Original Articles

Reproductive Biology Section

Isolating sperm by selecting those with normal nuclear morphology prior to intracytoplasmic sperm injection (ICSI) does not provide better pregnancy rates compared to conventional ICSI in women with repeated conception failure with in vitro fertilization

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Summary

Purpose: To determine if isolation of sperm by nuclear characteristics using high magnification offers any advantage over the normal morphologic methods when performing intracytoplasmic sperm injection (ICSI) in refractory cases in which the male partner had an abnormal DNA fragmentation index. *Materials and Methods:* Women aged ≤ 39 with failure to have a successful conception after three consecutive embryo transfers whose male partner had a DNA fragmentation index > 30% were randomly assigned to isolation of sperm for ICSI by a new high magnification procedure to evaluate nuclear morphology and the usual method with normal magnification where nuclear characteristics were not seen. Women 40-43 were not randomized and were given the option after hearing pros and cons. *Results:* Overall the live delivered pregnancy rates were similar in the high vs normal magnification groups (33.3% vs 36.3%). *Conclusions:* Isolation of sperm with normal nuclei with high magnification does not offer any advantage over conventional IVF for refractory cases where the male partner has a high DNA fragmentation index.

Key words: DNA fragmentation index; Intracytoplasmic sperm injection; Nuclear morphology; IVF failure.

Introduction

Repeated failure to conceive despite the transfer of morphologically normal embryos could be related to: bad luck, poor transfer technique, an occult oocyte factor (possibly genetic), an occult male factor (possibly genetic), an occult immunological factor, an occult endometrial factor, or an adverse effect of controlled ovarian hyperstimulation on the endometrium.

Options to consider would be to either try another IVF-ET cycle with lower dosage of gonadotropins, purposely freeze all embryos and defer fresh embryo transfer (ET) in favor of a future frozen ET, use donor oocytes, use donor sperm, pretreat with intravenous immunoglobulin or use lymphocyte immunotherapy, or use a gestational carrier.

The cheapest of all new options if one is guessing would be to use donor sperm. However, if donor oocytes is the wrong guess then the couple is spending money on another expensive IVF-ET cycle that may fail again.

Is there any evidence that a sperm factor could allow fertilization of the egg with apparent development of normal morphologic embryos that have low implantation potential? Such a defect has been found in males with hypoosmotic swelling tests < 50% when testing their semen [1]. Embryos formed by conventional insemination with low HOS test scores fail to implant [2, 3]. However, intractyoplasmic sperm injection (ICSI) fully corrects the problem [4].

Two sperm tests that at least in some studies have suggested that abnormalities of the tests are associated with low embryo implantation potential despite ICSI are: the sperm chromatin structure assay (SCSA) with DNA fragmentation indices DFI > 30% [5] and a high percentage of sperm with abnormal nuclear morphology [6]. Though initial studies with sperm with abnormal SCSA tests found no live babies despite ICSI, subsequent studies did not confirm the absolute failure to conceive but suggested high DFI index might be associated with higher miscarriage rates [7].

Studies of sperm highly magnified to 6000x have suggested that in some cases failure to conceive despite multiple failed IVF-ICSI cycles was related to the fact that the majority of the sperm had abnormal nuclei which could not be determined with an ordinary microscope [6, 8]. The authors suggested that the problem could be corrected by isolating sperm with normal nuclei and then performing ICSI [8].

The objective of the present study was to evaluate the pregnancy rates with a fourth cycle of IVF-ET in couples where the male partner had a DFI score > 30% and no

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live pregnancies had been achieved in three previous IVF-ET cycles where the "treated" group had IVF-ET performed with ICSI using high powered magnification to isolate sperm with normal nuclei. In contrast, the control group had IVF-ET with ICSI using a conventional microscope and injecting sperm with normal morphology as determined by strict criteria.

Materials and Methods

The male partner of couples with failure to have a successful pregnancy despite three previous embryo transfers (fresh or frozen) had their semen sent to Dr. Evenson's laboratory in South Dakota to perform the SCSA test. Only couples where fertilization was by ICSI were selected.

Those couples in whom the male partner had DFI scores > 30% and in whom the female partner was aged ≤ 39 were randomly assigned to have the next cycle performed with isolation of sperm using a high powered microscope to select one sperm with normal nuclear morphology vs conventional ICSI, where the sperm was selected with normal morphology based on strict criteria.

Couples aged 40-43 were informed that there was only a theoretical possibility but no evidence to support the contention that there could be an association with abnormal nuclei and abnormal SCSA test. They were advised that it was possible that the prolonged exposure to polyvinylpyrrolidone to slow the sperm sufficiently to allow evaluation of the nucleus could possibly have an adverse effect on the sperm. Thus this older group was given the option to use high magnification or not.

Only couples providing informed consent were evaluated. Thus the randomization was only given to couples in whom the female partner was aged ≤ 39. Couples in whom the female partner was aged 40-43 were allowed to choose between high magnification ICSI or conventional ICSI.

For the fourth IVF cycle all women were purposely treated with low dosage gonadotropins starting at no more than 150 IU per day. Live delivery rates were then compared in the younger group randomly assigned to high magnification ICSI vs conventional ICSI (in the older group where the choice of high magnification ICSI was decided by the couples).

Some women, especially those with borderline endometrial thickness chose to purposely freeze their embryos. They were not counted in the evaluation of pregnancy rates per fresh embryo transfer.

Results

In the randomized group (age ≤ 39) there were 12 randomly assigned to high magnification ICSI and 12 to conventional ICSI. There were six women who had high magnification ICSI and had fresh embryo transfer of at least two embryos vs nine with conventional magnification ICSI. Three women in the high magnification ICSI group cancelled the retrieval because of an inadequate number of follicles and three women froze all embryos because of borderline endometrial thickness. Two women in the normal magnification ICSI group cancelled the cycle because of inadequate response and one froze all embryos.

There were three live healthy deliveries in both groups (50.0% vs 33.3%). For women age 40-43, there was one live delivery of six choosing high magnification ICSI (16.7%) vs one of two (50%) choosing conventional ICSI.

Table 1. — *Comparison of IVF outcome following intracytoplasmic sperm injection using high magnification to isolate sperm with normal nuclei vs isolation by normal morphology using strict criteria with a conventional microscope in refractory IVF cases.*

	ICSI without high magnification			ICSI with high magnification		
	≤ 35	36-39	40-43	≤ 35	36-39	40-43
# retrievals	5	7	4	5	7	10
# transfers ≥ 2 embryos	5	4	1	3	3	2
# eggs retrieved	85	65	11	48	27	27
# insemination	76	52	9	40	27	37
# fertilized	50	29	4	13	12	15
% fertilized	65.8	55.8	44.4	32.5	52.2	48.4
# cryopreserved	31	17	0	5	0	3
# pregnancies	4	1	1	1	2	1
% pregnancy/transfer	80.0	25.0	50.0	25.0	50.0	16.7
# clinical pregnancies	2	1	1	1	2	1
% clinical pregnancy/ transfer	40.0	25.0	50.0	25.0	50.0	16.7
# chemical pregnancy	2	0	0	0	0	0
# ectopic	0	0	0	0	0	0
# viable	2	1	1	1	2	1
% viable/transfer	40.0	25.0	50.0	25.0	50.0	16.7
# miscarriages	0	0	0	0	0	0
% miscarriage/clin. preg.	0.0	0.0	0.0	0.0	0.0	0.0
# deliveries	2	1	1	1	2	1
% delivered	40.0	25.0	50.0	25.0	50.0	16.7
# transferred	17	10	4	8	9	10
Average # embryos transferred	3.4	2.5	4.0	2.7	3.0	5.0
# sacs implanted	3	1	1	1	2	1
Implantation rate	17.6	10.0	25.0	12.5	22.2	10.0

Combining both randomized treatment groups, the live delivery rate was 40.0% (6/15) in the 4th IVF cycle with failures in the previous three which is approximately the normal live delivery rate for this IVF center even for 1st and 2nd embryo transfer cycles. For women aged 40-43 combining both groups the live delivery rate was 25% (2/8) which is also similar to what is found in cycles one and two for this age group in this IVF center.

Combining both age groups there were four live deliveries in 12 fresh embryo transfers using high magnification ICSI with a live delivery rate per transfer of 33.3% vs four of 11 (36.3%) with conventional ICSI.

Conclusions

Based on these pregnancy rates achieved in the fourth IVF cycle of women failing to conceive after three IVF attempts, it can be concluded that if an abnormal DNA fragmentation index is associated with male infertility it seems to be correctable by ICSI.

Isolation of sperm with normal nuclei using high magnification does not seem to be a method that will markedly improve pregnancy rates in refractory IVF

cases compared to isolation of sperm for ICSI without the benefit of nuclear characteristics.

Since no other modifications other than using lower dosages of gonadotropins were performed in these couples, it appears that failure to conceive despite three previous failed IVF-ET with ICSI cycles is not usually related to an occult genetic defect in oocyte or sperm, or an occult endometrial or immunological factor.

One possible contributing factor to previous IVF-ICSI failures was the adverse effect on the endometrium or embryos by controlled ovarian hyperstimulation since all of these couples were treated in the 4th cycle with lower dosages of gonadotropins [9-12]. However, it is possible that merely bad luck explains the previous failure.

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