Conventional IVF vs ICSI (Intra Cytoplasmic Sperm Injection) for Patients Requiring Microsurgical Epididymal Sperm Aspiration (MESA)

S.J. Silber, A.C. Van Steirteghem and P. Devroey

St. Luke's Hospital, 224 S. Woods Mill Road, Saint Louis, MO 63017, USA
Centre for Reproductive Medicine, Academisch Ziekenhuis Virje Universiteit Brussel
Laarbeeklaan 101, 1090 Brussels, Belgium

Congenital absence of the vas deferens accounts for 11-50% of obstructive azoospermia[6]. This is a large and frustrating group of patients who have been shown on countless testicle biopsies to have normal spermatogenesis, and are theoretically making sperm quite capable of fertilizing an egg[23]. Yet treatment until recently has been very dismal[25].

We originally developed a treatment protocol for such patients, involving microsurgical aspiration of sperm (MESA) from the proximal region of the epididymis (where the most active sperm were always located) and in vitro fertilization (IVF). We reported the first successful fertilizations and pregnancies with this approach in 1987 in this previously frustrating group of couples[16, 17, 18, 22]. Actually this technique is actually applicable to all cases of obstructive azoospermia including vasectomy reversal and epididymal obstruction. The problem however has been the very low fertilization rates and pregnancy rates noted by everyone

attempting this procedure. A preliminary series with Dr. Van Steirteghem and Dr. Devroey in Brussels using ICSI now shows that the fertilization rates and pregnancy rates can be improved to the level that should be expected in routine IVF patients.

Induction of Follicular Development and Oocyte Retrieval

The female partners of men with azoospermia caused by congenital absence of the vas undergo induction of multiple follicular development with the following protocol: leuprolide acetate (Lupron, TAP Pharmaceuticals, North Chicago, IL) 1 mg subcutaneously daily until the day of follicular aspiration. Patients then receive human follicle stimulating hormone (FSH) (Metrodin, Serono Laboratories, Inc., Randolph, MA) and/or human menopausal gonadotropins (hMG) (Pergonal, Serono) until many follicles of 2.0 cm are noted on ultrasound, when human chorionic gonadotropin (Profasi, Serono, Randolph, MA) 10,000 IU is then administered intramuscularly.

Thirty-six hours after hCG administration, the patients undergo transvaginal follicular aspiration. The follicular fluids and washings are given immediately to the embryology laboratory adjacent to the operating room.

Epididymal Sperm Aspiration, Washing Methodology, and IVF

At the same time, the husband undergoes scrotal exploration with the intention of aspirating sufficient numbers of motile spermatozoa to utilize for IVF with the wife's eggs, with transfer of subsequent embryos into the wife's Fallopian tube, or uterus.

The surgical technique in the male is as follows: scrotal contents are extruded through a small incision, the tunica vaginalis is opened and the epididymis is exposed. Under 10-40 x magnification with an operating microscope, a tiny incision is made with microscissors into the epididymal tunic to expose the tubules in the distal-most portion first of the obstructed epididymis. Sperm are aspirated directly from the opening in the epididymal tubule with a micropipette. Great care is taken not to contaminate the specimen with blood, and careful hemostasis is achieved with microbipolar forceps. The epididymal fluid is immediately diluted in HEPES buffered media,

and a tiny portion examined for motility and quality of progression. If there is no motility or poor motility, another aspiration is made one-half centimeter more proximally. We thus obtain sperm from successively more and more proximal regions until progressive motility is found. We have found that motile sperm are not obtained until we reach the proximal-most portion of the caput epididymis or even the vasa efferentia, the inverse of what might have been anticipated[4, 3, 10, 21, 22].

Probably the major breakthrough in achieving fertilization and pregnancy with these patients using conventional IVF is the discovery that distal epididymal sperm (which we would think are the only ones capable of fertilization) are usually non-motile *not* because of immaturity, but rather (in the obstructed state) because they are just dead from 'old age'. The discovery that, in order to obtain live sperm, we had to go to the most proximal regions of the epididymis, even vasa efferentia, was the major factor resulting in our success in some patients.

In the laboratory, the epididymal sperm is concentrated into a volume of 0.3 ml, layered on a discontinuous mini-Percoll gradient, and centrifuged for 30 minutes. The entire 95% fraction is then washed x 2 and inseminated with all of the eggs in a Falcon mini-test tube with 1 ml of HTF culture media and incubated at 37°C with 5% CO_2 in air [11].

Two days after insemination, embryos are transferred to the Fallopian tubes or uterus. The wives receive progesterone in oil, 50 mg intramuscularly per day beginning with the day of egg aspiration.

Methodology for MESA with ICSI

Because of the relatively poor results (to be discussed later) using conventional IVF with epididymal sperm, our center in St. Louis has now collaborated with Dr. Van Steirteghem and Dr. Devroey in Belgium. We wished to see whether direct injection of epididymal sperm into the ooplasm could improve the meager results that were obtained in the first 167 cycles using conventional IVF methodology.

The technique of sperm and oocyte preparation, microinjection, and culture were the same as recently described by Van Steirteghem et al. for severe male factor cases[26, 27]. All embryos were transferred to the uterus via a Frydman catheter atraumatically. Stimulation

protocol, luteal support, and methodology for retrieval of eggs and sperm were all the same as in previous conventional IVF series.

Patient Population

167 cycles of MESA with conventional IVF were performed strictly on couples with CAV (congenital absence of vas). These were divided into five early series totaling 100 cycles, and then four later series totaling 67 cycles. MESA procedures were also performed on a very small number of men with other causes of epididymal obstruction (the results were similar) but this group is excluded for the sake of homogeneity. Our studies on the genetics, sperm motility, quantitative spermatogenesis, ultrastructure, and immunology, of these patients have already been reported[3, 4, 5, 12, 15, 19, 20, 26. 27].

Another group of 17 patients (15 with CAV) underwent MESA using intracytoplasmic injection (ICSI) instead of conventional IVF. 9 of the 17 had already undergone multiple previous attempts at MESA and conventional IVF in our hands in the United States with poor or no fertilization. We have reported that poor fertilization with conventional MESA in any given patient results also in poor fertilization in all subsequent conventional MESA - IVF cycles.

For 8 of the 17 patients this was their first MESA attempt, but for various reasons, these were also considered very poor candidates. Four patients had either no epididymis, or no sperm whatsoever in the epididymis or rete testis despite normal spermatogenesis, thus requiring the use of sperm extracted from a testicle biopsy. Two patients (one with congenital vas blockage) had undergone a previous vasoepididymostomy. One patient and his wife were both heterozygous carriers for DF 508. In three cases, the wife was over 40 years old.

All 17 MESA - ICSI cycles were performed during a one week period at the Free University Academic Hospital in Brussels, Belgium by Dr. Van Steirteghem, Dr. Devroey, myself, and co-workers. The patient population in 15 cases came from our usual base in the United States, and in 2 cases were generated from the Belgium area. In short, this group of 17 was intentionally selected as a fairly dismal group of candidates for MESA out of our usual population base.

Results With Conventional IVF

Our results with the first 100 cycles are summarized in Table I. The pregnancy rate and fertilization rate are highly variable from cycle to

Table I First 100 cases of in vitro fertilization for congenital absence of the vas: pregnancy rates

Series number	Number or sperm aspiration cycles	Number pregnant (term pregnancy)	Pregnancy rate per cycle (term pregnancy
1	32	10 (7)	31% (22%)
2	16	2 (1)	12% (6%)
3	21	5 (4)	24% (19%)
4	13	0 (0)	0% (0%)
5	18	5 (4)	28% (22%)
Totals	100	22 (16)	22% (16%)

cycle, but not from patient to patient. Table II demonstrates that in only 38% of the cycles were more than one embryo obtained, and if only one embryo was obtained, there was no pregnancy. Table III (the first 115 cycles) shows that 65% of patients either do not fertilize, or only result in one poor embryo, with no pregnancy and a 1.4% fertilization rate.

Table II First 100 cases in vitro fertilization for congenital absence of vas: results when more than two embryos are produced

Series number	Number or sperm aspiration cycles	Number of cycles producing two embryos	Pregnancy rate per transfer of two embryos
1	32	12 (38%)	9/12 (75%)
2	16	7 (44%)	2/7 (29%)
3	21	8 (38%)	5/8 (63%)
4	13	2 (15%)	0/2 (0%)
5	18	9 (50%)	5/9 (56%)
Totals	100	38 (38%)	21/38 (55%)

Table III

Patient Group According To Number of Embryos	Number of Patients	Fertilization Rate In These Patients	Pregnancy Rate Per Patient
0 - 1	65%	1.4%	0
2 - 4	16%	15.4%	26%
5 - 7	9%	36.4%	55%
> 7	10%	58.4%	67%
Total 115	100%	15.7%	16%

Thus, it appears that there are two sub populations of patients with congenital absence of the vas with regard to fertilizing ability. There are a small number (20%) who fertilize many of their wives' eggs and have a high pregnancy rate. Conversely there is a large number (80%) who fertilize only a few or none of their wives' eggs, and have a low pregnancy rate. Sixty percent of embryos came from 10% of the patients.

Table IV summarizes the results of 24 patients undergoing repeat attempts at conventional MESA (2-5 cycles). It is clear that there is a predictable pattern of modest to good fertilization in subsequent

Table IV

Patient's Pattern of Fertilization in Repeated Cycles	No. of Patients	No. of Embryos/ Eggs	Fertilization Rate	Cumulative Pregnancy Rate (2-5 Cycles)
"Good" Fert. Rate (10-90%)	8 (33%)	155/360	43%	5 (62%)
"Poor" Fert. Rate (0-9%)	16 (67%)	9/610	1%	0 (0%)
Overall Results	24	164/970	17%	5 (21%)

cycles of those that fertilize well in the first cycle, and predictably poor fertilization in those who did not fertilize well in the first cycle.

Table V summarizes the deteriorating results in the last four series of MESA cycles involving 67 cases. It appears as though 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.

Table V

Series Number	No. of Sperm Aspiration Cycles	Fertilization Rate	Term Pregnancy Rate
6	15	6.8%	6.7%
7	18	9.9%	5.6%
8	22	5.0%	4.5%
9	12	5.8%	0 %
Total	67	6.9%	4.5%

Our term pregnancy rate in the last 67 conventional MESA cycles has been only 4.5%, with an overall fertilization rate of 6.9%. The term pregnancy rate of all 167 conventional MESA/IVF cycles is now 11%.

Results With MESA + ICSI

Table VI summarizes the results of fertilization and pregnancy in the most recent series of 17 MESA cases utilizing intracytoplasmic injection as compared to conventional IVF. Over 80% of patients had good fertilization rates with over two to nine good quality embryos

Table VI Comparison of MESA - ICSI Results to Conventional MESA - IVF Results in a Similar Patient Population

Number of Cycles	Number of Mature Eggs	Number of Embryos	Fertilization Rate	Number of Cycles With >1 Embryo	Pregnancy Rate (Ongoing)
ICSI-MESA					
17	207	77	37%	14/17 (82%)	47% (30%)
IVF-MESA					
67	1,427	98	6.9%	13/67 (19%)	9% (4.5%)

resulting. Eight-eight percent went to embryo transfer. Forty-seven percent became pregnant, and 30% have healthy ongoing pregnancies. Three of the patients with three to nine embryos

produced were the result of testicular biopsy obtained sperm, who had complete absence of the epididymis. These results represent a dramatic change from previous experience with such patients.

Conclusion

It is clear from our published results that distal epididymal sperm in obstructed males are either non-motile or totally degenerate, due to senescence[3, 13, 22]. One has to go to the proximal caput or vasa efferentia to obtain motile sperm, because these sperm have been produced more recently. Yet these sperm still seem to fall into two categories: those that fertilize well (20%) and those that do not (80%). With intra cytoplasmic injection, this low success rate is completely reversed. That is, 80% are good fertilizers and only 20% are not. Using ICSI, it appears that MESA pregnancy rates are not much different from ordinary IVF cases.

It is remarkable that epididymal sperm which look adequate often do not fertilize, and those which look poor may very well have high fertilizing capacity. It is very difficult with conventional IVF to predict which sperm will and which sperm will not fertilize. The presence or absence of sperm antibodies seems to have no impact[1, 2, 7, 8, 9, 15, 24]. Computerized motion analysis, although not easily practicable, suggests that there may be only a very tiny sub population of sperm, present only in some of the patients, out of all of the millions of non-fertile sperm, that are actually responsible for the fertilization in successful patients[22]. Thus a routine glance at the sperm often shows no difference, but it may just be that only a tiny occasional fraction of all the sperm present may be necessary to produce fertilization in conventional IVF.

In any event, because of the consistently improved results with ICSI over regular IVF, and indeed the equally good results with testicular sperm (if epididymal sperm are not obtainable), mandates the use of ICSI for all future IVF treatments for MESA patients. In fact with this technology, we suggest that if we don't have to resort to testicular biopsy sperm, we can freeze enough epididymal sperm that the man will never require more than one operation for sperm retrieval. Yet his wife can undergo multiple cycles of IVF - ICSI until she finally gets pregnant.

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