Vasectomy and vasectomy reversal : development of newly designed nonabsorbable polymeric stent for reconstructing the vas deferens

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Chapter 5

Influence of postvasectomy spermgranulomas, antisperm-antibodies formation and histological alterations of testicular/epididymal tissue on the outcome of vasectomy reversal.

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Introduction

The success of a vasectomy reversal depends on several factors. A well known factor is the obstructive interval which is the time period between vasectomy and reversal. Other factors are length of the vas resection during vasectomy and occurrence of vasal fluid from the testicular end at the time of reversal as well as fertility status of the partner and the partner's age. In this review article we will focus on the influence of sperm granuloma, antisperm antibody formation and tissue damage to epididymis and testis on the final outcome of a vasectomy reversal. Many studies were contradictory on this subject raising uncertainty on their importance. Ligation of the testicular end at the time of vasectomy will lead to an enhancement of epididymal pressure sometimes resulting in granuloma formation as well as antisperm antibody production. This raise in pressure will eventually lead to tissue damage of epididymis and testis. What is the impact of a pressure release valve like a sperm granuloma on this sequence and what is the influence of this tissue damage on the vasovasostomy result? Do open-end vasectomies at the testicular side prevent these histological alterations and how do open-end vasectomies versus obstructive vasectomies affect antisperm antibody formation?

Role of sperm granuloma

Sperm granulomas are present in 10-30% of men undergoing a reversal procedure [1]. Sperm granulomas play an important role in the regulation of chronic obstruction in the male reproductive tract. A sperm granuloma is a complex network of multiple minimal epithelialized channels that help to prevent high intraluminal pressure in the obstructed ducts. Obstructive vasectomy induces a rise in pressure affecting the epididymis and efferent ductules. These structures become markedly distended and then adapt to reabsorb large volumes of testicular fluids and sperm products. In time all vasectomized men will develop blow outs of the epididymis or efferent ducts. Sperm granulomas may develop at the site of the disrupture and secondary epididymal or efferent duct obstruction may result. These events are the reason why vasectomy reversal is less successful if the obstruction interval increases. Sperm granulomas on the other hand may prevent this progressive damage to the epididymis and efferent ducts because these granulomas may act as pressure valves protecting the epididymis and efferent ducts from further deterioration. Above mentioned processes are extensively described in numerous animal studies [2-7]. From these studies there is little doubt that epididymal and testicular obstruction due to vasectomy will eventually lead to rupturing of epididymal tubules and formation of granulomas in these animals. Because it is difficult to obtain human tissue specimens there is only limited information on morphologic changes of human testes and epididymidis after vasectomy, but spermatic granulomas are reported [8].

Spermatic granulomas do probably have an impact on the development of immobilizing antibodies. Alexander and Schmidt [9] performed a study on 77 vasovasostomized men and found more sperm-immobilizing antibodies in patients with granulomas than in those without (67% versus 48%). In contrast, in a study performed by Broderick et al.[10], only 2 out of 12 patients with significant sperm-surface antibodies had granulomas. In a review article [11], the

presence of a sperm granuloma at the site of ligation after vasectomy might be interpreted as a pressure release valve. Those patients, who underwent a reversal procedure, including removal of their granulomas before reanastomosis, had an explicit good quality sperm in their ejaculum. These findings that men with sperm granuloma at the site of ligation had better intraoperative sperm quality and improved patency (81%) and pregnancy rates (53%) were previously described by Silber [12,13] . These outcomes were conflicting with the results of the Vasovasostomy Study Group [14] who presented the results of 1469 microsurgical vasectomy reversals. The presence of bilateral sperm granulomas at the vasectomy sites had no beneficial effect on the patency (p=0.050) or pregnancy rates (p=0.150). In a more recent paper by Boorjian et al. [15] the role of sperm granulomas on the outcome of 213 vasectomy reversals was investigated. They described better patency rates in men with granulomas then in those without (95% versus 78%). It is remarkable that there are no more recent literature data (pub med search 1998-2005) on the role of sperm granuloma as a possible mediator in successful vasectomy reversals. If the presence of sperm granulomas is important for increased success rates after vasovasostomy remains a question.

Could open-ended vasectomies induce sperm granuloma formation at the testicular end and could it thus have a beneficial effect on vasectomy reversals as it works as a pressure release valve? If so, could open-ended vasectomies be a solution to prevent increased pressure in the epididymis and subsequent bursting of epididymal tubules leading to granuloma formation within the epididymis and ductul obstruction. Shapiro and Silber [16] studied the development of granuloma formation in larger series of patients with open ended vasectomy. In a group of 410 patients from Ottawa, 97% developed a sperm granuloma at the non-ligated testicular vas end. In a second group of 23 patients from St Louis, all patients developed sperm granulomas at the testicular end. Despite this possible beneficial granuloma effect, Goldstein [17] stressed the fact

that open ended vasectomies could lead to higher vasectomy failure rates and therefore should be applied with the utmost precaution. In a larger series by Errey and Edwards [18] this risk of spontaneous recanalization was weakened. They compared 4330 open-ended vasectomies with 3867 standard vasectomies and spontaneous recanalization was rare in both groups. Essential was that the abdominal end was covered by vas sheath. All studies [19-23] published in the last fifteen years on the subject of open-ended vasectomy indicated reduction of epididymal congestion and post vasectomy orchialgia, but none of these studies gave definitive prove of a beneficial effect on vasovasostomy results in terms of patency and pregnancy rates. A well designed comparative study, between patients who had closed-ended or open-ended vasectomies before vasectomy reversal, is mandatory on this subject, taking into account that the outcome of vasectomy reversal is multifactorial.

Development of antisperm-antibodies

Presence of T suppressor/cytotoxic cells and formation of antisperm antibodies

Vasectomy may also induce local and systemic immune effects. Regarding the local effects, chronic epididymal obstruction results from inspissated sperm, damaging the efferent ducts leading to leakage, granuloma formation and fibrosis. Intraductal phagocytosis (with degradation of sperm) produces soluble antigens that pass the damaged transverse epithelial tight junctions due to distension and leakage. Inoculations of the immune system with these sperm antigens will result in activation of the humoral immunity system leading to antisperm antibody production. Obstructive vasectomy results in such a violation of the blood-testis barrier resulting in detectable levels of serum antisperm antibodies in 60-80 percent of men after vasectomy. Some data indicate diminishing of antibody titers in serum two or more years after vasectomy, others indicate persistent titers [24-27].

Next to the humoral immune system there is a cell mediated immune system. This involves the killing of antigens by phagocytes, cytotoxic lymphocytes and natural killer cells. The T cell lymphocytes are responsible for cell mediated immunity. After recognizing the antigen, the T cell lymphocytes order the B cell lymphocytes to produce antisperm antibodies. There are two subtypes of T cells (helper and suppressor) which are of importance in cell mediated immunity at the male genital area. The most important are the T suppressor/cytotoxic cells which occur mainly at the area of rete testis and efferent tubules. The existence of the T suppressor /cytotoxic cells at these locations could, as a theory, suppress any immune reaction and could probably play an important role in the prevention of antisperm antibody production [28]. In a study by Witkin and Goldstein a group of non vasectomized men were compared with men who had undergone both a vasectomy and a microsurgical vasovasostomy. Antisperm antibodies were detected in sperm, in seminal fluid and/or in serum of all the vasovasostomy patients but in none of the controls. Damage to the integrity of the excurrent ducts may induce alterations in T cell regulation, leading to a decrease in T suppressor/cytotoxic cells creating formation of auto antibodies to sperm-specific antigens [29].

Consequences of antisperm antibodies in semen

Only few (4%) vasectomized men have antibodies detectable in seminal plasma [30].

After vasovasostomy sperm agglutinins may appear in the seminal plasma of some men, provided that antisperm antibodies were detectable in serum before operation. Thus, systemically and/or locally produced antisperm antibodies do enter the obstructed part of the male genital tract in vasectomized men. This was confirmed in a study on motile epididymal spermatozoa obtained from men during vasovasostomy [31]. It was found that the antisperm antibodies detected with the mixed antiglobulin reaction (MAR) were bound to the sperm

surface at the epididymis level of the male genital tract. One year after operation the MAR-results on ejaculates were almost identical to the results obtained with epididymal spermatozoa. Thus, the binding of antisperm antibodies, takes place primary at the level of the epididymis. A study with split ejaculates from different compartments, did not exclude however, that additional binding may take place at the level of the prostate and the seminal vesicles [32].

Linnet et.al. [33] pointed out the significance of antisperm antibodies for pregnancy rates. They found a pregnancy rate of 85% among vasovasostomized men without antisperm antibodies in seminal plasma; however the mere presence of antisperm antibodies in seminal fluid, using the tray agglutination test (TAT), reduced the pregnancy rate to 14%. These results were confirmed by several other studies on this subject. Parslow et.al [34] found that the presence of antisperm antibodies in seminal plasma after vasectomy reversal was associated with diminished fertility only if the titer in the TAT exceeded 16, nevertheless even with higher titers, conceptions occurred. Studying serum antisperm antibodies, the same group [35] observed that pregnancy was significantly less likely when the preoperative serum antisperm antibody titer in the TAT was high, 512 or more.

Matson and co-workers [36] found reduced conception rates in those couples in whom the presence of IgG or IgA+IgG antisperm antibodies occurred in seminal fluid. Meinertz et al. [37] reported on 216 vasovasostomized men who were tested with the mixed antiglobulin reaction for IgA, IgG and secretory IgA antisperm antibodies bound to the sperm membrane. Free antisperm antibodies were analyzed with the gelatine agglutination test and the TAT. In a subgroup with only IgG response, the rate of conception was 85.7%, while 42.9% of men, who also had IgA on the sperm, achieved pregnancy. When 100% of the sperm was covered with IgA, the conception rate decreased to 21.7%. The combination of IgA on all sperm and a serum titer ≥ 256 was associated with zero conception. Aitken et al. [38] described the impact of

antibodies to stimulate or suppress sperm/oocyte fusion. In vasovasostomized patients they saw a higher stimulating effect on this fusion than in patients with primary infertility. In their series they observed no correlation between the antisperm antibodies titers in serum and seminal plasma and the ability of these antibodies to influence sperm function.

The significance of white blood cells in the ejaculate after vasovasostomy remains a point of further investigation. Barratt et al. [39] documented the white blood cell types in the ejaculates of vasovasostomized men and noticed that those men without antisperm antibodies had a predominance of suppressor/cytotoxic T cells over helper/inducer T cells. As previously said, Witkin and Goldstein [29] also viewed the fact that a decrease in suppressor/cytotoxic T cells may lead to a condition in which the formation of antisperm antibodies is stimulated.

Histological changes of testis and epididymis after vasectomy and vasectomy reversal

Animal studies

Postvasectomy histological changes in rats were discussed by Turner et al. [40] who described the importance of certain proteins in testis and epididymis (cysteine-rich secretory protein, prostaglandin D2 synthetase, phosphatidylethanolamine-binding protein) in the maturation and possible agglutination of sperm cells after vasovasostomy. The interstitial tissue in the epididymis was much more densely occupied by lymfocytic cells than in sham operated controls, suggesting that sperm material might have escaped the lumen, provoking an inflammatory reaction.

The relationship between development of antisperm antibodies after unilateral vasectomy and the occurrence of testicular damage on the non-vasectomized side was studied by Chehval et al. [41]. They performed a unilateral vasectomy in rats and described a relationship between the onset of antisperm antibodies after 30 days of obstruction and the occurrence of testicular

changes on the contra lateral side. They concluded that epididymal and/or testicular tissue damage after vasectomy does not only depend on high pressure in the epididymis but also on deposition of systemic circulating immune complexes as seen in the healthy non-obstructed side.

Johnson and Howards [42] investigated the effect of increased intraluminal pressure in the testis and epididymis after vasectomy in hamsters. They described mainly an increase in pressure in the epididymis and not in the testis. The pressures measured in the cauda epididymis, two weeks after vasectomy, were significantly higher ($p<0.0005$) compared to normal controls. Due to distension of the epididymis, ruptures were seen, illustrating the limitation in distensibility of the epididymis and its reabsorptive capacity. They could not find change in weight of the testes compared to normals four weeks after vasectomy. The effect of vasectomy on the epididymis in rats was also extensively studied by Flickinger et.al. [3]. Increased pressure in the epididymis is not generally transmitted towards the seminiferous tubules. The epididymal interstitium shows microscopic changes indicative of chronic inflammation with infiltration of lymphocytes, macrophages and plasma cells. Rats that have these tissue changes also have higher antisperm antibodies in serum. Due to these pressures the epididymal ducts will burst with escape of spermatozoa leading to an immune response and granuloma formation.

Whether testicular alterations after vasectomy are reversible by vasovasostomy was studied by Flickinger et.al. [43]. A group of Lewis rats was vasectomized and testicular weight and morphological alterations were described. Especially as time after vasectomy went by the testicular alterations enhanced. As a vasovasostomy procedure was advanced, the progression of these testicular changes was reduced but not reversed. Smaller testicles after vasectomy showed severe alterations in the seminiferous tubules compared to heavier testicles that presented with a normal morphology. Testicular alterations were graded for light microscopy

by use of a semiquantitative testicular biopsy score count (TBSC). As a result, the degree of morphologic changes corresponded closely to the testicular weight. A similar study in Lewis rats was performed by Herr et al. [44]. Testicular weight, histology and antisperm antibodies were studied, using enzyme-linked immunosorbent assay (ELISA). Animals with altered testes after vasectomy showed significantly greater mean absorbance values in ELISA, compared to animals without testicular alterations. Animals with a positive antisperm antibody response were seen in the group with testicular alterations but also in the group without testicular damage. Compared to the sham group, the level of antisperm antibodies in the vasectomized and vasovasostomized animals was significantly higher. Animals that had a successful reversal procedure (in terms of sperm count) showed even after 4 months similar elevated concentrations of antisperm antibodies compared to the vasectomized rats. The group of animals with small testes after vasectomy showed severe microscopic alterations consisting of depletion of germ cells. Seminiferous tubules lacked all stages of germ cells and thus were composed almost entirely of Sertoli cells. Animals with normal sized testes showed no morphological changes and resembled those of the sham group. These findings were confirmed by Neaves [45], who also examined histological changes on the testes in rats after vasectomy.

Vasovasostomy results, in terms of semen quality, were significantly better in animals that had a vasocystostomy as a contraceptive method compared to animals that had a vasectomy (100% versus 36 %). This is due to the epididymal and testicular changes after vasectomy. In 19 out of 26 rabbits (73%) some degree of disruption of the germinal epithelium of the testis was seen, however no testicular alterations were seen in the vasocystostomy group. Also the epididymis of vasectomized rabbits showed significant tissue changes in 42%. Especially fibrosis was seen in the caput and corpus area. In the vasocystostomy group only one granuloma was seen in the

cauda epididymis. Pressure release thanks to the vasocystostomy was of importance in the prevention of epididymal and testicular tissue damage [46].

Human studies

Studies on the effect of interstitial testicular fibrosis on vasovasostomy results in men are sparse. Jarow et al. [47] took testis biopsies from men undergoing vasectomy reversal and healthy volunteers. The morphometric analyses of these specimens revealed a significant increase in thickness of seminiferous tubular walls mean cross-sectional tubular area and a reduction in the mean number of Sertoli cells and spermatids. Focal interstitial fibrosis was only seen in the vasectomy group and not in the controls. They observed a significant ($p<0.01$) correlation between interstitial fibrosis and successful vasectomy reversal. In continuation of this study, Jarow et al. [48] pointed out that there was no association between testicular histologic changes and immune status of vasectomized men.

In two more or less identical studies by Shiraishi et al. [49,50] men obtained a testis biopsy at the time of vasectomy reversal. Significant increase in interstitial fibrosis was seen as obstructive intervals became longer. Interstitial fibrosis and not the extent of germ cell differentiation, measured by the proliferative cell nuclear antigen expression (PCNA), were important in the successful outcome of the vasectomy reversal. In a recent study by Raleigh et al. [51] 34 men receiving testicular biopsies at the time of vasectomy reversal were compared with 10 normal controls. Biopsies were examined for testicular germ cell populations and testicular fibrosis. Patients undergoing a vasectomy reversal had a significant decrease in germ cells in the later stages of spermatogenesis and had a significant increase (2.7 fold) in interstitial fibrosis. Both changes correlated strongly with the obstructive interval and the final outcome of the

vasectomy reversal. From these studies it seems obvious that testicular change do occur after vasectomy and that these changes do have an influence on the outcome of vasectomy reversal.

Discussion

Sperm granulomas, antisperm antibodies and tissue damage to epididymis and/or testis after vasectomy are all related to one another. Nevertheless each anomaly itself has a certain impact on the results of vasectomy reversal.

Regarding the literature, sperm granuloma will develop eventually in all men who had a vasectomy. From animal studies it is clear that sperm granulomas act as pressure relieve valves preventing further tissue damage to epididymis and testis. Otherwise the appearance of sperm granuloma induces the production of antisperm antibodies that still might damage the epididymal and testicular tissues. No study in men has been published with prove that the existence of sperm granuloma do improve vasovasostomy results. Several studies on the subject of open-ended vasectomies look promising on the reduction of epididymal pressure rise preventing damage to the ductules and thus antisperm antibody formation.

Concerning antisperm antibodies, a strong relationship between the appearance of sperm granuloma and antisperm antibodies is apparent. The importance of these antisperm antibodies in vasectomy reversal remains unclear. It is clear from studies published in literature, that higher antisperm antibodies titers in serum before vasectomy reversal do have an adverse effect on sperm quality. It is obvious that antisperm antibodies in serum do not influence the sperm motility. It is the antibodies in seminal plasma that interfere with sperm motility. Measuring of antisperm antibody titers in serum does not automatically indicate that ‘immobilizing antisperm antibodies’ are present in semen. Unfortunately we can not determine the influence of antisperm antibodies on sperm as long as the patient is still in a vasectomy status.

When considering vasectomy reversal, the relevance of these antisperm antibodies in daily practice is still unclear. Do higher antisperm antibody titers restrain physicians from performing a reversal procedure? We think not, because the only alternative is assisted reproductive techniques (ART). This alternative treatment is invasive for wife and man and much more expensive [52,53].

From animal studies we know that tissue damage is mainly occurring in the epididymis due to tubular rupturing, fibrosis and eventually obstruction. The testis is not always protected from these increased pressures. Interstitial fibrosis and increased seminiferous wall thickness are the most frequently described changes in testicular parenchyma in men. Despite the fact that the numbers of studies (and patients in these studies) are limited, we are convinced that testicular damage eventually occurs. With longer obstructive intervals more damage to the tissues will be seen, with an adverse effect on patency and pregnancy rates. But then again, who takes testis biopsies before vasectomy reversal and measures the amount of interstitial fibrosis and germ cell differentiation? Our knowledge of the influence of tissue changes in the testis on the outcome of vasectomy reversal is still limited. The importance of these tissue changes in men remains unclear due to the fact that from an ethical point of view these studies are hard to perform.

Reviewing the literature, some form of pressure release of the epididymis after vasectomy will have a beneficial effect on vasovasostomy results. The fear that open ended vasectomy results in a higher failure rate (in terms of azoospermia) seems not justified. If so, open-ended vasectomy could be the technique of choice in an era with growing numbers of divorces and renewed wish to have a child. But how can we be sure that if we leave the testicular end open that reactive fibrosis will not completely occlude this testicular end? How can we investigate the development of a possible sperm granuloma at this end and how can we be sure that this

works as a pressure relieve valve? A comparative study between open-ended and closed-ended vasectomy has to be performed to prove this hypothetical beneficial effect of open-ended vasectomy on vasovasostomy outcomes.

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