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Some semen abnormalities may cause infertility by impairing implantation rather than fertilization

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Summary The hypo-osmotic swelling (HOS) test measures the functional integrity of the sperm membrane. Although, the sperm membrane is essential for the fertilization of oocytes, several clinical studies suggest that abnormally low HOS test scores do not predict poor or failed fertilization in human in-vitro fertilization trials. However, in-vivo and in-vitro studies clearly demonstrate that a low HOS score is associated with poor pregnancy rates suggesting this sperm defect causes implantation problems rather than fertilization problems. The problem of implantation could be caused by the supernumerary sperm attached to the zona pellucida. Supporting evidence for this theory was demonstrated by finding high pregnancy and implantation rates despite low HOS scores following intracytoplasmic sperm injection which avoids the presence of supernumerary sperm on the zona pellucida. These data thus support theories that some sperm abnormalities may reduce fertility potential by causing implantation disorders rather than problems with fertilization. © 2001 Harcourt Publishers Ltd

PROBLEM OF IDENTIFYING SUBFERTILE MALES

The classic concept is that when sperm is the cause of infertility it is related to failure to fertilize the oocyte. Unfortunately it is not very easy to detect male subfertility by motile density (1), standard morphology (2), or morphology by strict criteria (3). Sometimes what appears to be normal sperm is determined to be subfertile (4). Thus there has been great interest in finding other tests that can help determine if a given sperm specimen is fertile or subfertile.

IMPORTANCE OF TESTS OF SPERM MEMBRANE FUNCTION

Jeyendran hypothesized that since membrane integrity is not only important for sperm metabolism, but it is required for successful union of the male and female gametes, i.e., for sperm capacitation, the acrosome reaction, and the binding of the spermatozoon to the egg surface (5), that tests designed to evaluate sperm membrane function may prove useful in detecting the subfertile male (5). Jeyendran pointed out that a property of the cell membrane is its ability to permit the transport of molecules selectively. Using bovine sperm, it had been shown that when exposed to hypo-osmotic solutions, water will enter the spermatozoon in an attempt to reach osmotic equilibrium (6–9). This test was modified for human sperm by Jeyendran et al. (5).

THE HYPO-OSMOTIC SWELLING TEST AND CORRELATION WITH OTHER SEMEN PARAMETERS

Once the test for humans was introduced, the first studies tried to see if subnormal hypo-osmotic swelling (HOS) test scores would correlate with other subnormal semen values. Chan et al. (10) found that semen samples with abnormal semen parameters had lower HOS scores, but

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only a weak correlation was seen with poor morphology and low motility, and no correlation was seen with in-vitro fertilization (IVF) as judged by the hamster ova penetration test assay (10). The same group subsequently found that there was a significant difference in HOS scores in fertile (72.4%) vs infertile (56.8%) couples (11). However, they did not find that the addition of this test improved fertility evaluation much more than the standard semen parameters (11). Another study found that the best correlation of the low HOS score is with poor sperm viability, only moderate correlation with normal sperm morphology, weak correlation with the sperm penetration assay, and the worst with IVF (12). Another study agreed that there was poor correlation with low HOS test scores and sperm penetration assay, but it did correlate with motility (13). Fuse et al. confirmed the lack of association of HOS test and sperm penetration assay but did find correlation with sperm concentration and motility (14).

HOS TEST AND FERTILIZATION OF HUMAN OOCYTES

A couple of studies found that low HOS scores correlated with the failure to fertilize human oocytes following IVF (15,16). Another study found the HOS test useful to predict failed fertilization only when used in the post-swim-up specimen (17). However several studies were subsequently published suggesting that there is very little if any correlation between low HOS scores and poor oocyte fertilization (18–20). Further studies confirmed that the other semen parameters of sperm concentration, motility and morphology (even though they have significant limitations as indicators of fertility) (1–3,21–25), correlated much better with oocyte fertilization than the HOS score (26,27).

DISSIMILITUDE BETWEEN FERTILIZATION AND PREGNANCY RATES

Though the majority of studies showed that low HOS scores did not correlate with poor fertilization (18–20,26,27) we found, at least in vivo, pregnancies were extremely rare when the male partner had HOS scores <50% (28). Two hypotheses could explain this apparent paradox. One possibility is that IVF is a very effective means of overcoming the HOS abnormality; a second theory is that the low HOS score is associated with subfertility but the defect causes the infertility problem in some other way than failure to fertilize the oocyte. The previous studies of HOS and fertilization rate did not include pregnancy rates (18–20). We thus performed a matched control study to determine if the pregnancy rate, even with IVF-embryo transfer (IVF-ET), suffers despite normal fertilization (29). The data confirmed previous studies that there is poor correlation with HOS scores and fertilization rates (29). However, despite the transfer of similar numbers and quality of embryos, we found a marked reduction in pregnancy rates in couples whose husbands had HOS scores <50% compared to those with higher scores (29).

At Cooper Center for IVF we have a shared oocyte system where in exchange for marked reduction in cost for IVF, an egg donor gives half of the oocytes to a recipient who has poor oocyte quality or quantity (30). Thus, we have an ideal situation to compare outcome in two women using the same egg pool but where one male partner had subnormal HOS scores and the other was normal (31). Once again a marked reduction in viable pregnancy rates was found in the couples where the male partner had an HOS score <50% (31). Another study confirmed that when comparing semen parameters of count, motility, morphology and HOS test scores, the latter was the least beneficial in predicting poor fertilization, but had the most efficacy in prognosticating failed conception despite ET (32).

TOXIC FACTOR CAUSING EMBRYO IMPLANTATION DEFECT

One proposed mechanism to explain how a male factor problem, as manifested by low HOS scores, could cause embryo implantation defects, is that it is not the one sperm fertilizing the oocyte that is responsible, but the supernumerary sperm that attach to the zona pellucida. Possibly some toxic factor associated with the sperm membrane gets transferred to the oocyte membrane, which, in turn, is reflected in the embryo membrane, and thus implantation is inhibited.

If the theory of a toxic factor is correct, then avoiding contact with the zona pellucida by fertilization with only one spermatozoon, i.e., as accomplished by intracytoplasmic sperm injection (ICSI), should overcome the problem. An anecdotal report supported this concept (33). A much larger series found that in contrast with previous studies using conventional insemination technique with IVF, where viable pregnancy rates approached 0%, the viable pregnancy rate per transfer in 52 couples where the male partner's HOS score was <50% was 45.3% and the implantation rate was 27.1% (34).

EVIDENCE FOR PROTEINACEOUS NATURE OF TOXIC FACTOR

The nature of the reported toxic factor is not known. Previous studies have found that pregnancy rates following intrauterine insemination (IUI) can improve when sperm bound with antisperm antibodies are first treated

by the protein digestive enzyme chymotrypsin-galactose (35). This treatment was also found helpful prior to the conventional insemination of oocytes with IVF when sperm was bound with antisperm immunoglobulins (36). Since occasionally sperm positive for antisperm antibodies also have HOS scores <50%, and the chymotrypsin-galactose therapy not only improves pregnancy rates but also frequently raises the HOS score, we considered that possibly chymotrypsin may improve the HOS scores even when antisperm antibodies were not present. Improvement of the HOS score with this enzyme would suggest that the toxic factor may be proteinaceous in nature. A small anecdotal series found that a majority of specimens with HOS scores <50% could improve to normal levels following chymotrypsin-galactose therapy, and this improvement results in a higher pregnancy rate (33).

CLINICAL USE

The HOS test is easy to perform and is inexpensive (5). In contrast to sperm count and motility which can fluctuate considerably, the HOS test scores are stable over time (37). However, this test is not included in most standard semen analyses and is rarely considered when there is unexplained infertility. There appears to be ample data that successful pregnancies are not likely with HOS scores <50% unless the sperm is treated with chymotrypsin-galactose or IVF with ICSI is performed. Certainly this test should be at least performed before undergoing expensive IVF. It is frightening to consider that many centers proceed to IVF for 'unexplained infertility', but if the low HOS test exists but remains undetected, the treating physician may be lulled into a false sense of security by creating what appears to be normal-looking embryos. The fact that these embryos are not likely to implant will be completely missed, and the patient may undergo repeated expensive failures which could have easily been corrected if this simple test had been performed.

Based on these data, treatment strategies when the HOS test is subnormal, are straightforward. First, the sperm specimen should be treated with chymotrypsin-galactose, and if the HOS score improves to greater than 50%, IUI and even intracervical insemination with the treated sperm should be performed. It would be important to stress that to prevent other competing sperm in the female's reproductive tract from being present that unprotected intercourse should be avoided until after ovulation. If the couple fails to conceive after several cycles of IUI with chymotrypsin-galactose treated sperm, or if the HOS does not improve >50%, then the couple should proceed to IVF with ICSI.

EVIDENCE FOR SPERM CAUSING IMPLANTATION DEFECTS DESPITE NORMAL HOS SCORES

Therapeutic strategies to cover the possibility of the male partner having sperm that could cause embryo implantation defects despite a normal HOS test are more difficult. Years ago, a study found a very high pregnancy rate when using therapeutic donor sperm insemination when a couple has had many cycles with failure to conceive even when all infertility factors were apparently corrected (4). The possibility exists that these males may have had subtle sperm defects resulting in inability to fertilize the oocyte, or possibly failure to conceive was related to failure to establish the proper immunological milieu that would favor the humoral immune system possibly related to sharing of major histocompatibility (MHC) antigens (e.g. HLA-E or G). However, it is also possible that the sperm was able to fertilize the oocytes but there were factors present leading to embryo implantation failure. The use of donor sperm is obviously not a simple solution for most couples who want a child with both of their gametes. A more recent study by Alvarez et al. suggested that a novel test called a stress test which when subnormal could also detect a sperm specimen that could cause implantation defects (38). The HOS test was not measured during that study so it is not known what overlap existed between subnormal stress tests and low HOS scores (38).

HOS ABNORMALITIES AND SPONTANEOUS ABORTION

There has been a suggestion that oligoasthenozoospermia may be associated with an increased risk of spontaneous abortion with IVF but not with ICSI (39). Another study also suggested that an increased risk of spontaneous abortions may be associated with lower HOS scores (40). We are conducting further studies to determine if there is a certain HOS level that permits pregnancies but is associated with a greater risk of spontaneous abortion. We are also exploring the possibility that if there is a large decrease from the viability (which measures the structural integrity of the sperm membrane) to the HOS score (which measures the functional integrity of the sperm membrane) that implantation failure may also occur even if the HOS score is >50%.

It should be noted that all the human studies we performed using the HOS test was approved by the Ethics Committee of the Cooper Center of IVF consisting of 12 members. IRB approval was not needed since no randomized studies were performed where one group may not have been given the best therapy or another group given a therapy that had potentially more side effects. Most of

the studies were observational; thus I believe that a high standard of ethics was applied in carrying out these investigations.

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