Clinical Outcome of Intracytoplasmic Sperm Injection in Infertile Men With Treated and Untreated Clinical Varicocele

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Abbreviations and Acronyms

2PN = 2 pronuclei
ART = assisted reproductive technology
ICSI = intracytoplasmic sperm injection
IVF = in vitro fertilization
IUI = intrauterine insemination

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Purpose: We evaluated the impact of varicocelectomy on intracytoplasmic sperm injection outcomes in infertile men with clinical varicocele.

Materials and Methods: We studied 242 infertile men with a history of clinical varicocele who underwent intracytoplasmic sperm injection. Of the men 80 underwent prior subinguinal microsurgical varicocelectomy (treated group 1) and 162 had any grade of clinical varicocele (untreated group 2) at sperm injection. We compared semen analysis results before and after varicocelectomy, and the sperm injection procedure outcomes. Mean time from surgery to sperm injection was 6.2 months. Logistic regression was done to verify whether varicocelectomy influenced the odds of clinical pregnancy, live birth and miscarriage.

Results: We noted an improved total number of motile sperm ($6.7 \times 10^6$ vs $15.4 \times 10^6$, $p < 0.01$) and a decreased sperm defect score (2.2 vs 1.9, $p = 0.01$) after vs before varicocele repair. The clinical pregnancy (60.0% vs 45.0%, $p = 0.04$) and live birth (46.2% vs 31.4%, $p = 0.03$) rates after the sperm injection procedure were higher in the treated than in the untreated group. The chance of achieving clinical pregnancy (OR 1.82; 95% CI 1.06–3.15) and live birth (OR 1.87, 95% CI 1.08–3.25) by the sperm injection procedure were significantly increased while the chance of miscarriage was decreased (OR 0.433, 95% CI 0.22–0.84) after varicocele was treated.

Conclusions: Results suggest that varicocelectomy improves clinical pregnancy and live birth rates by intracytoplasmic sperm injection in infertile couples in which the male partner has clinical varicocele. The chance of miscarriage may be decreased if varicocele is treated before assisted reproduction.

Key Words: testis; infertility, male; sperm injections, intracytoplasmic; varicocele; abortion, spontaneous

The complaint of infertility is common in the urological office. Approximately 8% of men of reproductive age seek medical assistance for fertility related problems, of whom 1% to 10% have conditions that compromise reproductive potential, including varicocele in 35%.1 Several studies show that surgical treatment for clinical varicoceles is highly effective to decrease seminal oxidative stress, increase seminal antioxidants, and improve sperm quality and the percent of spermatozoa with intact DNA.2–4 A recent meta-analysis of varicocelectomy demonstrated the benefit of treating clinical varicocele in infertile men with abnormal semen analysis.5 In that study the unassisted pregnancy rate was significantly higher after varicocelectomy than in patients without surgery (33% vs 15.5%). The chance of spontaneous conception was 2.8 times higher in the varicocelectomy group.
than in the patients who received no treatment or medication.

The benefit of repairing clinical varicocele in infertile men undergoing assisted conception is unclear. To date few studies have been published on this topic. In a small series the IUI success rate was higher in patients with treated varicocele. In a larger cohort of men Cayan et al reported that surgical repair improved semen parameters in about half and, thus, they suggested that varicocelectomy may rescue a number of couples from complex ART. To our knowledge it is still unknown whether varicocelectomy may improve the outcomes of invasive ART, such as IVF associated with ICSI. In clinical practice many patients with clinical varicocele and impaired sperm function are referred to ICSI without treatment due to the general belief that ICSI is highly successful independent of the cause of male infertility. We compared ICSI clinical and laboratory outcomes in infertile men with treated and untreated clinical varicocele.

MATERIALS AND METHODS
We reviewed the charts of all infertile men with clinical varicocele who underwent ICSI from January 2002 to July 2008. Varicocele was identified at physical examination and graded with the patient standing as large—grade 3 and visible, moderate—grade 2 and visible with the Valsalva maneuver, and small—grade 1 and palpable with the Valsalva maneuver. Only men with a complete infertility evaluation, including history, physical examination showing a clinical unilateral or bilateral varicocele, at least 2 semen analyses, hormone profile and genetic screening as appropriate were included in study.

Testicular volume was estimated with a Prader orchidometer. The hormonal profile included serum testosterone and follicle-stimulating hormone. Patients with a history of cryptorchidism, testicular trauma, orchitis, systemic or hormonal dysfunction and genetic abnormalities were excluded from study as well as those with azospermia/cryptozoospermia and ICSI cycles involving egg donation.

A total of 242 men were included in analysis and grouped by varicocele repair history, including group 1—80 with prior successful varicocelectomy, defined as absent palpable varicocele, and group 2—162 with any grade of clinical varicocele at ICSI. Signed informed consent was obtained from patients to use clinical and laboratory data for analysis with guarantees of confidentiality. The study was approved by our institutional review board.

Indications for ICSI were in accordance with the guidelines of the II Brazilian Consensus of Male Infertility even if the indication of IVF was a female factor. Only couples with primary infertility and the first ICSI cycle of each patient were included in study. No patients in the untreated or treated group underwent IUI before ICSI because they did not meet institutional criteria for this type of ART. In group 1 mean time from varicocelectomy to ICSI was 6.2 months (range 4 to 13). In group 2 mean time from the first appointment of the couple to ICSI was 4.2 months (range 3 to 15).

In the treated group patients underwent subinguinal microsurgical varicocelectomy on an outpatient basis, as previously described. Postoperative semen analysis was done at 3-month intervals. At least 2 semen analyses were obtained in each patient and evaluated by WHO criteria. Sperm parameters below normal values, ie sperm count less than 20 × 10^6/ml (oligozoospermia), progressive motility less than 50% (asthenozoospermia) and strict morphology less than 9% (teratozoospermia), were considered sperm defects. A score of 1 was assigned to each defect and the sum of the defects was scored. Maximum and minimum possible sperm defect scores were 3—oligoasthenoteratozoospermia and 0—no defects, respectively. Multiple semen analysis results in an individual were pooled. Mean values were calculated for each seminal parameter and used for statistical purposes.

Ovarian stimulation was done using a long down-regulation gonadotropin-releasing hormone agonist protocol, followed by the administration of human menopausal gonadotropin or recombinant human follicle-stimulating hormone. Human chorionic gonadotropin was administered when 2 or more ovarian follicles achieved a mean diameter of 18 mm. Oocyte retrieval, sperm processing and ICSI were done in clean room environments. Briefly, transvaginal ultrasound guided oocyte retrieval was performed 34 to 36 hours after human chorionic gonadotropin administration. ICSI was done for all metaphase II oocytes. Sperm samples were obtained by masturbation and processed by the 2-layer discontinuous colloidal gradient. Fertilization was considered normal when oocytes with 2PN and 2 polar bodies were seen 16 to 18 hours after ICSI. Fertilized oocytes were cultured until embryo transfer to the uterine cavity, which was guided by abdominal ultrasound on day 3 of embryo culture. Embryos were graded morphologically using a light inverted microscope 48 and 72 hours after ICSI. High quality embryos had 3 or 4 and 7 or 8 symmetrical blastomeres on days 2 and 3 of culture, respectively, with no multinucleation, grade I or II fragmentation, or zona pellucida abnormalities. Clinical pregnancy was confirmed by a gestational sac with an embryo showing cardiac activity on ultrasound at weeks 5 to 6. Miscarriage was considered when nonviable clinical pregnancy was noted on ultrasound followup.

Qualitative variables are expressed as the number and percent. Quantitative variables are shown as the mean ± SD. We applied the Kolmogorov-Smirnov test to assess the normal distribution by numerical variables. The Student t test was used to compare quantitative variables and the Pearson chi-square test was used to compare ICSI outcomes between the groups. We performed logistic regression analysis to verify whether varicocele repair increased the odds of clinical pregnancy and live birth or decreased the odds of miscarriage with p <0.05 considered significant. All statistical data were processed with SPSS®, version 13.0.

RESULTS
From January 2002 to July 2008 at our institution 476 couples, in whom the male partner had a history of varicocele, underwent ICSI. Of these men 242
(50.8%) with treated or untreated clinical unilateral or bilateral varicocele met study inclusion criteria and were assessed.

The distribution of varicocele grades in groups 1 and 2 did not differ, including grades 1 to 3 in 18.8% and 20.4%, 43.7% and 40.1%, and 37.5% and 39.5% cases, respectively. The proportion of patients with bilateral varicocele in groups 1 and 2 was also similar (61.2% and 50.6%, respectively). Groups 1 and 2 were homogeneous in mean age of the patients (35.8 ± 9.0 ml), history of infertility (3.7 ± 7.1 and 4.2 ± 6.8 years) and the proportion of females with a fertility associated problem (22.0% and 16.6%, respectively). Table 1 lists group 1 semen parameter results. Compared to before varicocele repair the postoperative motile sperm count (6.7 ± 9.1 vs 15.4 ± 9.1 ml), history of infertility (3.7 ± 7.1 and 4.2 ± 6.8 years) and the proportion of females with a fertility associated problem (22.0% and 16.6%, respectively). Table 1 lists group 1 semen parameter results. Compared to before varicocele repair an improved total sperm count (14.1 ± 10⁶ vs 34.9 ± 10⁶, p = 0.02) and number of motile sperm (6.7 ± 10⁶ vs 15.4 ± 10⁶, p < 0.01), and a decreased sperm defect score (2.2 vs 1.9, p = 0.01) were observed after varicocele repair.

There was no statistically significant difference in the total number of retrieved and mature oocytes between the groups. The normal 2PN fertilization rate was significantly higher in the treated than in the untreated group (78% vs 66%, p = 0.04). There was no difference in the rate of high quality embryos between the groups. The mean number of transferred embryos was also similar. The clinical pregnancy rate after ICSI in the treated and untreated groups was 60.0% (48 of 80 cases) and 45.0% (73 of 162, p = 0.04). The miscarriage rate after achieving clinical pregnancy by ICSI did not differ between the groups (table 2). The live birth rate in the treated and untreated groups was 46.2% (37 of 80 cases) and 31.4% (51 of 162), respectively (p = 0.03). The chance of achieving clinical pregnancy (OR 1.82, 95% CI 1.06–3.15; p = 0.03) and live birth (OR 1.87, 95% CI 1.08–3.25; p = 0.03) by ICSI were increased but the chance of miscarriage after achieving pregnancy by ICSI was decreased (OR 0.433, 95% CI 0.22–0.84; p = 0.01) when varicocele was treated before assisted conception. In patients with varicocele repair the postoperative motile sperm count and fertilization rate by ICSI were significantly higher in those who achieved live birth compared to those in whom impregnation failed (table 3).

**DISCUSSION**

Varicocele, the most common cause of male infertility, is associated with decreased sperm quality. Spontaneous conception is significantly increased after varicocelectomy in couples in whom the male partner has clinical varicocele and abnormal semen analysis. Although spontaneous pregnancy remains the litmus test to evaluate varicocele treatment success, many men with varicocele related infertility require ART due to severe sperm abnor-

<table>
<thead>
<tr>
<th>Mean ± SD Group 1</th>
<th>p Value</th>
<th>Mean ± SD Group 2</th>
<th>p Value vs Group 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol (ml)</td>
<td>3.1 ± 1.2</td>
<td>3.0 ± 1.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Sperm count (×10⁶)</td>
<td>4.3 ± 5.3</td>
<td>12.1 ± 31.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Total sperm count (×10⁶)</td>
<td>14.1 ± 23.8</td>
<td>34.9 ± 61.3</td>
<td>0.02</td>
</tr>
<tr>
<td>% Progressive motility</td>
<td>40.7 ± 23.9</td>
<td>46.5 ± 16.6</td>
<td>0.34</td>
</tr>
<tr>
<td>Total motile sperm count (×10⁶)</td>
<td>6.7 ± 5.5</td>
<td>15.4 ± 9.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>% Strict morphology</td>
<td>5.5 ± 5.9</td>
<td>5.7 ± 4.4</td>
<td>0.89</td>
</tr>
<tr>
<td>Sperm defect score</td>
<td>2.2 ± 0.5</td>
<td>1.9 ± 0.7</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 2. ICSI outcome in infertile couples in whom male partner had treated or untreated clinical varicocele**

<table>
<thead>
<tr>
<th>Mean ± SD No. oocytes:</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrieved</td>
<td>12.4 ± 7.5</td>
<td>12.3 ± 6.8</td>
<td>0.91 (unpaired Student’s t test)</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>10.2 ± 6.4</td>
<td>10.0 ± 5.5</td>
<td>0.46 (unpaired Student’s t test)</td>
</tr>
<tr>
<td>Mean ± SD % 2PN fertilization</td>
<td>78.0 ± 20.0</td>
<td>66.0 ± 22.0</td>
<td>0.04 (unpaired Student’s t test)</td>
</tr>
<tr>
<td>Mean ± SD % high quality embryo</td>
<td>47.0 ± 29.0</td>
<td>49.9 ± 29.0</td>
<td>0.76 (unpaired Student’s t test)</td>
</tr>
<tr>
<td>Mean ± SD No. embryos transferred</td>
<td>3.1 ± 1.4</td>
<td>3.1 ± 1.5</td>
<td>0.96 (unpaired Student’s t test)</td>
</tr>
<tr>
<td>No. clinical pregnancy (%)</td>
<td>48 (60.0)</td>
<td>73 (45.0)</td>
<td>0.04 (Pearson’s chi-square test)</td>
</tr>
<tr>
<td>No. miscarriage (%)</td>
<td>11 (22.9)</td>
<td>22 (30.1)</td>
<td>0.46 (Pearson’s chi-square test)</td>
</tr>
<tr>
<td>No. live birth (%)</td>
<td>37 (46.2)</td>
<td>51 (31.4)</td>
<td>0.03 (Pearson’s chi-square test)</td>
</tr>
</tbody>
</table>
malities and/or a significant problem affecting the female partner.

An indication for varicocele repair before IVF/ICSI is unusual but in certain cases varicocele treatment should be considered. Men with nonobstructive azoospermia may experience restored sperm to the ejaculate after repair of clinical varicocele. Despite these limitations demographic studies are not randomized to treatment or no treatment to attempt natural conception. However, our rationale for varicocele repair was to achieve overall improvement in sperm quality to optimize the reproductive outcome of ICSI treatment, as previously suggested, rather than achieve pregnancy by natural intercourse or less invasive ART modalities.

To our knowledge our study adds new evidence by showing that treatment for clinical varicocele in men with markedly decreased semen quality before IVF/ICSI increases the ability of the couple to conceive even in the presence of female factor infertility. The reasons for these findings may be related to the overall improvement in sperm quality. We observed significant improvement in the total number of motile sperm and a decrease in sperm defects. A higher fertilization rate was also achieved after ICSI, suggesting that the improved pregnancy rate after varicocelectomy may also have been due to functional factors that were not tested at standard semen analysis, such as seminal oxidative stress and sperm DNA integrity.

Men with increased radical oxidative stress in semen may have decreased potential for in vitro and in vivo fertility with negative effects on embryo growth. Oxidative stress parameters are significantly increased in men with varicocele compared with that in sperm donors. High radical oxidative stress production in the reproductive tract attacks the fluidity of the sperm plasma membrane and the integrity of DNA in the sperm nucleus. Excessive DNA damage is associated with a decrease in several fertility indexes, including the fertilization, embryo cleavage, implantation and pregnancy rates.

In our study the mean time from varicocelectomy to ICSI was 6.2 months. It may be argued that couples should wait longer before proceeding to ICSI to attempt natural conception. However, our rationale for varicocele repair was to achieve overall improvement in sperm quality to optimize the reproductive outcome of ICSI treatment, as previously suggested, rather than achieve pregnancy by natural intercourse or less invasive ART modalities. Semen was improved at a postoperative followup of approximately 6 months, comprising 2 or 3 spermatogonic cycles. Couples were then free to proceed to ICSI or wait longer if desired. Nonetheless, patient motivating factors cannot be entirely controlled and many couples simply do not wait longer for natural conception after varicocelectomy. Although we cannot exclude that further improvement in semen quality or spontaneous pregnancies would be achieved if a longer interval was allowed before ICSI, our patient population included men with markedly impaired semen quality even after varicocele repair. Female factor infertility was present in a significant proportion of couples and none met the minimum criteria for IUI according to our institutional guidelines. Men who achieve a postoperative total motile sperm count of greater than 20 million are more likely to initiate pregnancy by intercourse or IUI. In our study the semen quality in men with treated varicocele was below this threshold.

Due to the retrospective design of our analysis the possibility of some inherent bias exists. Patients were not randomized to treatment or no treatment and the decision in regard to varicocele treatment was made by the couples. Also, the indication for ICSI may not have been based only on varicocele related infertility since about a third of the couples had significant female infertility problems that were likely to have influenced the decision making process. Moreover, couples with a good response to surgery who achieved early spontaneous pregnancy were not included in analysis, although it is likely that excluding those with a good prognosis favored the untreated group rather than benefiting the treated group. Despite these limitations demo-
graphic characteristics were homogeneous in the 2 groups, as was the seminal profile in treated and untreated men. It is our practice to advise patients with clinical varicocele who enroll in our ART program to consider varicocele treatment before assisted conception but most elect not to undergo varicocele repair. Factors influencing patient decision making were difficult to ascertain but it appeared that there were 3 main reasons to refuse treatment, including 1) the costs associated with microsurgical repair, which are not reimbursed by most health care plans in our country and would add to the already high costs of ICSI, 2) the time required to improve sperm and the uncertainty of the beneficial effect of treatment, and 3) the general belief of health care professionals and general community that ICSI is highly successful independent of the cause of male infertility.

CONCLUSIONS

Our data suggest that varicocelectomy improves the clinical pregnancy and live birth rates by ICSI in infertile couples in whom the male partner has a clinical varicocele. Also, the chance of miscarriage may be decreased if varicocele is treated before assisted reproduction. Efforts should be made to maximize the fertility potential of the couple before assisted conception. Varicocele repair should be offered before IVF/ICSI to infertile men with clinical varicocele.

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