# Histological study of testis in Quail (Coturnix coturnix japonica)

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## Abstract

The present work were aimed to study the histology of testis in male of quail (C.C. japonica). Ten adult male of quails, nine weeks old, were used in this study. Experimental birds were anesthetaized by using chloroform inhalation in closed champers and then the necropsed technique were applied to remove the testis and study their histological characteristics.

The histological examination were showed that the male of Quail birds has a compound tubular testis. It covers by dense irregular connective tissue which called Tunica albuginea. The testis did not clearly divided into lobes because there is thin interstitial connective tissue septa between the adjacent seminiferous tubules. The convoluted seminiferous tubules surrounded by thin sheath consist of connective tissue fibers, myoid cells and smooth muscle fibers. Epithelium lining of semniferous tubules have two types of cells: supporting cells (Sertoli) and spermatogonium cells which produce the sperms. The extension of these tubules toward the epidydimus became like striated tubules (tubuli recti) less in diameter and lined by sertoli cells only. The striated tubules were anastomosing with each other to form rete testis which is directly contact with efferent ducts. The histological study also showed that the testis of quail has no mediastinum testis.

# دراسة نسيجية للخصية في طيور السمان (Coturnix coturnix japonica)

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## الخلاصة

يستهدف البحث الحالي دراسة التركيب النسيجي للخصية في ذكور طيور السمان الياباني. استخدم في هذه الدراسة عشرة ذكور بالغة بعمر تسعة أسابيع حيث خدرت الطيور بواسطة استنشاق الكلوروفورم الموضوع في حجرة مغلقة. بعد إجراء التقانة التشريحية تم عزل الخصية من كل الطيور وذلك لأجل إجراء اختبار الفحص النسيجي.

الفحص النسيجي اظهر بان ذكور السمان تمتلك خصيتين تصنف على أنها غدد نبيبية معقدة، وهي محاطة بمحفظة من نسيج ضام كثيف غير منتظم تسمى بالغلالة البيضاء. متن الخصية غير مقسم بشكل واضح إلى فصوص بسبب قلة النسيج الضام الخلالي (الحاجز) الذي يفصل بين النبيبات المنوية المتجاورة. تحاط النبيبات المنوية بغشاء رقيق يتكون من خلايا المايويد وألياف عضلية ملساء، تتكون الظهارة المبطنة للنبيبات المنوية من نوعين من الخلايا هي: الخلايا الساندة (سرتولي) والخلايا الوراثية (سليفات النطف)، امتداد النبيبات باتجاه البربخ تصبح على شكل نبيبات مستقيمة اقل قطرا ومبطنة بالخلايا الساندة فقط، تتقمم هذه النبيبات وتتداخل مع بعضها مكونة الشبكة الخصوية التي تتصل مباشرة مع القنوات الخارجية. كما وان نتائج الدراسة النسيجية أظهرت بان خصية ذكور السمان لا تحتوي على منصف خصوي.

## Introduction

Birds have two testes, one on either side of the midline of the body (1). Testis is a compound tubular gland with an exocrine and an endocrine functions. The exocrine function is the production of spermatozoa by the lining epithelium of the seminiferous tubules. The endocrine function is the production of the male sex hormone, testosterone, by the specific interstitial (leyding) cell in the intertubular connective tissue (2).

The testis is cover by two layers of tunica vaginalis (parietal and vasceral layers) and one layer of tunica albuginea (testis capsule) which consist of dense irregular connective tissue contain mainly collagen fibers and less number of elastic fibers, and the deep part of it rich by dens branches of testicular blood vessels. T.albuginea contact with testicular septa (spatula testis) which divide the testis into about 250 lobes, each testicular lobule is occupied by 1-4 U-shaped, double-ended seminiferous tubules (3).

Epithelium of the convoluted seminiferous tubule includes supporting cells and spermatogenic cells. The supporting cells (Sertoli cells), located in the basal region of the epithelium, surround the adjacent spermatogenic cells. Tightly bound to other supporting cells, they create a blood-testis barrier which shields the developing spermatogenic cells from blood-borne influence. Spermatogenic cells in various stages of development comprise the balance of the cell population. The basally located spermatogonium is capable of mitosis. The largest cell, the primary spermatocyte, undergoes the first meiotic division while the secondary spermatocyte completes the second meiotic division. The resulting spermatid is haploid and becomes the spermatozoon which represents the final developmental stage (4,5).

The seminiferous tubules, at their terminal segments, are joined by a transitional zone, lined by sustentacular cell, to straight tubules (tubuli recti), which are continuous with a network of anastomosing channels that form the rate testis. The rate testis has a simple squamous or cuboidal epithelium. It is surrounded by the loose connective tissue of the mediastinim and is drained between seven to 20 efferent ducts. The efferent ducts are lined with simple columnar or pseudostratified epithelium, and some of the cells are ciliated. The lumen of each duct is wide and the lamina propria is sparse connective tissue (2). Interstitial tissue of the testis consists of two main components, the first is that compact layer of myofibroblasts and connective tissue which closely surrounds the seminiferous tubule, and known as the *boundary tissue*. The other is the loose connective tissue, the *interstitium*, which lies between seminiferous tubules, with full expression in the angular areas or wedges between 3 or more adjacent seminiferous tubules (6). The aim of present study to illustrate the histological picture of the testis of male quail.

## Materials and Methods

Ten adult male of quails, nine weeks old, were used in this study. Experimental birds were anesthatized by using chloroform inhalation in closed champers and then the necropsy were applied to removed the testis. Testis were immediately fixed by formalin (10%) for 24 hours. After dehydration with ethyl alcohol in increasing concentration (70-100%) and passed in two content of xylol the samples were embedded in paraffin , sectioned by the rotary microtome at 5μm. After slides samples were passed through the decreasing concentration (100-70%) of ethylic alcohol and in xylol. The histological slides were stained by Hematoxylin and Eosin stain (7).

An ocular micrometer were used to measured the thickness of testicular capsule.

## Results and Discussion

The testes are compound tubular glands situated in the abdominal cavity of the quail. unlike in most mammals (8).

Testis of Quail is surrounded by dense irregular connective tissue capsule, the tunica albuginea, which is covered by a peritoneum (Fig.1). While Hodges (9) suggested that the testicular capsule in birds is composed of three main tissue layers: an outer, thin *tunica serosa*, a thick *tunica albuginea* and the innermost, very thin *tunica vasculosa* .

The histological study for the quail testis showed that the thickness of the testicular capsule were 91 µm and the capsule between testis and epididymis became much more thicking on the average 245 µm. Testicular capsule thickness in certain species of birds are, on the average, 578.1 µm in the ostrich, 81.5 µm in the rooster, 91.7 µm in the Japanese quail and 91.8 µm in the drake (*Anas platyrhynchos*). However, at the interface between the testis and epididymis, the capsule is much thicker, and has been found to be, on the average, 1215.9 µm thick in the ostrich, 515.0 µm in the rooster, 255.4 µm in the Japanese quail, and 233.7 µm in the drake (1).

Histological results disclosed that the quail testis contains two types of parenchymal tissue: the interstitial tissue and the seminiferous epithelium. The interstitial tissue contains blood, lymphatic vessels and Leydig cells. There are no well-developed interstitial tissue (septa) to divide the testes into lobules because there were very little connective tissue between adjacent seminiferous tubules and the Leydig cells are sparse. The leyding cells were found singly or in small clusters, primarily in the larger interstitial spaces, they are recognized by their small, round nucleus and an acidophilic, often foamy cytoplasm (Fig. 2,3). Aire (10) suggested the interstitium is relatively compact in birds except in the ostrich in which it forms a loose and ‘oedematous’ connective tissue. Seminiferous tubules of birds are dissimilar to those of mammals by forming highly and complexly anastomotic, non-blind-ending network of tubules and this probably responsible for the lack of connective tissue septa, as well as the non lobulation of the avian testis (11,12). Leydig cells of birds are generally similar, structurally, to those of mammals (13,14) and it seem to form columns of cells in the interstices in the most birds (15,16), The interstitial tissue of the testis has been described in a number of mammals (17,18), but reports on this tissue in birds are rather scanty, and are, in the main, on domestic species of birds, such as the rooster (19,20), Duck (21), Guineafowl (*Numida meleagris*) and Japanese quail (10).

The results of present Observation regarding that there were Single layer of flattened polygonal cells myoid or peritubular cells with smooth muscle characteristics forming a continuous sheet surrounding the tubules (Fig.4), and that agreed with Rothwell and tingari (19) they suggest of present thin concentric layers of myoepithelial cells, fibroblasts, and connective tissue fibrils overlie the basal lamina of the seminiferous tubule.

Convoluted seminiferous tubules of quail testis lined by a stratified epithelium of spermatogenic cells and Sertoli cells. The spermatogenic cells give rise to spermatozoa. Spermatogonia are the most immature spermatogenic cells, are small, round cells with dark, round nuclei that lie adjacent to the basement membrane. These cells undergo mitotic divisions and produce primary spermatocytes, larger cells whose nuclei often show distinct chromatin. The primary spermatocytes are undergo the first meiotic division, giving rise to smaller, secondary spermatocytes. The secondary spermatocytes are rarely observed because they undergo the second meiotic division shortly after they arise, forming haploid spermatids. The early spermatids are round cells with pale nuclei that occur in clusters toward the lumen of the somniferous tubule. Late spermatids are

characterized by small, oval to elongated, dark heads and long, faint tails that project into the lumen. They are eventually released from the somniferous epithelium as spermatozoa (Fig.5). These result were in agreement with William and linda (22) how described that the spermatogenesis in birds, as in mammals, involves a series of divisions of spermatogonia, resulting in primary spermatocytes and secondary spermatocytes, both of which undergo meiotic divisions, culminating in the evolution of spermatids. The latter differentiate to form motile, itinerant spermatozoa.

The results impression suggests that the sertoli cells are tall, columnar and extend from the basal lamina to the luminal border of the seminiferous epithelium, and that agree with Cooksey and Rothwell (4) and Osmant et. al. (24) they suggest that the avian sertoli cells are generally similar to those described for the mammal. It is usually irregular, has a prominent nucleolus, and it is situated close to the basal lamina in most birds except in the ostrich where it lies in the middle part of the cell , in the rooster it have fewer broad base contacts with the basement membrane than in mammals, and similar observation has been made in the Japanese quail.

The results show that the convoluted somniferous tubule are continuous with straight tubules (tubuli recti), which are lined by Sertoli cells. Straight tubules lead into the anastomosing channels of the rete testis, which is lined by a simple cuboidal to squamous epithelium . There is no mediastinim. The rete testis lies outside the tunica albuginea below the epididymis, excurrent ducts of the testis which comprise various ducts that transport spermatozoa and fluid produced by the seminiferous epithelium, in birds, have, surprisingly, been described in only a few species: the rooster (25,26), Turkey (27,28), Guineafowl (29,30,31), Japanese quail (32,33), Ostrich (34,35), passerine birds (36), Pigeon (37), Mute Swan (38,39) and drake (21). Excurrent duct system of birds, as in mammals, comprises (a) the *rete testis* (RT) unit, (b) the efferent duct unit and (c) the epididymal duct unit (28). The rete testis (RT) of birds is connected to the seminiferous tubules whose semen transports to the succeeding segment of the excurrent duct system, the occurrence of divisions, based on their location, of the RT has been a controversial subject and (40) considers that the rete is located entirely outside the testis but it is generally agreed that there are intracapsular (intratunical) and extratesticular portions of this duct unit. In addition, Budras (41), Aire (30) also Barker and Kendall (38) have described an intratesticular portion which is made up of rete channels that are similar in epithelial lining to those in the intracapsular and extratesticular segments, or appear in the form of tubuli recti in various species of birds (42,38). The surface of the epithelial lining of the RT ductules, except for a few minor details, is generally regular, but may bear a few shallow grooves in the ostrich (34). In birds, the RT lacunae are continued by the efferent duct unit (43,44), as in mammals (45,46).

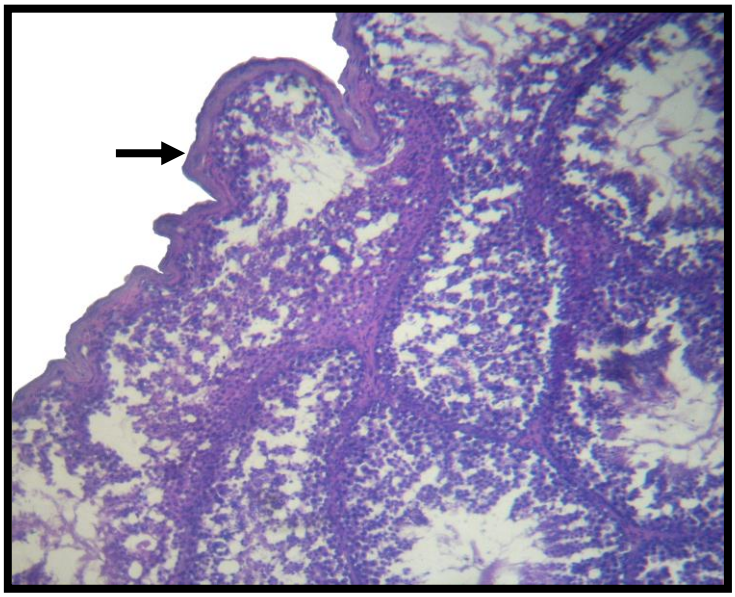

**Fig.(1) Dense irregular connective tissue capsule (Tunica albuginea) (⟶) (H&E) ×400**

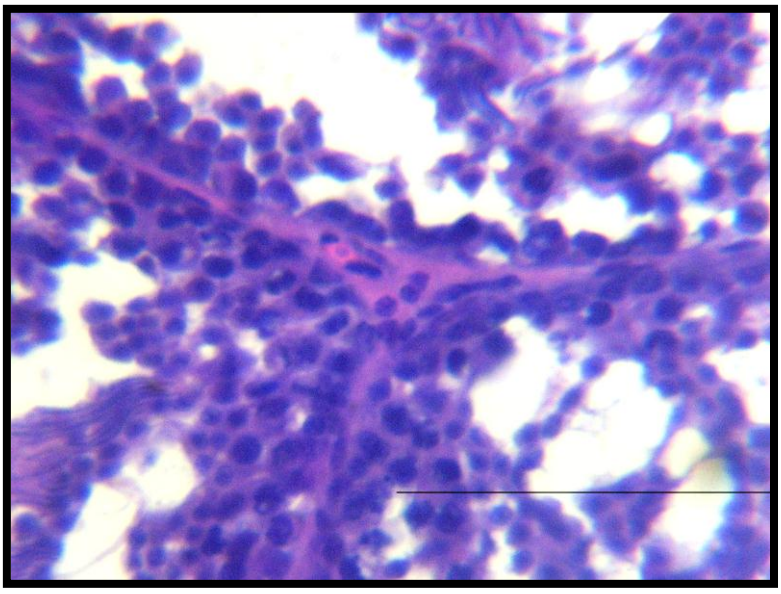

**Fig. (2) Thin septa between adjacent seminiferous tubules. (H&E) ×400**

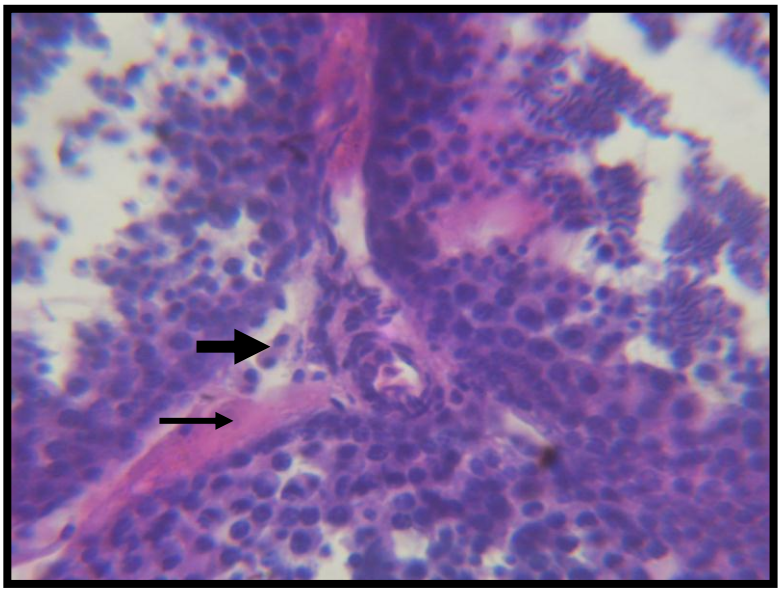

**Fig.(3) Leyding cells (➡) and interstitial connective tissue (→) (H&E) ×400**

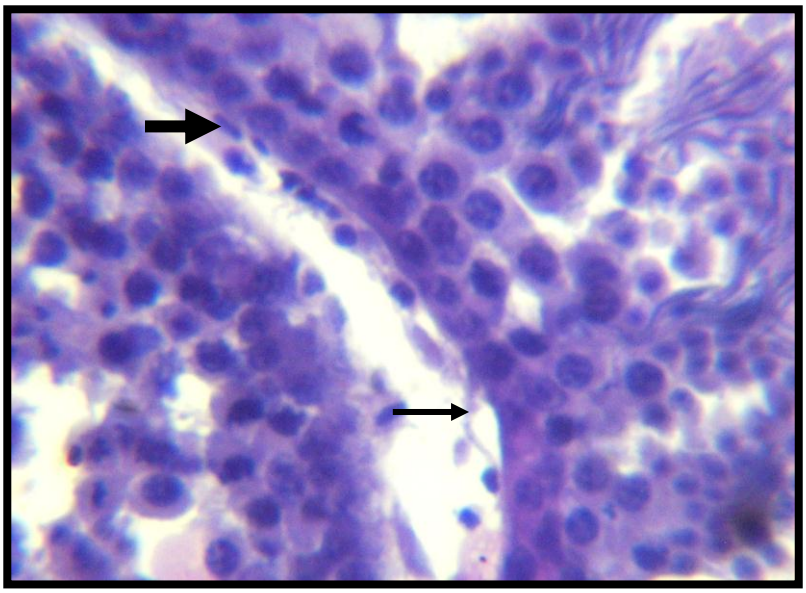

**Fig. (4) Nucleus of myoid cells (➡) and smooth muscle fibers (→) (H&E) ×400**

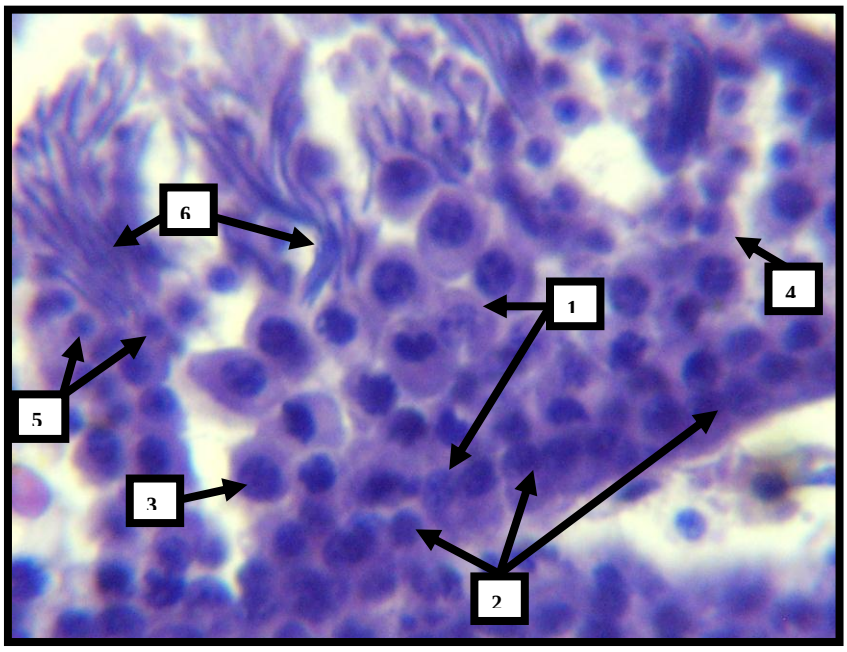


**Fig. (5) Epithelium lining of seminiferous tubules:**
**1. Sertoli cells**
**2. spermatogonium cells**
**3. primary spermatocyte cells**
**4. secondary spermatocyte cells**
**5. spermatid cells**
**6. spermatozoa cells (H&E) ×400**

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