Reprod. Fertil. Dev., 1994, 6, 93–104

A Modern View of Male Infertility

Sherman J. Silber

St Luke's Hospital, 224 South Woods Mill Road, St Louis, Missouri 63017, USA.

Abstract: It is archaic to view male factor infertility today separately from *in vitro* fertilization (IVF) and treatment of the female partner. Oligoasthenozoospermia may be an inherited condition (most likely on the Y chromosome), and is refractory to any treatment of the male including hormones and varicocelectomy. IVF technology is the only justifiable approach for achieving a pregnancy in these couples. The reasons for this view and the suggested modern approach to couples with oligoasthenozoospermia are outlined in this review. However, obstructive azoospermia is different as it can be successfully corrected with microsurgery in over 90% of men. When it cannot be corrected, as in congenital absence of vas, microsurgical sperm retrieval combined with IVF can still be highly effective in producing pregnancy with sperm from the husband. The most important arena for research into male infertility in the next decade will be to map out the deletions on the Y chromosome that might result in defective spermatogenesis, and which probably cause most cases of non-obstructive male factor infertility.

Extra keywords: congenital absence of vas, IVF.

Introduction

Male factor infertility has undergone revolutionary changes in the last decade. It is now clear that hormonal therapy (Clomid, Pergonal, human chorionic gonadotrophin, testosterone-rebound, tamoxifin, etc.) has no beneficial effect on the infertile male, except for the rare case of Kallman's Syndrome or pituitary deficiency (Ronnberg 1980; Tyler *et al.* 1982; Wang *et al.* 1983; Baker *et al.* 1984; Baker and Kovacs 1985; Baker 1986). Nevertheless, infertile men who are referred to urologists regularly receive such treatment.

It has also been shown by several large, well controlled studies supervised objectively that varicocelectomy has no beneficial effect on male infertility (Nilsson *et al.* 1979; Vermeulen and Vandeweghe 1984; Baker *et al.* 1985) but very few infertile men who are referred to a urologist escape this procedure. Countless papers by clinical urologists attest to the supposition that over 40% of male factor problems are caused by varicocele. However, there is no evidence for this contention and, in fact, no beneficial effect of varicocelectomy has ever been demonstrated in controlled studies. Indeed, 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 little evidence for antibodies having a major role, and immunological treatment of such men other than with *in vitro* fertilization (IVF) or gamete intrafallopian transfer (GIFT) has been largely ineffective (Silber 1989*a*; Patrizio *et al.* 1992).

So what does this leave for the treatment of male infertility? The most significant advance came from gynaecological endocrinologists who began to call the condition 'male factor' instead of 'male infertility'. We must leave the 1980*s* behind to realize how very far we have come in treating couples with male factor infertility. This brief review concerns: *(1)* the causes of male factor infertility, and its simplified evaluation; *(2)* the treatment of male factor 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 mammal studied so far, and the condition is evolutionary, genetic, and unremediable. Whereas most animals produce 20–25x10^6 sperm g^{-1} of testicular tissue per day, a human produces only 4x10^6 (Austin and Short 1976; Chowdbury and Marshall 1980). The gorilla has an even poorer production and his testes and penis are so tiny that they can barely be seen. Even the very 'fertile' human male with over 60x10^6 sperm mL^{-1} has very poor sperm compared with other mammals. The large number of abnormal forms, debris, and non-motile sperm found in human semen does not occur in most other mammals except, of course, the gorilla. Comparative biologists believe that this is due to the lack of 'sperm competition' in monogamous animals. Over many thousands of years, the fact that the female will only become impregnated by the sperm of one partner means that there is no sperm competition and,

therefore, no selection for greater sperm production in subsequent male offspring. Thus, oligoasthenozoospermia may simply be a genetically transmitted condition, worse in some than in others, but invariably a part of being human (Silber 1990). One need not search for a hormonal or other pathologic aetiology, because it is simply an unalterable genetic problem, and pathological testicular changes that can be detected histologically are generally not seen in men with oligoasthenozoospermia.

Since poor sperm is a characteristic of the human species, even those males without an infertility problem, we must start with the difficult issue of how to 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 this review.

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 most dramatic demonstration of this confusion is the case by Sokol and Sparkes (1987) in which a wife became pregnant naturally from her husband who had only 50 000 sperm mL^{-1} with poor motility. The husband, the mother, and the baby were carefully genetically screened by blood typing, and it was determined with 99·99% certainty that the husband was indeed the father. So if only 50 000 sperm mL^{-1} with less than 10% motility are adequate for a natural pregnancy (without IVF) what is 'male factor'? It had been thought that any sperm concentration under $20x10^6$ sperm mL^{-1} indicated infertility. This conclusion was clearly quite tenuous, but only recently has the weakness of this assumption been recognized. The possibility needed to be addressed that low sperm concentrations, like high sperm concentrations, might occur at either end of the bell-shaped population curve and might perhaps be unrelated to the fertility of the man. It is therefore essential to be cautious when suggesting to any infertile couple with a poor semen analysis that the husband is infertile, since severely low sperm concentrations and motility are simply associated with decreased fertility on a large statistical population basis.

The author has also reviewed sperm count and motility indices in men following vasovasostomy whose wives became pregnant, in comparison with those whose wives did not become pregnant (Silber 1989*a*; Table 1). Of the 'successful' vasovasostomy patients whose wives became pregnant, 11% had total sperm counts per ejaculate of fewer than $10x10^6$ sperm and 64% had more than $40x10^6$ sperm mL^{-1}. If this is compared with the group of 'infertile' men after vasovasostomy, there are no significant differences except that in the group of men whose wives did not become pregnant 23% had fewer than $10x10^6$ sperm mL^{-1}.

Table 1. Frequency distribution of motile sperm count following vasovasostomy in men whose wives did or did not become pregnant (10-year follow-up study; from Silber 1989*a*)

Values in parentheses are percentages of total no. in each column

Total motile sperm count ($x10^6$)	No. of patients	No. pregnant	No. not pregnant
0–10	32	25 (11)	7 (23)
10–20	31	27 (12)	4 (13)
20–40	32	30 (13)	2 (7)
40–80	79	68 (30)	11 (37)
>80	84	78 (34)	6 (20)
Total	258	228 (100)	30 (100)

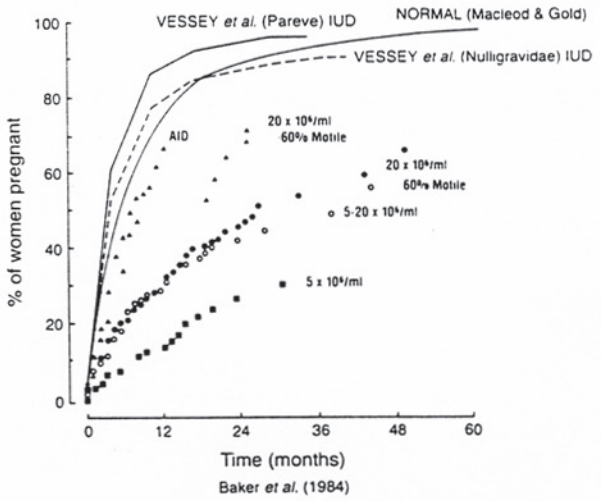

Fig. 1. Cumulative and life-table pregnancy rates in relation to sperm count. From Baker *et al.* (1986).

Relationship of Sperm Count to Pregnancy Rate

Using data from several sources (MacLeod and Gold 1953; Vessey *et al.* 1976; Kovacs *et al.* 1982), Baker *et al.* (1986) constructed a life-table pregnancy curve for infertile couples with varying degrees of oligozoospermia compared with controls (Fig. 1). The wife was treated

Table 2. Pregnancy rates using donor insemination in wives of azoospermic *v.* oligozoospermic men (from Emperaire *et al.* 1982)

Husband	No. of patients	Pregnancy rate (%)	Pregnancy rate per cycle (%)
Azospermic	95	61	11·6
Oligospermic	95	29	4·9

no matter how poor the semen from the husband. Pregnancy rates were compared for couples in whom the male had a sperm concentration of $<5x10^6$ mL^{-1}, $5–20x10^6$ mL^{-1}, $>20x10^6$ mL^{-1} with <60% sperm motility, and $>20x10^6$ mL^{-1} with >60% sperm motility. These four groups were then compared with an artificial donor insemination group (presumed to be the highest quality semen), and the pregnancy rate table from Vessey *et al.* (1976) of the mean time to pregnancy in women discontinuing the intrauterine device (IUD). The spontaneous pregnancy rate reported by MacLeod and Gold (1953) was assumed to be normal. They concluded that women may become pregnant with extremely low sperm counts, but the higher the motile sperm count, the greater are the chances of pregnancy.

Emperaire *et al.* (1982) published a remarkable observation from their donor insemination programme. Women undergoing artificial insemination by donor (AID) were separated according to their husband's infertility. The pregnancy rate per cycle in women whose husbands were azoospermic was 11·6% compared with only 4·9% in women whose husbands were oligozoospermic. 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 oligozoospermic (Table 2). The only reasonable explanation for this finding was that an oligozoospermic 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, whereas 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 concentrations 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.

IVF and GIFT for the Treatment of Male Factor Infertility

Great excitement has been generated about the use of IVF in couples with very low sperm concentrations, and good fertilization rates have been obtained in many severely oligozoospermic couples (Cohen *et al.* 1985). Theoretically, IVF or gamete intrafallopian transfer (GIFT) allows the small number of available sperm a greater opportunity for direct contact with the oocyte (McDowell *et al.* 1985; Matson *et al.* 1987).

In cases of congenital absence of the vas, we originally aspirated relatively poor sperm from the epididymis, and to our surprise fertilized oocytes *in vitro*; a good number of pregnancies in this group made it clear that sperm with severely reduced motility can often fertilize the oocyte if other obstacles are eliminated (Silber *et al.* 1988, 1990*a*).

Kruger *et al.* 1987 reported the importance of sperm morphology for IVF success, and they considered any deviation from a perfect oval head, neck and tail to be abnormal. Remarkably, provided that at least 4% of sperm demonstrated perfectly normal morphology, fertilization *in vitro* was achieved. Once again, this suggests that a critical visual examination of the spermatozoa is a good predictor of the likelihood of fertilization, and that only a small population of 'fertile' sperm within a poor semen sample is necessary for fertilization *in vitro*. The great success in treating many severely oligozoospermic couples with IVF, GIFT or zygote intrafallopian transfer (ZIFT) has required a new definition of 'male factor' so as to distinguish those men whose sperm are capable of fertilizing despite oligozoospermia from those who are infertile.

When the total number of motile spermatozoa in the ejaculate is $<5x10^6$ (severe oligoasthenozoospermia; Yovich *et al.* 1987), most methods of sperm preparation such as swim-up (Wong *et al.* 1986), washing and resuspension (Schlaff 1987) and sedimentation (Purdy 1982) fail to recover a sufficient number of normal, motile spermatozoa. This, in turn, is correlated with poor IVF results (Yates and de Kretser 1987). Resuspension does not remove cells and debris at all and, although swim-up is effective, Percoll gradient techniques are most effective in filtering out debris and other contaminants, although they often yield a low rate of sperm recovery in very poor samples (Hyne *et al.* 1986; Gellert–Mortimer *et al.* 1988; Guérin *et al.* 1989). However, the use of a mini-Percoll gradient achieves a greater sperm recovery with poor samples since a smaller volume can be used (Ord *et al.* 1990).

Because the definition of 'male infertility' is so broad, it is risky to present IVF and GIFT pregnancy rates for the 'male factor' category. With IVF, the pre-wash motile sperm count in the semen is not a significant determinant of fertilization or pregnancy. The pregnancy rate seems to be determined by the number of morphologically normal, motile sperm present after the sperm is washed to remove seminal plasma. In our experience, when the total motile sperm count recovered from a Percoll or mini-Percoll preparation is $>1·5x10^6$, the fertilization rate is not significantly different from couples without male factor infertility. When the post-wash recovery is $<1·5x10^6$ motile sperm, the fertilization and pregnancy rates are very poor; nevertheless, if fertilization occurs, the implantation rate per embryo transferred is the same. With the advent of intracytoplasmic sperm injection (Van Steirteghem *et al.* 1993), IVF will clearly be the only treatment for such cases.

Microsurgery for Obstructive Azospermia

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 a popular method of birth control (Liskin *et al.* 1983). For many years the pregnancy rate after surgical re-anastomosis of the vas had been very low and a variety of explanations were offered for the relatively poor success rates (O'Connor 1948; Phadke and Phadke 1967; Middleton and Henderson 1978). With the advent of microsurgical techniques, pregnancy rates improved considerably, suggesting that purely micro-mechanical factors were responsible (Silber 1977*a*, 1978*a*, 1978*b*, 1978*c*). Yet there were still many cases of technically perfect vasovasostomies followed by complete azoospermia or oligoasthenozoospermia with no pregnancy. On further investigation, we found that the pressure increase in these patients after vasectomy had led to secondary epididymal obstruction which was the cause of failure of otherwise successful vasovasostomies. Thus, vasoepididymostomy is required in most cases of vasectomy reversal in order to obtain a high success rate.

We have questioned (Silber 1989*a*) any major correlation between sperm antibodies or testicular damage and subsequent fertility after vasovasostomy (Linnet and Hjort 1977; Sullivan and Howe 1977; Thomas *et al.* 1981; Brickel *et al.* 1982; Jarow *et al.* 1985). We have established that the deleterious effect of pressure increase subsequent to vasectomy was not on the testis, but rather an effect on epididymal dilatation, perforation and sperm inspissation and blowouts in the epididymis, causing secondary epididymal obstruction which is the major problem in readily retrieving the fertility of vasectomized men (Silber 1977*a*, 1978*c*, 1979; Silber and Rodriguez-Rigau 1981). In humans, the deleterious effect of pressure increase is always on the epididymis, not on the testis. The secondary epididymal obstruction caused by vasectomy leads us to recommend that when vasectomy is performed the testicular end of the vas should not be sealed, so as to lessen the pressure build-up and thereby increase the ease of reversibility later (Silber 1977*b*; Shapiro and Silber 1979).

What is the fertility rate after vasovasostomy in patients who have suffered no secondary epididymal damage, as evidenced by sperm present in the vas fluid at the time of vasovasostomy? In such a favourable group of patients (Silber 1977*a*, 1979, 1989*a*), 98% of cases had sperm in the ejaculate and 88% successfully impregnated their wives. However, none of the wives of azoospermic patients became pregnant. This compares with the expected pregnancy rate of 96% for previously fertile couples discontinuing contraception (Vessey *et al.* 1976). The frequency distributions of post-operative semen parameters for males whose wives did or did not become pregnant is summarized in Tables 1 and 3. There was remarkably little difference in pregnancy rate among men with low or high sperm concentrations. Similar findings were seen with sperm motility provided it was greater than 20%. However, the pregnancy rate was lower when sperm motility was less than 20%. Above these lower limits, the pregnancy rate was not seriously affected by poor semen values. These post-operative semen parameters in patent cases were not very different from previously reported pre-vasectomy semen parameters (Zuckerman *et al.* 1977).

Table 3. Pregnancy rate according to percentage sperm motility in men with patency following vasovasostomy (10-year follow-up study; from Silber 1989*a*)

Values in parentheses represent percentages of total number

Sperm motility	No. of patients	No. pregnant	No. 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	228 (88)	30

Table 4. Lack of effect of varicocele (not operated on) on pregnancy rate following vasovasostomy

Values in parentheses are percentages

	No. of patients	With varicocele	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)	42 (14·8)	240 (85·2)

Table 5. Relationship of serum antisperm antibody titres to pregnancy rate after vasovasostomy

Values in parentheses are percentages

	No. of patients	Immobilizing titre[A]		Agglutinating titre[B]	
		>0	>10	>0	>20
Not azoospermic:					
Pregnant	75	29 (39)	18 (24)	42 (56)	30 (40)
Not pregnant	11	4 (36)	2 (16)	6 (54)	6 (54)
Azoospermic	12	5 (42)	3 (25)	7 (58)	5 (42)
Total group	98	38 (39)	23 (24)	56 (57)	41 (42)

A Determined using the Isojima sperm immobilization test.
B Determined using the Kibrick agglutination test.

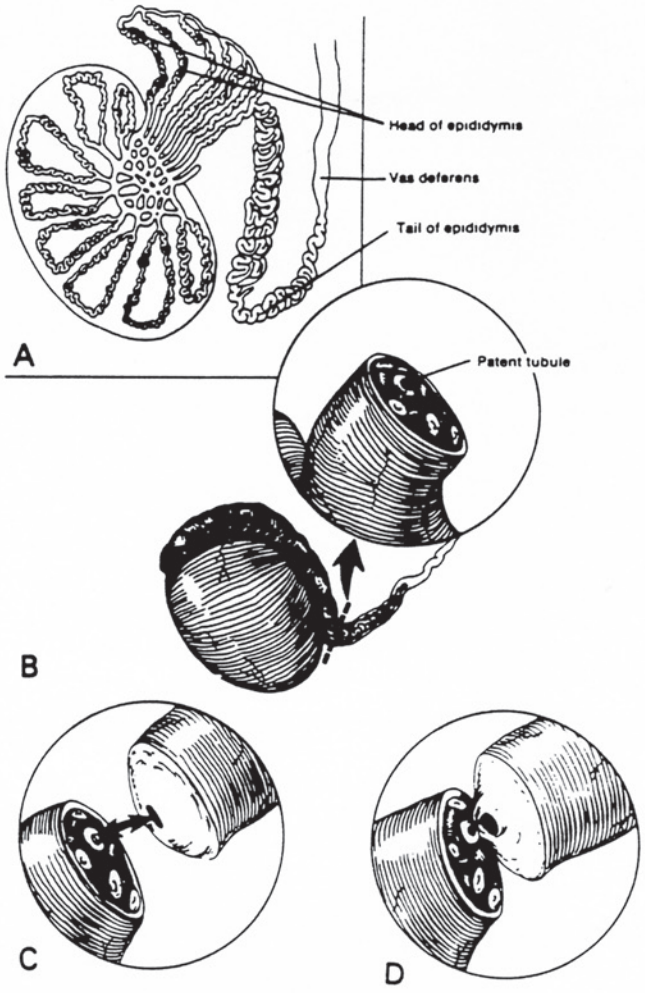

Fig. 2. Specific tubule end-to-end anastomosis of the vas lumen to the epididymis, proximal to the site of obstruction.

As summarized in Table 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 5 shows that the presence of high serum titres of immobilizing or agglutinating antisperm antibodies did not influence pregnancy rates. The data in fertile vasectomy-reversal patients substantiate our perhaps divergent view that sperm antibodies and varicocele, and even sperm concentration, have little to do with male infertility. All men have varying percentages (0–20%) of potentially fertile sperm in their ejaculate which may be genetically determined, and the only advisable treatment is IVF, except for obstruction and a few other rare cases.

Reasons for High Pregnancy Rate in Patients with no Secondary Epididymal Blockage

The high pregnancy rate in this group of patients requires some explanation. Our study suggested that the pregnancy rate in patients who have patency accurately re-established without epididymal damage is eventually not significantly lower than in a normal population of couples. Vessey *et al.* (1976) demonstrated that 96·5% of couples with proven prior fertility conceive within four years of discontinuing contraception. In our couples with patency after vasovasostomy who had no evidence of epididymal pressure damage, 88% had conceived after long-term (10 years) follow-up. However, patients with secondary epididymal blockage require a completely different approach.

The poor success with vasovasostomy 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 (Silber 1979). When there is no sperm in the vas fluid, vasoepididymostomy proximal to the site of epididymal blockage is required (Silber 1978*a*, 1984, 1986).

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. However, a practical understanding of epididymal physiology is as important as precise microsurgical techniques.

In every mammal that has been studied, spermatozoa from the caput epididymidis are capable of only weak circular motion at most, and are not able to fertilize (Orgebin-Crist 1969). In earlier studies, spermatozoa from the corpus epididymidis could occasionally fertilize, but the pregnancy rate was still low. Spermatozoa were simply aspirated from specific regions of the epididymis and then promptly inseminated (Gaddum and Glover 1965; Paufler and Foote 1968). However, previous experiments in guinea pigs (Young 1931) 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'. In fact, Young observed the same 'inversion' of regions of sperm motility and non-motility in the obstructed epididymis that we have noted in clinical obstructive azoospermia; sperm in the more distal regions have the poorest motility and those present in the more proximal regions have the best motility. He concluded that in an obstructed epididymis the more distal sperm are senescent, whereas the more proximal sperm

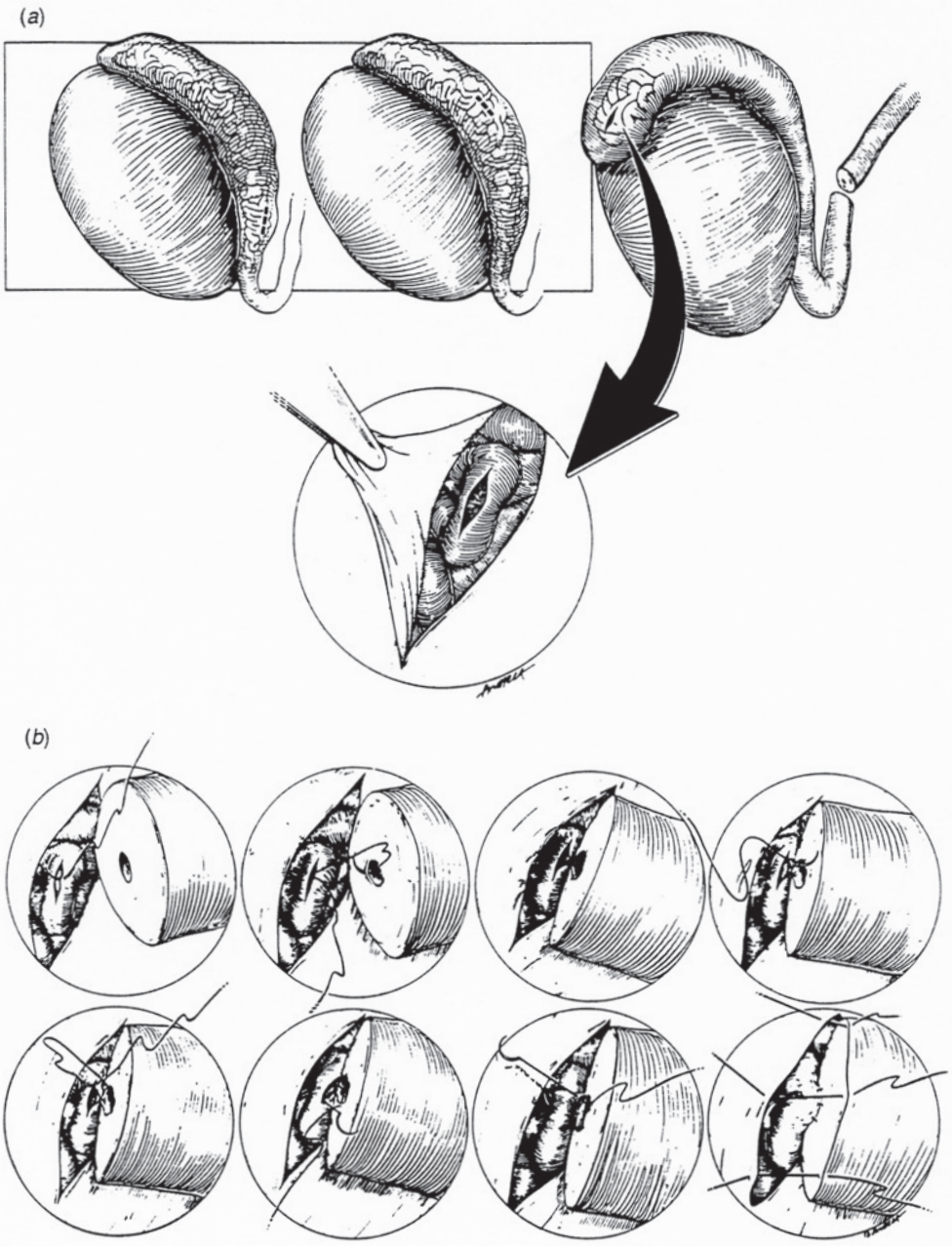

Fig. 3. *(a)* Small openings are made in the epididymal tunic beginning distally and moving proximally. After a longitudinal slit is made in the epididymal tubule with microscissors, the distal-most level at which motile sperm are found is used for the anastomosis; *(b)* the end-to-side specific tubule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

have had time to mature despite not having traversed the epididymis (Young 1931). Our clinical experience with vasoepididymostomy supports Young's original thesis (Silber 1989*b*).

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

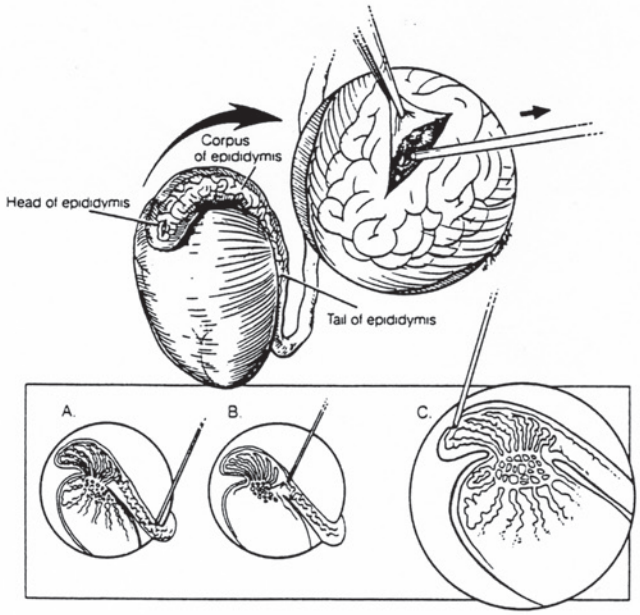

Fig. 4. Technique for epididymal sperm aspiration which begins in the distal corpus region of the epididymis, and moves proximally (*A* to *C*) until motile sperm are recovered. In most cases, motility is observed only in the most proximal region of the epididymis.

tubule, mucosa-to-mucosa in a leakproof fashion (Silber 1977*a*, 1978*a*, 1986). 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 where there are no spermatozoa to where there is an abundant number of spermatozoa in the fluid coming from the epididymal tubule (Figs 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.

Seventy-two percent of the cases of epididymal anastomosis have resulted in pregnancy (Silber 1989*b*). 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 of the sperm. The fact that a 43% pregnancy rate occurred with caput patency indicates that transit beyond the head of the epididymis is not an absolute requirement for sperm to attain fertilizing capacity. Recent clinical cases have demonstrated that it is even possible, in some circumstances, for spermatozoa that have never passed through the epididymis to fertilize the human egg. In two reported cases of vasa efferentia to vas deferens anastomosis, the post-operative ejaculate contained normally motile sperm and the wives became pregnant (Silber 1988*a*). In addition, pregnancy from aspiration of epididymal sperm combined with IVF and ZIFT in cases of irreparable obstruction provides further evidence that transit through the epididymis is not a mandatory requirement for fertilization (Silber *et al.* 1987; Silber 1988*b*).

Recent studies of epididymal sperm transport in the human indicate that the human epididymis is not a

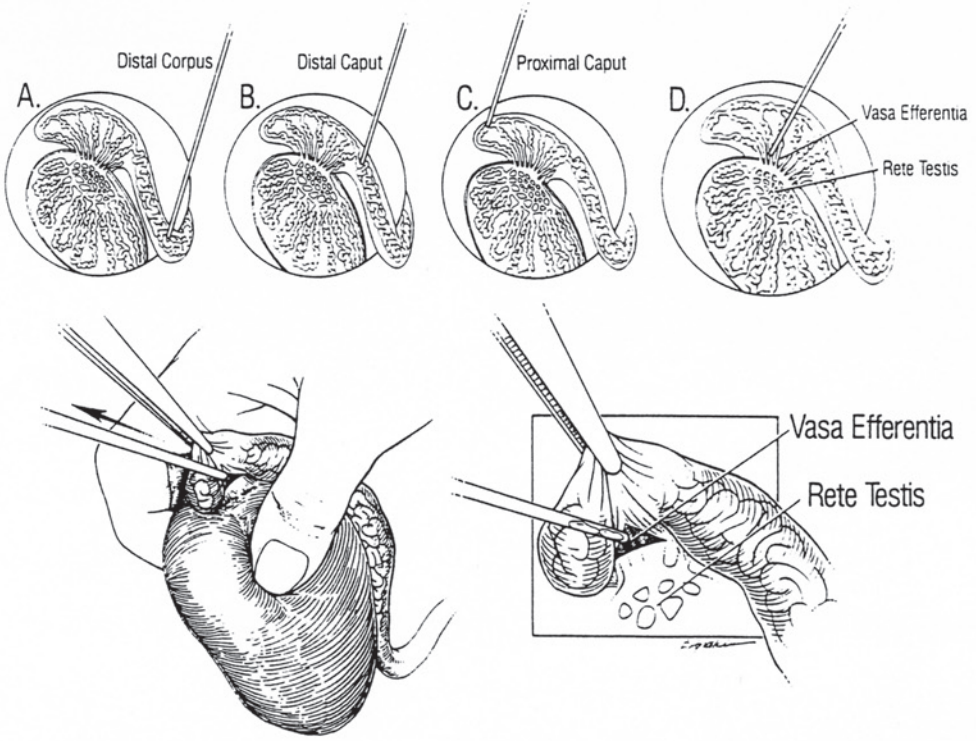

Fig. 5. The most motile sperm are found very proximally, usually in the vasa efferentia or rete testis.

storage area, and sperm pass through the entire human epididymis very quickly, in a mere two days, not eleven days as was previously thought (Johnson and Varner 1988). Thus, it is possible that the epididymis in the human may not be as essential to sperm development and fertility as it appears to be in most other 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 previously has been considered untreatable (El-Itreby and Girgis 1961). This large group of patients, frustrating to treat, has been shown on countless testis biopsies to have normal spermatogenesis, and are theoretically making sperm quite capable of fertilizing an egg (Silber *et al.* 1990*a*, 1990*b*). Treatment until now has been largely unsuccessful (Temple-Smith *et al.* 1985). However, a treatment protocol involving microsurgical aspiration of sperm from the proximal region of the epididymis, combined with IVF, has been developed; this now offers very good results in this previously frustrating group of couples (Silber 1988*b*; Silber *et al.* 1990*a*).

Epididymal Sperm Aspiration, Washing Methodology, and IVF

At the same time as the eggs are aspirated from the wife for IVF, the husband undergoes scrotal exploration. The surgical technique (Fig. 4) in the male is as follows: scrotal contents are extruded through a very small incision, the tunica vaginalis is opened and the epididymis is exposed. Under 10–40x magnification with an operating microscope, a tiny incision is made with microscissors into the epididymal tunic to first 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 is examined for sperm 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 detected. In contrast to what might be expected, we have found that motile sperm are not obtained until we reach the proximal-most portion of the caput epididymidis or even the vasa efferentia (Figs 4 and 5).

It was discovered that distal epididymal sperm (which one would think are the only sperm capable of fertilization) are usually non-motile, not because of immaturity but because of senescence. The discovery that in order to obtain live sperm we had to sample the most proximal regions of the epididymis, even vasa efferentia, was the major factor resulting in our success with many early patients. However, the results were still highly unpredictable and often inexplicably poor.

In the laboratory, the epididymal sperm are concentrated into a volume of 0·3 mL, layered onto a discontinuous mini-Percoll gradient, and centrifuged at 300*g* for 30 min. The entire 95% fraction is then washed twice and inseminated

Table 6. Pregnancy rates for the first 100 cases of IVF for congenital absence of the vas

Series	No. of cycles[A]	No. of pregnancies[B]	Pregnancy rate per cycle (%)[C]
1	32	10 (7)	31 (22)
2	16	2 (1)	12 (6)
3	21	5 (4)	24 (19)
4	13	0	0
5	18	5 (4)	28 (22)
Total	100	22 (16)	22 (16)

[A] Cycles in which sperm aspiration was successful.
[B] Values in parentheses represent term pregnancies.
[C] Values in parentheses are term pregnancy rates (%).

Table 7. Fertilization rates for the first 100 cases of IVF for congenital absence of the vas

Series	No. of cycles[A]	No. of cycles with ≥1 embryo[B]	No. of mature eggs	No. 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
Total	100	59 (59)	1326	324	24

[A] Cycles in which sperm aspiration was successful.
[B] Values in parentheses are percentages.

Table 8. Results when >2 embryos were produced in the first 100 cases of IVF for congenital absence of the vas

Series	Total no. of cycles[A]	No. of cycles with >2 embryos[B]	No. of pregnancies[C]
1	32	12 (38)	9 (75)
2	16	7 (44)	2 (29)
3	21	8 (38)	5 (63)
4	13	2 (15)	0
5	18	9 (50)	5 (55)
Total	100	39 (39)	21/38 (55)

[A] Cycles in which sperm aspiration was successful.
[B] Values in parentheses are percentages.
[C] Values in parentheses represent pregnancy rate (%) per transfer.

Table 9. Fertilization and pregnancy rates in cycles with ≥10 eggs in the first 100 cases of IVF for congenital absence of the vas

Series	No. of cycles[A]	No. of mature eggs	No. of embryos[B]	No. of pregnancies[C]
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
5	14 (78)	260	104 (40)	5 (36)
Total	65 (65)	1153	286 (25)	22 (34)

[A] Values in parentheses are percentages of the total number of cycles in the series.
[B] Values in parentheses represent percentage of oocytes fertilized.
[C] Values in parentheses are percentages.

Table 10. Fertilization rates in cycles with >1 embryo in the first 100 cases of IVF for congenital absence of the vas

Series	No. of cycles[A]	No. of mature eggs	No. 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
Total	45 (45)	693	306	44

[A] Values in parentheses are percentages of the total number of cycles in the series.

with all of the eggs in a Falcon mini-test tube in 1 mL of human tubal fluid (HTF) culture medium and incubated at 37°C with 5% CO_2 in air (Ord *et al.* 1990). Two days after insemination, embryos are transferred to the Fallopian tubes or the uterus, and the patients are discharged the next day and undergo a fairly painless post-operative recovery. The wives receive progesterone in oil, 50 mg per day intramuscularly, beginning on the day of embryo transfer.

Results with Conventional IVF

There were 24 pregnancies from the first 115 cases following conventional IVF, with 6 miscarriages. Thus, the pregnancy rate was 21% and the live baby rate per mature egg was 16%. Data from the first 100 cases are presented in Tables 6–10. The overall fertilization rate per mature egg was 24% and embryos were obtained in 59% of the cases (Table 7). However, more than two embryos were produced in only 38% of cycles (Table 8). With regard to fertilizing ability, there appeared to be two subgroups of patients with congenital absence of vas: those who fertilized many eggs and had a high pregnancy rate, and those who fertilized only a few eggs or none at all and had a low pregnancy rate (Tables 9 and 10).

Pregnancies that have occurred readily after vasoepididymostomy to the caput epididymidis, and even in some cases to the vasa efferentia, indicate that immature sperm that have not had a chance to pass through the epididymis might mature on their own during storage in the vas deferens (Silber 1988*a*, 1989*b*). If this theory were true, it might explain why we have been able to achieve pregnancy by aspirating sperm more proximally.

It is clear from our published results that distal epididymal sperm in obstructed males are either nonmotile or totally degenerate as a result of senescence (Silber *et al.* 1990*a*; Asch *et al.* 1992). Motile sperm have to be obtained from the proximal caput or vasa efferentia 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 how epididymal sperm that appear adequate often do not fertilize, and those that look poor may have high fertilizing capacity. A routine glance at the sperm often reveals no differences, but sometimes only a very small subpopulation are actually capable of fertilization (Silber *et al.* 1990*a*; Davis *et al.* 1991). The presence or absence of sperm antibodies has no apparent effect (Patrizio *et al.* 1989).

For the moment it is safe to conclude that: *(1)* sperm from the proximal-most caput epididymidis are capable of fertilizing the human egg *in vitro*; *(2)* passage of time after emergence from the testis may be adequate for sperm maturation in some cases without the absolute need for transit through the rest of the epididymis; and *(3)* the fertilization and subsequent pregnancy rates obtained with conventional IVF using epididymal sperm are very unpredictable.

Table 11. A comparison of results of micro-epididymal sperm aspiration (MESA) using intracytoplasmic sperm injection (ICSI) and conventional IVF in a similar patient population

No. of cycles	No. of mature eggs	No. of embryos	Fertilization rate (%)	No. of cycles with >1 embryo[A]	Pregnancy rate (%)[B]
ICSI–MESA					
17	197	80	41	14/17 (82)	47 (30)
IVF–MESA					
67	1427	98	7	13/67 (19)	9 (4·5)

[A] Values in parentheses are percentages.
[B] Values in parentheses are ongoing pregnancy rates (%).

Addendum 24 November 1993: Because of the poor results using conventional IVF and micro-epididymal sperm aspiration (MESA), we set up a collaborative study with André Van Steirteghem and Paul Devroey of the Free Dutch-speaking University in Brussels, Belgium to examine the potential for combining MESA with intracytoplasmic sperm injection (ICSI; Van Steirteghem *et al.* 1993). When we took the poorest candidates, whose epididymal sperm fertilized poorly if at all using conventional IVF, almost all achieved fertilization with ICSI and the pregnancy rate increased from 9% to 47% (Table 11). Even in patients that were so scarred that no epididymal sperm could be obtained, sperm (virtually non-motile) were collected from testicular biopsies and yielded a fertilization rate of 46% after ICSI. It is very clear to us now that conventional IVF has no role in MESA and that all these epididymal sperm cases should be treated using ICSI.

References

Asch, R., Patrizio, P., and Silber, S. (1992). Ultrastructure of human sperm in men with congenital absence of the vas deferens: clinical implications. *Fertil. Steril.* **58**, 190–3.

Austin, C. R., and Short, R. V. (1976). 'The Evolution of Reproduction.' (Cambridge University Press: Cambridge.)

Baker, H. W. G. (1986). Requirements for controlled therapeutic trials in male infertility. *Clin. Reprod. Fertil.* **4**, 13–25.

Baker, H. W. G., and Kovacs, G. T. (1985). Spontaneous improvement in semen quality: regression towards the mean. *Int. J. Androl.* **8**, 421–6.

Baker, H. W. G., Straffon, W. G. E., McGowan, M. P., Burger, H. G., de Kretser, D. M., and Hudson, B. (1984). A controlled trial of the use of erythromycin for men with asthenospermia. *Int. J. Androl.* **7**, 383–8.

Baker, H. W. G., Burger, H. G., de Kretser, D., Hudson, B., Rennie, G. C., and Straffon, W. G. E. (1985). Testicular vein ligation and fertility in men with varicoceles. *Br. Med. J.* **291**, 1678–80.

Baker, H. W. G., Burger, H. G., and de Kretser, D. (1986). Male infertility. In 'Reproductive Medicine'. (Eds E. Steinberger, G. Fraiese and A. Steinberger.) pp. 187–97. (Raven Press: New York.)

Brickel, D., Bolduan, J., and Farah, R. (1982). The effect of vasectomy–vasovasostomy on normal physiologic function of the vas deferens. *Fertil. Steril.* **37**, 807–10.

Chowdbury, A. K., and Marshall, G. (1980). Irregular pattern of spermatogenesis in the baboon and its possible mechanism. In 'Testicular Development, Structure, and Function'. (Eds A. Steinberger and E. Steinberger.) pp. 129–37. (Raven Press: New York.)

Cohen, J., Edwards, R., Fehilly, C., Fishel, S., Hewitt, J., Purdy, J., Rowland, C., Steptoe, P., and Webster, J. (1985). *In vitro* fertilization: a treatment for male infertility. *Fertil. Steril.* **43**, 422–32.

Davis, R. O., Overstreet, J. W., Asch, R. H., Ord, T., and Silber, S. J. (1991). Movement characteristics of human epididymal sperm used for fertilization of human oocytes *in vitro*. *Fertil. Steril.* **56**, 1128–35.

El-Itreby, A. A., and Girgis, S. M. (1961). Congenital absence of the vas deferens in male sterility. *Int. J. Fertil.* **6**, 409–16.

Emperaire, J. C., Gauzère-Sonmireu, E., and Audebert, A. J. (1982). Female fertility and donor insemination. *Fertil. Steril.* **37**, 90–3.

Gaddum, P., and Glover, T. D. (1965). Some reactions of rabbit spermatozoa to ligation of the epididymis. *J. Reprod. Fertil.* **9**, 119–30.

Gellert–Mortimer, S. T., Clarke, G. N., Baker, H. W. G, Hyne, R. V., and Johnson, W. I. H. (1988). Evaluation of Nycodenz and Percoll density gradients for the selection of motile human spermatozoa. *Fertil. Steril.* **49**, 335–41.

Guérin, J. F., Mathieu, C., Lornage, J., Pinatel, M. C., and Boulieu, D. (1989). Improvement of survival and fertilizing capacity of human spermatozoa in an IVF programme by selection on discontinuous Percoll gradients. *Hum. Reprod. (Oxf.)* **4**, 798–804.

Hyne, R. V., Stojanoff, A., Clarke, G. N., Lopata, A., and Johnston, W. I. H. (1986). Pregnancy from *in vitro* fertilization of human eggs after separation of motile spermatozoa by density gradient centrifugation. *Fertil. Steril.* **45**, 93–6.

Jarow, J. P., Budin, R. E., Dym, M., Kirkin, B. R., Noren, S., and Marshall, F. F. (1985). Quantitative pathologic changes in the human testis after vasectomy: a controlled study. *New Engl. J. Med.* **313**, 1252–6.

Johnson, L., and Varner, D. (1988). Effect of age and daily spermatozoa production on transit time of spermatozoa through the human epididymis. *Biol. Reprod.* **39**, 812–17.

Kovacs, A. T., Leeton, J. F., Matthews, C. D., Steigrad, S. J., Saunders, D. M., Jones, W. R., Lyneham, R., and McMaster, R. (1982). The outcome of artificial donor insemination compared to the husband's fertility status. *Clin. Reprod. Fertil.* **1**, 295–9.

Kruger, T. F., Acosta, A., Simmons, K. F., Swanson, R. J., Matta, J. F., Veeck, L. L., Morshedi, M., and Brugo, S. (1987). A new method of evaluating sperm morphology with predictive value for human *in vitro* fertilization. *Urology* **30**, 248–51.

Linnet, L., and Hjort, T. (1977). Sperm agglutinins in seminal plasma and serum after vasectomy: correlation between immunological and clinical findings. *Clin. Exp. Immunol.* **30**, 413–20.

Liskin, L., Pile, J. M., and Quillin, W. F. (1983). Vasectomy —safe and simple. *Popul. Rep.* **4**, 63–100.

MacLeod, J., and Gold, R. Z. (1953). The male factor in fertility and infertility. VI. Semen quality and other factors in relation to ease of conception. *Fertil. Steril.* **4**, 10–33.

Matson, P. L., Blackledge, D. C., Richardson, P. A., Turner, S. R., Yovich, J. M., and Yovich, J. L. (1987). The role of gamete intrafallopian transfer (GIFT) in the treatment of oligospermic infertility. *Fertil. Steril.* **48**, 608–12.

McDowell, J. S., Veeck, L. L., and Jones, H. W. J. (1985). Analysis of human spermatozoa before and after processing for *in vitro* fertilization. *J. In Vitro Fert. Embryo Transfer* **2**, 23–6.

Middleton, R. G., and Henderson, D. (1978). Vas deferens reanastomosis without splints and without magnification. *J. Urol.* **119**, 763–4.

Nilsson, S., Edvinsson, A., and Nilsson, B. (1979). Improvement in semen and pregnancy rate after ligation and division of the internal spermatic vein: fact or fiction. *Br. J. Urol.* **51**, 591–595.

O'Connor, V. J. (1948). Anastomosis of the vas deferens after purposeful division for sterility. *J. Am. Med. Assoc.* **136**, 162–3.

Ord, T., Patrizio, P., Marello, E., Balmaceda, J. P., and Asch, R. H. (1990). Mini-Percoll: a new method of semen preparation for IVF in severe male factor infertility. *Hum. Reprod. (Oxf.)* **5**, 987–9.

Orgebin-Crist, M.-C. (1969). Studies of the function of the epididymis. *Biol. Reprod.* **1**, 155–75.

Patrizio, P., Moretti-Rojas, I., Ord, T., Balmaceda, J., Silber, S., and Asch, R. H. (1989). Low incidence of sperm antibodies in men with congenital absence of the vas deferens. *Fertil. Steril.* **52**, 1018–21.

Patrizio, P., Silber, S., Ord, T., Moretti-Rojas, I., and Asch, R. (1992). Relationship of epididymal sperm antibodies to their *in vitro* fertilization capacity in men with congenital absence of the vas. *Fertil. Steril.* **58**, 1006–10.

Paufler, S. K., and Foote, R. H. (1968). Morphology, motility and fertility in spermatozoa recovered from different areas of ligated rabbit epididymis. *J. Reprod. Fertil.* **17**, 125–37.

Phadke, G. M., and Phadke, A. G. (1967). Experiences in the reanastomosis of the vas deferens. *J. Urol.* **97**, 888–90.

Purdy, J. M. (1982). Methods for fertilization and embryo culture *in vitro*. In 'Human Conception *in Vitro*'. (Eds R. G. Edwards and J. M. Purdy.) pp. 135–48. (Academic Press: London.)

Ronnberg, I. (1980). The effect of clomiphene citrate on different sperm parameters and serum hormone levels in preselected infertile men: a controlled, double blind crossover study. *Int. J. Androl.* **3**, 479–86.

Schlaff, W. D. (1987). New ways to prepare semen for IUI. *Contemp. Obstet. Gynaecol.* **4**, 79–86.

Shapiro, E. I., and Silber, S. J. (1979). Open-ended vasectomy, sperm granuloma and post-vasectomy orchalgia. *Fertil. Steril.* **32**, 546–50.

Silber, S. J. (1977*a*). Microscopic vasectomy reversal. *Fertil. Steril.* **28**, 1191–202.

Silber, S. J. (1977*b*). Sperm granuloma and reversibility of vasectomy. *Lancet* **ii**, 588–9.

Silber, S. J. (1978*a*). Microscopic vasoepididymostomy: specific micro-anastomosis to the epididymal tubule. *Fertil. Steril.* **30**, 656–76.

Silber, S. J. (1978*b*). Vasectomy and its microsurgical reversal. *Urol. Clin. North Am.* **5**, 573–84.

Silber, S. J. (1978*c*). Vasectomy and vasectomy reversal. *Fertil. Steril.* **29**, 125–40.

Silber, S. J. (1979). Epididymal extravasation following vasectomy as a cause for failure of vasectomy reversal. *Fertil. Steril.* **31**, 309–15.

Silber, S. J. (1984). Microsurgery for vasectomy reversal and vasoepididymostomy. *Urology* **5**, 504–24.

Silber, S. J. (1986). Diagnosis and treatment of obstructive azoospermia. In 'Male Reproductive Dysfunction'. (Eds R. J. Santen and R. S. Swerdloff.) pp. 479–517. (Marcel Dekker: New York.)

Silber, S. J. (1988*a*). Pregnancy caused by sperm from vasa efferentia. *Fertil. Steril.* **49**, 373–5.

Silber, S. J. (1988*b*). Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. *Fertil. Steril.* **50**, 525–8.

Silber, S. J. (1989*a*). Pregnancy after vasovasostomy for vasectomy reversal: a study of factors affecting long-term return of fertility in 282 patients followed for 10 years. *Hum. Reprod. (Oxf.)* **4**, 318–22.

Silber, S. J. (1989*b*). Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. *Hum. Reprod. (Oxf.)* **4**, 298–303.

Silber, S. J. (1990). 'How to get Pregnant with the New Technology.' (Warner Books: New York.)

Silber, S. J., and Rodriguez-Rigau, L. J. (1981). Quantitative analysis of testicle biopsy: determination of partial obstruction and prediction of sperm count after surgery for obstruction. *Fertil. Steril.* **36**, 480–5.

Silber, S. J., Ord, T., Borrero, C., Balmaceda, J., and Asch, R. (1987). New treatment for infertility due to congenital absence of the vas deferens. *Lancet* **ii**, 850–1.

Silber, S. J., Asch, R., Balmaceda, J., Borrero, C., and Ord, T. (1988). *In vitro* fertilization and pregnancy with sperm aspirated from the proximal head of the epididymis: a new treatment for congenital absence of vas deferens. *Fertil. Steril.* **50**, 525–8.

Silber, S. J., Ord, T., Balmaceda, J., Patrizio, P., and Asch, R. H. (1990*a*). Congenital absence of the vas deferens: the fertilizing capacity of human epididymal sperm. *New Engl. J. Med.* **323**, 1788–92.

Silber, S. J., Patrizio, P., and Asch, R. H. (1990*b*). Quantitative evaluation of spermatogenesis by testicular histology in men with congenital absence of the vas deferens undergoing epididymal sperm aspiration. *Hum. Reprod. (Oxf.)* **5**, 89–93.

Sokol, R. Z., and Sparkes, R. (1987). Demonstrated paternity in spite of oligospermia. *Fertil. Steril.* **47**, 356–8.

Sullivan, N. J., and Howe, G. E. (1977). A correlation of circulating antisperm antibodies to functional success of vasovasostomy. *J. Urol.* **117**, 189–91.

Temple-Smith, P. D., Southwick, G. J., Yates, C. A., Trounson, A. O., and de Kretser, D. M. (1985). Human pregnancy by *in vitro* fertilization (IVF) using sperm aspirated from the epididymis. *J. In Vitro Fert. Embryo Transfer* **2**, 119–22.

Thomas, A. J., Pontes, J. E., Rose, N. R., Segal, S., and Pierce, J. M. (1981). Microsurgical vasovasostomy: immunological consequences in subsequent fertility. *Fertil. Steril.* **35**, 447–50.

Tyler, J. P. P., Crockett, N. G., and Driscoll, G. L. (1982). Studies of human seminal parameters with frequent ejaculation. *Clin. Reprod. Fertil.* **1**, 273–85.

Van Steirteghem, A. C., Nagy, Z., Joris, H., Liu, J., Staessen, C., Smitz, J., Wisanto, A., and Devroey, P. (1993). High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod. (Oxf.)* **8**, 1061–6.

Vermeulen, A., and Vandeweghe, M. (1984). Improved fertility after varicocele correction: fact or fiction? *Fertil. Steril.* **42**, 249–56.

Vessey, M. P., Wright, N. H., McPherson, K., and Wiggins, P. (1978). Fertility after stopping different methods of contraception. *Br. Med. J.* **276**, 265–7.

Vessey, M. P., Lawless, M., McPherson, K., and Yeates, D. (1983). Fertility after stopping use of intrauterine device. *Br. Med. J.* **286**, 106.

Wang, C., Chan, C.-W., Wong, K.-K., and Yeung, K.-K. (1983). Comparison of the effectiveness of placebo, clomiphene citrate, mesterolone, pentoxifylline and testosterone rebound therapy in the treatment of idiopathic oligospermia. *Fertil. Steril.* **40**, 358–65.

Wong, P. C., Balmaceda, J. P., Blanco, J. D., Gibba, S., and Asch, R. H. (1986). Sperm washing and swim-up technique using antibiotics remove microbes from human semen. *Fertil. Steril.* **45**, 97–100.

Yates, C. A., and de Kretser, D. M. (1987). Male-factor infertility and *in vitro* fertilization. *J. In Vitro Fert. Embryo Transfer* **4**, 141–7.

Young, M. C. (1931). A study of the function of the epididymis. III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea pig. *J. Exp. Biol.* **8**, 151–62.

Yovich, J. L., Blackledge, D. G., Richardson, P. A., Matson, P. L., Turner, S. R., and Draper, R. (1987). Pregnancies following pronuclear stage tubal transfer. *Fertil. Steril.* **48**, 851–7.

Zuckerman, Z., Rodriguez-Rigau, L. J., Smith, K. D., and Steinberger, E. (1977). Frequency distribution of sperm counts in fertile and infertile males. *Fertil. Steril.* **28**, 1310–13.

Manuscript received 14 December 1993

Open Discussion

Peter Temple-Smith (Melbourne):

What sort of success rates have you had with GIFT?

Silber:

Actually, we haven't done GIFT on any of these cases because we always thought it was critical to know if we had fertilization. But I recognize the point of view from the Monash study, and if co-culture is good for embryos and can allow a larger percentage of them to develop to blastocyst, then certainly there has to be some benefit to GIFT in having an immediate intratubal environment.

Gary Clarke (Melbourne):

Do you have any more details on the breakdown of the immunoglobulin classes for the antibody results?

Silber:

The paper is in *Fertility and Sterility*. The immunoglobulin class didn't appear to matter. Bronson was convinced that this is a different group of patients, because we know that >80% binding is associated with a severe fertilization problem. But in this group, we know that the poor fertilization results in 65% of patients don't correlate with antibody formation.

Gordon Baker (Melbourne):

Can you comment on the results of patients with epididymal obstruction not due to congenital absence of the vas and what sort of overall results you're getting in those?

Silber:

The overall results have been similar but we have only done a small number of cases. We originally wanted to restrict ourselves to cases where I have done a vasoepididymostomy which failed, and a second vasoepididymostomy was being considered. In fact, if there has been a previous vasoepididymostomy that failed (10%), our results are really so good with repeat vasoepididymostomy that it is quite ethical just to do a repeat vasoepididymostomy without adding microepididymal sperm aspiration (due to the cost of IVF in the United States). The problem is that in a large number of these cases we derive absolutely no sperm because the whole rete testis is messed up on biopsy. But if you look at those cases where we do retrieve epididymal sperm, we find the quality of sperm is better in vasoepididymostomy cases for inflammatory disease. Therefore, I would anticipate a better result with conventional IVF if you are actually able to retrieve sperm.

The Infertile Male

Advanced Assisted Reproductive Technology

Editors: Sean P. Flaherty and Colin D. Matthews