Original Article

The Effects of Vasectomy on the Testes of Albino Rats: A Histological Study

Anatomy Section

GOLGHATE TARKESHWAR D., NAIDU SUNETRA S., SATHE VIVEK M., FULPATIL MILIND P.

ABSTRACT

Context: Population explosion has become one of the most challenging problems which are being faced by the entire world. Vasectomy is a simple, safe, quick and effective but vital method for male sterilization. In the recent years, a large number of vasectomies are being performed in India, since the inception of the National Family Planning Program in 1956. Thus, the number of requests for the restoration of fertility is also being increased.

Aim: The present study was carried out to know whether the histology of the testes and spermatogenesis remains normal after vasectomy.

Materials and Methods: The histology of the testes of 50 male albino rats was studied one, two, three and four months after vasectomy in 10 male albino rats of each group and 10 male albino rats served as the controls.

Results: After vasectomy, the seminiferous tubules were cut in various planes, which were covered externally by connective tissue stroma with a normal histological picture. The spermatogonia, spermatids, Sertoli cells, spermatozoa and Leydig cells showed a normal histological picture, with normal sized blood vessels in the interstitial connective tissue in the vasectomised and the control rats. In some seminiferous tubules, granular cytoplasmic vesicle like structures were found to fill the lumen and the spermatids which were metamorphosing to spermatozoa. Many round or elongated heads of the spermatozoa were present at the apical portion of the Sertoli cells. In some seminiferous tubules, disorganization of the seminiferous epithelium was found after the second month of the vasectomy.

Conclusions: On critical analysis, it was concluded that the histology of the testes and the spermatogenesis remained normal at four months after the vasectomy.

Key Words: Vasectomy, Histology, Spermatogenesis.

INTRODUCTION

Population explosion has become one of the most challenging problems which are being faced by the entire world. Therefore, in recent years, reproductive biology has received great attention from numerous scientists. Especially the testes and the vas deferens have important functional roles in reproduction and these have been extensively studied by a number of researchers in this field.

India is the second largest country after China in population. To control this population explosion, the National Family Planning Program was introduced in India in 1956 [1]. Vasectomy is a simple, safe, quick, effective and vital method for male sterilization. Vasectomy has been a popular means of male sterilization [2,3,4,1]. A large number of vasectomies have been performed till today.

Recent surgical techniques have shown that this procedure can be reversed [5,6]. Scrotal vasectomy, when it is performed away from the epididymis in the routine way, it can be recanalised. This fact, when made public, could further well accelerate the trend of vasectomy, especially when men become aware that their sexuality remains unimpaired by either operations [7].

The frequency with which vasectomy reversal was performed has increased dramatically, because many of the vasectomised men desire to regain their fertility [8,9]. The most common reason for requesting a vasovasostomy was divorce, followed by remarriage and the desire to have children with a new wife. Other motivating factors included the desire for more children, religious conversion, and change of heart about the idea of being sterile. Rarely could the reason be the loss of a child [8].

For the above mentioned reasons, the present study was carried out to know whether the histology of the testes and the spermatogenesis remained normal after the vasectomy.

MATERIALS AND METHODS

The study material consisted of the testes of 50 male albino rats in the age group of 4 to 14 months and their weights ranged from 100-250 gm. Out of which 50 rats in the study, 40 rats were vasectomised and 10 rats taken as controls.

All the rats were kept in different cages in the animal house. They were caged with a light: dark cycle, and were permitted for at least 2 weeks to acclimatize to their surroundings [10]. The choice of male albino rats for the present study was because of their easy availability. They are easy to feed, raise and mate. They have a short gestation period of 18-21 days and a long period of reproductive activity from 2 to 14 months of age. They can produce too many litters and hundreds of offspring. They are easy to handle [11]. Albino rat, “is a well established experimental animal model for the study of the testes after vasectomy” [12].

Out of the 50 male albino rats in the study, 40 rats in the study group were vasectomised by standard methods [13] and 10 rats of the control group were left un-vasectomised. The male albino rats were anaesthetized by the inhalation of ether. The vasectomy was performed under aseptic conditions through a vertical midline abdominal incision. The urinary bladder was displaced forward and the vasa deferentia were located at their point of entry into the posterior aspect of the urethra. Both the vasa deferentia were taken out of the abdominal cavity and were held taut by means of

curved forceps. Each vas deferens was divided between two 4-0 silk ligatures which were approximately 1.5 cm proximal to the end of the vas at the base of the bladder. The scrotal contents were not disturbed during the operation. Special attention was paid to not disturb the spermatic vessels. The cut ends were replaced in the abdomen along with the vas deferens. The incision was closed in two layers by using a 3-0 chromic catgut for the peritoneum and muscles, and 4-0 silk sutures for the skin. The scrotal sacs were checked on alternate days for two weeks and then at weekly intervals to ascertain that the testes remained in the scrotum. The albino rats in which the testes were withdrawn into the abdominal cavity were eliminated from the experiment [13,14].

The testes were collected for histological studies one, two, three and four months after the vasectomy (10 each). Similarly, the testes of the proportionate control rats were collected for comparison [Table/Fig 1].

The testes were fixed in Bouin's fluid and the processing of the tissue was done for dehydration, clearing and paraffin embedding. The paraffin blocks were prepared according to standard procedures [15].

The sections (7 microns) were prepared and stained with haematoxylene and eosin as per the standard histological procedures [15]. In each case, the serial sections were carefully observed under a light microscope by using the X10, X45 and X100 magnifications and the observations were concentrated on the interstitial stroma which contained the Leydig's cells and on the blood vessels and the seminiferous tubules including the cells of the spermatogenic series (spermatogonia A, spermatogonia B, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa) along with the Sertoli cells.

RESULTS

The normal histological findings in the testes of healthy, adult, male albino rats as per Rugh Roberts' study were used as a reference for this study [11]. The seminiferous tubules which were convoluted within the loculi were cut into multiple sections. The seminiferous epithelium of the tubules lay against a basement membrane that was surrounded by thin, fibrous connective tissue. Between the seminiferous tubules there was interstitial stroma which consisted of clumps of Leydig's or interstitial cells. The interstitial cells of the testes had large round nuclei, with each one or more nucleoli containing course granules. Their cytoplasm was eosinophilic. The seminiferous epithelium also had sustaining nutritive or nurse (Sertoli) cells. The Sertoli cells were attached by their bases to the basement membrane and they projected towards the lumen of the seminiferous tubules. They were elongated cells with large oval nuclei that appeared to be intended. Within the nucleus of the Sertoli cells was a compound or multiple nucleolous. These cells could assume several forms depending upon their activity. In the resting state, they were closely associated with the basement membrane to which they were attached and their oval nuclei were parallel to that membrane. As supporting cells for the metamorphosis of the spermatids to spermatozoa and for the temporary retention of the mature spermatozoa, they had an elongated, pyramidal shape and their nuclei lay perpendicular to the basement membrane. Their cytoplasm near the lumen generally contained the heads of many mature spermatozoa, the tails of which lay free within the lumen.

Period after Vasectomy	Study Group	Control Group
1 month	10	02
2 month	10	02
3 month	10	03
4 month	10	03
Total	40	10

[Table/Fig-1]: Schedule for collection of testes from Male Albino Rats. *Age of albino rats was 4-14 months and weight 100-250 gm.*

The potential (primodial) germ cells are the ancestors of all the millions of spermatozoa which would be produced by males. Shortly after birth, larger cells, the spermatogonia are seen. The spermatogonia A are capable of mitosis or of giving rise to the other types of cells and ultimately to spermatozoa. The spermatogonia A are the largest cells, and they contain fine dust like particles of nuclear chromatin and a single eccentrically placed chromatin nucleolous. They may give rise through intermediate spermatogonia to spermatogonia B. The spermatogonia B are smaller and more numerous and contain nuclear chromatin in a coarse flake of clumps, on or near the inner surface of the nuclear membrane. There is a centrally placed plasmosome like nucleus. The spermatogonia B may divide to give rise to more type B cells or they may change into primary spermatocytes, further from the basement membrane.

The first indication that the spermatogonia B will metamorphose into primary spermatocytes is that they enlarge noticeably and move away from the basement membrane, with deeply stained cytoplasm having prominent centrally placed nuclei which contain clumped and coiled chromatin material. The primary spermatocytes divide into two smaller secondary spermatocytes which are smaller than the primary spermatocytes, with dense, coiled chromatin material in the round and prominent nuclei. The secondary spermatocytes divide into four spermatids. They undergo a radical metamorphosis into an equal number of mature spermatozoa, losing most of their cytoplasm and changing their forms characteristically.

The spermatids lie inner to the spermatocytes which are round in shape, near the lumen. The transformation of the spermatids into spermatozoa involves no division. Most of the cytoplasm disappears, carrying with it certain residual bodies. The cytoplasm of the spermatids which is to be sloughed off contains lipid droplets, mitochondria, ribosomes, endoplasmic reticulum, the golgi apparatus and numerous multicellular and multigranular bodies. The spermatozoa vary in length, width and shape with the mouse strain. They generally have a hooked head with a length of about 0.0080mm, a short middle piece and a very long tail, for an overall length which averages 0.1226mm. The head of many such spermatozoa lie embedded in the cytoplasm of a Sertoli cell [11].

The histological findings in the control and the vasectomised rats were noted. The testes of the control and the vasectomised rats which were studied upto four months showed the following histological findings.

The testes of the control group had a normal histological pattern which showed normal spermatogonia, spermatocytes, spermatids, spermatozoa, and Sertoli cells in the well defined, intact and regular seminiferous tubules which were cut in various planes. The interstitial stroma which contained the Leydig's cells in clumps, and the blood vessels showed a normal histological picture.

The testes of the vasectomised rats showed seminiferous tubules which were cut in various planes, which were covered externally by connective tissue stroma, which contained the Leydig cells in clumps, and blood vessels embedded in it. The basement membrane was intact, well defined and regular and it was lined by

germinal epithelium. [Table/Fig 2] The spermatogonia A and B were found to be lying on the basement membrane, interspersed with each other and they could be clearly marked out. The spermatogonia A were large round cells which lay on the basement membrane, they were few in number and showed pink, homogeneously stained cytoplasm with prominent, round, centrally placed nuclei with granular chromatin material. The spermatogonia B were predominantly seen, they were smaller than the spermatogonia A and were interspersed with them. The nuclei of the spermatogonia B were very prominent and round and they contained chromatin material in coarse flakes near the inner surface of the well defined nuclear membrane. [Table/Fig 3]

The spermatocytes also lay on the basement membrane, but away from the spermatogonial layer. The primary spermatocytes were noticeably larger than the spermatogonia, with deeply stained cytoplasm which had prominent, centrally placed nuclei which contained clumps and coiled chromatin material. Some cells in meiotic division could be clearly marked out. The secondary spermatocytes were few in number and were smaller than the primary spermatocytes, with dense, coiled chromatin material in round prominent nuclei. The spermatids are found to be inner to the spermatocytes, round in shape and near the lumen, with a normal fine structure. [Table/Fig 4] Some cells were seen in the cytoplasmic crevices of the Sertoli cells. [Table/Fig 4] Some cells which were metamorphosing to spermatozoa were also seen.[Table/Fig 3] The Sertoli cells which lay on the basement membrane were polyhedral in shape, with ill defined margins and nuclei. The round or elongated heads of the spermatozoa were found to be clinging to the apex of the Sertoli cells and their tails were in the lumen [Table/Fig 2]. The numerous detached tails of the spermatozoa were seen as clumps in the lumen of the seminiferous tubules. The interstitial stroma covered the seminiferous tubules and embedded in it were blood vessels and round or oval Leydig's cells in clumps, with prominent large, round nuclei [Table/Fig 2].

In some seminiferous tubules, granular, cytoplasmic vesicle like structures, which more or less filled the lumen were clearly seen [Table/Fig 4].

In the testes, in the 2nd month after the vasectomy, in some seminiferous tubules, the disorganization of the seminiferous epithelium was found, but all the components of the seminiferous epithelium showed a normal histological picture [Table/Fig 5].

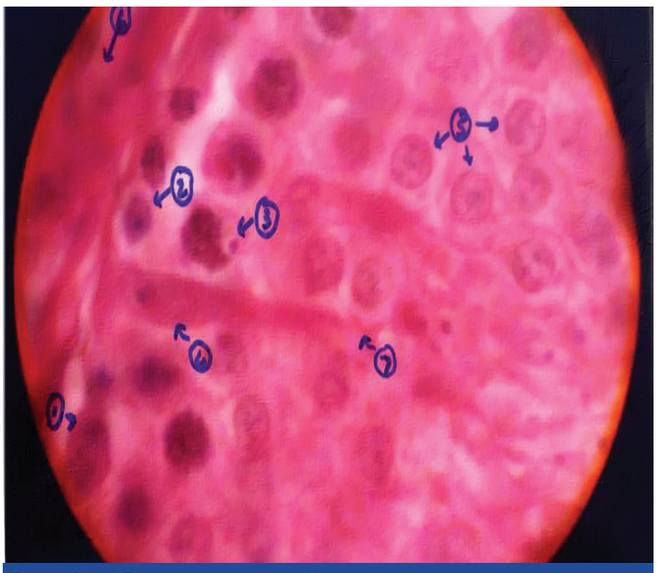
These findings in the connective tissue stroma which contained the Leydig's cells, which surrounded the seminiferous tubules and all the components of the seminiferous epithelium, showed a normal histological picture, as was described by Rugh Roberts [11].

The spermatozoa which were present in the lumen of the seminiferous tubules and all the components of the seminiferous epithelium showed a normal histological picture, which indicated that the process of spermatogenesis was not affected by vasectomy and that spermatogenesis continued at the 4th month of vasectomy. There was no difference between the histological profile of the control rats and the vasectomised rats at all the stages of the investigation.

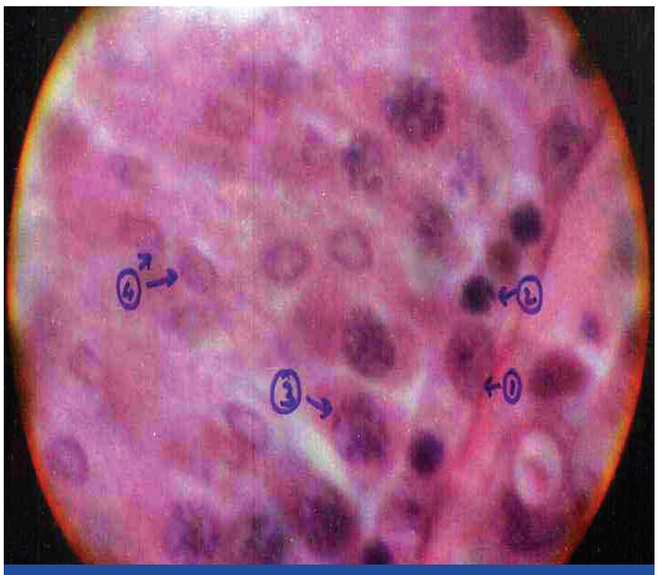
DISCUSSION

The testes and its histology after vasectomy is a subject of diversified interest, as is evident from the voluminous literature which is available on this subject all over the world.

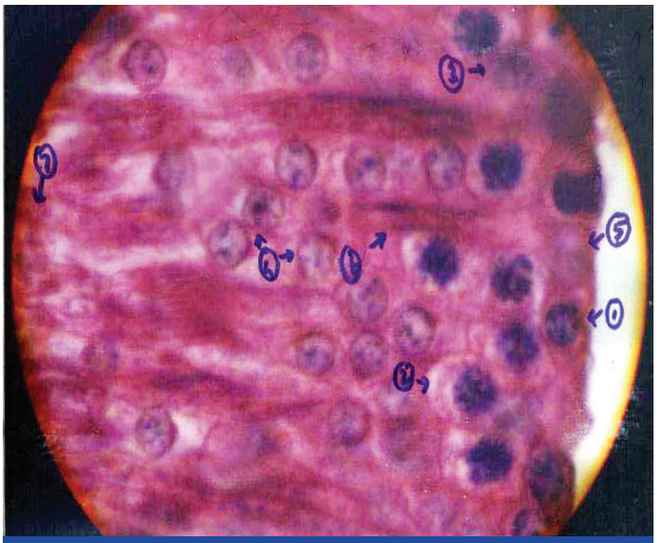
Vasectomy is becoming an important method of contraception. It



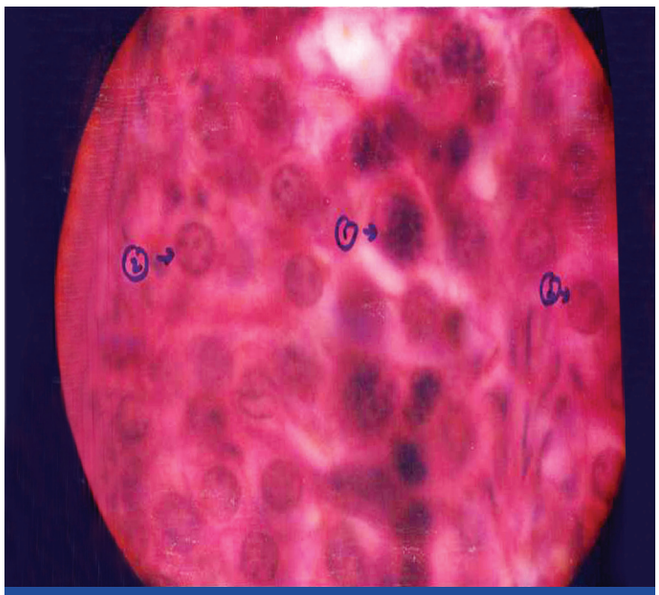
[Table/Fig-2]: Histology of testes 3 month after vasectomy.
1. Spermatogonia A, 2. Spermatogonia B, 3. Primary spermatocytes 4. Sertoli cells, 5. Spermatids, 6. Leydig cells, 7. Heads of spermatozoa



[Table/Fig-3]: Histology of testes 4 month after vasectomy
1. Spermatogonia A, 2. Spermatogonia B 3. Primary spermatocytes 4. Spermatids in metamorphosis phase



[Table/Fig-4]: Histology of testes 1 month after vasectomy
1. Spermatogonia B, 2. Primary spermatocytes, 3. Secondary Spermatocytes, 4. Spermatids, 5. Sertoli cells, 6. Heads of spermatozoa in apical portion of Sertoli cells, 7. Granular cytoplasmic material like vesicles.



[Table/Fig-5]: Histology of testes 2 month after vasectomy
1. Primary spermatocytes, 2. Disorganized Spermatids

is a simple, safe, quick and effective method of male sterilization which has been accepted by a majority of the population [1]. With the increasing public knowledge through media exposure, sex education classes in schools, enlightened health personal and more pressure from the female population, the trend of an increased demand for vasectomy will accelerate [16].

The histology of the testes of albino rats was included in this study to prove that the spermatogenesis remains normal and that the fertility can be restored even after vasectomy. The age of the male albino rats which were selected for this study was 4 months to 14 months, because albino rats have a long period of reproductive activity from 2 months to about 14 months of age [11].

The comparison of the findings from the control and the experimental (vasectomised) groups showed that the testicular features of the vasectomised group were similar to those which were seen in the control group. The spermatogonia, spermatocytes, spermatids, spermatozoa and the Sertoli cells were structurally normal. The interstitial stroma of the testes which contained the Leydig cells and the blood vessels were structurally normal after the vasectomy. These findings in our study suggested that the fine structure of the testes in rats which remained normal at four months of vasectomy showed the normal histology of the testes.[11] Similar findings were seen in some other studies also. [17-23,1,13,14]

In the present study, the spermatozoa were seen in the lumen of the seminiferous tubules. The many round or oblong heads of the spermatozoa were also seen in the lumen. Some spermatozoa were found to be clinging to the apex of the Sertoli cells with their tails towards the lumen. Many detached tails of the spermatozoa were also seen in the lumen. The testes revealed all the stages of spermatogenesis. These findings in our study suggested that the process of spermatogenesis continued at four months of vasectomy. Our findings correlated with the findings of previous studies [1,14,20].

In our study, the Sertoli cells were very large and polyhedral with ill defined margins and nuclei. But the many elongated and normal heads of the spermatozoa were found to be clinging to the apex, which showed their active phase. The Sertoli cells are capable of phagocytosing the material from the lumen of the seminiferous tubules and this activity of the Sertoli cells increases after vasectomy.[22] Some Sertoli cells have well defined margins and nuclei, and they touch the basement membrane. Our findings co-related with the findings of previous studies [24,22].

The findings of the present study were compared with a study on men who underwent vasectomy and thereafter, vasovasostomy (vasectomy reversal) for clinical application. The spermatozoa are present in the lumen of the seminiferous tubules, with round or oblong heads. These results correlated with the findings of Gupta Indrayani, Dhawan Sushama and Goel GP, thus indicating that spermatogenesis is not affected after vasectomy [25]. However, their findings were derived from semen analysis after vasovasostomy. They found that 52% subjects had a sperm count of at least 20 million/ml and that in all subjects, the sperms appeared in the ejaculate as early as 2 months after the vasovasostomy and continued thereafter.

In our study, the vasectomy was performed under aseptic conditions and the histology of the testes was studied, which appeared to be normal at four months of vasectomy. Similar findings were noted by Hellar GV and Rothchild I [26].

In some seminiferous tubules, granular, cytoplasmic vesicle like structures, which more or less filled the lumen, were clearly seen. These vesicles were the cytoplasm which was shed off during the metamorphosis of the spermatids into spermatozoa [11].

On the basis of critical analysis, it was concluded that the histology of the testes remained normal at 4 months after the vasectomy and that the spermatozoa continued to be produced. So, it definitely showed that the spermatogenesis was not affected by vasectomy. Hence, fertility could be re-established in vasectomised individuals by vasovasostomy if the need arose.

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AUTHOR(S):

1. Dr. Golghate Tarkeshwar D.
2. Dr. Naidu Sunetra S.
3. Dr. Sathe Vivek M.
4. Dr. Fulpatil Milind P.

PARTICULARS OF CONTRIBUTORS:

1. MS (Anatomy), Assistant Professor: Anatomy, SBH Government Medical College, Dhule
2. MS (Anatomy), Associate professor: Anatomy, Government Medical College, Nagpur
3. M.S. (Anatomy), Professor & Head: Anatomy (retired), Government Medical College, Nagpur
4. MS (Anatomy), Professor & Head: Anatomy, SBH Government Medical College, Dhule

PLACE OF STUDY:

SBH Government Medical College, Dhule

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. T.D. Golghate
Department of Anatomy,
Shri Bhausaheb Hire Govt. Medical College,
Chhakarburdi, Bypass road, Dhule, Maharashtra State.
Tel: 9422802401
Fax: 02562239207, 02562239407
Email: tarkeshwargolghate@yahoo.co.in

DECLARATION ON COMPETING INTERESTS:

No conflicting Interests.

Date of Submission: **Mar 29, 2011**
Date of peer review: **Aug 02, 2011**
Date of acceptance: **Sep 25, 2011**
Date of Publishing: **Dec 25, 2011**