

MICROSURGERY OF THE MALE GENITALIA FOR INFERTILITY

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MICROSURGERY FOR OBSTRUCTIVE AZOOSPERMIA

An understanding of how to obtain high success rates with vasectomy reversal will eventually lead to more successful vasoepididymostomy results in postinflammatory obstruction and finally to success with sperm aspiration and in vitro fertilization (IVF) for congenital absence of the vas.

Vasectomy Reversal

Vasectomy is the most popular method of birth control in the world.¹⁷ For years the pregnancy rate after surgical reanastomosis of the vas was low. Many explanations were offered for the relatively poor success in reversing vasectomy.^{18,21,27} With the advent of microsurgical techniques, pregnancy rates improved considerably, suggesting that purely micromechanical factors were responsible.^{30,33,34} Yet there still were many cases of technically perfect vasovasostomies followed by complete azoospermia or oligoasthenospermia with no pregnancy. The pressure increase after vasectomy led to secondary epididymal obstruction, which caused the failure of otherwise successful vasovasostomies. Thus vasoepididymostomy is required in most cases of vasectomy reversal in order to obtain a high success rate.

After questioning any major correlation between sperm antibodies or testicular damage and subsequent fertility after vasovasostomy,* we established that the deleterious effect of pressure increase subsequent to vasectomy is not in the testis but rather on epididymal dilation, perforation, and sperm inspissation and blowouts in the epididymis. This causes secondary epididymal obstruction, which is the major problem in returning fertility to vasectomized men.^{30,34,35,41,45}

Because of the secondary epididymal obstruction, the testicular end of the vas should not be sealed when performing vasectomy. This lessens the pressure buildup and thereby increases the ease of reversibility.^{1,28,31}

Ten years ago we studied a group of patients^{30,40} undergoing vasovasostomy who suffered no secondary epididymal damage (as evidenced by sperm being present in the vas fluid at the time of vasovasostomy) to find the fertility rate. In such a favorable group (sperm in vas fluid and no epididymal damage), 98% had sperm in the ejaculate and 88% got their wives pregnant. None of the azoospermic patients got their

wives pregnant. This compares to Vessey's expected pregnancy rate of 96% for previously fertile couples discontinuing contraception.⁵⁰

The frequency distribution of semen parameters postoperatively in men who did and did not get their wives pregnant is summarized in Tables 59-1 and 59-2. There was remarkably little difference in the pregnancy rate among men with low or high sperm counts. Similar findings were seen with sperm motility that was greater than 20%. However, the pregnancy rate was somewhat lower with motility of less than 20%. Above those lower limits, the pregnancy rate was not seriously affected by low semen parameters. These postoperative semen parameters in patent cases were not very different from previously reported prevasectomy semen parameters.⁵¹

As summarized in Table 59-3, a left-sided varicocele was clinically apparent in 42 of the 282 patients (14.8%), these varicoceles were not operated on. The pregnancy rate was not significantly different in patients with a varicocele as opposed to patients without a varicocele. Table 59-4 summarizes the relationship between preoperative serum antisperm antibody titers and the pregnancy rate after vasovasostomy. The presence of high serum immobilizing titers or agglutinating titers also had no influence on the pregnancy rate. These data verify that sperm antibodies and varicocele and even sperm count have nothing to do with male infertility. All men have varying percentages of potentially fertile sperm in their ejaculate (probably from 0% to 20% maximum); this is genetically determined, and the only advisable treatment for poor semen quality is IVF.

Reason for High Pregnancy Rates in Patients with No Secondary Epididymal Blockage

The high pregnancy rate in patients with no secondary epididymal blockage requires some explanation. Our study suggests that the pregnancy rate in patients who have patency accurately reestablished without epididymal damage is eventually not significantly less than a normal population of couples. Vessey demonstrated that among couples with previous fertility, 96.5% conceive within 4 years of discontinuing contraception.⁵⁰ Of our couples who had patent results after vasovasostomy and who had no evidence of epididymal pressure damage, 88% conceived with long-term (10 years) fol-

*References 2, 6, 9, 14, 16, 19, 23, 40, 46, 47.

Table 59-1 Pregnancy Rate According to Distribution of Motile Sperm Count in Men With Sperm Patency Following Vasovasostomy (10-Year Follow-up)

Total Motile Sperm Count (Per Ejaculate)	Total Patients	Pregnant	Not Pregnant
>0-10,000,000	32 (12%)	25 (78%)	7
10-20,000,000	31 (12%)	27 (87%)	4
20-40,000,000	32 (12%)	30 (93%)	2
40-80,000,000	79 (31%)	68 (86%)	11
80,000,000	84 (33%)	78 (92%)	6
TOTALS	258	228	30

Table 59-2 Pregnancy Rate According to Percentage of Sperm Motility in Men With Sperm Patency Following Vasovasostomy (10-Year Follow-up)

Motility	Total Patients	Pregnant	Not Pregnant
0-20	24	18 (75%)	6
20-40	70	66 (94%)	4
40-60	82	71 (86%)	11
60-80	62	55 (88%)	7
80	20	18 (90%)	2
TOTALS	258	228	30

low-up. However, patients with secondary epididymal blockage require a completely different approach.

The poor success of vasovasostomy is directly related to the absence of sperm in the vas fluid at the time the procedure is performed; this is because of the interruption of epididymal patency by pressure-induced sperm extravasation and by inspissation.³⁵ When there is no sperm in the vas fluid, vasoepididymostomy proximal to the site of the epididymal blockage is required.^{32,36}

Vasoepididymostomy

When vasectomy has produced secondary epididymal blockage or in cases of postinflammatory obstructive azoospermia, precise microsurgical tubule-to-tubule vasoepididymal anastomosis is required. Equally important is a practical understanding of epididymal physiology.^{32-34,36,37}

In every animal studied, spermatozoa from the caput epididymidis are capable only of weak circular motion at best and are not able to fertilize.²² In previous studies, spermatozoa from the corpus epididymidis could occasionally fertilize, but the pregnancy rate was still low. Spermatozoa were simply aspirated from specific regions of the epididymis and then promptly inseminated.^{12,13,26}

As far back as 1931, however, Young's experiments with ligation at various levels of the epididymis in guinea pigs indicated "that the time consumed by spermatozoa in passing through the epididymis is necessary for a completion of their development; that the changes undergone during this period

Table 59-3 Lack of Effect of Varicocele (Not Operated on) on Pregnancy Rate Following Vasovasostomy

	Number of Patients	Patients With Varicocele	Patients Without Varicocele
Pregnant	228 (80.9%)	33 (78.5%)	195 (81.2%)
Not pregnant	54 (19.1%)	9 (21.4%)	45 (18.8%)
TOTALS	282	42	240

represent a continuation of changes which start while the spermatozoa are still attached to the germinal epithelium, and are not conditioned by some specific epididymal secretion."²⁵ In fact, he observed the same "inversion" of regions of sperm motility and nonmotility in the obstructed epididymis that was noted in clinical obstructive azoospermia. The more distal regions have the poorest motility and the more proximal regions the best. Young concluded that in an obstructed epididymis the more distal sperm are senescent, whereas the more proximal sperm have had time to mature despite having not traversed the epididymis. Our clinical experience with specific tubule vasoepididymostomy supports Young's original thesis.³²

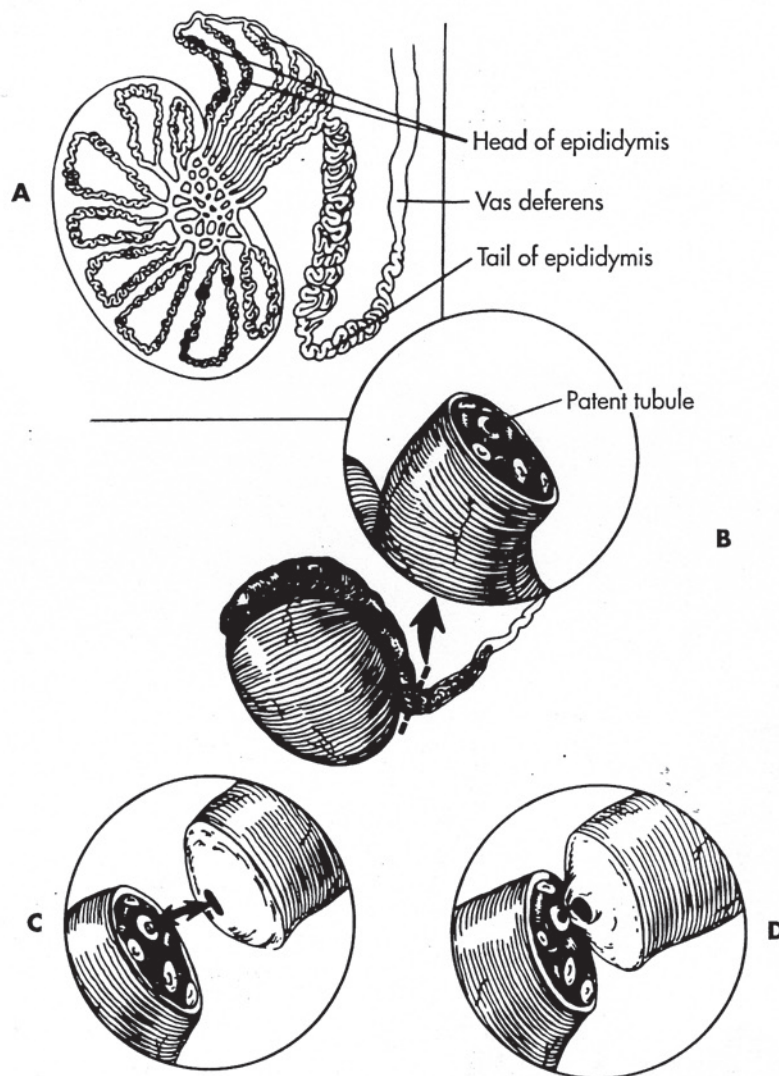
All vasoepididymostomies are performed with the "specific tubule" technique already described, which involves either an end-to-end or end-to-side anastomosis of the inner lumen of the vas to the epididymal tubule mucosa to mucosa in a leakproof fashion.^{30,32,37} Because of the high rate of technical failure with older surgical methodology, reliable data from the past on the fertility of spermatozoa from the epididymis have been difficult to obtain.

The anastomosis of the vas to the epididymis is performed at the transition point from where there is an abundant amount of spermatozoa in the fluid coming from the epididymal tubule (Figs. 59-1 and 59-2). Usually five to six 10-0 nylon interrupted sutures complete the leakproof end-to-end anastomosis; then the outer muscularis of the vas is separately sutured to the outer epididymal tunic with 9-0 nylon interrupted sutures.

About 72% of the cases of epididymis anastomosis have resulted in eventual pregnancy.⁴⁰ The younger the wife, the higher the pregnancy rate. The pregnancy rate was related to

Table 59-4 Relationship of Serum Sperm Antibody Titers to Pregnancy Rate After Vasovasostomy

	Total Studied	Immobilizing Titer (Isojima)		Agglutinating Titer (Kibrick)	
		>2	>10	>0	>20
Husband not azoospermic					
Wife pregnant	75	29 (39%)	18 (24%)	42 (56%)	30 (40%)
Wife not pregnant	11	4 (36%)	2 (16%)	6 (54%)	6 (54%)
Husband azoospermic	12	5 (42%)	3 (25%)	7 (58%)	5 (42%)
Entire group studied	98	38 (39%)	23 (24%)	56 (57%)	41 (42%)

**Fig. 59-1** A to D, Specific tubule end-to-end anastomosis of the vas lumen to the epididymis proximal to site of obstruction.

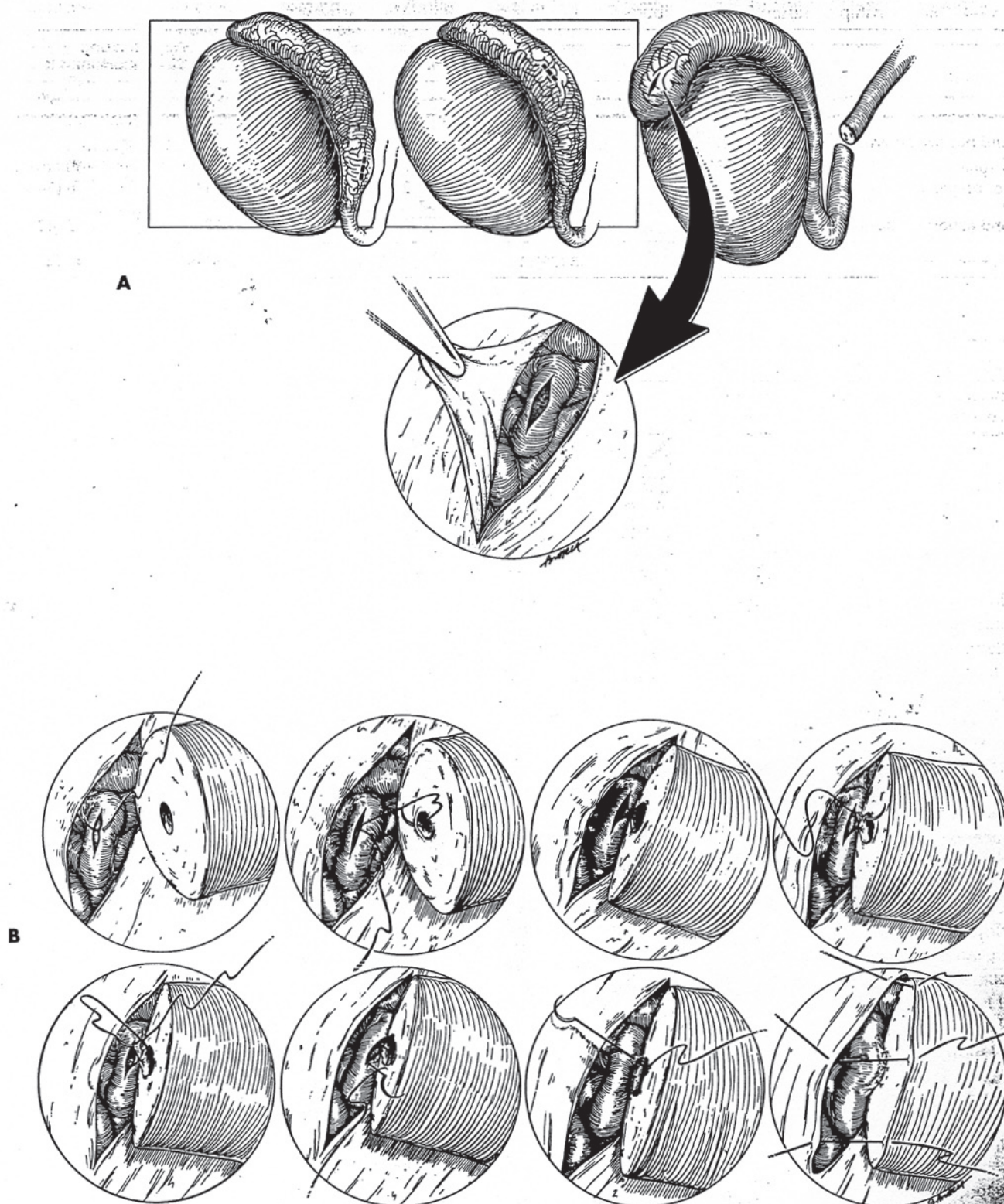


Fig. 59-2 **A**, Small openings are made in the epididymal tunic beginning distally and moving proximally. After a longitudinal slit is made in the epididymal tubule with the microscissors, the distal-most level at which motile sperm are found is used for the anastomosis. **B**, The end-to-side specific tubule anastomosis of the vas lumen to the epididymal tubule requires first a posterior row of three 10-0 nylon interrupted sutures followed by an anterior row of three 10-0 nylon interrupted sutures. The muscularis of the vas is then sutured to the outer epididymal tunic with 9-0 nylon interrupted sutures.

the motility but not to the numerical sperm count. The fact that a 43% pregnancy rate occurred in patent cases to the caput indicates that transit beyond the head of the epididymis is not an absolute requirement for spermatozoa to attain fertilizing capacity.

Recent clinical cases have demonstrated that it is possible in some circumstances for spermatozoa that have never transited any length of epididymis to fertilize the human egg. In two cases reported concerning anastomosis of vasa efferentia to vas deferens, the postoperative ejaculate contained normally motile sperm, and the wives became pregnant.³⁸ In addition, pregnancy from aspiration of epididymal sperm combined with IVF and zygote intrafallopian transfer (ZIFT) in cases of unrepairable obstruction give further evidence that transit through the epididymis is not a mandatory requirement for fertilization.⁴⁴

Newer studies of epididymal sperm transport in the human indicate that the human epididymis is not a storage area, and spermatozoa transit the entire human epididymis in 2 days, not the 11 days previously thought. Thus the epididymis may not be as essential to spermatozoan development and fertility in the human as it appears to be in most animals.

Finally, as long as adequate patency is achieved, IVF with or without micromanipulation can now obtain pregnancy in virtually all such cases even if the wife does not spontaneously become pregnant after a reasonable waiting period.

MICROSURGICAL EPIDIDYMAL SPERM ASPIRATION (MESA)

In men with uncorrectable obstructive azoospermia, the only hope for fathering a child is epididymal sperm aspiration combined with IVF. We have employed this technique in 167 cases using conventional IVF, and by evaluating results, have learned a great deal about maturation and fertilizing ability of human epididymal sperm. In addition, we have recently been the first to use intracytoplasmic sperm injection (ICSI) into the egg rather than conventional IVF in 48 recent patients. The results demonstrate that virtually *all* such patients can impregnate their wives.

The motility of sperm retrieved from the obstructed epididymis is better at more proximal levels because of senescence of the distally stored sperm. Although the conventional IVF fertilization rate of human epididymal sperm cannot be predicted based on either motility, antibody, or electron microscopy (E/M) studies or morphology, it is predictable and repeatable in multiple cycles of IVF in any given subject. About 10% of patients 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 cannot father a child without micromanipulation. The reason may be either poorly defined sperm maturation defects or senescent and pathologic changes caused by the obstruction.

ICSI has been successful in cases of extreme oligoasthenospermia, achieving pregnancies via IVF with the lowest imaginable sperm counts.^{24,47} The most severe case of male factor infertility is perhaps absence of the epididymis whereby the only sperm available are from macerated testic-

ular biopsy specimens. In such cases, all that can be seen in testicular tissue are free-floating Sertoli cells with many spermatids attached and occasional spermatozoa per high power field that have only the barest, slightly twitching motion. Centrifugation of the supernatant at 1800G usually yields about 10 to 25 of these poor sperm for ICSI.

Even in such "hopeless" cases, direct ICSI of an individual sperm into a mature oocyte from the wife achieved fertilization and normal embryos in 75% of cases, with an overall fertilization rate of 45%, with 85% going on to normally cleaving embryos. With epididymal sperm, *all* patients' wives had normal fertilization. Although complex mechanisms are required for conventional fertilization of human oocytes, whether in vivo or in vitro, none 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.

In 1988 and 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.^{42,43} (Fig. 59-3 and 59-4). This work established a treatment for male infertility caused by a previously untreatable condition and demonstrated that in the human, sperm passage through the epididymis was not always a mandatory requirement for fertilization. This conclusion verified what had been speculated on in previous reports on vasoepididymostomy.^{38,41}

However, subsequent to the initial enthusiasm for using IVF with aspirated sperm, it soon became apparent that epididymal sperm often did *not* fertilize. Furthermore, there appeared to be no easily recognizable difference between the quality of epididymal sperm that did and did not fertilize.

To relate the historic progression leading to our present breakthrough we will

1. Review the results of my first 115 consecutive patients who underwent IVF with micro surgically aspirated sperm and 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 MESA and IVF.)
2. Review the poorer results of the next 67 cases at the same laboratory with the same workers to demonstrate the extreme variability of results.
3. Demonstrate the repeatability of success with treatment of this problem using ICSI into the wife's eggs.

Level of Epididymis from Which Sperm Was Aspirated

Of the first 32 cases, progressive motility was found in the sperm of 20 cases (63%), and some motility was found in all cases.⁴³ The percent motility was always low (1% to 30%), but the greatest motility was always found, ironically, in the most proximal region of the epididymis. Table 59-5 shows data demonstrating in the original series of 32 cases that sperm with the greatest motility were found most proximally

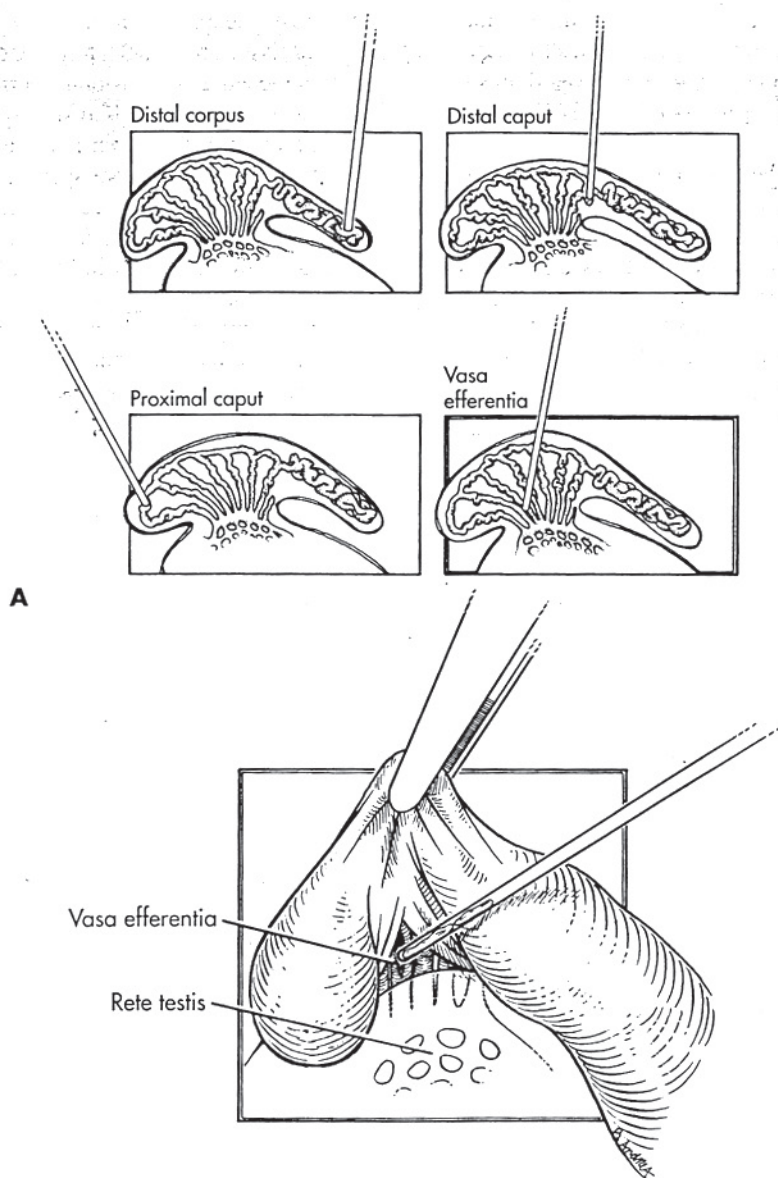


Fig. 59-3 A, Sperm were microsurgically aspirated from distal and proximal regions of the epididymis. The samples with the best quality motility were used for IVF. *Continued*

in the epididymis. The most distal site from which progressively motile sperm were recovered was the proximal corpus epididymidis (3 of the 32 procedures [9%]). 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. Of 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 epididymidis or vasa efferentia rete testis fluid. Rates of fertilization and pregnancy were similar whatever the site from which motile sperm were obtained.⁴³

The most striking finding in the retrieval of sperm from the chronically obstructed epididymis is the inversion of the

usual pattern of motility one would have expected in a nonobstructed 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.

The fertilization capacity of spermatozoa that 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 epididymidis are capable of weak circular motion at best and are not able to fertilize.² Spermatozoa from the corpus epididymidis can occasionally fertilize but the pregnancy rate is low. However, few of these

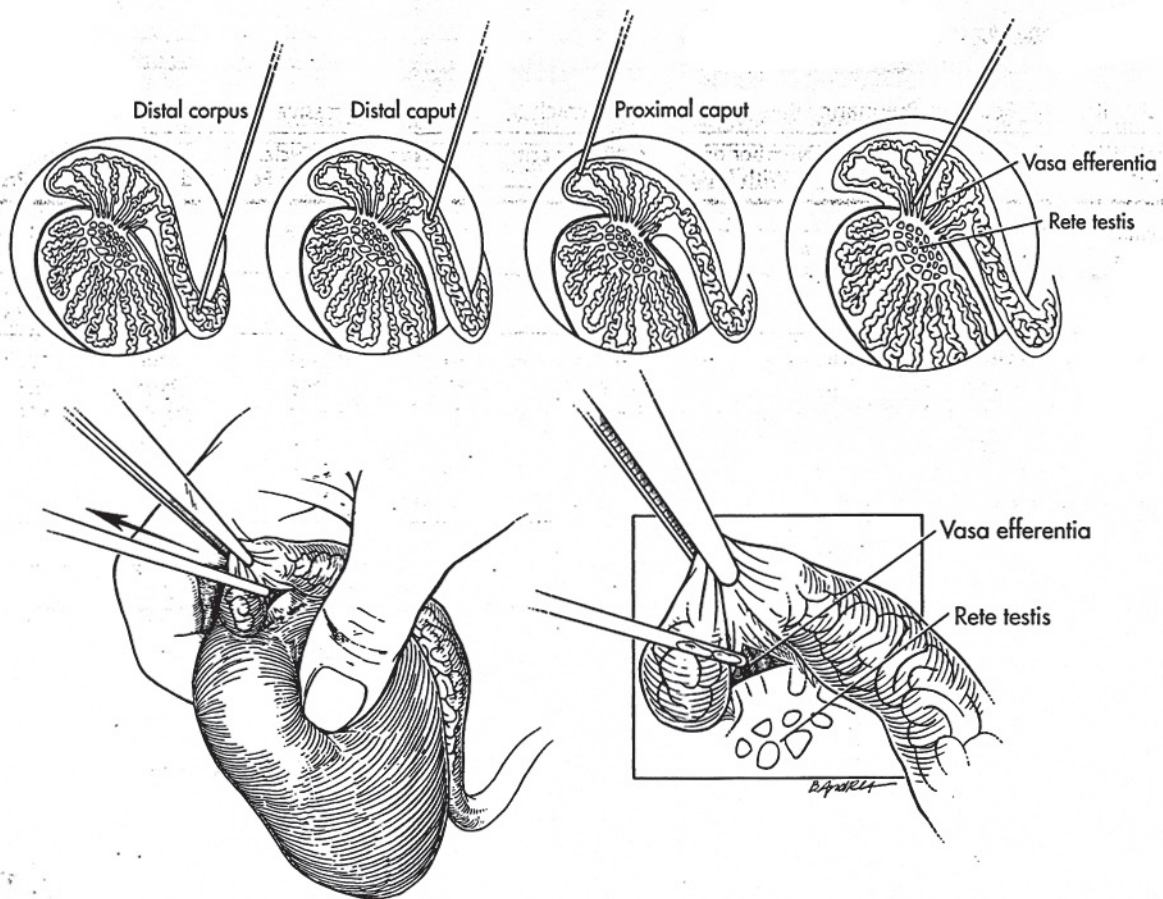


Fig. 59-3, cont'd B. Sperm in distal and often proximal epididymis were often nonmotile or poorly motile; in these cases vasa efferentia fluid usually had the most motile sperm.

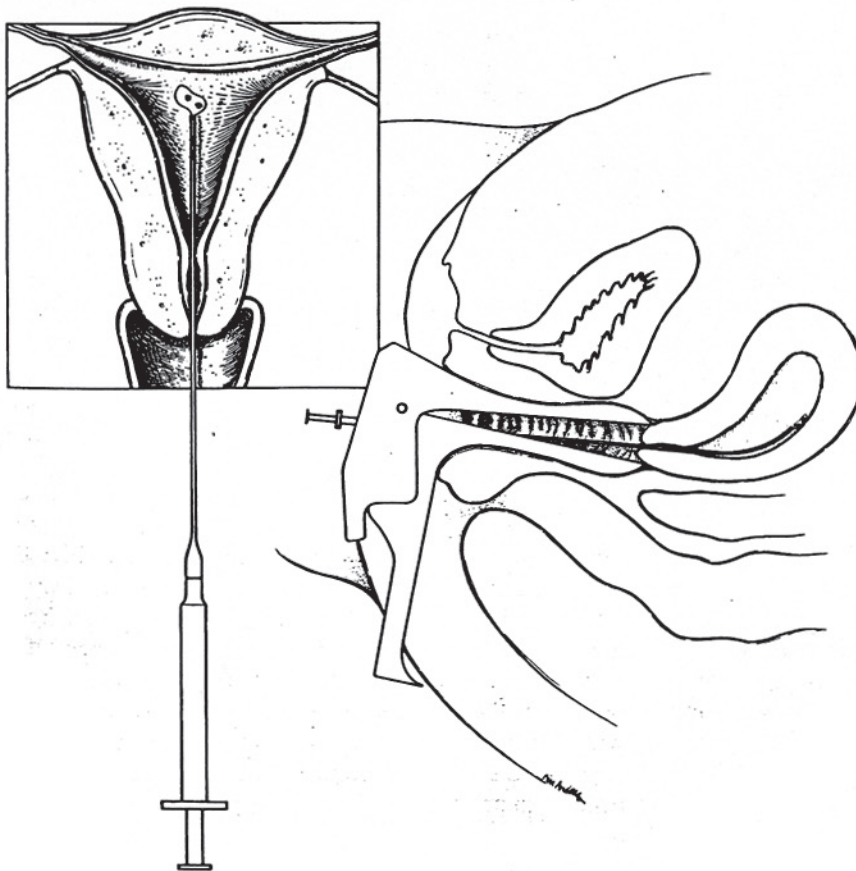


Fig. 59-4 IVF was performed with test tube methodology to assure the greatest approximation of eggs with the greatest number of sperm.

Table 59-5 Relation Between Pregnancy Rate and Site from Which Sperm With Progressive Motility Were Recovered

Site of Aspiration for IVF*	Number of Cycles	Number of Cycles With Sperm With Progressive Motility	Number of Cycles With at Least One Egg Fertilized	Number of Cycles With Pregnancy
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%)

*Most distal site in which motile sperm were present.
IVF, In vitro fertilization.

Table 59-6 Incidence of Antisperm Antibodies on Epididymal Sperm, Fluid, and Serum

	Direct IBT on Epididymal Sperm	Indirect IBT	
		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%)

*In two patients specimens were not available. A total of 45 patients were examined.

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 in which the epididymis was ligated to determine if time alone could allow spermatozoa to mature, the obstructed environment was so pathologic that no firm conclusion about fertility could be reached, and the initial increase in motility was followed by sperm stagnation and poor motility associated with obstruction.^{4,11-13} 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.

This finding should not be as surprising as it may seem. In a large series of men undergoing vasoepididymostomy for noncongenital obstructive azoospermia, 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.³⁹ Krylov and Borovikov have made similar observations in Moscow.¹⁵

The pattern of motility of sperm under obstructive conditions is obviously the inverse of that previously described for nonobstructed conditions.^{5,20} In most cases, sperm from the proximal epididymis of these obstructed patients does not fertilize with conventional IVF, and the reasons are not clear. Only in a minority of patients has good fertilization been achieved. Nonetheless, if motile sperm are obtained (usually in proximal regions), the level of the epididymis from which sperm are obtained has not been shown to be related to the

ability of that sperm to fertilize (see Table 59-5). Finally, with ICSI (to be discussed later), so long as any motility is present, however weak, fertilization can be achieved in all cases.

Sperm Antibodies

Since the level of epididymis from which sperm were retrieved had no effect on their IVF ability (provided we went proximally enough to obtain motile sperm), the next step was to see whether sperm autoimmunity resulting from the obstructive process might be detrimental to the fertilizing ability of epididymal sperm in men with obstruction.²⁵ 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 testing (IBT) on epididymal sperm and indirect IBT in epididymal fluid and serum.^{7,29} Results were expressed as the percentage of motile sperm showing at least two beads attached to the surface; a minimum binding of 20% was required to be positive.

Of the 45 patients, 35% demonstrated antibodies on epididymal sperm, 16% in the epididymal fluid, and 29% in the serum (Table 59-6).²⁵ 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 five 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.

Table 59-7 Correlation of Immunologic Details of the Positive Cases for Antisperm Antibodies and Their Fertilization Rate

	Direct IBT		Indirect IBT				Fertilization Rate	Pregnancy
	Epididymal Sperm		Epididymal Fluid		Serum			
	IgG	IgA	IgG	IgA	IgG	IgA		
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.

†Tail.

‡Tailtip.

§Midpiece.

||Head.

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 (Table 59-7).

Despite the vast literature clearly demonstrating a lower fertilization rate when more than 70% of ejaculated sperm are coated with either IgG or IgA (based on an interference with zona binding), no earlier data are available with epididymal sperm. Our studies show no significant relationship between class of immunoglobulin, 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.⁴¹ Thus we had to seek some other reason why we achieved excellent fertilization in some cases but in most cases did not.

Ultrastructure of Human Epididymal Sperm

We previously demonstrated with E/M studies that after vasectomy, sperm proximal to the site of occlusion undergo senescence and degeneration into globules of broken-down sperm heads DNA.¹⁰ We wanted to see whether the reason for poor sperm motility in the obstructed epididymis was due to such a senescent phenomenon.

The ultrastructural morphologic appearance of sperm from different levels of the epididymis demonstrated that spermatozoa aspirated from the rete testis, vasa efferentia, and caput

were markedly superior to that of sperm aspirated from the corpus and cauda epididymidis³ (Fig. 59-5). In distal epididymis, there were mostly degenerating and necrotic sperm, along with large numbers of giant sperm-engulfing macrophages; in proximal epididymis, the sperm were normal on E/M. This is similar to the senescent changes we found 15 years earlier in sperm proximal to a vas occlusion. Sperm aspirated from the vasa efferentia, rete testis, and caput epididymidis were normal and indistinguishable from ejaculated sperm in normospermic subjects.

Sperm Motility

The most baffling observation throughout our first 167 cases of epididymal sperm aspiration and IVF was the apparent inability to predict fertilization from sperm quality or percent motility. The quality of motility was not a good predictor for fertilization, the exception being that with less than 10% motility there were few fertilizations or pregnancy.^{42,43} Beyond that, there were no correlations (Table 59-8).

We attempted to refine that observation with computer-assisted sperm movement analysis.⁸ This provided a potentially useful retrospective theory; however, it is mathematically convoluted, and the explanation that follows does not change the fact that routine observation of the motility in epididymal sperm does not reveal anything obvious (Fig. 59-6). Still 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.

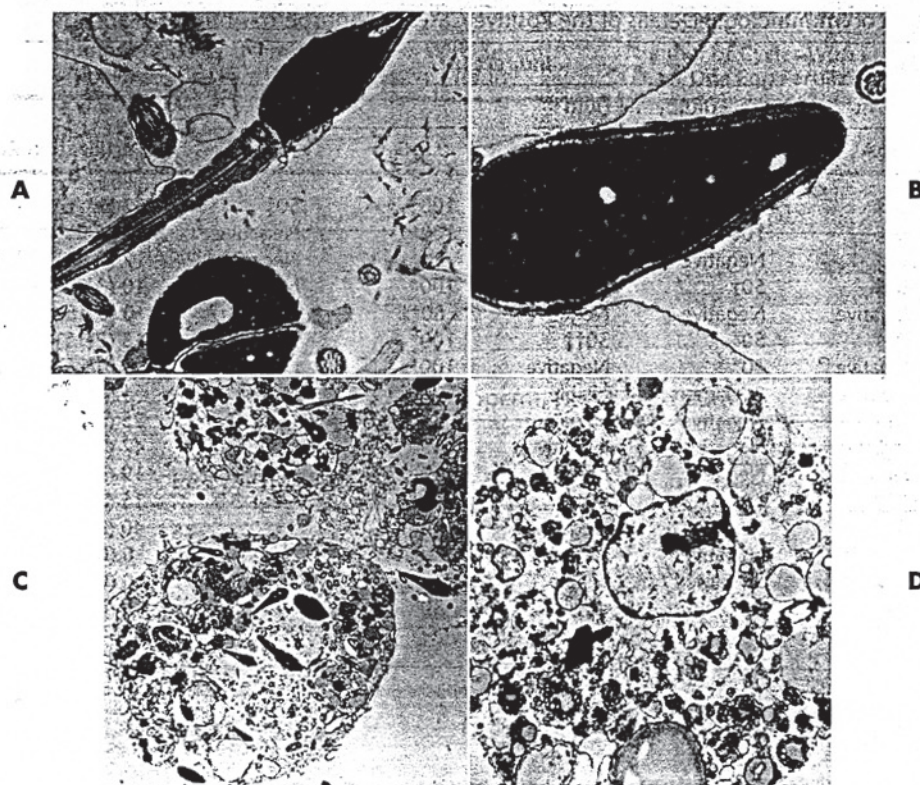


Fig. 59-5 **A**, Ultrastructure of sperm obtained from the rete testis from a man with congenital absence of the vas deferens. Note the similarities in the organization of the nucleus acrosome and flagella to normal ejaculated sperm. **B**, Ultrastructure of sperm obtained from the vasa efferentia of a man with congenital absence of the 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 epididymidis in a man with congenital absence of the vas deferens. Note the macrophages with their cytoplasm occupied by very large amounts of phagocytized sperm remnants in different degrees of degradation and digestion. **D**, Ultrastructure of macrophage obtained from the cauda epididymidis from a man with congenital absence of the vas deferens. Note the presence of prominent whorls of membranes and numerous lipid droplets indicating advanced stages of sperm degradation.

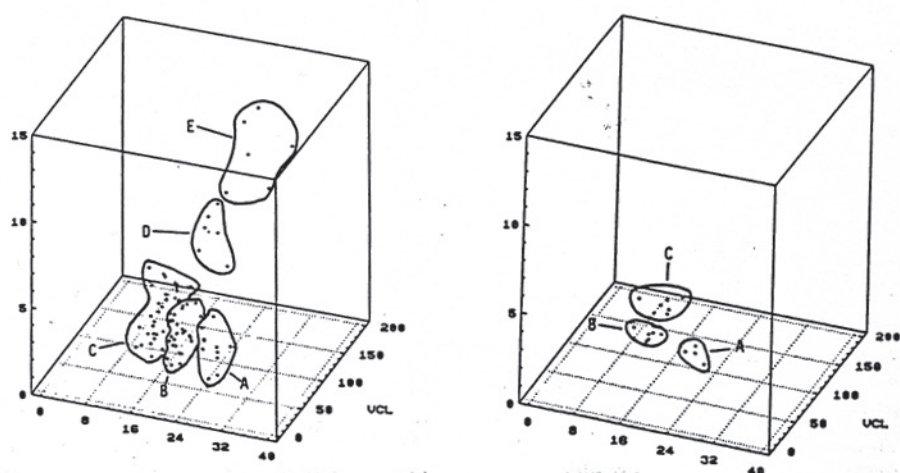


Fig. 59-6 Three-dimensional plots illustrating subpopulations of epididymal spermatozoa in the inseminates of two cases of IVF. One has five subpopulations of sperm (labeled A, B, C, D, and E); the other has three subpopulations (labeled A, B, and C).

Table 59-8 Summary of Fertilization and Pregnancy Results* (First 32 Reported Cases)

Patient Cycle Number	Age of Wife (Years)	Number of Mature Oocytes	Number of Embryos	Total Sperm Retrieved × 10 ⁻⁶		Motility (%)		Progression		Embryo Transfer	Outcome
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	—	0.48	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 born
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, miscarried
30	34	11	5	—	47.0	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.

OVERALL FERTILIZATION AND PREGNANCY RATES IN FIRST 115 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 59-9 and 59-10 summarize the results of the first five series, totaling 100 cases. Our protocol was to find a group of couples with congenital absence of the vas and perform sperm aspiration with IVF on them every 6 months over a 1- to 2-week period. There was no selection involved in any of the series; we took the patients as they came. In some groups our pregnancy rate was as high as 31%, and in others there were no pregnancies. The overall pregnancy rate for the first 100 cycles was 22%, with 16% term live baby deliveries; how-

ever, this hides the more dismal results endured in some other large groups of patients.

Clearly, the patients represented two obviously different populations (Tables 59-11 to 59-13). In 65% of the couples, the husband's sperm either failed to fertilize or produced no more than a single embryo. None of these couples achieved pregnancy. When two to four embryos were obtained, 26% of the couples got pregnant. When more than four embryos were obtained, over half the couples achieved pregnancy in that cycle. There appears to be one population of men whose epididymal sperm simply won't fertilize or fertilizes very poorly; this population represents the clear majority (65%). The other population (35%) fertilizes, but only 10% to 20% fertilize very well, and the pregnancy rate per transfer of such embryos is extraordinarily high. About 18% of patients fer-

Table 59-9 First 100 Cases In Vitro Fertilization for Congenital Absence of Vas: Pregnancy Rates

Series Number	Number of Sperm Aspiration Cycles	Number 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 59-10 First 100 Cases In Vitro Fertilization for Congenital Absence of Vas: Fertilization Rates

Series Number	Number of Sperm Aspiration Cycles	Number of Cycles Providing at Least One Embryo	Total Number of Mature Eggs	Total Number of 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%
TOTALS	100	59 (59%)	1339	324	24%

Table 59-11 Analysis of First 115 Cases of Epididymal Sperm Aspiration and IVF for Patients With Congenital Absence of the Vas (CAV)

Fertilized Per Patient	Number of Patients	Total Number of Eggs	Total Number of Embryos	Fertilization Rate	Percentage of Patients	Live Birth Pregnancy Rate
0-1	75	1074	15	1.4%	65.2%	0
2-4	19	336	52	15.4%	16.5%	5
5-7	9	137	50	36.4%	7.8%	5
>7	12	296	173	58.4%	10.4%	8
TOTAL	115	1843	290	15.7%	100%	18

Table 59-12 Analysis of First 115 Cases of Epididymal Sperm Aspiration and IVF for patients With Congenital Absence of the Vas (CAV)

Patient Group According to Number of Eggs Fertilized	Fertilization Rate in These Patients	Percentage of Patients	Percentage 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	15.7%	100%	100%	16%

Table 59-13 Analysis of First 115 Cases of Epididymal Sperm Aspiration and IVF for Patients With Congenital Absence of the Vas (CAV)

Patient Group According to Number of Eggs Fertilized	Percentage of Patients	Pregnancy Rate Per Patients
0-1	65.2%	0%
2-4	16.5%	26%
5-7	7.8%	55%
7	10.4%	67%
TOTAL 115	100%	16%

tilize more than seven eggs with the husband's epididymal sperm; 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 CAV undergoing sperm 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 fertilize well.

MESA with ICSI

Because of the relatively poor results using conventional IVF with epididymal sperm, the Infertility Center of 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 obtained in the first 167 cycles using conventional IVF methodology (Figs. 59-7 to 59-9).

The techniques of sperm and oocyte preparation, microinjection, and culture were the same as recently described by Van Steirteghem for severe male factor cases.^{48,49} 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 ICSI instead of conventional IVF. Nine of the 17 had previously undergone multiple attempts at MESA and conventional IVF 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 IVF/MESA cycles.

This was the first MESA attempt for eight of the 17 patients; for various reasons, these eight were also considered poor candidates. Four of them had either no epididymis or no sperm in the epididymis or rete testis despite normal spermatogenesis, thus requiring sperm extracted from a testicle biopsy. Two patients (one with congenital vas blockage) had undergone a previous vasopidymostomy. One patient *and his wife* were *both* heterozygous carriers for ΔF -508. In three cases, the wife was over 40 years old.

All 17 MESA/ICSI cycles were performed during a 1-week period at the Free University Academic Hospital in Brussels, Belgium, by Dr. Van Steirteghem, Dr. Devroey, myself, and coworkers. The patient population in 15 cases came from our usual base in the United States, and two cases were generated from Belgium. In short, this group of 17 was intentionally selected out of our usual population base because of their poor prognosis.

Table 59-14 summarizes the deteriorating results in the last four series of conventional IVF/MESA cycles involving 67 cases, a regression toward the mean that agrees with the low results of many other groups. Most programs performing MESA and IVF report no greater than a 5% to 10% pregnancy rate. The term pregnancy rate of all 167 of the conventional IVF/MESA cycles was only 11%.

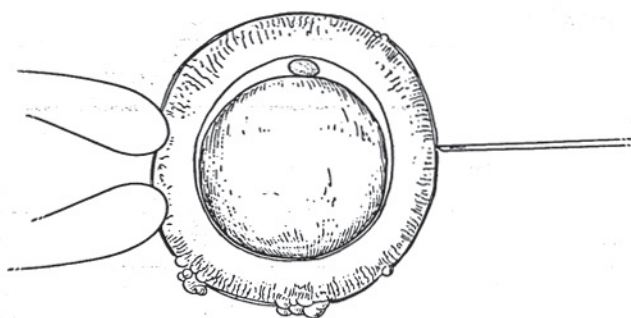


Fig. 59-7 Pick-up of weakly motile sperm from Petri dish microdroplet.

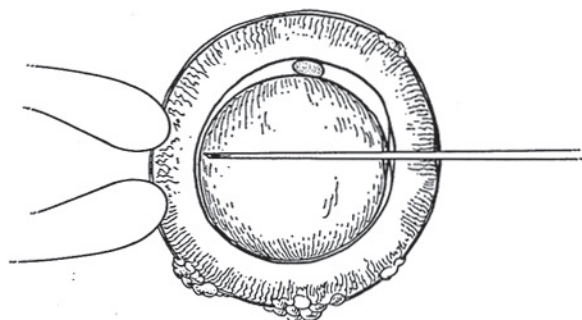


Fig. 59-8 Preparing to inject sperm into egg.

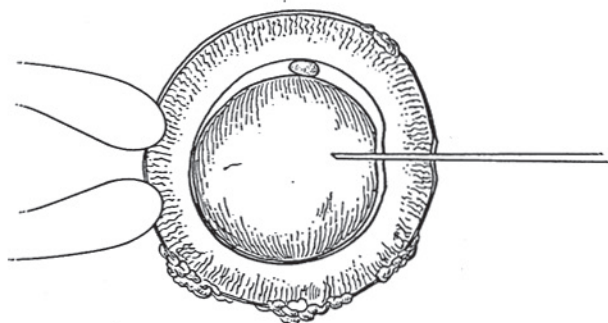


Fig. 59-9 Direct intracytoplasmic injection of sperm into egg.

Results with MESA/ICSI

Table 59-15 summarizes the results of fertilization and pregnancy rates in the most recent series of 17 MESA cases utilizing ICSI compared with the previous 67 cases of conventional IVF. Over 80% of patients utilizing ICSI had good fertilization rates with two to nine good quality embryos resulting. Some 88% went to embryo transfer. About 47% became pregnant, and 30% have healthy ongoing pregnancies. Three of the patients who produced three to nine embryos required testicular biopsy to obtain sperm because of complete absence of the epididymis (Table 59-16). These results represent a dramatic change from previous experience with such patients.

Table 59-14 Summary of Results of 67 Cases of Conventional IVF/MESA

Series Number	Number 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%

Table 59-15 Comparison of MESA/ICSI Results to Conventional IVF/MESA 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)
Mesa/ICSI					
17	197	80	41%	14/17 (82%)	47% (30%)
IVF/MESA					
67	1427	98	6.9%	13/67 (19%)	9% (4.5%)

Table 59-16 Fertilization of Human Oocytes by Testicular Tissue Spermatozoa (TESE) Using ICSI in Three Patients Who Had no Epididymis

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

Table 59-17 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 59-18 Results of MESA/ICSI in Nine Prior Recurrent IVF/MESA Failures

Patient	Cystic Fibrosis Genotype	Number Previous Unsuccessful IVF/MESA Cycles	Total Eggs Retrieved in All Previous IVF/MESA Cycles	Number Fertilized In Previous IVF/MESA Cycles	%	Number Eggs Retrieved in First MESA/ICSI Cycle	Number Fertilized in MESA/ICSI Cycle	Fertilization Rate	Wife's Age	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/R117H	2	68	0	(0%)	14	6	(43%)	31	Yes
6	ΔF-508/N	2	51	4	(7.8%)	18	6	(33%)	28	Yes
7	W1281X/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%)

Table 59-17 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 59-18 details the results in the specific group of nine patients who were previous IVF/MESA failures on multiple attempts. In conventional IVF these patients had a 3% fertilization rate with no pregnancy; however, 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%. A subsequent series of 31 cases has yielded a pregnancy rate of over 50% per cycle.

Conclusion

Distal epididymal sperm in obstructed males are either non-motile or totally degenerate, a result of senescence.^{3,25,43} Motile sperm must be obtained in the proximal caput or vasa efferentia because these sperm have been produced more recently. Yet these sperm still fall into two categories: those that fertilize well (20%) and those that do not (80%). With ICSI, this low success rate is completely reversed; epididymal sperm fertilize well with ICSI. When there is no epididymal sperm retrievable, testicle biopsy sperm yields good fertilization in 75% of cycles.

The consistently improved results with ICSI over regular IVF, and the good results using ICSI with testicular sperm, mandates the use of ICSI for all future IVF treatments for patients with obstructive azoospermia requiring microsurgical sperm retrieval.

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