Infertility

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With 89 Figures

Chapter 8

Epididymal Surgery

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Male factor infertility has undergone revolutionary changes in the last decade. The human male, and the gorilla, have the poorest sperm production of any animal. Whereas most animals produce 20 to 25 million sperm per gram of testicular tissue per day, the human produces only 4 million [1,2]. Even the very fertile human male has terrible sperm when compared to most other animals. The large number of abnormal forms, debris, and non-motile sperm found in human semen is not seen in other animals. This chapter will be divided into three sections: (a) the treatment of male factor infertility with IVF or GIFT; (b) microsurgery for obstructive azoospermia; and (c) IVF with epididymal sperm for irreversible obstructive azoospermia and congenital absence of the vas.

IVF and GIFT for "Male Factor" Infertility

Great excitement has been generated about the use of IVF in couples with very low or poor quality sperm counts. Fertilisation rates in most IVF centres are clearly poorer in couples with oligospermia, but fertilisation and pregnancy have been regularly obtained in severely oligospermic couples using IVF, GIFT and ZIFT [3]. Theoretically IVF or GIFT allows the fewer number of available sperm a greater opportunity for direct contact with the ovum [4,5]. In fact, if the partner's eggs fertilise in vitro with the husband's few sperm, the pregnancy rate is no different from what it is in couples with normal semen analysis.

In cases of congenital absence of the vas, a collaborative study has been made to aspirate sperm from the blocked epididymis, fertilise the partner's oocytes in vitro, and place the resultant embryos into the fallopian tubes (Zygote Intrafallopian Transfer, ZIFT) [6–8]. Resultant pregnancies led to the conclusion that even sperm with severely reduced motility can often fertilise the oocyte if other obstacles are eliminated.

Rodriguez-Rigau et al. [9] assessed the semen of a large number of couples who did or did not achieve a pregnancy with GIFT (Table 8.1). They found a correlation of the pregnancy rate with standard semen parameters such as count and motility. A similar finding was also reported by Kruger et al [10]. Morphology of spermatozoa was examined very carefully in partners of patients undergoing IVF. Any spermatozoa with a slight defect such as neck droplets, bent necks, abnormal heads, etc., were considered to be abnormal. Remarkably, provided that at least 4% of spermatozoa demonstrated perfectly normal morphology, fertilisation in vitro was achieved. Once again, this suggests that a critical visual exmination of the husband's spermatozoa is a predictor of the likelihood of fertilisation.

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 fertilise their partner'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 fertilisation, 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 fertilisation rate is not significantly different from patients with higher numbers of recoverable sperm. When the motile sperm count after washing is less than this, the fertilisation rate and pregnancy rate are greatly reduced.

Table 8.1. Relationship of motile sperm count and linearity of sperm movement to GIFT pregnancy rate

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

From Rodriguez-Rigau et al. [9].

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) [11], the conventional methods of sperm preparation such as swim-up [12], wash and resuspension [13], sedimentation [14] and Percoll [15] 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, leads to very poor IVF results [16–18]. We use a modified mini-Percoll technique [19,20], which consists of a reduced volume, discontinuous, Percoll gradient.

Semen samples are diluted 1:2 with culture medium and centrifuged at 200 g for 10 min. After 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% layers, this isotonic solution is diluted with human tubal fluid (HTF) and HEPES. The discontinuous gradient is established by carefully pipetting 0.3 ml of 95%, 70% and finally 50% isotonic Percoll into a 15-ml centrifuge tube, centrifuging 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% human cord serum (HCS) and incubated until the time of insemination.

Several advantages can be associated with the use of a mini-Percoll 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 all the cells and debris that are usually present in severe oligoasthenozoospermic samples and represent one of the limiting factors for successful fertilisation; and third, the use of mini-Percoll allows recovery of a high proportion of the normal and motile spermatozoa present in the sample.

Other aspects of IVF are beyond the scope of this chapter except for epididymal sperm aspiration, which will be discussed at the end.

Microsurgery for Obstructive Azoospermia

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

Vasectomy Reversal

Vasectomy is the most popular method of birth control in the world today [21]. For many years the pregnancy rate after surgical reanastomosis of the vas had

been very low, and a variety of explanations has been offered for this [22–24]. With the advent of microsurgical techniques pregnancy rates improved considerably, suggesting that purely micromechanical factors were associated with the low success rates [25–27]. Yet there were still many cases of technically perfect vasovasostomies followed by complete azoospermia or severe oligoasthenospermia. It was then found that the pressure increase after vasectomy had led to secondary epididymal obstruction which was the cause of failure of otherwise successful vasovasostomy. The greater the duration of time since vasectomy the greater the chance of either "blowouts" or inspissation in the epididymis with failure to achieve fertility. Thus, vasoepididymostomy was 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 [28–35]. However, any major correlation between sperm antibodies and subsequent fertility after vasovasostomy has been questioned [36,37]. It has been established that the deleterious effect of pressure increase subsequent to vasectomy was not in the testis, but 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 vasectomised men [25,27]. Despite the finding of no sperm in the vas fluid at the time of vasovasostomy the testicular biopsy of such patients had always appeared normal [38,39]. 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 build-up, and possibly increase the ease of reversibility later (notwithstanding the potentially damaging immunological consequences) [40–42].

What is the fertility rate in the favourable group of patients undergoing vasovasostomy who have suffered no secondary epididymal damage (as shown by sperm being present in the vas fluid at the time of vasovasostomy)? Ten years ago a group of such patients was studied [37]. A total of 326 men who had been previously vasectomised underwent vasovasostomy and received extensive long-term follow-up. In 44 of those men, 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 [25]. Almost all the patients had proven prior fertility as evidenced by previous fatherhood. All patients were followed for nine or ten years.

The overall, long-term pregnancy rate is summarised in Table 8.2. None of the azoospermic patients got their partners pregnant. If azoospermic patients are excluded, 88.4% of patients with sperm patency postoperatively eventually impregnated their partners. This compares to Vessey's expected pregnancy rate of 96% for previously fertile couples discontinuing contraception (1978).

The frequency distribution of semen parameters postoperatively in men who did and did not get their partners pregnant is summarised in Tables 8.3 and 8.4. There was remarkably little difference in pregnancy rate among men with low or high sperm counts. The pregnancy rate was somewhat lower with

Table 8.2. 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 8.3. 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) (10^{-6})	Total patients	Pregnant	Not pregnant
>0–10	32 (12%)	25 (78%)	7
10–20	31 (12%)	27 (87%)	4
20–40	32 (12%)	30 (93%)	2
40–80	79 (31%)	68 (86%)	11
80	84 (33%)	78 (92%)	6
Totals	258 (100%)	228 (88%)	30

Table 8.4. 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 (86%)	11
60–80	62	55 (88%)	7
80	20	18 (90%)	2
Total	258 (100%)	228 (88%)	30 (100%

motility of less than 20%. Above 20% motility, 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 [43].

As shown in Table 8.5, 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 8.6 summarises the relationship

Table 8.5. Lack of effect of varicocele (not operated on) on pregnancy rate following vasovasostomy

	No. 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%)

Table 8.6. Relationship of serum sperm antibody titres to pregnancy rate after vasovasostomy

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

of preoperative serum antisperm antibody titres to the pregnancy rate after vasovasostomy. As with varicocele, the presence of high immobilising or agglutinating titres in serum 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. Many reasons have been suggested for the failure to achieve fertility after reversal of vasectomy, including autoimmune changes and damage to the testis. Our study suggested that the eventual pregnancy rate in patients who have patency accurately re-established without epididymal damage is not significantly less than a normal population of couples. Vessey demonstrated that among couples with proven prior fertility, 96.5% conceive within four 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 shown that the success rate of vasovasostomy decreases with the duration of time since vasectomy [25]. This decrease 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 [38]. 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 [27,40,41]. When there are no spermatozoa in the vas fluid, vasoepididymostomy proximal to the site of epididymal blockage is required [44,45].

It thus appears that the fertility rate and pregnancy rate are quite high in patients with no epididymal blockage who undergo technically "successful" vasovasostomy.

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. But as important as precise microsurgical technique is a practical understanding of epididymal physiology [26,27,44–46].

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 fertilise [47]. In previous studies, spermatozoa from the corpus epididymis could occasionally fertilise, but the pregnancy rate was still low. Spermatozoa were simply aspirated from specific regions of the epididymis, and then promptly inseminated [48–50]. In some studies where the epididymis was ligated to determine if time alone could allow spermatozoal maturation, the obstructed environment was so pathological that no firm conclusions could be reached. Thus the outlook seemed theoretically poor for vasoepididymostomy.

In 1969, Orgebin-Crist [47] pointed out that it was not known 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. It was entirely possible that ageing 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 fertilise. Yet because of the animal studies, and poor results in humans using non-microsurgical techniques, it has always been assumed that epididymal blockage carries a poor prognosis [51–54].

However, as far back as 1931, Young's experiments in guinea pigs with ligation at various levels of the epididymis indicated "that the time consumed by spermatozoa in passing through the epididymis is necessary for a completion of their development; that the changes undergone during this period 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" [55]. 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, 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 [56].

All vasoepididymostomies are performed with the "specific tubule" technique 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 [25,44,46]. 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 has been difficult to obtain.
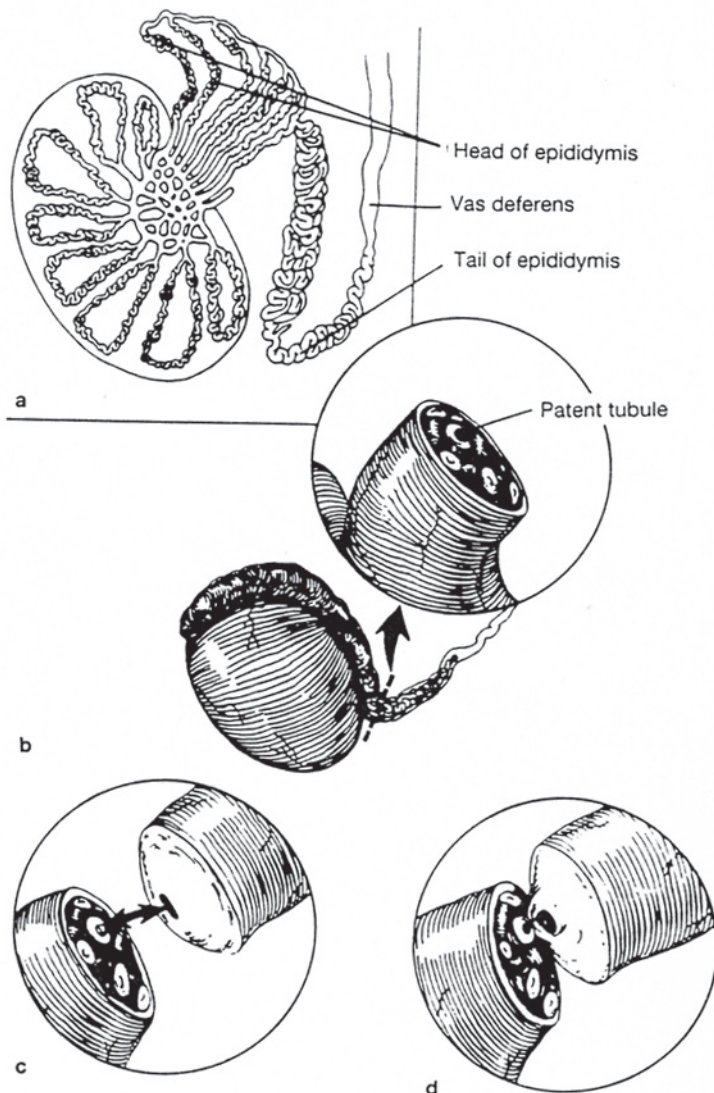
Fig. 8.1. Specific tubule-to-end anastomosis of the vas lumen to the epididymis proximal to site of obstruction.

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 epididymis tubule (Figs. 8.1 and 8.2a and b). Usually five or six 10-0 nylon interrupted sutures complete the leakproof end-to-end anastomosis, and then the outer muscular layer of the vas is separately sutured to the outer epididymis tunic with 9-0 nylon interrupted sutures.

Of the cases of epididymal anastomosis 72% have resulted in eventual pregnancy [56]. The younger the partner, 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 fertilising capacity.

Recent clinical cases have demonstrated that it is possible in some circumstances for spermatozoa which have never transited any length of epididymis to fertilise the human egg. In two cases of anastomosis between the vasa efferentia and the vas deferens, the postoperative ejaculate contained normally motile sperm, and the partners became pregnant [57]. In addition, pregnancy from aspiration of epididymal sperm combined with in vitro fertilisation and ZIFT in cases of unrepairable obstruction gives further evidence that transit through the epididymis is not a mandatory requirement for fertilisation [6,7].

Newer studies of epididymal sperm transport in the human indicate that the human epididymis is not a storage area, and spermatozoa transit the entire human epididymis very quickly, in two days, not eleven days as was previously thought [58]. Thus, it is possible that in the human, the epididymis may not be as essential to spermatozoal 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 has until now been considered untreatable [59]. This is a large and frustrating group of patients who have been shown on testicular biopsies to have normal spermatogenesis, and are theoretically making sperm quite capable of fertilising an egg. Yet treatment up until now has been very poor [60].

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 frustrating group of couples [7,8,25,57].

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
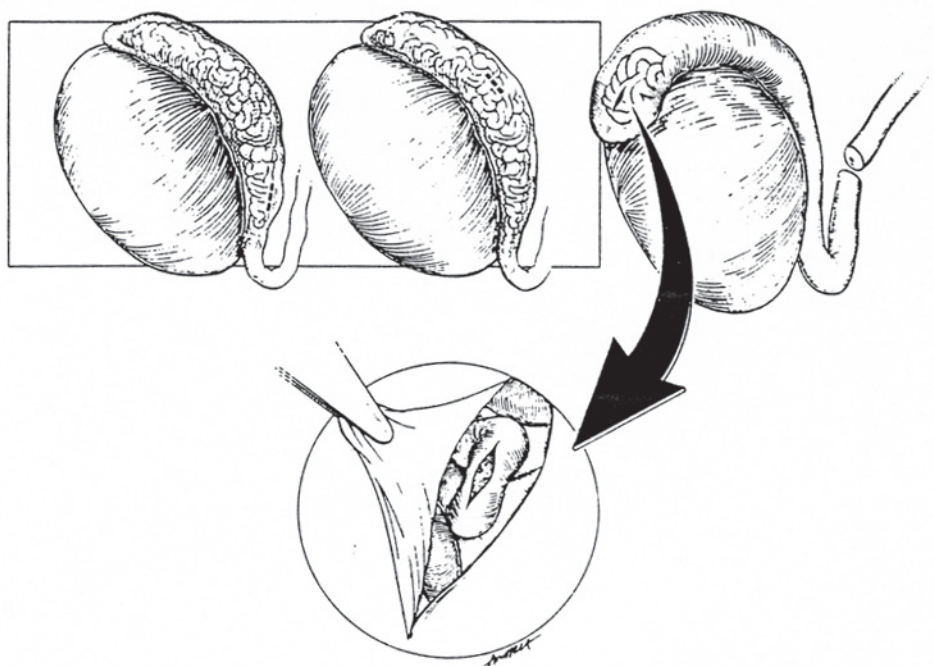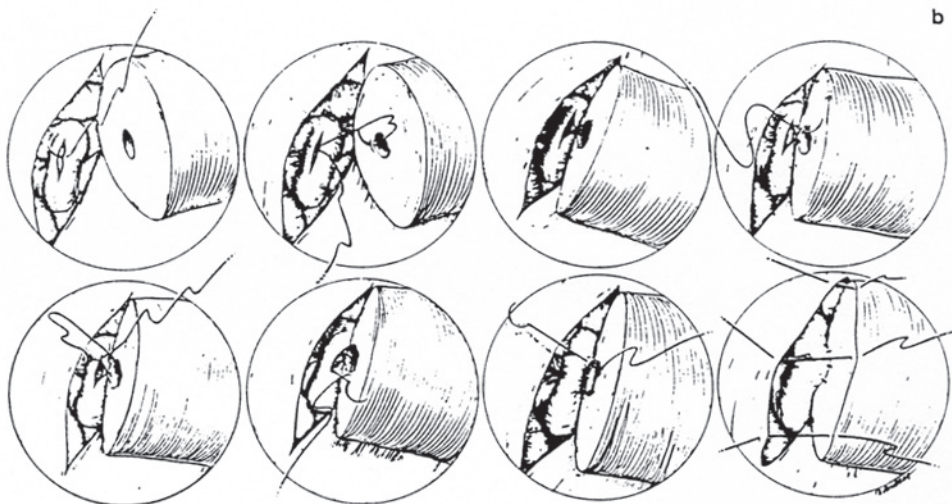
Fig. 8.2. a Small openings are made in the epididymal tunic beginning distally and moving proximally. After a longitudinal slit is made in the epididymal tubule with the microscissors, the distal-most level at which motile sperm are found is used for the anastomosis. **b** The end-to-side specific tubule anastomosis of the vas lumen to the epididymal tubule requires first a posterior row of three 10-0 nylon interrupted sutures followed by an anterior row of three 10-0 nylon interrupted sutures. The muscularis of the vas is then sutured to the outer epididymal tunic with 9-0 nylon interrupted sutures.

protocol: leuprolide acetate (Lupron, TAP Pharmaceuticals, North Chicago, IL) 1 mg subcutaneously daily until the day of follicular aspiration (Fig. 8.3). Patients then receive human follicle stimulating hormone (FSH) (Metrodin, Serono Laboratories, Inc., Randolph, MA) and human menopausal gonadotropins (hMG) (Pergonal, Serono) 150 IU intramuscularly (IM) daily from day 2 of the menstrual cycle until many follicles of 2.0 cm were noted on ultrasound. Then human chorionic gonadotropin (hCG) (Profasi, Serono, Randolph, MA) 10 000 IU is administered IM.

At 36 h after hCG administration, the patients undergo follicular aspiration 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) (no. 33-100) at a maximum vacuum pressure of 120 mmHg. The follicular fluid is given immediately to the embryology laboratory adjacent to the operating room.
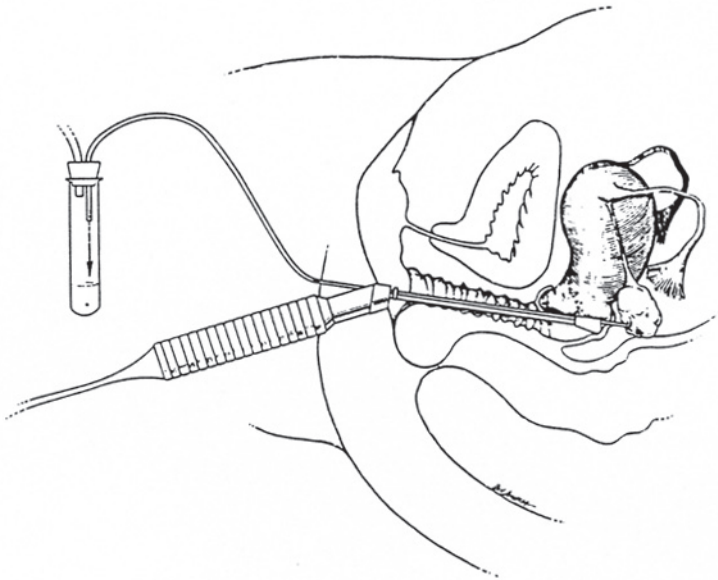
Fig. 8.3.   Illustration of placement of the ultrasound probe for transvaginal needle aspiration of eggs.

Epididymal Sperm Aspiration, Washing Methodology, and IVF

At the same time the husband undergoes scrotal exploration in order to aspirate sufficient numbers of motile spermatozoa for use for IVF of the aspirated eggs, with subsequent transfer into the partner's fallopian tube.

The surgical technique (Fig. 8.4) 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 into the epididymal tunic
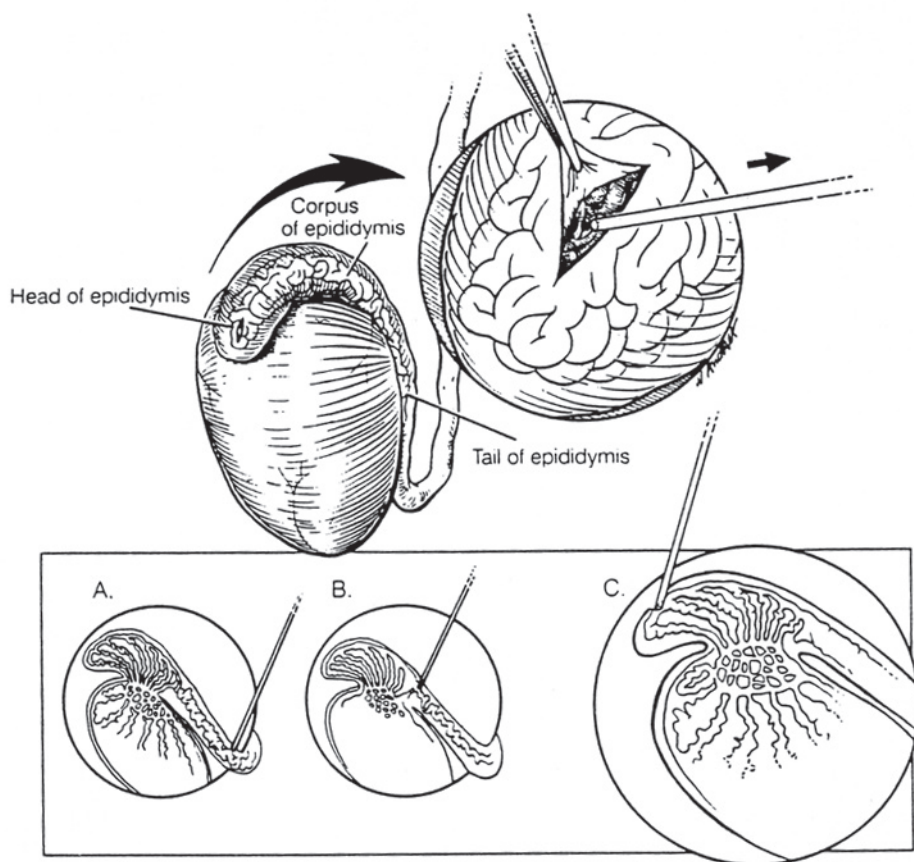
Fig. 8.4. Technique for epididymal sperm aspiration which begins in the distal corpus region of the epididymis, and moves proximally until motile sperm are recovered. In most cases, motility is observed only in the most proximal region of the epididymis.

to expose the tubules in the distal-most 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 haemostasis is achieved with microbipolar forceps. The epididymal fluid is immediately diluted in Hepes-buffered medium, and a tiny portion examined for motility and quality of progression. If there is no motility or poor motility, 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 the proximal-most portion of the caput epididymis or even the vasa efferentia was reached. This is the inverse of what might have been anticipated (Fig. 8.5).

In the laboratory the epididymal sperm are concentrated into a volume of 0.3 ml, layered on a discontinuous mini-Percoll gradient, and centrifuged for 30 min. The entire 95% fraction is then washed twice and inseminated with all
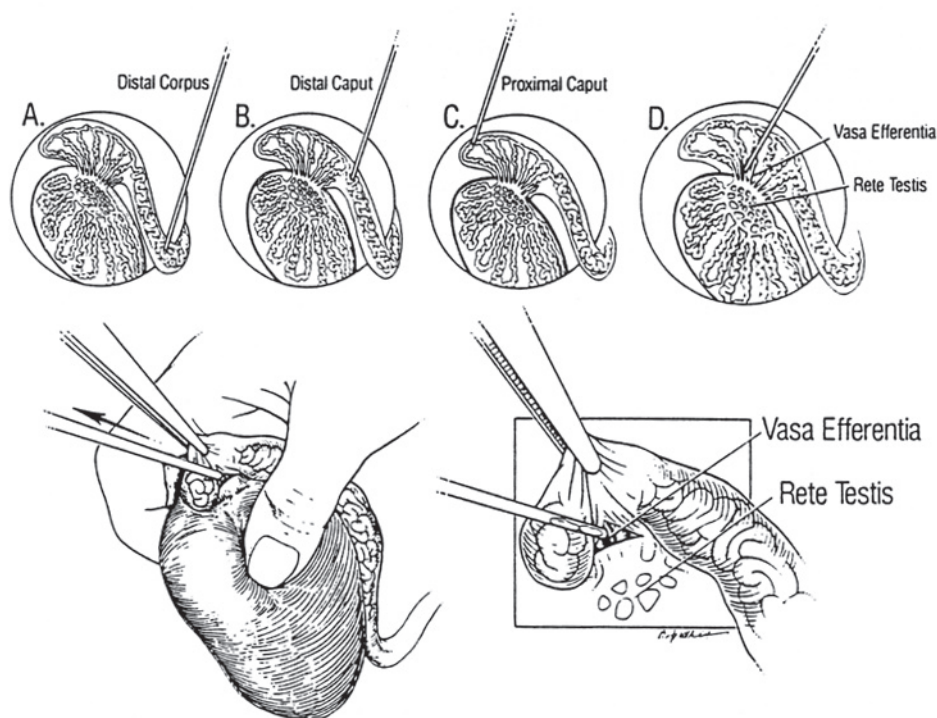
Fig. 8.5. The most motile sperm are found very proximally, usually in the vasa efferentia or rete testis.

of the eggs in a Falcon mini-test tube with 1 ml of HTF culture medium and incubated at 37°C with 5% CO_2 in air [47] (Fig. 8.6).

Two days after insemination, embryos are transferred to the fallopian tubes, via minilaparotomy using a technique similar to that used for gamete intrafallopian transfer (GIFT), via a Tomcat catheter (Monoject, St Louis, MO) 2.5 cm inside the fimbrial ostium (Fig. 8.7). The patients are discharged the next day and undergo fairly painless postoperative recovery. The wives receive progesterone in oil, 50 mg IM/day beginning with the day of embryo transfer.

Results

At present, of 115 cases, there have been 24 pregnancies, with six miscarriages, i.e. a pregnancy rate of 21% and a live-baby rate of 16% (Table 8.7).

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 [6,8]. 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
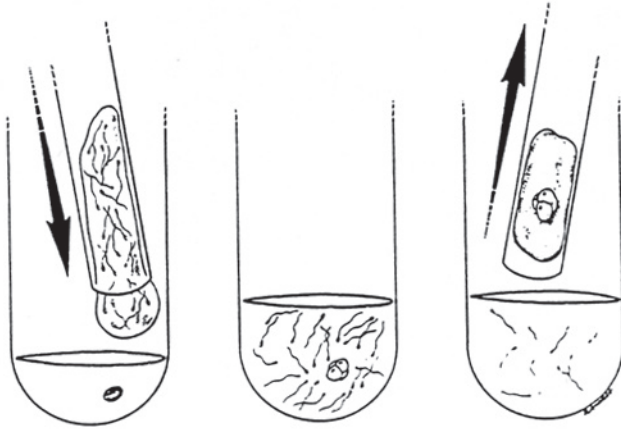
Fig. 8.6. Sperm being placed in test tube with egg, resulting in 4-cell embryo two days later, and retrieval of 4-cell embryo for transfer back to patient.
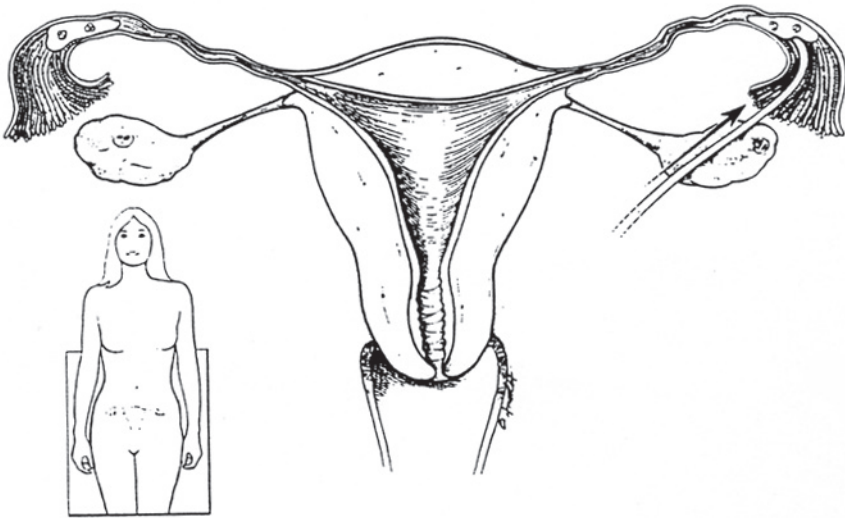

Fig. 8.7. In the ZIFT procedure eggs would have already been fertilised and growing for the previous 2 days in the laboratory and placed in the fallopian tube in a similar fashion to the GIFT procedure.

considerations) to distal regions of the epididymis where the sperm are generally senescent and non-motile in the chronically obstructed state.

Other factors in the success of this technique which may be equally important are: (1) obtaining large numbers of oocytes in order to increase the odds of fertilisation; (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.

Table 8.7. First 100 cases of in vitro fertilisation 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 (4ª)	28% (22%)
Totals	100	22 (16)	22% (16%)

ª Ongoing pregnancies not yet delivered.

Problems that remain in the way of achieving a higher pregnancy rate are that even the most proximal epididymal sperm with the best motility, have poor fertilisation rates in 70% of cases, and no fertilisation in 40% of cases. It is our suspicion that these obstructed sperm are senescent, but slightly less senescent than the dead, distal epididymal sperm. If that hypothesis is true, better fertilisation rates might be achieved by first aspirating the epididymis of all old sperm, and then at a later interval, re-aspirating to get sperm for IVF.

Although improvements are needed, for the moment it is safe to conclude that:

1. Sperm from the proximal-most caput epididymis are capable of fertilising 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.
3. There is now a non-experimental, valuable approach for achieving pregnancy in couples with a previously dismal condition, congenital absence of the vas deferens.

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Discussion

Aitken: I would like to know the mechanism by which cooling has an effect on sperm–oocyte fusion. If we freeze and store cells, they are much more fusogenic than the fresh cell. What I have always imagined to be the mechanism is that when a cell is cooled and it gets down below its phase transition temperature, big areas of lipid crystallise out and push the intercalating proteins

to one side, and when the cell is thawed out or warmed up, those intercalating proteins do not flow back into the lipid-rich areas of membrane, they stay as just areas of lipid.

Watson: Not all data confirm that. Some studies suggest there is a phased separation, which is reversed when the cells have been warmed. So that may be part of the story. Our thawing rates are much more rapid than our cooling rates, and it may well be that there is not the time during thawing for that redistribution to occur, so that we may well get cells at body temperature which do have these large areas. It may also be that the process of fusion takes a very short time.

Aitken: I am sure that is the case. How is it possible to differentiate in those cooled/warm cells between increased permeability to calcium and a decreased ability to pump out calcium? I had always imagined that the sperm cell was relatively permeable to calcium. What the intact cell does is to continually bail out the cation.

Watson: I think that is right. As the cell cools below 15°C or so, the pumps shut off and the cell then accumulates calcium simply because it is stopping the process of bailing out.

Abdalla: In our clinic, patients who have artificial insemination with frozen donor sperm have a low pregnancy rate, compared to those where GIFT is carried out using frozen sperm.

Watson: The GIFT procedure provides the optimal position for fertilisation. If the sperm are closer to that fertilisation event in terms of the membrane maturational changes, they may be able to achieve that fertilisation before they run out of steam. An acrosome reacted sperm has a relatively short lifespan. We have also referred to the importance of the acrosome reaction taking place close to or attached to the zone surface.

Kerin: We are told that the frozen–thawed sperm have a short time in which to fertilise the egg. Is there evidence from clinical studies that intrauterine insemination for these reasons using frozen sperm might be better than pericervical insemination?

Watson: I can only answer in terms of my reading, the literature is conflicting.

Asch: What is Dr Watson's impression, based on the physiology of how cryoinsemination works, of freezing severely oligoasthenospermic specimens? Is there any technique that would allow concentration of many specimens from an individual.

Watson: I do not have any specific techniques for dealing with that situation, but I do believe if we can come to grips with the causes of cryoinjury in normal sperm to get a higher percentage of cells that survive the freeze–thaw process, then that will have its repercussions for a situation where only a few cells are present. But the fertility, as I understand it, of oligospermic individuals, is

lower in terms of function and therefore the chances of achieving reasonable fertility by that procedure is reduced.

Fishel: It strikes me from the data just presented on behalf of Dr Silber that the pregnancy rate is probably influenced by the number of embryos replaced. But the incidence of implantation or pregnancy, given the low fertilisation rate, may also be a function of the type of sperm – as we also see in microinsemination. Are there data to suggest a higher miscarriage rate or an earlier miscarriage rate among these patients?

Asch: We have very few miscarriages in these patients. I do not know why. Perhaps we take particularly good care of them. The take home lesson for me is that if we take sperm from the same place, in patients with the same condition, we can get four totally different populations of sperm, from sperm that are severely asthenospermic all the way to sperm that are almost like ejaculated sperm, those that fertilise 66% of the time.

Winston: Surely the problem could be solved by letting us know the implantation rate per single embryo transferred. That should be a useful statistic for us to have and we can then evaluate this. It is a statistic in general by which we should be trying to evaluate IVF more and more, because that would certainly answer that problem.

Dr Fishel reported on 307 patients. How many cycles? Of the 31 clinical pregnancies, how many of those have progressed to something looking like a pregnancy which will establish successfully.

Fishel: I did not have time to show all the data. The 307 is all cycles and it also represents patients. Most of the microinsemination data that are being produced at the moment are cycles and patients. However, the miscarriage rate is definitely high.

Aitken: Professor Asch mentioned that they stimulated the sperm; he mentioned pentoxyfilline and I think they also use 2-deoxyadenosine. Do they do that globally or do they have some sort of selection criteria by which to apply these stimulants?

Asch: We do it in all cases, but we do not do it in all sperm.

Aitken: Has Dr Fishel tried any stimulatory drugs with these sperm?

Fishel: Yes, I have. It is a little preliminary and I do not have too much to show at the moment. Actually I am disappointed with pentoxyfilline. My finding at the moment is that there is some increase in the incidence of fertilisation with IVF, but once we start to move to microinsemination techniques, they will either fertilise or they will not, and pentoxyfilline at the moment, from the data that I have, does not seem to make that much difference.

We are discussing the use of drugs for stimulating fertilisation. It is my understanding that these drugs still have to be used in the UK on a named patient basis, but that the HFEA may give clarification on their use.

Whittall: The line that the authority takes on drugs such as pentoxyfilline and their clinical use in IVF is that if a centre intending to use it can demonstrate effectiveness, either through literature or through its own work, then the authority would consider it. Generally it will take the line on the basis of an individual centre applying for use, but there will come a point eventually with a particular aspect of treatment that it will become generally accepted. I do not think that we want to put down a set of rules, but just to recognise that there is a process that each particular drug will go through in becoming accepted.

Hull: My reading of the information available seems to show a rapid change in the last few years in which fertilisation rates by standard IVF – this is in controlled studies, sister oocytes– have been of the order of 20%–30% and higher. Jacques Cohen has published fertilisation rates of 20% or 30% with other methods of micromanipulation [1]. What is becoming apparent is that by better methods of sperm preparation, we are now doing a lot better. Certainly we have controlled studies, as yet unpublished, showing marked improvement with Percoll.

Fishel: Only a few people are doing microdrop IVF, but to do this with Percoll-type preparations increases the chances of IVF fertilisation. One of the big problems with comparative data is that there are enormous differences in the populations of males being assessed. Certainly the high incidence of fertilisation in some of the controlled studies says a lot about the types of males that are undergoing micro manipulation.

Braude: Dr Fishel was rather disparaging about direct intracytoplasmic injection, yet four pregnancies in patients with two failed SUZI attempts have been reported.

Fishel: I do not view it disparagingly. I think it is a valuable technique which will come. But at this stage, apart from Palermo's data there are no other studies to suggest an advantage.

Braude: If one was summarising the state of the art in terms of micro-assisted fertilisation, then surely the way we should be progressing is towards direct intracytoplasmic injection, which eliminates all the other problems if it can be made to succeed. I cannot see the disadvantage of direct injection over any of the other methods, but I can see advantages.

Fishel: I do not think I am arguing against it at this stage but there will have to be a far greater evaluation of that technique. I do not deny it may be of use and eventually better than SUZI.

Templeton: Dr Fishel, I wanted clarification of Group 1, which included patients with extremely low sperm motility. Was it always necessary that there was at least some residual motility for fertilisation to take place, or were there patients with no sperm motility where fertilisation occurred?

Fishel: It would only be fair to assess patients that have zero motility as a separate group, which I have done. Again I have been singularly unsuccessful in achieving fertilisation with 100% immotile sperm.

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