PRE-AND POST-HATCHING DEVELOPMENTAL STUDIES ON THE TESTIS OF DOMESTIC FOWL

El-Morsi, S. E.; Selim, A. A.*; Ebada, S. M. and El. Doumani, H. A. A.

Dept. of Anatomy, Faculty of Veterinary Medicine, Mansoura University

*Dept. of Anatomy, Faculty of Vet. Med., Zagazig University

ABSTRACT

The present study was carried out on 20 testes specimens (8 specimens from 10- and 20-day-old chick embryos and 12 specimens from 1, 6 and 17-week-old chicks) of domestic fowl, Cobb breed. At 10-day-old chick embryo, seminiferous cords lined by many pre-Sertoli cells and few primordial germ cells (PGCs), spermatogonia appeared at 20-day-old chick embryo. These cords are tubulated at one-week-old chick and reach the sexual maturity by appearance of free spermatozoa at 17-week-old chick. The early development of contractile elements was detected by distinct ?SMA immunoreaction in the testis of 10-day-old chick embryo. The immunoexpression of CK were firstly demonstrated in the supranuclear region of lining epithelium of seminiferous tubule at 6-week-old chicks.

INTRODUCTION

Poultry as an important source for protein production have gained much attention and the development of their male reproductive organs is of great concern to poultry breeders **(Kirby and Froman, 2000)**. Many developmental studies were carried out on different breeds of fowl and birds as White Leghorn chick **(Venzke, 1954; Romanoff, 1960; Stahl and Carlon**, 1973; **Van Krey**, 1990; **Morrish** and **Sinclair, 2002** and **González-Morán** and **Soria-Castro, 2010)**; Fayoumi chick **(Kamar**, 1960 and **Abd-Elhaseep (1994); pigeon (Bhujle et al., 1979)** and turkey **(Noirault et al., 2006 and Bakst et al., 2007)**. Moreover, other studies were investigated the testis of sexually mature male Japanese quail **(Yamamoto et al., 1967; Clulow** and **Jones, 1982** and **Lin et al., 1990); drake (Marchand** and **Gomot, 1973)**; fowl **(Gunawardana, 1977** and **González-Morán** and **Soria-Castro, 2010)**; guinea fowl **(Aire et al., 1980 and Brillard, 1986)**; in pigeon **(Orsi et al.**, 1984) and in turkey **(Aire, 2003** and **Bakst et al., 2007)** concluded that, the seminiferous epithelium represented by spermatogonia, primary and secondary spermatocytes, round and elongated spermatids. Unfortunately, only preliminary notes were provided by recent few immunohistochemical studies that were demonstrated on the testis of sexually mature Japanese quail **(van Nassauw et al., 1993)**; domestic fowl **(Maretta and Marettovà, 2004)**; mallard duck and turkey **(Aire and Ozegbe, 2007)**. Therefore, the present study was carried out to complete our knowledge by giving an overview on development of

testis in fowl during the pre-and post-hatching life by using different histological and immunohistochemical techniques. In order to provide more understanding for researchers who deal with the organogenesis among the chick embryo and poultry reproduction.

MATERIAL AND METHODS

The present study was carried out on 20 testes specimens (8 specimens from 10-and 20-day-old chick embryos and 12 specimens from 1, 6 and 17-week-old chicks) of domestic fowl, Cobb breed. For the pre-hatching studies, the specimens were collected from embryos obtained from incubated fertilized eggs at commercial incubator of Malaa, Mansoura, Dakahlia, Egypt. For the post-hatching studies, the specimens were collected from chicks raised under the scientific information essential for breeders. The allover collected specimens were fixed in Bouin's fluid and/or 10% neutral buffered formalin solution, processed and treated under the normal histological technique and sectioned at 5-7 µm. Some of obtained sections were utilized for staining with iron haematoxylin and eosin, PAS, Alcian Blue (pH 2.5) and Crossman's trichrome stains according to **Bancroft and Stevens (1990)**. As well as, the other obtained sections were used for immunohistochemical staining of both α smooth muscle actin (α SMA) and cytokeratin (CK) according to **Kumar and Rudbeck (2009)**. The all histological and immunohistochemical stained sections were investigated under the light binuclear microscope and photographed by Olympus CX41 photomicroscope. The nomenclatures used during this work were adapted to Nomina Anatomica **Avium (1993)**, Nomina Histologica **Veterinaria (1994)** and Nomina Embryologica **Veterinaria (2006)** and the available literature whenever it was possible.

RESULTS

At 10-day-old chick embryo, the testis appeared at the ventromedial aspect of mesonephros, protruded into the coelomic cavity in the space between the mesonephros and the dorsal mesentery of the gut with narrow attachment on both sides (Fig. 1). The testicular capsule consisted of tunica serosa and tunica albuginea. The tunica serosa is outer covering layer and representing the coelomic epithelium. It comprised of single thin layer of flattened or squamous cells with elongated oval nucleus and dense cytoplasm. The primitive tunica albuginea appeared more cellular and formed by circularly oriented condensation of mesenchymal cells under the surface epithelium to form with small blood spaces. There were no clearly defined branches of tissue leaving the tunica albuginea to enter the testicular parenchyma as testicular septa. The cells of testicular cords are basally located and forming a complete row at the periphery of the cords. They rested on a well-developed basal lamina delineating them from the surrounding interstitium. The pre-Sertoli cells are often radially oriented within the cords. Their nuclei are large, rounded with peripheral clumps of heterochromatin associated with the nuclear membrane. The nucleolus is often present. The PGC nucleus showed some mitotic figure. The interstitium are mesenchymal tissue with mesenchymal cells (Fig. 2).

At 20-day-old chick embryos, the spermat-

ogonia were observed at this stage of development as a result of mitotic activity of PGCs. The spermatogonia appeared smaller than the PGCs and has rounded euchromatic nucleus with nucleoli (Fig. 3).

The interstitium has significantly expanded in this age and their component more clearly differentiated including; premature Leydig cells, peritubular cells, loose network of undifferentiated mesenchymal cells, connective tissue cells, interstitial macrophages, blood spaces and degenerated cells. The premature Leydig cells are large cells with large oval nucleus contains one or 2 nucleoli. The interstitial macrophages are much smaller than the premature Leydig cells. They appeared oval, rounded or irregular in shape with oval deeply stained eccentric nuclei and lightly stained cytoplasm with characteristic strong PAS materials. The testicular cords are rapidly surrounded by a marked PAS positive basal lamina and elongated peritubular cells. The degenerated cells were appeared as densely stained cells showed condensation of nuclear chromatin and irregular nuclear membrane. (Fig. 4).

At one-week-old chick, the testis showed marked thickening in the testicular capsule due to thickening of tunica albuginea that represented by several layers of circularly oriented fibrous connective tissue with strong PAS positive reaction at its outer half. The testis showed characteristic evidence that some seminiferous cords luminated and become tubules. The lumen is extending for some distance but not continuous (Fig. 5).

At 6-week-old chick, spermatogonia were defined based on their nuclear morphology and chromatin distribution into; the dark (A) and pale (B) types. Type (A) was close to the basement membrane, have relatively large, ovoid and darkly stained nucleus. Its chromatin is homogeneous, finely granulated and appears dust-like. Type (B) was characterized by a relatively large round-to oval nucleus containing dispersed chromatin (hence the pale appearance) with 0 to 3 prominent nucleoli. The resting or preleptotenic primary spermatocytes have large, rounded euchromatic nucleus. These cells entering the stages of first meiotic prophase that subdivided into the leptotene, zygotene, pachytene and diplotene stages according to the characteristic changes in nuclear chromatin. At leptotene stage, the chromosomes began to condense and arranged in thread like strands dispersed throughout the nucleus. At zygotene stage, chromosomes began to thicken and become polarized. At pachytene stage, the synaptic homologous chromosomes appear denser and were irregularly distributed in the nucleus. Diplotene nuclei possessed more compact chromosomes evenly dispersed throughout the nuclei (Fig. 6).

At 17-week-old chick, the gradual cellular arrangement of spermatogenesis is now completed by appeared of secondary spermatocytes, round and elongated spermatids. The primary spermatocytes are the largest spermatogenic cells in the tubular epithelium that located in an intermediate position between the spermatogonia and secondary spermatocytes. The round spermatids are the smallest round cell, lay closer to the lumen with spherical nuclei in which a few chromatin masses are scattered in a fine network. The elongated

spermatids attached to the free apical border of the seminiferous epithelium. The mature elongated spermatid eventually gives rise to the mature free spermatozoa in the tubular lumen (Fig. 7).

The testicular capsule was wrinkled and the outer half of the tunica albuginea was almost exclusively populated by circularly arranged, compactly packed, coarse bundles of collagenic fibers. Large lymphatic and blood spaces mostly veins ran circularly through the tunica albuginea. The peritubular tissue showed very few thin collagenic fibers with no evidence for testicular septa (Fig. 8).

The immunoexpression of αSMA is firstly detected in the testis of 10-day-old chick embryo with weak immunopositive reaction at peritubular, intertubular tissue and the tunica albuginea of testicular capsule. The tunica media of interstitial blood vessels is immunoreacted strongly (Fig. 9). The testis of 20-day-old chick embryo and one-week-old chick showed the same immunolabelling expression of that of 10-day-old chick embryo. Increasing the intensity of immunoexpression of αSMA to moderate degree in all parts of the testis including the inner half only of testicular capsule of 6-week-old chick were clearly observed (Fig. 10), that reach its peak with strong immunopositive reaction at 17-week-old chick (Fig. 11).

Concerning the immunoexpression of CK, all parts of the testis at 10-and 20-day-old chick embryos and one-week-old chick showed negative CK immunoreactions. The immunoexpression of CK were firstly demonstrated in the supranuclear region of lining epithelium of seminiferous tubule in 6-and 17-week-old chicks (Fig. 12). In this study, the testis showed negative reaction to the Alcian blue stain (pH 2.5) in all ages.

DISCUSSION

The present study revealed that, the testis was formed at the ventromedial surface of the mesonephros at 10-day-old chick embryo. This finding is correlated with the statement of **Venzke (1954); Romanoff (1960); Stahl and Carlon (1973)** and **Smith (2007)** in fowl. In agreement with **Narbaitz and Adler (1968) and Van Krey (1990)** in White Leghorn chick embryo, the testis covered with single layer of squamous cells representing the coelomic epithelium and the primary sex cords formed mainly of mesenchymal cells with few PGCs inbetween. Furthermore, At 10-day-old chick embryo, the cells of testicular cords (pre-Sertoli cells and PGCs) are basally located and forming a complete row at the periphery of the cords, rested on a well-developed basal lamina delineating them from the surrounding interstitium. Several previous studied **(Romanoff, 1960; Van Krey, 1990; Abd-Elhaseep, 1994; Kirby and Froman, 2000 and González-Morán and Soria-Castro, 2010)** have been shown the same findings.

In our investigation, the seminiferous cords luminated and became tubules at one-week-old chick while the same finding observed at 12-day-old White Leghorn chick embryo **(Venzke, 1954)**; 19-day-old Fayoumi chick embryo **(Romanoff, 1960 and Abd-Elhaseep, 1994)** and at 30-day-old pigeon **(Bhujle et al., 1979)**. At 6-week-old chick, the seminiferous tubules characterized by

taller and more complex seminiferous epithelium internally and externally by one or two layers of peritubular myoid cells. These findings are coincided with that reported in fowl by **(Aire, 2007 and González-Morán and Soria-Castro, 2010)**.

In agreement with **(Marvan, 1969** and **Hodges, 1974)** in fowl and **Ghattas (1989)** in migratory birds, the testicular capsule was consisted of two tunica; outer thin tunica serosa that represented by flat or squamous cells and inner thick tunica albuginea that represented by circularly arranged bundles collagenic fibers with spindle-shaped, overlapping smooth muscle cells with blood and lymphatic spaces in between. However, Aire and **Ozegbe (2007)** in fowl, turkey, Japanese quail and duck observed that, the testicular capsule displayed three interconnected tunica; an outer tunica serosa, an intermediate tunica albuginea and inner tunica vasculosa.

Similar to that reported in fowl **(Hodges, 1974; Lake, 1981 and Maretta and Marettovà, 2004)**; in guinea fowl **(Aire et al., 1980)**; in Japanese quail **(van Nassauw et al., 1993 and Aire and Ozegbe, 2007)**; in turkey and mallard duck **(Aire and Ozegbe, 2007)**; in ostrich and emu **(Ozegbe et al., 2008)**; in Sudani duck and pigeon **(Abd-Elmaksoud, 2009)**, no obvious branching of testicular septa from the testicular capsule had been observed. However, **Bell and Freeman (1971)** in domestic fowl and **Ghattas (1989)** in migratory coot observed short and thick testicular septa were sent out from the testicular capsule to form supporting skeletal framework.

In this study, the inner half of testicular capsule showed αSMA positive reaction that coincided with that described in Japanese quail **(van Nassauw et al., 1993)**; in domestic fowl **(Maretta and Marettova, 2004)**; in turkey and mallard duck **(Aire and Ozegbe, 2007)** and emu **(Ozegbe et al., 2008)** while the outer half of the testicular capsule in ostrich **(Ozegbe et al., 2008)** and whole testicular capsule in Sudani duck and pigeon **(Abd-Elmaksoud, 2009)** showed the same αSMA positive reaction. Moreover, the peritubular tissue are found to contain myoid cells by its immunopositive reaction to αSMA in our study that parallel to that observed in fowl, turkey, Japanese quail and mallard duck **(Aire and Ozegbe, 2007)**; in ostrich and emu **(Ozegbe et al., 2008)**.

Van Nassauw et al. (1993) in quail reported that, smooth muscle actin is specific for contractile cells. Therefore, the peritubular cells in quail **(van Nassauw et al., 1993)**, fowl, turkey and duck **(Aire and Ozegbe, 2007)** and those of our study were of one type, i.e. myoid or smooth muscle cells, unlike the observations made by **Rothwell and Tingari (1979)** in fowl that both fibroblast-like and myoid cells were present into inner and outer layers, respectively in the peritubular tissue of the domestic fowl. The avian testes compared with those of mammals, produce large quantities of both fluid and spermatozoa in fowl **(Aire, 2007)**. Therefore, both the testicular capsule and peritubular tissue are capable of propelling the enormous amount of testicular fluid produced in the seminiferous tubules into the excurrent ducts of the testis by their contractile activities **(Aire and Ozegbe, 2007)**. Moreover, **Abd-Elmaksoud (2009)**

in fowl concluded that, tunica albuginea and peritubular smooth muscle dysfunction may be implicated in male infertility. The same author added that, away from its contractile ability, myoid peritubular cells engage in a dialogue with Sertoli cells which is crucially important for the structural formation of the blood-testis barrier. Peritubular myoid cells are known to stimulate total protein production by Sertoli cells and to increase the Sertoli cell production of androgen-binding protein and transferrin.

The tunica media of the blood vessels in the testis showed strong αSMA immunoreaction that coincided with that described in sexually mature male birds as quail **(van Nassauw et al., 1993)**, domestic fowl **(Maretta and Marettova, 2004)**; turkey and mallard duck **(Aire and Ozegbe, 2007)**; ostrich and emu **(Ozegbe et al., 2008)**; Sudani duck and pigeon **(Abd-Elmaksoud, 2009)**. Moreover, the distinct αSMA immunoreaction in the blood vessels of 10-day-old chick embryo in our study as an early embryonic age, revealing a good indicator for rapid early nutritional and/or functional supply and early development of contractile elements of the male genital system in chick embryo, otherwise the infertility will be occurred.

In agreement with **Aire and Ozegbe (2007)** in fowl, turkey, Japanese quail, duck and **Ozegbe et al. (2008)** in ostrich and emu, cytokeratin is not immunoexpressed neither in the testicular capsule nor peritubular tissue. The cytokeratin was immunoexpressed positive in the supranuclear region of the epithelial cells of the seminiferous tubules at 6-week-old chick. These findings are parallel with that described in fowl and turkey **(Aire and Ozegbe, 2007)** but disagree with that described in Japanese quail and duck **(Aire and Ozegbe, 2007)**, where the cytokeratin was absent. The last author added that, cytokeratin provide significant structural support correlated with increase of intraluminal pressure in the seminiferous tubules.

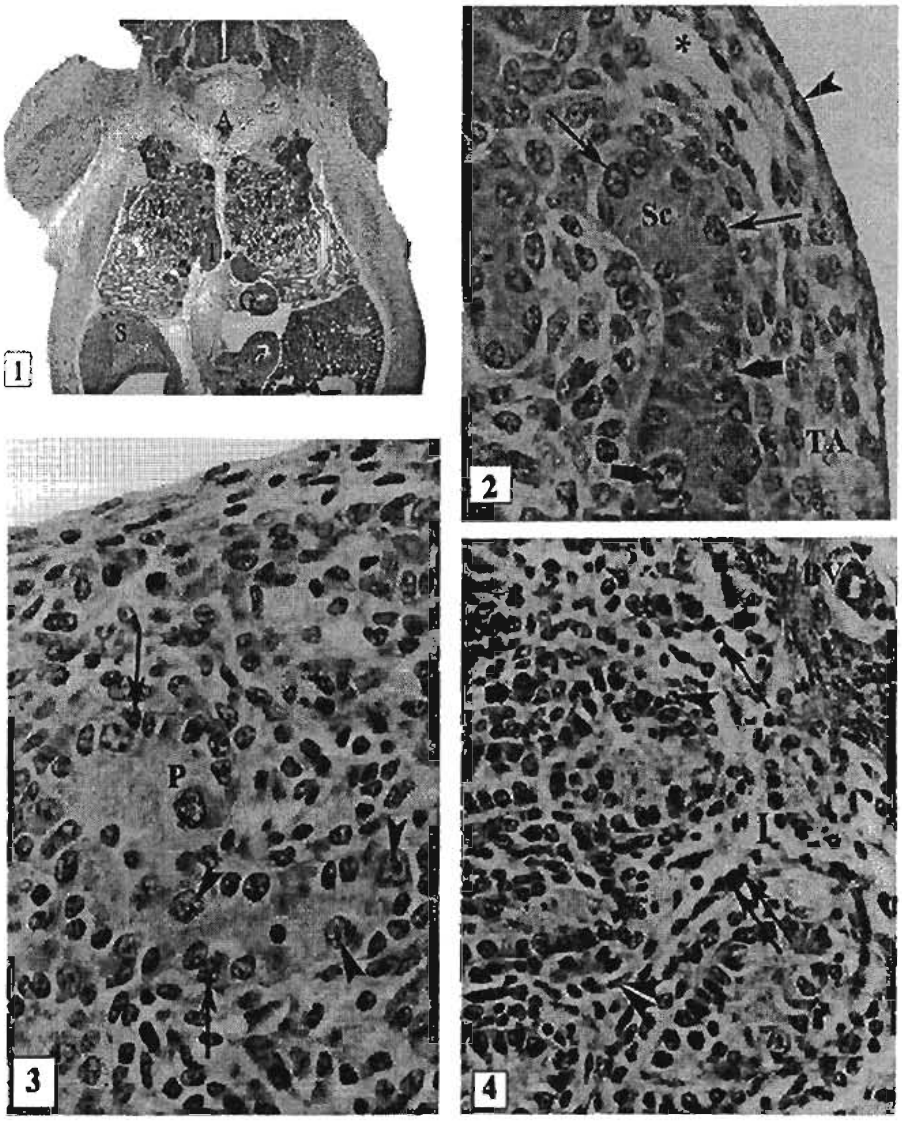

Fig. (1): A photomicrograph of in 10-day-old chick embryo showing the testis (T) at the ventromedial aspect of mesonephros (M), on either side of the dorsal mesentery (asterisk) of the gut (G). Dorsally the aorta (A) and ventrally, the liver (L) and stomach (S) on the right and left sides respectively, were identified. Stain; H&E. X 4.

Fig. (2): A photomicrograph of testis in 10-day-old chick embryo showing the tunica serosa (arrow head), tunica albuginea (TA) with its blood vessel (asterisk), seminiferous cord (Sc), PGCs (thick arrow) and Sertoli cells (thin arrow), and lined the. Stain: PAS/Haematoxylin. X 100.

Fig. (3): A photomicrograph of testis in 20-day-old chick embryo showing the Sertoli cells (arrow), spermatogonia (arrow head) and POC (P). Stain: PAS/Haematoxylin. X 100.

Fig. (4): A photomicrograph of testis in 20-day-old chick embryo showing the premature Leydig cells (double thin arrow), degenerated cell (thin arrow), interstitial macrophage (thick arrow), fibroblast (double arrow head), peritubular cells (arrow head) and blood vessel (BV) appeared in the interstitium (I). Stain: PAS/Haematoxylin. X 100.

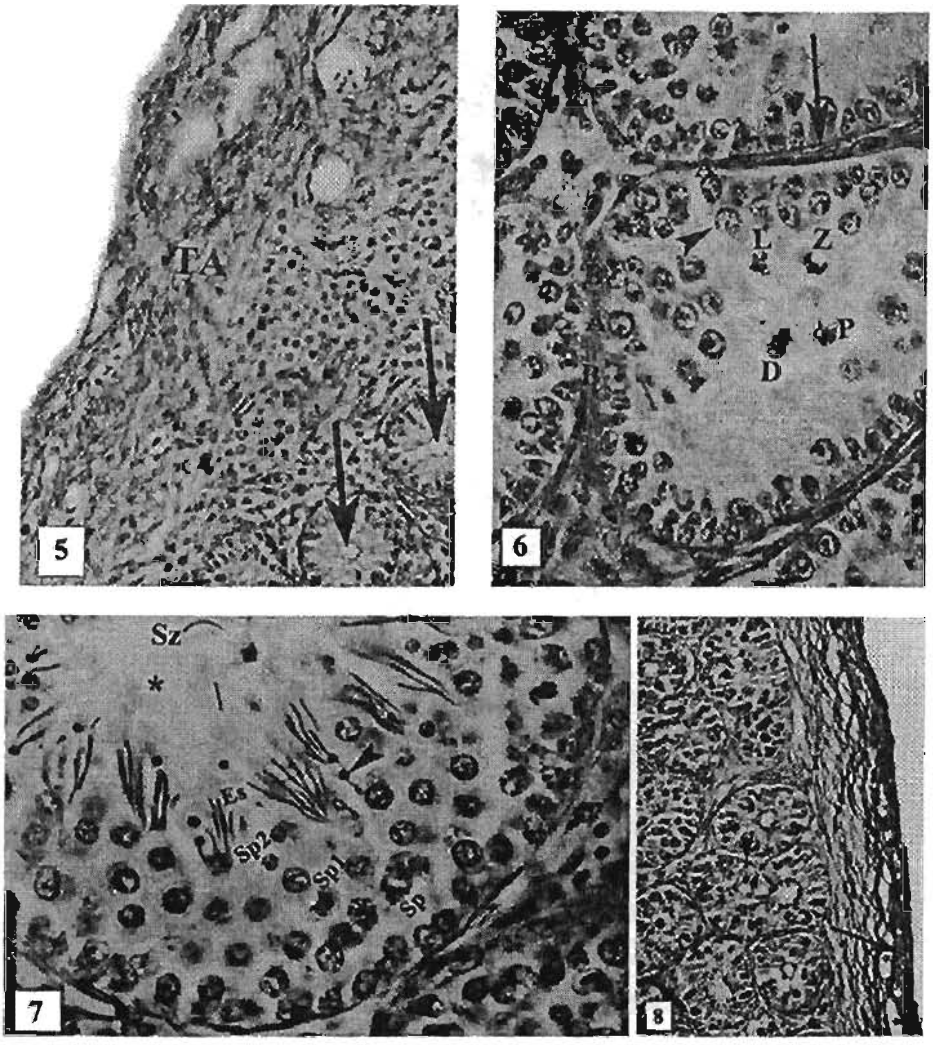

Fig. (5): A photomicrograph of testis in one-week-old chick showing the PAS positive reaction at the outer half of tunica albuginea (TA) and the lumen of the seminiferous tubules (arrow). Stain: PAS/ Haematoxylin. X40.

Fig. (6): A photomicrograph of testis in 6-week-old chick showing the spermatogonia type A (A) and type B (B), preleptotene primary spermatocytes (arrow head), leptotene (L), zygotene (Z), pachytene (P) and diplotene (D) primary spermatocytes and peritubular cell (arrow). Stain: PAS/Haematoxylin. X 100.

Fig. (7): A photomicrograph of testis in 17-week-old chick showing the gradual arrangement of the spermatogonia (Sp), primary spermatocytes (Sp1), secondary spermatocytes (Sp2), Round spermatid (arrow head), elongated spermatids (Es) and free spermatozoa (Sz) in the lumen of seminiferous tubule (asterisk). Stain: PAS/Haematoxylin. X 100.

Fig. (8): A photomicrograph of testis in 17-week-old chick showing thick collagenic fibers at the outer half (O) of tunica albuginea rather than its inner half (I), peritubular fine collagenic fibers (arrow head), blood (arrow) and lymphatic (asterisk) spaces were observed. Stain: Crossman's trichrome. X 40.

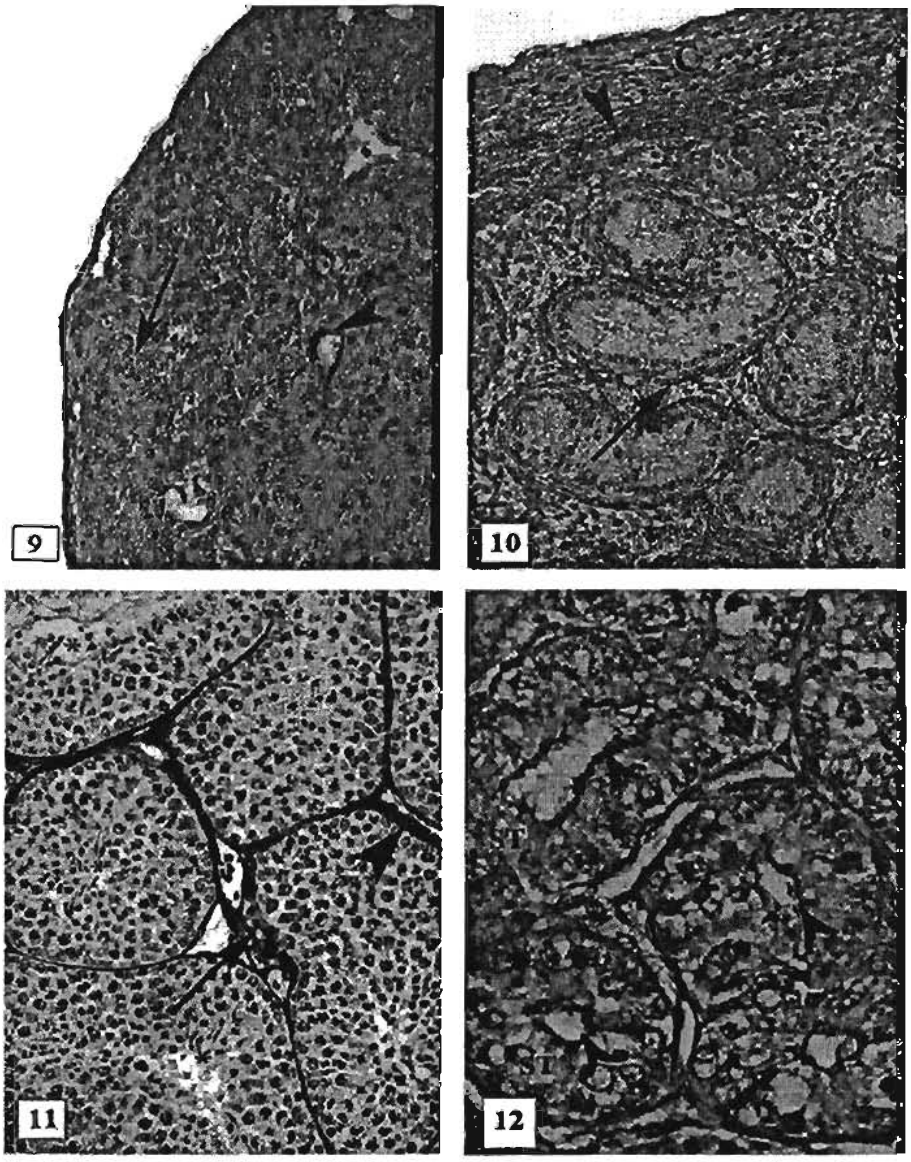

Fig. (9): A photomicrograph of the testis in 10-day-old chick embryo showing weak immunopositive reaction at the interstitial tissue (arrow) and strong immunopositive reaction at interstitial blood vessels (arrow head) to ?SMA. X 40.

Fig. (10): A photomicrograph of the testis in 6-week-old chick showing moderate immunopositive reaction at inner half (arrow head) of the testicular capsule (C), peritubular (arrow) and intertubular tissue (asterisk) to ?SMA. X 40.

Fig. (11): A photomicrograph of the testis in 17-week-old chick showing strong immunopositive reaction at the peritubular (arrow head) tissue with its blood vessel (arrow) to ?SMA. Note the elongated spermatid (asterisk). X 40.

Fig. (12): A photomicrograph of the testis in 6-week-old chick showing strong immunopositive CK reaction (arrow head) at the supranuclear region of the epithelium of the seminiferous tubule (ST). X 100.

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الملخص العربى

دراسات على نمو الخصية فى الدجاج المستانس قبل وبعد الفقس

صلاح المرسى فـــرج عاطف عبدالعزيز السيد سليم*
صفوت عباده محمـد هيثم عبد اللـــه الدومـــانى

قسم التشريح و الأجنة ، كلية الطب البيطرى ، جامعة المنصورة

قسم التشريح و الأجنة ، كلية الطب البيطرى، جامعة الزقازيق*

لقد أجريت هذه الدراســة على عـدد 20 خصية من الدجاج المستأنس سلالة القاب ثمانية منها من أجنة أعمارهم 10 و 20 يوم بينما أثنى عشر منها من ديوك أعمـارهم 1 و 6 و 17 أســبوع. وقد لوحظ أن الأحيـال المنويـة فى خصية جنين عمـر 10 أيام مبطنة بنوعين من الخلايـا: الأولـى و الأكثر هى مولـدات خلايا سرتولى و الثانية الأقل هى الخلايـا الجرثومية البدائية. ظهـرت الخلايا المنوية الأم عند عمر 20 يوم قبل الفقس. تتحول هذه الأحيال الى قنيات عند عمر اسبوع بعد الفقس والتى تصل الى مرحلة النضج الجنسى بظهور أول حيوان منوى حر عند عمر 17 اسبوع. وقد لوحظ أيضا النمو المبكر للخلايـا العضلية الناعمـة ذات وظيفة انقباضية فى خصية جنين عمر 10 أيام بظهور الرد الناعى الابجابى لمادة الاكتين بها. بينما بدأ ظهـورالسينوكيراتين كمدعـم ومقوى لخلايا الحبل المنوى عند عمـر 6 أسابيع.