Histomorphological and Histochemical Investigation of The Vagina of Adult Guinea Pigs (*Cavia porcellus*)

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ABSTRACT

This study was carried out to identify the morphological, histological and histochemical features of the vagina of the adult guinea pig (*Cavia porcellus*). To perform such project, 14 adult guinea pigs at their diestrous period were bought from the local breeders directly. Animals were euthanized, dissected and subsequently specimens from the cranial, middle and caudal portions of the vagina were collected and fixed. Fixation was implemented by using 10% neutral buffered formalin and Bouin's solution. Post routine processing such as dehydration, clearing, embedding and blocks preparation, tissue sections of 6 µm were prepared and stained by using hematoxylin-eosin, Masson's Trichrome, Alcian blue and Periodic acid shiff stains. Gross findings revealed that the vagina in the adult guinea pigs was characteristically very long tube-like structure with wide diameter. The vagina entirely was running ventral to the colon and then under the rectum in the pelvic cavity. It was terminated caudally by the vaginal orifice independent to the urethral orifice of the urinary system. Microscopic findings revealed mucous columnar lining epithelium which was folded at the vaginal fornix decreased caudally toward the external vaginal orifice where the epithelium changed into stratified squamous epithelium not keratinized. Thin dense lamina propria was continuous with loose connective tissue of the submucosa. Thickness of tunica muscularis was decreased which was surrounded with thick adventitia. Histochemically, the non-ciliated mucous columnar cells present in the lining epithelium of the cranial and middle regions of the vagina were positively stained with AB (pH 2.5) and PAS stains. The reaction with AB (pH 2.5) was more intense compared to the staining with PAS so that it indicated that mucin was more acidic than neutral in nature. Characteristic conclusions include that the vagina opening was U-shaped not circular and closed by transparent closure during the diestrous period. Histologically, the vagina also lined with mucoid lining and only the orifice and adjacent area lined with stratified squamous epithelium. Moreover, current study recorded differences in both macroscopic and microscopic aspects of the vagina in the guinea pigs compared to other animal considered laboratory species.

Keywords: Vagina, Guinea pig, Histochemistry, Genital tract, Diestrous

Introduction

Domestic guinea pig (*Cavia porcellus*) is descendant of the wild cavy (*Cavia porcellus*) which is one of the common rodents lived in South America. It is herbivorous rodent characterized by stocky body, short neck and limbs and it is more closely related to porcupine than mouse and rat (1, 2). Guinea pigs are now widely distributed because of their popularity as a pet and a food source.

Usually, guinea pig is used in biomedical research, for example in studies of the human immune system, since its immunological genes are more similar to human than those of the mouse so that it is considered a very important model organism for toxicology and vaccine testing (3).

Usually, the vagina is considered that part of the reproductive duct positioned between the cervix cranially and the vestibule caudally and it is

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entirely located within the pelvic cavity. The organ serves as the site of copulation in the female which receives the male penis during this activity and acts as a passageway for the foetus during parturition (4). It is usually used to diagnose some general and reproductive diseases and genital tract abnormalities (5-7).

In contrast to the considerable number of studies conducted on the reproductive tract of domesticated animals, few studies and paucity of works were focused on the female reproductive organs of the guinea pigs especially the vagina. In domestic animal; studies were focused on histomorphology of some organs of the female reproductive tract such as vagina of the dogs (8), oviducts of mouse (9), porcupine (10), bitch (11), mouse (12) and golden hamster (13), Similarly, few researches dealt with birds reproductive organs such as ovary and reproductive tract in mallard (14, 15).

There are few studies in the literatures investigated of the vagina in female guinea pigs. According to the importance of this animal species as one of good experimental model, the project was performed to add new data on both histomorphology and histochemical aspects of the vagina in adult female guinea pigs at their diestrous stage of the estrous cycle to provide better basic data to other fields such as pathology, physiology and pharmacology. Hence, the objective of this study was to identify the morphological, histological and histochemical features of the vagina of the adult guinea pig (*Cavia porcellus*).

Materials and Methods

Experimental design

This study was carried out after approval of the scientific committee of department of Anatomy and Embryology, College of Veterinary Medicine, University of Baghdad and accordance the international standards of animal welfare.

Animal's Collection and Dissection

Fourteen adult female Guinea pigs (*Cavia porcellus*) were selected to conduct the present project. Animals in apparent healthy condition were purchased directly from the local breeders. They were left under supervision to insure their good healthy condition before their euthanasia and subsequent dissection. Each animal weighed with a sensitive weighing balance and euthanized by intramuscular injection of sodium Phenobarbital (140 mg/kg body weight) (16).

Then each animal was placed on the dorsal recumbency to view its ventral aspect. Thereafter, a mid-line abdominal incision was performed cranio-caudal from the xiphoid cartilage to the pubic symphysis in order to expose the organs in the abdominal cavity. After partial removal of the skin covering the thigh, the inner thigh muscles were severed on both sides of the pelvic arch. The pubic symphysis disarticulated or cut through with bone cutter and pulled apart by lateral traction on either side. The intra-pelvic vagina was exposed and photographed in situ and later dissected out. The organ was weighted by sensitive balance then washed by physiological saline solution and subsequently immersed in fixatives (10% formalin, Bouin's solution).

Macromorphometric measurements such weight and length of the vagina as well as weight and length of the animal were listed in table.

Preparation of Histological Specimens

The specimens were selected from the cranial, middle and caudal portions of the vagina. All specimens were washed with normal saline and then immersed in 10% neutral buffered formalin for 48 hrs. For future staining with histochemical stains, some specimens were fixed by Bouin's solution for 16 hr. Next to fixation, specimens were dehydrated through ascending series of ethyl alcohol (70%, 80%, 90%, and 100%) each for 2 hours, then cleared with xylene for ½ hour. Specimens were infiltrated with paraffin wax (58 – 60 ºC) then embedded with new paraffin wax to obtain blocks of paraffin. Paraffin sections of six microns were prepared by using rotary microtome.

General histological staining procedure was performed by Hematoxylin and eosin (H&E). Special histochemical procedures were conducted by the following stains: Masson's trichrome stain (MTC) a special stain used for the staining of the collagenous connective tissue and the smooth muscle fibers constituting the muscular bundles. Alcian blue (AB) (pH 2.5) technique for the identification of secretory cells of acidic mucopolysaccharides that were observed in the lining epithelium of the vagina.

Periodic acid and Schiff (PAS) stain to identify secretory cells of neutral mucopolysaccharides in

nature. Histological slides were photographed using the Colour USB 2.0 digital image system (Scope Image 9.0) which was provided with image processing software.

Results

Gross Findings

Gross examination found that the vagina was characteristically very long and have large diameter in the adult studied guinea pigs. The vagina was entirely running ventral to the colon in the pelvic cavity, whereas, dorsal to the pelvic urethra (Figure 1).

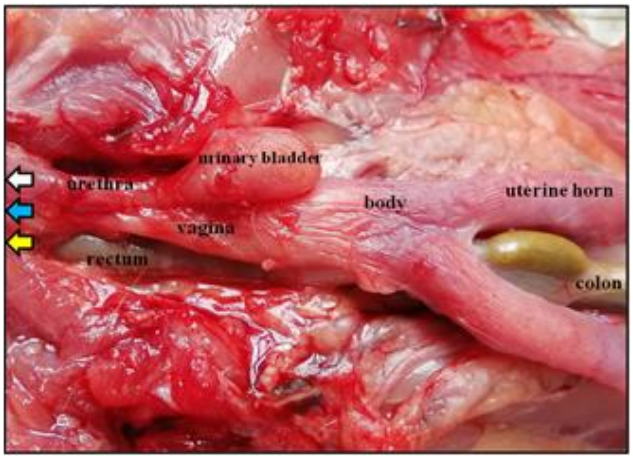

Figure 1. Uterus and vagina were dissected out. showed vagina, urethra, rectum, sites of openings of urethra (white arrow), vagina (blue arrow) and anus (yellow arrow)

The vagina was ended caudally by U-shaped vaginal orifice. It was closed by a thin transparent membrane during the diestrous studied stage currently. The vaginal orifice appeared between the anal opening dorsally and the urethral orifice ventrally (Figure 2). Morphometrical measurements of the vagina such as lengths and weights were listed in table 1. The means of lengths and weights of the vagina were 23 ± 0.12 mm and 0.47 ± 0.002 gm, respectively. Accordingly, the relative length and weight were 0.102 and 0.00086, respectively. The means diameter of vagina was 9.0 mm.

Figure 2. Sites of openings of urethra (white arrow), vagina (blue arrow) and anus (yellow arrow)

Table 1. Macromorphometric measurements of

Organs	Diameter (mm)	Length (mm)	Relative length	Weight (gm)	Relative weight
Vagina	9.0	23	0.102	0.47	0.00086
Body weight (mean ± SE)	545 gm ± 6.03				
Body length (mean ± SE)	225 mm ± 4.94				

Microscopic Findings

Microscopic examination of the wall of the vagina in the currently studied of guinea pigs revealed four different tunicae. They were mucosa, submucosa, muscularis and adventitia. Cranially, the vagina formed the fornix around the portio vaginalis uteri of the cervix. At such region, the mucosa was much folded (Figure 3).

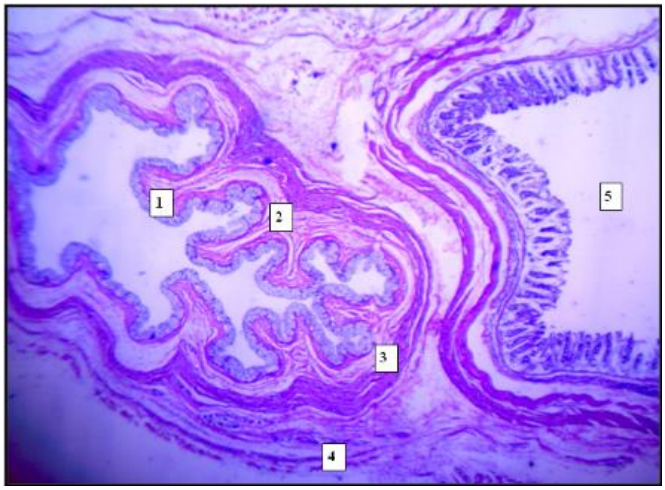

Figure 3. Vagina at fornix region showed folded mucosa lined with simple columnar mucous epithelium (1), propria-submucosa (2), internal layer of tunica muscularis (3), external layer of tunica muscularis (4), colon (5). 25X, H&E

The lining was simple mucous columnar epithelium characterized with basal nuclei and the whole cytoplasm filled with mucous materials. The underlying lamina propria was formed of dense irregular connective tissue. Absence of muscularis mucosa caused the continuity of propria with submucosa. The latter was formed of loose connective tissue rich with blood vessels. Tunica muscularis was formed of inner circular and an outer longitudinal layer of smooth muscle bundles (Figure 4).

Staining by AB (pH 2.5) and PAS revealed positive reactions toward both (Figures 5 and 6).

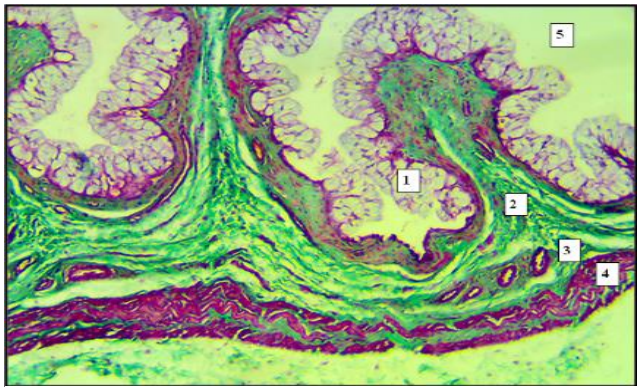

Figure 4. Vagina at fornix showed folded mucosa lined with simple columnar mucous epithelium (1), lamina propria-submucosa (2 and 3) and internal layer of tunica muscularis (4) and vaginal lumen (5). 100X, Masson's trichrome stain

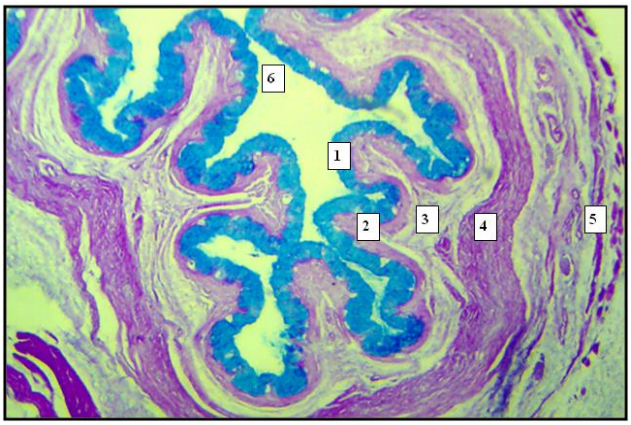

Figure 5. Vagina at fornix region showed folded mucosa lined with simple columnar mucous epithelium stained positively with Alcian blue stain (1), propria-submucosa (2& 3), internal layer of tunica muscularis (4), external layer of tunica muscularis (5), vaginal lumen (6). 40X, Alcian blue

Microscopic examination of vaginal wall at the mid-way between the cranial and caudal ends of the vagina showed similar histological features but with lesser folded mucosa (Figure. 7, 8, 9, 10).

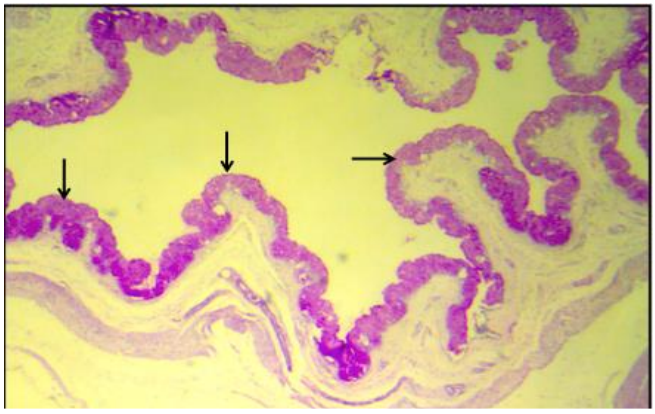

Figure 6. Vagina at fornix region showing folded mucosa lined with simple columnar mucous epithelium stained positively with PAS stain (black arrows). 40X, PAS

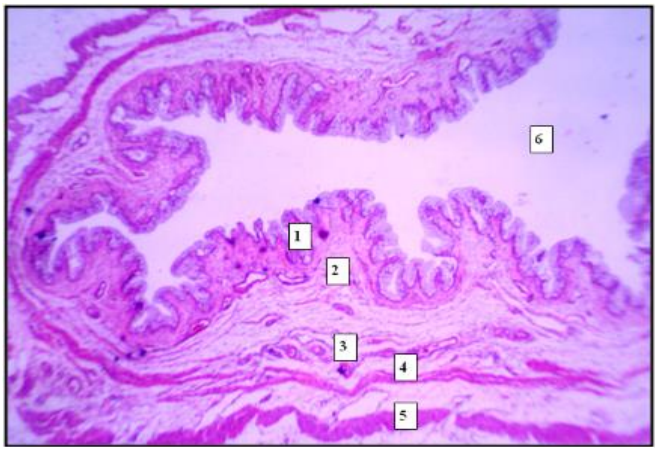

Figure 7. Vagina at its middle region showing short mucosal folds (1), propria- submucosa (2& 3), internal circular layer of tunica muscularis (4), external longitudinal layer of tunica muscularis (5), vaginal lumen (6). 40X, H&E

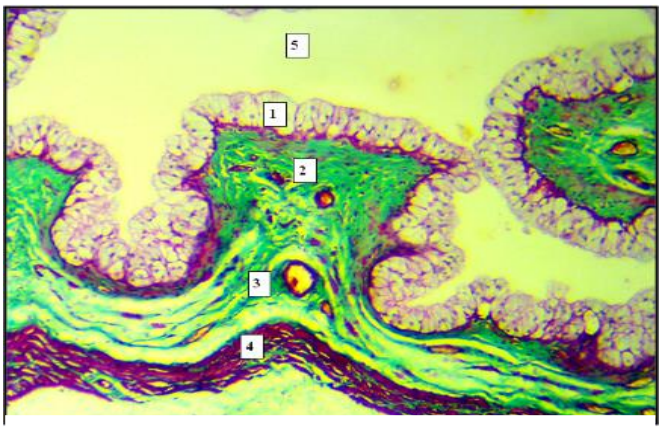

Figure 8. Vagina at its middle region showing short mucosal folds (1) covered with simple columnar mucous epithelium, propria submucosa (2), internal circular layer of tunica muscularis (3), vaginal lumen (4). 100X, Masson's trichrome stain

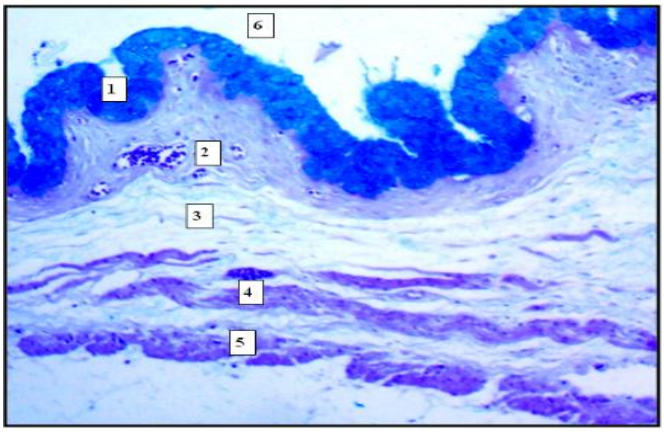

Figure 9. Vagina at its middle region showing short mucosal folds (1) covered with simple columnar mucous epithelium positively stained with Alcian blue, propria – submucosa (2& 3), internal circular layer (4) and external longitudinal (5) layers of tunica muscularis, vaginal lumen (6). 100X, Alcian blue stain

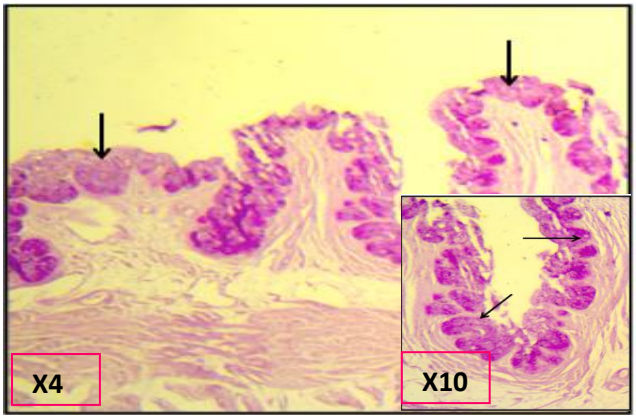

Figure 10. Vagina at its middle region showing short mucosal folds covered with simple columnar mucous epithelium positively stained with PAS (black arrows)

Sections examined at the caudal end near the vaginal opening showed the changes of lining epithelium from simple mucous columnar into stratified squamous epithelium not keratinized. Prominent increase in the connective tissue at propria- submucosa and between muscular layers which were appeared thinner in thickness compared to those observed in the middle and cranial ends (Figure. 11).

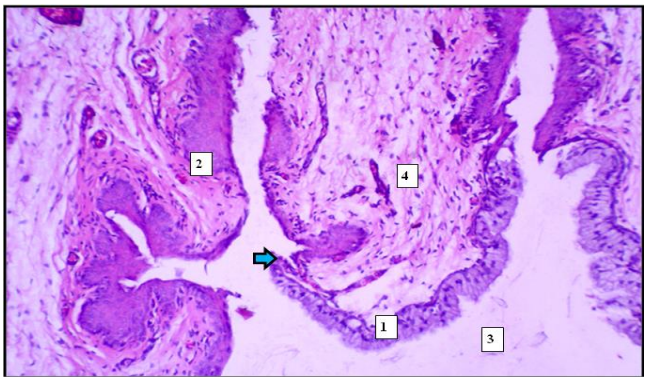

Figure 11. Vagina wall near the vaginal orifice showing changing of the simple columnar mucous (1) into stratified squamous epithelium (2). The figure showed area of junction (blue arrow) and vaginal lumen (3) and propria-submucosa (4). 100X, H&E

Discussion

A. Grossly

The vagina was characteristically very long tube-like organ in the studied guinea pig. It was entirely situated in the pelvic cavity running ventral to the rectum and such observations were in accordance with those described by (4) in the female bovine genital organ and vagina of the west african dwarf goat (17) and in the local does (18). The latter references also observed that the cranial end, the vagina was coiled around the *Portio vaginalis* of the cervix as a vagina fornix which gave rise to mucosal folds as recorded in the first cranial portion of guinea pigs vagina. But differently, the caudal end of the vagina was connected to the vestibule which was not found in the present studied animals.

The vagina was ended caudally by U-shaped vaginal orifice closed by a thin transparent membrane during the diestrous studied stage. The vaginal orifice was located between the anal opening dorsally and the urethral orifice ventrally so that the urinary and genital tracts were independent and such observation also recorded in the female agouti (19) but not in does (18) in which differently, urinary tract joint the genital one through the urethra- vaginal connection.

The means of length the vagina was 23 ± 0.12 mm which was shorter compared to other animal species such as the vagina of female West African dwarf goat (5.07 ± 0.39 cm) (17), local does (8.3 cm) (18) and female agouti (72.8 ± 11.4 mm) (19). The vagina in guinea pigs was long and wide in diameter and markedly of three regions similar to that documented in the vagina of local does

whereas in other species such as the golden hamster it composed of two distinct regions i. e. the upper vagina adjacent to the cervix and the lower vagina from which distinct pair of pouches were recorded protruding from the lateral walls that were extended caudally and also laterally and ventrally before terminating as close as to the vaginal orifice (20). In fact, the three characterized regions of the vagina recorded in the present study were in accordance with the descriptions of rat's vagina in many previous and recent investigations (21).

Absence of the vestibule in the current studied guinea pigs confirmed previous data commented such findings in the female guinea pig by (22) whom recorded that the end of the reproductive tract i.e. the vagina was ended directly by the external vaginal orifice.

B. Microscopically

The lining epithelium start cranially at fornix by simple mucous columnar and remain at the middle region then caudally near vaginal orifice it was changed into stratified squamous not keratinized. These findings were in a good agreement with previous researches stated that the lining epithelium of the cranial part of the vagina of adult animals was different than that of the caudal part (23). Differently, current findings were not comparable with those in female rabbits where the lining epithelium was ranged from simple columnar (cranially) to pseudostratified columnar or stratified squamous non keratinized (caudally) which gave negative reactions toward the histochemical staining procedures (18). Differently in guinea pigs vagina, the mucous cells were stained positively with histochemical stains such as PAS and AB (pH 2.5).

The vaginal musculature of guinea pigs tapers caudally toward the vaginal orifice but remain of two muscular layers (inner circular and an outer longitudinal layer of smooth muscle bundles). Oppositely, three distinct tunicae in the vagina of other species were recorded such as female rabbits (24), female of mini pigs (25), female agouti (19), West African dwarf goat (17) and female rats (26). In conclusion, current study recorded differences in both macroscopic and microscopic aspects of the vagina in the guinea pigs compared to other animal considered laboratory species, the reason by which guinea pigs were important model to be used in various medical experiments and researches. The vagina was long and wide in diameter with no connection with the urinary system as in other species and it ended by separated vaginal opening from the urethral orifice. Characteristically, vagina opening was U-shaped not circular and closed by transparent closure during the diestrous period. Histologically, the vagina also lined with mucoid lining and only the orifice and adjacent area lined with stratified squamous epithelium.

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Conflict of Interest

The authors declare there is no conflict of interest.

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دراسة شكلية- نسجية وكيميائية- نسجية للمهبل في خنازير غينيا البالغة (*Cavia porcellus*)

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الخلاصة

أجريت هذه الدراسة للتعرف على التركيب الشكليائي-النسيجي والكيمياء-نسيجي للمهبل في خنازير غينيا البالغة، ولغرض أجراء هذه الدراسة،تم جمع ستة عشر من خنازيرغينياالبالغةمن المربين المحليين مباشرةفي فترة سكون دورتها الشبقية.

تم قتل الحيوانات بشكل رحيم وتم بعد ذلك تشريحهاوبعدذلك تم جمع عينات المهبل وتم تثبيتها بالمحاليل المثبتة. إذ تمت عمليةالتثبيت باستخدام محلول 10 ٪ من الفورمالين المتعادل المخزن ومحلول البوينز لغرض اجراء عمليات الصبغ بالصبغات الكيمائية – النسيجية لاحقاً. ثم بعد ذلك اخضاع النماذج للمعالجات التقنيةالروتينيةمثلاً لتجفيف فالترويق،والطمر بالشمع ومن ثم صبها في قوالب لغرض التقطيع لاحقاً. أخيرًاتم تحضيرمقاطع بسمك 6 مايكرون وصبغها بصبغة الهيماتوكسيلين-ايوسين،وماسونثلاثيالكروم،وصبغة الباس وصبغة الالشيان الزرقاء. دلت النتائج العيانيه ان المهبل في خنازير غينيا البالغة عباره عن انبوب طويل جداً وواسع القطر بشكل متميز، ووجد المهبل بشكل كامل ممتداً بطنياً تحت القولون ومن ثم تحت المستقيم في تجويف الحوض. ينتهي المهبل خلفياً بفتحة المهبل بشكل مستقل عن فتحة الاحليل للجهاز البولي. بينت النتائج المجهرية وجود ظهارة مبطنة عمودية مخاطية ذي طيات مخاطية عند بداية المهبل وتبدأ بالاختفاء تدريجياً خلفيا باتجاه فتحة المهبل حيث تتغيرعندها الظهارة الى مطبقة حرشفية. طبقة خفيفة من الصفيحة الاعتيادية المكثفة تندمج مع النسيج الضام الرخو في تحت المخاطية. يقل سمك الغلالة العضلية التي يحيطها الطبقه البرانية السميكة.بينت خطوات التصبيغ بالمواد الكيميائية – النسيجية ان الخلايا العمودية غير المهدبة في الظهارة المبطنة للاجزاء الامامية والوسطى من المهبل ايجابياً بصبغتي الالشيان الزرقاء ذي حامضية بدرجة 2.5 وبصبغة الباس، وكان التفاعل مع صبغة الالشيان الزرقاء اكثر شدة مقارنة مع صبغة الباس. وهذا دليل على ان طبيعة المخاط كان حامضياً اكثر من المتعادل. تتضمن الاستنتاجات في هذه الدراسه, أن فتحة المهبل كانت على شكل حرف U وليست دائرية ومغلقة بإغلاق شفاف خلال الفترة المشوشة. من الناحية النسيجية ، يصطف المهبل أيضًا ببطانة مخاطية وفقط الفتحة والمنطقة المجاورة المبطنة بظهارة حرشفية طبقية. علاوة على ذلك ، سجلت الدراسة الحالية اختلافات في كل من الجوانب الميكروسكوبية والميكروسكوبية للمهبل في خنازير غينيا مقارنة بالأنواع المختبرية الحيوانية الأخرى.

الكلمات المفتاحية:المهبل،التركيب الشكليائي، خنزير غينيا ، الغلالةالعضلية، الظهارة المبطنة