Experimental vasectomy and testicular structure

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Summary. We have performed an experimental study on rats and dogs to evaluate the long term effects (from 1 to 12 months) of vasectomy on the structure of the testis.

From four months after vasectomy onwards, the specimens showed very important changes in the seminiferous epithelium and Sertoli cells, with an obvious thickening of the basement membrane that supports the epithelium. The deterioration depended on the time passed and, over six months after vasectomy, the alterations were very clear and the seminiferous tubules became atrophic and shrunken, sometimes without any remains of seminiferous epithelium and with an important hypertrophy of the interlobular interstitial tissue, although we did not see an increase in the number of Leydig cells.

Alterations due to vasectomy depend on the animal species, the peculiarities of techniques and, of course, the time passed after surgery.

Key words: Vasectomy, Testicle, Structure

Introduction

Although vasectomy, as a male sterilization technique, has been performed for time immemorial (Cooper, 1827; Curling, 1843; Oslund, 1924). Its repercussion on the testis structure was only considered much later by Flickinger (1972), Alexander (1972), Kothari and Mishra (1973), McGlynn and Erpino (1974), Gupta et al. (1975), Varma et al. (1975), Neaves (1978) and Ureña and Malavasi (1980). Authors do not agree that this technique is really innocuous and some of them assert that it could damage the structure of the testis. These assertions are important since the procedure has great social and economic repercussions, and as an example we can cite the USA, where the utilization of this technique moves a great deal of money with the possibility of vasovasostomy, which allows for reversibility, attributing to the extension of the intervention.

In the last few years, some negative aspects of vasectomy have been reconsidered, and especially its influence over lipid metabolism. Alexander and Clarkson (1978) already said that vasectomy could cause metabolic changes which could increase the formation of atherosclerotic lesions. Two years later, Clarkson and Alexander (1980), after causing atherosclerosis in monkeys (M. mulatta) subjected to severe hypercholesterolemic diets, noticed that vasectomized specimens developed atherosclerotic disease earlier and more seriously than controls. As did Westerfield (1983), Bridges and Westerfield (1984), Guate et al. (1989), Nih (1984) and Perrin et al. (1984). More recently some authors have written about the possible relation between vasectomy and the development of a genital neoplasm.

We have been studying the morphology of the vasectomized testis, in different species and periods of times, for many years (Núñez, Sarrat and Whyte). We have proved severe alterations in the structure of the gonad that we want to systematize in the present paper.

Materials and methods

We have studied the effects of vasectomy on 90 adult Wistar rats and 26 Beagle dogs. Twenty-two animals were used as controls at every stage.

We performed bilateral vasectomy according to the classic technique with extremely careful dissection in order to avoid damaging the deferent duct vascularization. A normal spermatogenesis was confirmed before surgery. Dogs and rats were housed under standard stable requirements.

After different periods of time, between 1 and 12 months, both testes of each animal were removed under superficial anaesthesia before sacrificing them with an anaesthetic overdose.

Following a lavage with warm physiological saline solution, we made a small incision on the albuginea of each testis and the whole piece was fixed in Bouin's fluid. After fixation, all specimens were dehydrated by routine means through graded alcohols and xylene and embedded in Paraplast. The tissues were sectioned with a Leitz microtome (7 μm) and were stained with Martin's trichrome.
Results

Normal animals

The main architecture of the testis, formed by its lobular organization, was basically composed of a number of convoluted seminiferous tubules (Fig. 1), among which islets of Leydig appeared as little bulges. The transverse section through the testis (Fig. 2) showed round-appearing tubules with germinal epithelium and Sertoli cells, outlined by a thin basement membrane. Successive divisions of maturation finished in the spermatozoa, which reached the end of its growth process over the sustentacular cells and whose tails formed the classic cowlick in the tubular lumen. The interstitial spaces were occupied by vessels, loose connective tissue and, interspersed among these elements, Leydig cells were seen.

We have found this same histological pattern in specimens, used as controls, subjected to surgery in which the scrotum was split open although they did not undergo vasectomy.

Vasectomized animals

After different periods of time after vasectomy, we noticed alterations in the structure of the testis. The longer the time after surgery, the more severe the changes.

In the first months, these lesions were focal but, as time went by, they became extensive and followed a sequence. The first step in this series of changes was the detention of the maturation process as shown by the increase in the number of tubules in stage 1, typified by the presence of round spermatids. As spermatozoa did not mature, the stop caused the spermatids to increase in number, lose their alignment and fuse their cytoplasm with each other, without fusing their nuclei, and, in the end, they were released into the lumen as multinucleate bodies (Fig. 3). As the process went on, the seminiferous epithelium degenerated and lost its different germinal elements. The degeneration was of a sclerotic type: the tubules had decreased diameters, thickened basement membranes and, at the end, the epithelium consisted of one peripheral row of spermatids and Sertoli cells (Fig. 4).

The cells of Sertoli showed great cytoplasmic vacuoles (Fig. 5), being more numerous in the cases in which the vasectomy was performed a longer time ago.

Degenerative findings were more severe as time of obstruction lapsed. Four months after vasectomy, the main testicular structure was composed of seminiferous tubules lacking in spermatozoa and only one row of spermatogonia and Sertoli cells, intermingled with other tubules with a thick basement membrane and no germinal elements that made the tubule shrink due to shortage of content. This degeneration was not generalized since there were some tubules such as the one on the left in Fig. 6, whose germinal epithelium seemed to be normal although with a severe decrease in the spermatic production, the tubular lumen being empty of spermatozoa.

The interstitial tissue (Fig. 7) underwent an important hypertrophy that gave the testis a parenchymal dense appearance. This hypertrophy was due to an increase in the collagen connective tissue content although we did not notice a proportional increase in the number of Leydig cells (Figs. 6, 7). This hypertrophied connective tissue seemed like a mould that drew the outline of the tubules in their first normal shape and

Fig. 1. Seminiferous tubules (panoramic view) after the section of the albuginea. Normal rat. Note their arrangement in different directions and the same diameter for all of them. x 25

Fig. 2. Transverse section through a seminiferous tubule, surrounded by interstitial tissue with many vessels, showing the normal sequence of maturation of the germinal epithelium, which finish in the spermatozoa, whose tails from a cowlick into the tubular lumen. Note the thin basement membrane that supports the seminiferous epithelium. x 480

Fig. 3. Transverse section through seminiferous tubules of the testis of a vasectomized dog (6 months). Note the thickening of the basement membrane, the detention of spermatogenesis in the spermatid stage (tubules in stage 1), and the formation of multinucleated cells. x 480

Fig. 4. A seminiferous tubule of the testis of a vasectomized dog (6 months). The great thickening of the basement membrane and the detention of spermatogenesis standing out, which reduce the germinal epithelium to one row of cells consisting of spermatids and Sertoli cells. The tubular lumen is occupied by desquamated degenerative cells. x 480

Fig. 5. Seminiferous tubules of the testis of a vasectomized dog (6 months), showing the destruction of the germinal epithelium and the great vacuolization of the Sertoli cells laying on a visibly thickened basement membrane. x 480

Fig. 6. Panoramic view of the testis of a vasectomized rat (4 months) showing the different stages of tubular degeneration with severe atrophy of the germinal epithelium and the loss of tubular morphology, which leaves great spaces among them. On the left a less altered tubule can be seen. x 240

Fig. 7. Seminiferous tubules of a vasectomized rat (9 months), showing the total degeneration of the germinal epithelium and the marked hypertrophy of the interstitial tissue of Leydig with predominance of collagen. x 320

Fig. 8. Seminiferous tubules of the testis of a vasectomized rat (6 months), showing the areas of degeneration that irregularly affects the different zones (the limit is marked by the arrow). Note how the vascularization is well preserved in the degenerated area, rejecting the vascular factor as the cause of the lesions. x 320
situation.

The vasculature underwent no alterations after vasectomy in any of our specimens, perfectly permeable vessels in the degenerative territories were seen (Fig. 8). There is no doubt that the severe lesions in the tests of the vasectomized animals were not due to ischaemia.

Discussion

The influence of vasectomy on the genital area and especially on the testicular structure has been described by many authors with unlike results. While some of them uphold the innocuousness of the method (Oslund, 1924; Nelson, 1952; Sniffen, 1954; MacMillan, 1953; Aman, 1962; Flickinger, 1972; Plaut, 1973; McGlynn and Erpino, 1974; Varma et al., 1975; Chapman, 1978), others, such as Laumas and Unival (1967), Grewel and Sachan (1968), Kubota (1969), Alexander (1972), Kothari and Mishra (1973), Kothari et al. (1973), Gupta et al. (1975), Neaves (1978), Ureña and Malavasi (1980), Nuñez et al. (1985, 1986a,b), Jarow et al. (1985), Lohiya et al. (1988), Lopez et al. (1988), Flickinger (1990) and Whyte et al. (1994) state they have found severe degenerative alterations in the vasectomized testis.

After vasectomy, we noticed alterations in the germinal epithelium, in the basement membrane that supports it, in the Sertoli cells and in the islets of Leydig. These alterations depended on the time lapsed, the technique used and the animal species considered.

The first fact recognized is the detention of spermatogenesis (Derrick et al., 1974), in the stage of spermatids, which increase in number, lose their alignment and fuse their cytoplasm with one another, being released into the lumen as multinucleate bodies. As Neaves (1978) shows, the progressive fusion of the spermatids is a characteristic quality of seminiferous epithelia in a degenerative stage and, according to Dym and Fawcett (1971), is due to the lengthening of the cytoplasmic bridges that connect the spermatids to each other. In the rat, we have very often noticed multinucleate bodies from the third month after vasectomy on. As time goes by, the germinal epithelium degenerates, initially in focal areas, but later the process becomes extensive to larger fields. Algaba (1991) explains the alterations of the Sertoli cells as a compression produced by the enlargement of the intersertolian space, because of the degeneration of the germinal epithelium, although its phagocytic function is not affected; however, we think that this alteration is due to the great vacuolization of these cells, since there is always a thin cytoplasmic lamina surrounding the vacuole. The basement membrane that supports the germinal epithelium also thickens, up to two or three times as thick as its normal size, and it is seen as a strong blue band in the samples stained with Martin’s trichrome. Similar alterations have been described by Alexander (1972), Kothari et al. (1973), Ureña and Malavasi (1980), Nuñez et al. (1985, 1986a,b), Lohiya (1988), Flickinger (1990) and Whyte et al. (1994).

Between six months and one year after vasectomy, the gonads of the rats undergo a process of generalized atrophy that gives the tubules an appearance of irreversible lesion. The alterations of the testes in the dogs are rather less severe, in this same period of time than those in the rats.

After vasectomy, as of 3 months in the rat and as of 6 in the dog, an increase in the interstitial tissue of Leydig is seen, as Steinach (1927) and Houssay (1955) described and Joshi et al. (1972), Nuñez et al. (1985, 1986a,b) and Geirhaas et al. (1991) corroborate; however, it does not seem that this hypertrophy is specific of the endocrine cells but of the connective tissue and especially of the collagen fibrillar material.

With ultrastructural studies, Geirhaas et al. (1991) describes, in vasectomized testes, an increase of 19% in the total cellular area of the islet, with a clear increase in the endoplasmic reticulum of the cells. These findings have no functional correlation, since between 4 weeks and 5 years after vasectomy, Rosenberg et al. (1974), Varma et al. (1975), Fisch et al. (1989) and Sanmartín (1991) did not find significative variations in the plasma testosterone levels in animals subjected to surgery and human vasectomy has been abandoned as a treatment for senile male impotence as having no demonstrable beneficial effects.

There are several theories about the possible causes of the damage of the testicular structure due to vasectomy, among which we can cite intratubular pressure (Flickinger, 1972; Marsh and Alexander, 1982; Kumar et al., 1990), vascular alterations (Flickinger, 1972; Neaves, 1975; Shivastava, 1979) or an immunological process (Samuel, 1975; Pedersen et al., 1983; Nih, 1984; Perrin et al., 1984; Fisch et al., 1989; Cafflisch and DuBose, 1990; Handley et al., 1991). We think that the thickening of the ductus deferens is the main cause of the testicular atrophy, due to an increase in the intraductal pressure, without rejecting the immunological factor. In fact, when the Silver technique, which leaves the testicular end free allowing the normal drain of seminiferous tubules into the interstitial space, is performed, the testicular alteration is much less, at least in the first months.

On the contrary, we think that the reabsorption by the phagocytic action of the macrophages of the spermatozoa released into the loose connective tissue can originate immunological mechanism with the development of antispermatoza antibodies.

We completely reject vascular causes, if the technique is correctly performed, because the vasectomy damages neither spermatic nor deferent vessels and our histological samples prove, that in territories of severe tissular damage, the vessels are completely normal.

A very important problem today is the possibility of association between vasectomy and the development of a male genital neoplasm. Thus, Cale et al. (1990), Moss et al. (1986), Thornhill et al. (1987) and Strader et al.
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(1988) assert that the vasectomy could accelerate the growth of testicular tumours and Jorgensen et al. (1993) assures that testicular cancer develops from a preexisting «in situ» carcinoma, but that vasectomy can speed up its development. We think, as Oliver (1991) does, that the tubular atrophy due to vasectomy is another factor to add to all the factors that cause a testicular neoplasm and, it cannot be forgotten that Sidney (1987), Honda et al. (1988), Hsing and Comstock (1989), Guess (1990), Rosenberg et al. (1990), Mettlin et al. (1990), Anonymou (1991), Peterson et al. (1992) and Venner and Berckel (1992) have noticed an increase in the risk of developing prostate cancer in vasectomized individuals.

Finally, we think that because of the variations found in different species, one must be cautious in the extrapolation of results in humans and in the evaluation of the successes of fertilization obtained after vaso-vasostomies.

References


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