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Histochemical study and spermatogenesis events in testis of adult camel Camelus dromedarius in AL-Muthanna province

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Abstract

Objective; This study aimed to explain the histological features and spermatogenesis events in testis of mature camel.
Materials and method; fourteen testes from adult camel collected from AL-Muthanna abattoir. The testis consists of a mass of seminiferous tubules, surrounded by fibrous capsule called tunica albuginea; which composed of dense collagenous tissue. A trabecula, pass inward from the tunica albuginea to form a framework for support of the seminiferous tubules gives off several trabeculae into the interior of the testis. These trabeculae divide the testis into lobules. Each lobule contains many seminiferous tubules. This capsule is covered by a tunica serosa, the connective tissue of which blends with the tunica albuginea. The tunica albuginea is continuous with the loose collagenous tissue of the mediastinum testis. Tunica albuginea is also continuous with the testis into the lobule testis; seminiferous tubules and rete testis are surrounded by loose collagenous tissue; Sertoli cells are elongated cells with irregular outlines extending from the basement membrane to the lumen. Leydig cells occurred in the interstitial space between the seminiferous tubules. The seminiferous tubules are associated with a specialized type of myoid cell. These cells are located within the surrounding basal lamina. Spermatogonia undergo mitosis to produce A-spermatogonia, Intermediate spermatogonia and B spermatogonia. This cyclic mitotic division activate B spermatogonia to produce primary spermatocyte that pass through six different phases; preleptotene, leptotene, zygotene, pachytene, diplotene and diakinesis phases. The secondary spermatocyte enters the second meiotic division which gives rise to spermatids. Differentiated by fourteen stages which lead to form sperm. We can conclude that the study has shown the Although some characteristics unique to camels were noted, spermatogenesis in camels was largely comparable to that of the majority of mammalian species. It was discovered that spermatogenesis occurred continuously all year long.

Key words; Testis, Camel, Seminiferous tubules, Leydig cell, Spermatogenesis.

INTRODUCTION

Camels are used for many purposes that are important in human life, including providing him with meat and milk (1). Camel breeding in the country did not receive its abundant share of scientific studies for the purpose of raising the efficiency of its production and improving its performance, especially in the southern region, which is characterized by the spread of a good breed in it. The male camel exhibits sexual activity on certain days of the year (the breeding or rutting phase), and camel known to breed seasonally (2,3). (4) said the exteriorization of soft palate, or dulah, is another characteristic of sexual behavior in camel, the soft palate protrudes at intervals of 15 to 30 minutes, accompanied by loud roaring and gurgling sounds. Increased excitation, including the existence of additional males and females, causes the protrusions to occur more frequently (5,6). The camel is known to be a seasonal breeder, the male camel shows sexual

activity during specific days of the year (breeding or rutting period), and have some aspects of the seasonal changes in the testis (6). Sexual behavior in male farm animals, including camel, is an expression of both sexual desire and the ability to mate. Male sexual behavior depends on the degree of sexual stimulation and preparation, which are greatly influenced by external stimuli such as sight, hearing, smell, and other stimuli (7-9). Increasing animal output is mostly dependent on management and reproduction. Because it affects the number of children produced each year, reproductive efficiency is therefore one of the most important economic characteristics (10,11). A prevalent issue is low reproductive efficiency. Maintaining optimal reproductive performance requires a thorough understanding of a woman's genital organs, and using modern reproductive technologies requires knowledge of the organs' measures (9,12). The knowledge of the reproductive increasing animal output is mostly dependent on management and reproduction (13). Reproductive efficiency is therefore one of the most important economic characteristics since it affects the number kids produced each year. Low reproductive efficiency is a common problem (14,15). Maintaining optimal reproductive performance requires a thorough understanding of genital organs, and using modern reproductive technologies requires knowledge of the organs' measures (16). Researching camel spermatogenesis is essential for understanding their distinct processes of reproductive and has implications for agriculture, veterinary care, and conservation, all of which could help develop efficient breeding and management plans for the species' sustainability and conservation.

MATERIALS AND METHODS

Animals: Fourteen testis from adult camel (6-7 years) from slaughter in the Muthanna. Take five samples (0.5-0.7 mm) from different regions from each testis and fixated with 10% formalin and the testis samples were taken for histological analysis and many stains used H@E, PAS, Masson Trichrome, Verhoef's and Van Gesion (17).

STATISTICAL ANALYSIS; A one way analysis of a variance (ANOVA) tests was used to examine the study at the 5% significant levels. Data were processed and controlled using social science statistical tools (2).

RESULTS AND DISCUSION

The tunica albuginea of testis forms a dense, asymmetrical capsule of connective tissue. Collagen fibers make up the majority of it, with a little amount of elastic fibers. There are sporadic smooth muscle cells. Trabeculae of connective tissue run continuously through the tunica albuginea. The mediastinum testis is where the septula testis converge. These thin, frequently incomplete trabeculae are made of the collagen fibers, contains nerves and arteries, and split the testis into a variety of testicular lobules, the septula are continuous with the intralobular connective tissues (Fig. 1,2), which is consistent in domestic animals as result of (2). The convoluted seminiferous tubule was surrounded with basal lamina followed with a layer of collagen fibers and elastic fibers. The seminiferous tubules, and the collecting ducts converge towards the mediastinum. The ducts anastamoses in their course to the mediastinum. Near the dorsal pole, the remaining efferent ducts unite to a single duct which passes through the tunica albuginea to join the epididymis. There are numerous reticular fibers and loose collagenous tissue around the tubules of the rete testis and seminiferous tubules (Fig. 3-7), this agree with results of (18,19), seminiferous tubule responsible to the production of gametes. This outcome was comparable to the one shown by (4,5) in camel and (16) in gazelle. Testicular tissue is covered by a thick layer of dense, fibrous tissue called the tunica albuginea. The left and right testis had significantly different mean tunic thicknesses ($p \leq 0.05$). The parenchyma was divided into lobules by thin septa that ran from tunica albuginea to mediastinum testis. Each lobule had tortuous seminiferous tubules. The left testicle's rounded seminiferous tubule had a significantly ($P<0.05$) greater mean diameter than the right testicle's (Table 1).

A layer of collagen fibers follows the basal lamina that envelops each convoluted seminiferous tubule. The parenchyma makes up the majority of the camel testis. The testis's parenchyma has a yellowish white hue. In the direction of the mediastinum, the collecting ducts and seminiferous tubules converge. On their way to the mediastinum, the ducts anastomose, efferent ducts merge near the dorsal pole to form a single duct

that connects to the epididymis by passing through the tunica albuginea. The tunica albuginea, a capsule made of thick collagenous tissue, encloses the testes. The connective tissue of the tunica serosa, which covers this capsule, melds with that of the tunica albuginea. The loose tissue of mediastinum is connected with the tunica albuginea. The testis and tunica albuginea continue into the lobules. There are numerous reticular fibers and loose collagenous tissue around the tubules of the rete testis and seminiferous tubules (Fig. 4-10), this agrees with results of (20-25). The seminiferous tubules are made up of seminiferous epithelium, a distinct basal lamina, and a tunica of connective tissue. Fibroblasts are found in multiple layers within the fibrous connective tissue tunic that envelops the seminiferous tubules. Flattened myoid cells with smooth muscle properties make up the innermost layer that adheres to the basal lamina (Fig. ,8). Spermatogenic cells and spermatozoa are found inside the seminiferous tubules. The heads of mature spermatozoa and spermatids are lodged in Sertoli cells, which also line the tubules. These cells have big, irregular nuclei with scattered chromatin and copious amounts of cytoplasm. There are several Leydig cells with round to oval nuclei and thin cytoplasmic sheets in between these seminiferous tubules. Little lipid droplets occupy the Leydig cell's little amount of cytoplasm. In the interstitial cell, several sizable lipid droplets also gather (Fig. 5,8,9), this agrees with (26,27).

The testis's interstitial tissue is formed by the connective tissue filling up the gaps between the seminiferous tubules. Numerous tiny blood vessels are also present in this interstitial tissue. interstitial tissue includes huge polyhedral cells, mast cells, macrophages, and fibroblasts in addition to tiny bundles of collagen fibers. These big cells have a single huge, spherical nucleus and are known as Leydig cells (Fig. 8). Sertoli cells have an oval nucleus and a broadly pyramidal shape (Fig. 9,10). The spermatogonia had round, black nuclei and were shaped like cubes or circles. Primary spermatocytes have granular chromatin aggregates and big spherical nuclei. The elongated spermatids developed from the little spherical spermatids, the spermatids and spermatozoa are present in certain seminiferous tubules. Up to three tubules may be separated by enormous quadrangular interstitial voids, or two seminiferous tubules may be separated by thin strands of interstitial tissue. blood capillaries, a small number of Leydig cells, a few fibroblast cells and blood vessels. Leydig cells were discovered either alone or in clusters within intertubular gaps (Fig10). In the direction of the basal lamina were the spermatogonia. Spermatogonia could be categorized as typical spermatogonia type A, typical spermatogonia type B, and "intermediate" types based on the length of the area of contact with the basal lamina, the shape of cell and nucleus, the number of chromatin encrustations in nucleus. Spermatogonia were primarily classified according to their cell and nucleus shapes as well as their area of contact with basal lamina (Fig. 8). this similar to results of (5) in camel and agrees with (22) in rams.

Spermatogonia type (A), these cells are large, flat, and feature a broad area that reaches the basement membrane. The surrounding Sertoli cells cover the remaining surface. Different cells have varying cytoplasmic densities. The nucleus is tiny and has an elliptical or circular shape, nuclear cytoplasm has a make up of soft granules and is uniform. One side of the nucleus contains a certain amount of heterochromatin. The cytoplasm will have a dusty appearance, and the nucleus will have a central location. The nuclear membrane of the nucleus is narrow and transparent (Fig 6). In general, the primordial spermatocytes are similar to those in the bull (9,29). (30-32) point out that the Golgi apparatus is highly noticeable in camel spermatocytes, and that proacrosomal granules are found in huge proacrosomal vesicles during the pachytene stage..

The conversion of undifferentiated diploid spermatogonia into highly differentiated haploid spermatozoa is known as spermatogenesis, and it is a complex process that takes place continually in the seminiferous tubules. This intricate process has three primary phases: the proliferative phase is marked by development a whip-shape tails covering a flagellum with densely packed mitochondria, nuclear DNA condensation, and spermiogenesis, that is morphological transformation of immature spermatids through spermatozoa. Together, the numerous unique animal spermatozoa that emerge from these stages demonstrate the significance of this process in reproductive biology. Important phases in the architectural development of the germ cells—specifically, spermatogonia, spermatocytes, and spermatids—have been made clear by us. Proper maturation of these germ cells is necessary to boost camel fertility, this precise structural configuration was also found by (7) in goat. Cells of the spermatogonia type (I) are regarded as

mediates. It has a varied form and size. Among the differences is the chromatin distribution pattern. Small granules or a few chromatin flakes in the nucleus' center are examples of the dispersed chromatin that can be observed in nuclear cytoplasm (Fig. 6,7). Spermatogonia type (B) is produced by the subsequent divisions at mitosis of intermediate spermatogonia. Because it has a large elliptical nucleus and more peripheral heterochromatin than spermatogonium type (A), it is comparatively larger than the latter. These chromatin flakes are distributed at various distances and form a crust along the nuclear membrane. The nuclear cytoplasm contains fragments of the chromatin of these big flakes near the nuclear membrane, there are just one or two nucleoli (Fig. 7) as result of (32).

Spermatogenic cells and mature spermatozoa are found inside the seminiferous tubules, heads of spermatozoa and spermatids are lodged in Sertoli cells, which also line the tubules. These cells have big, irregular nuclei with scattered chromatin and copious amounts of cytoplasm. There are several Leydig cells with round to oval nuclei and thin cytoplasmic sheets in between these seminiferous tubules. Little lipid droplets occupy the Leydig cell's little amount of cytoplasm. Within the interstitial cell, several sizable lipid droplets also gather (Fig.3).Sertoli cells have an oval nucleus and a roughly pyramidal shape. The spermatogonia had round, black nuclei and were shaped like cubes or circles. Primary spermatocytes have granular chromatin aggregates and big spherical nuclei. The elongated spermatids developed from the little spherical spermatids, spermatids and spermatozoa are present in certain seminiferous tubules. Up to three tubules may be separated by enormous triangular interstitial voids, or two seminiferous tubules may be separated by thin strands of interstitial tissue. In addition to blood capillaries, it also contains a large number of Leydig cells, fibroblast cells, and Leydig cells. In the gaps between tubula, Leydig cells were found as large masses in intertubular areas (Fig. 8).

Spermatogenesis, or the process of producing spermatozoa, is a crucial part of male genital system. In order to assess the reproductive systems of dromedary camels for potential and health, During various phases of growth and activity, the testes display a variety of physical characteristics, such as sexually matured testes, sexually matured even functional testes, as well as mature although resting testes (3,7). These morphological variations are shown by the histomorphological alterations observed in the seminiferous tubules. Cross-sectional analysis of these tubules in most animals reveals the presence of multiple generations of the germ cells, which are arranged clearly and successively from basement membrane to tubule lumen. Every generation of the germ cells has precisely timed developmental stages, and they typically coexist with earlier generations at the predictable as well as species-specific manner. This synchronization is crucial to spermatogenesis since mistakes during any of those developmental stages could significantly impact the overall process (9,25, 26).

Spermatogonia type (A) or dusty type; These cells are considered as a big size cell with a plane surface and have wide area which touches the basement membrane and rest of surface are covered with the adjacent Sertoli cells, and density of cytoplasm is different from cell to another. The shape of the nucleus is either elliptical or round with small size. The nuclear cytoplasm is homogenous and consists of gentle granule. There is a quantity of heterochromatin in one side of the nucleus. The nucleus has neuclous with a central location and the nuclear cytoplasm will be a dusty shape. The nucleus has a clear and limited nuclear membrane (Fig. 2,4,6). The primary spermatocytes resemble those of the bull (29). (9) mention in the camel spermatocyte the Golgi apparatus is very prominent and in pachytene stage large vesicles contain the proacrosomic granules. Spermatogonia type (I) It is different in size and shape. The difference includes the pattern that distributes of the chromatin. The distributed chromatin are found in nuclear cytoplasm in form of a small granules or a little number of flakes chromatin in the center of the nucleus, and subsequent mitotic divisions of intermediate spermatogonia give spermatogonia type (B). (Fig.8). This coincides with view of (12) in goat.

Spermatogonia type ; Relatively it is bigger than the spermatogonia type (A) because it has a big elliptical or round nucleus and it has much quantity of peripheral heterochromatin compared with spermatogonium type (A). These flakes chromatin were gathered in the crusts shape along nuclear membrane and in different distances. Parts of these large flakes chromatin are spread in the nuclear cytoplasm. The chromatin is less homogenous. There is only one or two nucleoli close to the nuclear membrane (Fig. 7), this similar into results of (19) in alpaca.

The later spermatogonia turned to primary spermatocytes through subsequent mitotic division and it comes through different six phases, These results were not similar to result of (3) who register four stages present in and in contrast to (5) in whom they register fifteen stages respectively. Preleptotene Phase; The primary spermatocytes, possess small round nuclei. Their nuclei have two or three large pieces of chromatin situated in the center. The nucleus gives up star shaped. Presence of delicate flakes of chromatin which associated with large pieces of chromatin. Chromatin granules are also found adhere to the nuclear membrane (Fig. 9,10), this agree with (28) in deer. The primary spermatocytes in leptotene phase are situated far from the membrane of seminiferous tubules. Lateral processes of sertoli cells surround these spermatogenic cells. In leptotene primary spermatocyte the nucleus is round in shape and has one or two nucleolus. Chromatin granules are also found aggregated at one pole of the nucleus. Chromatin filament extend from the granules towards nuclear membrane. The chromatin granules will be organized and pale (Fig. 9,10), this precise structural configuration was also found by (25) in goat.The zygotene primary spermatocyte possess dark looser chromatin filaments which distributed through most of the nuclear matrix Some of these chromatin flakes form a network, others positioned in one pole of their nuclei (Fig. 8,9,10). this precise structural configuration was also found by (22,31) in rams. the nuclei of pachytene spermatocyte increases in size. The mass of chromatin begins to spread throughout the nucleus. The chromatin filaments appear beaded with chromatin granules. These filaments progressively become more thick and short. Heavily stained band cytoplasms are very often seen around the nuclei (Fig. 7). This similar to (19) in alpaca. During diplotene phase, formations of two chromatids are completed, but they remain attached through the centromere. The nucleoli are clearly observed (Fig. 8). This agreement with (22) in ram. Diakinesis Phase;The crusts of the chromatin in this stage of spermatocytes become contracted and coiled arounded their self. The two chromatids shorten and begin to move a part and move further a part. The nucleolus and the nuclear membrane also disappeared (Fig.4), this precise structural configuration was also found by (7) in goat.

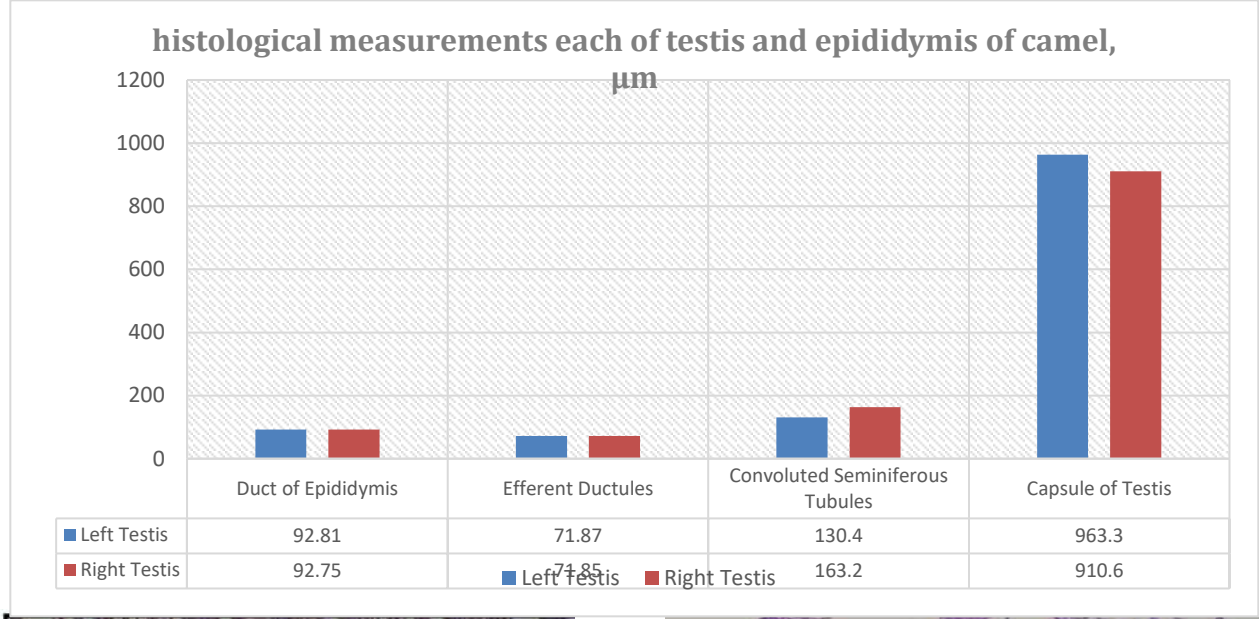
Spermatocytogensis, the process during which spermatogonia develop into spermatocyte, meiosis, the maturation division of spermatocytes that results in spermatid with a reduced (hapliod) number of the chromosomes. spermiogensis, the process of the transformation of spermatid for the spermatozoa. Thus the sequence events of spermatogensis and spermiogensis in the camel seminiferous tubule beings with primitive germ cell. The spermatogonium revealed a series of mitosis and give rise to type A-spermatogonia which are present through out the cycle A few intermediat spermatogonia are observed. Type B – spermatogonia are resulted from multiplication of an intermediate spermatogonia (Fig. 7). this precise structural configuration was also found by (12) in goat.

Table, (1): Measurements each of testis camel, µm Mean ± standard error

Tunica albugenia		Tunica vascularis		Tunica vaginalis		Diameter of convoluted Seminfeorus	
Left	Right	Left	Right	Left	Right	Right	Right
172.7±0.4 A	157.1±0.6B	70.3±0.4A	63.1±0.2B	30.3±0.2A	27.1±0.5B	158.1 ±0.5B	158.1± 0.5B

Values with capital letters in same measure denote to significant differences (P>0.05) between left and right testis

histogram (1): Thickness of epithelium in parts of epididymis of camel, µm Mean



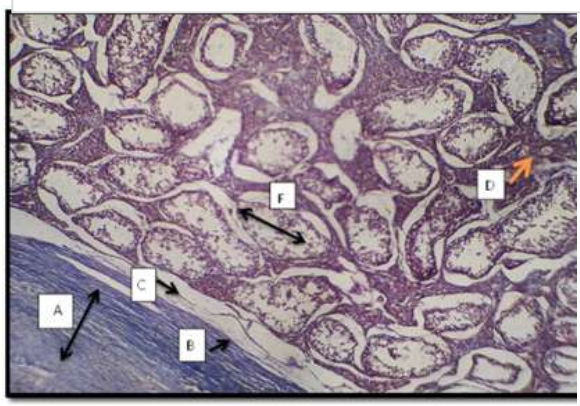


Fig. (1): Microscopic section of the Right testis in camel shows the : A. Capsule, B.irregularly collagenous, C. elastic fiber, D.loose connective tissue ,E. seminiferous tubule Masson

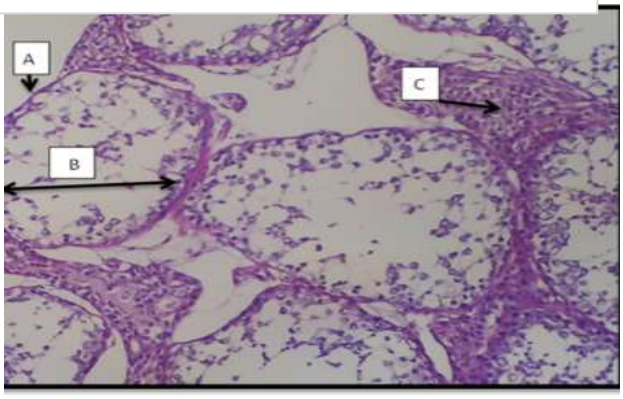
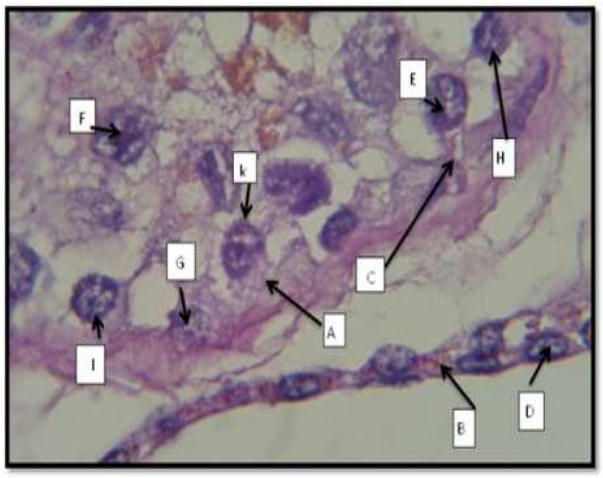


Fig.2. Microscopic section of th Right testis in camel shows the : A. basement membrane, B. seminiferous tubules, C Interstitial tissue**,** D**.**blood vessels.E.collagen fiber PAS. Stain(X100).



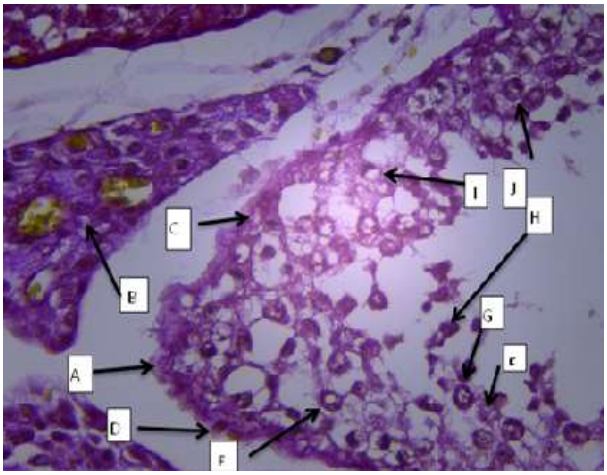


Fig. (3): Microscopic section of the testis in camel shows: A. seminiferous tubules, B. basement membrane , C. Interstitial tissue, D. Septa , E. Lumen of Seminiferous Tubule Masson stain (X100)

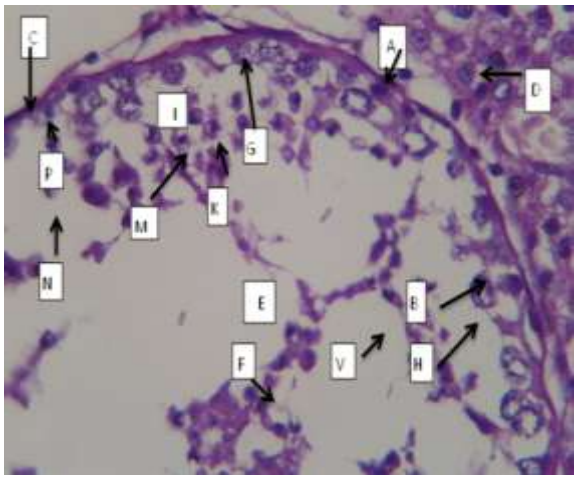


Fig. (4): Microscopic section of the testis: **A**. Basment membaren,B. Intersitial cell, C.sertoli cell , D.Myoid cell, F.Zygotene, G. Second Stage , H. Fourteen Stage , I. Fourth Stage , j.secondary spermatocytes, Weigert's iron hematoxylin, (X400)

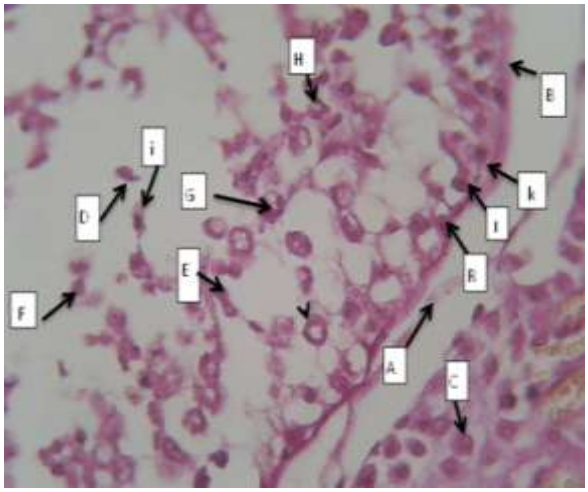
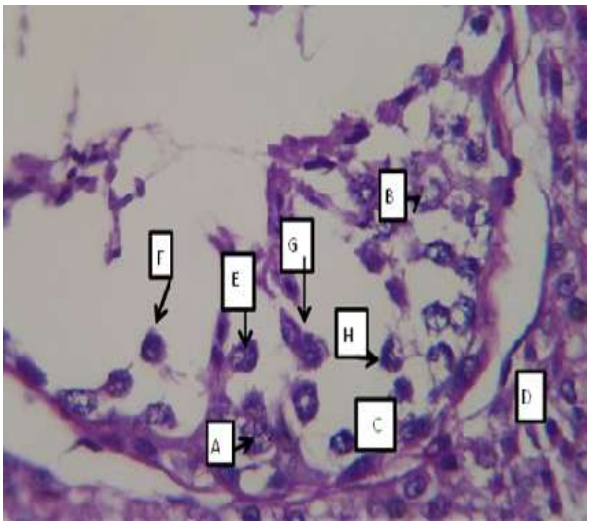


Fig. (5). Microscopic section of testis : A. basement membrane, B.Sertoli cell ,C.Myoid cell, D. Leyding cell**,** E**.** seminiferous tubules, F.Eleven Stage , G.Type I, Type B, P.Type A, H. second stage, K. Six stage, M. Seven stage .N. Stage five, V. Thirteen stage PAS Stain(X400).

Fig. (6): Microscopic section of the testis : A. Spermatogonium type A, B. Basement membrane, C. Sertoli cell**,** D. Myoid cell, E. Zygotene phase, F. diplotene phase, G.Type B, H. Perteptotene phase, I. Type I, K, Pachytene, Verhoeff,s stain (X400)



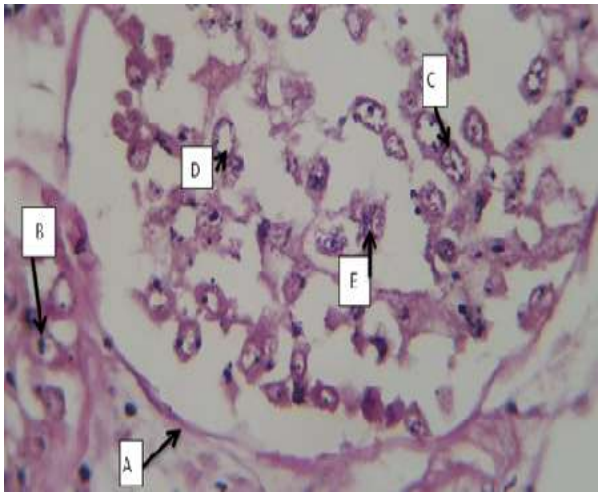


Fig.(7): Microscopic section of the testis shows: A. Preteptotene, B. Third Stage, C. Sertoli cell**,** D. leydig cell, E. Secondary spermatocytes, F. Second Stage , G. Thirteen stage, H. Six stage, H&E (

Fig.(8). Microscopic section of the testis shows the : **A.** basement membaren, B. leydig cell , C. Diplotene, D. Nine Stage , E. Eight Stage , Verhoeff,s stain (X100)

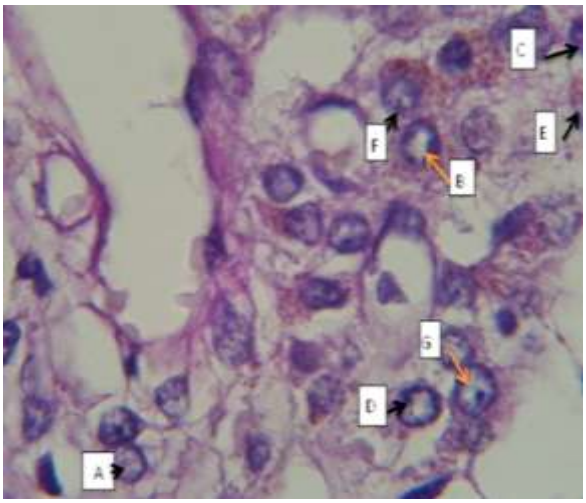


Fig.(9): Microscopic section of the testis: A, pachytene, B. Nine stage, C. Spermatogonia type B, D, First Stage, E. Elaventh Stage, F. Seventh Stage, G. Four stage, Van Gieson stan (X400)

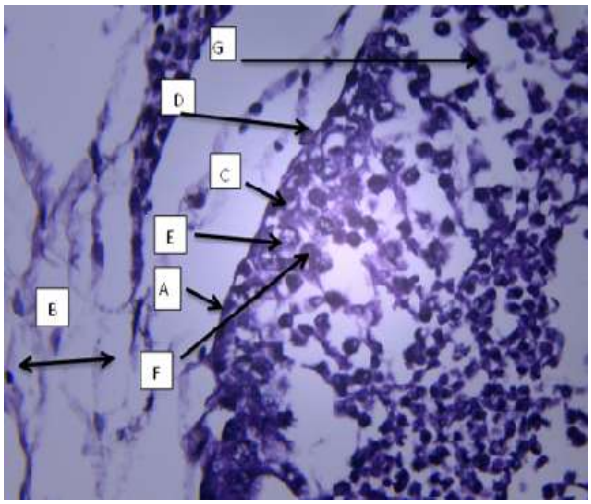


Fig.(10): Microscopic section of testis shows **A**. Basement membrane, Connective tissue, C.sertoli cell, D. Myoid cell, E. Spermatogonia type A, F.Type B, G. Seventh Stage, Weigert's iron hematoxylin stain, (X400)

CONCLUSION:

The study has shown the Although some characteristics unique to camels were noted, spermatogenesis in camels was largely comparable to that of the majority of mammalian species. It was discovered that spermatogenesis occurred continuously all year long.

Funding

The author funds this study alone rather than through outside financing.

Availability of data and materials

Data for the current study is available upon an adequate request.

Competing interest

None of the writers have any conflicts of interest.

Ethical consideration

Study conduct at AL Muthanna University in Iraq had been conducted in accordance with ethical standards.

Author contributions

Every author makes an equal contribution.

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