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Seasonal alterations in the contents of ovary and oviduct of common Indian rock lizard, *Psammophilus blanfordanus*

D. K. Singh[1], N. Jena[2] and N. Mohanty[3]*

[1] Department of Zoology, Govt. Autonomous College, Rourkela-769004
[2] P. G. Department of Life Sciences, Govt. Autonomous College, Phulbani-762001
[3]P. G. Department of Zoology, North Orissa University, Baripada-757003
**Corresponding author: nakulanandamohanty@gmail.com*

Abstract

Studies on reproductive organs which correlate the acid hydrolase and phosphatase enzyme activities and macromolecular concentrations with reproductive status of the female are mostly confined to mammalian systems. No report is available for ovary and oviduct on these parameters in case of a tropical common Indian rock lizard, *P. blanfordanus*. The present study has been undertaken to quantify the acid and alkaline phosphatases, â-D-galactosidase enzymes, and protein, DNA and RNA contents in the ovary and oviduct, of *P. Blanfordanus*, to know whether they have any role in reproductive activities, and will be useful in comparative study of vertebrates from the evolutionary point of view. The enzyme activities as well as macromolecular contents are high during breeding period and low during non-breeding period. The results of the present study reveal that these biochemical parameters may have a role in folliculogenesis, vitellogenesis, egg shell formation, oviposition and other reproductive activities.

Keywords: Agamidae, female *Psammophilus blanfordanus*, acid and alkaline phosphatases, â-D-galactosidase, macromolecule

Introduction

Reptiles are positioned centrally in vertebrate phylogeny as they took the advantage to become the first animal to survive and reproduce on land [1]. Reptiles show a great variability in their reproductive patterns, i.e., in female the oviduct (genital tract) is well developed to support for internal fertilisation and also the site for egg shell formation. The duct system probably evolved as a homeostatic mechanism to facilitate fertilisation and some embryological development under conditions protected from the external environment [2]. Therefore, the internal milieu of the reproductive organs and tracts are more complex. Different glycosidases, acid phosphatase (AcP) and alkaline phosphatase (AlkP) are associated with the reproductive organs [3].

â-D-galactosidase is important as it is involved in the catabolism of glycoproteins, glycolipids and proteoglycans containing terminal galactose or sugar residues[2]. Glycosidases could be involved in the control of polyspermy[4], interaction between spermatozoa and oviductal epithelium [5], binding of spermatozoa to the zona pellucida [6], capacitation of spermatozoa[7] and dispersion of cumulus cells[8] . So, glycosidases are important components of oviduct as they are capable of modifying the carbohydrate moieties of glycoproteins.

Phosphatase enzymes (acid and alkaline phosphatases) are influenced by the steroid hormones [9]. Alkaline phosphatase activity is seen in the theca interna of the ovarian follicles during follicular development[10]. It is also associated with the transfer of solutes across cell membrane [11]. Acid phosphatase contributes in ovarian metabolic functions such as oocyte maturation, resumption of meiotic divisions, germinal vesicle breakdown and ovulation, digests the corpus luteum and helps the atresia of follicles by autophagia and heterophagia activities [12]. It has been reported that lecithotrophic species show specialisation for nutrient transfer and other physiological exchange [13].

The concentration of DNA provides a good estimate of total number of cells. The ratio of DNA and RNA varies widely among different animals and tissue types because they reflects the metabolic activity of the constituent cells [14]. In reptiles the growing oocyte primarly accumulates RNA, protein and combined ribonucleoprotein (RNP) [15]. Even the yolk granules show activity of RNA and protein [15]. In *Hemidactylus flaviviridis* higher concentration of proteins, lipoproteins, RNA and other macromolecules are observed in the cortical zone of the oocyte [16]. In this animal, Sharma and Grewal [17] have demonstrated that DNA, RNA and protein contents increase with the advancing stages of follicular growth. The proteins are androgen dependent in the reproductive organ and these require androgens to maintain their normal level of expression [18].

There is limited study on different glycosidases and phosphatases on the reproductive organ of lizards [19, 20, and 21] in comparison to mammalian system. Further, no report is available on the activities of â- D- galactosidase , AcP and AlkP enzymes, and protein/DNA/RNA contents in the ovary and oviduct of the female during pre-breeding, breeding and post-breeding sesaons of the tropical common Indian rock lizard, *Psammophilus blanfordanus* (Reptilia: Diapsida: Agamidae). Thus, these parameters have been studied on the ovary and oviduct to know their role during different phases of reproductive cycle of this animal. It will be helpful to enumerate the evolutionary tactics in lizards and studying vertebrate phylogeny.

Materials and Methods

Animal: Eightyseven live specimens (Fig. 3) were collected from Baripada, Mayurbhanj, Odisha (21° 6' and 22°34' N and 85°40' and 87°11' E) and adjoining areas during morning hours (7-9 a.m.). Sexual maturity of the animal was ascertained following Pradhan [22] as mentioned below. Sexual dimorphism exists in this animal. The head and the anterior parts of the body of matured male appear scarlet or brilliant red colour (Figs. 1-3) while adult female, juvenile and sub-adult are not (Fig. 4) . Another criterion for identification of male is the presence of hemipenis musculature. The size of the female is smaller than the male. In matured female the snout to vent length (SVL) ranges from 8-11 cm, tail length (TL) ranges from 17-21 cm and body weight ranges from 32-48 g. The lizards were acclimatised to the laboratory conditions by keeping them in clean cages fed with insects at least for 15 days. Water was given to the animal *ad libitum.*

Biochemical analysis: The animals were killed by decapitation. The ovary and oviduct were dissected out and freed from fats and connective tissues and weighed in monopan balance (Shimazdu, Japan). The above tissues were homogenised (1:9 w/v) separately in ice cold 0.25 M sucrose solution. Proteins were precipitated from the homogenate with 0.1% Triton x100 and centrifuged at 800 x g. The protein contents were estimated as per Lowry *et al.* [23]. The extraction and estimation of DNA and RNA were done with some modifications as per Schneider [24]. The macromolecular contents of the ovary and oviduct were expressed as ìg/g wet weight of the organ.

The activities of the enzymes were assayed directly from the crude extract obtained after homogenisation. á-D-galactosidase activity was measured at pH 4.5 taking 4-nitrophenylgalactopyranoside as substrate[25]. Acid (pH 4.8) and alkaline (pH 10.1) phosphatase activities were estimated as per Brambley [26] taking p-nitrophenol as substrate. The enzyme activity was expressed as ìmol of substrate liberated/ minute/mg protein of the organ.

EDTA was added to the extraction medium as chelating agent. The above study was undertaken for each month of the year.

Results

Enzyme activity: The lowest activity of ovarian acid phosphate (AcP) in December (non-breeding period) (24.38±0.75) was increased gradually from January to March (pre-breeding period) and the maximum value was in July (breeding period from April to July) (451.24±1.14) (Table 1). Similarly, the AcP activity decreased gradually from August to November (post-

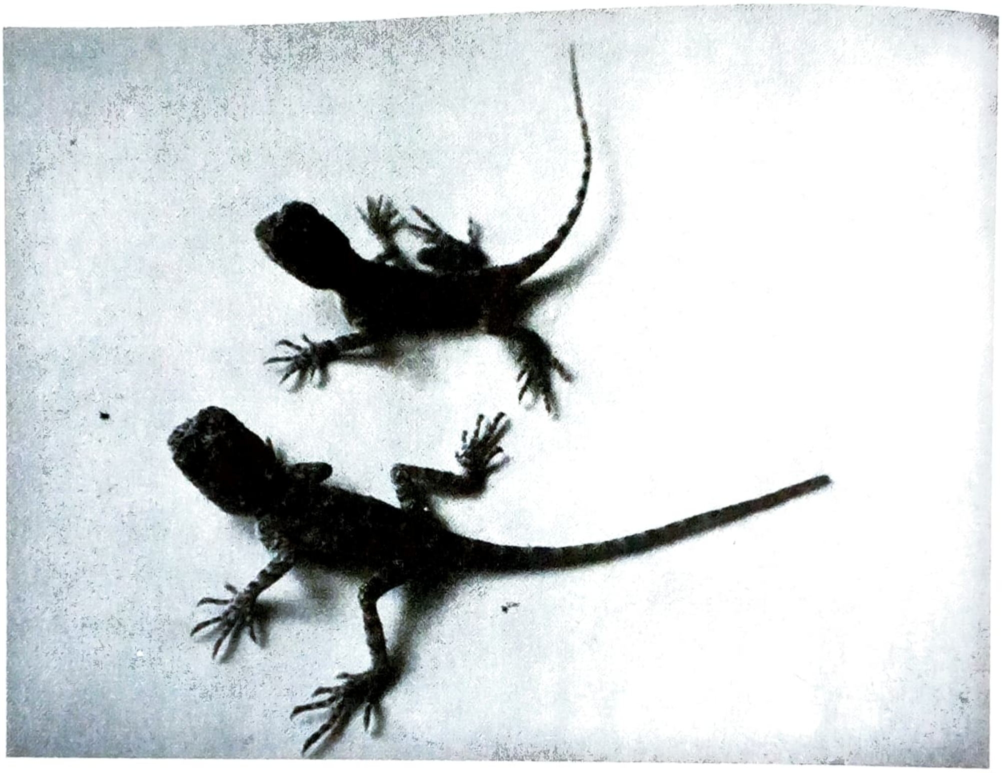

Figure 1. Juveniles of P. blanfordanus

Figure 2. Sub-adult female of P. blanfordanus

Figure 3. Gravid P. blanfordanus

Figure 4. Adult male P. blanfordanus displaying in breeding season

Table 1: Seasonal variation in the activities of acid phosphatase (AcP), Alkaline Phosphatase (AlkP) and â-D-galactosidase in the ovary of *Psammophilus blanfordanus*. Unit of measurement is expressed as µmol paranitrophenol liberated / minute /mg protein and data is represented as mean ± standard deviation

Month	Number of animals (n)	AcP	AlkP	β-D-galactosidase
January	6	56.08±1.15	65.89±1.32	83.52±2.01
February	9	26.55±4.30	75.04±1.25	96.01±1.03
March	5	110.29±1.31	132.48±1.82	119.36±3.62
April	5	133.33±3.32	142.27±1.96	396.95±3.85
May	7	207.38±1.36	188.58±2.24	543.36±4.47
June	7	431.68±1.54	232.21±2.15	703.35±2.66
July	7	451.24±1.14	536.39±2.78	803.71±2.25
August	8	152.77±2.83	324.64±2.95	623.03±2.07
September	9	121.66±1.93	189.26±1.44	349.31±1.68
October	9	74.25±1.15	112.94±1.91	126.00±1.79
November	8	53.74±0.77	77.86±0.87	61.65±2.88
December	7	24.38±0.75	39.54±1.48	40.89±0.87

Table 2: Seasonal variation in the activities of acid phosphatase (AcP), Alkaline Phosphatase (AlkP) and â-D-galactosidase in the oviduct of *Psammophilus blanfordanus*. Unit of measurement is expressed as µmol paranitrophenol liberated / minute /mg protein and data is represented as mean ± standard deviation

Month	Number of animals (n)	AcP	AlkP	β-D-galactosidase
January	6	52.87±0.82	42.86±1.74	3.89±0.20
February	9	27.32±1.17	37.58±1.57	4.27±0.34
March	5	78.79±0.73	44.07±2.83	7.29±0.31
April	5	121.72±1.33	45.92±1.94	9.26±0.46
May	7	127.44±0.79	44.51±2.18	8.64±0.55
June	7	131.61±1.54	45.98±2.26	10.30±0.69
July	7	196.58±2.65	71.77±0.98	21.78±1.09
August	8	171.29±0.79	53.53±1.37	10.91±0.78
September	9	52.68±0.82	48.26±0.55	6.99±0.42
October	9	35.24±0.79	46.51±0.44	5.30±0.48
November	8	24.42±1.32	25.73±0.57	3.88±0.51
December	7	23.09±1.58	20.51±1.12	1.74±0.14

Table 3: Seasonal variation in protein, DNA and RNA contents in the ovary of *Psammophilus blanfordanus*. Unit of measurement is given in the parentheses and data is represented as mean ± standard deviation

Month	Number of animals (n)	Protein	DNA	RNA
		(µg/g of ovary wet weight)		
January	6	62.06±1.38	0.09±0.021	0.28±0.031
February	9	60.91±1.75	0.09±0.007	0.25±0.022
March	5	78.28±1.70	0.12±0.010	0.77±0.039
April	5	83.04±1.35	0.53±0.022	1.30±0.089
May	7	120.79±1.96	0.66±0.033	2.17±0.172
June	7	153.18±1.22	0.76±0.071	2.36±0.230
July	7	174.12±1.90	0.87±0.062	1.20±0.201
August	8	100.80±1.03	0.44±0.034	0.78±0.052
September	9	60.93±2.90	0.39±0.023	0.42±0.019
October	9	56.51±1.49	0.27±0.039	0.35±0.037
November	8	39.83±0.99	0.16±0.015	0.26±0.035
December	7	50.65±1.30	0.15±0.028	0.18±0.006

Table 4: Seasonal variation in protein, DNA and RNA contents in the oviduct of *Psammophilus blanfordanus*. Unit of measurement is given in the parentheses and data is represented as mean ± standard deviation

Month	Number of animals (n)	Protein	DNA	RNA
		(µg/g of oviduct wet weight)		
January	6	8.04±2.16	0.05±0.008	0.07±0.017
February	9	6.61±0.81	0.04±0.004	0.06±0.010
March	5	13.54±2.04	0.07±0.008	0.10±0.009
April	5	16.84±2.14	0.11±0.007	0.12±0.012
May	7	19.84±2.09	0.18±0.008	0.15±0.008
June	7	34.10±2.21	0.20±0.010	0.17±0.011
July	7	74.66±1.74	0.36±0.006	0.48±0.005
August	8	37.58±1.73	0.22±0.009	0.12±0.003
September	9	23.57±2.25	0.10±0.008	0.10±0.005
October	9	12.56±1.77	0.07±0.005	0.08±0.004
November	8	9.63±1.04	0.06±0.004	0.05±0.006
December	7	6.33±0.99	0.03±0.02	0.04±0.006

breeding) (Table 1). The oviductal AcP activity followed the same trend of ovarian AcP. The highest value in July was 196.58±2.65 and the lowest value of 23.09 ± 1.58 in December (Table 2). The ovarian and oviductal alkaline phosphatase (AlkP) and â-D-galactosidase enzyme activities showed almost similar patterns of increase or decrease like that of AcP activities (Tables 1 and 2). The activities of AlkP in the month of December in ovary (39.54 ± 1.48) and oviduct (20.51 ± 1.12) were lowest (Tables 1 and 2). The highest ovarian and oviductal AlkP activities in July were 536.39±2.78 and 71.77±0.98, respectively (Tables 1 and 2). The lowest ovarian â-D-galactosidase activity in December was 40.89±0.87 and the highest in July was 803.71±2.25 (Table 1). The â-D-galactosidase activity of oviduct was very low in December (1.74±0.14) and the highest value was in July (21.78±1.09) (Table 2).

Macromolecular contents: The macromolecular contents of ovary and oviduct (Tables 3 and 4) showed more or less similar cyclical changes as that of enzyme activities. The protein and DNA contents of ovary in July (highest value) were 174.12±1.90 µg and 0.87±0.062 µg, respectively (Table 3). The lowest value of ovarian protein 50.065 ±1.30 µg and DNA 0.15±0.028 µg was observed in December (Table 3). The RNA content in the ovary was lowest in December (0.18± 0.006 µg) (Table 3). There was sudden increase in its content in March (0.77± 0.039 µg) in comparison to February value (0.25 ±0.022 µg) (Table 3). These values for the months of May, June and July were 2.17± 0.172 µg, 2.36± 0.230 µg and 1.20±0.201µg, respectively (Table 3). The RNA content of oviduct is highest in July (0.48±0.005 µg) and lowest in December (0.04±0.006 µg) (Table 4). Similar is the case for oviductal protein and DNA contents (Table 4).

Discussion

Lysosomal enzymes are regarded as having the housekeeping function and are widely distributed in nature. These lysosomal enzymes have important roles in ovarian physiology as they are involved in follicular atresia, regression of corpora lutea and ovulation [27]. Acid and alkaline phosphatases are essential enzymes in spermatogenesis and steroidogenesis [28]. The increase activity of AlkP during breeding season (March/April-June/July) in the ovary and oviduct (Tables 1 and 2) of *P. blanfordanus* may be attributed to the transport of materials across cell membrane to meet the demand during highly proliferative state of the ovary (breeding season). The enzyme activity is also gradually increases during recrudescence phase (pre-breeding) over quiescence phase (post-breeding). It has been suggested that AlkP is associated with transfer of solutes across cell membrane [29]· transport of glucose [30] and lipid

[11]. Alkaline phosphatase is localised in theca interna cells of growing follicles and after ovulation with the formation of corpora lutea, the granulosa lutein cells also showed this activity [15]. McNatty [31] has suggested that AlkP is associated with follicular atrophy. Further, the growing follicles have less AlkP activity than the atretic follicles [32] Marked activity of this enzyme has been observed in the blood vessels of corpus luteum and interstitial glands [33] suggesting its role in growing of capillaries. Alkaline phosphatase activity is present in the uterine glandular epithelium of gravid as well as oviparous skinks [34]. They have suggested that the glands in the uterus of skink are continuously involved in active secretion or absorption. This enzyme is localised on the surface of epithelium lining the lumen of uterus in swine (gilt) during oestrus cycle and plays an important role in placental transfer mechanism [29].They have demonstrated that the enzyme activity is greatly increased during breeding and decreased during luteal phase. The AlkP activity in the uterus is associated with nutrient transport [34], helps in its activity, embryo survival and development [29]. Alkaline phosphatase may participate in intracellular processes such as cellular differentiation and growth [35].

Gonadotropic treatments causes a decrease in autophagic vacuoles associated with atretic follicles and increase the AcP activity in steroidogenic cells, like theca of antral follicles, granulosa of Graafian follicles and luteal cells. Theca cells of the growing follicles showed strong AcP activity compared to the oocyte and granulosa cells [15]. In small antral follicles its activity is also high [36]. Acid phosphatases are known to provide phosphate to tissues that show high energy requirements, especially during development, growth and maturation [37] and their possible role in ovulation [38].The increased activity of AcP in the ovary and oviduct during breeding season of *P. blanfordanus* may be attributed to folliculogenesis, vitellogenesis, ovulation and functions of the oviduct which role is as that of uterus for this animal.

From maintenance of spermatozoa to its penetration into oocyte during fertilisation are the carbohydrate mediated events [39]. All these processes are modulated in the oviduct and in fact there are active enzymes present which are able to modify the structure of important oligosaccharides involved. It is hypothesised that the â-D-galactosidase in the oviduct participates in the release of spermatozoa which requires further study [40].â-D-galactosidase could release â-D-galactose into oviduct by removing it from oviduct epithelial cells making it available for metabolism by sperm. Presence of galactose in the bovine zona pellucida has also been reported [41].A possible role of hydrolases in the oviduct (especially in the sperm storage pockets) in female is to maintain the dormancy of sperm [21]. The increased â-D-galactosidase in the oviduct in the present study (Table 2) may have these functional roles during breeding season. Similarly, the activity in the ovary (Table 1) of this animal may be involved in the modification of galactose residues contained in zona pellucida glycoproteins and it is likely helping in remodelling of spermatozoon-oocyte or oocyte-oviduct interaction. But this has to be established. However, as in the present study crude extracts of whole organs were taken for quantification of these three enzyme activities, it is not possible to know the contribution of a particular tissue type of the ovary and oviduct.

The increase in protein, DNA and RNA contents in ovary and oviduct during breeding season (Tables 3 and 4) is in good agreement as reported for *Hemidactylus flaviviridis*. Sharma and Grewal [17] demonstrated in this lizard that the DNA, RNA and protein increases with the advancing stages of follicular growth. Saxena [16] have also showed higher concentration of protein, lipoprotein, RNA and other macromolecules in the cortical zone of the oocytes in *H. flaviviridis*. The increase in RNA to DNA ratio and these macromolecular contents of the ovary during breeding period may be due to increased metabolic activities.

Conclusion

It may be concluded that there is a correlation between enzymatic activities and macromolecular contents of the ovary and oviduct with that of the different breeding phases of this seasonal breeder. The above parameters are higher in breeding period (April to July) than non-breeding period (August to March). They may have a role in follicular growth, vitellogenesis, egg shell formation and oviposition.

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