

# Comparison of the efficacy of selecting sperm with normal nuclei by high magnification for intracytoplasmic sperm injection (ICSI) according to age in refractory in vitro fertilization (IVF) cases

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## Summary

**Purpose:** To determine the pregnancy rates according to age in women failing to conceive after three previous embryo transfers or having a husband whose sperm shows a DNA fragmentation index (DFI) > 30% when performing the sperm chromatin structure assay. **Methods:** Women up to age 45 were included and there was no restriction for low egg reserve. Live delivered pregnancy rates were determined according to three age groups:  $\leq 34$ , 35-38, 39-45. The data were also analyzed in a group comparable to previous publications using high magnification ICSI, i.e., younger women with normal egg reserve. Pregnancy rates following frozen embryo transfer were also evaluated. **Results:** Using all 86 in vitro fertilization-embryo transfer (IVF-ET) cycles the live delivered pregnancy rates were 40% (10/25) for women  $\leq 34$ , 24% (6/25) in women age 35-38, and 13.8% in women aged 39-45. Evaluating the younger group with normal egg reserve with  $\geq 3$  previous failed IVF-ET cycles the live delivered pregnancy rate per transfer was 38% (16/42). If one adds the additional six live deliveries from subsequent frozen embryo transfer (6 of 17, 35.3%) this group of women had a 52.3% (22/42) live delivered pregnancy rate from one egg retrieval. **Conclusions:** These data were uncontrolled and thus conclusions should be viewed with caution. The results are sufficiently encouraging to warrant a prospective controlled trial and possibly encourage a company to consider commercially manufacturing high magnification microscopes.

**Key words:** High magnification; Nuclear morphology; DNA fragmentation index; Intracytoplasmic Sperm Injection.

## Introduction

A manuscript published in December, 2003 in *Fertility and Sterility* found that in couples failing to conceive despite at least two previous cycles of in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI), very high pregnancy rates of 66% per transfer could be achieved by designing a special microscope set-up so that there could be sufficiently high magnification to allow viewing nuclear details and injecting into the oocytes only those sperm with normal nuclei [1]. In fact, the couples had failed to conceive after a mean of 4.1 previous IVF-ICSI attempts [1].

These findings were corroborated by a different group in which a 43.5% clinical pregnancy rate/transfer was achieved despite several previous ICSI failures even when the male partner had a high level of DNA fragmentation as determined by TUNEL assay (2).

## Materials and methods

The criteria for selection was a minimum of at least three previous IVF cycles or a DNA fragmentation index (DFI) > 30% on the sperm chromatin structure assay.

Pregnancy outcome was evaluated according to the reason for high magnification ICSI performed, i.e., multiple failed cycles vs high DNA fragmentation score.

After oocyte retrieval, sperm were selected with the aid of a specially designed microscope that magnifies sufficiently to observe nuclear detail. Sperm were immobilized by polyvinylpyrrolidone (PVP) and then selected according to the criteria of normal established by Bartoov *et al.* (1).

## Results

There were a total of 86 IVF-ET cycles evaluated. Only one cycle per couple was evaluated. There were 54 women failing at least three IVF-ET cycles (mean  $3.2 \pm 0.9$ ) and 32 women whose male partners had DFI scores > 30%.

The live delivery rates according to age combining refractory IVF cases with previous three or more failures and couples with male partners with high DFI scores are shown in Table 1.

If one evaluates women more comparable to those in the reports by Bartoov *et al.* [1] and Hazout *et al.* [2] i.e., women with sufficient egg reserve having three or more previous embryo transfers (ETs) that failed to achieve a live pregnancy, there were 42 ETs. The clinical pregnancy rate per transfer was 42.8% (18/42) and the live delivered pregnancy rate/transfer was 38.0% (16/42).

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Table 1. — Live delivery rates following IVF-ET according to age using high magnification ICSI (using all patients, i.e., multiple failed IVF cycles or high DFI scores).

Age	Number transfers	Live deliveries	% live deliveries/transfer
< 34	25	10	40
35-38	25	6	24
39-45	36	5	13.8

These data include women with poor egg reserve and even only one embryo transferred.

There were 17 frozen ETs (all ages) resulting in six (35.3%) live deliveries in women who were good responders. Thus if one adds these six cases to other 16 live deliveries from fresh transfers the overall live delivered pregnancy rates per retrieval in this group of refractory cases using high magnification ICSI was 52.3% (22/42).

## Conclusions

The study by Bartoov *et al.* evaluated women age 36 and under and the 66% quoted rate was close to the delivery rate [1]. The study by Hazout *et al.* [2] did not specify the age studied or the live delivered pregnancy rate. At least in the younger group that we studied (age  $\leq 34$ ) the present data would be consistent with the Hazout *et al.* study since it would be safe to assume at least a 10% miscarriage rate.

The present data found a significantly lower live delivery rate ( $p < .01$ ) in women 35-40 compared to 34 and under. This contrasts with our normal IVF statistics where the 35-40 year-old group only has a non-significant trend for lower live delivery rates compared to the younger group.

The possibility still exists that similar delivery rates could have been achieved by performing ICSI without high magnification since there were no controls. The cost for building the microscope was over 55 thousand dollars. Furthermore it takes much longer to perform ICSI using this technique and there is much greater exposure of the sperm to PVP and possible increased exposure to reactive oxygen species. A prospective controlled study is greatly needed before the true benefit of this new sperm selection technique can be determined.

These data do support that in multiple failed cycles of IVF a sperm factor may be the more likely etiologic factor in younger women but possibly the oocyte factor may be more important in women aged 35-45. Despite live delivery rates less than half compared to the younger group, the possibility exists that prospective studies could still demonstrate some benefit for selecting sperm by nuclear characteristics using high magnification even in somewhat older patients.

When we first built the microscope there had been reports of practically no pregnancies even with ICSI

when the DFI score was  $> 30\%$  [3]. Subsequently several articles refuted this claim [4-8]. At present it is not clear whether high DFI scores are associated with a higher miscarriage rate [4, 5] or not [6, 7] when performing ICSI.

Initially thinking that the live pregnancy rate was zero, it was exciting to find ongoing pregnancies by performing ICSI with selection of sperm by nuclear characteristics. Thus a prospective controlled study would be needed to determine if high magnification ICSI improves outcome when the male partner has a high DFI score. Nevertheless, we frequently did not perform the SCSA test unless there had been previous IVF failures. Though having three previous failures was not a prerequisite for obtaining a SCSA assay, it should be noted that the mean number of previous IVF cycles with failure to have a successful pregnancy was  $2.7 \pm 8$  in those with high DFI scores.

These data despite the caveats are sufficiently adequate to possibly encourage a company to commercially manufacture these high magnification microscopes.

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