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# ANATOMICAL AND HISTOLOGICAL STUDY OF THE UTERUS IN ADULT FEMALE ALBINO RAT

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**ABSTRACT : Anatomical and histological process was used to investigate walls structure of uterus albino rat, total ten health albino rat was used for this study the animals were euthanized, dissected and subsequently uteruses were collected and fixed with 10% neutral buffered formalin then subjected to routine processing such as dehydration, clearing, embedding and block preparation, epithelial lining of uterine horn and body examined by H&E stain , PAS and masson trichrome stain by using routine tissue process technique. The uterus was show as being duplex, having two separate uterine horns and two cervices. Glandular mucosa consisting of lamina epithelialis and lamina propria mucosae. Its epithelium varied between simple and pseudostratified columnar and the tunica mucosa characterized by development of furthermore of simple mucosal folds in addition to elongation of these folds and the tunica muscularis was differentiated into two layers: thick inner circular and thin outer longitudinal smooth muscle fiber. *Tunica serosa* was cellular loose connective tissue covered by mesothelium. The two cervices were partially fused and separated by a mid-saggital septum. Their thick musculatures projected caudally into the vagina as the portio *Vaginalis uteri*. The uterine glands occurred in variable numbers and sizes and thickness of the uterus wall appear endometerium were 151.35±17.88, myometerium 103.91±5.60 and perimeterium 47.81±1.47 while thickness of cervix endometerium 117.85±12.27, myometerim 111.87±6.03 and permeterium 12.85±0.8.**

***Key words :*** Anatomy, histology, uterus, albino rat.

## INTRODUCTION

The uterus is the major female reproductive organ of most mammals, one end, the cervix, opens into the vagina; the other is connected on both side to the fallopian tubes. The main function of the uterus is to accept a fertilized ovum, which becomes implanted into the endometrium. The uterine wall in the adult female consist from endometrium with its mucosal surface facing the uterine lumen and thrown into longitudinal folds. Also observe the relatively thick myometrium interspersed with blood vessels and surrounded by serosa, the perimetrium. (Papka *et al*, 1985). The uterus of female reproductive tract of Rat have two separate uterine horns, (Labor *et al*, 1996). The uterus was bicorn type in Rat, and after birth, the uterus lacked the endometrial glands. However, the uterine mucosa had lined by a simple epithelium supported by undifferentiated mesenchymal tissue (Brody and Cunha, 1989). In neonatal rat the endenogenesis proceeded after 9 days until 15 days and that resulted in the development of simple tubular glands (Branham *et al*, 1985). While the endometrium was observed to be a glandular mucosa consisting of lamina epithelialis and lamina propria mucosae. Its epithelial lining varied between simple columnar and pseudostratified columnar epithelia that formed low longitudinal folds. The lamina propria consisted of loose connective tissues, medium and small blood vessels as well as tubular glands. The tubular glands were observed to extend from the endometrium into the thick lamina propria and displayed little branching. The endometrial layer acts as a lining to the myometrium (Jones and Lopez, 2006). It is composed of 2 layers, the stratum functionalis and the stratum basalis. The stratum functionalis consists of epithelial cells and uterine glands; blood vessels are concentrated in the stratum basalis. The myometrium consists of layers of longitudinal and circular smooth muscle cells (Figs. 1, 2); a layer of connective tissue containing blood vessels lies between the two smooth muscle layers. The inner smooth muscle layer borders the endometrial layer (Zoubina and Smith, 2000; Latini *et al*, 2008). The perimetrium contained simple squamous epithelium.

## MATERIALS AND METHODS

### Animals and study design

Twenty female albino rat were selected to process this study. The collected animals were mature rats.

### Dissection of animals

Each selected animals was euthanized by intramuscular injection of ketamin. The animals was placed on dorsal recumbency to view the ventral aspect, thereafter; a mid-line abdominal incision was made to expose the structures in the peritoneal cavity. The reproductive organ exposed and both uterine horns and cervices were observed and photographed insitu and later dissected out. The lengthand diameters were measured using Vernier calipers.

### Preparation of histological specimens

The uteruses as a whole were dissected and their different regions such as horns and cervices were cut out and washed with normal saline and then by 10% formalin fixed for 72 hrs.

Next to fixation, specimens were dehydrated through ascending series of ethyl alcohol (70%, 80%, 90% and 100%) each for 2 hrs , then cleared with xylene for ½ hr. Specimens were infiltrated with paraffin wax (58 – 60ºC) then embedded with paraffin wax to obtain blocks of paraffin. Paraffin sections of six microns were obtained by using rotary microtome. General and special stains were used to stains the tissue sections such as hematoxyline-eosin (H&E), Masson Trichrome (MTC), Periodic acid schiff (PAS) (Culling, 1986).

### Micromorphometric measurements

Thickness of *Tunica mucosa* and muscularis in uterine horns and cervices were measured using the colour USB 2.0 digital image system, which is provided with image measurement software.

### Statistical analysis

All data of both macromorphometric and micromorphometric measurements were analyzed by ANOVA and t-test using SPSS software (version 14).

## RESULTS

Macroscopic examination of the present study revealed duplex uterus in the albino rat, having two separate uterine horns ended by twounited cervices. Uterine horns having larger diameters compared with the uterine tubes. They were held distinctly by the mesometrium to the dorsal abdominal wall through which passed the supplying blood vessels. It seemed that these ligaments connected or continuous with those of uterine tubes (mesosalpinx) on both right and left sides of the abdominal cavity. Both of uterine horns and large parts of united cervices were situated in the abdominal cavity, whereas the small remaining parts were inside the pelvic cavity together with the vagina (Fig. 1).

### Morphometric measurements

Uterine size length and diameters was significantly increased during life progress such as lengths and diameters were listed in Table 1.

### Microscopic examination

In the mature theuterine horns and cervices were showed three distinct tunicae that were from inward to outward endometrium, myometrium and perimetrium.

### Uterus of mature female rats

In the mature rats, the simple columnar epithelial lining of the endometrium in the uterine horns the secretory cells that located in the surface of the epithelium were less numerous and increased slightly to luminal surface and theinvaginated into the underlying lamina propria forming uterine glands some of them goes deeply and distributed within the loose connective tissue adjacent to the inner layer of myometrium. The lamina propria consisted of loose connective tissues, medium and small blood vessels as well as tubular glands. The tubular glands were observed to extend from the endometrium into the thick lamina propria and displayed little branching and coiling (Fig. 2). The myometrium consisted of inner circular and outer longitudinal smooth muscles with medium sized arteries and veins in-between. The perimetrium contained simple squamous epithelium. It was filled with numerous blood capillaries surrounding the initiated up uterine glands. The myometrium was made of two layers of smooth muscle fibers but the inner longitudinal muscle layer was prominently thicker than the outer circular one.

In the cervices the lining epithelium was simple columnar (Fig. 1). The subepithelial connective tissue was vascular with many eosinophils with the presence of many small cervical glands distributed in the lamina propria surrounded by blood capillaries. The greater proportion of the cervical wall was composed of dense fibrous connective tissue consisting of compactly and regularly

**Table 1 :** The means of lengths and diameters of the uterine horns.

| Uterine horn | Animal weight | Length (mm) | Diameter (mm) |
|---|---|---|---|
| **Adult** | 221.1±24.34** | 21.37±0.68** | 2.45±0.21** |

**Table 2 :** Thickness (mm) of the uterine wall and cervix during rat development.

| | Cervix thickness (mm) | Uterus thickness (mm) |
|---|---|---|
| | **Mature** | **Mature** |
| **Endometrium** | 117.85±12.27** | 151.35±17.88** |
| **Myometerium** | 111.87±6.03** | 103.91±5.60** |
| **Perimeterium** | 12.85±0.83 | 47.81±1.47** |

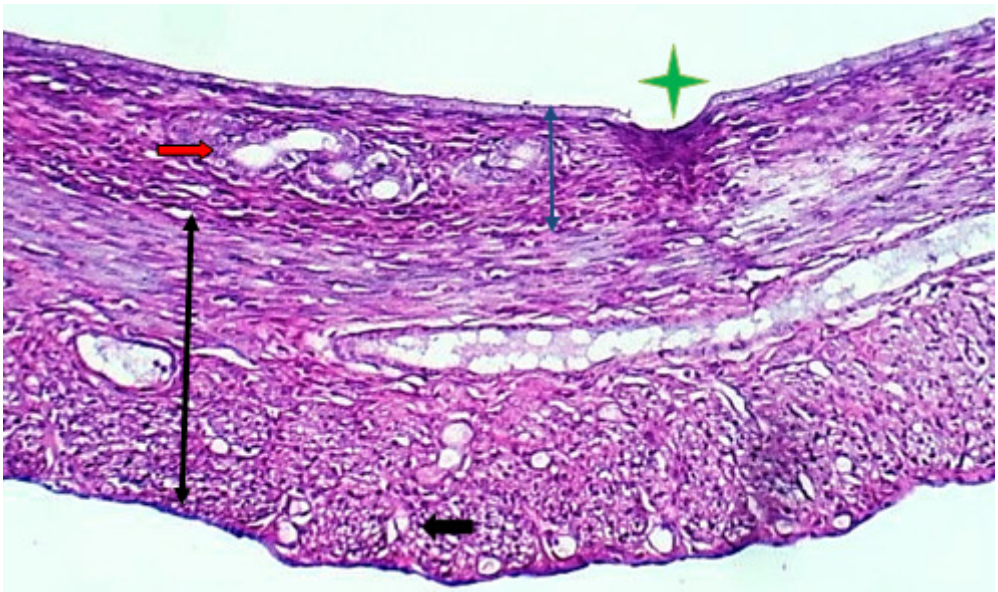

**Fig. 1 :** Uterine horn of mature showed endometrium (blue arrow), myometrium (double heads black arrow), uterine lumen (green star), uterine glands formation (red arrows), connective tissue lamina propria (blue stars), blood vessels (black arrows). X10, H&E

**Fig. 2 :** Mature cervix showed endometrium (blue arrow), myometrium (yellow arrow), smooth muscle fibers in the broad ligament (red arrow), cervical canal (green stars), cervical folds (yellow stars), X10, H&E.

arranged collagen fibers with fibroblasts and eosinophils embedded in sparse ground substance. The smooth muscle fibers formed an incompletemuscularis in the middle and deeper layers of the cervical wall (Figs. 1, 2). Blood vessels were present throughout the depth of the tissue, but were most numerous in the deepest layers (Fig. 2). The compactly and regularly arranged collagen fibers taking the blue colour in Masson trichrome stained sections (Fig. 3). The endocervical glands were well observed inMTC stained semithin sections. A variety of connective tissue cells including mast cells, neutrophils and eosinophils were also observed (Fig. 4). Cervical folds were longer prominently compared to the previous ages and going deep into the lumen of the cervical canal (Fig. 7).

## Histochemical staining techniques

THE uterus that was conducted by the staining with either Mason Trichrome for the connective tissues on the uteruses of female rats showed that the walls of their uterine horns were constructed of inner endometrium, middle myometrium and outer perimetrium.

The stains were successfully stained the loose connective tissues constituted the lamina propria of endometrium, loose connective tissue intervening between muscular bundles of myometrium and the loose connective tissue of the perimetrium which was thin at the free borders of the uterine horns and wide and thick at their

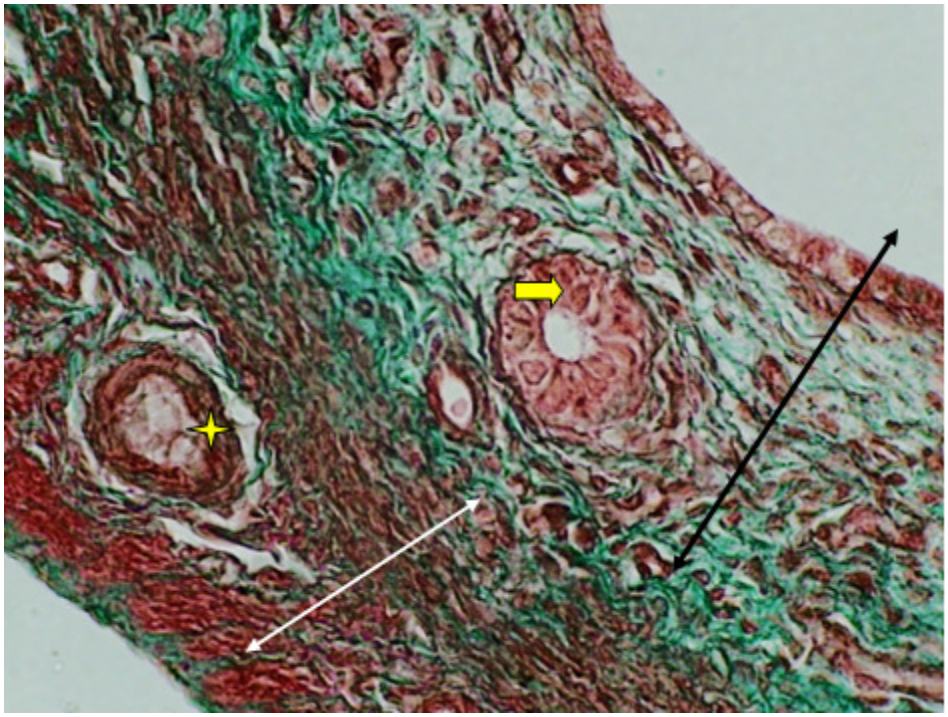

**Fig. 3 :** Uterine horn of mature female rat showed endometrium (black double arrow), myometrium (white double arrow), uterine glands (black arrows) and blood vessels (yellow star). X10, X20, MTC.

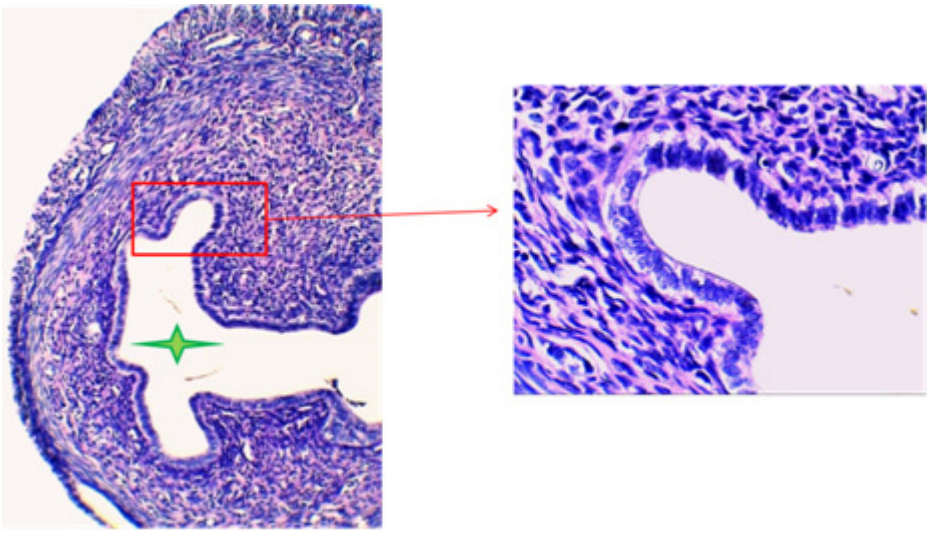

**Fig. 4 :** Uterine horn of mature female rat showed endometrium (double heads black arrow), myometrium (double heads blue arrow), lamina propria showed uterine glands and blood vessels (blue stars), lumen (green star). 10X ,X20, Gomori Trichrome.

attached borders to the related mesometrium. Most of the fibers were collagenous that were stained green or blue when the compositions of the above stains included light green. The stains showed numerous blood capillaries and lymphocytes infiltrated in the lamina propria. The myometrium appeared clearly of inner circular and outer longitudinal muscular bundles with presence of many blood capillaries inside and between these muscular layers of smooth muscle fibers (Figs. 6 & 7). In the cervices, similar results to those ofuterine hornswere obtained, but it showed widerlamina propria in the endometrium and thinner myometrium compared to those recorded in their related uterine horns (Figs. 4 & 8).

### Micromorphometric data of the uteruses

Morphometrical datain Table 2 revealed that the thicknesses of the wall of uterus were endometerium 151.35±17.88, myometerium 103.91±5.60 and perimeterium 47.81±1.47 whereas in their cervices the same measurements were in order 117.85±12.27, 111.87±6.03 and 12.85±0.83. Thicknesses of the wall and lamina propria of the cervices were higher than those of the uterine horns in all studied ages.

## DISCUSSION

### Macrocopic and microscopic examination of uterus

Macroscopically, present findings revealed that the type of uterus in the white albino rat was duplex which was similar to other small laboratory species such as guinea pigs, mice and rabbits but was different than others documented types that were bipartite (in cows), bicornuate in pigs (Hafez, 1979).

Macroscopic examination of the present study revealed uterus of adult rat was reported to about 2-3cm in length (Kayanja and Jarvis, 1971) and this report is agreement with our report, duplex uterus, having two separate uterine horns ended by two united cervices that were projected into the vagina as portio vaginalis uteri. Both of uterine horns. Similarly in African giant rat, the uterus was described as uterus duplex in which the two uteri were suspended by the mesometrium which is originated from the dorsolateral pelvic wall and the lumber region (Ali *et al*, 2010; Akinloye and Oke, 2010).

Such observations were also described in the female genitalia of other species such as laboratory rabbet (Hebel and Stromberg, 2001) the *Mongolian gerbil* (Camila *et al*, 2001) and female agouti (Singh *et al*, 2014). But in the mouse, the uterus was described differently because it composed of a cranial part contained two cavities separated by a median septum and undivided caudal portion named the cervix (Claudio *et al*, 2009). Uterine horns in female rat showedthe two uterine horns in the uterus of the rat and hamster, respectively leading to the cervix, which in turn leads to the vagina and the vulva but in these species (Labor *et al*, 1996). The cervix composed of upper dual cervix part leading to the two uterine horns and a lower cervix part composed of a single canal that leads to the upper vagina. Current measurements showed that the of mean of lengths of the uterine horns were 21.37±0.68, which appeared longer than those recorded in the female agouti (10.4 ± 5.5 mm) by Singh *et al* (2014). Previously, it was recorded that the mean length of the uterus in African giant rat was 4.877 ± 0.011 cm. Microscopic finding showed the histological developmental changes were recorded in their endometrium and myometrium was gradually changed from the isthmus to the cranial end of the uterine horn in which the junction appeared very short canal without folds or crypts that were noted in both joined organs. The wall appeared well developed characterized,In rats recorded that, the uterine mucosa was lined with simple columnar epithelium that composed of a mixture of ciliated cells and the non-ciliated cells. The lamina properia contained the uterine glands (Johnson and Foley, 1992). The endometrium showed invaginations into underlying *Lamina propria* agreed with Brody and Cunha (1989). The myometrium was thickened with the diminishing of the vascular layer into numerous blood vessels with the presence of nerve plexuses intervening between its internal and external layers. These result agreed with Arnold *et al* (2001). These changes were more advanced subsequently in the mature stage showing many uterine glands grew deeply in the endometrium. The type of the endometrial gland was the secretory substance collectively consisted of a complex array of protein and related substance termed histotrophic, it was an extra cellular material derived from the endometrium and the uterine glands (Burton *et al*, 2002; Spencer and Bazer, 2004). The myometrium was thickened specially its *Tunica externa*. The lining simple columnar epithelium was secretoryfunction and due to the mammalian species have estrous cycle in which these gonadotrophic hormones and subsequent release of ovarian hormones will control the changes in the wall of the reproductive tract one of them is the uterus (Gu *et al*, 2005). Present postulation comes parallel and agreement with recent knowledge that estrogens and progesterone are contributed in the structural integrity maintenance of the oviduct, uterine and vaginal tissues by regulating activities of the epithelial cells existed in the reproductive tract such as growth, proliferation and secretion.

Accordingly, the changes in their concentrations during the oestrus cycle will induce hemodynamic changes in the vascular tissue which caused an increment in the permeability of the blood vessels and humid weight of the uterus (Kim et al, 2004; Vinci *et al*, 2010). The important hormone estrogen is usually manufactured by the granulosa cells of the ovarian follicles which convert androgens synthesized by the theca interna in response to blood levels of the anterior pituitary hormones (Young and McNeilly, 2010). In fact results and hypothesis of the present study regarding the hormonal effects on the wall structures of the uteruswere highly agreed and confirmed the recent findings and postulations of Abd-Elkareem (2017).

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