Morphologic evaluation of spermatozoa is an integral part of the semen analysis work-up for infertility. Kruger et al. proposed that sperm classification on the basis of stricter morphologic criteria could be useful in predicting successful fertilization (1). In a meta-analysis of outcomes in intrauterine insemination cycles, a trend toward better pregnancy rates was observed in samples with severe teratozoospermia with >4% normal forms in the semen sample (3). Severe teratozoospermia with <4% normal forms in the semen sample was also associated with poorer fertilization outcomes during IVF (3, 4). A meta-analysis of sperm morphology and IVF outcome parameters in 49 different studies using conventional IVF similarly concluded that sperm morphology assessment may be valuable as a diagnostic tool in evaluating male infertility (5). This, however, is still an area of considerable debate. Numerous other reports suggest that abnormal morphology is not an accurate predictor of pregnancy or cycle outcome parameters (6–11).

The relevance of the assessment of sperm morphology of the overall semen sample as it pertains to IVF with intracytoplasmic sperm injection (ICSI) is even less clear. Oocyte fertilization through ICSI circumvents many problems, such as poor motility, poor zona binding, and poor acrosome reaction, which are frequently found in association with specimens having abnormal sperm morphology. Multiple studies have shown that with IVF/ICSI, semen samples with poor Kruger morphology have similar fertilization and pregnancy rates to those with normal morphology (8, 9, 11–13). McKenzie et al. (14) analyzed ICSI outcomes in a subset of severe teratozoospermic patients with 0% normal sperm. They concluded that sperm morphology had little predictive value regarding fertilization and pregnancy outcome in men undergoing ICSI for severe teratozoospermia. Some of the studies analyzing morphology and ICSI outcomes were small and not all of them included live birth rates or examined detailed subsets of patients across the spectrum of morphologic impairment.
The role of paternal factors in embryonic development, as well as the effect of the ICSI procedure itself remains controversial. Several studies have suggested that ICSI-derived embryos have reduced developmental capacity (15–18). In contrast, others using severely teratozoospermic sperm have seen no difference in blastulation rates with ICSI (19, 20).

The objective of the present study was to determine if critical assessment of sperm morphology during the pre-IVF semen analysis correlated with ICSI cycle outcome parameters. We analyzed the impact of severe teratozoospermia on fertilization, embryonic development to the blastocyst stage, and pregnancy. To our knowledge, this is the first large ICSI series that examines live birth outcomes in sperm morphology subgroups ranging from severely teratozoospermic to normal. This information should help us to gain a better understanding of paternal effect on embryonic development and the influence of the ICSI technique itself on embryonic development.

**Materials and Methods**

**Patients**

The IVF cycles with ICSI were performed at the Cleveland Clinic Fertility Center from January 2004 to December 2006 and retrospectively analyzed. Day 5 transfers accounted for 65 cycles, and these were not included in the present analysis. During the time interval encompassed by this study, ICSI was the laboratory’s preferred method of fertilization unless the patient specifically requested standard insemination. Cases involving some or all conventional IVF were eliminated (~5%). Cycles in which semen specimens had insufficient sperm (usually <1 × 10⁶/mL) to allow morphologic assessment were also excluded. Only women <37 years of age were included in the analysis, to minimize the contribution of maternal age as a confounding variable. As a result, 1,074 ICSI cycles were available for analysis. Patients were divided into subgroups based on sperm morphology.

**Sperm Morphology Assessment**

Semen samples were evaluated for total count, percentage motile sperm, forward progression, and sperm morphology. Sperm were stained using Diff-Quik (Dade Behring, Newark, Delaware). Sperm morphology was assessed using Kruger’s strict criteria (21). A total of 100 sperm were graded at each evaluation and percentage normal forms determined. Sperm morphology grading was performed by one of two andrology technicians, and uniformity of grading between the two andrology technicians was routinely monitored using control slides.

**IVF Procedure**

Down-regulation with leuprolide acetate (Lupron; TAP Pharmaceuticals, Lake Forest, IL) followed by ovulation induction with gonadotropins was the primary stimulation protocol used. Oocytes were recovered by transvaginal aspiration of follicles under ultrasound guidance. Oocytes were cultured in microdrops of human tubal fluid (HTF) medium (LifeGlobal, Guilford, CT) supplemented with 10 mg/mL human serum albumin (Cooper Surgical, Trumball, CT) under an oil overlay. Culture dishes were incubated at 37°C with 5.5% CO₂. Mature oocytes were fertilized by ICSI 3–4 h after the retrieval. Fertilization check was performed 18–20 h after the ICSI procedure. Normally fertilized zygotes were subsequently moved to microdrops containing HTF medium with 10% synthetic serum substitute (SSS, Irvine, CA) and cultured for an additional 2 days.

Embryo transfer was performed on day 3 under ultrasound guidance using a Wallace catheter. Embryo selection for day 3 transfer was based on morphologic parameters. Embryos were graded on the basis of cell number, regularity of blastomeres, good blastomere expansion, fragmentation level, and signs of embryonic compaction. Spare embryos not transferred or frozen on day 3 were kept in culture and frozen at the blastocyst stage. Pregnancy testing was performed 15 days after the embryo transfer. Clinical pregnancy was confirmed by the presence of a fetal heart on ultrasonic examination at 6–8 weeks of pregnancy.

**Data Collection and Statistical Analysis**

Cycle outcome data was collected under Institutional Review Board approval from the IVF lab data registry. The primary outcome measures tabulated were fertilization rate, blastocyst formation, embryos transferred, pregnancy rate, and implantation rate. The implantation rate was calculated by dividing the number of fetal heart tones by the total number of embryos transferred. Multiple pregnancy and live birth rates were also monitored. Patients were stratified into groups based on their sperm morphology assessment. Using Kruger’s strict criteria, eight subgroups were analyzed: 0%, 1%, 2%, 3%, 4%, 5%–7%, and >7% normal sperm morphology. Differences in outcome measures between groups were compared using the Chi-squared test and the Student t test. P values of <.05 were considered to be statistically significant.

**Results**

A total of 1,074 ICSI cycles were included in this analysis. The semen characteristics in each morphology subgroup are portrayed in Figure 1. The percentage of patients in each morphology group with either a compromised sperm count or overall motility of <40% is shown. As might be expected, patients in the more teratozoospermic morphology subgroups (<4% normal sperm) also exhibited deficits in other sperm parameters.

Table 1 depicts the relationship between sperm morphology based on Kruger’s strict assessment and cycle outcome parameters. Fertilization rates were high regardless of sperm morphologic parameters. Intracytoplasmic sperm injection resulted in 74%–77% of oocytes being fertilized. The average
number of oocytes retrieved, mature oocytes for injection, number of embryos transferred, and patient age were similar across morphology subgroups.

Clinical pregnancy outcomes were excellent, ranging from 60% in the subgroup with the most severe teratospermia (0% normal sperm) to 56% in the patient set with the highest percentage normal spermatozoa. The implantation rate per embryo transferred ranged from 31% to 41% (Table 1). The variations in the main outcome parameters of fertilization, pregnancy, and embryo implantation did not correlate with sperm morphology. Our data suggest that in cycles in which oocyte fertilization is mediated through ICSI, poor sperm morphology at the time of semen analysis has no significant impact on clinical outcome parameters. This is further shown by the fact that the highest pregnancy and live birth rates were observed in the group of patient eggs fertilized with sperm from specimens with the most severe teratozoospermia, i.e., the subgroup with 0% normal sperm morphology.

Culturing the “spare” embryos not transferred on day 3 for an additional 3 days gave us the opportunity to further observe development to the blastocyst stage and to assess the quality of the blastocysts. Table 1 summarizes both the percentage of blastocysts formed and the percentage of high-quality blastocysts deemed to be suitable for freezing based on morphology. Blastocyst quality assessment was based on the degree of expansion, presence of an adequate cell number, and differentiation of the inner cell mass and the trophoderm. The blastulation rate was not different among the different sperm morphology subgroups ($P=.07$). We did, however, find that the percentage of high-quality blastocysts was significantly greater in the severely teratozoospermic patients with 0% normal sperm compared with patients with $\geq 5\%$ normal sperm (37% vs. 28%; $P<.005$).

To better understand these data, we looked at patient diagnosis across the different morphology subgroups. Figure 2 depicts the percentage of patients with each diagnosis for each sperm morphology subgroup. From these data it would appear that in the lower morphology subgroups, female factors are less prevalent and the primary infertility problem is male factor. In the 0%–1% normal sperm subgroup, approximately 38% of couples had male factor as their primary diagnosis. At the other extreme, in the subgroup with $>7\%$ normal sperm, female factors such as ovulatory dysfunction, tubal factor, polycystic ovary, and endometriosis were the primary diagnosis in 45% of couples. Another 41% of patients had unexplained infertility. Only 14% of couples in this subgroup had male factor as the primary diagnosis based on low sperm count, motility, or progression.

Severe teratozoospermia did not negatively affect ICSI success. The live birth rate per transfer ranged from 44% to 56%. The observed variation in live birth rate among the different morphology subgroups was not found to be significant. The miscarriage rate was 4%–5% for all but one of the subgroups (Table 1). Among the 774 live births, only four birth defects were reported to the clinic—three were cardiac problems and one was classified as genetic. Follow-up beyond the immediate neonatal period was not conducted.

DISCUSSION
This study represents the first large ICSI series to analyze embryonic development, pregnancy outcomes, and live birth rates in different sperm morphology subgroups based on Kruger’s classification system. Stratification of data in this manner allowed for evaluation and comparison of outcomes based on degree of teratozoospermia. In this study, fertilization, implantation, pregnancy, and live birth rates were not
statistically different among various morphology subgroups, even including those with severe teratozoospermia. Moreover, a negative paternal effect on blastocyst development as a result of ICSI and/or use of sperm from patients with severe teratozoospermia was not observed.

Although strict sperm morphology may affect fertilization rates in intrauterine insemination (2) and cycles with conventional IVF insemination (3), this association is not seen when ICSI is used. Numerous studies, including the present work, support the conclusion that poor sperm morphology on pre-IVF semen analysis using Kruger’s strict criteria does not correlate to either poor fertilization and/or pregnancy rates in ICSI cycles (8, 9, 11–14).

The most likely explanation for this is that during ICSI the embryologist can microscopically select individual sperm that appear morphologically “normal,” from even the most impaired specimens. Thus, fertilization occurs with sperm that may not be representative of the sperm population within the entire sample, making the initial semen morphology assessment irrelevant. This concept has been taken to the subcellular level in new techniques such as intracytoplasmic morphologically selected sperm injection and motile sperm organellar morphology examination (MSOME). In these techniques, investigators select sperm for ICSI using a high-power ×1,000 objective to magnify and allow more critical assessment of sperm morphology. Sperm nucleus morphology by the MSOME method has positively correlated with fertilization and pregnancy rates (22, 23). Experience with these methods, however, is too limited to draw definitive conclusions regarding efficacy and potential for augmenting the birth rate of healthy infants after ICSI.

Looking at how sperm morphology correlates with sperm function, DNA damage, and chromosomal status may help in understanding the prognostic value of strict morphology assessment. Data from several studies suggest that abnormal sperm morphology does not necessarily translate into

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Kruger strict morphology and ICSI cycle outcome.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% normal sperm morphology</td>
<td>0%</td>
</tr>
<tr>
<td>Transfers</td>
<td>138</td>
</tr>
<tr>
<td>Maternal age (mean ± SD)</td>
<td>32.3 ± 3.2</td>
</tr>
<tr>
<td>No. of oocytes (mean ± SD)</td>
<td>13.3 ± 6.1</td>
</tr>
<tr>
<td>No. of oocytes injected (mean ± SD)</td>
<td>10.2 ± 5.0</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>75%</td>
</tr>
<tr>
<td>No. of embryos transferred (mean ± SD)</td>
<td>2.3 ± 0.48</td>
</tr>
<tr>
<td>Blastulation rate with spare embryos</td>
<td>50%</td>
</tr>
<tr>
<td>Blastocysts of high quality suitable for freezing</td>
<td>37%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>60%</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>36%</td>
</tr>
<tr>
<td>Live birth rate per transfer</td>
<td>56%</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>4%</td>
</tr>
<tr>
<td>Total deliveries</td>
<td>77</td>
</tr>
<tr>
<td>Singleton</td>
<td>55</td>
</tr>
<tr>
<td>Twins</td>
<td>19</td>
</tr>
<tr>
<td>Triplets</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly different from other morphology subgroups (P<.05).

abnormal chromosomal content (24–26). Celik-Ozenci et al. (27), using fluorescent in situ hybridization, found that 10% of sperm with disomic nuclei were categorized as normal by strict morphology. Sperm morphology defects may serve more as a barrier to reaching and penetrating the oocyte zona. Sperm DNA fragmentation may affect cycle outcome, embryonic development, and blastocyst formation (28–31). However, no clear relationship has been shown between morphologic attributes of sperm and degree of DNA damage (32–34).

Despite its efficacy as a tool for oocyte fertilization, there is continued concern that the use of the ICSI technique eliminates any opportunity for “natural selection” of the sperm that will ultimately fertilize the oocyte. Although the risk of birth defects in children conceived through ART is increased over that of natural conception, the fear that ICSI will result in higher rates of major birth defects compared with conventional IVF has not been substantiated by current data. A recent meta-analysis showed a 30%–40% increased risk of major birth defects with assisted reproduction (either IVF or ICSI) compared with natural conception (35). A study comparing 1,462 children conceived through IVF or intrauterine insemination with 8,422 naturally conceived children found the incidence of birth defects to be 6.2% with IVF or IUI versus 4.4% with natural conception. However, no difference was seen in the risk of birth defects when comparing traditional IVF and ICSI (36). A meta-analysis of four studies with a total of 5,395 children born after ICSI confirms a lack of statistically significant increase in birth defects with ICSI compared with conventional IVF (37).

Another concern has been that the ICSI process itself causes damage to imprinted genes (38, 39). These methylated genes are particularly susceptible to damage and are labeled such that only the maternal or paternal copy is expressed. It has been suggested that the ICSI process itself disrupts these fragile genes, leading to their loss of function and in turn manifestation of imprinting disorders, such as Angelman syndrome and Beckwith-Wiedemann syndrome. Among children with Beckwith-Wiedemann syndrome, 4.6% were identified as having been conceived by ART (mostly ICSI), which was an increase from the baseline rate of 0.76% of live births resulting from ART in the general population (40). Although no such cases were noted in the present series, developmental disturbances are clearly more difficult to track than congenital anomalies.

Paternal effect on blastocyst quality has been a question of great interest. Janny and Menezo (41) suggested a correlation between impaired semen parameters and reduced blastocyst development in conventional IVF cycles. Others have reported poorer blastocyst development in embryos derived from ICSI versus conventional IVF (15). Miller and Smith (16) observed that a greater number of ICSI-derived embryos arrested at the 5–8-cell stage, corresponding to the time of paternal genome activation. Moreover, fewer ICSI-derived embryos (14%) developed to blastocyst compared with IVF embryos (28%). Kihaile et al. (18) compared sibling oocytes inseminated or injected with sperm from patients with severe teratozoospermia. Despite a higher fertilization rate, development was compromised in the ICSI group, with fewer embryos progressing to the blastocyst stage. Both groups had severe teratozoospermia, suggesting that something inherent in the ICSI process itself affects blastocyst development. Contradictory results were obtained by Van Landuyt et al. (20), who found that sibling oocytes from ICSI versus...
conventional IVF showed similar rates of embryonic development and blastocyst formation.

The present findings did not indicate a negative effect of the ICSI technique on blastocyst formation. We achieved a blastulation rate of between 41% and 50% among the different sperm morphology subgroups using ICSI. We did not observe a correlation between severe teratozoospermia and poorer blastocyst quality. A significantly greater percentage of high-quality blastocysts was obtained in the most severely teratospermic subgroup, with 0% normal forms, probably owing to a lower contribution of female factor infertility in this group. These data suggest that Kruger’s strict morphology was not useful in predicting either the rate of blastocyst development or the morphologic characteristics of the resulting blastocysts in ICSI cycles.

We acknowledge several limitations of this report. The contribution of female diagnosis to cycle outcome could not be completely assessed. Even though the most important variable affecting oocyte quality, maternal age, was controlled for in our data set, we recognize that genetic factors and ovarian response to stimulation also play significant roles. The relationship between sperm morphology, ICSI, and birth defects could not be adequately addressed in this investigation. A more controlled study with ICSI and IVF of sibling oocytes is needed to confirm our observation that the intracytoplasmic sperm injection technique itself was not disruptive to subsequent normal embryonic development and live birth rate. Also, long-term follow-up at multiple IVF centers and a larger live birth data set will be necessary to truly assess the impact of ICSI on birth defects and imprinting disorders. Finally, this study excluded the subgroup of men with semen densities of under 1 million, owing to logistical problems performing morphology assessments on small numbers of spermatozoa. Further study of this subgroup is certainly warranted. Teratozoospermia combined with severe oligozoospermia could potentially affect ICSI outcomes, by reducing the likelihood of locating a “normal” sperm for injection.

In conclusion, the present data suggest that, in ICSI cycles, Kruger strict morphology is not predictive of fertilization rates, clinical pregnancy rates, blastulation, or blastocyst quality. Microscopic selection of sperm with “normal” morphology during the ICSI procedure allowed excellent outcomes even in samples with severe teratozoospermia. The live birth rate was similarly unaffected by sperm morphology parameters. Kruger strict criteria, as paternal factor, and/or the ICSI procedure itself did not appear to negatively impact blastocyst development or pregnancy outcome.

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