

Histological Feature of Uterus and Uterine Tube During Late Period of Pregnancy in Rabbits

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Abstract

Current work was conducted to investigate the histological architecture of the uterine tube and uterus during the period of late pregnancy in rabbits. Twelve adult local breed rabbits were used. The samples from different parts of the uterine tube were prepared for histological study after staining with H&E, Masson's trichrome and combine Alcian blue (pH2.5)-PAS. The pre ampulla was a narrow tube and its tunica mucosa had slightly long simple mucosal folds lined by pseudostratified columnar epithelium, the ampulla had a wide lumen and its tunica mucosa displayed several highly tall branched mucosal folds with few short simple folds while the isthmus was the narrowest region and its tunica mucosa displayed few of tall and short simple mucosal folds. The mucosa of three parts of the uterine tube was lined by pseudostratified columnar epithelium which is composed of three types of cells: mucous secretory cells, non-secretory cells and basal cells, the mucous secretory cells were the predominant type and revealed secretory activities. The lamina propria-submucosa of the uterine tube was composed of cellular connective tissue and tunica muscularis. The uterus had a very thick wall with well-recognized endometrium and myometrium, the endometrium was composed of many-branched and simple endometrial folds that were covered by multinucleated syncytial cells and simple cuboidal epithelial cells. Lamina propria was composed of loose connective tissue had scattered groups of well growing simple uterine glands that showed secretory activities. The current result showed that the uterus during the late period of pregnancy was differed from those of non-pregnant rabbits, while the uterine tube at late pregnancy has a structure that appeared prepared for the next physiological period.

Keywords: Histology, Uterine tube, Uterus, Rabbits.

الوصف النسيجي للرحم والأنبواب الرحمي خلال فترة الحمل المتأخر في الأرانب الخلاصة

تم إجراء العمل الحالي للكشف عن الوصف الخلوي النسيجي للرحم والأنبواب الرحمي خلال فترة الحمل المتأخر في الأرانب. تم استخدام اثني عشر أرنبًا من السلالات المحلية البالغة. تم اخذ عينات من اجزاء مختلفة من أنبواب الرحم وتم تحضيرها بتقنية الطمر الاليشان الازرق. اظهرت منطقة قبل الأمبولة بالبرافين وصبغت بملون الهيماتوكسلين والايوسين وملون ماسون ثلاثي الصبغ وملون عن أنبواب ضيق وكان الغشاء المخاطي للغلالة يحتوي على طيات مخاطية طويلة وبسيطة قليلة العدد واخرى قصيرة وكانت الطيات مبطنة بظهارة عمودية طبقية زائفة، اظهرت الأمبولة تجويف عريض وتكونت الغلالة المخاطية من عدة طيات مخاطية متفرعة عالية الارتفاع مع عدد قليل من الطيات البسيطة القصيرة. اظهر البرزخ تجويف ضيق جدا واحتوت الغلالة المخاطية على القليل من الطيات المخاطية الطويلة والقصيرة البسيطة. اظهر الرحم ذات جدار سميك للغاية مع بطانة الرحم وطبقة عضلة واضحة التركيب، تكونت بطانة الرحم من العديد من الطيات الرحمية المتفرعة والبسيطة التي تغطيها خلايا مخلوية متعددة النوى وخلايا ظهارية مكعبة بسيطة. يستنتج من الدراسة الحالية أن التركيب النسيجي للرحم خلال فترة الاخيرة من الحمل اختلف عن الرحم في الأرانب غير الحامل، بينما اظهر الأنبواب الرحمي في أواخر الحمل بنية نسيجية ظهرت معدة للفترة الفسيولوجية التالية.

Introduction

Rabbits are heavily used as an experimental animal in research field of basic and applied science. They have been used as suitable model in inducing many diseases and subsequent treating approaches focused on many aspects such as toxicology, pharmacology and surgery (1). On the other hand rabbits are considered once important income sources, in addition as a source of protein as meat for poor rural and developing countries, rabbits have small body size and unique reproductive potential, fast growth rate, genetic diversity, and the ability to utilize different types of forages components (2). Rabbit's does reproductive system is classified as a duplex type, it is composed of two cervixes, left and right uterus, left and right uterine tubes and left and right ovaries (3, 4). Many of morphological, histological and histochemical studies were investigate and described the doe reproductive organs and the different periods of postnatal growth of the reproductive organs does (5). On the other hand Al DIAhhan was describing the doe female reproductive organs in different periods and under the circumstances of ovariectomy and exogenous applied of steroid hormone (6). Microscopic structure of different organs of does is required furthermore investigation of the uterine tubes and uterus as an important organs of the reproductive system during different physiological status, so the current study was aimed to investigate the histochemical architectures of the uterine tubes and uterus at late period of pregnancy.

Materials and Methods

Ethical approval

Current study was under approval of Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad. Baghdad- Iraq.

Experimental animals

Twelve healthy, pregnant rabbits were obtained from the animal's house of college of veterinary medicine-university of Baghdad. The animals

aged approximately 6 months and weight about 1500-1800g. Animals were maintained in the room temperature 25C°, have fed with standard pellet dry diets, and tap water.

Histological preparation

Animal were euthanize by over dose sodium pentobarbital. The abdomen of animal was incisioned and samples of uterus and uterine tubes were collected, washed up with normal saline twice and fixed in 10% neutral buffer formalin for 48h. The tissue specimens were trimmed, processed with the paraffin technique, sectioned at 5-6 μm , and stained with hematoxylin and eosin, combine Alcian blue (pH2.5)-PAS stain and Masson's trichrom stain (7). Tissue sections were examined by light microscopy and microphotography has been done by using Future Win Joe microscopic camera, the images have been analyzed and scored by using Fiji image analyzer system (5). The statistical analysis was done by using SPSS (Version-24) and data were represented by Mean \pm Se.

Results and discussion

Pre ampulla

The pre ampulla was tubular structure which measured $402.41\pm 8.83\mu\text{m}$ in diameter and its mucosa measured $184.89\pm 50.64\mu\text{m}$ that composed of few slightly tall simple pyramidal mucosal folds with few of short folds in between (fig.1 & 2). The mucosal folds were lined by pseudo stratified columnar epithelium which composed of three types of epithelial cells; mucous secretory cells, non-secretory cells and basal cells. The mucous secretory cells were the predominant type, had oval-round shaped nuclei with lightly stained cytoplasm. The non-secretory cells were little had elongated shaped nuclei, eosinophilic cytoplasm and showed no cilia. The basal cells were few restricted at the basement membrane characterized by eosinophilic stained round nuclei (fig.2). The lamina propria-submucosa was constructed of highly vascular and cellular connective tissue. The thickness of tunica

muscularis was measured $77.25 \pm 7.32 \mu\text{m}$, composed of well distinct two layers of smooth muscles: thick inner circular and thin outer longitudinal layers (fig.1).

Ampulla

The ampulla had a wide lumen diameter which measured $632.94 \pm 15.93 \mu\text{m}$ and its tunica mucosa measured $200.61 \pm 5.80 \mu\text{m}$ which composed numerous highly tall branched mucosal folds with few of short simple folds (fig.3). The mucosal folds were lined by pseudo stratified columnar epithelium which composed of three types of epithelial cells; mucous secretory cells, non-secretory cells and basal cells. The mucous secretory cells have showed highly vesicular cytoplasm which revealed secretory activities. The non-secretory cells were little had elongated shaped nuclei, eosinophilic cytoplasm and showed no cilia. The basal cells were few restricted at the basement membrane characterized by darkly stained round nuclei (fig.4). The lamina propria-submucosa was constructed of highly vascular and cellular connective tissue (fig.4). The thickness of tunica muscularis was $57.82 \pm 5.35 \mu\text{m}$, composed of two layers of smooth muscles; thin inner circular layer and thick outer longitudinal layer (fig.3).

Isthmus

Isthmus appeared the narrowest region which measured $329.20 \pm 16.8 \mu\text{m}$ in diameter, and thickness of tunica mucosa was $123.23 \pm 5.14 \mu\text{m}$ which displayed few of tall and short simple mucosal folds (fig.5). The mucosal folds were lined by pseudo stratified columnar epithelium which composed of three types of epithelial cells; mucous secretory cells, non-secretory cells and basal cells. The mucous secretory cells have seen in the lining epithelium at base of mucosal fold while the non-secretory cells were predominately lined the middle and top of the mucosal folds. The main features of this period the epithelial cells displayed no cilia (fig. 6). The lamina propria-submucosa was constructed of cellular connective tissue composed mainly of fibrocytes (fig.6). The thickness of tunica muscularis was $136.12 \pm 11.93 \mu\text{m}$, composed of two layers of smooth muscles; very thick inner circular layer & thin outer longitudinal layer (fig.5).

The current results of three parts of uterine tube were revealed branched and simple mucosal

folds within mucosa of uterine tube, the distribution and the ratio of these folds in different regions of uterine tube are similar to those recorded by (9, 10). This result suggests that the branching of mucosal folds of uterine tube is correlated with preparation for next period of post pregnancy and that under the control of adniiophypophysis. Other researchers who revealed that the meeting of ovum and sperm take place in ampulla and comprise half-length (11), while at the isthmus the lumen became narrow and has well developed muscular layers with few folds, so the secretory cells are predominate in ampulla. The tubal pseudo stratified columnar epithelium is differed from those recorded by (12) who referred to simple cuboidal. While this result is similar that recorded by (7, 10). The variable types of epithelial cells of uterine tube are correlated with the various phases of estrus or period of pregnancy which denoted that the resting of stimulating status of these epithelial types. Types of epithelium is associated with events occurred along the length of the uterine tube under the control of sex hormones during ovulation at which the sperms and ovum must be transport to ampulla, the modulation of epithelial cells was turned into ciliated cells be the predominate, this suggestion had supported by (6,10,13 and 14). Secretion of non-ciliated secretory cells is nourished the ovum during passage through the oviduct, they are particularly prominent at day 14 of the estrous cycle around the time of ovulation (15). The oviductal glycoproteins aid in embryonic development, sperm capacitation, and hyper activation, the steroid hormones regulated the secretion of these glycoproteins. The epithelial cells are capable to secrete fluid which are significantly vital for survival of gametes it provide basic medium for sperm capacity and subsequent fertilization of the ova (16, 17). The structures of the lamina propria and tunica muscularis during period of late pregnancy were similar to those recorded by (3, 7, 18).

Uterus

During late period of pregnancy the uterus was measured 24.70 ± 0.83 mm in diameter, revealed very thick uterine wall with well recognized endometrium, myometrium and perimetrium (fig.7). The thickness of endometrium was 332.34 ± 15.90 μ m, composed of many branched simple endometrial folds, the mucosa was covered by two different types of epithelium: the first type of the epithelial cells was the syncytial cells of the placenta which characterized by a large size, oval-dump shape cells and had a feature of multi nucleated, most of these cells were showed eosinophilic cytoplasm and some of them had clear cytoplasm, the second type of the epithelial cells was simple cuboidal cells which had large rounded nuclei with dark stained cytoplasm (fig.8). The lamina propria of the endometrium has composed of loose connective tissue characterized by scattered groups of well growing simple uterine glands which lined by simple cuboidal cells and showed secretory activities (fig.8 & 9). The thickness of myometrium was 474.41 ± 10.79 μ m, composed of well-organized double layer of smooth muscle fibers; inner circular layer and thin outer longitudinal layer (fig.8). The current result revealed marked development and growth of uterine gland which are also recorded at functional status of uterus by (7, 19). The finding revealed that the endometrium and myometrium thickness were significantly increased during late pregnancy as compared to other results recorded by (6,10) in not pregnant rabbits and rat respectively, this was associated with increase in population of uterine glands and sizes of muscle fibers of myometrium, similar finding are reported in the humans (20, 21). On the other hand, the lamina propria consists of the collagen reticular fibers network with many fibroblast-like cells, which known as fusiform cells and highly vascularized (22).

Histochemical results

The pre ampulla, ampulla and isthmus were revealed two types of secretory cells; neutral mucopolysaccharid secreting cells and acidic

mucopolysaccharid secreting cells, during late period of pregnancy the acidic secreting cells was the predominant type (fig.10). The epithelium of endometrium was and uterine glands were revealed weak neutral mucopolysaccharid secretions and there was thin layer of acidic mucopolysaccharid covered the apical surface of epithelium (fig.10).

The current results revealed variable contents of neutral and acidic mucopolysaccharids in three regions of uterine tube as those observed by (23,24). Result of pre ampulla and isthmus are similar to those recorded by (25) in immature and mature does, this result suggest that these regions play an important role in both nutrition of ovum and zygote and antimicrobial action. However, the ratio of non-secretory cells to secretory are different in compared among three regions of uterine tube and are according the predominated hormones, for example non-secretory cells are numerous in fimbriae epithelial cells, less in ampulla, and fewer in the isthmus (26). Types of secretion are slightly constant in ampulla during periods of late and post pregnancy the ampulla which revealed three types of secretory cells (Neutral mucopolysaccharid secreting cells, acidic mucopolysaccharid secreting cells and the third type showed mixed secretory granules). The endometrial epithelium and glands revealed neutral mucopolysaccharids, such type of secretion are also recorded by (6, 10). The secretions are accumulated in the apical surface along the border of the epithelia which indicated apocrine mode of secretion. Strong PAS reaction in the apical part of lining epithelium during follicular and luteal phase (26).

Conclusion; The current result showed that the histological cytoarchitecture of uterus during period of late pregnancy were differed from those of non-pregnant rabbits, while the uterine tube at late pregnancy has structure which appeared prepared for next physiological period.

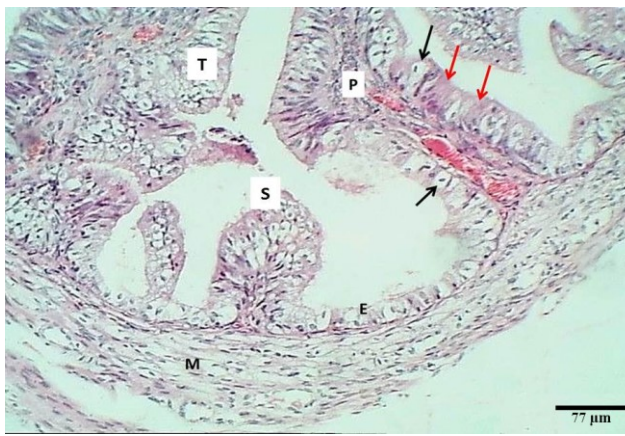


Figure 1: Section through wall of pre ampulla (Late pregnancy) shows: tall folds (T), short fold (S), epithelium (E) lamina propria (P), secretory cells (Black arrows), non-secretory cells (Red arrows) & tunica muscularis (M).H&E stain.

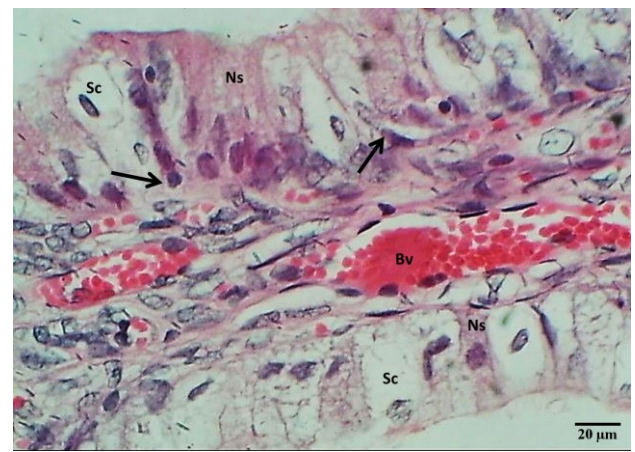


Figure 2: Section of mucosal fold of the pre ampulla (Late pregnancy) shows: secretory cells (Sc), non-secretory cells (Ns), basal cells (Arrows) & blood vessels (Bv).H&E stain.

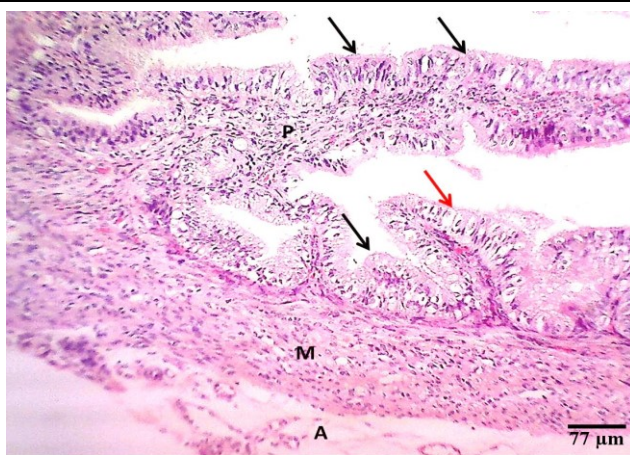


Figure 3: Section through wall of fallopian tube- ampulla (Late pregnancy) shows: lamina propria (P), secretory cells (Black arrows), non-secretory cells (Red arrows), tunica muscularis (M) & tunica serosa (A).H&E stain.

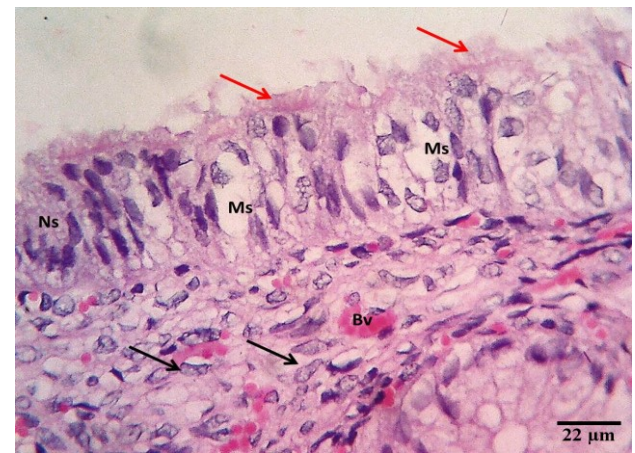


Figure 4: Section of mucosal fold of ampulla (Late pregnancy) shows: secretory cells (Ms), non-secretory cells (Ns), cilia (Red arrows), fibroblast (Black arrows) & blood vessels (Bv).H&E stain.

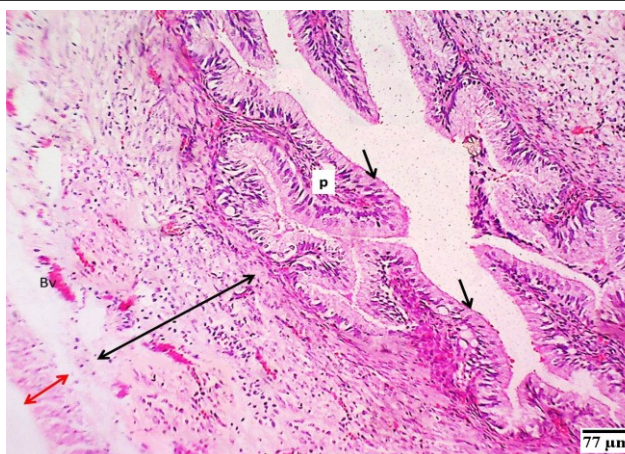


Figure 5: Section of isthmus (Late pregnancy) shows: lamina propria (P), epithelium (Black arrows), blood vessel (Bv), inner circular layer of tunica muscularis (Black double head arrow) & longitudinal layer of tunica muscularis (Red double head arrow).H&E stain

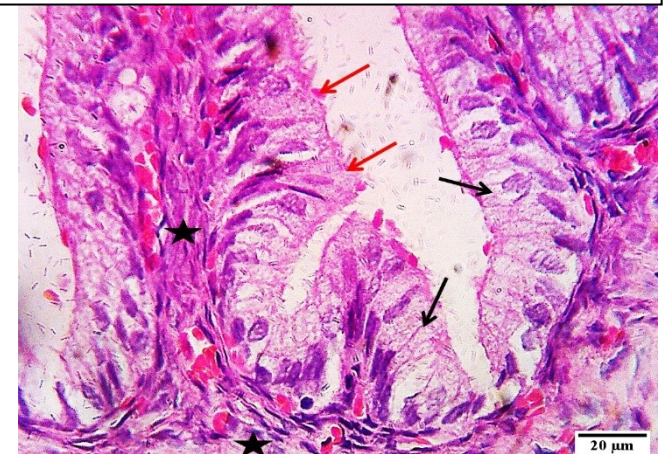


Figure 6: Section of mucosal fold of the isthmus (Late pregnancy) shows: secretory cells (black arrows), non-secretory cells (Red arrows), cellular loose connective tissue (Asterisks).H&E stain.

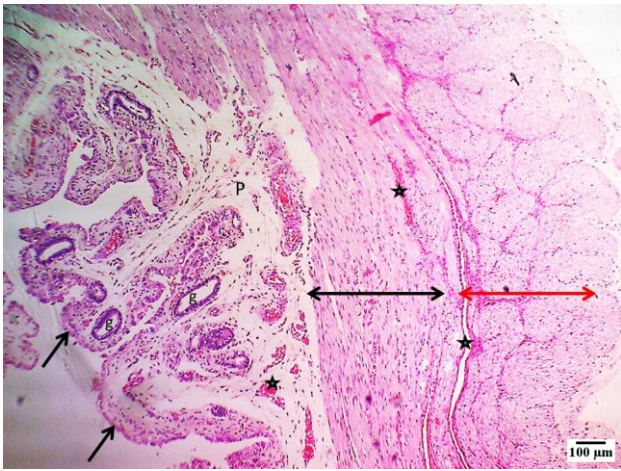


Figure 7: Section of uterus (Late pregnancy) shows: few of branched endometrial folds (Black arrows), uterian glands (g), lamina propria (P), blood vessels (Asterisks), inner circular of myometrium (Black double head arrow) & outer longitudinal layer of myometrium (Red double head arrow) .H&E stain.

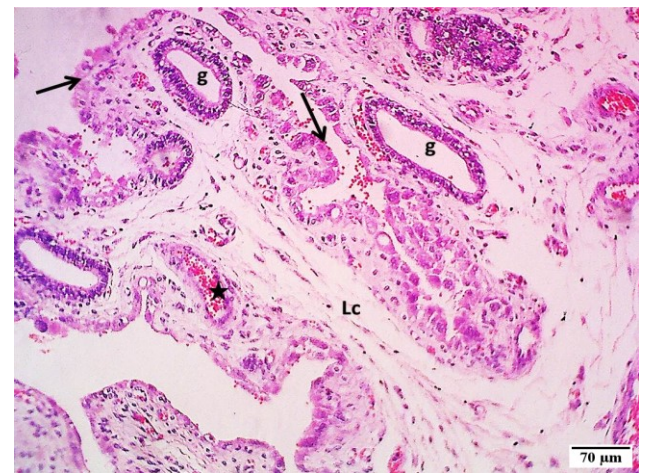


Figure 8: Section through the wall of uterus (Late pregnancy) shows: loose connective tissue (Lc), epithelium (Black arrows) & blood vessels (Asterisks) .H&E stain.

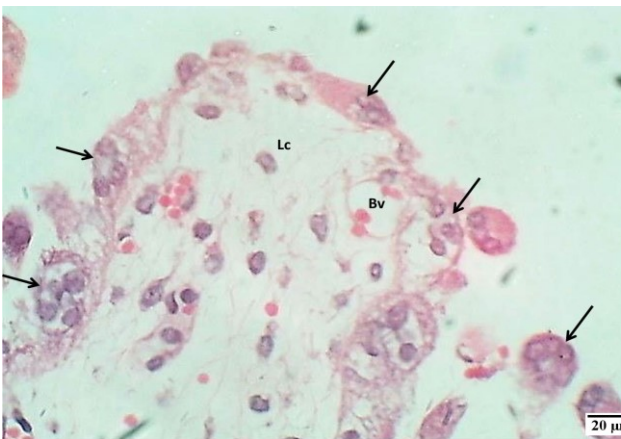


Figure 9: section of endometrium (Late pregnancy) shows: multi nucleated cyncicial cells of placenta (Black arrows), (Black arrows), cuboidal epithelial cells (Red arrows), loose connective tissue (Lc) & blood vessels (Bv) .H&E stain

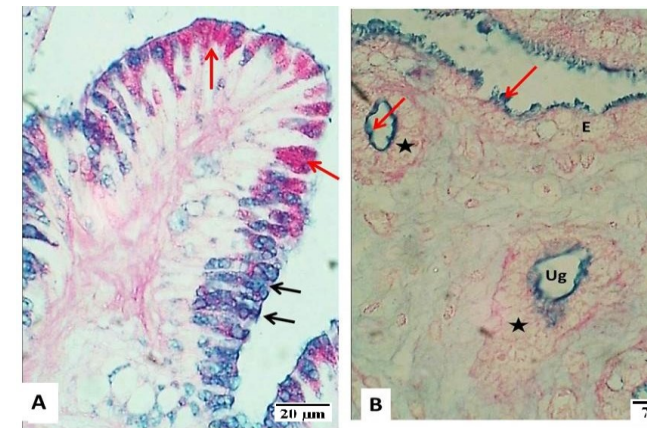


Figure 10: Micrograph (A) mucosal folds of ampulla shows neutral mucopolysaccharid secreting cells (Red arrows), acidic mucopolysaccharid secreting cells (Black arrows). Combine AB (pH2.5) & PAS stain (400x).Micrograph (B) uterus shows weak neutral glycoprotein in the cytoplasm of uterine glands (Ug) and epithelium (E) of endometrium and acidic mucopolysaccharid layers (Red arrows) .(4Combine AB

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