Postnatal Development of the Rabbit Uterine Tube (*Oryctolagus cuniculus*)

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With 3 figures & 1 table **Received September, accepted for publication October 2025**

Abstract

The uterine tubes receive the ovum and provide the appropriate en-vironment for its fertilization. The female reproductive tracts of 18 completely healthy white New Zealand rabbits, from postnatal day one (P1) to P120 were used. At P1 the reproductive tract appeared as a thin, straight tube without any line of demarcation between the uterine tube and the uterus. However, at P7, this demarcation was observed. At P7, the uterine tube started to differentiate histologically into an infundibulum, ampulla and isthmus. The mucosal folds started to develop at P7. They were represented by few and short folds which increased in number, height and branching with the advancement of age. At P1, the uterine tube was lined with short columnar showing few mitotic figures. At P7, various degrees of epithelial cell differentiation were demonstrated by the presence of ciliogenic activity in all regions. At P14, ciliated cells with long cilia could be detected. In addition, the secretory cells were demonstrated first in the ampulla and the isthmus. At P120, the ciliated cells represent the majority of the infun-dibular epithelium; however, the secretory cells were predominantly at the isthmus. Scanning electron micro-scope was applied to elucidate the 3D structural features of the ampulla. We observed that the surface epithelium at P7 showed few ciliogenic cells and abundant sec-retory cells. With the progress of age, the number of ciliated cells increased.

keywords: uterine tube; rabbit; postnatal; SEM

Introduction

The rabbit is one of the most commonly used animal models in developmental experimental studies. Uterine tubes play an essential role in reproduction, as it provides an appropriate microenvironment for oocyte maturation, sperm capacitation, fertilization and early embryonic development. The rabbit uterine tube is divided into infundibulum, ampulla and isthmus (Hafez 1993). The epithelial lining of the uterine tube shows marked regional variations in ultrastructural, histochemical and physiological features in many mammals. The epithelium of the uterine tube in mammals consists mainly of ciliated cells and secretory non-ciliated cells. The ciliated cells aid in the transportation of both gamete and embryo, while the secretory cells produce a secretion necessary for proper sperm functions and early embryonic development (H Abe 1996). The secretory product is mainly produced by the ampulla, the site of fertilization (Odor et al. 1980). A detailed histomorphological description of the rabbit uterine tube during the post-natal life is missing. The present study aimed to investigate the morphological changes in the uterine tube of rabbits during the postnatal development using light and scanning electron microscopy.

Materials and Methods

Samples collections:

The study was approved by the Ethics Committee of Assiut University, Egypt. Three completely healthy New Zealand white female rabbits were used for each age examined. The studied ages were postnatal day 1 (p1), P7, P14, P28, P60, P90 and P120 after birth. The rabbits were obtained from the animals' house at the Faculty of Medicine, Assiut University. The animals were kept on a diet freely and at 25 ^{o}C. Before scarification, the animals were weighed and anes-thetized by ketamine (35 mg/kg).

Biometrical measurements:

The reproductive tract and the uterine tubes were dissected and photographed. The average lengths of the uterine tubes (right and left) were measured using imageJ.

Histological preparation:

Specimens from infundibulum, ampulla and isthmus were washed by physiological saline and imme-diately fixed in Bouin's solution for 14-24 hours, dehydrated in graded series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Cross sections were obtained at 3-6μm in thickness and stained with Harris's Haematoxylin and Eosin for general histological examination, Weigert's Resorcin Fuchsin stain for demonstration of elastic fibers, Crossmon's trichrome for demons-tration of muscle fibers and collagen fibers and the combined periodic acid Schiff's (PAS) and alcian blue (AB) (pH 2.5) for demonstration of neutral and acidic mucoploysaccharides, respectively. Staining protocols and used procedures were carried out according to (Bancroft, Layton, and Suvarna, 1996.)

Semithin sections:

Small pieces from the ampullae at P7, P28 and P120 were fixed in cold 5% gluteraldehyde in cacodylate buffer, washed several times in 0.1M cacodylate buffer PH 7.3, dehydrated in ethanol. Tissues were embedded in Epoxy resin of low viscosity (ERL, 4206) (Spurr, 1969), sectioned at 1 um thickness with LKB ultramicro-tome and stained with toludine blue.

Scanning electron microscopy (SEM)

To study the epithelial surface of the female reproductive tract, scanning electron microscopy was applied. The previously fixed ampulla tissues were further fixed in 1% buffered osmic acid for 2 hours and dehydrated in alcohols followed by amyl acetate. They were dried by critical point drying using liquid CO_2 and mounted on stubs. The specimens were sputter coated with gold, examined and photographed at JEOL 5400 LV scanning electron microscope.

Morphometerical studies

The morphometric dimensions of the uterine tube were measured from the stained histological sections and semithin sections using the Leica Q 500MC image analyzer. The measurements were 1) The diameter of each segment and the thickness of its wall; 2) The height and number of mucosal folds; 3) The height of the lining epithelium; 4) The number of ciliated and non-ciliated cells. These measurements were calculated from ten random fields and the mean ± SE.

Results

Gross anatomy

Topographically, the rabbit's female reproductive tract extended from the sublumbar region of the abdominal cavity to the outlet of the pelvic cavity. At P1, the uterine tubes were located at the level of the caudal pole of the left kidney (Fig 1A). With the advancement of age, the distance between the uterine tubes and the level of the caudal pole of the left kidney increased gradually till P120 (Fig 1B). Anatomically, at P1, the female reproductive tract appeared as a thin, simple tube having the same diameter along its length. The demarcation between the uterine tube and uterus was unclear (Fig 1C); however, it could be distinguished after one week (Fig 1D). The growth pattern of the uterine tube was slow over the first 90 days (Fig 1C-F) and showed a pronounced increase from P90 to P120 (Table 1 and Fig 1G). The uterine tube is a highly tortuous muscular tube. It had two extremities; one of them opens into the peritoneal cavity next to the ovary and the other connects with the uterus. The mean length of the uterine tubes was about 13.46 mm at P7, and then increased to 31.44 mm at P28, reaching 83.84 mm at P120 (Table 1 and Fig 1).

Table (1): Biometrical measurements of the rabbit female reproductive tract of the rabbit during the postnatal development.

Age	Body weight/ gm	Tract weight/ gm	Ut. tubes weight/ gm	Ut. tubes length/ mm
1 d	41 ±3.8	0.06 ±0.0067	______	______
1 w	114.33 ±10.9	0.193 ±0.035	0.01 ±0.003	13.46 ±0.59
2 w	268.33 ±3.3	0.42 ±0.023	0.02 ±0.0047	20.52 ±0.5
4 w	405 ±10.4	0.62 ±0.044	0.04 ±0.0079	31.44 ±1.46
8 w	1656.7 ±34.8	2.01 ±0.087	0.11 ±0.011	76.38 ±5.53
12 w	2003.3 ±57.8	7.28 ±0.32	0.42 ±0.024	68.57 ±3.15
16 w	2533 ±185.6	10.73 ±0.50	0.34 ±0.4	83.84 ±1.25

Data (mean) ± Standard error of mean

Histomorphology of the Uterine tube

Just after birth, the uterine tube was a thin tube which could not be differentiated morphologically into the infundibulum, ampulla and isthmus. The average thickness of its wall was 113.04±15.5 µ. Its wall showed no mucosal folds (Fig. 2A). It consisted of a single layer of epithelial cells resting on a highly cellular connective tissue layer which was surrounded by a poorly developed muscular layer (Fig 2B). The lamina epithelialis consisted of short columnar cells or pseudo-stratified undifferentiated cells of one type (Fig 2B, C). Their cytoplasm was highly basophilic, and the nuclei were large, round or oval, vesicular and contained distinct large nucleoli. The lamina propria was highly cellular. The muscular layer was poorly developed with circular smooth muscle fibers (smf) and covered by a layer of flattened mesothelial cells (Fig 2B, C).

At P7, the uterine tube started to differentiate into the infundibulum, ampulla and isthmus. Some mucosal folds started to appear as a few and short thick folds (Fig 2D). The number and the height of the folds of the infundibulum were 10.67±0.33 folds and 92.73±5.21 µ, respectively. They decreased towards the isthmus reaching 3.33±0.33 folds and 58.53± 3.02 µ, respectively. The uterine tube was lined with columnar or pseudo-stratified columnar cells (Fig 2E). Various degrees of epithelial cell differentiation were observed in some cells. The ciliogenic activity was represented by the appearance of the basal bodies at the apical regions of the cells just below the cell membrane or the presence of very short and few cilia at the free apical border (Fig 2E, At P14, all parameters slightly increased and the lumen became narrower than at P7. The number and height of the folds concurrently decreased from the infundibulum towards the isthmus. The ciliogenic activity had achieved further differentiation, with some ciliated cells having long ciliary shafts at the infundibular region (Fig 2H). At the ampulla and isthmus, differentiated secretory cells were observed for the first time, however, many cells were still un-differentiated. The secretory cells of the ampulla reacted positively to PAS stain and PAS/AB combination (Fig H-J). Many apoptotic cells with deeply stained basophilic cytoplasm and darkly stained fragmented nucleus were observed (Fig 2G). The cytoplasm of the ciliated cells appeared lighter stained than that of the secretory cells. The nuclei of the ciliated cells were vesicular and located centrally or apically. However, those of the secretory cells were denser, located basally with oval to irregular shape (Fig 2I).

At P28, all parameters increased slightly more than P14. These parameters were higher in the infundibulum and decreased towards the isthmus (Fig 3A-C). Secondary mucosal folds were first observed in the infundibulum (Fig 3A). The number of the ciliated cells slightly increased more than P14, and secretory cells started to release their secretion (Fig 3D). Besides, many undifferentiated (basal) cells were devoid of cilia and secretory granules (Fig 3E). We could not detect any differentiated secretory cells in the infundibulum yet. Moreover, many apoptotic cells were observed in the infundibulum and ampulla (Fig 3E).

Both P60 and P90 were nearly similar with just more increase in different parameter At P90. The external diameter and the wall of the uterine tube were nearly twice as that of P28. At the infundibulum, the mucosa was characterized by a large number (20.33±0.88) of well defined, long branched mucosal folds (Fig 3F), which decrease in number and height towards the isthmus (Fig 3F-H). Moreover, secondary folds were observed at the ampulla (Fig 3G). The differentiation of the secretory cells appeared for the first time at the infundibulum at P60. The lining epithelium of the infundibulum reacted positively to PAS (PAS) technique (Fig 3J), and reacted slightly to PAS/AB combination (Fig 3K). The number of the ciliated cells was more than that of the secretory cells at the infundibulum, the reverse at the isthmus and nearly equal at the ampulla. The elastic fibers were absent from the wall of the uterine tube except in the wall of the blood vessels (Fig 3L). The muscular layer became more developed than in previous ages.

At P120, the wall's thickness was greatly increased compared to P90. The higher number of mucosal folds 28±0.58 and the highest folds were observed in the infundibulum leaving a narrow irregular lumen (Fig 3M) and decrease towards the isthmus (Fig 3M-O). The ciliated cells represented most of the infundibular lining epithelium (Fig 3P), while the secretory cells predominated in the isthmus. Neither mitotic divisions nor apoptotic cells could be observed. Basal cells with a rounded to oval darkly stained nucleus, surrounded by a clear halo were observed at different levels of epithelial lining of the infundibulum (Fig 3P). At the infundibulum and ampulla, the muscular layer appeared as a thin layer of circular smooth muscle fibers followed by thick layer of serosa containing collagenous fibers and a large number of blood vessels. At the isthmus, the muscular layer was formed of thick layer of circular smf supported by dense connective tissue (Fig 3Q).

Scanning Electron Microscopy (SEM)

SEM studied the surface organization of the ampulla of the uterine tube of rabbits at P7, P28 and P90. At P7, the mucosa of the ampulla showed a series of unbranched longitudinal folds, separated by narrow slits (Fig 3R). The most abundant cell was the non-ciliated, whereas the ciliogenic cells was singly scattered. The later had numerous long, slender cilia. The non-ciliated cells had a dome-shaped surface of variable sizes. It was heavily invested with numerous short microvilli, with an occasional single cilium projecting from its surface (Fig 3S). At P28, the number of the ciliated cells was obviously increased, but still less than that of non-ciliated cells (Fig 3T). The number of single cilia was markedly decreased (Fig 3T). At P90, an acute alteration in the number of both ciliated and non-ciliated (microvillus) cells was observed. The number of the ciliated cells was obviously increased compared to the non-ciliated ones (Fig 3U).

Discussion

In the present study, the biometrical data revealed that the uterine tube continues to growth and histomorphologic changes after birth. There are two stages of the growth pattern during the postnatal development. First, a slow growth pattern over the period from birth to P90. Second, at P120, the rabbit uterine tube showed a pronounced increase in the weight and length. The rabbit's neonatal uterine tube appeared as a thin, simple tube. The subsequent differentiation into infundibulum, ampulla and isthmus occurred at P7, similar to that in rats (Okada et al. 2004). At P1, the mucosa showed no mucosal folds, however at P7, a few and short thick mucosal folds appeared (Konsowa 2007). The lamina epithelialis was formed of undifferentiated short columnar cells with large, round or oval and vesicular nuclei similar to those of mice (Lauschova, 2003) and golden hamsters (Hiroyuki Abe and Oikawa 1989). On the contrary, the oviductal epithelial cells were fully differentiated into ciliated and secretory cells in the fetuses of rhesus monkey (Brenner

1971) and human (Patek, Nilsson, and Johannisson 1972). The surface pattern of the mucosal folds acted as a sperm passage to the site of fertilization and also as a sperm reservoir (Kanagawa et al. 1972). At P7, the lamina epithelialis showed supranuclear ciliogenic activity, similar to that reported in rabbit from P3-20 (Mccarron and Anderson 1973). The steroid hormones affected on both ciliated and secretory cells in the oviduct epithelium (Anzaldúa, Camacho-Arroyo, and Cerbón 2002). At P14, the ciliated cells with long cilia could be demonstrated at different segments. Although former publications reported that the secretory cells appeared at P24- 26 days, first in the isthmus then in the ampulla (Mccarron and Anderson 1973). We could detect the first PAS-positive secretory cells at P14 at different segments, simultaneously. The ciliated cells appeared earlier than the secretory cells in the infundibulum, like the golden hamster (Hiroyuki Abe and Oikawa 1989). The late differentiation of secretory cells might be due to the shorter period required for them (Hiroyuki Abe and Oikawa 1989). We observed many apoptotic cells and mitotic divisions during the early stages of the postnatal development. These processes are essential for proper construction of the developing epithelium, where the overproduced epithelial cells degenerate and new healthy cells are born (Elmore 2007). Basal cells were demonstrated in the lamina epithelialis during different stages of the postnatal development. Basal cells were demonstrated at this study and also called reserve cells that were described by Mokhtar (2015); Özen et al. (2010) who suggested that the basal cells are undifferentiated cells that give rise to secretory and ciliated ones, as the nuclei of the basal cells stained intensely with acridine orange suggesting more than a diploid content of DNA (Woodruff and Pauerstein 1969). The ciliated cells were more abundant in the infundibulum than in the ampulla and the isthmus (Morita et al., 1997). Together with the muscle contraction and the flow of tubal secretions, the ciliary activity of the ciliated cells helps in the propulsion of the gametes and embryo (Lyons, Saridogan, and Djahan-bakhch 2006). The secretory cells predominated in the isthmus over the ampulla. The secretory granules contained neutral and acidic mucopolysaccharides, which could be reacted positively with PAS and Alcian Blue. This observation was supported by (Lyons, Saridogan, and Djahan-bakhch 2006; Konsowa 2007; Rodriguez-Martinez 2007), who stated that the secretory cells synthesized and released nutrients and growth factors which constitute a suitable environment for gametes, the sperm transport, storage and capacitation, fertilization and early embryonic cleavage. Özen et al., (2010) interpreted the higher secretory activity in the isthmus compared to the ampulla, as the spermatozoa are stored and attained their motility and fertilizing capability in the isthmus. Oviductins were a family of glycoproteins which were synthesized and secreted by the oviductal secretory cells around the time of ovulation. They are asso-ciated with the postovulatory oocytes and they had a role in embryo development (McBride et al. 2004). Our study demonstrated that the tunica muscularis within the walls of all three segments varies in thickness, which suggests the different functions of each segment. The thin to moderate layers observed in the infundibulum and ampulla may be related to the mild contractions within these segments. However, the contractions of the isthmus may result in stenosis of the flexure at the utero-tubal junction leading to restriction and regulation of the upward passage of sperm cells or downward transport of the fertilized zygote (Abiaezute, Nwaogu, and Igwebuike 2017).

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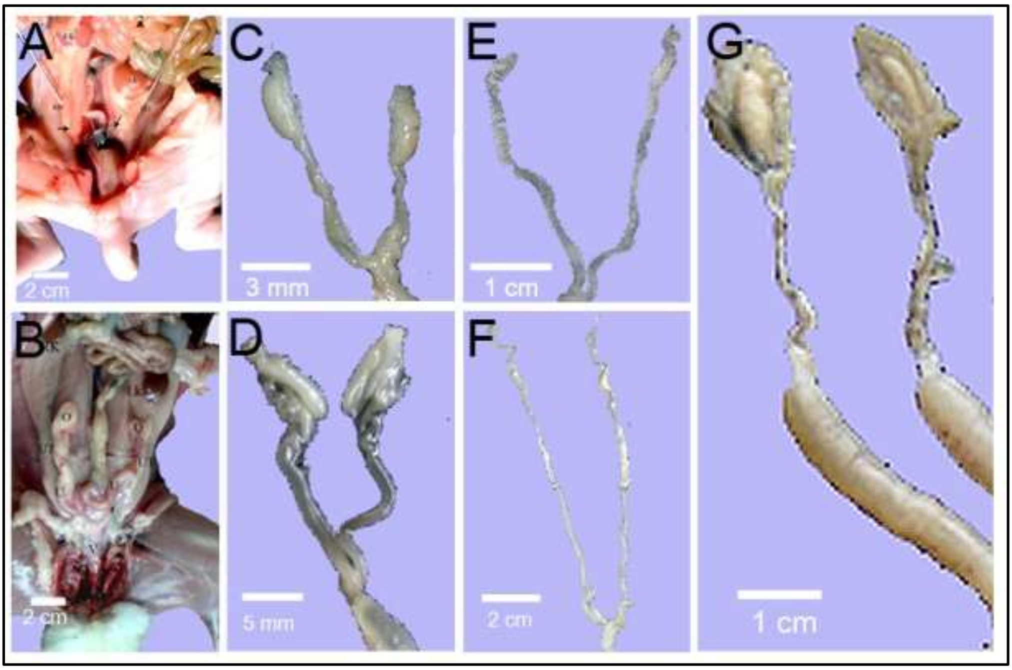

Fig (1): Anatomy of the uterine tube

(A) the uterine tubes were located next to the caudal pole of the left kidney.

(B) the distance increased gradually till P120.

(C) at P1, the reproductive tract appears thin and having the same diameter along its length.

(D) The demarcation between the uterine tube and uterus could be distinguished at P7.

(C-F) The uterine tube grows slowly over the first 90 days.

(G) The pronounced increase in uterine tube size was from P90 to P120, as a highly tortuous muscular tube.

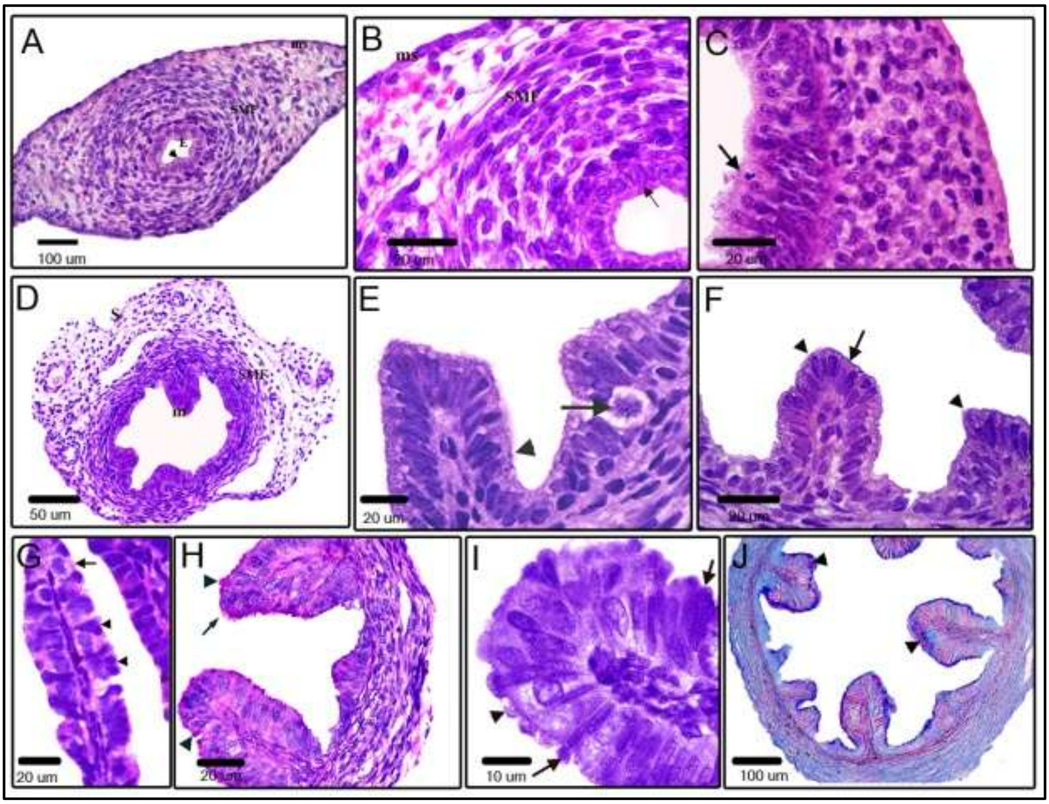

Fig (2): The uterine tube at P1-14; 1st row= P1, 2nd row =P7, 3rd row =P14.

A) lamina epithelialis (E), smooth muscle fibers (SMF) and the mesothelium (ms).

B) The mucosa lined with simple columnar epithelium (arrow)

C) mitotic activity among the epithelial cells (arrow).

D) the appearance of folds.

E) basal bodies (arrowhead) and mitotic activity (arrow).

F) a pseudostratified columnar epithelium with the basal bodies (arrow) and the short cilia (arrowhead).

G) ciliated cells (arrow) and the apoptotic cells (arrowhead).

H) PAS- positive reaction of the secretory cells (arrowhead) and the ciliated cells (arrow).

I) ciliated cell (arrowhead), a secretory cell (arrow) and many apoptotic cell (∗).

J) PAS/AB positive reaction within the lamina epithelialis of the mucosal folds.

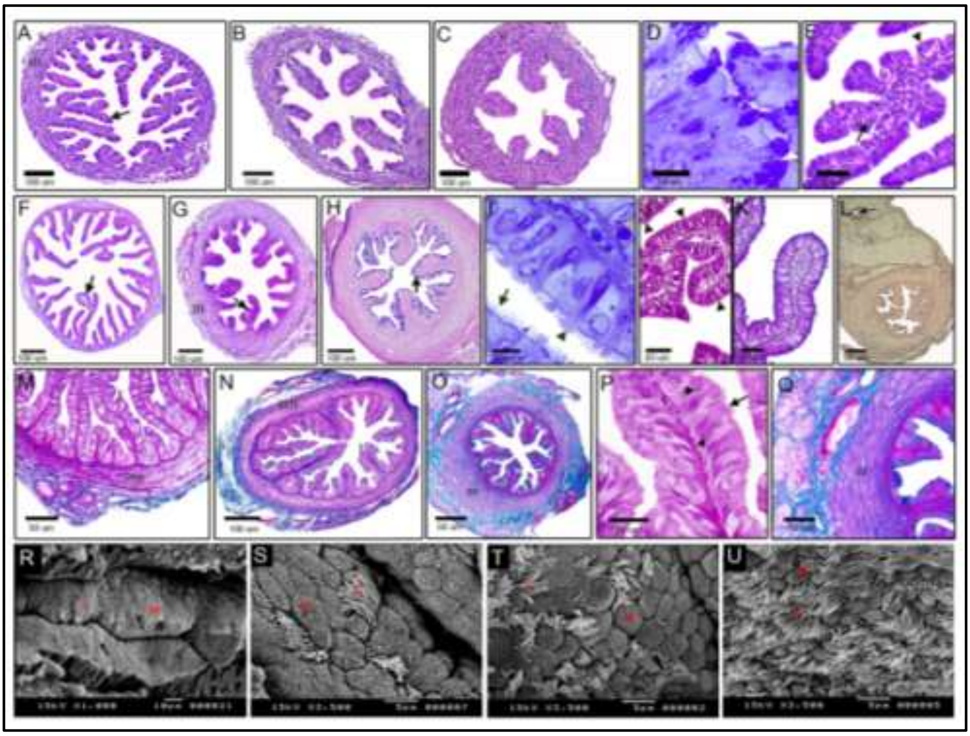

Fig (3): The uterine tube at P28-120; 1st row= P28, 2nd row =P90, 3rd row =P120 and 4th row= SEM.

The first row, at P28, (A) the infundibulum, (B) Ampulla, (C) isthmus. (A) Secondary mucosal folds (arrow). (D) ciliated cells (C) secretory cells (S). (E) undifferentiated (basal) cells (arrow) and apoptotic cells (asterisk).

The second row, at P90, (F) the infundibulum, (G) Ampulla, (H) isthmus. (F) long branched mucosal folds (F-H). (G) Secondary folds were observed at the ampulla (arrow). (I) More ciliated cells (arrowhead) than secretory cells (arrow) at the infundibulum. The lining epithelium reacted positively to PAS (J) and reacted slightly to PAS/AB combination (K). (L) The elastic fibers in the wall of the blood vessels and the muscular layer more developed.

The third row, at P120, (M) the infundibulum, (N) Ampulla, (O) isthmus. The thickness of the wall was greatly increased. (M) More and higher mucosal folds in the infundibulum, and a narrow irregular lumen and decrease towards the isthmus (M-O). (P) more ciliated cells in the infundibulum, while the secretory cells were predominating in the isthmus (Q). (P) Basal cells (arrow) and the muscular layer (m) contain collagenous fibers and large number of blood vessels in the infundibulum (P) and at the isthmus (Q).

The fourth raw (SEM), at P7, in the ampulla mucosa, the longitudinal folds separated by narrow slits (R). numerous non-ciliated, whereas the ciliated cells were singly scattered (S). At P28, the ciliated cells increased, but still lesser than that of non-ciliated cells (T). At P90, the ciliated cells were more than the non-ciliated ones (U).

Animals of this issue

Rabbit *(Oryctolagus cuniculus)*

Kingdom: Animalia & Phylum: Chordata & Class: Mammalia & Order: Lagomorpha & Family: Leporidae (in part) & Genus: *Oryctolagus*

The rabbit's long ears, which can be more than 10 cm (4 in) long, are probably an adaptation for detecting predators. They have large, powerful hind legs. The two front paws have 5 toes, the extra called the dewclaw. The hind feet have 4 toes. They are plantigrade animals while at rest; however, they move around on their toes while running, assuming a more digitigrade form. Wild rabbits do not differ much in their body proportions or stance, with full, egg-shaped bodies. Their size can range anywhere from 20 cm (8 in) in length and 0.4 kg in weight to 50 cm (20 in) and more than 2 kg. The fur is most commonly long and soft, with colors such as shades of brown, gray, and buff. The tail is a little plume of brownish fur (white on top for cottontails). Rabbits can see nearly 360 degrees, with a small blind spot at the bridge of the nose.

Source: Wikipedia, the free encyclopaedia