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The sequence events of spermatogenesis and spermiogenesis in adult Perdix (*PerdixPerdix)*

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Summary

Someanatomical remarks of male reproductive system of adult perdix, histological structure of testes of perdix and the sequence events of spermatogenesis and spermiogenesis are dealed in this research. The anatomical study of the male reproductive system in perdix include: the paired testes which remain within the abdominal cavity and connect with the dorsal wall via mesorchium. The testes are covered by a thin tunica albuginea. The spermatic cord, tunica vaginalis, and scrotum are lacking. the seminiferous tubule has seminiferous epithelium; spermatogenic cells and sertoli cells lie on the basement membrane and that whole is circumscribed by interstitial tissue which contain leydig cells. The sequence events of spermatogenesis and spermiogenesis in seminiferous tubule of adult perdix start with spermatogonia. Three types of spermatogonia are present which include: A-spermatogonia, B-spermatogonia and Intermediate spermatogonia. The B-spermatogonia give rise to primary spermatocytes. The primary spermatocytes appear six different phases. Which are include: (preleptotene, leptotene, zygotene, pachytene, diplotene,anddiakinesis phases). The secondary spermatocytes being short - living cells and are derived from the primary spermatocytes. With the appearance of spermatids, spermatocytogenesis end. The spermatids undergo complex processes of differentiation depending on changes in the acrosome and nuclei. These changes show 17 phases lead to form sperms.

The sertoli cells possess elongated nuclei which are often triangular in shape. The sertoli cells form an extensive ramification around and between the germinal cells making

intimate contact with them. A cycle of stages in sertoli cells structure are not identified.

The leydig cells are scattered singly or in small groups and are chiefly found in the larger intertubular space. The basement membrane of convoluted seminiferous tubule is comprised of a layer of connective tissue and myoepithelial cells. The myoid cells are elongated cells, with oval nuclei, surrounded by a small amount of cytoplasm.

Introduction

Birds are underveiw of many researchers, like Austen (1961) , Brown and Amadon (1968). Order Galliformes are considered as an economic birds and also as laboratory animals for the purpose of research.Theperdix(*Perdix P.*), are related to this order. There are many studies on the male reproductive system of the Order Galliformes; Stoll and Maraud (1955); Marvan (1969), Hussin (1996), Alsamarrae et.al (2000) and Al-shamary (2001).It is considered that the testes have fulfill two important functions within the male body: Spermatogenesis takes place in the tubules of the testes (kirby and Froman 2000). and the production of androgens secretion of testosterone which occurs in the leydig cells of the interstitial tissue (Lofts and Murton, 1973).

Theaim of this study is to build up some anatomical remarks and to document the gonadal cycles of perdix which support also the physiological and pathological fields.

MATERIALS AND METHODS

Ten adult male perdix are purchased from Baghdad city, Iraq. These adult perdix are kept under light (14 hours light/10 hours' dark light cycle) and are given feed and tap water adlibitum. Abdominal laboratomy is done for each perdix.

The testes are obtained immediately after euthanasia of these perdixthey fixative , appropriate with of normal saline (about 50 ml), which used to wash out residual blood or debris. Each testis is transferred to a large volume of fixing formalin fluid (formalin 10%), in labeled containers (Luna, 1968).There by keeping postmortem changes at a minimum. The containers preferably shaked gently several times to make certain that the fixative has reached all surface of the testis. The period of fixation is for 24h. After fixation with 10%formalin is accomplished, washing out of

the testis with running water for 2-3 hours in order to prevent interference with subsequent processes. Dehydration of testis samples are to remove of all extractable water. The dehydration process is done by using a graded series of ethanol (60, 70, 80, 90, 95 and 100%) . Xylene is the most widely used as clearing reagent and is important to clear opacity from dehydrated tissue and making them transparent. The testis of perdix are placed in small container and filled immediately to orient the samples within the containers in order to determined the proper surface for sectioning (Edward, 1962).The next subsequent process is to cut the testis by using rotary microtome. The thickness of cutting is about (5-7) micrometer .There are three kinds of stains were using:
Harris heamatoxylin and Eosin, Weigarts iron hematoxylin and Periodic acid Schiff stain (Luna, 1968).

RESULTS

The current study confirm that the male reproductive system of perdix consists of paired testes, epididymides, deferent ducts, and a single phallus which is the copulatory organ. The testes remain within the abdominal cavity and connect with the dorsal wall via mesorchium . Spermatic cord, tunica vaginalis, and scrotum are lacking. There is no accessory genital glands and urethra. The result showed that testis of adult perdix was covered by tunica albuginea, which resemble the capsule.(fig.1)Without septa, to divide the testis into separated lobules. The sequence events of spermatogenesis and spermiogenesis in adult perdix seminiferous tubules start with spermatogonia. (Spermatogonia type (A) or dusty type, Spermatogonia type (B) or Crusty type and Spermatogonia type (I) or Intermediate type).
The our result appear that Spermatogonia type (A) lie on the basement membrane of the seminiferous tubules and adjacent to the lateral surfaces of sertoli cells. With cytoplasm is different in density from cell to another and appears as dusty shape. The nuclear chromatin seen in the form of heterochromatin located on one side of the nucleus. While B-spermatogonia is rather bigger than the spermatogonia type -A. with big elliptical or somewhat round nuclei and it’s chromatin is somewhat less homogenous

and some flakes of chromatin are grouped in a crusty shape along the nuclear membrane.(fig.2)

Intermediate spermatogonia (I-spermatogonia)., represent the intermediate stage between the dusty and crusty type spermatogonia .which are smaller than A- spermatogonia located far from basement membrane of seminiferous tubules. Coarsely clumped chromatin are found in their nuclei.

The results of Subsequent mitotic division of B- spermatogonia give rise to primary spermatocyte. Which appear six different phases. As the following.

The primary spematocytes in Perleptotene phase posses small round nuclei. And the chromatin of their nuclei show star shaped represented by two or three large pieces of chromatin. Delicate flakes of chromatin are associated with these chromatin.

The primary spermatocytes appear far from the basement membrane of the convoluted seminiferous tubules. Round nuclei are present in these leptotene primary spermatocytes. The chromatin content of these nuclei is in the form of granules which aggregated in one pole of each nucleus. Filaments of chromatin protruded from these granules towards the nuclear membrane. (fig.2)Zygotene phase of primary spermatocytes have dark looser chromatin filament distribute throughout the most of the nucleoplasm. Chromatin flakes form also a network. In pachytene phase the chromatin mass distributed throughout the nuclei. The nucleoplasm has also chromatin filament. Presence of heavily stained band of cytoplasm often seen around the nuclei. In diplotene phase, two chromatids are well observed which remain in relationship with the centromere.(fig.3)The nuclear membrane and nucleolus in diakinesis phase are disappeared. The two chromatids begin to move a part and more further from each other. Crusts of chromatin in diakinesis primary spermatocytes become coiled aroundtheirself.(fig.3) The chromatin material of the secondary spermatocytes are scattered evenly throughout their nuclei in the form of flakes and fine granules.The secondary spermatocytes are nearly centrally located within the seminiferous tubules.With the appearance of the spermatids, spermatocytogensis end.

Based on change in the acrosomes and nuclei, the spermatids, develop into mature sperms. These change show 17 phases. Which are.

1- The First Stage:
The spermatid is small in size and has round nucleus. The chromatin contains small granules. Chromatin flakes are also present distributed within the nucloplasm. (fig.2)

2- The Second Stage:
Few small flakes positioned near to the centre of the spermatid nucleus. Moreover, small chromatin flakes adhere to the nuclear membrane.

3- The Third Stage:
Crust of chromatin is found nearer to the nuclear membrane of the spermatid. Chromatin filaments extend from the center of spermatid nucleus and reach its periphery.

4- The Fourth Stage:
The spermatid has round nucleus whichposses three to four chromatin masses. Beside, differentiated pieces of chromatin crust are found in adherence or nearer to the nuclear envelope. (fig.2)

5- The Fifth Stage:
The nuclear chromatin aggregated at one pole of the cell with Elongation of the nucleus is happened and appears as a ring or triangular in shape.(fig.3)

6- The Sixth Stage:
More elongation of spermatid nucleus is noticed. Two protrution from the aggregated chromatin are present and look like two horns.

7- The Seventh Stage:
Three chromatin horns are formed in the spermatid nucleus.

8- The Eight Stage:
The elongated nucleus becomes irregular in shape and slender in appearance. Condensation of some of nuclear chromatin at one pole, rest of the nuclear chromatin become blended to attach the other pole of the nucleus. (fig.2)

9- The Ninth Stage:
The nucleus has new oval shape and surrounded by thick cellular cytoplasmic band.

10- The Tenth Stage:
In this stage, the nuclear spermatids appear more flat and elongated. Disappearance of cytoplasmic band is also happened.

11- The Eleventh Stage:

Can be considered as the beginning of the maturation of the spermatids.The elongated nuclei occupy the most spaces of the cells and the Flagellum appears clearly in each spermatid.

12- The Twelve Stage:(Golgi phase)

The anterior end of the spermatid nucleus is wide in appearance, while posterior end is more slender. Homogenous chromatins with one or two vacuoles in the cytoplasm are present.(fig.4)

13- The Thirteen Stage:(Cap phaes)

The end of each nucleus expanded to the exterior and represent as converged slimy horns.

14- The Fourteen Stage:

The nuclear chromatin has the form of U**-**shape and posses large granular chromatin.(fig.4)

15- Fifteen Stage:

In this stage, the nuclear chromatin is dense and show five short chromatin protrutions.(fig.4)

16- Sixteen Stage:(Acrosomal phase)

The nuclear chromatin changed to V-shape which look like two short slimy horns. Each horn is covered by arch of chromatin which extends outside the nuclear membrane.(fig.4)

17- Seventeen Stage:(Maturation phase)

The result in current study appear that the sperms within the lumen of the seminiferous tubule or attached to the sertoli cells are seen The spermatozoa of pigeon is an elongated, flagellate cell which is classically divided into three parts, the head, the middle-piece and the tail. While the sperm head of pigeon is a long, cylindrical object with a slightly taper towards the anterior end.

Sertoli Cells in the Seminiferous Tubules of Pigeon Testes:

Sertoli cells possess elongated nuclei which are often triangular in outline. And its nuclei exhibit little hetrechromatin. With foamy cytoplasm .They form an extensive ramification around and between the germinal cells. The bases of the sertoli cells adhere to the basal lamina of seminiferous tubules. (fig.3)

Interstitial Tissue and Leydig Cells:

It consists of loose connective tissue with scattered, elongated fibroblast, blood vessels and interstitial cells of leydig. Leydig cells are scattered singly and in small groups and are chiefly found in

the larger intertubular space. They may bepolyhedral or irregular, somewhat flattened or even elongated.

Basement Membrane of Seminiferous Tubule ofTestes:

The germinal epithelium and sertoli cells of the seminiferous tubule are supported by a wall or the basement membrane.The basement membrane is composed of a layer of collagen fibers, associated cells of fibroblast and myoepithelial cells or myoid cells. Myoid cells are usually elongated cells with oval nuclei. (fig.2)

Discussion

The capsule or tunica albuginea of the testis of perdix is very thin and do not send septa. In this case, no separated lobules are present, and no mediastinum is observed in the testis of perdix. This coincides with the observations of De Reviersand Williams (1984) in his study on the testes of cockerel and agreed with Hussin (1996) in Turkey (Meleagrisgallopavo). The convoluted seminiferous tubules of perdix testis is supported by a basement membrane. The cytology of germ cells present in the seminiferous tubules of adult perdix testis revealed three types of spermatogonia; A-spermatogonia, B-spermatogonia and intermediate (I) spermatogonia. The spermatogonia in perdix in this research revealed mitotic activity in several stages of the cycle to give rise to primary spermatocytes. The primary spermatocytes are large and spherical in outline. They are usually found in the adluminal part of the seminiferous epithelium. The primary spermatocytes show six different phases including (preleptotene, leptotene, Zygotene, Pachytene, Diplotene, Diakinesis).

The secondary spermatocytes are about one_halve the size of primary spermatocytes and being short lined cells. These cells occur nearer to the lumen of the seminiferous tubule. This description of primary and secondary spermatocytes is in agreement with the finding of Yamamato*et al*., (1967) in male Japanese quail ,with the finding ofHussin (1996) in his study in Turkey and with Al-Shamary (2001) in common quail.

The meiosis of the secondary spermatocytes in this result lead to seventeen stages. This result are not similar to the result of

Gunowardana and Scott (1977) who registed four stages present in adult white Leghorn cocks and in contrast to Hussin (1996) in adult male Turkey and Al-Shamary (2001) in common quail whom they registed fifteen stages respectively. The more important signes of differentiation steps of the sequence events in the seminiferous epithelium of perdix testes which result in spermatozoa include: Golgi phase or (stage 12), Cap phase or (stage 13), Acrosome phase or (stage 16), Maturation phase or (stage 17). The nucleus of Golgi phase is wide anteriorly and more slender posteriorly. In Cap phase, the nucleus posses two converged slimy horns. The nuclear chromatin changed to a V_shape in acrosome phase. The final stage of differentiation represented by the appearance of elongated, flagellated sperm which resemble the maturation stage. These previous stages of differentiation in pigeon are also similar in common quail studied by Al-Shamary (2001) and in ram studied by Al-Khuzae (2007) and in goat studied by Al-Hamery (2008).

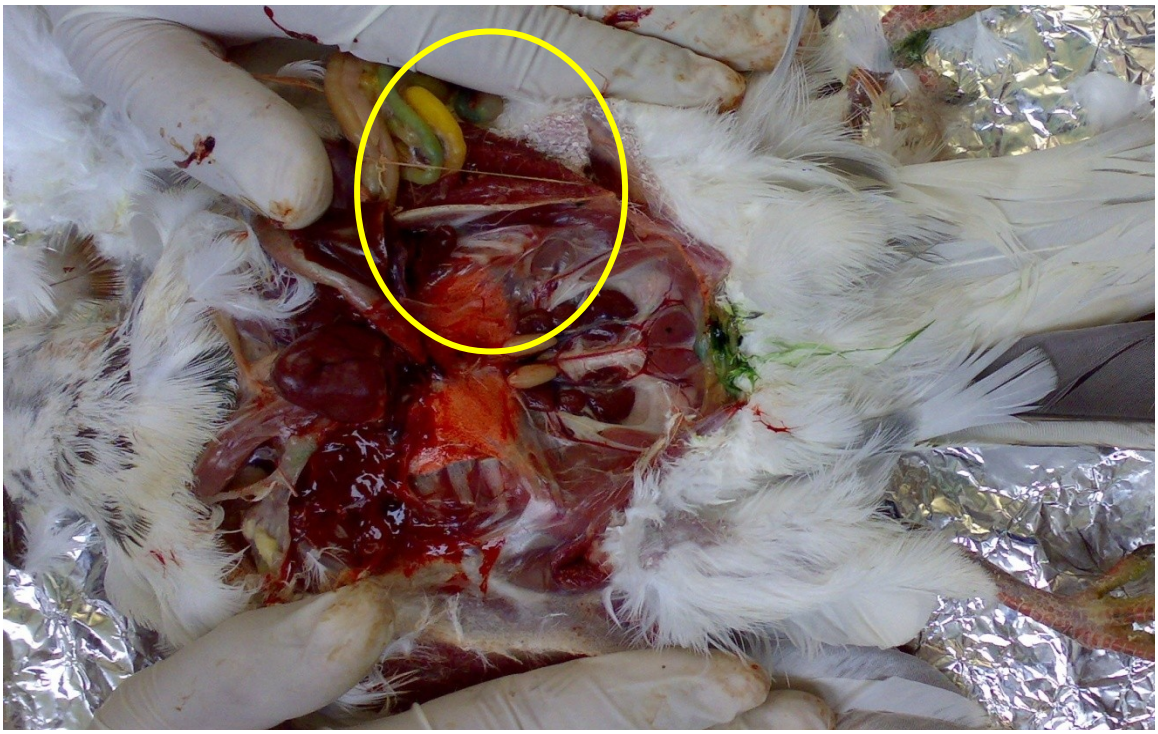

Figure (1)Abdominal cavity and right and left testis of adult male perdix

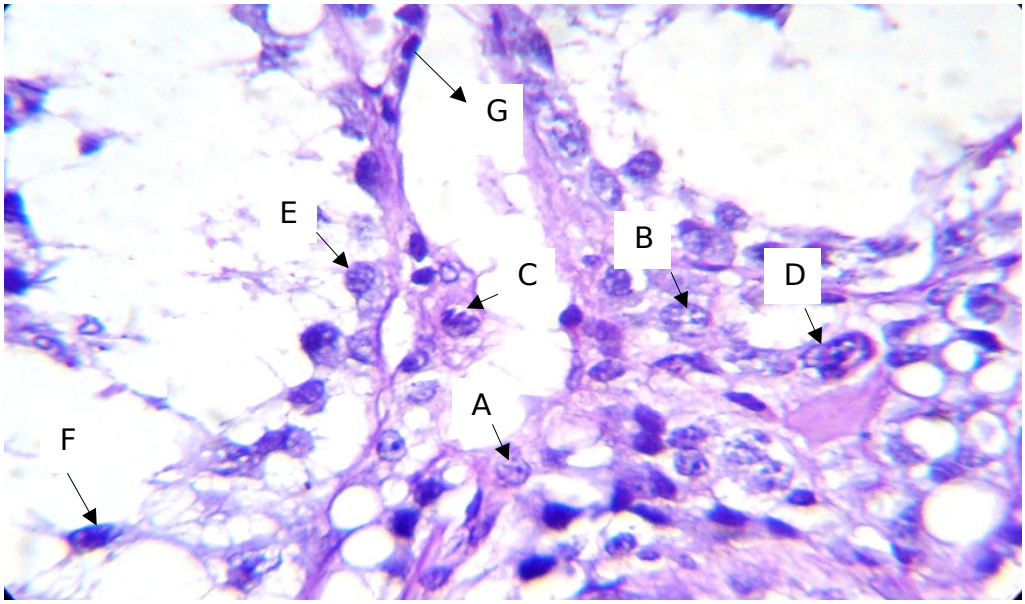

Figure 2: A: First Stage .. B: ZygotenePhase .. C: Fifth Stage .. D: Eighth Stage ..E: LeptotenePhase .. F: Fourth Stage ..

G:MyiodCell ..PAS stain (X100)

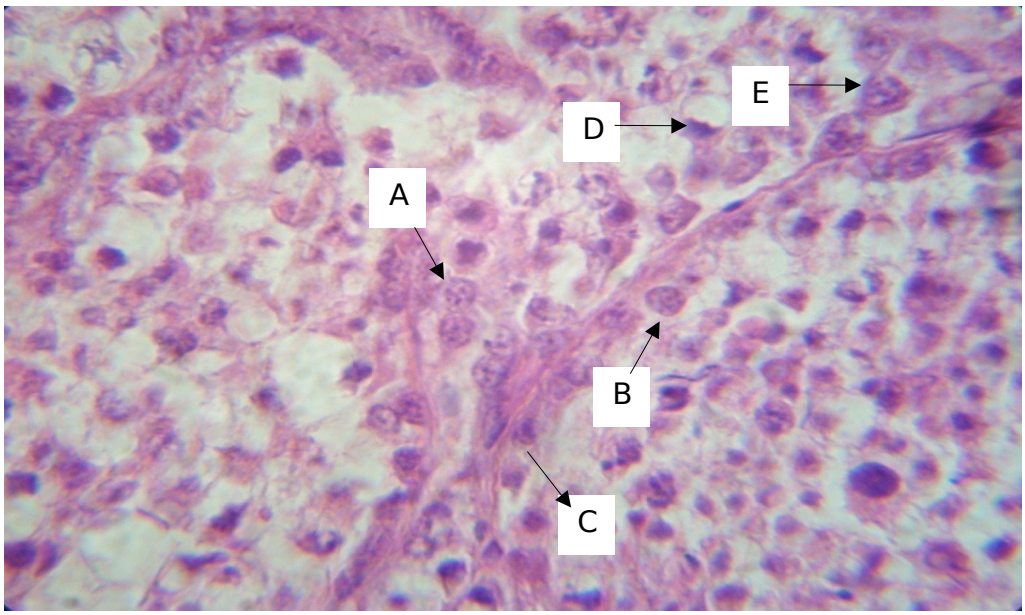

Figure 3: A: Diakinesis Phase .. B: diplotene Phase .. C: B-Spermatogonia .. D: Fifth Stage.. E: Sertoli cell

Harris hematoxylin and eosin stain (X100)

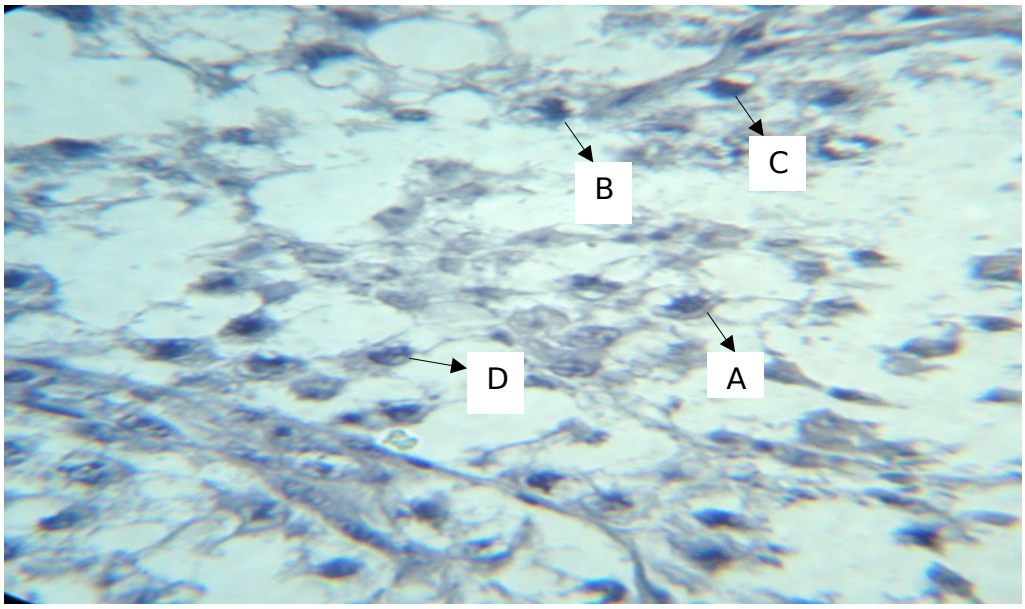

Figure 4: A: Fifteen Stage .. B: Fourteen Stage .. C: Twelve Stage .. D: Sixteen Stage ..
Weigerts iron hematoxylin stain (X100)

Reference

AL-Hameary, H. D. (2008). The sequence event of spermatogenesis and spermiogenesis in adult goat (Carpus Hericus). Ms c. a thesis submitted to the college of veterinary medicine university of Baghdad ,Baghdad –Iraq .

AL-Khuzae ,B. A. (2007). Study of seminferous epithelium and spermatogensis in adult (indogenous) ram . Ms c. a thesis submitted to the college of veterinary medicine university of Baghdad ,Baghdad –Iraq .

Al-Samarrae, N.S; Al-Jobori, K.A; Al-Hathry,S.A.and Al-Shamary, B.F. (2000). Topographical and histological structure of ducts epididymids of common quail (Coturnix C. Coturnix L). The Iraq J. Vet. Med. 24: 169-178.

Al-shamary, B.F. (2001).Anatomical and histologicl study of the male genital system and some ultrastructure studies of spermatogenesis in common quail (coturnix c. coturnix L)Ms.Cthesis.College of Vet. Med.BaghdadUniversity.Iraq.

Austen, O.L. (1961). Birds of the fowl 1st ed., Golden press, New York, U.S.A.

Brown, L. and Amadon, D. (1968). Eagles, Haks and Falcons of the word.1st ed. McGrawn-Hill New York, U.S.A.
De Reviers M, Williams JB. (1984). Testis development and production ofspermatozoa in the cockerel (Gallus domesticus). In"Reproductive Biology of Poultry" (F. J. Cunningham, P. E. Lake,and D. Hewitt, eds.), pp. 183-202.
Edward ,G. (1962).Staining animal tissue. Practical 1 steds .Leandard hill (Books),LTD. London .U.K.
Gunawardana, V.K., and Scott, M.G (1977). Ultrastructure studies on the differentiation of spermatids in the domestic fowl. J. Anat. 124,: 741-755.
Hussin, A.M. (1996).Gross anatomical and Histological study of the Male reproductive system in Turkey Meleagrisgallopavo,Ms.Cthesis.College of Vet. Med.BaghdadUniversity.Iraq.
Kirby JD, Froman DP (2000) Reproduction in male birds. In:Whittow GC (ed) Avian physiology. Academic, London, U.K.pp597–615.
Lofts B, Murton RK (1973) Reproduction in birds. In: Farner DS,King JR, Parkes KC (eds) Avian biology. Academic, New York, U.S.A.PP 1-107.
Luna, L.G. (1968): Manual of histologic staining method of armed forces institute of pathology 3ed. New York, U.S.A. P.:123.
Marvan, F.(1969).Postnatal development of the male genital tract of the Gallus domesticus.Anat.Anz.,124,443-462.
Stoll, R. and Maraud, R. (1955).Sur la constitution de 1’epididyme du cop.G.r.Soc.Biol.,Paris, 149,687-689.
Yamamoto, S. Tamate,H. and Itikawa,O.1967.Morphological studies on the sexual maturation in the male Japanese quail, coturnixcoturnixJapanica .II.The germ cells types and cellular association during spermatogenesis. Tohoku J.Agr.Res.18,27-30.

الحوادث الدورية لنشأة و حؤول النطفة في الحنجلالبالغجنس (***PerdixPerdix***.)

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الخلاصة
استهدفت الدراسة بعض الملاحظات التشريحية للجهاز التناسلي الذكري في الحنجل البالغ والتركيب النسيجي لخصى الحنجل والحوادث الدورية لنشأة وحوؤل النطف. شملت الملاحظات التشريحية للجهاز التناسلي الذكري للحنجل وجود زوج من الخصى داخل التجويف ألبطني ومعلقة بالجدار الظهري بواسطة مسراق الخصية. تغلف الخصية بالغلالة يفتقد الجهاز التناسلي الذكري في الحنجل إلى الحبل المنوي و الغلالة البيضاء وكيس الصفن.يمتلك النبيب المنوي ظهاره منوية تشمل الخلايا النطفية وخلايا سرتولي. تقع الخلايا النطفيه على الغشاء القاعدي والذي يحاط بنسيج خلالي يحتوي على خلايا لايدك. تبدأ الحوادث الدورية لنشأة وحوؤل النطف في النبيبات المنويه للحنجل البالغ من سليفات النطف. يوجد ثلاثة انواع من سليفات النطف تشمل سليفات النطف نوع -أ وسليفات النطف الوسطيه وسليفات النطف نوع ب. تنشأ الخلايا النطفيه الابتدائيه من سليفات النطف نوع ب. تمر الخلايا النطفيه الابتدائيه في ستة اطوار تمايزيه وتشمل هذه الاطور (الطور قبل الخيطي,الطور الخيطي ,الطور الاعتناقي,الطور التغلصي,الطور التضاعفي والطور الحركي). تشتق الخلايا النطفيه الثانوية من الخلايا النطفيه الابتدائية وتتميز بقصر عمرها. تنتهي مرحلة نشووء النطف حين ظهور الطلائع النطفيه. تمر الطلائع النطفيه بمراحل معقدة تعتمد على التغيرات في الجسيمات الطرفيه ونوى الخلايا. تشمل هذه المراحل سبعة عشر مرحله لتكوين الحيامن. تمتلك خلايا سرتولي على نواى متطاولة او غالبا تكون مثلثة الشكل. تمتد من خلايا سرتولي استطالات تتصل وتحيط الخلايا الجرثومية بشدة. لم تشخص مراحل حوادث خلايا سرتولي. تنتشر خلايا لايدك بصوره مفردة ومجاميع صغيره وتوجد بصوره رئيسة في النسيج بين النبيبات المنويه الكبيرة. يتكون الغشاء القاعدي للنبيب المنوي الملفوف من طبقة من النسيج الضام والخلايا الظهارية العضلية. تكون الخلايا العضلية متطاولة وذات نوى بيضويهتحاط بكمية ضئيلة من الهيولي.