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Original Article

Macroscopic and microscopic study of the testis after vasectomy and ligation of vasefferentia of testis in adult male albino rats.

Pradeep Kumar murudkar [a]*, Ratnapriyanka janamala [b], Tejovathi Bandike [c] Archana.M.Hatti [d] Ashwini.H.Pundalik [e], Jyothinath kothapalli [f]

[a*] *Professor and HOD, Department of Anatomy, Khaja BandaNawaz Institute of Medical Sciences, Gulbarga, Karnataka.*
[b] *Tutor,Department of Anatomy, Khaja BandaNawaz Institute of Medical Sciences, Gulbarga, Karnataka.*
[c] *Tutor,Department of Biochemistry, Khaja BandaNawaz Institute of Medical Sciences, Gulbarga, Karnataka*
[d] *Assistant proffesor, Department of Anatomy, Khaja BandaNawaz Institute of Medical Sciences, Gulbarga, Karnataka.*
[e] *Assistant proffesor ,Department of Anatomy, Khaja BandaNawaz Institute of Medical Sciences, Gulbarga, Karnataka.*
[f] *Tutor,Department of Anatomy, Khaja BandaNawaz Institute of Medical Sciences, Gulbarga, Karnataka*

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ABSTRACT

The present study is done to reassess the effects of ligation of excurrent duct system of testis at different levels in adult male albino rats and to study the long term effects of vasectomy which has been studied only by few people, and to answer some of the usual questions posed by someone before undergoing vasectomy. In the present experimental series effects were studied from 30 days onwards, as the main aim was to see the long term effects in adult male albino rats. The animals selected were albino rats because of their continuous spermatogenesis and ease of availability and can withstand the chloroform anaesthesia and operative procedure. The observations revealed that the vasectomy does not cause any change in the testis and there should not be any change in the sexual potency, since there are no atropic changes either in the seminiferous tubules or in the interstitial tissue. Since the spermatogenesis is not affected if the anatomical re-anastomosis of the vas is done, when needed, fertility can be restored and the operation is reversible and also concluded that ligation of vasa efferentia caused more damage in the testis. With this observation the study suggests that the ligation of excurrent duct system of testis at a lower level i.e. vasectomy does not cause any adverse changes in the testis but ligation at a higher level i.e. vas efferentia results in back pressure and causes progressive degenerative changes in the testis.

1. Introduction

Vasectomy or ligation of ductus deferens is performed in India, mainly as permanent family planning measure to prevent further conceptions. Vasectomy is also proposed for alleviating senile prostatic hypertrophy, and has been used in connection with prostatectomy to prevent retrograde infection going to epididymis. It is also indicated for disputed rejuventive effects in males.

In all five year plans specially starting from the fourth five year plan much importance was given for family planning programme and incentives were provided to men who undergo vasectomy operations are done on mass scale.

* Corresponding Author : Dr. Pradeep Kumar murudkar
Department of Anatomy,
Khaja BandaNawaz Institute of Medical Sciences,
Gulbarga, Karnataka.
Email id: pradeepkumar.murudkar@gmail.com

Every man who undergoes operations will have a number of doubts in his mind and often, asks the doctor about the consequences of the operation. The questions usually posed were

1. What happens to the sperms produced if they are prevented from ejaculation?

2. What happens to sexual potency after operation?

3. Unfortunately if the children die is the operation reversible?

Elaborate experimental work was done to study the effects of ligation of excurrent duct system of testis, by ligating at different levels. Experiments were performed in different species, such as rabbits, mice guinea pigs and dogs. And also testicular and epididymal biopsies were studied after vasectomy in men. The literature on the effects on vasectomy is not unanimous. In the

recanalisation operation also, even though the anatomical reanastomosis of the vas deferns is possible, it is not possible to restore normal fertility in all the cases. In only 5 to 10 % of cases it proved to be successful. The present work is done to reassess the effects of ligation of excurrent duct system of testis at different levels to study the long term effects of vasectomy which has been studied only by few people, and to answer some of the usual questions posed by some men before undergoing vasectomy. In the present experimental series, the effects were studied 30 days onwards, as the aim was to see the long term effects.

The animals selected for the present study were albino rats, because the spermatogenesis in them is a continuous process and they are easily available and they also with stand the chloroform anaesthesia and operative procedure well.

2. Materials and method

The present study was based on the experiments performed on 35 mature male albino rats weighing 150-200 gms. Two experiments were performed by ligating the duct system of the testis at various levels as follows

A. Ligation of vas efferentia in 10 rats

B. Ligation of vas deferens nearer the cauda epididymis in 25 rats.

Ligation was done on the right side so that left testis and epididymis could serve as control, the rat undergoing operation was weighed and weight recorded. The operations were performed under sterile conditions using chloroform anaesthesia. The rat was anaesthetized by keeping it under glass bell jar putting a small piece of cotton soaked in chloroform. Within few minutes the rat becomes unconscious.

The anterior abdominal wall was shaved and cleaned with spirit. The operation was performed by taking a median suprapubic incision 1.25 long and muscular and peritoneal layers were cut. The right testis was gently squeezed out of scrotum and delivered through the abdominal wound and ligatures were put as per type of operation.

Experiment A-Ligature was put including all the ductuli efferents.

Experiment B-Ligatures were applied at a distance of 1/2'" on the vas deferens closer to the cauda epididymis and a piece of vas about 1/4" was cut between the sutures.

After ligation the testis was replaced in to scrotum, care was taken not to allow the testis to remain in the abdominal cavity. Then peritoneal and muscular layers of the anterior abdominal wall were sutured, using '00' chromic cat gut. Skin was closed by interrupted sutures using black cotton thread.

Animal recovered from anaesthesia within few minutes after closing the abdomen .Then it was transferred to its cage where sterile cotton was spread. After one hour, water was given to animal; it started taking its regular feed after 3 hours.

Skin sutures were removed after 8 days, in the animals which were observed for more 9 days, wound infection was seen in only 3-4 rats.

In experiments number 'A' animals were sacrificed at the interval of 3 days up to 30 days, considering the day of operation as '0'. In the experiment number 'B' effects were studied at the interval of 10 days from 30-120 days, the animals weight was recorded before the sacrifice. The gonads and the epididymis were separated trimmed free of adipose tissue and connective tissue and the length and volume of testis were recorded. The volume and weight of the epididymis were also recorded. The testis and epididymis were fixed in 10% formal saline.

For histological investigation, the tissues were processed and blocks were prepared using paraffin wax. The tissues were cut at 6-7 microns thickness and stained with Haematoxylin and eosin. The sections of testis and epididymis were examined,

1. To see the diameter of seminiferous tubules:

2. To the study the changes if any in germinal epithelium, interstitial tissue in the testis and

3. To see the tubular diameter and content of the epididymal tubules.

4. To study the lining epithelium and intertubular tissues in the epididymis

The sections of cysts were also taken and stained to study the histological appearance. The above findings were compared with sections of the control testis and epididymis.

3. Results

In the present study 35 mature male albino rats were subjected to various levels of ligation and following observations were recorded at the intervals of 10 days from 30-120 days after vasectomy were represented in Table no.1 and observations after ligation of vas efferentia were represented in Table no.2.

Statistical application

The present work could be worked out statistically to prove any hypothesis related to two experiments conducted. For this purpose the t test for independent samples can be applied and could be correlated to the effects observed. The observations of the different experiments could be worked out stastically, to prove whether there is any significance or not.

The hypothesis is:

1. Ligation of vasa efferentia will affect the testis during the early post operative period.

2. Vasectomy affects the epididymis.

These hypotheses could be negative or positive. In order to prove these hypotheses each experiment can be worked out statically by applying t test for independent samples.

The formulas to be used are:

1. $\delta\ X_1\text{-}X_2 = \sqrt{\frac{X_1^2 + X_2^2}{n_1 + n_2}\ (1/n\ 1+1/n2)}$

Where

δ X1-X2 = the standard error of the difference between two means.

n1 = the number of cases in group 1

n2 = the number of cases in group 2

$\sum X1^2$ = the sum of squared deviation scores in group 1.

$\sum X2^2$ = the sum of squared deviation scores in group 2

And

2 $\delta\ X_1\text{-}X_2$

$\delta\ X_1\text{-}X_2$

Where,

$\delta X_1\text{-}X_2$ = the observed difference between two means

$\delta\ X_1\text{-}X_2$ = the standard error of the difference between two means.

Or,

The t-ratio formula in more comp form by including the above two formulas:

$$3.\ t = \frac{X_1 - X_2}{\sqrt{\frac{(\sum X1^2 + \sum X2^2)}{n1 + n_2 - 2}\ (1/n_1 + 1/n_2)}}$$

The observed ratio should be compared with the expected and conclusion of significance can be drawn.

Expected ratio can be had from the t-table, by reading the columns of different levels, i.e., 0.1, 0.05, 0.01 and 0.001, against the row of degrees of freedom.

The degrees of freedom can be calculated as follows.

$df = n_1 + n_2 - 2$

Where,

df = degrees of freedom

n_1 = number of cases in group 1

n_2 = number of cases in group 2

Fig:(1) 30 days after vasectomy along with control showing No change in size

Fig:(2) 30 days afer vasectomy

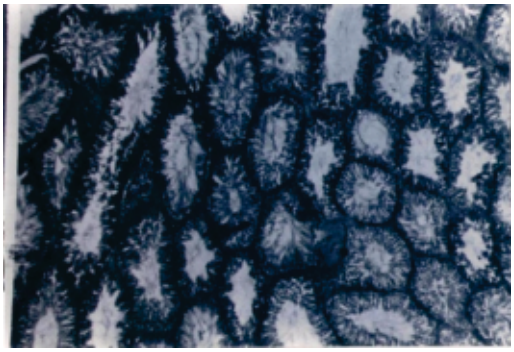
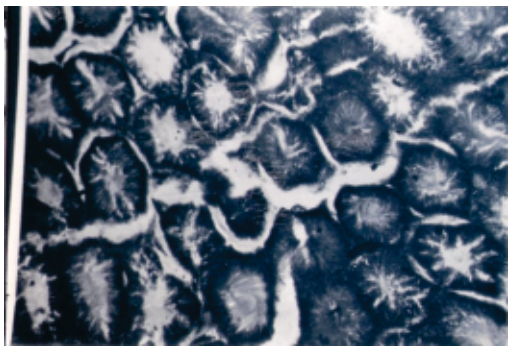
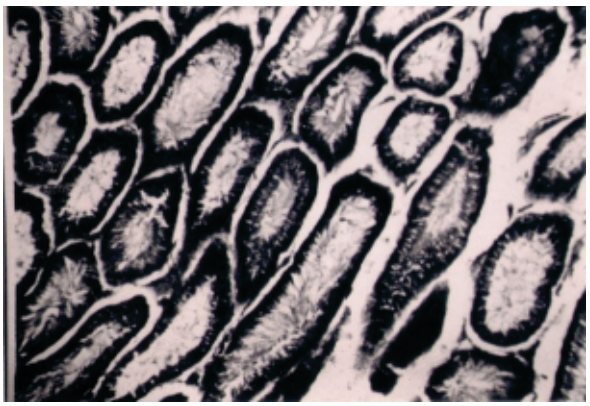
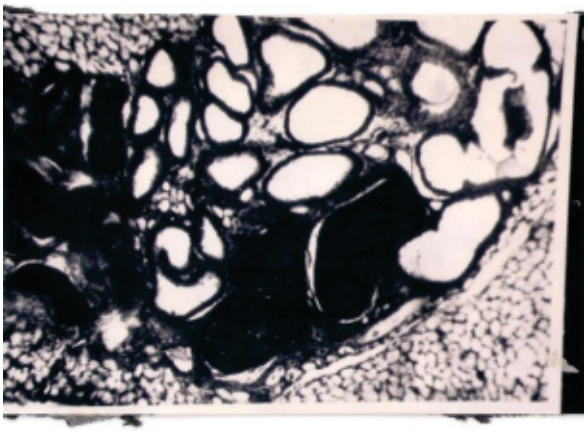


Fig:(3) 120 days after vasectomy showing hypermia and hypertrophy of interstitial cells



Fig(4) 3 days after ligation of vas efferentia showing distended seminiferous tubules





Fig(5) 30 days after ligation of vas efferentia showing distended seminiferous tubules

Table 1.showing the observations at the intervals of 10 days after vasectomy

Days	Macroscopic changes			Microscopic changes				
	Testis	**Cauda epididymis**	**Cyst**	**Testis**	**Epididymal tubules**	**Lining epithelium**	**Interstitial tissues**	**Cyst**
30	No changes Fig:(1)	Dilated	Small cyst proximal to site of ligation	No histological changes Fig:(2)	Dilated &studded with sperms	Flattened	-	Had a fibrous capsule, studded with sperms and infiltration of lymphocytes
60	No changes	Dilated	Firm in consistency proximal to site of ligation	No histological changes	Dilated &studded with sperms	Flattened	Intertubular connective tissue was thickened	Had a fibrous capsule, studded with sperms and infiltration of lymphocytes
70	No changes	Dilated	Cyst formation of vas was seen	No histological changes	Dilated &studded with sperms	Flattened	-	Had a fibrous capsule, studded with sperms.
80	No changes	Dilated	Cyst formation of vas was seen	No histological changes	Dilated &studded with sperms	Flattened	Hypertrophic and hypermic	Lumen of cyst was filled with degenerated sperms with infiltration of lymphocytes
90	No changes	Dilated	Cyst formation of vas was seen proximal to ligation	No histological changes	Dilated &studded with sperms	Flattened	Hypertrophic and hypermic	Lumen of cyst was filled with degenerated sperms with infiltration of lymphocytes
100	No changes	Dilated	Irregular hard cyst was seen on vas proximal to ligation	No histological changes	Dilated &studded with sperms	-	Hypermia of interstitial tissue	Cyst was studded with sperms and infiltrated by lymphocytes
110	No changes	Dilated	Cyst formation of vas was seen proximal to ligation	No histological changes	Dilated &studded with sperms	Flattened	Hypertrophic and hypermic	Cyst showed increased phagocytic activity by lymphoid tissue. Loculation and cavity formation was seen.
120	No changes	Dilated	Cyst formation of vas was seen.	No histological changes	Dilated &studded with sperms	Flattened	Hypertrophic and hypermic Fig:(3)	Cyst was studded with degenerated sperms and infiltrated by lymphocytes

Table 2.showing the observations after the ligation of vasa efferentia

Days	Macroscopic changes			Microscopic changes		
	Size of testis	**Seminiferous tubules**	**Reduction in vol(Table No-5)**	**Seminiferous tubules Central & peripheral**	**Interstitial tissue**	**Epididymis**
3	Decrease	Dilated	12%	Increase in diameter of the tubule, central tubules appearing normal, degeneration of tubules peripherally, the changes include the shedding of an epithelium in lumen. Fig:(4)	Homogenous	Lining epithelium appeared thickened, there was perivascular infiltration of lymphocytes
6	Reduced and congested	-	33%	Degeneration in central &peripheral(more).germinal epithelium was disturbed and shed in lumen of many tubules	Blood vessels were enlarged and showed perivascular infiltration of lymphocytes.	Tubules were empty
9	Reduced, less turgid, in consistency	Dilated and tortuous	51%	Lining epithelium almost restricted to a single layer and many appeared to be collapsed and empty in varying shapes and sizes	Hypertrophied	Lining cells of epididymis were hypertrophied, there was perivascular infiltration of lymphocytes
12	Reduced, less turgid, in consistency	-	55%	Peripheral region showed severe type of degeneration, seminiferous tubular outline could not be made out. Many giant cells could be seen. Thickening of tunica albugenia with infiltration of lymphocytes	Hypertrophy of Intertubular tissue	Reduction in tubular diameter, adjacent tubules were coming together and anastomosing.
15	Reduction in size	-	14%	Lining epithelium was restricted to a single layer and contained a homogenous material.	Hypertrophic and hypermic	Degenerative changes in tubules
18	Reduction in size	-	45%	Lining epithelium was restricted to a single layer and contained a homogenous material	Hypertrophic and hypermic	Degenerative changes in tubules
21	Reduction in size	-	30%	Testis showed thickening of tunica albugenia with marked infiltration of lymphocytes in periphery. Lumina of tubules were filled eosinophilic material and appeared to be homogenous	-	Epididymis showed Intertubular fibrosis and degenerative changes.
24	Reduction in size	-	40%	Testis showed thickening of tunica albugenia with marked infiltration of lymphocytes in periphery. Lumina of tubules were filled with eosinophilic material and appeared to be amorphous	Homogenous	Epididymis showed Intertubular fibrosis and degenerative changes.
27	Reduction in size	-	60%	Testis showed marked and complete degeneration of seminiferous tubules with homogenous pink mass inside them.	Intertubular tissue was hypertrophic	Epididymis showed degeneration of epididymal tubules. The cilia of cells were lost
30	Reduction in size	-	60%	Lining epithelium was reduced to a single layer and contained a pink homogenous mass. Fig:(5)	Hypermia	Epididymal tubules were distorted and Intertubular fibrosis was seen.

Table No-3. Showing the mean weight and volume of Testis following vasectomy

		Weight in gms			Volume in ml		
P.o period In days	No of Rats	control L	operate D	Differ.	control L	operate D	Differ.
30	1,16&17	1.180	1.200	0.020	1.10	1.25	0.15
40	2,3&18	1.395	1.435	0.040	1.40	1.40	--
50	4,5&19	1.345	1.275	0.070	1.20	1.20	--
60	11,12&20	1.135	1.226	0.091	1.10	1.10	--
70	14&15	1.195	1.106	0.089	1.10	1.10	
80	6&10	1.300	1.240	0.060	1.20	1.20	--
90	21&22	1.215	1.165	0.050	1.30	1.30	--
100	7,13&23	1.225	1.175	0.050	1.10	1.10	--
110	8&24	1.070	1.045	0.025	1.00	1.00	--
120	9&25	1.370	1.406	0.036	1.20	1.20	--

Table No-4 Showing percentage of increase or decrease of epididymis and proximal end of Vas Deferens on the operated side in volume in comparison with control, following vasectomy

No of days	Volume of Epididymis and Proximal end of Vas Deferens in ml		Percentage of increase in size
	Control	Operated	
30	0.50	0.6	20
40	0.50	0.7	40
50	0.50	0.7	40
60	0.40	0.6	50
70	0.40	0.7	60
80	0.40	0.6	50
90	0.45	0.9	110
100	0.45	1.1	150
110	0.40	0.7	75
120	0.50	0.7	40

Table No-5 Showing percentage of increase or decrease in the volume of Testis on the operated side in comparison to contralateral control following ligation of Vasa Efferentia

No of days	Volume of Testis in ml		Volume of the experimental Testis expressed as% of control	Percentage of increase or decrease in size
	Control	Operated		
3	1.5	1.32	88.00	-12.00
6	1.5	1.00	66.66	-33.33
9	1.6	0.78	49.00	-51.00
12	1.6	0.68	45.00	-55.00
15	1.6	0.96	60.00	-40.00
18	1.5	0.83	55.00	-45.00
21	1.5	0.90	70.00	-30.00
24	1.6	1.00	60.00	-40.00
27	1.5	0.60	40.00	-60.00
30	1.6	0.80	50.00	-50.00

4. Discussion

The purpose of the study is to determine that vasectomy does not cause any adverse changes in the testis but ligation at a higher level at vas efferentia results in back pressure and causes progressive degenerative changes in the testis. so in order to bring the awareness of vasectomy as a permanent family planning measure to prevent further conceptions and to study the changes effecting after vasectomy the study has been undertaken.

The degeneration of seminiferous tubules consequent upon the ligation of vas associated with decline in the spermatogenesis and hyperplasia of interstitial elements were observed by Bouin and Ancel ,Housseay, steinach,white [8,17,45,50]. In the present study the observations made on vasectomised rats for a period of 30/120 days, did not coincide with the opinion expressed by the above workers.

Cunning-ham [10] noticed the occurrence of degeneration of seminiferous tubules, following vasectomy and also observed regeneration after 6 weeks. In the present study the author did not notice any degenerative changes of seminiferous tubules and the question of regeneration does not arise.

Kar et al,Macmillion,Moore,Nelson,Poynter,Reddy and Wheelman[21,26,27,28,31,39,42,49], have observed the normal spermatogenesis after vasectomy. phadke [36,37] in his studies of testicular biopsies of vasectomised persons and in cases of obstructive azospermia could find normal spermatogenesis. The present study showed normal histological structure of testis which is in complete agreement made by these workers.

Rangam et al [40] noticed early degeneration of the seminiferous tubules at the end of first week and advanced degeneration at the end of 2nd week, after vasectomy in the 4 week and restoration to normality in 6th week. But in the present study there was no evidence of either degeneration or regeneration at the end of 5th week.

The authors observations were not in concurrence with observations of Rathore and Chaturvedi [41] as they observed that vasectomy results in progressive degeneration of seminiferous tubules and complete degeneration of tubules after 5 months in dogs. The dogs are seasonal breaders and there is no continous spermatogenesis in them where as rats show continuous spermatogenesis as in man.The author observations were not in agreement with those of Joshi et al [20], who in dogs have observed progressive arrest of spermatogenesis up to 8 weeks.The authors observations were in complete agreement with findings of Reddy and Smith [42,44], seen in rat testis after vasectomy. They have mentioned that vasectomy resulted in temporary increase in the weight of epididymis and development of spermatocoele on the cut testicular end of the vas.

In the present study the histological changes in the epididymis such as dilation of the tubules, flattening of lining epithelium with loss of cilia, clumps of spermatozoa in lumen, evidence of phagocytosis were similar to those observed by Reddy and Smith [42,44].

The observations of Amman Almquist and Amman [3,4] in dairy bulls similar to those observed by author in rats. In dairy bulls there was no development of cyst on vas, instead there was marked dilation of vas just proximal to the site of vasectomy.

Paufler and Foote[33,34] in their series of experiments studied the effect after ligating the vas at the junction of ducts deferens and cauda epididymis in rabbits. Their results were similar to those observed by author in the present study.

Authors observations of the effects of vasectomy in rats were not similar to those noticed by Swanson and Hafe[46] in rabbits. They have noticed increased cauda epididymal weight and reduction in the rate of speramatogenesis. In the authors experiments there was increase in the cauda epididymal weight but there was no interference with spermatogenesis. The cysts were developed on the vas which were not seen in rabbits.

Longterm effects of vasectomy, observed by Iqboeli and Rakha in dairy bulls and Kar et al [19,21] in rats, were similar to the observations made by author in rats.

Observations made by Alexander [1] in vasectomised monkeys for a period of 1-7 yrs as regards to the structure of epididymis were similar to those of the authors observations in vasectomised rats.those of the authors observations in vasectomised rats.The author noticed no significant change in size, length and weight of testis compared to the control side which was in agreement with Plaunt[38] who has studied morphological changes in the testis for a period of 28-58 days.

Electron microscopic study of testis,epididymis, ductli efferent's and vas deferens following vasectomy was conducted in rats, men and monkeys by Alexander,Flickinger and Kubota [2,11,12,23].There were structural changes in the testis excepting the presence of vacuoles and residual bodies in the sertoli cells and degenerative and fine alterations in the spermatids.

Flickinger [11] in his series of experiments has noticed development of spermatogranulomas in 20 out of 21 rats which reached their maximum size by about 40 days, the author in his experiments has noticed the development of the cyst,in all experimental animals,except in 2 rats and cyst reached its maximum size in 60 days specimen.Histologically the structure of the cyst was similar to the description given by Flickinger[11].

Reddy [42] in his series experiments had noticed development of spermatogranulomas in 23 out of 25 rats,near the site of ligation of vas. The author in his experimental animals also noticed granulomas except in two animals. Histologically structure of the cyst was similar to the description given by Reddy [42].

Ligation of vas efferentia

With ligation of vas efferentia there was constant decrease in testicular weight, 9 days after the operation the testicular size was halved.

Effect of ligation of vas efferentia on the testis were studied by many investigators like Macmillan,Oslaund , Paufler and Foote,Reddy ,Van Wagenon and White [24,32,33,34,42,47,50].

The results of the author confirmed the findings of Harison ,Iqboeli&Foote ,Macmillan ,Oslaund ,Reddy,Van Wagan,White[16,18,24,32,42,47,50], and the observations noticed by author being progressive (1) Testicular atrophy (2) Degeneration of seminiferous tubules and its epithelium (3) Interstitial cells and hypermia of Interstitial tissue following ligation of vasa efferentia.

Smith observed initial increase in the diameter of the seminiferous tubules and also increase in the testicular weight with in 36 hrs after ligation of vasa efferentia, it was followed by progressive decrease in the diameter of seminiferous tubules and testicular weight and degeneration of seminiferous epithelium which was limited to a single layer by 28 days.The observations of the author seen after ligation of vasa efferentia confirmed the results of Smith[44].

The histological changes found after ligation of vasa efferentia observed by Barrak [7]were related to the present study.

The results of Paufler and Foote [33,34]who have studied the effects of ligation of vasa efferentia noticed moderate and transient disturbance in the spermatogenesis,were in partial agreement with the observation made by author.

The epididymis in present studies was smaller in size on the operated side. Histologically lumen was reduced in size,appeared empty, the cells of the lining epithelium were hypertrophied and vacuolated. The cell margin became indistinct,some of the tubules became thin walled.Inter tubular fibrosis was seen,with perivascular infiltration of lymphocytes.Author confirmed the findings of Reddy[42] as his finding were similar to authors findings.

5. conclusion

Two groups of rats were subjected to 2 experimental procedures by ligating the excurrent duct system of testis at different levels and effects studied both macroscopically and microscopically in testis and epididymis. The operations were performed unilaterally on the right side,the contralateral left testis and epididymis serving as control.

The following are the conclusions observed by the author 1.Vasectomy does not cause any change in the testis and there should not be any change in the sexual potency, since there are no atropic changes either in the seminiferous tubules or in the interstitial tissue. The spermatozoa produced by continuous spermatogenesis are accommodated in the cauda epididymis tubules and they are regenerated and phagocytised in the distended epididymal tubules. Because the sperms are accommodated in the cauda epididymis and cyst there is no resulting back pressure to cause degenerative changes in the testis. Since the spermatogenesis is not affected if the anatomical re-anatomises of vas is done, when needed, fertility can be restored and the operation is reversible.

2. Ligation of vasa efferentia caused progressive atrophy of the testis and degeneration of seminiferous tubules and seminiferous epithelium and relative prominence of interstitial tissue.

The final conclusion of the present study suggests that the ligation of excurrent duct system of testis at a lower level i.e Vasectomy does not cause any adverse changes in the testis ,but ligation at a higher level ,i.e vasa efferentia results in back pressure and progressive degenerative changes in the testis. .

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