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Histomorphological and histochemical study of the fallopian tube during pregnancy and post-partum of the cape hare rabbit (Lepus Capensis)

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***Abstract*---**This research was investigate the Anatomical and Histochemical structure of uterine tube during period of pregnancy and postpartum in cap hare rabbits. Eighteen female rabbits were used. The target samples were processing with paraffin embedding technique the tissue sections stained with H&E stain, and Masson's trichrom stain. Results; The length and weight of the uterine tube were (91.31 ± 0.72mm), (0.53 ±0.01g) at pregnancy. While. its (84.76 ±0.22mm), (0.41 ±0.01g) at post-partum. The infundibulum has a funnel-shaped end. Simple mucosal folds are bordered with pseudo stratified columnar epithelium. The lamina propriea-sub-mucosa is made up of connective tissue that is both vascular and cellular. The ampulla was highly vascularized and had a twisted appearance. The mucosal folds of the ampulla were lined by pseudo stratified columnar epithelium, which consisted of three cell types: mucous secretory cells, non-secretory cells and basal cells. The lamina propria was a thin layer of fibrous connective tissue that exposed a significant

amount of fibroblast. The isthmus was short and straight at post-partum, and it was held in place by the mesosalpinx. Mucosal folds in the tunica mucosa are mild and short. The tunica muscularis is made up of two layers: a dense inner circular layer and a thin longitudinal layer that followed externally by tunica serosa.The infundibulum gives a weak positive reaction to (PAS Stain), which reveals neutral mucopolysaccharid secretion, and a positive reaction to (AB Stain), which reveals acidic mucopolysaccharid secretion, during pregnancy and postpartum. During pregnancy and after delivery, the ampulla and isthmus secrete both neutral and acidic mucopolysaccharides.

***Keywords*---**uterine tube, infundibulum, ampulla, isthmus, histology, rabbits, postpartum.

Introduction

The female reproductive system of rabbits is duplex, with two cervixes, left and right uterus, left and right uterine tubes, and left and right ovaries (Muna *et al*, 2016; Salih *et al*, 2020). The mesosplenx fixes the wall of the fallopian tube and supports the pelvis of the nearby rabbit. There are two layers to the tunica muscularis (inner circular and outer longitudinal) (aldahhan, 2015). The fallopian tube, also known as the uterine tube or oviduct, is one of the most functionally important and competent organs of the reproductive apparatus, functioning as a bridge between the ovary and the uterus. It involves peristaltic transport to the site of fertilization (ampulla) and the provision of a microenvironment for fertilization as well as the transfer of the embryo to the uterus for implantation (Hunter, 2012).

Both the gametes and the embryo must be transferred within a specific time range in order for intrauterine implantation to be effective. Tubal musculature contractions, ciliary activity, and tubal secretion flow all influence this perfectly timed process (Lyons *et al*,2006).

Materials and Methods

Experimental animals: Eighteen female rabbits were used to investigate the histological and Histochemical structure of fallopian tubes during pregnancy and post-partum. The rabbits were obtained from the Baghdad spinning market and were 6 months old, weighing around 1600-1900g. The rabbits were authorized by using sodium pentobarbital overdose. Then the fallopian tube was dissected from the abdomen wall, washed with distal water, and fixed in 10% neutral buffer formalin that changed after 24 hours. The uterus and uterine tubes samples were taken, washed twice with normal fluid, and fixed in 10% neutral buffer formalin for 48 hours. The tissue specimens were trimmed, paraffin treated, sectioned at 5-6 m, and stained with hematoxylin and eosin and Masson's trichrom stain (Suvarna *et al*, 2018). Light microscopy was used to analyze tissue slices. Future Win Joe microscopic camera was used for microphotography, and the photos were evaluated and graded using the Fiji image analyst system. The data were

represented by Mean SE and statistical analysis was performed using SPSS (Version-24).

Results and Discussion

During pregnancy The uterine tubes were elongated, coiled, and positioned in the abdominal cavity, characterized by numerous blood arteries. The length and weight of the uterine tube were (91.31 ± 0.72mm), (0.53 ±0.01g). While. The uterine tube is longer, less torsioned, and positioned in the pelvic inlet at post-partum. The length and weight of the uterine tube were (84.76 ±0.22mm), (0.41 ±0.01g). The uterine tube is divided into three parts (infundibulum, ampulla, and isthmus) (Fig. 1,2). (Table . 1).

The infundibulum is the cranial portion of the uterine tube that connects the ovary cranially to the ampulla caudally. It has a funnel-shaped end that connects to the ovary. The length, weight, and diameters are (6.22±0.31mm), (1.44±0.1g), and (5.88± 0.21 mm) accordingly throughout pregnancy (Fig. 1,2). The infundibulum is a broad portion of the uterine tube that occupies the majority of the ovary at post-partum. (6.87±0.12 mm), (1.73±0.1g), and (4.93±0.2mm) are the length, weight, and diameters, respectively (Table. 2). Simple mucosal folds are bordered with pseudo stratified columnar epithelium in the tunica mucosa (mucous secretory cells, non-secretory cells and basal cells). (Figures 3,4,5). The lamina propria-sub-mucosa is made up of connective tissue that is both vascular and cellular. The tunica muscularis was made up of two thick thick inner circular and thin outer longitudinal layers, while, the tunica serosa was thin layer (Fig. 6),(Table. 3).

The ampulla was the middle section of the body that joined the infundibulum with the isthmus. It was highly vascularized and had a twisted appearance (Fig. 7). The length (15.43±0.31mm), weight (1.1±0.02 g), and diameters (3.21±0.12 mm) were measured during pregnancy. At post-partum, the central part of the body was extremely torturous. (16.21±0.01 mm), (1.8±0.01g), and (4.1±0.021 mm) are the length, weight, and diameters, respectively (Table. 2). The mucosal folds of the ampulla were lined by pseudo stratified columnar epithelium, which consisted of three cell types: mucous secretory cells, non-secretory cells and basal cells. The lamina propria was a thin layer of fibrous connective tissue that exposed a significant amount of fibroblast. There are two layers to the tunica muscularis: a thin inner circular layer and a thick outer longitudinal layer. Externally, the tunica serosa surrounds muscularis (Fig.8,9,10), (Table. 3).

At pregnancy, the isthmus was the third and smallest section of the uterine tube that straightened the longest (Fig. 1,2). (53.64±0.34mm), (2.8±0.03g), and (2.12±0.21 mm) are the length, weight, and diameters, respectively. The isthmus was short and straight at post-partum, and it was held in place by the mesosalpinx. (55.72±0.17 mm), (3.1±0.01g), and (2.87±0.3 mm) are the average length, weight, and diameters, respectively(Table. 2). Mucosal folds in the tunica mucosa are mild and short. The pseudo stratified columnar epithelium, which consisted of three types of epithelial cells, lined the mucosal folds (mucous secretory cells, ciliated non-secretory cells and basal cells). The lamina propria-submucosa is made up of fibrocyte-rich cellular connective tissue. The tunica

muscularis is made up of two layers: a dense inner circular layer and a thin longitudinal layer that followed externally by tunica serosa (Fig. 11,12), (Table. 3). The infundibulum gives a weak positive reaction to (PAS Stain), which reveals neutral mucopolysaccharid secretion, and a positive reaction to (AB Stain), which reveals acidic mucopolysaccharid secretion, during pregnancy and postpartum. During pregnancy and after delivery, the ampulla and isthmus secrete both neutral and acidic mucopolysaccharides (Fig. 13,14).

The neck of the infundibulum controls the abdominal ostium of the oviduct, and the funnel part of the infundibulum is associated with the initial purpose of the oviduct, which is to catch the ovum, as indicated in previous studies (Felipe *et al.*, 1998; Lyons *et al.*, 2006). The distribution and ration of these folds in different parts of the fallopian tube are similar to those documented by Pereda *et al.* (2006), Hagiwara *et al.* (2008), Sokol (2011), and Al-Dahhan (2015). and rabee and Ahmed (2019). Flamini (2014), as well as (kalaf M.H. 2018). demonstrated that ovum and sperm meet in the ampulla and are half-length, but the lumen narrows at the isthmus and has well-developed muscle layers with minimal folds, indicating that secretory cells predominate in the ampulla (kalaf M.H. 2018). According to prior findings, the pseudo stratified columnar epithelium lined the uterine tube in the current investigation, as it did in most animals (Al Dahhan, 2015; . Ahmed and rabee 2019). Multiple proteins are produced and released by the epithelial cells of the uterine tube, which govern fertilization as well as embryonic development (Avilés *et al.*, 2010). Insulin-like growth factor-1 is a secretory protein that responds to estrogen's cyclic impact to aid normal embryo implantation. IGF-1 is regulated by estrogens through a direct transcriptional mechanism (Gabler *et al.*, 1998; Shao *et al.*, 2012). These cells are hypothesized to enable spermatozoa to fertilize an ovum in the wide section of the uterine tube (ampulla); in addition, their secretion lubricates the uterine tube, facilitating ovum passage and providing protection and nourishment. Mucin in the three regions (infundibulum, ampulla, and isthmus) can play a vital role in ovum and zygote feeding, as well as having antibacterial properties. The ratio of non-secretory cells to secretory cells, on the other hand, varies between the three areas of the uterine tube. Non-secretory cells are abundant in infundibulum epithelial cells, less in ampulla, and less in the isthmus (Ozen *et al.*, 2010; Saleem *et al.*, 2016; kalaf M.H. 2018).

Table 1: measurements of length and weight of the uterine tube during pregnancy and post-partum period

	Uterine tube	
Periods	At pregnancy	At Post-partuim
Lengths /mm	91.31 ± 0.72 mm	84.76± 0.22 mm
Mean±SE	A	A
Weights /g	0.53 ± 0.021 g	0.41± 0.1 g
Mean±SE	A	B

• Similar litters revealed no significant differences ($P > 0.05$) between periods.
• Different litters revealed significant differences ($P < 0.05$) between periods.

Table 2: measurements of length, weight and diameter of the infundibulum , ampulla and isthmus during pregnancy and post-partum period.

	Infundibulum		Ampulla		Isthmus	
	At pregnancy	At postpartum	At pregnancy	At postpartum	At pregnancy	At postpartum
Lengths /mm Mean ± SE	6.22±0.31 A	6.87±0.12 B	15.43±0.31 A	16.21±0.01 B	53.64±0.34 A	55.72±0.17 B
Weights /gr Mean ± SE	1.44±0.1 A	1.73±0.1 A	1.1±0.02 B	1.8±0.01 B	2.8±0.03 A	3.1±0.01 A
Diameter /mm Mean ± SE	5.88±0.21 B	4.93±0.2 A	3.21±0.12 A	4.1±0.021 A	2.12±0.21 B	2.87±0.3 A

- Similar capital litters revealed no significant differences ($P < 0.05$) between periods.
- Different capital litters revealed significant differences ($P < 0.05$) between period.

Table 3: measurements of tunica mucosa thickness and thickness of tunica muscularis of the infundibulum , ampulla and isthmus during the two period (pregnancy and post-partum)

	Infundibulum		Ampulla		Isthmus	
	At pregnancy	At postpartum	At pregnancy	At postpartum	At pregnancy	At postpartum
Thickness of mucosa Mean ± SE	194.65±41.28 A	213.21±12.53 A	198.72±6.31 B	231.54±9.21 B	138.62±8.34 A	112.84±6.35 A
Thickness of muscularis Mean ± SE	82.62±5.44 A	61.54±8.62 A	53.64±6.66 B	61.84±5.31 B	141.84±10.31 B	138.54±3.12 B

- Similar capital litters revealed no significant differences ($P < 0.05$) between periods.
- Different capital litters revealed significant differences ($P < 0.05$) between periods.
- Similar small litters revealed no significant differences ($P > 0.05$) between left & right tubes.

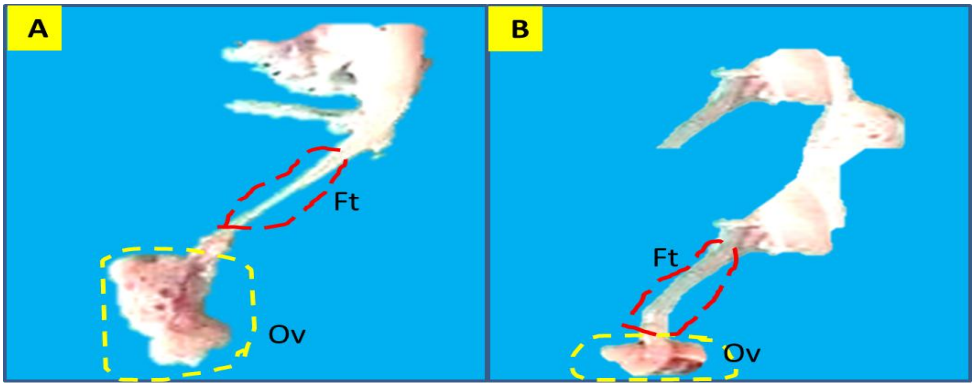

Figure 1: A : Cape Hare Rabbit (during pregnancy) shows: left fallopian tube (Ft) and left ovary (Ov). B: Cape Hare Rabbit (during pregnancy) shows: Right fallopian tube (Ft) and left ovary (Ov).

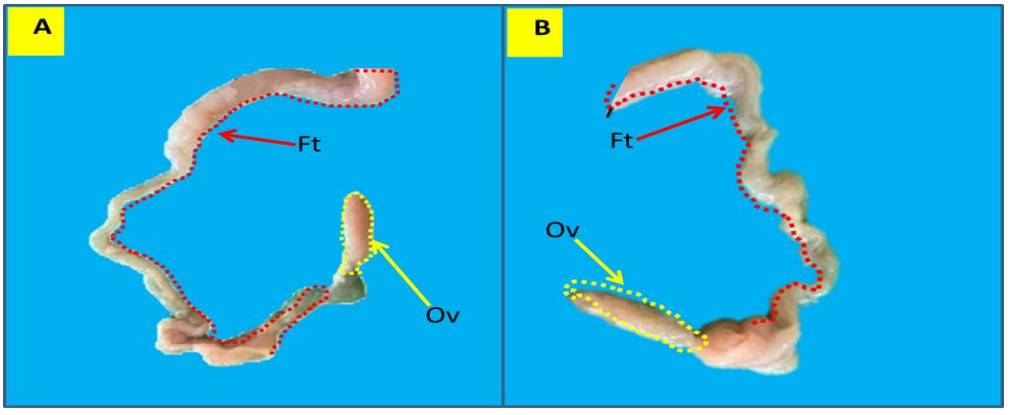

Figure 2: Female cape Hare Rabbit uterine tube (post-partum) shows: left ovary and fallopian tube (A), right ovary and fallopian tube (B), ovary (Ov), fallopian tube (Ft), infundibulum (In), ampulla (AM), isthmus (Is).

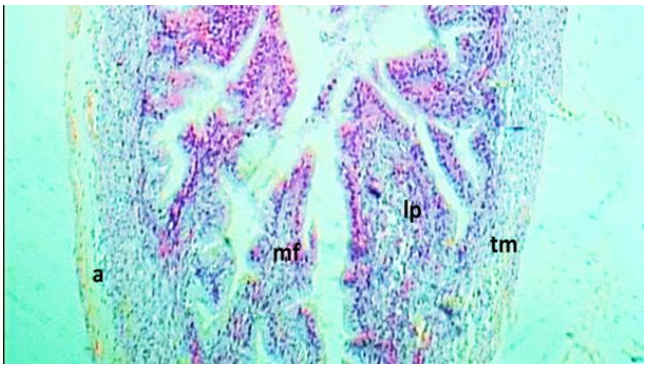

Figure 3: Histological section of Female cape Hare Rabbit uterine tube infunbibulum (during pregnancy) shows: lining epithelia (E), lamina properia (lp), tunica muscularis (tm), adventatia (a). Alcian blue stain. 10X.

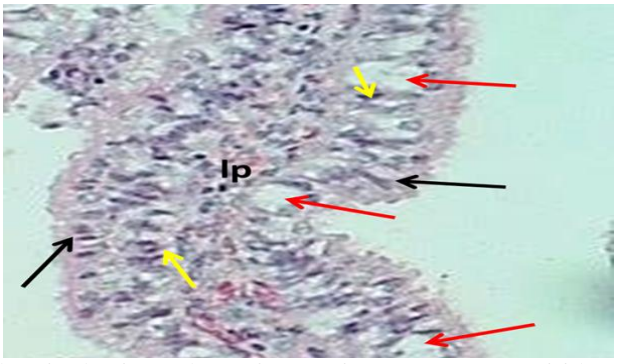

Figure 4: Histological section of Female cape Hare Rabbit uterine tube infundibulum (during pregnancy) shows: lamina properia (lp), basal cell (blue arrow), non-secretory cell (black arrow), secretory cell (red arrow). H&E stain. 20X.

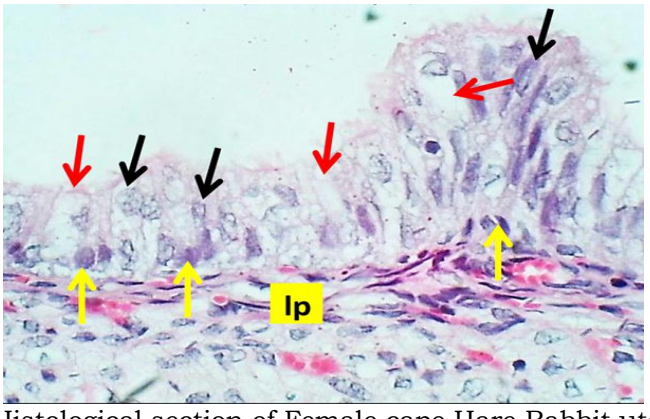

Figure 5: Histological section of Female cape Hare Rabbit uterine tube infundibulum (during pregnancy) shows: lamina properia (lp), basal cell (blue arrow), non secretory cell (black arrow), secretory cell (red arrow). H&E stain. 40X.

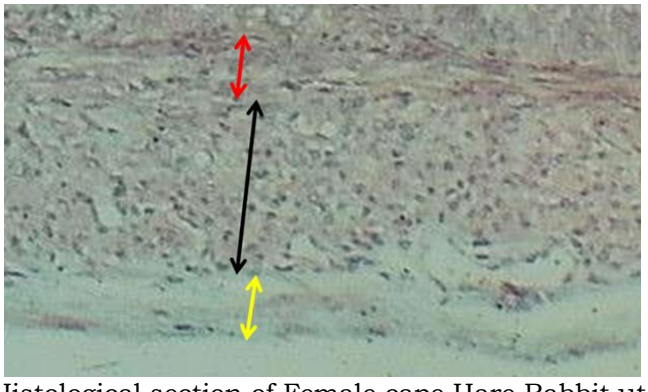

Figure 6: Histological section of Female cape Hare Rabbit uterine tube infundibulum (post- partum) shows: inner muscularis layer (red double head arrow), outer muscularis layer (black double head arrow), adventitia (yellow double head arrow). H&E stain. 40X.

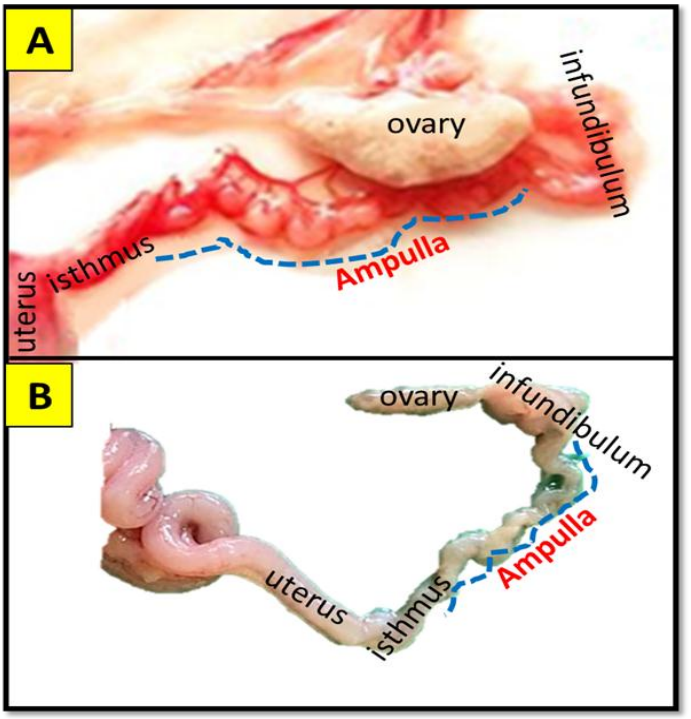

Figure 7: photo-macrograph of the cape Hare Rabbit uterine tube shows: The ampulla at period of pregnancy (A) and the ampulla at post partum period (B).

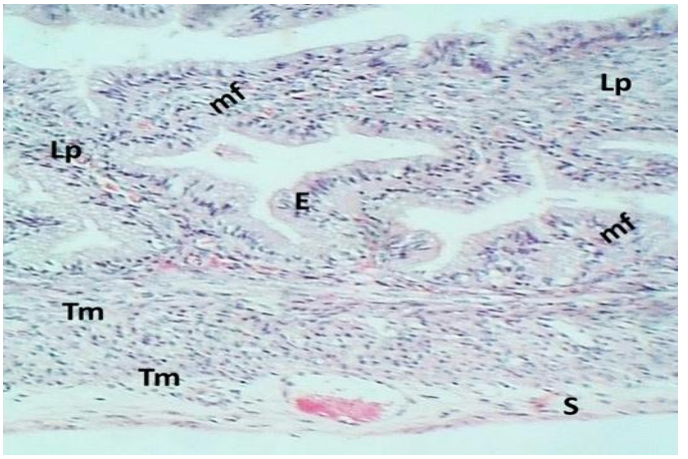

Figure 8: photo-micrograph of the ampulla of cape Hare Rabbit uterine tube during pregnancy shows: mucosal fold (mf), lamina properia (Lp), epithelia (E), tunica muscularis (Tm), serosa (S). H&E stain 20X.

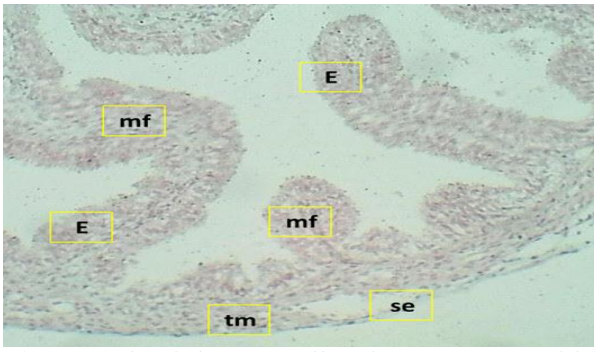

Figure 9: photo-micrograph of the ampulla of cape Hare Rabbit uterine tube at post- partum shows: mucosal fold (mf), epithelia (E), tunica muscularis (Tm), serosa (Se). H&E stain 10X.

Figure 10: photo-micrograph of the ampulla of cape Hare Rabbit uterine tube at post- partum shows: secretory cell (blue arrow), ciliated non secretory cell (red arrow), basal cell (black arrow), blood vessels (Bv). H&E stain 400 X.

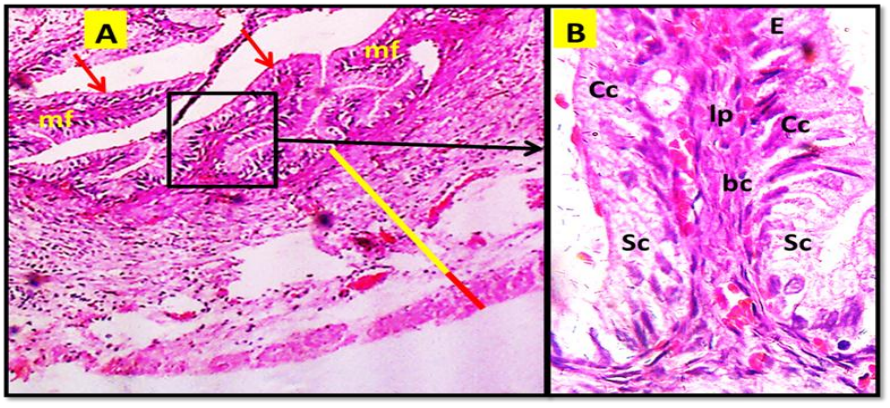

Figure 11: Histological section of the fallopian tube (isthmus) of cape Hare Rabbit during pregnancy shows:
A: mucosal fold (mf), epithelia (red arrow), tunica muscularis inner layer (blue line), tunica muscularis outer layer (red line). basal cell (black arrow), blood vessels (Bv). H&E stain 400 X.H&E stain 10X.
B: magnified section shows: secretory cell (Sc), ciliated cell (Cc), basal cell (bc), lamina proprea (lp), epithelia (E). H&E stain 400X.

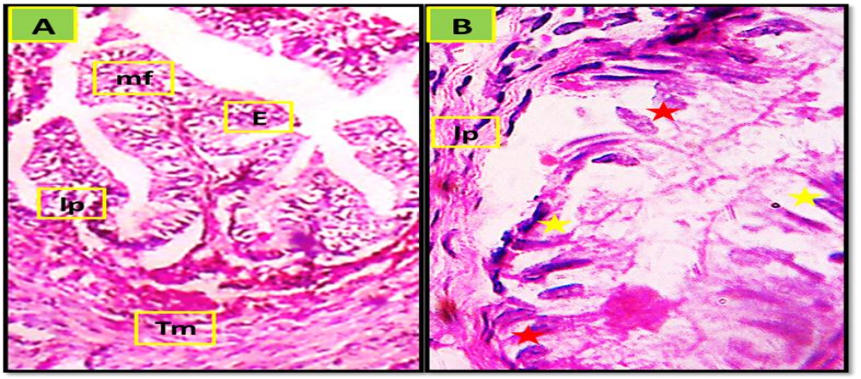

Figure 12: Histological section of the fallopian tube (isthmus) of cape Hare Rabbit during post-partum shows: A: mucosal fold (mf), epithelia (E), lamina proprea (lp), tunica muscularis (Tm). H&E stain 20X.
B: magnified section shows: secretory cell (blue star), ciliated cell (red star). H&E stain 400X.

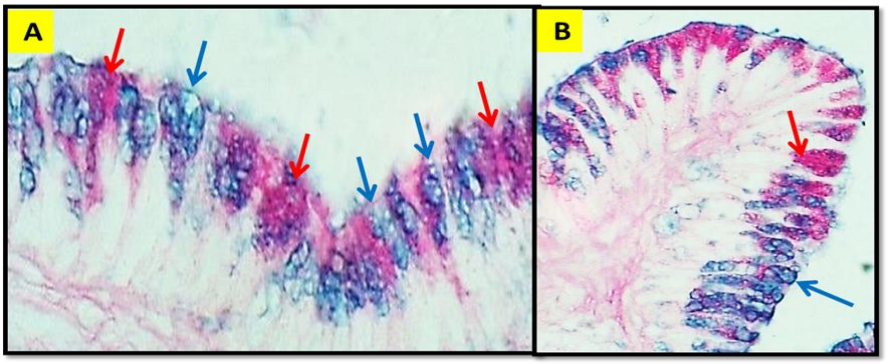

Figure 13: Histological section of infundibulum A: (Post-partum) and B (during pregnancy shows neutral mucopolysaccharid secreting cells (Red arrows) & acidic mucopolysaccharid secreting cells. 400x. Combine AB (pH2.5) & PAS stain.

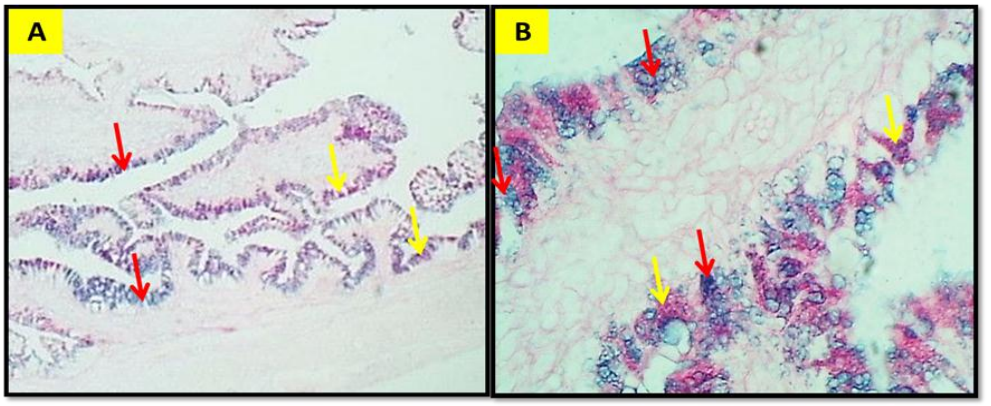

Figure 14: (A)Histological section of the fallopian tube (ampulla) of cape Hare Rabbit give positive reaction for both periodic acid shife and alcian blue stain during pregnancy and post- partium. Combination (PAS&AB) stain 10X. (B) magnified section of isthmus that give the same reaction of ampulla. 400X.

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