Induction of spermatogenesis in azoospermic men after internal spermatic vein embolization for the treatment of varicocele

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BACKGROUND: To evaluate the improvement in semen quality and pregnancy rate after internal spermatic vein (ISV) embolization in men with nonobstructive azoospermia virtual azoospermia, or extremely severe oligotesta-theno-azooospermia (OTA). METHODS: A prospective cohort of 101 azoospermic or severe oligotesta-theno-azooospermic men of mean (±SD) age 34.1 ± 7.7 years who underwent ISV between September 1998 and June 2003 were evaluated for semen characteristics, endocrinology profile, and conception rate. RESULTS: Significant improvement was noted in mean sperm concentration, motility, and morphology in 83 men (82%). Mean sperm concentration increased from 0.22 ± 0.30 x 10⁶/ml total sperm in the ejaculate to 9.28 ± 1.2 x 10⁶/ml after embolization (P < 0.001); mean sperm motility rose from 8.78 ± 1.59 to 29.56 ± 2.0% (P < 0.001), and mean sperm morphology rose from 3.79 ± 0.74 to 13.72 ± 1.37% (P < 0.005). Pregnancy was achieved in 34 cases (34%), 20 (20%) unassisted and 14 (14%) assisted. CONCLUSIONS: Based on our findings, the following statements can be made: (i) Varicocele may cause any variation of severity in OTA, including azoospermia. (ii) Since male fertility is preserved with only one testis, OTA, azoospermia or virtual azoospermia represent bilateral testicular dysfunction. (iii) Treatment of bilateral varicocele may reverse testicular dysfunction and improve spermatogenesis and testosterone production in men with extremely severe OTA and induce sperm production in men with azoospermia and virtual azoospermia. (iv) If azoospermia is not too long-standing, the treatment of varicocele may significantly improve spermatogenesis and renew sperm production. (v) Adequate treatment may spare in >50% of azoospermic patients the need for testicular sperm extraction as preparation for ICSI. (vi) Since achievement of pregnancy in IVF units is higher when spermatogenesis is better, the treatment of varicocele (bilateral) is an effective medical adjunct for the IVF units prior to the treatment. We recommend that infertile men with azoospermia or virtual azoospermia or extremely severe OTA be evaluated for varicocele, with special attention to its bilateral nature.

Key words: azoospermia/embolization/oligozoospermia/sperm/varicocele

Introduction

Varicocele is a major cause of male infertility (Greenberg, 1977; Gonzalez et al., 1983; Saypol, 1986; Gorelick and Goldstein, 1993; Gat et al., 2003). Recent improvements in the diagnosis of the disorder with the introduction of colour Doppler ultrasonography, thermography and internal spermatic vein (ISV) venography have raised the incidence to 40% in men with primary infertility and up to 80% in men with secondary infertility (Gorelick and Goldstein, 1993). Varicocele causes a progressive deterioration in semen quality and testicular function, ranging from oligozoospermia to complete azoospermia (Saypol, 1986; Gorelick and Goldstein, 1993; Matthews et al., 1998; Kadioglu et al., 2001). The benefits of varicocele repair to sperm concentration, motility, and morphology have been well established in oligozoospermic men (Madgar et al., 1995), but its effect in azoospermic men remains unclear. Although two small series yielded poor results in this patient group (Mehan, 1976; Czaplicki et al., 1979), several others reported improved sperm parameters after microsurgical varicocelectomy in 40–60% of men with azoospermia or severe oligoasthenoteratospermia (OTA) (Matthews et al., 1998; Kim et al., 1999; Kadioglu et al., 2001; Pasqualotto et al., 2003). The vast majority of these patients were treated bilaterally. The fact that both OTA and azoospermia indicate bilateral testicular dysfunction is in agreement with recent reports that...
varicocele is mainly a bilateral disease (Gat et al., 2003, 2004a). This assumption is further supported by reports of pregnancies in partners of azoospermic men after varicocele repair (Kim et al., 1999; Kadioglu et al., 2001; Pasqualotto et al., 2003). The aim of the present study was to evaluate whether it is feasible to improve semen quality and conception rate after ISV embolization in men with non-obstructive azoospermia, virtual azoospermia or extremely severe OTA. To our knowledge, the present study is the first large series characterizing treatment outcome after ISV embolization in men with azoospermia or extremely severe OTA.

Materials and methods

Patients

From September 1998 to June 2003, 101 men patients aged 18–55 years (mean ± SD 34.1 ± 7.7) with azoospermia or virtual azoospermia and extremely severe OTA were offered ISV embolization for the treatment of varicocele in our tertiary referral centre. A minimum of ≥12 months duration of infertility was required for enrolment in the study. All patients underwent a basic infertility evaluation, including a detailed history, complete physical examination, hormone profile and genetic testing if needed (karyotype or Y microdeletion). Possible obstructive factor was ruled out in all cases by normal volume of ejaculate, elevated levels of FSH, and absence of epididymitis in the past medical history or by palpation. Patients with cryptorchidism or testicular trauma, and patients after surgery of the urogenital tract, and those with genetic problems were excluded from the study. The patients’ female partners were aged 20–38 years; all had normal ovulation and normal findings on pelvic imaging. All patients gave informed consent to participate prior to the procedure.

Procedure

Patients were examined in a warm room after standing for 5 min. The volume, position and consistency of the testes and epididymis were checked, and each spermatic cord was palpated in the standing position and during the Valsalva manoeuvre. Findings were graded according to the system of Dubin and Amelar (1971) as follows: grade I, varicocele palpable only during Valsalva manoeuvre; grade II, varicocele palpable in standing position; grade III, varicocele detectable by visual scrutiny alone. All patients underwent contact thermography using a flexible liquid crystal thermostrip ‘Varicoscreen’ (FertiPro; Beernem, Belgium). ISV sclerotherapy was performed after venography as described in the study of Gat et al. (2004a).

Evaluation

Seminal analysis was performed twice before embolization and at least twice 4, 6, 9 or 12 months after (except for nine patients who agreed to only one analysis before, and one 6 months after, embolization). Patients were instructed to abstain from sexual intercourse for 3 days before semen collection. The samples were assessed within 1 h of collection for sperm concentration, motility and morphology according to the World Health Organization criteria (Aboughar, 1997). The pre- and post-procedural seminal values were averaged separately.

Endocrinological evaluation was performed before embolization and at ≥6 weeks after, including assays of serum FSH, LH, testosterone and free testosterone. Testosterone was measured in all cases between 08:00 and 10:00. The normal ranges at our laboratory are as follows: FSH, 1–8 mIU/l; LH, 4.9–25 IU/l; testosterone 8.5–38.8 nmol/l; free testosterone, 4.3–14.9 nmol/l.

Statistical analysis

Two-sample paired Student’s t-test was used for statistical analyses. Values were expressed as means ± SD. Type I error α = 0.05 and P < 0.05 were considered significant.

Results

The study group consisted of 32 men with azoospermia, 31 men with virtual azoospermia (sperm concentration ≤0.1 × 10^6), and 38 men with extremely severe OTA (sperm concentration ≤1 × 10^6). Varicocele was detected by non-invasive methods and confirmed by venography in all 101 patients. Ninety men (89%) underwent a bilateral procedure (28 with azoospermia, 26 with virtual azoospermia, and 36 with extremely severe OTA), nine (8.9%) a unilateral left-sided procedure, and three (2.9%) a unilateral right-sided procedure.

Table 1 shows the changes in sperm parameters for the whole cohort and by group. In the azoospermia group, significant improvement was noted in mean (± SD) sperm concentration, motility and morphology in 18 of the 32 men (56.2%). Mean sperm concentration increased from zero total sperm in the ejaculate to 3.81 ± 1.69 × 10^6/ml after embolization (P < 0.03); mean sperm motility rose to 1.20 ± 0.362% (P < 0.001), and mean sperm morphology to 8.30 ± 2.64 (P < 0.005). A mean post-embolization sperm count of >1 × 10^6 was achieved in seven men (22%), and of >5 × 10^6 in four men (13%).

Four of these 18 patients with improvement in semen quality relapsed into azoospermia 2–5 months after the induction of spermatogenesis.

In the virtual azoospermia group, significant improvement in sperm parameters was noted in 29 of the 32 men (94%). Mean sperm concentration increased from 0.054 ± 0.007 × 10^6/ml sperm in the ejaculate to

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-embolization</th>
<th>Post-embolization</th>
<th>P</th>
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<tbody>
<tr>
<td>All patients (n = 101)</td>
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<tr>
<td>Testosterone (nmol/l)</td>
<td>9.81 ± 5.67</td>
<td>18.23 ± 8.09</td>
<td>&lt;0.001</td>
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<td>Free testosterone (nmol/l)</td>
<td>5.10 ± 2.37</td>
<td>10.59 ± 5.13</td>
<td>&lt;0.001</td>
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<td>Sperm count (×10^6/ml)</td>
<td>0.22 ± 0.30</td>
<td>9.28 ± 1.2</td>
<td>&lt;0.001</td>
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<td>Motility (%)</td>
<td>8.78 ± 1.59</td>
<td>29.56 ± 2.0</td>
<td>&lt;0.001</td>
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<tr>
<td>Morphology (%)</td>
<td>3.79 ± 0.74</td>
<td>13.72 ± 1.37</td>
<td>&lt;0.001</td>
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<tr>
<td>Azoospermia (n = 32)</td>
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<tr>
<td>Sperm count (×10^6/ml)</td>
<td>0</td>
<td>3.81 ± 1.69</td>
<td>&lt;0.03</td>
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<tr>
<td>Motility (%)</td>
<td>0</td>
<td>1.20 ± 3.62</td>
<td>&lt;0.001</td>
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<tr>
<td>Morphology (%)</td>
<td>0</td>
<td>8.30 ± 2.64</td>
<td>&lt;0.005</td>
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<tr>
<td>Virtual azoospermia (n = 31)</td>
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<tr>
<td>Sperm count (×10^6/ml)</td>
<td>0.054 ± 0.007</td>
<td>10.31 ± 1.87</td>
<td>&lt;0.001</td>
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<tr>
<td>Motility (%)</td>
<td>6.07 ± 2.69</td>
<td>35.8 ± 2.76</td>
<td>&lt;0.001</td>
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<tr>
<td>Morphology (%)</td>
<td>1.96 ± 0.56</td>
<td>15.25 ± 2.10</td>
<td>&lt;0.001</td>
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<tr>
<td>Extremely severe oligozoospermia (n = 38)</td>
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<tr>
<td>Sperm count (×10^6/ml)</td>
<td>0.54 ± 0.04</td>
<td>12.11 ± 1.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>13.96 ± 3.06</td>
<td>33.24 ± 3.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>4.90 ± 1.03</td>
<td>15.85 ± 2.28</td>
<td>&lt;0.001</td>
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10.31 \pm 1.87 \times 10^9/ml after embolization (P < 0.001); mean sperm motility rose from 6.07 \pm 2.69 to 35.8 \pm 2.76% (P < 0.001), and mean sperm morphology from 1.96 \pm 0.56 to 15.25 \pm 2.10% (P < 0.001). A mean post-embolization sperm count of \( >1 \times 10^8 \) was achieved in 23 men (74%), and of \( >5 \times 10^6 \) in 17 men (55%).

In the extremely severe OTA group (sperm concentration up to \( 1 \times 10^5 \)), significant improvement was noted in mean (\( \pm SD \)) sperm concentration, motility and morphology in 36 of the 38 men (94.7%). Mean sperm concentration increased from 0.54 \pm 0.04 total sperm in the ejaculate to 12.11 \pm 1.85 \times 10^6/ml after embolization (P < 0.001); mean sperm motility rose from 13.96 \pm 3.06 to 33.24 \pm 3.13% (P < 0.001), and mean sperm morphology from 4.9 \pm 1.03 to 15.83 \pm 2.28% (P < 0.001). A mean post-embolization sperm count of \( >5 \times 10^6 \) was documented in 22 men (58%).

Mean serum testosterone concentration for the study group was 9.81 \pm 5.67 nmol/l (range 2–27 nmol/l) pre-operatively and 18.23 \pm 8.09 nmol/l (range 3–37 nmol/l) after ISV embolization (P < 0.001), for an increase of 86%. Corresponding values of mean serum free testosterone were 5.10 \pm 2.37 nmol/l (range 1–12 nmol/l) and 10.59 \pm 5.13 nmol/l (range 4–23 nmol/l) (P < 0.001), for an increase of 108% (Figure 1). Mean serum FSH concentration for the study group was 5.67 nmol/l (range 2–27 nmol/l) pre-operatively and 8.09 nmol/l (range 3–37 nmol/l) after ISV embolization (data not shown).

In 34 cases (34%), the patient’s partner became pregnant after ISV embolization. Twenty of the pregnancies (19.8%) were unassisted and 14 (13.8%) were assisted, 4 with intrauterine insemination (IUI), and 10 with intracytoplasmic sperm injection (ICSI). All ended in a live birth. By group, there were nine pregnancies (26%) in the azoospermic group, four (12%) unassisted and five (15%) by ICSI (one was a twin pregnancy), and 12 (35%) in the virtual azoospermia group, 8 (24%) unassisted and 4 (11%) assisted. In the severe OTA group, there were 13 pregnancies (34%), 8 unassisted (24%) and 5 (15%) assisted.

### Discussion

Azoospermia is considered a significant barrier to unassisted pregnancy. Nowadays, the only option for men with azoospermia is testicular sperm extraction (TESE) with ICSI (Belenky et al., 2001).

There is growing evidence that patients with varicocele-induced severe OTA and even azoospermia may benefit from varicocele repair (Matthews et al., 1998; Kim et al., 1999; Kadioglu et al., 2001; Pasqualotto et al., 2003), though the findings remain controversial. Tulloch (1952) was the first to report a spontaneous post-varicocelectomy pregnancy in a couple with an azoospermic male. Since then, varicocelectomy has become the most commonly performed surgery in the treatment of male infertility. Several studies reported that varicocelectomy improved serum testosterone level and spermatogenesis (Su et al., 1995; Cayan et al., 1999); however, in other studies, these findings were not statistically significant (Evers and Collins, 2003). In a recent report on IUI, Daitch et al. (2001) found that varicocele repair improved pregnancy and live birth rates in couples in which the male had varicocele and the woman was healthy. On the other hand, in their systematic review, Evers and Collins (2003) stated in a comprehensive meta-analysis that ‘Varicocele repair does not seem to be an effective treatment for male subfertility.’ Their stated findings are not surprising, since nearly all the patients in their meta-analysis were treated according to the usual practice, on the left side only. A recent study demonstrated that varicocele is a predominantly bilateral disease (Gat et al., 2004a) and the diagnosis of bilaterality is missed by physical examination in >90% of the cases (Gat et al., 2004b). In essence, the patients in Evers’ meta-analysis were treated only partially.

The present study agrees with previous reports indicating that men with azoospermia, virtual azoospermia or extremely severe OTA can benefit from varicocele repair. In our series, semen parameters were improved in 83 of 101 patients (82%), (Figure 1) and 34% of the couples achieved pregnancy, 58% of them unassisted. A positive effect of varicocelectomy in this patient population also has important implications for assisted reproduction technologies. First, even modest improvements in sperm motility and quality in the ejaculate can reduce the need for TESE by open or needle biopsy, an invasive and potentially damaging procedure, in ICSI procedures (Aboulghar et al., 1997; Belenky et al., 2001). Second, in men with spermatogenic failure, freshly ejaculated sperm are easier to use, and fertilization ability in ICSI is higher with normal semen than with sperm retrieved by TESE (Aboulghar et al., 1997).
In our earlier study, we observed that bilateral varicocele was present in 81% of 255 consecutive infertile men presenting for infertility evaluation (Gat et al., 2004a). In the present series, 90 of the 101 patients had bilateral disease: 28 of 32 men (87.5%) with azoospermia, 26 of 31 men (84%) with virtual azoospermia, and 35 of 38 men (92%) with extremely severe OTA. The difference in rates of bilaterality between the three groups was not statistically significant. Similar findings were noted by Matthews et al. (1998), namely, an 82% rate of bilateral varicocele in 78 infertile patients: 77% for those with severe OTA and 84% for those with azoospermia. Accordingly, Kadioglu et al. (2001) reported that all the azoospermic men in their series with bilateral high-grade (2 or 3) varicocele improved after bilateral surgery, and all the spontaneous pregnancies that occurred were in this subgroup.

In the present study, semen quality improved significantly in 18 patients (56.2%) in the azoospermia group, 29 patients (94%) in the virtual azoospermia group, and 36 patients (94.7%) in the extremely severe OTA group. Mean post-embolization counts of $>1 \times 10^6$ were achieved in seven azoospermic men (22%) and 23 virtual azoospermic (74%) men, and of $>5 \times 10^6$ in four men (13%) and 17 men (55%) respectively. In the extremely severe OTA group, a count of $>5 \times 10^6$ was achieved in 22 men (58%). Thirty-four pregnancies (34% of cases) were documented, including 20 unassisted. Thus, 56.2% of our patients were spared TESE procedures, which is usually the initial therapeutic option in azoospermic men.

Although we had no control group, our results can be compared to those of Madgar et al. (1995), who reported only a 10% spontaneous pregnancy rate at 12 months in partners of men with OTA and untreated unilateral left-side varicocele. Moreover, our results are in agreement with those of Matthews et al. (1998) who reported that after microsurgical varicocelectomy, 55% of the 78 infertile men in their series had motile sperm in the ejaculate, and the partners of 24 (31%) became pregnant (15 unassisted and nine assisted). Separate analysis of the azoospermia/zero motile sperm group yielded a 69% rate of patients with motile sperm and a 24% pregnancy rate (seven unassisted, five assisted). Among the improved patients, elevated serum FSH level (up to 150% above normal) or smaller testicular volume (but with elastic consistency) did not block the induction of spermatogenesis and OTA grade was not associated with the outcome. Kim et al. (1999) demonstrated motile sperm in the ejaculate in 12 of 28 patients (43%) with complete azoospermia after microsurgical inguinal varicocelectomy, but no pregnancies by natural intercourse within 24 months. Others reported a 20.8% rate of motile sperm in the ejaculate in 24 men with complete azoospermia treated by microsurgical inguinal varicocelectomy repair (Kadioglu et al., 2001), and induction of spermatogenesis in seven of 15 azoospermic men (47%) after treatment (Pasqualotto et al., 2003).

In our study, the large majority of patients responded favourably to ISV embolization in terms of concentrations of serum testosterone (86% increase) and free testosterone (108% increase). Microsurgical techniques for varicocele, such as our procedure, successfully occlude all potential collateral venous channels (Kunnen and Comhaire, 1992) have similarly successful results. Su et al. (1995) reported a 28% increase in serum testosterone concentrations (from 319 ± 12 to 409 ± 23 ng/dl) in 53 infertile men with varicocele, and Cayan et al. (1999) reported a 49% increase in serum testosterone (from 5.63 to 8.37 ng/ml) and 42% increase in free testosterone (from 23.1 to 32.8 pg/ml) in 78 infertile patients. Comhaire and Vermeulen (1975) were the first to demonstrate significant increase in serum testosterone in patients with sexual inadequacy and infertility. These data are further supported by our previous study, demonstrating elevation of testosterone after bilateral embolization of spermatic veins (Gat et al., 2004c).

It should be noted that once sperm is detected in the semen analysis after varicocelectomy repair, the patient needs to be warned of the possibility of relapse and offered the option of sperm cryopreservation.

Based on our findings, the following statements can be made. (i) Varicocele may cause any variation of severity in OTA, including azoospermia. (ii) Since male fertility is preserved with only one testis, OTA, azoospermia or virtual azoospermia represent bilateral testicular dysfunction. (i) Treatment of bilateral varicocele may reverse testicular dysfunction and improve spermatogenesis and testosterone production in men with extremely severe OTA and induce sperm production in men with azoospermia. (iv) If azoospermia is not too long-standing, the treatment of varicocele may significantly improve spermatogenesis and renew sperm production. (v) Adequate treatment may spare the need for TESE as preparation for ICSI in >50% of azoospermic
patients. (vi) Since achievement of pregnancy in IVF units is higher when spermatogenesis is better, the treatment of varicocele (bilateral) is an effective medical adjunct for IVF units prior to the treatment.

We recommend that infertile men with azoospermia or virtual azoospermia or extremely severe OTA be evaluated for varicocele, with special attention to its bilateral nature.

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