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Transfer of cryopreserved embryos improved pregnancy rates in patients with damage to the functional integrity of the sperm membrane as measured by the hypo-osmotic swelling test

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Objective: To compare the pregnancy rates (PRs) after transfer of cryopreserved embryos in patients who have damage to the functional integrity of the sperm membrane as measured by the hypo-osmotic swelling test to those without this defect.
Design: Prospective clinical study.
Setting: University-associated IVF center.
Patients: Fifty-four patients enrolled in a matched prospective study to evaluate the effects of low HOS scores (<50%) on PRs after IVF-ET were followed to determine the PR after transfer of cryopreserved embryos.
Main Outcome Measure: Clinical PRs and implantation rates.
Results: Fourteen patients with low hypo-osmotic swelling test scores underwent 21 frozen ET cycles, achieved four clinical pregnancies for a PR per cycle of 19.0% and an implantation rate of 7.1%. Twelve patients with normal hypo-osmotic swelling test scores underwent 21 frozen ET cycles, achieved five pregnancies for a clinical PR per cycle of 23.8% and an implantation rate of 9.3%.
Conclusion: Previous studies have demonstrated an adverse effect of low hypo-osmotic swelling test scores on PRs after IVF-ET despite normal fertilization. This adverse effect was not found in the transfer of cryopreserved embryos from males with low hypo-osmotic swelling test scores. Further investigation is required to determine how cryopreservation improves the chances of implantation of these embryos. Fertil Steril 1996;65:1241–4

Key Words: Embryo freezing, implantation rates, IVF

The functional integrity of the sperm membrane is believed to be an important factor in the acrosome reaction, sperm capacitation, sperm metabolism, and the binding of the spermatozoon to the egg surface. This factor can be measured by the hypo-osmotic swelling test.

Functional damage to the sperm membrane as measured by low scores on the hypo-osmotic swelling test has been associated with reduced pregnancy rates (PRs) in vivo (1) and in vitro (2). Several in vitro studies have failed to find a negative correlation between fertilization rates and hypo-osmotic swelling test scores (3). The objective of the current study was to compare the relationship of hypo-osmotic swelling test scores with PRs when cryopreserved supernumerary embryos are thawed and transferred in patients who failed to conceive after transfer of fresh embryos in a standard IVF cycle.

MATERIALS AND METHODS

The hypo-osmotic swelling test was performed on two unprepared semen specimens from each patient presenting for treatment with IVF-ET during standard semen analysis. The test was performed by combining 0.1 mL of ejaculate with 1.0 mL of hypo-osmotic solution (fructose-sodium citrate) after the technique of Jeyendran et al. (4). After incubation of the mixture for ≥30 minutes at 37°C, 100 sperma-

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tozoa were observed with a phase-contrast microscope for tail swelling changes typical of a reaction in the hypo-osmotic swelling test. Based on our previous study (1), scores <50% were considered abnormal instead of the 60% cutoff reported by Jeyendran et al. (4).

In the previously reported study, when a patient with low hypo-osmotic swelling test was identified, he was matched with the next available patient undergoing IVF who had a normal hypo-osmotic swelling test score but similar motile density and strict normal morphology using Tygerberg's strict criteria (2). The hypo-osmotic swelling tests had been performed on two semen specimens but were not performed on the actual specimens used to fertilize the oocytes. Thus, 27 pairs of patients had been enrolled in a prospective matched study to compare the effects of low hypo-osmotic swelling test scores on PRs after IVF-ET (2).

In the current follow-up study, those patients that failed to conceive were followed to compare the PRs of frozen ET using cryopreserved supernumerary embryos. The embryos were cryopreserved after the oocyte retrieval in the original study so that the embryos used for frozen ET in the study presented herein were fertilized by the same sperm that had been used in the study reporting poor PRs after fresh ET when the hypo-osmotic swelling test score was <50%. Twenty-six of the patients had subsequent ETs with cryopreserved embryos, 14 from the group with low hypo-osmotic swelling test scores, and 12 from the group with normal hypo-osmotic swelling test scores. The ovarian stimulation protocol used in the IVF cycles was the luteal phase leuprolide acetate (LA)-hMG protocol.

Hormonal therapy for frozen ET cycle followed one of three protocols. In the down-regulated protocol, 1 mg SC LA was given on day 21 of the patient's cycle for 10 days. When the P was <3.18 pmol/L, oral E_2 was given to stimulate the endometrial lining. When the lining reached a thickness of ≥10 mm, P in oil was administered (50 mg IM daily). Frozen ETs were scheduled for the 3rd day of IM P.

In the natural protocol, the patient's endogenous LH surge as measured by sera and urine levels were used to determine the time of ET (5). Progesterone was started on day of ET. The choice between natural versus down-regulation with estrogen replacement was based on the patient's ability to produce a mature follicle (>18 mm in diameter in conjunction with an E_2 level >734 pmol/L) and have a natural LH surge.

In the third protocol, estrogen supplementation was given without down-regulation to promote proper development of the endometrial lining. In this protocol, the E_2 dosage schedule is one 2 mg tablet on days 1 to 5, two 2 mg tablets on days 6 to 9, and three 2 mg tablets on days 10 to 14. If the endometrial lining was <10 mm on day 14, the E_2 dosage could be increased at the physician's discretion to improve endometrial lining.

The embryos were cryopreserved and thawed according to a technique that was developed at the Cooper Center for IVF and has been described previously (5). It is the policy at out center to allow a maximum of six embryos to cleave. After 48 hours, three or four embryos are chosen for transfer based on morphology and the remaining are cryopreserved at the multicellular stage. Any embryos in excess of six are cryopreserved at the pronuclear stage.

Before transfer, embryos were examined at 200× magnification using an inverted microscope. One numeric grade was given for the evenness of blastomeres and a separate letter grade for degree of cellular fragmentation. The grades for blastomeres were 1, embryos with even-sized round cells; 2, slightly uneven-sized round cells; or 3, uneven sized and or irregularly shaped cells. The grades for fragmentation were grade A, 0% fragmentation; B, 1% to 25% fragmentation; C, 26% to 50% fragmentation; and D, >50% fragmentation.

The transfer catheter was a Set De Frydman (FET Set; Fertility Technologies, Natick, MA). The transfer medium consisted of HEPES buffered human tubal fluid + 20 mg/mL bovine serum albumin.

Clinical pregnancy was defined as sonographic demonstration of a gestational sac and presence of a heartbeat. Implantation rates were computed as number of gestational sacs per embryo transferred.

Statistical analysis included Fisher's exact test to compare pregnancy and implantation rates between the low hypo-osmotic swelling test group and the normal hypo-osmotic swelling test group. The *t*-test was used to compare the mean number of embryos transferred in the two groups. All tests were done at the 0.05 level of significance.

RESULTS

Fourteen patients in the low hypo-osmotic swelling test group had 21 transfer cycles using cryopreserved embryos and 12 patients in the normal hypo-osmotic swelling test group had 21 frozen ET cycles. In the low hypo-osmotic swelling test group, the age of the patients ranged from 28 to 46 years with a median age of 37 years. In the normal hypo-osmotic swelling test group, the age ranged from 28 to 50 years with a median of 34 years. The etiology of infertility in the low hypo-osmotic swelling test group included tubal factor (n = 2), endometriosis (n = 1), male factor (n = 4), ovulatory dysfunction (n = 3), unexplained (n = 1), and multiple factors

Table 1 Outcome of Frozen ETs According To Hypo-Osmotic Swelling Test Score of Male Partner

	Low hypo-osmotic swelling test group (<50%)	Normal hypo-osmotic swelling test group (≥50%)
No. of ETs	21	21
Stage embryos thawed*		
Two pronuclear (%)	39 (31/80)	52 (43/82)
Multicell (%)	61 (49/80)	48 (39/82)
Survival rates*		
Two pronuclear (%)	90.3	93.5
Multicell (%)	86	87
Mean number of embryos transferred*	3.3 ± 1.1	3.6 ± 1.1
Viable pregnancy*	4	5
Rate per transfer (%)	19.0	23.8
Implantation rate* (%)	7.1 (5/70)	9.3 (7/75)

* P = not significant.

(n = 3). In the normal hypo-osmotic swelling test group, the etiologies were tubal (n = 3), endometriosis (n = 2), male factor (n = 3), ovulatory dysfunction (n = 1), and unexplained (n = 3).

Eighty embryos were thawed for transfer in the low hypo-osmotic swelling test group and 82 embryos were thawed for transfer in the normal hypo-osmotic swelling test group. A comparison of the results of the embryos thawed and subsequent transfers are presented in Table 1. There was no difference in the stage at which the embryos were thawed (pronuclear or multicellular) nor the embryo survival rate in the two groups.

Thirty-one of the embryos in the low hypo-osmotic swelling test group and 35 of the embryos in the normal hypo-osmotic swelling test group were graded using the grading system described above before transfer. In the low hypo-osmotic swelling test group, the grades for evenness of blastomeres were 4 (12.9%) grade 1, 13 (41.9%) grade 2, and 14 (45.2%) grade 3. In the normal hypo-osmotic swelling test group, the grades were 7 (20.0%) grade 1, 22 (62.9%) grade 2, and 6 (17.1%) grade 3. There were significantly more grade 3 embryos in the low hypo-osmotic swelling test group ($P < 0.05$, χ^2).

The fragmentation grades were 6 (19.3%) grade A, 13 (41.9%) grade B, 8 (25.8%) grade C, and 4 (12.9%) grade D in the low hypo-osmotic swelling test group and 2 (5.7%) grade A, 17 (48.7%) grade B, 11 (31.4%) grade C, and 5 (14.3%) grade D in the normal hypo-osmotic swelling test group. There was no difference in the fragmentation rates by hypo-osmotic swelling test group. There was no difference in the mean number of embryos transferred in the two groups, 3.3 in the low group, and 3.6 in the normal group ($P > 0.05$, t-test).

Four clinical pregnancies occurred in the low hypo-osmotic swelling test group and five in the normal hypo-osmotic swelling test group for clinical PRs per transfer of 19.0% and 23.8%, respectively ($P > 0.05$, Fisher's exact test). All the clinical pregnancies were viable at the end of the first trimester. The implantation rates were also similar in the two groups (7.1% in the low group, 9.3% in the normal group).

The pregnancies in the low hypo-osmotic swelling test group resulted from three ETs involving a mixture of embryos cryopreserved at both pronuclear stage and multicellular stage and one transfer involved embryos all frozen at the multicellular stage. Thus, the four pregnancies resulted from the transfer of seven embryos cryopreserved at the pronuclear stage and eight embryos cryopreserved at the multicellular stage. Similarly, in the normal hypo-osmotic swelling test group, the five pregnancies resulted from the transfer of embryos cryopreserved at the pronuclear stage and eight embryos cryopreserved at the multicellular stage.

DISCUSSION

The hypo-osmotic swelling test is the first semen parameter found that demonstrates a dissimilitude between fertilization and PRs in IVF-ET when subnormal (2). The mechanism to explain the adverse effect on pregnancy outcome despite normal cleavage is unknown. One theory may be that the exposure of the oocyte to large numbers of abnormal sperm during oocyte insemination may have an adverse effect. Methods for testing this theory would include reducing the incubation time of sperm and oocytes or testing the fertility potential of each individual sperm through the use of intracytoplasmic sperm injection (ICSI).

We have some preliminary data from our center that ICSI allows normal PRs with fresh ETs, thus suggesting that it is the number of abnormal sperm rather than the individual sperm that has a negative effect on the embryo. To date we have not found any reports on ICSI in the English literature in which hypo-osmotic swelling test was measured and pregnancy rates evaluated. Further study is needed.

These data failed to demonstrate any association between PRs and hypo-osmotic swelling test scores in frozen ET in women failing to conceive after transfer of fresh embryos. Further investigation is required to determine why the association between PR and hypo-osmotic swelling test differs when transferring fresh versus cryopreserved embryos. A comparison of embryo quality at time of frozen ET did not find any difference in the degree of fragmentation of embryos by hypo-osmotic swelling test score. However, there was an increase in the number of

embryos with uneven blastomeres in the low hypo-osmotic swelling test group but that would not explain why the PRs were good after frozen ET. There was no difference in the stage at which the embryos were cryopreserved in the two groups nor the stage of embryos that resulted in the pregnancy in the two groups.

The attainment of a successful pregnancy through frozen ET after failure to achieve a pregnancy after fresh ET has been attributed to either an adverse effect of controlled ovarian stimulation or inadequate endometrial development. The data presented herein suggest that having an abnormal hypo-osmotic swelling test score is another explanation for this phenomenon.

The hypo-osmotic swelling test is a simple, inexpensive test that could help explain the failure of some couples to achieve a pregnancy despite adequate fertilization. Although subnormal hypo-osmotic swelling test scores have only been found in approximately 5% of all men with normal standard semen parameters (1), we recommend that it be performed for all couples electing to undergo IVF. Additionally, the evaluation of other tests of sperm function that may be able to differentiate between fertilization and pregnancy potential is highly recommended.

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