Effects of vasectomy on spermatogenesis and fertility outcome after testicular sperm extraction combined with ICSI

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BACKGROUND: Each year 40 000 men have a vasectomy in the UK whilst another 2400 request a reversal to begin a second family. Sperm can now be obtained by testicular biopsy and subsequently used in assisted conception with ICSI. The study aims were to compare sperm yields of men post-vasectomy or with obstructive azoospermia (OA) of unknown aetiology with yields of fertile men and to assess any alteration in the clinical pregnancy rates after ICSI.

METHODS: Testicular tissue was obtained by Trucut needle from men who had undergone a vasectomy >5 years previously or had OA from other causes and from fertile men during vasectomy. Seminiferous tubules were milked to measure sperm yields. Numbers of Sertoli cells and spermatids and thickness of the seminiferous tubule walls were assessed using quantitative computerized analysis.

RESULTS and CONCLUSIONS: Sperm yields/g testis were significantly decreased in men post-vasectomy and in men with OA, relative to fertile men. Significant reductions were also observed in early (40%) and mature (29%) spermatid numbers and an increase of 31% was seen in the seminiferous tubule wall (basal membrane and collagen thickness) of vasectomized men compared with fertile men. Clinical pregnancy rates in couples who had had a vasectomy were also significantly reduced.

Key words: clinical pregnancy rate/ICSI/spermatids/sperm yield/vasectomy

Introduction

The first report of a human vasectomy was in 1899 (Jhaver et al., 1952). Over the past 30 years there has been a marked increase in vasectomy, particularly in more affluent parts of the world. Currently, over 40 000 men have a vasectomy in the UK every year; accounting for >50% of sterilizations. Traditionally, vasectomy has been considered an irreversible form of contraception. However, increasingly men are requesting reversal to have a second family, and now 2400 (~6%) men request reversal in the UK (Potts et al., 1999). Even when surgery is successful, antisperm antibodies impair sperm function in up to 50% of cases, thus reducing fertility. In addition, Kolettis et al. (1999) found that vasectomy reversal is associated with seminal oxidative stress, which can impair sperm structure (Hughes et al., 1998) and function (de Lamirande et al., 1998). There is evidence that men who have had a vasectomy less than 2 years previously have a 50% chance of a pregnancy, whereas those who have had their vasectomy more than 15 years previously have a 30% chance of a spontaneous pregnancy (McLachan and Royce, 1996). However, as yet, the long-term consequences of vasectomy for testicular and sperm function and future fertility have not been well documented.

For post-vasectomized men wishing to have second families, vasectomy reversal has been replaced in part by testicular biopsy via fine-needle testicular sperm aspiration (TESA) or percutaneous epididymal sperm aspiration, performed at an outpatient clinic and subsequently used in ICSI (Abdelmassih et al., 2002).

The aims of this study were to determine the effects of vasectomy on sperm yield and testicular histology. Secondly, we aimed to compare the fertility outcomes (cumulative embryo score and clinical pregnancy rates) after assisted conception of normal female partners of vasectomized men and men with obstructive azoospermia (OA).

Materials and methods

Testicular biopsies

Testicular biopsies were obtained from three groups of subjects: men who had had a vasectomy >5 years previously (Group 1, n = 24); men with OA resulting from congenital absence of the vas deferens, either through cystic fibrosis or an idiopathic cause (Group 2, n = 32); and men of proven fertility biopsied as they were undergoing vasectomy (Group 3, n = 39). Mean age was 46.53 ± 0.77 years for Group 1, 39.15 ± 1.10 for Group 2 and 39.00 ± 0.70 years for Group 3. Written consent for participation was obtained and the project was approved by the Queen’s University Belfast Research and Ethics Committee.
**Anaesthesia**

The left spermatic cord was located, and 10 ml of 0.5% bupivacaine (without adrenaline) was injected on either side of it with use of a 21-gauge needle. After 10 min, anaesthesia was confirmed by firm testicular palpation: if the patient was aware of pain or pressure, a further bolus was injected. The scrotal skin over the lower pole of the testicle was anaesthetized with 0.5 ml of 1% lignocaine.

**Testicular biopsy**

The skin and tunica albuginea of the testis were punctured with a disposable scalpel. Two biopsies were taken from the lower pole of the testis, opposite to the pole of the epididymis with the direction of the biopsy along the long axis of the testis. A 14-gauge Trucut needle (Baxter Healthcare, Thetford, UK) was inserted through the puncture site into the testicle and advanced 1 cm, taking the tissue from the middle of the testis. The biopsy was taken by advancing the inner core of the needle and then closing the outer sheath rapidly over it. A core of tissue about the size of a grain of rice was trapped in the specimen notch. The tissue was then transferred into culture medium [Biggers–Whitten–Whittingham (BWW) medium] (Biggers et al., 1971). The procedure was repeated at a different site to obtain the second sample. The subject maintained pressure over the skin wound for 30 min before discharge from the clinic. Diclofenac sodium (100 mg) (Novartis Pharmaceuticals UK, Camberley, UK) was given at the time of the procedure and 12 h later for analgesia.

**Sperm yield**

The first biopsy was weighed before sperm were ‘milked’ from the seminiferous tubules. Milking was performed by stabilizing the left end of each seminiferous tubule with forceps and drawing the tubule through a second pair of closed forceps in a left-to-right direction to milk the contents from the free end into the culture medium (BWW). The material obtained was centrifuged at 1000 g for 10 min. Subsequently the pellet was resuspended in 200 µl BWW, and 10 µl was placed on a haemocytometer to calculate sperm/g for the testis.

**Spermatid and Sertoli cell number per unit area of biopsy**

A second biopsy was not milked but was immersion-fixed intact without milking in Bouin’s fixative and stained with haematoxylin and eosin for histological assessment. From a transverse section of the intact biopsy, 10 tubules were assessed for each subject. The numbers of Sertoli cells, round spermatids and mature spermatids were counted per cm² of tissue in round or slightly oval-shaped transverse tubules using a Leica IM measurement module (Northern Microsystems, Cambridge, UK). The spermatids were classified and assessed according to Clermont (1963), and divided into the following groups: the most immature generation of spermatids with spherical nuclei (Sa); darker round spermatids (Sb), also with spherical nuclei, typically pointed elongated spermatids (Sc); and spermatids with smaller, more compact structures (Sd) where the chromatin was condensed into a form similar to that in mature sperm (Figure 1). The ratio of the spermatid count per target area of biopsy to Sertoli cell count per unit area of biopsy was calculated.

**Seminiferous tunica propria thickness**

As the thickness of the wall depends on the angle of sectioning we measured only the thinnest part of each tubule so that any difference in thickness in any group was not protocol induced. The tubule wall (tunica propria) included the lamina propria, peritubular myoid cells and the collagen layer. Each biopsy was also assessed by standard pathology techniques for inflammation or neoplasia.

![Figure 1. Representative picture of a counted seminiferous tubule where the intact biopsy was fixed in Bouin's fluid.](image)

**Cumulative embryo score (CES)**

A cumulative embryo score (CES) was used to calculate the embryo quality (Steer et al. 1992). Two days after oocyte recovery, prior to embryo transfer, the embryos were graded as follows: grade 4, equal-sized symmetrical blastomeres; grade 3, uneven blastomeres with <10% fragmentation; grade 2, 10–15% blastomeric fragmentation; and grade 1, >50% blastomeric fragmentation or pronucleate single-cell embryos. The morphological grade of the embryo was then multiplied by the number of blastomeres to produce a quality score for each embryo. The scores of all embryos transferred per patient were summed to obtain the CES, to be used as an index of the total embryonic contribution to pregnancy.

Only partners of women less than 40 years of age with a normal endocrine profile and normal spontaneous ovulatory cycle were used in this study. The mean female age at the time of the ICSI cycle was 34.20 ± 0.75 years (range 23–39) for the men who had been vasectomized ≥10 years and 32.90 ± 0.43 (range 24–39) years for Group 2.

**Assisted conception outcome**

Clinical pregnancies per embryo transfer were calculated following pregnancy confirmation by ultrasonic fetal heart detection at 6 weeks.

**Statistical analysis**

Data were analysed using SPSS 10 for Windows. The non-parametric Kruskal–Wallis test was employed to determine the differences in sperm yield between the men in each group and the CES value of the embryos. In addition, the non-parametric Mann–Whitney test was employed to determine the differences in spermatid number and the width of the tubule wall between fertile and post-vasectomized men. Differences in cumulative embryo scores and pregnancy rates in Groups 1 and 2 were also determined using the non-parametric Mann–Whitney test.

**Results**

The testicular biopsies had a mean (±SEM) of 28.8 ± 2.67 mg of tissue.
Effects of vasectomy on sperm yield

The sperm yields/g of both treated groups of men were significantly lower than those obtained from fertile men ($P < 0.001$, Table I).

Effects of vasectomy on Sertoli and germ cells and the thickness of the tubule wall (tunica propria)

There was no significant reduction in the Sertoli cell number per unit area of biopsy in the men vasectomized for ≥10 years compared with previously fertile men. In contrast, there was a marked reduction in the number of early round spermatids (Sa) and dark mature spermatids (Sb) ($P < 0.05$) and in the number of mature spermatids (Sc and Sd) in the men vasectomized ≥10 years relative to the fertile men ($P < 0.01$). The ratio of round spermatids per target area of biopsy to Sertoli cell count per unit area was reduced from 3.46 to 2.86 and the elongated spermatids were reduced from 3.50 to 2.86 in the post-vasectomized men relative to the fertile men. A significant increase in the thickness of the tunica propria was also observed in the men vasectomized for ≥10 years compared with the fertile men ($P < 0.001$) (Table II). There was no evidence of an increase in the incidence of carcinogenesis or infection in the pathologists’ reports (data not included in Table II).

Effects of vasectomy on the CES and clinical pregnancy rate

There was no difference between the age of the female partners in Groups 1 and 2 ($P = 0.124$) and no difference in the CES of the embryos transferred to the partners in Groups 1 and 3 (Table III). However, there was a significant reduction in the clinical pregnancy rate of partners of men vasectomized for ≥10 years relative to the men with non-surgically induced OA ($P < 0.05$, Table IV).

Discussion

This is the first study, to our knowledge, to report reduced sperm yields from men who have had vasectomies and also from men with OA from other aetiologies undergoing assisted conception compared with sperm yields from fertile men. It is also the first report showing a decrease in the numbers of specific spermatogenic stages—early round (Sa), late round (Sb) and mature spermatids (Sc and Sd). Our results confirm those of Dym et al. (1982), who suggested that there was a reduction in spermatogenesis after vasectomy. The reduction in sperm numbers/g testis caused either by vasectomy (Group 1) or chronic obstruction (Group 2) is not a function of a reduction in the Sertoli cell population, since we found that there was no significant reduction in the numbers of Sertoli cells per unit area of biopsy in Group 1. However, the ratio of spermatid count per unit area of biopsy to Sertoli cell count per unit area of biopsy was reduced after vasectomy, suggesting that support given by the number of Sertoli cells was suboptimal. This is not surprising since Sertoli cell number is already defined by puberty (Orth et al., 2000). This study supports that of Kubota et al. (1969), who reported vacuolation of Sertoli cells in post-vasectomized men, showing that the reduced sperm yield is associated with Sertoli cell dysfunction.

Such testicular dysfunction could be a result of endocrine disruption. Spermatogenesis is primarily controlled by the endocrine effects of FSH and the paracrine effects of testosterone on Sertoli cells. An increase in FSH has been observed in men following vasectomy (Mo et al., 1995), and Smith et al. (1976) also found that, 1 year after vasectomy, levels of LH and testosterone were increased. However, this was not confirmed by Naik et al. (1976), who found that there were no significant changes in FSH, LH and testosterone after vasectomy. Further, Kothari and Gupta (1974) reported that Leydig cell columns increased after vasectomy.

Interstitial fibrosis may also affect the paracrine functions of the seminiferous tubules after vasectomy (Shiraishi et al. 2003), leading to a reduction in spermatogenesis. We noted a marked thickening in seminiferous tubule walls, in agreement with Gupta et al. (1975), who reported widespread degeneration of the germinal epithelium, including thickening of the basement membrane and interstitial fibrosis as early as 1 month after vasectomy. Howards and Johnson (1979) reported an increase in testicular hydrostatic pressure 4 months after vasectomy using a guinea-pig model. Fibrosis may also be the result of inflammation, and inflammatory reactions have been reported after vasectomy in guinea-pigs (Alexander, 1973). This is supported by the knowledge that up to 50% of men have antisperm antibodies in serum and/or semen within a year of vasectomy (Linnet, 1983). However, no antibodies have been detected on

Table I. Effects of vasectomy on sperm yield

<table>
<thead>
<tr>
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<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vas &gt;5 years</td>
<td>OA</td>
<td>Fertile</td>
</tr>
<tr>
<td>$n$</td>
<td>24</td>
<td>32</td>
<td>39</td>
</tr>
<tr>
<td>Sperm yield ($10^6$/g) ± SEM</td>
<td>3.95 ± 0.79*</td>
<td>3.00 ± 0.43*</td>
<td>11.18 ± 1.56</td>
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* $P < 0.001$ each compared with Group 3.

Table II. Effects of vasectomy on Sertoli cells, germ cells and the seminiferous tunica propria

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Vasectomized ≥10 years ($n = 8$)</th>
<th>Group 3 Fertile ($n = 9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli cells/cm²</td>
<td>2.35 ± 0.18</td>
<td>2.71 ± 0.21</td>
</tr>
<tr>
<td>Round spermatids (total Sa and Sb)/cm²</td>
<td>6.72 ± 0.57</td>
<td>9.36 ± 0.93</td>
</tr>
<tr>
<td>Total elongated spermatids (total Sc and Sd)/cm²</td>
<td>6.72 ± 0.49</td>
<td>9.48 ± 0.80</td>
</tr>
<tr>
<td>Round spermatids (total Sa and Sb)/cm²/Sertoli cells/cm²</td>
<td>2.86 ± 0.63</td>
<td>3.46 ± 1.17</td>
</tr>
<tr>
<td>Total elongated spermatids (total Sc and Sd)/cm²/Sertoli cells/cm²</td>
<td>2.86 ± 0.85</td>
<td>3.50 ± 0.86</td>
</tr>
<tr>
<td>Seminiferous tunica propria thickness (µm)</td>
<td>7.63 ± 0.42</td>
<td>4.86 ± 0.34</td>
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$P = 0.21$; $P = 0.05$; $P = 0.01$; $P = 0.19$; $P = 0.17$; $P < 0.001$.
Effects of vasectomy on cumulative embryo score

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasectomized &gt; 5 years</td>
<td>OA</td>
</tr>
<tr>
<td><strong>Embryo transfer at day 2</strong> (n = 14)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>CES value ±SEM</td>
<td>25.0 ± 2.2</td>
</tr>
<tr>
<td><strong>Embryo transfer at day 3</strong> (n = 12)</td>
<td>(n = 22)</td>
</tr>
<tr>
<td>CES value ±SEM</td>
<td>39.0 ± 4.8</td>
</tr>
</tbody>
</table>

Table III. Effects of vasectomy on cumulative embryo score

Table IV. Effects of vasectomy on the clinical pregnancy rate

<table>
<thead>
<tr>
<th>No. of cycles</th>
<th>Group 1</th>
<th>Group 2 OA</th>
</tr>
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<tbody>
<tr>
<td>Vasectomized ≥ 10 years (n = 25)</td>
<td>(n = 68)</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancies per cycle (%)</td>
<td>9.1</td>
<td>28.4</td>
</tr>
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</table>

The role of reactive oxygen species (ROS) and oxidative stress in the sperm yield of post-vasectomy patients has been a subject of interest in recent years. We now know that the production of ROS is a necessary function of sperm (Aitken et al., 2003), but one that must be tightly regulated to prevent damage (Aitken and Krausz, 2001). Testicular germ cells have been shown to generate hydrogen peroxide, a process that continues as sperm progress through the epididymis (Fisher and Aitken, 1997). This group has also detected superoxide generation in epididymal sperm. Regulation occurs through a balance of ROS generation and the protective influence of preventative, chain-breaking and scavenger antioxidants, whose enzyme activity in testes has been well documented (Peltola et al., 1992; Ochsendorf, 1999; Mruk et al., 2002; Fujii et al., 2003). With this innate redox potential, an accumulation of sperm in the epididymis following vasectomy may lead to deregulation of ROS generation, causing oxidative stress. Sperm after vasectomy reversal (Shapiro et al., 1998) has been shown to have higher ROS levels than semen of fertile men. In further support, in another study in which testicular damage was induced in rats by unilateral torsion, levels of superoxide dismutase and catalase were reduced in the ipsilateral testis but were not altered in the contralateral testis (Saba et al., 1997).

Another study (Aydos et al., 1998), on the generation of ROS in vasectomized rat testes and its relationship with the histological alterations in the testis following vasectomy, showed that output of malondialdehyde (a biomarker for oxidative stress) from vasectomized rats had a direct correlation with tissue destruction. This may be due to a reduced total antioxidant capacity (TAC), since Kolettis et al. (1999) found that the seminal ROS-TAC score (a novel indicator of oxidative stress) was lower in men who were infertile after vasectomy reversal rather than in those who were fertile after vasectomy reversal. This is supported by a study reporting a reduction in key antioxidants in seminal plasma of infertile men from our group (Lewis et al., 1995).

A final mechanism for spermatogenic impairment could be elevated rates of apoptosis. Apoptosis plays an important role in the normal testis (Lee et al., 1997; Rodriguez et al., 1997), eliminating 50–70% of germ cells at different stages of spermatogenesis prior to spermiogenesis (Billig et al., 1995; Henriksen et al., 1995). Apoptosis in the testis can be initiated through the Fas/FasL pathway (Lee et al., 1997) and p53 upregulation (Yin et al., 2002). It is regulated by many protein interactions, including those of the Bcl-2 family, before the cells commit to the process via the caspases and endonucleases. The p53–Bax pathway has been shown to increase in the vasectomized rat, and the Bax:Bcl-2 ratio has also been shown to be significantly increased 8 weeks after vasectomy (Shiraishi et al., 2001). Lue et al. (1997) have even reported a significant increase in germ cell apoptosis 3 weeks after vasectomy when the spermatocytes are dividing (stage XIII) in hamsters.

In this study we observed no reduction in embryo quality at day 2 or day 3 in Groups 2 or 3 (CES) (Steer et al., 1992). This is to be expected as Braude et al. (1998) have shown that the paternal genome does not influence embryo cleavage until after the third cleavage division. Further, sperm quality is almost irrelevant for fertilization when the ICSI procedure is used (Twigg et al., 1998).

One confounding factor in this study might be the difference in age between groups: the mean age of men was 46 years in Group 1 and 37 in Group 2. However, we do not believe this to be the case. There is little in the literature, in terms of sperm quantity or quality, to suggest that men <50 years have impaired spermatogenesis. Limited data are available; most studies have chosen greater or less than 50 years arbitrarily but the ranges in each group are usually very wide [31–40 versus 41–50 versus 51–64 (Gallardo et al., 1996) and 30–39 versus 40–49 (Brzechffa et al., 1998)]. In 2001, Kidd et al. (2001) conducted a comprehensive review of all human age and fecundity status studies between 1980 and 1999. Of the 21 studies comparing sperm concentration with increasing age, five reported decreases, six found little or no association and eight reported an increase in sperm concentration with age. Few of the studies controlled for the duration of abstinence or other potential confounding factors. Thus Kidd et al. (2001) concluded that the weight of evidence from the literature did not suggest that increased male age is associated with a significant reduction in sperm count.

This is also the first study to assess the clinical pregnancy rate of the partners of men who have undergone a vasectomy using TESA followed by ICSI. We found a significant reduction...
(from 28 to 9%) in the clinical pregnancy rate of the partners of the men who had had a vasectomy ≥10 years relative to those of men with OA from other aetiologies. Our work confirms that of Abdelmassih et al. (2002), who have recently reported a negative correlation between pregnancy rates after ICSI using epididymal sperm and an increasing time interval after vasectomy. This is supported by Borges et al. (2003), who used sperm retrieved from the epididymis followed by ICSI after vasectomies 14 years previously. They reported an increase in miscarriage rates. Further, Kubota (1969) reported degenerative changes in the spermatids and sperm of vasectomized men. All these studies suggest that the quality of post-vasectomy sperm may be compromised and that they are less capable of achieving a pregnancy.

The results reported in this paper raise significant concerns about the effects of vasectomy on both testicular and spermatogenic function. We would conclude that the success of assisted conception is compromised when using sperm from men vasectomized more than 10 years previously. Further multicentre studies are needed to assess the appropriateness and efficiency of fertility treatments for this patient group.

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