9

Treatment of Male-Factor Infertility

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Male-factor infertility has undergone revolutionary changes in the last decade. It is now clear that hormonal therapy (clomiphene citrate, menotropins [Pergonal], human chorionic gonadotropin, testosterone-rebound, tamoxifen, etc.) has no beneficial effect on the infertile male, except for the rare case of Kallmann's syndrome or pituitary deficiency [1–7]. Nonetheless, infertile men who are referred to a urologist regularly receive such treatment. It has also been shown by several large, well-controlled studies supervised by those with no vested interest in the outcome that varicocelectomy has no beneficial effect on male infertility [8–10]. Still, very few infertile men who are referred to a urologist escape this hopeless procedure. The role of sperm antibodies in male infertility remains highly controversial, but if there is a role, evidence does not point to its being a major one, and treatment of such men (other than in vitro fertilization (IVF) or gamete intrafallopian transfer [GIFT]) has been dismally ineffective anyway [11; P Patrizio and colleagues, unpublished manuscript].

Nonetheless, we have made great strides in treating *couples* with male-factor infertility. The advances of the last decade will be reviewed, including the evaluation of male-factor infertility and its causes; treatment of male-factor infertility with IVF or GIFT; microsurgery for obstructive azoospermia; and IVF using epididymal sperm for irreversible obstruction and congenital absence of the vas.

Evaluation of Male-Factor Infertility and Its Causes

The male human, and the gorilla, have the poorest sperm production of any animal on the face of the earth. Whereas most animals produce 20 to 25 million sperm per gram of testicular tissue per day, the human produces only 4 million [12,13]. The gorilla is even less prolific, and his testicles are incredibly tiny. Even the very fertile male human has terrible sperm when compared to other animals. The large number of abnormal forms, debris, and nonmotile sperm found in human semen is not seen in other animals. It is believed, by comparative biologists, that this is due to the lack of "sperm competition" in monogamous animals such as humans. Over many thousands of years, the fact that the female will become impregnated by the sperm of only one partner

means there is no sperm competition and, therefore, no selection for greater sperm production in subsequent male offspring. Thus oligoasthenospermia appears to be simply a genetically transmitted condition, worse in some than others, but invariably a part of being human [14]. A hormonal or other pathologic etiology need not be sought; no pathologic testicular changes generally are seen in men with oligoasthenospermia or azoospermia.

Because poor sperm is to some extent the lot of all humans, even for those without an infertility problem, we must explore first the difficult issue of how we decide whether there actually is a male-factor problem in the couple.

THE SEMEN ANALYSIS: DEFINING MALE FACTOR

To what extent do standard semen analysis parameters reflect male fertility? In truth, when a couple has been unable to achieve pregnancy over a certain period of time, all we really know is that the *couple* is infertile. The time-honored method of evaluating the male partner has been the semen analysis. This method has been much maligned of late and replaced with very expensive tests such as the hamster egg penetration and sperm antibody tests, but these tests have actually confused rather than clarified the picture. There remains a frustrating discrepancy between the results of hamster egg penetration tests, sperm antibody tests, and fertilization of human oocytes via IVF or GIFT [15].

The exemplary case, documented by Sokol and Sparkes [16], involves a wife who, remarkably, became pregnant naturally by a husband who had only 50,000 spermatozoa per milliliter with terrible motility. The husband, the wife, and the baby were carefully blood-typed genetically, and it was determined with 99.99% certainty that the husband was indeed the father. If only 50,000 spermatozoa per milliliter are required for a natural pregnancy (*without IVF*), what is *male factor*?

The Controversy Over Low Sperm Counts
The correlation of sperm count with fertility was originally presented in the famous article by MacLeod and Gold in 1951 [17]. These authors stud-

ied sperm counts in 1000 fertile and 1000 infertile men (Table 9-1). Their results clearly indicated that 16% of infertile men had sperm counts of fewer than 20×10^6 per milliliter, and only 5% of fertile men had counts in that range. It was therefore concluded that any count of fewer than 20×10^6 spermatoza per milliliter indicated infertility. This conclusion was tenuous, but only recently has the weakness of this assumption been recognized.

Rehan and colleagues [18] reported results similar to those of MacLeod and Gold [17] and also found that 17% of fertile men had sperm with very poor motility (grade 1 or 2 at most). David and associates [19], in 1979, reported similar results in almost 3000 infertile men; their control group consisted of 190 fertile men. Neither of these studies addressed the possibility that low sperm counts, like high sperm counts, might occur at either end of the bell-shaped population curve and might be unrelated to the man's fertility.

In 1974, Nelson and Bunge [20] reported on 386 fertile men and found that 20% had sperm counts of fewer than 20×10^6 per milliliter, and only 28% had sperm counts greater than 60×10^6 per milliliter. Thus these investigators concluded that fewer than 20×10^6 sperm per milliliter does not indicate a male factor.

In 1977, Zuckerman and colleagues [21] along with Smith's group [22] studied several thousand fertile men presenting for a vasectomy (Table 9-2). They did show a direct relationship of the numeric sperm count to the chances of pregnancy for the couple. The motile sperm count correlated not only directly with the

Table 9-1. *Frequency Distribution of Sperm Counts in 1000 Fertile Men and 1000 Infertile Men*

Sperm Count (million/ml)	Fertile Men (%)	Infertile Men (%)
20	5	16
20–39	12	13
40–59	12	11
60	71	60

Source: Reprinted with permission from J MacLeod, RZ Gold, The male factor in fertility and infertility: II. Sperm counts in 1000 men of known fertility and in 1000 cases of infertile marriage. *J Urol* 66:436, 1951.

Table 9-2. *Frequency Distribution of Sperm Counts in Infertile Couples Compared to Fertile Couples*

Sperm Count (millions/ml)	Infertile Couples (%)	Fertile Couples (%)
10.1	28.5	11.7
10.1–20.0	14.0	11.2
20.1–40.0	16.7	22.0
40.1–60.0	12.0	14.8
60.1–100.0	12.7	19.4
100	16.1	20.9

Source: Reprinted with permission from Z Zuckerman et al, Frequency distribution of sperm counts in fertile and infertile males. *Fertil Steril* 28: 1310, 1977.

chances of pregnancy but also inversely with the number of cycles required for the female partner to conceive (Table 9-3). When the motile sperm count was fewer than 5×10^6 per milliliter, the female partner required a mean of 11 months to become pregnant, whereas when the motile count exceeded 60×10^6 spermatozoa per milliliter, she required a mean of 6 months to achieve pregnancy. However, despite a lower motile sperm count being associated with a longer mean time until pregnancy, 11% of men of proved fertility presenting for a vasectomy were found to have sperm counts of fewer than 10×10^6 per milliliter. It is therefore essential to be cautious when suggesting to any infertile couple with a poor semen analysis that the husband is infertile.

The author has also reviewed sperm count and motility indices in men after vasovasostomy whose wives became pregnant and compared this information to those whose wives did not become pregnant (Table 9-4) [11]. The distribution of sperm counts, percent motility, and total motile sperm per ejaculate were similar to the

Table 9-3. *Mean Number of Cycles to Conception Related to Sperm Motility*

Motile Count (million/ml)	Mean No. of Cycles to Conceive
5	11
5–20	9.4
20–60	8
60	6.0

findings of Zuckerman's and Smith's groups [21,22]. Eleven percent of the successful vasovasostomy patients whose wives became pregnant had total sperm counts per ejaculate of fewer than 10×10^6. Sixty-four percent had more than 40×10^6 spermatozoa per milliliter. If this is compared to the group of infertile men after vasovasostomy, there are no significant differences except for sperm counts of fewer than 10×10^6 per milliliter. In the group of men whose wives did not become pregnant, 23% had counts fewer than 10×10^6, as opposed to 11% in the group of men whose wives did become pregnant.

Relationship of Sperm Count to Pregnancy Rate

Smith and colleagues [22], in 1977, compared the average motile sperm count of the husband in their infertile couples to the ultimate pregnancy rate over many years of follow-up (assuming the wife was treated) (Table 9-5). When there were fewer than 5 or 10×10^6 motile spermatozoa per milliliter, approximately 30% of the couples eventually achieved a pregnancy. When the motile sperm count per milliliter was greater than 100×10^6, 70% of the couples became pregnant. In general, the higher the motile sperm count, the greater the chance that an infertile couple ultimately would conceive.

Baker and coworkers [23], in 1986, compiled their data on a graph in combination with the data of Kovacs and associates [7] (1982), Vessey and colleagues [24] (1976), and MacLeod and Gold [25] (1953), and constructed a life table pregnancy curve on infertile couples with varying degrees of oligospermia compared to controls (Fig. 9-1) [7,23–25]. In this clinic, the wife was treated no matter how poor the husband's semen. Pregnancy rates were compared for couples in whom the man had a sperm count lower than 5×10^6, 5 to 20×10^6, greater than 20×10^6 with fewer than 60% motile, and greater than 20×10^6 per milliliter with more than 60% motile. These four groups were then compared to an artificial donor insemination group (presumed to be the highest-quality semen) and to the pregnancy rate table from Vessey's study of the mean time to pregnancy in women discontinuing use of an intrauterine device. Normal was

Table 9-4. *Frequency Distribution of Motile Sperm Count Following Vasovasostomy in Men Who Did or Did Not Impregnate Their Wives (10-year Follow-up)*

Total Motile Sperm Count (millions/ejaculate)	Total No. of Patients (%)	No. Pregnant (%)	No. not Pregnant (%)
0–10	32 (12)	25 (11)	7 (23)
10–20	31 (12)	27 (12)	4 (13)
20–40	32 (12)	30 (13)	2 (7)
40–80	79 (31)	68 (30)	11 (37)
80	84 (33)	78 (34)	6 (20)
Total	258 (100)	228 (100)	30 (100)

Source: Reprinted with permission from SJ Silber, Pregnancy after vasovasostomy for vasectomy reversal: A study of factors affecting long-term return of fertility in 282 patients followed for 10 years. *Hum Reprod* 4(3):318–322, 1989.

Table 9-5. *Relationship of Sperm Count to Pregnancy Rate Among Infertile Couples*

Motile Sperm Count (millions/ml)	Pregnancy Rate (%)
5.1	33.3
5.1–10.0	27.8
10.1–20.0	52.9
20.1–40.0	57.1
40.1–60.0	60.1
60.1–100.0	62.5
100.0	70.0

Source: Reprinted with permission from KD Smith et al, Relation between indices of semen analysis and pregnancy rate in infertile couples. *Fertil Steril* 28:1314, 1977.

assumed to be the spontaneous pregnancy rate reported in 1951 by MacLeod and Gold [17].

Again, quite remarkably, with fewer than 5×10^6 spermatozoa per milliliter, regardless of motility, the pregnancy rate at 2 years was almost 30% and at 3 years was more than 30%. When the sperm count was between 5 and 20×10^6 per milliliter, the pregnancy rate at 2 years was approximately 43% and at 3 years was 50%. When the sperm count exceeded 20×10^6, although fewer than 60% were motile, the pregnancy rate at 2 years was similar to the results obtained when the count was between 5 and 20×10^6. When the sperm count was essentially normal— that is, more than 20×10^6 per milliliter with greater than 50% motility—the pregnancy rate at 2 years approached 72%. In conclusion, women may become pregnant by partners with extremely low sperm counts, but the higher the motile sperm count, the greater are the chances of pregnancy.

PREGNANCY RATES WITH DONOR INSEMINATION IN WIVES OF OLIGOSPERMIC MEN VERSUS WIVES OF AZOOSPERMIC MEN

In 1982, Emperaire and colleagues [26] published a remarkable observation from their donor insemination program. Women undergoing artificial insemination by donor (AID) were divided into those whose husbands were azoospermic and those whose husbands were oligospermic. The pregnancy rate per cycle in women whose husbands were azoospermic was 11.6%. However, the pregnancy rate per cycle in women whose husbands were oligospermic was only 4.9%. Overall, 61% of women whose husbands were azoospermic became pregnant by donor insemination, but the success rate was only 29% in those whose husbands were oligospermic (Table 9-6).

The only reasonable explanation of this finding was that an oligospermic man might have initiated a pregnancy in his wife with his small number of spermatozoa if the wife herself did not also have reduced fertility. The wives of azoospermic husbands were less likely to have reduced fertility and were more likely to be a normal population. This constitutes indirect evidence that low sperm counts can initiate pregnancies in fertile women and that infertility is commonly caused not simply by male factor alone but by a combination of male and female factors in the couple.
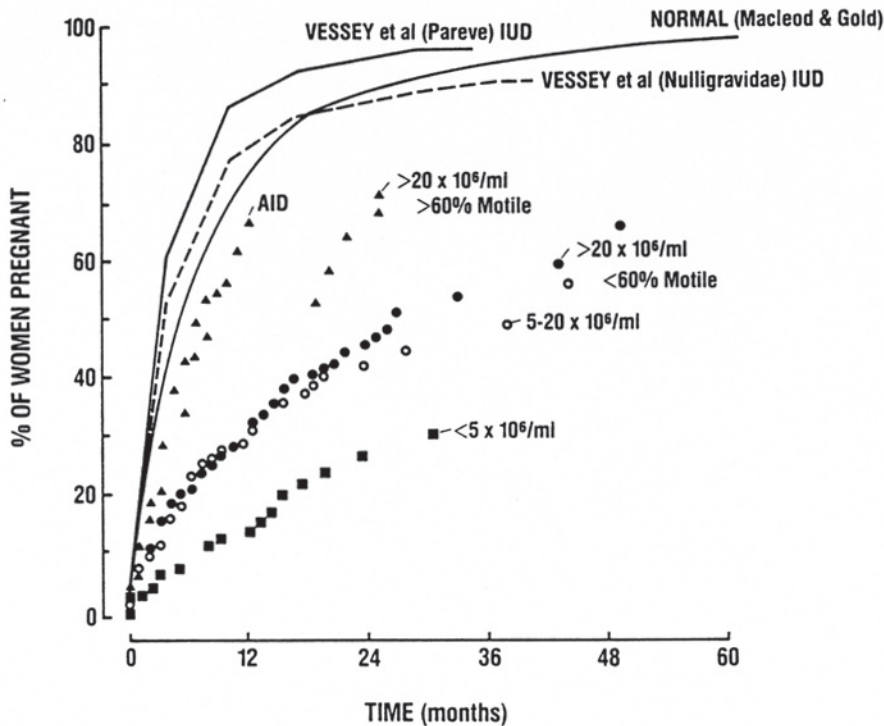
Fig. 9-1. *Cumulative and life table pregnancy rates in relation to sperm count. AID = artificial insemination by donor; IUD = intrauterine device. (Data from Vessey and colleagues [24] and MacLeod and Gold [25]. Reprinted with permission from Baker, deKretser, 1984.*

Table 9-6. *Pregnancy Rate Using Donor Insemination in Wives of Azoospermic Versus Oligospermic Men*

Husband	Pregnancy Rate	Pregnancy Rate per Cycle
Azoospermic (N = 95)	61%	11.6%
Oligospermic (N = 95)	29%	4.9%

Source: Reprinted with permission from JC Emperaire, E Gauzere-Sonmireu, AJ Audebert, Female fertility and donor insemination. *Fertil Steril* 37:90, 1982.

In our experience with infertile couples, an inverse relationship was found between the sperm count in the husband and the degree of fertility of the wife [27]. The lower the sperm count in an infertile couple, the lower is the chance that the wife will be subfertile, and vice versa.

IVF and GIFT for Male-Factor Infertility

BACKGROUND

Great excitement has been generated about the use of IVF in couples with very low sperm counts. Fertilization rates in most IVF centers are clearly poorer in couples with oligospermia compared to other populations, but good fertilization rates have been obtained in oligospermic couples [28]. Theoretically, IVF or GIFT allows the fewer number of available sperm a greater opportunity for direct contact with the ovum [29,30].

In cases of congenital absence of the vas, a collaborative study has evaluated aspiration of sperm from the epididymis, fertilization of the

wife's oocytes in vitro, and placement of the resultant embryos into the fallopian tubes (zygote intrafallopian transfer [ZIFT]) [31–33]. The pregnancies that ensue lead us to conclude that sperm with severely reduced motility can fertilize the oocyte if other obstacles are eliminated.

Rodriguez-Rigau and colleagues [15], in 1989, evaluated the semen parameters of a large number of couples who did or did not achieve a pregnancy with GIFT (Table 9-7). They found a correlation between the pregnancy rate with GIFT and standard semen parameters such as count and motility. The hamster egg penetration test was much poorer in predicting the success or failure of GIFT. The linearity of forwardly progressive sperm movement was a useful predictive indicator, although the velocity of movement was not. This suggests that microscopic observation of the husband's spermatozoa is useful in determining whether pregnancy is likely. If there are any sperm with linear forward progression, fertilization may be possible.

A similar finding was reported by Kruger and associates [34]. The morphologic features of spermatozoa were examined very carefully in husbands of patients undergoing IVF. Any spermatozoa with a slight defect, such as neck droplets, bent necks, or abnormal heads, was considered abnormal. Remarkably, provided that at least 4% of spermatozoa demonstrated perfectly normal morphology, fertilization in vitro was achieved. Once again, this suggests that a critical

Table 9-7. *Relationship of Total Motile Sperm Count and Linearity of Sperm Movement to GIFT Pregnancy Rate*

Semen Parameter	Clinical Pregnancy (%)
Total motile sperm count (× 10 ^{6}) per ejaculate	
25	7.7
25–100	15.2
100	24.1
Motility Index	
0.8	12.2
0.8	27.6

GIFT = gamete intrafallopian transfer.
Source: Reprinted with permission from LJ Rodriguez-Rigau et al, Relationship between the results of sperm analysis and GIFT. *J Androl* 10(2): 141–143, 1989.

visual examination of the husband's spermatozoa can be a good predictor of the likelihood of fertilization.

The great success in treating many severely oligospermic couples with IVF, GIFT, or ZIFT has required a whole new definition of *male factor* so as to distinguish those who readily fertilize their wife's oocytes despite oligospermia from those who do not. With IVF in male-factor cases, the prewash motile sperm count in the semen is not a heavily significant determinant of fertilization or pregnancy. The pregnancy rate with IVF or GIFT is, of course, lower in men with low sperm counts than in men with high sperm counts, but what really determines the pregnancy rate is the total motile sperm count after washing. In our experience, when the total motile sperm count recovered from a Percoll or mini-Percoll preparation is greater than 1.5 × 10^6 million motile sperm, the fertilization rate is not significantly different from that of patients with higher numbers of recoverable sperm. When the postwash motile sperm count is lower than this, the fertilization rate and pregnancy rate are greatly reduced.

PROCESSING MALE-FACTOR SPERM FOR IVF, GIFT, AND ZIFT

When the total number of motile spermatozoa in the ejaculate is lower than 5 × 10^6 (classified as severe oligoasthenozoospermia) [35], the conventional methods of sperm preparation—such as swim-up [36], wash and resuspension [37], sedimentation [38], and Percoll [39]—are not very effective in allowing the recovery of a sample that is clean with a sufficient number of normal motile spermatozoa. This, in turn, is correlated with very poor IVF results, as has been confirmed by other authors [40–42].

The resuspension method does not remove cells and debris, whereas Percoll gradient techniques, although very effective in filtering out debris and other contaminants, often give a low rate of sperm recovery in very poor samples [43]. In an attempt to identify a method that could be used for oligoasthenozoospermic patients, a modified Percoll technique, the mini-Percoll,

which consists of a reduced volume, discontinuous Percoll gradient, was developed by Ord and coworkers [44,45].

Semen samples are diluted 1:2 with culture medium and centrifuged at 200 g for 10 minutes. Following centrifugation, pellets are suspended in 0.3 ml medium and layered on a discontinuous Percoll gradient consisting of 0.3 ml each of 50, 70, and 95% isotonic Percoll (mini-Percoll). An isotonic solution of Percoll is obtained by mixing nine parts of Percoll (Pharmacia, Sweden) with one part Ham's F-10 (10×) (Gibco, RI), and adding 2.1 g/liter of sodium bicarbonate. To obtain the 95, 70, and 50% layers, this isotonic solution was diluted with HTF and HEPES. The discontinuous gradient is established by carefully pipetting 0.3 ml of 95, 70 and, finally, 50% isotonic Percoll in a 15-ml centrifuge tube. The gradient is centrifuged at 300 g for 30 to 45 minutes. After centrifugation of the gradient, the 95% Percoll layer is removed, washed twice, and resuspended in 1 ml HTF and 10% HCS and incubated until the time of insemination.

It is recognized that the fertilization rate is greatly reduced when severe qualitative and quantitative alterations exist in the male partner's semen parameters [46]. It has been reported by Hyne and colleagues [43] that fractionation of human spermatozoa by centrifugation on a discontinuous density gradient of Percoll can improve the capacity for IVF of spermatozoa from men with oligospermia (<40% motility). However, in our laboratory, the use of this discontinuous gradient in cases of severe oligoasthenozoospermia ($<5 \times 10^6$ total motile count) is ineffective because of poor or no recovery. For IVF with sperm samples containing a very low number of motile spermatozoa combined with a high percentage of abnormal forms, we recommend this modification of the Percoll method.

The mini-Percoll gradient seems to offer not only a high recovery of motile spermatozoa from oligoasthenozoospermic subjects but also a good fertilization and pregnancy rate. There are several advantages of this gradient. First, the reduced volume of each Percoll layer allows better "migration" of spermatozoa. Second, the volume of 0.3 ml per layer still retains the cleaning function, as in other reported techniques using Percoll, filtering out literally all the cells and debris that are usually present in severe oligoasthenozoospermic samples and that represent one of the limiting factors for successful fertilization. Third, the use of mini-Percoll allows recovery of a high proportion of the normal and motile spermatozoa present in the sample. It is clear that for the present decade, IVF technology will be the only valid treatment for most cases of male-factor infertility except those involving obstruction of the vas or epididymis.

Microsurgery for Obstructive Azoospermia

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

VASECTOMY REVERSAL

Vasectomy is the most popular method of birth control in the world today [47]. For many years, the pregnancy rate after surgical reanastomosis of the vas had been very low, and a variety of explanations had been offered for the relatively poor success in reversing vasectomy [48–50]. With the advent of microsurgical techniques, pregnancy rates improved considerably, suggesting that purely micromechanical factors were associated with the low success rates [51–53]. However, there still were many cases of technically perfect vasovasostomies followed by complete azoospermia or severe oligoasthenospermia.

Theories for the consistently poor results with vasectomy reversal had included development of sperm antibodies, damage to the deferential nerve, and testicular damage [54–61]. We have questioned any major correlation between sperm antibodies and subsequent fertility after vasovasostomy [11,62]. The deleterious effect of pressure increase subsequent to vasectomy is not in the testis but rather on epididymal dilatation, perforation, and sperm inspissation. These complications were more likely to result in "blowouts" in the epididymis as the time interval from vasectomy increased. Such blowouts result

in epididymal obstruction, which is the major problem in readily restoring fertility to vasectomized men [11,51,53]. Thus vasoepididymostomy is required in many cases of vasectomy reversal in order to obtain a high success rate. Despite the dismal finding of no sperm in the vas fluid at the time of vasovasostomy, the testicle biopsy of such patients had always appeared normal [63,64]. This deleterious effect of pressure increase is *always* on the epididymis, not on the testis, in humans. In fact, the secondary epididymal obstruction caused by vasectomy leads us to recommend that the testicular end of the vas not be sealed at the time of vasectomy, so as to lessen the pressure buildup and possibly increase the ease of reversibility later (notwithstanding the potentially damaging immunologic consequences [65–67].

What is the fertility rate in the favorable group of patients undergoing vasovasostomy who have suffered no secondary epididymal damage (as evidenced by sperm being present in the vas fluid at the time of vasovasostomy)? Ten years ago, we studied such a group of patients [11]. Three hundred twenty-six men who had been previously vasectomized underwent vasovasostomy and received extensive long-term follow-up. In 44 of those men, no sperm were found in the vas fluid. All such patients have been determined to be azoospermic after vasovasostomy, and all required vasoepididymostomy later.

The vasovasostomy involved a meticulous, two-layer microsurgical technique performed by the same surgeon with accurate mucosa-to-mucosa approximation [51]. Almost all the patients had proved prior fertility, as evidenced by previous fatherhood. All patients were followed for 9 or 10 years.

Table 9-8. *Overall Long-Term Pregnancy Rates in Patients Undergoing Vasovasostomy in whom Sperm Were Seen in Vas Fluid (10-year Follow-up)*

	Combined 1975 and 1976–77 Series	Original 1975 Series
No. of patients (%)	282 (100)	42 (100)
No. pregnancies (%)	228 (81)	32 (76)
No. azoospermic (%)	24 (9)	5 (12)

The overall, long-term pregnancy rate and sperm patency rate is summarized in Table 9-8. None of the azoospermic patients were able to impregnate their wives. If azoospermic patients are excluded, 88.4% of patients with sperm patency postoperatively eventually impregnated their wives. This compares to Vessey's expected pregnancy rate of 96% for previously fertile couples who discontinue contraception (1978).

The frequency distribution of semen parameters postoperatively in men whose wives did or did not achieve pregnancy with them is summarized in Tables 9-9 and 9-10. There was remarkably little difference in the pregnancy rate among men with low or high sperm counts. Findings were similar with sperm motility of 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 [21].

As summarized in Table 9-11, a left-sided varicocele was clinically apparent in 42 of the 282

Table 9-9. *Pregnancy Rate According to Distribution of Motile Sperm Count in Men with Sperm Patency After Vasovasostomy (10-year Follow-up)*

Total Motile Sperm Count (per ejaculate)	Total No. of Patients (%)	No. Pregnant (%)	No. 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
Total	258 (100)	228 (88)	30

Table 9-10. *Pregnancy Rate According to Sperm Motility in Men with Sperm Patency After Vasovasostomy (10-year Follow-up)*

Motility	Total No. of Patients (%)	No. Pregnant (%)	No. not Pregnant
0–20	24 (9)	18 (75)	6
20–40	70 (27)	66 (94)	4
40–60	82 (32)	71 (86)	11
60–80	62 (24)	55 (88)	7
80	20 (8)	18 (90)	2
Totals	258 (100)	228 (88)	30

Table 9-11. *Lack of Effect of Unoperated Varicocele on Pregnancy Rate After Vasovasostomy*

	No. of Patients (%)	No. of Patients with Varicocele	No. of Patients Without Varicocoele
Pregnant	228 (80.9)	33 (78.5)	195 (81.2)
Not Pregnant	54 (19.1)	9 (21.4)	45 (18.8)
Totals	282 (100)	42 (14.9)	240 (85.2)

Table 9-12. *Relationship of Serum Antisperm Antibody Titers to Pregnancy Rate After Vasovasostomy*

	Total No. of Patients 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 (18%)	6 (55%)	6 (55%)
Husband azoospermic	12	5 (42%)	3 (25%)	7 (58%)	5 (42%)
Entire group studied	98	38 (39%)	23 (23%)	55 (56%)	41 (42%)

patients (14.9%). Varicoceles were not operated on, and yet the pregnancy rate was not significantly different in patients with as opposed to those without varicocele. Table 9-12 summarizes the relationship of preoperative serum antisperm antibody titers to the pregnancy rate after vasovasostomy. Similarly to varicocele, the presence of high immobilizing titers or agglutinating titers had no influence on the pregnancy rate.

The high pregnancy rate in this group of patients requires some explanation. There have been many speculations about the causes of failure to achieve fertility after reversal of vasectomy, including autoimmune changes and damage to the testis. Our study suggested that the pregnancy rate in patients in whom patency is accurately reestablished without epididymal damage is ultimately not significantly less than in a nor-

mal population of couples [11]. Vessey demonstrated that among couples with proved prior fertility, 96.5% conceive within 4 years of discontinuing contraception (1978). In our couples with patent results after vasovasostomy who had no evidence of epididymal pressure damage, 88% conceived with long-term follow-up. Patients with secondary epididymal blockage require a completely different approach.

It has been previously shown that the success rate of vasovasostomy decreases with the duration of time since vasectomy [51]. This decreased success rate over time is directly related to the absence of sperm in the vas fluid at the time of vasovasostomy, which is caused by the interruption of epididymal patency by pressure-induced sperm extravasation and inspissation [63]. The incidence of this pressure-mediated interruption

of epididymal patency is reduced dramatically by the presence of a sperm granuloma at the vasectomy site, which serves as a release valve to prevent the pressure increase that would otherwise occur proximal to the vasectomy site [53,65,66]. When there is no sperm in the vas fluid, vaso-epididymostomy proximal to the site of epididymal blockage is required [68,69].

VASOEPIDIDYMOSTOMY

When vasectomy has produced secondary epididymal blockage, or in cases of postinflammatory obstructive azoospermia, very precise microsurgical tubule-to-tubule vasoepididymal anastomosis is required. A practical understanding of epididymal physiology [52,53,68–70] is as important in this undertaking as is precise microsurgical technique.

In every animal that has been studied, spermatozoa from the caput epididymis are capable of only weak circular motion at most and are not able to fertilize [71]. In previous studies, spermatozoa from the corpus epididymis could occasionally fertilize, but the pregnancy rate was still low. Spermatozoa were simply aspirated from specific regions of the epididymis and then promptly inseminated [72–74]. Some of these previous animal studies allowed the spermatozoa time to mature and, thereby, possibly develop fertilizing capacity, yet where the epididymis was ligated to determine if time alone could allow spermatozoa maturation, the obstructed environment was so pathologic that no firm conclusions could be reached. Thus the outlook seemed poor for vasoepididymostomies.

In 1969, Oregebin-Crist pointed out that we still did not know with certainty from any of these animal studies whether the factors governing the maturation process of spermatozoa are intrinsic to the spermatozoa themselves and merely require time or whether spermatozoa must travel through most of the epididymis in order to mature. It was entirely possible that aging alone might mature the spermatozoa and that spermatozoa might not need to pass through all the epididymis to develop the capacity to fertilize. Nonetheless, because of the animal studies already mentioned, as well as the poor results in humans using nonmicrosurgical techniques, it

has always been assumed that epididymal blockage carries a poor prognosis [75–78].

As far back as 1931, however, Young's experiments in guinea pigs [79] with ligation at various levels of the epididymis indicated the contrary: ". . . 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 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." In fact, Young [79] observed the same "inversion" of regions of sperm motility and nonmotility in the obstructed epididymis that we have noted in clinical obstructive azoospermia. The more distal regions have the poorest motility and the more proximal regions the best. Young [79] 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 [80].

All vasoepididymostomies are performed with the specific tubule technique we have already described, which involves either an end-to-end or an end-to-side anastomosis of the inner lumen of the vas to the epididymal tubule, mucosa to mucosa, in a leakproof fashion [51,68,70]. Because of the high rate of technical failure with older surgical methods, reliable data on the fertility of spermatozoa from the epididymis in the past was difficult to obtain.

The anastomosis of the vas to the epididymis is performed at the transition point between the location where there are no spermatozoa and where there is an abundant amount of spermatozoa in the fluid emanating from the epididymal tubule (Figs. 9-2 through 9-4). Usually, five to six 10-0 nylon interrupted sutures complete the leakproof end-to-end anastomosis, and then the outer muscularis of the vas is sutured separately to the outer epididymal tunic with 9-0 nylon interrupted sutures.

Seventy-two percent of the cases of epididymal anastomosis have resulted in eventual pregnancy. The younger the wife was, the higher was the pregnancy rate. The pregnancy rate was not
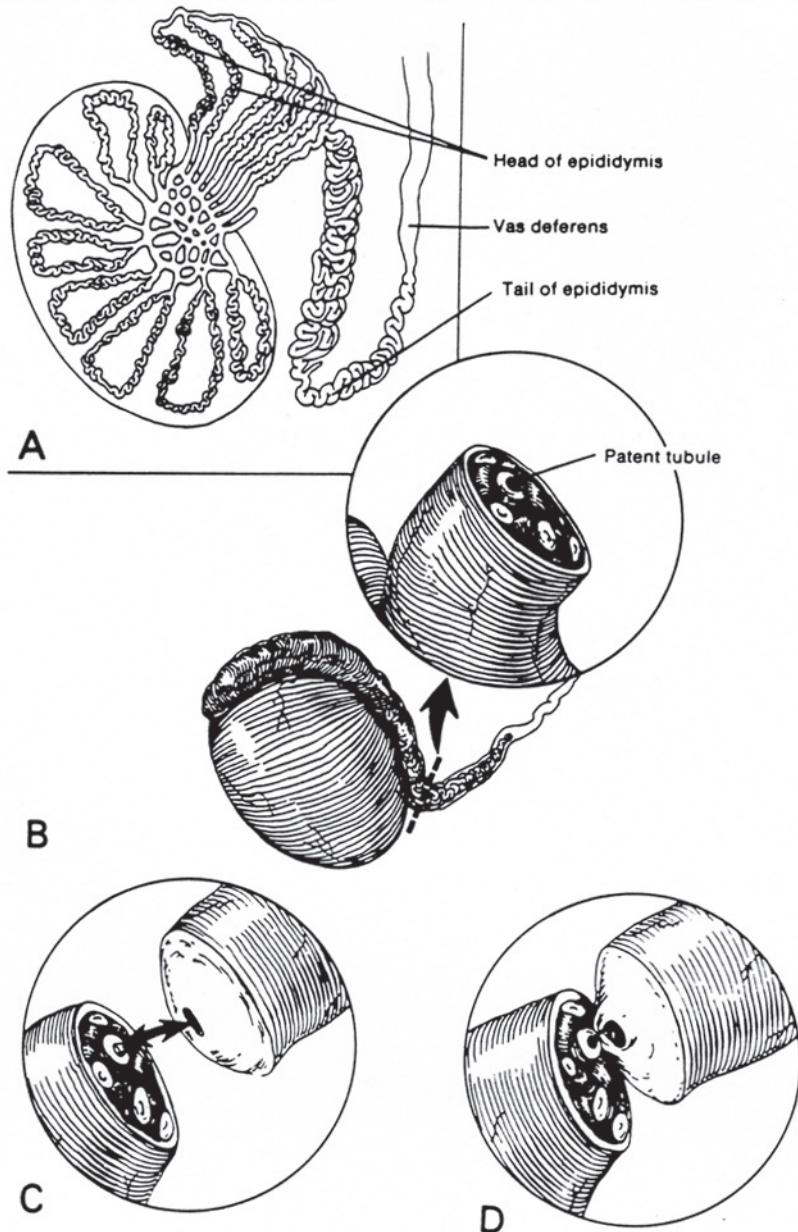
Fig. 9-2. *Specific tubule end-to-end anastomosis of the vas lumen to the epididymis proximal to the site of obstruction.*

related to the numeric sperm count but was related to the sperm's motility. The fact that pregnancy 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 even possible, in some circumstances, for spermatozoa that have never traversed any length of epididymis to fertilize the human egg. In 2 reported cases of vasa efferentia–to–vas deferens anastomosis, the postoperative ejaculate contained normally motile sperm, and the wives became pregnant [81]. In addition, pregnancy achieved by aspiration of epididymal sperm combined with IVF and ZIFT in cases of irreparable obstruction gives further evidence that transit through the epididymis is not mandatory for fertilization [31,82].
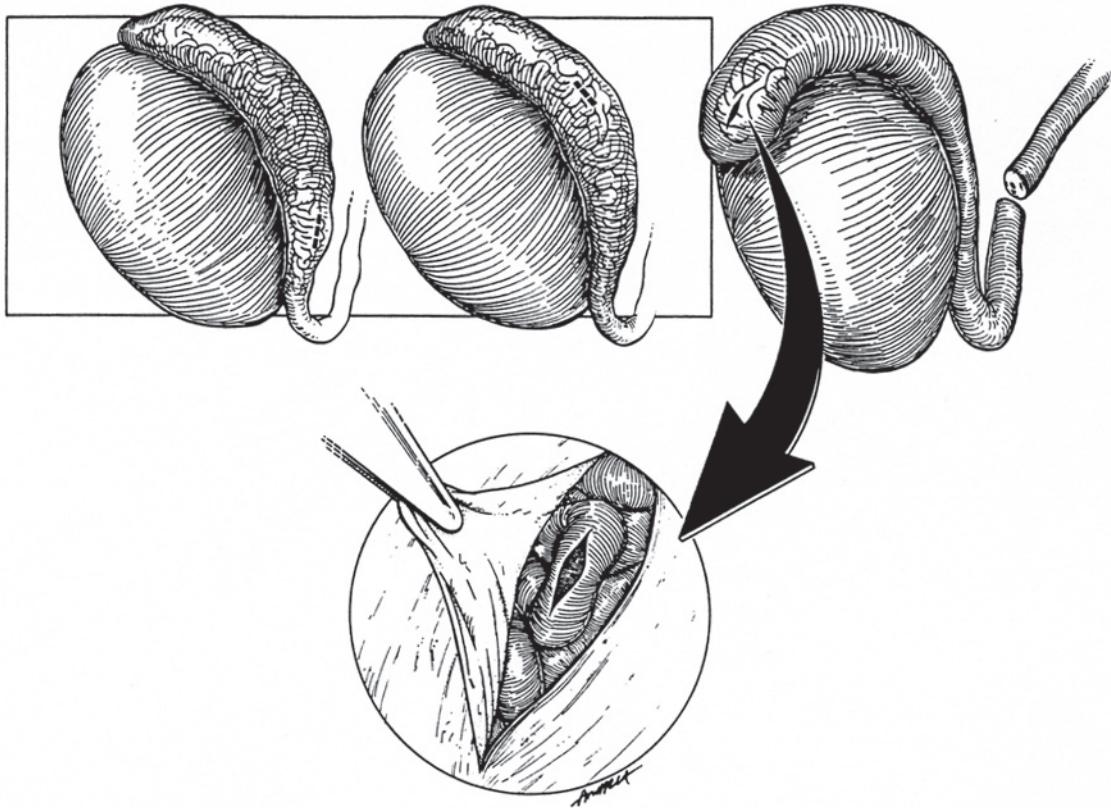
Fig. 9-3. *Small openings are made in the epididymal tunic beginning distally and moving proximally. After a longitudinal slit is made in the epididymal tubule with the microscissors, the distalmost level at which motile sperm are found is used for the anastomosis.*
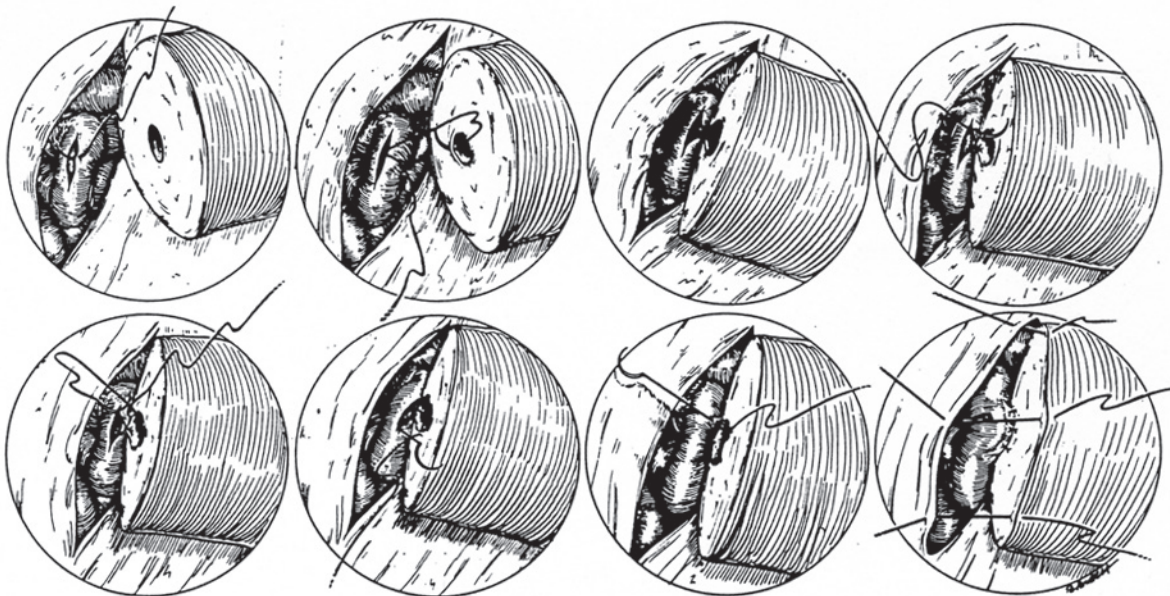
Fig. 9-4. *The end-to-side specific tubule anastomosis of the vas lumen to the epididymal tubule requires first a posterior row of three 10-0 nylon interrupted sutures, followed by an anterior row of three 10-0 nylon interrupted sutures. The muscularis of the vas is then sutured to the outer epididymal tunic with 9-0 nylon interrupted sutures.*

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Newer studies of epididymal sperm transport in the human indicate that the human epididymis is not a storage area, and spermatozoa traverse the entire human epididymis very quickly, in a mere 2 days, not 11 days, as was previously thought [83]. Thus it is possible that in the human, the epididymis may not be as essential to spermatozoa development and fertility as it appears to be in most animals.

Congenital Absence of the Vas Deferens and Sperm Aspiration with IVF

Congenital absence of the vas deferens accounts for 11 to 50% of cases of obstructive azoospermia and heretofore has been considered basically untreatable [84]. This group of patients is large and frustrated; on countless testicle biopsies,

these patients have demonstrated normal spermatogenesis and, theoretically, are making sperm capable of fertilizing an egg. Nonetheless, treatment to date has been dismal [85].

Dr. Ricardo Asch and I have collaborated to develop a treatment protocol involving microsurgical aspiration of sperm from the proximal region of the epididymis, combined with IVF and ZIFT, which now offers very good results in this previously frustrated group of couples [33,51,81,82].

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, 1 mg/day subcutaneously, is administered until the day of follicular aspiration (Fig. 9-5). Patients
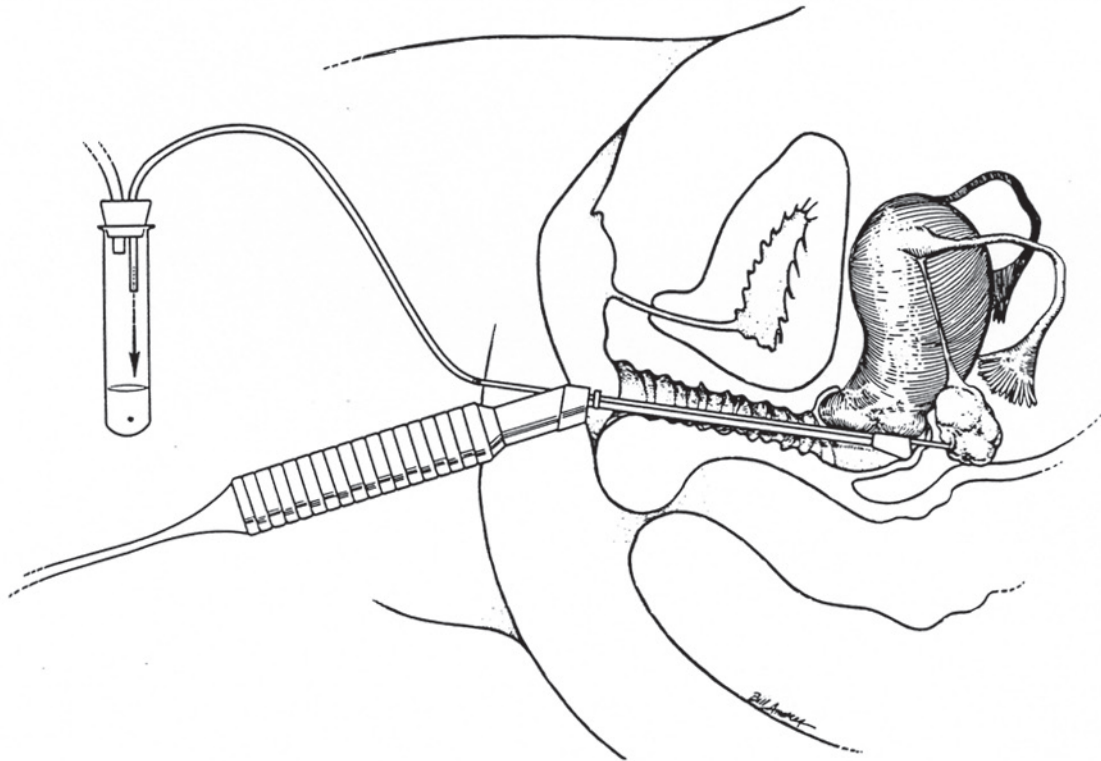

Fig. 9-5. *Placement of the ultrasound probe for transvaginal needle aspiration of eggs.*

receive human follicle-stimulating hormone (FSH) and human menopausal gonadotropins (Pergonal), 150 IU/day intramuscularly, from day 2 of the menstrual cycle until follicles of 2.0 cm are noted on ultrasonography. Human chorionic gonadotropin (hCG), 10,000 IU, is then administered intramuscularly.

Thirty-six hours after hCG administration, the patients undergo follicular aspiration in the operating room under intravenous sedation with titrating doses of 0.1 to 0.25 mg Fentanyl and 5 to 7 mg midazolam hydrochloride.

Follicular aspiration is performed using a transvaginal probe (GE H4222 TV) adapted to an ultrasound system (GE RT 3000; General Electric Company, Milwaukee, WI) with a needle set for ovum aspiration and connected to a Craft Suction Unit (#33-100, Rocket USA, Branford, CT) at a maximum vacuum pressure of 120 mmHg.

Each case of follicular aspiration has been performed without complications in less than 30 minutes, and patients are discharged 2 hours after the outpatient procedure. The follicular fluids and follicular washings are given immediately to the embryology laboratory adjacent to the operating room.

EPIDIDYMAL SPERM ASPIRATION, WASHING METHODOLOGY, AND IVF

At the same time that multiple follicular aspiration is being performed on his partner, the man undergoes scrotal exploration with the intention that sufficient numbers of motile spermatozoa
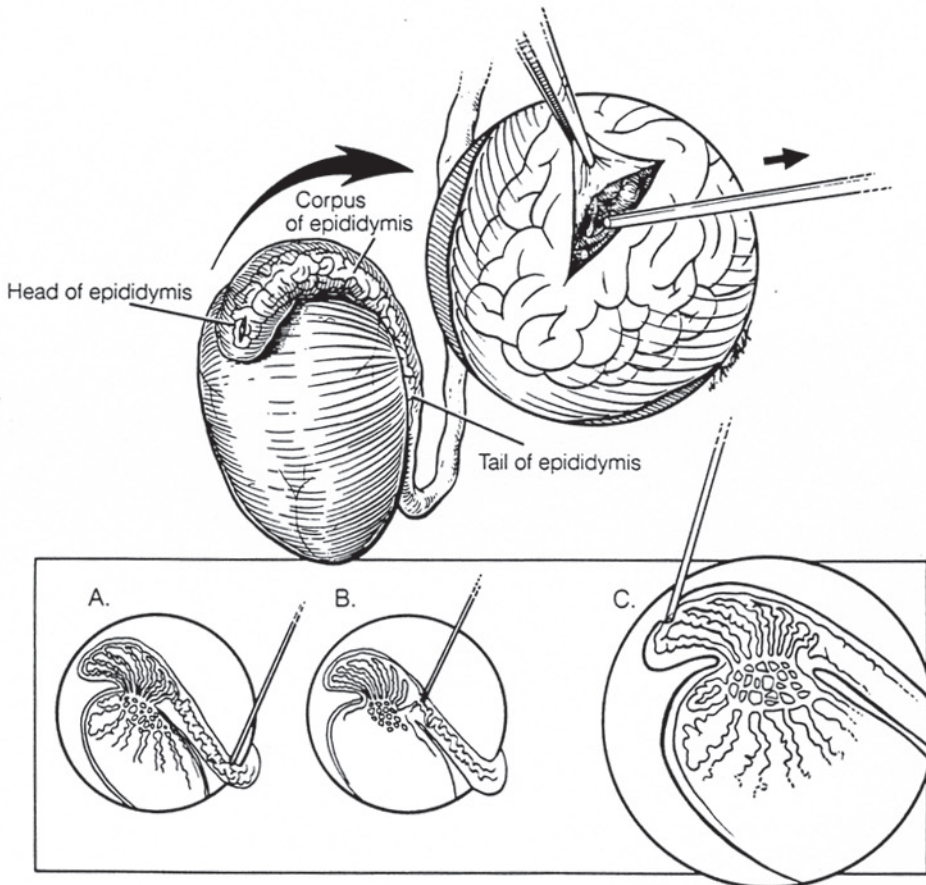

Fig. 9-6. *Technique for epididymal sperm aspiration, which begins in the distal corpus region of the epididymis and moves proximally until motile sperm are recovered. In most cases, motility is observed in only the most proximal region of the epididymis.*

will be aspirated to use for IVF of the aspirated eggs, with subsequent transfer into the partner's fallopian tube. The surgical technique in the male partner is as follows (Fig. 9-6): Scrotal contents are extruded through a small incision, the tunica vaginalis is opened, and the epididymis exposed. Under magnification of 10 to 40 power with an operating microscope, a tiny incision is made with microscissors into the epididymal tunic to expose the tubules in the distalmost portion of the congenitally blind-ending epididymis. Sperm are aspirated with a no. 22 Medicut on a tuberculin syringe directly from the opening in the epididymal tubule. Great care is taken not to contaminate the specimen with blood, and careful hemostasis is achieved with microbipolar forceps. The epididymal fluid is diluted immediately in HEPES buffered media, and a tiny portion is ex-amined for motility and quality of progression. If there is no motility or if it is poor, another aspiration is made 0.5 cm more proximally. We thus obtain sperm from successively more and more proximal regions until progressive motility is found. In all cases, motile sperm were not obtained until we reached the proximalmost portion of the caput epididymis or even the vasa efferentia, the inverse of what might have been anticipated (Figs. 9-6, 9-7).

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 twice; all eggs are insem-inated in a Falcon mini–test tube with 1 ml of HTF culture media and incubated at 37°C with 5% CO_2 in air [45] (Fig. 9-8).
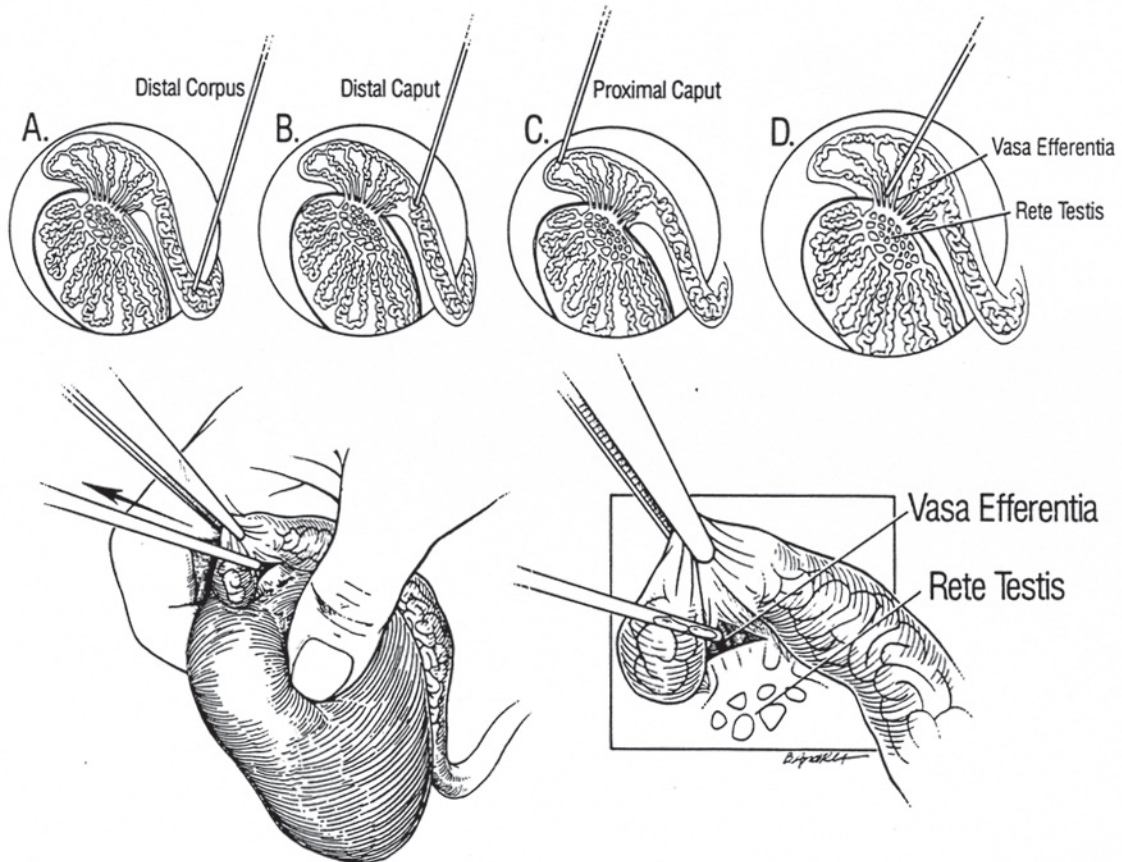

Fig. 9-7. *The most motile sperm are found very proximally, usually in the vasa efferentia or rete testis.*
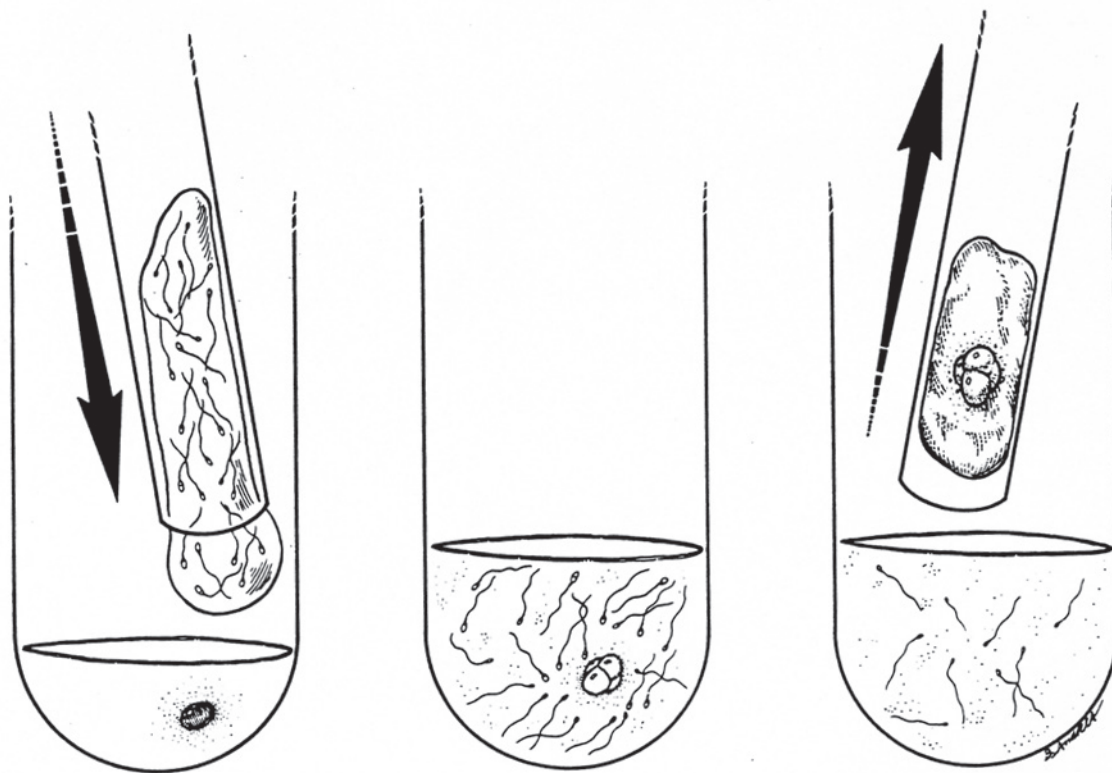
Fig. 9-8. *Sperm being placed in a test tube with egg, resulting in a four-cell embryo 2 days later, and retrieval of the four-cell embryo for transfer back to patient.*

Two days after insemination, embryos are transferred via minilaparotomy to the fallopian tubes of each patient, using a technique similar to the one for GIFT and employing a Tomcat catheter (Monoject, St. Louis, MO) 2.5 cm inside the fimbrial ostium (Fig. 9-9). The patients are discharged the next day and undergo fairly painless postoperative recovery. The wives receive progesterone in oil, 50 mg/day intramuscularly, beginning with the day of embryo transfer.

RESULTS

At present, of 115 cases, there have been 24 pregnancies, with 6 miscarriages, which reflects a pregnancy rate of 22% and a live baby rate of 16% (Table 9-13). Pregnancies that have occurred readily after vasoepididymostomy to the caput epididymis (and even, in some cases, to the vasa efferentia) suggest that immature sperm which have not had a chance to traverse the epididymis might mature on their own during storage in the vas deferens [31,33]. If this theory proves true, it might explain why we have been able to achieve success by aspirating more proximally, not being limited (because of theoretic considerations) to distal regions of the epididymis where sperm are generally senescent and nonmotile in the chronically obstructed state.

Other factors in the success of this technique that may be equally important are (1) obtaining large numbers of oocytes to increase the odds of fertilization, (2) obtaining sperm that are clean and free of erythrocytes, (3) incubation of sperm outside the milieu of the obstructed epididymis,
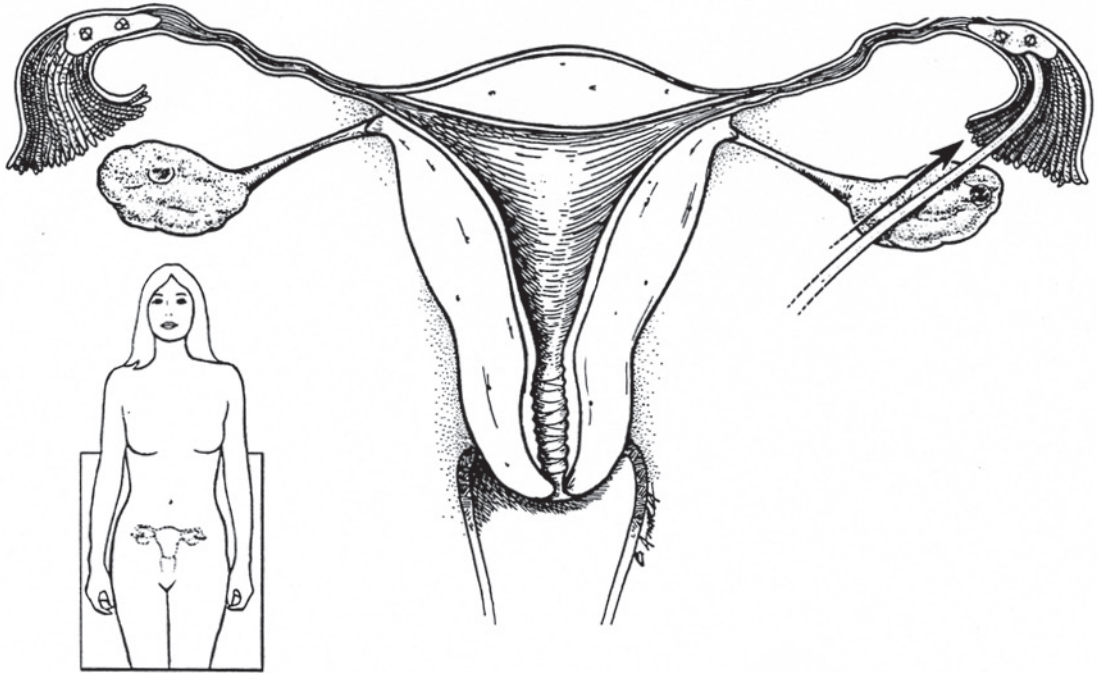
Fig. 9-9. *In the zygote intrafallopian transfer procedure, eggs would have already been fertilized and growing for the previous 2 days in the laboratory and placed in the fallopian tube in a fashion similar to the gamete intrafallopian transfer procedure.*

Table 9-13. *First 100 Cases of In Vitro Fertilization for Congenital Absence of Vas: Pregnancy Rates*

Series No.	No. of Sperm Aspiration Cycles	No. 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%)
Total	100	22 (16)	22% (16%)

*Ongoing pregnancies, not yet delivered.

and (4) transfer of the embryos into the fallopian tube (ZIFT) rather than into the uterus.

Although these results must be considered preliminary until greater numbers are obtained, for the moment it is safe to draw the following conclusions:

1. Sperm from the proximalmost caput epididymis are capable of fertilizing the human egg in vitro.

2. The passage of time after sperm's emergence from the testicle may be adequate for sperm maturation in some cases, without the absolute need for transit through the rest of the epididymis.

3. We now have an approach for achieving pregnancy in couples with a heretofore dismal condition, congenital absence of the vas deferens.

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Progress in Infertility

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