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SHORT COMMUNICATION

LEXINGTON, KENTUCKY

Intracytoplasmic Injection Using Spermatids and Subsequent Pregnancies: Round Versus Elongated Spermatids

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The purpose of this clinical trial study was to report the outcome of intracytoplasmic injection of round (RS) and elongated (ES) spermatids retrieved from the testis of nonobstructive men. Seven and three cycles using RS and ES injections were performed, respectively. Only one cycle utilizing the late stage of ES (Sd2) resulted in an on-going pregnancy. The remarkable low success rate following RS microinjection was established in this study.

KEY WORDS: Nonobstructive azzospermia; spermatid; ICSI.

INTRODUCTION

With the advent of IVF and other assisted reproductive technologies (ARTs), both male and female infertilities can be treated quite effectively. Specifically, in severe male infertility with nonobstructive azoospermia, patients may benefit the most from employment of intracytoplasmic spermatid injection, which, at present, is the only available treatment protocol. In recent years, there have been several reports on human spermatid microinjections into oocytes that resulted in successful pregnancies. The birth of two healthy babies from ejaculate round spermatids (1), a healthy female from testicular elongated spermatid (2), and also four on-going pregnancies with testicular spermatid injection (3) brought about some hope for azoospermic men who wish to become the genetic fathers.

Overall, the aforementioned reports from human studies using spermatid injections via intracytoplasmic sperm injection (ICSI) technology are encouraging for use of spermatids in clinical settings in cases of nonobstructive azoospermia. However, the success rate of ICSI with testicular spermatid remain far below those obtained using mature spermatozoa. Furthermore, the lack of correct classification and proper identification of the spermatid stages employed in the overall ART arena today lead to inadequacies in predicting the conception potential for each of those different spermatid stages. Therefore, the objective of this study was to evaluate the outcome of ICSI using round (RS) and elongated (ES) spermatids of different maturation stages, retrieved from testicular biopsies of nonobstructive azoospermic males with previously known maturation arrest.

MATERIALS AND METHODS

The protocols for ovarian stimulation of the female partners have been previously described, in addition to the details of the ICSI procedure (4). For the performance of ICSI, we have employed micropipettes with internal diameter of 5 and 9 μm for ES and RS, respectively. Fertilization was assessed 15–19 h post-ICSI, and embryo evaluation and grading was carried out as described by Hill *et al.* (5). Embryos were transferred into the uteri of recipients at 48 h after the microinjection with the use of standard methodology.

The harvesting of spermatids was carried out via testicular biopsy and the samples were collected and processed in Ham's F10 media with 10% synthetic serum substitute (SSS; Irvine Scientific Co., CA). The samples were vortexed for 30 s and teased with surgical blades, and the resultant fluid was then washed in culture media via centrifugation for 5 min at 1000*g*. All biopsies were carried out immediately following the oocyte aspirations.

The RS were classified into two distinct groups; the first group consisted of round spermatids (Sa) and the second group was of round spermatids with small flagellum (Sb). The ES were also classified into those with fully elongated nucleus (Sc), fully elongated with head attached to midpiece (Sd1), and mature with large cytoplasmic sheath in midpiece (Sd2) (6). The integrity of RS was assessed via visual appraisal and by aspiration through the injecting needle. Intact RS were classified as those that maintained their integrity and round shape when expelled from the needle.

RESULTS

We performed seven treatment cycles of round spermatid injection (ROSI) and three treatment cycles using elongated spermatid injection (ELSI). The mean ages of the male and female partners were

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Table I. Results from Microinjections of Round and Elongated Spermatids Retrieved from Testis from Men with Nonobstructive Azoospermia

Case no.	No. of oocytes	Type of spermatids injected[a]	No. of injected oocytes	No. of fertilized oocytes	Embryo grade (A–D)				No. of transferred embryos/cycle
					A	B	C	D	
1	14	Sb	9	5		1	1	2	4
2	6	Sa + Sb	6	2		1			1
3	2	Sa	2	0					
4	12	Sa	8	2			1		1
5	14	Sa + Sb	11	0					
6	4	Sb	3	0					
7	3	Sa	3	0					
8	2	Sd2	2	2	2				2
9	4	Sd1 + 2	4	3		1	2		3
10	14	Sd1	9	4		1	1	2	3
Total	75		57	18	2	4	5	4	14

[a] Sa: round spermatid; Sb: round spermatid with small flagellum; Sd1: elongated spermatid with head attached to midpiece; Sd2: elongated spermatid with large cytoplasmic sheath in midpiece.

32.1 (27–38) and 29.2 (25–36) years, respectively; the duration of infertility for those couples was 8.2 (4–14) years. Table I presents the clinical results from the 10 treatment cycles. The fertilization rates obtained for ROSI and ELSI procedures were 21.4 and 100%, respectively. Four cycles of ROSI resulted in total fertilization failure (TFF). Only one cycle (cycle # 8) utilizing Sd2 spermatids resulted in an on-going pregnancy. The results showed that injection of more advanced stage ES could yield higher pregnancy rates. This observation, however, does not seem to be evident for cases utilizing RS (Table I).

DISCUSSION

The introduction of ICSI combined with spermatids is a novel technology that makes it possible for nonobstructive azoospermic men to become fathers of their own biological children. The first human live birth resulting from spermatid microinjection was reported by Tesarik *et al.* (1) and Fishel *et al.* (2). Since then, several clinics have reported their successes, however, on a case by case basis. Our experience with ROSI in achieving fertilization and pregnancies has been very disappointing. However, we observed that injection of mature ES (Sd2) resulted in an on-going pregnancy, even though only two MII oocytes were available for microinjection in this particular case.

Our ROSI results are in agreement with those reported by Bernobeu *et al.* (7) who achieved 33% fertilization rate, but no pregnancy was noted. They concluded that poor clinical prognosis should be expected when ROSI is employed. Furthermore, in a large series of patients, Amer *et al.* (8) reported 34 cycles where ICSI was performed with RS and ES retrieved from testicular biopsies of nonobstructive men with complete arrest of spermiogenesis. They reported a fertilization rate of 24% and a pregnancy rate of only 3%. Remarkably, Vanderzwalmen *et al.* (9) showed the influence of the type of spermatid injection on clinical results. They reported one and four pregnancies when RS and ES were employed. Their observations are also in agreement with ours, showing that injection of early stage spermatids retrieved from testes markedly worsen the prognosis. On the other hand, we noted that injection of advanced level of ES could enhance embryo formation and subsequent onset of pregnancy (one pregnancy in three cycles).

On the basis of available informations on ROSI and the low success rates associated with it, one can deduct that these difficulties could be due to identification of RS; gamete injury during microinjection; cytoplsmic immaturity; and genetic abnormalities (10). In order to overcome those difficulties, Zavos *et al.* (11) developed and applied the hyperosmotic shrinkage (HYOS) test for proper selection of viable RS. They established that the majority of RS (87%) which were retrieved from testes exhibited cell membrane damage. This may explain, as they proposed, the disappointing results of ROSI when RS from testicular retrievals are used.

Currently, we are in the process of evaluating the effect of Pentoxifylline on late stage of RS as well as on ES. Pentoxifylline is considered as an effective motility enhancer that may improve the spermatid flagella beating (12). This may not only assist in the identification of viable spermatids, but may also improve the clinical outcomes of spermatid injection. However, until further improvements on the clinical outcome are established, especially for ROSI, patients should be clearly informed of the remarkably low success rates.

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