The Use of Epididymal Sperm in Assisted Reproduction

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In men with uncorrectable obstructive azospermia, the only hope for gathering a child is epididymal sperm aspiration combined with IVF. We have employed this technique in 167 cases using conventional IVF, and by evaluating the relatively poor results, have learned a great deal about the physiology, maturation, and fertilizing ability of human epididymal sperm. More recently, we have been the first to use ICSI rather than conventional IVF with epididymal and even testicular biopsy retrieved sperm, with success no different from IVF with ejaculated normal semen.

We have discovered the following with conventional IVF and epididymal sperm: 1) The motility of sperm retrieved from the obstructed epididymis is better at more proximal levels because of senescence of the distally stored sperm; 2) The conventional IVF fertilization rate of human epididymal sperm is not predictable based on either motility, antibody, or M/M studies or morphology; 3) Yet, it is quite predictable and repeatable in multiple cycles of IVF in any given subject. A minority of patients

(10%) have repeatably high fertilization rates with conventional IVF and should eventually father a child, if they go through enough cycles. The majority, however, have consistently poor or no fertilization, and will not father a child without micromanipulation. The reason for this problem may be either sperm maturation defects that are poorly defined, or senescent and pathologic changes caused by the obstruction.

Intracytoplasmic sperm injection has been successful in cases of extreme oligoasthenospermia in achieving pregnancies via IVF with the lowest imaginable sperm counts (13, 22). Therefore, we wanted to see if ICSI could overcome the poor fertilization results using conventional IVF with epididymal sperm. The most severe case of obstructive male factor infertility is perhaps absence of the epididymis whereby the only sperm available are from macerated testicular biopsy specimens. In such cases, all that can be seen in testicular tissue are free-floating Sertoli cells with many spermatids attached, and an occasional rare spermatozoa per high power field that has only the barest, occasional, slightly twitching motion. Centrifugation of the supernatant at 1800G usually yields about 10-25 such poor sperm for ICSI.

In our experience with 167 cases of MESA/IVF and 48 cases of MESA/ICSI, direct intracytoplasmic injection of an individual such sperm into mature oocytes from the wife achieved fertilization and normal embryos in 90% of cases, with an overall fertilization rate of 45%, with 85% going on to normally cleaving embryos. We conclude that although complex mechanisms are required for conventional fertilization of human oocytes whether in vivo or in vitro, none of these mechanisms are required for fertilization after direct microinjection into the egg. Because of the consistently good results using epididymal sperm with ICSI over regular IVF, and the moderately good results when having to resort to testicular tissue sperm, ICSI is mandated for all future MESA patients.

INTRODUCTION

In 1988, and later in 1990, the first successful series of pregnancies and normal live births resulting from IVF using sperm from the proximal epididymis or vasa efferentia of men with congenital absence of the vas (CAV) were reported (20, 21). Two major conclusions from this work were: 1) the establishment of a treatment for male infertility caused by a condition that had previously been untreatable; 2) a demonstration that in the human, sperm passage through the epididymis was not (as had been previously thought) always a mandatory requirement for

fertilization. This conclusion verified what had been speculated upon from previous reports on vasoepididymostomy (16, 20).

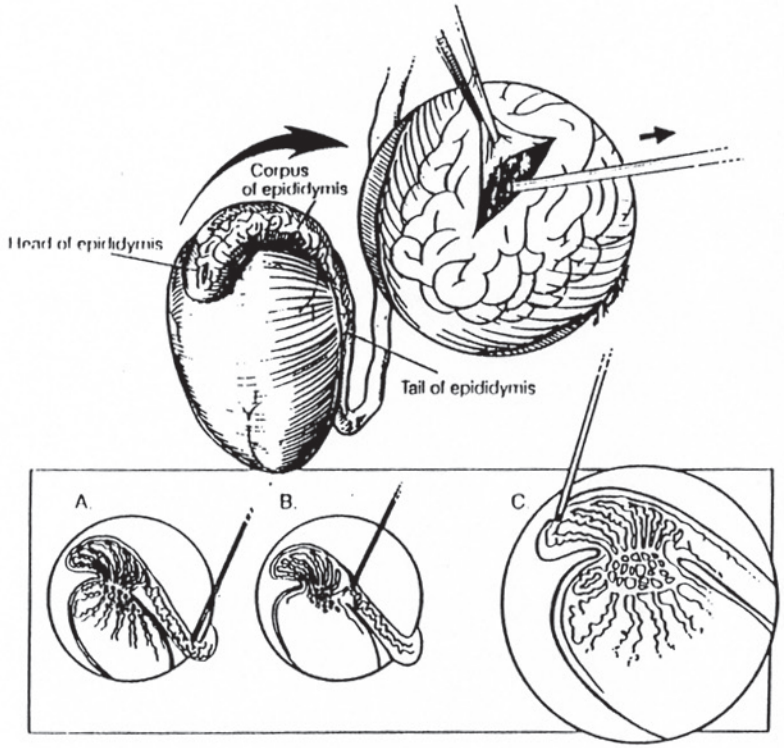

FIG. 1a. Sperm were microsurgically aspirated from distal and proximal regions of the epididymis. The samples with the best quality motility were used for IVF.

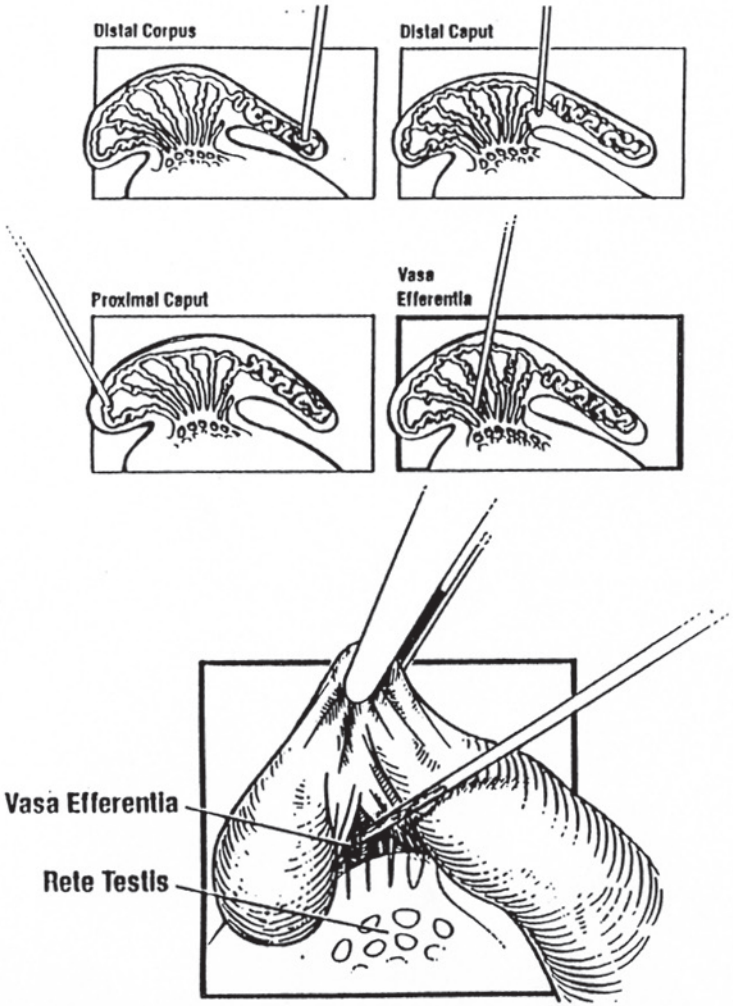

FIG. 1b. Sperm in distal and often proximal epididymis were often nonmotile or poorly motile, and in these cases vsa efferentia fluid usually had the most motile sperm.

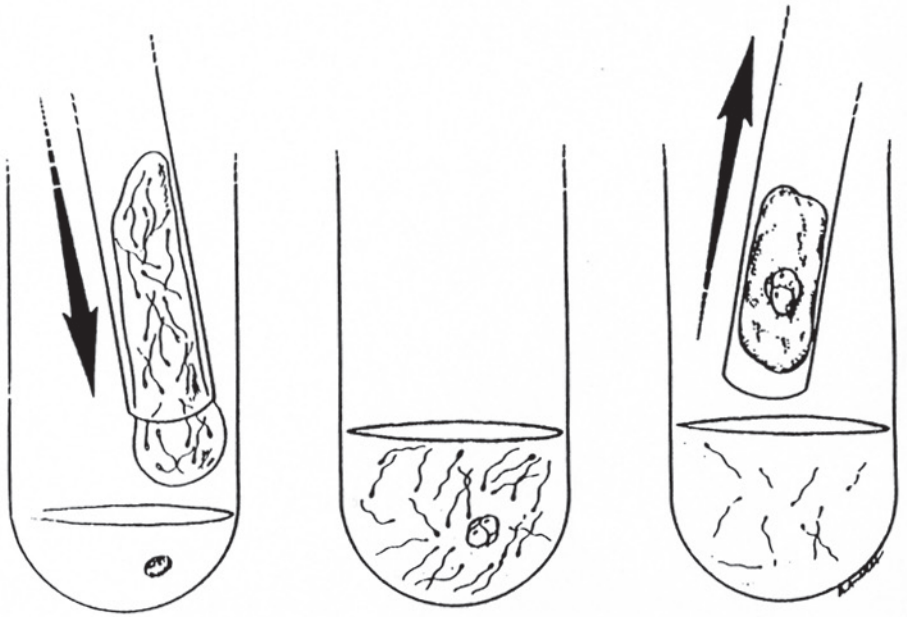

FIG. 2. IVF was performed with test tube methodology to assure the greatest approximately of eggs with the greatest number of sperm

However, subsequent to the initial enthusiasm for using IVF with aspirated sperm, it soon became apparent that epididymal sperm often did not fertilize, and the reason was not easily apparent. There appeared

to be no easily recognizable difference between the quality of epididymal sperm that fertilized, versus epididymal sperm that did not fertilize.

The purpose of this paper is to A) Review the results of the first 115 consecutive patients who underwent IVF with microsurgically aspirated sperm by me, and to discuss the studies employed to try to discern what factors might predict failure or success of fertilization with human epididymal sperm. I limit this review to congenital absence of the vas, which is the purest indication for microsurgical epididymal sperm aspiration and IVF. B) The paper then reviews the poorer results of the next 67 cases at the same lab with the same workers to demonstrate the extreme variability of results. C) Finally, suggestions are made for improving the repeatability of success with treatment of this problem using ICSI.

LEVEL OF EPIDIDYMIS FROM WHICH SPERM WAS ASPIRATED

Of the first 32 cases (21), progressive motility was found in the sperm of 20 cases (63%), and some motility was found in all cases. The percent motility was always low (from 1% to 30%), but the greatest motility was always found, ironically, in the most proximal region of the epididymis. Table 1 shows the data demonstrating in the original series of 32 cases that sperm with the greatest motility were found most proximally in the epididymis. The most distal site from which progressively motile sperm were recovered was the proximal corpus epididymis (3 of the 32 procedures, or 9 percent). In all the other cases (91%), progressively motile sperm were not recovered from the distal epididymis; it was only in the caput or rete testis fluid or from the vasa efferentia that the motility was the greatest. In the 10 procedures in which sperm were obtained exclusively from the vasa efferentia, fertilization was achieved in 5 (50%) and pregnancy in 4 (40%). All but one pregnancy occurred with sperm from either the caput epididymis or vasa efferentia rete testis fluid. Rates of fertilization and pregnancy were similar whatever the site from which motile sperm were obtained (21).

The fertilization capacity of spermatozoa which have not traversed all sections of the epididymis can ideally be studied with this human clinical model. In every animal that has been studied, spermatozoa from the caput epididymis are only capable of weak circular motion at most and are not able to fertilize (12). Spermatozoa from the corpus epididymis can occasionally fertilize but the pregnancy rate is low. However, few of these previous animal studies allowed the spermatozoa time to mature and thereby possibly develop the capacity for fertilization. In our patients,

spermatozoa were aspirated from specific regions of the obstructed epididymis and then promptly inseminated. In animal studies where the epididymis was ligated to determine if time alone could allow spermatozoa to mature, the obstructed environment was so pathological

TABLE 1

RELATION BETWEEN PREGNANCY RATE AND SITE FROM WHICH SPERM WITH PROGRESSIVE MOTILITY WERE RECOVERED

Site of Aspiration for IVF	Cycles	Cycles with Sperm with Progressive Motility	Cycles with at Least 1 Egg Fertilized	Cycles with Pregnancy
	no. (%)	*no.*	*no. (%)*	
Vasa efferentia	10 (31)	6	5 (50)	4 (40)
Proximal caput	14 (44)	9	9 (64)	3 (21)
Distal caput	5 (16)	2	2 (40)	2 (40)
Corpus	3 (9)	3	1 (33)	1 (33)

that no firm conclusion about fertility could be reached, and the initial increase in motility was followed subsequently by sperm stagnation and poor motility associated with obstruction (2,7,8,9). This phenomenon observed in animal epididymal ligation models may explain why we had to go to extreme proximal levels of the epididymis to find adequately motile sperm.

The most striking finding in the retrieval of sperm from the chronically obstructed epididymis is this inversion of the usual pattern of motility one would have expected in a non-obstructed epididymis. Sperm in the distal regions of an obstructed epididymis have the weakest motility, and sperm in the proximal regions have the strongest. One may speculate that the distal sperm are aging, and the more recently produced proximal sperm have had time to mature on their own. In fact, the problems with fertilization with these epididymal sperm may be aging as well as immaturity.

This finding should not be as surprising as it may seem. In a large series of men undergoing vasoepididymostomy for noncongenital obstructive azospermia, sampling of sperm from various levels of the epididymis has consistently demonstrated that highly progressive motile sperm can usually be found in the most proximal region of the epididymis, even though only poorly motile or nonmotile sperm are found in the distal epididymis (17). Krylov and Borovikov have made similar observations in Moscow (10).

There is strong experimental support for this concept, dating as far back as 1930 with Young's studies in guinea pigs (25). Young ligated the epididymis at various levels and examined the proximal sperm that had been trapped for varying periods of time. Contrary to expectation, the more distal sperm had the poorest motility and the proximal sperm had the best. He concluded that in the obstructed epididymis the more distal sperm are senescent, whereas the more proximal sperm have had time to mature despite not having passed through the epididymis. Our study in humans confirms Young's original studies in animals -- some elements of sperm maturation are intrinsic in nature.

Clinical studies with vasoepididymostomy in humans have demonstrated equivalent rates of pregnancy whether the sperm pass through a long or short length of the corpus epididymis (16,17). Even when sperm have only passed through a portion of the caput, there have been reasonable rates of pregnancy. In fact, two pregnancies have been decomented (with proven paternity) after end-to-end anastomosis of the vas deferens to the vasa efferentia, with normal motile sperm found in the postoperative ejaculate. It was, therefore, suggested that sperm do not require transit through the epididymis in order to fertilize an oocyte (16). Our work with IVF and epididymal spem now offer direct proof of this concept.

However, the interpretation of these findings must be severely limited. The fertilizing capacity of sperm in various regions of an obstructed epididymis is not likely to be the same as that in a nonobstructed epididymis. The pattern of motility of sperm under obstructive conditions is obviously the inverse of that previously described for nonobstructed conditions (3,11). More importantly (and to be disucssed later in this paper), in most cases, sperm fro the proximal epididymis of these obstructed patients does not fertilize at all with conventional IVF, and the reasons are not clear. It is only in a minority of patients that good fertilization is achieved. Nonetheless, it is safe to conclude (see Table 1) that so long as motile sperm are obtained (usually in proximal regions),

the level of the epididymis from which sperm are obtained has not shown a relationship to the ability of that sperm to fertilize.

SPERM ANTIBODIES

Since the level of epididymis from which sperm were retrieved had no effect on their in vitro fertilizing ability (provided we went proximally enough to obtain motile sperm), the next thought was to see whether sperm antoimmunity resulting from the obstructive process might be detrimental to the fertilizing ability of epididymal sperm in men with obstruction (14). We evaluated the incidence of antisperm antibodies in serum, epididymal fluid, and directly on epididymal sperm in 45 couples undergoing IVF with sperm aspiration for congenital absence of vas. Testing was carried out using direct immunobead binding (IBT) on epididymal sperm and indirect IBT in epididymal fluid and serum (4,15). Results were expressed as the percentage of motile sperm showing at least two beads attached to the surface, and a minimum binding of 20% was required to be positive.

Thirty-five percent of the 45 patients demonstrated antibodies on epididymal sperm, 16% in the epididymal fluid, and 29% in the serum (see Table 2) (14). Five of the patients with positive IBT for IgG had 100% binding with beads attached all over the surface of the sperm (head, midpiece, tail, and tailtip). Yet all four of them fertilized, and one resulted in pregnancy. Of the 16 patients who tested positive, 11 (69%) fertilized at least one oocyte that cleaved, and five (31%) resulted in pregnancy with documented fetal heart motion. Of the 29 patients with negative IBT, 15 (52%) achieved fertilization, and five (18%) resulted in pregnancy.

The overall fertilization rate was not greatly different in the two groups. When the men were antibody positive, 58 embryos resulted from 546 oocytes (16%), and when the men were antibody negative, 113 embryos resulted from 546 oocytes (21%), yielding no major or statistically significant difference (see Table 3).

TABLE 2

INCIDENCE F ANTISPERM ANTIBODIES ON EPIDIDYMAL SPERM, FLUID, AND SERUM

	Direct IBT on Epididymal Sperm		Indirect IB T Epididymal Fluid		Serum	
Total Positive	16	(35) *	7	(16) †	13	(29)
For IgG Only	6		1		10	
For IgG and IgA	10		6		3	
Fertilization	11	(69)	4	(57)	10	(77)
Pregnancy	5	(31)	3	(43)	5	(39)

*Values in parentheses are percents.
†In two patients specimens were not available. A total of 45 patients were examined.

TABLE 3
CORRELATION OF IMMUNOLOGICAL DETAILS OF THE POSITIVE CASES FOR ANTISPERM ANTIBODIES AND THEIR FERTILIZATION RATE

	Direct IBT		Indirect IBT					
	Epididymal Sperm		Epididymal Fluid		Serum		Fertilization	
	IgG	IgA	IgG	IgA	IgG	IgA	Rate	Pregnancy
	%							
1	100*	50†‡	40*	90†‡	100*	50‡	52 (11/21)	Yes
2	100*	90†§	100*	40†§	100*	Negative	27 (6/22)	Yes
3	100*	60†‡	Negative	Negative	50†‡	Negative	17 (2/12)	No
4	80*	80†‡	50†	35†	100*	Negative	10 (3/32)	No
5	40†	Negative	Negative	Negative	80†‡	Negative	0 (0/18)	—
6	60*	40†‡	50*	30†‡	100*	90†	13 (2/15)	Yes
7	100*	Negative	40*	Negative	100*	Negative	0 (0/25)	—
8	85†‡	Negative	Specimen not available		90†‡	Negative	4 (1/22)	No
9	80†§	30†	Negative	Negative	90†§	Negative	25 (3/12)	No
10	40†§	Negative	Negative	Negative	65†	Negative	47 (17/36)	Yes
11	80†‖	Negative	Negative	Negative	Negative	Negative	19 (3/16)	No
12	100*	80†‡	40*	40†‡	100*	Negative	15 (2/13)	No
13	70†‡	30†	Negative	Negative	95*	35†	40 (8/20)	Yes
14	50*	Negative	Negative	Negative	Negative	Negative	0 (0/50)	—
15	60†‖	60†‡‖	Specimen not available		Negative	Negative	0 (0/18)	—
16	90*	60†‖	35†	40†	80*	Negative	0 (0/35)	—

* All over.
† Ir
‡ Tailtip.
§ Midpiece.
‖ Head.

Despite the vast literature which clearly demonstrates a lower fertilization rate when greater than 70% of ejaculated sperm are coated with either IgG or IgA (based on an interference with zona binding), ther has been no prior data available with epididymal sperm. Our studies show no significant relationship between class of Ig, site of antibody location, titer of binding, and the subsequent IVF outcome using

epididymal sperm. This is similar to observations we made 15 years ago with vasectomy reversal using cruder immunologic techniques (18). Thus, we had to look for some other reason why in some cases we achieve excellent fertilization, and in others, we don't.

ULTRASTRUCTURE OF HUMAN EPIDIDYMAL SPERM

We had previously demonstrated with E/M studies that after vasectomy, sperm which are proximal to the site of occlusion undergo senescence and degeneration into what finally appears on light microscopy to be debris and dead sperm, but is in fact globules of broken-down sperm heads and tails (6). This "debris" and dead sperm is initially seen in the ejaculate after vasovasostomy. However, if there is no secondary epididymal occlusion, the ejaculate eventually has "fresh" (normal-appearing on E/M) new sperm, and subsequent to that occurrence, the wife is then likely to get pregnant. We wanted to see if the reason for the poor sperm motility in the epididymis of these patients was due to such a senescent phenomenon and if there was some reason we could discern for the failure in many cases of this sperm to fertilize.

The ultrastructural morphologic appearance of sperm from different levels of the epididymis were identical among the 13 patients with congenital absence of the vas that we studied (1). The E/M observed quality and integrity of spermatozoa aspirated from the rete testis, vasa efferentia, and caput was always markedly superior to that of sperm aspirated from the corpus and cauda epididymis. In distal epididymis, there were mostly degenerating and necrotic sperm, along with large numbers of giant sperm-engulfing macrophages. This is similar to the senescent changes we found 15 years earlier in sperm proximal to a vas occlusion. However, sperm aspirated from the vasa efferentia, rete testis, and caput epididymis were quite normal, and indistinguishable from ejaculated sperm in normospermic subjects. The one exception is that some of the proximal epididymal sperm possessed a small cytoplasmic droplet around the posterior portions of the head and the initial segment of the flagellum. With that exception, regardless of IVF success or failure, all of the proximal epididymal sperm samples were basically normal-appearing on ultrastructure.

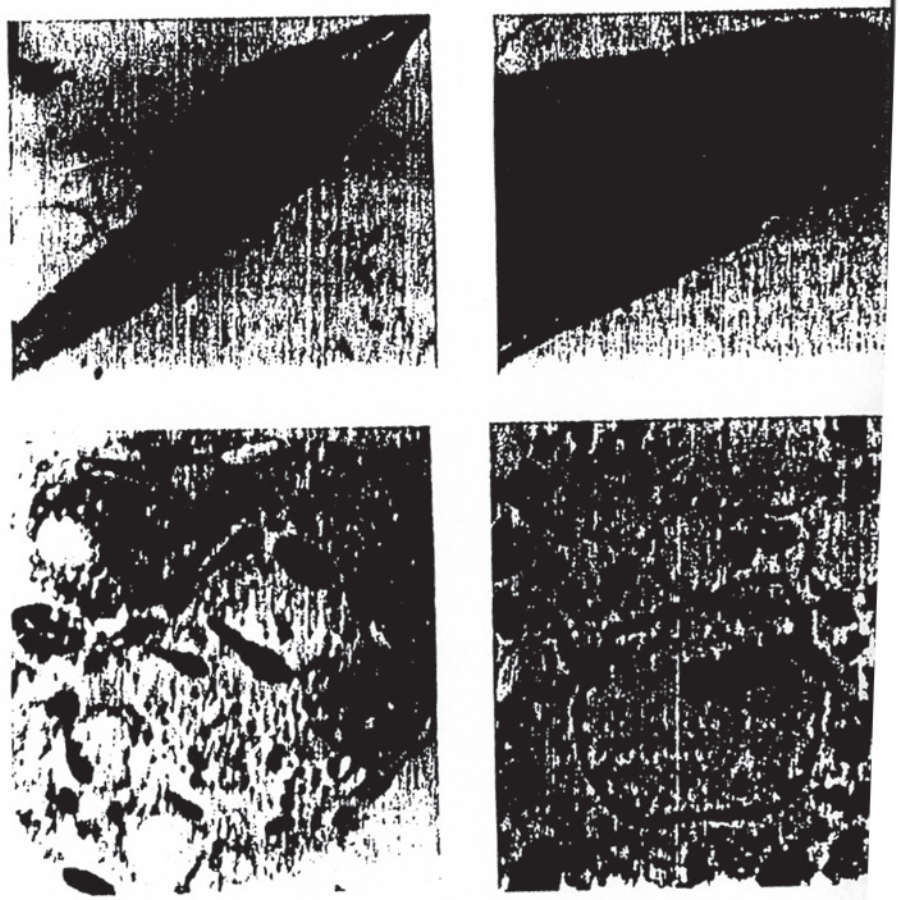

FIG. 3. (A) Ultrastructure of sperm obtained from the rete testis from a man with congenital absence of the vas deferens. Note similarities in the organization of the nucleus acrosome and fla to normal ejaculated sperm. (B) Ultrastructure of sperm obtai from the vasa efferentia from a man with congenital absence o vas deferens. Note the similarities in the organization of the nucleus acrosome and flagella to normal ejaculated sperm. (C Ultrastructure of cells obtained from the corpus epididymis ir man with congenital absence of the vas deferens. Note the ma phages with their cytoplasm occupied by very large amounts phagocytized sperm remnants in different degrees of degrada and digestion. (D) Ultrastructure of macrophages obtained fr the cauda epididymis from a man with congenital absence of vas deferens. Note the presence of prominent whorls of membranes and numberous lipid droplets indicating advanc stages of sperm degradation.

SPERM MOTILITY

The most baffling observation throughout all of our first 115 cases of epididymal sperm aspiration and IVF is the apparent inability to predict fertilization from the sperm quality or percent of motility. We used to be very excited for every patient at the time of surgery when we found sperm of exceptionally good motility. Then, often we became dismal when there was no fertilization with as many as 40 or 50 eggs from the wife. Yet in other cases with apparently poor motility, we achieved good fertilization rates and many embryos.

In our original papers (20,21), we simply made the observation that quality of motility was not a good predictor for fertilization, with the exception that with less than 10% motility, there were exceptionally few fertilizations or pregnancy. Beyond that, there were no correlations (see Table 4).

We attempted to refine that observation with computer assisted sperm movement analysis (5). This provided a potentially useful retrospective theory, but it is mathematically convoluted, and the explanation which follows does not change the fact that routine observation of the motility in epididymal sperm does not reveal anything obvious. Yet our mathematical retrospective analysis suggested that there are many different sperm subpopulations within any sample. It is only a small fraction of sperm, or a certain subpopulation in a sample, that may actually be able to fertilize. All epididymal aspirates had four basic subpopulations of sperm, but those most likely to fertilize had a fifth subpopulation.

Fertilization was more likely to occur when the sperm populations that were aspirated, before the wash preparation, contained a higher proportion of motile cells that were capable of capacitation in vitro, as suggested by specific changes in their movement characteristics. The most relevant factor characterizing the subpopulation of sperm that fertilized was curvilinear velocity of post-wash sperm and the change in lateral head displacement after sperm preparation. Percent motility seemed to matter only in respect to increasing the likelihood of having a larger number of motile sperm subpopulations.

TABLE 4 SUMMARY OF FERTILIZATION AND PREGNANCY RESULTS* (FIRST 32 REPORTED CASES)

Patient Cycle No.	Age of Wife	No. of Mature Oocytes	No. of Embryos	Total Sperm Retrieved	Motility	Progression	Embryo Transfer	Outcome
	yr			$\times 10^6$	%			
1	37	28	6	—	—	1-2	Tubal	Pregnant, miscarried
2	26	24	15	—	—	1-2	Tubal	Pregnant, girl born
3	34	7	2	—	5	1	Uterine	—
4	33	3	2	Very low	10	1	Tubal	—
5	22	20	0	—	0	0	—	—
6	29	6	1	1.68	10	1	Uterine	—
7	24	8	0	26.0	10	1-2	—	—
8	31	3	0	9.4	20	1	—	—
9	36	4	1	—	—	—	Uterine	—
10	35	13	2	16.4	20	1	Tubal	—
11	30	9	2	—	—	—	Tubal	—
12	26	11	0	—	20	1	—	—
13†	37	20	9	—	2	1-2	Tubal	Pregnant, girl born
14	38	13	0	Very low	5	1	—	—
15	32	11	0	—	10	1-2	—	—
16	26	2	1	—	1	1	Uterine	—
17	35	2	0	—	5	1	—	—
				Pre Post	Pre Post	Pre Post		
18	26	14	4	— 10.2	10 10	1-2 3-4	Tubal	—
19	25	8	0	59.0 —	1 1	1 1	—	—
20	28	12	5	24.8 5.3	15 20	1 2	Tubal	Pregnant, miscarried
21	33	11	2	37.2 3.4	10 10	1-2 1-2	Tubal	Pregnant, girl born
22	35	10	3	— 0.68	30 60	1-2 3	Tubal	—
23‡	22	14	0	13.7 6.9	1 1	1 1	—	—
24	26	6	0	— 13.0	5 20	1 2	—	—
25	31	10	7	47.4 9.7	30 60	1-2 3-4	Tubal	Pregnant, boy born
26§	35	25	3	37.8 10.9	10 30	1-2 2-3	Tubal	Pregnant, boy born
27¶	26	10	8	119.0 34.0	20 30	1-2 1-2	Tubal	Pregnant, twin girls b
28	37	10	5	101.2 10.2	10 20	2 2	Tubal	Pregnant, girl born
29	40	11	9	34.8 3.8	30 60	2-3 3-4	Tubal	Pregnant, miscarrie
30	34	11	5	— 67.0	1 10	1 1-2	Tubal	—
31	24	12	0	31.0 11.5	10 30	1 1-2	—	—
32	40	4	1	— 1.1	1 10	1 1-2	Uterine	—

* Pre and post denote number of sperm, percent motility, and progression before and after preparation with Percoll. Before cycle 18, such data were not available.

† Repeat of cycle 1. ‡ Repeat of cycle 5. § Repeat of cycle 10. ¶ Repeat of cycle 12.

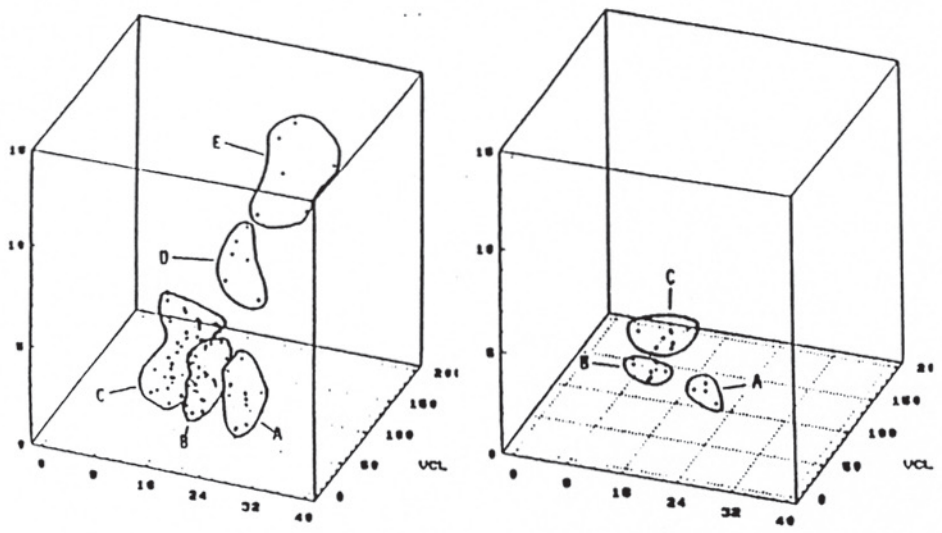

FIG. 4. Three-dimensional plots illustrating subpopulations of epididymal spermatozoa in the inseminates of two cases of IVF. One has five subpopulations of sperm; the other has three subpopulations.

This was only a retrospective, highly convoluted study, which will be valid only if it can prospectively predict fertilization in the future. What is clear, however, is that there is nothing obvious about the motility of epididymal sperm having any effect on fertilization. This will be discussed further in relation to Tables 5 and 6 at the end of the paper.

OVERALL FERTILIZATION AND PREGNANCY RATES IN FIRST 11 CONVENTIONAL IVF/MESA CYCLES: UNPREDICTABILITY OF FERTILIZATION

It became apparent after doing a fairly large number of cases that fertilization and pregnancy rates with epididymal sperm using conventional IVF are completely unpredictable. Tables 7 and 8 summar the results of the first five series, totalling 100 cases. Our protocol was t accumulate a group of couples with congenital absence of the vas, and then perform sperm aspiration with IVF on them every six months over one-to-two week period. There was no selection involved in any of the series. We just took the patients as they came. In some groups our pregnancy rate was as high as 31%, and in others there were no pregnancies at all. The overall pregnancy rate for the first 100 cycles wa very respectable 22%, with 16% term live baby deliveries, but that hides the more dismal results which were endured in some other rather large groups of patients.

Clearly the patients divide into two obviously different populations (See Tables 9, 10, and 11). In 65% of the couples, the husband's sperm either failed to fertilize, or produced no more than a single embryo. No of these couples achieved a pregnancy. When 2 to 4 embryos were obtained, 26% got pregnant. When more than 4 embryos were obtained over half the couples achieved pregnancy in that cycle. There appears t be one population of men whose epididymal sperm simply won't fertili or fertilizes very poorly, and this population represents the clear majorit (65%). The other population (35%) fertilize, but only 10-20% fertilize ve well, and the pregnancy rate per transfer of such embryos is extraordinarily high. Eighteen percent of patients fertilize more than 7 eggs with the husband's epididymal sperm, and these couples have a pregnancy rate per transfer of over 55%.

Interestingly, 60% of the embryos were produced by 10% of the couples, and 77% of the embryos by 18% of the couples. Thus, only a minority of couples with congenital absence of the vas undergoing sper aspiration with conventional IVF have a chance of getting pregnant. Furthermore, it is impossible to predict by looking at sperm quality, motility, antibody formation, or site of epididymal aspiration, which couples will, and which couples won't, fertilize well.

TABLE 5

STANDARD GRADE OF MOTILITY PATTERNS IN RELATION TO FERTILIZATION OR LACK OF FERTILIZATION WITH EPIDIDYMAL SPERM

# Embryos Per Patient	Grade Motility		
	1	2	3
0-1	46 (63%)	25 (35%)	1 (2%)
2-4	12 (60%)	7 (35%)	1 (5%)
5-7	4 (40%)	6 (60%)	0
8 or more	2 (29%)	4 (57%)	1 (14%)

TABLE 6

STANDARD % MOTILITY PATTERNS IN RELATION TO FERTILIZATION OR LACK OF FERTILIZATION WITH EPIDIDYMAL SPERM

# Embryos Per Patient	% of Motile Sperm			
	0-4%	5-10%	11-20%	Over 20%
0-1	20 (28%)	20 (28%)	13 (18%)	19 (26%)
2-4	5 (25%)	5 (25%)	5 (25%)	5 (25%)
5-7	2 (20%)	4 (40%)	2 (20%)	2 (20%)
8 or more	0	2 (29%)	0	5 (71%)

TABLE 7

FIRST 100 CASES IN VITRO FERTILIZATION FOR CONGENITAL ABSENCE OF VAS: PREGNANCY RATES

Series #	# Sperm Aspiration Cycles	# Pregnant (Term Pregnancy)	Pregnancy Rate Per Cycle
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%)

* Ongoing pregnancies not yet delivered.

TABLE 8

FIRST 100 CASES IN VITRO FERTILIZTION FOR CONGENITAL ABSENCE OF VAS: FERTILIZATION RATES

Series #	# Sperm Aspiration Cycles	# Cycles Providing at Least One Embryo	Total # Mature Eggs	Total # Embryos	Fertilization Rate Per Mature Egg
1	32	21 (66%)	352	93	26%
2	16	9 (56%)	198	53	27%
3	21	13 (62%)	326	60	18%
4	13	6 (46%)	170	11	6%
5	18	10 (56%)	293	107	37%

TABLE 9

ANALYSIS OF FIRST 115 CASES OF EPIDIDYMAL SPERM ASPIRATION AND IVF FOR PATIENTS WITH CONGENITAL ABSENCE OF THE VAS (CAV)

Fertilized Per Patient	# Patients	Total # Eggs	Total # Embryos#	Fert Rate	Percent of Patients	Live Birth Preg Rate
0-1	75	1074	15	1.4%	65.2%	0
2-4	19	336	52	15.4%	16.5%	5 (26%)
5-7	9	137	50	36.4%	7.8%	5 (55%)
>7	12	296	173	58.4%	10.4%	8 (67%)
Totals	115	1843	290	15.7%	100%	18

Silber, Patrizio, Ord, Asch (1992)

TABLE 10

ANALYSIS OF FIRST 115 CASES OF EPIDIDYMAL SPERM ASPIRATION AND IVF FOR PATIENTS WITH CONGENITAL ABSENCE OF THE VAS (CAV)

Patient Group According to # Eggs Fertilized	Fertilization Rate in These Patients	% of Patients	% of Embryos	Pregnancy Rate Per Patient
0-1	1.4%	65.2%	5.1%	0
2-4	15.4%	16.5%	17.9%	26%
5-7	36.4%	7.8%	17.4%	55%
>7	58.4%	10.4%	59.6%	67%
Total 115	15.7%	100%	100%	16%

TABLE 11

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2-4	16.5%	26%
5-7	7.8%	55%
>7	10.4%	67%
Total 115	100%	16%

Methodology for MESA with ICSI

Because of the relatively poor results using conventional IVF with epididymal sperm, our center in St. Louis has now collaborated with Dr. Andre Van Steirteghem and Dr. Paul 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 (See Figures 5, 6, and 7).

The technique of sperm and oocyte preparation, microinjection, and culture were the same as recently described by Van Steirteghem for severe male factor cases (23,24). 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

A group of 17 patients (15 with CAV) underwent MESA using intracytoplasmic injection (ICSI) instead of conventional IVF. Nine 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 found that poor fertilization with conventional MESA in any given patient results also in poor fertilization in all subsequent conventional MESA /IVF cycles.

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 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, myelf, and co-workers. The patient population in 15 cases came from our usual base in the United States, and in two cases were generated from the Belgium area. In short, this group of 17 was intentionally selected as a fairly dismal group of candidtes for MESA, selected for study out of our usual population base because of their poor prognosis.

Table 12 summarizes the deteriorating results in the last four series of MESA/Conventional IVF cycles involving 67 cases, a regression toward the mean which agrees with the low results of many other groups. Most programs performing MESA and IVF report no greater than a 5-10% pregnancy rate. The term pregnancy rate of all 167 of our conventional MESA/IVF cycles was only 11%.

TABLE 12

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%

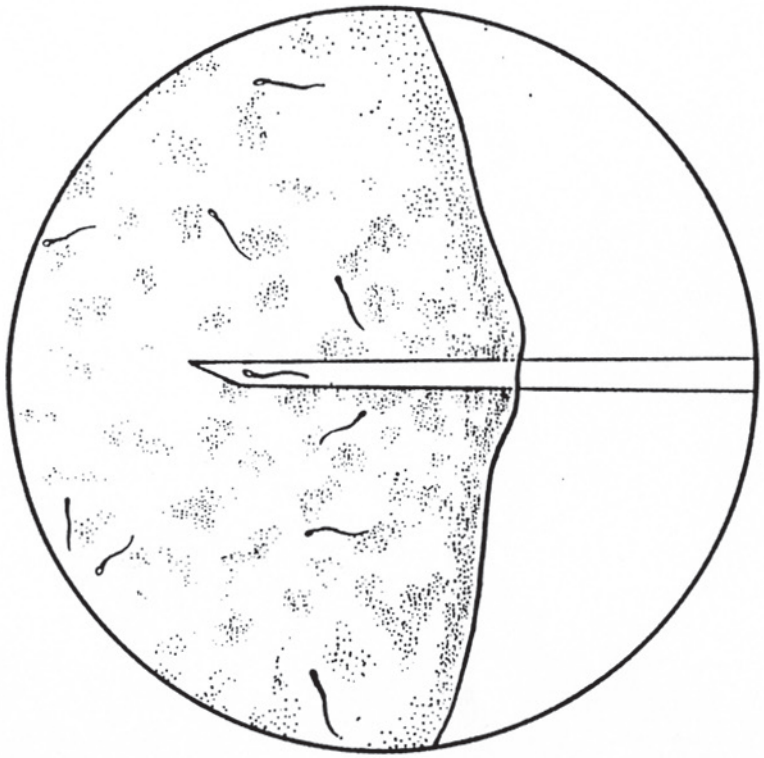

FIG. 5. Pick-up of weakly motile sperm from petri dish microdroplet.

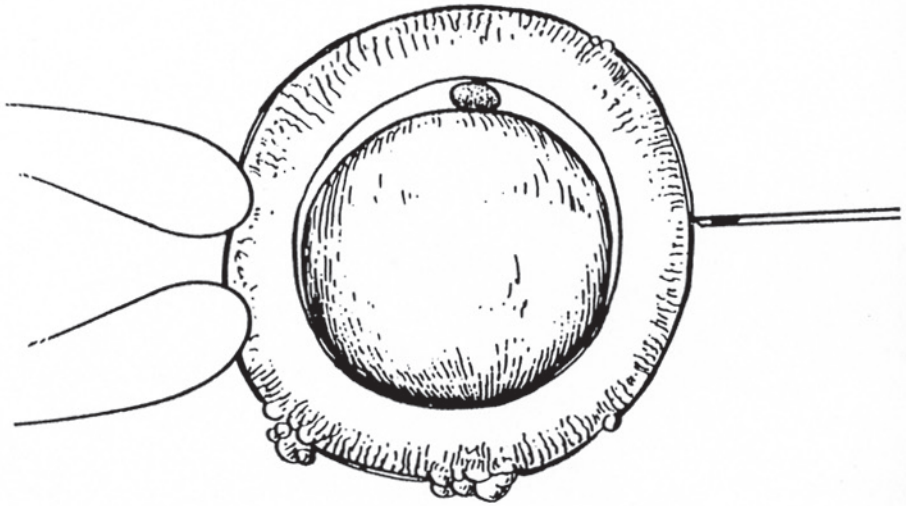

FIG. 6. Preparing to inject sperm into egg.

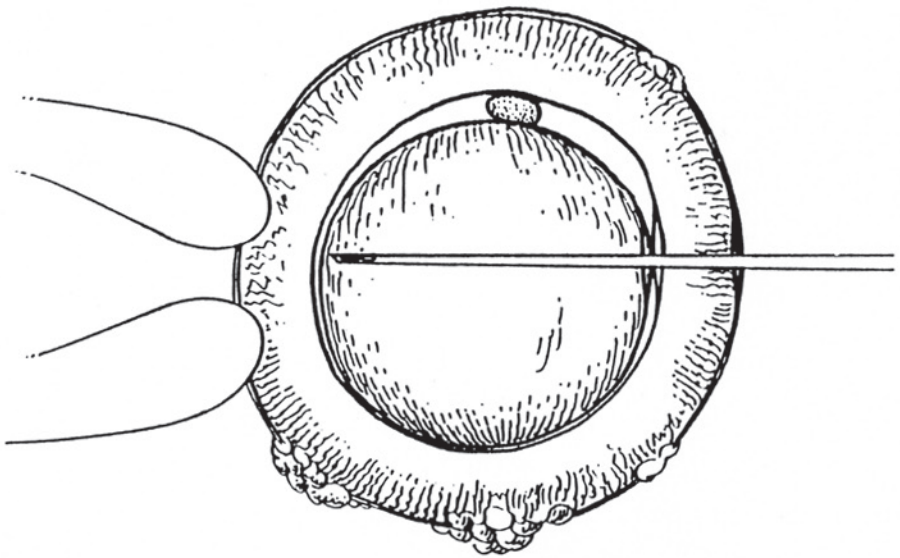

FIG. 7. Direct intracytoplasmic injection of sperm into egg (ICSI).

Results with MESA & ICSI

Table 13 summarizes the results of fertilization and pregnancy rates i the most recent series of 17 MESA cases utilizing intracytoplasmic injection (ICSI) as compared to the previous 67 cases of conventional IVF Over 80% of patients utilizing ICSI had good fertilization rates with over two to nine good quality embryos resulting. Eighty-eight percent went t embryo transfer. Forty-seven percent became pregnant, and 30% have healthy ongoing pregnancies. Three of the patients who produced three nine embryos required testicular biopsy to obtain sperm because of complete absence of the epididymis (Table 14). These results represent a dramatic change from previous experience with such patients.

TABLE 13
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	197	80	41%	14/17 (82%)	47% (30%)
IVF-MESA					
67	1,427	98	6.9%	13/67 (19%)	9% (4.5%)

TABLE 14
Fertilization of Human Oocytes by Testicular Tissue Spermatozoa (TESE) Using ICSI in 3 Patients Who Had No Epididymis

Patient	# of MII Oocytes	# Intact After ICSI	# 2PN Fertilization		# of Embryos Obtained	
1	9	9	4	(44%)	3	(33%)
2	14	14	6	(43%)	5	(36%)
3	22	21	10	(48%)	9	(43%)
Totals	45	44	20	(45%)	17	(38%)

Table 15 gives the details of all 17 patients in this first MESA/ICSI series. Note that 47% of these patients became pregnant and 30% held their pregnancy. The overall fertilization rate was 41% despite previous failure to fertilize. The cystic fibrosis genotype of the husband had no relationship to the results.

TABLE 15 Results of All 17 Patients In First MESA/ICSI Series **

Patient	Genotype If CAV	Number Mature Eggs (MII)	Number Fertilized (2PN)	Number Embryos Transferred	Fertilization Rate (%)	Age of Wife	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	ΔF508/N	18	6	4	(33%)	28	Yes
7	W1281X/N	16	5	3	(31%)	36	Yes
8	ΔF508/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		22	10	3	(45%)		No
17		8	5	2	(63%)		Yes
Totals		197	80	50	(41%)		8/17
				47%	Pregnant		

Table 16 details the results in the specific group of 9 patients who were previous IVF/MESA failures on multiple attempts. In conventional IVF these patients had a 3% fertilization rate with no pregnancy, but with ICSI, the fertilization rate was 39%, and 33% got pregnant. Again, there was no correlation to the cystic fibrosis genotype. The embryo transfer rate was a remarkable 89%.

TABLE 16 Results of MESA-ICSI In Nine Prior Recurrent MESA/IVF Failures

Patient	Cystic Fibrosis Wife's Genotype	Age	Number Previous Unsuccessful MESA/IVF Cycles	Pregnant	Total Eggs Retrieved In All Previous MESA/IVF Cycles	Number Fertilized In Previous Previous MESA/IVF Cycles	Percentage	Number Eggs Retrieved In First MESA/ ICSI Cycle	Number Fertilized In MESA/ ICSI Cycle	Fert Rate
1	ΔF-508/N		5		78	0	(0%)	9	3	(33%)
2	N/N		5		127	5	(3.9%)	14	5	(36%)
3	ΔF-508/N		2		69	0	(0%)	22	11	(27%)
4	ΔF-508/N		2		23	5	(21%)	10	5	(50%)
5	R117H/R117H		2		68	0	(0%)	14	6	(43%)
6	ΔF-508/N		2		51	4	(7.8%)	18	6	(33%)
7	W1282X/N		1		24	0	(0%)	16	5	(31%)
8	ΔF-508/N		2		23	0	(0%)	3	2	(66%)
9	N/N		2		8	0	(0%)	3	0	(0%)
Total			23		471	14	(3%)	109	43	(39%)

1) TRANSFER RATE: 89%

2) ALL CASES WERE CONGENITAL ABSENCE OF VAS AND EPIDIDYMIS (CBAVD).

3) 2 CASES WERE TOTAL ABSENCE OF EPIDIDYMIS ((BOTH OF THESE CASES FERTILIZED WITH TESTICULAR TISSUE SPERM: 33% & 36%.

Conclusion

It is clear from our published results that distal epididymal sperm in obstructed males are either non-motile or totally degenerate, due to senesence (1,14,21). 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 intracytoplasmic 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. Computerized motion analysis, although not easily practicable, suggests that there may be only a very tiny subpopulation 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. 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, 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.

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