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Edited by

George Webster
MB, FRCS, FACS
Professor of Urologic Surgery
Duke University Medical Center
Durham, North Carolina, USA

Roger Kirby
MA, MD, FRCS (Urol)
Consultant Urologist
St Bartholomew's Hospital
London, UK

Lowell King
MD, FACS
Head, Section of Pediatric Urology
Duke University Medical Center
Durham, North Carolina, USA

Benad Goldwasser
MD
Chairman, Department of Urology
Chaim Sheba Medical Center
Tel-Hashomer, Israel

Foreword by

Richard Turner-Warwick
CBE, BSc, MCh, DM(Oxon), DSc(Hon),
FRCP, FRCS, FRCOG, FACS, FRACS(Hon)
Senior Lecturer
Institute of Urology
London, UK

Chapter 78

Techniques for the Resolution of Testicular Obstruction

Sherman J. Silber

Introduction

Testicular outflow obstruction as a cause of male infertility can be divided into three categories: iatrogenic (e.g. vasectomy reversal and complications of hernia repair, etc.), postinflammatory, and congenital. If high pregnancy rates are to be obtained in treating such patients, technically competent surgery is no more important than a full understanding of the physiology of sperm outflow obstruction. It is easier to begin with vasectomy reversal, because an understanding of how to obtain high success rates with these patients will aid tremendously in proceeding to the more complex issues of vasoepididymostomy for postinflammatory obstruction, and sperm aspiration with *in vitro* fertilization for congenital absence of the vas.

Vasectomy reversal

Vasectomy is the most popular method of birth control in the world today [1]. For many years the pregnancy rate after surgical reanastomosis of the vas has been very low, and a variety of explanations had been offered for the relatively poor success in reversing vasectomy [2–4]. With the advent of microsurgical techniques pregnancy rates improved considerably, suggesting that purely micromechanical factors were associated with the low success rates, but long-term follow-up on large numbers of patients were not available and the matter remained somewhat controversial for years.

Theories for the consistently poor results with vasectomy reversal have included development of sperm antibodies, damage to the deferential nerve, and testicular damage [5–12]. Yet some investigators questioned any correlation between sperm antibodies in the serum and subsequent fertility after vasovasostomy [13], and the effect, if any, of vasectomy on the testis in humans and animals has also been very controversial [14]. Segregating the various studies by species has not cleared up the confusion. If any testicular damage occurs, the generally agreed upon mechanisms would be either autoimmune, or pressure-related [14–19].

The author's group has fully established the deleterious effect of pressure increase subsequent to vasectomy, and the effect of this pressure on epididymal dilatation, perforation, and sperm inspissation and blowouts in the epididymis, causing secondary epididymal obstruction [14,20,21]. Despite the dismal finding of no sperm in the vas fluid at the time of vasovasostomy the testicle biopsy of such patients always appeared normal [22,23]. This deleterious effect of pressure increase was always on the epididymis. The secondary epididymal obstruction after vasectomy led to the group's suggestion that the testicular end of the vas should not be sealed at the time of vasectomy, so as to lessen the pressure build-up, and

possibly increase the ease of reversibility later (notwith-standing the potentially damaging immunologic consequences) [21,24,25].

The first question to be answered is, 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? We attempted to relate in these patients presence or absence of varicocele, postoperative semen analyses, preoperative sperm antibody titers, and quantitative evaluation of testicular biopsy to the chance for pregnancy. Firstly, results in these patients who were thought to have no epididymal blockage are reviewed [26]. Patients with no sperm in the vas fluid, all of whom exhibited secondary epididymal obstruction, will be discussed later.

Patient study group

A group of 326 men who had been previously vasectomized underwent vasectomy reversal and received extensive long-term follow-up. All such patients have been found to be azoospermic after vasovasostomy and require vasoepididymostomy instead. In 44 men no sperm was found in the vas fluid, and a vasovasostomy was performed. The vasovasostomy involved a meticulous, two-layer microsurgical technique performed by the same surgeon with accurate mucosa-to-mucosa approximation [20]. Almost all of the patients had proven fertility as evidenced 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 78.1. None of the azoospermic patients got their wives pregnant. If azoospermic patients are excluded, 88.4% of patients with sperm patency postoperatively eventually impregnated their wives. This compares to Vessey et al.'s expected pregnancy rate of 96% for previously fertile couples discontinuing contraception [27].

The frequency distribution of semen parameters postoperatively in men who did and did not get their wives pregnant is summarized in Tables 78.2 and 78.3.

There was remarkably little difference in pregnancy rate among wives of men with low or high sperm counts.

Table 78.1 Overall long-term pregnancy rates in patients undergoing vasovasostomy: 10-year follow-up (sperm seen in vas fluid)

	Combined 1975 and 1976–7 series	Original 1975 series
Total patients	282 (100%)	42 (100%)
Total pregnant	228 (81%)	32 (76%)
Azoospermic	24 (9%)	5 (12%)

Table 78.2 Pregnancy rate according to distribution of motile sperm count in men with sperm patency following vasovasostomy (10-year follow-up)

Total motile sperm count (per ejaculate)	Total patients	Pregnant	Not pregnant
0–10 000 000	32 (12%)	25 (78%)	7
10–20 000 000	31 (12%)	27 (87%)	4
20–40 000 000	32 (12%)	30 (93%)	2
40–80 000 000	79 (31%)	68 (86%)	11
>80 000 000	84 (33%)	78 (92%)	6
Total	258 (100%)	228 (88%)	30

Table 78.3 Pregnancy rate according to 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%)

Similar findings were seen 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 [28].

As summarized in Table 78.4, 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 78.5 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 results of quantitative analysis of testicle biopsy are summarized in Table 78.6. In the 16 patients biopsied who had sperm present in the vas fluid, postoperative

Table 78.4 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%)
Total	282 (100%)	42 (14.8%)	240 (85.2%)

Table 78.5 Relationship of serum sperm antibody titers to pregnancy rate after vasovasostomy

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

Table 78.6 Quantitative study of testicle biopsy in patients who had sperm patency

Patient	Years since vasectomy	Number of mature spermatids per seminiferous tubule		Ratio of pachytene spermatocytes to mature spermatids	Expected sperm count (per ml)	Actual sperm count (per ml)	Pregnant
		Right	Left				
GB	9		24.0	1.19	19 000 000	20 000 000	Yes
RD	4		17.2	1.17	7 000 000	6 600 000	Yes
WB	10	43.3		0.89	78 000 000	82 000 000	Yes
CB	16	30.3		0.91	33 000 000	37 200 000	Yes
MB	2		27.7	0.96	26 000 000	35 800 000	Yes
RC	5		18.0	1.33	8 000 000	5 800 000	Yes
SC	9	31.8	27.8	1.06	31 000 000	30 000 000	Yes
MC	1	28.6		1.04	30 000 000	24 850 000	No
AB	1	22.6		1.15	17 000 000	24 500 000	No
PC	5	14.0		1.64	4 000 000	3 000 000	Yes
AC	6	30.1	27.9	1.16	32 000 000	30 000 000	Yes
RC	5	29.9	27.8	1.11	30 000 000	38 000 000	Yes
RB	22	40.4		1.18	63 000 000	70 000 000	Yes
MC	5		36.1	1.01	50 000 000	63 000 000	Yes
MC	4		30.9	1.28	37 000 000	40 800 000	Yes
AF	6	33.2		1.11	41 000 000	36 000 000	Yes

sperm count was fairly well-predicted by the number of mature spermatids (Sc and Sd) per seminiferous tubule in the testicle biopsy. The close correlation (exponential curve) of mature spermatids per tubule to sperm count has been well-demonstrated in a previous study and is corroborated by these data [24]. Of these 16 patients, two did not get their wives pregnant. The semen parameters in these two were no different from patients who did get their wives pregnant. The ratio of pachytene spermatocytes to mature spermatids showed a close one-to-one correlation in all patients and was not significantly different in the two patients who failed to impregnate their wives.

Reason for high pregnancy rate from 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. This study suggests that the pregnancy rate in patients who have patency accurately reestablished without epididymal damage is eventually not significantly less than a normal population of couples. Vessey demonstrated that, among couples with proven prior fertility, 96.5% conceive within 4 years of discontinuing contraception [27]. In our couples with patent results after vasovasostomy who had no evidence of epididymal pressure damage, 88% conceived with long-term follow-up. Of course, these men had demonstrated prior fertility. Patients with secondary epididymal blockage, of course, require a completely different approach.

It has been previously shown that the success rate of vasovasostomy decreases with the duration of time since the vasectomy [21]. 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 [22]. 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 [14,21,24]. When there is no sperm in the vas fluid, vasoepididymostomy proximal to the site of epididymal blockage is required [29,30].

It thus appears that the fertility rate and pregnancy rate may be higher than previously expected 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 once again, a precise microsurgical technique is no more important than a practical understanding of epididymal physiology [14,29–32].

In every animal that has been studied, spermatozoa from the caput epididymis are only capable of weak circular motion at most, and are not able to fertilize [32]. In previous studies, spermatozoa from the corpus epididymis can occasionally fertilize, but the pregnancy rate is low.

But few of these previous animal studies allowed the spermatozoa time to mature and thereby possibly develop fertilizing capacity. Spermatozoa were simply aspirated from specific regions of the epididymis, and then promptly inseminated. In most studies 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 [34–36].

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 [32]. 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 nonmicrosurgical techniques, it has always been assumed that epididymal blockage carries a poor prognosis [37–40].

As far back as 1931, however, Young's experiments in guinea-pigs with ligation at various levels of the epididymis [41] 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." In fact, he 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 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 [42].

The diagnosis of obstructive azoospermia in nonvasectomy reversal cases was made by testicle biopsy, demonstrating quantitatively normal spermatogenesis, a palpable vas deferens on physical examination, normal semen volume, and azoospermia [23].

The localization of the site of epididymal obstruction was determined at the time of surgery by proximal serial sectioning of the epididymis until normal spermatozoa were found in the fluid coming from the epididymal tubule. Histologic sections obtained in the process of transecting proximally up the epididymis confirmed the area of transition from no spermatozoa in the epididymal lumen to an epididymal lumen dilated and packed with spermatozoa. We now use a specific tubule microsurgical end-to-side vasoepididymostomy with somewhat better results, but the long-term data presented here are based on our end-to-end specific tubule method.

Factors related to pregnancy and patency

Classical postoperative semen parameters (including numerical count, morphology, percentage motility, quality of motility, velocity of motility, and direction of motility) were ascertained at intervals of 3 months, if possible, to yearly. Many patients had as many as 10–15 semen analyses performed, and the mean of the semen analyses obtained after a leveling off of the rise after surgery was used in tabulation. The area of the epididymis, the degree of dilatation of the epididymal tubule, volume of fluid efflux, and the quality of spermatozoa in the fluid proximal to the obstruction were recorded. The epididymis was serially sectioned proximally until there was a good volume of fluid efflux, and a copious number of long-tailed spermatozoa (whether motile or nonmotile) in the epididymal fluid. Postoperative spermatozoa count and directional motility were related to pregnancy rate, mean time till pregnancy, and the level of the caput or corpus epididymis (proximal, mid, or distal) at which the anastomosis was performed.

All vasoepididymostomies were performed with the

specific tubule technique we have described, which involves an end-to-end anastomosis of the inner lumen of the vas to the epididymal tubule, mucosa-to-mucosa in a leakproof fashion [29,30,32]. 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 have 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 (Figs 78.1 and 78.2). Usually four to five 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.

Seventy-two per cent of the cases of corpus epididymis anastomosis resulted in pregnancy. Forty-three per cent

(a)

Head of epididymis

Vas deferens

Tail of epididymis

Patent tubule

(b)

(c) (d)

Fig. 78.1 Specific tubule end-to-end anastomosis of the vas lumen to the epididymis proximal to the site of obstruction.

of caput epididymis cases resulted in pregnancy. 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.

Such a vasoepididymostomy model in which spermatozoa cannot traverse the full length of epididymis would none the less allow maturation to occur with time only. The fact that pregnancy occurred in almost half of the 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.

It should be emphasized that none of these patients underwent any special treatments such as *in vitro* fertilization or gamete intrafallopian transfer, and these pregnancies all occurred simply with natural intercourse. With *in vitro* techniques, more than half of these patients with spermatozoa from the caput epididymis will be able to accomplish fertilization.

Recent clinical cases have demonstrated that it is even possible in some circumstances for spermatozoa which have never traversed 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 [43]. In addition, pregnancy from aspiration of epididymal sperm combined with *in vitro* fertilization and zygote intrafallopian transfer in cases of irreparable obstruction gives further evidence that transit through the epididymis is not a mandatory requirement for fertilization [44,45]. Finally, newer studies of epididymal sperm transport in the human indicate that the human epididymis is not a storage area, and indeed spermatozoa transit the entire human epididymis very quickly in a mere 2 days, not 11 days as was previously thought [46]. 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 *in vitro* fertilization

Congenital absence of the vas deferens accounts for 11– 50% of cases of obstructive azoospermia, and heretofore has been considered basically untreatable [47]. 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. Yet treatment up until the present time has been very dismal [48].

Dr Ricardo Asch and the author 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 [20,45,49].
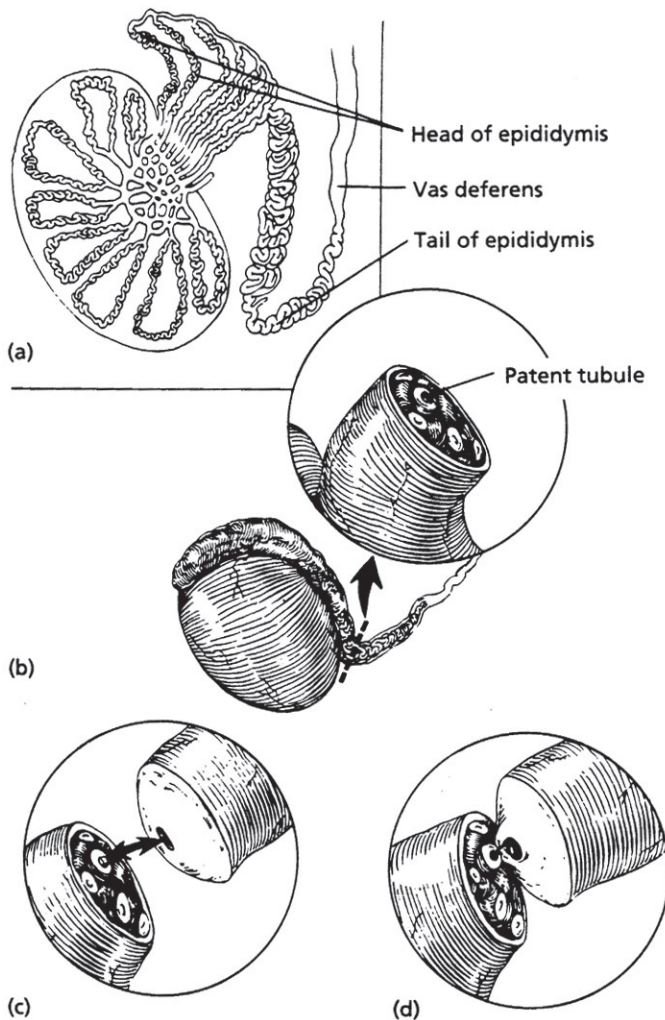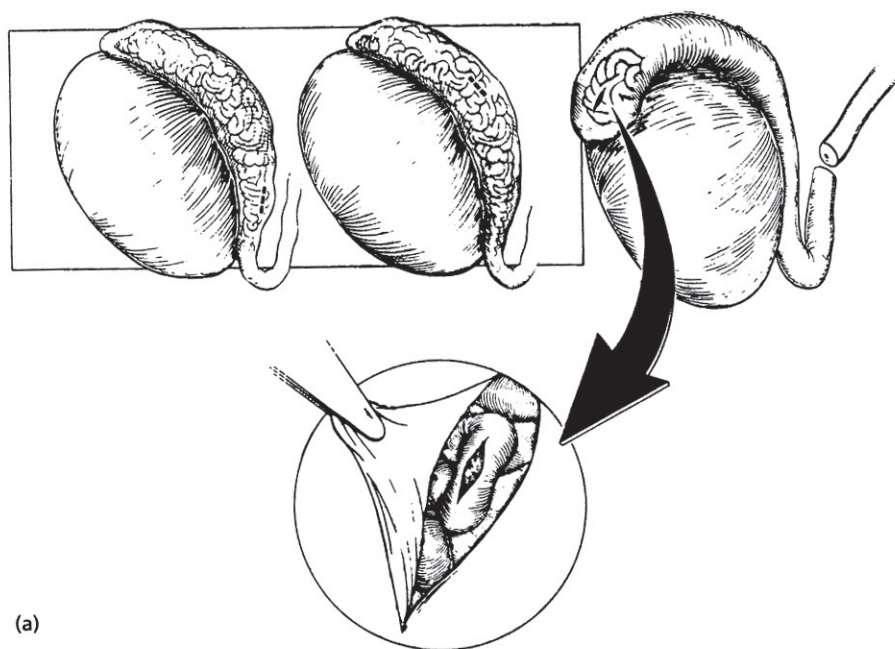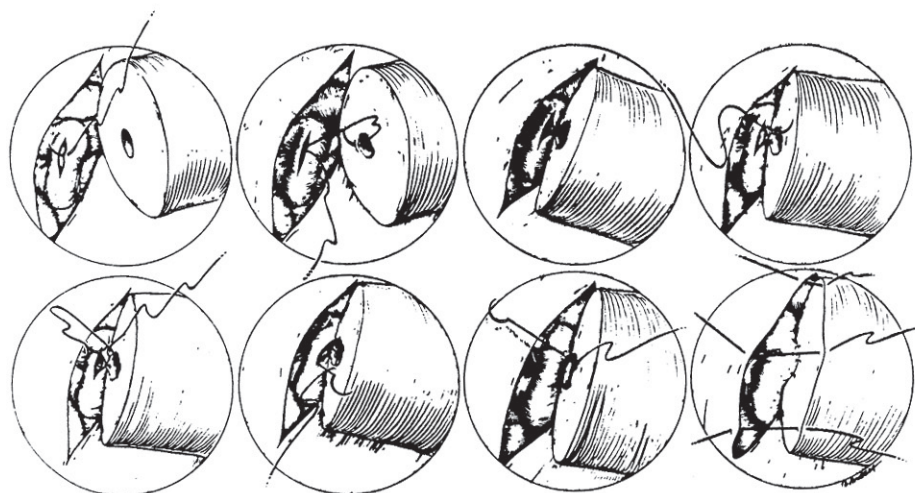
(a)

(b)

Fig. 78.2 (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 distalmost 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.

They now have a method for microsurgical sperm aspiration from the proximalmost region of the head of the epididymis, combined with *in vitro* fertilization, with the first documentation of fertilization and pregnancy utilizing this approach for the treatment of congenital absence of the vas deferens. At the time of writing, we have performed 115 such cases and had 24 pregnancies [43].

Induction of follicular development and oocyte retrieval

The female partners of men with azoospermia caused by congenital absence of the vas undergo induction of mul-

tiple follicular development with the following protocol: leuprolide acetate (Lupron) 1 mg subcutaneously daily until the day of follicular aspiration. Patients received human follicle-stimulating hormone (FSH; Metrodin) and human menopausal gonadotropins (hMG; Pergonal) 150 iu intramuscularly 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), 10 000 iu was administered intramuscularly.

Thirty-six hours after hCG administration, the patients underwent follicular aspiration in the operating room under intravenous sedation with titrating doses of 0.1–0.25 mg fentanyl (Sublimaze) and 5–7 mg midazolam HCl (Versed).

Follicular aspiration was performed using a transvaginal probe (GE H4222 TV) adapted to an ultrasound system (GE RT3,000) (General Electric Company, Milwaukee, Wisconsin) with a needle set for ovum aspiration and connected to a Craft Suction Unit (33-100) (Rocket USA, Branford, Connecticut) at a maximum vacuum pressure of 120 mmHg.

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

Epididymal sperm aspiration, washing methodology, and *in vitro* fertilization

At the same time the husband underwent scrotal exploration with the intention of aspirating sufficient numbers of motile spermatozoa to utilize for *in vitro* fertilization of the aspirated eggs, with subsequent transfer into the wife's fallopian tube.

The surgical technique (Fig. 78.3) in the male is as follows: scrotal contents were extruded through a small incision, the tunica vaginalis was opened, and the epididymis was exposed. Under ×10−40 magnification with an operating microscope, a tiny incision was made with microscissors into the epididymal tunic to expose the tubules in the distalmost portion of the congenitally blind-ending epididymis. Sperm were aspirated with a number 22 Medicut on a tuberculin syringe directly from the opening in the epididymal tubule. Great care was taken not to contaminate the specimen with blood, and careful hemostasis was achieved with microbipolar forceps. The epididymal fluid was immediately diluted in HEPES-buffered media, and a tiny portion examined for motility and quality of progression. If there was no motility or poor motility, another aspiration was made 0.5 cm more proximally. We thus obtained sperm from successively more and more proximal regions until progressive motility was 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 78.3 and 78.4).

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 min. The entire 95% fraction is then washed twice 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% carbon dioxide in air.
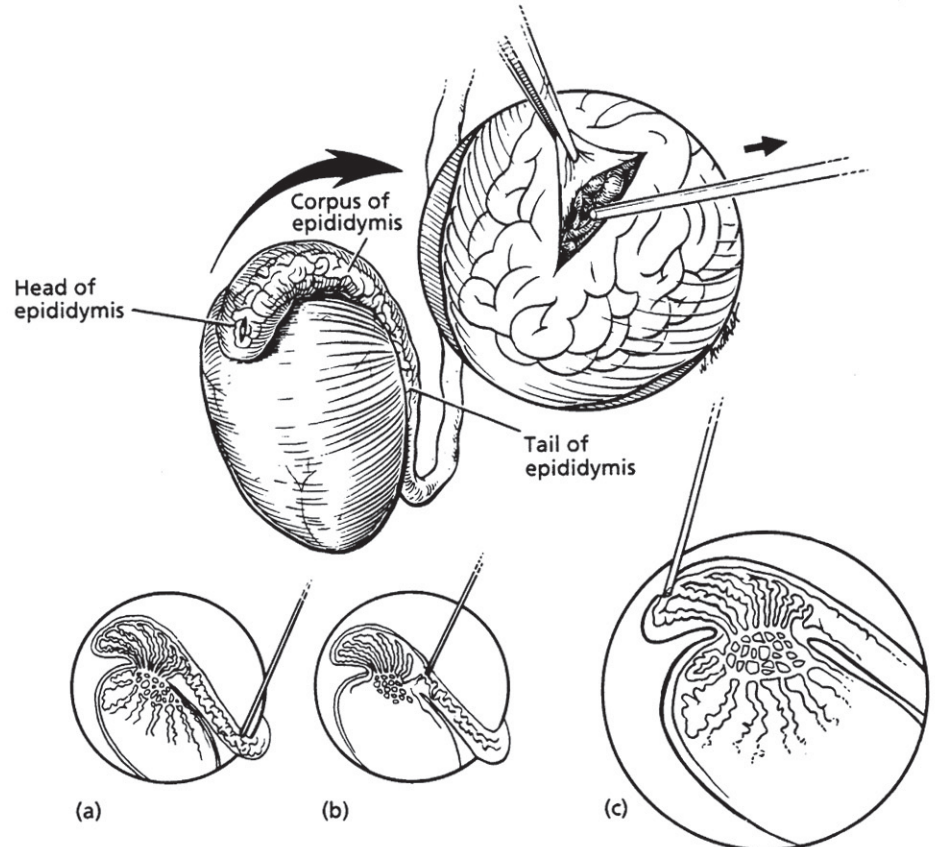

Fig. 78.3 Technique for epididymal sperm aspiration which begins in the distal corpus region of the epididymis, and moves proximally until motile sperm are recovered. In most cases, motility is observed only in the most proximal region of the epididymis.

Corpus of epididymis

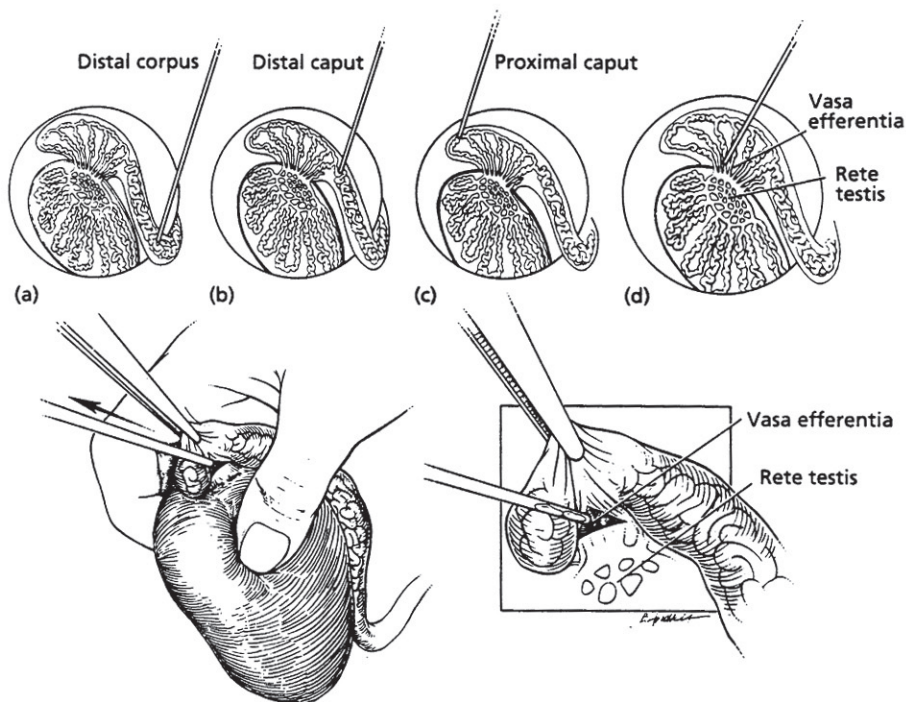Head of epididymis

Tail of epididymis

(a)　　　　(b)　　　　(c)

Fig. 78.4 The most motile sperm are found very proximally, usually in the vasa efferentia or rete testis fluid.

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, Montana) 2.5 cm inside the fimbrial ostium. The patients are discharged the next day and undergo fairly painless postoperative recovery. The wives receive progesterone in oil, 50 mg day^{-1} intramuscularly beginning with the day of embryo transfer.

Results

At present, of 115 cases, there have been 24 pregnancies, with six miscarriages. That is a pregnancy rate of 21% and a live baby rate of 16%.

Pregnancies which have occurred readily after vaso-epididymostomy 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 [43]. If this theory is 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 the sperm are generally senescent and nonmotile in the 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 fertilization; (2) incubation of sperm outside of the milieu of the obstructed epididymis; and (3) transfer of the embryos into the fallopian tube rather than into the uterus.

Although these results will have to be considered preliminary until greater numbers are obtained, for the moment it is safe to conclude: (1) sperm from the proximalmost 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 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.

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