ORIGINAL ARTICLE

The Effects of Vasectomy on Testicular Tissue of Mice: Histological Changes and DNA Fragmentation Study

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ABSTRACT

Introduction: We aim to investigate the effect of vasectomy on the histology of the testis as well as to evaluate DNA fragmentation in testicular tissue of male mice. **Methods**: Bilateral vasectomy was performed on 20 mature male mice; 10 control mice underwent sham-operation. After 6 weeks, the testes were evaluated for histological changes and DNA fragmentation by single cell gel electrophoresis (comet assay). **Results:** Marked alterations were observed in the testes of vasectomized mice, including degeneration of spermatids, thickened basement membrane, dilatation of the seminiferous tubules, exfoliation of germ cells, reduction in the seminiferous cell population, vacuolated appearance of the epithelium in the tubules and marked interstitial fibrosis. Single cell gel electrophoresis showed a highly significant (P<0.0001) increase in DNA damage among vasectomized mice (46.02%) compared with control group (%27.17) after six weeks of operation. **Conclusion**: Vasectomy induced deterioration in the seminiferous tubules associated with increased testicular cell's DNA fragmentation.

KEYWORDS: Vasectomy, comet assay, DNA degeneration, vas deferens.

INTRODUCTION

Vasectomy is an established method of male sterilization, and permanent birth control in which the vas deferens is surgically clamped, cut, or otherwise sealed and thus prevents the release of sperm when a man ejaculates. It is one of the most common methods of male contraception used worldwide, with the number of sterilized men estimated to be between 40 and 60 million, and is thought to account for between 5% and 10% of all contraceptive methods used.[1,2] Family planning, male vasectomy, contraception, female sterilization and abortion have different issues, religious and ethical challenges in different countries and religions.[3,4]

Vasectomy is a highly effective contraceptive method that can play an important role in reducing a country's population growth. According to United Nations estimates, in 2005, 37 million of reproductive ages were using vasectomy as their method of contraception, accounting for 5.6%, of all contraceptive use.[5] Over 40,000 men have a vasectomy in UK every year.[6] Since 1974, over 18 million Chinese men have undergone vasectomy by the no-scalpel technique.[7] Today, India is one of the leading nations across the world with regards to the use of no-scalpel vasectomy;[8] almost 7% of all Indian couples were sterilized with a total of 6.2 million vasectomies were done in 1976.[9] Between 1993 and 2004 an estimated 375,000 Iranian underwent the no-scalpel procedure, raising the prevalence of vasectomy from 0% to 3.5% in the national contraceptive mix.[10]

It is well known that genetic material for producing protein is located in the deoxyribonucleic acid (DNA). DNA damage may be induced by variety of exogenous and endogenous agents. Single-strand breaks are often a result of attack by reactive oxygen species, and double strand breaks, which are dangerously cytotoxic but occur much less often, can be generated by ionizing radiation[11] and by the antitumor agent, bleomycin.[12] Oxidative DNA damage is caused by reactive oxygen species, "ROS". ROS can induce strand breaks, modifications of bases and basic sites in DNA.[13]

Attention has been paid to study the effects of vasectomy on the spermatogenesis; there are different opinions among authors to the effect of vasectomy on the testis. Significant reductions were observed in early and mature spermatid numbers and an increase in the thickness of the seminiferous tubule wall associated with reduced fertility in vasectomized men,6 with inadequate concentration and motility in human ejaculated sperm.[14] Several experimental studies in the monkeys, guinea pigs,

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and rats have shown that vasectomy resulted in severe morphological changes in the testis, spermatogenesis deterioration, testicularb degeneration, testicular lesions, absence of spermatids, depleted germ cells and thickening of the basement membrane.[15-1] Furthermore, there were observed exfoliation of germ cells and vacuolated appearance of the tubular epithelium in mice;[19] and vacuolization and marked reduction of the germinal epithelium, thickening of the tunica albuginea and widening of the interstitial space of the Mongolian gerbil.[20] However, other authors did not find alterations in the testes of vasectomized rabbits;[21,22] rhesus monkeys[23] and rats.[24,25]

Increased DNA fragmentation was observed in semen samples of men after vasectomy.[26] Kubota et al,[27] observed damaged seminiferous tubules, disorder of cellular arrangement, depletion of the germ cells, and local interstitial fibrosis of vasectomized rats. These changes were associated with apoptotic germ cells exhibiting cellular DNA fragmentation. O'Neill et al,[28] observed reduced sperm yields after vasectomy in men associated with increased apoptosis and increased DNA fragmentation.

The single-cell gel electrophoresis assay (comet assay) was developed as a method to examine DNA integrity and measuring DNA breakage in individual mammalian cells.[29,30] Heaton et al,[31] applied comet assay on canine and feline leucocytes and concluded that this method provides a way of assessing levels of DNA damage. They further suggested that it was a useful tool for determining the optimal effects of dietary antioxidants on a reliable biomarkers of oxidative stress such as cellular DNA status in cats and dogs. Simon et al.[32] applied comet assay to evaluate DNA fragmentation and semen parameters and found that men with sperm DNA fragmentation at more than a diagnostic threshold of 25% had a high risk of infertility.

The objectives of this study were to investigate the effects of vasectomy on histological changes that possibly occur in the mice testes and to evaluate DNA damage in the testes using a single cell gel electrophoresis assay.

MATERIALS AND METHODS

Animals and procedure

Thirty healthy mature Swiss albino strain male mice (age: 8-12 weeks) were enrolled into this study; they were kept under suitable environmental conditions such as a room temperature that was maintained at about 24±2oC and exposed to 12 hour/day light program. Standard chew of pellets and water was provided ad libitum.

The mice were divided into two groups; Control group of 10 mice with sham operation, and 20 mice for the vasectomy group (case). Histological and biochemical evaluations of the testes were demonstrated in both groups.

The vasectomy procedure was performed according to Foley.[33] After six weeks, experimental and control animals were anaesthetized by single intraperitoneal dose of 0.2 ml of ketamine (hydrochloride) 50 mg/ml concentration and 0.05 ml Xylazine (Rompun 2%) 23.32 mg/ml concentration. The abdomens of each group were immediately opened and both testes were removed. Each testiswas bisected into two halves; one half used for gel electrophoresis (comet assay) and the other half processed for morphological study. The experimental animal protocol was conducted in compliance with humane animal care standards outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental study was approved by Al-Nahrain University with ethic number of 000 (M) 00.

Cell isolation

Tissue used for gel electrophoresis was minced (after removing the testicle's capsule) into fine pieces in 1 ml cold HBSS (Hank's Balanced Salt Solution) containing 20 mM Ethylenediaminetetraacetic acid (EDTA)/10% Dimethyl Sulfoxide (DMSO). The settled tissues were removed (5-10 µl) and mixed with 75 µl LMPA (Low Melting Point Agarose), and centrifuged at 4000 rpm for 30 minutes 2-3 times using cooled centrifuge. For visualization of DNA damage, observations were made of Ethidium bromide (EtBr)-stained DNA using a X40 magnification on a fluorescent microscope.[29]

Histological study

Tissue was fixed in Bouin's solution for 12-16 hours. After fixation, they were washed using many changes of 50%, ethanol to remove the yellow colour of picric acid, dehydrated through graded alcohols and cleared using two changes of xylene and embedded in paraffin wax. A serial sections of 4-5µm thickness was stained by Harris's hematoxylin and eosin.

Statistical Analysis

Statistical analysis was performed using SPSS software, version 18 (Chicago, Illinois). Results were analyzed using Student's t-test and paired sample t-test to compare the differences between the means of tests and control studies. The results were expressed as mean ± standard deviation(SD).

RESULTS

Histological observation

The seminiferous tubules of the control group show normal components of spermatogenesis lineage cells and supporting Sertoli cells and normal interstitial tissue (Figure 1)

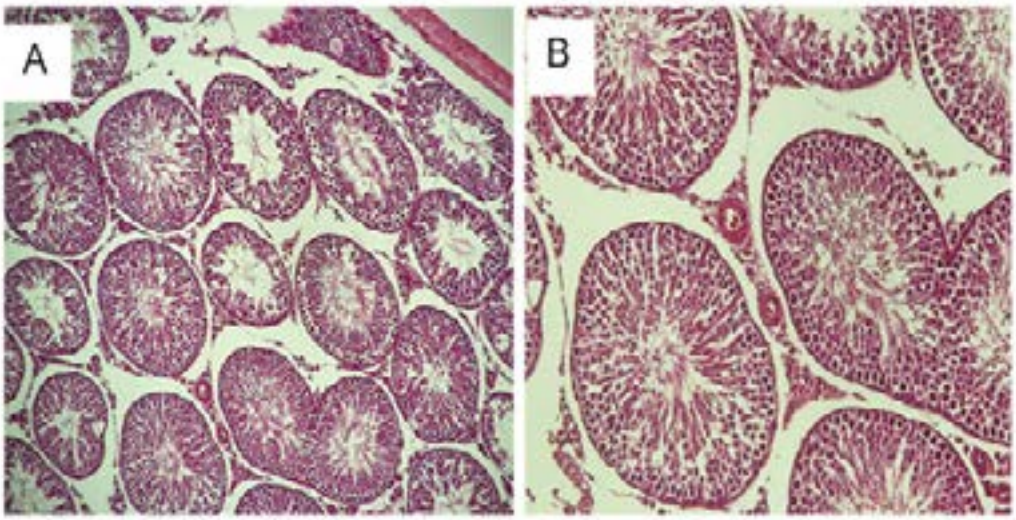

Figure 1. Histological sections of normal mouse testes showing normal seminiferous tubules with spermatogenic cells, Sertoli cell and interstitial cells. (H &E stain, original magnification "A, x 10; B, x20").

Vasectomized mice showed degenerative changes within the seminiferous tubules; these changes include sloughing of immature germ cells in the tubules, thickening of the basement membrane and vacuolations of the tubules. Some seminiferous tubules had large numbers of spermatids and sloughed spermatogenic cells in their lumen (Figure 2-A). The interstitial tissue of few sections had highly congested blood vessels and large necrotic areas covering some of the tubules together with intertubular eosinophilic material (edema) with scant mononuclear cell's infiltration (Figure 2-B). Disarrangement of some tubules associated with lacking of some stages of germ cells was observed (Figure 2-A &B). Some tubules were almost completely destroyed (with vacuolations in the spermatogenic cells) leaving large area of interstitial space associated with edema (Figure 2-C). Some testes showed increased amounts of interstitial fibrosis with thickening of the basement membrane and vacuolations of the tubules (Figure 2-D).

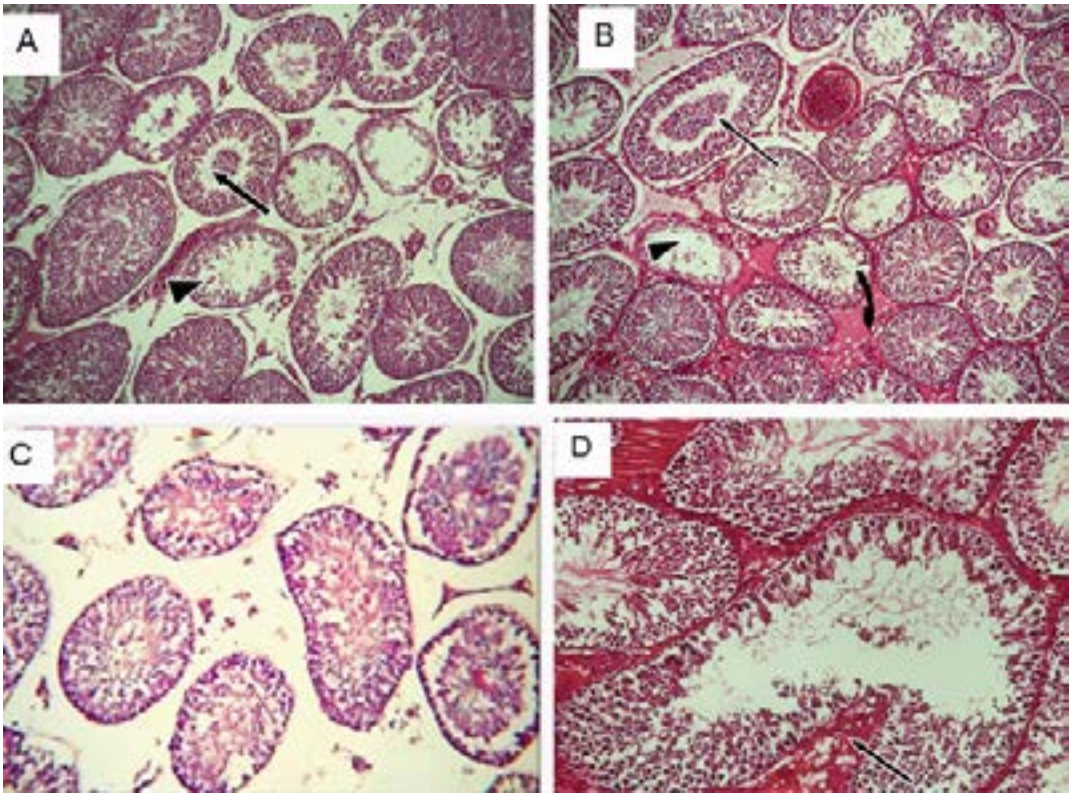

Figure 2. Sample photographs from vasectomized mice testis showing vacuolations of the tubules "head arrow", tubules with sloughed spermatogenic cells and spermatids "arrow" (A &B), congested blood vessels and large necrotic areas with intertubular eosinophilic material "curved arrow" and scant mononuclear cells infiltration (B), necrotic tubules lacking of some stages of germ cells with vacuolations in the spermatogenic cells (C), interstitial fibrosis "arrow" and thickened basement membranes of seminiferous tubules (D).("H & E stain" original magnification "A, B, C x 10; D, x20").

Single cell gel electrophoresis (comet assay)

After six weeks of vasectomy, there was an increase in the percentage of DNA damage in the testicular cells of the vasectomized group compared with the control group. In the present work, 500 cells were counted from the control group, the percentage of cells that showed DNA damage was 27.17%, while in 1000 cells that obtained from the vasectomized group was 46.02% (Table 1).

Table 1. The means of cells with DNA damage in control and vasectomized groups.

Group	No. of counting cells	Percentage of cells with DNA damage	SD	t-test	P value
Control	500	27.17%	1.515	13.518	0.0001*
Vasectomy	1000	46.02%	2.268		

*P value reveals highly significant difference of vasectomized groups to their control.

Vasectomized mice showed a significant increase in the mean (±SD) comet tail moment compared to their control mice (Table 2). Statistical analysis using independent t-test showed highly significant elevation in the mean comet tail length level in the total cells of the vasectomized group compared to their control group (Table 2).

Table 2. The parameters (tail length, tail moment and percentage of DNA in tail) of comet assay in the vasectomized and control group.

Parameter / Groups	Tail Length (px) X ± SD	Tail Moment X ± SD	%DNA in Tail X ± SD
Control	5.71±1.36	0.566±0.36	5.295±2.805
Vasectomy	33.371±25.541	8.2±2.183	16.394±6.372
Independent t test	13.528	7.471	14.182
P value	0.0001*	0.0001*	0.0001*

*P value reveals highly significant difference of vasectomized groups to their control

To support these results, other parameters were used from the comet software (TriTek Comet ScoreTM). Table 3 shows the mean ±SD of these parameters that include comet length, comet area, comet mean intensity; tail area and tail mean intensity of both two groups. Statistical analysis showed a significant increase in the vasectomized group to all of these parameters of the control group. One parameter that was used to evaluate the intact DNA in the cells obtained from the testes of the control and vasectomized groups, incorporated in the head of comet was the DNA in head %.

Table 3. The parameters of comet assay in the vasectomized and control groups.

Parameter / Groups	Comet Length (px) X ± SD	Comet Area (px) X ± SD	Comet Mean Intensity X ± SD	Tail Area (px) X ± SD	Tail Mean Intensity X ± SD
Control	41.158± 13.202	2144.05± 161.719	24.717± 11.387	83.131± 22.872	253.241± 105.334
vasectomy	81.872± 36.937	4488.358± 1126.580	30.201± 10.058	730.71± 234.543	1326.495± 342.506
Independent t test	14.754	7.652	6.119	5.795	4.694
P value	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*

*P value reveals highly significant difference of vasectomized groups to their control.

Table 4 shows the mean ±SD of this parameter, and the statistical analysis showed a highly significant decrease ($p<0.0001$) of DNA in the head of comet in the vasectomized group as compared with the control group.

Table 4. Percentage of DNA in head of comet assay in the vasectomized and control groups.

Parameter / Groups	%DNA in Head X´ ± SD
Control	94.71±8.805
Vasectomy	83.61±16.372
Independent t test	14.182
P value	0.0001*

*P value reveals highly significant difference of vasectomized groups to their control.

DISCUSSION

The present study has demonstrated an elevation in the percentages of DNA fragmentation associated with abnormal morphology of testicular tissue in vasectomized mice; after six weeks of surgery, there was a significant increase ($P< 0.0001$) in DNA fragmentation of vasectomized group (46.02%) compared with the control group (27.17%).

It is well known that the testis is characterized by very high rates of cell proliferation. Since developing testicular sperm need supportive Sertoli cells, an overabundance of cell proliferation requires elimination of excess germ cells by apoptosis. One characteristic of apoptosis in eukaryotic cells is endonuclease cutting of DNA into discrete sizes.[34,35] This may explain the presence of low level (27.17%) of DNA fragmentation among control mice of the present study. The presence of high rate of DNA fragmentation may be related to oxidative stress that is provoked during vasectomy in the case group; this is in accordance with Yamanaka et al,[36] who related the testicular tissue damage during vasectomy to reactive oxygen species (ROS) exposure. Oxidative stress may significantly impair spermatogenesis and sperm function, which may lead to male infertility.[37,38] The role of ROS and oxidative stress in the sperm yield of post-vasectomy patients has been shown to be an important cause of male infertility.[14,39] Seminal oxidative stress is associated with vasectomy reversal, and the ROS-TAC "total antioxidant capacity" score is a possible predictor of infertility after vasectomy reversal.[40]

Jarow et al,[41] observed focal interstitial fibrosis in men underwent vasectomy; they observed a significant ($P<0.01$) correlation between interstitial fibrosis and successful vasectomy reversal. Shiraishi et al,[42,43] detected significant increase in interstitial fibrosis in testicular biopsy; the severity was associated with the period of the obstructive intervals. The present study has demonstrated certain histological changes after vasectomy represented by lacking some stages of germ cells, increase in the sloughing cells, degeneration of spermatids, thickened basement membranes; damaged Sertoli cells, dilatation and necrosis of the seminiferous tubules. Ligation of the vas deferens should increase the pressure within the distal structures and the structure of the tunica albuginea may not allow for expansion to decrease the pressure. The increased pressure may therefore decrease the blood flow to the ipsilateral testis through increasing the intratesticular pressure.[44] The present histological features may be related to an increase in oxygen free radical, which damage the testicular cells and their DNA, and/or to mechanical effects on the lining of the seminiferous tubule due to increase in hydrostatic pressure. Electron microscopic investigation is in progress to study in detail the ultrastructural changes that occurred in the vasectomized testes.

CONCLUSION

It is concluded that vasectomy induces histological alteration associated with an increase of DNA damage in the testes and related that to stress.

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