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A Retrospective Comparison of Pregnancy Outcome Following Conventional Oocyte Insemination vs Intracytoplasmic Sperm Injection for Isolated Abnormalities in Sperm Morphology Using Strict Criteria

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Abstract

Initial in vitro fertilization (IVF)–embryo transfer studies found poor fertilization and pregnancy rates following conventional fertilization of oocytes when using sperm with <4% normal morphology using strict criteria. Some consider today that sperm with only ≤5% normal morphology using strict criteria are associated with infertility. However, other studies have disputed the diagnostic potential of low strict morphology in identifying subnormal male fertility. Based on the original studies most IVF centers perform intracytoplasmic sperm injection (ICSI) when the sperm shows low morphology using strict criteria to allow selection of normal sperm. However, ICSI adds extra time for the embryologist and extra expense for the infertile couple. The present study retrospectively compared fertilization, pregnancy, and implantation rates according to the 2 methods of oocyte fertilization with sperm having normal morphology using strict criteria of ≤5% in women ≤39 years. All fresh embryo transfers were performed on day 3. There was a significantly higher fertilization rate with ICSI. However, there were significantly higher clinical and delivered pregnancy and implantation rates following conventional insemination. The rate of canceled transfers due to no available embryo was 1.9% with conventional insemination vs 1.5% for ICSI in women with adequate egg reserve. Hopefully, this retrospective study will generate interest in a prospective study.

Key words: In vitro fertilization, zona pellucida, subfertile male

In 1988 a new method of evaluating sperm morphology using strict criteria was described (Kruger et al, 1988). A score <4% normal predicted very poor fertilization and pregnancy rates following conventional oocyte insemination (Kruger et al, 1988). Although adjusting the concentration of sperm helped with the fertilization rates, the pregnancy rate per embryo transfer was still poor when sperm with <4% normal morphology was used for oocyte fertilization (Oehninger et al, 1988). Today many have even raised the discriminatory level to ≤5% (Coetzee et al, 1998; Nikolettos et al, 1999; Van Waart et al, 2001; Ombelet et al, 2003; van der Merwe et al, 2005).

However, some in vivo studies failed to find any association of low pregnancy rates with subnormal morphology using strict criteria (Check et al, 1992; Check et al, 2002). Furthermore, a study of in vitro fertilization (IVF) using conventional oocyte insemination failed to find any relationship between subnormal sperm morphology using strict criteria and pregnancy rate per transfer (Kiefer et al, 1996).

The majority of the world's IVF centers supported the concept that poor strict morphology led to infertility. With the development of intracytoplasmic sperm injection (ICSI), a method to overcome this problem of poor morphology was established by simply selecting only normal-shaped sperm and injecting 1 normal sperm into each egg (Oehninger et al, 1998; De Vos et al, 2003).

Although our own data did not support the importance of strict normal morphology as a discriminatory test of male subfertility, those couples undergoing IVF were counseled that the majority of the world's IVF centers believe that sperm with poor morphology is a cause of infertility. Thus, couples undergoing IVF–embryo transfer (ET) for various reasons (eg. fallopian tube problems, unexplained infertility, male factor problems) were given the option of ICSI so that a sperm with normal morphology could be selected for oocyte injection or the option of conventional insemination, which would also save money.

The present study retrospectively compared fertilization rates and pregnancy outcome following IVF-ET with oocytes fertilized by ICSI vs conventional insemination in couples with a male partner who had normal semen parameters except subnormal sperm morphology ≤5% normal using strict criteria.

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A retrospective review over a 7-year time period was performed. This review evaluated pregnancy outcome in all IVF cycles in women ≤39 years with male partners who had normal semen parameters except that normal morphology using strict criteria was ≤5%.

To be considered normal, the male partner had to have a motile density >8 x 10^6/mL, a hypo-osmotic swelling test >50%, and anti-sperm antibodies <20% determined by a direct immunobead test (Keel and Webster, 1990; World Health Organization, 1999). Sperm morphology was performed using the World Health Organization fourth method. Abnormal results were checked in duplicate. All morphology results of 0% to 4% were checked in duplicate by 2 of 4 andrologists to ensure accuracy of the results. Disagreement by 2 andrologists were settled by the results being given to a third, and the final answer would be whichever of the results of andrologist 1 or andrologist 2 were closest to those of andrologist 3. Rarely was a third andrologist needed.

There were no exclusions for day 3 serum follicle-stimulating hormone (FSH) levels including women with marked decreased oocyte reserve. Since women with decreased oocyte reserve (day 3 FSH >15 mIU/mL) who often end up doing single embryo transfers are not treated in most IVF practices, pregnancy outcome was also analyzed with this group of women excluded. Women aged ≤39 years were used for this study.

The oocyte stimulation regimen used for women with decreased egg reserve has been published in detail (Check et al, 2007). Some with normal FSH levels were treated with the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate 0.5 mg from mid-luteal phase for approximately 10 days and then reduced to 0.25 mg thereafter after gonadotropins were started. Some women were treated with all FSH, and some were treated with combinations of FSH and human menopausal gonadotropin (hMG). The usual starting dosage of gonadotropins was 300 IU per day and the amount of hMG varied from 75 IU to 150 IU per day. Frequently the dosage was reduced according to response. An injection of 10 000 U human chorionic gonadotropin (hCG) was given when at least 2 lead follicles reached 20 mm average diameter (ie, in good responders). Oocyte retrieval was then scheduled 35 hours later. Another protocol involved starting gonadotropins on day 2 but added a GnRH antagonist of either cetrorelix or ganirelix 250 µg daily when a dominant follicle reached 14 mm.

The pregnancy outcome parameters evaluated were clinical pregnancy rate per transfer (ultrasound showing a gestational sac at 8 weeks), live delivered pregnancy rate per transfer, and implantation rate. The clinical pregnancy rate per transfer (ie, those women having ß-hCG >100 mIU/mL that never progressed to ultrasound evidence of pregnancy) was also evaluated. Fertilization rates, failure to generate any embryos, and distribution of morphology per transfer (0—1%, 2%—3%, and 4%—5%) between ICSI and standard insemination were determined. The miscarriage rate per clinical pregnancy (ultrasound evidence of pregnancy) was also compared. At recent meetings, some experts have stated that they consider the sperm morphology abnormal up to 5% so the group of 4% to 5% was also analyzed.

Oocyte retrieval cycles were excluded if there was no transfer due to freezing all embryos because of the potential risk of the ovarian hyperstimulation syndrome or inadequate endometrial ultrasound parameters at the time of hCG injection. If, however, there was failure to fertilize, those cycles were included in the data analysis for fertilization rates and the number of cycles with failed fertilization. The rate of failure to produce any embryos for transfer was analyzed with the decreased ovarian reserve group left in and then with that group left out.

All COH protocols were used in the data analysis, including luteal-phase leuprolide acetate or GnRH antagonists (ganirelix or cetrorelix). Various combinations and brands of gonadotropins were used. Only the first IVF-ET cycle was evaluated per patient.

The ICSI technique was performed on all mature oocytes (Palermo et al, 1993). For each oocyte, a motile sperm was immobilized with an injection pipette in a drop of polyvinylpyrrolidone (Conception Technologies, San Diego, Calif) and then injected into the ooplasm. The injected oocytes were placed in Quinn fertilization media (Sage BioPharma, Pasadena, Calif) and 10% serum protein substitute (Sage BioPharma) and incubated for ≥16 hours before evaluation for signs of fertilization (2 pronuclei).

For conventional insemination, semen samples were prepared with a 3-layer isolate gradient initially (in later years switched to Pureconception [Sage, Trumbull, Conn]). Oocytes were inseminated with 25 000 normal motile sperm per oocyte and incubated for ≥16 hours before evaluation for signs of fertilization. Each specimen was centrifuged at 800 x *g* for 20 minutes. The resulting pellet was

removed and washed in 2 mL of medium (modified Quinns Advantage/0.5% serum substitute supplement [Irvine Scientific, Santa Ana, Calif]). Each egg was inseminated with 25 000 motile sperm.

This was a retrospective study. Thus, decisions to use ICSI or conventional insemination of oocytes was not influenced by the study design but was decided on an individual basis by the couple after consulting with a physician. All patients signed a consent on their initial visits giving permission to use their results without using their names in future studies. This study was approved by the Institutional Review Board of Cooper Health System, Camden, NJ.

It should be noted that the 7-year period was chosen because that is when the transfer catheter was changed to the Wallace with ultrasound guidance. There was no change in embryo media and the pregnancy rates were consistent over the time span. There were no additional embryologists used over this time. A couple of new doctors were added, but they were not the ones deciding on ICSI or conventional insemination and thus did not bias the results.

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A comparison of fertilization rates, pregnancy, and implantation in all patients according to insemination method is shown in Table 1. The fertilization rate was significantly higher with ICSI vs conventional insemination ($P < .001$). However, the clinical pregnancy rate was >50% higher with conventional insemination of oocytes vs ICSI ($P = .05$) as was the delivered pregnancy rate ($P = .038$). The implantation rate was almost twice as high with conventional insemination ($P < .001$).

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Table 1. *Comparison of outcome variables by method of insemination for cycles using sperm with normal morphology ≤5% using strict criteria*

In the conventional fertilization group, 17 women (8.3%) had a single embryo transfer in which the egg was fertilized by ICSI and 6 (11.5%) had a single embryo transfer. We defined a poor responder as one who had only 1 embryo transferred because only 1 was available. Thus, 23 of the 252 transfers (9.1%) were in poor responders.

Table 1 shows that if one eliminates the group of poor responders treated with minimal or no gonadotropins (Check et al, 2004), the clinical and delivered pregnancy rates were almost twice as high as those with conventional insemination ($P = .004$ and $P = .006$, respectively) and the implantation rates was more than twice as high ($P < .001$). Miscarriage rates were similar. Only 4.9% of women having oocytes fertilized by ICSI that did not proceed to a clinical pregnancy had ß-hCG levels over 100 mIU/mL, and there were no such women in the conventional group.

Table 2 evaluates the data according to ICSI vs conventional insemination in women stimulated with the luteal-phase leuprolide acetate protocol exclusively. Antagonists were not used in the early part of this study so we wanted to evaluate a more uniform group. Significant differences were noted in clinical pregnancy rates ($P = .019$), delivered rates ($P = .006$), and implantation rates ($P = .006$), again favoring conventional oocyte insemination. However, the fertilization rate was higher

with ICSI (*P* = .008).

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Table 2. *Comparison of outcome of IVF-ET by insemination method in women treated with uniform protocols**

In the group with uniform COH protocols (luteal-phase leuprolide acetate), 28 of 86 (32.5%) deferred fresh for frozen ET as did 6 of 26 (23.0%) using conventional insemination, as seen in Table 2. The category of clinical and delivered pregnancy rates per retrieval includes the first frozen ET if fresh was deferred (Katsoff et al, 2005). Again, conventional insemination resulted in higher pregnancy rates then ICSI.

Table 1 suggests that failure to perform ICSI for poor morphology using strict criteria could lead to twice the risk of not having an embryo to transfer (9.6% vs 4.9%). However, those data include the marked decreased oocyte reserve group using minimal stimulation. For conventional insemination, if one eliminates the low ovarian oocyte reserve group, there were 51 retrievals in which oocytes were retrieved and failure to produce an embryo only occurred in 1 (1.9%). Failed fertilization occurred in only 1.5% (2/128) of ICSI cycles. Two or more embryos were transferred in 88% of the ICSI cycles vs 82% of the conventional insemination cycles (*P* = NS). These data include poor responders.

To eliminate the possibility that there was a learning curve for ICSI during the early part of the study (although this procedure was performed routinely since 1994), we evaluated the pregnancy rates for each year for all types of male factor infertility for this 7-year period. The clinical and delivered pregnancy rates were stable over the 7 years with either conventional insemination or ICSI, as seen in Table 3. In 1999, 2002, and 2005 we added new doctors, but the data did not show that a learning curve for performing ICSI may have biased the outcome.

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Table 3. *Comparison of clinical and delivered pregnancy rates for conventional insemination vs intracytoplasmic sperm injection for all types of male fertility factor during a 7-year period*

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These data clearly show that in contrast to early studies, poor normal morphology using strict criteria does not result in a high fertilization failure rate with conventional insemination (Kruger et al, 1988; Oehninger et al, 1988). Possibly this could be related to improvements in the IVF laboratory and improvements in techniques and media. Although there seems to be a higher fertilization rate with ICSI, the fertilization rate with conventional insemination was sufficient at 61.8%. The increase in the number of embryos produced does not seem to favor the choice of ICSI over conventional insemination

because of the decrease in the pregnancy and implantation rate with the ICSI procedure.

These data support the conclusion that subnormal morphology using strict criteria does not accurately identify male subfertility at least according to IVF outcome. These data support the possibility that we may be doing a disservice to couples by choosing the method of fertilization based on these morphologic criteria and then performing ICSI rather than just using conventional fertilization. Perhaps there are much more important factors that allow the proper sperm to attach to the zona pellucida. Nevertheless, one should be cautious about conclusions from a retrospective comparison, especially with unequal numbers of patients in each group. At a minimum, these data should prompt a prospective study comparing conventional oocyte insemination vs ICSI for isolated sperm morphology defects. A multicenter study would help mitigate the possibility that some procedural nuances in a particular center lower the pregnancy and implantation rates following ICSI rather than the ICSI process itself.

Having sperm with 0% to 1% normal morphology using strict criteria seems to be very uncommon, especially if other semen parameters are normal. Thus, since there were no couples or doctors choosing conventional insemination when the levels were 0% to 1%, it is possible that ICSI could prove superior to conventional insemination for this minority subgroup. Determining this might require a large cooperative study amongst many IVF centers. In our center, we only found 8 such cases over a 7-year period.

Perhaps one could target the group with 2% to 3% normal morphology. Although there were only 9 cases using conventional insemination in this category, there was a large discrepancy in fertilization rates (44.1% vs 77.2%), as seen in Table 1. The clinical pregnancy rates in these groups were 22.5% (18/80) for ICSI and 22.2% (2/9) using conventional insemination. For delivery rates, the comparable statistics were 17.5% (14/80) for ICSI vs 22.2% (2/9) using conventional insemination.

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