**Proceeding of the Eleventh Veterinary Scientific Conference, 2012; 248-253**

# Spermatogenesis and spermiogenisis in the testes of local Iraqi breed cat (*Felis catus*)

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## Summary

The seminiferous epithelium of the testes of cat consists of two groups of cells; Spermatogenic cells and Sertoli cells. The interstitial areas are filled with Leydic cells, blood and lymph vessels, and connective tissue. Germ cells in the Spermatogenic process of the testis of cat can be classified into ten steps, based on the pattern degree of nuclear chromatin condensation. Primary spermatogonia contain large spherical nuclei with mostly euchromatin. Spermatogonia proliferate to give rise to spermatogonia type –A; Intermediate or type-I spermatogonia, and spermatogonia type-B. Type–B spermatogonia yield primary spermatocyte at the end of mitosis. The primary spermatocyte is transformed into secondary spermatocyte during meiosis I. These cells are converted into spermatid during meiosis II. Metamorphosis of spermatids shows: Golgi step, Cap step, Acrosomal step, Maturation step.

# دراسة مجهرية لمراحل نشاة وحوؤل النطف في خصي القط العراقي المحلي

**نعمان سلمان السامرائي وصلاح حسن بداي و ايمان موسى خليل و فراس عباس السعدي**

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## الخلاصة

تتكون ضهارة النبيبات المنوية لخصية القط من مجموعتين من الخلايا :خلايا نشاة النطف وخلايا سرتولي .تشغل الباحة بين النبيبات ,نسيج ضام , خلايا ليدك , واوعية دموية ولمفية . تصنف الخلايا الجرثومية في عملية نشاة النطف على طراز ودرجة تكثيف الصبغين النووي وتشمل عشرة مراحل . تحتوي سليفات النطف الاولية على نوى دائرية وصبغين متجانس . تتكاثر سليفات النطف وينشا منها سليفات نوع (ا ) وسليفات نوع ( و) وسليفات نوع ( ب) . تنشا الخلايا النطفية الابتدائية من سليفات نوع ب في نهاية الانقسامات الخيطية . تتحول خلايا النطف الابتدائية الى خلايا نطف ثانوية خلال الانقسامات الاختزالية الاولى .تتحول الخلايا الاخيرة الى طلائع النطف خلال الانقسامات الاختزالية الثانية . تمر الطليعة النطفية بمرحلة كولجي , ومرحلة القلنسوى , ومرحلة الجسيم الطرفي , ومرحلة الطور النضوجي .

## Introduction

Some researchers has been mentioned a criteria for the steps of seminiferous epithelium in the testes of some vertebrates .It can be divided into eight steps in rat (*Rattus norvegicus*) (1), Rabbit (*Oryctolagus cuniculus L.*) (2), fourteen steps for camel (*Camelus dromedaries*) (3), fifteen steps in goat (*Capara L*), (4) and seventeen steps for dog (5). As consequence, the number of steps and their cellular configuration make up varies between animals which depend on the morphological criteria used (9). The goat of this research is to clarify and fix the steps of seminiferous epithelium in cat testes and these steps can have a significant impact on histopathological evaluation (5).

## Materials and methods

Ten indigenous cats aged between (1-2) years and range weight (2-4) were used in this research. Bilateral closed orchidectomy was done for all these animals according to the technique described by (6). The obtained testicles were used for histological examination. The testes were immediately immersed into a large amount of 0.9% physiological saline to wash out the residual debris and to preserve their constituently. Each testis was cut from the anterior pole to the posterior pole and immersed again deeply in a large volume of 10% formalin. Trimming was done to smaller sizes. The testicular samples were cut into slices of about two to three millimeters in thickness. The samples were put in a labeled containers contained also 10% formalin fixative. Washing out of the tissue samples with running water

followed the fixation. The subsequent processes include dehydration by upgrading alcohol from 50%, 70%, 80%, 90%, and 100%. Then, clearing in xylene, clearing was followed by embedding in melted paraffin at 60C .The small blocks containing the testicular tissue were sectioned by a microtome to thickness of five micrometers. These sections were stained by Harris haematoxyline and eosin and Periodic acid schift (7).Photographs of the examined slides were carried out with Olympus microscope that posses 1.25 tube factor and supplied with a digital camera with resolution power of two Mega Pixel.

## Results

The male testicular germ cells of local Iraqi breed cats were classified into various steps based on the pattern of nuclear chromatin organization and other features including the acrosome and tail formations. The seminiferous epithelium of the testes of cats consists of two groups of cells; Spermatogenic cells and Sertoli cells (fig.1). Sertoli cells are triangular cells in which the developing gametes appear embedded within the Sertoli cell membrane. Sertoli cell extends from the basement membrane of the seminiferous tubule to the luminal compartment. Its nucleus is irregular in outline and its nuclear membrane has a longitudinal folds. The interstitial spaces between the seminiferous tubules are filled with connective tissue which womb Leydic cells, nerves, blood and lymph vessels. Leydic cells typically have round to oval nuclei. They are distinguished from other interstitial and peritubular cells by their darker staining cytoplasm. They are frequently clustered tightly together in groups (fig.2). The steps of Spermatogenic cells could be clearly identified, but the mechanism of cellular renewal are not totally understood. It appears that stem cells divided into various types of spermatogonia (fig.3) and an operative copy of itself become dedicated to spermatocytogenesis and the other cell is kept in reserve for future division. Therefore, the first step consists of a single spermatogonium or type A-Spermatogonium. This cell contains a large spherical nucleus with mostly euchromatin and presence of few blocks of heterochromatin in the center. Each nucleus contains one or two prominent nucleoli. The spermatogonium type-A give rise by successive mitotic division to a spermatogonium type-B. This type of cell still lying close to the basement membrane .Its nucleus is round and small blocks of heterochromatin are present within it when compared with spermatogonium type-A , which are distributed along the inner facet of nuclear envelope as well as in the central region . The mitotic division of type-B spermatogonium lead to form intermediate type or type-I spermatogonium. The intermediate spermatogonium appears as small cell and can be identified upon the level of A-spermatogonium but far from the basement membrane of the seminiferous tubule (fig.4). The nucleus of this cell passes coarsely clumped chromatin which lie peripherally. The mitotic division of type-B spermatogonium yields the primary spermatocyte. There are six steps of primary spermatocyte; Leptotene, Zygotene, Pachytene, Diakinesis, and Metaphase steps, each with distinctive patterns of chromatin organization. The cells in Leptotene step are spherical in shape, but with a large spherical nuclei. Most of the chromatin is in the euchromatin form and there is no thin rim of condensed chromatin along the nuclear envelope. The nucleolus is still present. The zygotene primary spermatocyte has a round nucleus. The distinguishing feature of zygotene primary spermatocyte is the increase in size and density of heterochromatin blocks and the nucleolus is completely disappears. The nucleus of pachytene primary spermatocyte is still round. It is characterized by the presence of long cords of heterochromatin, some of which are attached at the end to the nuclear membrane. In diplotene step, the nucleus of this cell has a round to oval shape with slightly smaller size than the nucleus of the previous step. The chromatin blocks become increasingly larger and attached to the nuclear envelope in a cart wheel pattern. Diakinesis primary spermatocyte is identified by the presence of long and large pieces of chromosomes that are distributed within the whole nucleus. Primary spermatocyte in Diakinesis step seems rapidly turns into the final step or metaphase primary spermatocyte whose chromosomes become aligned in a row along the equatorial region. The nuclear membrane disintegrates and

completely disappears. The first meiotic division of a primary Spermatocyte generates two secondary spermatocyte. The duration of a Spermatogenic cell in the secondary spermatocyte step is relatively short. The nucleus of a secondary spermatocyte is round and it contains about five large clumps of heterochromatin blocks along the inner facet of the nuclear envelope, with usually one block located in the center of the nucleus. The second meiotic division lead to form the spermatid. The spermatid passes through metamorphosis. The metamorphosis pictures lead to classify the spermatids according to their nuclear pattern of chromatin condensation, formation of acrosome, the nuclear size and shape. Four successive steps of spermatids are identified. The nuclei of successive steps vary from round to oval, and finally to cylindrical shape. Each spermatid cell in the first sub step is characterized by the presence of a round to oval shaped nucleus which is reduced in size and the cytoplasm is a relatively large in its mass. The acrosome starts to appear as a short thick but in the form of flat segment on the nuclear envelope in the second sub step. In the third sub step, the cytoplasmic spermatid is relatively clear in comparison with earlier spermatid. The cytoplasm shows progressive migration to the caudal part of the nucleus and become more elongated and the nuclear chromatin becomes almost uniformly dense. The acrosome appears as an enlarged thickened plate in close opposition to the nuclear envelope at one pole of the nucleus. The spermatids in the fourth sub step are usually lying close to the lumen of seminiferous tubule, where the heads are usually embedded in the cytoplasm of Sertoli cell. The nucleus is more elongated and the chromatin is completely condensed throughout the nucleus. The cytoplasm in this sub step appears pushed back to the opposite side to where the acrosome is positioned and only the caudal end still posses a substantial amount of cytoplasm. The acrosome appears as a flat cap-like structure over the pointed anterior end of the nucleus. The last step is the real construction of spermatozoa. The mature spermatozoa are recognized by their highly elongated cylindrical nuclei. The nucleus occupies vituely the entire head region. It contains completely opaque chromatin.

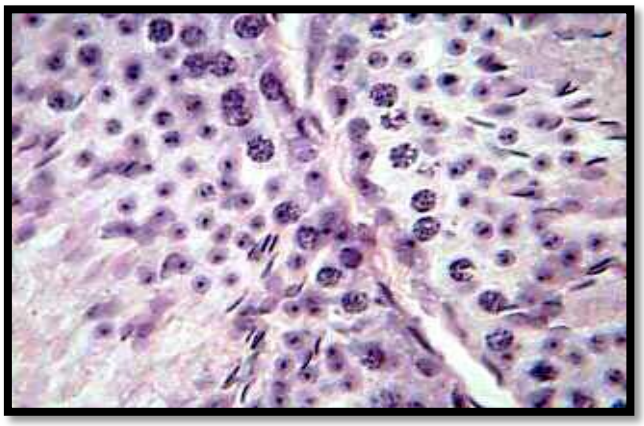

**Fig. (1): Transverse section of adult cat testis showing Spermatogenic cells and Sertoli cells. Harris haematoxylin &eosin stain(x100).**

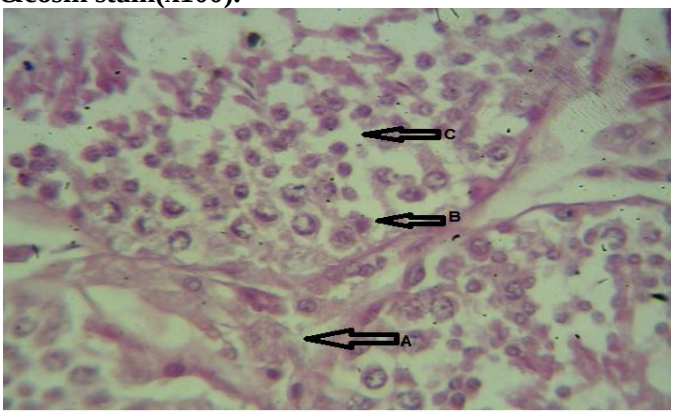


**Fig. (2): Transverse section of seminiferous tubule of adult cat testes. A-Leydic cell.B-sertoli cell. C.spermatogenic cells.H&E stain. (x40).**

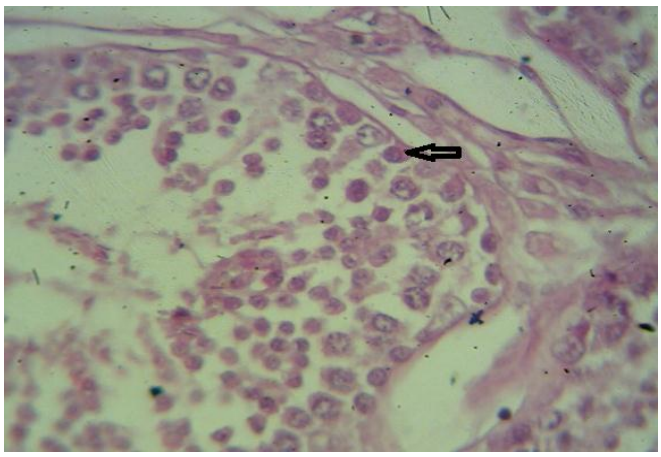

**Fig. (3): Transverse section of seminiferous tubule of adult cat testis showing spermatogonia cells.H&E. stain (x40).**

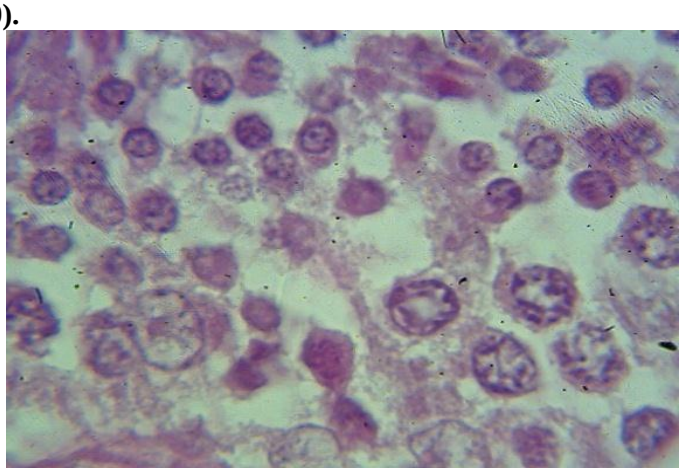

**Fig. (4): Transverse section of seminiferous tubules of adult cat testis showing various types of spermatogonia.PAS stain. (x100).**

## Discussion

Generally, the testes of local Iraqi cat breed are combined exocrine and endocrine organs. The exocrine portion is a compound, coiled tubular gland that produces spermatozoa as it's holocrine secretory product. The endocrine portion is represented by the cells of Leydic and cells of Sertoli. The functional unite of the testis is the lobuli testis which contains the seminiferous tubules. These tubules radiate from the mediastinum testis. They are lined by stratified epithelium that consist of basal, Intermediate, and superficial zones. The stratified cells consist of spermatogonia, primary spermatocytes, secondary spermatocyte, spermatid, and spermatozoa. However, not all the epithelium of the seminiferous tubules are in the same period of activity, nor different portions of the same tubules are characterized by cells with the same degree of differentiation. This information's are also coincided by (8) and (19). Sertoli cells displayed normal indented nuclei. Their cytoplasm was highly vacuolated and contained many lipid droplets. It has been proposed that the Spermatogenic associations can be regulated by Androgen-binding protein (10, 11). Follicle-stimulating hormone is also participates in spermiogenesis (12). Concomitantly, hormonal effects on sperm cells are not direct, but are mediated through Sertoli cells (12, 13, 14). Blood-testis barrier also acts to conserve certain products of Sertoli cells within the seminiferous tubule (15). The endocrine component of the testes contains Leydic cells. The Leydic cells are foamy in appearance and are abundant polyhedral with large spherical nuclei and a distinct nucleoli. This is coincided with (16). These Leydic cells are responsible for the elaboration of testosterone. Morphology of Leydic cell has been described in a number of mammalian species (17). There are thought to be two distinct populations of Leydic cell that arise at different times during the development of the testis (18, 19). It is also thought that Leydic cells are derived from undifferentiated mesenchymal cells of the testis interstitium. Some of these mesenchymal

cells become putative Leydic cells, while others become myoid cells and fibroblasts (18, 20).These information's also supported by van et al. (21) in pig testes and in the differentiating mesenchymal cells of the hamster testis studied by Gondos et al. (22). Spermatogenesis occurs within the seminiferous tubules of the testis of cat which are responsible for the production of gametes; Whereas (9, 23) denoted that the ductus epididymidis aid in their maturation process. A series of nuclear and cytoplasmic changes involves the Spermatogenic lineage within the seminiferous tubules of cat testis. The progression begins with spermatogonia and terminates with spermatozoa. The most primitive cells are located near the periphery of the seminiferous tubule or basal compartment; whereas, the more developmentally a advanced cells are located on the luminal compartment. The first step of spermatogenesis is characterized by an increase in the number of spermatogonia through successive mitotic divisions. Al-Samarrae et al. (24) reported that not all spermatogonia differentiate simultaneously, but some are retained as stem cells for future differentiation. Spermatogonia within the seminiferous of cat testis a actually represent many generations of cells. These include: A-Spermatogonia, I-Spermatogonia, and B-Spermatogonia. Subsequent division of B-Spermatogonia lead to the differentiation of primary spermatocytes. The later cells marks also the differentiation of the secondary spermatocytes at the end of the first division of meiosis and when they enter the second meiotic division lead to form the spermatids. This is in agreement with Rathi et al. (25). The Spermatids comprise the most developed, most numerous of largest layer of the seminiferous tubular epithelium and located in the zone of metamorphosis. The organelles involved in metamorphosis are the nuclei, Golgi apparatus, and centrioles. AL-Maliki (5) registers that proacrosomal granules appear in the vesicles of the Golgi apparatus. The vesicles coalesce to form a single large acrosomal vesicle that contains the acrosome. The acrosomal vesicle enlarges and extends itself over half the nucleus to form the head cap. The spermatid nucleus in cat testis seen to be highly dense and more elongated. In this case, the spermatid itself becomes elongated. At this time, the centrioles and developing flagellum migrate toward and come in contact with the nuclear membrane. Indeed, in this work and various works revealed that the numbers of steps of Spermatogenic wave are species variable especially during non breeding season which reveal the presence of scattered spermatogonia and Sertoli cells and also, the tubule could be involuted and replaced by connective tissue. This is also coincided by (5, 26).

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