A comparative analysis of embryo implantation potential in patients with severe teratozoospermia undergoing in-vitro fertilization with a high insemination concentration or intracytoplasmic sperm injection

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The objective of this study was to assess fertilization, implantation and pregnancy rates in infertile patients with severe teratozoospermia [P (poor prognosis) pattern sperm morphology assessed by strict criteria] treated by in-vitro fertilization (IVF) using a high insemination concentration (HIC), or by intracytoplasmic sperm injection (ICSI). This was a retrospective cohort study performed in an academic tertiary institution. The outcome of 115 consecutive ICSI cycles was compared to that of a similar number of cycles of IVF with HIC performed during a similar time frame and matched by woman’s age and basal serum (cycle day 3) follicle stimulating hormone concentrations. The inclusion criteria were sperm morphology ≤4% normal forms (P pattern) and ≥1×10^6 total motile spermatozoa per ejaculate. The diploid fertilization rate in the HIC-IVF group was 86% and in the ICSI group 68% (P < 0.05). Importantly, an equal number of embryos was transferred to both groups of patients. The morphological quality of the embryos (proportion of transfers having superior morphology embryo scores) was significantly better in the ICSI group than in the patients receiving HIC-IVF. Although there was a clear trend for better implantation and pregnancy rates in the ICSI group, these differences were not statistically significant. We conclude that, although HIC-IVF resulted in a higher fertilization rate than ICSI in patients with severe teratozoospermia, ICSI produced a significantly higher proportion of morphologically superior embryos with a tendency towards a higher implantation potential. Therefore, teratozoospermic patients having adequate numbers of motile spermatozoa should be offered ICSI as an alternative to modified (HIC) IVF treatment.

Key words: fertilization/ICSI/implantation/strict morphology/teratozoospermia

Materials and methods
All IVF patients who presented to The Jones Institute for Reproductive Medicine, Norfolk, VA, USA, during the period January 1994 to March 1995 were included in the analysis. All couples gave their informed consent for ICSI according to the guidelines of the Institutional Review Board of Eastern Virginia Medical School. Inclusion criteria were (i) severe teratozoospermia [sperm morphology of ≤4% normal forms (P pattern or poor prognosis factor group as assessed by strict criteria)] and (ii) ≥1×10^6 total motile spermatozoa per ejaculate (before semen processing). This was required in order to be able to compare similar groups of male infertility cases who could have been offered HIC-IVF or ICSI (typically, patients with <1×10^6 motile spermatozoa per ejaculate are treated by ICSI). Allocation of patients into HIC-IVF or ICSI therapy was performed in a non-randomized fashion.

In our programme, clinical and laboratory data are stored for individual patients in a Pentium computer (Zios International Ltd., Minneapolis, MN, USA) using a customized software program written in APL Plus (originally developed by K. Iverson of Harvard University, Boston, MA, USA). A total of 115 ICSI cycles fitting the inclusion criteria were identified. These patients underwent ICSI therapy due either to poor sperm parameters or prior cycles with failed fertilization.
This was followed by identification of 115 HIC-IVF cycles using the database, selected using similar sperm criteria and matched by women's age and basal serum FSH concentrations. Except for the knowledge of these parameters, the computerized selection of patients remained blind.

Basal serum FSH concentrations were measured by a microenzymatic immunological assay (Abbott Pharmaceutical, Chicago, IL, USA). Ovarian stimulation protocols (using a combination of a gonadotrophin-releasing hormone agonist and gonadotrophins), oocyte retrieval, IVF and ICSI, embryo cryopreservation and uterine embryo transfer procedures were performed as previously described (Veeck, 1991a; Grow et al., 1994; Oehninger et al., 1995). For HIC-IVF, oocytes were inseminated individually or using the multiple oocytes per dish technique in 3, 1 or 0.3 ml total medium volume, depending on the total number of motile spermatozoa available (Veeck, 1991a). After overnight gamete co-incubation, fertilized oocytes were transferred to embryo growth medium (Veeck, 1991a).

The morphological condition of cleaving embryos was assessed immediately before transfer (2 days after oocyte insemination) using the criteria outlined by Veeck (1991b).

At the time of IVF, semen samples were assessed for sperm concentration and motile characteristics with an automated computerized sperm analyser (Hamilton-Thorn Research, Danvers, MA, USA), using fixed parameter settings (Oehninger et al., 1990). The total number of motile spermatozoa per ejaculate was calculated using the formula: semen volume × percentage sperm progressive motility × sperm concentration. Sperm morphology evaluation using strict criteria has been fully described in previous publications (Kruger et al., 1988; Menkveld et al., 1990). The oocyte insemination concentration was based on the diagnosis of sperm morphology pattern: for samples presenting with a P pattern and in accordance with published publications, oocytes were inseminated with ~500 000 motile spermatozoa/ml/oocyte (after a three-layer Percoll gradient separation) (Oehninger et al., 1988; Grow et al., 1994). Results using only mature oocytes (metaphase I and II at the time of aspiration) were considered.

The fertilization rate was calculated based on the number of normally fertilized (diploid fertilization) oocytes divided by the total number of oocytes inseminated or micromanipulated. The implantation rate, expressed as fetal poles per number of embryos transferred, was determined after ultrasonic detection of a fetal pole with heartbeat in the first trimester. Ongoing pregnancy referred to any pregnancy progressing past 20 weeks gestation. A miscarriage referred to the loss of a pregnancy confirmed by first trimester ultrasound.

The unpaired, Student's t-test and χ² test were used to analyse the data as appropriate. P values < 0.05 were considered significant. Results are shown as the mean ± SE. A power analysis was performed in order to determine the likely sample size needed based on these assumptions: a prior large cohort study of P pattern patients showed implantation and delivery rates of 7 and 14% respectively (Grow et al., 1994). Assuming a 100% increase in implantation rate (7 to 14%) and in delivery rate (14 to 28%), a sample size of 212 embryos transferred per group and 82 cycles of transfer per group would reach significance with an estimated α level of 0.05 and a calculated β error of 0.20.

Results

Sperm parameters for the HIC-IVF and ICSI groups were respectively as follows: concentration, 106 ± 7×10⁶ and 30 ± 4×10⁶/ml (t-test, P < 0.01); progressive motility, 49 ± 2 and 29 ± 2% (P < 0.01); total motile spermatozoa per ejaculate, 150 ± 12×10⁶ and 24 ± 4×10⁶ (P < 0.01); normal morpho-

logly, 2.5 ± 0.1 and 3.6 ± 0.2% (P < 0.05) (range 0–4% in both groups). The mean sperm insemination concentration used for HIC was 504 300 ± 43 000 motile spermatozoa/oocyte/ml of insemination medium.

The mean woman's age for the HIC-IVF and ICSI groups was 35.3 ± 0.3 and 35.2 ± 0.3 years, and the basal serum FSH concentrations were 6.5 ± 0.4 and 7.3 ± 0.2 mIU/ml respectively (not significant). The mean number of embryos transferred was 3.8 ± 0.1 and 4.0 ± 0.1 for the HIC-IVF and ICSI groups respectively (not significant).

Table I summarizes the results for both study groups. The fertilization rate was significantly higher in the HIC-IVF group compared to the ICSI group (t-test, P < 0.001). The implantation and the clinical, ongoing and multiple pregnancy rates were higher in the ICSI group but the differences did not attain statistical significance. Embryo grading score [proportion of transfers with grade 1 and 2 embryos (best morphological condition of cleaving embryos)] was significantly higher in the ICSI group: 47% of the embryos in the ICSI group were grade 1 versus 24% in the HIC-IVF group (χ², P = 0.01), and 87 and 72% respectively of the two groups were grades 1 and 2 embryos (χ², P = 0.01). Cleavage rates (99.1 and 98.0%) and mean number of blastomeres/embryos transferred (4.1 ± 0.1 and 3.9 ± 0.1) were not significantly different for the ICSI and HIC-IVF groups respectively.

Because the fertilization rate was significantly higher in the HIC-IVF group it was relevant to examine whether this would represent an advantage after embryo cryopreservation. This was not the case, since an equal number of cycles with embryo freezing was performed after HIC-IVF (51/115 or 44%) and ICSI (49/115 or 43%). Furthermore, the mean number of embryos cryopreserved per cycle and the implantation and pregnancy rates were similar for those patients who returned for subsequent cycles of embryo thaw and uterine transfer (47 and 28 cycles for HIC-IVF and ICSI respectively; data not shown).

Discussion

In this study we compared the outcome of HIC-IVF and ICSI in patients with severe teratozoospermia (P pattern by strict
criteria). To be able to examine similar groups of patients, we evaluated infertile men whose semen samples had a minimal acceptable concentration of motile spermatozoa (and who therefore could have been candidates for modified IVF using HIC). Typically, in our programme, patients with \( <1 \times 10^6 \) total motile spermatozoa per ejaculate are treated by ICSI, irrespective of sperm morphology. Although patients were selected on the basis of defined parameters (i.e. \( \leq 4\% \) normal forms and \( \geq 1 \times 10^6 \) total motile spermatozoa), the HIC-IVF and ICSI groups differed significantly in their basic sperm parameters. The ICSI group had a slightly higher morphology score but markedly reduced motility and total motile fraction. Importantly, the range of normal sperm morphology was 0–4% in both groups. In order to eliminate other confounding variables, we matched the study groups by those factors known to have a major impact on implantation and pregnancy outcome, i.e. a woman’s age and basal serum FSH concentrations (Muasher et al., 1988; Toner et al., 1991). More importantly, patients had a similar number of transferred embryos per attempt. Therefore, although retrospective in its nature, this study had the advantage of assessing the impact of teratozoospermia on modified IVF (using HIC) and ICSI results in an adequately matched cohort of patients.

The fertilization rate was significantly higher in the HIC-IVF group (86 versus 68% in the ICSI patients). This high fertilization rate using HIC-IVF in patients with severe teratozoospermia is similar to previously reported findings and confirms the beneficial effects of this modified IVF technique (Oehninger et al., 1988; Grow et al., 1994). We and others have previously reported a fertilization rate following ICSI of \( \sim 60\% \), similar to that shown in this study (Van Steirteghem et al., 1993b; Oehninger et al., 1995). This rate of fertilization was independent of the type and degree of sperm abnormalities present in the ejaculates (Nagy et al., 1995; Oehninger et al., 1995; Sherins et al., 1995).

Although more oocytes were fertilized by HIC-IVF, this positive result was offset by a deleterious effect on embryo quality. Embryos produced by ICSI had a significantly higher morphological score and, after uterine transfer, showed a tendency toward superior implantation and pregnancy rates than HIC-derived embryos. The observed differences in implantation and pregnancy rates favouring ICSI were relatively small and not statistically significant, and only a multicentre trial evaluating >500 pregnancies would resolve whether these disparities are clinically significant. The question arises whether the use of a HIC in cases of severe teratozoospermia perhaps affects oocyte/embryo quality by some unknown (toxic?) mechanism. Cohen et al. (1992) reported a compromised implantation rate in cases of severe teratozoospermia when partial zona dissection (PZD) was utilized. This observation prompted Hall et al. (1995) to suggest that the defects observed in embryo quality in PZD cases might have been due to the high concentration of motile spermatozoa, immotile spermatozoa and other seminal debris present in the insemination medium.

The apparently lower implantation and pregnancy rates observed here for HIC-treated teratozoospermic cases (compared to cases with normal morphology) are similar to those previously reported (Baker et al., 1993; Grow et al., 1994). Hall et al. (1995) also compared the use of HIC-IVF and ICSI for a similar group of teratozoospermic patients diagnosed using strict criteria. Their study had the merit of a sibling oocyte comparison between the two techniques; however, the number of patients receiving a 'pure' transfer of embryos derived either from HIC-IVF or ICSI and the total number of embryos examined were small. These authors communicated similar fertilization (67 versus 59%), implantation (9 versus 12%) and pregnancy (18 and 22%) rates for HIC-IVF and ICSI respectively. We agree with these authors that 'when concerns of safety are fully allayed and the cost of ICSI can be brought into line with IVF, then ICSI may become the first option' (Hall et al., 1995). However, in our opinion, ICSI still remains an experimental clinical procedure and patients should be adequately counselled about the possibility of risks which are currently unforeseeable (therefore requiring patients to receive adequate information and counselling).

Here, we have shown that in patients with a relatively adequate concentration of motile spermatozoa but with severe teratozoospermia, the ICSI procedure is an excellent treatment alternative to HIC-IVF. This micromanipulation technique offers another dimension of therapy for these patients, with a potential for a better implantation rate per embryo transfer. In this setting, sperm morphology assessed by strict criteria can be used as a simple diagnostic tool to direct patients with severe teratozoospermia into the ICSI programme. Bioassays that assess and reflect multiple sperm functions (like the hemizona assay) and that are highly predictive of fertilization success/failure in the IVF setting should be added to the diagnostic armamentarium to enable the prompt identification of cases of dysfunctional spermatozoa (Oehninger et al., 1992; Oehninger, 1995).

In conclusion, this study demonstrated that ICSI is at least as efficient as HIC-IVF in cases with severe teratozoospermia and adequate numbers of motile spermatozoa. Because a significantly superior embryo (morphological) quality and a trend toward better implantation and pregnancy rates were observed with ICSI, this new, formidable technique should be offered to this category of patients. HIC-IVF remains a viable alternative for programmes that have not developed the ICSI technique in their laboratories.

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References
Implantation rate in HIC-IVF and ICSI


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