



Histomorphometrical and Histochemical Seasonal Variations in the Epididymis of Meriz in Kurdistan Region of Iraq



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THIS STUDY investigated the histomorphometrical and histochemical characteristics of the epididymal duct in 24 sexually mature male Merize bucks across seasons. This duct was divided into six segments; IS of the caput, PS, MS and DS of the corpus and PS and DS of the cauda. The maximum total diameter, epithelial height and luminal diameter of all epididymal segments were significantly higher ($p < 0.05$) in autumn (October) compared to other seasons. The epithelial lining of the epididymal duct consisted of a pseudostratified columnar encompassing five distinct cell types: PCs, BCs, ACs, NCs, and CCs. Intraepithelial glands were notably present exclusively in the IS. PCs had lightly stained nuclei and abundant cytoplasm indicating increased activity during autumn compared to darkly stained nuclei and scant cytoplasm in other seasons. The cytoplasmic granules, apical blebs, stereocilia and secretion of the PCs as well as cytoplasmic granules and globules of the BCs along with the secretion of intraepithelial glands exhibited a strong positive reaction for PAS and Diastase-PAS stains during autumn and a moderate reaction with same stains during other seasons indicating the presence of neutral glycoproteins. Furthermore, the stereocilia showed a strong reaction with AB (pH 2.5) in autumn and a moderate reaction during other seasons showing the presence of acid mucopolysaccharides. ACs, NCs and CCs were more numerous in autumn, winter and spring but less in summer. Immune cells were consistently present throughout the epididymal duct in all seasons. The PMC was thick and the ICT contained numerous blood vessels in autumn.

Keywords: Meriz buck's, Epididymal duct, Histomorphometric, Histochemical, Seasonal changes.

Introduction

The Meriz goats are considered the only main breed in Kurdistan region produced the cashmere [1]. This breed is smaller in size than the local mountain goat breed, easy to handle and it is regarded the most favorable goat breeds for the mount due to the adaptation well for the elevation conditions and also is a source of meat and milk production [2]. The Kurdistan Organization for Animal Protection (2012) has

called for the protection of Meriz goats from the threat of extinction. The organization conducted a statistical study to determine the number of these animals in Duhok Governorate which revealed that the population of these goats had decreased to 4,000 in 2010 down from more than 10,000 in 2007. (<https://www.iraqhurr.org/a/25735228.html>). The epididymis serves as a tubular structure linking the testis to the vas deferens encompassing four distinct anatomical segments: the initial

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segment, caput, corpus and cauda. Throughout the transit within the epididymal passages sperm maturation takes place through the complex interaction of sperm cells with the distinctive luminal environment in each of these regions. The epididymis a vital component of the reproductive system plays a pivotal role in tasks such as sperm concentration, maturation, safeguarding sperm and providing storage facilities [3]. Similar to other economically valuable livestock, the examination of the reproductive organ morphology of Meriz bucks is crucial for enhancing and optimizing their reproductive functions. This research is vital for the improvement and effective utilization of their reproductive capabilities. After reviewing existing studies on Meriz goats, it is evident that no research had been conducted on the morphology of the epididymis in this animal. In light of these findings, so this study aimed to inspect histomorphometric and histochemical characteristics of the epididymis throughout various seasons.

Material and Methods

Animals

The epididymal ducts from twenty-four sexually mature 2-year-old Meriz bucks gathered from Sumel town, Duhok Province, Kurdistan Region of Iraq throughout the year (Table 1). Age determination in the Meriz bucks involved examining the goat deciduous teeth dental formula: $2(Di\ 0/4; Dc\ 0/0; Dp\ 3/3) = 20$. Furthermore, the permanent teeth were inspected using the dental formula: $2(I0/4; C0/0; P3/3; M3/3) = 32$ for thorough age estimation.[4] Animal's health was clinically examined by testing their blood and fecal samples at the Medicine and Surgery Department of College of Veterinary Medicine, Duhok University to ensure disease-free status.

Tissue samples

The animals were first castrated under the influence of local anesthesia using 10 ml of 2% lidocaine [5] in the previous department. Subsequently the testes and epididymis were cleaned from blood and debris using normal saline. After that, the testes and epididymis were carefully separated. The epididymis divided into six segments as shown in (Fig.1). Each segment cut with as a size of 0.5 to 1 cm in size and there was an interval about 2 cm between each segment. [6]

Preparation of tissue specimens

Some epididymis samples were fixed in Bouin's solution for 4-18 hours, while others were

kept for (24-48) hours in 10% neutral buffered formalin. After fixation, the samples were dehydrated in increasing ethanol concentrations, cleared in xylene, and then embedded in paraffin wax at 58-60°C. A rotary microtome was used to create successive tissue sections with a thickness of 4-5 μm . Subsequent staining of the tissue sections utilized the following dyes, and all methodologies followed the procedures outlined in [7].

1. The general tissue structure was illustrated using Harris hematoxylin and eosin stain (H&E).
2. Masson's trichrome stain (MTS) was used to confirm the existence of collagen and muscle fibers.
3. Identification of reticular fibers was accomplished through the application of silver impregnation.
4. Periodic Acid Schiff (PAS) staining was conducted to demonstrate neutral mucopolysaccharides.
5. Diastase-Periodic Acid Schiff (Diastase-PAS) stain was implemented to distinguish glycogen from other PAS-positive elements.
6. To identify acidic mucopolysaccharides, use the Alcian blue pH 2.5 (AB pH 2.5) stains.

The first stain was carried out at the Duhok Central Laboratory in Duhok City; while the remaining stains were carried out at the RNA Research Laboratory in Mosul City and the Vajeen Hospital in Duhok City.

Morphometrical and Statistical analysis

Stained slides with H&E and MTS were prepared for general structure and morphometrical analysis. Image measurements were captured and examined using a light microscope (Olympus CX22, Japan) equipped with a digital microscopic USB 3.0 camera (SWIFT, 18MP, China). All microscope objective lenses were calibrated with the aid of stage micrometer (μm). The measurements included the following parameters: epididymal ductular diameter, epididymal luminal diameter and epididymal epithelial height. Approximately ten tubular cross-sections were measured from each part of the epididymis using a medium-power lens (10 x magnifications) for each of the mentioned parameters. One-way analysis of variance (ANOVA) was employed to analyze all the data. Using JMP Pro 14.3.0 (<https://>

www.jmp.com/content/jmp/en_us/home.html), a Tukey test (was applied to identify any specific differences between the various segments. The mean \pm SD was used to indicate the accepted level of significance, which was established at $p < 0.05$.

Ethical Considerations

The approval of the ethics committee of the college of veterinary medicine at the University of Duhok was obtained to conduct this study (references no; VM2020/0112UD), issue date: 1-12-2020.

Results

Histomorphometrical findings

As shown in Tables 2, 3 and 4, the epididymal duct in Meriz buck's revealed significant variations in total diameter, epithelial height and luminal diameter in all seasons. The total diameter showed a distinct pattern gradually decreasing from the initial segment of the caput to the middle segment of the corpus. Subsequently, it increased steadily in the distal segment of the corpus getting the extreme diameter in the distal segment of the cauda. Epithelial height exhibited a different trend increasing in the initial segment of the caput and then decreasing towards the distal segment of the corpus. A marked decrease was observed in the proximal and distal segments of the cauda. The luminal diameter displayed an increase in the initial segment of the caput followed by a decrease from the middle segment of the corpus. A marked increase was noted in the distal segment of the corpus the maximum diameter was gutted in both segments of the cauda. All three parameter was significant difference at p -value 0.05. Notably, the maximum total diameter, epithelial height and luminal diameter across all segments of epididymal duct was significantly higher ($p < 0.05$) during the breeding season (autumn) particularly in October compared to the non-breeding seasons.

Histological and histochemical findings

Firstly, the histological and histochemical description of epididymal segments was discussed particularly in autumn and these characteristics became more obvious in October. The epididymal duct was lined by pseudostratified columnar epithelium. Five types of cells were observed in this epithelium: principal (PCs), basal (BCs), apical (ACs), narrow (NCs), and clear (CCs). Additionally, immune cells such as intraepithelial lymphocytes (IELs) and macrophages that including intraepithelial macrophages (IEMs)

and intraluminal macrophages (ILMs) were also noted.

The initial segment of the caput

The epithelium in this segment exhibited undulating appearance (rising and falling). The epithelial crypts were frequently found in this segment (Fig. 2). Furthermore, the epithelium of this segment revealed numerous intraepithelial glands that were characteristic of this segment (Fig.3). These glands were lined with a simple columnar epithelium that released its contents into the lumen of the epididymal duct. The luminal secretion from these glands exhibited a robust response to PAS (Fig.4) and Diastase-PAS stains.

Principle cells (PCs)

The (PCs) predominated as the most prevalent cell types along the entire length of the epididymal duct, extending from the basal lamina to the lumen. The nuclei of (PCs) exhibited an oval to elongated shape, predominantly located adjacent to the basal lamina of the epithelium. Sometimes, the nuclei could be located most apically, especially in the hill of the epithelium. The nucleus of PCs was lightly stained and possessed one or more nucleoli. The cytoplasm of PCs was abundant and contained fine granules that faintly stained pink with eosin and strongly reacted with PAS. In addition to that, different sizes of vacuoles were noted in the supranuclear and infranuclear regions (Fig.6). The boundaries of these cells were not clearly defined along the entire epididymal duct. The apical edges of the PCs were adorned with stereocilia, exhibiting a pronounced positive response to PAS, Diastase-PAS, and AB (pH 2.5). (Fig.7). Different sizes of the apical blebs projected from the apical borders of the PCs into the lumen that stained pink (Fig.8) and a strong PAS, Diastase -PAS.

Basal cells (BCs)

The BCs were regarded as the second most common cells along the whole epididymal duct. The BCs were situated adjacent to the basal lamina of the ductal epithelium in close association with neighboring PCs (Fig. 8). The nuclei of the BCs possessed different shapes: rounded, elongated, triangular and pear-shaped. The cytoplasm of some BCs contained strongly PAS (Fig.9) and Diastase -PAS positive globules and granules.

Apical cells (ACs)

The ACs were wedge-like cells with a wide portion toward the apex and a narrow base distally. They were observed in the apical portion

of the ductal epithelium close to the lumen. These cells were present singly among PCs, and their numbers ranged from one to three in each tubular cross-section. The luminal border of the ACs lacked stereo cilia and had a somewhat dome-shaped appearance. The nucleus was triangular in shape with its base directed apically and its apex directed basally (Fig. 7).

Narrow cells (NCs)

NCs were slender, elongated cells situated at intervals (Fig. 10) within the ductular epithelium. These cells were narrower than the PCs and were situated between them. They possessed darkly stained, elongated, spindle-shaped nuclei located apically or distally. The NCs contained few cytoplasmic vacuoles and granules that strongly stained with PAS and Diastase- PAS.

Immune cells

IELs located at various positions of the ductal epithelium, their nuclei were frequently dark, rounded in shape and Enclosed by a slim, lightly colored cytoplasmic border. (Fig. 11). IEMs were observed at various levels of the epididymal epithelium (Fig 12), while ILMs were located inside the lumen (Fig.13). Both cell types predominantly exhibited nuclei that were eccentric, with a rounded to oval shape, surrounded by acidophilic cytoplasm. Additionally, some of the IEMs displayed nuclei with a kidney-shaped appearance. (Fig.12).

The proximal segment of the corpus

In this section, the lumens were consistently oval or rounded, enclosed by a uniform epithelium containing concentrated masses of spermatozoa. (Fig.14). The cytological characteristics of PCs were similar to those of the initial segment of the caput, except that the nuclei of PCs were elongated oval in shape and situated toward the middle of the cells. The shape, position and characteristic features of BCs, NCs, ACs and immune cells were similar to those of the previous segment. However, the numbers of BCs were fewer when compared with those in the initial segment of the caput.

The middle segment of the corpus

All the cells in this segment were similar to those of the initial segment of the caput and proximal segment of the corpus, despite that the BCs became more numerous and contained large PAS and Diastase-PAS positive globules (Fig.15). The ACs and NCs were more recurrent in this segment comparing with those of the previous segments.

The distal segment of the corpus

The lining epithelium of this segment had slight folding that gave the lumen an irregular oval to triangular or hexagonal appearance. The lumina of the tubules might contain condensed or dispersed masses of spermatozoa (Fig.16). The cytological features of all cells in this segment were similar to those of the initial segment of the caput, excluding that the luminal secretion of the PCs revealed strong PAS (Fig.17) and PAS-Diastase positive reaction.

The proximal segment of the cauda

The lumina of the epididymal duct in this segment revealed irregularity in shape due to abundant epithelial folds. All cells of this segment were identical to the cells of the initial segment of caput with the exception that the nuclei of the PCs were large and more crowded. They seem to form a single row just above the level of the BCs leaving a wider supranuclear zone than infranuclear one. The apical border of PCs was equipped with short, crowded stereocilia. Large cytoplasmic vacuoles were observed at the apical region of the PCs as well as numerous apical blebs that displayed more intense PAS and Diastase -PAS-positive reaction compared to the preceding segments (Fig.18). The luminal secretion of the PCs became more abundant and showed positive reaction with PAS (Fig. 19) and Diastase-PAS stains. The BCs formed a distinct layer (Fig.20). Most of them revealed numerous granules (Fig .20) and fewer globules than those in the previous segments. The ACs and NCs were rare present. In addition to the aforementioned cells, another cell type could be seen in this segment known as CCs. The CCs were large cells located between the PCs and extended from the basal lamina to the epididymal lumen. In this segment, these cells were more prominent in the cauda when compared to the caput and corpus. The CCs exhibited cytoplasm with a light stain and a sizable oval nucleus positioned centrally with an open face. (Fig.20).

The distal segment of the cauda

The lumina were round or oval and wider than any other former segments (Fig.21). In this segment the lumina were filled with abundant condensed masses of spermatozoa. The lining epithelium of this segment tended to be regularly uniform (Fig.21) and lower than any other preceding segments. Further distally, near the vas deferens the lumen of this segment became very wide and irregular due to the presence of large folds. Each fold comprised of the epithelium,

peritubular connective tissue and smooth muscle which protruded into the ductal lumen (Fig.22). The previous cytological differences that had been mentioned in the proximal segment of the cauda were also observed in this segment except, the stereocilia became shorter and more intensely reacted with both PAS and Diastase -PAS as well as AB (pH 2.5) stains and the nuclei of the PCs were located in the middle of the cell, although some of them were displaced more apically. Additionally, an intraluminal multinucleated giant cell was observed only within the lumen of this segment (Fig. 23).

Peritubular connective tissue (PCT) and peritubular muscular coat (PMC)

A dense network of reticular fibers which stained black with silver impregnation stain formed the reticular lamina of the basement membrane along all segments of the epididymal duct (Fig. 24). The PMC consisted of several layers of circularly arranged smooth muscle fibers that stained pink with eosin and red with Masson's trichrome (Fig.25). The thickness of this muscular coat varied from one segment to another, being slightly thin in the initial segment (Fig.25) and gradually became thick towards the distal segment of the cauda (Fig.26). Fine collagen fibers and reticular fibers were found among the smooth muscle fibers (Fig.25).

Interstitial connective tissue (ICT)

The ICT in the initial segment of the caput and the proximal segment of the corpus was loose connective tissue (Fig. 25), while in another segments of the corpus and cauda it was dense connective tissue (Fig. 26). Furthermore, the ICT of the distal segment of the cauda possessed numerous blood vessels including arterioles, venules and a network of capillaries (Fig.26) more than in any other segments of the epididymal duct.

Histological and histochemical seasonal changes

The current study revealed some seasonal variations in the PCs and BCs along the epididymal duct in non-breeding seasons; winter, spring and summer. The PCs exhibited darkly stained nuclei with scant cytoplasm and mostly located basally (Fig .27). The number of BCs with their granules and globules became fewer. All histochemical stains of the PCs and BCs that had been mentioned in the breeding season became moderately staining in non-breeding seasons (Fig.28). The number of the ACs, NCs and CCs cells were numerous in autumn followed by

spring (Fig.29), winter and became less common in summer. These cells did not show significant cytological changes in and out of the seasons. Both IELs and IEMs were found along the entire length of the epididymal duct throughout the seasons. The intraepithelial glands became fewer in non-breeding seasons (Fig. 30) and their secretion showed moderately reaction with PAS and Diastase-PAS stains. In non-breeding season, the luminal content of the spermatozoa became less in all the segments, except the cauda. The fold of the distal segment of the cauda became smaller in non-breeding seasons. The PMC exhibited an increase in their thickness from the caput towards the cauda across all seasons. The thickness of the PMC, connective tissue density and vascularity of the ICT were reduced during non-breeding seasons (Fig.31).

Discussion

The epididymal duct of the Meriz bucks revealed significant seasonal variations in total diameter, epithelial height and luminal diameter. The gradual decline in epithelial height was observed from the initial segment towards the distal segment of the cauda in all seasons demonstrating statistical significance at $p < 0.05$. This gradual decrease in epithelial height might facilitate the mechanical passage of sperm towards the terminal segment [8]. Furthermore, the higher epithelium in the initial segment might suggest a greater absorptive capacity of the epithelium in this region. In the present work, the maximum epithelial height in all segments was observed during autumn that significantly differences ($p < 0.05$) when compared with other seasons. In the current study, the distal segment of the cauda exhibited the widest lumen, the lowest epithelial height and the highest sperm concentration. A possible explanation for these changes was thought to be adaptations to maximize the storage and maturation of sperm during the breeding season. [9] in bulls stated that the proximal portion of the tail exhibited slightly folded epithelium and these folds gradually faded as they extended towards the ductus deferens. Our findings support partly with this result, since we observed these folds in the distal segment of the cauda near the vas deferens. These folds may potentially play a role in delaying the passage of epididymal fluid and spermatozoa to the vas deferens. The epididymal duct of Meriz buck was lined by pseudostratified columnar epithelium which was similar to findings reported by [10] in Gaddi goat, [11] in Marwari goat, [12]

in Assam goat and [13] in Marwari sheep. In the current study, this epithelium comprised mainly of five cell types; PCs, BCs, ACs, NCs, and CCs. However, [14] and [15] reported four cell types; PCs, BCs, NCs and ACs [12] observed three cell types; PCs, BCs and ACs in the same species. This variation in cell types was due to different goat breeding in various geographical territories.

In the present study, the PCs were primarily responsible for the absorption and secretion of materials into the epididymal lumen, so they exhibited high secretory and endocytic activity. They were also modulating luminal pH [16] and [17]. During the breeding season the PCs showed lightly stained oval nuclei and abundant cytoplasm indicating increased activity compared to the darkly stained nuclei and scant cytoplasm observed in non-breeding seasons. These findings were similar to [8] and [18] in camel bulls.

Our results revealed that the apical blebs and cytoplasmic granules of the PCs as well as the luminal secretion reacted strongly with PAS, Diastase-PAS during breeding seasons and moderately reacted out the season. These reactions indicated for presence neutral glycoproteins. [19] described that these glycoproteins contributed to the spermatozoa maturation, capacitation, and fertilization. In the current study, the stereocilia reacted strongly with PAS, Diastase-PAS, and AB pH (2.5) stains during breeding season and moderately with same stains during the rest of the seasons. This result indicated the presence of glycoprotein and acid mucopolysaccharides materials. Our results revealed that the BCs showed some cytological changes across the seasons. However the number of these cells became more numerous in autumn and their granules and globules increased in autumn, winter than in summer and spring. [18] and [20] found increased activity in BCs during the breeding season indicating their potential role as stem cells for PCs. Furthermore, our findings mentioned that these granules and globules reacted strongly with PAS and Diastase-PAS stains in breeding season. In the same respect, [21] mentioned that the globular structures were considered PAS-positive residual bodies following the reabsorption and digestion of ductular fluid.

In the present study, the ACs, NCs and CCs were numerous in autumn followed by spring, winter, and they became less common in summer. These cells were different in shape, number and position within the ductal epithelium. [22] and [23] had

been mentioned that each of these cells possessed a unique function. Furthermore, the coordinated interactions between spermatozoa, PCs, BCs, NCs, CCs and immune cells form a complex process by which the epididymal epithelium established and managed the conditions for sperm maturation, protection, selection and storage [24]. So, for these facts, our opinion documented that these cells were regarded as an independent cells [25].

The current study pointed out that both IELs and IEMs were present throughout the entire epididymal epithelium in all seasons. Similar observations had been documented in goats [26, 14, 27] and bulls [9]. According to previous reports, immunological tolerance may be induced by macrophages and lymphocytes, preventing the animal's body from mounting an immune reaction against spermatozoa [28]. Epididymal epithelium of Merize buck contained numerous intraepithelial glands in the initial segment of the caput only in all seasons. These findings were inconsistent with those of [29] and [18] in camels as well as [30] in boars who mentioned that these glands were more numerous in the distal portion of the middle segment. Our findings revealed that the lumen of the intraepithelial glands were filled with strong PAS, Diastase - PAS positive material in breeding season and moderately reacted with the same stains in non-breeding seasons. [31] stated that these glands potentially play a role in epididymal function, including the establishment of a secretory microenvironment and the recycling of damaged organelles or unused proteins for reuse or to provide energy under stress conditions. Additionally, they might serve as regulatory sensors for seasonality.

Our results indicated that the PMC increased distally during both breeding and non-breeding seasons especially in cauda. These changes were more pronounced in autumn compared to other seasons. Similarly, [32] in camel bulls showed that the thicker muscular coat in the terminal segment of the epididymis during the breeding season might contribute to powerful ejaculation. The present investigation indicated that the ICT became progressively denser and contained numerous capillaries, arterioles, venules and lymphatics in the distal segment of the cauda during breeding season comparing to other seasons. These findings were agreed with the researchers conducted by [9] in bulls and [29] in camels. The unique and densely layered arrangement of capillaries

beneath the lamina epithelialis in the bovine cauda epididymidis provided a strong morphological basis for intensive metabolic processes [25] Bucks were considered as seasonal breeders being short or long day breeders, the sexual activity of the bucks varies among breeds and the animal's geographical location [33]; [34]; [35] The researches of the animals management mentioned that the optimum seasonality was seen in late summer and autumn [36]; [37]. Furthermore, some studies on Meriz bucks indicated that the highest rates of reproduction occurred in August followed by September [38]. Histomorphometric and histochemical of our studies documented that the breeding season occurred in autumn particularly in October. Our disagreement with the researchers above was attributed to the impact of global warming on some countries worldwide including Iraq [39]. This phenomenon had affected the seasonality of mating, as previously, two decades ago, mating occurred at the end of summer and beginning of autumn. Since goats had the ability to adapt to changing conditions and

environments [40] therefore the animals breeding season had extended to the October aligning with the altered patterns caused by global warming.

Conclusion and Recommendations

The results of our histological, morphometrical and histochemical studies proved that the breeding season for Meriz bucks occurred in autumn and specifically in October. . Therefore, we recommend that the breeders of these animals rely on this month for mating, as it has been shown to yield the best results compared to other months.

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Conflicts of interest

The authors declare no conflict of interest.

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TABLE 1. Distribution of epididymal samples used in different seasons for histomorphometric and histochemical studies.

Seasons	Months	Number of animals
Winter	December (2020)	2
	January (2021)	2
	February	2
Spring	March	2
	April	2
	May	2
Summer	June	2
	July	2
	August	2
Autumn	September	2
	October	2
	November(2021)	2

TABLE 2. Comparisons of total diameter among different segments of Merize buck epididymal duct in each month

	Month 11	Month 10	Month 9	Month 8	Month 7	Month 6	Month 5	Month 4	Month 3	Month 2	Month 1	Month 12
Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
127.444±24.890*	204.076±19.313	167.744±13.297*	194.392±12.271	167.304±14.757*	145.222±7.612*	125.539±11.265*	171.965±18.277	165.134±18.535*	162.136±18.455*	159.137±18.555*	142.425±15.129*	Caput Initial segment
122.204±12.768*	184.895±16.806	160.583±14.206	181.040±11.496	123.677±8.646*	110.987±8.593*	120.395±4.955*	157.247±4.282	144.082±20.275*	141.072±20.285*	143.082±21.295*	107.882±7.347*	Proximal segment
100.478±12.338*	175.965±21.133	157.429±17.192	142.320±16.249	106.840±10.182*	108.358±6.808*	113.809±5.691*	149.094±13.371	134.410±24.458	137.309±24.458	135.510±24.458	99.986±9.001*	Corpus Middle segment
135.374±21.724*	184.895±16.806	160.583±14.206	164.090±12.940	116.129±4.564*	132.762±20.110*	121.447±3.519*	159.964±6.835	165.054±10.660	166.054±11.760	167.044±12.760	130.323±12.893*	Distal segment
154.592±25.713*	285.206±16.423	191.532±13.675*	187.955±25.127*	137.623±5.172*	142.097±9.918*	124.738±24.632*	187.734±19.157*	282.394±20.645	280.391±14.636	281.392±23.624	163.395±21.612*	Cauda Proximal segment
220.288±54.351*	342.425±15.336	292.755±35.152	239.151±44.412*	257.008±38.685*	151.965±15.306*	159.197±4.375*	223.037±46.635*	294.927±34.863	295.825±37.782	293.826±37.982	168.700±46.426*	Distal segment

The values were expressed as mean ± SD., significant level (*) was considered at p<0.05 compared to month 10.

TABLE 3. Comparisons of epithelial height among different segments of Merize buck epididymal duct in each month

	Month 11	Month 10	Month 9	Month 8	Month 7	Month 6	Month 5	Month 4	Month 3	Month 2	Month 1	Month 12
Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
35.748±5.399*13	46.898±6.266	39.654±8.229	44.412±5.052	45.378±11.629	34.772±6.385*	31.227±3.906*	44.691±4.466	34.215±5.125*	33.225±4.225*	32.235±5.225*	31.879±5.490*	Caput Initial segment
29.383±4.114*	44.665±2.177	36.992±3.421*	33.609±4.308*	33.764±1.282*	30.838±3.310*	28.499±3.747*	32.442±6.056*	27.711±2.650*	30.621±2.651*	31.721±2.652*	29.552±3.110*	Proximal segment
25.215±4.697*	40.325±2.806	37.435±5.155	29.861±1.457*	32.129±4.588*	28.098±1.377*	21.564±3.283*	30.461±5.451*	24.609±2.092*	22.619±2.191*	21.016±2.026*	23.664±2.222*	Corpus Middle segment
16.748±5.399*	38.860±5.123	27.889±3.663*	27.479±5.784*	23.559±2.153*	23.503±1.522*	18.008±4.019*	17.799±1.769*	22.014±2.027*	20.015±2.156*	20.629±2.091*	21.826±1.452*	Distal segment
14.876±1.688*	20.028±2.251	16.066±2.367*	18.014±4.919*	15.277±2.560*	19.079±4.988*	16.986±1.685*	13.365±4.337*	18.977±1.424*	18.979±1.414*	17.879±1.433*	11.064±2.073*	Cauda Proximal segment
..250±1.461*	18.703±5.558	12.660±2.703*	16.069±2.556	14.356±2.841	10.378±1.864*	13.382±0.650*	11.953±1.024*	14.558±3.271	15.559±3.260	16.549±3.270	10.020±1.988*	Distal segment

The values were expressed as mean ± SD., significant level (*) was considered at p<0.05 compared to month 10.

TABLE 4. Comparisons of luminal diameter among different segments of Merize buck epididymal duct in each month

Month 11	Month 10	Month 9	Month 8	Month 7	Month 6	Month 5	Month 4	Month 3	Month 2	Month 1	Month 12
Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
70.69±20.144*	129.77±27.043	91.94±13.366	114.57±9.273	93.11±24.904	95.64±4.495	76.89±14.893*	117.599±15.178	106.816±12.257	108.818±12.248	109.817±11.258	69.205±8.828*14
69.429±8.808*	119.990±15.914	84.937±24.932	95.624±26.938	56.729±8.268*	63.278±9.105*	73.568±6.375*	107.420±8.135	94.327±19.658	95.327±20.559	97.327±20.659	66.644±6.003*
54.174±2.849*	98.497±22.936	82.413±17.429	87.973±12.892	45.300±6.919*	47.058±7.348*	59.252±7.649*	92.762±6.469	94.086±20.797	91.086±22.687	95.086±21.697	53.127±5.624*
60.658±11.873*	102.124±23.870	93.173±17.232	96.865±16.977	61.930±10.633*	69.221±15.231*	65.441±5.609*	93.651±8.583	97.844±15.644	99.844±13.743	100.843±14.744	68.086±9.793*
126.378±25.738*	269.296±13.051	139.049±35.026*	136.814±22.367	106.197±8.315*	70.075±11.739*	76.193±22.965*	161.160±19.900	264.739±20.534	260.737±20.523	262.738±20.524	141.992±23.182*
204.69±56.116*	323.334±18.857	153.520±20.948*	212.619±47.417*	229.488±40.840	112.888±22.902*	133.506±7.106*	198.455±6.887*	273.544±41.036	269.544±42.037	270.543±43.038	146.393±11.157*

The values were expressed as mean ± SD, significant level (*) was considered at p<0.05 compared to month 10.

- I. The initial segment of the caput(IS)
- II. The proximal segment of the corpus(PS)
- III. The middle segment of the corpus(MS)
- IV. The distal segment of the corpus(DS)
- V. The proximal segment of the cauda(PS)
- VI. The distal segment of the cauda(DS)

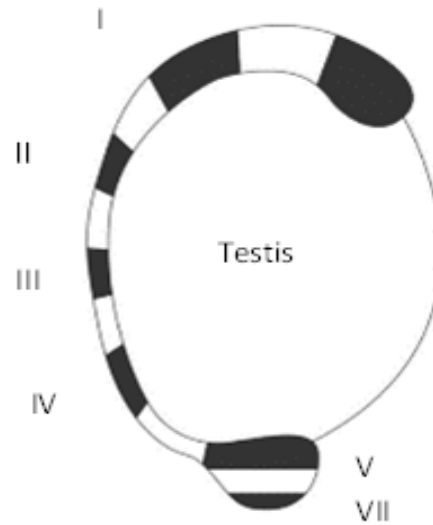


Fig.1. A diagram of the testis and epididymal duct of Meriz buck's illustrated the different segments from which the samples were obtained

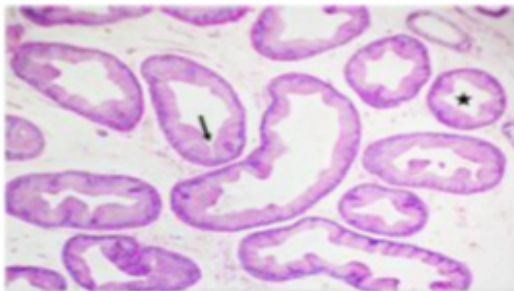


Fig.2. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: Lumen(star) Epithelial crypt (arrow) Stain:H&E (x4)

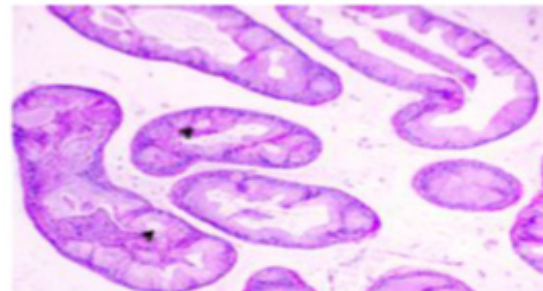


Fig.3. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: intraepithelial glands (arrows head) stain: H&E (x4)

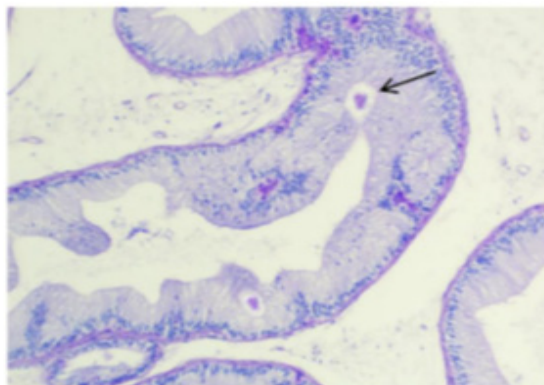


Fig.4. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: strong secretion (arrow). Stain: PAS (x10)

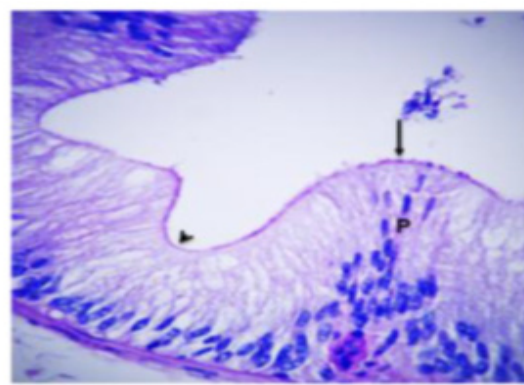


Fig.5. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: Principle cell (P), rising (arrow), falling (arrow head). Stain: Diastase-PAS (x40)

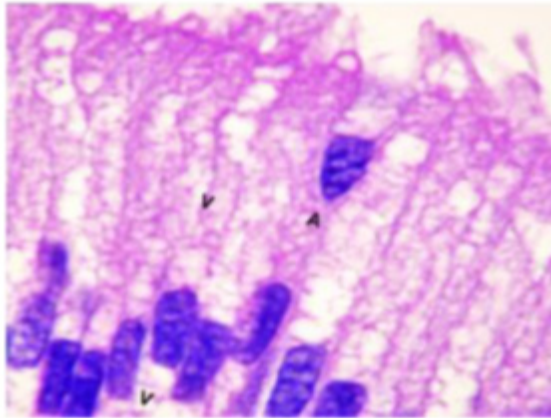


Fig.6. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: Apical cell (A), Vacuoles (V) Stain: H&E

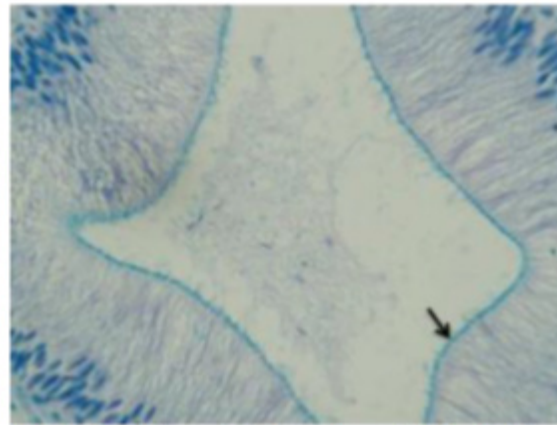


Fig.7. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: stereocilia (arrow) Stain: AB pH2.5(x40)

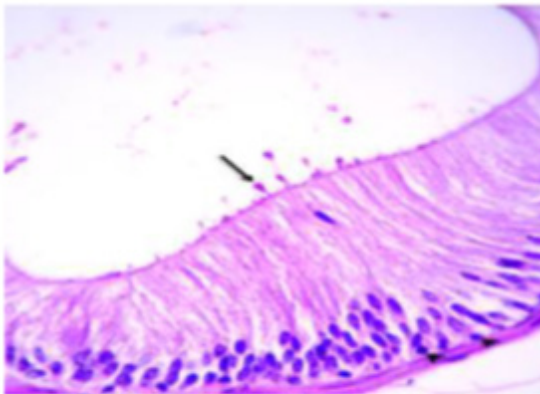


Fig.8. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: Apical bleb (arrow), Basal cells (arrows head). Stain: H&E (x40)

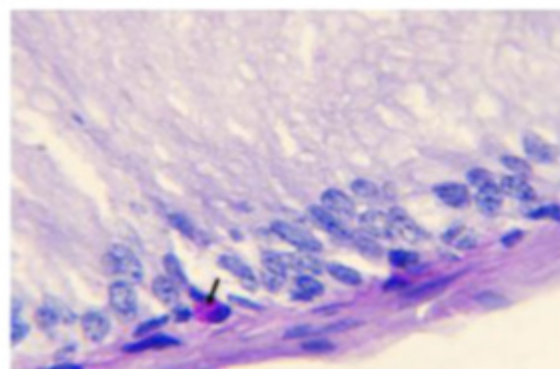


Fig.9. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: Basal cell with its globules (arrow) Stain: PAS (x 100)

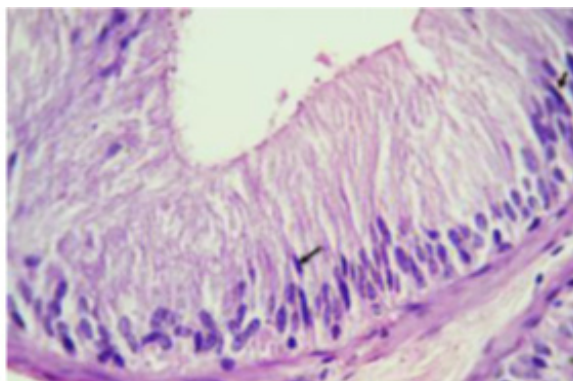


Fig.10. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: Narrow cells (arrows) stain: H&E(x40)

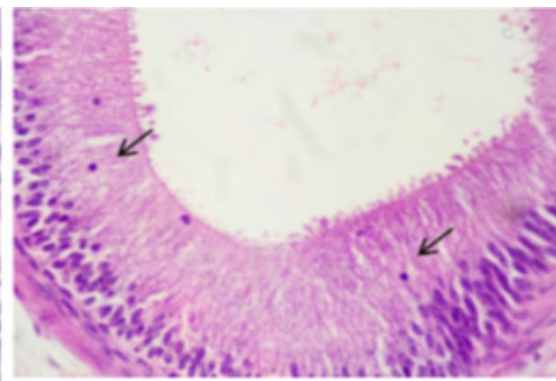


Fig.11. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: IEL (arrows) Stain: H&E (x40)

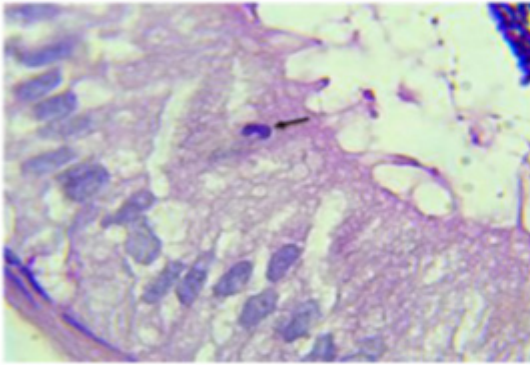


Fig.12. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: Nucleus (arrow) of IEM. Stain: PAS (x100)

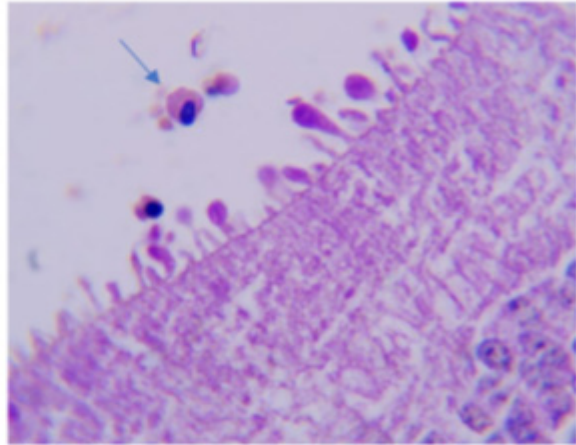


Fig.13. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during autumn showing: ILM (arrow) Stain H&E (x100)

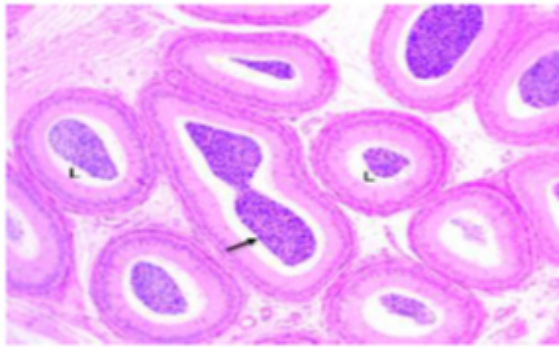


Fig.14. Photomicrograph of a section at the level of the proximal segment of corpus of Meriz buck's epididymal duct during autumn showing: lumina (L), Spermatozoa (arrow) Stain: H&E (x10).

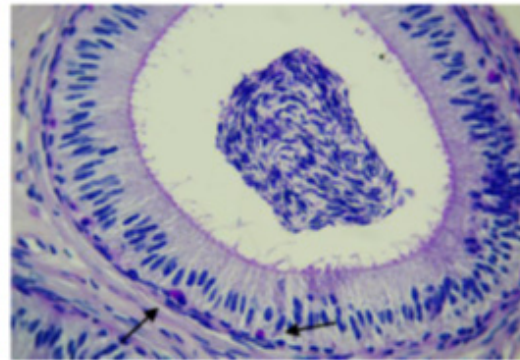


Fig.15. Photomicrograph of a section at the level of the middle segment of the corpus of Meriz buck's epididymal duct during autumn showing: Globules of basal cells (arrows).Stain: Diastase -PAS (x40)

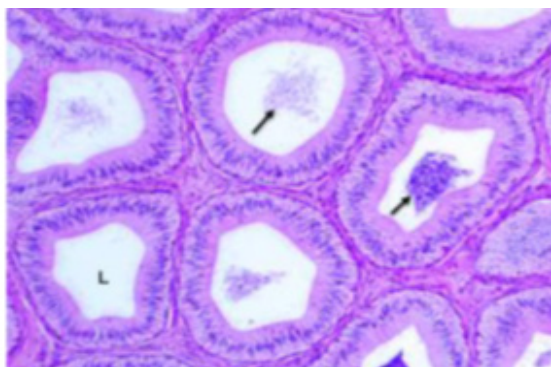


Fig.16. Photomicrograph of a section at the level of the distal segment of the corpus of Meriz buck's epididymal duct during autumn showing: lumen (L) with spermatozoa (arrows). Stain. H & E (x10)

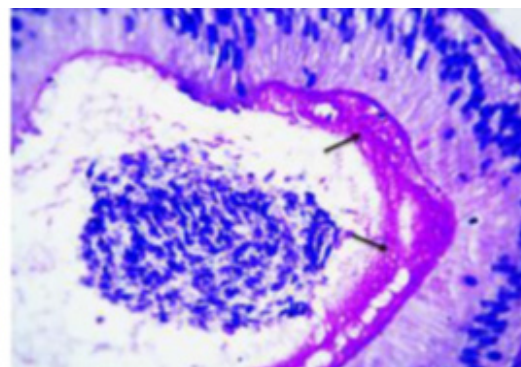


Fig.17. Photomicrograph of a section at the level of the distal segment of the corpus of Meriz buck's epididymal duct during autumn showing: luminal secretion (arrows) .Stain: PAS (x40)

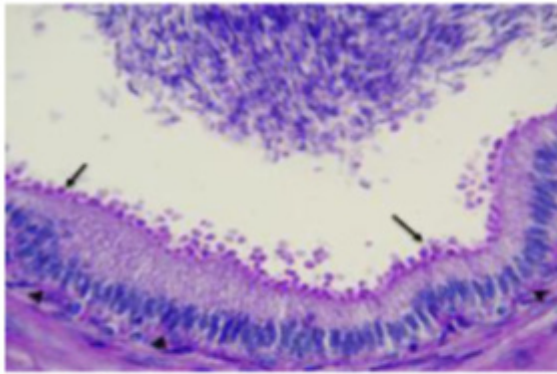


Fig. 18. Photomicrograph of a section at the level of the proximal segment of the cauda of Meriz buck's epididymal duct during autumn showing: Apical blebs (arrow). Stain: Diastase-PAS (x40)

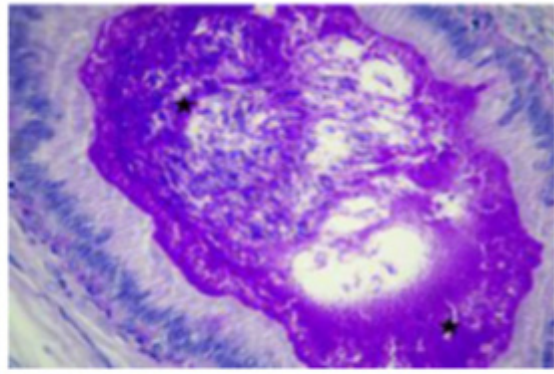


Fig.19. Photomicrograph of a section at the level of the proximal segment of the cauda of Meriz buck's epididymal duct during autumn showing: luminal secretion (stars). Stains (x10)

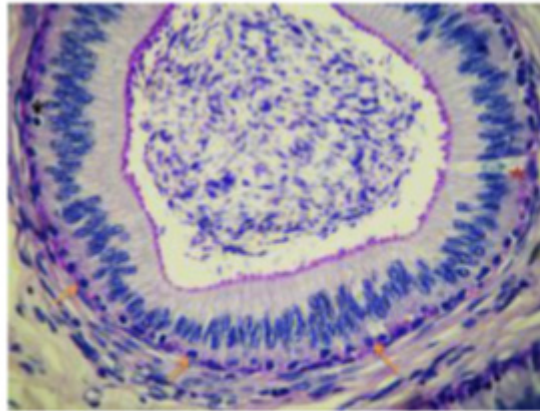


Fig. 20 Photomicrograph of a section at the level of the proximal segment of the cauda of Meriz buck's epididymal duct during autumn showing: Basal cells granules (arrows), Clear cell (star), IEL (arrow head). Stain: Diastase -PAS (x40).

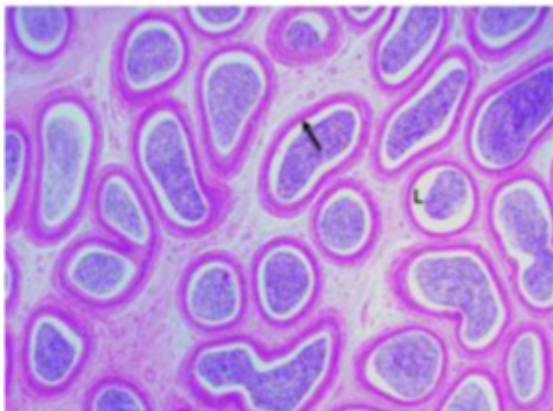


Fig.21. Photomicrograph of a section at the level of the distal segment of the cauda of Meriz buck's epididymal duct during autumn showing: lumina (L) Epithelium (arrow). Stain: H & E (x4)

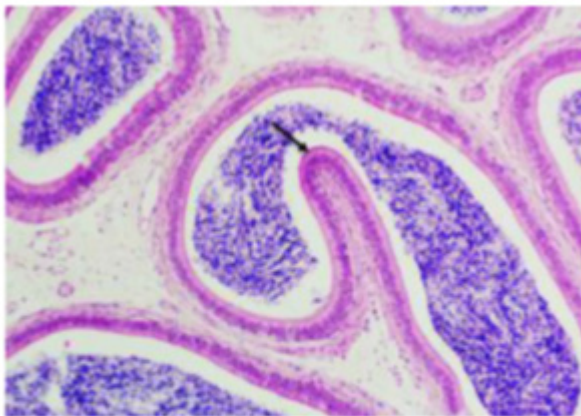


Fig.22. Photomicrograph of a section at the level of the distal segment of the cauda of Meriz buck's epididymal duct during autumn showing: Fold (arrow) Stain: H& E (x10)

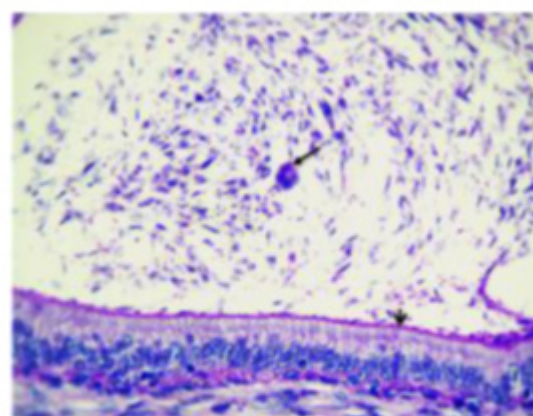


Fig.23. Photomicrograph of a section at the level of the distal segment of the cauda Meriz buck's epididymal duct during autumn showing: Intra luminal multinucleated giant cells (arrow), stereocilia (arrow head). Stain: PAS (x40)

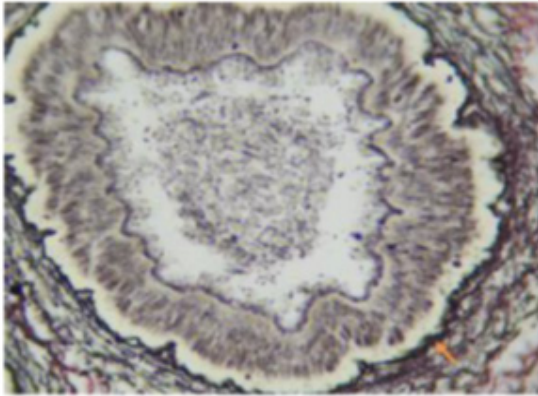


Fig.24. Photomicrograph of a section at the level proximal segment of the cauda of Meriz buck's epididymal duct during autumn showing: Peritubular reticular fibers (arrow) Stain: Sliver impregnation (x40)

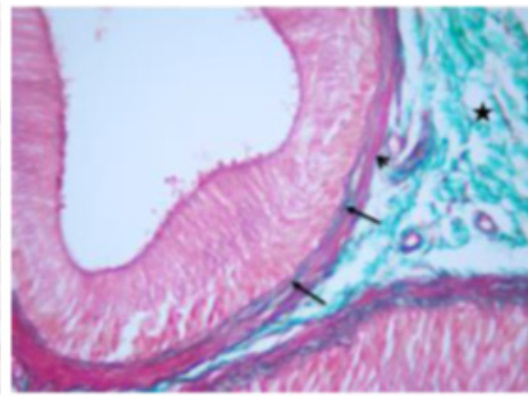


Fig.25. Photomicrograph of a section at the level of the initial segment of Meriz buck's epididymal duct during autumn showing: PMC (arrow head), collagen fibers (arrows), ICT (star). Stain: Masson Trichrom (x40)

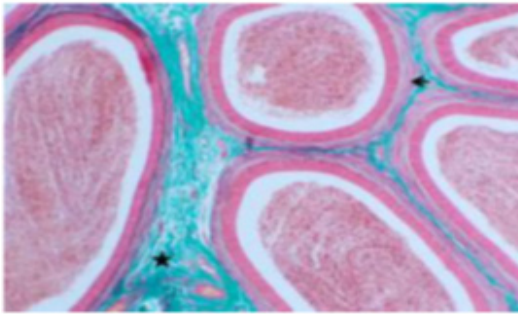


Fig.26. Photomicrograph of a section at the level of distal segment of the cauda of Meriz buck's epididymal duct during autumn showing: PMC (arrow), highly vascularized ICT (Star). Stain: Masson Trichrom (x10).

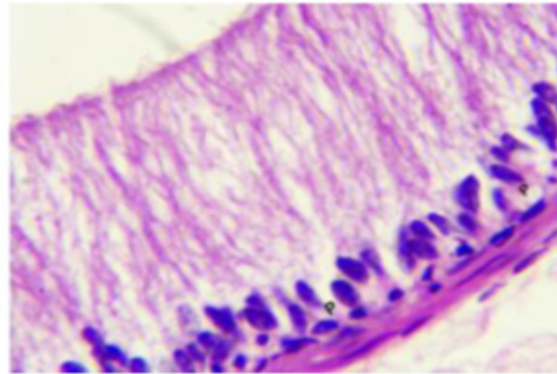


Fig.27. Photomicrograph of a section at the level of the proximal segment of the corpus of Meriz buck's epididymal duct during winter showing :Principle cells (arrows head) Stain : H&E (x40)

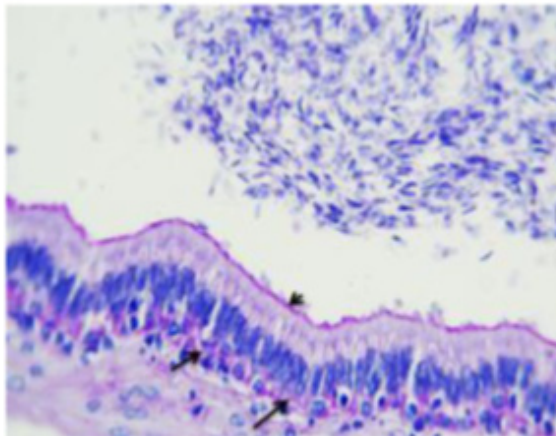


Fig.28. Photomicrograph of a section at the level of the proximal segment of the cauda of Meriz buck's epididymal duct during summer showing: stereocilia (arrow head) granules (arrows) Stain : PAS (x40)

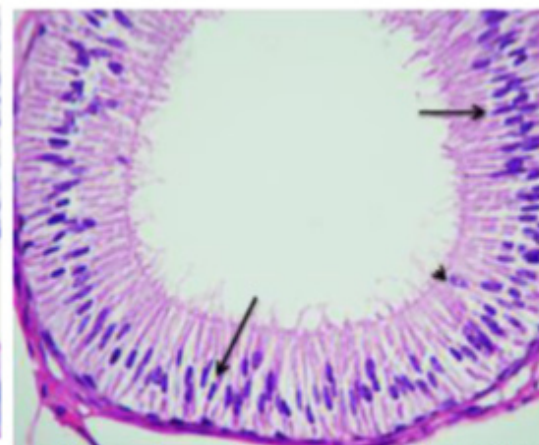


Fig.29. Photomicrograph of a section at the level of the proximal segment of the corpus of Meriz buck's epididymal duct during spring showing: apical cells (arrows head) and narrow cells (arrows). Stain : H&E X40

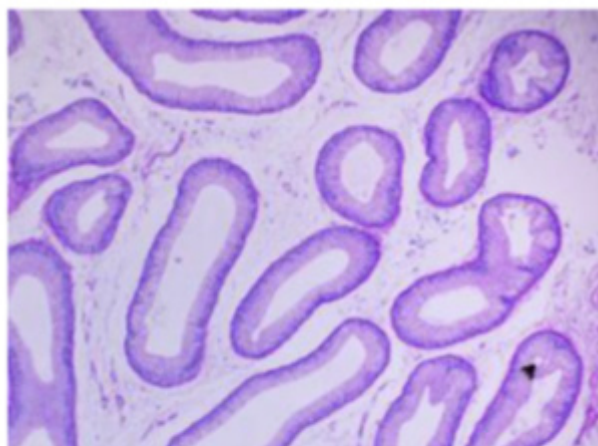


Fig.30. Photomicrograph of a section at the level of the initial segment of the caput of Meriz buck's epididymal duct during summer showing Intraepithelial glands(Arrows) Stain: PAS (x4)

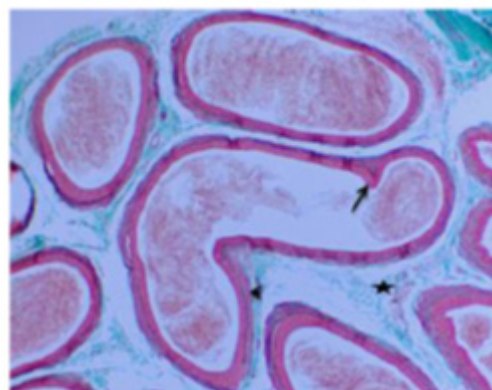


Fig.31. Photomicrograph of a section at the level of the distal segment of the cauda of Meriz buck's epididymal duct during winter showing: ICT (Star), PMC (arrow head), Fold (arrow head) Stain: Masson Trichrom (x4)

Discussion

The epididymal duct of the Meriz bucks revealed significant seasonal variations in total diameter, epithelial height and luminal diameter. The gradual decline in epithelial height was observed from the initial segment towards the distal segment of the cauda in all seasons demonstrating statistical significance at $p < 0.05$. This gradual decrease in epithelial height might facilitate the mechanical passage of sperm towards the terminal segment [8]. Furthermore, the higher epithelium in the initial segment might suggest a greater absorptive capacity of the epithelium in this region. In the present work, the maximum epithelial height in all segments was observed during autumn that significantly differences ($p < 0.05$) when compared with other seasons. In the current study, the distal segment of the cauda exhibited the widest lumen, the lowest epithelial height and the highest sperm concentration. A possible explanation for these changes were thought to be adaptations to maximize the storage and maturation of sperm during the breeding season. [9] in bulls stated that the proximal portion of the tail exhibited slightly folded epithelium and these folds gradually faded as they extended towards the ductus deferens. Our findings support partly with this result, since we observed these folds in the distal segment of the cauda near the vas deferens. These folds may potentially play a role in delaying the passage of epididymal fluid and spermatozoa to the vas deferens. The epididymal duct of Meriz buck was lined by pseudostratified columnar epithelium

which was similar to findings reported by [10] in Gaddi goat, [11] in Marwari goat, [12] in Assam goat and [13] in Marwari sheep. In the current study, this epithelium comprised mainly of five cell types; PCs, BCs, ACs, NCs, and CCs. However, [14] and [15] reported four cell types; PCs, BCs, NCs and ACs [12] observed three cell types; PCs, BCs and ACs in the same species. This variation in cell types was due to different goat breeding in various geographical territories.

In the present study, the PCs were primarily responsible for the absorption and secretion of materials into the epididymal lumen, so they exhibited high secretory and endocytic activity. They were also modulating luminal pH [16] and [17]. During the breeding season the PCs showed lightly stained oval nuclei and abundant cytoplasm indicating increased activity compared to the darkly stained nuclei and scant cytoplasm observed in non-breeding seasons. These findings were similar to [8] and [18] in camel bulls.

Our results revealed that the apical blebs and cytoplasmic granules of the PCs as well as the luminal secretion reacted strongly with PAS, Diastase-PAS during breeding seasons and moderately reacted out the season. These reactions indicated for presence neutral glycoproteins. [19] described that these glycoproteins contributed to the spermatozoa maturation, capacitation, and fertilization. In the current study, the stereocilia reacted strongly with PAS, Diastase-PAS, and AB pH (2.5) stains during breeding season and moderately with same stains during the rest of

the seasons. This result indicated the presence of glycoprotein and acid mucopolysaccharides materials. Our results revealed that the BCs showed some cytological changes across the seasons. However the number of these cells became more numerous in autumn and their granules and globules increased in autumn, winter than in summer and spring. [18] and [20] found increased activity in BCs during the breeding season indicating their potential role as stem cells for PCs. Furthermore, our findings mentioned that these granules and globules reacted strongly with PAS and Diastase-PAS stains in breeding season. In the same respect, [21] mentioned that the globular structures were considered PAS-positive residual bodies following the reabsorption and digestion of ductular fluid.

In the present study, the ACs, NCs and CCs were numerous in autumn followed by spring, winter, and they became less common in summer. These cells were different in shape, number and position within the ductal epithelium. [22] and [23] had been mentioned that each of these cells possessed a unique function. Furthermore, the coordinated interactions between spermatozoa, PCs, BCs, NCs, CCs and immune cells form a complex process by which the epididymal epithelium established and managed the conditions for sperm maturation, protection, selection and storage [24]. So, for these facts, our opinion documented that these cells were regarded as an independent cells.

[25] The current study pointed out that both IELs and IEMs were present throughout the entire epididymal epithelium in all seasons. Similar observations had been documented in goats [26]; [14]; [27] and bulls [9]. According to previous reports, immunological tolerance may be induced by macrophages and lymphocytes, preventing the animal's body from mounting an immune reaction against spermatozoa [28]. Epididymal epithelium of Merize buck contained numerous intraepithelial glands in the initial segment of the caput only in all seasons. These findings were inconsistent with those of [29] and [18] in camels as well as [30] in boars who mentioned that these glands were more numerous in the distal portion of the middle segment. Our findings revealed that the lumen of the intraepithelial glands were filled with strong PAS, Diastase - PAS positive material in breeding season and moderately reacted with the same stains in non-breeding seasons. [31] stated that these glands potentially play a role in epididymal

function, including the establishment of a secretory microenvironment and the recycling of damaged organelles or unused proteins for reuse or to provide energy under stress conditions. Additionally, they might serve as regulatory sensors for seasonality.

Our results indicated that the PMC increased distally during both breeding and non-breeding seasons especially in cauda. These changes were more pronounced in autumn compared to other seasons. Similarly, [32] in camel bulls showed that the thicker muscular coat in the terminal segment of the epididymis during the breeding season might contribute to powerful ejaculation. The present investigation indicated that the ICT became progressively denser and contained numerous capillaries, arterioles, venules and lymphatics in the distal segment of the cauda during breeding season comparing to other seasons. These findings were agree with the researchers conducted by [9] in bulls and [29] in camels. The unique and densely layered arrangement of capillaries beneath the lamina epithelialis in the bovine cauda epididymidis provided a strong morphological basis for intensive metabolic processes [25]. Bucks were considered as seasonal breeders being short or long day breeders, the sexual activity of the bucks varies among breeds and the animal's geographical location [33]; [34]; [35]. The researches of the animals management mentioned that the optimum seasonality was seen in late summer and autumn [36]; [37]. Furthermore, some studies on Meriz bucks indicated that the highest rates of reproduction occurred in August followed by September [38]. Histomorphometric and histochemical of our studies documented that the breeding season occurred in autumn particularly in October. Our disagreement with the researchers above was attributed to the impact of global warming on some countries worldwide including Iraq [39]. This phenomenon had affected the seasonality of mating, as previously, two decades ago, mating occurred at the end of summer and beginning of autumn. Since goats had the ability to adapt to changing conditions and environments [40-43] therefore the animals breeding season had extended to the October aligning with the altered patterns caused by global warming.

Conclusion and Recommendations

The results of our histological, morphometrical and histochemical studies proved that the breeding season for Meriz bucks occurred in autumn and specifically in October. . Therefore,

we recommend that the breeders of these animals rely on this month for mating, as it has been shown to yield the best results compared to other months.

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Conflicts of interest

The authors declare no conflict of interest.

Funding statement

The study was funded by authors only.

Authors contributions:

The authors contributed to the conception, study design, and methodology of the work. Bayan.S.Saadi executed sampling and laboratory techniques. The authors collectively interpreted the statistical analyses and examined the histological and histochemical microscopic slides .Additionally; the authors collaborated on composing the main text. Yahya. A. Mohammed and Zeravan A. Mohammed supervised the study and revised the text. The authors reviewed and approved the final version of the manuscript for publication.

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دراسة موسمية للتغيرات النسجية القياسية والانسجية الكيميائية لقناة البربخ لذكور المرعز في إقليم كردستان العراق

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اجريت هذه الدراسة علي اربعة وعشرون قناة بربخ لذكور مرعز بالغه جنسيا في جميع فصول السنة لتبيان خصائصها النسجية القياسية والانسجية الكيميائية. قسمت تلك القناة الى ستة اجزاء؛ الجزء البادي من الرأس، الجزء الداني والجزء الاوسط والجزء القاصي من الجسم وكذلك الجزء الداني و القاصي من الذيل. أظهرت قياسات القطر الكلي وارتفاع الظهارة و قطر تجويف القناة البربخية لجميع اجزئها فروقا معنويا ذات معنى إحصائي (p-value) عند مستوى (0.05) في فصل الخريف (أكتوبر) مقارنةً بالفصول الأخرى. تبطن قناة البربخ بظهارة عمودية مطبقة كاذبة تشمل خمسة انواع من الخلايا؛ اساسية، قاعدية، قمية، نحيفة و صافية. اضافة لهذه الخلايا تم ملاحظة الخلايا المناعية والتي تشمل؛ الخلايا اللمفية داخل الظهارة، البلعيمات الكبيرة داخل الظهارة وضمن تجويف القناة. وجدت عديد من الغدد داخل ظهارة الجزء البادي من الرأس فقط. تمتاز الخلايا العمودية بانويتها فاتحة الصبغة وسائتوبلازمها الوفير وهذا يعد مؤشرا على زيادة نشاط تلك الخلايا في فصل الخريف مقارنة مع الانوية الداكنة والسائتوبلازم الضئيل للخلايا نفسها في المواسم الاخرى. تظهر الحبيبات السائتوبلازمية، الفقاعات القمية، الزغابات و افرازات الخلايا الاساسية وكذلك الحبيبات و كريات الخلايا القاعدية مع افرازات الغدد داخل الظهارة تفاعلا ايجابيا شديداً مع صبغة الحمض شيف البيريودي و صبغة خميرة الدايستاز- حمض شيف البيريودي خلال فصل الخريف وتفاعلاً معتدلاً مع الصبغات نفسها في بقية المواسم مما يدل على وجود البروتينات السكرية المتعادلة، علاوة على ذلك اظهرت الزغابات تفاعلاً ايجابياً شديداً مع صبغة الالشيان الزرقاء ذات الاس الهيدروجيني (2.5) خلال فصل الخريف وتفاعلاً معتدلاً مع الصبغة نفسها خلال المواسم الاخرى مما يشير على وجود عديدات السكريات الحمضية. ان عديد الخلايا النحيفة والقمية و الصافية يكون كثيراً في فصل الخريف، الشتاء والربيع لكنها تقل في فصل الصيف، تتواجد الخلايا المناعية على طول القناة البربخية في كل مواسم السنة. امتاز الغلاف العضلي للقناة بسمكه والنسيج الضام الخلالي بغزارة او عيته الدموية اثناء فصل الخريف. كل المؤشرات والتغيرات المذكورة اعلاه تثبت ان موسم تزواج المرعز يكون خلال فصل الخريف وخصوصاً في شهر اكتوبر.

الكلمات الدالة: ذكور المرعز، القناة البربخ، النسجية القياسية، النسجية الكيميائي، تغيرات الموسمية.