Fate of Spermatozoa Following Vasectomy

KEYWORDS

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ABSTRACT   *Vasectomy with its reversibility problem has been worked upon at almost all possible levels; the consequences of it have revolved around the fate of spermatozoa produced after vasectomy. The study concentrated on the fate of spermatozoa disposal following vasectomy as its path is obstructed not production. Observations revealed that the mode of sperm disposal is intraluminal endocytosis by macrophages and in the surrounding connective tissue of tubules in the vicinity of blood capillaries.*

Introduction

Vasectomy despite being a highly successful method of male contraception, still lies behind for the couples wanting to keep the reversibility option open. Since from long time this problem has been worked upon at almost all possible levels, the consequences of vasectomy have revolved around the fate of spermatozoa produced after vasectomy.

Objective

The aim of the study was to investigate the mechanism of sperm disposal following vasectomy which may lead to physiological and immunological problems.

Animal Model

Adult male langurs (Presbytis entellus entellus Dufresne) were used in the study.

Experimental Design
Surgery

Intact Control: Unoperated three animals served as control.

Sham Operations: Six animals were sham operated. It involved immolilization of vas without cutting and ligating the vas.

Vasectomy: Bilateral vasectomy was performed in 6 six adult langurs under sterile conditions. Animals were anaesthetized with intravenous injection of intraval sodium (20 mg/kg/b.wt) through saphenous vein puncture with the help of hypodermic syringe. Vas deferens of both side were exposed and a piece of 0.5 – 1 cm was removed from each side of vas and both the cut ends were ligated with black non absorbable sterilized 4.0 silk sutures . The vas was allowed to retract at its original position and the wound was closed in layers using 4.0 chromic cat gut.

Histopathology

Epididymis and vas deferens were fixed from 3 intact, 3 sham operated animals & 3 vasectomized animals following 6 months and 24 months of vasectomy in Bouin's fixative and Karnorsky's fixative for light and electron microscopy respectively. Three animals were used as intact control.

Light Microscopy

For light microscopic studies epididymis and vas deferens fixed in Bouin's fixative for 24 hours were dehydrated in graded alcohol and embedded in paraffin wax. These paraffin blocks were cut at 5µ thickness and stained with haemtoxylin and eosin **(Mcmanus and Moury, 1960).** Observations were made under light microscope.

Electron Microscopy

For electron microscoy tissues fixed in Karnovsky's fixative were post fixed in 1% osmium tetraoxide. After osmification these tissues were trasfered to series of graded alcohol and acetone. Tissue samples were infilterated and embedded in epon and tissue blocks were prepared. Semithin sections were taken on the LKB III ultramicrotome at 0.5µ thickness and stained with 1% toludine blue. Ultrathin sections of sliver color (60-75mm thick) were cut and lifted on to copper grids, stained with saturated uranyl acetate and lead citrate, dried and examined with Philips CM10 electron microscope using 60KV accelerating voltage.

Results
Sham Operated Animals

Sham operated animals resembled exactly the unoperated control in the light microscopic features of epididymis and vas deferens.

Vasectomized animals

Light microscopic studies in vasectomized animals revealed no alteration in cell morphology.

Lumen throughout the duct was found to be occupied by sperm laden macrophages and closely packed sperms in the fluid following vasectomy. Lymphocytes were also found in the surrounding connective tissue.

Ultra structural studies revealed a common increase in the number of multivesicular bodies following both durations of vasectomy in the cytoplasm of the Principal cells. Numerous lysosome like structure were observed in the principal cell cytoplasm. Macrophages with sperm remnants were seen in the lumen. Macrophages often develop narrow finger like pseudo pods and contain numerous sperm remnants in them, probably to engulf the sperms.

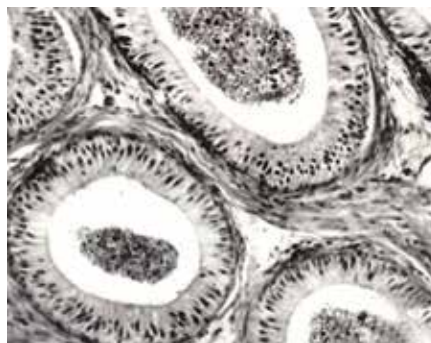Principal mechanism for sperm disposal thus appears to be intraluminal endocytosis by macrophages.

Figure 1

Fig. 1 Photomicrograph illustrating cytology of zone IV of epididymis. Note pseudostratified epithelium with short, bended stereocilia. Lumen diameter is appreciably greater than in zone III with decrease in the epithelial cell height. Spermatozoa with few macrophages in the lumen may be seen. HE x 100.
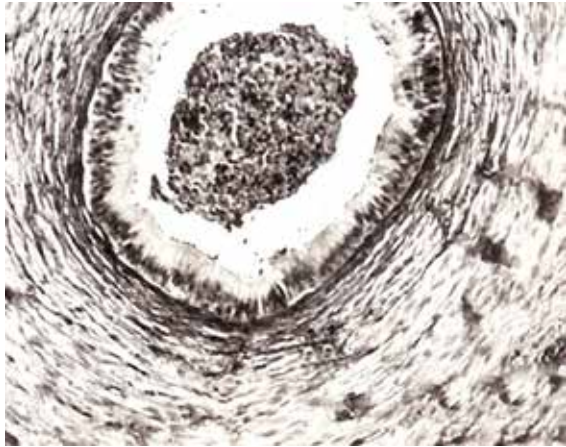

Figure 2

Fig.2 Photomicrograph of the proximal vas deferens. Note almost circular contour of epithelium and accumulation of spermatozoa in the lumen. HE x 100.
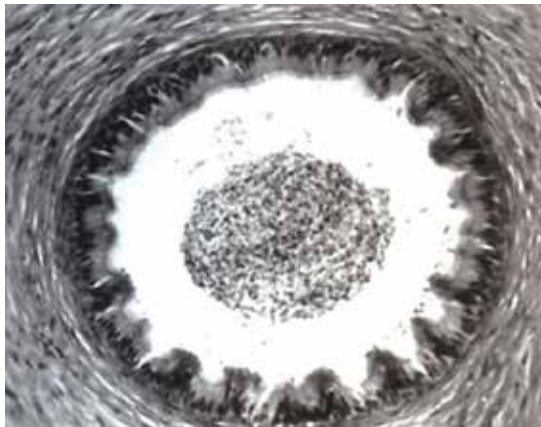

Figure 3

Fig. 3 Photomicrograph of the middle vas deferens. Festooned and wavy epithelium. Spermatozoa are seen in the lumen. HE x 100.
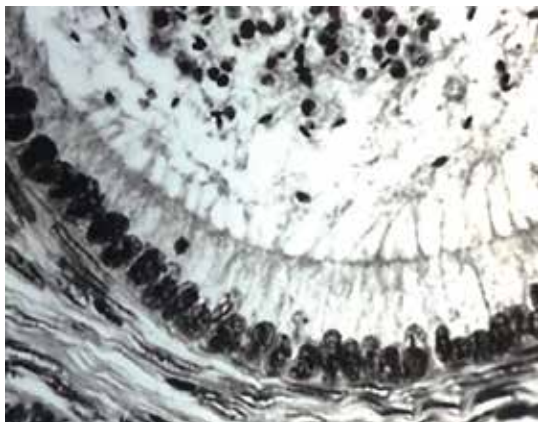

Figure 4

Fig. 4 Light micrograph of the zone I of epididymis of six months vasectomized monkeys showing normal but reduced epithelium. Numerous macrophages with fluid and few sperms may be noted in the lumen. HE x 400.
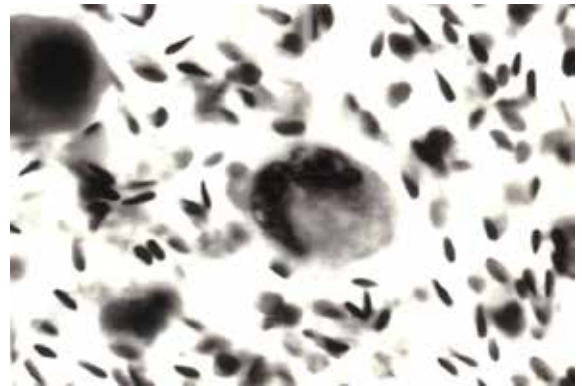

Figure 5

Fig. 5 Micrograph of the zone II of epididymis of two years vasectomized monkey showing numerous sperm parts and macrophages in the lumen. Macrophages containing sperm parts are visible. HE x 400.
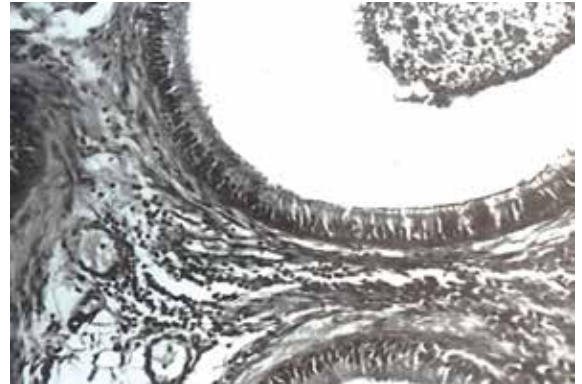

Figure 6

Fig. 6 Light micrograph of the zone VI of epididymis of six months vasectomized monkey showing numerous macrophages with reduced epithelium. Numerous macrophages in the connective tissue in the vicinity of capillaries. Macrophages and sperms are also present in the lumen. HE x 400.
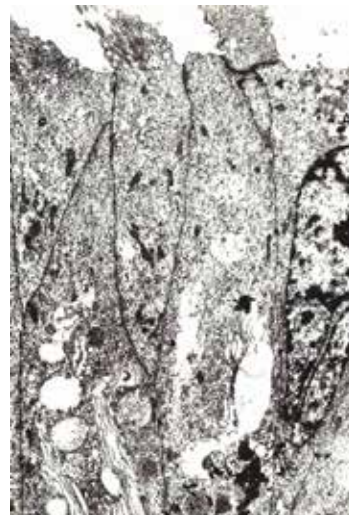

Figure 7

Fig.7 Electron micrograph of the Epididymal epithelium of epididymis showing few principal cells and an apical cells (AC). Various cellular organelles are visible in the cell cytoplasm. TEM × 10,450.
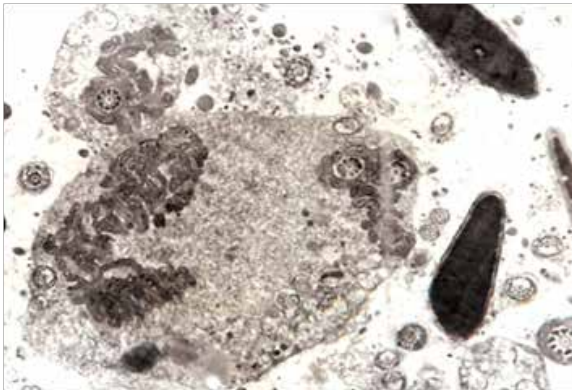

Figure 8

Fig.8 Electron micrograph of the apical portion of the caput epididymis of two years vasectomized monkey showing macrophages containing sperm remnants in the lumen. TEM x 15,200.
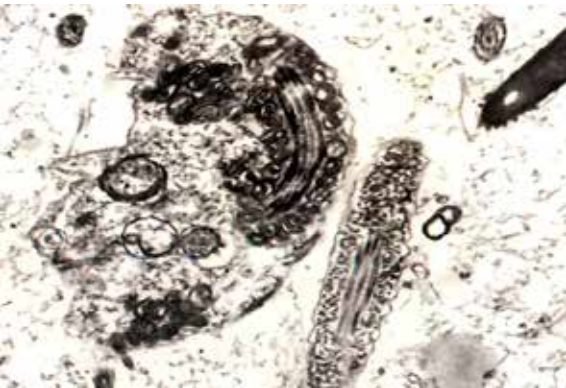

Figure 9

Fig. 9 Electron micrograph of the lumen of corpus epididymis of two years vasectomized monkey. Sperms cut in various sections are visible in a macrophage. TEM x 26,500.k
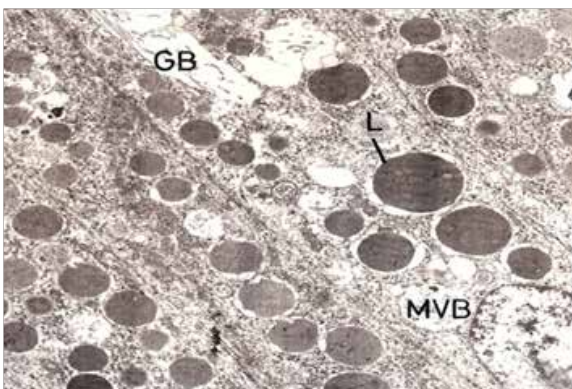

Figure 10

Fig. 10 Electron micrograph of the corpus epididymis of two year vasectomized moneky showing many lysosome like bodies (L), multivesicular bodies (MVB) and dilated Golgi bodies (GB). Recognizable parts of sperms are not detectable in these lysosome like figure but they contain

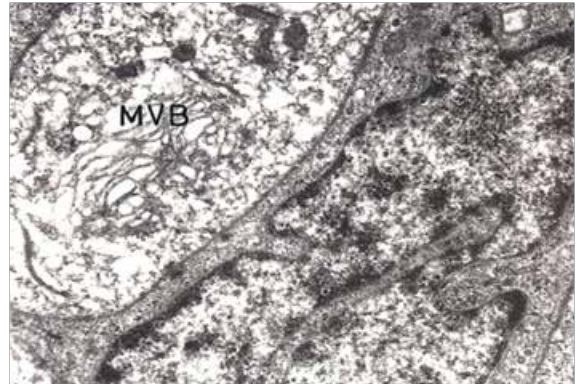some dense substance including some membranous material in them x 57,630.

Figure 11

Fig. 11 Electron micrograph of a principal cell of cauda epididymis, following two years of vasectomy, indicating large multivesicular body (MVB) in the perinuclear region containing recognizable cellular organelles in them x 31,000.
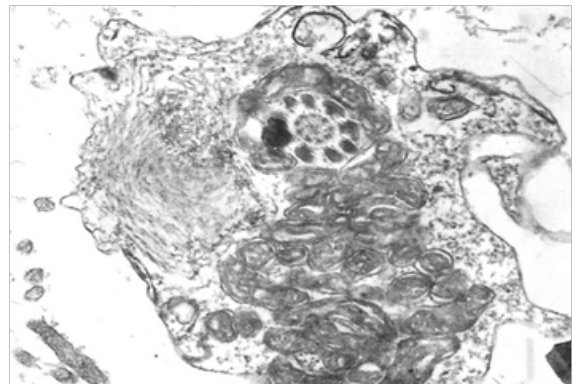

Figure 12

Fig. 12 Luminal area of cauda epididymis of six months vasectomized langur monkey. Lumen contain macrophages with finger like pseudopods & sperm remnants in it. TEM x 37,800.

Discussion

Vasectomy is a safe and effective method of male contraceptive rather a permanent form of birth control. It is a short surgical procedure which interrupts the route that the sperm take from the testicles, where they are produced to move out to the penis.

Besides not affecting the sperm production, vasectomy also does not affect male hormone, sex determination, and sex drive. As a result spermatozoa accumulate in and began to distend the vas deferens and the distal portion of the epididymis, but the response there after varied from species to species and sometimes among individuals within the species.

Schmidt and Morris (1973) Neaves (1975) and Bedford (1976) reported that most notable change, in excurrent duct following vasectomy in several species is the formation of spermatic granuloma. Vasal and epididymal spermatic granulomate may result from enlargement of the epididymis and vas deferens with sperms to the degree that distensive capacity of the duct is exceeded and the wall ruptures. In the species where granuloma accumulates spermatozoa in turns lessens the increase of tubular diameter. Baratt and Cohen (1986) reported in mouse that vasectomy does not affect the rates of sperm production or transport until just before the blockage

in the swollen cauda epididymis. According to them radioactivity appeared in the caudal and 'para aortic' lymph nodes as the radioactive spermatozoa passes from the corpus showing that this is one route of disposal of spermatozoa or sperm products after vasectomy. Some loss of super flows spermatozoa in the normal male tract therefore occurs naturally by this route, and they suggest that vasectomy further exploits this physiological pathway. This accounts for the finding that many males do not make antisperm antibodies after vasectomy, just as normal males do not even though their lymph nodes normally receive spermatozoa/sperm product.

We did not observe any granuloma formation in vasectomized Langurs but a marked increase in lumen diameter was observed following six months and two years of vasectomy. Marsh and Alexander (1982) have reported two to four fold increase in the diameter of the efferent duct in the rhesus monkey that remained vasectomized for one year. Under normal conditions, luminal macrophages appear to participate in the removal of decapitated spermatozoa from the epididymis of the rhesus monkey. (Russel et al., 1967) In non-vasectomized animals spermatozoa are absorbed as a part of normal process. Resorption mainly occurs in the caudal portion of the epididymis as   suggested, by the presence of broken spermatozoa most common in the body of the epididymis.

As the production of spermatozoa is continuous after vasectomy and outlet is blocked, they must be absorbed somewhere between the testis and vas deferens.

Flickinger (1975) suggested four different mechanisms for the disposal of sperm in male genital tract. (1) Phagocytosis of sperm by epithelium of the epididymis and vas deferens. (2) Migration of phagocytes through the epithelium and phagocytosis of sperm in the lumen, possibly following alteration and dissolution in the ductal epithelium. (3) Degeneration and dissolution of sperm in the lumen of the excurrent ducts, with uptake of soluble material by the epithelium, and (4) Rupture of the duct system with formation of spermatic granuloma and phagocytosis of sperm by macrophages outsides the confine of the male reproductive system. In primates intraluminal destruction of spermatozoa by macrophages has been reported more frequently than uptake by epithelial cells. In the present study we observed numerous macrophages in the lumen as well as in the connective tissue with sperm remnants in them. Suggesting that excessive sperms were also digested outside the lumen in the connective tissue. Samuel and Rose (1980) also suggested that extraluminal phagocytosis may also play role in sperm removal. Flickinger (1982) in hamsters found that after vasectomy sperm are disposed of by phagocytosis in spermatic granulomas, intraluminal phagocytosis and dissolution in the lumen of the male duct which may be incomplete. Wang and Holstein (1983) demonstrated that in men macrophages with sperm fragments were found to be escaping from the lumen to the ductus epididymis and penetrating the epithelium towards the sub epithelial tissue layers. Chatterjee et al., (2001) also suggested that spermatic granuloma is believed to maintain physiological harmony in the male reproductive tract by maintaining the balance of hydrostatic pressure post vasectomy. Ratnakumar et al., (1990) in rhesus monkey, reported that the principal mechanism of sperm disposal after vasectomy is the intraluminal endocytosis by macrophages. He also stated that it occurs throughout the duct as macrophages are present in all zones of epididymis and that too with greater number in the cauda region. In a similar study Mc Donald (2000) reported that in many species, spermatozoa in the obstructed duct are destroyed by intraluminal macrophages and degradation products, rather than whole sperms are absorbed by the epididymal epithelium. Similarly, Tait et al., (2000) reported the macrophages in the granuloma wall external to the central mass of extravasated spermatozoa in swiss albino rats following 3 months after vasectomy and suggests the evidence of phagocytic activity in the form of sperm fragments observed.

Sarda et al., (2010) studying the effects of obstruction to sperm on the male testis and epididymis in 50 men patients reported the presence of plasma cells and lymphocytes (31 patients) and macrophages (11patient) in the interstitial considering effects of antigenic sperm extravasations in the interstitium. He considered these effects as local cell mediated immune response.

Conclusion
Study concludes that the mode of sperm disposal is intraluminal endocytosis by macrophages and in the surrounding connective tissue of tubules in the vicinity of blood capillaries.

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