

Morphological, Histological, and Ultrastructural Changes in Epididymis after a Vasectomy

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Abstract: The long-term effect of a vasectomy was investigated in this work. Twenty sexually mature New Zealand white male rabbits (*Oryctolagus cuniculus*) of proven fertility were used. The maturity of these rabbits was determined by the age of about 14 months from birth, palpation of genitalia, and examination of semen samples. The animals were divided into four groups: four rabbits were used as controls, and sixteen rabbits were vasectomized. After six months after vasectomy, we start taking a sample by exposing each group in one month after the previous one. The operated ducts revealed an increase in size in 7-8 months after vasectomy. The initial and terminal segments appeared greatly swollen and distended with fluid, resulting in the whole epididymis's increasing weight. Histological changes included a drop in the height of the lining epithelium and microvilli of the epididymis. After eight months of vasectomy, dilatation in the epididymal tubules increased its diameters, especially in the terminal segment. This caused an increase in the thickness of intertubular connective tissue. In addition, there was distension due to the intratubular pressure increase. This resulted in the tubular wall rupture and extravasation of spermatozoa into the interstitial tissue after 6-7 months from vasectomy. Eight months later, the epithelial lining was returned. The examination of morphological changes due to vasectomy was also investigated.

Keywords: vasectomy, excurrent duct, epididymis, lumen, epithelium.

输精管结扎术后附睾的形态学、组织学和超微结构变化

摘要：这项工作调查了输精管结扎术的长期影响。使用了20只已证明生育能力的性成熟新西兰雄性白兔(鹧鸪)。这些兔子的成熟度由出生后约14个月的年龄、生殖器触诊和精液样本检查确定。将动物分为四组：四只兔子作为对照，十六只兔子进行输精管切除术。输精管结扎术后6个月后，我们开始取样，在前一组后的一个月对每组进行暴露。输精管结扎术后7-8个月，手术后的输精管体积增大。始端和末节明显肿胀并充满液体，导致整个附睾重量增加。组织学变化包括内膜上皮和附睾微绒毛的高度下降。输精管结扎八个月后，附睾小管的扩张增加了其直径，尤其是在末端段。这导致管间结缔组织的厚度增加。此外，由于管内压力增加而出现扩张。这导致输精管结扎术后6-7个月后管壁破裂和精子外渗到间质组织中。八个月后，上皮衬里恢复了。还研究了输精管结扎引起的形态变化检查。

关键词：输精管切除术、外流管、附睾、管腔、上皮。

1. Introduction

Population explosion has become one of the chief inspiring problems the entire world faces. An extensively applied and accepted practice of vasectomy

is a means of male contraception to regulate the population explosion. The vasectomy is used by 60-100 million men worldwide for birth control [1-4]. Vasectomy has great potential to be recommended in

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investigating the structure and performance of epididymis. The mechanism is associated clearly with understanding the previous data on the basic controlling of the structure process. After leaving the testis, the spermatozoa pass along the epididymis to gain full fertilizing potential [5, 6]. Due to metamorphosis, the immature and immotile sperm becomes mature and capable of progressive motility and fertility. This may result in highly regulated and complex sequential events in the epididymis. In addition, it has been pointed out that the testicular sperms cannot move and fertilize an ovum. They have acquired these properties during their passage through the epididymis for 1–2 weeks in most species [7-9]. The epithelial lining of the epididymis of mammals, keeping a proper environment for the maturation of spermatozoa, is the goal of numerous morphological and histochemical investigations [10-15]. This work aims to examine the morphological, histological, and ultrastructural changes due to vasectomy.

2. Material and Methods

A sample of twenty New Zealand sexually mature white male rabbits, verified for fertility and weighing 2.6-3.2 kg, was used in this study. Rabbit maturity was determined by age, 14 months from birth, palpation of genitalia, and examination of semen samples. The animals were kept in separate cages under standard laboratory conditions, given dry straw, concentrated food, water, and ad libitum feeding. The rabbits were kept in the animal house of the Faculty of Medicine, University of Al-Ameed, Karbala. They were left to adapt to the new atmosphere environment for about one week at a minimum before beginning the experiment. The animals were divided into four groups. The groups were as follows: 4 rabbits were used as normal controls, and 16 were vasectomized. The interval between the groups was one month, starting after six months from the vasectomy (Table 1).

Table 1 The groups and their duration

Group	No.	Duration of vasectomy month	Time duration/months
Normal control	4		10
Vasectomized	16		
Group A	4	6	6
Group B	4	7	7
Group C	4	8	8
Group D	4	9	9

2.1. General Histology

Samples from the different segments of the epididymis were obtained from the experimental and control sides (right and left). The small pieces of tissue were fixed in 10% formal saline and buffered formalin. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in several changes of xylene, and impregnated and embedded in paraffin wax following

the procedure in [16]. The tissues were blocked and sectioned at 5-7 μm using a rotary microtome (Leica, Germany) and then subjected to the subsequent treatments. The general histological observations were made on paraffin sections stained with hematoxylin and eosin (H & E). Special stains included Van Gieson's stain to demonstrate collagenous fibers, Aldehyde fuchsin for elastic fibers, and Gomori's silver nitrate for type III collagen fibers (reticular fibers) [17].

2.2. Electron Microscopy

The samples were fixed in cold 5% glutaraldehyde, washed in phosphate buffer (pH 7.2) 3-4 times for 20 minutes each time, and postfixed in the following sequence on the rotator: cacodylate buffer for 10 min., two changes in the laminar flow cabinet, 1% OsO₄ in cacodylate buffer for 1 hour, and then they were washed in the same buffer for 10 min. and in distilled water for 20 min. The specimens were dehydrated in ascending grades of ethanol (50%-70%-96%) for 15 min. each and then in 100% ethanol (with an added molecular sieve) for two changes for 10 min each. The specimens were then embedded in propylene oxide for two changes for 10 min. each. The blocks were oriented using the stereomicroscope, embedded in 100% epoxy resin in molds, and labeled with the specific specimen number. Semithin sections (0.5 μm thick) were cut in an LKB ultramicrotome and stained with toluidine blue. The desired areas were selected, and then ultrathin sections (from pale gold to silver) 500-700 Å were prepared in a Reichert Austria ultramicrotome, counterstained in uranyl acetate and lead citrate, and examined via a TEM Philips CM10 Transmission Electron Microscope with Mega View III Imaging system.

3. Result

Macroscopically, the epididymis of control rabbits displayed features of normal animals and appeared cream in color. After 4-6 months, the tail of epididymis of the vasectomized rabbits was distended. These organs increased progressively in size in the vasectomized animals. In rabbits exposed after 6, 7, and 9 months, there was a progressive distention in the cauda epididymis, which became remarkably distended with fluids. In the rabbit exposed seven months after vasectomy, soft yellow material of rubbery consistency was present between the epididymis and the testis.

3.1. Histological Remarks

In six months after the vasectomy, there were a drop in the heights of cells and microvilli ($30.693 \pm 1.267 \mu\text{m}$) compared with the normal control ($52.201 \pm 1.78 \mu\text{m}$), many cytoplasmic vacuoles, and intertubular connective tissue increase. The tubular lumina were more or less rounded or oval, and a few tubules had irregular luminal surfaces. There were no luminal spermatozoa. After seven months following the

vasectomy, most of the tubules still appeared dilated ($270.432 \pm 4.412 \mu\text{m}$), with a slight increase in the epithelial height ($36.286 \pm 0.939 \mu\text{m}$), and there was no uniformity in height as compared with the vasectomized animals which were exposed in earlier intervals. Also, a few tubules became highly dilated, and the epithelial lining folded, resulting in irregular lumina (Fig. 1).

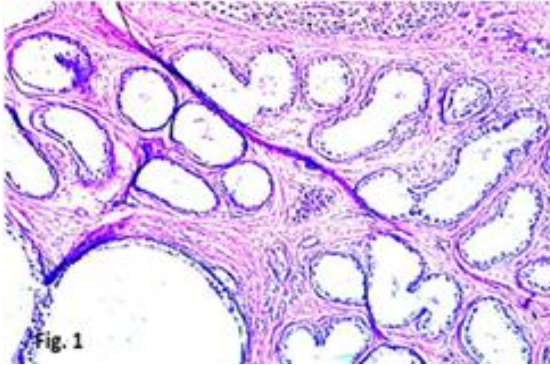


Fig. 1 Initial segment of epididymis after seven months following the vasectomy

Most of the tubules still appeared dilated with a slight increase in the epithelial height with no uniformity in height compared to the vasectomized animals exposed at earlier intervals ((H&E) X 40). After eight months following the vasectomy, the tubular lumina were more or less rounded or oval. Epithelial cells were more uniform in height so was the apical surface which had appeared smooth. However, a few tubules showed some epithelial surface damage and contained a few luminal spermatozoa, lymphocytes, and macrophages (Fig. 2).

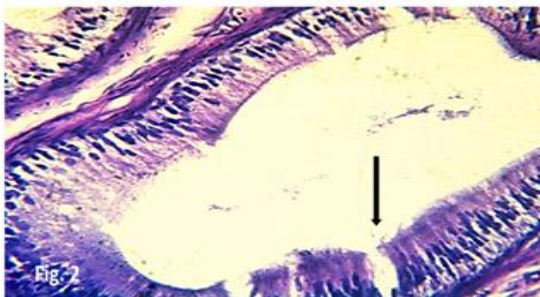


Fig. 2 Initial segment of epididymis after eight months following the vasectomy. Epithelial cells were more uniform in height, so the apical surface had a smooth profile, with few tubules showing some epithelial damage (arrow) ((H & E) X 400)

The intertubular connective tissue underwent reduction compared to the vasectomized animals exposed in earlier intervals. In addition, there was a remarkable accumulation of adipose tissue in the intertubular tissue. After nine months following the vasectomy, the epithelial height was low in height about ($36.043 \pm 0.522 \mu\text{m}$), with the changed feature of microvilli. A small amount of intertubular connective tissue and adipose tissue accumulation became more remarkable. The tubular lumina, however, remained rounded or oval. Furthermore, the proximal part of the middle segment was characterized by variable epithelial height and a small amount of adipose tissue,

and a few luminal spermatozoa (Fig. 3).

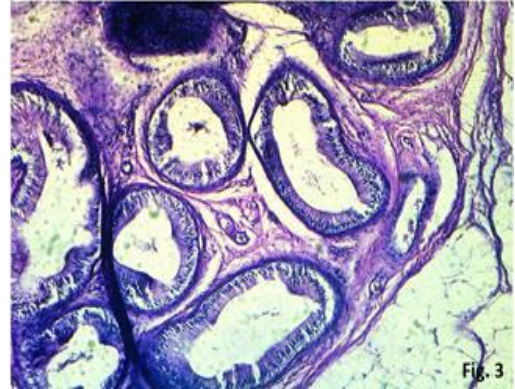


Fig. 3 Initial segment of epididymis after nine months following the vasectomy. In the proximal part of the head, characterized by less amount of adipose tissue, the epithelium varied in the height with the presence of a few intraluminal spermatozoa ((H&E) X100)

3.2. Proximal Part

In six months after the vasectomy, the tubules were dilated and lost their regular oval shape. The luminal diameter was large ($246.478 \pm 1.254 \mu\text{m}$), the epithelial lining was intact but low in height ($20.606 \pm 2.423 \mu\text{m}$), and microvilli were absent in most tubules. Also, there was an increase in the number of lymphocytes in the tubular stroma (Fig. 4).

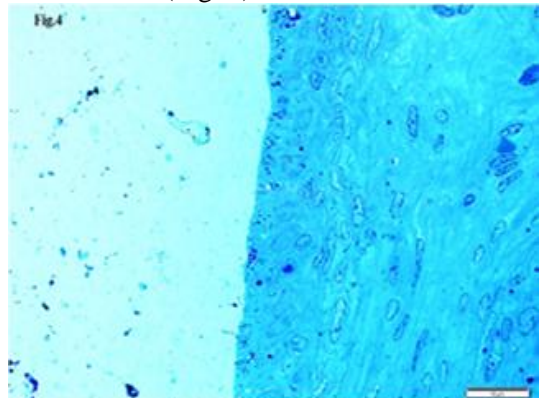


Fig. 4 Proximal part of the middle segment of epididymis after six months following the vasectomy. The epithelium was low. Microvilli were absent (Toluidine blue, X400 A. 1000 B)

The connective tissue was dense in the area of the contracted tubules and loose in other areas where tubules were dilated. In the seventh month following the vasectomy, a few tubules appeared contracted ($226.964 \pm 2.025 \mu\text{m}$), with narrow lumina. Other tubules, however, appeared less dilated with low epithelial lining ($19.528 \pm 2.223 \mu\text{m}$) of variable heights. Dense circumtubular connective tissue and some spermatogenic granuloma were seen in a few of them. Tubules experienced epithelial surface damage with some intraepithelial vacuoles and the absence of intraluminal spermatozoa. After eight months following the vasectomy, the tubules were more or less rounded or oval. The epithelial lining was folded, resulting in irregular lumina. Most tubules were lined with tall epithelium ($31.807 \pm 1.500 \mu\text{m}$) (Fig. 5).



Fig. 5 Proximal part of the middle segment of epididymis after eight months following the vasectomy

Most tubules showed tall epithelia; microvilli appeared, and luminal spermatozoa were absent ((H&E) X100). Circumtubular connective tissue was denser than that of the control ducts; a single subepithelial spermatogenic granuloma was observed in one animal. It consisted of numerous spermatozoa, lymphocytes, and macrophages. After nine months following the vasectomy, the tubular lumina were more rounded and contained spermatozoa. The lining epithelium was more uniform in height, and the apical surface had a smooth profile. A few tubules showed some epithelial surface damage and intraepithelial vacuoles with a small amount of circumtubular connective tissue (Fig. 6).

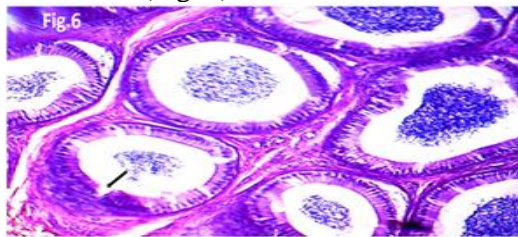


Fig. 6 Proximal part of the middle segment of epididymis after nine months following the vasectomy. Epithelial cells were more uniform in height, and hence the apical surface showed a smooth profile. A few tubules showed some epithelial surface damage (arrow) ((H & E) X100)

3.3. Distal Part

Following six months of vasectomy, the luminal diameter of tubules was larger ($254.874 \pm 1.056 \mu\text{m}$) than that of the normal control ($185.282 \pm 1.456 \mu\text{m}$), but they lost their regular oval shape. There was a drop in the height of the epithelium ($15.896 \pm 0.895 \mu\text{m}$) and microvilli. Also, the number of lymphocytes in the intertubular tissue increased, and there were no luminal spermatozoa. In the seventh month after vasectomy, there was a slight decrease in the height of the lining epithelium ($27.543 \pm 2.253 \mu\text{m}$) and microvilli. A striking observation was that the epithelial surface underwent damage and the cell became detached from the basal lamina. Many tubules were contracted, and their epithelial lining underwent degeneration. Tubules contained no spermatozoa and were surrounded by thick connective tissue with significant fibrosis. After eight months following the vasectomy, the tubules were more or less rounded, oval, or irregular in shape. The

epithelial lining became somewhat folded, resulting in irregular lumina with less epithelial surface damage and the cells detached from the basal lamina. Most tubules were lined by tall epithelium, and the microvilli reappeared. There were no luminal spermatozoa. The tubules were surrounded by thick connective tissue. Subepithelial spermatogenic granulomas were observed. After nine months following the vasectomy, the tubular lumina were of variable shapes and sizes, and the luminal diameter became wider ($248.180 \pm 0.778 \mu\text{m}$). Epithelial cells were more uniform in height, and the apical surface had a smooth profile. Most of the tubules contained masses of luminal spermatozoa (Fig. 7) and were surrounded by a small amount of connective tissue.

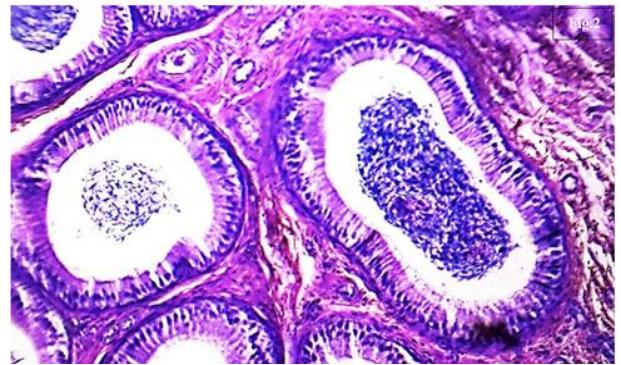


Fig. 7 Distal part of the middle segment of epididymis after nine months following the vasectomy: the variable shapes and sizes of tubules, the increased luminal diameter, the epithelial height, and large masses of spermatozoa ((H & E) X200)

3.4. Terminal Segment

In six months after the vasectomy, many histological changes were observed in most (vasectomized) ducts, including dilatation of tubular lumina, luminal accumulation of spermatozoa, epithelium damage, cellular height reduction, absence of microvilli, and break in the basal lamina (Fig. 8).

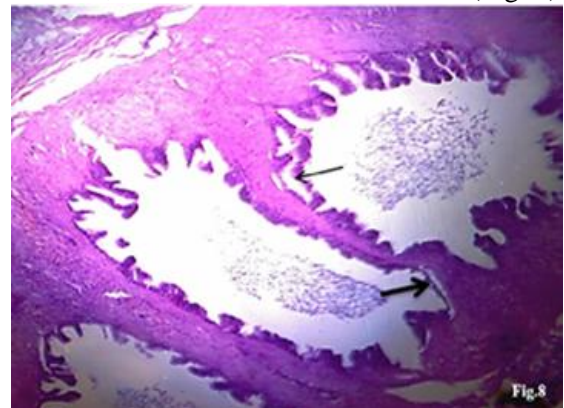


Fig. 8 Terminal segment after six months following the vasectomy: Damage of the epithelium, reduction in the cellular height, basal lamina (arrow), and dilatation of tubular lumina ((H&E) X40)

These tubules were surrounded by thick connective tissue rich in smooth muscle fibers. Some luminal spermatozoa appeared in close contact with the luminal cell membrane or occasionally invaded the damaged epithelial lining. The lumina of these tubules were filled with macrophages together with remnants of

spermatozoa and damaged epithelial cells (Fig. 9).

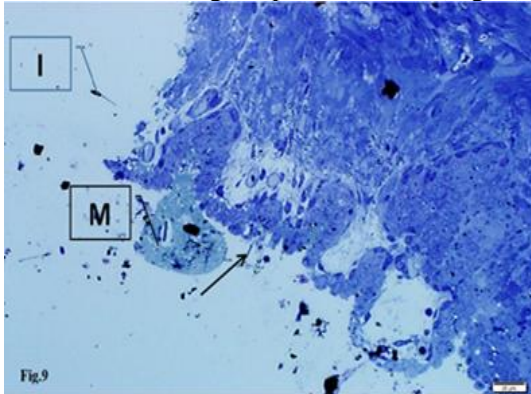


Fig. 9 Terminal segment after six months following the vasectomy. Spermatozoa appeared in contact or even adhering to the luminal cellular membrane of the tubules (arrow). Spermatozoa were either seen intact (I) or phagocytized within macrophages (M) (Toluidine blue, X400)

After seven months of vasectomy, some ducts experienced marked morphological changes. Many ducts showed a few epithelial lining folds, cell damage, cellular height reduction ($16.456 \pm 1.914 \mu\text{m}$), absence of microvilli, and the basement membrane discontinuity (Fig. 10).

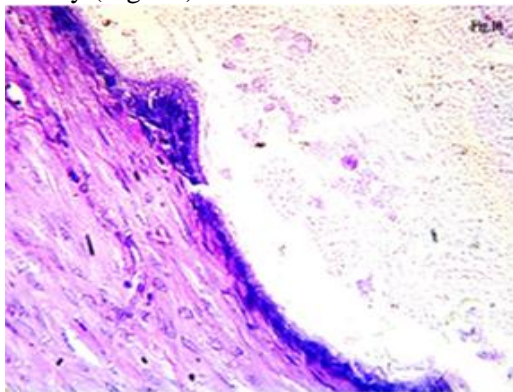


Fig. 10 Terminal segment after seven months following the vasectomy. More epithelial damage, reduction in the cellular height, absence of microvilli, and breaks in the basement membrane ((H&E) X400)

A large number of intraepithelial vacuoles have appeared. The lumina contained macrophages and some spermatozoa. In eight months after the vasectomy, most tubules were less folded. They showed the cellular height reduction ($27.673 \pm 0.620 \mu\text{m}$), the absence of microvilli (same as the previous group), the basement membrane breakage, and a smaller number of intraepithelial vacuoles. Some tubules showed damaged epithelium, and the lumina contained macrophages with some spermatozoa. The tubules were surrounded by a thick layer of connective tissue and some smooth muscle fibers. After nine months of vasectomy, most of the tubules had the same epithelium height as the previous group, with some broken parts. The epithelial lining was less folded and with a small number of intraepithelial vacuoles. The lumen contained many spermatozoa and some macrophages (Fig. 11). Tubules were surrounded by a thick connective tissue with a low amount of smooth

muscle fibers. A feature of this stage was a thickened intertubular connective tissue.

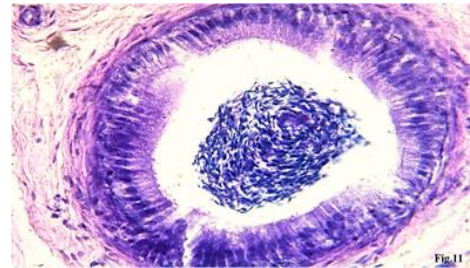


Fig. 11 Terminal segment after nine months following the vasectomy. The tubules showed brooked epithelium with a large number of spermatozoa ((H&E) X400)

3.5. Ultrastructure

3.5.1. Initial Segment

After six months following the vasectomy and compared with normal control animals, there was a drop in the epithelial height and damage to both cells and their microvilli. There was a reduction in the number of mitochondria, amount of endoplasmic reticulum, and size of the Golgi complex. A variable number of vacuoles, vesicles, and electron-dense bodies were seen in the supra- and intranuclear cytoplasm. The nuclei became elongated with dispersed chromatin content (Fig. 12). The basal plasma membrane was folded, the lateral plasma membranes were fairly irregular throughout their length, and junctional complexes joined apposed plasma membranes. Large intraepithelial macrophages with many mitochondria, the well-developed Golgi apparatus, RER, lipid droplets, vacuoles, residual bodies, and a relatively small heterochromatic nucleus were also observed. The cytoplasm contained various profiles of electron-dense bodies, probably lysosomes, and dark globules, which may represent phagosomes, sperm fragments, and large vacuoles.

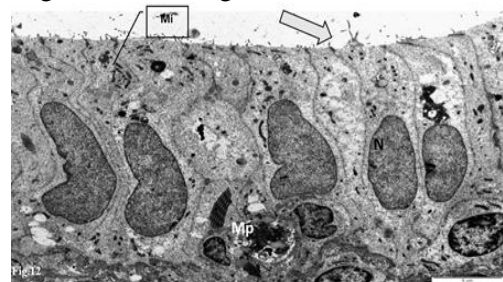


Fig. 12 Electron micrograph of the initial segment of epididymis after six months following the vasectomy, showing short cells with elongated nuclei (N) and damaged microvilli (arrow). Mitochondria (Mi). Scale bar X 3400

After 7-8 months following the vasectomy, the effect was relatively less marked than that of six months. The principal cells increased in height, and the microvilli became taller. The cellular membranes appeared somewhat folded. Vesicles were observed in the luminal cytoplasm, and there was an increase in the number of mitochondria in the principal and basal cells. The nuclei were more or less round or irregular in

shape with many indentations. Golgi apparatus and content of RER were relatively more than those of the previous group (Fig. 13).

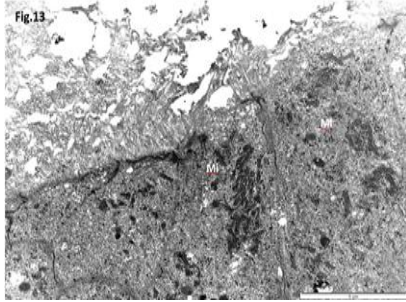


Fig. 13 Electron micrograph of the initial segment of epididymis after seven months following the vasectomy: increase in the number of mitochondria (Mi) in the principal (a) and basal cells (b). Scale bar X3400

After nine months following the vasectomy, the duct showed variations in the height of both epithelium and microvilli. Large amounts of mitochondria, vacuoles, vesicles, and electron-dense bodies were observed in the supra- and intranuclear cytoplasm. The nuclei were more or less round with a variable amount of chromatin content (Fig. 14). The Golgi apparatus and RER were seen but not remarkable. Dark cells were also present (Fig. 15).

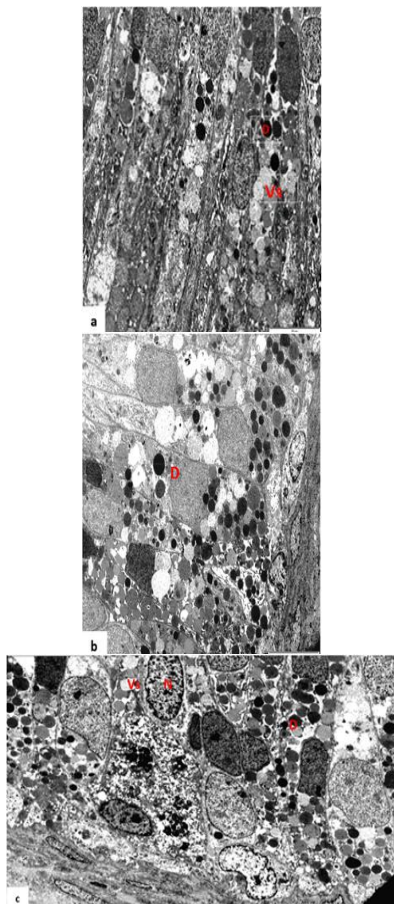


Fig. 14 Electron micrograph of the initial segment of epididymis after nine months following the vasectomy: a large number of vacuoles (Vs), vesicles (V), electron-dense bodies (D), and lysosome (L) in the supra- and intranuclear cytoplasm. Nucleus (N). Scale bar: a) X1950, b) X1950, c) X 1950

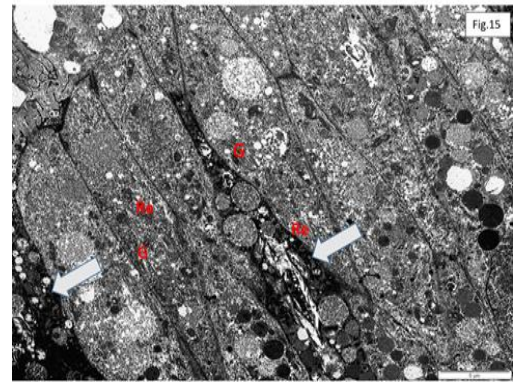


Fig. 15 Electron micrograph of the initial segment of epididymis after nine months following the vasectomy: the Golgi apparatus (G) and rER(Re). Dark cells were present (arrow). Scale bar X3400

3.5.2. Middle Segment

From 6 to 8 months following the vasectomy, remarkable changes have been noted in the principal cells of the proximal and distal parts of the middle segment of the duct. There was a drastic drop in the epithelial height, and the cell became damaged, including the microvilli. The cell membranes were also damaged and completely absent in some cells. There was a reduction in the number of mitochondria, endoplasmic reticulum, and size of the Golgi complex. A variable number of vacuoles, vesicles, and electron-dense bodies were seen in the supra- and intranuclear cytoplasm. The basal cell became somewhat flattened and irregular in shape with deep enfolding. The basal plasma membrane was wavy, and the lateral plasma membranes were fairly irregular (Fig. 16).

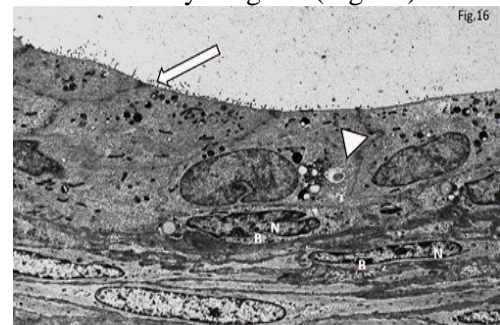


Fig. 16 Electron micrograph of the middle segment of epididymis after six months following the vasectomy: short cells and damaged microvilli (arrow), basal cell (B) with flattened nucleus (N). Lateral plasma membranes were slightly irregular (head arrow). Scale bar X 3400

3.5.3. Terminal Segment

After 6-7 months following the vasectomy, many epithelial cells were regressed and damaged with a drop in the height of the cells and microvilli. The principal cells became cuboidal, and the basal cells were small. There was a reduction in the number of mitochondria, endoplasmic reticulum, and size of the Golgi complex of principal cells. A variable number of vacuoles, vesicles, and electron-dense bodies were seen in the supra- and intranuclear cytoplasm. The nuclei became large and irregular in shape with dispersed chromatin content. The basal plasma membrane became wavy, while the lateral plasma membranes

were fairly irregular throughout their length (Fig. 17).

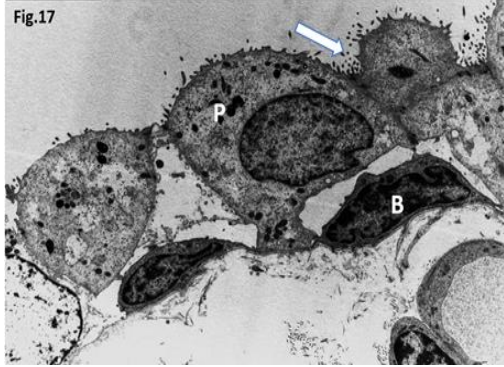


Fig. 17 Electron micrograph of the terminal segment after six months following the vasectomy illustrating the drop in height and damage of epithelium and microvilli (arrow). Scale bar X 3400

After eight months of vasectomy, the effect was relatively less marked than that of previous groups. The epithelial lining and microvilli attained an increase in height, and the cells became columnar. Cellular membranes appeared somewhat folded. Vacuoles and vesicles were observed in the supranuclear cytoplasm. There was an increase in the content of mitochondria, endoplasmic reticulum, and the size of the Golgi complex in principal and basal cells. The nuclei in both principal and basal cells were more or less round or irregular in shape with some indentations (Fig. 18a, b).

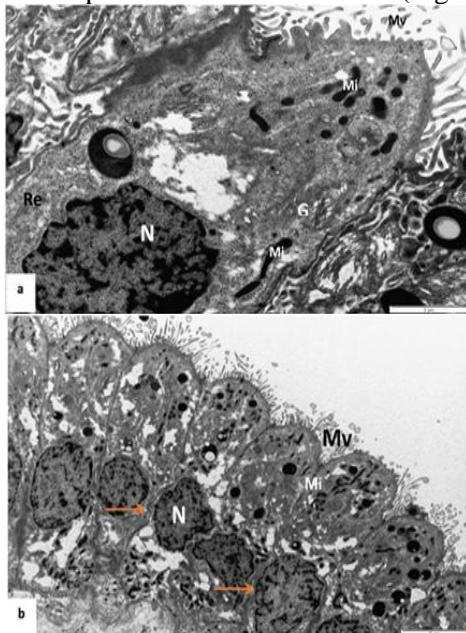


Fig. 18 Electron micrograph of the terminal segment after eight months following the vasectomy, demonstrating an increase in the height of the cells, microvilli (Mv), and the cells became columnar. Cellular membranes appeared somewhat folded (arrow). There was an increase in the number of mitochondria (Mi), endoplasmic reticulum (Re), and the Golgi complexes (G) in the principal and basal cells. The nuclei (N) were more or less round or irregular in shape with some indentations. Scale bar: a) X 3400, b) X7900

After nine months following the vasectomy, the cells became more organized. The epithelial cells were small, although, in some parts, they were tall with irregular luminal surfaces. The principal cells were low in height, the cellular membranes were somewhat folded, and intraepithelial cytoplasmic vacuoles were

present. A remarkable observation was that the microvilli became tall and crowded, almost occluding the lumen. There was an increase in the number of mitochondria, vacuoles, and vesicles in the cytoplasm. The nuclei of both cells (principal and basal) were more or less round or irregular in shape with many indentations and contained heterochromatin. Membrane-bounded bodies, probably cytoplasmic fragments, were seen in the lumen with macrophages and spermatozoa (Fig. 19).

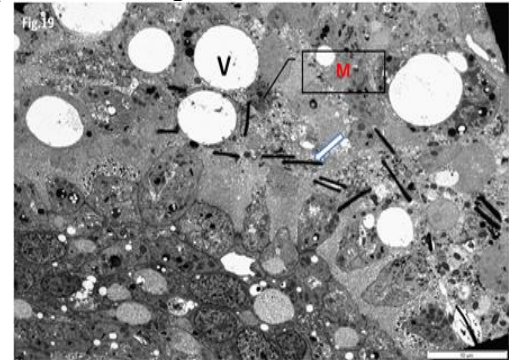


Fig. 19 Electron micrograph of terminal in nine months after the vasectomy showing the lumen with numerous vacuoles (V), vesicles, sperm fragment (arrow), and macrophages (M). Scale bar X1950

4. Discussion

According to the results of this study, four months after vasectomy, the proximal part of the excurrent duct system of the testis was enlarged and progressively became distended, notably after 6 and 9 months; this was especially true of the cauda epididymitis because of the accumulation of fluid therein. In the distal part, however, no significant change has been noticed. Turgidity and distension with fluid as a consequential increase in size and weight of the whole epididymal duct have been reported in the rabbit [18-20], ram, bull, rat, man, fowl, and adult male balb. [13] reported no change in testis and epididymis weights of the dog after 30 days following bilateral vasectomy. However, a significant increase was noted after 60 and 120 days following unilateral and bilateral vasectomy [14]. Similarly, reported changes in weights of testis and epididymis of the rabbit after eight weeks from vasectomy, but a significant decrease was recorded beyond 16 weeks of bilateral vasectomy. In the current study on rabbits exposed after 6 and 9 months following a vasectomy, there was a progressive distention in the epididymis cauda [15]. The histological observations have shown that the entire epididymis was lined by a pseudostratified columnar epithelium with long microvilli. The epithelium gradually became shorter in height as we proceeded caudally from the initial segment towards the terminal segment. According to the present study, a significant drop in the heights of the epithelium and microvilli contributed to an increase in the luminal diameter of the epididymal segments of the most vasectomized rabbits. This was noted during the sixth month and

reached its peak during the seventh month following the vasectomy [16]. This agrees with the findings reported in the rhesus monkey. Dilated tubules with the increased luminal diameter and decreased epithelial height of vasectomized rabbits could result from a high intraluminal pressure caused by the accumulation of the non-ejaculated spermatozoa and fluids within the obstructed duct, especially in the cauda epididymidis, where spermatozoa are normally stored. This agrees with the findings reported in the rhesus monkey, rat, dog, rabbit, and man [17]. These authors reported tubular distension with wide lumina and low epithelial height observed after eight weeks following the vasectomy in the initial segment and proximal part of the middle segment.

After 6-8 months from control vasectomy in two rabbits, the spermatozoal accumulation in the epididymal tubules seen in this study caused intertubular pressure followed by rupture of tubular wall and extravasation of spermatozoa into the interstitial tissue forming spermatic granulomas. The latter was in the form of a mass of spermatozoa surrounded by macrophages and other leukocytes involved in the defense mechanism [18, 19]. The present findings confirm those on man, rat, and rabbit. These granulomas have been observed mainly in the proximal and distal parts of the middle and terminal segments of epididymis after seven months following the control vasectomy. In a few cases, spermatic granulomas were seen in the initial segment [20]. Spermatic granulomas were also reported in cauda epididymidis of the ipsilateral side of lamb [21] and rabbit, the caput epididymidis of goat [22], and domestic fowl (*Gallus domesticus*) after long-term vasoligation. Vasectomy in the monkey for 6-12 months induced sperm granuloma formation in the epididymis but had no considerable impact on the testicular structures [23].

Granulomas were present in the rabbit epididymis after the vasectomy. A similar observation was noted in mice examined for five weeks after the vasectomy [24]. In the current study, increased pressure may have physiological effects on epithelial cell morphology, cellular ultrastructure in the epididymis, and vas deferens. Moreover, sperms cannot traverse the intentionally obstructed vasal lumen. Instead, they accumulate and die within the proximal part of the vas deferens and the epididymis, resulting in increased intraluminal pressure, distension of tubules, the tubular wall rupture, and extravasation of spermatozoa. Following vasectomy, a severely damaged epithelial wall with extravasated spermatozoa was observed in many tubules, especially in the terminal segment. The pressure seems relieved after 8-9 months from vasectomy, perhaps because the epididymis possesses limited distensibility and reabsorptive capacity. This explanation has been suggested by Zarog [14]. When this pressure drops, the epithelium and lumen return to

normal features. The epithelium could be identified as pseudostratified columnar. The present study shows that, after nine months from vasectomy, there were a variable decrease in the heights of principal cells and microvilli, many mitochondria, vacuoles, vesicles, and electron-dense bodies. This was also observed in the hamster, langur monkey, man, rabbit, and mice [17]. The study has shown that the principal and basal cells, after vasectomy, were functionally active as indicated by the characteristic content of cellular organelles, including vacuoles and Golgi complex, mitochondria, vesicles, and lysosomes. The well-developed rough endoplasmic reticulum, the Golgi complex, and mitochondria indicated the normal secretory and absorptive functions after the vasectomy. This is in agreement with the observation by Zarog [14]. The morphology of the epididymal epithelium and its intracellular organelles suggests a high level of activity after vasectomy. An increased number of electron-dense bodies probably suggests an increased resorption capacity of the initial segment of epididymidis after the vasectomy. In the present study, demonstrating the numerous cytoplasmic vacuoles in the lining epithelium of rabbits' vasectomized epididymis suggests enhanced absorptive and digestive mechanisms.

5. Conclusions

A drop in the height of principal cells and the absence of microvilli after 6-8 months of vasectomy was confirmed ultrastructurally. Fluid accumulation in the vasectomized epididymis increased the epididymis size and weight. The fibrosis, especially in the terminal segment, increased the thickness of the intertubular connective tissue. The distension of tubules resulted in reduced lining epithelium height, luminal spermatoceles, ruptured tubular walls, and extravasation of spermatozoa into the subepithelial tissue forming spermatic granulomas. An increased number of macrophages, lymphocytes, and other defensive cells and spermatozoal fragments after the vasectomy are probably signs of phagocytic activity and disposal of unejaculated spermatozoa in the lumina and subepithelial tissue.

References

- [1] AMORY J. K. Development of Novel Male Contraceptives. *Clinical and Translational Science* 2020, 13(2): 228–237. <https://doi.org/10.1111/cts.12708>
- [2] OLIVEIRA F. B., PEREIRA V. X., OLIVEIRA F. R., ABREU L. C., DABOIN B. E. G., NORBERTO A. R., ALCANTARA SOUSA L. V., TAVARES L. F. B., and GLINA S. Effect of ductus deferens lavage on the time to achieve azoospermia in patients undergoing vasectomy. *Clinics*, 2018, 73: e504. <http://dx.doi.org/10.6061/clinics/2018/e504>
- [3] AMORY J. K. Development of Novel Male Contraceptives. *Clinical and Translational Science* 2020, 13(2): 228–237. <https://doi.org/10.1111/cts.12708>

- [4] MEYER M. L. *What to Expect with a Vasectomy*. Vital Record News, 2019. <https://vitalrecord.tamhsc.edu/what-to-expect-with-a-vasectomy/>
- [5] JAMES E. R., CARRELL D. T., ASTON K. I., JENKINS T. G., YESTE M., and SALAS-HUETOS A. The Role of the Epididymis and the Contribution of Epididymosomes to Mammalian Reproduction. *International Journal of Molecular Sciences*, 2020, 21(15): 5377. <https://doi.org/10.3390/ijms21155377>
- [6] ROBERTS K. P. What are the components of the male reproductive system. In: ROBAIRE B., CHAN P., and LAWRENCE K. S. (eds.) *Handbook of Andrology*. Allen Press, 2010: 1-5.
- [7] ARRIGHI S. Primary cilia in the basal cells of equine epididymis: A serendipitous finding. *Tissue and Cell*, 2013, 45: 140–144. <https://doi.org/10.1016/j.tice.2012.10.003>
- [8] BRETON S., NAIR A. V., and BATTISTONE M. A. Epithelial dynamics in the epididymis: role in the maturation, protection and storage of spermatozoa. *Andrology*, 2019, 7(5): 631–643. <https://doi.org/10.1111/andr.12632>
- [9] BRETON S., NAIR A. V., and BATTISTONE M. A. Epithelial dynamics in the epididymis: role in the maturation, protection, and storage of spermatozoa. *Andrology*, 2019, 7: 631–643. <https://doi.org/10.1111/andr.12632>
- [10] ZHOU W., DE IULIIS G. N., DUN M. D., and NIXON B. Characteristics of the Epididymal Luminal Environment Responsible for Sperm Maturation and Storage. *Frontiers in Endocrinology*, 2018, 9: 59. <https://doi.org/10.3389/fendo.2018.00059>
- [11] PERRY C., CHUNG J.-Y., YLAYA K., CHOI C. H., SIMPSON A., MATSUMOTO K. T., SMITH W. A., and HEWITT S. M. A Buffered Alcohol-Based Fixative for Histomorphologic and Molecular Applications. *Journal of Histochemistry & Cytochemistry*, 2016, 64(7): 425–440. <https://doi.org/10.1369/0022155416649579>
- [12] JAMES E. R., CARRELL D. T., ASTON K. I., JENKINS T. G., YESTE M., and SALAS-HUETOS A. The Role of the Epididymis and the Contribution of Epididymosomes to Mammalian Reproduction. *International Journal of Molecular Sciences*, 2020, 21(15): 5377. <https://doi.org/10.3390/ijms21155377>
- [13] ZHOU W., DE IULIIS G. N., DUN M. D., and NIXON B. Characteristics of the Epididymal Luminal Environment Responsible for Sperm Maturation and Storage. *Frontiers in Endocrinology*, 2018, 9: 59. <https://doi.org/10.3389/fendo.2018.00059>
- [14] ZAROOG H.M. *The Morphology and Histochemistry of Rabbit Epididymis before and after Vasectomy*. Ph.D. thesis. University of Khartoum, 2010.
- [15] REZIGALLA A. A. *Correlation between the Morphology and Histochemistry of the Rabbit Testes and Anterior Lobe of the Pituitary Gland before and after Vasectomy*. Ph.D. thesis. University of Khartoum, 2011.
- [16] MENEZES T. P., HILL E., DE ALENCAR MOURA A., LOBO M. D. P., MONTEIRO-MOREIRA A. C. O., BRETON S., and MACHADO-NEVES M. Pattern of protein expression in the epididymis of *Oligoryzomys nigripes* (Cricetidae, Sigmodontinae). *Cell and Tissue Research*, 2018, 372(1): 135–147. <https://doi.org/10.1007/s00441-017-2714-9>
- [17] BERNIE A. M., OSTERBERG E. C., STAHL P. J., RAMASAMY R., and GOLDSTEIN M. Vasectomy reversal in humans. *Spermatogenesis*, 2012, 2(4): 273–278. <https://doi.org/10.4161/spmg.22591>
- [18] ZHENG W., ZHANG S., CHEN X., JIANG S., LI Z., and LI M. Case Report: Dendritic Cells and Macrophages Capture Sperm in Chronically Inflamed Human Epididymis. *Frontiers in Immunology*, 2021, 12: 629680. <https://doi.org/10.3389/fimmu.2021.629680>
- [19] PADILLA L., MARTÍNEZ-HERNÁNDEZ J., BARRANCO I., LUCAS X., PASTOR L. M., RODRIGUEZ-MARTÍNEZ H., ROCA J., and PARRILLA I. Granulocyte-macrophage colony stimulating factor (GM-CSF) is fully expressed in the genital tract, seminal plasma and spermatozoa of male pigs. *Scientific Reports*, 2020, 10: 13360. <https://doi.org/10.1038/s41598-020-70302-9>
- [20] MA L., GUO Y., YUAN Y., LI Y.-G., DENG X.-Z., and YANG Z.-W. Morphometric study of the testis and reproductive tract (including sperm granuloma) after vasectomy in mature rats. *Asian Journal of Andrology*, 2016, 18(1): 66–73. <https://doi.org/10.4103/1008-682X.150038>
- [21] FIJAK M., PILATZ A., HEDGER M. P., NICOLAS N., BHUSHAN S., MICHEL V., TUNG K. S. K., SCHUPPE H.-C., and MEINHARDT A. Infectious, inflammatory and ‘autoimmune’ male factor infertility: how do rodent models inform clinical practice? *Human Reproduction Update*, 2018, 24(4): 416–441. <https://doi.org/10.1093/humupd/dmy009>
- [22] JIN G., GUO L., ZHANG Y., XUE Y., ZHANG X., WANG X., WANG D., LI B., ZHAO P., XU F., and CHENG J. Expression and localization of lipocalin-type-prostaglandin D synthase in the goat testis, epididymis and sperm. *Small Ruminant Research*, 2017, 154: 1-4. <https://doi.org/10.1016/j.smallrumres.2017.06.020>
- [23] SEPPAN P., and KRISHNASWAMY K. Long-term study of vasectomy in *Macaca radiata* – histological and ultrasonographic analysis of testis and duct system. *Systems Biology in Reproductive Medicine*, 2014, 60(3): 151–160. <https://doi.org/10.3109/19396368.2014.896957>
- [24] YANG F., LI J., DONG L., TAN K., HUANG X., ZHANG P., LIU X., CHANG D., and YU X. Review of Vasectomy Complications and Safety Concerns. *The World Journal of Men's Health*, 2021, 39(3): 406–418. <https://doi.org/10.5534/wjmh.200073>

参考文献:

- [1] AMORY J. K. 新型男性避孕药的开发。临床与转化科学 2020, 13(2): 228–237. <https://doi.org/10.1111/cts.12708>
- [2] OLIVEIRA F. B., PEREIRA V. X., OLIVEIRA F. R., ABREU L. C., DABOIN B. E. G., NORBERTO A. R., ALCANTARA SOUSA L. V., TAVARES L. F. B. 和 GLINA S. 输精管灌注对输精管结扎术患者无精子症时间的影响。诊所, 2018年, 73 : e504. <http://dx.doi.org/10.6061/clinics/2018/e504>
- [3] AMORY J. K. 新型男性避孕药的开发。临床与转化科学 2020, 13(2): 228–237. <https://doi.org/10.1111/cts.12708>
- [4] MEYER M. L. 输精管切除术的期望。重要记录新闻, 2019. <https://vitalrecord.tamhsc.edu/what-to-expect-with-a-vasectomy/>

- [5] JAMES E. R., CARRELL D. T., ASTON K. I., JENKINS T. G., YESTE M. 和 SALAS-HUETOS A. 附睾的作用和附睾对哺乳动物繁殖的贡献。国际分子科学杂志, 2020, 21(15): 5377. <https://doi.org/10.3390/ijms21155377>
- [6] ROBERTS K. P. 男性生殖系统的组成部分是什么。在: ROBAIRE B., CHAN P. 和 LAWRENCE K.S. (编辑) 男科手册。艾伦出版社, 2010: 1-5.
- [7] ARRIGHI S. 马附睾基底细胞中的初级纤毛: 一个偶然的发现。组织与细胞, 2013, 45: 140-144. <https://doi.org/10.1016/j.tice.2012.10.003>
- [8] BRETON S., NAIR A. V. 和 BATTISTONE M. A. 附睾中的上皮动力学: 在精子成熟、保护和储存中的作用。男科, 2019, 7(5): 631-643. <https://doi.org/10.1111/andr.12632>
- [9] BRETON S., NAIR A. V. 和 BATTISTONE M. A. 附睾中的上皮动力学: 在精子成熟、保护和储存中的作用。男科, 2019, 7: 631-643. <https://doi.org/10.1111/andr.12632>
- [10] ZHOU W., DE IULIIS G. N., DUN M. D. 和 NIXON B. 负责精子成熟和储存的附睾腔环境特征。内分泌学前沿, 2018, 9: 59. <https://doi.org/10.3389/fendo.2018.00059>
- [11] PERRY C., CHUNG J.-Y., YLAYA K., CHOI C.H., SIMPSON A., MATSUMOTO K.T., SMITH W.A. 和 HEWITT S.M. 用于组织形态学和分子应用的缓冲酒精基固定剂。组织化学与细胞化学杂志, 2016, 64(7): 425-440. <https://doi.org/10.1369/0022155416649579>
- [12] JAMES E. R., CARRELL D. T., ASTON K. I., JENKINS T. G., YESTE M. 和 SALAS-HUETOS A. 附睾的作用和附睾对哺乳动物繁殖的贡献。国际分子科学杂志, 2020, 21(15): 5377. <https://doi.org/10.3390/ijms21155377>
- [13] ZHOU W., DE IULIIS G. N., DUN M. D. 和 NIXON B. 负责精子成熟和储存的附睾腔环境特征。内分泌学前沿, 2018, 9: 59. <https://doi.org/10.3389/fendo.2018.00059>
- [14] 扎鲁格 H.M. 兔输精管结扎前后附睾的形态学和组织化学。博士论文。喀土穆大学, 2010年。
- [15] REZIGALLA A. A. 输精管切除术前兔睾丸和垂体前叶的形态学和组织化学之间的相关性。博士论文。喀土穆大学, 2011年。
- [16] MENEZES T. P., HILL E., DE ALENCAR MOURA A., LOBO M. D. P., MONTEIRO-MOREIRA A. C. O., BRETON S., 和 MACHADO-NEVES M. 黑寡头鲟(蟋蟀科, 鼠尾草科)附睾中的蛋白质表达模式。细胞和组织研究, 2018, 372(1): 135-147. <https://doi.org/10.1007/s00441-017-2714-9>
- [17] BERNIE A. M., OSTERBERG E. C., STAHL P. J., RAMASAMY R. 和 GOLDSTEIN M. 人类输精管切除术逆转。精子发生, 2012, 2(4): 273-278. <https://doi.org/10.4161/spmg.22591>
- [18] ZHENG W., ZHANG S., CHEN X., JIANG S., LI Z., 和 LI M. 案例报告: 树突状细胞和巨噬细胞在慢性发炎的人附睾中捕获精子。免疫学前沿, 2021, 12: 629680. <https://doi.org/10.3389/fimmu.2021.629680>
- [19] PADILLA L., MARTÍNEZ-HERNÁNDEZ J., BARRANCO I., LUCAS X., PASTOR L.M., RODRIGUEZ-MARTÍNEZ H., ROCA J. 和 PARRILLA I. 粒细胞-巨噬细胞集落刺激因子(通用脑脊液)在公猪的生殖道、精浆和精子中充分表达。科学报告, 2020年, 10: 13360. <https://doi.org/10.1038/s41598-020-70302-9>
- [20] 马立, 郭瑛, 袁瑛, 李瑛-G., 邓新志, 杨志伟. 成熟大鼠输精管结扎后睾丸和生殖道(包括精子肉芽肿)的形态学研究。亚洲男科学杂志, 2016, 18(1): 66-73. <https://doi.org/10.4103/1008-682X.150038>
- [21] FIJAK M., PILATZ A., HEDGER M.P., NICOLAS N., BHUSHAN S., MICHEL V., TUNG K.S.K., SCHUPPE H.-C. 和 MEINHARDT A. 传染性、炎症性和“自身免疫性”男性因素不孕症: 啮齿动物模型如何为临床实践提供信息? 人类生殖更新, 2018, 24(4): 416-441. <https://doi.org/10.1093/humupd/dmy009>
- [22] 金刚, 郭丽., ZHANG Y., XUE Y., ZHANG X., WANG X., WANG D., LI B., ZHAO P., XU F., 和 CHENG J. 表达与本地化脂质运载蛋白型前列腺素D合酶在山羊睾丸、附睾和精子中的含量。小反刍动物研究, 2017, 154: 1-4. <https://doi.org/10.1016/j.smallrumres.2017.06.020>
- [23] SEPPAN P. 和 KRISHNASWAMY K. 放射猕猴输精管结扎的长期研究——睾丸和导管系统的组织学和超声分析。生殖医学系统生物学, 2014, 60(3): 151-160. <https://doi.org/10.3109/19396368.2014.896957>
- [24] YANG F., LI J., DONG L., TAN K., HUANG X.,

ZHANG P., LIU X., CHANG D., 和 YU X. 志, 2021年, 39 (3) : 406-
输精管切除术并发症和安全性问题回顾。世界男性健康杂 418。 <https://doi.org/10.5534/wjmh.200073>