Recent advances in male reproductive surgery

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Male factor infertility has undergone revolutionary changes in the last decade. It is now clear that hormonal therapy (such as clomid, pergonal, human chorionic gonadotropin (hCG), testosterone-rebound and tamoxifen) has no beneficial effect on the infertile male, except of course for the rare case of Kallman's syndrome or pituitary deficiency[1–7]. None the less, 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]. Yet very few infertile men who are referred to a urologist escape this hopeless procedure. Countless papers by clinical urologists attest to the illusion that over 40% of male factor problems are caused by varicocele. Yet there is no shred of evidence for such absurdly high numbers and, in controlled studies, no beneficial effect of varicocelectomy has ever been demonstrated. In fact, 15% of all men, fertile or infertile, have a varicocele and it is of no consequence.

The role of sperm antibodies in male infertility remains highly controversial, but there is very little evidence for its having any major role; immunologic treatment of such men (other than with *in vitro* fertilization (IVF) or gamete intrafallopian transfer (GIFT) has been dismally ineffective anyway[11,12].

So what does that leave us to discuss for the treatment of male infertility? Quite a lot. The most significant advance really came from gynecological endocrinologists who began to call the condition not 'male infertility', but just 'male factor'. We must leave the 1980s forever behind to realize how very far we have come in treating *couples* with male factor infertility. This chapter will be divided into: (1) the causes of male factor infertility, and its simplified evaluation; (2) the processing of male factor sperm for treatment with IVF or GIFT; (3) microsurgery for obstructive azoospermia; and (4) IVF using epididymal sperm for irreversible obstruction and congenital absence of the vas.

EVALUATION OF MALE FACTOR INFERTILITY AND ITS CAUSES

The human male, and the gorilla, have the poorest sperm production of any animal on the face of the earth, and the condition is evolutionary, genetic and unremediable. Whereas most animals produce 20–25 million sperm/g of testicular tissue per day, the human produces only 4 million[13,14]. The gorilla is even worse than that and his testicles and penis are incredibly tiny; they can barely be seen. Even the very 'fertile' human male with over 60 million sperm/ml has terrible sperm when compared to other animals. The large number of abnormal forms, debris, and non-motile sperm found in human semen is just not seen in other animals except, of course, the gorilla. It is thought by comparative biologists that this is due to the lack of 'sperm competition' seen in monogamous animals. Over many thousands of years, the fact that the female will be 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[15]. One need not search for a hormonal or other pathological etiology,

because it is simply an unalterable genetic problem, and no histologically pathological testicular changes are generally seen in men with oligoasthenospermia.

Since poor sperm, to a greater or lesser degree, is the lot of all humans, even those without an infertility problem, we must start with the difficult issue of how we decide if there actually is a male factor problem in the couple. Of course, azoospermia is clearly a different issue, and will be discussed later in the chapter.

What is male factor? The semen analysis

To what extent do standard semen analysis parameters reflect male fertility? In truth, when a couple has been unable to achieve a 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 has been the semen analysis. This method has been much maligned and replaced with very expensive tests such as the hamster egg penetration and sperm antibodies tests, but these tests have actually added more confusion than clarity to 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[12,16].

The most dramatic demonstration of this confusion is the case reported by Sokol and Sparkes where a wife became pregnant naturally from a husband who had only 50 000 spermatozoa/ml with terrible motility and with no treatment[17]. The husband, the mother, and the baby were carefully blood-typed genetically, and it was determined to be 99.99% certain that the husband was indeed genetically the father. So if only 50 000 spermatozoa/ml with less than 10% motility are adequate for a natural pregnancy (without IVF), what, after all, is 'male factor'?

Studies which suggest that low sperm counts indicate 'male factor'

The correlation of sperm count with fertility was originally presented in the famous article by MacLeod and Gold in 1951[18]. These authors studied sperm counts in 1000 'fertile' and 1000 'infertile' men (see Table 1). Their results indicated that 16% of 'infertile' men had sperm counts under 20×10^6/ml, and only 5% of 'fertile' men had counts in that range. It was therefore concluded that any count under 20×10^6 spermatozoa/ml indicated infertility. This conclusion was clearly quite tenuous, but none the less only recently has the weakness of their unfounded assumption been recognized.

Table 1 *Frequency distribution of sperm counts in 1000 fertile men and 1000 infertile men*[18]

Sperm count (millions/ml)	*Fertile men* (%)	*Infertile men* (%)
20	5	16
20–39	12	13
40–59	12	11
60	71	60

Table 2 *Frequency distribution of sperm counts in husbands of infertile couples*[20]

Sperm count (millions/ml)	*Infertile couples* (%)	*Fertile couples* (%)
< 10.01	28.5 } (42%)	11.7 } (23%)
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

The possibility needed to be addressed that low sperm counts, like high sperm counts, might occur at either end of the bell-shaped population curve and might perhaps be unrelated to the man's fertility. In 1974, Nelson and Bunge reported on 386 'fertile' men and found that 20% had sperm counts of less than 20×10^6/ml, and only 28% had sperm counts greater than 60×10^6/ml. Thus, less than 20×10^6 sperm/ml does not indicate a male factor[19]. However, in 1977, Zukerman and colleagues, along with Steinberger's group studied several thousand 'fertile' men presenting for a vasectomy[20,21] (see Table 2). They did show a direct relationship of the numerical sperm count to the chances of pregnancy for the couple. Also, the motile sperm count not only correlated directly with the chances of pregnancy, but inversely with the number of cycles required for the wife to con-

Table 3 *Mean number of cycles to conception related to sperm motility*[37]

Motile count (millions/ml)	*Mean number of cycles to conceive*
< 5	11.0
5–20	9.4
20–60	8.0
> 60	6.0

ceive (see Table 3). When the motile sperm count was less than 5×10^6/ml, the wife required a mean of 11 months to become pregnant, whereas when the motile count was over 60×10^6 spermatozoa/ml, the wife required a mean of 6 months to get pregnant. But despite a lower motile sperm count being associated with a longer mean time until pregnancy, 11% of men of proven fertility presenting for a vasectomy were found to have sperm counts below 10×10^6/ml. It is therefore essential to be cautious when suggesting to any infertile couple with a poor semen analysis that the husband is infertile. But severely lower sperm counts and motility are simply associated with decreased fertility on a large statistical population basis.

The author has also reviewed sperm count and motility indices following vasovasostomy in men whose wives became pregnant, in comparison to those whose wives did not become pregnant[11] (see Table 4). The distribution of sperm counts, percentage motility, and total motile sperm per ejaculate were quite similar to that of Zukerman and Steinberger's group[20,21]. Of the 'successful' vasovasostomy patients whose wives became pregnant, 11% had total sperm counts per ejaculate of less than 10×10^6; 64% had more than 40×10^6 spermatozoa/ml. If this is compared to the group of 'infertile' men after vasovasostomy, there are no significant differences except for sperm counts below 10×10^6/ml. In the group of men whose wives did not become pregnant, 23% had total sperm counts per ejaculate of less 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 co-workers 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)[21] (see Table 5). When there were fewer than 5 or 10×10^6 motile spermatozoa/ml, approximately 30% of the couples eventually achieved a pregnancy. When the motile sperm count/ml 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 would ultimately conceive.

Baker and colleagues in 1986[22] compiled data on a graph in combination with the data of

Table 5 *Relationship of sperm count of the male to pregnancy rate among infertile couples*[21]

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

Table 4 *Frequency distribution of motile sperm count following vasovasostomy in men who did or did not impregnate their wives (10-year follow-up)*[11]

Total motile sperm count (millions per ejaculate)	*Total patients* (%)	*Number pregnant* (%)	*Number 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)
Totals	258 (100)	228 (100)	30 (100)

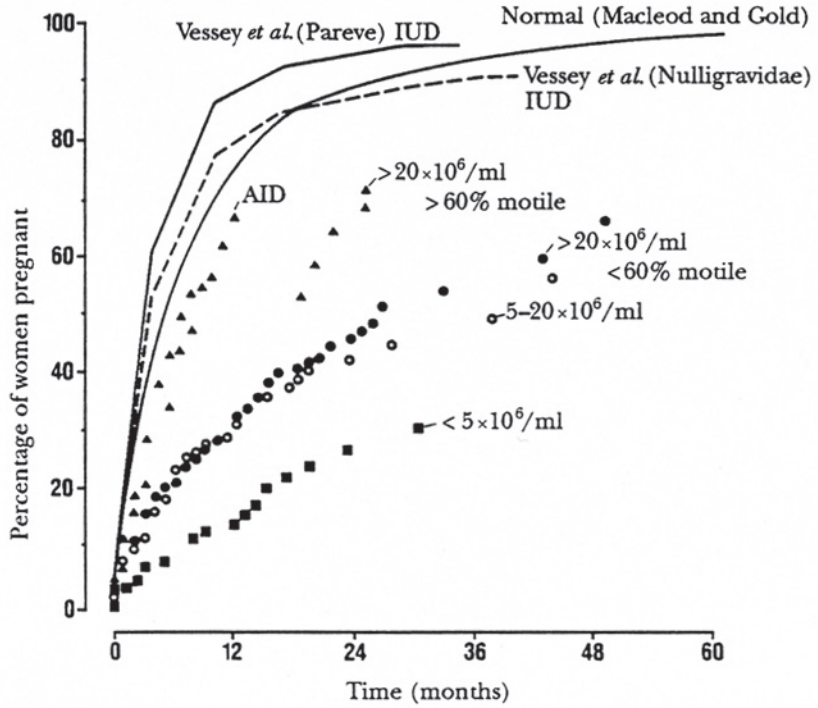

Figure 1 *Cumulative and lifetable pregnancy rates in relation to sperm count (from reference 22); IUD = intrauterine device; AID = artificial insemination by donor*

Kovacs and co-workers in 1982[7], Vessey and colleagues in 1976[23] and MacLeod and Gold in 1953[24], and constructed a life table pregnancy curve for infertile couples with varying degrees of oligospermia compared to controls (see Figure 1). 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 of less than 5×10^6/ml, 5–20×10^6/ml, greater than 20×10^6/ml with less than 60% motile, and greater than 20×10^6/ml 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 the pregnancy rate table from Vessey's study[23] of the mean time to pregnancy in women discontinuing the intrauterine device. Normal was assumed to be the spontaneous pregnancy rate reported in 1951 by MacLeod and Gold[18].

Again, quite remarkably, with fewer than 5×10^6 spermatozoa/ml 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/ml, the pregnancy rate at 2 years was about 43%, and at 3 years was 50%. When the sperm count was over 20×10^6/ml, though less than 60% motile, the pregnancy rate at 2 years was similar to the results obtained when the count was between 5 and 20×10^6/ml. When the sperm count was essentially 'normal' (that is, over 20×10^6/ml with greater than 50% motility), the pregnancy rate at 2 years was about 72%. In conclusion, women may become pregnant when sperm counts are extremely low, but the higher the motile sperm count, the greater are the chances of pregnancy.

Pregnancy rates with donor insemination in wives of oligospermic men vs. wives of azoospermic men

In 1982, Emperaire and colleagues published a remarkable observation from their donor insemination program[25]. Women undergoing

Table 6 *Pregnancy rate using donor insemination in wives of azoospermic vs. oligospermic men*[25]

Husband	*n*	*Pregnancy rate*	*Pregnancy rate per cycle*
Azoospermic	95	61%	11.6%
Oligospermic	95	29%	4.9%

artificial insemination by donor 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 from donor insemination, but the success rate was only 29% in those whose husbands were oligospermic (see Table 6).

The only reasonable speculation to explain 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 was 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.

IN VITRO FERTILIZATION AND GAMETE INTRAFALLOPIAN TRANSFER WITH 'MALE FACTOR'

Great excitement has been generated over the use of IVF in couples with very low sperm counts, and good fertilization rates have been obtained in many severely oligospermic couples[26]. Theoretically, IVF or GIFT allows the fewer number of available sperm a greater opportunity for direct contact with the ovum[27,28].

In cases of congenital absence of the vas, a collaborative study has been reported by the author and Ricardo Asch's group to aspirate relatively weak sperm from the epididymis, fertilize the wife's oocytes *in vitro*, and place the resultant embryos into the Fallopian tubes (zygote intrafallopian transfer (ZIFT)[29–31]. A good number of resultant pregnancies in this group has led us to conclude that sperm with severely reduced motility can often fertilize the oocyte if other obstacles are eliminated.

Rodriguez-Rigau and co-workers, in 1989, evaluated the semen parameters of a large number of couples who did or did not achieve a pregnancy with GIFT[16] (see Table 7). They found a correlation of the pregnancy rate with GIFT to standard semen parameters such as count and motility. The hamster 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. However, in our patients with epididymal sperm, the correlations were much more subtle. There was some correlation to computerized analysis of sperm motion, but it was very complex[32].

In 1987, Kruger and colleagues first reported the importance of morphology of spermatozoa in husbands of patients undergoing IVF[33]. Any spermatozoa with a slight defect such as neck droplets, bent necks, abnormal heads and so on was considered to be abnormal. Also, any deviation from a perfect oval head was considered to be abnormal. Remarkably, provided that at least 4% of spermatozoa demonstrated perfectly normal morphology, fertilization *in vitro* was

Table 7 *Relationship of total motile sperm count and of linearity of sperm movement to GIFT pregnancy rate*[16]

	Clinical pregnancy (%)
Total motile count ($\times 10^6$) per ejaculate	
< 25	7.7
25–100	15.2
> 100	24.1
Motility index	
< 0.8	12.2
> 0.8	27.6

achieved. Once again, this suggests that a critical visual examination of the husband's spermatozoa is a good predictor of the likelihood of fertilization, and not a very large population of 'fertile' sperm within a poor semen sample is necessary.

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 pre-wash motile sperm count in the semen is not a heavily significant determinant of fertilization, or of 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 morphologically normal, motile sperm count 'post-wash'. In our experience, when the total motile sperm count recovered from a Percoll or mini-Percoll prep post-wash is greater than 1.5×10^6 motile sperm, the fertilization rate is not significantly different from patients with higher numbers of recoverable sperm. When the post-wash motile sperm count is less 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 less than 5×10^6 (classified as severe oligoasthenozoospermia)[34], the conventional methods of sperm preparation such as swim-up[35], wash and resuspension[36] and sedimentation[37] 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 poor IVF results[38–40].

The resuspension method does not remove cells and debris, while Percoll gradient techniques, although very effective in filtering out debris and other contaminants, often give a low rate of sperm recovery[41] in very poor samples.

In an attempt to identify a method that could be used for these difficult cases, a modified Percoll technique, mini-Percoll, which consists of a reduced volume, discontinuous Percoll gradient, was developed by Ord and colleagues[42,43]. Semen samples are diluted 1 : 2 with culture medium and centrifuged at 200 g for 10 min. Following centrifugation, pellets are suspended in 0.3 ml of 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/l of sodium bicarbonate. To obtain the 95, 70 and 50% laters, this isotonic solution is 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 30–45 min. Following centrifugation of the gradient, the 95% Percoll layer is removed, washed twice and resuspended in 1 ml of HTF and 10% serum and incubated until the time of insemination.

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. Several advantages can be associated with the use of a mini-Percoll gradient: (1) the reduced volume of each Percoll layer allows better 'migration' of spermatozoa; (2) 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; and (3) the use of mini-Percoll allows recovery of a high proportion of the few normal and motile spermatozoa present in the sample.

Because the definition of 'male infertility' is so blurred, it is absurd to give 'pregnancy rates' for male infertility using IVF or GIFT, as all of the blurred urological varicocele papers do. However, it can be said that in infertile 'couples' whose post-wash motile sperm recovery is

greater than 1.5×10^6 motile sperm, the pregnancy rate with IVF, GIFT or ZIFT in patients with 'male factor' is no different from whatever that center is obtaining otherwise with its infertile couples who don't have 'male factor'.

Beyond the preparation of sperm for IVF, GIFT and ZIFT for cases with severe 'male factor' infertility, other chapters in this book will go into further detail regarding IVF and GIFT which need not be elaborated here. But it is clear that for the present decade, IVF technology will be the only valid treatment for most cases of male factor infertility, except for 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 eventually lead to more successful vasoepididymostomy results in post-inflammatory 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[44]. For many years the pregnancy rate after surgical reanastomosis of the vas had been very low. A variety of explanations has been offered for the relatively poor success in reversing vasectomy[45–47]. With the advent of microsurgical techniques, pregnancy rates improved considerably, suggesting that purely micro-mechanical factors were responsible[48–50]. Yet there still were many cases of technically perfect vasovasostomies followed by complete azoospermia or oligoasthenospermia with no pregnancy. We then found that the pressure increase after vasectomy had led to secondary epididymal obstruction which was the cause of failure of otherwise perfectly successful vasovasostomies. The greater the duration of time since vasectomy the greater the chance of either 'blowouts' or 'inspissation' in the epididymis, with subsequent failure to achieve fertility. Thus, vasoepididymostomy is required in many cases of vasectomy reversal in order to obtain a high success rate.

Theories for the consistently poor results with vasectomy reversal had included development of sperm antibodies, damage to the deferential nerve, and testicular damage[51–58]. Yet we have questioned any major correlation between sperm antibodies and subsequent fertility after vasovasostomy[59]. We have established that the deleterious effect of pressure increase subsequent to vasectomy was not in the testis, but rather on epididymal dilatation, perforation and sperm inspissation and blowouts in the epididymis, causing secondary epididymal obstruction which is the major problem in readily returning fertility to vasectomized men[11,48,50]. 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[60,61]. In humans, this deleterious effect of pressure increase is *always* on the epididymis, not the testis. In fact, the secondary epididymal obstruction caused by vasectomy leads us to recommend that, when performing vasectomy, the testicular end of the vas should not be sealed, so as to lessen the pressure build-up and possibly increase the ease of reversibility later (notwithstanding the potentially worrisome immunological consequences)[62–64].

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]. A total of 326 men who had been previously vasectomized underwent vasovasostomy and received extensive long-term follow-up. In 44 of those men who underwent vasovasostomy, no sperm was found in the vas fluid. All such patients have been found to be azoospermic after vasovasostomy and required vasoepididymostomy later.

The vasovasostomy involved a meticulous, two-layer microsurgical technique performed by the same surgeon with accurate mucosa-to-mucosa approximation[48]. Almost all of the patients had proven prior fertility as evidenced

Table 8 *Overall long-term pregnancy rates in patients undergoing vasovasostomy; 10-year follow-up (sperm seen in vas fluid)*

	Combined 1975 and 1976–77 series	*Original 1975 series*
Total patients	282 (100%)	42 (100%)
Total pregnant	228 (81%)	32 (76%)
Azoospermic	24 (9%)	5 (12%)

Table 9 *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*
$0–10 \times 10^6$	32 (12)	25 (78)	7
$10–20 \times 10^6$	31 (12)	27 (87)	4
$20–40 \times 10^6$	32 (12)	30 (94)	2
$40–80 \times 10^6$	79 (31)	68 (86)	11
$> 80 \times 10^6$	84 (33)	78 (93)	6
Totals	258 (100)	228 (88)	30

Table 10 *Pregnancy rate according to percentage 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 (87%)	11
60–80	62	55 (89%)	7
> 80	20	18 (90%)	2
Totals	258 (100%)	228 (88%)	30

by previous fatherhood. All patients were followed for 9 or 10 years.

The overall, long-term pregnancy rate and sperm patency rate are summarized in Table 8. None of the wives of azoospermic patients became pregnant. If azoospermic patients are excluded, 88.4% of patients with postoperative sperm patency eventually impregnated their wives. This compares to Vessey's expected pregnancy rate of 96% for previously fertile couples discontinuing contraception[23].

The frequency distribution of postoperative semen parameters in men who did and did not impregnate their wives is summarized in Tables 9 and 10. There was remarkably little difference

Table 11 *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 (100.0)	42 (14.8)	240 (85.2)

in pregnancy rate among men with low or high sperm counts. Similar findings were seen with sperm motility so long as it 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 pre-vasectomy semen parameters[20].

As summarized in Table 11, a left-sided varicocele was clinically apparent in 42 of the 282 patients (14.8%). Varicoceles were not operated on, and yet the pregnancy rate was not significantly different in patients with varicocele as opposed to patients without varicocele. Table 12 summarizes the relationship of preoperative serum antisperm antibody titers to the pregnancy rate after vasovasostomy. Similar to varicocele, the presence of high serum immobilizing titers or agglutinating titers had no influence on the pregnancy rate.

Reason for high pregnancy rate in patients with no secondary epididymal blockage

The high pregnancy rate in this group of patients requires some explanation. There have been many speculations for the 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 who have patency accurately re-established without epididymal damage is eventually not significantly less than a normal population of couples. Vessey demonstrated that among couples with proven prior

Table 12 *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 (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)

fertility, 96.5% conceive within 4 years of discontinuing contraception. In our couples with patent results after vasovasostomy who had no evidence of epididymal pressure damage, 88% conceived with long-term (10 years) follow-up. However, 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[48]. The decrease of success with longer duration of time since vasectomy is directly related to the absence of sperm in the vas fluid at the time of vasovasostomy, and this is caused by the interruption of epididymal patency by pressure-induced sperm extravasation and inspissation[60]. 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[50,62,63]. When there is no sperm in the vas fluid, vasoepididymostomy proximal to the site of epididymal blockage is required[65,66].

It thus appears that the fertility rate and pregnancy rate are considerably higher in those patients with no epididymal blockage who undergo technically 'successful' vasovasostomy.

Vasoepididymostomy

When vasectomy has produced secondary epididymal blockage, or in cases of post-inflammatory obstructive azoospermia, very precise microsurgical tubule-to-tubule vasoepididymal anastomosis is required. But just as important as precise microsurgical technique is a practical understanding of epididymal physiology[49,50,65–67].

In every animal that has been studied, spermatozoa from the caput epididymis are capable only of weak circular motion at most, and are not able to fertilize[68]. 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[69–71]. Some of these previous animal studies allowed the spermatozoa time to mature (by ligating the epididymis in these animals) and thereby possibly develop fertilizing capacity. Yet when the epididymis was ligated to determine if time alone could allow spermatozoa maturation, the obstructed environment was so pathological (and the sperm became so senescent) that no firm conclusions could be reached. Thus, the outlook for vasoepididymostomy seemed theoretically poor.

In 1969, Orgebin-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 just require time, or whether spermatozoa must transit through most of the epididymis in order to mature[68]. It was entirely possible that aging alone might mature the spermatozoa, and that spermatozoa might not need to pass through all of the epididymis in order to develop the capacity to fertilize. Yet because of the animal studies alluded to, and poor results in humans using non-microsurgical techniques, it has always been assumed that epididymal blockage carries a poor prognosis[72–75].

As far back as 1931, however, Young's experiments in guinea pigs with ligation at various levels of the epididymis indicated to 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[76]. In fact, he observed the same 'inversion' of regions of sperm motility and non-motility 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 have the best motility. Young concluded that, in an obstructed epididymis, the more distal sperm are senescent, while 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[77].

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[48,65,67]. Because of the high rate of technical failure with older surgical methodology, reliable data on the fertility of spermatozoa from the epididymis in the past had been difficult to obtain.

The anastomosis of the vas to the epididymis is performed at the transition point from no spermatozoa to the point where there is an abundant amount of spermatozoa in the fluid coming from the epididymal tubule (Figures 2 and 3). 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 separately sutured to the outer epididymal tunic with 9-0 nylon interrupted sutures.

Of the cases of epididymis anastomosis, 72% have resulted in eventual pregnancy[77]. The younger the wife, the higher was the pregnancy rate. The pregnancy rate was not related to the numerical sperm count, but was related to the motility. The fact that pregnancy occurred in cases patent to the caput indicates that transit beyond the head of the epididymis is not an absolute requirement for spermatozoa to attain fertilizing capacity.

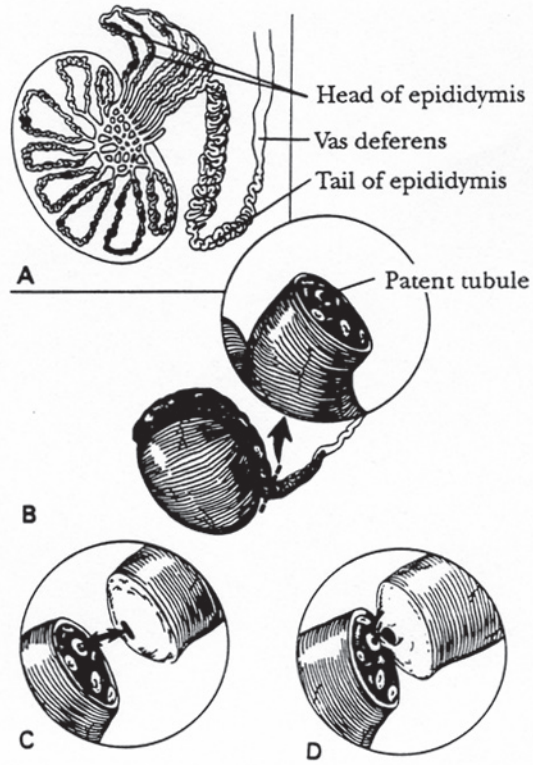

Figure 2 *Specific tubule end-to-end anastomosis of the vas lumen to the epididymis proximal to site of obstruction*

Recent clinical cases have demonstrated that it is even possible in some circumstances for spermatozoa which have never transited any length of epididymis to fertilize the human egg. In two cases reported of vasa efferentia to vas deferens anastomosis, the postoperative ejaculate contained normally motile sperm, and the wives became pregnant[78]. In addition, pregnancy from aspiration of epididymal sperm combined with *in vitro* fertilization and ZIFT in cases of irreparable obstruction gives further evidence that transit through the epididymis is not a mandatory requirement for fertilization[29,79].

Newer studies of epididymal sperm transport in the human indicate that the human

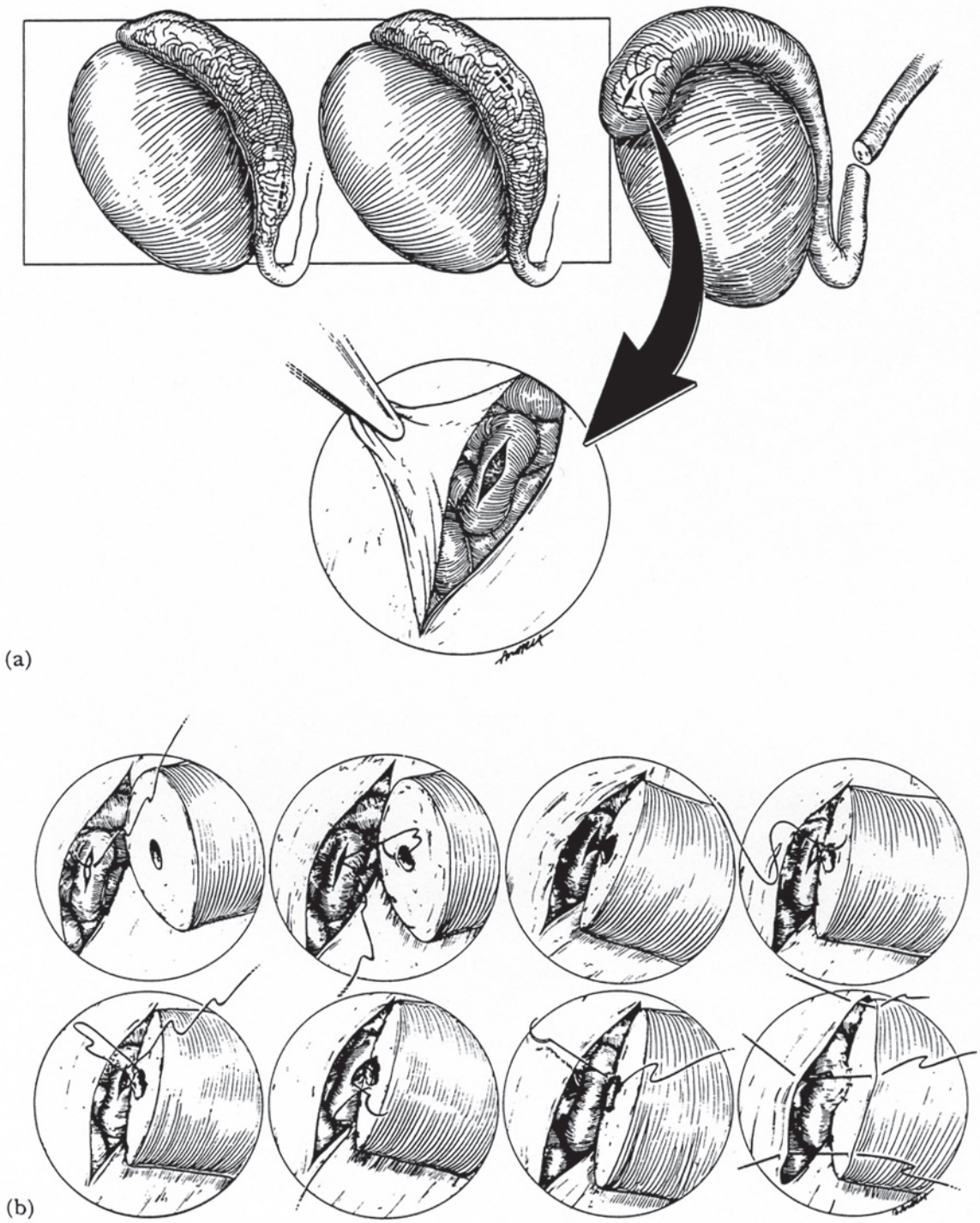

Figure 3 *(a) Small openings are made in the epididymal tunic beginning distally and moving proximally. After a longitudinal slit is made in the epididymal tubule with the microscissors, the distal-most level at which motile sperm are found is used for the anastomosis; (b) The end-to-side specific tubule 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*

epididymis is not a storage area and spermatozoa transit the entire human epididymis very quickly, in a mere 2 days, not 11 days as was previously thought[80]. 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 11–50% of cases of obstructive azoospermia, and heretofore has been considered basically untreatable[81]. This is a large and frustrating group of patients who have been shown on countless testicle biopsies to have normal spermatogenesis, and are theoretically making sperm quite capable of fertilizing an egg[82]. Yet treatment up until the present time has been very dismal[83].

Dr Ricardo Asch and the author have collaborated to develop a treatment protocol involving microsurgical aspiration of sperm from the proximal region of the epididymis, combined with *in vitro* fertilization and zygote intrafallopian transfer, which now offers very good results in this previously frustrating group of couples[31,48,78,79].

Induction of follicular development and oocyte retrieval

The female partners of men with azoospermia caused by congenital absence of the vas undergo induction of multiple follicular development with the following protocol: leuprolide acetate (Lupron, TAP Pharmaceuticals, North Chicago, IL) 1 mg subcutaneously daily until the day of follicular aspiration. Patients then receive human follicle stimulating hormone (FSH) (Metrodin, Serono Laboratories, Inc., Randolph, MA) and human menopausal gonadotropins (hMG) (Pergonal, Serono) 150 IU intramuscularly daily from day 2 of the menstrual cycle until many follicles of 2.0 cm are noted on ultrasound. Then human chorionic gonadotropin (Profasi, Serono, Randolph, MA) 10 000 IU is administered intramuscularly.

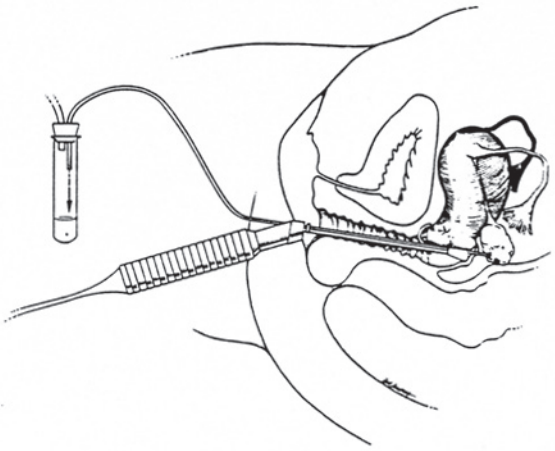

Figure 4 *Illustration of placement of the ultrasound probe for transvaginal needle aspiration of eggs*

Thirty-six hours after hCG administration, the patients undergo follicular aspiration in the operating room (see Figure 4). 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 (Rocket USA, Branford, CT) (33-100) at a maximum vacuum pressure of 120 mmHg. 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, the husband undergoes scrotal exploration with the intention of aspirating sufficient numbers of motile spermatozoa to utilize for IVF with the wife's eggs, with transfer of subsequent embryos into the wife's Fallopian tube.

The surgical technique (Figure 5) in the male is as follows: scrotal contents are extruded through a small incision, the tunica vaginalis is opened and the epididymis is exposed. Under 10–40 × magnification with an operating microscope, a tiny incision is made with microscissors

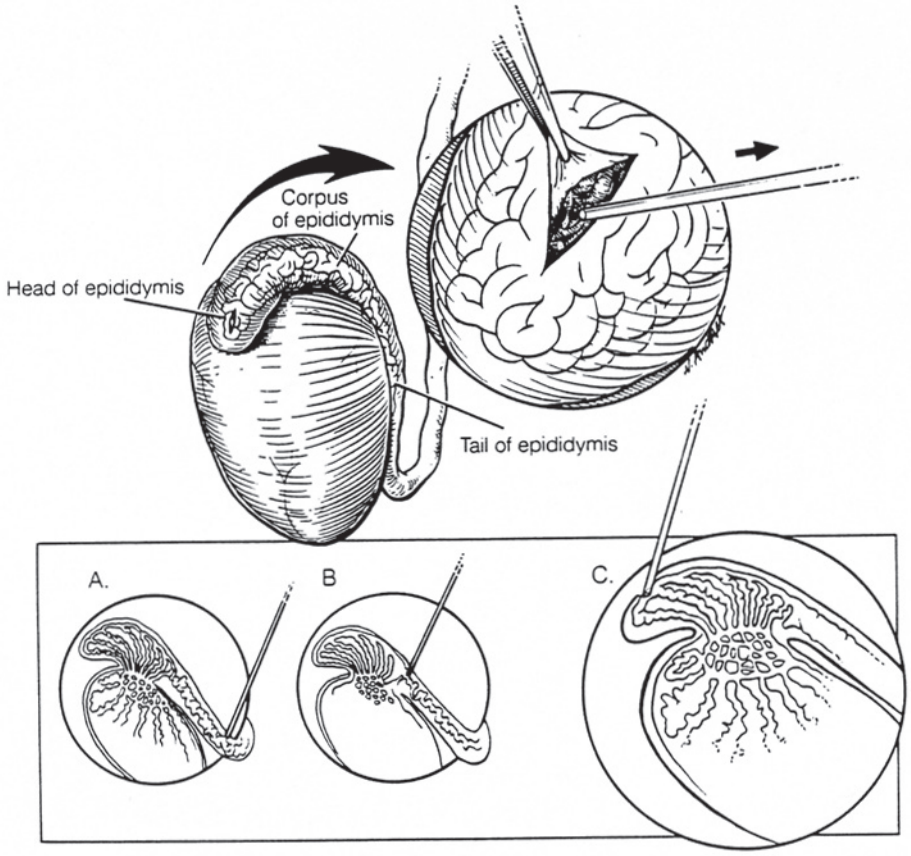

Figure 5 *Technique for epididymal sperm aspiration which begins in the distal corpus region of the epididymis, and moves proximally until motile sperm are recovered. In most cases, motility is observed only in the most proximal region of the epididymis*

into the epididymal tunic to expose the tubules in the distal-most portion first of the congenitally blind-ending epididymis. Sperm are aspirated directly from the opening in the epididymal tubule with a 22 medicut on a tuberculin syringe. Great care is taken not to contaminate the specimen with blood, and careful hemostasis is achieved with microbipolar forceps. The epididymal fluid is immediately diluted in HEPES buffered media, and a tiny portion examined for motility and quality of progression. If there is no motility or poor motility, another aspiration is made one-half centimeter more proximally. We thus obtain sperm from successively more and more proximal regions until progressive motility is found. We have found that motile sperm are not obtained until we reach the proximal-most portion of the caput epididymis or even the vasa efferentia, the inverse of what might have been anticipated (Figures 5 and 6).

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

In the laboratory, the epididymal sperm is concentrated into a volume of 0.3 ml, layered on a discontinuous mini-Percoll gradient, and

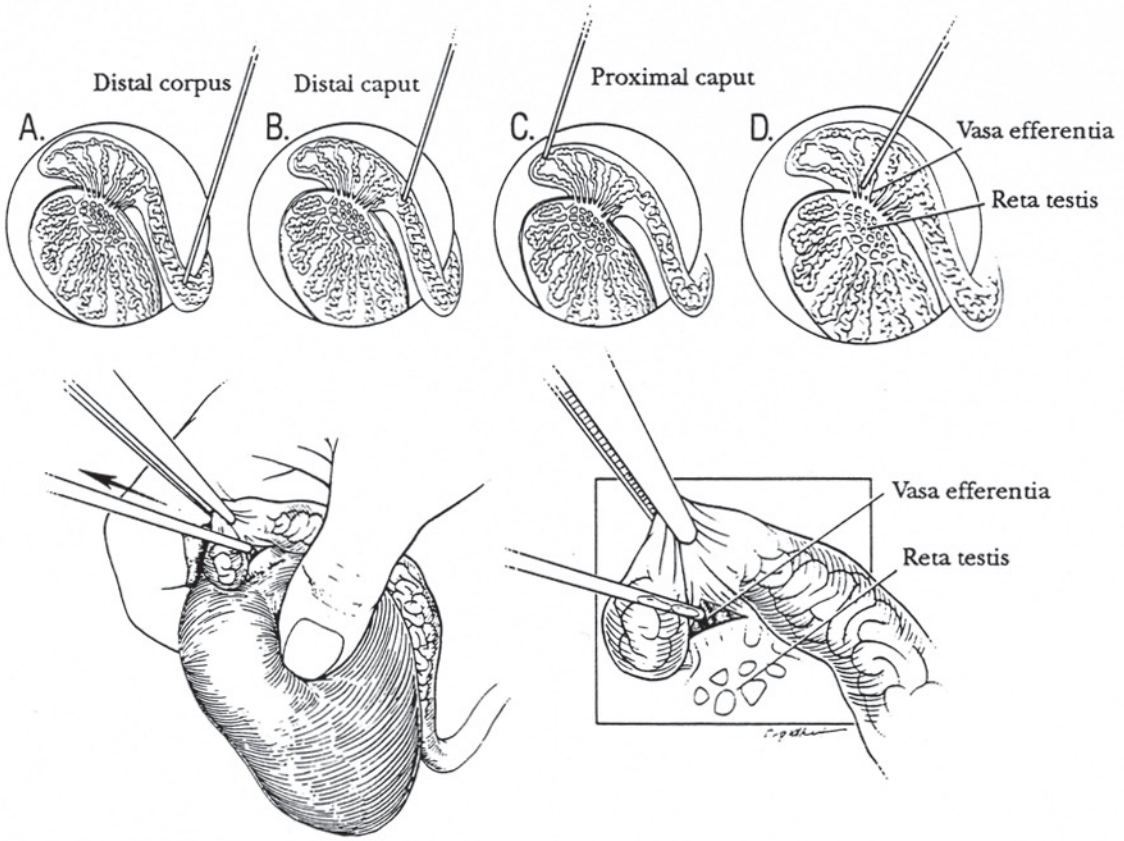

Figure 6 *The most motile sperm are found very proximally, usually in the vasa efferentia or rete testis*

centrifuged for 30 min. The entire 95% fraction is then washed × 2 and inseminated with all of the eggs in a Falcon mini-test tube with 1 ml of HTF culture media and incubated at 37°C with 5% CO_2 in air[43] (see Figure 7).

Two days after insemination, embryos are transferred to the Fallopian tubes of each patient via minilaparotomy using a technique similar to the one for gamete intrafallopian transfer, via a Tomcat catheter (Monoject, St Louis, MO) 2.5 cm inside the fimbrial ostium (see Figure 8). The patients are discharged the next day and undergo fairly painless postoperative recovery. The wives receive progesterone in oil, 50 mg intramuscularly per day beginning with the day of embryo transfer.

Results

At present, of 115 cases, there have been 24 pregnancies, with 6 miscarriages. That is a pregnancy rate of 21% and a live baby rate of 16% (see Table 13). Embryos are obtained in 59% of the cases (see Table 14), but in only about half of those cases are more than two embryos achieved. The overall fertilization rate is only 24% per mature egg. In patients where more than two embryos are achieved, the pregnancy rate per tubal transfer is very high (55%) (see Table 15). When the wife produces more than

Table 13 *First 100 cases of in vitro fertilization for congenital absence of the vas: pregnancy rates*

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

Table 14 *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%

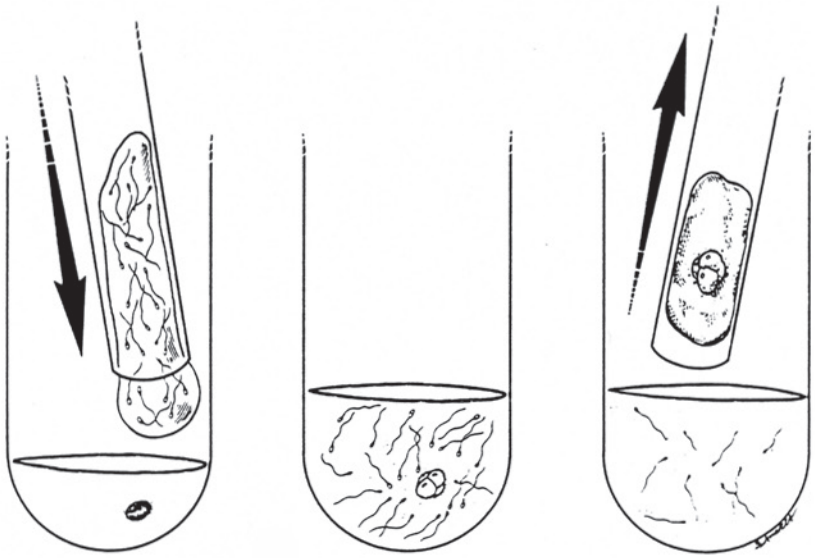

Figure 7 *Sperm being placed in test tube with egg, resulting in 4-cell embryo 2 days later, and retrieval of 4-cell embryo for transfer back to patient*

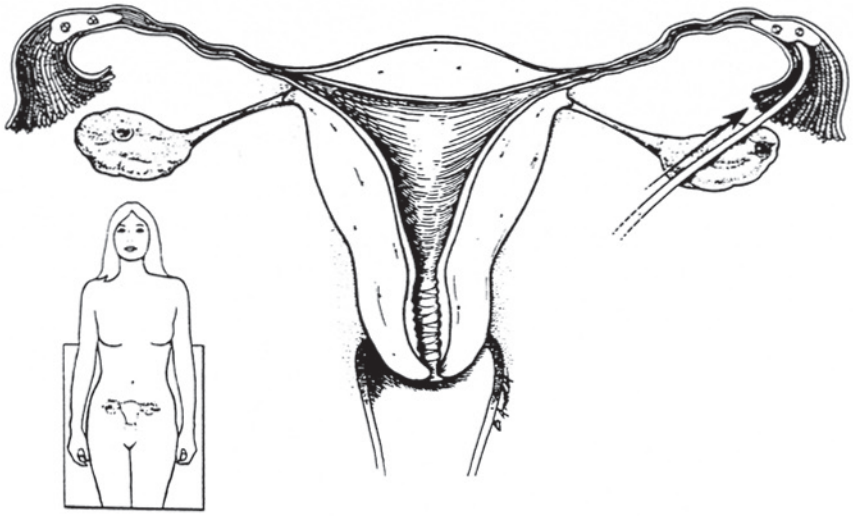

Figure 8 *In the ZIFT procedure eggs would have already been fertilized and growing for the previous 2 days in the laboratory and placed in the Fallopian tube in a similar fashion to be GIFT procedure*

Table 15 *First 100 cases in vitro fertilization for congenital absence of vas: results when more than two embryos are produced*

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

Table 16 *Fertilization rates in congenital absence of vas in cycles producing ten or more eggs (first 100 cases)*

Series number	*Number of cycles (%)*	*Number of mature eggs*	*Number of embryos (%)*	*Pregnancy (%)*
1	20 (63)	290	82 (28)	10 (50)
2	7 (44)	132	43 (33)	2 (29)
3	13 (62)	266	51 (19)	5 (38)
4	11 (85)	205	6 (3)	0 (0)
5	14 (78)	260	104 (40)	5 (36)
Totals	65 (65)	1153	286 (25)	22 (34)

ten eggs in her stimulation cycle, the overall fertilization rate is still only 25%, but the pregnancy rate is 34% per cycle. In those patients who yield more than one embryo, the fertilization rate is 44% per cycle (see Tables 16 and 17).

Thus, it appears that there are two subpopulations of patients with congenital absence of the vas with regard to fertilizing ability: there are those who fertilize many of their wives' eggs and have a high pregnancy rate, and those who fertilize only a few or none of their wives' eggs, and have a low pregnancy rate.

Pregnancies which 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 transit the epididymis might mature on their own during storage in the vas deferens[31,78,79]. If this theory were true, it might explain why we have been able to achieve success by aspirating more proximally, not being limited (because of theoretical considerations) to distal regions of the epididymis where the sperm are

Table 17 *Fertilization rates in congenital absence of vas in cycles producing more than one embryo (first 100 cases)*

Series number	*Number of cycles (%)*	*Number of mature eggs*	*Number of embryos (%)*	*Fertilization rate*
1	17 (53)	222	89	40%
2	7 (44)	125	51	41%
3	11 (52)	181	56	31%
4	2 (15)	15	7	47%
5	8 (44)	150	103	69%
Totals	45 (45)	693	306	44%

generally senescent and non-motile in the chronically obstructed state.

Other factors which may be equally important in the success of this technique are: (1) obtaining large numbers of oocytes in order to increase the odds of fertilization, (2) obtaining sperm which are clean and free of erythrocytes, (3) incubation of sperm outside the milieu of the obstructed epididymis, and (4) transfer of the embryos into the Fallopian tube (ZIFT) rather than into the uterus.

It is clear from our published results that distal epididymal sperm in obstructed males is either non-motile or totally degenerate, due to senescence[31,84,85]. 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, and those that do not. It is remarkable that epididymal sperm that look adequate often do not fertilize, and those that look poor may very well have high fertilizing capacity. It is very difficult to predict which sperm will and which sperm will not fertilize. The presence or absence of sperm antibodies seems to have no impact[51,52,54,55,58,64,86]. However, 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[31]. Thus a routine glance at the sperm often shows no difference, but it may just be a tiny occasional fraction of all the sperm present that may be necessary to produce fertilization.

For the moment it is safe to conclude that: (1) sperm from the proximal-most caput epididymis are capable of fertilization of the human egg *in vitro*; (2) passage of time after 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; and (3) we now have an approach for achieving pregnancy in couples with a heretofore dismal condition, congenital absence of the vas deferens.

There are two possible explanations for the low fertilization rates obtained with epididymal sperm from men with congenital obstruction, and one of those explanations yields great hope for improving the results further. The first possibility is that proximal epididymal sperm have not had a chance to be matured by the epididymis. Yet we were forced to use proximal sperm, because distal epididymal sperm had weak or no motility due to 'old age'. This explanation of poor sperm maturation does not seem to reconcile with the remarkable fertilizing ability of many samples obtained from as far proximal as the vasa efferentia, which implies that sperm can mature with time on their own.

Another possible explanation, which offers great therapeutic possibilities, is that even the proximal epididymal sperm we obtain must of course be fairly senescent. They are produced recently enough to be alive, but they are still obviously old, stagnant sperm. By way of example, we know that with routine IVF, a processed sperm sample can fertilize for about 24 h. At 72 h of incubation, the sperm still look reasonably good (with respect to motility and morphology), but they certainly cannot fertilize very well.

This could be the phenomenon we are witnessing with aspirated epididymal sperm in men with congenital absence of the vas. A therapeutic option to solve this problem for that population of patients with low fertilization rates is to first aspirate their sperm several days before obtaining the wife's eggs, and then to aspirate again on the day of egg retrieval. Perhaps then we would have a sample of sperm more recently produced, which has not lost its fertilizing capacity due to senescence.

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