Original Articles

Reproductive Biology Section

About 13% of women may have the wrong method of oocyte insemination when undergoing in vitro fertilization by failure to evaluate the abnormal hypo-osmotic swelling test score

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Summary

Purpose: To determine the percentage of males with a low hypoosmotic swelling (HOS) test score in couples having in vitro fertilization-embryo transfer (IVF-ET) where the male partner had normal motile densities and no antisperm antibodies. *Methods:* The results were also analyzed according to the percentage of HOS scores < 50% when morphology using strict criteria was ≤ 4% or > 4%. A retrospective review was performed. *Results:* There were 250 subnormal HOS scores out of 1993 otherwise normal semen specimens. *Conclusions:* 12.5% of couples would have had conventional oocyte inseminations rather than ICSI if the HOS test had not been not performed (it is rarely performed by other IVF centers). Without ICSI expected live delivered pregnancy rates would be close to zero for males with low HOS tests < 50%. Intracytoplasmic sperm injection allows pregnancy rates almost as good as completely normal sperm.

Key words: Hypoosmotic swelling test; Intracytoplasmic sperm injection; Conventional oocyte insemination; Implantation defects.

Introduction

The development of intracytoplasmic sperm injection (ICSI) has been of monumental importance in allowing males to fertilize oocytes whose semen parameters were insufficient to achieve fertilization even through in vitro fertilization (IVF) with conventional oocyte insemination. Because ICSI adds additional expense, more embryologist time, and even may reduce the chance of pregnancy compared to embryos fertilized through conventional means, most IVF centers do not perform ICSI routinely but only when it seems necessary [1, 2].

The hypoosmotic swelling (HOS) test which measures the functional integrity of the sperm membrane was found to be able to detect males with marked subfertility [3]. However some studies concluded that IVF with conventional oocyte insemination eliminated any contributions of this abnormality (defined as males with < 50% tail swelling following exposure to a hypo-osmotic medium) to male subfertility as determined by fertilization rates [4-8]. Interestingly these studies neglected to provide pregnancy rates [4-8].

Subsequently, it was found that a subnormal HOS test does allow normal fertilization of oocytes and produce embryos with normal morphology but that the embryos are severely impaired from implanting [9-12]. It has been demonstrated that the defect of embryo implantation because of fertilization with sperm with HOS test scores < 50% can be obviated by ICSI [13, 14]. The concept is that the abnormality of embryo implantation defects is created by the transfer of the toxic product (probably a protein) from the sperm to the zona pellucida which then becomes incorporated into the embryo membrane [12, 15].

It is not clear why this simple very inexpensive semen test is rarely performed by most IVF centers. The frightening thing about this abnormality is that in contrast to sperm binding abnormalities or oocyte activation defects where the problem would be discovered after just one IVF cycle because of low or failed oocyte fertilization, the couple with an undetected male with a subnormal HOS test problem could be undergoing many expensive and risky IVF procedures with little chance of successful embryo implantation.

The objective of the present study was to determine how common this sperm abnormality is in a population of couples undergoing IVF-ET where other semen parameters are normal, such that except for the low HOS test score, ICSI would not have been suggested.

Materials and Methods

All first IVF-ET cycles from January 1, 1997 to March 31, 2008 were evaluated. Excluded from this study were IVF cycles where the male partner had a motile density < 8 x 10^6/ml or > 50% antisperm antibodies detected by the direct immunobead assay. Two age groups were evaluated: age < 39 and age 40-42.

As mentioned in the introduction, some recent studies suggest that ICSI may lower rather than help the odds of a successful pregnancy when using sperm with a low percentage of

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normal morphology using strict criteria [1]. Therefore some clinicians would use ICSI for low morphology. Thus the data were evaluated both with the elimination of IVF cycles with strict morphology ≤ 4% and were also evaluated with all morphology included for those who do not think that poor morphology correlates with low fertilization rates with conventional oocyte insemination.

Clinical and live delivered pregnancy rates were also determined for those with low HOS test scores. The oocytes from women whose male partners had low HOS tests were fertilized by ICSI.

The couples used for the study whose male partners all had normal semen parameters could still have had ICSI performed because of fear of failed fertilization but most would have had conventional oocyte insemination. Probably about half of the group having poor morphology even with normal HOS test scores would have ICSI performed.

The HOS test was performed as described by Jeyendran *et al*. [16].

Results

One can assume that most IVF centers will do ICSI for poor morphology. Therefore Table 1 includes all couples undergoing their first IVF-ET cycle at our institution where all semen parameters were normal and the female partner was ≤ age 42. The frequency of males with low HOS test scores and normal semen parameters was 12.5% (250/1993).

Couples with male partners with normal semen parameters except those possibly having < 4% normal morphology using strict criteria were separately evaluated (Table 2). There were 14.8% (26/174) of the male partners with abnormal morphology who also had low HOS test scores.

The clinical and live delivered pregnancy rates according to the two age groups in males with low HOS test scores are shown in Tables 1 and 2.

Table 1. — *Frequency of low HOS test scores in male partners of couples undergoing in vitro fertilization – all semen parameters normal (strict morphology > 4%).*

Ages of women	≤ 39	40-42
# pts w/normal semen parameters including strict morphology > 4%	1663	330
# pts w/normal semen parameters that have low HOS test	206	44
% of patients with low HOS test scores	12.4	13.3
# transfers with low HOS scores	54	14
# pregnancies	22	7
% pregnancies/transfer	40.7	50.0
# clinical pregnancy	19	5
% clinical pregnancy per transfer	35.2	35.7
# live deliveries	18	3
% live delivered pregnancy/transfer	33.3	21.4

Discussion

The frequency of low HOS test scores in males participating in IVF-ET (where semen parameters show normal motile density and there is not a high level of antisperm antibodies) was higher than the frequency of subnormal morphology (12.7%, 276/2,170 vs 8.1%, 177/2,170) ($p < 0.0001$).

Table 2. — *Frequency of low HOS test scores in male partners of couples undergoing in vitro fertilization – semen parameters normal except morphology ≤ 4.*

Ages of women	≤ 39	40-42
# pts w/normal semen parameters and strict morphology ≤ 4%	144	30
# pts w/normal semen parameters that have low HOS test scores	19	7
% of patients with low HOS test scores	13.2	23.3
# transfers of patients with low HOS test scores	15	5
# pregnancies	6	2
% pregnancies/transfer	40.0	40.0
# clinical pregnancies	4	2
% clinical pregnancy per transfer	26.7	40.0
# live deliveries	4	1
% live delivered pregnancy/transfer	26.7	20.0

The pregnancy rates demonstrated that fertilization of oocytes by ICSI overcomes the severe implantation defect associated with conventional fertilization of oocytes with sperm with low HOS test scores.

Thus based on previous data showing terrible pregnancy rates with conventional insemination of oocytes with sperm with low HOS test scores, failure to perform this simple test prior to oocyte insemination could lead to repeated failures in about 12% of couples undergoing IVF-ET [9-14].

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