

## THE VALUE OF MOTILE DENSITY, STRICT MORPHOLOGY, AND THE HYPOOSMOTIC SWELLING TEST IN IN VITRO FERTILIZATION-EMBRYO TRANSFER

D. KIEFER  
J. H. CHECK  
D. KATSOFF

University of Medicine and Dentistry of New Jersey,  
Robert Wood Johnson Medical School at Camden,  
Cooper Hospital and University Medical Center, Department  
of Obstetrics and Gynecology, Division of Reproductive  
Endocrinology and Infertility, Camden, New Jersey, USA

This study was conducted to evaluate the effect of a single abnormal semen parameter on subsequent implantation and pregnancy rates (PRs) following in vitro fertilization-embryo transfer (IVF-ET). The parameters evaluated were motile density (MD), strict normal morphology (SNM), and the hypoosmotic swelling (HOS) test. A total of 592 IVF cycles were evaluated. Patients were divided into four groups each with one abnormal semen parameter except for the control: group 1 ( $n = 509$ ), control group: MD  $\geq 10 \times 10^6/\text{mL}$ , HOS  $\geq 50\%$ , SNM  $\geq 4\%$ ; group 2 ( $n = 51$ ): HOS and SNM normal, MD  $< 10 \times 10^6/\text{mL}$ ; group 3 ( $n = 14$ ): MD and SNM normal, HOS  $< 50\%$ ; group 4 ( $n = 18$ ): MD and HOS normal, SNM  $< 4\%$ . The implantation rate was 10.2% for the control group. The implantation rate was similar for the low MD (9.0%) and the low SNM (16.7%) groups. However, the low HOS group had a significantly lower implantation rate (0%). The clinical PRs are similar in the control group and low MD and SNM groups, but decreased in the low HOS group (21.5, 15.0, 30.8, and 0%, respectively). Ongoing PRs were also similar with the exception of the low HOS group (0%). This comparative study supports previous conclusions that the subnormal HOS test is the best semen parameter available that predicts poor PRs. It also suggests that some qualitative defect in the embryos may result from defective sperm membranes, resulting in an apparently normal appearance but physiologically defective embryo.

**Keywords** hypoosmotic swelling test, IVF-ET, motile density, strict morphology

The hope exists that one semen parameter can be identified that is most predictive of pregnancy rates (PRs). Abnormal semen parameters have been associated with reduced PRs in vitro. However, when evaluating a semen analysis, there are often multiple factors that could cause, or at least contribute to, the infertility problem. Thus, it is frequently difficult to determine which parameter, if any, has the greatest impact (i.e., lowest PR) on subfertility. One of the best ways to determine the importance of a semen parameter is to examine its effect on in vitro fertilization (IVF) outcome.

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Address correspondence to Jerome H. Check, MD, 7447 Old York Road, Melrose Park, PA 19027, USA.

Sperm motile density (MD) is a common criterion used to detect the subnormal male. Others have found strict morphology to be an important predictor of subfertility. With IVF, adjustments can be made for low MD and strict normal morphology (SNM) leading to normal fertilization rates [6, 10, 11]. Oehninger et al. reported that adjusting the number of motile sperm per oocyte led to improved fertilization rates but not PRs [10]. In 1994, the Norfolk group published a study showing a normal fertilization rate after adjusting sperm concentration, but still a decreased PR with low SNM, and thus concluded that morphology is the best parameter predictive of subfertility [5]. However, several studies were not able to confirm this conclusion [2, 4].

It would appear that pregnancy rate correlates directly to the fertilization rate and that the PR/cycle would be similar as long as a minimal number of embryos are transferred. However, some studies, including Oehninger's, have challenged this conclusion [4, 10]. Another test that has been used in evaluating possible male factor infertility is the hypoosmotic swelling (HOS) test [1, 3]. In contrast to sperm concentration, which may fluctuate over time, the HOS score is relatively stable [12].

This study was carried out to evaluate single abnormal semen parameters by evaluating couples where only a single factor was subnormal and evaluating subsequent implantation and PRs following in vitro fertilization-embryo transfer (IVF-ET).

## MATERIALS AND METHODS

Patients were enrolled in the study from 1 November 1991 through 30 October 1995. A total of 592 IVF cycles were evaluated. Patients were divided as follows into four groups with one parameter abnormal except for the control group: group 1 control ( $n = 509$ ): MD  $\geq 10 \times 10^6/\text{mL}$ , HOS  $\geq 50\%$ , SNM  $\geq 4\%$ ; group 2 ( $n = 51$ ): HOS and SNM normal, MD  $< 10 \times 10^6/\text{mL}$ ; group 3 ( $n = 14$ ): MD and SNM normal, HOS  $< 50\%$ ; group 4 ( $n = 18$ ): MD and HOS normal, SNM  $< 4\%$ .

Patient selection was as follows: ET with at least three embryos; no donor sperm; data available for all three semen parameters prior to the day of retrieval: MD, HOS score, and SNM; abnormal results were repeated and were still abnormal; no oocyte recipients included; no intracytoplasmic sperm injection (ICSI) or assisted embryo hatching used.

Normal morphology was measured using Tygerberg strict criteria [9]. The HOS test was performed as previously described by Jeyendran et al. [7].

## RESULTS

The mean number of embryos transferred, implantation rate, clinical PR, and ongoing/delivery rates for the four groups are shown in Table 1. The implantation rate was 10.2% for

TABLE 1 Comparison of IVF outcome according to a single abnormal semen parameter

Group	Implantation rate	Clinical PR	Ongoing delivery rate
1. All normal ( $n = 509$ )	10.2% (213/2091)	25.7% (131/509)	21.8% (111/509)
2. MD low ( $n = 51$ )	9.0% (19/211)	25.5% (13/51)	15.7% (8/51)
3. HOS low ( $n = 14$ )	0% (0/61)	0% (0/14)	0% (0/14)
4. SNM low ( $n = 18$ )	16.7% (12/72)	44.4% (8/18)	38.9% (7/18)

Note. PR, pregnancy rate.

the normal group. The implantation rate was similar in the low MD and low SNM groups, but decreased (0%) in the subnormal HOS group ( $p = .014$ , chi square). The clinical PRs were similar for the control group and low MD and SNM groups (25.7, 25.5, and 44.4%, respectively), but decreased for the low HOS group (0%,  $p = .043$ , chi square). The ongoing/delivery rates follow the same pattern, with the HOS at 0% ( $p = .043$ , chi square).

## DISCUSSION

The Cooper Center for IVF previously published data from a matched controlled study concluding that low HOS scores do not predict decreased fertilization but do identify couples with poor PRs following IVF [4]. All 14 couples with low HOS scores used in the present study were also part of the aforementioned report of 26 matched pairs where the male had an HOS score <50%. The other 12 were not included in this study because they had a subnormal semen parameter other than the HOS score. Thus this study was aimed more at determining which single subnormal semen parameter other than the HOS score could predict poor PRs despite normal fertilization. The data indicate that only the HOS score could identify this unusual group of patients. Our results contrast those of Oehninger et al., which suggest that strict morphology <4% could also demonstrate dissimilitude between fertilization and PRs [5, 10].

There have been many previous studies concerning poor fertilization rates according to abnormal semen parameters. This study differs by requiring for inclusion a minimum of 3 embryos transferred, thereby eliminating cases of poor fertilization. In fact, fertilization rates were not provided. Instead, the study was designed to see if any single semen parameter could determine a male factor predictive of subfertility despite embryo replacement. The data presented herein agree with the concept that a sperm abnormality may exist that allows normal fertilization following IVF but yet there are still poor subsequent PRs following ET.

The parameters evaluated in this study can be routinely performed in most laboratories. Furthermore, they are inexpensive and therefore, unlike the SPA and acrosome reaction, are available to nearly all patients. Normal implantation and PRs can be achieved by adjusting for the number of morphologically normal motile sperm. However, the low HOS group had the lowest implantation rate, thus indicating that defective sperm membranes may cause some qualitative defects in the oocyte, resulting in a visibly normal embryo but yet apparently physiologically defective.

A recent *in vivo* study by Katsoff and Check suggests that the effect of sperm membrane damage may be reversible with the use of chymotrypsin/galactose [8]. Further study is required to determine if the same effect can be seen *in vitro*. It is also possible that micromanipulation techniques such as ICSI could prevent damage to the embryo. The basis for these suggestions are the possibility that abnormal sperm may release some toxic product that not only damages the sperm membrane but may also damage the oocyte in such a way that fertilization and cleavage are not affected but the implantation is impeded.

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