

MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF THE EVENTS CYCLE OF SPERMATOGENESIS IN THE TESTES OF ADULT MALE COMMON QUAIL

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ABSTRACT

Twenty adult common quail male obtained from Soq-Al-Shiyookh local market .They were kept under light and feeding conditions adopted by it. The anatomical study was appeared that the reproductive system in common quail consist of paired testes ,efferent ducts, epididymis, vasdeference and recetaculum .The testes are lying within the abdominal cavity and are joined to the dorsal body wall by short connective tissue . They are paired oval-shaped structure .The average of the left testicular size is ($2 \pm 0.04 \text{ cm}^3$) and the average of the right testicular size is ($1.85 \pm 0.01 \text{ cm}^3$).The average of the left testicular weight is ($1.9 \pm 0.01 \text{ gm.}$) and the average of the right testicular weight is ($1.7 \pm 0.01 \text{ gm.}$). The testes of quail are long axis is oblique and directed dorso caudally .The epididymis is relatively large and is firmly attached to the dorso lateral surface of the testes. The histological study was showed that the spermatogonia are identified adjacent to the basement membrane of the seminiferous tubules either singly or in cluster but they do not form a complete basal layer. The primary spermatocyte are the largest cells of the spermatogenic population . Their nuclei are notable for both their size and appearance ,being big and vesicular is due to the presence of condensed chromatin. The secondary spermatocyte enter the second meiotic division ,the subsequent cells formed from this division are spermatids. Spermatocytogenesis ends when spermatids appears .The spermatids undergo a complex process of differentiation leading to the transformation of these spermatids into sperm . Based on changes in the acrosomes and nuclei during the development of spermatid will give rise mature spermatozoa.

INTRODUCTION

The testes in domestic fowl (*Gallus domesticus*) are even organs , internal ,patellar, diplaced at the side of the body median line , presenting rounded surface ,however ,with varied shape until the 20 the week. They were oval , elongated extremity .The testes presented a central area slightly depressed through which the testicular arteries from the abdominal aorta artery supply them being fixed to the body dorsal wall by

meso extension ,permitting a certain buoyancy ,reminding that the adjacent organs contribute to their position maintenance (1). In the birds as in mammals ,the testes are responsible for the production of spermatozoa and the section of androgen .spermatocytogenesis consist of the mitotic division involving proliferation and maintenance of spermatogonia(2). Spermatogonia then undergo meiosis to form primary spermatocyte and then secondary spermatocyte ,which differentiate into spermatids, morphological, histochemical characterization of the seminiferous epithelial and leyding cells of the turkey were observed (3).Germ cell transfer is becoming an important technique for the study of spermatogenesis and potential can be used for the production of transgenic progeny (4).The morphological characteristic of the testes in the same species always change according to age and sexual activity cycle .The cytological relationships of the cells comprising the seminiferous epithelium of several avian have already been examined , with the most comprehensive studies done on Japanese quail (5). The reproductive cell continuously profile rated and at the same time it degenerated during the source of differentiation and development (6).Cell apoptosis , the process of cellular self-destruction ,also involves active process of intracellular synthesis , and is controlled by cellular genes (7),(8) . Cells with a high regeneration and division rate , or under endocrine control , are particularly susceptible to apoptosis (9).(10) were described the ducts succession from the seminiferous tubules to the ducts deferens papilla , as well as the microanatomy of the epididymis region and the ducts deferens in the common quail (*cotunrix coturnix L.*).The testes of adult ram (*ovis ovis*) is situated within the scrotum ,each testes is enclosed ,by the tunica albugina of dens collagenous tissue which continues with the loose connective tissue of mediastinum testes and divide the testes into lobule .These lobules have seminiferous tubules (11). .The aim of this study was to investigate microanatomical and macro anatomical features of the reproductive organs of the quail, which is one of the most reared birds in the word . The entire reproductive system of the birds are necessary for breeding ,but the testes ,epididymis and ductus deference are the most important functional regions. Therefore this present study aim to investigate the reproductive system in common quail.

MATERIAL AND METHODS

We obtained from Soq-Al-Shiyookh local market on twenty male common quail ,where they were kept under light and feeding conditions adopted by it .Initially ,the birds were anesthetized with ketamine and diazepam (25 mg/kg) for weight (12).The testes being removed are weighed on a sensitive balance .The reproductive organs ,including the testes ,ductus deference and epididymis of the birds were dissected and placed into 10% formalin solution for fixation .Washing out of the tissue sample with running water followed after fixation with formalin to prevent interference with subsequent processes .The dehydration process was continued by upgrading the alcohol from 50%, 70% , 80%,90%,100% . Clearing in xylene in order to impregnate the sample with paraffin .This was followed by embedding in melted paraffin at(58-60 c). The small blocks were sectioned by microtome to thickness of (5) micrometer

(13) .The sample were stained with Harris-heamatoxylin-eosin stain (H&E),Periodic acidSchiff(PAS),Wiegerts iron heamatoxylin stain(14).

Dimension of the testis

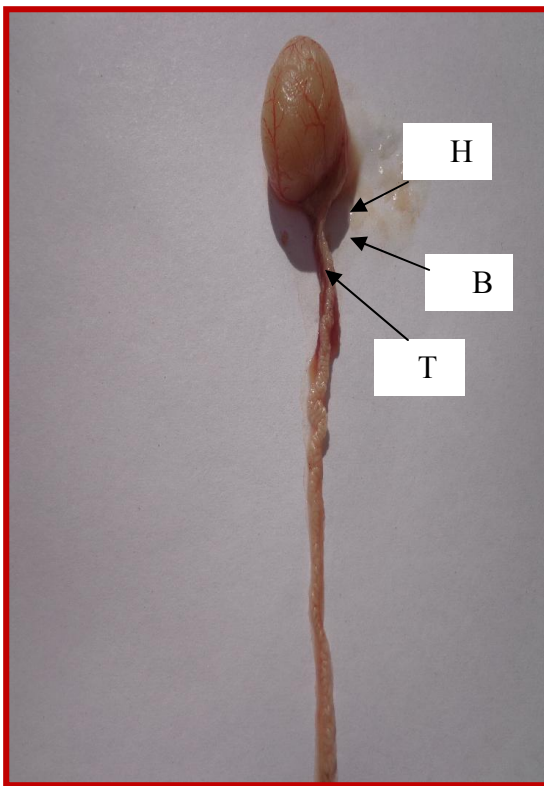
The measurement of length of the testis was taken from the cranial pole to the caudal pole .The measurement of width was taken from the middle(15).Gonadosomatic index (GSI) was taken which represents the body mass percentage allocated in testes .The GSI is calculated by dividing the weight of both testes by the body weight (16).

Tools:

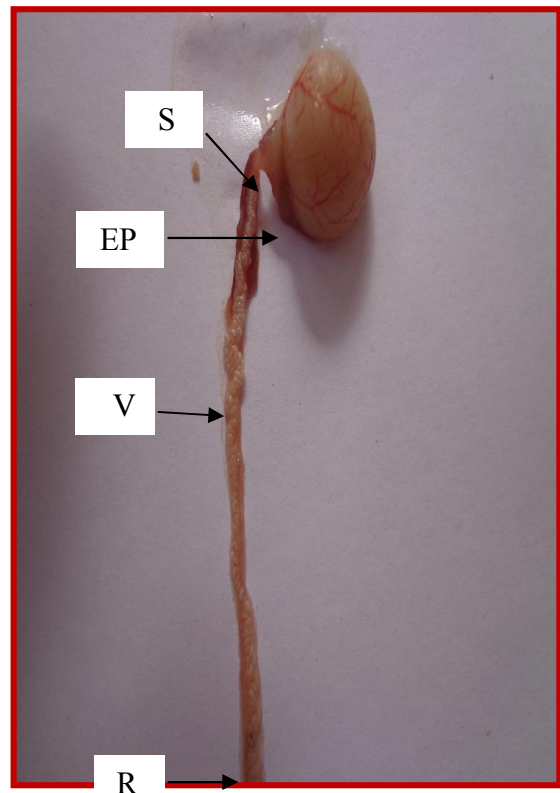
- 1-Photographs of the examined slides were carried out with a motic microscope which is supplied with a digital camera with resolution power of two mega pixel .
- 2-Digital caliper .
- 3-Digital balance.

RESULTS AND DISCUSSION

The anatomical observation was showed that the reproductive system in common quail consist of paired testes, efferent ducts, epididymis, vasdeference and receptaculum.Figure(1,2). The

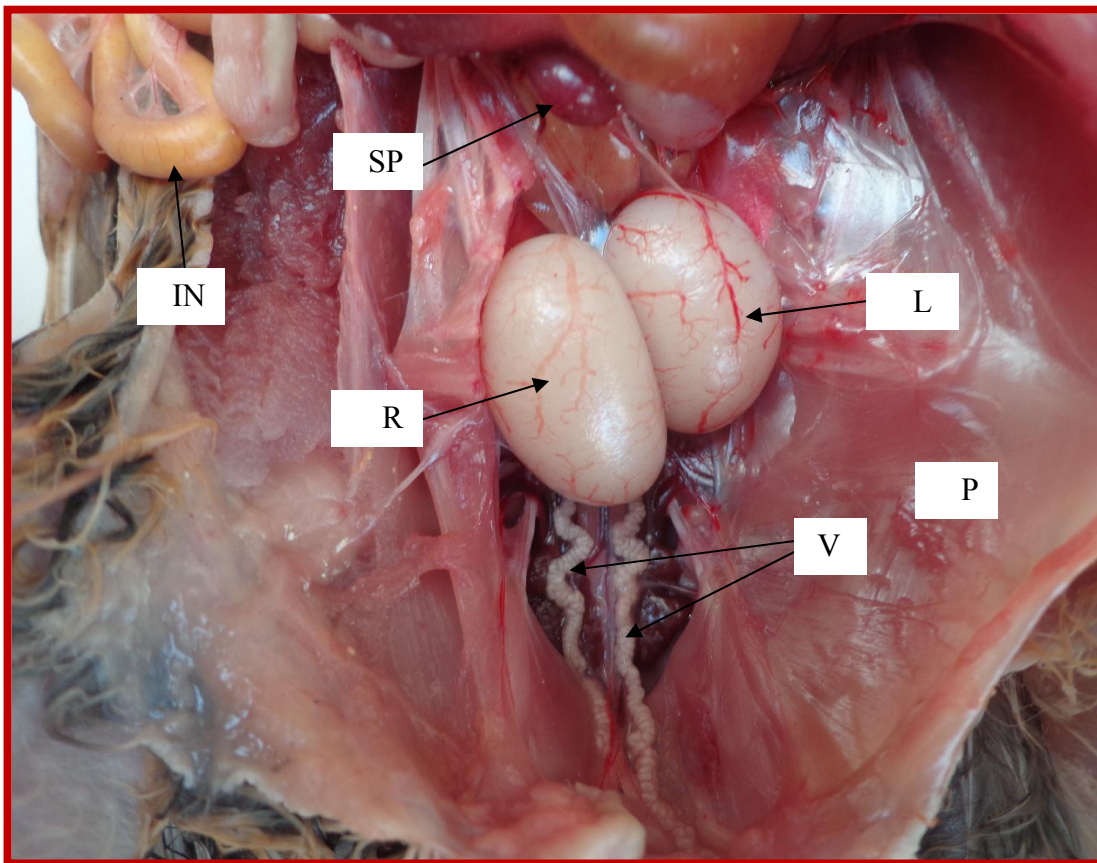


Figure(1).The right testes of common quail ,(H) head of epididymis,(B) body of epididymis, (T) tail of epididymis,



Figure(2).The left testes of common quail ,(S) spermatic cord,(EP)epididymis. (V)vasdeferance, (R)receptaculum

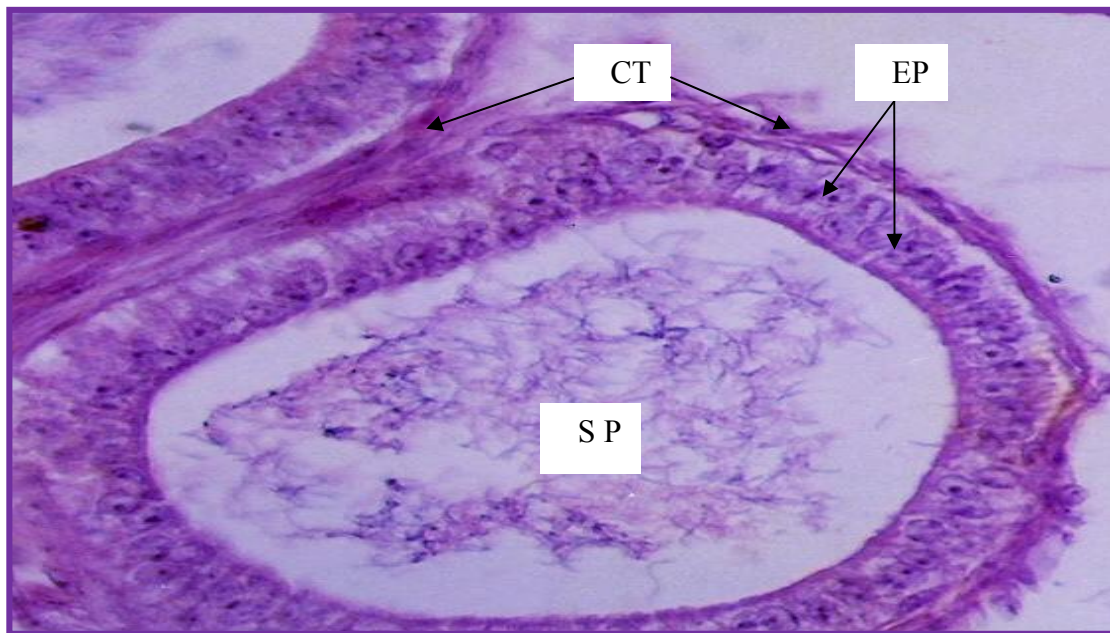
cavity and displaced at the sides of the body median line. They are joined to the dorsal body wall by short connective tissue cord are mesorchium. The testes of common quail are long axis is oblique and directed dorso caudally. They are paired oval-shaped structure.(figure.3). The average of the right testicular size is ($1.85 \pm 0.01 \text{ cm}^3$) and the average of the left testicular size is ($2 \pm 0.04 \text{ cm}^3$). The average of the right testicular weight is ($1.7 \pm 0.01 \text{ gm.}$) and the average of the left testicular weight is ($1.9 \pm 0.01 \text{ gm.}$). The measurement of the length ,width of right testes of common quail are($1.7 \pm 0.01 \text{ cm}$), ($1.4 \pm 0.003 \text{ cm}$),while the average length of the left testes is ($2.12 \pm 0.03 \text{ cm}$),width is ($1.5 \pm 0.003 \text{ cm}$).Table (1). Our results is similar to the finding (10), (17).



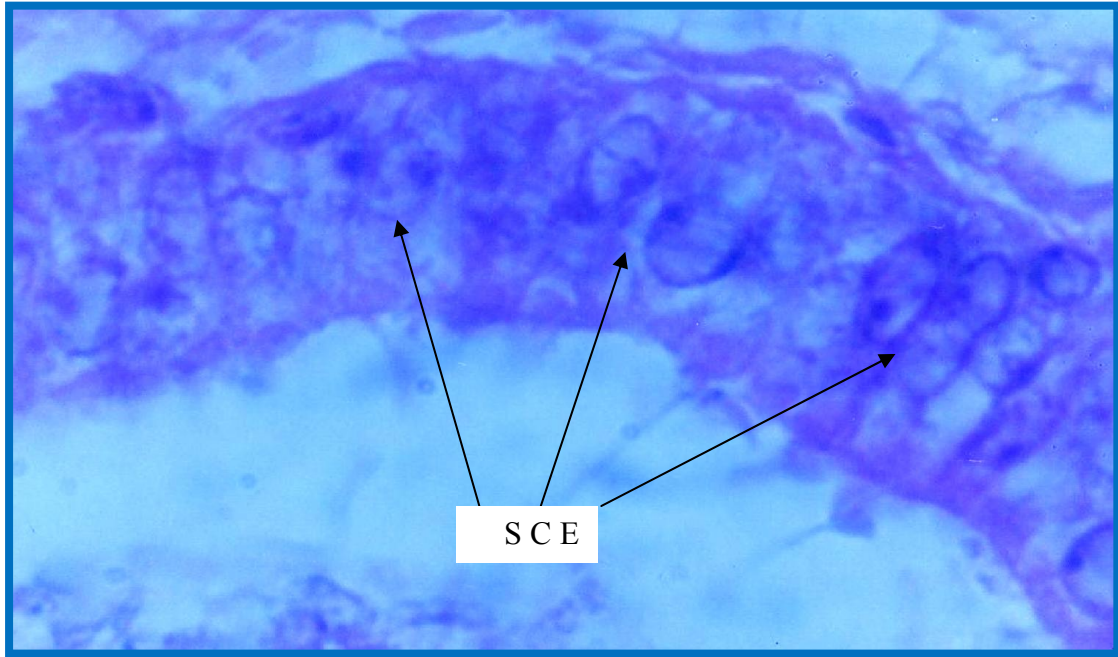
Figure(3).Showing the testis of common quail are lying within the abdominal cavity and displaced at the sides of the body median line.(R)right testes,(L)left testes,(V) vasdeferance, (p)peritoneum, (IN)Intestin.

These anatomical observation coincide with the description reported by (1). The epididymis is relatively large and is firmly attached to the dorso lateral surface of the testes. Each testes is covered by capsule (tunica albugina) of dense irregular connective tissue contained collagen fibers. The epididymis is a well developed structure laying closely applied to the median border of the testes and running approximately the full length of the organ. The average length of the left epididymis is ($1.6 \pm 0.05 \text{ cm}$), while the right epididymis is ($1.4 \pm 0.04 \text{ cm}$).Table(1). Our study is

in agreement with the finding (10) . The epididymis in common quail was attached very intimately and extending from the cranial pole to the caudal pole of the testes ,histological observation of the ductus epididymis showed a series of colloid ductules which were closely bounded to the testis by connective tissue .The lining epithelium of these ductules were simple columnar epithelium .(Figure.4,5) These results is similar to the finding (18). (19) described the epithelium of the epididymis of the white rooster was pseudostratified and columnar ,embedded in a loose connective tissue ,the proximal part of the ductus deference was covered with pseudostratified columnar epithelium, which was continuous with simple cuboidal epithelium towards the distal portion .The light microscopic study on spermatogenic lineage in the testes of common quail revealed that the chief cell of the seminiferous epithelium is the spermatogenic cell.

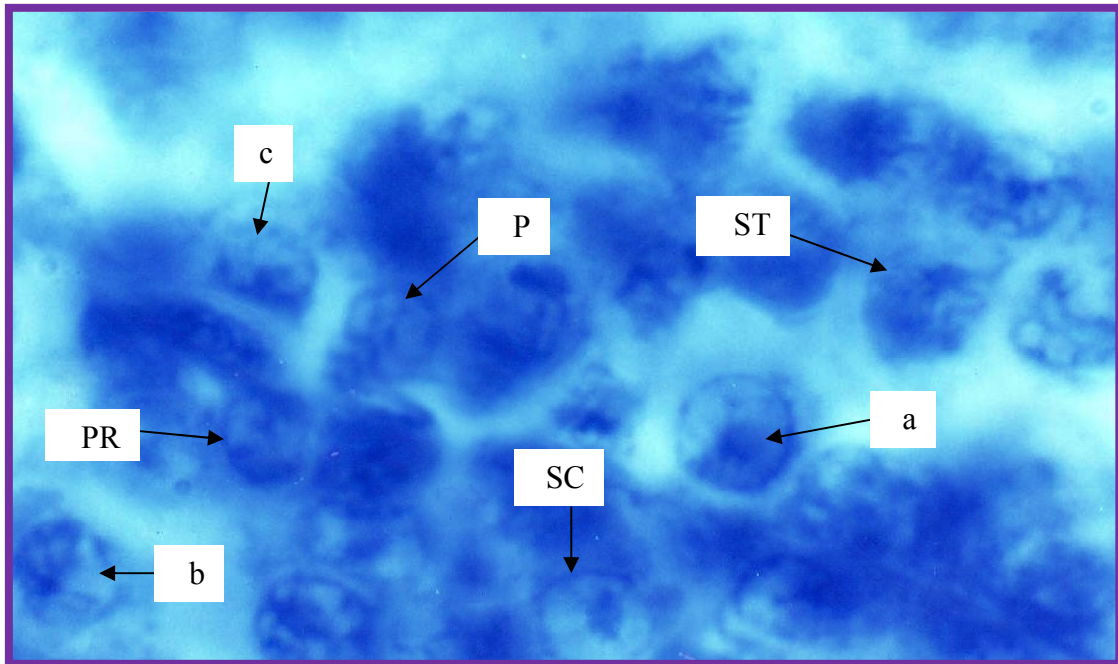


Figure(4) .Transverse section of epididymis of testes .showing,(SP) spermatozoa.(CT)Connective tissue,(EP)Epithelial cells(simple columnar epithelium).H&E stain(400 x).



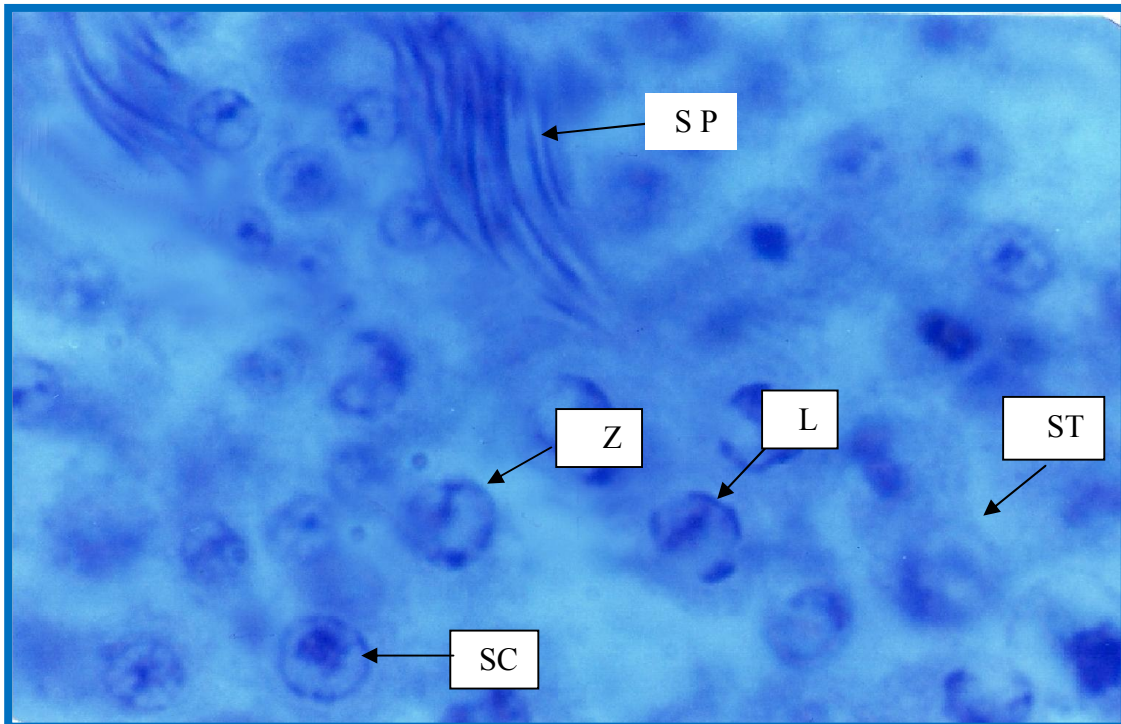
Figure(5)Cross section of epididymis showing epithelial cells lining epididymis(simple columnar epithelium).(PAS)periodic acid Schiff stain(1000 x).

This cell gives rise to the rest of cell population began with spermatogonium. The spermatogonia lie next to the basement membrane of the seminiferous epithelium between adjacent sertoli cells. The adluminal compartment of the seminiferous tubules extends also to the lumen of the tubule and is involved with different stages of the spermatogenic cell development .The seminiferous tubules are also lined by sertoli cells in which the heads of mature spermatozoa beside spermatids are embedded . Spermatogonia are identified adjacent to the basement membrane of the seminiferous tubules either singly or in clusters but they do not form a complete basal layer. In the present study was revealed that There are three types of spermatogonia are (A- spermatogonia , I – spermatogonia, B-spermatogonia) figure (6).These data is similar to observation of (3), (19), They were reported that there are three type of spermatogonia in turkey are the dark type A(Ad),the pale type A(Ap) and the type B .(5) were demonstrated that the spermatogonia in Japanese quail differs from the process described in mammals in that there are fewer mitotic division and they are all synchronized with the cycle of seminiferous epithelium. Indeed subsequent mitotic division of B-spermatogonia leads to the formation of primary spermatocyte.



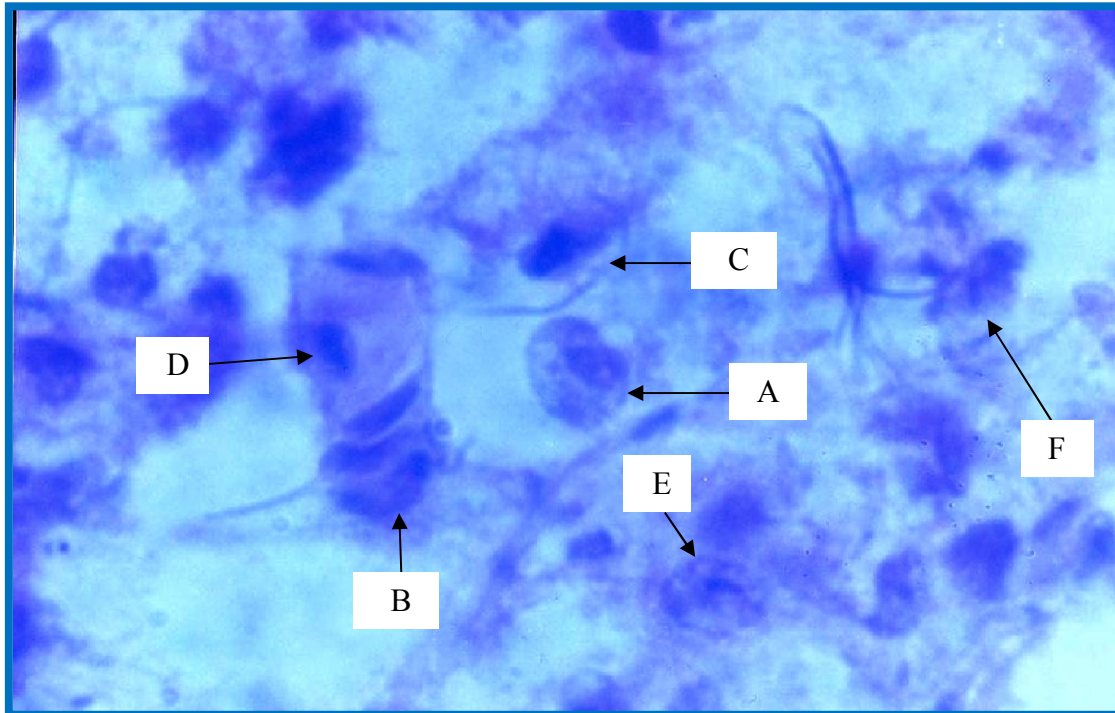
Figure(6).Transvers section of seminiferous tubules .showing,(a)A-spermatogonia ,(b)I-spermatogonia ,(c)B-spermatogonia(PR)Preleptoten(P)Pacheten.(SC)Secondary spermatocyte. (ST) Sertoli cell Wiegerts iron heamatoxylin stain.(1250 x).

. The primary spermatocyte are located more from the basal compartment to the adluminal compartment. The primary spermatocyte are the largest cells of the spermatogenic population , their nuclei are notable for both their size and appearance ,being big and vesicular is due to the presence of condensed chromatin .The primary spermatocyte pass through the first maturation division ,this stage of division includes six phases (1-Preleptoten,2-Leptoten, 3-zygoten, 4-Pachyten, 5-Diploten and 6-Diakinesis).Figure(6,7). Our data was found to be similar to that described in previous study (20) .The first meiotic division of the primary spermatocyte lead to the formation of seconday spermatocytes (Figure,6,7)



Figure(7).Transvers section of seminiferous tubules .showing ,(SP)sperms , ,(D) diploten, (L)leptoten, (Z)zygoten,(SC) secondary spermatocyte.(ST) Sertoli cell. Periodic acid Schiff stain .(1000 x).

These newly formed cells have one -half of the number of chromosomes .These cells are small when compared with the primary spermatocyte and spend only a short time within the seminiferous epithelium and for that reason are not usually encountered when examining the testis histologically .The secondary spermatocyte enter the second meiotic division .The subsequent cells formed from this division are spermatids . .The spermatocytogenesis ends when the spermatid appears . The spermatid undergo a complex process of differentiation leading to the transformation of these spermatids into sperm. Our study showed that there are seventeenth stage of spermatids .Figure(8,9)



Figure(8).Cross section of seminiferous tubules .showing spermatids,(A) stage 4,(B) stage 16 ,(C)stage 11, (D)stage 9, (E)stage 8, (F)stage 15 .Periodic acid Schiff stain (1000 x).

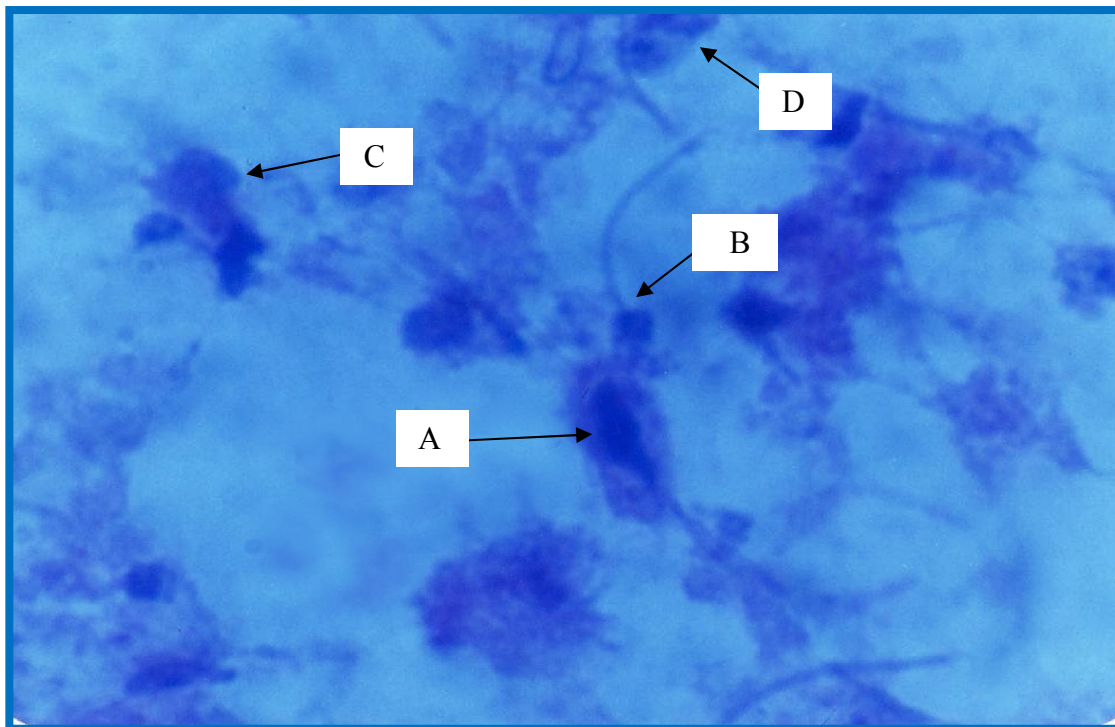


Figure (9) .Cross section of seminiferous tubules .showing spermatids,(A) stage 13,(B) stage 17,(C)stage 12, (D)stage 6 .Periodic acid Schiff stain (1000 x).

These result is in agreement with (21). These changes in spermatids are from fifteenth stage to seventeenth stage in many studies(22) ,(23) . Based on changes in the acrosomes and nuclei during the development of spermatids will give rise mature

spermatozoa (Figure.7). Our data agreement with results of (18) they were studies on the common quail testes (*coturnix c. coturnixL*).

In conclusion the structure of testes common quail were found to be similar to that of other avian species except few minor differences, and compared to the studies published on other birds and mammals ,the morphological features and apoptosis of the common quail testes were examined to provide morphological evidence for the comparative histology ,the developmental biology , and the reproductive biology ,as well as to provide data for the formation and prevention of disease in common quail and other birds .

Table(1):Measurement of Size ,Weight, Length, Width of the Right and Left testes and Length of Epididymis of Common Quail (Means ± SD).

TESTIS(MEANS±SD)	LEFT	RIGHT
Size	(2 ± 0.04 cm ³)	(1.85± 0.01 cm ³)
Weight	(1.9± 0.04 gm.)	(1.7 ± 0.01 gm.)
Length	(2.12 ±0.03cm)	(1.9 ± 0.01 cm)
Width	(1.5± 0.003 cm)	(1.4 ± 0.001 cm)
Length of Epididymis	(1.6± 0.05cm)	(1.4 ± 0.04cm)

There are no significant differences between the left and right testes of common quail in all traits which

دراسة مورفولوجية وكيمياء نسجية لدورة حوادث النطاف في خصى ذكور طائر السمان

البالغ

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الخلاصة

عشرون طيرا من ذكور طائر السمان تم الحصول عليها من السوق المحلي لمدينة سوق الشيوخ، وضعت تحت ظروف الضوء والتغذية المعتمدة طبيعيا. اظهرت الدراسة التشريحية بأن الجهاز التكاثري في طائر السمان يتكون من زوج من الخصى،القنوات الناقلة، البربخ، الوعاء الناقل والصهرج. تقع الخصى في التجويف البطني

ويكون شكل الخصى بيضويا. كان معدل حجم ($2 \pm 0.04 \text{ cm}^3$) ومرتبطة بالجدار الظهري للجسم بواسطة نسيج ضام الخصية اليسرى ومعدل حجم الخصية اليمنى ($1.85 \pm 0.01 \text{ cm}^3$). ومعدل وزن الخصية اليسرى ($1.9 \pm 0.01 \text{ gm.}$) ومعدل وزن الخصية اليمنى ($1.7 \pm 0.01 \text{ gm.}$). تكون الخصى في طائر السمان ذات محور طولي مائل ويتجه مباشرة ظهريا . كما يظهر المحور الطولي لخصى طائر السمان قحافيا مع المنطقة الظهرية. يكون البربخ طويل نسبيا مرتبط بشدة مع الجانب الظهري للخصى. اظهرت الدراسة النسجية بان سليفة النطفة تكون قريبة من الغشاء القاعدي للنبيبات المنوية اما ان تكون منفردة او بشكل تجمعات لكن غير مستمرة مع الطبقة القاعدية . الخلية النطفية الاولى خلية كبيرة الحجم ذات نواه واضحة قياسا بحجمها وتكون كبيرة الحجم حويصلية الشكل ويرجع ذلك الى وجود الكروماتين الكثيف. تدخل الخلية النطفية الثانوية الانقسام الاختزالي الثاني والخلايا المتكونة نتيجة هذا الانقسام هي ارومات النطاف. تمر ارومات النطاف بسلسلة من العمليات المعقدة لتتمايز وتتحول من ارومات النطاف الى نطاف. اساس التغيير هو في الاكروسوم والنواة خلال تطور ارومات النطاف التي تعطي فيما بعد الحيوانات المنوية.

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