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Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration

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Intracytoplasmic sperm injection (ICSI) has been successful in cases of extreme oligoasthenozoospermia in achieving pregnancies via in-vitro fertilization (IVF) with the lowest imaginable sperm counts. In azoospermia caused by congenital bilateral absence of the vas deferens (CBAVD), it has been shown that epididymal spermatozoa can be retrieved in large numbers, but fertilization rates using conventional IVF are low. Furthermore, no fertilization has ever been possible using testicular spermatozoa with conventional IVF. In the most extreme case of absence of the epididymis, spermatozoa can only be retrieved from macerated testicular biopsy specimens. In such cases, all that can be seen are free-floating Sertoli cells with many spermatids attached, and only occasional spermatozoa per high power field which have only the barest, occasional, slightly twitching motion. The objective of the present study was to determine whether ICSI could achieve better results than conventional IVF with microsurgical aspiration of spermatozoa (MESA). ICSI (using epididymal or testicular spermatozoa) from men with CBAVD or irreparable obstructive azoospermia, achieved good fertilization and normal embryos in 82% of cases, compared to 19% with conventional IVF. There was an overall fertilization rate of 45%, with 85% progressing to normally cleaving embryos using ICSI, compared to 6.9% using conventional IVF. The pregnancy rate with ICSI/MESA was 47% per stimulated cycle (normal delivery rate was 30%), compared to 4.5% with conventional IVF. These results were achieved in patients who had consistently failed to fertilize in previous cycles with MESA and conventional IVF. We conclude that although complex mechanisms (facilitated by epididymal passage) may be required by spermatozoa for conventional fertilization of human oocytes (whether *in vivo* or *in vitro*), no such mechanisms are required for fertilization after direct microinjection. Because of the consistently good results using epididymal spermatozoa with ICSI in comparison to conventional IVF, and also the good results in extreme cases requiring testicular tissue spermatozoa, ICSI may be mandated for all future MESA patients with CBAVD, or with irreparable obstructive azoospermia.

Key words: epididymal/fertilization/injection/intracytoplasmic/sperm

Introduction

Congenital bilateral absence of the vas deferens (CBAVD) and irreparable obstructive azoospermia are the cause of male infertility in a large and frustrating group of patients who have normal spermatogenesis, and from whom large numbers of spermatozoa are surgically retrievable for in-vitro fertilization (IVF) (Temple-Smith *et al.*, 1985; Silber *et al.*, 1990a).

A treatment protocol for such patients, involving microsurgical aspiration of spermatozoa (MESA) from the proximal region of the epididymis (where the most active spermatozoa were always located) and in-vitro fertilization (IVF), was developed to solve this problem. The first successful fertilizations and pregnancies with this approach were achieved in CBAVD patients in 1988 (Silber, 1988; Silber *et al.*, 1990a,b). This technique is applicable to all cases of obstructive azoospermia including vasectomy reversal and epididymal blockage.

The problem, however, has been that fertilization rates with epididymal spermatozoa have been very low. Sixty-five per cent of patients get no viable embryos at all despite large numbers of eggs, and pregnancy rates noted by most centres attempting this procedure have never exceeded 9%, with term pregnancy rates being even lower (Belker, 1994).

The objective of this study was to see whether micromanipulation specifically using intracytoplasmic sperm injection (ICSI) could improve the poor fertilization and pregnancy rates obtained when attempting IVF in such patients with microsurgically retrieved spermatozoa. This present collaboration indicates that ICSI using epididymal or testicular spermatozoa in men with CBAVD does give reliable, and much higher, fertilization and pregnancy rates than conventional IVF with MESA.

Materials and methods

Induction of follicular development and oocyte retrieval

The female partners of men with azoospermia caused by CBAVD underwent induction of multiple follicular development with the following protocol: leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL, USA) 1 mg s.c. was administered daily until the day of follicular aspiration. After desensitization, patients received human follicle stimulating hormone (FSH)

(Metrodin; Serono Laboratories Inc., Randolph, MA, USA) and/or human menopausal gonadotrophins (HMG) (Pergonal; Serono) until many follicles of 2.0 cm were noted on ultrasound. Human chorionic gonadotrophin (HCG; Profasi; Serono) 10 000 IU was then administered i.m., and 36 h later, the patients underwent transvaginal follicular aspiration. The follicular fluids and washings were given immediately to the embryology laboratory adjacent to the operating room.

Epididymal sperm aspiration

The male partners underwent microsurgical scrotal exploration with the intention of aspirating sufficient numbers of motile spermatozoa to utilize for IVF with the wife's eggs. The surgical technique in the male was as follows: scrotal contents were extruded through a small incision, the tunica vaginalis was opened and the epididymis exposed. Under 10–40× magnification with an operating microscope, a tiny incision was made with microscissors in the epididymal tunic to expose the tubules in the proximal portion of the obstructed epididymis. The reason for proceeding proximally was that our previous studies have shown that this is where spermatozoa with the greatest motility are located (Silber *et al.*, 1990a). Spermatozoa were aspirated directly from the opening in the epididymal tubule with a micropipette. Great care was taken not to contaminate the specimen with blood, and careful haemostasis was achieved with microbipolar forceps. The epididymal fluid was immediately diluted in HEPES-buffered Earle's medium and a tiny portion examined for motility and quality of progression. If there was no motility or poor motility, another aspiration was made more proximally. We have found that motile spermatozoa were not obtained until we reached the proximal-most portion of the caput epididymis or even the vasa efferentia, the inverse of what might have been previously anticipated (Silber *et al.*, 1988, 1990a; Davis *et al.*, 1991; Asch *et al.*, 1992).

Methodology of sperm treatment and conventional IVF

Probably the major breakthrough in achieving fertilization and pregnancy with these patients using conventional IVF was the discovery that distal epididymal spermatozoa (which we would think are the only ones capable of fertilization) are usually non-motile because in the obstructed state they are dead owing to senescence. The discovery that, in order to obtain live sperm, one had to go to the most proximal regions of the epididymis (even vasa efferentia) was the major factor resulting in success with conventional IVF in some patients (Silber *et al.*, 1990a).

When no spermatozoa were retrievable from the epididymis or vasa efferentia on either side, or when the epididymis was absent, a testicular biopsy was performed in an effort to retrieve any spermatozoa at all. Invariably, only occasional, small numbers of spermatozoa were obtainable from minced testicular tissue, along with large numbers of spermatid-laden Sertoli cells. The free spermatozoa had only weak, slowly twitching motility.

In the laboratory, the epididymal sperm was first diluted and examined in a volume of several millilitres and then concentrated into a volume of 0.3 ml, layered on a discontinuous mini-Percoll gradient, and centrifuged for 30 min. The entire 95% fraction was then washed twice and inseminated with all of the eggs in a Falcon mini-test tube with 1 ml of HTF culture medium and incubated at 37°C with 5% CO_2 in air (Ord *et al.*, 1990).

Two days after insemination, embryos were transferred to the Fallopian tubes or uterus. The female partners received progesterone in oil, 50 mg i.m. per day beginning with the day of egg aspiration.

Methodology for MESA with ICSI

The technique of spermatozoa and oocyte preparation, microinjection, and culture were the same as recently described by Palermo *et al.* (1992, 1993) and by Van Steirteghem *et al.* (1993a,b) for several male factor cases. All embryos were atraumatically transferred to the uterus via a Frydman catheter. Stimulation protocol, luteal support and methodology for retrieval of eggs and spermatozoa were all the same as in previous conventional IVF series (Silber *et al.*, 1990a).

Using the ICSI technique for MESA, several differences in approach were used. Since only very small numbers of spermatozoa were required for ICSI, the majority of the retrieved epididymal spermatozoa were frozen using standard techniques, with small numbers in each straw. Since for ICSI only a minor degree of motility was required (for vitality assessment only), freezing these fragile epididymal spermatozoa was not a serious difficulty. All of the epididymal fluid that was not used fresh for ICSI was diluted 50:50 by volume with spermatozoa freezing medium, drawn into 25 μl straws, and suspended over liquid nitrogen vapour for several hours and then plunged into liquid nitrogen.

Epididymal spermatozoa were prepared for microinjection in the same manner as for conventional IVF using Percoll. However, testicular tissue spermatozoa were retrieved in such small numbers, and with such minimum motility, that the only method of preparation was to mince the tissue, centrifuge the effluent at 300 *g* for 5 min and resuspend in 100 μl of media.

Patient population

Sixty-seven cycles of MESA with conventional IVF were performed on couples with CBAVD or irreparable obstructive azoospermia. Sixty-two were CBAVD and five were epididymal obstruction cases.

A group of 17 similar patients (15 with CBAVD and two with epididymal obstruction) underwent MESA using ICSI instead of conventional IVF. Nine of the 17 had already undergone multiple previous attempts at MESA and conventional IVF with poor or no fertilization. We have found previously that poor fertilization with conventional MESA in any given patient results also in poor fertilization in all subsequent conventional MESA–IVF cycles (Silber, 1993).

For eight of the 17 patients, this was their first MESA attempt, but for various reasons, these were also considered very poor candidates. Four of these eight patients had either no epididymis, or no spermatozoa whatsoever in the epididymis or rete testis despite normal spermatogenesis, thus requiring the use of spermatozoa extracted from a testicular biopsy. Two patients (one with congenital vas blockage) had undergone a previous vasoepididymistomy. In one case, both partners were both heterozygous carriers for ΔF508. In three cases, the female

partner was over 40 years old. In short, these 17 patients were intentionally selected as a fairly dismal group of candidates for MESA, because of their poor prognosis with conventional IVF.

Results

Results with MESA and conventional IVF

Previous work with conventional IVF and MESA showed that 65% of patients either do not fertilize, or have only poor quality embryos. This group does not get pregnant and has only a 1.4% fertilization rate (Silber, 1993). Thirty-five percent of MESA patients do fertilize, but only 19% have a good fertilization rate. It seems that there were two populations of patients with obstructive azoospermia. There was a small number (19%) who fertilized their partners' eggs moderately well and had an acceptable pregnancy rate, and conversely, a much larger number (81%) who fertilized only one or none of their partners' eggs, and had no pregnancy.

Table I summarizes the typically poor results in 67 consecutive MESA/conventional IVF cycles, and compares those results to MESA/ICSI cycles. It appears that a regression toward the mean has brought our pregnancy rate using MESA with conventional IVF in line with the low results of many other groups (Belker, 1993). No programme performing MESA and conventional IVF has >10% pregnancy rate over the long haul. Note that our ongoing and term pregnancy rate in the last 67 cycles was only 4.5% with an overall fertilization rate of only 6.9%.

Table I. Comparison of MESA–ICSI results to MESA–conventional IVF results in a similar patient population

No. of cycles	No. of mature eggs	No. of embryos	Fertilization rate (%)	No. of cycles >1 embryos	Pregnancy rate (ongoing)
ICSI–MESA					
17	197	80	41	14/17 (82%)	47% (30%)
IVF–MESA					
67	1427	98	6.9	13/67 (19%)	9% (4.5%)

Results with MESA and ICSI

Table I also summarizes the results of fertilization and pregnancy in the series of 17 consecutive MESA cases utilizing ICSI. Over 82% of patients had good fertilization rates with over two to nine good quality embryos resulting. Eighty-eight per cent went to embryo transfer. Ninety-three per cent (12 out of 13) with epididymal spermatozoa had fertilization and transfer of normal embryos with ICSI. Forty-seven per cent became pregnant, and 30% have healthy ongoing pregnancies (5 months). Three of the patients with three to nine embryos had complete absence of the epididymis and, therefore, resulted from testicular-biopsy-obtained spermatozoa. This represents a dramatic change from previous experience with MESA patients.

Table II gives the details of all 17 patients in this first MESA/ICSI series. The overall fertilization rate was 41%. The pregnancy rate was 47%. The cystic fibrosis genotype of the husband had no relationship to the results.

Table III details results in the specific group of nine patients who were previous conventional IVF/MESA failures on multiple attempts. With conventional IVF these patients had a 3% fertilization rate, but with ICSI the fertilization rate was 39% and 33% became pregnant. Again, there was no correlation to the cystic fibrosis genotype. The embryo transfer rate was a remarkable 89% in this group of MESA patients with previous failure to fertilize.

Discussion

The critical goal of this series was to differentiate clearly between results with MESA using standard IVF procedures versus those

Table II. Results of all 17 patients in first MESA–ICSI series

Patient	Genotype if CBAVD	No. mature eggs (MII)	No. fertilized (2PN)	No. embryos transferred	Fertilization rate (%)	Age of female partner	Pregnant
1	ΔF-508/N	9	3	3	33	42	no
2	N/N	14	5	5	36	39	no
3	ΔF-508/N	22	11	6	50	31	no
4	ΔF-508/N	10	5	5	50	33	no
5	R117H/R117H	14	6	4	43	31	yes
6	ΔF-508/N	18	6	4	33	28	yes
7	W1282X/N	16	5	3	31	36	yes
8	ΔF-508/N	3	2	2	67	25	no
9	N/N	3	0	0	0	40	no
10	N/N	7	3	3	43	28	yes
11	N/N	10	8	3	80	38	no
12	ΔF-508/N	8	1	1	13	35	yes
13	ΔF-508/N	12	5	3	42	36	yes
14	ΔF-508/N	10	0	0	0	35	no
15	R117H/N	11	5	3	45	29	yes
16	N/A	22	10	3	45		no
17	N/A	8	5	2	63		yes
Totals		197	80	50	41		8/17 (47%)

Table III. Results of MESA–ICSI in nine CBAVD patients with prior recurrent MESA–IVF failures

Patient	Cystic fibrosis genotype	Number previous failed MESA–IVF cycles	Total eggs retrieved in all previous MESA–IVF cycles	Number fertilized in previous MESA–IVF cycles	Fertilization rate (%)	Number eggs retrieved in first MESA–ICSI cycle	Number fertilized in 1st MESA–ICSI cycle	Fertilization rate (%)	Age of female partner	Pregnant
1	ΔF-508/N	5	78	0	0	9	3	33	42	no
2	N/N	5	127	5	3.9	14	5	36	39	no
3	ΔF-508/N	2	69	0	0	22	11	27	31	no
4	ΔF-508/N	2	23	5	21	10	5	50	33	no
5	R117H/117H	2	68	0	0	14	6	43	31	yes
6	ΔF-508/N	2	51	4	7.8	18	6	33	28	yes
7	@1282X/N	1	24	0	0	16	5	31	36	yes
8	ΔF-508/N	2	23	0	0	3	2	66	25	no
9	N/N	2	8	0	0	3	0	0	40	no
Totals		23	471	14	3	109	43	39		33

using ICSI. It is still not clear to what extent the severely diminished fertilization rate with epididymal spermatozoa is the result of lack of maturation which should normally have occurred during epididymal transport or the senescence of spermatozoa in an obstructed system. In rabbits, artificial obstruction of the epididymis improves the motility dramatically for caput spermatozoa, but still results in a low pregnancy rate (Bedford, 1966; Paufler *et al.*, 1968).

It is clear from our published results that distal epididymal spermatozoa in obstructed males are either non-motile or totally degenerate, due to senescence (Silber *et al.*, 1990a,b; Asch *et al.*, 1992). One has to go to the proximal caput or vasa efferentia to obtain motile spermatozoa, where they have been produced more recently and where the best motility is found in an obstructed system.

MESA cases with standard IVF seem to fall into two categories: those that fertilize (20%) and those that do not (80%). With ICSI, this low success rate is completely reversed. That is, 90% are good fertilizers and only 10% are not. Using ICSI, it appears that MESA pregnancy rates are not much different from ordinary IVF cases. With ICSI, as well as with conventional IVF, it is still necessary to use proximal, motile spermatozoa. Senescent distal epididymal spermatozoa may fertilize and result in a pregnancy (Tournaye *et al.*, 1994). However, the low pregnancy rate and the high rate of miscarriage with distal spermatozoa still mandates the use of the more viable proximal spermatozoa. Even testicular biopsy spermatozoa, with the barest discernible motility, yield good fertilization rates with ICSI. But senescent or 'dead' spermatozoa, do not (Van Steirteghem *et al.*, 1993a,b).

It is remarkable that epididymal spermatozoa which look adequate often do not fertilize in standard IVF, and those which look poor may very well have high fertilizing capacity with standard IVF. It is very difficult with conventional IVF to predict which spermatozoa will and which will not fertilize. The presence or absence of sperm antibodies seems to have no impact (Silber, 1993). Computerized motion analysis, although not easily practicable, suggests that there may be a very tiny subpopulation of spermatozoa, present only in some of the patients, out of all the millions of non-fertile spermatozoa, that are actually responsible for the fertilization in successful patients (Silber *et al.*, 1990a,b; Davis *et al.*, 1991). Thus, a routine glance at the spermatozoa often shows no difference, but it may just be that only a tiny occasional fraction of all the spermatozoa present are necessary to produce fertilization in conventional IVF. None the less, we have found it impossible for practical purposes to determine which MESA cases would do well with standard IVF and which would require ICSI.

Therefore, because of the consistently improved results with ICSI over regular IVF and, indeed, the good results with testicular spermatozoa (if epididymal spermatozoa are not obtainable), we feel that the use of ICSI may be mandated for all MESA patients. The consistently good fertilization rates with epididymal and testicular biopsy spermatozoa using ICSI seem to highlight the possible role of the epididymis in normal fertilzation as having nothing to do with the intrinsic ability of the spermatozoa to fertilize and result in a normal embryo once penetration is completed. Most likely, the function of the epididymis is to give the already genetically completed spermatozoon the ability to fuse with the oolema. Zona penetration and, perhaps more importantly, membrane fusion capacity may be what the spermatozoon develops as it is processed through the epididymis.

An additional benefit of this MESA/ICSI technology is that epididymal spermatozoa can be frozen and stored so that the male partner will not require more than one operation for sperm retrieval. Yet his partner can undergo multiple cycles of IVF/ICSI with thawed epididymal spermatozoa until she finally becomes pregnant. However, if testicular biopsy spermatozoa are required because of absence of the epididymis or absence of epididymal spermatozoa, then the numbers and motility may be too low to allow this additional benefit of freezing. None the less, the apparent efficacy of testicular spermatozoa (when no epididymal spermatozoa are available) using ICSI would imply that there may be no case of obstructive azoospermia that cannot be treated with this methodology.

Addendum

In a subsequent series of 31 consecutive MESA/ICSI cycles, 28 patients achieved fertilization and embryo transfer (90%), and 65% became pregnant with 48% ongoing pregnancies.

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