Effect of varicocelectomy on the abnormal retention of residual cytoplasm by human spermatozoa

A.Zini¹, M.Buckspan, M.Jamal and K.Jarvi

Division of Urology, Department of Surgery, Mount Sinai Hospital, University of Toronto, 600 University Avenue, Suite 455, Toronto, Ontario, Canada, MSG 1X5

¹To whom correspondence should be addressed

Abnormal retention of cytoplasmic residues by human spermatozoa is associated with the generation of reactive oxygen species (ROS) in semen and defective sperm function. We have examined the effect of varicocelectomy on the retention of residual cytoplasm by human spermatozoa. Clinical reports of 43 men who underwent microsurgical varicocelectomy at our institution during a 1 year period beginning July 1996 were reviewed. Standard semen parameters (concentration, motility and morphology) and residual cytoplasm retention (monitored by Papanicolaou stain) were assessed before and 6 months after varicocelectomy. The percentage of spermatozoa with residual cytoplasm decreased significantly following varicocelectomy compared to pre-operatively (25.8 versus 18.1% respectively). The percentages of motile spermatozoa and normal forms increased significantly (P = 0.0003, P = 0.005 respectively) following varicocelectomy (22.6 versus 32.9% and 46.4 versus 54.4% respectively). Our data suggest that varicocelectomy can improve the disposal of residual sperm cytoplasm by the testis and/or epididymis in infertile men with varicocele. These data also suggest that varicocelectomy reduces the potential for ROS generation by human spermatozoa in these men.

Key words: cytoplasmic droplet/residual cytoplasm/spermatozoa/varicocele/varicocelectomy

Introduction

Spermatogenesis is an orderly process whereby male germ cells in the testis pass through sequential phases of differentiation to develop subsequently into mature spermatozoa (Clermont, 1972). During the process of spermiogenesis (differentiation of the haploid spermatid to a mature spermatozoon), extensive nuclear remodelling and cytoplasmic reduction occur (Clermont, 1972). Any residual cytoplasm is then normally removed from spermatozoa prior to their release from the germinal epithelium of the testis (spermatiation) or during early epididymal transit (Clermont, 1972; Cooper, 1986). Failure to release excess cytoplasm results in retention of a cytoplasmic mass in the mid-piece region of the spermatozoon.

Recent work has shown that spermatozoa with retained excess cytoplasm have defective sperm function (Aitken et al., 1994; Gomez et al., 1996; Keating et al., 1997). This excess cytoplasm contains a number of enzymes, many of which are probably detrimental to normal sperm function (Huszar and Vigue, 1993, 1994; Gomez et al., 1996). It has been shown that the retention of residual sperm cytoplasm is negatively correlated with sperm motility (Gomez et al., 1996; Zini et al., 1998). It has also been reported (Gomez et al., 1996) that in a subset of men with unproven fertility, the retention of residual sperm cytoplasm is positively correlated with reactive oxygen species (ROS) production in semen (Gomez et al., 1996). This has important clinical implications because semen ROS have been clearly shown to impair multiple aspects of sperm function, including fertilizing capacity (Aitken et al., 1989, 1991; Iwasaki and Gagnon, 1992; Aitken and Fisher, 1994).

The effect of adult varicocelectomy on male fertility remains controversial. However, it is reported that overall, varicocele repair results in improved semen quality in 60–80% of infertile men (Schlesinger et al., 1993). These data suggest that spermatogenesis is improved following varicocele repair. However, it is not known whether varicocele repair can specifically influence the normal disposal of residual sperm cytoplasm in the testis and/or epididymis. As such, the purpose of this study was to examine the possible effect of varicocelectomy on the retention of residual sperm cytoplasm.

Materials and methods

The clinical reports were reviewed of men presenting for infertility evaluation at our andrology clinic from July 1996 to July 1997. A total of 43 consecutive men who underwent varicocelectomy for clinically detectable varicocele was identified. None of the patients was azoospermic or had evidence of genital infection. All men underwent microsurgical subinguinal varicocelectomy as previously described (Goldstein et al., 1992). All procedures were performed by the same surgeon (A.Z.). The pre-operative evaluation included a history, physical examination, scrotal ultrasound and a standard semen analysis including a Papanicolaou smear. At 6 months (range 4–8) after varicocelectomy another semen analysis was obtained.

Semen samples were obtained by masturbation after 3–5 days of sexual abstinence. After liquefaction of semen, standard semen parameters (volume, concentration, motility, morphology) were obtained according to World Health Organization (WHO, 1992) guidelines. Retention of residual cytoplasm was assessed by examination of the Papanicolaou sperm smear microscopically (magnification ×400) for the presence of light blue-green cytoplasmic staining along the sperm mid-piece or tail. A mid-piece cytoplasmic droplet greater than one-third the size of the sperm head was considered significant (WHO, 1992). A minimum of 200 spermatozoa was examined per sample. The evaluation of semen analyses (including assessment of sperm smears) was blinded.

Differences between the pre- and post-varicocelectomy parameters
with residual cytoplasm (prior to varicocelectomy) and the magnitude of reduction in cytoplasm retention following varicocelectomy ($r = 0.67$, $P < 0.01$).

**Discussion**

The association between a clinical varicocele and impaired spermatogenesis is well described (Dubin and Hotchkiss, 1969; Johnsen and Agger, 1978; Terquem and Dadoune, 1981). Most studies have reported varying degrees of hypospermatogenesis, as well as Sertoli cell changes and premature sloughing of germ cells into the seminiferous tubule lumen. These histological changes are manifested by the well-described semen abnormalities associated with varicocele (Macleod, 1965). Although several studies have reported improved semen parameters and pregnancy rates after varicocele repair (Laven et al., 1992; Madgar et al., 1995), other studies have not shown a beneficial effect (Nilsson et al., 1979; Nieschlag et al., 1998). Thus, the effect of varicocelectomy on male fertility potential remains controversial.

The present study was designed to evaluate the ability of varicocelectomy to improve the normal disposal of residual sperm cytoplasm by the testis and/or epididymis. We have observed a decrease in the mean percentage of spermatozoa with residual cytoplasmic droplets following microsurgical varicocelectomy. This was also accompanied by an increase in the percentage of sperm motility and the percentage of normal forms (WHO, 1992). Based on work by Gomez et al. (1996), the observed reduction in residual sperm cytoplasm retention would translate into improved sperm function (i.e. increased sperm motility) and a decreased potential to generate ROS and other cytotoxic products (Gomez et al., 1996). In theory, this may also reduce the risk of oxidative spermatozoa DNA damage and the possible transmission of genetic mutations (Twigg et al., 1998).

There is preliminary evidence to suggest that varicocele is associated with increased production of reactive oxygen species (ROS). It has been reported (Wesse et al., 1993) that fertile men with varicocele have an increased level of stimulated ROS production in semen compared to men without varicocele (Wesse et al., 1993). It has been demonstrated (Lenzi et al., 1993) that men with varicocele and germ-free genital tract inflammation can benefit from antioxidant therapy (glutathione), suggesting that these men produce high amounts of ROS (Lenzi et al., 1993). These authors have also shown that it is specifically the oxidative sperm membrane damage that is partially reversed by glutathione therapy in men with varicocele (Lenzi et al., 1994).

There is indirect and direct evidence to suggest that monitoring the retention of residual sperm cytoplasm may be used to predict fertility in vitro and in vivo. It has been shown that the retention of residual sperm cytoplasm is correlated with ROS in semen, and that these ROS levels are negatively correlated with sperm fertilizing capacity (Aitken et al., 1991; Alvarez et al., 1996). It has been demonstrated that semen ROS levels are negatively correlated with hamster oocyte penetration test scores and pregnancy rates in vivo (Aitken et al., 1991). Recently, it has been demonstrated that the

### Results

Of the 43 men who underwent varicocelectomy, 30 were evaluable. Of these, 17 underwent left and 13 bilateral varicocelectomy. The mean age of the 30 men was 34.5 years (range 28–46) with a mean duration of infertility of 2.2 years (range 1–8); 24 men presented with primary infertility and six had secondary infertility. The 13 men excluded from this study did not return for their post-varicocelectomy semen analysis.

An example of a spermatozoon with retained cytoplasm is shown in Figure 1. The mean (±SD) percentages of spermatozoa with residual cytoplasm before and at a mean of 6 months following microsurgical varicocelectomy are shown in Table I (25.8 ± 11.8 versus 18.1 ± 9.2% respectively; $P = 0.0003$). The percentage of motile spermatozoa increased significantly following varicocelectomy ($P = 0.003$). In addition, the percentage of normal forms (WHO, 1992) increased significantly after varicocelectomy ($P = 0.005$). However, the sperm concentration did not change significantly following varicocelectomy (Table I).

We detected a significant negative correlation between the percentage of spermatozoa with residual cytoplasm and sperm motility ($r = -0.36$, $P = 0.02$). We also observed a significant positive correlation between the percentage of spermatozoa with residual cytoplasm (prior to varicocelectomy) and the magnitude of reduction in cytoplasm retention following varicocelectomy ($r = 0.67$, $P < 0.01$).

**Table I.** Semen parameters before and after microsurgical varicocelectomy

<table>
<thead>
<tr>
<th></th>
<th>Pre-operative</th>
<th>Post-operative</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% spermatozoa with residual cytoplasm</td>
<td>25.8 ± 11.8</td>
<td>18.1 ± 9.2</td>
<td>0.0003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sperm concentration ($\times 10^6$/ml)</td>
<td>34.8 ± 41.4</td>
<td>33.6 ± 55.9</td>
<td>0.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% motile spermatozoa</td>
<td>22.6 ± 11.0</td>
<td>32.9 ± 19.2</td>
<td>0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>% normal forms (WHO, 1992)</td>
<td>46.4 ± 14.7</td>
<td>54.4 ± 14.5</td>
<td>0.005&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD.
<sup>a</sup>Paired $t$-test.
<sup>b</sup>Wilcoxon signed-ranks test.

were estimated by parametric and non-parametric tests as appropriate. Pearson’s correlation coefficient was used to evaluate the association between individual parameters (SAS Institute, Cary, NC, USA).

**Discussion**

The association between a clinical varicocele and impaired spermatogenesis is well described (Dubin and Hotchkiss, 1969; Johnsen and Agger, 1978; Terquem and Dadoune, 1981). Most studies have reported varying degrees of hypospermatogenesis, as well as Sertoli cell changes and premature sloughing of germ cells into the seminiferous tubule lumen. These histological changes are manifested by the well-described semen abnormalities associated with varicocele (Macleod, 1965). Although several studies have reported improved semen parameters and pregnancy rates after varicocele repair (Laven et al., 1992; Madgar et al., 1995), other studies have not shown a beneficial effect (Nilsson et al., 1979; Nieschlag et al., 1998). Thus, the effect of varicocelectomy on male fertility potential remains controversial.

The present study was designed to evaluate the ability of varicocelectomy to improve the normal disposal of residual sperm cytoplasm by the testis and/or epididymis. We have observed a decrease in the mean percentage of spermatozoa with residual cytoplasmic droplets following microsurgical varicocelectomy. This was also accompanied by an increase in the percentage of sperm motility and the percentage of normal forms (WHO, 1992). Based on work by Gomez et al. (1996), the observed reduction in residual sperm cytoplasm retention would translate into improved sperm function (i.e. increased sperm motility) and a decreased potential to generate ROS and other cytotoxic products (Gomez et al., 1996). In theory, this may also reduce the risk of oxidative spermatozoa DNA damage and the possible transmission of genetic mutations (Twigg et al., 1998).

There is preliminary evidence to suggest that varicocele is associated with increased production of reactive oxygen species (ROS). It has been reported (Wesse et al., 1993) that fertile men with varicocele have an increased level of stimulated ROS production in semen compared to men without varicocele (Wesse et al., 1993). It has been demonstrated (Lenzi et al., 1993) that men with varicocele and germ-free genital tract inflammation can benefit from antioxidant therapy (glutathione), suggesting that these men produce high amounts of ROS (Lenzi et al., 1993). These authors have also shown that it is specifically the oxidative sperm membrane damage that is partially reversed by glutathione therapy in men with varicocele (Lenzi et al., 1994).

There is indirect and direct evidence to suggest that monitoring the retention of residual sperm cytoplasm may be used to predict fertility in vitro and in vivo. It has been shown that the retention of residual sperm cytoplasm is correlated with ROS in semen, and that these ROS levels are negatively correlated with sperm fertilizing capacity (Aitken et al., 1991; Alvarez et al., 1996). It has been demonstrated that semen ROS levels are negatively correlated with hamster oocyte penetration test scores and pregnancy rates in vivo (Aitken et al., 1991). Recently, it has been demonstrated that the
retention of residual sperm cytoplasm (monitored by Papanicolaou stain) is highly negatively correlated with fertilization rates in vitro (Keating et al., 1997). Taken together with the demonstration that the retention of residual sperm cytoplasm is negatively correlated with sperm fertilizing capacity, the results of this study provide a possible mechanism for the observed improvement in pregnancy rates after varicocelectomy repair. However, the lack of a fertile control group in this study limits our ability to comment on the possible correlation between the retention of residual sperm cytoplasm and male fertility.

In summary, we have shown that in infertile men with varicocele, varicocelectomy can improve the normal disposal of residual sperm cytoplasm in the testis and/or epididymis. These data suggest that varicocelectomy reduces the potential for ROS generation by human spermatozoa in these infertile men. These data provide an additional mechanism for the reported improvement in pregnancy rates after varicocelectomy repair.

References

Received on November 13, 1998; accepted on March 11, 1999