

Effect of vasectomy on testis and epididymis in adult male Swiss albino mice: Histological and histometric study

Umarani S, Sivaraj R¹, Parasuraman S² and Gunasekaran JP

Division of Anatomy, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, Chidambaram - 608002. India

¹Division of Pharmacology, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, Chidambaram - 608002. India

²Pharmacology Unit, Faculty of pharmacy, Asian Institute of Medicine, Science and Technology (AIMST) University, Bedong, 08100, Kedah, Malaysia

Submission Date: 1-11-2013; Review completed: 20-1-2014; Accepted Date: 4-4-2014

ABSTRACT

Objective: To study the short- and long-term effects of vasectomy on the testis and epididymis in adult male Swiss albino mice.

Methods: Healthy adult male Swiss albino mice were divided into three groups, viz., control group (n=6), sham control group (n=6) and vasectomized experimental group (n=12). Vasectomy was performed on the mice in the vasectomized experimental group, and the sham-operated control group were made to undergo a surgical procedure but not vasectomy. Half of the animals in the vasectomized experimental group were sacrificed on the 20th day, and the remaining mice were sacrificed on the 70th day after vasectomy. The testis and epididymis were collected and preserved in Bouin's fluid. The control and sham control animals were sacrificed on the 70th day of the experiment, and the testis and epididymis were collected and fixed using Bouin's fluid over 24 hours. Qualitative evaluations of testicular sections were supplemented by the semi-quantitative testicular biopsy score count (TBSC) and histometric assessments (volume of parenchyma and stroma, surface area of lining epithelium, diameter of seminiferous tubules and ductus epididymis, height of epithelium) of various tissue components. Stained sections were subjected to stereological procedures using a microscope.

Results: Vasectomy did not affect the weight of the testis and epididymis, but it significantly reduced the tissue component of the seminiferous tubules in short-term vasectomized mice. The same was observed in normal and long-term vasectomized mice. A reduction in diameter of the seminiferous tubules and ductus epididymis was observed in short-term vasectomized mice, whereas long-term vasectomized mice showed a significant increase in cauda of the epithelium of the ductus. Only long-term vasectomized mice showed a significant decrease in epithelial height compared with control and sham control animals.

Conclusion: The reduction in spermatogenesis observed in short-term vasectomized mice could be due to fluid pressure acting back on the testis, and it is purely a temporary phenomenon. This effect was reversed, and renewal of spermatogenesis occurred in long-term vasectomized mice.

Keywords: Spermatogenesis, vasectomy, histometric analysis.

DOI: 10.5530/ijper.48.2.8

Address for correspondence:

Dr. S. Umarani,
Division of Anatomy,
Rajah Muthiah Medical
College,
Annamalai University,
Annamalai Nagar,
Chidambaram-608002.
India.

Phone: +91 996564888;
+91 995283999

E-mail:
drsumarani@gmail.com

INTRODUCTION

Vasectomy is a procedure that is commonly performed for birth control, mainly as a per-

manent family planning measure to prevent further conceptions and retrograde infection of the epididymis.¹ It is also indicated for



www.ijper.org

disputed rejuvenating effects in males. In all the five-year plans, much importance was given to the family planning programme, and incentives were provided to men who had undergone vasectomy operations. The operations are done on a mass scale. Every man who undergoes vasectomy will have a number of doubts regarding sperms production, sexual potency and the reversibility of the operation. Sexual potency is not affected by a vasectomy, and with the advancement of surgical techniques, methods have been developed to reverse this previously permanent procedure.² In approximately 90% of cases, a vasovasostomy restores the potency of the vas deferens, as measured by return of sperm to ejaculate.³ Many factors may contribute to persistent infertility after successful anatomical reanastomosis of the vas deferens, including immunologic responses and the presence of high titres of serum antisperm antibodies.⁴ The reversal may be affected by the length of time the vas deferens has been disrupted, and it also possible that are changes in the functions of the epididymal epithelium after a vasectomy.⁵⁻⁷ The epididymis has two primary functions: (a) the cauda epididymis serves as the primary storage site for sperm prior to ejaculation; and (b) the epididymal epithelium of the caput epididymis secretes proteins and other components required for maturation of sperm.^{8,9} Ligation of the vas deferens in a vasectomy may disturb the microenvironment of the epididymis through mechanical pressure on the epithelial cells.¹⁰ The short- and long-time effects of a vasectomy on the testis and epididymis are not clear. Hence, the present work is undertaken to investigate the short- and long-term effects of a vasectomy on the testis and epididymis in adult male Swiss albino mice.

MATERIALS AND METHOD

Animals

Healthy adult male Swiss albino mice (25–30 g) were used in the experiment. The experimental animals were obtained from the central animal house of the Rajah Muthiah Medical College, Annamalai University. After randomization into various groups, the mice were acclimatized for a week to adapt to the experimental laboratory conditions. All the animals were maintained according to the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The mice were fed with standard rodent pellets (Hindustan Limited, Bangalore, India) and water *ad libitum*. The study was approved by the Institute Animal Ethics Committee, and all the animal experiments were performed in accordance with the guidelines of the CPCSEA.

Method

Twenty-four healthy adult male Swiss albino mice were divided into three groups as follows:

Group I: Normal control (6 animals)

Group II: Sham-operated control (6 animals)

Group III: Vasectomized experimental group (12 animals)

The normal control group were not subjected to any surgical procedure, and the sham-operated control group was subjected to a surgical procedure without a vasectomy. The vasectomized experimental group of animals was sacrificed 20 days and 70 days after the vasectomy, with the day of the operation being treated as day 0. The animals in groups I and II were sacrificed on the 70th day of the experiment. The testis and the epididymis were separated and trimmed free of adipose tissue and connective tissue. The weights of the testis and epididymis were recorded. They were fixed in Bouin's fluid for a total period of 24 hours. After fixing, the tissues were processed for light microscopy, and blocks were prepared using paraffin wax. The tissues were cut with a thickness of ^{6,7} mm and stained with haematoxylin and eosin and connective tissue stains. The stained sections of testis and epididymis were examined under a light microscope at low power (×100) and high power (×400). A qualitative study was made of the tissue components and significant alterations recorded. The qualitative evaluations of the testicular sections were supplemented with the semi-quantitative testicular biopsy score count (TBSC) to estimate the extent of testicular alteration.^{11,12}

To perform histometric assessment of the tissue components, the stained sections were subjected to stereological procedures using a microscope. The volume of parenchyma and stroma, surface area of the lining epithelium, diameter of the seminiferous tubules and ductus epididymis and the height of the epithelium were measured.¹³

Operative procedure

All surgical procedures were performed with sterile precautions, under xylazine (16 mg/kg, i.m.) + ketamine (60 mg/kg, i.m.) anaesthesia.¹ The anterior abdominal wall was shaved and cleaned with spirit. The operation was performed by a 1.25 cm long midline incision in the suprapubic region, and the muscular and peritoneal layers were cut. The right testis was gently squeezed out of the scrotal sac and delivered through the abdominal wound. The vas deferens was identified along with the vassal artery, and it was dissected free from the vas deferens. Two ligatures were made on the vas deferens 0.5 cm apart with 4-0 non absorbable suture thread (about 1 cm proximal to the base of bladder). The vas deferens was

then transected between the ligatures, and a 0.4 cm long segment was removed. After the vasectomy, the testis was replaced in the scrotum. Care was taken not to allow the testis to remain in the abdominal cavity. The same procedure was performed on the left testis. Then the peritoneal, and muscular layers of the anterior abdominal wall were sutured, using '00' chromic catgut. The skin was closed by interrupted sutures using black cotton thread. The wound site was cleaned and disinfected with betadine and soframycin, respectively. After recovery from anaesthesia, the animal was transferred to a hygienic cage contains fasting grill. After 1 hour, the animal was allowed free access to drinking water, and 3 hours after the operation, it was fed standard rodent pellets.

The procedure was duplicated for the sham-operated control animals. In brief, the incision was made, and the vas deferens was exposed but not ligated or transected. The incision was closed in the same manner.

The post-operative period was uneventful. Post-operatively, the scrotal sacs were checked on alternate days for 2 weeks. No animals developed cryptorchidism after surgery. The sutures were removed on the 8 after the operative day. The wounds healed well in all the animals. None of the animals had a wound infection.

Volume estimation

The volume of the paranchyma and stroma were estimated by a point count using the eyepiece reticule at low magnification. The reticule was mounted on the eyepiece diaphragm, which is situated at the focal plane of the eyepiece lens. The reticule consisted of 100 small squares formed by 11 horizontal and 11 vertical lines. The points of intersections of lines were referred to as 'hits' or 'points'.

The volume was calculated using the formula $V_i = P_i / PT$, where V_i = volume of tissue component per unit volume of tissue, P_i = number of points touching the tissue component and PT = total number of points in the reticule.

Surface area estimation

The surface area of the lining epithelium per unit volume of tissue was measured by an intercept count using the same reticule at low magnification. There were 22 lines in the reticule. The length of each line was 1 mm.

The surface area was calculated using the formula $S_v = 2P/L$, where S_v = surface area of the tissue component (epithelium) per unit volume of tissue, P = number of points of intersection between the lines of the reticule and the free surface of the tissue component (epithelium) and L = sum of the length of all the lines in the reticule.

Epithelial height estimation

The height of the secretory epithelium was measured using an ocular micrometer at high magnification.

Diameter of tubules

The diameter of the tubules was measured using an ocular micrometer. The diameter of the tubules was calculated using the formula $D = (L+B)/2$, where D = diameter of the tubule, L = length of tubule and B = breath at right angle to length of tubule (L).

Results

The weights of the testis and epididymis of the control and experimental animals are provided in Table 1. There was no significant change in the experimental group compared with the control or sham-operated controls.

Normal histological features of intact control mouse testis: The testis of normal animals was covered by a thick, white, fibrous connective tissue capsule. Internal to the tunica albuginea there was a layer of vascular loose connective tissue that surrounded and supported the seminiferous tubules inside. This interstitial loose connective tissue contained clusters of eosinophilic endocrine cells, the interstitial cells of Leydig, which produce testosterone. The seminiferous tubules were lined with a modified stratified cuboidal epithelium, called the seminiferous epithelium. This germinal seminiferous epithelium contains two cell types, the spermatogenic cells, which produce sperm, and the supportive Sertoli cells, which nourish the developing sperm. This epithelium was resting upon the basement membrane of the seminiferous tubules. Spermatogenic cells were arranged in developmentally higher order from the basement membrane to the lumen, namely spermatogonia, spermatocytes, spermatids and spermatozoa (Plate 1a).

Normal histological features of intact control mouse epididymis: The epididymis of normal animals has a dumbbell-shaped structure on the dorsolateral aspect of the testis. It is made of a long, highly coiled tube, the ductus epididymis, supported by vascular connective tissue. The epididymis was conventionally divided into three parts, namely the caput (head), corpus (body) and cauda (tail). Each of the parts can further be divided into proximal and distal segments, based on the structure and function. There is also

Table 1. Weights of mouse testis and epididymis

Animal group	Weight of testis (g)	Weight of epididymis (g)
Intact control	0.08 ± 0.01	0.06 ± 0.01
Sham-operated control	0.09 ± 0.00	0.06 ± 0.01
Vasectomized short-term effect	0.11 ± 0.02	0.05 ± 0.01
Vasectomized long-term effect	0.10 ± 0.08	0.06 ± 0.01

All values are mean ± SEM (n=6).

another segment, called the initial segment, between the efferent ductules and the caput, which appears as a small rounded eminence on the caput. The ductus epididymis of the initial segment is lined by pseudostratified columnar epithelium (Plate 1b), which is mainly made of tall columnar principal cells and occasional small basal cells. The tall columnar cells bear long microvilli called stereocilia. The epithelium gradually decreases in thickness and becomes columnar in the caput (Plate 1c), low columnar in the corpus and cuboidal in the cauda (Plate 1d).

Beneath the epithelium there is a layer of circularly arranged smooth muscle fibres. This muscle layer increases in thickness gradually from the head to the tail. The lumen of the ductus epididymis gradually increases in width from the proximal end to the distal end, and it reaches the maximum size in the distal part of the cauda and is loaded with sperms (Plate 1d).

Histological features of vasectomized mouse testis: There was no testicular alteration, and the epithelium was intact, with normal spermatogenesis, in testis from sham-operated control mice compared with intact controls (TBSC=10). However, in the vasectomized (short-term) group, the seminiferous tubules showed thinning of the seminifer-

ous epithelium, affecting spermatogenesis. These testes also showed a reduction in number of spermatozoa and the presence of a conspicuous lumen in the seminiferous tubules. The spermatids were also scanty, giving a TBSC score of 7. Many spermatids were found inside the lumen of the seminiferous tubules (Plate 5a).

The histological appearance of testis from the vasectomized (long-term) group is shown in Plate 5b. These animals showed restoration of the seminiferous epithelium and spermatogenesis. Many spermatozoa were seen attached to the Sertoli cells. The TBSC score was 9.

Histological features of vasectomized mouse epididymis: The initial segment of the epididymis of all vasectomized (short-term) animals showed a dilated lumen (Plate 6a) and reduced epithelial height compared with control animals (Plate 6b). In the proximal part of the caput and cauda epididymis the lumen contained many spermatids and few sperms. It also contained eosinophilic globules (Plate 7) and occasional multinucleated giant cells (Plate 8). It was interesting to note that there were structural and functional differences between the proximal and distal parts of the cauda in short-term animals. The proxi-

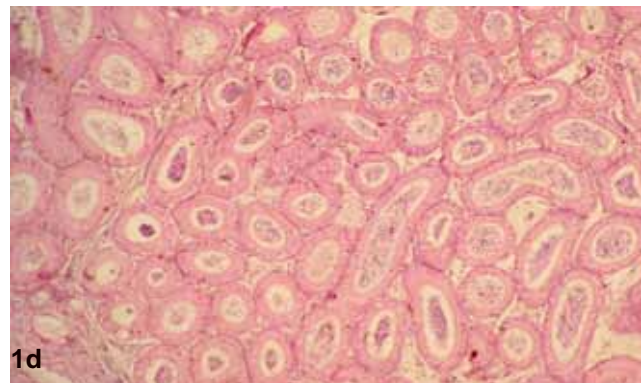
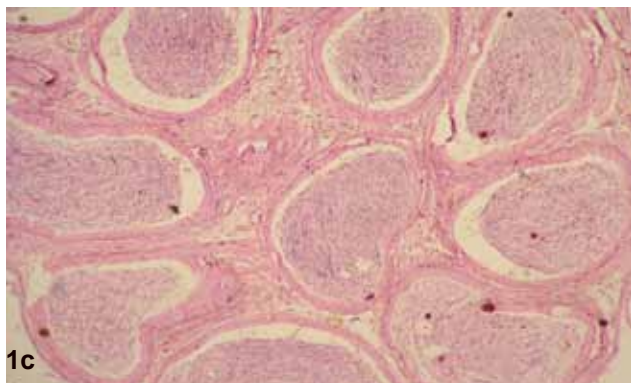
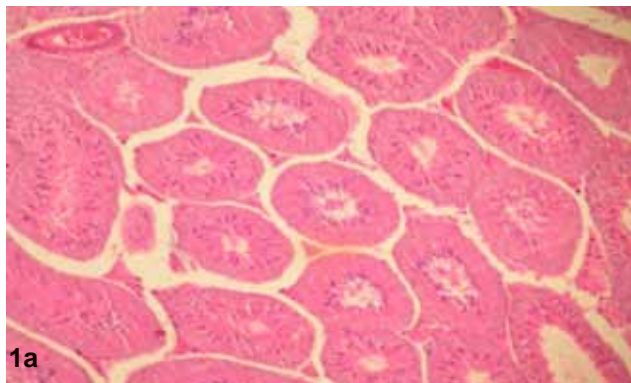


Plate-1: Sections from testes of control animal shows normal histological features of (a) clumps of spermatozoa attached to sertoli cells, H&E, 100 X; (b) columnar epithelium with no sperms in the lumen, H&E, 100 X; (c) caput epididymis which has low columnar ciliated epithelium with few sperms in the lumen, H&E, 100 X; (d) cauda epididymis which has cuboidal ciliated epithelium and the lumen is loaded with plenty of sperms, H&E, 100 X

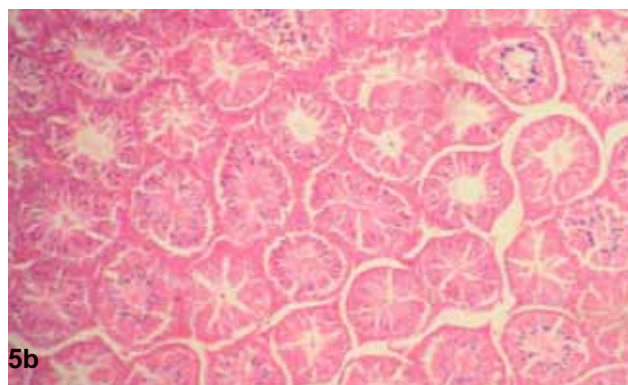
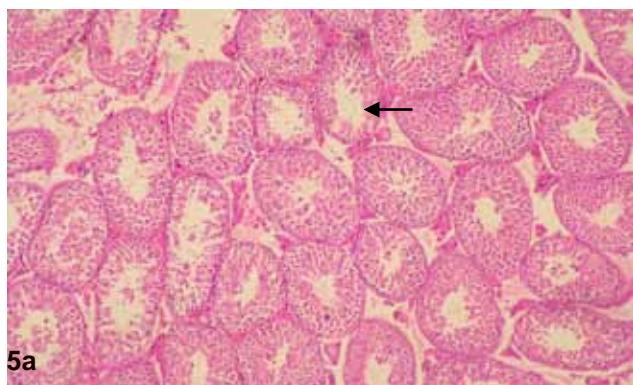


Plate-5: (a) Sections from testes of vasectomized (short term) mice shows very few sperms, exfoliated spermatids in the lumen (arrow), thin and degenerating seminiferous epithelium which affecting spermatogenesis, H&E, 100X. (b) Sections from testes of vasectomized (long term) mice shows presence of more sperm cells and well defined epithelium, H&E, 100X.

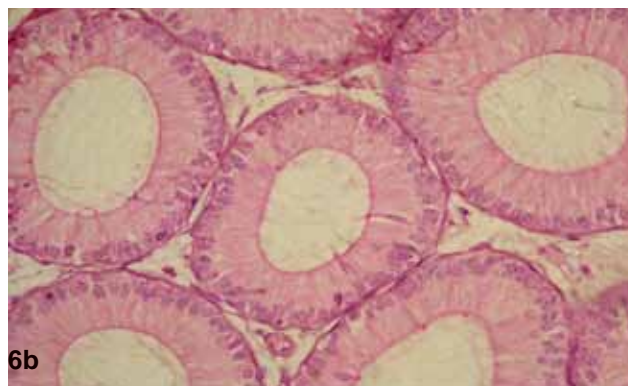
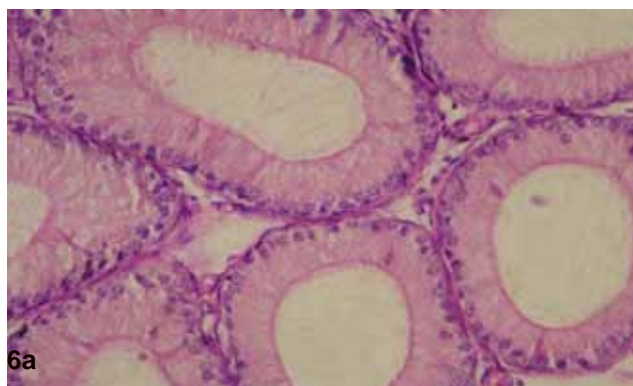


Plate-6: (a) Sections from epididymis (initial segments) of vasectomized (short term) mice shows dilated lumen and reduced epithelial height H&E, 400X. (b) Sections from epididymis (initial segments) of control mice shows normal histological features H&E, 400X.

mal cauda contained more eosinophilic bodies and few sperms, whereas the distal cauda had plenty of normal, healthy sperms (Plate 9). similar observations could be made regarding the corpus epididymis (Plate 10).

Another interesting observation about these vasectomized (short-term) animals was the presence of vacuolated cells spread among the tall columnar principal cells in the epithelium of the caput and corpus (Plate 11).

Restoration of normal histological features of the epididymis was noticed in the long-term vasectomized animals. This included epithelial height restoration and storage of a greater number of spermatozoa (Plates 12).

Histometric analysis of testis: Quantitative analysis of the various tissue components of the testis showed that there was a significant increase ($p < 0.05$) in the volume of the seminiferous tubules at the expense of the connective tissue component in the vasectomized (short-term) group of animals (Table 2). The increase in volume of the seminiferous tubules was due to the

dilatation of the lumen. This is also evident from Table 3, which shows an increase in the diameter of the seminiferous tubules. However, in the vasectomized (long-term) group of animals, the volume of seminiferous tubules was not significantly altered compared with the intact or sham-operated controls.

Histometric analysis of epididymis: The morphometric data of the epididymis are presented in tables 4 and 5 (volume of tissue components and epithelial surface area and height, respectively). No marked change in the experimental animals is discernible from Table 4, which shows the volumes of various tissue components of the epididymis. On the other hand, the surface area of the ductus epididymis of the caput and cauda showed a significant increase in vasectomized (short-term) experimental animals. The height of the epithelium of the cauda was reduced, whereas the diameter of the caput was increased in these animals (Table 5).

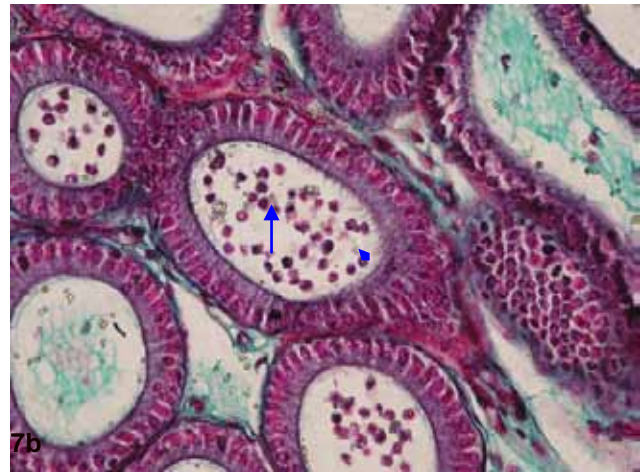
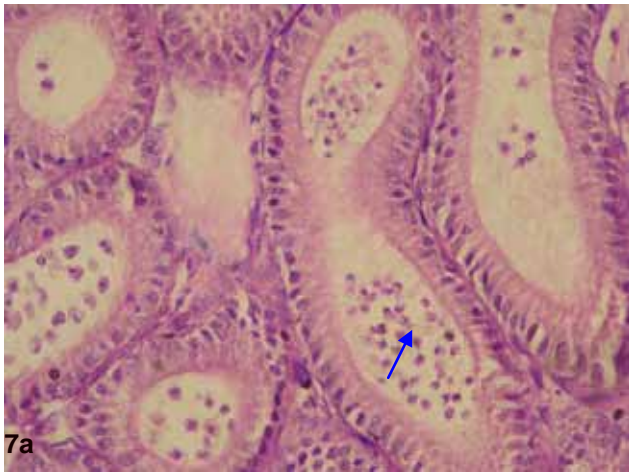


Plate-7: Sections from epididymis- caput of vasectomized (short term) mice shows lumen of the ductus contains more spermatids (arrow) and few sperms (arrow head) (a) H&E, 400X. (b) Masson's trichrome stain; 400X.

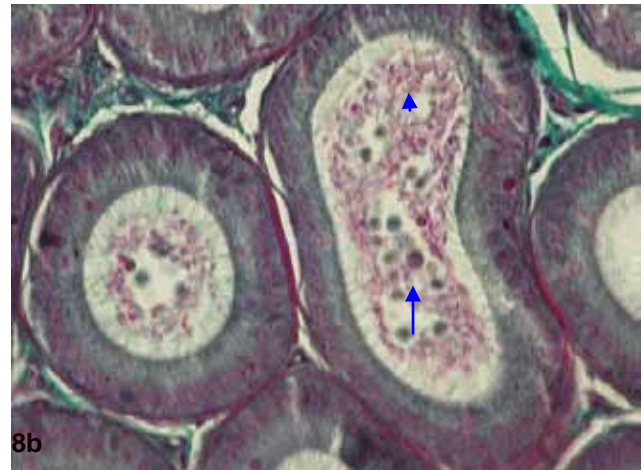
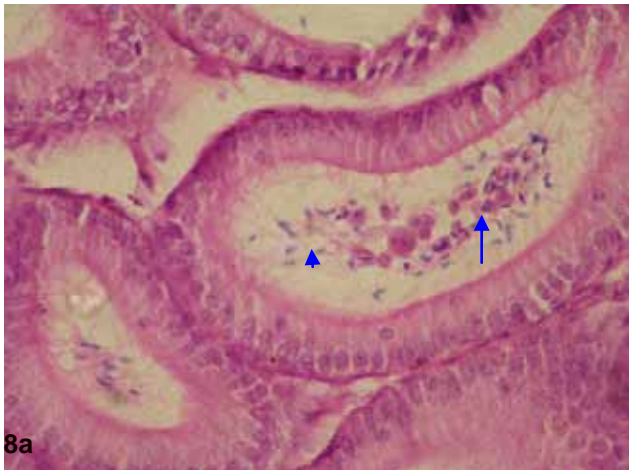


Plate-8: Sections from epididymis- caput of vasectomized (long term) mice shows lumen of the ductus contains sperms (arrow head) and few spermatids (arrow). (a) H&E, 400X. (b) Masson's trichrome stain; 400X.

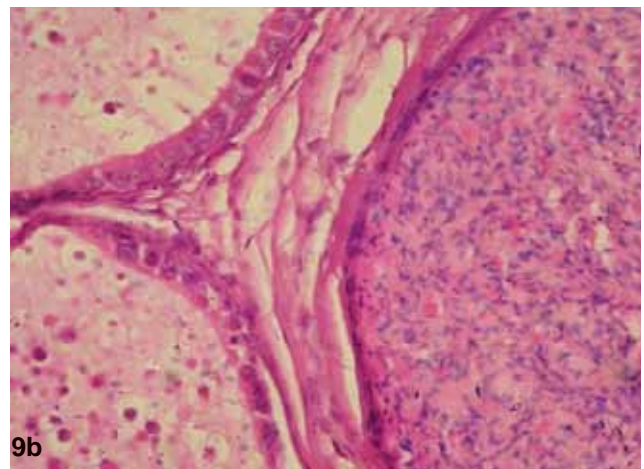
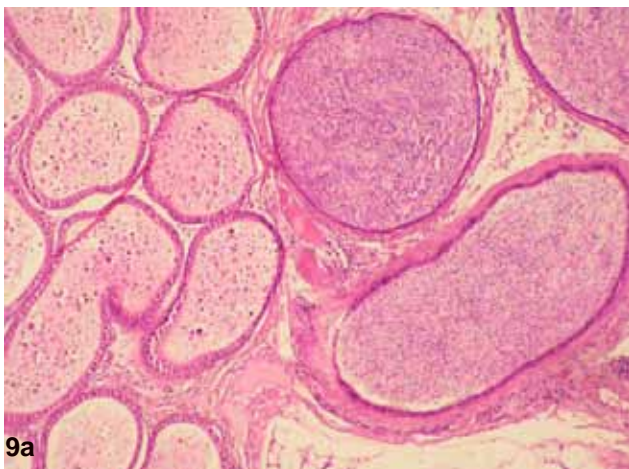


Plate-9: Sections from epididymis- caput of vasectomized (short term) mice shows (a) difference in size of the lumen and contents in these segments H&E, 100X (b) proximal cauda contains eosinophilic mass, few sperms and spermatids. Whereas the adjacent distal cauda contains normal healthy sperms H&E, 400X.

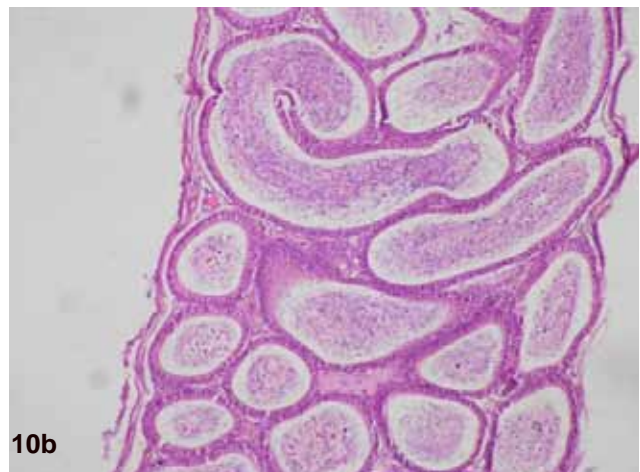


Plate-10: Sections from epididymis- corpus of vasectomized (short term) mice shows (a) the lumen contains eosinophilic substance in which few sperms and spermatids are embedded. H&E, 100X (b) healthy spermatozoa in the lumen H&E, 100X.

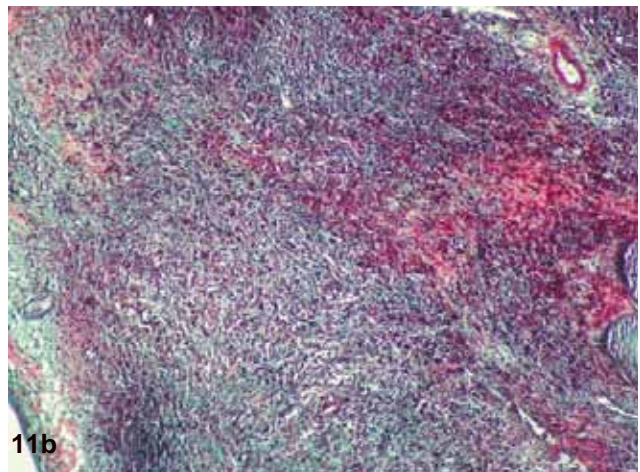
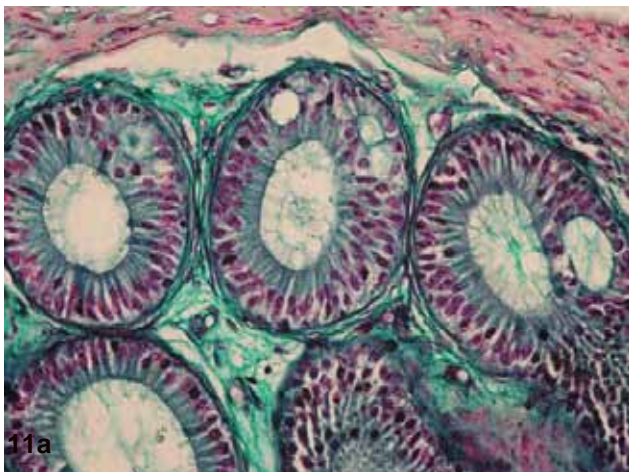


Plate-11: Sections from (a) initial segment of ductus epididymis from vasectomized (short term) mice shows vacuolated epithelial cells; Masson's trichrome stain; 400X (a) H&E, 100X (b) cauda epididymis of a vasectomized (short term) mice shows spermatic granulomas; Masson's trichrome stain; 100X.

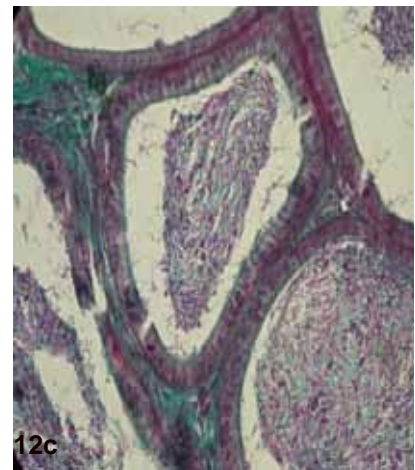
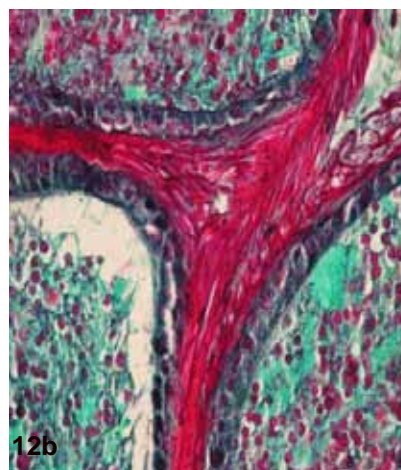
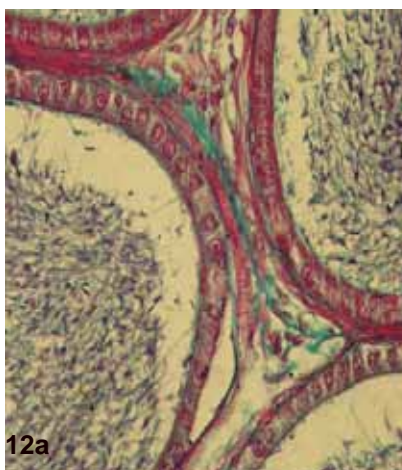


Plate-12: (a) Sections from epididymis- cauda of control mice shows normal histological features with plenty of sperms in the lumen Masson's trichrome stain; 400X, (b) Sections from epididymis- cauda of vasectomized (short term) mice lumen contains a mixture of spermatids, sperms and eosinophilic globules, Masson's trichrome stain; 400X. (c) Sections from epididymis- cauda of vasectomized (long term) mice lumen contains both sperms and eosinophilic globules, Masson's trichrome stain; 400X.

Table 2. Volumes of tissue components of the testis

Animal group	Volume of seminiferous tubules (mm ³ /mm ³)	Volume of interstitial tissues (mm ³ /mm ³)
Intact control	0.72 ± 0.01	0.28 ± 0.01
Sham-operated control	0.74 ± 0.00	0.22 ± 0.01
Vasectomized short-term effect	0.84 ± 0.02*	0.16 ± 0.13
Vasectomized long-term effect	0.68 ± 0.32	0.22 ± 0.03

All the values are mean ± SEM (n=6). *p<0.05 compared with intact control group (one-way ANOVA followed by Bonferroni test).

Table 3. Diameters of semiiferous tubules and ductus epididymis

Animal group	Diameter of seminiferous tubules (µm)	Diameter of ductus epithelium	
		Caput (µm)	Cauda (µm)
Intact control	220.80 ± 1.86	113.75 ± 2.41	185.0 ± 1.454
Sham-operated control	221.50 ± 1.96	110.25 ± 1.88	182.0 ± 1.253
Vasectomized short-term effect	230.40 ± 1.99	153.05 ± 5.71*	174.18 ± 1.52**
Vasectomized long-term effect	182.15 ± 4.35	130.54 ± 2.08	191.04 ± 3.73**

All the values are mean ± SEM. *p<0.05; **p<0.01 compared with intact control group (one-way ANOVA followed by Bonferroni test).

Table 4. Volumes of tissue components of epididymis

Animal group	Caput		Cauda	
	Volume of ductus (mm ³ /mm ³)	Volume of connective tissue (mm ³ /mm ³)	Volume of ductus (mm ³ /mm ³)	Volume of connective tissues (mm ³ /mm ³)
Intact control	0.76 ± 0.00	0.24 ± 0.01	0.77 ± 0.02	0.23 ± 0.02
Sham-operated control	0.76 ± 0.02	0.25 ± 0.14	0.75 ± 0.02	0.25 ± 0.03
Vasectomized short-term effect	0.75 ± 0.01	0.25 ± 0.01	0.76 ± 0.03	0.22 ± 0.03
Vasectomized long-term effect	0.77 ± 0.04	0.25 ± 0.04	0.81 ± 0.04	0.19 ± 0.04

All the values are mean ± SEM (n=6).

Table 5. Epithelial Surface area and height of ductus epididymis

Animal group	Epithelial surface area		Epithelial height	
	Caput (mm ² /mm ³)	Cauda (mm ² /mm ³)	Caput (µm)	Cauda (µm)
Intact control	18.30 ± 0.48	8.56 ± 0.80	25.45 ± 0.75	17.30 ± 0.48
Sham-operated control	18.04 ± 0.57	9.25 ± 0.70	24.32 ± 0.68	18.25 ± 0.56
Vasectomized short-term effect	22.122 ± 1.12	14.52 ± 1.72*	21.29 ± 0.50	14.35 ± 0.24
Vasectomized long-term effect	18.81 ± 1.50	10.09 ± 0.63	27.00 ± 0.52	13.86 ± 0.21*

All the values are mean ± SEM (n=6). *p<0.05 compared with intact control group (one-way ANOVA followed by Bonferroni test).

Discussion

Adult male albino mice were used in this study because the spermatogenesis in these animals is a continuous process, resembling that of the human model, they are easy to handle and have good immunogenic power and their cost is low compared with other animals. These mice also withstand ketamine anaesthesia and operative procedure well.

The effect of vasectomy on the testis varied from (a) complete degeneration of the seminiferous tubules, including hypertrophy and hyperplasia of interstitial tissues, (b) early degeneration, followed by regeneration, and (c) complete arrest of spermatogenesis to (d) no effect on spermatogenesis. Mice sacrificed 20 days (short term) after vasectomy showed a reduction in the number of spermatozoa, depletion of spermatids and thinning of the germinal epithelium, affecting spermatogenesis. The spermatids were shed into the lumen of the seminiferous tubules, and the same were transported into the epididymis, as evident from the histological observations. So it can be inferred that the spermiogenesis process of the spermatogenesis process was affected in these cases.

The present finding is in agreement with the findings of Peng *et al.*, who studied the effect of vasectomy on spermatogenesis in rhesus monkeys. They observed sperm granuloma, reduced diameter of seminiferous tubules and increased the number of type A spermatogonia in vasectomized animals.¹⁵

Singh and Chakravarty, observed a generalized depletion of germinal cells in mouse testis 30 and 45 days after bilateral vasectomy even though the testis presented a normal histological appearance.¹⁶ According to Handley *et al.*, the testicular changes after vasectomy may be secondary, a consequence of an inflammatory reaction or obstruction of the excurrent duct system.¹⁷

The second part of the present investigation is the long-term study. Testes obtained from mice sacrificed 70 days (long term) after vasectomy displayed restoration of the seminiferous epithelium and spermatogenesis, as evident from the increased number of spermatozoa seen attached to the Sertoli cells. Restoration of the epithelium is further supported by the presence of a large number of spermatozoa in the epididymis.

Rangam *et al.*, demonstrated that the structural and functional changes produced in the testis after a vasectomy are purely transient in rats. They noticed testicular degeneration in rats in the first few weeks after a vasectomy, followed by regenerative changes resulting in restoration of the testicular structure to normality in six weeks.¹⁸

The primary function of epididymis is storage of sperms prior to ejaculation, and this function is carried out especially by the cauda (tail). Recent evidence suggests that the epididymis participates in the maturation of spermatozoa by secreting some glycoproteins that are incorporated into the plasma membrane of the maturing spermatozoa.^{8,9-19} The epididymis also creates the luminal microenvironment required for maturation of sperms by secreting some substances into and absorbing other substances from the lumen of the epididymal duct.

Many workers have studied the effect of vasectomy on the epididymis and tried to find out the mechanism by which sperms are disposed in the epididymis. From a study conducted on dairy bull Amann and Almquist reported that most of the spermatozoa produced by the testis are reabsorbed primarily by the cauda epididymis and not by the ductus deferens.²⁰

Although the aforementioned investigators have proposed many sites for sperm disposal, they have not convincingly proven them. In the present short-term study, the lumen of the caput epididymis contained lot of spermatids and cellular debris as well as a few sperms. But when these reached the cauda, the number was reduced, indicating there is luminal breakdown of these structures, mainly spermatids. It is possible that they are endocytosed by the vacuolated cells or clear cells observed among the lining epithelial cells. These types of cells were noticed earlier by Moore and Bedford and by Hermo *et al.* and were involved in disposal of cytoplasmic droplets shed by the luminal sperms.^{21,22}

The other interesting finding of the present investigation is the presence of two different types of luminal contents in two adjacent segments of the cauda in experimental animals. The proximal cauda is filled with a lot of cellular debris and eosinophilic globules as well as a few spermatids and sperms and occasional macrophages, whereas the distal cauda is filled with a lot of healthy sperms (Plate 12b). This observation leads us to postulate that the proximal cauda is involved in intraluminal breakdown and phagocytosis, while the distal cauda is principally involved in storage of spermatozoa, where the micro-environment is congenial for this.

CONCLUSION

The disruption/thinning of the seminiferous epithelium, with reduction in spermatogenesis, observed in short-term vasectomized mice could be due to fluid pressure acting back on the testis, and it is purely a temporary phenomenon. Restoration of the seminiferous epithelium to the original thickness and renewal of spermatogenesis occur 70 days after vasectomy or even earlier. The proximal part of the epididymis may be involved in luminal breakdown of trapped spermatozoa, apart from transport and maturation of spermatozoa, in vasectomized animals.

REFERENCES

1. Murudkar PK, Janamala R. Macroscopic and microscopic study of the testis after ligation of head and body of epididymis in adult male albino rats. *IOSR J Pharm.* 2013;3:16–23.
2. Kabalin JN, Kessler R. Macroscopic vasovasostomy re-examined. *Urology.* 1991;38:135–8.
3. Feber KM, Ruiz HE. Vasovasostomy: Macroscopic approach and retrospective review. *Tech Urol.* 1999;5:8–11.
4. Abdelmassih V, Balmaceda JP, Tesarik J, Abdelmassih R, Nagy ZP. Relationship between time period after vasectomy and the reproductive capacity of sperm obtained by epididymal aspiration. *Hum Reprod.* 2002;17:736–40.
5. Flickinger CJ, Howards SS, Herr JC. Effects of vasectomy on the epididymis. *Microsc Res Tech.* 1995;30:82–100.
6. Doiron K, Légaré C, Saez F, Sullivan R. Effect of vasectomy on gene expression in the epididymis of cynomolgus monkey. *Biol Reprod.* 2003; 68:781–8.
7. Légaré C, Verville N, Sullivan R. Vasectomy influences expression of HE1 but not HE2 and HE5 genes in human epididymis. *J Androl.* 2004;25:30–43.
8. Hinton BT, Palladino MA. Epididymal epithelium: Its contribution to the formation of a luminal fluid microenvironment. *Microsc Res Tech.* 1995;30:67–81.
9. Kirchhoff C, Pera I, Derr P, Yeung CH, Cooper T. The molecular biology of the sperm surface: Post-testicular membrane remodelling. *Adv Exp Med Biol.* 1997;424:221–32.
10. Johnson AL, Howards SS. Intratubular hydrostatic pressure in testis and epididymis before and after vasectomy. *Am J Physiol.* 1975 Feb;228(2):556–64.
11. Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. *Hormones.* 1970;1:2–25.
12. Glander HJ, Horn LC, Dorschner W, Paasch U, Kratzsch J. Probability to retrieve testicular spermatozoa in azoospermic patients. *Asian J Androl.* 2000;2:199–205.
13. Elias H, Pauly JE. *Histology and Human Microanatomy.* 3rd ed., F.A. Davis Company, U.S.A., 1966.

14. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 2010;1:87–93.
15. Peng B, Zhang RD, Dai XS, Deng XZ, Wan Y, Yang ZW. Quantitative (stereological) study of the effects of vasectomy on spermatogenesis in rhesus monkeys (*Macaca mulatta*). *Reproduction*. 2002;124:847–56.
16. Singh SK, Chakravarty S. Histologic changes in the mouse testis after bilateral vasectomy. *Asian J Androl*. 2000;2:115–20.
17. Handley HH, Flickinger CJ, Herr JC. Post-vasectomy sperm autoimmuno-gens in the Lewis rat. *Biol Reprod*. 1988;39:1239–50.
18. Rangam CM, Arora MM, Gupta JC. Testicular changes in rats following vasectomy. *Indian J Med Sci*. 1957;11:717–21.
19. Kohane AC, Cameo MS, Piñeiro L, Garberi JC, Blaquier JA. Distribution and site of production of specific proteins in the rat epididymis. *Biol Reprod*. 1980;23:181–7.
20. Amann RP, Almquist JO. Reproductive capacity of dairy bulls. VI. Effect of unilateral vasectomy and ejaculation frequency on sperm reserves: Aspects of epididymal physiology. *J Reprod Fertil*. 1962;3:260–8.
21. Moore HD, Bedford JM. The differential absorptive activity of epithelial cells of the rat epididymus before and after castration. *Anat Rec*. 1979;193:313–27.
22. Hermo L, Dworkin J, Oko R. Role of epithelial clear cells of the rat epididymis in the disposal of the contents of cytoplasmic droplets detached from spermatozoa. *Am J Anat*. 1988;183:107–24.