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**THE EFFECT OF SEASONAL CHANGES ON THE STRUCTURE
OF THE INTRATESTICULAR EXCURRENT DUCTS
OF GOAT IN SINAI**
(With 8 Figs.)

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التغيرات الموسمية في تكوين الانبيبات الاخراجية داخل خصية الماعز البدوي

في سيناء (دراسة بالميكروسكوب الضوئي)

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اظهرت الدراسة أن للانبيبات الاخراجية داخل خصية ذكر الماعز البدوي يتكون من الجزء المبتدى وهو الجزء الذي يربط المناطق النهائية الانبيبات الخصية اللتوية مع الانبيبات المستقيمة وقد أمكن تمييز ثلاثة مناطق مختلفة به: منطقة تحويلية، مبطنه بطبقة مضروبة من النسيج الطلائي المولد للحيوانات المنوية، منطقة وسيطة مبطنه بخلايا سرتولي وهذه الخلايا تتميز بوجود منطقة خيطية كثيفة في جزئها العلوي والتي تؤدي الى تكوين سداة داخل تجريف الانابيب، منطقة نهائية مبطنه بطبقة واحدة من خلايا مكعبه مع بعض خلايا الليمفوسيت والمكروفاج وهذا الجزء الأمامي نادرا ما يظهر أثناء فصل الصيف.

الانبيبات المستقيمة: التي تربط المنطقة النهائية للجزء الأمامي مع الشبكة الانبوية داخل الخصية ظهرت هذه الانبيبات مستقيمة ولها تجريف ضيق خالي من أي محتويات مبطن بواسطة نسيج طلائي مضعف ويزداد تجريف هذه الانبيبات وتظهر خلايا مولده للحيوانات المنوية في فصل الشتاء والربيع.

الشبكة الانبوية: وتنقسم الى جزء كبير داخل الخصية وجزء أصغر خارج الخصية، في فصل الشتاء والربيع تبدو مبطنه بخلايا مكعبه أو عموديه. بالإضافة الى أعداد قليلة من الليمفوسيت والمكروفاج، وتمتلى تجاوبها بالخلايا المولده للحيوانات المنوية وخلايا المكروفاج.

SUMMARY

The micromorphology of the intratesticular duct system has been investigated with emphasis on the effect of the seasonal variations. The period between September and April was considered as the active period and that between May and August as the inactive one. The active period was characterized by regional differentiation of the duct system which comprised: 1) initial segment, which was delineated from the terminal portion of the seminiferous tubule by a sphincter-like constriction. This segment was subdivided into; transitional zone lined with reduced spermatogenic epithelium, intermediate zone lined with modified Sertoli cells and finally

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a terminal zone representing an epithelial dilatation, 2) tubulus rectus, which was not always straight and lined with cuboidal epithelium and some lymphocytes and macrophages, 3) rete tubules, which were highly branched and anastomosed epithelial chambers lined with "3" types of cells. In some animals the chambers got fused forming a huge labyrinthine chamber designated as mediastinal labyrinth. The boundary tissue surrounding the epithelial elements was easily demarcated from the loose interstitial tissue. The inactive period showed the following changes: a) attenuation of the epithelial lining of the different segments, b) high reduction in the luminal content of the sperms, degenerating sperm components and luminal macrophages, c) diminution of the size of the rete tubules which became separated with wide interstitial spaces made up of homogeneous dense fibrous tissue, d) the boundary tissue could not be demarcated from the interstitial tissue.

INTRODUCTION

Although a lot has been reported on the seminiferous tubules, the seat of spermatogenesis, and the structure and function of the extratesticular ducts, the data on the architecture of the intratesticular ducts are sparse in domestic animals. Scattered histological studies were reported by PEREY, *et al.* (1961), ROSEN-RUNGE (1961), LEESON (1962), MARIN-PADILLA (1964), DYM (1972, 1974), GOYAL and DHINGRA (1973), DELLMANN and WROBEL (1976) and DHINGRA (1977, 1980). Spermiophagy in the testicular excurrent ducts has been described by HOLSTEN (1978), SINOWATZ, *et al.* (1979) and GOYAL (1982).

The available informations have been clarified that the structure of the intratesticular duct system of the Bedouin goat during the different seasons could have escaped detection until now.

The aim of the present investigation is a trial to reveal a correlation between the structure of the various segments of the intratesticular excurrent ducts of the Bedouin goat in Sinai and the effect of different climatic conditions during the four seasons of the year.

MATERIAL and METHODS

The material comprised the testes of 60 sexually mature male goats (bucks) collected from Al-Arish slaughter-house at regular monthly intervals (5 each month) over a period of twelve months. The animals were 1-2.5 years old and they were all apparently healthy. The testis was dissected and small pieces from the entire length of the mediastinum testis and the surrounding tissue were fixed in Bouin's fluid for

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24 hours and processed for paraffin embedding. Serial sections of 5 μ thickness were stained with H & E, Van Gieson, Weigert elastic stain, PAS, Best's carmine, Alcian blue and Alcian blue-Van Gieson (BANCROFT and COOK, 1984).

RESULTS

The present study has revealed that the intratesticular excurrent ducts of the Bedouin goat in Sinai comprised three histologically discrete components; an initial segment connecting the terminal end of the tortuous seminiferous tubule with the tubulus rectus, tubulus rectus and finally the rete testis which was differentiated into a major intratesticular portion occupying the mediastinum testis and a minor extratesticular one communicating with the efferent ductules. For convenience, two periods of structural activity will be described; active period (between September and April) and inactive one (between May and August).

Active period:

On piercing the mediastinum testis, the terminal portion of the tortuous seminiferous tubule tapered down to about one third or half of its diameter forming a narrower tubular segment, designated as the initial segment. The latter, was delineated by a prominent constriction demarcating its connection with the seminiferous tubule (Fig. 1).

The initial segment was differentiated into three distinct zones: 1) transitional zone, which was short and lined with two or three layers of spermatogenic epithelium encircling a narrow lumen, 2) intermediate zone, which was the longest of the three zones and it was lined with a single layer of modified Sertoli cells which were larger in size than those lining the seminiferous tubules, with hazy contours, their basal ovoid nuclei were euchromatic and multinucleolated and their clear cytoplasm was lightly eosinophilic, moderately PAS-positive and showed weak positivity to Best's carmine. The apical portions of such cells exhibited a characteristic filamentous extensions inclining in one direction toward the lumen of the third zone where a plug-like mass was constructed (Fig. 2), 3) terminal zone, which was shorter and smaller in diameter than the previous zone but its lumen was the widest. This receptacular zone was lined with simple cuboidal epithelium with an average height 5.5-8 μ . The cells showed no reaction to PAS, Best's carmine and Alcian blue. Sporadic lymphocytes and macrophages were insinuated between the epithelial cells. The lumen, in some terminal zones, showed a number of sperms and exfoliated spermatogenic cells.

The straight tubule (tubulus rectus) was the second segment of the duct system representing the direct continuation of the terminal zone into the mediastinum testis (Fig. 3). They were not always straight and showed wide variations in length and diameter. They were lined with a single layer of brick-like to high cuboidal cells

with an average height 4-8 μ . The nucleus was oval, moderately basophilic and occupied most of the cell (high nuclear/cytoplasmic ratio). The eosinophilic cytoplasm was moderately PAS reactive with the exception of the luminal surface which was strongly positive. Sporadic lymphocytes and macrophages were observed among the epithelial cells. Some luminal sperms and other spermatogenic cells were present.

The present investigation has revealed that the rete testis of goat is constructed of a major intratesticular portion, occupying most of the mediastinum testis, and a minor extratesticular one lying beyond the capitulum testis (vascular pole) of the testis and communicating with the efferent ductules.

The intratesticular rete testis was made up of highly branched and anastomosed epithelial chambers separated by narrow interstitial tissue spaces and oriented axially in the mediastinum testis (Fig. 4). A huge labyrinthine epithelial chamber was observed in some animals, instead of the individual smaller chambers, occupying the mediastinum testis and draining the peripheral tubuli recti (Fig. 5). It is suggested to designate this single chamber as the mediastinal labyrinth. The rete testis was lined with three distinctive cell types arranged in a single layer; principle cells, clear cells and columnar cells. The principle cells were brick-like with an average height about 4 μ . Their ovoid, moderately basophilic nuclei were multinucleolated and their cytoplasm was eosinophilic, weakly PAS-positive, alcian blue and Best's carmine negative. The luminal surface was exceptionally showing a strong PAS film. The clear cells were in the form of patches among the principle ones. They were cuboidal with an average height about 7.5 μ . The spherical, centrally located nucleus was euchromatic with a prominent central nucleolus. The cytoplasm was characteristically clear and showed no affinity for the used stains with the exception of the luminal surface which showed a strong PAS reactivity. The cytoplasmic/nuclear ratio was high. The columnar cells were also arranged in small patches among the principle ones. The cells were closely adjacent, their oval nuclei were heterochromatic and their little cytoplasm was basophilic, weakly PAS positive and showed no reaction to both alcian blue and Best's carmine. Their luminal surface was slightly bulging into the lumen and was strongly PAS-positive. The nuclear/cytoplasmic ratio was high. The average height of the cell was 10.5 μ .

Occasional basal cells were also observed among the principle cells especially during February and March. Sporadic lymphocytes and macrophages insinuating among the epithelial lining was a constant feature. The lumina of the rete tubules (or the labyrinth) were overloaded with sperms, exfoliated spermatogenic cells, degenerated cellular elements and luminal macrophages (Fig. 6).

The boundary tissue of the initial segment, tubuli recti and rete testis was more or less similar. It was easily demarcated from the rest of the interstitial tissue by its density (Fig. 6). It was made up of a basal membrane and a fibrocellular coat. The former, was characterized by prominent infoldings with the basal portions of

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the overlying epithelial cells (Fig. 6) and its average thickness was about 2-3.5 μ . The fibrocellular coat was made up 2 to 3 layers of elongated spindle cells morphologically identical to the peritubular myoid cells found inbetween the seminiferous tubules, followed by a fibrillar sheath constructed entirely of fine collagenic fibers. Both the cells and fibers were parallel to the basal membrane and the overlying epithelium. The total thickness of such coat was ranging between 9 to 21 μ .

The interstitial tissue separating the epithelial components of the duct system was exclusively made up of highly vascular loose connective tissue. It was characterized by the presence of thickwalled lymphatic vessels whose lumina were communicating with those of the rete tubules and the mediastinal labyrinth (Fig. 7) and showed the presence of enormous number of degenerated spermatogenic cells.

Inactive period:

The intratesticular duct system, during this period, showed the following micro-morphological variations: 1) the majority of the seminiferous tubules were directly communicating with the tubuli recti, where the initial segments were missing, 2) the initial segment, when present, has lost its sphincter-like constriction delineating its connection with the terminal portion of the seminiferous tubule, 3) the filamentous apical portions of the Sertoli cells lining the intermediate zone were ill-defined, therefore, their plug-like masses were missing, 4) the epithelial lining of the of the different segments of the duct system became attenuated, the nuclei became heterochromatic and the cytoplasm became scanty, 5) the tubuli recti were always straight taking a penicillus form, their lumina were much narrower and showed the presence of scarce if no sperms (Fig. 8, 6) the rete tubules were decreased in both number and size, their branching and anastomosing pattern was very much reduced, they were separated by wide interstitial tissue spaces and the mediastinal labyrinth could not be observed in any of the examined animals (Fig. 8, 7) the characteristic boundary tissue surrounding the epithelial components could not be differentiated from the adjacent interstitial tissue. The basal membrane has been lost its characteristic infoldings with the basal portions of the overlying epithelial cells and it became thinner. The surrounding myoid cells were missing, 8) the interstitial tissue became exclusively made up of dense fibrous tissue with minimal vascular components.

DISCUSSION

Considering the voluminous literature on the structure of the extratesticular duct system of experimental and domestic animals, it is quite extraordinary that the intratesticular portion has been taken little attention. PEREY, *et al.* (1961), demonstrated that the rat intratesticular duct system comprised of a transitional region lined exclusively of Sertoli cells, a tubulus rectus and the rete testis. An additional glycogen-rich segment was described in guinea pigs connecting the proximal portion of the

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tubulus rectus with the rete testis (FAWCETT and DYM, 1974). DHINGRA (1980) subdivided the goat intratesticular duct into; transitional zone, receptacle, tubulus rectus and rete tubules.

Although there is no adequate information pertaining to the structure of the intratesticular ducts of goat in general and those of the Bedouin one in particular, the present investigation has revealed that there are two periods of morphological activity affecting the epithelial components, the boundary tissue and the interstitial tissue. For convenience, the period between September and April was considered as the active period and that between May and August was the inactive one.

The active period was characterized by the regional differentiation of the intratesticular duct system. The latter, was including; the initial segment, tubulus rectus and rete testis. At its connection with the terminal portion of the seminiferous tubule, the initial segment was delineated by a prominent constriction demarcating the beginning of the duct system. It is suggested that this constriction, which was not described in any of the available data, represents a sphincter-like structure controlling the passage of the cellular and fluid components from the seminiferous tubules.

A distinctive feature of the filamentous apices of the Sertoli cells lining the intermediate zone of the initial segment was their entire inclination toward the lumen of the terminal zone where they form a plug-like mass. It is suggested that this passage may function as a valve-like structure allowing the passage of the cellular products of the seminiferous tubules in one direction toward the duct system preventing their reflux. Similar plugs have been described in monkey (DYM, 1974) and rabbit (DHINGRA and BARNWALL, 1977).

The terminal zone of the initial segment in guinea pig was considered as a glycogen-rich segment (FAWCETT and DYM, 1974). In the present study, no glycogen could be testified. In this respect, buck is similar to ram and monkey (DYM, 1972, 1974).

The tubuli recti, during the active period, were not always straight and their comparatively wider lumina showed the presence of a number of sperms and degenerated sperm components. The absence of these components in the lumina of the initial segments may suggest that the latter are designed to eliminate the sperms and other cellular components to the lumina of the tubuli recti and the rete tubules at a higher rate than the other segments of the duct system do.

The rete testis was comprised of a major intratesticular portion distributed axially in the mediastinum testis and a minor extratesticular one communicating with the efferent ductules. The intratesticular portion was made up of highly branched and anastomosed epithelial chambers separated by narrow interstitial spaces. The testes collected during Winter and Spring have clarified that the mediastinum testis was

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occupied by a single huge epithelial chamber draining the tubuli recti. This structure was not described in any of the available data, and therefore, the present study suggests to designate it as MEDIASINAL LABYRINTH. It is suggested that this single labyrinthine chamber was constructed as a result of the fusion of many rete tubules in order to receive the enormous number of sperms and other cellular components liberating from the seminiferous tubules during this period. HAFEZ (1987) has mentioned that the rate of spermatogenesis in buck reaches its peak during Autumn and Winter and the concentration of spermatozoa/1 ml ejaculate is 2-6 billion. It was also observed that the mediastinal labyrinth was communicating with thick-walled lymphatic vessels whose structure was similar to the veins with thick muscular media. The lumina of such vessels were heavily loaded with degenerated cellular components like those found in the lumen of the mediastinal labyrinth. It is suggested that this communication is essential in the elimination of a certain percentage of the degenerated cellular elements via the lymph nodes draining the testis and prevent their discharge in the ejaculate. In the other hand, it is suggested that the presence of luminal and intraepithelial macrophages in the various segments of the duct system may selectively engulf the degenerated cellular elements without any harmful effect on the normal spermatozoa. Selective phagocytosis by luminal macrophages was also observed in the epididymis of bull, rabbit and monkey (ROUSSEL *et al.*, 1967) and in the bull rete testis (GOYAL, 1982). The latter author has been confirmed that the epithelial lining of the bull rete testis has also a selective phagocytic activity.

A discrete boundary tissue was observed surrounding the epithelial components of the duct system. It was made up of a glycoprotein basal membrane, concentrically arranged layers of collagenic fibrils and vermiform cells which were morphologically identical to those termed peritubular contractile cells in rat and mouse testes (ROSS, 1967) and as myoid cells in guinea pig testis (FAWCETT *et al.*, 1969). A characteristic feature of the basal membrane during the active period, was its infolding with the basal portions of the overlying epithelial cells. similar infolding has been described in the rat rete testis (LEESON, 1962) where it is considered to represent a firm attachment of the epithelial lining to the surrounding interstitial tissue. The present investigation suggests that these irregularities may help in the distension of the duct system to receive increasing cellular and fluid discharges from the seminiferous tubules. The entire thickness of the boundary tissue may act as a barrier to the diffusion of certain molecules from the interstitial tissue to the ductal spermatozoa. JOHNSON and SETCHELL (1968) had observed that the ram rete testis fluid contained very little protein and only traces of immunoglobulin, meanwhile, their blood level was much higher.

The inactive period "May to August" was characterized by many involutionary changes affecting the various segments of the intratesticular ducts. These changes could be summarized in the following items: a) the regional differentiation of the different segments of the duct system became ill-defined, b) most of the initial segments

were missing, and in such conditions, the seminiferous tubules were directly communicating with the tubuli recti. When it was present, the intitial segment has been lost its sphincter-like constriction which may explain its regulatory action on the passage of sperms and other cells from the seminiferous tubules to the duct system, c) the apical plugs of the cells lining the intermediate zones which were considered to act as valves preventing the reflux of sperms to their site of production, were entirely indistinct. This indicates that the presence of such plugs is correlated with the spermatogenic activity, d) the tubuli recti decame in the form of penicillus-like long tubes with narrow lumina because they receive minimal secretory products, e) the rete tubules became much narrower and were separated by wider interstitial spaces; moreover, the mediasinal labyrinth was missing in all of the examined testes. This could be discussed on the basis that the growth and differentiation of the rete testis is directly proportional to the increased secretory activity of the seminiferous tubules, f) the epithelial lining of the different segments became attenuated which may indicate a diminution in its cellular metabolic activity. g) disappearance of the boundary tissue surrounding the epithelial components because of the great increase in the density of the interstitial tissue which makes both structures homogeneous.

The aforementioned data are entirely different from those of DHINGRA (1980) who mentioned that no appreciable difference in the histology of the intratesticular excurrent ducts of the Indian goat could be observed in different seasons of the year.

It is concluded that the hot climate of Sinai during summer has an inhibitory effect on the growth and differentiation of the intratesticular duct system of buck. Moreover, the structure of these ducts reflects the functional activity of the associated seminiferous tubules and could be taken as a morphological guide to determine such activity.

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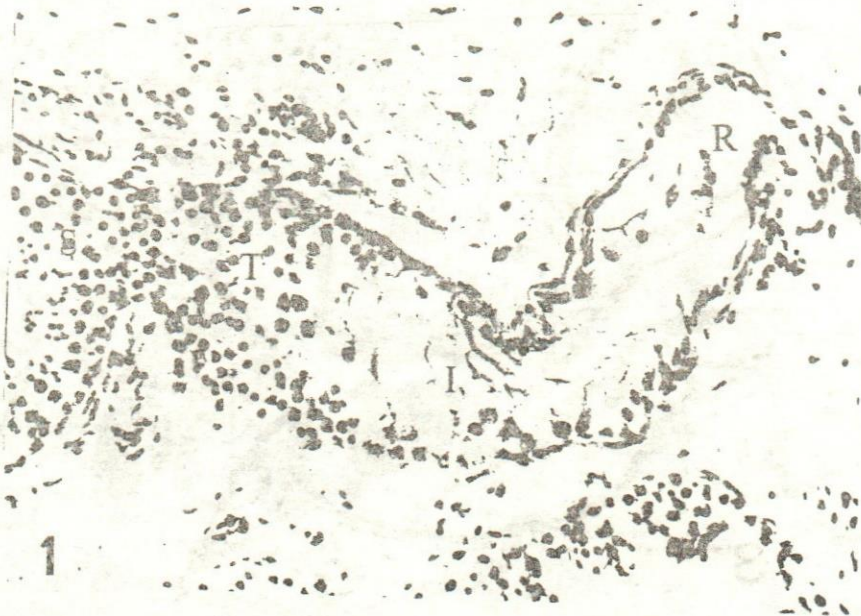
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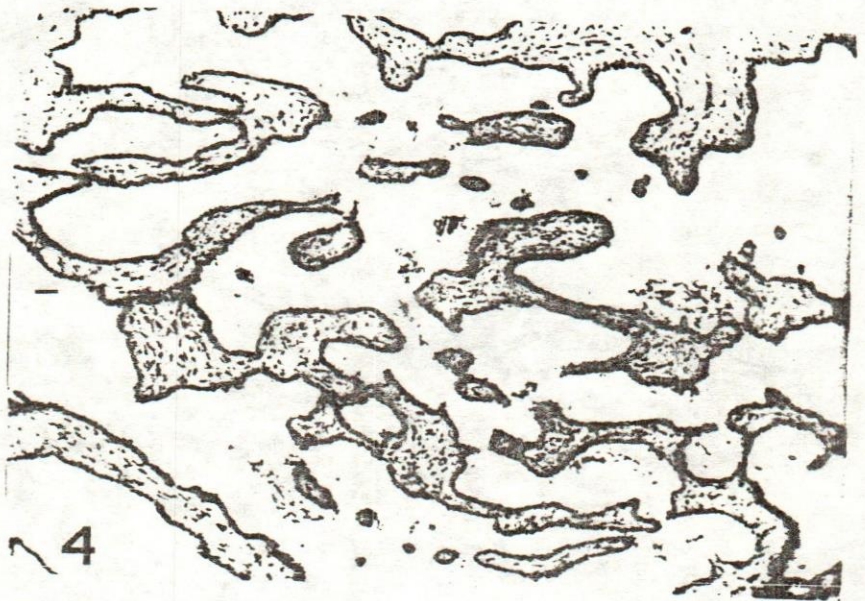
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- Fig. (1):** The initial segment during March with a prominent constriction delineating its connection with the seminiferous tubule (S). The segment includes; transitional zone (T), intermediate zone (I), and terminal zone (R). Hematoxylin and eosin. x 250.
- Fig. (2):** The initial segment during February. The intermediate zone (I) has a plug-like mass (P) projecting into the lumen of the terminal zone (R). PAS-hematoxylin. x 400.
- Fig. (3):** A straight tubule (T) communicating with a rete tubule (R) during April. Note the luminal degenerated sperm components. Hematoxylin and eosin. x 250.
- Fig. (4):** Rete testis during March, constructing of highly branched and anastomosed tubules separated with narrow interstices. Hematoxylin and eosin. x 125.
- Fig. (5):** Mediastinal labyrinth during November. It is lined with principle cells (P), columnar cells (L) and some basal mononuclear leucocytes (arrow head). The boundary tissue (B) around the epithelial lining is denser than the interstitial tissue (T). PAS-hematoxylin. x 400.
- Fig. (6):** Rete tubules during March. A patch of clear cells (C) among the principle ones (P). The lumen contained degenerated sperm components and luminal macrophages (M). The boundary tissue (B) is clearly demarcated from the loose interstitial tissue. Note the undulations of the basal membrane (arrow head). PAS-hematoxylin. x 400.
- Fig. (7):** Thick-walled vessel (V) communicating with the rete tubules. This communication may be artifactual. Hematoxylin and eosin. x 250.
- Fig. (8):** Mediastinal testis during July. A tubulus rectus (T) with straight course and narrow lumen; the rete tubules (R) are separated by wide, dense and homogeneous interstitial tissue (H). Hematoxylin and eosin. x 125.

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