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Histomorphological and Histochemical study of the uterine tubes in the premature female goats

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Abstract

This study aimed to examine the histomorphological and histochemical characteristics of the uterine tubes in premature female local goats. For this particular investigation, a total of 10 samples of uterine tubes were used. These samples were obtained from slaughterhouses and were taken from individuals aged between 3 and 5 months. The histological technique involved the execution of a standard approach to prepare histological sections. This was achieved by applying both general and particular Histochemical stains. Uterine tube samples were obtained and stored using a solution of 10% neutral buffered formalin for preservation. Immediately after collection, we conducted measurements of the uterine tube's gross morphology, weight, and length. By employing the methodology of standard histology procedure. Histochemical sections were prepared using both general and specific histochemical stains. The gross examination showed the presence of bilateral uterine tubes consisting of a thick, short, coiled preampulla, a thin, long ampulla, and the isthmus, which is the smallest and straightest section connecting to the uterus. Under microscopic examination, three distinct regions were identified based on variations in their histological characteristics. The data showed that the uterine tubes in kittens were not fully grown, while immature does exhibited significant histological and histochemical changes compared to kittens. On the other hand, mature does indicated that the post-weaning phase plays a crucial role in the development of the animals. The histochemical method specifically identified changes, with a particularly significant positive reaction observed in the isthmus region, which clearly reacted with PAS, AB, and a combination of PAS-AB. To summarise, the latest data revealed distinct variations in the uterine tubes of local rabbits compared to other mammals, particularly those belonging to the rodentia species.The uterine tube exhibited a statistically significant increase in thickness ($P<0.05$).The uterine tube was subjected to histochemical analysis utilising Haematoxylin and eosin, Alcian blue, Masson trichrome, Periodic acid, and Schiff reagent (PAS) stains.

I. INTRODUCTION

Goats are considered to be one of the important livestock species that play a crucial role in the economics of various nations, including Iraq. Due to their ability to yield milk, meat, and skin for the production of leather and fibres, goats are a superior source of resources compared to other animals. The goat is an excellent subject for studying and doing biological research. **(Smith and Sherman, 2009; Eesa, 2016; Ali and Ibrahim, 2022).** The significance of uterine tubes in the reproductive process of domestic mammals is widely acknowledged. **(Bosch and Wright, 2005).** The uterine tube was partitioned into three distinct segments: the infundibulum, the ampulla, and the isthmus. **(Abiaezute et al .,2017).** The uterine tubes are bilateral, tortuous structures that transport ova from the ovaries to each uterine horn. In domestic animals, spermatozoa fertilise the oocytes at the location of the uterine tubes. The infundibulum and fimbriae extended from the unattached edge of each tube segment adjacent to its ovary. The fimbriae aid in guiding the oocyte towards the infundibulum during the process of ovulation. **(Reece and Rowe ,2017**)

The main goal of the present study is to examine the histomorphological and histochemical features of the uterine tubes (ampulla, infundibulum, and isthmus) in premature female goats. The aim is to provide

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comprehensive data on the morphology, macromorphometric, and micromorphometric properties of the uterine tubes in premature goats.

II. MATERIALS AND METHODS

This experiment utilised a sample of ten female indigenous goats, all of which were in good health and aged between 3 and 5 months. The goats were acquired from a slaughterhouse. All the goats were purchased from the slaughterhouses.The butcher performed a midline abdominal incision on the goats to expose the reproductive organs, the structure of the abdominal and pelvic cavities, and both uterine tubes. These tubes were subsequently extracted and studied in their original position.The reproductive organ was exposed, and both uterine tubes were observed, documented, and then extracted for dissection.The uterine tube was rinsed with buffered saline solution. Subsequently, each tube was placed on filter paper to air-dry prior to being weighed.The length and weight of the extracted object were measured using a vernier calliper, a weighing balance with a sensitivity of 0.0001 grammes, and a ruler and thread, respectively (Table 1).The standard error of the mean (mean±SEM) represents the statistical measure of the variability in the gathered data.Significance was attributed to p-values below 0.05.

The entire uterine tube was dissected and its distinct portions, namely the infundibulum, ampulla, and isthmus, were excised. Subsequently, these sections were cleansed using normal saline and subsequently immersed in 10% neutral buffered formalin for a whole duration of 72 hours. Several samples were conserved using Bouin's solution in order to facilitate staining with histochemical dyes. The histological slices were created using a conventional histology method. The tissue slices were stained using various general and specific stains, including Hematoxylin-eosin (H&E), Masson Trichrome (MTC), Alcian blue (AB) at pH 2.5, Periodic acid schiff (PAS), combined PAS-AB at pH 2.5, and combination AB at pH 2.5. The utilisation of periodic acid and Schiff reagent (PAS) facilitates the identification of neutral mucopolysaccharide secretory cells observed in the lining epithelium. **(Mirhish,2018; Reshag and Khalaf,2021)**.

Micromorphometric measurements of shape and size The colour USB 2.0 digital imaging equipment (Scope Image 9.0) was utilised to evaluate the thickness of the tunica mucosa and muscularis in uterine tubes, with the assistance of image processing software. In order to enable comparisons between different postnatal ages, all of the parameters described above were incorporated into a table. The tissue slices, with a thickness of 6 mm, were stained using the Masson Trichrome dye to facilitate the identification of different tissues during micromorphometric measurements. Statistical analysis The data from macromorphometric and micromorphometric measurements were analysed using the Oneway ANOVA method in the SPSS software.

Macroscopic findings:

The premature female local goats exhibited bilateral uterine tubes on both the left and right sides, as observed through a gross examination. These tubes appeared as long tubular structures, coiled in their cranial regions, very long and twisted in their middle regions, and shorter and more straightened in their caudal regions. The cranial ends of the respective uterine horns were gradually connected to them caudally. The uterine tube is divided into three sections: the infundibulum, the ampulla, and the isthmus (fig1.A). Additional sources, such as **(Dyce et al., 2002)** The mammalian uterine tube is anatomically separated into three distinct sections: the infundibulum, ampulla, and isthmus.These areas are associated with a wide range of physiological functions. Based on the reference, it appears that the fimbriae located in the front part of the infundibulum are responsible for transferring the oocyte into the uterine tube after ovulation. Moreover, distinct appellations were assigned to the various sections of the oviduct in mammalian species, including fimbriae, ampulla, isthmus, and utero-tubal junction. **(Nickel et al.,1979; Abe, 1996).** The ovarian end of the uterine tube is connected to the abdominal cavity through a small aperture called the ostium abdominale, which is shaped like an infundibulum. In female horses and dogs, the uterine horn ends with a little papilla, which serves as the opening of the uterus.

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(Getty, 1975). The female goats have distinct characteristics in their reproductive system, unlike other animal species such as rabbits. Specifically, their uterine tube is composed of the preampulla, ampulla, isthmus, and tubule-uterine junction**(Al-Saffar and Almayahi,2019)** The ovary is anterior to the opening of the tube. The path is intricate, curving widely from the centre to the outside side of the ovary's upper end, then passing through the outer wall of the bursa and terminating in the uterine horn. Fertilisation takes place in the oviduct, which receives ovulated eggs from the ovaries through the fimbria. This part is located between the uterine connection and the ovary.The text refers to figure 1, specifically to the part labelled B. The outcome of the female goat that was examined was comparable to the outcome of the **(Hena et al ,2019)** The uterine tubes in sheep are observed as paired convoluted tubes that extend from the ovaries to the uterine body. These tubes are characterised by their tortuous, wiry, and hard nature, and are embedded in mesosalpinx fat. The mesometrium and the mesovarium were anatomically linked to the mesosalpinx, which served as the housing for the uterine tube, both towards the tail and towards the head. The outcomes of **(Khaton et al ,2015)** The oviduct of female cows exhibits comparable characteristics to the current results, where it appears as intricate tubes that connect the ovaries to the pushed ends of the uterine horns. The oviduct is twisted, thin, and inflexible, with fatty tissue called mesosalpinx embedded inside it. **(Pérez etal,2013)** The deer's uterine tubes were characterised as small and winding, situated within the two folds of the mesosalpinx, which connected them to other female reproductive organs through the mesosalpinx portion of the wide ligament.. **(Mustafa and Reshag, 2018)**.

Lengths of uterine tubes of the female premature goats were 8.72±0.38cm for the right and 9.80±0.52 cm for the left one. while the mean weight of uterine tubes in this animal species was 0.50±0.05gm for the right and left was 0.47±0.05 gm. which were recorded in table(1) .These results were different compared to those recorded in the West African Dwarf goat In this animal species the mean length of right and left uterine tubes was 19.05± 0.39 and 21.10 ± 0.42 cm for the right and left respectively. Also the mean weight were different to the results with previous measurements conducted on the right and left uterine tubes in West African Dwarf goat which showed 0.22±0.02 g and 0.22±0.03g, respectively **(Abiaezute et al.,2017).**

Microscopic findings:

The classification was based on the distinct morphological and micromorphometric variations of their walls, specifically categorised as infundibulum, ampulla, and isthmus. The uterine tubes were composed of three layers: mucosa, muscularis, and serosa, as seen in (fig 2) The obtained results exhibited a resemblance to the outcomes of **(Saleem et al ,2016; Mokhtar, 2015)** The wall of the oviduct in the Bakerwali goat is described as consisting of four layers arranged from the inside outwards: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. The thickness of these tunica varied throughout the three distinct zones. The tunica mucosa of the uterine tube contains a combination of ciliated columnar and non-ciliated columnar cells within its epithelial layer. The subsequent non-ciliated cells were classified as either secretory or non-secretory, also known as peg cells. The mucosal folds exhibited a complicated pattern, as is typical. The three separate regions of the uterine tube (fig3) exhibited distinct variations.

The uterine tubes can be categorised into three divisions based on their unique histological characteristics in each area. The identification of these structures as infundibulum, ampulla, and isthmus was determined by analysing the morphological and micromorphometric differences of their walls. The uterine tube wall consisted of three tunicae: mucosa, muscularis, and serosa, as observed under a microscope. The tunica had different thicknesses in each of the three zones. The epithelial cells of the tunica mucosa of the uterine tube consisted of a combination of ciliated and non-ciliated columnar cells. The cells lacking cilia were designated as peg cells and might exhibit either secretory or non-secretory functions (fig2,3). The mucosal folds exhibited intricate arrangements that varied across the three distinct regions (fig4). In fact **(Abe et al., 1999; Al-Saffar and Almayahi, 2019**) The goat oviduct's epithelial lining is composed of a single layer of columnar cells, which includes both ciliated and non-ciliated cell types. These cell types display distinct physical characteristics and have microvilli.

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Infundibulum

The histological study of the uterine tube showed a consistent composition across all three sections, including the tunica mucosa submucosa, tunica muscularis, and tunica serosa. The epithelium of the infundibulum consists of two types of cells: ciliated cells and non-ciliated cells (known as peg cells), and it is classified as simple columnar epithelium. Most of the cells exhibited cilia, as seen in (Fig4). **Mokhtar, (2015)** In bovine, the epithelium in this region had a simple pseudostratified columnar organization and comprised two main cell types: ciliated and non-ciliated secretory cells. **(Gandolfi, 1995**) The non-ciliated cells engage in the production and release of glycoproteins, which play a crucial role in fertilisation and the early phases of embryonic development. The ciliated cells had a centrally located nucleus that was rounded in shape, along with an extended and lightly pigmented cytoplasm. The nuclei of non-ciliated cells were elongated, darkly pigmented, and located near the base. They had a columnar shape. The lamina propria, composed of abundant vascularized loose connective tissue, provides support to the epithelium (fig5). The tunica mucosa displayed small, thin, and extensively branched infundibular folds. The primary mucosal fold exhibited a distinctive shape, with a rounded tip and a broader centre section, resembling a papilla. The primary mucosal fold consisted of a lax connective tissue core that originated from the lamina propria below. The infundibulum in ruminants, especially in cows, has a highly folded mucosa with distinct primary and secondary folds. Displayed significant verticality and a noticeable level of asymmetry **(Bacha and Bacha, 2012)**.

The study found that the mucosa was thinner than the tunica muscularis, which consisted of a few concentric layers of smooth muscle fibres. The tunica serosa, located at the open end of the tube, consisted of a thin layer of loose connective tissue covered by mesothelium. In contrast, the part of the tube linked to the mesosalpinx displayed a dense layer of loose connective tissue including numerous blood vessels and adipose tissue(Fig4). Whereas, **(Abiaezute *et al.*, 2017**) West African dwarf goats The tunica muscularis of the infundibulum is thin and composed of an inner layer of circular smooth muscle and a few outside bundles of longitudinal smooth muscle. The muscle layer in the infundibulum showed minimal growth. The lamina propria demonstrates a higher density of connective tissue, as visualised with masons' trichrome stain. The mucosal epithelial cells, basement membrane, and blood vessel walls all demonstrate a robust positive response to PAS staining (fig 5). The lamina propria exhibits an acidic character and shows a favourable response to AB stain (fig 6).

Ampulla:

The epithelium of the ampulla resembled that of the infundibulum, being simple, columnar, and consisting of both ciliated and non-ciliated cells. Moreover, the vast majority of the cells exhibited cilia. The number of main folds in the tunica mucosa was lower compared to the infundibulum, but the diameter was slightly less than that of the infundibulum (fig2). **(Mokhtar, 2015; Reshag and Khalaf, 2021)** The lamina propria–submucosa is composed of loose connective tissue containing a significant quantity of collagen fibres. The lining epithelium was comprised of a simple columnar type, which consisted of two separate cell types: ciliated cells and non-ciliated secretory cells (as seen in fig 7). (**Abiaezute *et al.*, 2017; Dellmann and Eurell, 1998)** The ampulla of West African dwarf goats is characterised by a thin tunica muscularis, which is composed of a single inner circular layer and a few exterior longitudinal strands of smooth muscle. The muscle layer in the ampulla of West African dwarf goats (C. hircus) undergoes minimal growth between birth and week 16 of age. The tunica muscularis of the ampulla, consisting of circularly organised smooth muscle fibres, had a greater thickness compared to the mucosa, similar to the infundibulum. The border of the ampulla, which was connected to the mesosalpinx, had a somewhat lower quantity of blood vessels (fig 8,9). The Masson's trichrome stain revealed a higher concentration of connective tissue in the lamina propria and tunica serosa, as well as in the mesosalpinx and the adjacent main blood arteries (fig 10). The PAS/AB stain exhibited a robust positive PAS response in the mucosal layer, epithelial cells, and basement membrane (fig11). The AB activity was moderately detected around blood vessels, specifically in the ground substance of the lamina propria and serosal layers. Furthermore, there was evident AB activity observed at the uppermost portions of the epithelial cells (fig12).

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Isthmus

While the number of primary mucosal folds in the ampulla was reduced, their size and length remained similar to the histological structure. Figures demonstrate that the muscularis was thicker than the mucosa, similar to the infundibulum (fig13).The diameter of the Isthmus was more than that of the infundibulum and certainly larger than the ampulla. Conversely, the border connecting to the mesosalpinx exhibited a compact layer of loose connective tissue, densely populated with many blood vessels and adipose tissue (fig 13). The tunica muscularis in the wall of the Isthmus was thicker and more prominent compared to the infundibulum and Ampulla. The mucosal fold heights decrease from the infundibulum to the isthmus. The data suggest that the main role of the infundibulum is to gather and direct oocytes from the ovary to the ampulla (fig7,8). Previous refrences such as **(Hafez and Hafez,2013;Hunter1981;Saif and Farhan, 2019; Yahia and Kadhim.2021)** It was shown that the thickness of the tunica muscularis varies across the three segments. This implies that the development of smooth muscles in certain parts of the oviduct is linked to their primary function, and that the thickening of the isthmus is crucial for the contraction of the muscular layer resulting from the narrowing of the bend at the utero-tubal junction. This contraction controls and restricts the upward movement of sperm cells. The Masson's trichrome stain exhibited a notable accumulation of collagen fibres in the lamina propria and serosal layer, particularly in close proximity to major blood arteries (fig13). The PAS/AB stain revealed positive PAS staining in the mucosal epithelium, basement membrane, and main arterial walls (fig14). AB activity is detectable in both the ground substance of the lamina propria and the serosal layers (fig15). The morphometric measurements, such as Mucosa and Muscularis, were documented in table 2.. Infundibulum of the uterine tube of premature goat were mucosa 32.20±2.87µm and the muscularis 4.00±0.89µm , in ampulla were the mucosa 35.60±3.6µm and the muscularis 4.00±0.63µm, isthmus were mucosa 8.00±0.63µm and the muscularis 17.20±2.41µm. In the premature goats the mucosa and muscularis uterine tube displayed statistically significant increases ($P < 0.05$) (Table 2).

Table1:Gross measurement of female uterine tube of local Iraqi goat at premature age (Mean ± SE)

Organ	Premature goat
Length of Uterine tube (cm)	Right B8.72±0.38b Left A9.80±0.52a
Relative Length of Uterine tube(cm)	Right A28.63±0.60a Left A32.22±1.48a
Weight of Uterine tube(gm)	Right B0.50±0.05a Left A0.47±0.05a
Relative Weight of Uterine tube (gm)	Right 1.36±0.11 Left 1.27±0.11

***Table (1) shows statistically significant differences (P≤0.05) in the Right and Length of Uterine Tube. Specifically, the Left (9.80±0.52 cm) is superior than the Right (8.72±0.38 cm).**

***The Relative Length of the Uterine tube shows a substantial difference between the two locations. The left side measures 32.22±1.48 cm, which is more than the right side measuring 28.63±0.60 cm.**

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***There were no significant differences seen between the Right and Left sides in terms of the Relative Weight of the Uterine tube.**

Table 2: Micromorphometric measurement showed thicknesses of mucosa, muscularis of uterine tube in premature of Iraqi local goat.(mean±SE).

Regions of uterine tube		Premature goat
Infundibulum	Mucosa (µm)	**A32.20±2.87a**
	Muscularis(µm)	**B4.00±0.89b**
Ampulla	Mucosa (µm)	**B35.60±3.65a**
	Muscularis(µm)	**A4.00±0.63a**
Isthmus	Mucosa (µm)	**B8.00±0.63a**
	Muscularis(µm)	**A17.20±2.41a**

The data presented in Table (2) clearly indicate that there are statistically significant differences (P≤0.05) between the Mucosa and Muscularis layers in the Infundibulum. The thickness of the Mucosa layer was found to be largest (32.20±2.87 µm), while the Muscularis layer had a lower thickness (4.00±0.89 µm).

Table (2) indicates that there were significant differences (P≤0.05) in the Ampulla values between the Mucosa and Muscularis. The Ampulla measurement was higher in the Mucosa (35.60±3.65 µm) and lower in the Muscularis (4.00±0.63 µm).

The data shown in Table (2) clearly indicate that there are statistically significant differences (P≤0.05) between the Mucosa and Muscularis layers in the Isthmus. The thickness of the Muscularis layer (17.20±2.41 µm) was found to be larger, whilst the Mucosa layer (8.00±0.63 µm) exhibited lower thickness.

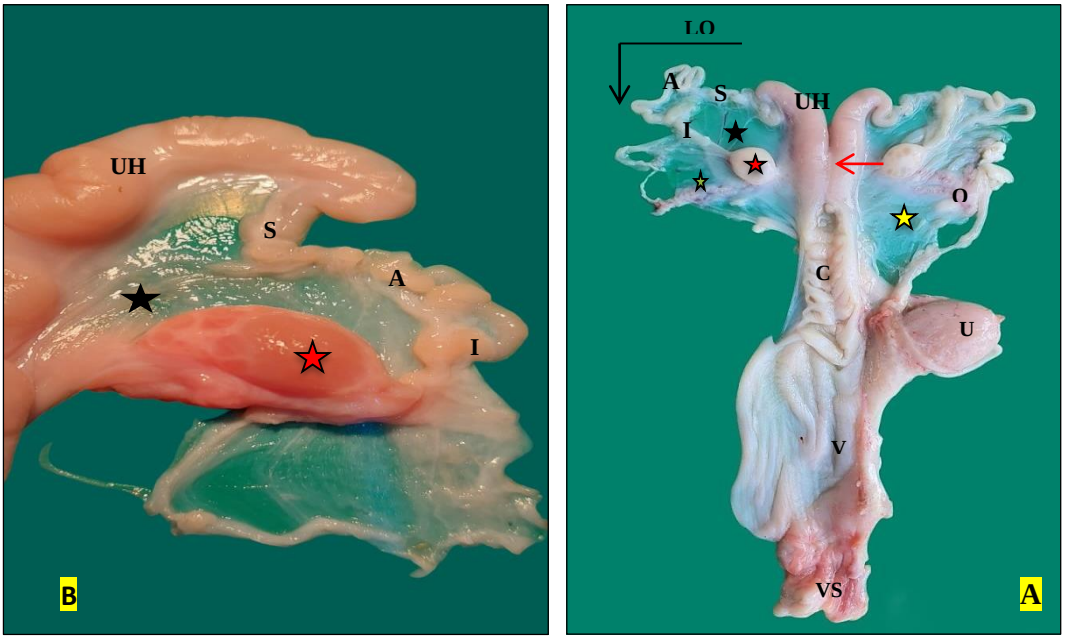

Figure. 1. Shows the female Reproductive system in local Iraqi goat. It showed left uterine horn (UH), left oviduct (LO), isthmus (S), ampulla (A), infundibulum (I), mesosalpinx (black star), mesovarium (green star), mesometrium (yellow star), ovarian artery (O), ,left ovary (red star), cervix (C), vagina (V), vestibule (VS), urinary bladder (U),and external uterine bifurcation (red arrow)

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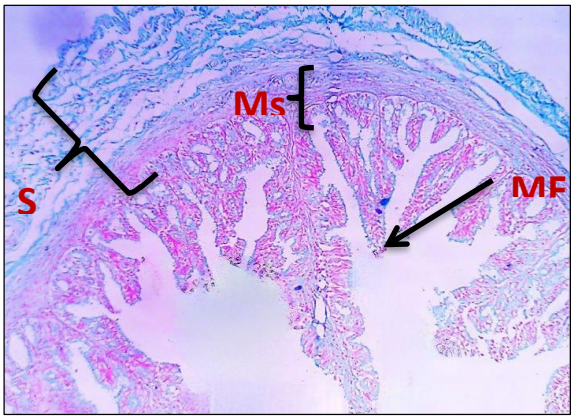

Figure4 : show the microstructure of the premature oviduct infundibulum (Mf) mucosal folds ,(Ms) muscular layer , (S) serosa , 10X, AB/PAS

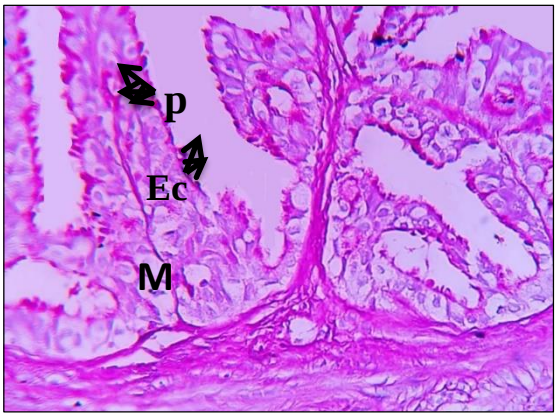

Figure5 : Cross section of the infundibulum of the premature female goat .It showed mucosa(Mc) ,(EC) ciliated epithelial cells , non-ciliated epithelial cells(P) peg cells , X400, PAS.

Figure 6 : show the microstructure of the premature oviduct infundibulum, (Mc) mucosa,(Ms) muscular layer , (Mf) mucosal fold . 400X, PAS

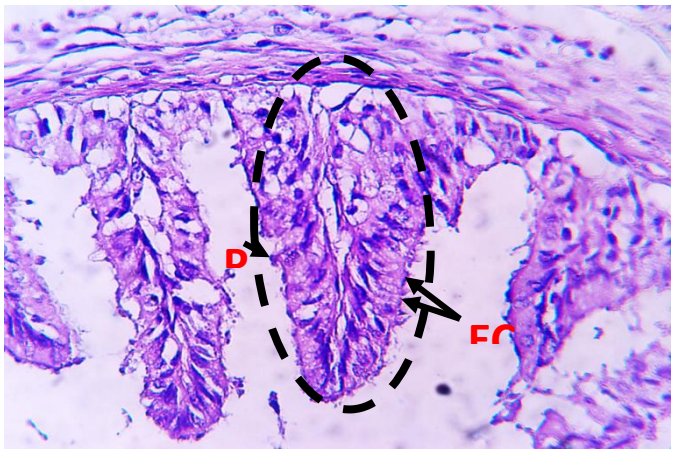

Figure 7 : show the microstructure of the premature oviduct ampullary mucosal folds (dashed circle) , epithelial cells (EC) peg cells , 400X, H&E

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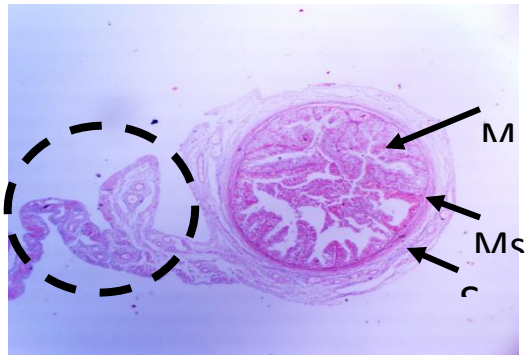

Figure 8: show the microstructure of the premature oviduct ampulla (dashed circle) Mesosalpinx ,(Mf) mucosal folds ,(M) muscular layer , (S) serosa , 4X, MTC

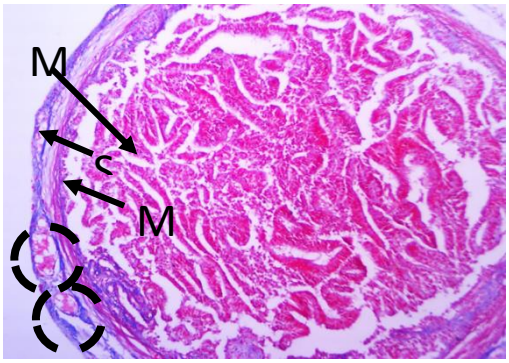

Figure 9: show the microstructure of the premature oviduct ampulla (dashed circle) serosal venules ,(Mf) mucosal folds ,(Ms)

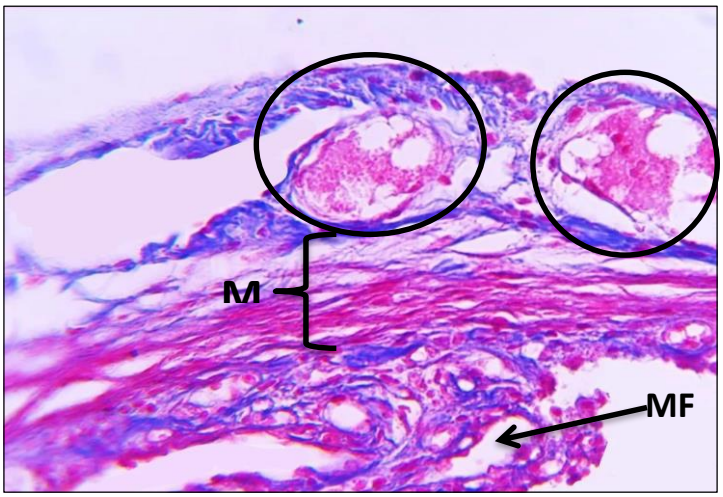

Figure10 : show the microstructure of the premature oviduct ampulla serosal venules (dashed circle) , muscular layer (Ms) , mucosal fold (Mf), 400X, MTC

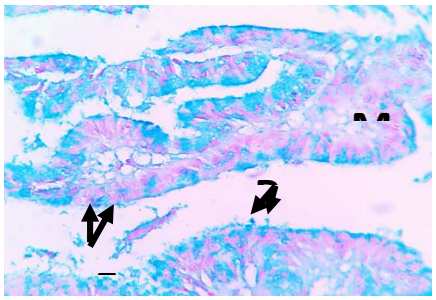

Figure 11: show the microstructure of the premature oviduct ampullary mucosa (Mc) , epithelial cells (EC),

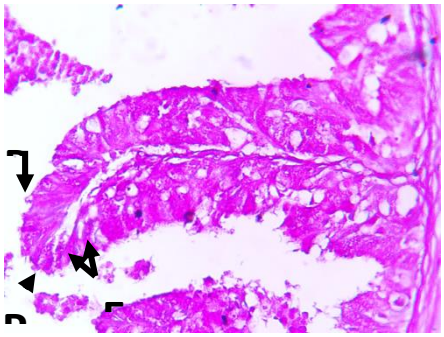

Figure 12 : show the microstructure of the premature oviduct ampullary mucosal

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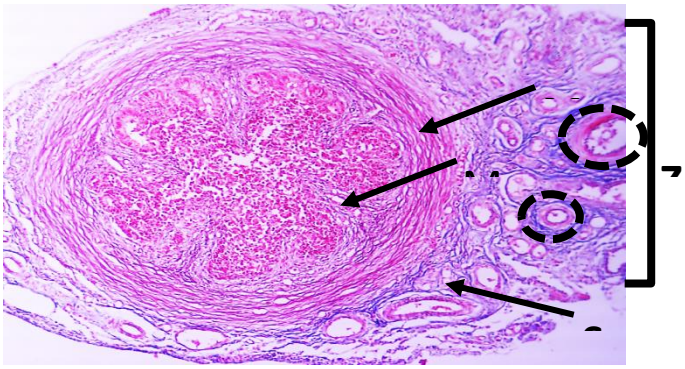

Figure 13: show the microstructure of the premature oviduct isthmus , (Mf) mucosal folds ,(M) muscular layer , (S) serosa, (Z) mesosalpinx ligament, (dashed circle) serosal arterioles , 10X, MTC

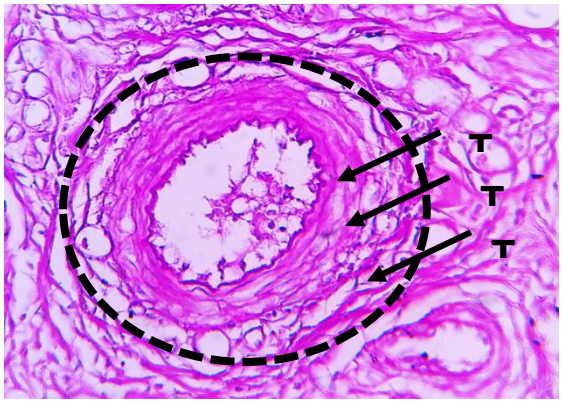

Figure 14: show the microstructure of the premature oviduct isthmus serosa , (dashed circle) serosal artery ,(TI) tunica intima , (TM) tunica media , (TA) tunica adventitia , 400X,

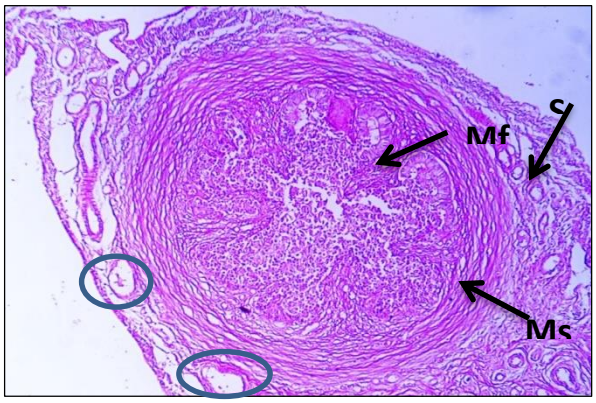

Figure15 : show the microstructure of the premature oviduct isthmus , (Mf) mucosal folds ,(M) muscular layer , (S) serosa, (dashed circle) serosal venules , 10X, H&E

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