

Histomorphological Study of Epididymis, ductus deferens and phallus in Adult Male Guinea Fowl (*Numida meleagris*)

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Summary

This study was conducted to investigate the anatomical, histological characteristic features of epididymis, ductus deferens and phallus of male guinea fowl (*Numidia meleagris*). Fifteen healthy male guinea fowl aged between 18-24 months, weighing 1280 - 1405 gram were used in current study, which brought from local market in Baghdad governorate. The epididymis of guinea fowl consisted from rete testis, efferent ductless, connecting duct and epididymal duct. The study revealed that the mean diameter of epididymal ducts was $285.17 \pm 2.43 \mu\text{m}$ lined with pseudostratified columnar epithelium. The epididymal duct continuous with ductus deferens which become wider caudally towards the cloaca, the length of right and left ductus deferens were 9.16 ± 1.20 cm and 9.80 ± 1.24 cm respectively. The copulatory organ (phallus) of guinea fowl was not well developed and non-intermittent type consist from right and left lateral lymphoid bodies with median eminences, the transverse width 5.01 ± 0.24 mm, the cranio-caudal width 3.51 ± 0.07 mm and dorso-ventral width 4.32 ± 0.25 mm. The lining epithelium of coprodeum was pseudo stratified columnar epithelium. This study concluded that the epididymis of male guinea fowl located on dorsomedial surface of the testes and without definite parts as in domestic animals, the ductus deferens of guinea fowl was tortuous, the phallus of guinea fowl is non - intermittent type..

Keywords: Epididymis, Ductus deferens, Guinea fowl.

Introduction

The Guinea fowl is the common name of the seven species of gallinaceous birds of the family Numididae, which is indigenous to Africa. It is well adapted to the realities of life on African continent (1).

The epididymis connects with the medial border of the testis. The epididymis of Japanese quail (*cotunix japonica*) is situated on the dorso-medial part of the testis, (2). Epididymis in birds continuous with ductus deferens which was closed ventrally to the epididymis, the epididymis histologically composed of stratified cuboidal epithelium with thicker muscular layer (3). The main components of epididymis are the extra testicular rete testis, efferent ducts, the connecting ducts and epididymal duct (2, 4, 5 and 6). The ostrich phallus is intermittent type and lacks an internal cavity its connected to the ventral wall of the cloaca and comprises two main parts: The basis phalli and apex phalli(7). The avian phallus studies have been

conducted in domestic anseriform galliform and ratite birds (8). In domestic fowl the phallus lies in the ventral midline of the proctodeum which consist from paired lateral phallic bodies and Lymphatic fold lies between the lateral phallic body and the papilla of the deferent duct (8 and 9). The aim of this study was conducted to investigate the anatomical, histological and characteristic features of epididymis, ductus deferens and phallus of male guinea fowl.

Materials and Methods

The study included fifteen apparent healthy clinically mature male guinea fowls (*Numida Meleagris*). Aged 18-24 months, the mean body weight was (1333 ± 2.09) gram. The birds were brought from local market and owners of Baghdad governorate and set in two groups randomly, eight birds for anatomical study and seven birds for histological approach.

The birds were euthanized by injecting over dose of ketamine dose 35mg/kg and xylazine dose 5mg/kg (10) in the thigh region muscles

,after that, the skin were dissected by using scalpel and complete incision by using straight scissor in the midline of abdominal muscle reaching the neck region and cutting the ribs and cricoid bone by bone cutter of each sides to the keel and pectoral muscles and make incision in abdominal muscle to open celomic cavity to exposure the epididymis, ductus deferens and cloaca as well as distinguishing papillae of vas deferens. After that relationship of the structures were described in situ then dissected, the samples of anatomical study were fixed by 10% formalin solution for 48 hours, while the samples of histological study were fixed by using bouins solution and a representative specimens of 1cm were taken and left in fixative for 24 hours(11).

Anatomical description include epididymal location and its bounders as well as the phallus. Gross morphometric measurements done by using measuring tape and Vernier calipers include:length of ductus deferens, Length of the right and left papilla / mm, Diameter of the right and left papilla /mm, Transverse width of the phallus/mm, Cranio-caudal width of the phallus/mm, Dorso-ventral width of the phallus/mm

Histological specimens of 1cm were process according to the routine histological techniques to get sections of 5-6 μm thickness and stained with Harris hematoxylin and eosin stain and mason's Trichrome stain to investigate the general histological features of epididymal parts and phallus (12). All the following parameters were done by using the ocular micrometer which calibrated to microscope lenses with aid of stage micrometer (13):

- Height of ductus epididymis epithelium / μm
- Diameter of ductus epididymis / μm
- Diameter of cranial, middle and caudal parts of ductus deference / μm
- Height of epithelium of cranial, middle and caudal parts of ductus deferens / μm

Ten randomly measurements for each parameter from each sample of testis from five

birds counting the arithmetic mean was done by (10 \times objective power).

Photographs of examined slides were carried out with a motic microscope which is supplied with a digital camera with resolution power two mega pixel.

Anatomical and histological parameters were analyzed statistically by using analysis of variance with ANOVA- test and t- test. The results were presented as means \pm SE. The level of significance $P>0.05$ was used for analysis of variance test (14).

Results and Discussion

The results of study showed that the epididymis in guinea fowl recognized as tubular structure and was not divided into recognized parts, its body situated and attached to the corresponding testes on dorsomedial surface and continuous with ductus deferens which run on midline of the body. The epididymis appeared as short-coiled white tubule, which represent the epididymal region, from this region the ductus deferens extend from caudal of epididymis to the urodeum region of cloaca, (Fig.1).The lining epithelium of epididymis was pseudostratified columnar epithelium consisting from columnar ciliated cells, non-ciliated cells and basal cells .

The ductus epididymis is a canal beginning from the cranial end of testes and passing along its border to continue as ductus deferens, the mean height of epididymis duct epithelium was $47.97\pm 0.56\mu\text{m}$ (Table,1).The ductus epididymis lined by pseudostratified columnar epithelium (Fig.2), the diameter of ductus epididymis was $285.17\mu\text{m}$ $2.43\mu\text{m}$ (Table,1). The ductus epididymis increased in diameter caudally and continuous with ductus deferens. Ductus epididymis is very tortuous tube begin from cranial end of epididymis these epididymis ductus are narrow and continues passing caudally as ductus deferens.

Ductus deferens was appeared as white tube due to presence of semen. The ductus deferens appeared coiled and was impossible to pull out into straight duct. The ductus deferens attached to the dorsal wall of the body by short folds of peritoneum due to this reasons the ductus deferens along its length attached and

lies dorsally to the testes (Fig.3). Mean lengths of the right and left ductus deference were (9.16 ± 0.30 and 9.80 ± 0.26 cm) respectively (Table,2). In outline, the course of both right and left deferens duct were lateral to the corresponding ureters.

There was non-significant differences at ($P>0.05$) between the length of the right and left ductus deference. The mean diameters of cranial, middle and caudal parts of the right ductus deference were (444.7 ± 1.67 , 645 ± 2.01 and $975.3\pm2.61\mu\text{m}$) respectively. The mean diameters of cranial, middle and caudal parts of the left ductus deference were (454.9 ± 1.92 , 659.8 ± 1.76 and $1046.5\pm21.0\mu\text{m}$) respectively (Table, 2). There was significant differences at ($P>0.05$) among the cranial part and the middle and caudal part also there was significant differences between the middle and caudal parts of ductus deference. The ductus deferens opened into ejaculatory apparatus which located at the end of ductus deferens especially at papillae. The two ductus deferens opened on the tip of obviously long conical slender duct of papillae inside the urodeum of the cloaca (Fig.4).

The wall of ductus deferens which is also lined by a pseudostratified columnar epithelium with longitudinal folds of mucosa. However, the lamina propria submucosa of the ductus deferens is areolar loose connective tissue, and the tunica muscularis is very thick and contains three layers of smooth muscle. The tunica muscularis is very thick and contains three layers of smooth muscle. The tunica serosa is present and typical (Fig.5). The wall of ductus deferens was made up of loose connective tissue and many mucosal fold was present in ductus deferens and projected inside the lumen of ductus deferens (Fig.5). The lumen of ductus deferens wider than the epididymal duct, the diameter increasing towards the caudal part, at the extreme caudal end there is dilation two times in diameter reached (975.3 ± 2.61) of right and ($1046.5\pm2.10\mu\text{m}$) of left ductus deferens (Table,2) which open into papillae, represent the ejaculating duct. The epithelium of ductus deferens was Pseudo-stratified columnar epithelium the mean epithelium height of ductus deferens at cranial part is $23, 8\pm0.83\mu\text{m}$ at the right side $22.10\pm0.92\mu\text{m}$ and the middle

part is $18.32\pm 1.81\mu\text{m}$ of right side and $18.93\pm0.72\mu\text{m}$ at left side and caudal part $36.73\pm3.08\mu\text{m}$ of right side and $35.98\pm4.02\mu\text{m}$ at the left side (Table,2)

The papillae had thicker base and pointed tip and situated laterally where the ductus deferens penetrate the cloacal wall (Fig.4). The result revealed that the mean length of the right and left papillae were ($1.297\pm0.08\text{mm}$ and $1.021\pm0.0621\text{mm}$) respectively (Table,3). There was non-significant differences at ($P>0.05$) between the mean length of the right and left papilla. The mean diameters of the right and left papillae were (730.5 ± 0.03) μm and $812.34\pm0.04\mu\text{m}$) respectively (Table,3). There was non-significant differences at ($P>0.05$) between the length of the right and left papillae. The epithelial lining of the lumen of papilla was pseudostratified epithelium (Fig.6).

The anatomical result of the not well developed phallus of guinea fowl (*Numidia meleagris*), it could be seen through the opening of vent (Cloaca). The organ appeared as two fused lips these lips moisten and white in color. The lips of vestigial phallus corresponding concave depression in the dorsal wall of the proctodeum of cloaca, also these lips surrounding laterally by pubic bones (eminencia vent), by other means, the phallus located definitely in the ventral wall of the cloaca and could be seen lies in the ventral mid line of proctodeum immediately caudal to the papillae of the ductus deferens (Fig.4 and 7).

The phallus composed anatomically from two obvious lateral phallic bodies and rudimentary median body of phallus like very small eminence and lymphatic folds these lymphatic folds situated in the distance between lateral phallic body and papillae of ductus deferens. The lymphatic fold responsible for phallic erection the type of phallus in guinea fowl was non-intermittent type (non-protruding) (Fig.4 and 7).

The anatomical parameters of the phallus in the guinea fowl (*Numidia meleagris*) showing the transverse, cranio-caudal and dorso-ventral widths of the phallus were ($5.01\pm0.24\text{mm}$, $3.51\pm0.07\text{mm}$ and $4.32\pm0.25\text{mm}$) respectively (Table,4) These were significant difference at ($p>0.05$) between the transvers and cranio-

caudal of widths and that of the dorso-ventral width .

The histological result of the phallus explained each lateral lymphatic body of the guinea fowl phallus (Fig.7) which consist from space between thin bundles of collagen

Fiber which filled by lymphatic fluid during erection of the phallus two lateral bodies (Fig.7). Well-developed of muscle fiber could be seen in the periphery of the lymphatic spaces (Fig.8).

Table, 1: Showing the diameter of ductus epididymis μm and height of epithelium of ductus epididymis μm

Parameter	Mean \pm S
Diameter of ductus epididymis	285.17 \pm 2.43
Epithelium of epididymis / μm	47.97 \pm 0.56

Table, 2: Shows the length and diameter of cranial, middle and caudal parts of the ductus deference.

Parameter of ductus deferens	Mean \pm SE	
Ductus deference	Right	Left
Length / cm	9.16 \pm 12.0	9.80 \pm 12.4 *
Diameter of cranial part of ductus deference / μm	444.7 \pm 16.7 ab	454.9 \pm 19.2 ab
Diameter of middle part of ductus deference / μm	645 \pm 20.1 ab	659.8 \pm 17.6 ab
Diameter of caudal part of ductus deference / μm	975.3 \pm 26.1 ab	1046.5 \pm 21.0 * ab
Height of epithelium of cranial part of ductus deferens	23.8 \pm 0.83	22.10 \pm 0.92
Height of epithelium of middle part of ductus deferens	18.32 \pm 1.81	18.93 \pm 0.72
Height of epithelium of caudal part of ductus deferens	36.73 \pm 3.08	35.98 \pm 4.02

(*) Represents significant differences at level of ($p > 0.05$) between the left and right ductus deference. different letters (a, b) represents significant differences at level of ($p > 0.05$) among the cranial, middle and caudal parts of ductus deference.

Table, 3: Shows the length and diameter of the right and left papilla.

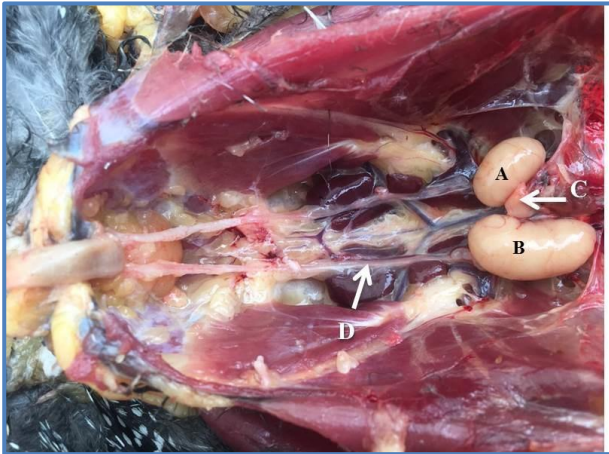
Papilla	Right	Let
Length / mm	1.297 \pm 0.08	1.21 \pm 0.062
Diameter/ μm	730.5 \pm 0.03	812.34 \pm 0.04

Non- significant differences at level of ($p > 0.05$).

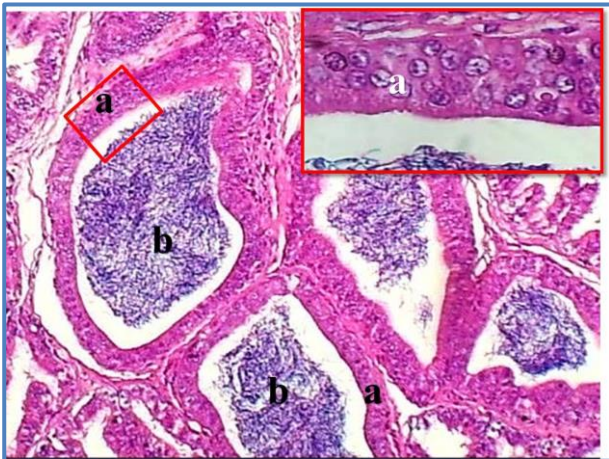
Table, 4: Shows the transverse, cranio-caudal and dorso-ventral widths of the phallus/mm.

Parameters	Mean \pm SE/mm
Transverse width	5.01 \pm 0.24 *
Cranio-caudal width	3.51 \pm 0.07 *
Dorso-ventral width	4.32 \pm 0.25

(*) represents significant differences at ($p > 0.05$).



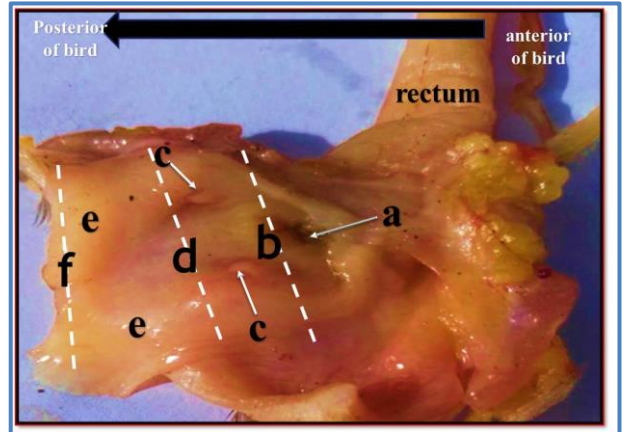
Figure,1: Gross appearance of celomic cavity of guinea fowl showing: right testis (A), left testis (B) epididymis (C) left ureter (D)



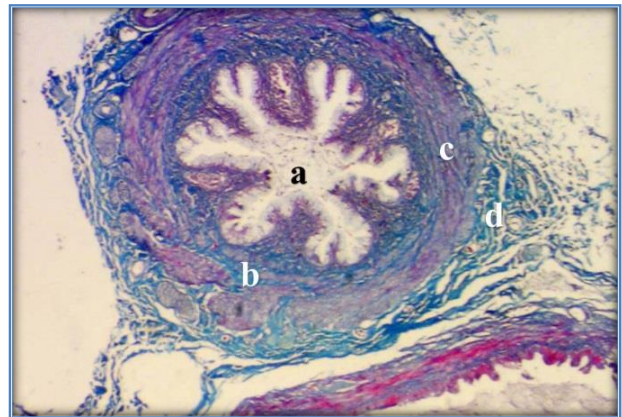
Figure,2: Histological section of epididymal duct showing a- pseudostratified columnar epithelium b- lumen of epididymal duct full with spermatozoa H&E stain (40x)



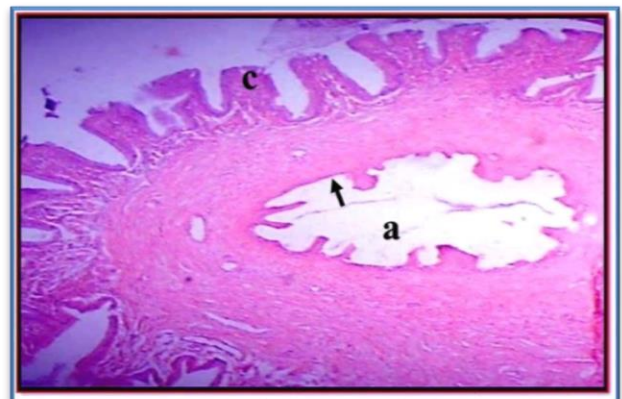
Figure,3: Gross appearance of celomic cavity of guinea fowl showing: right testis (A), left testis (B), right and left ductus deference (yellow arrow), kidney (black arrow)



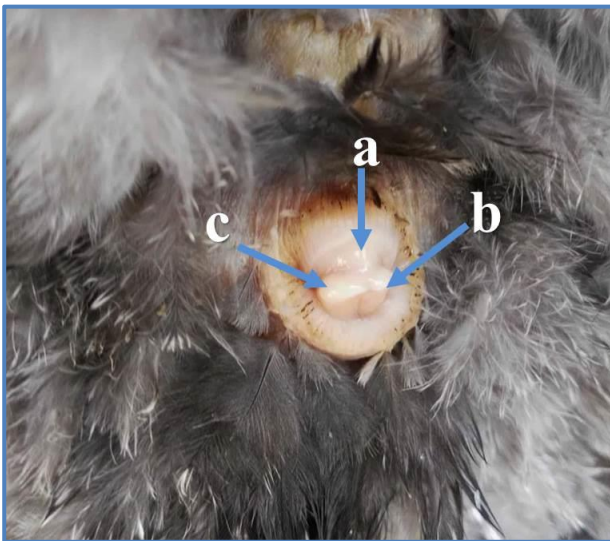
Figure,4. Gross appearance of phallus of male guinea fowl showing: orifice of rectum (a), coprodeum (b) bright and left papillae (c), urodeum (d), right and left lymphatic body (e), proctodeum (f)



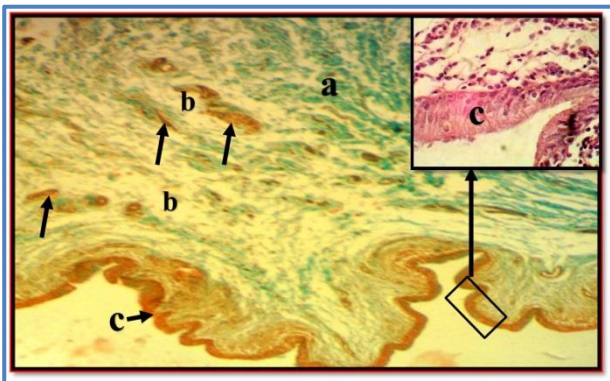
Figure,5: Histological section of ductus deferens: a- lumen, b- sub mucosa, c- tunica muscularis d- adventitia. Masson's Trichrome stain (40x).



Figure,6 : Histological section of papillae showing a- lumen of papillae duct, c- stratified squamous epithelium of urodeum. (arrow) - Non ciliated pseudostratified columnar epithelium H&E stain (40x)



Figure,7: Gross appearance of copulatory organ of guinea fowl showing: dorsal lip of wall (a) right lateral body (b), left lateral body (c)



Figure,8: Histological section in the lateral lymphatic body of phallus of guinea fowl showing a- collagen fiber b- space between collagen fibers c- lining epithelium of proctodeum (pseudostratified columnar epithelium), (arrows)-muscle fibers. Masson's Trichrome stain (40x).

The present study distinguished the anatomical and histological structures of the epididymis of the guinea fowl (*Numida melegris*). The results of the connecting ductules of guinea fowl which continues to opened into the ductus epididymis which continuous with ductus deferens, this duct gradually increases in diameter from the beginning at the cranial end of the epididymal region to ductus deferens, its diameter at the beginning was narrow and gradually become wider in the middle diameters of cranial, middle and caudal parts of the ductus deference were $(454.90 \pm 23.1 \text{mm}$, $659.80 \pm 22,3 \text{mm}$ and $949.49 \pm 111.9 \text{mm}$). There was significant differences at ($P > 0.05$) among the cranial part and the middle and caudal part

also there was significant differences between the middle and caudal parts of ductus deference these findings were similar to (15) in domestic fowl and (16) Aseel and Vanarja breeds of poultry. The length of the right and left papilla were $(1.297 \pm 0.08 \text{mm}$ and $1.21 \pm 0.0621 \text{mm}$) respectively. There was non-significant differences at ($P > 0.05$) between the length of the right and left papilla, the diameters of the right and left papilla were $(730.5 \pm 0.03 \mu\text{m}$ and $812.34 \pm 0.04 \mu\text{m}$) respectively. There was non-significant differences at ($P > 0.05$) between the length of the right and left papilla. These results agree with (17) domestic fowl. The study revealed that the epithial lining of the coprodeum, urodeum and proctodeum of guinea fowl to the urodeum were simple columnar epithelium with goblet cells are more numerous in coprodeum compared to the urodeum same result sated by (18) in Ratite birds.

In the floor of the proctodeum a non-intermittent type of phallus of male guinea fowl which represented the copulatory organ. Anon- intermittent phallus are short and cannot enter the fowl cloaca but the two lymphatic folds of inner cloaca with enhance the median furrow formed from erected of two lateral lymphatic bodies which collect the semen and placed it's in the proper position it can be readily take up by the female during cloacal opposition. This process is assisted by the eversion of two cranial papillae from the wall of the males proctodeum this fact not corresponding with result of (19-21).

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دراسة شكلية و نسجية قياسية للبربخ والقناة الدافعة والعضو التكاثري لذكور الدجاج الفرعوني البالغ
(Numida Meleagris)

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الخلاصة

أجريت الدراسة لبحث المميزات التشريحية والنسجية للبربخ، القناة الدافعة والعضو التكاثري للذكور للدجاج الفرعوني وشملت الدراسة خمسة عشر من ذكور الدجاج الفرعوني سليمة من الأمراض وبعمر (18-24) شهر ووزنها بين 1280-1405غم جلبت من الأسواق المحلية في محافظة بغداد إن البربخ في الدجاج الفرعوني يتألف من الشبه الخصويه، والقنويات الواصلة التي ترتبط بالقناة البربخية تبين ان معدل قطر القناة البربخية (2.43 ± 285.17) مايكرون. ومبطنة بظهارة مطبقة عمودية وقد تكون مهدبة وغير مهدبة. والقناة البربخية تستمر لتتصل بالقناة الدافعة للحيامن التي تتوسع باتجاه المجمع حيث كان معدل طول القطر للقناة الدافعة اليمنى واليسرى (1.20 ± 9.16) و (1.24 ± 9.80) سم على التوالي. أما العضو التكاثري للذكور الدجاج الفرعوني فيكون غير متطور ويصف بغير البارز ويتألف من جسمين لمفاويين وحشبيين أيمن وأيسر وبينهما ميزاب حيث كان معدل عرض العضو التكاثري للذكور المستعرض (0.24 ± 5.01) ملم في حين كان عرضه امامياً خلفياً (0.07 ± 3.51) ملم والعرض الظهري البطني (0.25 ± 4.32) (ملم وتكون ظهارة العضو التكاثري للذكور مغطاة بخلايا طلائية متعددة الطبقات العمودية. أستنتج من هذه الدراسة أن البربخ في ذكور الدجاج الفرعوني يقع على الجهة الظهرية الانسيبه للخصيه وبدون تميز الاجزاء كما في الحيوانات المستأنسه، والقناة الدافعة لذكور الدجاج الفرعوني متعرج والعضو التكاثري للذكور بالدجاج الفرعوني من النوع غير البارز.

الكلمات المفتاحية: البربخ ، القناة الدافعة، الدجاج الفرعوني .