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A scanning electron microscopic study of the oviduct of the toad, before and during ovulation

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Summary. The surface features of the oviduct of the toad, *Bufo melanostictus*, was studied under the scanning electron microscope. The epithelial folds show regional variations, being longitudinally disposed in the upper part of the oviduct, spirally arranged in the middle and convoluted in the lower part. There is an abundance of cilia through the oviduct except in the ovisac. Goblet cells are interspersed among the epithelial cells. In between the epithelial folds are openings of tubular secretory glands. During ovulation, there is patchy loss of cilia throughout the oviduct. Most of the goblet cells are empty. On the surface, the cilia are matted together by secretory material which are laid down in layers.

Key words: Scanning EM, Toad oviduct

Introduction

The earliest description of the amphibian oviduct was given by Ecker and Wiedersheim (1904); they described the frog oviduct as being made up of three parts: *pars recta*, *pars convoluta* and *pars uterina*. This was followed by studies of the general morphology of the oviduct by Holmes (1934) and Rugh (1961). Such a subdivision of the oviduct on the basis of regional differences has been further supported by observations from morphological (Low et al., 1976), histological (Suvarnalatha, 1975; Low et al., 1976), histochemical (Yoshizaki, 1985) and ultrastructural (Fawcett and Porter, 1954; Lee, 1967; Fasolo and Franzoni, 1970) studies.

The present paper presents the results of a scanning electron microscopic (SEM) study of the features of each region of the oviduct of the toad, *Bufo melanostictus*, before and during experimentally-induced ovulation which have not been reported earlier.

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Materials and methods

Sex and size of toads used

60 mature male and 6 mature female toads with well-developed ovaries of the species, *Bufo melanostictus* Schneider, measuring about 70 mm from snout to cloaca and weighing between 70 to 130 g were used. The 6 female toads were separated out and kept in a holding tank as «recipient» toads which will receive an injection of pituitary glands. The female toad was differentiated from the male by the absence of a yellowish band on the undersurface of its throat. The male toads were used as «donor» toads from which the pituitary gland was obtained.

Assessment of the status of the ovaries

The state of the ovaries was assessed by holding up the toad with its abdomen against a table lamp. The presence of a large opacity in the abdomen indicated that the ovaries were well-developed.

Artificial induction of ovulation

Ovulation was induced by injecting pituitary glands from «donor» toads of either sex into the abdominal cavity of the «recipient» toads.

Removal of pituitary gland from the «donor» toads

The small, pink, pea-sized pituitary gland from the pithed, «donor» toad was removed according to the method described by Lee and Chen (1970) following decapitation and splitting of the cranium and forebrain in the midsagittal plane. The pituitary gland was picked up with the watchmaker's forceps and placed in Holtfreter's solution.

Injection of the pituitary gland into the «recipient» toads

Each «recipient» toad was injected with 10 pituitary

glands as described by Rugh (1962). The glands were drawn up into the barrel of a 20 ml syringe together with about 10 ml of Holtfreter's solution and injected into the lower, ventrolateral region of the abdominal cavity of the «recipient» toad with an 18-gauge needle.

After the injection, each toad was kept in a plastic container which was covered with a wire net. Each container contained a little tap water at room temperature (28 °C). When abdominal contractions were observed - about 6-8 hours after the injection - the toads were sacrificed by intracardiac perfusion with 50 ml of toad Ringer solution followed by 10% formalin for about 30 minutes.

Protocol for SEM

The fixed tissues were cut into small pieces and kept in formalin for 2-3 days after which they were osmicated in 1% osmium tetroxide solution in 0.1M phosphate buffer at pH 7.3 for 2 hrs. The tissues were then dehydrated through an ascending series of ethanol and dried off liquid carbon dioxide in a Polaron E 3100 Series II critical point dryer (Polaron Equipment Ltd., U.K.). In order to study the tissues in cross-section, some pieces were fractured transversely. The tissues were then mounted on to stubs with silver paint and coated with gold in a Polaron E 5100 Series II «Cool» sputter coater (Polaron Equipment Ltd., U.K.). The specimens were then viewed through a Philips SEM 505 scanning electron microscope.

Results

Nomenclature

The nomenclature used for the six regions of the oviduct of the toad in the present study will be that introduced by Low et al. (1976), namely: fimbrium, infundibulum, upper magnum, middle magnum, lower magnum, isthmus and ovisac.

Oviduct before ovulation

Epithelial folds

Longitudinally-disposed epithelial folds which run parallel to each other are a prominent feature throughout the oviduct except the ovisac. From the fimbrium, they converge as they enter the ostium which leads into the infundibulum (Fig. 1). In the infundibulum, these folds are much taller than those in the fimbrium and vary in length. Some are straight whereas others are convoluted. In the magnum, most of them run spirally, obliquely or transversely across the long axis of the oviduct. In the ovisac, the epithelium is folded and convoluted along its length.

Epithelial cells

Throughout the extent of the oviduct, except the ovisac, the epithelial folds are covered with a thick carpet of fine cilia (Fig. 2). In the ovisac, only tufts of cilia are seen dispersed randomly on the surface. Most of the epithelial cells are ciliated (Fig. 3) and each ciliary shaft measures about 7-8 µm in length. These cells are columnar and in fractured specimens, their cytoplasm shows numerous fine granules (Figs. 3, 4).

Dotted irregularly over the surface of the epithelial folds are cilia-free areas which are the luminal surfaces and openings of goblet cells (Fig. 5). Globules of secretory material are often seen on them (Fig. 6). Such cells are particularly numerous in the isthmus.

Glands

Many glandular openings are present in the trough between adjacent folds (Fig. 7). On the surface, the ostium of each gland appears as a small opening on the summit of a round to oval, button-shaped structure. In fractured specimens, the glands have a tubular appearance (Fig. 8).

Oviduct during ovulation

Epithelial folds

The epithelial folds in the oviduct, except in the ovisac of the ovulating toad, show the same general features as in the pre-ovulating ones. In contrast, the surface of the ovisac is covered by a layer of secretory material which obscures the epithelial surface. This layer of secretion is perforated in many places, giving it a fenestrated appearance (Fig. 9). Here and there, tufts of cilia protrude above the layer of secretion into the lumen of the ovisac (Fig. 9).

Cilia

A characteristic feature of the oviduct in ovulating toads is the loss of cilia which occur in patches on the epithelial folds throughout the length of the oviduct (Figs. 10, 11).

Fig. 1. Oviduct before ovulation. Photograph of the infundibulum (*) leading into the ostium (O). x 45

Fig. 2 Oviduct before ovulation. Photograph of the upper magnum showing the epithelial folds which are densely covered with cilia. x 380

Fig. 3. Oviduct before ovulation. A high power micrograph of a fractured specimen of the epithelial fold showing numerous cilia and intracellular granules. x 5,200

Fig. 4. Oviduct before ovulation. Photograph of a fractured specimen of an epithelial fold. x 1,500


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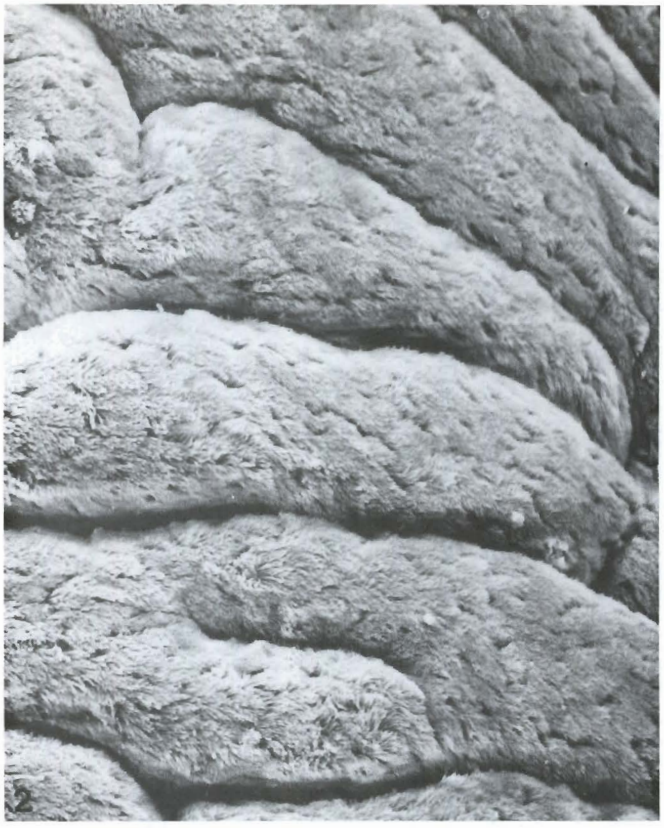
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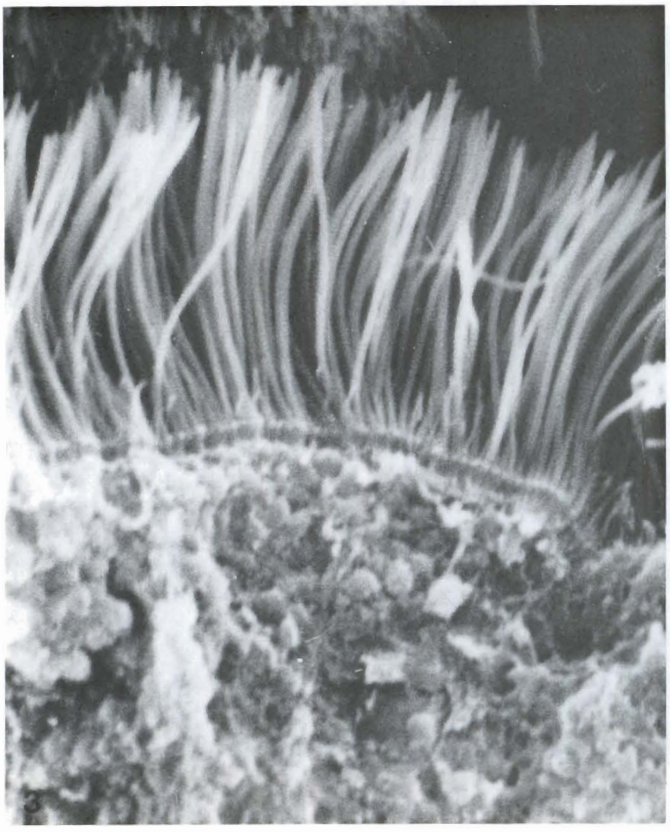
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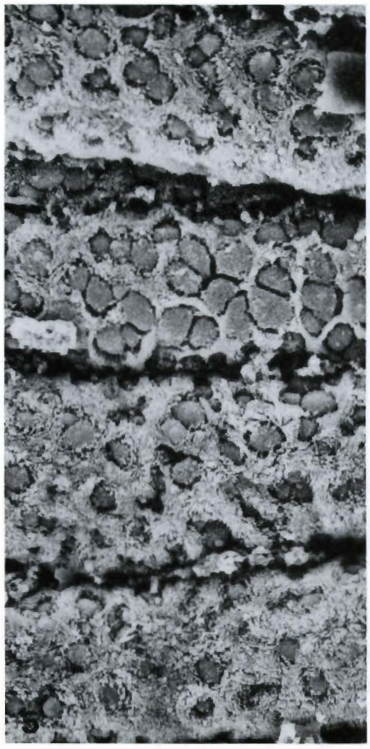
Oviduct of the toad



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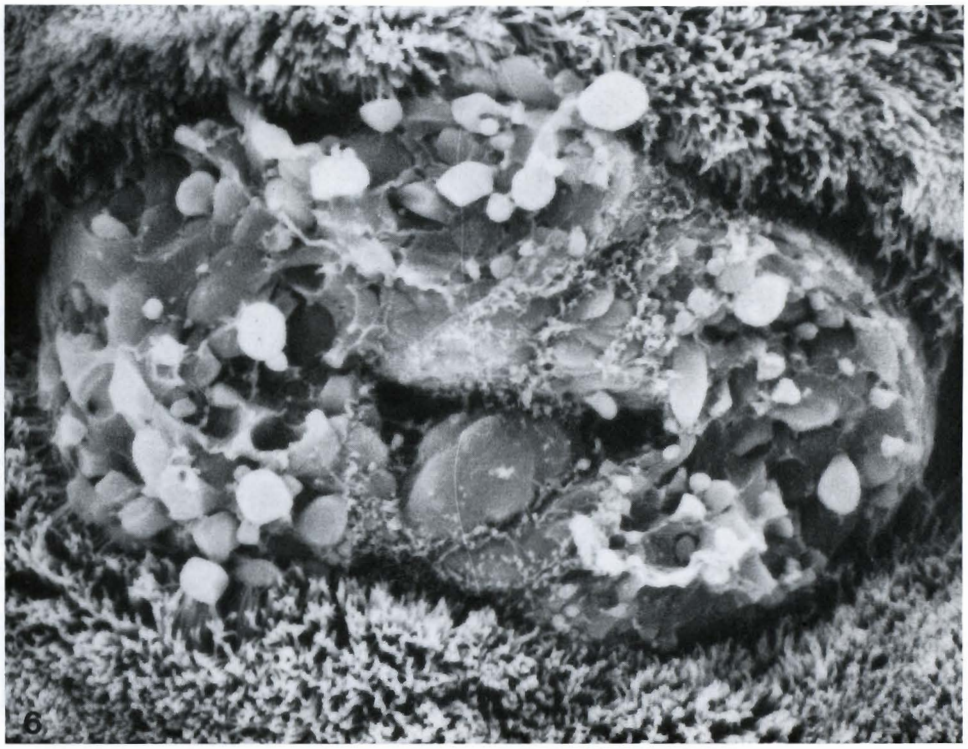

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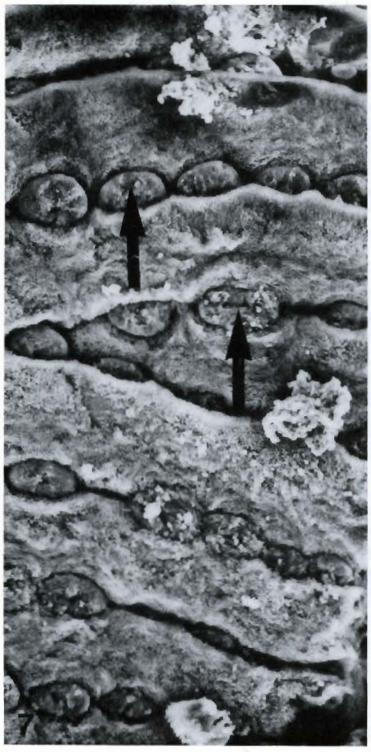

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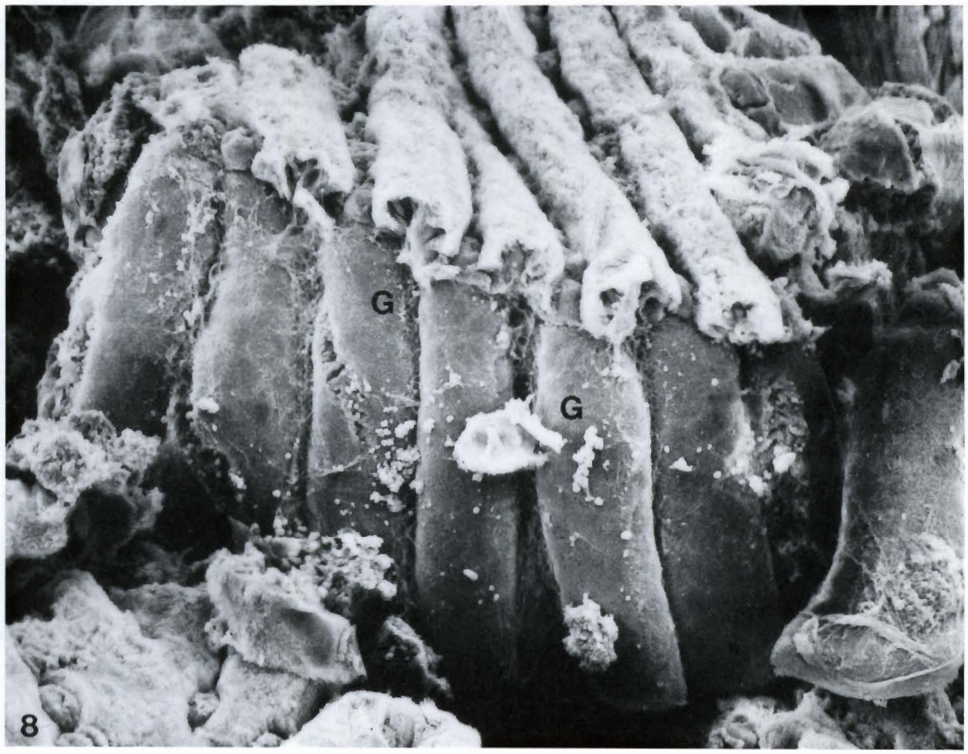

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Fig.5. Oviduct before ovulation. Photograph of the surface of the epithelial folds of the magnum showing the luminal surfaces of goblet cells interspersed among the cilia. x 380

Fig. 6. Oviduct before ovulation. A high power micrograph of the luminal surface of a goblet cell showing secretory material leaving its ostium. x 2,300

Fig. 7. Oviduct before ovulation. Photograph of the magnum showing the openings of gland cells (arrows) in the troughs between the epithelial folds. x 210

Fig. 8. Oviduct before ovulation. Fractured specimen of the magnum showing the tubular gland cells (G) below the level of the epithelial folds. x 220

Goblet cells

As a result of the loss of cilia, the luminal surfaces of groups of goblet cells bearing microvilli are now more obvious. In the isthmus, many cilia-free openings of goblet cells are seen. Some of these goblet cells appear to be empty (Fig. 12) whereas others appear to exude an amorphous substance. In the magnum, the cilia appear to be matted or glued together by secretory material (Fig. 13).

Egg jelly

In specimens containing an egg, the features of the egg jelly can be observed. Figure 14 shows an egg together with its covering of egg jelly in the lower magnum. The latter forms a continous sheet or layer over the epithelium. Under high power SEM, this sheet is seen to be made up of several layers (Fig. 15). The superficial part of the jelly is made up of several layers which have an undulating appearance while the deeper layers, which come into contact with the epithelium, are compact.

Discussion

Epithelial folds

The present study has shown that in the epithelium throughout the entire length of the oviduct of *Bufo melanostictus* is made up of a single layer of ciliated, columnar cells interspersed with goblet cells. But in the magnum, simple tubular glands are present beneath the epithelium; these glands open into the lumen of the oviduct through ostia which are located in the troughs between adjacent folds of the epithelium. A similar epithelial structure has also been reported in some species of frogs, namely, *Rana pipiens* (Shivers and James, 1970) and *Rana cyanophylctis* (Suvarnalatha et al., 1975). However, such a feature is not present in the oviduct of all anurans. In *Rana japonica* (Yoshizaki and Katagiri, 1981), *Bufo bufo japonicus* (Katagiri et al., 1982) and *Xenopus laevis* (Yoshizaki, 1985), saccular outgrowths instead of tubular glands are present at the bases of the epithelial folds.

It has been reported in *Rana japonica* (Yoshizaki and Katagiri, 1981) and *Xenopus laevis* (Yoshizaki, 1985) that the ridges of the epithelial folds do not run longitudinally but spiral down the magnum. The present study also suggests a similar arrangement of the epithelial folds in *Bufo melanostictus*.

The folds help to greatly increase the surface area of the epithelial surface so that a greater volume of secretion can be produced. This is especially pronounced and perhaps more important in the distal part of the oviduct since it has been suggested that one of the important functions of such secretions is their ability to cause a structural transformation of the vitelline coat which can assist sperm penetration and therefore fertilization (Grey et al., 1977; Katagiri et al., 1982).

Lofts (1974) has suggested that the folds may serve an important function in causing the egg to rotate as it is propelled down the oviduct by the action of the cilia. This would enable at least the first layer of jelly envelope of the egg to be laid down evenly.

Epithelium

Ciliated cells

In the present study, two types of cells, namely ciliated, non-secretory, columnar cells and non-ciliated, secretory cells have been observed in the epithelium. The ciliated cells bear long cilia which are densely crowded together and project prominently into the lumen of the oviduct. In *Rana japonica*, microvilli have been reported in addition to cilia (Yoshizaki and Katagiri, 1981). Since the oviduct has only a very thin layer of smooth muscle, it has been suggested that in ovulating toads, ciliary action is important in propelling the eggs down the tract (Low et al., 1976).

Histochemical studies (unpublished data), have shown that glycogen granules are present in these cells in the non-ovulating toads but they are greatly reduced

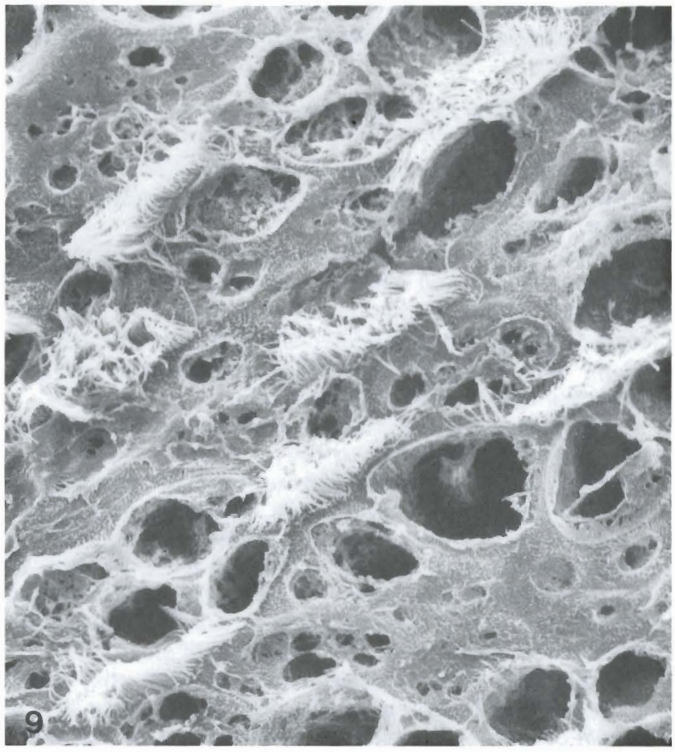
Fig. 9. Oviduct during ovulation. Photograph of the surface of the ovisac which is covered by a thick, fenestrated layer of secretory material. Scattered tufts of cilia are seen. x 1,180

Fig. 10. Oviduct during ovulation. Photograph of the upper magnum showing extensive loss of cilia. x 310

Fig. 11. Oviduct during ovulation. A high power view of the surface of the upper magnum showing an area denuded of cilia. x 5,900

Fig. 12. Oviduct during ovulation. Photograph of a fractured specimen of an epithelial fold in the upper magnum showing empty goblet cells (*). x 700

Oviduct of the toad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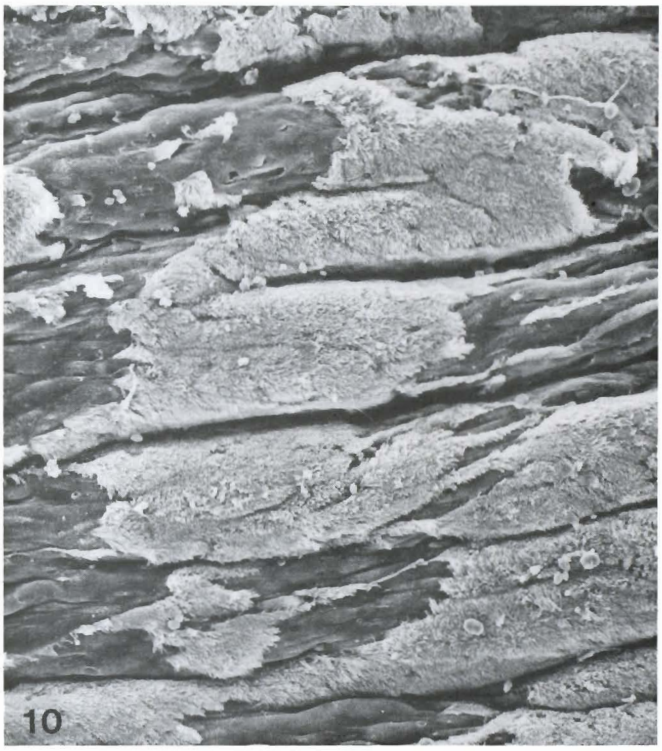
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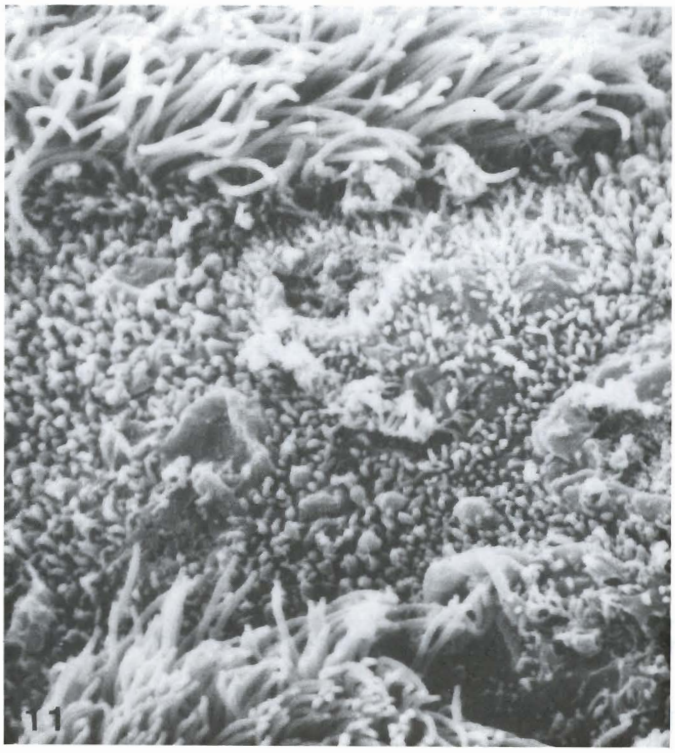
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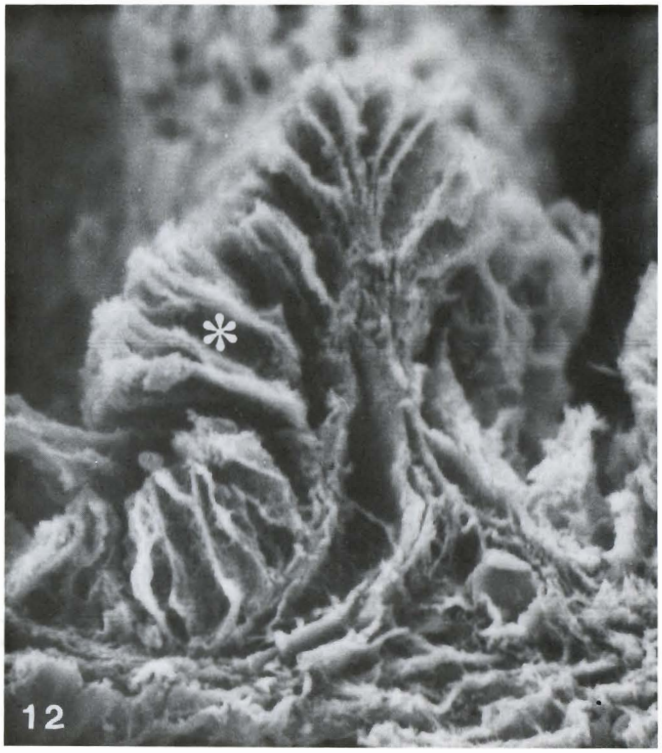

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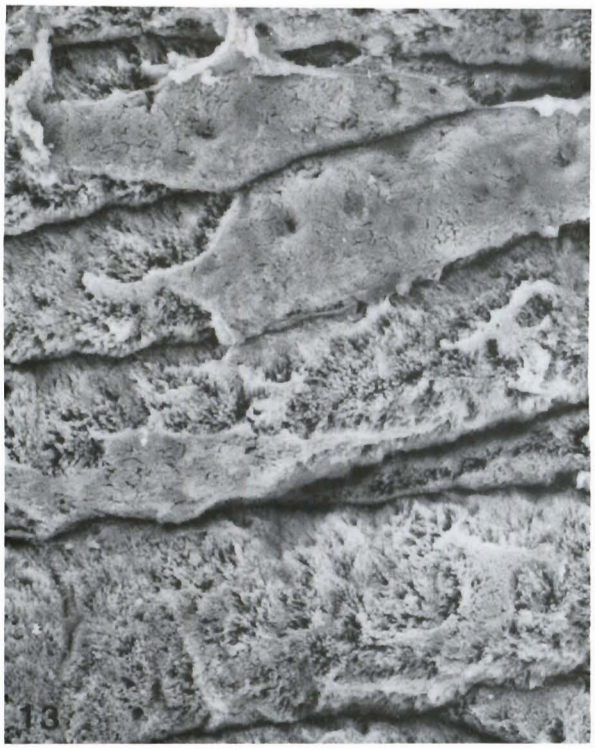

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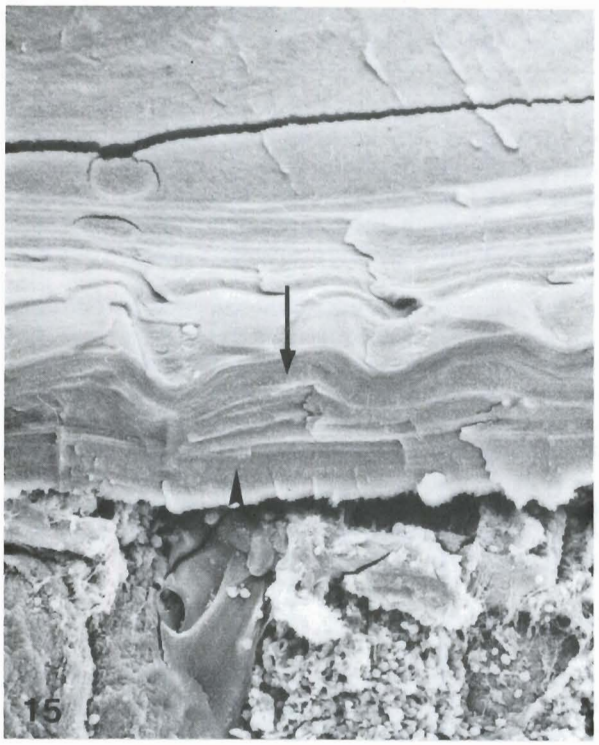

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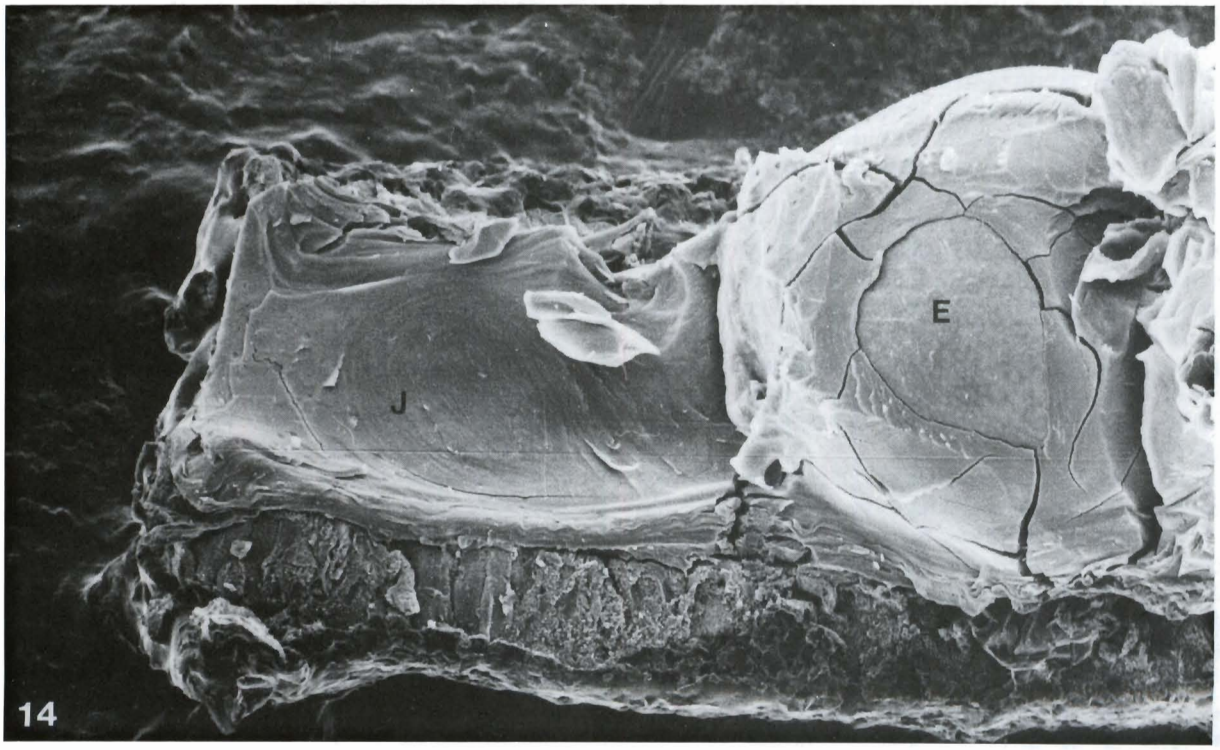

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Fig. 13. Oviduct during ovulation. Photograph of the magnum showing cilia being matted together by secretory material. x 365

Fig. 14. Oviduct during ovulation. A photograph showing an egg (E) with its covering of jelly (J) in the lower magnum. x 70

Fig. 15. Oviduct during ovulation. A high power photograph showing the multiple layering of the egg jelly lying on top of the epithelium. (arrow = superficial layer of jelly; arrow-head = deeper layer of jelly). x 520

in amount or depleted in the ovulating toad. This suggests that glycogen in these cells could have been depleted quickly due to the activity of the cilia. This is not unlikely as Fredricsson (1959) has observed that in the human fallopian tube, a decrease in glycogen paralleled the known increase in ciliary activity during ovulation.

The paucity of cilia in the ovisac suggests that ciliary action is less important near the end of the tract since the eggs there are released into an easily distensible ovisac. Furthermore, the egg at the end of the oviduct would have been coated by a thick and slippery layer of jelly which would enable it to pass down a narrow tube with much less friction than an egg without jelly in the upper regions of the duct. Furthermore, ciliary action is not necessary for oviposition, which is controlled by muscles (Lofts, 1974; Low et al., 1976).

Secretory cells

Secretory cells bearing microvilli have been reported in *Rana pipiens* (Lee, 1967), *Rana japonica* (Yoshizaki and Katagiri, 1981) and *Xenopus laevis* (Yoshizaki, 1985). In the present study, microvilli-bearing cells, which occur either singly or in small groups, were observed only in the infundibulum. In the frog, Lee (1967) has described mucus-secreting and ciliated epithelial cells capping the tubular glands of the *pars convoluta*. The goblet cells in *Bufo melanostictus*, however, have been observed to contain muco-polysaccharides (unpublished data).

In *Xenopus laevis*, Yoshizaki (1985) has shown that the secretory cells in the proximal two-thirds of the pars recta differ from those in the posterior third. Furthermore, in the distal third, the secretory cells on the ridges of the epithelial folds contain electron-lucent granules which are different in size from those in the secretory cells at the bases of the ridges. Such differences could not be observed in the present study with the SEM.

Yoshizaki and Katagari (1981) have suggested that the secretory cells do not produce the egg jelly and that their secretions are responsible only for altering the properties of the vitelline coat so as to enhance the fertilizability of the egg. In a latter study, Yoshizaki and Katagiri (1984) suggested that another type of secretory cell is involved in the formation of a pre-fertilization layer for the eggs.

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References

Ecker A. and Widersheim R. (1904). Anatomie des Frosches auf Grund Eigener Untersuchungen durchaus neu Bearbeitet von Dr Ernst Grupp. 2nd ed. Druck und Verlag Frierich Viewes und Son. Braunschweig.

Fasolo A. and Franzoni M.F. (1970). Cyclic modifications of the ciliated cells in the oviduct of the crested newt. Boll. Zool. 37, 161-167.

Fawcett D.W. and Porter K.R. (1954). A study of the fine structure of ciliated epithelial. J. Morphol. 94, 221-274.

Fredricsson B.G. (1959). Histochemical observations on the epithelium of human fallopian tubes. Acta Obst. Gynecol. Scand. 38, 109-134.

Grey R.D., Working P.K. and Hedrick J.L. (1977). Alteration of structure and penetrability of the vitelline envelope after passage of eggs from coelom to oviduct in *Xenopus laevis*, J. Exp. Zool. 201, 73-84.

Holmes S.J. (1934). Biology of the frog. 4th edition. Macmillan Co. New York.

Katagiri C., Iwao Y. and Yoshizaki N. (1982). Participation of oviductal pars recta secretions in inducing the acrosome reaction and release of vitelline coat lysin in fertilizing toad sperm. Dev. Biol. 94, 1-10.

Lee P.A. (1967). Studies of frog oviductal jelly secretion. II. Cytology of secretory cycle. J. Exp. Zool. 166, 107-120.

Lee S.H. and Chen T.W. (1970). Artificial breeding and early development of the tadpoles of *Rana limnocharis* Boie. J. Sing. Nat. Acad. Sci. 2, 59-67.

Lofts B. (1974). Physiology of the amphibia. Academic Press. New York.

Low K.L., Chen T.W. and Tan C.K. (1976). The acquisition of egg jelly and its effect on fertilizability and hatchability in *Bufo melanostictus*. Copeia 1976, No. 4, 684-689.

Rugh R. (1961). The frog: Its reproduction and development. Rev. Edition. McGraw-Hill, New York.

Rugh R. (1962). Experimental embryology: Techniques and procedures. 3rd edition. Burgess Publishing Co.

Shivers A.C. and James J.M. (1970). Morphology and histochemistry of the oviduct and egg-jelly layers in the frog, *Rana pipiens*. Anat. Rec. 166, 541-556.

Suvarnalatha M., Sarkar H.B.D. and Pilo B. (1975). Histopathology of the oviduct in the skipper frog, *Rana cyanophlyctis* (Schn). J. Anim. Morphol. Physiol. 22, 174-183.

Yoshizaki N. (1985). Fine structure of oviductal epithelium of *Xenopus laevis* in relation to its role in secreting egg envelops. J. Morphol. 194, 155-169.

Yoshizaki N. and Katagiri C. (1981). Oviductal contribution to alteration of the vitelline coat in the frog, *Rana japonica*. An electron microscopic study. Dev. Growth Differen. 23, 495-506.

Yoshizaki N. and Katagiri C. (1984). Necessity of oviductal pars recta secretions for the formation of the fertilization layer in *Xenopus laevis*. Zool. Sci. 1, 255-264.

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