

## Editorial Articles

# Sperm may be associated with subfertility independent of oocyte fertilization

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## Summary

In vitro fertilization-embryo transfer seems to be an effective treatment for unexplained infertility. Some IVF centers always perform intracytoplasmic sperm injection in these circumstances being concerned that fertilization failure may occur by conventional oocyte insemination. However, other IVF centers perform intracytoplasmic sperm injection on half of the oocytes and do conventional insemination on the other half. However, if the group with conventional oocyte insemination had a good fertilization rate, in the future intracytoplasmic sperm injection would not be performed. Other IVF centers would inseminate all the oocytes with conventional insemination and not consider intracytoplasmic sperm injection in the future unless there were poor fertilization rates. The aforementioned studies suggest that prior to considering conventional insemination that as a minimum the simple inexpensive hypo-osmotic swelling test be performed and strong consideration also be given to the sperm stress test and SCSA.

Similarly, even though IUI is less risky and costly than IVF-ET, there still is a moderate expense and risk involved, especially when superovulation is used. Thus, consideration for performing these tests should also be given even prior to IUI. This is especially important for subnormal HOST scores where pretreatment of the sperm with the protein digestive enzyme chymotrypsin when preparing the sperm has been demonstrated to markedly improve pregnancy rates [12].

## Introduction

The traditional concept is that sperm concentration, motility, and even morphology are needed to allow a normal sperm to reach the oocyte, attach to the zona pellucida, traverse the zona, and fertilize the oocyte. The traditional concept is that once fertilization takes place the job of the sperm is finished. Another way of stating this is that once an egg is fertilized any subsequent problem in embryo development or implantation is either related to the oocyte or the uterine environment. The purpose of this editorial is to supply evidence that the sperm factor may cause infertility by inhibiting implantation, or cause infertility by creating embryos with less quality morphology, or create embryos that are more likely to die after a pregnancy is established, i.e., lead to a higher rate of miscarriage.

## Implantation defects

### *The hypoosmotic swelling test:*

The most important of the sperm abnormalities associated with implantation disorders is the subnormal hypoosmotic swelling test (HOST). This test was modified for human use in 1984 by Jeyendren *et al.* [1]. They considered that abnormalities in this factor cause a functional impairment of the integrity of the sperm membrane [1]. Abnormal was considered if < 50% of the sperm demonstrated sperm tail swelling after being exposed to a hypoosmotic environment and 50-59% was considered the grey zone. One study found no pregnancies following natural intercourse despite correction of female factors in couples where the male partner had a HOST score < 50% even if the motile density was normal [2]. The same group subsequently found that scores in the grey zone had no clinical significance [3]. Another important characteristic of males with low HOST scores was that once it was low, it stayed low over time [4].

The prevailing concept at this time was that HOST score defects in sperm probably lead to poor fertilization of the oocyte since the functional integrity of the sperm membrane is important for the acrosome reaction, sperm capacitation, sperm metabolism, and the binding of the sperm to the egg surface [1]. If this theory was true, then failure to achieve fertilization and subsequent pregnancies would be found following in vitro fertilization (IVF) following conventional fertilization of the oocytes. However, the effect on failed fertilization by sperm with low HOST scores was challenged by several studies showing no decrease in fertilization rates or embryo quality following IVF-ET [5-7].

These aforementioned studies [5-7] did not mention pregnancy rates, thus the possibility did exist that low HOST scores could be associated with normal fertilization, but nevertheless, poor pregnancy rates. Very poor pregnancy rates despite normal fertilization and embryo quality were, in fact, subsequently demonstrated in a matched controlled study where the clinical pregnancy rates for couples with males with normal HOST scores was 25.9% per transfer vs 3.7% with low HOST scores [8]. These data were consistent with the concept that sperm with low HOST scores could lead to subfertility not by inhibiting fertilization but in some way lead to embryo implantation defects [8].

The concept of sperm with HOST scores < 50% causing embryo implantation disorders was supported by subsequent studies. One study evaluated donor-recipient pairs sharing a common pool of oocytes; the female partners of males with completely normal semen parameters showed a 50% clinical pregnancy rate per embryo transfer (ET) but the female partners of males with normal semen parameters but HOST scores < 50% had a zero % clinical pregnancy rate [9]. The number and quality of embryos transferred were very similar in both groups as were fertilization rates [9].

Another study also supported the concept of implantation disorders by comparing pregnancy outcome in women transferring embryos with fertilization achieved by sperm with single semen parameter abnormalities [10]. The clinical pregnancy rates when motile density alone was <  $10 \times 10^6/\text{ml}$  (25.5% per transfer) and when strict normal morphology was < 4% (44.4%) were no lower than when all semen parameters were normal (25.7%); however, there were no pregnancies when the HOST score was < 50% [10].

The factor causing the HOST score was found to be cryolabile [11]. This factor seemed to be proteinaceous in nature not only as evidenced by improving HOST scores with treatment of the sperm with the protein digestive enzyme chymotrypsin, but also the demonstration of improved pregnancy rates following chymotrypsin treatment after either intrauterine insemination or IVF after conventional insemination of oocytes [12, 13].

Finally, evidence was provided that the implantation defect was caused by the supernumerary sperm attaching to the zona pellucida rather than the one sperm fertilizing the oocyte by demonstrating a 49.0% and 45.3% clinical and viable pregnancy rate per transfer despite low HOST scores by fertilizing the oocytes by intracytoplasmic sperm injection [14]. Thus, one hypothesis to explain these observations is that a toxic protein attached to the sperm causes a defect in the functional integrity of the sperm membrane. This protein can be transferred to the zona pellucida after normal fertilization by intercourse, intrauterine insemination, or IVF with conventional insemination. The zona pellucida becomes part of the embryo membrane. This toxic factor now in the embryo membrane causes a functional impairment of the embryo membrane thus inhibiting implantation.

#### *Sperm stress test:*

The sperm stress test was designed to measure endogenous lipid peroxidation [15]. This process results in extensive damage to the plasma and acrosomal membranes, leading to loss of permeability and leakage of pyridine nucleotides that ultimately renders the sperm immotile [16-18]. The sperm stress test combines the stress of a shaking water bath and incubation at 40°C for four hours to cause loss of motility [19]. The sperm stress test score was equal to the initial percent motility divided by the motility after four hours [19]. A stress score < 75% was considered abnormal.

Though the number of embryos transferred were not significantly different in those with scores  $\geq 75\%$  (mean = 3.4) vs those with scores < 75% (mean 2.6), 23 of 64 (35.9%) conceived with normal scores vs only one of 55 (1.8%) with subnormal scores [19].

Thus, this test also seems to predict implantation defects rather than fertilization failure similar to the HOST. However, since 55 of 119 (46.2%) were subnormal, this abnormality seems to be more prevalent than low HOST scores. These findings need to be corroborated by another IVF center.

#### *Sperm chromatin structural assay (SCSA):*

This test measures the percentage of sperm with a high susceptibility to low pH-induced DNA denaturation and is expressed as the DNA fragmentation index (% DFI) [20-22]. Some studies found that even despite transferring embryos following conventional IVF-ET there were no live pregnancies when the DFI was > 27% [21-23]. However, in contrast to HOST abnormalities, these studies found that ICSI did not correct the problem [21-23]. Thus, this appeared to be another test that could detect implantation defects.

However, two recent studies questioned whether abnormal SCSA abnormalities have any predictive value in predicting implantation abnormalities. One study only showed no lowering of pregnancy rates following IVF with ICSI [24]. This nonetheless left the possibility that high DFI scores could still inhibit implantation when sperm attach to the zona pellucida, but similar to the HOST abnormality, is correctable by avoiding this contact by performing ICSI [24]. Another recent study, however, even questioned any predictability of lowered pregnancy rates following conventional insemination of oocytes [25]. The fact that this same study found very poor pregnancy rates with intrauterine insemination with sperm with high DFI scores would suggest that this defect inhibits natural fertilization more but can be overcome by adding excessive sperm [25].

Our own experience involving refractory IVF cases suggests that high DFI scores may lower clinical pregnancy rates slightly (36.1% for low DFI scores vs 27.6% for high scores, and may also increase miscarriage rates (46.1% vs 62.5%) thus leading to half the number of live pregnancies (19.4% vs 10.3%) [26]. The same group that initially found no pregnancies with IVF and high DFI scores studying a larger group of first IVF cycles concluded that high DFI scores do allow live pregnancies. However, there was a 40% lower chance of success [27]. Similar to our findings, the lower live pregnancy rate was more related to a higher miscarriage rate than to a lower clinical pregnancy rate [27]. It is not clear from this study if lower success would have been seen if conventional insemination had been performed.

## Sperm factor as a cause of spontaneous abortion

### *Oligoasthenozoospermia:*

A study evaluated the outcome of pregnancies achieved by men with oligoasthenozoospermia vs normal following IVF-ET after conventional insemination of oocytes and found similar implantation rates but higher miscarriage rates in those females whose male partners had low motile densities (40.0% vs 11.7%) [28]. This was not related to teratozoospermia because only males with normal morphology were evaluated in this study [28].

In another study evaluating single sperm parameter abnormalities with conventional oocyte insemination for IVF, the miscarriage rate when all parameters were normal was 15.2%, 38.4% when motile density was low, and 12.5% when strict normal morphology was < 4% [10]. Though one could consider that subnormal quality sperm as manifested by low motile densities could allow a greater chance for fertilization by sperm with chromosome abnormalities, the fact that low strict morphology does not seem to increase miscarriage rates, suggests the possibility that some other factor exists that causes the miscarriage rather than a genetic explanation. The miscarriage rate not being lowered by males with teratozoospermia was confirmed by another study of pregnancies achieved through normal intercourse where the miscarriage rate in female partners of males with low strict morphology was only 7% [29].

### *Abnormal sperm chromatin structure assay:*

As mentioned earlier in this editorial, sperm from males with high DFI scores seem to result in pregnancies that are more likely to abort [26, 27].

## Sperm factor as a cause of poor embryo morphology

There are some data in humans that suggest that poor sperm quality can be associated with poor embryonic development [30, 31]. A case report of a woman who consistently made highly fragmented embryos illustrated this possibility [32].

The male partner had a normal spermiogram except for the presence of antisperm antibodies (82% IgG and 77% IgA using the immunobead assay) and a low HOST score of 47%. The female partner of this male became a shared egg donor where she underwent oocyte retrieval but shared half of the eggs with a recipient. The donor's half of the eggs were fertilized by her male partner's sperm and the recipient's half were fertilized by the male partner for the recipient.

The 49 oocytes retrieved were divided with 25 going to the recipient. The fertilization rate was 70.8% (17 of 24) for the donor following ICSI. The embryos were all cryopreserved. All 17 of the donor's embryos were allowed to cleave and 13 were transferred over three cycles. All of the embryos had > 25% fragmentation and 30.7% had more than 50% fragmentation. She failed to conceive.

The recipient used conventional insemination and 52% fertilized. Five of the embryos were allowed to cleave and two were discarded because of cleavage arrest. None of the embryos transferred had > 50% fragmentation and only 33.3% had > 25% fragmentation. She conceived and delivered. The remaining eight embryos that had been cryopreserved were donated to another woman. All eight were allowed to cleave; none had > 50% and only 12.5% had > 25% fragmentation.

## Conclusion

In vitro fertilization-embryo transfer seems to be an effective treatment for unexplained infertility. Some IVF centers always perform intracytoplasmic sperm injection in these circumstances being concerned that fertilization failure may occur by conventional oocyte insemination. However, other IVF centers perform intracytoplasmic sperm injection on half of the oocytes and do conventional insemination on the other half. Nonetheless, if the group with conventional oocyte insemination had a good fertilization rate, in the future intracytoplasmic sperm injection would not be performed. Other IVF centers would inseminate all the oocytes with conventional insemination and not consider intracytoplasmic sperm injection in the future unless there were poor fertilization rates. The aforementioned studies suggest that prior to considering conventional insemination that as a minimum the simple inexpensive hypo-osmotic swelling test be performed and strong consideration also be given to the sperm stress test and SCSA.

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