CHAPTER IV

OVIDUCT

***

The oviducts in submammalian vertebrates are derived from the Mullerian ducts and develop to form oviducts under the influence of estrogens. They open into the cloaca. The oviducts are surrounded by a sheath of smooth muscle fibers and by rhythmical contractions, can propel objects like eggs towards the exterior, which is also true for reptilians.

In reptiles as in other vertebrates the ovariectomy resulted in regression of the oviducts, whereas administration of estrogens stimulated the growth and development of these structures (Miller 1948). Panigel (1956) showed that although progesterone stimulated the oviduct, it is less effective than estrogen. He also believed that estrogens might act most effectively on the glandular portion of the oviduct and progesterone on the muscular wall. The effects of female sex-harmones in reptiles have been reported by Turner, 1935; Mellish and Meyer(1937); Fobes (1938); Gorbman (1939); Evans and Clapp (1940), Mead Rodney et al. (1981); Botte and Granata (1977), isolated proteins from Lacerta sicula which could be compared with the sex-harmone receptors in target organs of mammals.

Goin and Goin (1962) have generalized the oviducal histology of different reptiles. The inner layer of the oviduct contains numerous glandular cells and may be modified in various ways so that it contributes to the well being of

the egg. In the oviparous species, segmental differences in function may occur along the length of the oviduct, such as one associated with the formation of albumen glands and the secretion of hard outer shell (Van Tienhoven, 1968). The oviducts of lizards and snakes lack well defined albuminous glands in the upper portions (Goin and Goin, 1962). These glands are involved in the development of egg. The egg yolk is the only source of nutriment to the young. In most species the eggs increase in size considerably due to water uptake from a network of maternal blood vessels in the area of oviduct adjascent to the eggs. The water intake takes place through the porus shell. (Goin and Goin, 1962).

The eggs of geckos are soft when laid with lower content of the calcium carbonate as compared to the bird egg shells, but harden after exposure to air (Sadleir, 1969).

The literature related to histochemical study of reptilian oviduct is very scanty. Botte(1973) distinguished four regions in oviducts of L. sicula viz., infundibulum, tuba, uterus and vagina. He reported two types of cells, ciliated and secreting in tuba, uterus and vagina; the latter type elaborated only acidic mucins in tuba but acidic and neutral mucins in uterus and vagina. The oviducal glands in the middle part of the tuba elaborated albumen, where as the glands in the terminal part of the tuba and uterus involved in shell organization. Callard and Leathem (1970) showed

seasonal variations in glycogen content in preovulatory animals and it was higher during pregenacy. Jahangeer et al., (1976) also studied seasonal alterations in oviducal glycogen in U. hardwickii. The glycogen was high during breeding and hibernation. Faratade (1981) also reported goblet cells containing acid mucopolysaccharides in oviduct of garden lizard; which showed seasonal variations.

The oviducts of this gecko were situated in the abdominal cavity. Morphological variations of oviduct throughout the year indicated, that it was related to the seasonal breeding activity of the animal. They were well developed during breeding season.

The oviducts from the sacrificed females in each month were cut sequentially in four parts (Botte, 1973; Faratade 1981) and were processed as mentioned in Chapter II. The histology of the above four parts was determined with the eosin-haematoxylene preparation.

OBSERVATIONS

A) HISTOLOGY

1) Infundibulum - This part of the oviduct showed very narrow lumen lined by mucosal layer formed of pseudostratified epithelium, very thin layer of submucosa and outermost layer of muscles. The cells from mucosal layer in this region showed cilia (Fig. 27,28).

2) Tuba - The lumen in this part was some-what dialated. All the other parts were same as in infundibulum region, but the submucosal region had oviducal glands which showed extensive basophilia in eosin-haematoxylene preparation. The mucosal layer showed pseudostratified epithelium, which contained number of goblet cells. Few cells in this region showed cilia (Fig. 29,30).

3) Uterus - The histological pattern was same as the tuba region. The oviducal glands observed in tuba region showed an increase in number during breeding season. In addition to these glands, pear shaped glands were noted at the base of the mucosal layer during breeding season only. In some sections, the confluency of these pear shaped glands with the oviducal glands and with the oviducal lumen was evident (Fig. 31,32,33).

4) Vagina - This part of the oviduct was thin walled. It lacked the oviducal glands. The mucosal part of the oviduct showed pseudostratified epithelium. Number of goblet cells was also evident (Fig, 34).

B) DISTRIBUTION OF MUCINS :

1) Infundibulum :

The connective tissue and muscle layer showed moderate PAS reactivity which could be partially blocked by phenylhydrazine pretreament and partially resistant to maltdiastase digestion

indicating presence of glycogen and neutral mucopoly--saccharides. Poor amount of acid mucopolysaccharides were also noted in the connective tissue. The mucosal layer showed presence of goblet cells which displayed an intense PAS reactivity intense alcianophilia at AB pH 2.5, weak acianophilia at AB pH. l. The PAS reactivity could be partially blocked by phenylhydrazine pretreatment showing presence of neutral mucins and carboxy mucins in them. The corboxy mucins were infered from their blue staining with AF-AB pH 2.5 sequence, enhanced metachromatia with azure A at pH 3.0 and above, the blockade of their alciano--philia after methylation and restoration of it following saponification. Their alcianophilia was sensitive to acid hydrolysis. All these results indicated presence of neutral mucosubstances and carboxy mucins in them (Fig. 28-G).

During breeding period all the parts of infundibulum were well developed and showed increased number and staining intensities of goblet cells in mucosa (Fig. 28-G).

2) Tuba

The connective tissue and muscle layer showed poor amount of glycogen, neutral mucopolysaccharides, and acid mucopolysaccharides in them, which could be judged by their poor staining intensities. The oviducal glands in the submucosal tissue did not exhibit any histochemical reactions indicating

absence of mucopolysaccharides. Their extensive basophilia observed in eosine-haematoxylene preparations might be due to protein; which was confirmed by an intense staining with bromophenol blue reaction; which was sensitive to pepsin digestion. The goblet cells from the mucosal layer showed PAS-reactive acidic mucins; which were infered by different methods employed, for the study. (Table NO. 2)

3) Uterus

As in infundibulum and tuba the connective tissue and muscle layer showed presence of glycogen, neutral mucins and acidic mucins in them. The oviducal glands in this region showed presence of proteins. The deeply seated pear shaped glands showed intense PAS reactivity which could be blocked by pepsin digestion indicating presence of PAS reactive proteins probably containing sufficient amount of hydroxylysin in them, These glands were unreactive to the remaining methods employed for the mucin detection. The goblet cells from mucosa showed presence of only acidic mucins in them, which were confirmed by the blue staining with AF-AB pH 2.5 sequence, enhanced metachromatia with azure A at pH 3.0 and above; the blockade of this alcianophilia after methylation and restoration of it following saponification. Their alcianophilia was sensitive to acid hydrolysis. (Table No. 2).

This part also showed hypertrophy of oviducal glands,

pear shaped glands and the goblet cells during breeding period showing increase in number of glands, goblet cells and increase in their staining intensities.

4) Vagina

The connective tissue and muscular layer showed moderate amount of glycogen neutral mucopoysaccharides and acid mucopolysaccharides. The mucosal layer showed goblet cells containing acid mucopolysaccharides. In this region also the staining intensities and number of goblet cells showed an increase during breeding period. (Table No. 2).

DISCUSSION

The above results show the presence of acid mucoplysaccharides, glycogen and neutral mucopolysaccharides in muscles and connective tissue of all the four parts of the oviduct, though poor in amount. The goblet cells from the mucosal layer of infundibulum, tuba, uterus and vagina showed acid mucopolysaccharides; while few goblet cells from infundibular region showed neutral mucopolysaccharides in addition to the acidic mucins. The goblet cells were maximum in number in tuba and uterus as compared to those in infundibulum and vagina region of oviduct. Oviducal glands containing proteins are also located in tuba and uterus regions of oviduct, as observed by Botte (1973).

The mucopolysaccharides found in infundibulum were acidic type and were present mainly in the goblet cells

where as goblet cells showed presence of neutral mucins. This is the nearest part to the ovary, and ova enters the oviduct through this passage. This region of oviduct showed presence of number of ciliary cells which suggests that the ciliated cells involved in the movement of ovum and the mucins probably participate in the lubrication of oviduct (Lison, 1960; Goudsmit, 1972; Varute and Nalawade, 1973).

The tuba region showed presence of the oviducal glands embeded in connective tissue, which were elaborated during breeding period. These cells displayed histochemical reactions indicating presence of proteins only. The carbohydrates were mainly found in the goblet cells. The number of goblet cells containing high amount of acid mucopolysaccharides could be identified in this region. This region showed number of ciliary cells.

All these results show that the acid mucopolysaccharides and proteins in this part may be sharing in egg growth and smooth passage of egg through the oviduct as suggested by Botte (1973); Van Tienhoven, (1968).

In the uterus region were noted the two types of glands, which with their staining reactions showed presence of proteins but those from pear shaped glands probably contain proteins with hydroxylysine (Glegg et al., 1953). The goblet cells from the mucosal layer showed large amount of acid mucopolysaccharides. The two types of glands containing proteins (oviducal and pear shaped) showed continuity with

the lumen (Fig. 33). Probably to discharge their secretion in the lumen, proteins containing hydroxylysine secreted by pear shaped glands and proteins from oviducal glands and mucins secreted by goblet cells. This region may be participating in the shell formation as suggested by Botte (1973) in L. Sicula.

The presence of low amount of calcium carbonate is noted in gecko egg shells as compared to that of birds (Sadleir 1969). The phenomenon of calcification of eggs of gecko may be taking place in some part of the oviduct. The Ca^{++} metabolism in relation to shell formation activities is discussed in turtles (Erben, 1970; Packard, ,1980; Simkiss, 1967) but its relationship, if any, with oviduct is not discussed. Such type of attempt is made only in the shell formation of birds. Aitken, (1971), has reviewed the details of oviducal histochemistry and composition of shell, in case of birds. The egg shell composition of birds indicate presence of ovakeratin, containing hydroxylysine (Candlish and Scougall, 1969) and the fibers observed in the outer sheath, showed proteins probably glycoproteins (Balch and Cook 1970). The calcium metabolism especially the levels of Ca^{++} in serum during breeding period are studied inbirds (Aitken, 1971; Simkiss, 1961; 1967; Simkiss and Taylor 1971; Sturkie and Mueller, 1976); and in reptiles especially in turtles. But actually the process of calcification takes place in the oviduct; and the presence of Ca^{++} in oviduct is important. The autoradiographical data showed the presence of Ca^{++} in mucosal epithelial

layer of oviducts (Johnston et al.,1963; Mccallion 1953) of birds. The bird oviduct also showed proteins containing hydroxylysine which were also found in the egg shell of birds (Gilbert et al., 1968). The presence of acid mucopolysaccharides was noted in bird oviduct (Aitken, 1971, Simkiss and Taylor, 1971) especially in tuba and uterus part.

When the above oviducal data of bird is compared with the present data of H. flaviviridis; it shows many similarities e.g. presence of hydroxylysine containing proteins which are also uterus region; presence of acid mucopolysaccharides in the muscosal goblet cells; and presence of Ca^{++} in the mucosal epithelial cells (which was carried out in present studies by Alizarin red staining). All these similarities in bird oviduct and wall lizard oviduct seem to be interesting.

The process of calcification is involved in many regions of the animal body and the deposition of Ca^{++} in soft tissues appears to be same in all the tissues under going calcification.

The process of calcification in cartilage has been described and studied by many workers (Dziewatkowski, 1964; Dziewiatkowski et al., 1970; Hirschman and Dziewatkowski, 1966; Hirschman, 1967; Hirschman, 1967; Hirschman and Silverstein, 1968; Jibril, 1967). The probable role of acid mucopolysaccharides (proteoglycans) in cartilage in relation to calcification of the cartilage is studied and discussed by Ali, (1964); Dziewiatkowski, (1964); Jibril, (1967); Hirschman

and McCabe, (1974); and Weinstein et al.,(1963); where they have suggested that the selective degradation of proteoglycans by proteases helps to move the Ca^{++} at the deposition site.

In case of H. flaviviridis presence of acid mucopolysaccharides in mucosal epithelium; presence of Ca^{++} at the border of the epithelium and presence of hydroxylysine containing protein in the same region of the oviduct are of suggestive importance; as they are also found in the bird oviduct which is actively involved in the shell formation.

But still, the exact role of oviduct in shell formation in the Indian gecko remains to be elucidated as the processes like calcium metabolism, formation of calcium carbonate and other metabolisms involved in shell formation are complex and need a detail study and different approach.

..