

قسم : التشريح والهستولوجيا .  
كلية : الطب البيطرى - جامعة أسيوط .  
رئيس القسم : أ.د. / حلمي محمد بدوى .

دراسات بالمجهر الضوئى وباستخدام الماسح الاليكترونى  
على الرحم فى الدجاج الفيومى

\* جمال كامل ، أحمد حسن ، عبدالهاده على ، علام نفاى

استخدم فى هذا البحث عدد ١٥ دجاجة فىومى وذلك لدراسة تركيب الرحم اثناء  
مرور الببضة فى قناة الببض .

وقد أمكن فى هذا البحث تميز نوعين من الخلايا فى النسيج الطلائى ، خلايا  
مهديه وخلايا غير مهديه وتزداد نشاط هذه الخلايا عندما يكون الرحم حاملا للببضة .

وقد لوحظت ظاهرة تكوين الأهداب فى الخلايا المبطنه لجدار الرحم .  
وقد أعطيت الغدد الأنبيبه الكيسيه الرحمية نتائج سالبة مع المواد الكريوهيدراتية  
والمواد الدهنية وطريقة فون كوسا للكشف عن الكالسيوم .

وقد تم مشاهدة الخلايا الصبغية الحاملة للميلانين فى الطبقة المخاطية للرحم مما  
يدل على أن هذه الخلايا ربما تكون هى المسئولة عن اعطاء اللون لقشرة الببضة .

Dept. of Anatomy & Histology,  
Faculty of Vet. Med., Assiut University,  
Head of Dept. Prof. Dr. H. Badawy.

**LIGHT AND SCANNING ELECTRON MICROSCOPICAL  
OBSERVATIONS ON THE SHELL GLAND OF FAYOMI FOWL**  
(With 13 Figs.)

By  
**G. KAMEL; A.H.S. HASSAN; A.M.A. ALI and A. NAFADY\***  
(Received at 4/12/1986)

**SUMMARY**

Light and scanning electron microscopic studies were carried out on the uterus (shell gland) of fifteen mature fayomi laying hens during egg producing cycle.

Two types of cells were recognized in the surface epithelium of the uterine mucosa, ciliated and non ciliated cells. These cells exhibited morphological signs of increased activity when the uterus contained an egg indicating that these cells were associated with the formation of the protein material of the shell as well as the cuticle of the egg.

In addition, these ciliated cells presents a real evidence for the process of ciliogenesis.

The uterine glands were of the branched tubulo-alveolar variety, negatively stained with PAS, AB, Sudan black and von Kossas method. They were markedly active when the uterus was distended with an egg.

Melanin containing cells were demonstrated in the lamina propria of the uterine mucosa. These cells might be responsible for colouration of the egg shell.

**INTRODUCTION**

SEM has been utilized extensively in evaluating mammalian oviduct (PATEK, *et al.* 1972; STALHEIM, *et al.* 1975 and ARNOLD and SHOREY, 1985). However, the avian oviduct, particularly during egg producing cycle have been received little attention.

The shell substance of the avian egg is secreted in the portion of the oviduct called uterus or shell gland.

The exact involvement of the various structure in the formation of the shell are not elucidated (RICHARDSON, 1935; JOHONSTON, *et al.* 1963; BREEN and DE BRUYN, 1969; AITKEN, 1971 and GAY and SCHARER, 1971). Therefore, the aim of the present work is to throw a light on the surface morphology and the structural changes which occur in the uterus of fayomi fowl during egg producing cycle.

---

\* Dept. of pathology, Fac. of Vet. Med., Assiut University.



## MATERIAL and METHODS

Fifteen mature fayomi laying hens were used throughout the experiment. The hens were slaughtered and the oviduct was dissected free after noting the position of the egg in the reproductive tract. Three of the hens had no egg in their oviduct (just after oviposition), three had an egg in the magnum, three had an egg in the isthmus, and the remainder had an egg in the uterus (shell gland).

For scanning electron microscopy mucus were removed by a gentle stream of normal saline. Small pieces from the uterus were fixed in cold 3% glutaraldehyde, then post-fixed in 2% buffered osmium tetroxide. The tissue was dehydrated, critical point dried (ANDERSON, 1951), and fastened to aluminum stubs with silver paste.

All specimens were then placed in a vacuum evaporator and carbon coated. This was followed with gold coating. Examination and photography was done with JSM 200 scanning electron microscope operated at 25 KV accelerating voltage.

After dehydration tissues were embedded in Epon, sectioned at 1  $\mu$ m thickness with LKB ultramicrotome and stained with toulidene blue.

For light microscopy paraffin sections were prepared from the uterine tissue fixed in Bouin's fluid and were stained with haematoxyline and eosin. Crossman's trichrome, periodic acid Schiff's method (PAS) and Alcian blue (AB) technique. Frozen sections from formalin fixed tissue were stained with Sudan black for neutral lipid. Von Kossa method for calcium was also used.

## RESULTS

When the egg was located in the magnum or isthmus, the uterine mucosal folds were long, leaf-like or tongue shaped and showed longitudinal grooves (Fig. 1). The surface epithelium of the uterine mucosa displayed both ciliated and non ciliated cells as well as the opening of the uterine glands (Fig. 2). The apical surface of the ciliated cells was covered with long closely packed cilia (Fig. 3) which may obscure most of the other surface details (Fig. 4). The non ciliated cells were slightly domed and their apical surface was covered with short microvilli (Fig. 3). The glandular openings (Fig. 5) were narrow and could be easily recognized. These opening were bounded by dense array of cilia.

Examination of the semi-thin sections of the uterine mucosa showed that the ciliated cells were long and tapered toward the basement membrane. Their nuclei were large, spherical vesicular and located at the apical part of the cell. The cytoplasm contained considerable amount of secretory granules which varying in size and number (Fig. 6). These cytoplasmic granules were negatively stained with Alcian blue or PAS technique. However, sudanophilic granules and melanin pigments were demonstrated in the cytoplasm of the ciliated cells.

When the uterus contained an egg, the uterine mucosa exhibited a relatively different morphological features. The mucosal folds appeared as bent tills and arranged parallel to the uterine surface (Fig. 7). The ciliated cells were wider in shape, faintly, stained and contained larger vesicular nuclei (Fig. 8). Most of these cells have long ciliary shafts, which appeared to be swollen, clumped and disarranged. On the other hand, some of these ciliated cells appeared lacking their cilia. However, the basal bodies were obviously demonstrated under their plasma



## SHELL GLAND OF FAYOMI FOWL

membrane or containing a very short and few ciliary shafts (Fig. 8 & 9).

The non ciliated cells were bulged into the uterine lumen. Several large vacuoles were found in both luminal and basal sides of the nuclei of the latter cells (Fig. 10). These vacuoles were relatively more prominent and the cytoplasmic PAS-positive materials were abundant. Small branched melanin-containing cells were distributed under the surface epithelium as well as in the interglandular connective tissue (Fig. 11 A). These melanin-containing cells have a long cytoplasmic processes projecting into the surface epithelium and containing abundant melanin pigments (Fig. 11 B). Abundant sudanophilic substances were demonstrated within the uterine epithelium (Fig. 12).

The uterine glands were of the branched tubuloalveolar type opened into the mucosal surface by a short duct. When the uterus was distended with the egg, the glandular cells were pyramidal in shape, their nuclei were large, spherical and vesicular occupied the basal half of the cells. Their cytoplasm was vacuolated, and appeared lightly stained. The lumen of the secretory end pieces were narrow (Fig. 13). After oviposition, the cells of secretory end pieces of the uterine glands were cuboidal or pyramidal with truncated apex and surrounded a relatively wide lumen.

## DISCUSSION

The scanning electron microscopy revealed that the two cell types which could be differentiated on the mucosal surface of the uterus showed certain morphological changes during egg producing cycle. These changes were similar to that obtained by BAKST and HAWARTH (1975) in hen's oviduct and CADDUM, *et al.* (1975) in human oviduct.

The morphological observations of the ciliated cells which had fewer and shorter cilia as well as those which lacked cilia but had a row of basal bodies under their plasma membrane, are probably a real evidence of ciliogenesis in the uterine mucosa of fayomi fowl (PALMITER and WRENN, 1971; SEGAL, 1974 and RICHARD, *et al.* (1976).

The increased cellular activity of the ciliated cells when the uterus contained an egg, indicating that these cells were associated with the formation of protein material of the shell (JOHNSTON, *et al.* 1963; BREEN and De BRUYN, 1969). Moreover, the non ciliated cells of the uterine mucosa also showed maximal activity when the uterus contained an egg. This is in accordance with the observation of RICHARDSON (1935) who reported that these cells might be responsible for the production of egg cuticle.

ROBINSON, *et al.* (1968) noted that the surface cells of the uterus stained with Alcian blue, however the present work showed that the non-ciliated cells contained only PAS positive materials.

The sudanophilic reaction observed in the surface epithelium of the uterine mucosa of fayomi fowl, suggesting that these epithelial cells were probably responsible for secretion of trace lipids contained in the egg shell (ROMANOFF and ROMANOFF, 1949).

The negative reaction of the shell glands of fayomi fowl with von kossa method indicating that these glands does not store calcium prior to the shell formation.

The colouration of the fayomi egg shell might be attributed to the presence of pigmented cells observed in the lamina propria, of the uterus. According to MAKITA and MOCLIZUKI



(1984) these pigmented cells develop from neural crest, immigrate to the skin and could be demonstrated by others organs.

### REFERENCES

- Aitken, R.N.C. (1971): The oviduct. In *Physiology and Biochemistry of the domestic fowl*. (Edited by D.J. Bell and B.M. Freeman, pp. 1237-1289. London; Academic press.
- Anderson, T.B. (1951): Techniques for the preservation of three dimensional structures in preparing specimens for electron microscope. *Trans. N.Y. Acad. Sci.*, 13: 130-134.
- Arnold, R. and Shorey, C.D. (1985): Structure of the oviductal epithelium of the brush-tailed possum. *J. Reprod. Fert.* 73: 9-19.
- Bakst, M. and B. Hawarth, (1975): SEM preparation and observations of hen's oviduct. *Anat. Rec.* 181: 211-226.
- Breen, P.C. and De Bruyn, P.P.H. (1969): The fine structure of the secretory cells of the uterus (shell gland) of the chicken. *J. Morph.*, 128, 35-66.
- Caddum-Rosse; P.R.E. Rumery, R.T. Blandau and J.B. Tiersch (1975): Studies on the mucosa of postmenopausal oviducts, Surface appearance, ciliary activity and the effect of estrogen treatment *Fertil. Steril.* 26: 951-969.
- Gay, Carol V. and Schraer, H. (1971): Autoradiographic localisation of calcium in the mucosal cells of the avian oviduct. *Calc. Tiss. Res.*, 7, 201-211.
- Johnston, H.S.; Aitken, R.N.C. and Wyburn, G.M. (1963): The fine structure of the uterus of the domestic fowl. *J. Anat.*, 97, 333-344.
- Makita, T. and Mochizuki, S. (1984): Distribution of pigment cells in tissues of silky fowl. I. light microscopic observations. *The remaguchi J. of Vet. Med.*, 11: 17-38.
- Odor, D.L.; Ross, P.G.; Rumery, R. and Blandal, R.J. (1980): Cyclic variations in the oviductal ciliated cells during the menstrual cycle and after estrogen treatment in the pig-tailed monkey. *Anat. Rec.* 198: 35-57.
- Palmiter, R.D. & Wrenn, J.T. (1971): Interaction of estrogen and progesterone in chick oviduct development. III. Tubular gland cell cytodifferentiation. *J. Cell Biol.* 50, 598-615.
- Patek, E.; L. Nilsson, and E. Johannisson (1972): Scanning electron microscope study of the human fallopian tube. Report 1. The proliferative and secretory stages. *Fertil. Steril.* 23: 459-465.
- Richard, G.; W. Anderson and Cheryl, E. Hein (1976): Estrogen Dependent cilliogenesis in the chick oviduct. *Cell tiss. Res.* 171: 459-466.
- Richardson, K.C. (1935): The secretory phenomena in oviduct of the fowl, including the process of shell formation examined by micronincineration technique. *Phil. Trans. Roy. Soc. B.*, 225, 149-196.
- Robinson, D.S.; King, N.R. and Bowen, D.J. (1968): The occurrence of neutral and acidic mucin in reproductive tract of laying hen. *Histochemical*, 13: 97-104.
- Romanoff, A.L. and Romanoff, A.J. (1949): *The Avian Egg*. New York: John Wiley.
- Segal, S.J. (1974): The primary effect of gonadal steroids on target cells. *Recent progress in reproductive endocrinology*. edit. by P.G. Crosignani and V.H.T. James. pp. 1-26. London Academic press.
- Stalheim, O.V.H.; Gallagher, J.E. and Deyol, B.L. (1975): Scanning electron microscopy of the bovine, porcine and caprine uterine tubes. *Am. J. Vet. Res.* 36, 1069-1075.

## SHELL GLAND OF FAYOMI FOWL

## LEGENDS

- Fig. (1):** Tongue shape uterine mucosal fold. (no egg in uterus) SEM (x35).
- Fig. (2):** The mucosal uterine surface showed clear uterine opening. (no egg in uterus) SEM (x 750).
- Fig. (3):** Surface epithelium note ciliated cells A and non ciliated cells B. (no egg in isthmus, SEM (x7500).
- Fig. (4):** Surface view of the uterine mucosa, note the ciliated cells with long cilia. SEM (x 7500).
- Fig. (5):** Opening of the uterine glands (arrowed) bounded by dense array of cilia. SEM (x 5000).
- Fig. (6):** Semithin section of uterine mucosa showing ciliated and non ciliated cells. Toulidine blue stain (x 1000).
- Fig. (7):** Bent tillis uterine fold arranged parallel to the uterine surface. SEM (x 100).
- Fig. (8):** Semithin section of the uterine mucosa; Note, prominent loss of cilia. Toulidine blue stain (x 1000).
- Fig. (9):** Uterine mucosal surface showing deciliation of the epithelial cells. (Egg in uterus) SEM (x 7500).
- Fig. (10):** Uterine mucosa showing vacuolation of the surface epithelial cells. PAS & H (x 400).
- Fig. (11):** Pigmented cells located in the lamina propria of the uterine mucosa. Crossman trichrome (x 400). b) Higher magnification of melanine containing cells.
- Fig. (12):** Surface epithelium of the uterine mucosa containing sudanophilic materials. Sudan black (x 400).
- Fig. (13):** Semithin section of the uterine glands showing vacuolated cytoplasm and narrow lumen. Toulidine blud stain (x 1000).











