



## EVIDENCE THAT SPERM WITH LOW HYPOSMOTIC SWELLING SCORES CAUSE EMBRYO IMPLANTATION DEFECTS

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A previous prospective study using matched samples found that sperm with low hypoosmotic swelling (HOS) scores had no adverse effect on fertilization rates but markedly reduced pregnancy and implantation rates. The present study attempted to corroborate or refute the aforementioned study by comparing pregnancy rates in donor-recipient pairs using shared oocytes where the sperm of one of the two males had low HOS scores. The results found no pregnancies from the sperm with low HOS scores versus a 41% live delivered rate for those with normal scores. However, fertilization rates were not affected. This retrospective study thus confirms that sperm with low HOS scores cause implantation defects of the embryos that are formed without affecting fertilization rate, embryo cleavage rate, or embryo quality.

**Keywords** defects, implantation rate, sperm membrane

The percentage of sperm demonstrating hypoosmotic (HOS) changes has been used as a test of the functional integrity of the sperm membrane [14]. A cutoff value of 50% has been found to establish a clear demonstration of infertile males [5].

The clinical usefulness of the HOS test was challenged by several studies failing to demonstrate any decrease in fertilization rates following in vitro fertilization-embryo transfer (IVF-ET) when the sperm has subnormal versus normal HOS scores [1, 2, 4, 17]. However, none of the 4 studies included pregnancy rates (PRs). A study by Check et al. confirmed that the low HOS test failed to predict poor fertilization rates but demonstrated that low scores did predict very poor viable PRs [12]. These data thus suggested that sperm with low HOS scores may cause infertility by interfering with successful implantation.

The aforementioned study by Check et al. used matched controls [12]. The study presented

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herein was established to either corroborate or refute the previous study in a different manner. The Cooper Center for IVF has established a shared oocyte program where half of the oocytes retrieved by women needing IVF to allow conception are given to an anonymous recipient in exchange for sharing of expenses [7]. We compared PRs in donor-recipient pairs using a shared oocyte pool when one male partner had a subnormal HOS score and the other male partner had a normal score.

## MATERIALS AND METHODS

A retrospective review of IVF cycles from January 1991 to July 1995 that involved donor-recipient pairs was conducted where either the male partner of the donor or recipient had an HOS score < 50%. No data beyond July 1995 were included because it was at this time that conventional insemination of oocytes with sperm from males with subnormal HOS scores was no longer performed. Instead, either the embryos were first cryopreserved prior to transfer or the sperm was treated with chymotrypsin/galactose prior to insemination of the oocytes [3, 10, 13, 15]. Since the beginning of 1996, intracytoplasmic sperm injection (ICSI) has been mostly used to inseminate the oocytes when the HOS score was below 50% [15].

All donors were stimulated with leuprolide acetate starting at the midluteal phase for 10 days, after which 300 units of gonadotropins were added [16]. No cycles were chosen if micromanipulation was performed for very severe oligoasthenozoospermia. If there was failed fertilization in either pair, the cycle was included in the investigation. However, if either a donor or recipient did not receive a fresh transfer (deferred for a frozen embryo transfer or because of inadequate endometrial thickness or homogeneous hyperechogenic endometrial echo pattern), that pair was not included in the study [6, 8, 9].

The HOS test was performed as previously described [14]. A score of <50% was considered subnormal [5]. Only cycles where the HOS test was subnormal on baseline and the day of oocyte retrieval were included. A clinical pregnancy was established if there was ultrasound evidence of pregnancy at 6 weeks gestation. Live delivered rates were also calculated and compared.

## RESULTS

There were 22 donor oocyte pairs evaluated. In no instance did both the donor and recipient male partner have subnormal HOS scores. There were 12 male partners of recipients with HOS test scores < 50% and 10 male partners of the donors.

The mean motile sperm concentration for donors was  $37.3 \pm 33.6 \times 10^6$  motile sperm/mL versus  $29.7 \pm 26.2 \times 10^6$  motile sperm/mL for recipients. The motile densities for males with normal HOS scores was  $38.7 \pm 33.2 \times 10^6$ /mL and  $27.5 \pm 25.8 \times 10^6$ /mL for males with subnormal HOS scores. Mean normal morphology for males with normal HOS scores was  $7.1 \pm 4.8\%$  versus  $6.4 \pm 5.3\%$  for males with HOS < 50%. The mean HOS scores were  $65.3 \pm 8.8\%$  for the normal group and  $38.2 \pm 9.4\%$  for the subnormal group.

The mean fertilization rate was similar in both groups (67.2% in the normal versus 60.9% in the subnormal HOS group,  $p > .05$ , paired  $t$  test). The number of embryos transferred were similar in both groups (3.3 for couples with a male partner with normal HOS scores versus 3.2 for those with subnormal HOS scores). Embryo morphology was also similar in both groups. The clinical pregnancy rates were 50% (11 of 22) for the normal group versus 0% for subnor-

mal group ( $p < .05$ , McNemar's test for matched proportions). The live delivery rate per transfer for female partners of males with normal HOS scores was 40.9% (9 of 22).

## DISCUSSION

The previous study of low HOS scores showing a dissimilitude between fertilization rates and subsequent pregnancy rates using prospective matched samples has not been either corroborated or refuted [12]. It would be difficult to perform any controlled studies since intracytoplasmic sperm injection is now used by The Cooper Center for IVF for patients undergoing IVF where the male partner has low HOS scores.

We thus thought that another way to refute or corroborate the aforementioned study was to evaluate a group of patients who were excluded in the previous study, i.e., those who were part of a donor-recipient pair. In fact, this group seems to be a particularly good group to evaluate for pregnancy rates according to HOS score, since by using a common oocyte pool, one could not consider that differences in pregnancy rates could be related to fortuitous differences in oocyte quality. There are data suggesting a significantly greater pregnancy and implantation rate in recipients vs donors despite using a shared oocyte pool [11]. However, there were slightly more recipients than donors with low HOS scores so the data cannot be explained on this basis.

The fact that ICSI allows a very high pregnancy rate despite low HOS scores [15] supports the concept that the defect in implantation may be related to some factor that is attached to the sperm membrane that functionally impairs the sperm membrane, but may transfer to the zona pellucida, and thus similarly impair the functional integrity of the oocyte membrane. This could be secondary to the supernumerary sperm attached to the zona pellucida, which would be bypassed by ICSI [15].

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