

## Some Studies on The Development of The Uterine Tubal Ampullary Epithelium in Baladi Rabbit

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### ABSTRACT

The present study was carried out on 25 embryos, fetuses and postnatal specimens till the age of maturity ( four months ) of Baladi rabbits. In the prenatal specimens, The whole embryos or the caudal half of the fetuses were used for light microscopical study. In the postnatal specimens, the ampullary regions of the uterine tubes were processed for light and electron microscopical examination. Prenatally, The uterine tube was developed from the cranial segment of the paramesonephric (müllarian) duct. It was lined with one layer of cuboidal epithelium with a highly vesicular nuclei, and surrounded by undifferentiated mesenchymal connective tissue in 15-18 days rabbit embryo. At 19-25 days of rabbit fetus, the primitive uterine tube had a star shaped lumen, The epithelial cells lined the primitive uterine tube were formed from undifferentiated columnar epithelium, surrounded by undifferentiated mesenchymal connective tissue. Postnatally, the mucosal folds were incompletely developed at birth. They appeared as a little folded mucosa without connective tissue cores. These folds increased in number and acquired connective tissue cores at one week. Then, they showed progressive increase in number and reduction in thickness with the progress of age. Branching of the folds was firstly observed at one month and increased at four months postnatally. The lamina propria appeared at birth as a connective tissue layer separating the epithelium from the muscular layer. It became more extensive at one week. Then its thickness was markedly reduced with the progress of age. The ampullary epithelium of the uterine tube consisted of undifferentiated cells at birth. These cells carried microvilli and solitary cilia. At one week, differentiated ciliated cells were detected. Few ciliated cells were observed at two weeks, and their number increased at one month. The secretory cells were firstly detected at one month. They were mainly seen near the basis of the folds. At two months, the secretory cells increased in number. but still less numerous than the ciliated cells. At four months, the ciliated and secretory cells were nearly equal in number at the tips of the folds, but the secretory cells predominated near the basis of the folds. Moreover, The secretory granules were seen within the uterine tube lumen for the first time at four months.

### INTRODUCTION

It is well known that, the adult uterine tubes of most mammals are lined with nearly equal numbers of ciliated and secretory cells (1-9). Moreover, the prenatal studies revealed that the uterine tubes of most mammals are lined with undifferentiated or incompletely differentiated epithelium at birth (10-12). Many authors reported that important cytological changes take place in the oviductal epithelium from birth till puberty. These changes transform the undifferentiated epithelium at birth into well differentiated ciliated and secretory cells at puberty (13-16). However, studies dealing with the development of the rabbit uterine tube are limited and do not cover all aspects of development (17-20). Consequently, this study

was undertaken in an attempt to increase the knowledge concerning this region, because the uterine tube is critical to normal reproduction (21), because it is a passage for the egg on the way to the uterus. Also, it is the site of fertilization. In addition to, it is the site of beginning of embryonic development.

### MATERIAL AND METHODS

The present study was carried out on ten embryos and fetuses and fifteen postnatal specimens till the age of maturity (four months) of Baladi rabbits. In the prenatal specimens, The whole embryos or the caudal half of the fetuses were sectioned for light microscopy. In the postnatal specimens, the ampullae of the uterine tube were obtained from each animals after slaughtering. The

obtained postnatal specimens were processed for light and electron microscopic examinations. The light microscopic specimens were fixed immediately by neutral buffered formalin for 48 hours. Then, processed by normal histological technique, paraffin wax sections were stained with H&E stain (22-23). The electron microscopic specimens were fixed in a solution of 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24-48 hours and postfixed in a 1% solution of phosphate buffered (pH 7.4) osmium tetroxide at 4°C for one hour. Then, the specimens were dehydrated through a series of graded ethanol solutions, infiltrated and embedded in Epoxy resin (24). Semithin and ultrathin sections were cut with ultramicrotome. The semithin sections were stained with toluidine blue and examined with light microscope. The ultrathin sections were stained with uranyl acetate and lead citrate. The specimens were examined with a JEOL JEM-100 sx transmission microscopic unit at Faculty of Science, Zagazig University. The nomenclature in this study was adopted as previously described (25), as if it was possible.

## RESULTS

The uterine tube (Tubae uterinae) developed from the cranial segment of the paramesonephric (Müllarian) duct. At 15-18 days rabbit embryo, the Müllarian duct was lined with a single layer of high cuboidal epithelium with a highly vesicular nuclei. It was surrounded by undifferentiated mesenchymal connective tissue, containing primitive blood vessels (Fig. 1).

At 19-25 days rabbit fetus, The primitive uterine tube had a star-shaped lumen, The epithelial cells lined the primitive uterine tube were formed from undifferentiated columnar epithelium, surrounded by undifferentiated mesenchymal connective tissue (Figs. 2,3).

One day postnatally, light microscopy revealed that the wall of the ampulla (Ampullae tubae) consisted of three tunics; mucosa, muscosa and serosa. The mucosa consisted of epithelium resting on a connective tissue lamina propria. The mucosal folds

(Plicae tubariae) were ill developed without connective tissue cores (Fig. 4). The epithelium consisted of undifferentiated columnar cells which presented apical bulgings into the lumen. The epithelial cell nuclei were lightly stained and had prominent nucleoli (Fig.5).

By electron microscopy: The undifferentiated columnar epithelium was separated from the connective tissue lamina propria by a basement membrane. They had slightly irregular euchromatic nuclei with prominent nucleoli (Fig.6). The cells were connected together by well developed junctional complexes. Their cytoplasm contained numerous ribosomes, few round to oval mitochondria, short profiles of rough endoplasmic reticulum and Golgi complexes. The apical parts of these cells had microvilli and solitary cilia (Fig.7).

One week postnatally, light microscopy showed that the lamina propria was more extensive than that in the previous stage, and was extended into the cores of the mucosal folds. The folding of the mucosa was increased and became more developed. Each fold consisted of a connective tissue core covered by the epithelial layer (Figs.8,9).

By electron microscopy: The epithelium consisted of undifferentiated columnar cells, resting on the basement membrane. The luminal surface of these cells had microvilli and solitary cilia. Their nuclei were euchromatic with multiple prominent nucleoli. The organelles observed in the cells of the previous age were observed (Fig.10).

Two weeks postnatally, the ampullary mucosa by light microscopy had more mucosal folds than that observed in the previous age. The lamina propria decreased in its thickness. The ciliated cells were firstly detected at this age (Figs.11,12).

By electron microscopy: The ciliated cells were clearly observed for the first time. The ciliated cells had cilia projecting from their luminal membranes. Their nuclei were irregular with clear nucleoli. These cells were characterized by the presence of numerous

mitochondria in their apical parts, near the basal bodies of the cilia (Fig.13).

At one month postnatally, by light microscopy the mucosal folds increased in number and height, but decreased in thickness. The thickness of the lamina propria was markedly decreased. Also, the ciliated cells increased in number, and carried well-developed cilia. The secretory cells were observed for the first time at this age, but they were fewer than the ciliated cells. The latter cells were observed near the basis of the folds (Figs.14-16).

By electron microscopy: The epithelium of the ampullary mucosa consisted of ciliated and secretory cells. The ciliated cells had well-developed cilia and irregular euchromatic nuclei in their apical parts. The secretory cells had irregular euchromatic nuclei with prominent nucleoli, and some chromatin condensation. Also, they had rounded electron dense secretory granules (Fig.17). Their cytoplasm contained numerous mitochondria and well-developed rough endoplasmic reticulum.

Two months postnatally, by light microscopy the epithelium of the mucosal folds appeared as that observed in the previous age. In addition, the branching of these folds was observed for the first time at this age. The secretory cells increased in number but were still less numerous than the ciliated cells (Figs.18,19).

By electron microscopy: The epithelium of the mucosal folds consisted of ciliated and secretory cells. The number of the secretory granules was increased, but they were still electron dense. The nuclei of the ciliated cells were more irregular than those of the secretory cells (Fig.20).

Four months postnatally, by light microscopy the thickness of the mucosal folds was reduced but their branching was greatly increased, it was formed from primary and secondary folds (Fig. 21). The ciliated and secretory cells were nearly equal in number at the tips of the folds, but the secretory predominated near the basis of the folds (Fig. 22).

Fig.1: A photomicrograph of a cross section of 18 days rabbit embryo showing the müllarian duct lined with a one layer of high cuboidal epithelium (a), surrounded by undifferentiated mesenchymal connective tissue (b) and the injected primitive blood vessels (arrow). H&E stain. Obj. X10.

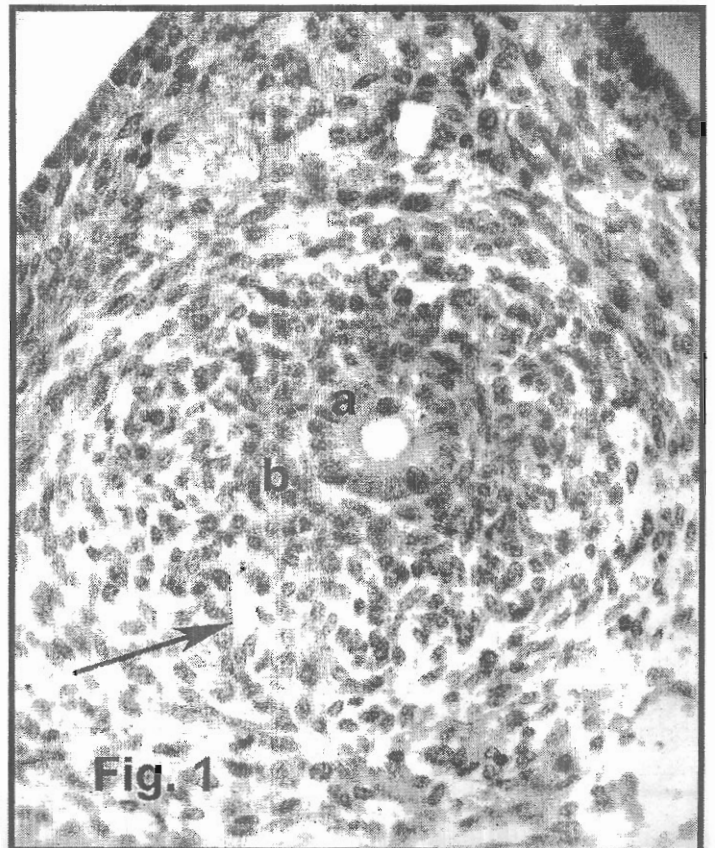


Fig. 2: A photomicrograph of a cross section of 23 days rabbit fetus, showing the two müllarian ducts (arrows). H&E stain. Obj. X4.

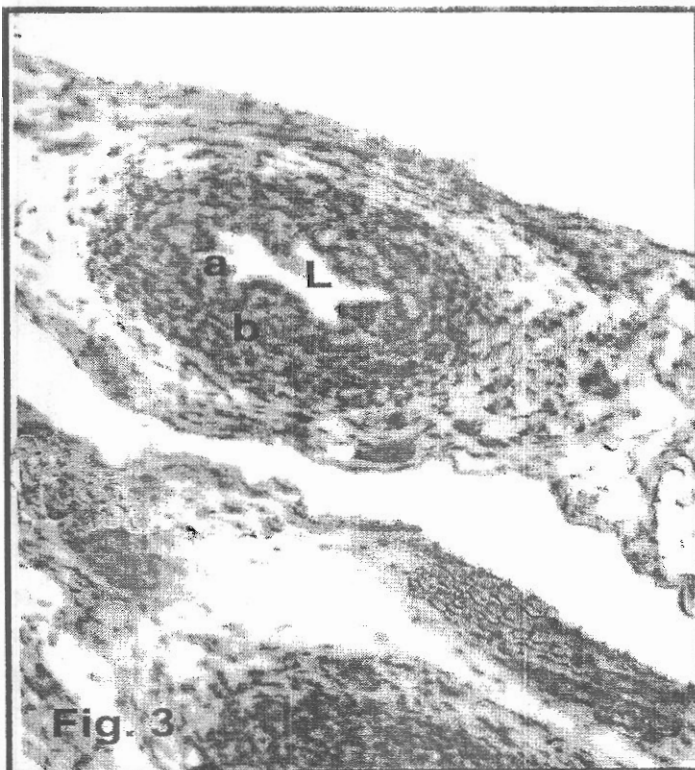
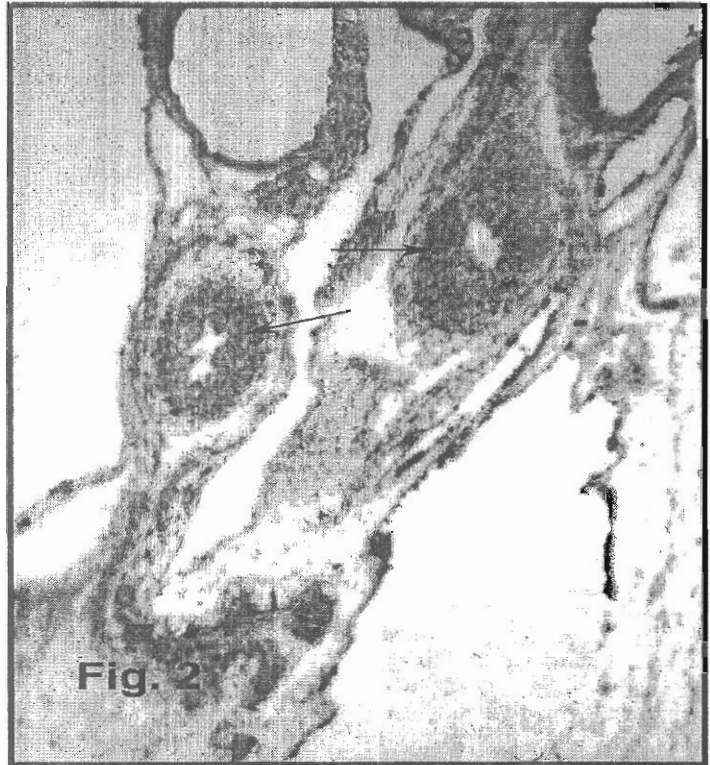


Fig. 3: Higher magnification of fig. 2 showing the lumen (L), mucosal epithelium (a) surrounded by undifferentiated mesenchymal connective tissue (b). H&E stain. Obj. X10.

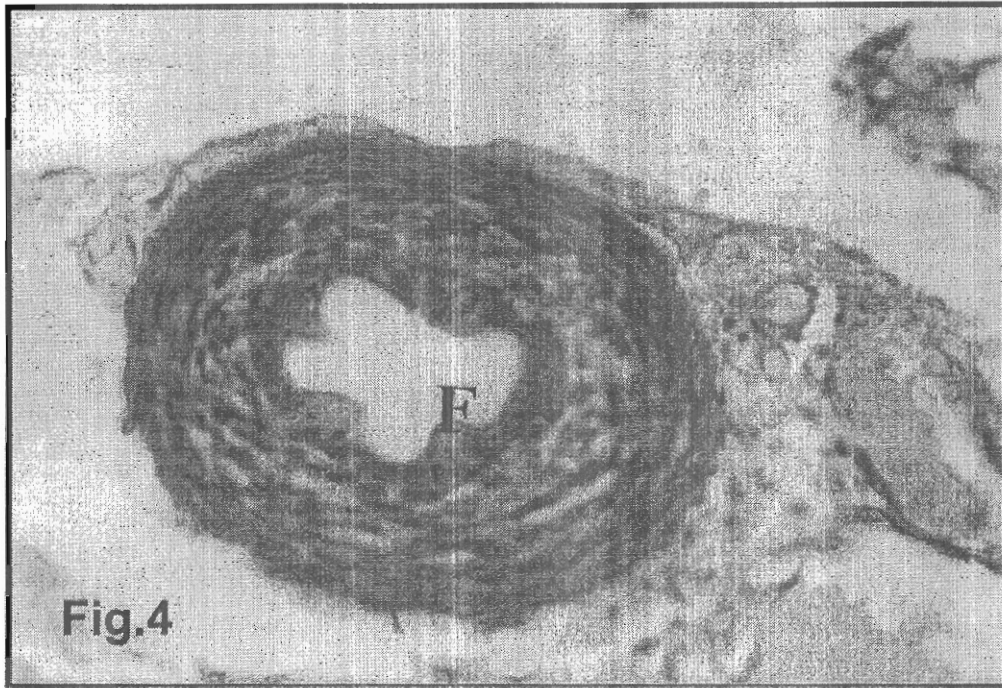


Fig. 4: A photomicrograph of a cross section of the ampullary region of the uterine tube in one day old rabbit, showing the beginning of folded mucosa (F), without connective tissue core. H&E stain. Obj. X20.

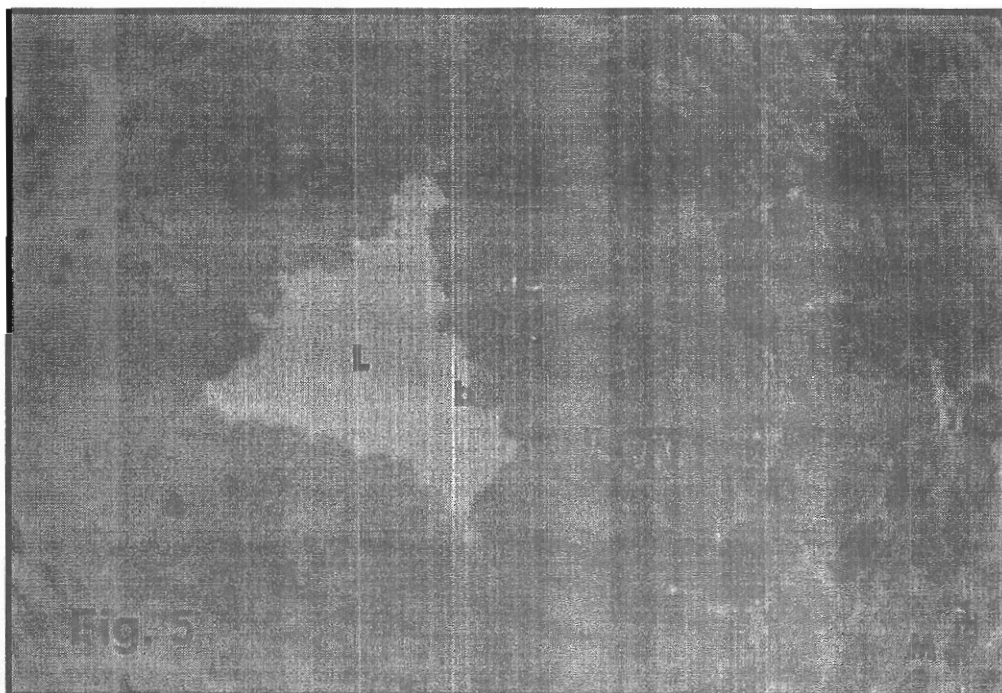


Fig. 5: A photomicrograph of a cross section of the ampullary region of the uterine tube in one day old rabbit showing that, the epithelium (E) consists of undifferentiated columnar cells. The nuclei (N) are lightly stained with prominent nucleoli (n). There is an epithelial bulging (b) into the lumen (L). The epithelium is separated from the muscular layer (M) by a developing connective tissue lamina propria (LP). Toluidine blue stain. Obj. X100.

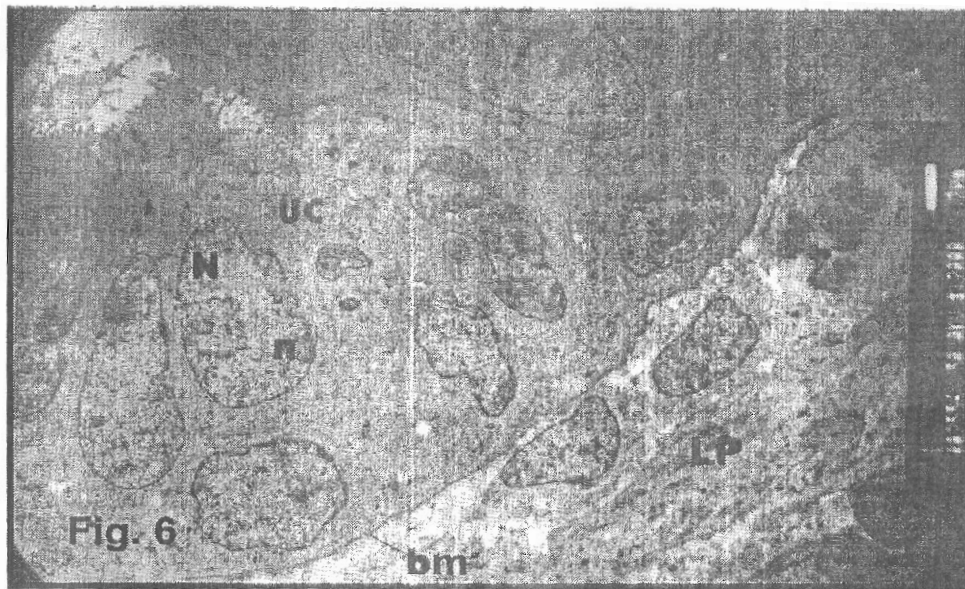


Fig. 6: An electron micrograph of a cross section of the ampullary epithelium of the uterine tube in one day old rabbit showing that the undifferentiated columnar cells (UC) rested on a basement membrane (bm) separating them from a connective tissue lamina propria (LP). They have slightly euchromatic nuclei (N) with prominent nucleoli (n). X8000.

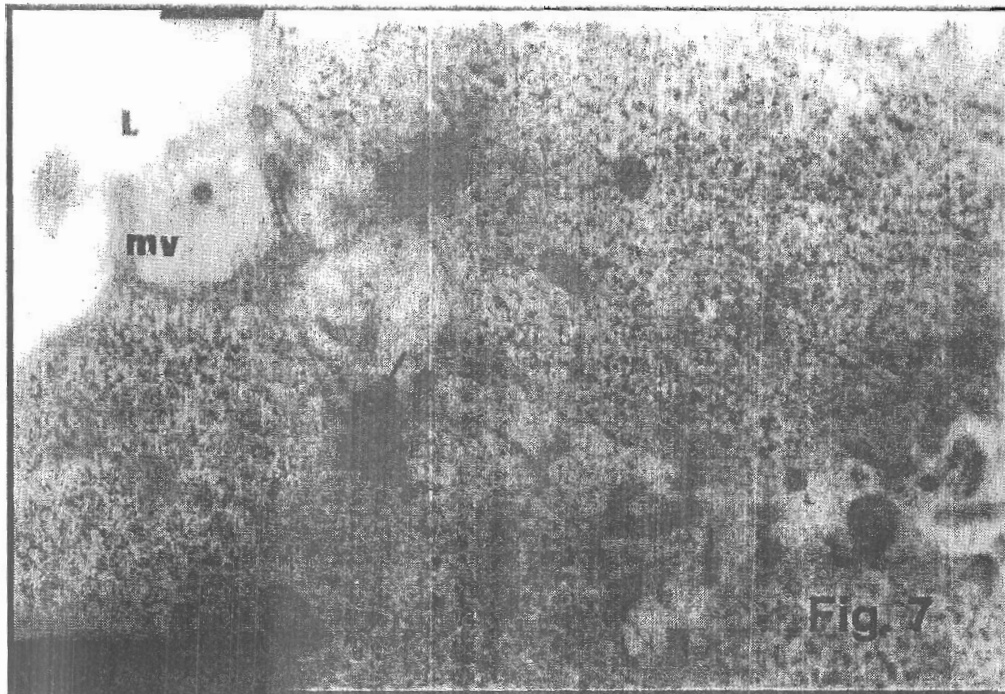


Fig. 7: An electron micrograph of the apical portion of the ampullary epithelium of the uterine tube in one day old rabbit showing that, the luminal surface of the epithelium having microvilli (mv) and occasionally solitary cilia (arrow), projecting into the lumen (L). X56,000

Fig. 8: A photomicrograph of a cross section of the ampullary region of the uterine tube in one week old rabbit showing that. The mucosa having a primary folds (F) covered with a clear pseudostratified columnar epithelium (a), with a clear connective tissue core (b) and lamina propria (LP). H&E stain. Obj. X40.

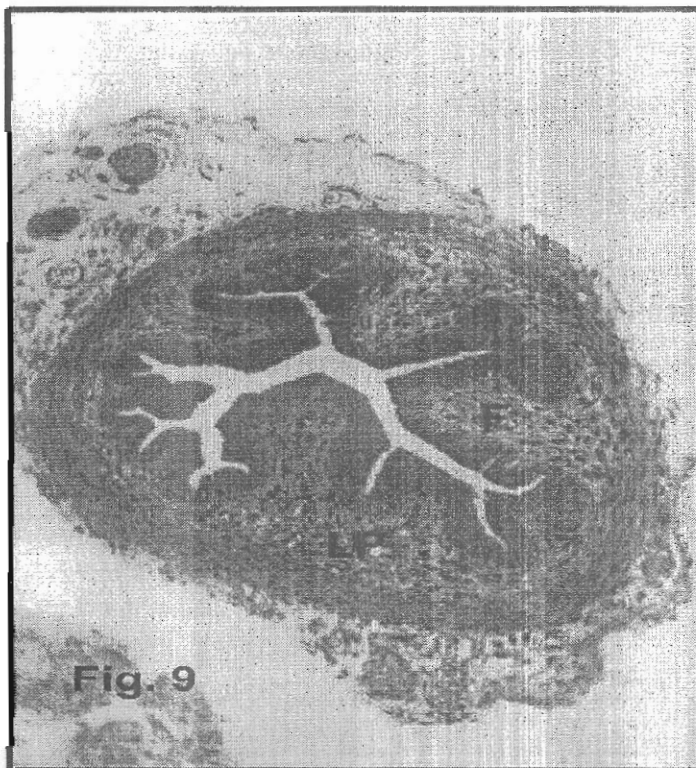
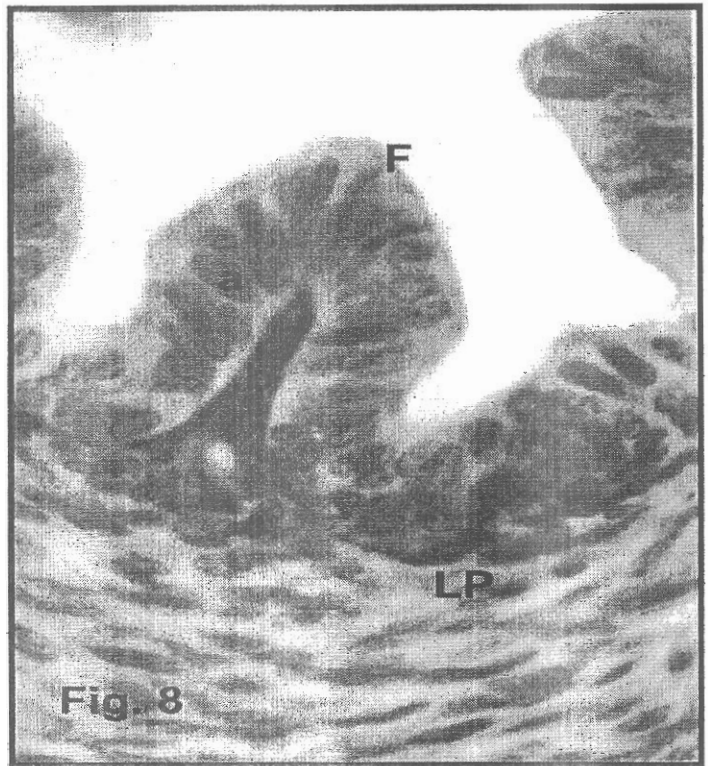


Fig. 9: A photo- micrograph of a cross section of the ampullary region of the uterine tube in one week old rabbit showing that, the ampulla having more extensive mucosal folds (F) and lamina propria (LP) than that of the previous age. Toluidine blue stain. Obj. X10.

Fig. 10: An electron micrograph of the apical portion of the ampullary epithelium of the uterine tube in one week old rabbit showing that, the epithelium consisted of undifferentiated columnar cells (UC) rested on a basement membrane (bm), their luminal surface having microvilli (mv) and occasionally solitary cilia (arrow). A clear nuclei (N) with multiple prominent nucleoli (n). X10.000.

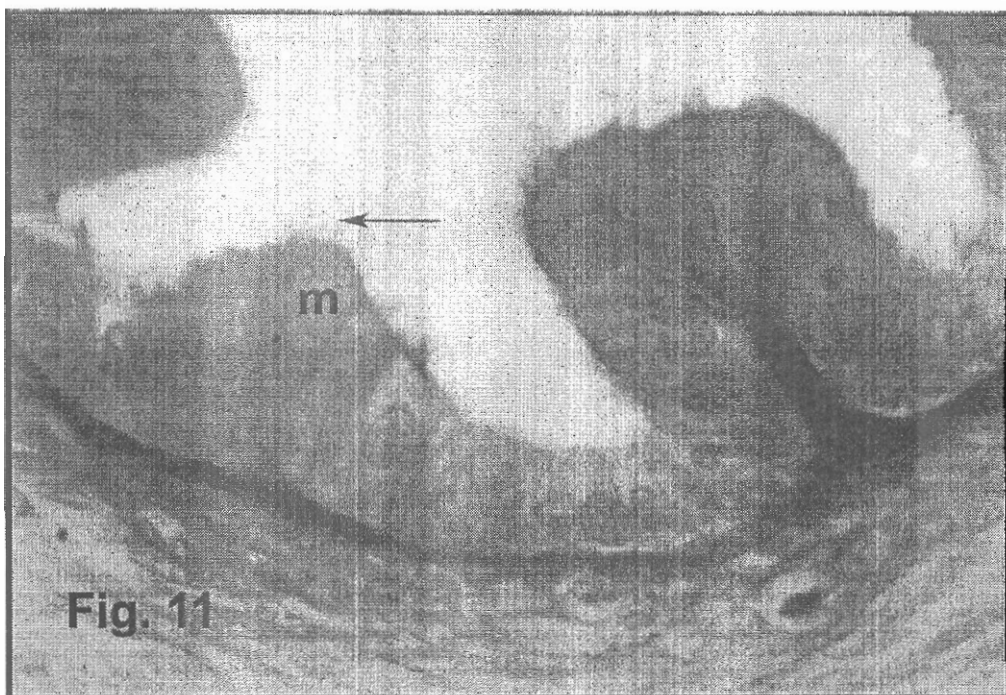
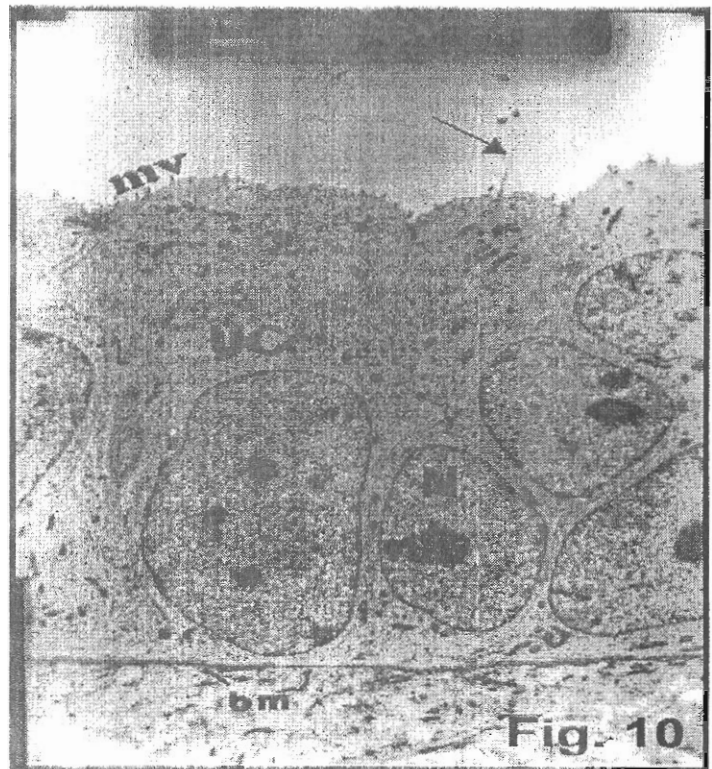


Fig. 11: A photomicrograph of a cross section of the ampullary region of the uterine tube in two weeks old rabbit showing that, the luminal surface of the mucosal cells (m) carrying few cilia (arrow). H&E stain. Obj. X40.



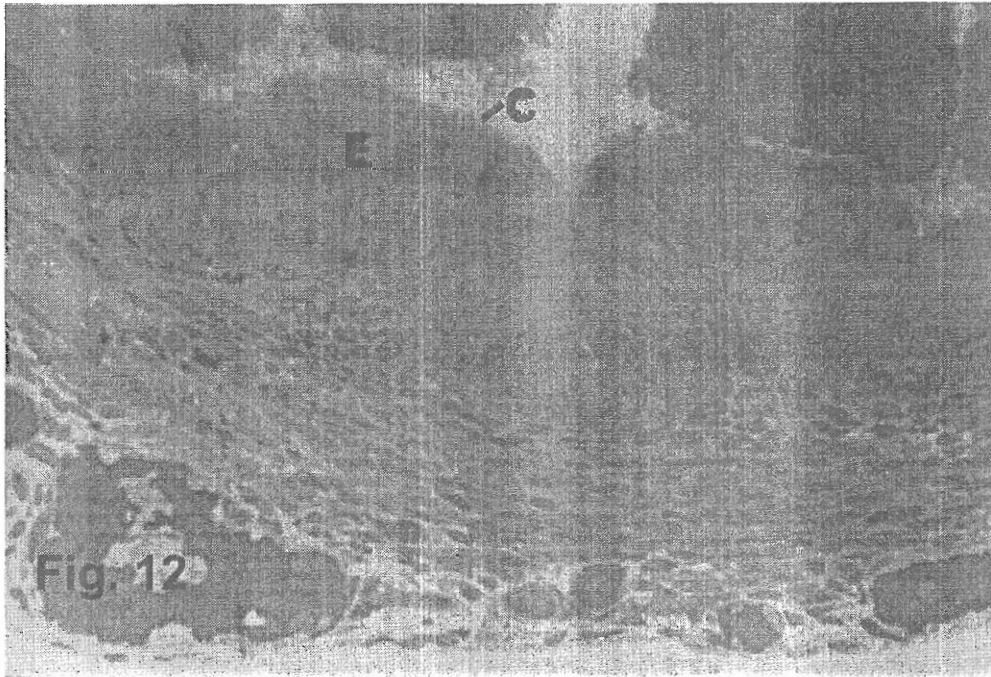


Fig. 12: A photomicrograph of a cross section of the ampullary region of the uterine tube in two weeks old rabbit showing that, the mucosal epithelium (E) contained few ciliated cells (C). Toluidine blue stain, Obj. X40

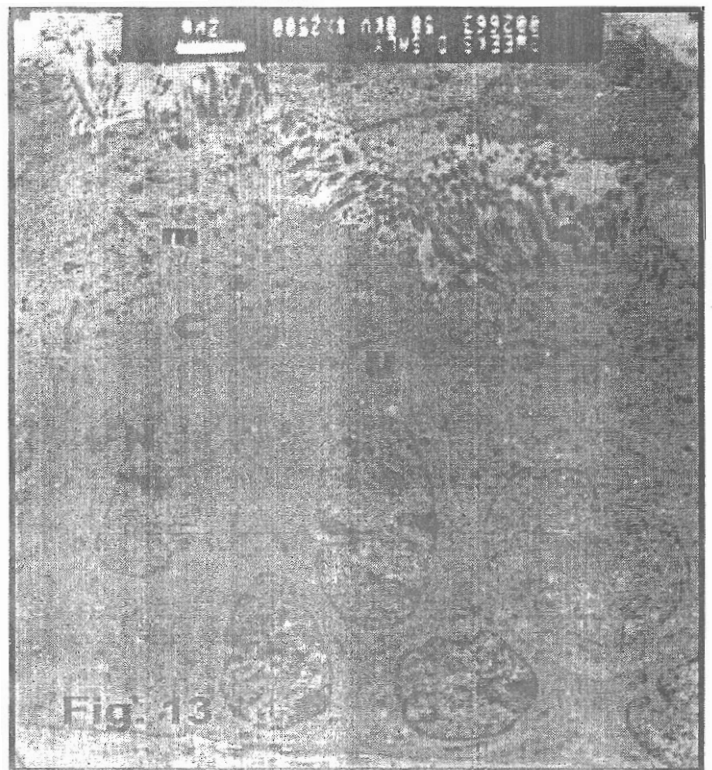


Fig. 13: An electron micrograph of the apical portion of the ampullary epithelium of the uterine tube in two weeks old rabbit showing that, the epithelium consisted of undifferentiated (U) and ciliated (C) cells projecting into the lumen. The ciliated cell nuclei (N) are euchromatic irregular with clear nucleoli (n), numerous mitochondria (m) are seen in the apical part of the ciliated cells. X 10,000.

Fig. 14: A photo- micrograph of a cross section of the ampullary region of the uterine tube in one month old rabbit showing that, the mucosal folds (F) decreased in thickness and covered with pseudostratified columnar epithelium, carrying clear cilia (arrow) towards the lumen (L). H&E stain. Obj. X20

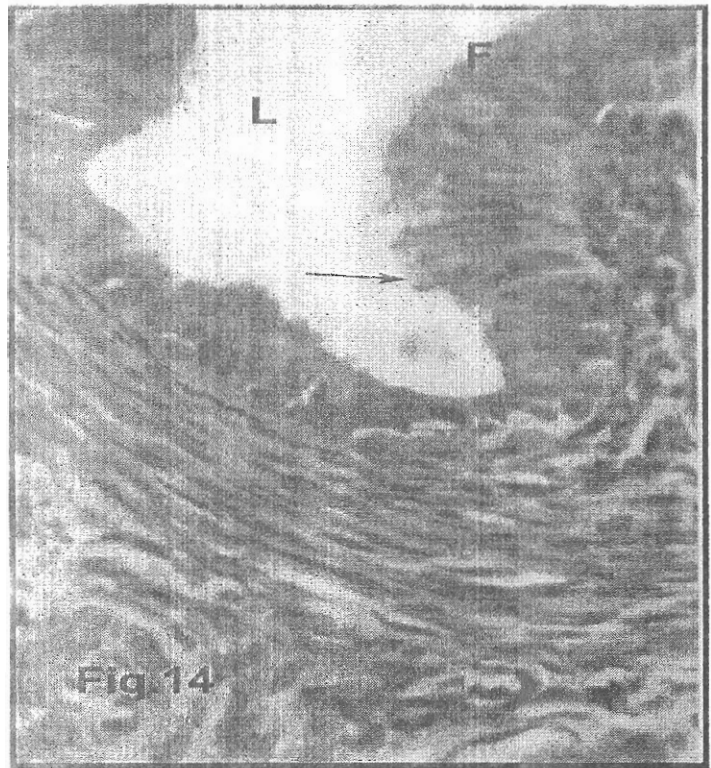


Fig.15: A photo- micrograph of a cross section of the ampullary region of the uterine tube in one month old rabbit showing that, the number and height of the mucosal folds (F) are greater but their thickness lesser. The thickness of the lamina propria (LP) is markedly reduced. Toluidine blue stain. Obj. X20.

Fig.16: Higher magnification of fig.15 showing marked increase in the number of the ciliated cells (C), with well developed cilia (c). The secretory cells (S) are fewer than the ciliated cells. Toluidine blue stain. Obj. X100.

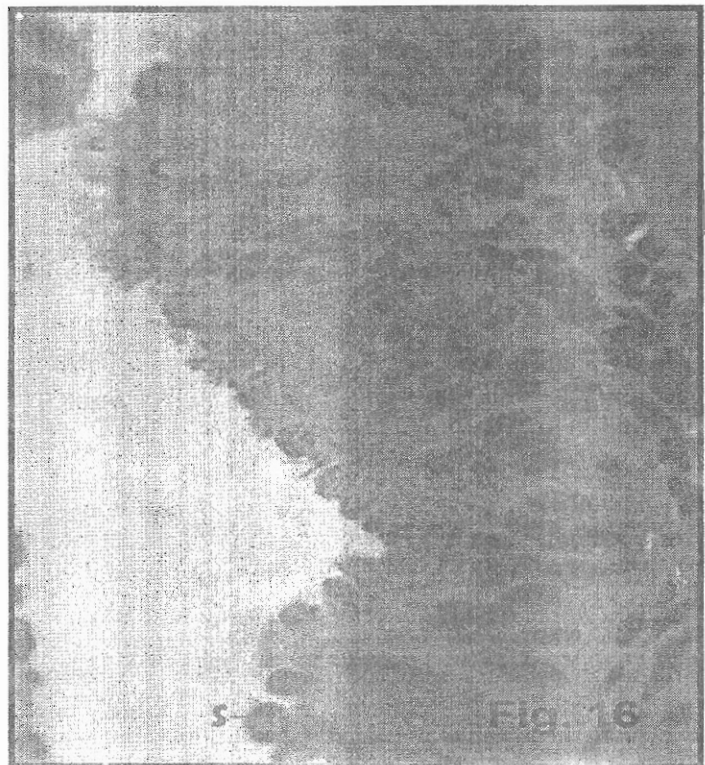
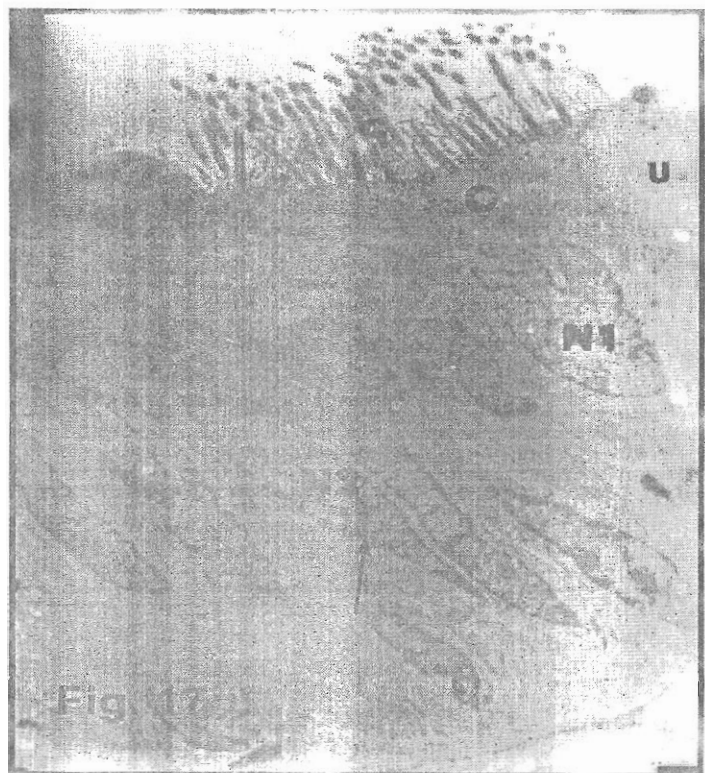


Fig. 17: An electron micrograph of the apical portion of the ampullary epithelium of the uterine tube in one month old rabbit showing that, The epithelium consisted of undifferentiated (U), ciliated (C) and secretory (S) cells. The ciliated cells having well developed cilia (c). Their nuclei (N1) lied in the apical part of the cell. The secretory cells having a single raw of rounded electron dense secretory granules (g). The secretory cells nuclei (N) having a clear nucleoli (n) and some chromatin condensation (arrow). X10,800.



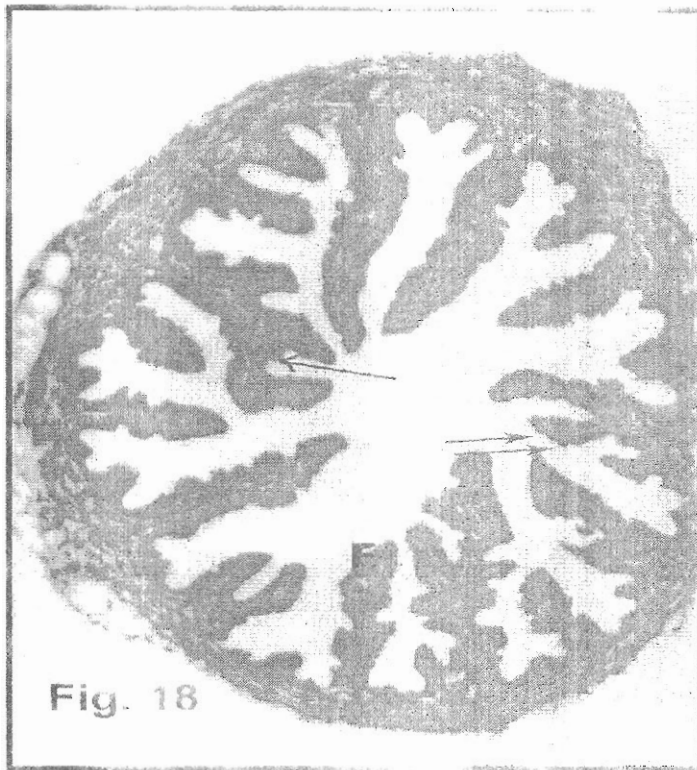


Fig. 18: A photomicrograph of a cross section of the ampullary region of the uterine tube in two months old rabbit showing that, the mucosal folds (F) are numerous and decreased in thickness. The branching of the folds into primary (arrow) and secondary (double arrow). H&E stain. Obj. X10.

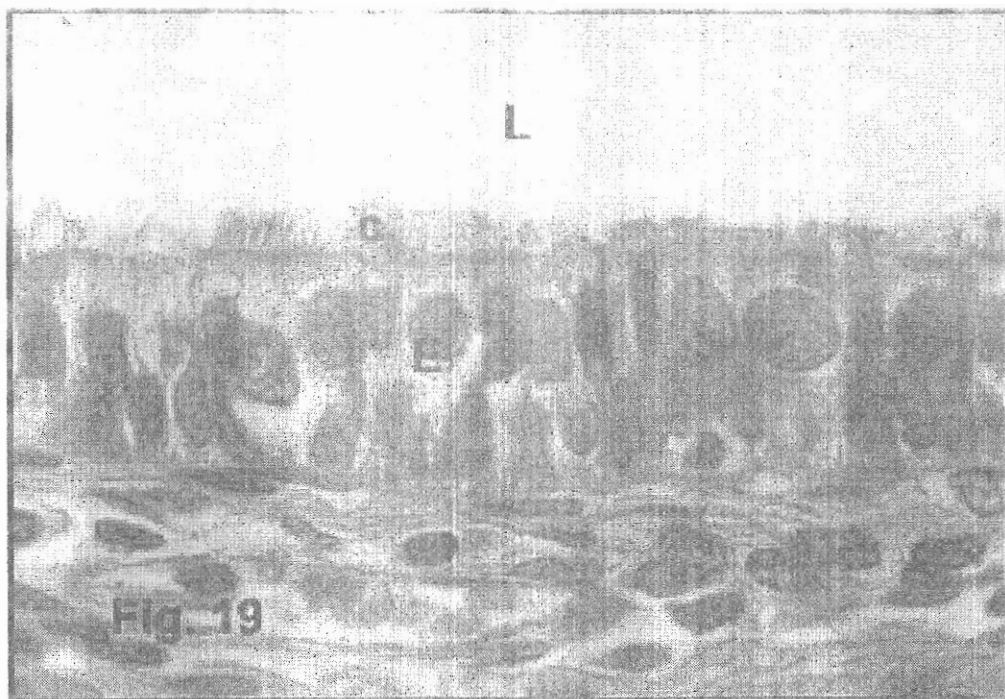
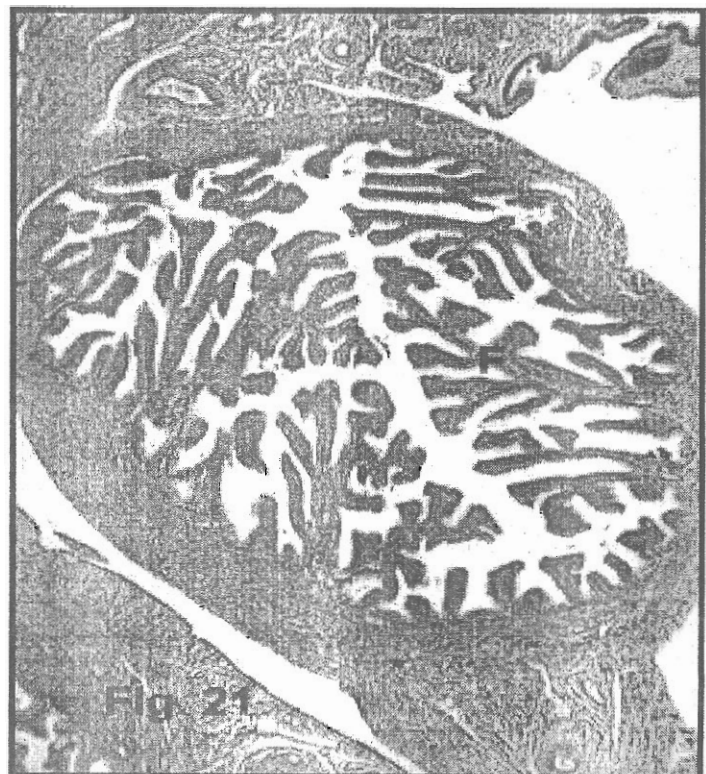


Fig 19: Higher magnification of Fig. 18 showing a clear pseudostratified columnar epithelium (E) with a clear cilia and the lamina propria (L). H&E stain. Obj. X40

Fig. 20: An electron micrograph of the ampullary epithelium of the uterine tube in two months old rabbit showing that, The epithelium consisted of secretory (S) with its nuclei (N1) and ciliated (C) cells with its nuclei (N2). The secretory cells contained more numerous electron dense secretory granules (g). X8000.



Fig. 21: A photo- micrograph of a cross section of the ampullary region of the uterine tube in four months old rabbit showing a clear numerous branching of the mucosal folds (F). H&E stain. Obj. X10.



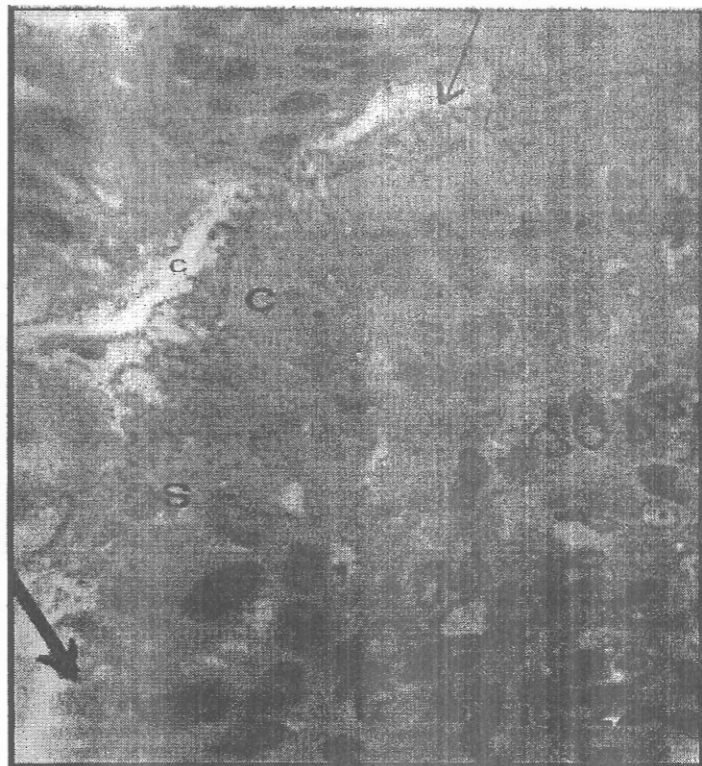


Fig. 22: A photomicrograph of a cross section of the ampullary region of the uterine tube in four months old rabbit showing that, the epithelium consisted of ciliated (C) with well developed cilia (c) and secretory cells (S) with clear secretory granules. The ciliated and secretory cells are nearly equal in number at the tip of the fold (thin arrow), but the secretory cells predominated near the base of the fold (thick arrow). Toluidine blue stain. Obj. X100.

## DISCUSSION

The present study revealed that, the uterine tube was originated from the Müllerian duct. This finding is similar to that previously recorded (26 & 27). Just after birth, the mucosal folds of the rabbit ampulla of the uterine tube are inadequately developed. They appeared as a less folded mucosa without connective tissue cores. At one week, the folds increased in number and acquired connective tissue cores. Then, they showed progressive increase in number and reduction in thickness with the progress of age. The progressive changes of the mucosal folds of the uterine tube were previously described in rabbit (28) and dog (8).

The formation of the mucosal folds begin by pushing of the epithelial basement membrane towards the lumen by the connective tissue lamina propria. Also, the folds increase in number by invagination of the mucosa into the entire length of the folds (8 & 28). However, the present work revealed that this step was preceded by epithelial thickenings at the site of the developing folds.

Also, the increase in fold number occurred by the formation of new folds and branching of the older one.

The ampullary epithelium of the rabbit uterine tube consisted of undifferentiated cells at birth. These cells were characterized by apical bulgings carrying microvilli and solitary cilia. Their cytoplasm contained euchromatic nuclei, numerous ribosomes, few mitochondria, short profiles of rough endoplasmic reticulum and Golgi complexes. These findings are similar to those found shortly after birth in the oviducts of the variety of mammals as mouse (29), human (10), rabbit (30), pig (16), golden hamster (13) and rat (15).

The function of the solitary cilia observed in the undifferentiated cells, lining the oviduct is unknown. Several authors described the solitary cilia to be immotile due to their abnormal structure (6,15,31,32). The cilia of the immature oviductal epithelium are shorter and have a slower beat than that of cilia in mature ciliated cells (32).

The presence of a solitary cilium in a cell could be considered as a sign of beginning of cell differentiation. This consideration depended on the present observation of solitary cilia in developing secretory cells, and on other recorded observations which reported that solitary cilia are frequently found in cells stopping mitosis in mouse (33 & 34).

In the present work the undifferentiated epithelium lined the rabbit ampulla underwent progressive changes with the progress of age. So that it was transformed into well-developed ciliated and secretory cells at four months postnatally. These changes began at one week postnatally by the appearance of proliferative elements, basal bodies and mitochondria in some cells. The appearance of these proliferative elements considered to be the first sign of ciliogenesis (29).

In this investigation, the ciliated cells were differentiated before the secretory cells. This observation is a general occurrence during the postnatal development of the oviducts of different mammals (13,15,16) in golden hamster, rat and pig respectively.

Few secretory cells were firstly detected in the current study at one month postnatally. Then, they increased in number up to four months postnatally. Similar findings were reported in the pig (16), golden hamster (13) and rat (15). Moreover, the present study revealed that at four months after birth, although the ciliated and secretory cells were nearly equal at the tips of the folds, the secretory cells were more numerous than the ciliated cells near the basal parts of the folds. This observation is not reported in the available literature.

The different types of secretory granules observed in the rabbit at four months after birth may be due to different stages of their maturation. This explanation is consistent with earlier observation (30), which recorded that the secretory granules are formed from two sources; Golgi complex and rough endoplasmic reticulum, which form electron lucent granules with electron dense cores.

Many authors described the important role of the secretion of the secretory cells. They produce specific glycoproteins, which affect sperm function, fertilization and embryo development (35). Moreover, it has been reported that the secretory cells release ovum specific glycoproteins, which play an important role in reproductive and developmental events in the oviduct (34,35).

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## الملخص العربي

### بعض الدراسات على تطور ظهارية أمبولة الأنبوب الرحمي في الأرانب البلدى

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تم عمل هذا البحث على عدد ٢٥ جنين واراناب بعد الولادة حتى عمر البلوغ من الأرانب البلدى. وقد تم أخذ الجنين كاملاً أو الجزء الخلفى منه فى العينات الحينية للفحص المجهرى الضوئى. أما فى العينات بعد الولادة، فقد تم أخذ أجزاء من أمبولة الأنبوب الرحمى للفحص المجهرى الضوئى والالكترونى.

وقد أثبتت النتائج أن الأنبوب الرحمى ينشأ من الجزء الأمامى من قناة مولاريان فى المرحلة الجنينية. وقد وجد أنها مبطنه بطبقة واحدة من الخلايا المكعبة، ومحاطة بنسيج رابط من اللحمة المتوسطة غير مكتمل عند ١٥-١٨ يوم قبل الولادة. أما عند عمر ١٩-٢٥ قبل الولادة، فإن تجويف الأنبوب الرحمى يكون نجمياً فى الشكل ويتكون من خلايا عمادية غير مكتملة. أما بعد الولادة مباشرة، فقد أثبتت النتائج عدم إكمال تكوين التثنيات المخاطية، حيث ظهرت كتسمك ظهارى بدون قلب من النسيج الضام. وقد زادت التثنيات عدداً واكتسبت قلباً من النسيج الضام عند عمر أسبوع بعد الولادة. ثم حدثت زيادة مطردة فى العدد واختزال فى السمك مع تقدم العمر. ولوحظ نقرع التثنيات لأول مرة عند عمر شهرين وزاد النقرع عند عمر أربعة أشهر. ظهرت الطبقة الأساسية عند الولادة كطبقة من النسيج الضام تفصل الظهارة عن الطبقة العضلية، وقد امتدت فى قلب التثنيات المخاطية عند عمر أسبوع. بعد ذلك قل سمك هذه الطبقة بوضوح مع تقدم العمر. وقد كانت ظهارة أمبولة الأنبوب الرحمى مكونة من خلايا غير مميزة عند الولادة. وتميزت هذه الخلايا ببروز قصى يحمل خملات دقيقة وأهداباً وحيدة. وقد احتوى السيترولازم على أنوية نشطة وريبوزومات عديدة وقليل من الحبيبات الخيطية وشبكة الجيلة الداخلية المحببة وتراكيب جولجى. واحتوت بعض الخلايا على عناصر تكاثرية وأجسام قاعدية وحبيبات خيطية منقسمة عند عمر أسبوع. وقد شوهدت الخلايا المهديبة لأول مرة عند عمر أسبوعين ثم زادت فى العدد عند عمر شهر. وقد ظهرت الخلايا الإفرازية لأول مرة عند عمر شهر وكانت أكثر عدداً عند قواعد التثنيات المخاطية. واحتوت كل خلية على صف واحد من الحبيبات الإفرازية. ثم زادت هذه الخلايا عند عمر شهرين ولكنها مازالت أقل من الخلايا المهديبة فى العدد. وعند عمر أربعة أشهر، كانت الخلايا الإفرازية و المهديبة متساوية تقريباً فى العدد عند قمم التثنيات المخاطية، بينما كانت الخلايا الإفرازية أكثر عدداً عند قواعد التثنيات، وزادت الحبيبات الإفرازية.