5-3 nm) or gold-palladium and then placed them in desicator all times.

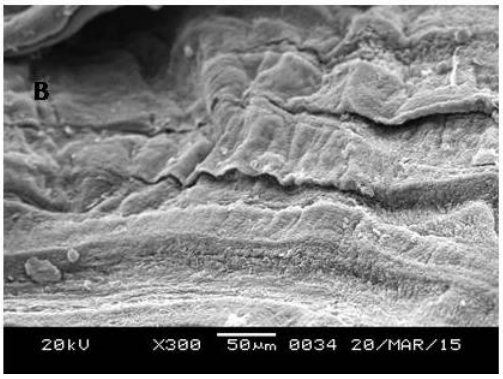
2. Result :

Fig1.1 : Collected in early March (rapid growth phase)

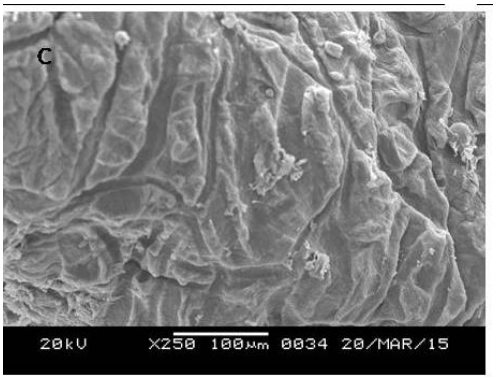
3.1 Superficial structure of ovary, oviduct and placental tissues through SEM :



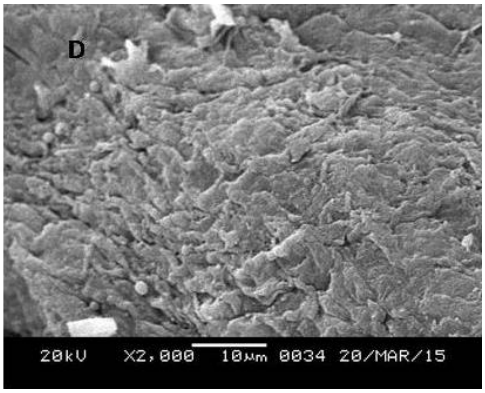
A. Developing ova of *Mabuya multifasciata*



B. External lamina of the allantois of *Mabuya multifasciata*



C. External surface of chorion of *Mabuya multifasciata*



D. Inner surface of uterine component of chorioallantoplacenta *of Mabuya multifasciata.* Bar= 500 µm

Fig 1.2 : (A) Developing ova of *Mabuya multifasciata;* (B) External lamina of the allantois of *Mabuya multifasciata;* (C) External surface of chorion of *Mabuya multifasciata; (D)* Inner surface of uterine component of chorioallantoplacenta *of Mabuya multifasciata.* Bar= 500 µm

The external surface of the chorion bears ridges (Fig 1.2- B, C) That correspond to similar ridges in the uterine lining (Fig 1.2- D).Under scanning EM, mouths of the uterine glands are visible opening into the uterine lumen (Fig 1.2 -D). The glands often open into the uterus on small hillocks that are situated on the peaks of the uterine ridges. Scanning electron microscopy reveals that the ridges of chorion are elongated and can be oriented in parallel (Fig 1.2- C). They are deep in some regions in late gestation (Fig 1.2.- D). The degree of interdigitation between the chorioallantois and uterus can make the two tissues difficult to separate.

3.2 Certain elements in the ovary

Table 1.1: Certain elements in the egg yolk during the study period 2011-2015

Elements	StageI (%) (August-January)	Stage II(%) (January-March)	Stage III(%) (March-May)	Stage IV to parturition (%) (May-July)
Mg	36.06±1.92	32.07±0.05	28± 0.36	6.08±0.65
P	58.07±2.37	45.00±0.05	35.89± 0.43	10.05±0.36

Ca	32.44±2.21	30.78±0.74	27.05±0.78	4.26±0.35
Zn	40.50±3.07	38.78± 0.86	23.56±0.59	2.08±0.22

Table 1.2 : Percentage of certain elements present in ovary through different stages

Parameter	Stage I (Aug-Jan)	Stage II (Jan-Mar)	Stage III (Mar-May)	Stage IV (May-Jul)
Ovary diameter(cm)	1.0cm ±1.62	1.7cm± 1.17	1.9sm±2.25	2 cm± 3.57
Elements %	41.5	36	28.2	3.25

The ovary diameter through different stages of development showed a negative correlation with the certain elements present in the eggs and ovaries (Correlation coefficient= -0.743). This shows a significant difference in presence of the elements in different developmental stages in the ovaries and eggs (P=0.00000128,n=30).

3. Disscussion :

The present study found that purturation occurred during May-July. However previous study reported that no *Mabuya mabouya* were found to be gravid from November to May in Peru. Most of the females studied during this study had one germinal bed .The result is supported by the study previously done on *Mabuya mabuya* (Gomez *et al*.,2004). They reported one to two germinal beds per ovary. The present investigation has been able to establish communication link, yet the presence of high lipid molecule perhaps render the communication sign. The ovarian with tandem to the follicle formation, since the time of formation of oocyte and granulose cells are working with tandem for follicle formation (Rochild, 2015). The squamate reptiles like *Mabuya* showed that the granulose layer surrounds the oocytes of previtellogenic follicles passes through several morphological changes at different stages of development (Viera *et al.,* 2010) along with the differentiation of pyriform cells.

Ultrastructural analysis of vitellogenic oocytes reveal a process of lysis and gradual fusion between small and large electron-lucent vesicles, from the periphery to the center of the oocyte, responsible for the lacunar appearance of the central ooplasm.

The nature of the material found in these membrane lacking lacunar spaces is unknown, as well as if their formation is the result of excess organelle recycling, degradation of macromolecules for posterior use during embryo development and/ or both. As in Mabuya, the advanced primary oocytes of most marsupials are characterized by a vacuolated cytoplasm (Falconnier and Kress, 1992; Kress et al., 2001). Falconnier and Kress (1992) found that in Monodelphis domestica, Golgi complexes contribute to the formation of multivesicular bodies, which then appear to coalesce and transform to large electron-lucent vesicles that contain osmotically active glycoproteins. Large electron-lucent vesicles are also found in large numbers in the central cytoplasm of eutherian oocytes (Cran et al., 1980). It is suggested that these vesicles observed during oogenesis in marsupials and in eutherian mammals are the residual elements of white yolk present in the larger yolky eggs of monotremes and sauropsids and their function is to provide extracellular matrix (ECM), especially hyaluronan containing stabilizing proteins, for expansion of the blastocyst and epithelial construction (Kress and Selwood, 2003; Menkhorst et al., 2009).

4. References :

1. Cran et al.,1980. Fine structure of the sheep oocyte during antral follicle development. J Reprod Fertil 59:125–132.
2. Dey et al. (1989). "Reproductive cycles in lizards and snakes. Univ. Kansas Mus. Nat. Hist. Misc. Pub.52, 1-247.
3. Falconnier C et al., 1992. Ultrastructural aspects of oocyte growth in the marsupial Monodelphis dosestica (grey shorttailed opossum). J Anat 181:481–49.
4.Gomez, D., Ramírez-Pinilla, M. (2004): Ovarian histology of the placentotrophic *Mabuya mabouya* (Squamata, Scincidae). J.Morphol. 259: 90-105.
5. Kress A et. Al., 2001. Oogenesis in the marsupial stripe-faced dunnart, Sminthopsis macroura.. Cells Tissues Organs 168:188–202.
6. Menkhorst E, Nation A, Cui S, Selwood L. 2009. Evolution of the shell coat and yolk in amniotes: A marsupial perspective. J Exp Zool B (Mol Dev Evol) 312:625–638.
7. Rothschild, B.M., Schultze, H.-P., and Pellegrini, R. 2015. Osseous and other hard tissue pathologies in turtles. In: D. Brinkman, J. Gardner, and P. Holroyd (eds.), Morphology and Evolution of Turtles, 501–534. Springer, New York.
8. Viera, G. H. C., Cunha, L. D., Scheltinga, D. M., Glaw, F., Colli, G. R. & Bao, S.

N. 2010. Sperm ultrastructure of hoplocercid and oplurid lizards (Sauropsida, Squamata, Iguania) and the phylogeny of Iguania. Journal of Zoological Systematics and Evolutionary Research, 45, 230–241.