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Secretion of mucins in the fallopian tube epithelium of the bat *Taphozous kachhensis* (Dobson)

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Abstract

Taphozous kachhensis is one of the largest Indian microchiropteran bats with a prominent gular pouch on the ventral side of the lower jaw. This is a seasonally monoestrous species and breeds once in a year. The Fallopian tube epithelium of *T. kachhensis* in both the segments, ampullary and isthumic, is lined by two cell types: ciliated and non-ciliated (secretory). The Fallopian tube shows alterations in its muco-subtances during estrus and pregnancy. The ciliated cells elaborate large quantity of glycogen, while non-ciliated cells elaborate high amounts of glycogen and neutral and acid mucins during estrus. Mucins are localized in the cytoplasm as well as cytoplasmic blebs. Glycogen and other muco-substances in non-ciliated cells decrease during pregnancy and the ciliated cells show a small amount of glycogen only. The concentration of muco-substances is highest during estrus, and the cytoplasmic blebs are numerous and prominent and are filled with secretion during this stage.

Key words: Bat, Estrus, Fallopian Tube, Pregnancy, Tubal Epithelium

Introduction

Reproduction in Chiroptera is of special interest due to numerous adaptive specializations exhibited by this diverse and successful group of mammals. Several authors have reviewed the progress of research on chiropteran reproduction (Bernard, 1985). Considering the various biological processes which take place leading to fulfillment of prerequisites of normal pregnancy, the Fallopian tube is of paramount importance. Several workers have studied the histology and histochemical features of the Fallopian tube in mammals (Gadegone et al., 1981; Louise et al., 1983; Yaniz et al., 2000; Ayen et al., 2012). The oviductal epithelium consists of two morphologically distinct cell types, ciliated and non-ciliated. The non-ciliated cells synthesize and release secretory materials. Secretory products originating from the oviductal epithelial cells have been identified and characterized in several mammalian species (Thomas and Thomas, 1982; Abe, 1996). Some of these oviduct-specific glycoproteins are associated with the zona pellucida of ova and or the surface of the spermatozoa and may play important roles in fertilization, early development and functions of spermatozoa (Hunter, 1994; Buhi et al., 2000; Abe et al., 1995; Gandolfi, 1995; Killian, 2004; Bhatt et al., 2004; Suarez and Pacey, 2006). Thus, it is tempting to speculate that oviductal secretions create an important microenvironment for fertilization and early embryonic development. Therefore, detailed study of the histological and functional characteristics of the Fallopian tube of *T. kachhensis* during estrus and pregnancy has been undertaken to find the probable role of tubal secretion in transport of gametes in this monoestrus bat.

Materials and Methods

T. kachhensis (Dobson) is an exclusive Indian Emballonuridae bat found in caves, tunnels and temples. The specimens were collected from Kampa-Tempa, District Chandrapur, Maharashtra State. Many collections were made during the breeding period and pregnancy. The specimens were netted at random with the help of butterfly net. The animals were brought alive to the laboratory with minimum stress and constant supply of food and glucose water. Ten specimens for each reproductive stage were used for the present study.

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Live animals were at once anesthetized with sodium pentabarbital and specimens were weighed immediately in a sensitive spring balance. The specimens were killed by decapitation and their Fallopian tubes were immediately dissected out and immersed in ice cold solution of 2% calcium acetate in 10% formalin (CAF) and Rossman's fixative. After prolonged fixation (24 hr), the tissues fixed in CAF were well washed in chilled distilled water, followed by washing in running tap water. After dehydration in various grades of alcohol, the tissue samples were cleared in xylene and embedded in paraffin. Sections at 3-5 µm thickness were cut with the help of Leica 2417 microtome. Some sections were routinely stained in Hematoxylene-Eosin (H&E) and Mallory's triple technique (M-T) for histological observations, while the adjacent sections were subjected to histochemical analysis for identification of mucosubstances in the Fallopian tube (Pearse, 1968). Photomicrographs were obtained with the help of a Karl Zeiss camera attached to the microscope and magnified to the required size.

Results

The details of the breeding habits of *Taphozous kachhensis* Dobson have already been described by Sapkal and Deshmukh (1984). Mating and fertilization took place during the last week of March and first week of April. Pregnant bats were observed from April to the middle of July followed by lactation from July to the middle of September.

Histology

The Fallopian tube of *T. kachhensis* can be divided into two distinct segments, the ovarian (ampulla) and the uterine (isthmus), on the basis of its muscular coat and its mucosal folds. The ampulla has a thin muscular coat, and the mucosal folds form a complicated structure dividing the lumen into irregular cavities. The uterine segment has a thick muscular coat and the mucosal folds are relatively simple.

During estrus, the tubal epithelium of ampullary and isthmic segments is of columnar type and consists of two types of cells, ciliated and non-ciliated secretory cells with round nuclei. The ciliated cells alternate irregularly with non-ciliated cells in the tubal epithelium. The nonciliated secretory cells show secretion in their cytoplasm and cytoplasmic blebs. The cytoplasm shows granular eosinophilic secretion. Prominent cytoplasmic blebs are seen attached to the epithelial cells, and some are lying free in the lumen (Fig. 1).

During pregnancy the mucosal folds in ampulla are long and elongated but the mucosal folds are short and blunt in the isthmus. The cytoplasm of epithelial cells shows eosiniphilic coarse granules. The cytoplasmic blebs are reduced and a few blebs are seen in the lumen. Increase in vacuolation and decrease in secretion in the isthumic epithelium are observed. Cilia of the ciliated cells are less prominent as compared to the previous period (Fig. 2).

Histochemical features

Table-1 shows the histochemical reactivities of various mucosubstances present in the Fallopian tube of *T. kachhensis* during estrus and pregnancy. These reactivities are indicated semiquantitatively with ++++ as the strongest activity as determined by visual comparison of the intensities of staining for various techniques.

During estrus, ciliated cells of ampullary and isthmic regions exhibit weak PAS positive reaction. After phenylhydrazine treatment and saliva digestion, the PAS-positive reaction in the ciliated cells is abolished indicating the presence of glycogen. These cells do not react with AB indicating absence of acidic mucins. In both the segments, the non-ciliated secretory cells show intense PAS positive reaction. The cytoplasmic blebs are also intensely positive to PAS (Fig. 3). After phenylhydrazine treatment and saliva digestion, the PAS positive reaction is partly reduced from the cytoplasm and the cytoplasmic blebs. The non-ciliated secretory cells are positive to alcian blue

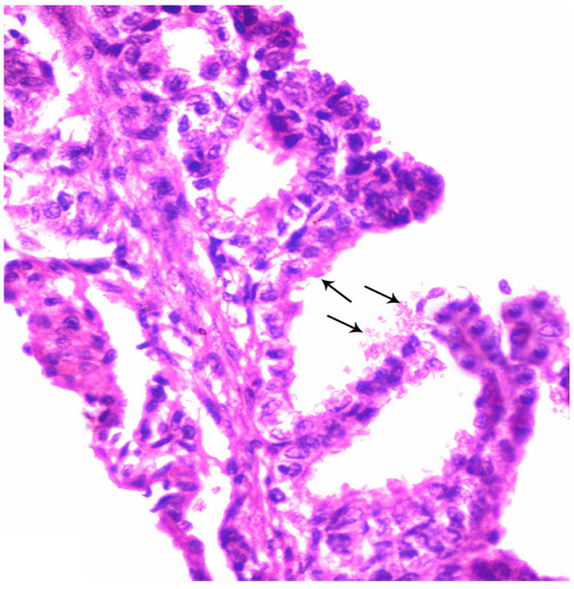

Fig. 1. Transverse section of ampulla of the Fallopian tube of T. kachhensis during estrus. Ampullary epithelium consists of ciliated and non-ciliated secretory cells. The non-ciliated cells show secretion in cytoplasm (arrow). Some blebs are attached to apical portion of cells. H&E; x 250.

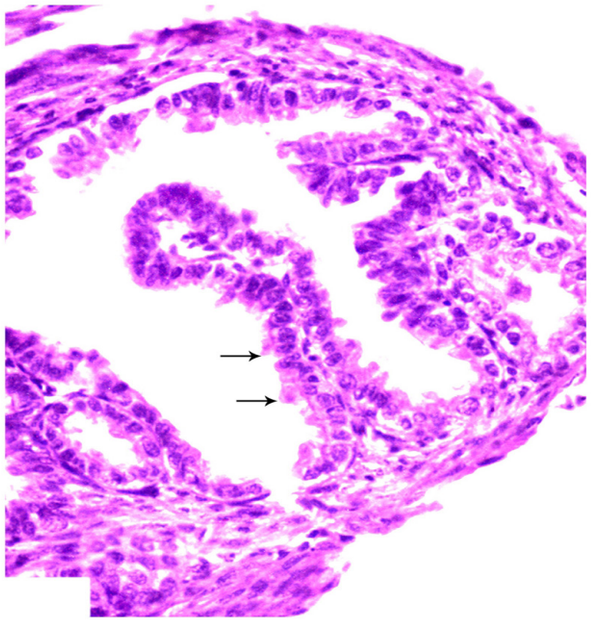

Fig. 2. Transverse section of ampulla of Fallopian tube during pregnancy. The epithelium shows prominent vacuolation. The amount of secretion decreases from epithelial cells. H&E; x 250.

Table 1: Histochemical reactions of mucosubstances in the Fallopian tube of *T. kachhensis* during estrus and pregnancy

Histochemical Reactions	Estrus		Pregnancy	
	Ciliated Cells	Non-Ciliated Cells	Ciliated Cells	Non-Ciliated Cells
PAS	+ + P	+ + + + P	+ P	+ + P
Phenylhydrazine- PAS	-	+ ± P	-	-
Saliva digestion- PAS	-	+ ± P	-	-
AB (pH 2.5)	-	+ + + B	-	+ +B
AB (pH 2.5)-PAS	+ + P	+ + + ± PB	+ P	+ + PB

Colors: P = Pink, B = Blue, PB= Purple Blue, BP = Bluish Purple
Abbreviations: PAS = Periodic Acid Schiff, PH-PAS = Phenylhydrazine- PAS, AB = Alcian Blue (8G x 300)
Intensity: + = Poor, + + = Weak, + + + = Moderate, + + + + = Intense

imparting blue color (Fig. 4). These cells are positive to PAS and AB imparting bluish purple color (Fig. 5). Thus, the non-ciliated cells elaborate glycogen, and neutral and acidic mucins.

During pregnancy, the ciliated cells contained a poor quantity of glycogen since they stained poorly with PAS. The reduced amount of the secretion of non-ciliated cells at ampullary and isthmic regions showed PAS-positive granules, AB-positive and PAS-AB (pH 2.5) positive granules in them. PAS- and AB-positive secretion was adherent to the luminal surface of the cells. The intensities of staining reactions were low during this period as compared to breeding period. The non-ciliated cells and secretion exhibited identical staining reactions to those observed for these cells and secretion during the breeding period (Figs. 6, 7, 8). Thus, the non-ciliated cells elaborated glycogen and less quantity

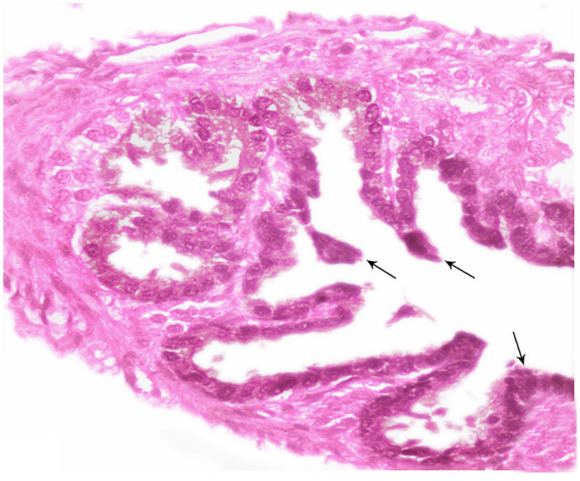

Fig. 3. T.S. of isthmus of Fallopian tube during estrus, stained with PAS showing PAS- positive material in the cytoplasm. A few blebs are seen (arrow). PAS; x 250.

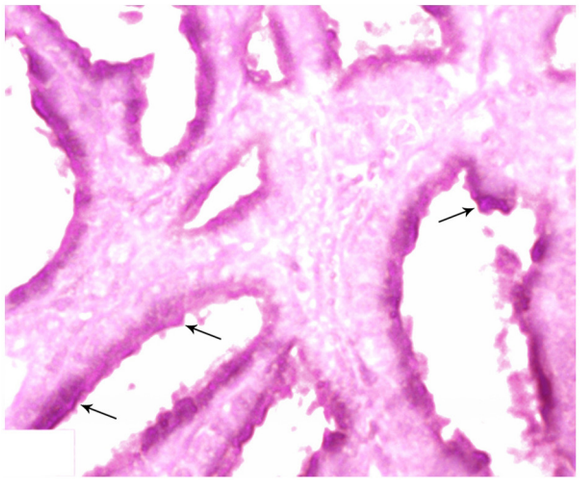

Fig. 4. T.S. of ampulla of Fallopian tube during estrus, showing secretary cells with AB- positive secretion. Some AB positive cytoplasmic blebs are attached to the apical portion (arrow). AB (pH 2.5); x 650.

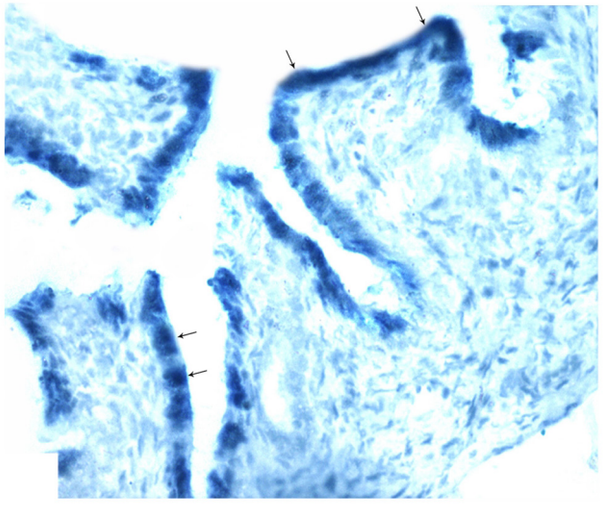

Fig. 5. T.S. of ampulla of Fallopian tube stained with AB-PAS showing bluish purple color in the cytoplasm. AB (pH 2.5)-PAS; x 250.

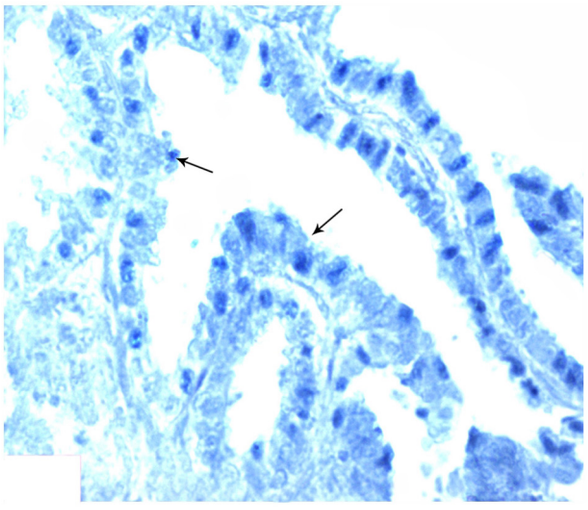

Fig. 6. T.S. of isthmus of Fallopian tube during pregnancy, stained with PAS showing weak reaction with PAS (arrow). PAS; x 250.

of mucosubstances during pregnancy than the breeding period.

Discussion

The tubal epithelium of *T. kachhensis* is composed of columnar ciliated and non-ciliated cells. Both the cell types alternate irregularly in the tubal epithelium. The non-ciliated secretory cells show secretory granules in their cytoplasm and secretion in the form of cytoplasmic projections or secretory blebs attached to the apical surface of the cell during estrus. There is a reduction in the secretion from the cytoplasm as well as reduction in the number of secretory blebs from the non-ciliated secretory cells during pregnancy. Ciliated and nonciliated secretory cells of the Fallopian tube of this bat show cyclic variations in their morphological and cytological characteristics as reported in *T. melanopogon* (Sapkal

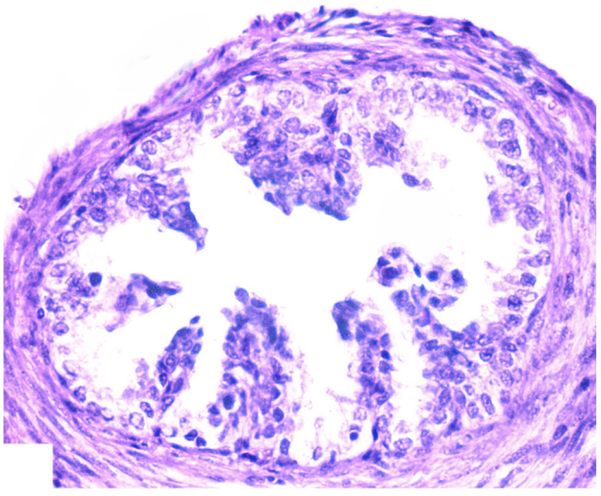

Fig. 7. T.S. of ampulla of Fallopian tube during pregnancy. Note AB-positive material in the cytoplasm. AB (pH 2.5); x 250.

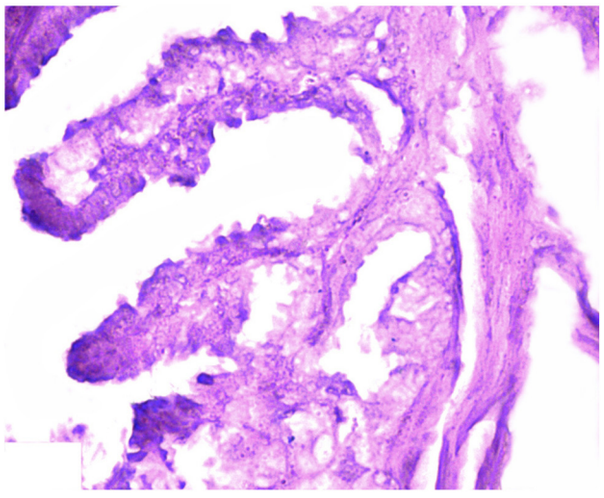

Fig. 8. T.S. of isthmus during pregnancy stained with AB (pH 2.5); x PAS showing weak bluish purple secretion. AB (pH 2.5)-PAS; x 250.

and Gadegone, 1980; Gadegone et al., 2000). Ciliated and non-ciliated secretory cells in the tubal epithelium have been reported in other mammals (Shapiro et al., 1974; Gadegone et al., 1981; Louise et al., 1983; Hollis et al., 1984).

A characteristic feature of *T. kachhensis* is the presence of cytoplasmic projections or blebs containing secretion. The presence of cytoplasmic projections, their significance and correlation with secretion in Fallopian tube have puzzled many workers. The apical portion of nonciliated secretory cells is filled with secretory granules which are later pinched off with secretion and are found lying free in the lumen. An occasional presence of nuclei in these projections suggested a possibly holocrine secretion in this species of bat. Occurrence of cytoplasmic projections or 'domes' containing secretion has been reported in non-ciliated cells of mammals (Hollis et al., 1984). Such cytoplasmic projections or secretory blebs with secretion are also reported in the non-ciliated cells of bats, *Glossophaga soricina* and *Carollia* (Rasweiler, 1972a, b), *T. longimanus* (Sapkal and Gadegone, 1980) and *T. melanopogon* (Gadegone et al., 2000) supporting the present observations.

In *T. kachhensis,* the ciliated cells elaborate large quantity of glycogen, while non-ciliated cells elaborate glycogen and neutral and acidic mucins during estrus. The concentration of mucosubstances is highest during estrus. Glycogen and other mucosubstances in non-ciliated cells decrease during pregnancy and the ciliated cells show only small amount of glycogen. Glycogen was demonstrated in the ciliated cells of the Fallopian tube in man (Thomas and Thomas, 1982), rabbit (Jansen, 1995), bovine (Nayak et al., 1976), goat (Gadegone et al., 1981) and bats (Sapkal and Gadegone, 1980). Glycogen was also reported in small amounts in the non-ciliated cells and during pregnancy in the basal region of cells of human Fallopian tube (Nayak et al., 1976), goat (Gadegone et al., 1981) and bats (Sapkal and Gadegone, 1980).

PAS-positive material was identified as a neutral mucopolysaccharide and acid glycoprotein in mammals (Fujita et al., 1984; Thomas and Thomas, 1982). The acid mucopolysaccharides were found to be sulfo-mucopolysaccharides in sialic acid and sulfomucins in rat (Schultka and Schmidt, 1971) and sialic acid, sulfomucins and neutral mucins in goat (Gadegone et al., 1981). The non-ciliated secretory cells of the tubal epithelium of bats elaborated sulfomucins, sialomucins, and neutral mucins in *Taphozous longimanus* (Sapkal and Gadegone, 1980) and *T. melanopogon* (Gadegone et al., 2000).

However, the non-ciliated cells of *Pteropus* and *Megaderma* elaborated all these mucins except sulfomucins (Sapkal and Gadegone, 1980).

The nonciliated cells showed glycogen, neutral, sialo- and sulfomucin during the estrous period. During early pregnancy there was overall decrease in mucin content of the infundibular region; acid mucins were not observed in the isthmus during early pregnancy (Sapkal and Gadegone, 1980; Gadegone et al., 2000). In the present study, the ciliated and non-ciliated secretory cells of the Fallopian tube of *T. kachhensis*, show glycogen during all the phases of the reproductive cycle. In addition, the non-ciliated cells show neutral and acidic mucosubstances during estrus. The highest concentration of these mucosubstances was seen at estrus which declined during pregnancy. Our observations are in conformity with the observations of other researchers on tubal epithelium of other bat species. From the forgoing report, it is clear that elaboration of mucosubstances seems to be under the influence of estrogen causing its initiation and increasing the concentration as estrogen increases. Progesterone seems to block further elaboration but causes release of the secretion in to the tubal lumen, as the latter becomes filled with secretion during early gestation.

In conclusions, the present study demonstrates that in *T. kachhensis* the oviductal secretion in ampulla and isthmus showed high amount of acid mucopolysaccharides as demonstrated histochemically during estrus. It is suggested that the acid glycoproteins secreted by the ampulla and the isthmus of bat Fallopian tube may be interacting with zona pellucida, perivitelline space and vitelline or blastomere membrane of ovulated eggs and early embryo. Mucus glycoproteins would exert their most important effects on spermatozoa ascending the reproductive tract and then on fertilized ova enroute to the uterus (Abe et al., 1995b; Gandolfi, 1995; Buhi et al., 2000; Killian, 2004; Bhatt et al., 2004; Suarez and Pacey, 2006). They may have a role in preimplantation events which would have far reaching implications in assisted reproductive technology and in the development of non-hormonal contraceptive vaccine. The mechanisms whereby the ampulla and the isthmus of bat contribute to the biochemical milieu of the Fallopian tube are now well documented. However, there is a need of further investigation on biosynthesis and molecular biological data to support the present observations

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