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Editorial Article

Sperm with an abnormal hypo-osmotic swelling test – normal fertilization, normal embryo development, but implantation failure

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Summary

Purpose: To review the clinical importance of including the hypo-osmotic swelling (HOS) test in routine male fertility testing which in general is not evaluated by most physicians dealing with infertility. *Materials and Methods:* Pregnancy rates were evaluated in patients with low HOS test scores. A low HOS test was specifically defined as having less than 50% of sperm exhibiting the normal physiologic response of tail swelling, when subjected to a hypo-osmolar solution. Pregnancy rates of patients with low HOS test were examined after intercourse, intrauterine insemination (IUI), conventional oocyte insemination, and in vitro fertilization (IVF). Patients with a low HOS test were also treated with a protein digestive enzyme chymotrypsin. Patients receiving intervention then underwent IUI, IVF with conventional oocyte insemination, or IVF with intracytoplasmic sperm injection (ICSI). Pregnancy rates of the cohort receiving intervention were then examined for comparison. *Results:* The HOS test abnormality leads to normal fertilization but almost invariably negatively effects embryo implantation. Treatment with chymotrypsin, or performing IVF with ICSI, can overcome the toxic protein causing the embryo implantation defect. This toxic protein may be cryolabile and freezing sperm or embryos may prove to be another mode of therapy. *Conclusions:* The HOS abnormality may be the most reliable semen abnormality predicting failure to conceive even with IVF unless the defect is negated. Therapy is very effective. Unfortunately this test is rarely evaluated by most infertility specialists but it should be. The frequency increases with age.

Key words: Hypo-osmotic swelling test; Semen analysis; Embryo implantation defects; Chymotrypsin; Intracytoplasmic sperm injection.

Introduction

Typically, a normal semen analysis is able to provide information regarding the sperm concentration and motility. Assuming normal quality of the mucous, at the proper time in the cycle, the sperm is able to traverse the cervix, proceed into the uterine cavity, then out to the fallopian tubes. Approximately 50-80 million motile sperm typically start along this trajectory. Only 400 will attach to the zona pellucida. Finally, only one sperm will reach the oocyte.

It is generally assumed that if a semen specimen is adequate to achieve fertilization, it is adequate or deemed a normal semen analysis. However, most physicians dealing with infertility do not measure the hypo-osmotic swelling (HOS) test. Sperm which exhibit an abnormal HOS test may allow normal fertilization, and even normal embryo cleavage and normal embryo morphology, however the result is still an extremely poor pregnancy rate. The succeeding pages will provide evidence-based data in support of this.

History

The HOS test was historically designed for bovine sperm, however the test was modified for human sperm by Jeyendran *et al.* [1-5]. The HOS test is based on osmosis. The general principal of osmosis is that water will move from an area of high concentration to an area of low concentration. Water will therefore move from a hypotonic to a hypertonic solution. The HOS test places the hyperosmotic sperm in a hypo-osmotic solution. The normal physiologic response should be water crossing into the sperm tail via active transport. If the sperm membrane is functioning normally, the tail should swell due to the transit of water into this region. Specific normal and abnormal values were developed by Jeyendran *et al.* by evaluating the semen specimens from fertile couples [5]. An abnormal value was established as < 50% tail swelling. Those with 50-59% of sperm with tail swelling were determined to be in the gray zone. Sperm membrane integrity is not only important for sperm metabolism, but it is also needed for other processes including suc-

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cessful sperm capacitation, acrosome reaction, and binding of the sperm to the egg surface. Jeyendran *et al.* therefore hypothesized that an abnormal HOS test should detect male subfertility [5].

Evidence for subfertility in males with apparently normal semen analysis but low HOS test

The first study to evaluate the influence of a subnormal HOS test in women not undergoing in vitro fertilization (IVF) was performed by Check *et al.* [6]. This study examined the pregnancy rates in those with normal semen parameters (motile density and morphology) *vs*. subnormal semen parameters. Additionally this study examined the pregnancy rates in those with HOS tests that were subnormal *vs.* normal. Those with HOS tests in the grey zone were included in the normal group [6]. Results of this study showed that when semen parameters were normal and when the HOS test was also normal, the pregnancy rate following normal intercourse was 89% (83/93). Interestingly, even when semen parameters were subnormal, (i.e. the motile density and morphology were abnormal), but the HOS score was normal, the pregnancy rate still remained high; 83% (24/29) [6]. These data suggested that the typical semen analysis parameters of motility, density, and morphology were not good predictors of male subfertility, since those with both normal and abnormal semen parameters approached the same pregnancy rate. This fact was confirmed by subsequent studies [7-10]. In contrast, males with normal sperm concentration and morphology, but HOS test score < 50%, had no pregnancies (0/7) with intercourse over eight months and those with subnormal standard semen parameters and a low HOS test score (0/6) also had no pregnancies [6]. The assumption made by us at that time was this defect of the functional integrity of the sperm membrane precluded normal fertilization leading to infertility. This was subsequently found not be true.

Effect of sperm with low HOS test scores on in vitro fertilization outcome

Prior to our publication in 1989 in Fertility and Sterility [6], there was one study that suggested that there was a correlation with reduced fertilization following IVF and low HOS scores [11]. As one frequently sees, once a given study is published, there are generally other studies supporting these findings, but then later others refute these findings. Subsequently, one needs a meta-analysis to resolve the difference. The following information is a summary of the data published on the HOS test. Liu *et al.* in 1988 found the HOS score to average 65% in males with < 50% fertilization *vs*. 77% with ≥ 50% [12]. Takahashi *et al*. in 1990 found that the HOS test showed a stronger correlation with fertilization rates than other semen parameters [13]. However, there were other, more convincing studies, that found the HOS test not to correlate with IVF fertilization rates including studies by Barratt *et al.* (1989), Sjoblum *et al.* (1989), Avery *et al.* (1990), and Chan *et al.* (1990) [14-17].

Since our study in 1989 [6], later studies were published showing no correlation between fertilization rates in patients undergoing IVF and having a low HOS test score. However, this disparity is likely accounted for given the fact that our study evaluated pregnancy rates following intercourse, whereas the later studies studied fertilization rates following IVF. Indeed, we also confirmed that low HOS test scores do not lower fertilization rates [18]. The 50,000 sperm added to the oocyte during IVF (as opposed to the 400 sperm reaching the oocyte following intercourse) could theoretically obviate the fertilization issue by contact with many more numbers of sperm. Thus, increasing the number of sperm in contact with the zona pellucida that is seen with conventional insemination could explain the reason why intercourse results in no pregnancies, but IVF allowed reasonable fertilization rates in those with low HOS scores. However, there was something suspicious about these latter four publications [14-17] in that none of them mentioned pregnancy rates. Thus, one other interpretation of the finding of no pregnancies in 13 female partners of males with HOS test scores <50% was that this defect could possibly allow normal fertilization, but somehow may interfere with embryo implantation. Indeed our matched controlled study also corroborating the inability of low HOS scores to predict low fertilization rates, did, in fact, demonstrate relatively very low pregnancy rates [18]. To confirm these findings suggesting that sperm with a subnormal HOS test could allow normal fertilization, but cause embryo implantation defects, we decided to retrospectively evaluate 592 IVF-ET cycles using conventional oocyte insemination to compare fertilization rates and pregnancy rates in males with low HOS test, as compared to abnormalities in motile density and low strict morphology [19]. In addition, this retrospective study also examined a new criteria that had been established suggesting that morphology at a level ≤ 4% predicted failure to conceive by intercourse, intrauterine insemination, and conventional IVF-ET [19]

For background information and to understand this study better, in 1989 Kruger *et al.* published their data suggesting that using a new strict criteria for evaluating sperm morphology that a level ≤ 4% predicted failure to conceive by intercourse, intrauterine insemination, and conventional IVF-ET [20]. They even suggested donor sperm to be used with morphology ≤ 4%! [20]. Even adjusting the sperm concentration led to improved fertilization rates, but low pregnancy rates were still found [21, 22].

When all semen parameters were normal, except for abnormal strict morphology at ≤ 4%, we did not find any predictability factor for this new test of morphology [23]. Thus, the retrospective study of 592 IVF-ET cycles was aimed to not only corroborate or refute our finding with

the matched controlled study that suggested a low HOS test score predicts poor embryo implantation rates, but also to determine the effect of low strict morphology on pregnancy rates following IVF-ET [18, 19] compared to cases where all semen parameters were normal. The clinical and live delivered pregnancy rates following conventional oocyte insemination was 25.7% and 21.8%, respectively, when all semen parameters were normal *vs*. 25.5% and 15.7% when motile density was subnormal *vs*. 44.4% and 38.9% with low strict morphology *vs*. 0% and 0% with HOS test scores < 50%. Thus, we corroborated our finding that sperm with low HOS scores cause embryo implantation defects, and confirmed that strict morphology is a poor test to detect male subfertility [19].

As mentioned, the aim of the matched controlled study was to not only determine if sperm with low HOS will result in normal or subnormal fertilization rates, but more importantly, the effect of low HOS test score on pregnancy rates [18]. A five-year prospective study was started from 1989 ending in 1994 [18]. There were 27 matched couples reaching our eligibility criteria. For normal HOS test scores, the mean number of oocytes retrieved was 11.0 *vs*. 11.2 for subnormal scores. The mean fertilization rates were very similar (55.5% *vs*. 56.0%) [18]. Thus, our results supported the data of Barratt, Sjoblum, Avery, and Chan [14-18]. These data strongly suggested that sperm with HOS test scores < 50% are not a cause of poor fertilization or poor embryo development but impairs fecundity by impairing implantation since the group has a far lower pregnancy rate then controls with HOS scores ≥ 50% [18].

In the late 1980s we did not use paid oocyte donors for recipients in premature ovarian failure or advanced reproductive age, but instead used half of the oocytes retrieved from an infertile donor needing IVF-ET in exchange for free medication and free IVF (the cost paid by the recipient). This shared model seemed to be a naturally controlled group to corroborate or refute the aforementioned study suggesting low HOS scores led to embryo implantation defects [24].

The outcome of shared oocyte cycles were determined where both male partners had normal standard semen parameters, but where one of the two partners had a low HOS test score, while the other one was normal. This was a retrospective study comparing donor oocyte recipient cycles from 1991 to 1995 where normal standard male semen parameters were found in both male partners, but where one of the two male partners had a low HOS test score [24]. The study was performed to evaluate whether low HOS tests do not impair IVF outcome, as suggested by the studies by Barratt, Sjoblum, Averym and Chan [14-17], and their omission of pregnancy rates was merely related to the fact that they were only interested on fertilization rates, or were the pregnancy rates possibly purposely omitted because they were embarrassingly low [14-17]?

In this study of 22 donor oocyte recipient pairs, the mean fertilization rate for normal *vs*. low HOS were similar (67.2% *vs*. 60.9%) using conventional oocyte insemination [20]. The 3.3 *vs*. 3.2 embryos transferred had similar morphology. However, whereas the pregnancy rate was 50% in females whose male partners had HOS scores ≥ 50%, it was 0% in those < 50% [24].

We thought that when we published our 1995 and 1996 articles that we would peak the interest of infertility specialists who seemed to be more influenced by the studies published that showed that sperm with subnormal HOS test scores do not necessarily effect fertilization rates, and therefore is a worthless test, not realizing the serious omission of pregnancy rates from these studies [14-19]. We thought if we could show, using a common pool of oocytes, that pregnancy rates would be markedly reduced by one male partner with sperm with a low HOS test score *vs*. another with normal semen parameters, despite normal fertilization rates, our concept that some sperm abnormalities can cause embryo implantation defects would promote interest in either the aforementioned authors (who had doubts about the importance of the HOS test) or other infertility specialists, to corroborate or negate our claims. However no such studies have been subsequently performed. It is evident from scanning the literature with computer searches, attending infertility meetings, and seeing a large variety of infertile couples who had previously consulted other infertility specialists, that this simple, inexpensive, extremely important sperm test is ignored by the large majority of the infertility specialists [25].

Stability of the subnormal HOS test over time

It is well known that some semen characteristics can change from sample to sample. This is especially true for motile densities, where some males who initially are found to have a subnormal motile density, can sometimes significantly improve the motile density in another specimen [10]. Even 25% of very normal sperm donors were found to have a higher motile density in a second specimen obtained within one hour [26]. This variation makes it difficult to interpret efficacy of some treatments, e.g., varicocelectomy, since if one sees improvement of motile density, it is difficult to tell if it was merely fortuitous or related to surgery [27].

In contrast to other semen parameters, the HOS test seems to remain stable over time [28]. In this study, 444 specimens were frequently observed for over two years. There were 34 males that initially had a low HOS test score (7.7%) of < 50% [28]. Fifteen males (44%) never improved the score ≥ 50% even once over a prolonged period of observation. However, 19 males did improve at least once ≥ 50% (55.9%). Interestingly 12 of the 19 went from subnormal HOS test < 50% but just into the grey zone (50-59%). Six of the seven who went from HOS test score < 50% to ≥ 60% may have had a false positive initial HOS test score related to one of the factors discussed in the next section [28].

There were 17 semen specimens that were initially ≥ 50% that changed to a low score when next tested. Four were in the grey zone initially. All 17 that changed remained low on subsequent testing [28].

Causes of falsely low HOS test scores

Plastic containers

Initially, semen specimens were collected in glass jars because it was realized that toxic factors from the plastic could leak into the semen specimen and adversely effect semen parameters. It was shown that the HOS test score is, in fact lower in semen specimens collected in plastic *vs*. glass [29]. In fact, in the aforementioned study of 444 semen specimens, the mean HOS test score was 68.5 for the first two years when glass containers were used, but dropped to 61.3 in the next year when plastic containers were used [28]. Because glass was expensive, the bottles were sterilized and re-used. However, with the concern for infection, a switch was made to disposable plastic. The adverse effect on HOS test is magnified by prolonged exposure to plastic toxic factors.

An additional factor that can affect the HOS test is the interval between ejaculations. In the previous study, some of the false scores that were initially low were related to producing the specimens at home with two hours or more intervals before the sperm specimen was evaluated. A long interval between ejaculation, e.g., > ten days can produce a falsely low HOS test score [30].

Effect of freezing on HOS score

A study of seven males with initial semen specimens showing HOS test scores > 50% had the HOS test repeated after freezing then thawing. Though there was good preservation of the motile density, all seven had HOS test scores < 50% [31]. The fact that freezing effects the HOS test does not have the same significance as when it occurs in the fresh specimen. Evidence will be provided in subsequent pages that the causative factor contributing to a low HOS test score of fresh sperm is a toxic protein possibly acquired as the sperm passes through the ejaculatory ducts. This toxic protein gets transferred to the oocyte membrane when the sperm attach to the zona pellucida. The zona pellucida becomes incorporated into the embryo membrane. This toxic protein, now having been incorporated into the embryo membrane, causes a functional impairment of the embryo membrane. Subsequently, this defective embryo membrane leads to an embryo implantation defect.

Since the frozen thawed sperm can still produce live pregnancies, it is suspected that freezing itself can damage the functional integrity of the sperm membrane in more than half the sperm membranes, but those not effected, can produce normal pregnancies (i.e., those sperm with freezing damage if they attach to the zona pellucida do not have the associated toxic protein and thus do not impair embryo implantation). Additionally, the toxic protein may be cryolabile, and therefore, the freezing and thawing process may select for those sperm without this protein. In the treatment section, we will show that the toxic protein may be cryolabile, and freezing can even be employed as a treatment option.

Correlation with low HOS test scores and other semen parameters

A study was performed evaluating single semen parameter abnormalities and presence of low HOS test scores [32]. Males (n=212) with low concentration of sperm had a low HOS test score in 1.9% of specimens. With abnormal strict morphology < 5% (n=407), low HOS test scores were found in 4.52% *vs*. 3.51% (n=57) for those < 2%. For presence of antisperm antibodies > 50%, the frequency of HOS test score < 50% was 5.3%. The best correlation was with lower percent progressive motility. Using 50% as a cut-off (n=443), the frequency of low HOS tests was 14.2%. This difference was found to be significantly different when the 63/443 males with low HOS tests and motility < 50% were compared to single defects combined (23/831) with chi-square analysis showing $p < 0.0001$ [32].

In fact, when percentage of motility was < 40%, the frequency of HOS test scores < 50% was 25.8% (29/112). The lower the percentage of motility, the greater the frequency of HOS test scores < 50: 24.1% for 30-39.9%, 30.0% for 20-29.9%, and 33.3 for 0-19.9% [32].

Sperm with high DNA fragmentation indices are more likely to have subnormal HOS tests also [33]. Viability (also called vitality) measures the structural integrity of the sperm membrane. If a sperm membrane is structurally damaged it will be functionally damaged also. We found that the majority of males with subnormal HOS test scores have normal viability (vitality) (12.5%, 45/361) [34].

HOS test abnormalities increase with advancing age of the male

It is quite clear that aging of the male is the most common association with subnormal HOS test scores [35]. In a study of over 4,000 semen specimens, the frequency of subnormal years was only 5.41% *vs*. 6.56% for males 30-34.9 years *vs*. 8.00% for males 35-39.9 years, *vs*. 9.7% for males 40-44.9 years, *vs*. 12.9% for males 45-49.9 years *vs*. 25.1% for males ≥ age 50 [35]. In contrast,standard semen parameters do not seem to change much with advancing age of the male [36].

Treatment of sperm with HOS test scores <50% to improve subfertility

Treatment with the protein digestive enzyme chymotrypsin

Sperm antibodies are a well known cause of male infertility [37, 38]. In 1994, a method to improve pregnancy

rates was described for those with sperm antibody associated infertility. This method entailed first treating the sperm with the protein digestive enzyme chymotrypsin (antibodies are proteins) to render the antibodies biologically inactive. This reaction was then stopped before the enzyme damaged the sperm. Once this process was completed, IUI was performed [39]. Subsequently, this method was also found effective for pretreating sperm with antisperm antibodies prior to conventional oocyte insemination during IVF-ET [40].

As previously mentioned, we hypothesized that there was a toxic factor added to the sperm during the time they traverse the ejaculatory ducts, causing the defect in the functional integrity of the sperm membrane, as evidenced by a low HOS test score. It seemed likely this toxic factor could be proteinaceous in nature. Thus, we considered treatment with chymotrypsin-galactose, similar to treating antisperm antibodies. The first study reporting benefits of treatment with chymotrypsin prior to IUI compared 38 cycles of IUI without enzymatic therapy *vs*. 12 cycles with chymotrypsin galactose therapy in males with low HOS test scores. To be sure the pregnancy was related to the IUI, they were advised to abstain from intercourse, but to keep the ejaculatory time period for the specimen used for IUI to be < four days. There were no pregnancies in 38 IUI cycles without chymotrypsin treatment *vs*. four of 12 cycles where IUI was preceded by chymotrypsin therapy [41]. Subsequently, chymotrypsin galactose therapy was found to improve live delivered pregnancy rates using sperm with HOS test scores < 50% when IVF-ET was performed using conventional oocyte insemination [41, 42].

Because the infertility seems to be related to sperm laden with the toxic protein attaching to the zona pellucida, it is important to emphasize the importance of avoiding unprotected intercourse prior to the IUI with chymotrypsin treated sperm. A matched controlled study of 135 cycles of IUI for decreased sperm concentration, motility, or morphology was compared to 135 cycles treated with chymotrypsin prior to IUI for HOS scores < 50%. The clinical pregnancy rates (ultrasound evidence of pregnancy at eight weeks) and live delivery rates for low HOS scores were 32.3% and 21.2%, respectively, *vs*. 21.9% and 15.4% for other sperm abnormalities [43]. This confirms the efficacy of pretreatment of sperm with low HOS test scores prior to IUI, and avoidance of unprotected intercourse, for treating males with low HOS test scores [43].

Correction of subfertility related to a subnormal HOS test by performing IVF with intracytoplasmic sperm injection (ICSI)

The first reported case of using ICSI for low HOS test scores found two pregnancies in four IVF-ET cycles [41]. The reason why ICSI should be effective, assuming the theory of the mechanism is correct, is that ICSI overcomes the attachment of sperm with the toxic protein to the zona pellucida. Subsequently, a series of publications demonstrated the efficacy of ICSI for sperm with low HOS test scores [44-47].

Naturally, IVF would be expected to achieve a higher pregnancy rate per given cycle than IUI, irrespective of the reason for performing the IUI. For cycles where the male partner had an HOS score < 50%, there were eight successful conceptions in 60 women having two IUI's (unless pregnancy occurred on first cycle). This was a pregnancy rate of 13.3% per patient. In contrast in one ICSI cycle, there were 79/248 (31.8%) having had a successful conception. Thus the pregnancy rate was 2.5-fold higher with one IVF with ICSI cycle *vs*. two IUI's with chymotrypsin [48]. In contrast, when the initial HOS test score was ≤ 39% there was only one pregnancy in 33 women having two IUI cycles (3.3%) *vs*. 34.4% (63/183) for one cycle of IVF with ICSI [48].

Evidence that ICSI completely overcomes the HOS defect was demonstrated in a study comparing outcome in donor-recipient pairs who were sharing the same pool of oocytes where one male partner had a low HOS test score and the other a normal value [49]. For low *vs*. normal HOS test scores, the fertilization rates were comparable 73.1% *vs*. 65.8%, the clinical and live delivered pregnancies were 53.1% and 49.0% *vs*. 55.8% and 50.0%. The implantation rates were 29.6% *vs*. 27.4% [49]. This is in sharp contrast to the aforementioned study of donor-recipient pairs where males with normal HOS achieved a 50% pregnancy rate following conventional insemination technique *vs*. no pregnancies with < 50% when conventional oocyte techniques were used.

With the advent of ICSI, fertilization and pregnancies have been achieved with sperm with such low motile densities that fertilization in the conventional manner may not have been possible. Similar pregnancies can be achieved with testicular sperm or sperm coated with antisperm antibodies that inhibit sperm from attaching to the zona pellucida. However, if oocytes can be fertilized by conventional oocyte insemination *vs*. ICSI, the resulting embryos have a greater chance of successful implantation and pregnancy [50-52]. Thus, ICSI should not be used routinely, not only because the procedure may lower pregnancy rates, but because it also adds additional expense. Thus, most IVF centers will be performing conventional oocyte insemination when semen parameters are normal in the first IVF cycle. They will continue the policy even if no pregnancy was achieved in previous IVF cycles when performing subsequent IVF cycles as long as the fertilization rate was good with the production of embryos with good morphology.

One study evaluated the percentage of male partners with low HOS test scores who had normal standard semen parameters and whose female partners were undergoing IVF-ET. There were 1,663 IVF-ET cycles evaluated for females

aged ≤ 39 and 330 IVF-ET cycles in women aged 40-42. Lower HOS test scores were found in 2.4% of the younger group and 13.3% of the older group [53]. Thus about 13% of these IVF-ET cycles in the older group would have been inseminated with the wrong method had we not checked the HOS test [53].

Unfortunately, despite the litany of published articles related to the importance of the HOS abnormality, most IVF centers and infertility specialists never perform this simple inexpensive test. This is the motivation for writing this editorial – to try to re-kindle interest in this extremely important test. For this editorial, we attempted to stay away from anecdotal experience but one anecdotal case (never reported) will drive our point home, i.e., that this single inexpensive test is generally ignored by the large majority of infertility specialists and its avoidance can be very costly.

A couple with unexplained infertility who had failed to conceive after seven IVF-ET cycles, and was in the midst of controlled ovarian hyperstimulation for another IVF-ET cycle, consulted our group to try IVF cycle number 9 if they failed again on number 8, and to try to determine the reason for their previous failures. The seven IVF cycles and the intention for number 8 was conventional oocyte insemination because the standard semen parameters were normal, the fertilization rate was quite good, and the resulting embryos looked normal. However, the HOS test was subnormal. We advised the couple not to blame their IVF center for not performing the HOS test because for some reason the majority of infertility specialists ignore this test. We assured them that performing ICSI should reverse their poor fortune. They were told that they should just advise their IVF doctor to perform ICSI. She called a couple days later stating that her doctor "did not believe in the test" and insisted on doing conventional oocyte insemination for number 8. We told her to tell the doctor that unless ICSI was performed, that she will come to our IVF center for this IVF cycle. The other IVF center reluctantly agreed and performed ICSI rather than lose their patient. She conceived and delivered a healthy baby. Could we now count on this infertility group to add this test to their infertility investigation? No! A few years later a couple sought our advice because of failing to conceive again with four IVF-ET cycles with the aforementioned IVF center. Again, a low HOS test was found. Again they refused ICSI! This time she came to us instead, had a successful fresh and subsequent successful frozen embryo transfer cycle producing two live babies.

As previously mentioned, the HOS test abnormality increases with advancing age [35]. Interestingly, based on finding an increase of meiosis errors in sperm from males ≥ aged 50, one group assumed that finding a 25% reduction in pregnancy rates using younger donor oocytes when fertilized by males aged ≥ 50 was related to the 25% increase in sperm with chromosome abnormalities [54]. However, the IVF center reporting these data did not perform the HOS test. In contrast, we found no decrease in live delivered pregnancy rates using sperm from males ≥ aged 50 in a younger donor egg model, but we perform ICSI for the 25% having low HOS tests [55]. Thus, our data suggests that the 25% reduction is from not detecting low HOS test scores, not from an increase in male aneuploidy [54, 55].

Cryopreservation to overcome the HOS test abnormality

In our previous matched controlled study showing no abnormality of fertilization with low HOS tests following conventional oocyte insemination, but extremely poor pregnancy rates, there were frozen embryos left over from the study [18]. We evaluated 21 frozen embryo transfer cycles in 14 patients that utilized extra embryos from the aforementioned study [18, 56]. The study was published in 1996 so pregnancy rates were inferior to those of today. There were four pregnancies in 21 frozen ETs for a rate of 19.0% per cycle with an implantation rate of 7.1%. When compared to the frozen ETs from the males with normal HOS tests, the pregnancy rate in 12 patients undergoing 21 frozen ET cycles was 23.8% and the implantation rate was 9.3% [56]. Thus, this study suggested that the toxic sperm factor may be cryolabile. However, because of the success with treating sperm with chymotrypsin before IUI, and performing ICSI rather than conventional oocyte insemination, and the indifference to this test in other IVF centers, the possibility of purposely freezing the embryos formed from sperm with low HOS test scores with subsequent transfers of frozen-thawed embryos has not been performed.

For a period of time, for unknown reasons, the manufacturing of chymotrypsin was temporarily stopped. Thus, some patients who preferred IUI with chymotrypsin-treated sperm for financial reasons, proceeded to IVF with ICSI. However, there were some patients who asked if another option can be tried with IUI. Considering the possibility that this toxic protein causing low HOS test scores may be cryolabile (i.e., negating the toxic factor incorporated in the embryo membrane and success with frozen ET), we offered one couple to try to freeze the sperm and then perform IUI. The couple had failed to conceive at another infertility center following five cycles of IUI and two cycles of IVF with conventional oocyte insemination. For financial reasons, she wanted to try chymotrypsin with IUI. This treatment did correct the HOS test from 32% to 75-80%. She wanted to do a fourth cycle, but the chymotrypsin was not available, and she could not afford IVF with ICSI [57]. Following insemination of cryopreserved sperm, she conceived in her first IUI attempt. Unfortunately, she had a late first trimester miscarriage (trisomy 14) [57]. Chymotrypsin became available again and she conceived on her four chymotrypsin cycle. She delivered a live baby [57]. Though the chymotrypsin treatment is a relatively simple procedure and inexpensive (for description of technique sees refer-

ence 43), some centers may be uncomfortable trying chymotrypsin treatment and may prefer to try sperm cryopreservation.

If women have embryos that were fertilized conventionally, and subsequently find that the oocytes were fertilized by sperm with low HOS test scores, they should consider transferring the remaining frozen embryos rather than trying another cycle of IVF with fertilization with ICSI.

Association of sperm with low HOS test scores and miscarriage

A priori, one might expect that if sperm with low HOS test scores can cause an embryo implantation defect, that it may not only cause infertility, but be a cause of miscarriage. Indeed, one of the early publications suggested that sperm with low HOS test scores could be a cause of recurrent miscarriage [58]. From our own personal experience, a high percentage of cases with a low HOS test score lead to failure to conceive. On occasion, we find a rare case that suggests that this defect can cause miscarriage. We had one unreported case of a couple whose female partner was a primary aborter with four previous miscarriages. Under our aggressive progesterone treatment she had two more. Her husband initially refused to do a semen analysis. He was subsequently found to have a low HOS test score, but otherwise normal semen parameters. With chymotrypsin pretreatment of sperm prior to IUI, they delivered a healthy baby. We evaluated that possibly the grey zone score for the HOS test (50-59%) can detect a milder group that may be more prone to miscarriage. However, we could not find any evidence that a sperm with an HOS test score in the grey zone leads to miscarriage [58-60].

Conclusions

In summary, the HOS test scores is an inexpensive and crucial test to include in the male infertility workup. In the past, studies have shown that the fertilization rates are comparable in those with abnormal and normal HOS test scores and this has largely been the stimulus for discounting the HOS test as part of the male infertility workup. However, these studies focused on fertilization rates and not implantation or pregnancy outcomes. Numerous studies have since been published indicating that those with low HOS test scores may indeed have similar fertilization rates, however they also routinely and reproducibly have decreased pregnancy outcomes. We have proposed that the causative factor contributing to a low HOS test score is a toxic protein, possibly acquired as the sperm passes through the ejaculatory ducts. When the sperm attaches to the zona pellucida, this toxic protein is subsequently transferred to the oocyte membrane then becomes incorporated into the embryo membrane. Once incorporated into the embryo membrane, it causes a functional impairment of the membrane resulting in embryo implantation defect. This defect may be easily corrected by treating sperm with the protein digestive enzyme chymotrypsin. Pregnancy outcomes for those who underwent treatment of sperm with chymotrypsin followed by IUI were significantly improved. If chymotrypsin is not readily available, other methods of treatment are available. We have also found that the toxic protein may be cryolabile and therefore, the freezing and thawing process selects for those sperm without this toxic protein, and ultimately results in improved pregnancy outcomes. Furthermore, our studies also indicate that ICSI is also an effective option for these patients, given that ICSI overcomes the attachment of sperm with the toxic protein to the zona pellucida. Unfortunately, despite the plethora of published articles related to the importance of the HOS abnormality, most IVF centers and infertility specialists never perform this simple inexpensive test.

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