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THESIS ENTITLED

"AUTO ANTIBODIES TO SPERMATOZOA IN SUBFERTILE HUMAN MALES"

(Volume 1 of 2 volumes)

presented by

William Forbes Hendry

MB, ChM (with commendation)

FRCS (Edinburgh and England)

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Doctor of Medicine

in the

University of Glasgow

Department of Urology

St. Bartholomew's Hospital,

London, EC1A 2BE.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

"Autoantibodies to Spermatozoa in Subfertile Human Males"

All of the clinical work with subfertile males described in this MD thesis was done by the author and his staff. The vasectomy reversals were done in collaboration with Mr M G Royle, Consultant Urologist in Brighton. All of the laboratory tests were done by Miss Stedronska or Dr Parslow and their staff at Chelsea Hospital for Women and St Bartholomew's Hospital respectively. The results, correlations and statistical analysis were all done by the author. All papers relating to the work included in the thesis were first written by the author, except for Stedronska-Clarke et al (1987) which was written by Prof David Clarke of Hamilton, Ontario. As Dr Parslow's PhD supervisor, I encouraged her to include our clinical results of vasectomy reversal in her thesis which was submitted to the University of London in 1985. No other work from this thesis has appeared in any other thesis, and the main part of Dr Parslow's PhD thesis relating to quantification of class of antibody on live spermatozoa by double antibody radio-immunoassay is only mentioned in the discussion part of this thesis.

All modern research depends on effective interdisciplinary collaboration. The considerable help afforded to the author by many other individuals is recorded in the Acknowledgements Section.

W F Hendry, 22 April 1992

PREFACE

Terminology

Although autoantibodies to spermatozoa is strictly correct, the term "antisperm antibodies" has come to be widely accepted: thus, in 32 papers listed in Index Medicus in 1989 and 1990, "antisperm antibodies" was used in the title of 24, "sperm antibodies" in 4 and "antibodies or autoantibodies to spermatozoa" in 4. In accordance with common usage, antisperm antibodies (usually abbreviated to ASA) is used throughout this thesis. Titres are shown as the reciprocal (4-1024) of two-fold dilution (1:4 to 1:1024). Other abbreviations are listed on page 21.

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SUMMARY

The diagnosis and treatment of immunological infertility in human males has been the subject of much controversy. Antisperm antibodies (ASA) have therefore been measured in all subfertile males seen by the author since 1975. A number of separate studies were completed, and these have been amalgamated and critically reviewed to form this thesis.

The first problem was to find out which tests gave results which were clinically significant. Sera from over 500 subfertile men were tested for circulating unbound antibody by gelatin agglutination test (GAT), sperm immobilisation test (SIT) and an indirect immunofluorescence test (IFT) using standardised internationally accepted laboratory methods. The results were compared with each other, and correlated with the results of sperm-cervical mucus contact (SCMC) tests, since failure of penetration was known to be associated with impaired fertility. Significant titres of GAT, which correlated with SIT and with failure of cervical mucus penetration were found in 8.5% of patients. IFT gave disparate results and was discontinued. Over the ensuing years GAT, a macroscopic spermagglutination test, was gradually superceded by the microscopic tray agglutination test (TAT) which was slightly more sensitive and allowed more tests to be done on serum and seminal plasma.

A screening test for ASA bound to patients' spermatozoa was also needed. The direct mixed erythrocyte-spermatozoa antiglobulin reaction (MAR) was carefully assessed in 2 separate studies and found to be

quick and technically simple, it gave reproducible results, and it was applicable to most semen samples. Furthermore, the results correlated well with estimations of serum unbound antibody measured by GAT or TAT. This technique was also used to define the class of antibody bound to the patients' spermatozoa. Failure of penetration of cervical mucus was found to be associated with IgA antibodies, which correlated with estimations of ASA in seminal plasma. A direct immunobead test (IBT) was compared with MAR and found to give similar results, but was more labour intensive and time consuming since the spermatozoa had to be washed free of unbound immunoglobulin in the seminal plasma.

Medical treatment was developed for men with ASA. Criteria for entry to treatment studies were as follows: GAT or TAT positive in serum at titre 32 or more, and/or in seminal plasma at any titre, plus a poor post coital test (PCT) in the female partner with demonstrably impaired sperm penetration of cervical mucus on crossed hostility testing. After preliminary medical checks, and after ensuring that the female partner was ovulating and had patent fallopian tubes, various corticosteroid regimes were used. Initially, long term low dose prednisolone (15mg daily for 6 months), was compared with cyclical high dose methylprednisolone (96mg daily from day 21-28 of female partner's cycle). Although the success rate was higher with the latter regime, serious side effects occurred and it was abandoned. An alternative cyclical regime was introduced (prednisolone 40/80mg daily from day 1-10 of female partner's cycle) which was as effective as the higher dose regime, and free of serious side effects. It was then subjected to prospective

multicentre double blind controlled trial and shown to be significantly more effective than placebo.

The relationship between ASA and testicular obstruction was studied in infertile men with unilateral or bilateral blocks, and in men undergoing vasectomy reversal. ASA were found, often in high titres, in three quarters of 80 men with unilateral blocks, half of whom had moderate or severe oligozoospermia despite having normal testicular biopsies. Following surgical and/or medical treatment one-third of those with adequate follow-up produced pregnancies; paradoxically, the best results occurred in those starting with the lowest sperm counts. Removal of irreparably blocked testes lead to profound falls in ASA titres in 10 men. Histological examination of the removed testes showed mononuclear cell infiltration in epididymes, rete testes and in some cases, seminiferous tubules. Amongst 370 infertile men with bilateral blocks, serum ASA were found in over half of those with acquired obstruction following infection, but in few (16%) of those with congenital absence of vasa. Follow up studies on 60 men after surgical correction of obstructive azoospermia showed that pregnancies were produced by a significantly higher proportion (60%) of those negative for ASA compared to those positive for ASA in any titre, only one-third of whom were successful. Unfortunately, the best surgical groups were those with post infective blocks, who also had the highest incidence of ASA.

Men undergoing vasectomy reversal were different. Although ASA were found in sera of almost 80% of men before reversal, seminal plasma ASA were present in only 10% before

reversal rising to almost 30% afterwards, which was significantly less than the 65% observed in an unselected series of spontaneously infertile men, and in the men with unilateral testicular obstruction. Patency was restored in over 90% of cases, and similar pregnancy rates were observed in the two collaborating centres (Brighton 44%, St. Bartholomew's 45%). No reduction in fertility was observed amongst partners trying to conceive until ASA titres in the men rose to very high levels (>512 in serum, > 32 in seminal plasma). Subsequent studies by Dr. J.M. Parslow (PhD thesis, University of London) showed that the vasectomy reversal men had much less sperm-bound IgA, and much better mucus penetration than spontaneously infertile men.

Evidence of focal mononuclear cell infiltration resembling auto-immune orchitis was found in a few severely oligozoospermic and azoospermic men with very high (>512) serum titres of ASA. Sperm concentration in these, and in some other men without these histological changes, improved with long term low dose prednisolone and 2 produced pregnancies.

In vitro fertilization studies are ongoing, but results so far indicate that the ovum fertilization rate is reduced with spermatozoa from men with ASA, but once fertilised the implantation rate is similar to that observed in other couples.

Autoantibodies to spermatozoa occur commonly in subfertile men, both as a primary local phenomenon and secondary to testicular obstruction. They can be detected sensitively, and their significance checked selectively by appropriate laboratory tests. Modern medical and surgical treatment is both effective and safe.

ABBREVIATIONS

ASA - antispermatozoal antibodies, antibodies to spermatozoa, sperm antibodies

Barts - St. Bartholomew's Hospital, London

CHW - Chelsea Hospital for Women, London

ELISA - enzyme linked immuno absorbent assay

FSH - follicle stimulating hormone

GAT - gelatin agglutination test

GIFT - gamet intrafallopian transfer

IBT - immunobead test

IFT - immunofluorescent test

IgA - immunoglobulin A

IgG - immunoglobulin G

IVF - in vitro fertilization

LH - lutenising hormone

MAR - mixed antiglobulin reaction

MP - methylprednisolone

PCT - post coital test

Pred - prednisolone

SCMC - sperm-cervical mucus contact

SIT - sperm immobilisation test

Sperm - spermatozoon, spermatozoa

Subfertile male - male referred because of subfertility (not vasectomised)

TAT - tray agglutination test

WHO - World Health Organisation

X-hostility - crossed hostility test

CHAPTER 1: Introduction

1.1 GENERAL STATEMENT OF PROBLEM

This work has been done to study the relevance of autoimmunity to spermatozoa in subfertile men. First the tests to detect antisperm-antibodies (ASA) and measure them - how reliable are they, do they give false positive results, and when are they significant? Next, medical treatment: which drug, what dose, when should it be given and for how long - is it safe, and is it as effective as it seems? Then testicular obstruction, naturally of interest to the surgeon - does the blockage provoke autoimmunity to spermatozoa? If unilateral, is this important in subfertile males? If bilateral, does the cause of the obstruction affect the nature of the ASA response, and is this relevant in determining whether the ASA impair fertility after successful surgical reconstruction? Finally cell mediated immunity - is there evidence that autoimmune orchitis occurs spontaneously in subfertile men? These are the questions to be addressed in this thesis, based on work carried out during the past 15 years.

1.2 HUMAN INFERTILITY

1.2.1. Incidence

From the point of view of the world population, there is no need to treat infertility; from the point of view of the couple concerned, who find themselves unable to have children, there may be the strongest compulsion to seek medical help. Infertility is a most potent cause of anxiety and emotional disturbance (Platt et al, 1973). It has been estimated that as many as 10% of couples may experience difficulty or delay in starting their families; amongst these couples, the abnormality lies solely with the husband

in 20%, but in a further 20% there is abnormality in both husband and wife (Buxton and Southam, 1958). Two more recent studies from the United Kingdom have shown that infertility is even more common now. Hull et al (1985) estimated that 1 in 6 couples in a single Health District needed specialist help because of infertility, the average duration of which was 2½ years. The commonest single cause was sperm defects or dysfunction, which were found in 24% of 708 couples investigated. Similarly, Templeton et al (1990) found that 14% of 766 women aged 46-50 had had infertility, defined as delay in production of pregnancy for more than 2 years: of these only half eventually conceived. There can be no doubt, therefore, about the need for an efficient and well coordinated infertility service to define the cause of the problem as clearly as possible, and thus provide the couple with guidance and counselling based on an accurate prognosis. This is particularly important nowadays since many couples leave starting their family until relatively late in life, and there are few babies available for adoption.

In considering the results of any treatment for infertility, it has to be remembered that pregnancy seldom occurs instantaneously even in normal couples - rather, there is a cumulative conception rate which rises asymptotically with the passage of time. Typical figures, derived by Cooke et al (1981) from data published by Vessey et al (1978) on outcome of pregnancies in parous women after stopping barrier contraception (UK data) indicate pregnancy rates of 0.32 at 3 months, 0.54 at 6 months, 0.82 at 1 year and 0.95 at 2 years. Even in this idealised setting, nature

takes its time for conception to occur, and it follows that much patience is required in treating infertility, and that the results of any treatment regime must be judged against a certain natural conception rate that would have occurred anyway.

1.2.2 Co-ordination of care

The diagnosis and treatment of subfertility is unusual in that the medical care of two separate adult individuals must be closely coordinated. Efficient communication between the doctors looking after the male and female partners is essential if confusion, and resulting loss of confidence, is to be avoided. This thesis is concerned with the diagnosis and treatment of immunological infertility in the male. Great care was taken throughout, however, to ensure that investigation and any necessary treatment proceeded simultaneously in the female partner, except when the male was azoospermic or presented for vasectomy reversal. This was easily achieved at Chelsea Hospital for Women, where much of the initial work was carried out in the specialised Fertility Clinics attended by both male and female partners. Both sets of notes were filed together, and gynaecologist and andrologist met regularly to discuss joint problems. Laboratory staff were included in all discussions relating to the ASA work at weekly joint meetings ("sandwiches in seminology"). In the busy setting of a general teaching hospital (St. Bartholomew's) this ideal was more difficult to achieve; however, full information was always obtained from gynaecological colleagues on the female partner and her problems, and included in all ASA treatment protocols, which did not

permit therapy to proceed in the male until certain basic preconditions had been fulfilled in the female (see section 2.4.1). Regular meetings were held with the staff of the Williamson Laboratory, where the ASA work was done.

Correspondence regarding the results of investigations and any recommendations for treatment (including entry into the clinical trials) was invariably sent to the general practitioner with a copy to the female partner's gynaecologist and a further copy was kept in the patient's notes. Separate personal records were kept on punch cards on all ASA and testicular obstruction patients.

1.2.3. Modern Technology

The advances in scientific technology that lead to the production of pregnancy by in vitro fertilisation (IVF) (Steptoe and Edwards, 1978) and by gamete intrafallopian transfer (GIFT)(Asch et al, 1986) raised hopes that any man with even a few motile spermatozoa in the ejaculate had a chance of fatherhood - a fact which could perhaps open the way to successful treatment of some subfertile males previously regarded as hopeless (Cohen et al, 1985). Does this mean that investigation and treatment of male subfertiliity is now unnecessary? Statistically, ovum fertilization is much less likely (39.6% versus 88.6% in the Norfolk, Virginia experience) if sperm quality is poor, and fertilization is unlikely to occur with less than 1.5×10^6 spermatozoa with rapidly progressive motility (van Vem et al, 1985). Similar experience has been reported from other centres (Sharma et al, 1988), and most will not accept couples for IVF or GIFT unless a reasonably adequate sperm preparation can be obtained from the male partner. The

effects of ASA on ovum fertilization are variable and unpredictable (see Section 4.5.1). Although IVF or GIFT offers an attractive alternative to other treatments for some couples where the man has ASA (Alexander, 1990), there are many others in whom fertilisation does not occur due to impaired sperm function (see section 4.5.2) even with sub-zonal insemination (Fishel, 1992; Fishel et al, 1992).

1.3 MALE INFERTILITY

1.3.1 The "Male Factor"

An air of pessimism still surrounds much of the treatment of male infertility (Wardle, 1990), with azoospermia in particular being widely regarded as virtually incurable (Thornton and Cooke, 1991); indeed, many practitioners feel that further investigation is simply a waste of time. Seminal analysis is the first investigation to be done in an infertile couple, and it is the easiest to criticise. Too little, too few, too sluggish, too abnormal - the cause of the problem is all-too-often assigned to the man's spermatozoa, with little or no attempt made to define the underlying pathology, and donor insemination on offer as a temptingly easy solution. The use of the term "male factor" as a diagnostic category is mentioned here only to be condemned, lumping together as it does so many different causes of impairment of male reproductive function. The elucidation of the cause of subfertility in an individual male requires a careful and methodical approach (Hendry et al, 1973, Hendry 1975, 1979 - see Volume 2 for reprints).

1.3.2 Incidence of problems

A survey of 1025 consecutive referrals of men who had been trying for a pregnancy for at least 1 year, seen by the

author between 1971 and 1978 at Chelsea Hospital for Women, revealed that a definable medical or surgical condition was present in only 38% (Stanwell-Smith and Hendry, 1984 - see volume 2). Varicocele was the most common, being found in 173 patients. There was a history of maldescent, or previous inguinal hernia in 73, of mumps or other orchitis in 35, of torsion or other trauma in 13, and there was testicular atrophy or aplasia in 16, absent testis or vas in 17. Dubin and Amelar (1971) analysed 1294 consecutive cases of male infertility and found varicoceles in 39%, endocrine abnormalities in 11.8%, sexual problems in 5.1%, ductal obstruction in 7.4%, testicular failure in 14%, cryptorchidism in 4.4% and miscellaneous other factors in a further 4%. Greenberg et al (1978) analysed 425 subfertile males and found varicoceles in 37.4%. ductal obstruction in 6.1%. cryptorchidism in 6.1%, testicular failure in 9.4% and true endocrine abnormalities in only 0.9%; 108 (25.4%) were considered to be idiopathic. The much lower incidence of varicoceles in our series (17%) resulted from our refusal to accept this diagnosis in the absence of demonstrable thermographic abnormality (Hendry and Jones, 1981).

In over 60% of our 1025 cases, the exact cause for the infertility could not be accurately defined. However, amongst 208 azoospermic men the cause was easier to define. In 103, there was severely depressed or absent spermatogenesis, and in a further 16 there was maturation arrest. Thus in 89, there was normal spermatogenesis with obstruction to the testicular outflow passages most commonly in the epididymes. There was oligozoospermia (less than 20 million spermatozoa per ml) in 459 patients and

asthenozoospermia (motility less than 30% moving actively) in the remaining 345 patients. The majority of patients therefore had idiopathic infertility in the period under study (1971-1978). Prospective study of 200 subfertile males showed somatic chromosome abnormalities in 3.5%, and definite abnormalities in meiotic chromosomes in 20%, including supernumerary chromosomes, low chiasma frequency and asynapsis and translocations; in 4 cases a previously unrecognised abnormality in pachynemas was defined (Hendry et al, 1976 - see volume 2). It was concluded that abnormalities in meiosis could account for the decreased spermatogenesis and poor response to treatment in at least 20% of subfertile men. Treatment was largely empirical: the men were encouraged to wear loose fitting underwear, to avoid excessive smoking or alcohol intake (so-called general measures) (Hendry et al, 1973 - see volume 2); varicoceles were treated by high ligation; patients with oligozoospermia were given mesterolone 100mg daily for 6-12 months, and those with asthenozoospermia had fluoxymesterone 5mg daily for 3-6 months (Hendry et al, 1973 - see volume 2). Patients with azoospermia underwent scrotal exploration and testicular biopsy, with relief of obstruction by reconstructive surgery whenever possible (Hendry et al, 1978; Hendry 1981 - see volume 2)..

1.3.3 Prognosis

Of the 1025 men whose records were examined (Stanwell-Smith and Hendry, 1984), 180 (18%) produced a pregnancy while still attending the clinic at Chelsea Hospital for Women. The proportion of men producing pregnancies rose with the mean sperm density on presentation

up to 20 million per ml, and then fell amongst those with asthenozoospermia (Table 1).

Mean Sperm Density (million/ml)	Number of Men	Number of Pregnancies
0	208	8 (4%)
1-5	185	29 (16%)
6-10	95	25 (26%)
11-20	179	49 (27%)
>20	345	64 (19%)
Unknown	13	5
Total	**1025**	**180**

TABLE: 1 Results at CHW 1971-1978 (Stanwell-Smith and Hendry 1984)

Amongst 131 operated for varicocele 25.2% produced pregnancies, whereas 9 (21.4%) of 42 with varicoceles not operated on also produced pregnancies (mostly whilst awaiting surgery).

Other workers have reported similar pregnancy rates of 0-27% in the partners of men with sperm defects or dysfunction (Hull et al, 1985). In a collaborative study, a 9-month course of mesterolone gave a pregnancy rate of 18% compared to 15% obtained with vitamin C (Scottish Infertility Group, 1984). Tamoxifen, known to increase serum FSH levels in oligozoospermic men (Vermuelen and Comhaire, 1978) gave similarly disappointing results after 9 months (pregnancy rate of 22% compared to 23% with vitamin C) (Hargreave et al, 1986).

Controversy continues to surround the role of varicocele in male infertility. There are many reports of improvement in seminal quality in around 70% of patients after high ligation, with pregnancies occurring in 40-50% of

female partners (Tulloch, 1955; Charny, 1962; Scott and Young, 1962; Hanley and Harrison, 1962; Brown et al, 1967; Macleod, 1969; Dubin and Amelar, 1977). However, three large series showed no difference in pregnancy rates in the partners of those who did or did not have the varicoceles ligated (Rodriguez-Rigau et al, 1978; Nilsson et al, 1979; Baker et al, 1985), and this question remains open at present. However, particularly good results have been observed after varicocele ligation for solitary, or functionally single left testis (Hendry, 1992a).

1.3.4 Need for specific diagnosis

It will be appreciated that the treatment for the majority of subfertile males was empirical and the results really rather disappointing in the period 1971-78 surveyed above. In 1976, the author heard a paper by Kremer and Jager indicating that autoimmunity to spermatozoa in the male could remain unrecognised until the sperm reached the female partner, where antibodies on the sperm surface became attached to receptors in the cervical mucus thus impeding their progress. The concept of "cervical hostility" was not new, but the idea that the antibodies were in fact auto-antibodies in the male rather than in the female where they had always been supposed to be, was intriguing. Detailed immunofluorescence studies amongst our infertile couples at CHW had shown no difference in incidence of ASA between pregnant, non-pregnant and infertile women (Wall et al, 1975A), although ASA were found significantly more often amongst subfertile males than amongst normal controls (Wall et al, 1975B). Could this be a specific immunological disorder, occurring chiefly in the male partners, leading to

interference in the normal process of fertilisation in the female? In order to answer this question, all subfertile males seen by the author since 1975 were checked for ASA, and analysis of the findings form the basis of this thesis.

1.4 ANTIGENICITY OF SPERMATOZOA

1.4.1 Historical aspects

Landsteiner (1899) and Metchnikoff (1899) showed that spermatozoa were antigenic when injected into another species:

> "La résorption des spermatozoides dans la cavité peritonéale est suivie de la formation dans la liquide du peritonie ainsi que dans le serum sanguin de ces animaux d'une substance qui immobilise les spermatozoides. Il se produit donc un anticorps".
>
> (Metchnikoff, 1899)

It is clear that production of an immobilising antibody by the guinea pig was recognised and recorded.

Metalnikoff (1900) extended these observations by showing that autoimmunisation also occurred:

> "la sérum du cobaye devient toxique non seulement pour les spermatozoides d'autres cobayes, mais ainsi pour ceux du cobaye inoculé lui-même"

Interestingly, Metalnikoff showed that the immobilising antibody was complement dependant:

> "en ajoutant 10 gouttes de sérum à une goutte de spermatozoides on les immobilise en 3-4 minutes. Elle perd ses propriétés toxique étant chauffée au 55°C, et les reacquient facillement apres l'addition du serum d'un cobaye neuf".

There followed numerous attempts to induce sterility

by injection of spermatozoa into a bewildering assortment of animals, documented by Katsh (1959). Baskin (1932) reported that women could avert pregnancy for about 1 year by intramuscular injection of whole human semen, and subsequently patented his vaccine (US Patent Number 2,103,240). However, the results were unpredictable and certainly not sufficiently reliable to catch on as a contraceptive.

Spermatozoa can be shown to acquire an antigen during maturation which is absent from immature germinal cells. Since antibodies can be induced by immunising with autologous spermatozoa, it is clear that the immune system does not regard sperm as part of self. Sperm antigens (and lens antigen of the eye) are in fact examples of hidden or sequestered antigens, not normally accessible to the cells of the immune system (Weir, 1988).

1.4.2 Blood Testis Barrier

Spermatozoa are normally hidden from the immune system in the male by the blood-testis barrier (Johnson 1970, 1973) formed in the seminiferous tubules by tight junctions between the sertoli cells (Fawcett, 1974). Elsewhere in the male reproductive tract the barrier is less well formed, and is probably at its weakest at the rete testis (Tung et al, 1970). In addition to the blood testis barrier, which may not provide a perfectly effective immunological screen, it is now believed that local tolerance to spermatozoal antigens may be induced by suppressor cells - thus local immuno-regulation may serve as a "back-up" system (Alexander and Anderson, 1987). Monoclonal antibody studies have shown that the majority of the mononuclear cells in the submucosa

of the male genital tract are suppressor T lymphocytes (El Demiry et al, 1985).

1.4.3 Induction of immune response

Voisin et al (1951) and Freund et al (1953) showed that if homologous or autologous testicular tissue is inoculated in the guinea pig together with complete Freund's adjuvant, a cell-mediated immune response is provoked which leads to immune orchitis and ultimately aspermatogenesis. Mononuclear cell infiltration commences in the rete testis and caput epididymis by the 9th day, leading to extensive atrophy by 28 days. The seminiferous tubules are empty by 48 days (Waksman, 1959; Brown et al, 1963; Brown and Glynn, 1969; Tung et al, 1970). Apparently both circulating antibody and delayed hypersensitivity (i.e. cellular immunity) are necessary to produce immune orchitis (Brown et al, 1967). Thus if incomplete Freund's adjuvant is used, specific circulating ASA are produced but immune orchitis does not occur. If testicular homogenate is replaced by autologous epididymal spermatozoa, however, the same responses occur i.e. circulating antibody if incomplete adjuvant is used, and immune orchitis with complete Freund's adjuvant. Although much of this work has been done using guinea pigs, the phenomenon also occurs in other species and has been the subject of numerous experiments carried out with the twin objectives of defining spermatozoal/testicular antigens more clearly, and studying how cell mediated immune response is stimulated and cytotoxic lymphocyte activity is transmitted (see Section 4.4).

1.4.4 Spermatozoal antigens

During spermatogenesis, unique antigens appear at spermatocyte stage which persist thereafter. Study of these antigens is extremely complex, not least because other antigens present in the seminal plasma may become absorbed onto the surface of the spermatozoa (O'Rand, 1980; Shulman, 1987). Nevertheless, it is important to recognise that certain surface antigens are relevant because they react with ASA in vivo, whereas other subsurface antigens are irrelevant because they are normally concealed, but may become evident during fixation of spermatozoa. The chief spermatozoal antigens are listed in Table 2, which is derived from Hjort et al (1982). It may be seen that the membrane antigens on the sperm surface are those detected by agglutination and immobilisation tests, cytotoxicity, MAR and IBT tests (i.e. those using live spermatozoa) whereas the various subsurface antigens are those demonstrated by IFT or ELISA on fixed spermatozoa. There is ample evidence to demonstrate that antibodies to these two sets of antigens are quite different, and the presence of one type of antibody may not correlate with the existence of the other, although both may properly be called ASA (see Section 3.1). The first and most important study at CHW was to find out which antibodies and which tests were clinically relevant, and to distinguish them from those that were simple epiphenomena. Although sperm antigens are complex, there does not appear to be continuing justification for the view that they still require further elucidation before treatment can be developed for these patients, as recently concluded by Hamilton (1992).

ANTIGENS	LOCALIZATION AND CHARACTERIZATION OF ANTIGEN	METHODS FOR DETECTION OF ANTIBODY
Membrane antigens	Two glycoproteins widely distributed in the membrane One antigen restricted to tail end piece	Agglutination tests Immobilization tests Cytotoxicity MAR, IBT Cellular radio immunobinding techniques
Sperm Specific enzymes:		
LDH-X (LDH-C4)	Post-acrosomal area	Electrophoretic RIA
Acrosin	Acrosome	Enzyme
Hyaluronidase	Acrosome	inhibition
DNA polymerase	Nucleus	test
Nuclear proteins	Protamine 1 & 2 in sperm nucleus	IFT on swollen sperm heads
Various subsurface antigens	Acrosome, equatorial, postnuclear, tail, tail end piece	IFT, immunoperoxidase techniques on fixed spermatozoa

TABLE 2 Spermatozoal antigens (from Hjort et al, 1982)

1.5 INVESTIGATION OF IMMUNOLOGICAL INFERTILITY

1.5.1 Agglutination and immobilisation tests for unbound ASA in serum and seminal plasma

(i) Gelatin Agglutination Test (GAT)

The existence of ASA had been recognised for more than 50 years before laboratory techniques were developed with sufficient sensitivity to define the titre of antibody accurately, yet with enough specificity to be reasonably sure that the observed effects were indeed immunologically based. Kibrick et al (1952) mixed a freshly prepared dilution of highly motile spermatozoa obtained from a fertile donor with an equal quantity of 10% gelatin solution, to obtain a final concentration of 20 million spermatozoa per ml. The gelatin increased the viscosity of the test solution so that at 37°C it was still perfectly usable, but clumps of spermatozoa agglutinated by antibody, once formed, were not easily broken up and indeed could be preserved indefinitely by simply cooling the solution. Equal quantities of the sperm preparation were mixed with test serum in serial double dilution, and incubated at 37°C for 2 hours. A positive result was indicated by grossly visible agglutinates, and the dilution before the one where the effect disappeared indicated the titre of antibody. Careful control studies indicated that non-specific agglutination was uncommon provided the serum was diluted to 1:4 or more. Known positive and negative controls were always included.

Within 2 years, the first reports appeared recognising that high titres of ASA could be identified in subfertile

human males. Rumke (1954) described 2 men with severe oligozoospermia. Wilson (1954) reported 2 men with normal sperm counts and agglutinating antibody in serum and seminal plasma; their female partners had poor post coital tests and demonstrably impaired sperm penetration of cervical mucus - one became pregnant promptly with donor insemination. Thus there appeared the first evidence that ASA occurred spontaneously in infertile men, and that ASA could be associated with either oligozoospermia or with apparently normal sperm counts. In the latter case the antibodies were associated with failure of penetration of cervical mucus under circumstances where other spermatozoa could apparently penetrate normally.

Rumke and Hellinga (1959) continued to evaluate the Kibrick test in a large series of men (Table 3).

Serum of:	Number Tested	Antibody Titre (Kibrick)			
		0	4-16	32-128	≥256
Men with azoo- or oligozoospermia	102	96	1	1	4
Male Infertility Clinic	1913	1836	15	26	36
Husbands of pregnant women	416	412	4	0	0

TABLE 3 Spermagglutinins in the serum of human males (from Rumke and Hellinga, 1959)

Comparing men whose women were pregnant with those attending a Male Infertility Clinic, it was concluded that a serum titre of 32 or more was likely to be the borderline below which the result was unlikely to be significant. Tests for specificity were done, and positive sera showed no cross reactivity with other antibodies, and sera from patients with other autoimmune diseases gave no reaction with spermatozoa. It was concluded the agglutinating factor was an autoantibody and was specific for spermatozoa.

Thereafter, Rumke et al (1974) tested several thousand serum samples from the Netherlands and Belgium, and investigated 254 patients found to have positive serum titres ranging from 4 to >1024. Following 137 men with normal sperm counts for at least 5 years indicated an inverse relation with the serum ASA titre (Table 4). These studies were of fundamental importance, showing

Serum Titre (Kibrick)	Number of Patients	Number (percent) who became Fertile
4	12	5 (42)
8	11	6 (55)
16	8	4 (50)
32	14	3 (21)
64	17	4 (24)
128	27	2 (7)
256	20	3 (15)
512	17	3 (18)
≥1024	11	0 (0)
Total	**137**	**30 (22)**

TABLE 4 Probability for Infertile Normospermic Men with different Serum ASA titres producing pregnancy within 5 years (from Rumke et al, 1974)

a relationship between increasing serum titre of ASA and diminishing likelihood of fertility. A serum titre of less than 32 was probably meaningless, and it was recognised that a few pregnancies were produced with titres above this level.

(ii) Sperm Immobilisation Test (SIT)

Fjallbrant (1965, 1968a,b, 1969) studied ASA in infertile men using both Kibrick's test and a sperm immobilisation test, in which fresh guinea pig serum of known complement activity was mixed with inactivated patient serum and the test suspension of spermatozoa on a slide. The proportion of motile sperm was estimated by examination under the microscope at intervals

Sperm Agglutinin Titre	Cervical Mucus Penetration		
	0	1	2
4-8	1	2	4
16-32	1	2	10
64-128	5	2	2
256-512	2	1	
≥1024	3	1	

Time to Sperm Immobilization	Cervical Mucus Penetration		
	0	1	2
>12	1	1	12
6-12		3	3
3-6	6	4	1
<3 hours	5		

TABLE 5 Cervical mucus penetration by capillary-tube test related to serum sperm agglutinin titres and time to sperm immobilization (from Fjallbrant, 1968b)

thereafter. The results were correlated with penetration of cervical mucus and a strong negative correlation was found, especially with ASA with immobilising activity (Table 5). The interrelation between penetration ability and fertility was also very good (Table 6).

	Cervical Mucus Penetration		
	0	1	2
Fertile	0	6	9
Sterile	12	2	7

TABLE 6 Distribution of subjects in sterile and fertile groups according to degree of cervical mucus penetration (from Fjallbrant, 1968b)

These studies were of great importance, as they indicated the way that ASA were affecting spermatozoal function - that is, by impeding their penetration of cervical mucus, and, not unexpectedly, this interfered with the process of fertilisation.

Sperm immobilisation by ASA in the presence of complement had also been demonstrated in 1 case by Rumke (1954), before the studies by Fjallbrant outlined above. Isojima et al (1968) modified the sperm immobilisation test by repeating it in serial dilution, and defining the titre at which motility in the test spermatozoa had dropped to a certain percentage of motility in a negative control - in practice this is generally taken as a drop in motility to

half or less that of the control. Husted (1975) studied the relationship between agglutinating and immobilising ASA and found a close correlation, in serum, although immobilisation titres were considerably lower. It was concluded that agglutination, immobilisation and cytotoxicity are probably caused by the same ASA, the type of reaction depending on the presence or absence of complement. However, it was also noted that immobilization and cytotoxicity was rarely observed in seminal plasma, presumably due to its anticomplementary activity.

(iii) Tray Agglutination Test (TAT)

Friberg (1974a) recognised that a shortcoming of the Kibrick technique was that only a relatively small number of tests could be done using the sperm from a single ejaculate, owing to the large quantity of reagent used in each test. A micro-agglutination technique was therefore developed, in which a single donor semen sample sufficed for 200-300 determinations. Furthermore, since the agglutination of the test spermatozoa was observed through an inverted microscope, the type of agglutination - tail-to-tail (T-T) or head-to-head (H-H) - could be distinguished; it was noted that the former was more common in sera from men, the latter in sera from women. However, amongst men H-H spermagglutinating activity was sometimes noted and further detailed immunological studies indicated that H-H activity was caused by IgM antibodies whereas T-T activity appeared to be due to IgG antibodies (Friberg, 1974b). The significance of the mode of agglutination is discussed in detail in Section 4.1.3. It should be noted that Friberg initially used the abbreviation H-T for the agglutination

commonly seen in men, and subsequently changed it to T-T (Friberg, 1980a,b). The latter abbreviation is used throughout this thesis to avoid confusion in terminology.

Friberg (1974c) also studied the relationship between antibody titres in serum and seminal plasma, and showed that in general agglutinating antibodies were only present in seminal plasma when the serum titre was high ($\geq$64) and when T-T agglutination was present. None of the men with pure H-H agglutination had seminal plasma ASA activity.

(iv) Tube-Slide Agglutination Test

Franklin and Dukes (1964) incubated 0.05ml of standardised suspension of sperm with 0.5ml of patient's serum (undiluted and diluted 1:5 with isotonic saline) in serological tubes for 4 hours at 37^{o}C, before examining samples on a slide ½, 1, 2 and 4 hours later for evidence of sperm agglutination. Two or more spermatozoa aggregated per high power field was considered a positive result. Head-to-head agglutination was most commonly observed. Amongst 89 women, antibody was detected in no less than 78.9% of those with unexplained infertility, and in 11.8% of fertile women. This test was obviously far too sensitive, and the results did not correlate well with the results of other tests in comparative studies (see Section 1.5.2). Boettcher et al (1970) subsequently showed that the spermagglutinins found in the sera of women commonly were not antibodies, but were in fact due to steroids bound to Beta-lipoprotein. This test fell into disuse with the development of the Tray Agglutination Test.

1.5.2 WHO Reference Bank

Concerned about the proliferation of tests for detection of ASA, and lack of standardisation of laboratory methods, the WHO gathered together 32 scientists - "knowledgeable and experienced specialists in the field of sperm immunology" - to a workshop held in 1974 to define techniques for the detection and study of ASA. The details of the tests outlined above were described, and published on behalf of the group by Rose et al (1976). The WHO Reference Bank for Reproductive Immunology was set up, and 36 sera were distributed to 38 participating laboratories all over the World for testing for ASA by the defined test methods. Comparison of the results showed that the gelatin agglutination test (GAT), tray agglutination test (TAT) and sperm immobilization tests (SIT) were reliable and reproducible procedures (WHO Reference Bank for Reproductive Immunology, 1977). The relative sensitivities of these various tests was defined, and this is shown in Table 7. ASA in sera from women appeared to be nearly exclusively head-to-head (H-H) agglutinins, whereas sera from men contained predominantly agglutinins reacting with tail antigens (T-T) or (TT-TT).

TYPES OF AGGLUTINATION

SENSITIVITY OF TESTS	H – H	T – T	TT – TT
GAT	+	++	++
TSAT	+++	–	–
TAT	++	++	++
SIT (I)	+	+	?

TABLE 7 Relative sensitivities of the various tests for detection of ASA of different specificities (from Shulman 1978)

These studies, sponsored by the WHO and based in Aarhus, Denmark, were of fundamental importance in defining the technical methods for detection and quantification of ASA. Organised by Dr. Tage Hjort, the conclusions remain valid to the present time (for a recent review see Hjort, 1987).

1.5.3 In vivo tests for ASA bound to sperm surface

The theoretical advantage of direct tests are that ASA bound to the patient's spermatozoa are detected and these are demonstrably in a position to exert a pathogenic effect.

The mixed antiglobulin reaction (MAR) was originally described as a direct test for detection of platelet

antibodies (Coombs et al, 1956). It was modified for use as an indirect test by Coombs et al (1973) to study the class of immunoglobulin in serum and seminal plasma from vasectomised and infertile men - indicating that ASA in serum were largely IgG, whereas in seminal plasma ASA were chiefly IgA. It was suggested that infertility was probably due to locally produced IgA antibody - a remarkably accurate prediction (see Sections 4.1 and 4.3).

The direct MAR test was developed as a screening test for ASA by Jager et al (1978). Patients spermatozoa (1 drop) are mixed with group O Rh D-positive red blood cells sensitised with incomplete human anti-D antibody (1 drop), to which 1 drop of undiluted monospecific anti-human IgG antiserum (Behring) is added. The three drops are mixed and the test is ready to read in 2 minutes. Of course the red cells must agglutinate, but a positive reaction is indicated if more than 10% of the patients' spermatozoa are involved in mixed agglutinates. This test proved to be extremely useful and has been extensively evaluated (see Sections 2.2 and 3.1). The test can also be modified to test for IgA antibodies. The red cells can be replaced by Latex particles sensitised with appropriate immunoglobulin with no loss of sensitivity (Sperm MAR - Hellstrom et al, 1989).

The immunobead test (IBT) was developed by Clarke et al (1982) and by Bronson et al (1982) and also tests for surface Ig on the patients' spermatozoa. The essential difference between IBT and MAR tests is that the commercially available immunobeads (BioRad, Richmond, California) have purified antihuman-Ig on their surface (anti-IgG, anti-IgM or anti-IgA), whereas the red cells in

the MAR test have incomplete Ig bound to their surface, and the antihuman-Ig has to be added separately. Since seminal plasma contains unbound immunoglobulin which would neutralise the IBT, the spermatozoa must be carefully washed prior to the IBT, but this is not necessary for the MAR. The two procedures are compared as routine direct screening tests for subfertile males in section 2.2, 3.1 and 4.1.4. The immunobeads have also been used by others for indirect tests for the presence of ASA in serum or seminal plasma, and to define the class of Ig, and these results will be discussed in Section 4.1.4.

1.5.4 ASA to subsurface antigens

Once spermatozoa are fixed, subsurface antigens are revealed. Methanol is the usual fixative, and then antibodies reacting with the subsurface antigens show characteristic immunofluorescent patterns - staining the acrosome, post nuclear cap, the equatorial segment, the main tail piece or the tail end-piece (Hjort and Hansen, 1971). At Chelsea Hospital for Women, we found that immunofluorescent antibodies in sera, seminal plasma and cervical mucus which reacted with these antigens occurred with equal frequency in fertile and infertile patients, male and female (Harrison et al, 1976). As a general rule it seemed best for clinical purposes to use live spermatozoa as the substrate for ASA testing, and to use tests that had been thoroughly assessed. Fortunately, ASA which had clinical significance had been shown to have an adverse effect on sperm penetration of cervical mucus, (Fjallbrant 1968b) (Table 6) and this had a close correlation with impaired fertility.

1.5.5 Sperm cervical-mucus contact tests

Kremer and Jager (1976) studied the interaction between spermatozoa sensitized by ASA and cervical mucus, and showed that progressive propulsion of the sperm was changed into stationary, shaking movement. This phenomenon was also observed when normal spermatozoa came into contact with cervical mucus from a woman whose serum contained ASA. It appeared that the spermatozoa coated with ASA stuck to the network of long glycoprotein micelles in the normal ovulatory cervical mucus. Investigation of 30 couples with poor cervical mucus penetration due to ASA revealed that the antibody was in the male partner in 25, and in the female in 5 (Kremer et al, 1978a).

The SCMC test could therefore serve as a 'test-bed' to see if the results of ASA testing were clinically relevant, and also provided an opportunity to see which partner was affected, so long as the test was repeated using normal donor sperm and normal cervical mucus as controls, thus forming a "crossed hostility test". Furthermore, it seemed that the phenomonon of cervical hostility might in fact be due to abnormality in the male more often than it had previously been suspected.

1.6 INCIDENCE OF ASA

Review of the literature to 1976 reveals a remarkable variety of different tests applied to a number of different populations. For the reasons stated above, it seems sensible to try to draw conclusions only from agglutination or immobilisation tests done on sera by recognisably standardised techniques. Three populations of men are of interest: fertile men, spontaneously infertile men, and men

known to have testicular obstruction (e.g. vasectomised men or those with obstructive azoospermia). These results obtained from published reports up to 1976 are presented in Tables 8,9 and 10 respectively. It may be seen that agglutinating ASA were found in 2% or less of fertile men, 3-13% of subfertile men, and 26-75% of men with known testicular obstruction. There therefore appeared to be good grounds for investigating further the relationship between ASA and infertility in men, with particular reference to its co-existence with testicular obstruction.

AUTHOR AND YEAR	NUMBER STUDIED	NUMBER POSITIVE (%) AGGLUTINATION
Rumke & Hellinga (1959)	416	0*
Fjallbrant (1968a)	500	4 (1)
Halim & Antoniou (1973)	100	2 (2)*
Ansbacher (1973)	106	1 (1)
Gupta & Garg (1975)	15	0
Gupta et al (1975)	50	1 (2)*

* Titre ≥ 32

TABLE 8 Incidence of ASA in Sera of Fertile Men

AUTHOR AND YEAR	NUMBER STUDIED	NUMBER POSITIVE (%) AGGLUTINATION
Rumke & Hellinga (1959)	1913	62 (3)*
Schwimmer et al (1967)	64	5 (8)
Fjallbrant (1968a)	400	17 (4)†
Hanafiah et al (1972)	124	11 (9)
Halim & Antoniou (1973)	144	19 (13)*
Ansbacher et al (1973)	554	27 (5)
Gupta & Garg (1975)	70	4 (6)
Hjort & Husted (1975)	165	16 (10)
Schoenfeld et al (1976)	165	9 (5)

* Titre $\geqslant 32$

† Titre $\geqslant 64$

TABLE 9 Incidence of ASA in Sera of Infertile Men

AUTHOR AND YEAR	NUMBER STUDIED	NUMBER POSITIVE (%) AGGLUTINATION	IMMOBILISATION
Rumke & Hellinga (1959)	61*	16 (26)	
Phadke & Padukone (1964)	50*	13 (26)	
Ansbacher (1973)	69†	39 (57)	19 (28)
Gupta et al (1975)	141†	86 (61)	56 (40)
Schoenfeld et al (1976)	45‡	29 (64)	
	4†	3 (75)	
	9*	6 (67)	
Alexander et al (1976)	87†	29 (33)	37 (43)
	94†	30 (32)	27 (29)

* Epididymal obstruction

† Vasectomised

‡ Absent vasa

TABLE 10 Incidence of ASA in Sera of men with Testicular Obstruction (Vasectomy or Obstructive Azoospermia)

CHAPTER 2: SUBJECTS, MATERIALS AND METHODS

2.1.1 Subjects and clinical diagnosis

The vast majority of the subjects described in this thesis were male partners of subfertile marriages seen, examined and treated by the author. The general approach to these patients remained the same throughout (Hendry et al, 1973; Hendry 1975, 1979). There were, in addition, studies of vasectomised men before and after reversal which were done in collaboration with Mr. Michael Royle, Consultant Urologist in Brighton. All subfertile males were fully investigated. The history covered the age, occupation and religion of the patient and his female partner, the time they had been trying to produce a family, and the frequency and technical success of sexual intercourse. Present medical conditions, especially endocrine disorders, and any current drug therapy or exposure to toxic chemicals were reviewed. Relevant past history included tuberculosis, adult mumps, venereal disease, maldescended testes, and hernia repairs. In the social history intake of drugs, tobacco and alcohol were recorded.

On examination the general body build, hair distribution, and type of underpants worn were checked. The penis, foreskin, external meatus, testicular size and consistency, epididymes and vasa were examined with the patient standing in a good light, to allow a thorough search to be made for a possible varicocele; the prostate and vesicles were examined digitally per rectum with the patient lying on the left lateral position. Scrotal thermography was done to settle any doubts about the possible presence of a small varicocele and repeated after surgical correction (Jones and Hendry, 1978).

Seminal analysis was always checked twice, irrespective of the findings reported in previous tests done elsewhere. Technical details of laboratory methods are described in section 2.2.

Hormone studies included measurement of plasma testosterone, FSH, LH and prolactin. Failure of spermatogenesis due to gonadotrophin deficiency was uncommon (Greenberg et al, 1978), although it was important to identify such cases because they could respond well to replacement therapy. Much more often the cause was a primary defect in spermatogenesis which was recognised because the serum FSH level was elevated. Indeed the combination of azoospermia, small testes and a grossly elevated serum FSH level was considered to be diagnostic of absent or seriously impaired spermatogenesis for which there was no treatment (Pryor et al, 1976). Full blood count (with sickle status if relevant) blood urea and liver function tests were usually checked. Urine was tested for glucose, protein and evidence of infection, and any co-existing urological abnormality was fully investigated.

In cases of oligozoospermia or azoospermia where the hormone levels were normal, and there was at least one testicle of normal size (15ml measured by orchiometer), exploration of the scrotum was carried out by the author to find out if there was testicular obstruction (see section 2.5) and take testicular biopsies. Spermatogenesis was rated from 0-10 by a mean score system (Johnsen, 1970).

Somatic chromosomes were checked in buccal smears and blood and meiotic chromosome studies were done on testicular biopsy material initially as part of a prospective study,

and subsequently when indicated by the results of clinical findings, (Hendry et al, 1975 - see volume 2).

2.1.2. ASA studies, Laboratories and staff

Early work at Chelsea Hospital for Women (CHW) on immunological infertility was based on immunofluorescence studies and gave disappointing results, failing to distinguish between fertile and infertile populations (Harrison et al, 1976). Simultaneous work at the London Hospital by Halim and co-workers (1974) appeared to indicate that a spermagglutination test might give better discrimination particularly in men. A film by Kremer and Jager seen by the author and subsequently published in 1976, drew attention to the fact that significant ASA in men produced alterations in sperm behaviour in cervical mucus. This lead directly to our first comparative studies and from September 1975, onwards all men attending the Male Fertility Clinic at CHW were tested for ASA by several different methods, by Miss Jitka Stedronska, who continued to work in the Seminology Laboratory there until 1987. ASA testing at St. Bartholomew's Hospital (Barts.) has been done since 1978 by Mrs. (now Dr.) Jaroslava Parslow who successfully presented her PhD thesis on autoimmunity to spermatozoa to the University of London in 1985. The published works on which this MD thesis is based are listed in Table 11, all of which depended on these laboratory services at CHW and Barts.

SECTIONS 2.3 AND 3.1	DIAGNOSIS IN SUBFERTILE MALES
GAT and clinical findings	Hendry et al (1977)
GAT and X-hostility	Morgan et al (1977)
GAT and MAR (IgG)	Hendry & Stedronska (1980)
MAR (IgG and IgA)	Hendry et al (1982a)
TAT and MAR (IgG)	Stedronska & Hendry (1983)
MAR and IBT (IgG & IgA)	Rajah et al (1992)
ELISA & TAT (IgG)	Stedronska-Clark et al (1987)

SECTIONS 2.4 AND 3.2	MEDICAL TREATMENT OF SUBFERTILE MALES
Continuous Pred.	Hendry et al (1979)
Intermittent Methylpred.	Hendry et al (1981)
Intermittent Pred.	Hendry et al (1986)
Pred. vs placebo	Hendry et al (1990a)

SECTIONS 2.5 AND 3.3	SURGICAL TREATMENT OF TESTICULAR OBSTRUCTION IN SUBFERTILE MALES
Surgery for testicular obstruction	Hendry (1987a,b)
Unilateral Obstruction	
- diagnosis	Hendry et al (1982b)
- results of treatment	Hendry (1986)
Obstructive Azoospermia	
(168 cases)	Hendry et al (1983a)
(370 cases)	Hendry et al (1990b)
Genital tract injuries in childhood	Parkhouse & Hendry (1991)

SECTIONS 2.6 AND 3.4	VASECTOMY REVERSAL
ASA before and after reversal	Royle et al (1981)
1 year follow up studies	Parslow et al (1983)
Comparison of vasectomy reversal and spontaneously infertile men	Hendry et al (1983b)
Failed vasectomy reversal	Royle & Hendry (1985)

TABLE 11 Published ASA Work (see volume 2)

2.2 Laboratory Methods

Standard laboratory methods recommended by the World Health Organization were used (WHO Laboratory Manual for the examination of the human semen and semen-cervical mucus interaction 1987; WHO Reference Bank for Reproductive Immunology 1977).

2.2.1 Seminal Analysis

Patients were asked to abstain from sexual intercourse for 3 to 4 days prior to semen collection. The semen specimens were produced in the hospital by masturbation into a sterile plastic container. In some cases the semen samples were collected at home but brought into the laboratory within one hour. Following liquefaction, the semen was examined for volume, pH and viscosity. The motility and morphology of spermatozoa was estimated microscopically in a drop of undiluted semen. The sperm count was determined in a Neubauer counting chamber after dilution with 1% formalin containing 5% $NaHCO_3$. The specimen was considered azoospermic if there were no spermatozoa seen in a wet film, oligozoospermic with less than 20 million of spermatozoa per ml, and normospermic when at least 20 million of spermatozoa were present in 1 ml of the ejaculate.

2.2.2 Post Coital Test (PCT)

PCT's were performed as close as possible to the time of ovulation as determined by clinical criteria (based on body temperature, cervical mucus changes and vaginal cytology). The couple were instructed to abstain from sexual intercourse for about 2 days prior to the day on which the test was to be performed. The test was performed

preferably 6-10 hours after intercourse, but was sometimes done after a longer interval (up to 18-20 hours) after coitus. A non-lubricated syringe was inserted into the vagina and a sample of cervical mucus obtained with a tuberculin syringe (without needle) from the cervical canal. This was placed on a glass slide and examined under the microscope at 400x. The number of motile spermatozoa per HPF was recorded: fewer than 5 was regarded as abnormal. Volume, consistency, Spinbarkeit, cellularity and pH of the cervical mucus was also recorded.

2.2.3 Cervical Mucus Penetration (Crossed Hostility)

This in vitro "cervical mucus hostility" test first described by Miller and Kurzrok in 1932 was used to study the effect of spermatozoa coated with ASA on penetration of cervical mucus. For this purpose husband's fresh semen and wife's cervical mucus were obtained. In order to determine whether the antibodies were located on the husband's spermatozoa or in the wife's mucus, the test was performed in a cross-test system, using fresh semen and cervical mucus of normal donors as a control.

A drop of preovulatory cervical mucus with signs of good oestrogenic stimulation and a pH greater than 6.7 was placed on the centre of a slide and flattened by a coverslip to about 2cm diameter. Fresh semen was applied to the edge of the coverslip to draw it into contact with the edge of the mucus. The slide was then placed (in a moist Petri dish) in an incubator at 37^{o}C and examined microscopically after 20 minutes. Penetration was assessed as 0= no penetration; 1= penetration not exceeding 2 mm into peripheral mucus; 2= sperm moving throughout the mucus.

Movement within the mucus was assessed as positive if there was forward progression of the sperm or negative if the sperms were immobile or shaking. In the tube test, a 1 cm column of semen was placed at the bottom of a plastic Luckam (L.P.3) test-tube and into this was placed the quill with 1 cm of cervical mucus at the lower end and physiological saline at the upper end. The tube was put in the incubator at 37°C. After one hour the saline and the upper, middle, and lower parts of the cervical mucus were examined for the presence or absence of sperms and motility was graded. Results were expressed descriptively according to the level of penetration of sperms.

2.2.4 Sperm Cervical Mucus Contact (SCMC) Test

In this technique the spermatozoa in a drop of semen are brought into contact with cervical mucus. Changes in the motility pattern of spermatozoa (due to antisperm auto- or isoantibodies) are observed when they are inside the mucus.

The SCMC test was carried out as described by Kremer and Jager (1976) and Jager et al (1979).

A fresh semen sample of a husband was placed into a narrow test tube which was left in an upright position for 30 minutes at room temperature in order to allow agglutinated spermatozoa to settle to the bottom. Approximately 0.1 ml of the semen was then withdrawn from a level about 0.5 cm under the fluid surface using disposable tuberculin syringe fitted with a very fine needle. One drop of the wife's preovulatory cervical mucus was placed on a glass slide next to a drop of semen. Both drops were then thoroughly and carefully mixed and covered with a coverslip.

A second drop of semen was placed on the same slide as a motility control. After 15 minutes of incubation at 37°C in a moist Petri dish, the slide was examined and the percentage of motile spermatozoa showing quickly shaking movements (shaking phenomenon expressed in %S) was estimated. The classification of the SCMC test resuls was as follows: grade negative with at least 75% of progressively motile spermatozoa, grade + with 26 to 50% of locally quickly shaking spermatozoa, grade ++ and grade +++ with 51 to 75% and with 76 to 100% of spermatozoa exhibiting the shaking phenomenon, respectively. The SCMC test was also performed with a donor's spermatozoa and, whenever possible, with a donor's cervical mucus.

2.2.5 Gelatin Agglutination Test (GAT)

The GAT introduced by Kibrick and his colleagues in 1952 was performed as described by Shulman (1975) with slight modifications.

Samples under investigation (serum or seminal plasma) were heat-inactivated by incubation at 56°C for 30 minutes in a water bath, to destroy complement activity that may otherwise cause (with the antibody) sperm immobilization. Samples were then serially diluted in serological test tubes (LP 3, Luckham), using Baker's buffer as the diluent and starting with dilution 1:4. Baker's buffer had the following composition: glucose 30.3g; $Na_2HPO_4.12H_2O$, 6.0g; NaCl 2.0g; KH_2PO_4, 0.1 g; distilled water to 1000ml. 200 µl of each of these dilutions were transferred to small Luckham LP 2 test tubes, which were then stored at 37°C until required.

A fresh semen specimen of good quality (i.e. sperm count of at least 50 million/ml, motility of at least 50%. good morphology and with neither agglutinates nor increased numbers of non-spermatozoal cells) was diluted with Baker's buffer, warmed to 37°C to a final concentration of 40 million/ml. An equal volume of 10% gelatin (Difco) in Baker's buffer was added to the diluted semen suspension and mixed gently. The gelatin medium prevented rapid sedimentation of the spermatozoa.

200 µl volumes of semen-gelatin mixture were added to each of the LP 2 test tubes containing the serum or seminal plasma dilutions and carefully mixed using disposable stirring rods. The test tubes were then incubated at 37°C and examined after 1 hour and after 2 hours against a dark background in front of a strong light. In positive cases an agglutination was observed by the appearance of white floccules along with clearing of the suspending medium (fig. 1). Sera showing the positive result at dilution 1:4 were studied again in the 2-fold dilution series. The antibody titre was defined as the hightest dilution which showed a positive result (precipitation), expressed as the reciprocal of this dilution. Control sera, both positive and negative, were always tested in parallel to evaluate the particular semen samples used in the test.

2.2.6. Tray Agglutination Test (TAT)

This microscopic test was performed according to Friberg (1974a) with some modifications by Hellema and Rumke (1976) and Jager et al (1978).

Samples (serum or seminal plasma) were inactivated as for GAT and diluted with Earle's balanced salt solution of

following constitution: 10ml 10x concentrated Earle's solution (Gibco); 3.3 ml of 30% bovine serum albumin (BSA) (Sigma); 3.0 ml of 7.5% $NaHCO_3$; distilled water to 100 ml; pH was adjusted prior to use to 7.2 with HCl. Dilutions of 4-, 8-, up to 128-fold were prepared in the tissue typing microtitre plates (Sterilin), using automatic 25 ul pipettes. Five µl volumes of the sample dilutions were transferred to the 60 ring microchambers (Medicel International) under paraffin oil, using a Hamilton micropipette with disposable tips (fig. 2).

A donor semen sample with good sperm concentration (at least 80 million/ml) and motility greater than 70% was aliquoted into narrow test tubes (Luckham LP 2) to a depth of about 1 cm. An equal volume of Earle's solution containing 1% BSA at 37°C, was carefully layered on top of the semen sample. The spermatozoa penetrated the layer of Earle's solution after an incubation for about 1 hour at 37°C, leaving behind any debris, non-spermatozoal cells and agglutinated or immotile spermatozoa. The top layer, which contained a high percentage of motile spermatozoa, was carefully removed and sperm concentration adjusted to between 30 and 40 million/ml (fig. 3).

One µl of the spermatozoa suspension was added to 5 µl drops of the diluted sample with the aid of a Hamilton microsyringe and mixed gently. The trays were subsequently incubated at 37°C and examined after 1 and 2 hours for the mode of agglutination (fig. 4) and the titre, using an inverted microscope (Olympus CK) (fig. 5). A known positive and negative control sera were always included in the test.

2.2.7 Sperm Immobilisation Test (SIT)

This test was carried out as described by Isojima et al (1968). Samples under investigation were pretreated by heating at 56°C for 30 minutes to inactivate complement. The test was normally carried out on undiluted serum only, unless GAT or TAT were positive, in which case 2-fold dilutions were made. Human spermatozoa of at least 70% motility and good forward progression were diluted to 60 x 10^6 cells/ml in Baker's buffer. Pooled fresh guinea-pig serum stored in small aliquots and frozen (-20° to -60°C) were used as the complement source. To the small tube was added: 0.25 ml of inactivated test serum or a control serum, 0.05 ml of complement solution and 0.025 ml of fresh human semen dilution. Known positive and negative sera were included in each test as controls. A similar mixture was made <u>without</u> the complement solution, to detect any non-specific sperm immobilizing activity, consisting of 0.25ml of test semen plus the semen sample. The mixtures were incubated at 37°C for 1 hour.

A drop of each mixture was examined on a microscope slide at 100 x to 250 x, studying several fields. The percentage of motility was measured, and a positive result was recorded if the sperm motility decreased to 50% or less of the motility in the negative control mixture. Positive sera were titrated from 1:4 to 1:128.

2.2.8 Mixed Antiglobulin Reaction for IgG antibodies (MAR-IgG)

The MAR-IgG test based on the method of Coombs et al (1956) was carried out as recommended by Jager and his co-workers (1978). The test was performed on all fresh

semen specimens as a part of a routine seminal analysis, provided that the sperm concentration was greater than 1.0 million/ml and the motility sufficient.

Sensitized red blood cells (RBC's) were prepared by the Blood Transfusion Laboratory according to the method of Chalmers et al (1959) or obtained commercially (Coombs Control, Baxter). Group O, Rh positive red blood cells were washed three times in phosphate buffered saline (PBS), pH 7.5. Five volumes of this suspension were mixed with 1 volume of a strong incomplete anti-D serum and incubated at 37^{o}C for 30 minutes. The cells were then washed three times with PBS to a haematocrit of between 5 to 10%.

One drop of fresh semen was placed on a microscope slide with one drop of the sensitized red blood cell suspension and one drop of undiluted monospecific anti-human IgG (Gamma-chains) antiserum (Dakopatts). The three small drops were thoroughly mixed and covered with a coverslip. The reaction was observed under a microscope within 5 minutes. The test was considered negative if at least 90% of spermatozoa were freely swimming between the clumps of agglutinated erythrocytes. The reaction was scored as + if up to 20% of the motile spermatozoa were involved with adhering blood cells, ++ with up to 80% of spermatozoa incorporated into RBC's agglutinates and +++ when more than 80% of motile spermatozoa were attached to the erythrocytes (fig. 6).

2.2.9 Mixed antiglobulin reaction for IgA antibodies (MAR-IgA) (Hendry et al, 1982)

The MAR tests were modified as follows for the addition of IgA testing: group O Rh-positive red blood cells

were washed three times in Alsever's solution and resuspended to a hematocrit of 50%. To one part of red cell suspension was added two parts of 1-in-5 dilution of serum containing anti-D, which was partly IgA, although mainly IgG (Serum Avgh., supplied by Professor P.L. Mollison). The red cells and serum were incubated at 37°C for 30 minutes. The cells were then washed three times again in Alsever's solution, and resuspended to a hematocrit of between 5% and 10%, and stored until required for use in small aliquots at 4°C.

One drop of fresh semen was placed on a microscope slide with one drop of sensitized red cell suspension and one drop of undiluted monospecific anti-human antiserum for either IgG or IgA (Behring Diagnostics, Somerville, N.J). The three drops were thoroughly mixed, and the reaction was read within 10 minutes. No interpretation was made unless agglutination of the red blood cells was observed. The test was read as negative (0) if no motile mixed agglutinates were seen and freely swimming spermatozoa could be observed between the clumps of agglutinated red cells. If motile mixed agglutinates were observed, the reaction was graded as follows: doubtful (±), only an occasional mixed agglutinate seen, with less than 10% of the motile spermatozoa involved with adhering red blood cells (this result is interpreted as negative); positive (++), 10% to 90% of the motile spermatozoa attached to the erythrocytes; strongly positive (+++), more than 90% of the motile spermatozoa incorporated into mixed agglutinates.

2.2.10 Immunobead Test (IBT)

This test was carried out as recommended by Bronson et al (1982) and Clarke et al (1985). Anti-IgG and anti-IgA immunobeads (BioRad Laboratory) were reconstituted in 10ml of Tyrode's solution or Bulbecco's phosphate buffer saline (PBS) which was passed through a millipore (22 μm filter) before use. 0.2 ml of antiIgG beads and 0.2ml of anti IgA beads were put into separate centrifuge tubes and made up to 10 ml with 0.4% bovine serum albumin (BSA) buffer. In a centrifuge tube the required amount of semen was added and made up to 10ml with 0.4% BSA buffer. The amount of semen required was determined by sperm count and motility (table 12).

Sperm Concentration (million/ml)	Motility (%)	Semen Required (ml)
>50	>40	0.2
20-50	>40	0.4
20-50	<40	0.8
<20	>40	1.0
<20	<40	2.5

TABLE 12 Volume of semen required for direct IB test

The three tubes were centrifuged at 500g for 5 minutes. The supernatant was decanted and the pellet was washed and resuspended in 10 ml of 0.4% BSA buffer. The washing of the semen was repeated and the semen pellet resuspended in 0.2 ml of 0.4% BSA buffer. 5 μl drops of antiIgG beads and anti

IgA beads were placed at either end of the glass slide and approximately 5 µl of the semen suspension was added to each of the bead drops and mixed well with the cover slip. The slide was left for 10 minutes in a moist chamber and observed microscopically at 400x magnification. The percentage of motile spermatozoa attached to the immunobeads were scored separately in the IgG and IgA containing mixture (fig. 7). The test was regarded as positive if 20% or more of the motile spermatozoa were attached to one or more bead.

2.2.11 Enzyme Linked Immunosorbent Assay (ELISA)

Test plates coated with sperm antigen were prepared by ZER (ZER Science Based Industries Ltd., Jerusalem) and were obtained through Horwell Ltd., U.K. These kits included positive and negative control serum and alkaline-phosphatase-coupled anti-human immunoglobulin. According to the protocol sheet accompanying the kit, we added various dilutions of company control reagent or our own set of sera known to be positive or negative by TAT to the assay wells. After incubation and washing, the anti-human immunoglobulin reagent was added, and after a further wash the p-nitrophenyl phosphate substrate was added. After 60 minutes, 3 N NaOH was added to stop the reaction, and colour development was measured by means of an ELISA plate reader. A positive result was one exceeding the negative control.

2.2.12 Immunofluorescent Test (IFT)

This test was done by the method of Hjort and Hansen (1971). The spermatozoa in 0.2 ml of donor semen were washed twice in 10ml buffered saline with centrifugation at 680g for 5 minutes. The washed spermatozoa were resuspended

in buffered saline, and the concentration adjusted to 10 x 10^6/ml. Single drops of this suspension were spread on slides and dried under a fan for 30 minutes. The dried spermatozoa were fixed in analytical reagent grade absolute methanol for 30 minutes, after which the slides were transferred to phosphate-buffered saline PBS (pH 7.2). A drop of serum or semen dilution was added, and the preparation left in a moist chamber for 1 hour at room temperature. The serum or semen was then rinsed away with PBS and the preparation washed for 20 minutes with one change of buffer at 10 minutes. The preparations were then incubated for 30 minutes with Fluorescein Isothiocyanate (FITC) conjugated antisera and after repeated washing were mounted in PBS in 10% glycerol. The slides were examined using a good fluorescent microscope equipped with interference filters. Staining patterns were defined as involving acrosome, equatorial segment, post acrosomal area (post nuclear cap), tail mainpiece and tail end piece.

2.3 INFERTILE MALES: DIAGNOSIS OF IMMUNOLOGICAL INFERTILITY

2.3.1 Experimental design

The first sections (2.3) are concerned with studies done to find out which ASA tests were clinically relevant and meaningful. Initially, over 500 subfertile men attending the Infertility Clinic at CHW had blood taken for testing for ASA by 3 different techniques (GAT, SIT and IFT). The results were correlated with each other and with clinical findings in the men and their female partners (PCT) (2.3.2). They were also correlated with the results of sperm-cervical mucus contact (SCMC) and X-hostility testing (2.3.3). The results showed that GAT was both sensitive and

reliable, though rather cumbersome.

Miss Stedronska and then Mrs. Parslow therefore went to work with Dr. Jager and Dr. Kremer in Groningen for 2 weeks to learn the details of the newer micromethod of testing (TAT). which could be used to test seminal plasma routinely as well as serum. Having mastered the necessary technology GAT and TAT were run in parallel for some years before ultimately dropping GAT. Dr. Jager also demonstrated MAR (IgG) testing, which was evaluated carefully at CHW and is now used routinely to screen for ASA (2.3.4; 2.3.5).

Dr. Kremer showed that the class of antibody on the spermatozoa was of fundamental importance. With the help of Professor Pat Mollison of St. Mary's Hospital, London, a new MAR test for IGA was devised and evaluated with serum and seminal plasma TAT (2.3.6). Subsequently Mrs. Parslow developed a sophisticated double antibody radioimmunoassay to quantify the class of antibody more accurately, and this formed the basis of her PhD thesis. These results will be alluded to in discussion.

Shortly before Miss Stedronska left to become Mrs. Stedronska-Clark, she conducted with her husband Dr. David Clark our last study evaluating a new ELISA test (2.3.8). CHW closed in 1989, and since then ASA work has continued solely in the Williamson Laboratory at Barts., where recently the direct IBT has been compared with direct MAR as a routine screening test for ASA in subfertile males and prior to IVF or GIFT (2.3.7).

2.3.2 Serum GAT, SIT, IFT, PCT and clinical findings (Hendry et al, 1977)

Between 1975 and 1977, sera from 591 male partners of infertile marriages were tested for the presence of ASA by GAT, SIT and IFT. All of the men submitted at least two specimens for seminal analysis. Those with counts of less than 20 m/ml, or motility less than 30% moving actively within 3 hours, were investigated as previously described. When positive antisperm antibody tests were found, both husband and wife were examined and repeated post-coital tests were done.

2.3.3 Serum GAT, SIT, IFT and X-hostility (Morgan et al, 1977)

Forty four couples were selected for the crossed hostility test on the basis of the husbands' antisperm antibody results. In 22 couples (group I) the husbands had high titres (more than 1/50) of both agglutinating and immobilising antibodies. In 10 couples (group II) the husbands' sera were negative for agglutinating and immobilising antibodies, but positive on immunofluorescent testing (acrosome pattern 2, equatorial segment 1, postnuclear cap 2, tail 3, tail + equatorial segment 2). In a further 12 couples (group III) all antibody tests were negative in both husband and wife.

2.3.4 Serum GAT and MAR (IgG) (Hendry & Stedronska, 1980)

In 1978 and 1979, 775 semen samples from 557 male partners in subfertile marriages were examined, and MAR IgG testing was completed with 664 specimens from 463 patients. In 213 patients, serum antisperm antibodies were measured in serial dilution by GAT and SIT. One hundred and seventy

eight of these patients were tested on more than one occasion.

2.3.5 Serum TAT and MAR (IgG) (Stedronska & Hendry, 1983)

In 1980 and 1981, 1279 semen samples from 720 male partners of infertile marriages were examined. MAR (IgG) testing was done in 651 patients with sufficient numbers of motile spermatozoa to allow interpretation of the results, and the fact that it was not possible in the remaining 69 patients was noted on the seminal analysis report. In 204 patients, antisperm antibodies were also measured by GAT, SIT and TAT. In 448 patients, the negative result obtained by MAR test was taken as sufficient to exclude the presence of antisperm antibodies, and no other immunological tests were done.

2.3.6 Serum and seminal plasma TAT, MAR (IgG and IgA) and X-hostility (Hendry et al, 1982a)

The presence or absence of IgA antibodies on spermatozoa was defined by the direct MAR test in 104 semen samples with counts of at least 1 million spermatozoa per milliliter, provided by 51 untreated subfertile males with positive MAR tests for IgG. In 34 cases the MAR tests for IgA were repeated on two or more occasions. The quantity of antisperm antibody in seminal plasma was measured in serial dilution by GAT in all cases and by TAT in 49 cases. The serum antibody titre was also defined in 47 cases by GAT, TAT and SIT. In 20 couples, the patients' spermatozoa were tested against preovulatory cervical mucus obtained from the wife and from donors of proven fertility, comparing the behaviour of patients' spermatozoa with those obtained from fertile donors by x-hostility test.

2.3.7 MAR (IgG) and IBT (IgG and IgA) (Rajah et al, 1992)

Spermatozoa from 109 male partners of infertile couples were tested for surface bound ASA by MAR (IgG), and by direct IBT for both IgG and IgA. Sperm concentrations ranged from 0.6 to 282 (median 55) million per ml, overall sperm motility was from less than 5 to 80 (median 60) per cent. In addition, ASA were measured by TAT in seminal plasma in 26, and in serum in 34.

2.3.8 Serum TAT and ELISA (Stedronska-Clarke et al, 1987)

Eight known positive and 4 known negative sera from infertile and control patients were tested by ELISA and the results compared with those obtained by serum TAT.

2.4 INFERTILE MALES: MEDICAL TREATMENT

2.4.1 Experimental design, patient selection and pretreatment checks

Sections 2.4 and 3.2 relate to the development of effective medical treatment for the subfertile male with immunological infertiity. Drug dosage and timing required careful sequential evaluation in searching for a treatment that was effective and safe, and this had then to be tested against placebo in a prospective double blind trial. Professor Michael Besser in the Endocrinology Department at Barts. provided valuable help throughout these studies, and with his guidance a protocol was drawn up to select patients suitable for treatment, and to define essential medical checks which had to be completed before treatment started. Dr. Louis Hughes, Clinical Assistant at CHW, provided great help in seeing patients on treatment, making sure that the protocols were followed carefully. Miss Gill Scammell, Lecturer at CHW, looked after many of the female partners.

Protocol for selection of patients for medical treatment for antisperm antibodies:

Patients

The patients should be males who have been trying unsuccessfully to produce a pregnancy for at least 1 year with:

1. Some spermatozoa in the ejaculate in at least two samples. (The MAR test should be positive if it is technically possible to do this test).
2. A positive serum GAT or TAT titre of 32 or more and/or a positive seminal plasma GAT or TAT titre of 4 or more.

3. An abnormal PCT (less than five progressively motile sperms per high power field) done between the 12-14th day of cycle after intercourse 8-12 hours previously.
4. An abnormal SCMC test with sperm 'shaking' on the 12-14th day of cycle.

If these criteria are satisfied, it is reasonable to conclude that autoimmunity to spermatozoa in the male partner is a significant factor in the couple's infertility. Female partners should have patent tubes (preferably demonstrated by laparoscopy) and have been documented to be ovulating adequately (two progesterone levels on days 16, 21 or 24 above 33 nmol/L). If the female partner is not ovulating adequately, then she should be appropriately treated and no therapy given to the husband until this has been shown to be effective by repeating the measurement of the progesterone levels.

Pretreatment Work-Up

Diastolic blood pressure should be less than 90mmHg, and chest x-ray and liver function tests should be normal. The blood sugar 2 hours after food should be less than 8.9 mmol/L, and there should be no glycosuria. There should be no first-degree family history of diabetes - if there is, then a glucose tolerance test should be done and found to be normal. Finally, there should be no significant history of dyspepsia. If these criteria cannot be fulfilled, the patient should be referred for a detailed medical work-up before treatment is commenced.

Informed Consent

Both partners should be interviewed together and the details of treatment explained in full. Precautions to be followed whilst on steroid therapy, and possible side effects, are detailed on an information sheet which is given to the couple to take away and keep. Mention of possible serious complications is made, and the final decision on whether to proceed or not is left to the couple. If necessary, a further consultation is arranged to give the couple time to consider their decision, and if they wish, discuss it with their general medical practitioner.

A copy of the Patients' Information Sheet used for the cyclical moderate dose prednisolone regime is reproduced in full:

INFORMATION FOR PATIENTS RECEIVING PREDNISOLONE TREATMENT FOR ANTISPERM ANTIBODIES

1. Prednisolone treatment can lower the quantity of antisperm antibodies in subfertile men and this may help them to produce a pregnancy.
2. Approximately one-third (33%) of wives can expect to become pregnant whilst their husband is receiving Prednisolone treatment.
3. The normal course of treatment lasts for nine months.
4. Couples are seen after the first course of treatment and then every three months to recheck antibody levels in blood and semen.
5. The usual dose of Prednisolone is four 5 mg tablets (20mg) twice daily taken with meals, by the husband, from day one to ten of the wife's cycle, followed by

one tablet (5mg) in the morning on days 11 and 12. This dose may be adjusted up or down, according to individual response or side-effects.

6. No alcohol should be taken whilst on treatment.

7. Side-effects occur in about 20% of patients taking Prednisolone. Many patients gain a little weight and develop a few spots on the face and body. Some experience indigestion, and this can usually be relieved by antacid treatment (e.g. Rennies). Some men become irritable and this may require patient understanding. There is a small risk of thinning of the bones and rarely joint damage may occur, especially if treatment goes on for longer than the recommended period.

8. It is essential that any other doctor who treats a patient who is, or has been on Prednisolone, is informed of the fact. For this reason, all patients should carry a Steroid Card whilst on treatment and for one year thereafter.

9. In the event of an emergency whilst on Prednisolone treatment, please contact your General Practitioner or:-

Mr. W.F. Hendry,
St. Bartholomew's Hospital, London, EC1.
(071 601 8394).

10. If pregnancy occurs, please be sure to inform your consultant.

2.4.2 Prednisolone - continuous low dose treatment (Hendry et al, 1979)

Group 1 - 15 patients had average pre-treatment sperm

counts of less than 20 million per ml; 4 had varicoceles which were ligated, and 1 had bilateral maldescended testes operated upon in late childhood. These patients were treated with prednisolone 5mg three times a day for 3-12 (average 6.1) months. Testicular biopsy specimens were obtained under general anaesthetic from 3 patients with severe oligozoospermia (2 of whom had varicoceles).

Group 2 - 14 patients with average pre-treatment sperm counts of more than 20 million sperms per ml received Prednisolone 5mg three times a day for 3-12 (average 7.1) months.

2.4.3 Cyclical high dose methyl prednisolone (Hendry et al, 1981)

Forty-five men, aged between 24 and 46 years (average 31.7), were seen because of infertility lasting from 2 to 10 years (average 5.3). Forty-two men were "naturally infertile" of whom 5 had a past history of epididymitis, 4 testicular injury, 2 venereal disease, 1 mumps orchitis, and 2 very tight foreskins removed as adults; in all, 14 (33%) of these 42 had a history of a possible predisposing factor. In addition, two men had had reversal of vasectomy, and one had had successful epididymovasostomy.

As recommended by Shulman (1976), Methylprednisolone (MP), 32 mg three times a day with meals, was given to the husband for 7 days from days 21 to 28 of his wife's menstrual cycle. If no serious side effects occurred, and if the wife did not become pregnant, the treatment was repeated in alternate months for 6 months. Antisperm antibody levels in serum and seminal plasma were rechecked as often as possible after treatment. In a few cases,

serial antibody estimations (every 2 or 3 days for 10 days) were made following the third course of MP to allow fine adjustment of the timing of future courses of treatment. Ten men received up to three additional monthly courses of MP at times ranging from days 1 to 8 to days 5 to 12 of successive cycles, depending on the results of these additional serial antibody tests. In two patients this was the primary treatment given. Enquiry regarding side effects or pregnancy in the spouse was made at each visit, and no patient was lost to follow-up.

2.4.4 Cyclical moderate dose prednisolone (Hendry et al, 1986)

Seventy-six men 27 to 47 years of age (mean, 33 years) complained of spontaneous involuntary infertility for 15 months to 21 years (mean, 4 years). In all cases initial semen analysis screening revealed a positive MAR test for IgG antibodies on spermatozoa. Sperm counts of 20 x 10^6/ml or greater were recorded in 65 patients; sperm motility was 40% or more in 36 patients. Serum titres of 32 or more were recorded by GAT or TAT in 70 patients; for the remaining 6 patients, seminal plasma antibody titres were positive at 4 or more. Crossed-hostility (SCMC) tests were carried out in 41 couples. In the women, apparent ovulation was confirmed by observation of luteal phase serum progesterone levels of at least 33 nmol/L, and in 29 women this was induced by medication, including clomiphene citrate and gonadotropin therapy.

Patients were advised of the risks and possible side effects of treatment and cautioned to abstain from alcohol during therapy. They were then given soluble prednisolone,

20mg twice daily (taken with meals), from days 1 to 10 of the partner's cycle, followed by 5mg in the morning of days 11 and 12. Serum and seminal plasma antibody levels were remeasured after three cycles of treatment, and the patients were assessed. If the antibody titres had fallen 2 or more dilutions, up to six more courses of treatment were given, bringing the total cycles of therapy up to nine. In 15 patients in whom the titres had not fallen, the dose of prednisolone was increased to 40mg twice daily taken, as before, from days 1 to 10 of the partner's cycles, followed by 5mg in the morning of days 11 and 12. The patient and the antibody titre were reassessed after three cycles at this increased dosage. The protocol permitted a final rise to 60mg prednisolone twice daily (the equivalent of methylprednisolone, 96mg daily), but this was required in only one patient. The protocol specified a limit of not more than 9 treatment cycles; however, for a variety of reasons, 14 men opted to continue for 12 cycles and 1 man went on for 17 cycles without ill effect.

2.4.5 Controlled clinical trial of prednisolone versus placebo (Hendry et al, 1990a)

These studies were done in collaboration with Mr. Tim Hargreave, Western General Hospital, Edinburgh, and Mr. John Pryor, St. Peter's and Kings College Hospitals, London.

Forty-three men with ASA, who had been trying to produce a pregnancy for at least a year, entered the trial after giving informed concent. The men were stratified into two groups according to whether they had been trying to produce a pregnancy for less than 3 years (16 men) or 3 or more years (27 men); they were then randomised to receive

double-blind either prednisolone (22 patients) or matching placebo (21 patients) for 9 months, before crossing over to the other treatment. The active treatment consisted of soluble prednisolone 20mg twice daily taken with meals on days 1-10 of the female partner's menstrual cycle, followed by 5mg in the morning of days 11 and 12. Alcohol was forbidden during this period. After three cycles the antibody levels were remeasured; if the serum or seminal plasma titres had dropped 2 or more dilutions the treatment was repeated for two more periods of 3 months to a total of nine cycles. If the titres were unchanged (10 patients) the dose of prednisolone (or placebo) was raised to 40mg twice daily, taken as previously, for the remaining 6 months.

An information sheet was given to each couple outlining the side-effects to be expected and precautions to be followed with steroid treatment, together with a card bearing their code number so that the tablets could be identified by the hospital pharmacy if necessary. Couples were seen every 3 months so that side-effects could be discussed, and seminal analysis and antibody tests were repeated. The trial was approved by the appropriate hospital ethical committees.

2.5 INFERTILE MALES: SURGICAL TREATMENT

2.5.1 Patient selection and operative techniques

The selection of subfertile males for exploration of the scrotum to confirm the presence of testicular obstruction, and correct it whenever possible, is never very easy, and has been the subject of a number of chapters written by the author (Hendry, 1987 a, b). The indications can be described as absolute or relative, and

contraindications are definable. These are summarised as follows:

Absolute indications:

(i) Vasectomy reversal.

(ii) Azoospermia with normal sized testes, palpable vasa and normal serum FSH levels.

(iii) Oligozoospermia (less than 20m/ml) with antisperm antibodies, and clinical evidence of unilateral testicular obstruction.

Relative indications

(i) Azoospermia or persistently severe oligozoospermia after a previous attempt at vasectomy reversal.

(ii) Azoospermia with small testes and low serum FSH levels, prior to gonadotrophin therapy.

(iii) Azoospermia or severe oligozoospermia with marked disparity in the size of the testes, even with slightly or moderately elevated serum FSH levels.

(iv) Normal sperm count and significant titre of antisperm antibodies, with some evidence of unilateral testicular obstruction.

Contraindications

(i) Azoospermia with small testes and grossly elevated serum FSH levels.

(ii) Bilateral absence of the vasa deferentia.

Surgical Technique

Under general anaesthesia, the scrotum was opened by a midline incision, the testes delivered and the vaginal spaces opened. The epididymes were examined and often photographed. If the epididymes were empty a testicular

biopsy was taken and nothing further was done. Provided that the epididymis contained distended tubules, attention was turned to the vasa. If they were absent nothing further was done. If they were present, the vas was picked up across the tunica vaginalis and a short longitudinal incision was made. The lumen was cannulated with a size 2F intravenous cannula (Portex) and 5ml 25% Hypaque injected before taking a vasogram x-ray film. The epididymis was then incised over the lowest part containing dilated tubules and side-to-side epididymovasostomy (Ep-Vas) was completed with a continuous suture of 6/0 Prolene and no splint (fig. 8). If vasography showed a block in the vas, the area was explored through a separate incision and the block was either corrected by division of the vas and side-to-side vasovasostomy (Vas-Vas) with 6/0 Prolene (fig. 9) or the testicular end of the vas was anastomosed to the opposite vas by end-to-side technique as a transvasovasostomy (Trans-Vas-Vas) (fig. 10). All patients wore a scrotal support lined with cotton wool for 7 days.

Most patients nowadays are done as Day Cases, although they generally stayed in for a day or two in years gone by.

Vasectomy reversal was done by overlapped side-to-side anastomosis (fig. 9) with 6/0 Prolene, usually via bilateral oblique incisions.

2.5.2 Unilateral testicular obstruction - diagnosis (Hendry et al, 1982b)

Thirty-two subfertile males were questioned about their past medical history, examined clinically and investigated by seminal analysis and measurement of hormone levels. Serum antisperm antibodies were assayed in serial

dilution and the mode of sperm agglutination was defined as head-to-head (HH), tail-to-tail (TT) or mixed by TAT. In 26 cases the scrotum was explored and unilateral obstruction was confirmed.

Three patients had had bilateral epididymo-vasostomies some years previously: they presented with positive sperm counts and continuing partial obstruction was suspected on clinical examination. Two patients refused surgical exploration - one had had epididymitis, the other had had a hernia repair twice on one side, and in both cases the epididymis on the affected side was distended and tender; they have therefore been included on clinical grounds. Finally, one patient had had epispadias repair as a child, followed by urethral stricture and urinary infection. The stricture was dilated and the infection was eradicated but the scrotum was not explored and once again unilateral obstruction was diagnosed clinically. In 2 additional patients, the scrotum was explored for suspected obstruction but this was not confirmed; testicular biopsies were taken.

The clinical and operative findings were correlated with the results of seminal analysis and antibody estimations. The antibody results were compared with those found in 160 unselected spontaneously infertile males, and those obtained in 162 vasectomised men in an attempt to define criteria that would draw attention to otherwise unsuspected testicular obstruction.

2.5.3 Unilateral testicular obstruction: results of treatment (Hendry, 1986)

These studies were subsequently extended to include 80 males aged 23 to 60 years (average 33) who were referred for

investigation of spontaneous infertility of 1 to 13 years' duration (average 4). No vasectomy reversal patients were included in this study. Nine gave a history of having produced children 4 to 18 years previously (average 10). The diagnosis of unilateral testicular obstruction was established in all cases by exploratory scrototomy. The sites of obstruction, and the causes so far as could be ascertained, are listed in Section 3.3.3.

In 48 patients, immediate reconstruction was done by epididymo-vasostomy or vaso-vasostomy. In 10 patients with impenetrable blocks or unilateral absence of vas, the obstructed testis was removed at a second operation and replaced with a prosthesis following full discussion with the patient and his wife. No surgical reconstruction was done in 22 cases. Sixty patients have been followed for at least 6 months, with repeated sperm counts and measurement of antisperm antibodies. Amongst these, 19 patients with significant titres of antisperm antibodies received prednisolone 5mg tds for 6 to 12 months and another nine received up to 9 courses of prednisolone 20mg twice daily, taken with meals from days 1 to 10 of the female partner's cycle, followed by 5mg daily on days 11 and 12.

All female partners were fully investigated by gynaecological colleagues to assess tubal patency and establish ovulation, with drug treatment if necessary.

2.5.4 Bilateral testicular obstruction - incidence of ASA (Hendry et al, 1983a)

In 168 azoospermic males with normal serum FSH levels, the site of obstruction (or exact nature of failure of spermatogenesis) was defined by exploration of the scrotum

and related to known or suspected aetiological factors, and to the presence or absence of ASA.

These studies were subsequently extended to 370 men, and are described in the next section.

2.5.5 Bilateral testicular obstruction results of surgery and ASA (Hendry et al, 1990b)

This work formed the basis of a Hunterian Lecture at the Royal College of Surgeons of England. Histopathological features of testicular obstruction were studied in collaboration with Prof David Levison (now at Guy's Hospital, London) and Dr. M.C. Parkinson of St. Peter's Hospitals, London. ASA Studies were done throughout. Fertility after successful reconstruction in 60 men with obstructive azoospermia was related to presence or absence of ASA. No vasectomy reversal men were included in this section.

2.5.6 Genital tract injuries in childhood (Parkhouse & Hendry, 1991)

Thirty men aged 20 to 41 years presenting with infertility were studied. All had undergone surgery or trauma before the age of 12 years and this was demonstrated by surgical exploration to have resulted in injury to the vas deferens or epididymis. Twenty patients had undergone correction of hydroceles or congenital hernias, 1 combined with contralateral orchiopexy; 3 had had orchiopexies only (1 unilateral and 2 bilateral), 4 had undergone rectal surgery for imperforate anus (3) or Hirschsprung's disease (1), 1 had undergone multiple bilateral ureteric reimplants, 1 had vasal damage due to an intra-abdominal drain following laparotomy in infancy, 1 had undergone cystoprostatectomy

for rhabdomyosarcoma, and 1 had sustained a ruptured urethra. Results of seminal analysis and serum TAT were compared in those with unilateral and bilateral damage.

2.6.1 Vasectomised males before and after reversal (Royle et al, 1981; Parslow et al, 1983; Hendry et al, 1983b)

These studies were done in collaboration with Mr. Michael Royle, Consultant Urologist in Brighton. Between September 1979 and December 1980, 130 vasectomised men underwent bilateral side-to-side vasovasostomies using 6/0 Prolene and no splints. Patients were randomised to receive either hydrocortisone 100mg intramuscularly at the time of operation, then prednisolone 10mg twice daily by mouth for 5 days, or no steroids. ASA were measured by GAT and TAT. Serum samples were taken before, 1 week and 3 months after operation. Seminal plasma antibodies were measured before and 3 months after reversal; sperm counts were checked at 3 months, and at intervals thereafter. Since many of the patients lived a considerable distance from the 2 centres carrying out this procedure, not all follow-up specimens could be obtained; however, attempts were made to contact all patients to encourage them to send the semen samples and to enquire about the occurrence of pregnancy in their partners.

The preliminary results were reported by Royle et al (1981) and the final definitive analysis with at least 1 year follow up of all possible cases by Parslow et al (1983).

The proportion of men with ASA in seminal plasma before and after vasectomy reversal was compared with an

unselected group of 146 spontaneously infertile men and published briefly in Hendry et al (1983b).

2.6.2 Failed vasectomy reversal (Royle & Hendry, 1985)

In 32 patients who had vasectomy reversal which failed to restore their fertility, we re-explored the scrotum, defined the cause of the problems as far as possible, and carried out corrective surgery. Twenty three patients had no spermatozoa in the ejaculate at all, and nine had severe persistent oligozoospermia with sperm counts < 10m/ml, generally 1 or 2m/ml. The age range of the patients was 26 to 54 (average 39). Vasectomy had been done between 4 and 17 years previously (average 8). One to 11 years (average 2.5) had elapsed since the previous vasectomy reversal.

The scrotum was opened by either midline or bilateral oblique incisions. The tunica vaginalis was opened on both sides and the epididymes were inspected to see whether they contained distended tubules. If no distension was seen, a testicular biopsy was taken and nothing further was done on that side. If, however, the epididymis showed distended tubules, the vas was located and incised immediately on the testicular side of the previous anastomosis. If clear or milky fluid ran out, this was taken as evidence of obstruction at the site of the previous anastomosis and the anastomosis was re-done by an overlapped side-to-side technique, using continuous 6/0 prolene. If, on the other hand, no fluid ran out at this point, this was taken as evidence of obstruction closer to the testicle. Patency of the previous anastomosis was demonstrated whenever possible by passage of a nylon splint or injection of a little saline through a size 2F Porges tube inserted into the vas.

Epididymo-vasostomy was then done. The epididymis was freed from the body of the testicle and transected repeatedly until a good flow of milky fluid was obtained. End-to-side epididymo-vasostomy was then carried out, using a continuous 6/0 prolene stitch. The anastomosis was done either below or above the previous vaso-vasostomy depending on the height at which the original vasectomy had been done.

2.7 AUTOIMMUNE ORCHITIS IN SUBFERTILE MALES (Hendry et al, 1979; Hendry 1987a,b; Hendry et al, 1990b)

Testicular biopsies were taken from all azoospermic and most oligozoospermic males at the time of scrotal exploration as a matter of routine, fixed in Bouin's solution, stained with haematoxylin and eosin, and examined by Consultant Histopathologists (Dr. R.C.B. Pugh, Dr. K.M. Cameron, Dr. M.C. Parkinson, Dr. P. Trott, Prof. D.A. Levison). Spermatogenesis was assessed by the score-count system of Johnsen (1970). Any evidence of mononuclear cell infiltration of seminiferous tubules was recorded descriptively.

In 10 patients with high titres of ASA and uncorrectable obstruction or absence of one vas, with a normal testis on the opposite side, and after full discussion with the patient and his female partner, the obstructed testis was removed and replaced with a prosthesis. The removed testis and epididymis were subjected to detailed histopathological examination. In some cases, testes and epididymes were sent fresh to Prof. Isaacson at University College Hospital for mononuclear cell studies (work still in progress).

Seven patients with azoospermia (4), severe oligozoospermia (less than 1 million spermatozoa per ml) (3) and very high serum ASA titres ($\geq$512), with no evidence of obstruction on scrotal exploration and evidence of reasonably normal spermatogenesis on testicular biopsy, were treated with prednisolone 5mg TDS for 6-12 months on a presumptive diagnosis of autoimmune orchitis, although in only 4 cases was there histological evidence of mononuclear cell infiltration of seminiferous tubules.

Sperm concentration and serum and seminal plasma ASA titres were followed 1-2 monthly for the duration of the prednisolone treatment and whenever possible for a month or 2 after stopping treatment.

CHAPTER 3: RESULTS

3.1 LABORATORY RESULTS

3.1.1 Continuous Appreciation of Results

Since 1975, every subfertile male seen by the author has had ASA checked as a matter of routine, by at least 2 different techniques. Initially serum GAT and SIT were used, then TAT replaced GAT, and routine testing of seminal plasma became feasible; as the value of the MAR-IgG test became established, direct MAR-IgG supplemented by serum and seminal plasma TAT became the standard form of testing. By always using at least 2 tests, a constant check was maintained on reliability of the tests, and any disparity was immediately discussed with the laboratory concerned. The results that followed were worked out at intervals during this 15 year period and were used to guide clinical practice.

3.1.2 Serum GAT, SIT, IFT, PCT and clinical findings - see 2.3.2 (Hendry et al, 1977)

Incidence and Correlation between Tests

Agglutinating or immobilising antibodies were found in 68 of 591 men (11.5%). The correlation bewteen the presence or absence of immobilising antibodies and the titre of agglutinating antibodies is shown in Table 13. Immobilisation was always produced by sera with positive agglutination titres of more than 64. Antibodies were detected by immunofluorescent testing in 139 of 448 patients (31%). Although these antibodies were found more commonly with increasing titre of agglutinating antibodies (Table 14), the overall correlation was poor.

GAT Titre	Number of Patients	Sperm Immobilisation Test		
		Negative	Doubtful	Positive
0	1			1
4-16	17	9	4	4
32-64	9	4	1	4
≥128	41			41

TABLE 13 Correlation between the Presence or Absence of Immobilising Antibodies and Titres of Agglutinating Antibodies.

GAT Titre	Number of Patients	IFT Positive
0	1	0
4-16	17	6 (35%)
32-64	9	4 (45%)
≥128	41	23 (56%)

TABLE 14 Correlation between the Presence of Immunofluorescent Antibodies and Titre of Agglutinating Antibodies

Analysis of 50 Patients with Agglutinating Antibodies (Titre ≥32)

The 50 patients (8.5%) with agglutinating antibodies in a titre of 32 or more were the subject of further detailed analysis.

(a) Clinical

The average duration of sterility was 6.3 years (range 2-18 years) and only 2 of 50 had ever produced a pregnancy in the past. Twenty patients (40%) gave a past history of genito-urinary infection (9), testicular swelling (3), injury (2) or ectopia (2), vasectomy (3) or haemospermia (1). Four patients had eczema.

(b) Seminal Analysis

Twenty seven patients had sperm counts of more than 20 m/ml, and in 18 of these the motility was greater than 30%. Eleven patients had oligozoospermia and 5 of these had a varicocele. All specimens showed agglutination of sperms and marked variability in the sperm counts was a noticeable feature. Twelve patients had azoospermia, which was obstructive in 7.

(c) Seminal Culture

Seventeen of 31 patients (55%) with antisperm antibodies had positive seminal cultures (Staphylococcus coagulase positive 8, Escherichia coli 8, Streptococcus group A, 1), compared with only 5 of 32 unselected patients (15%) without antisperm antibodies. None of the patients with positive cultures had any symptoms of genito-urinary infection.

(d) Findings in their Wives

Three patients had wives with blocked tubes which were treated surgically. Post-coital tests were never normal.

Of 31 couples in whom the result was known and the husband had sperms in the ejaculate, many "dead" sperms were seen in 4, a few "dead" sperms in 12, and no sperms were seen at all in 15. Cervical "hostility" was observed in all 8 couples in whom it was sought.

3.1.3 Serum GAT, SIT, IFT and X-hostility (see 2.3.3) (Morgan et al, 1977)

The results are summarised in Table 15. Good correlation was found between the slide and tube methods of determining cervical hostility. High titres of both immobilising and agglutinating antibodies were present in all the husbands' sera in group I, but in only 8 was immunofluorescent testing also positive (acrosome 3, equatorial 2, post nuclear cap 1, tail and equatorial 2).

In 21 of the couples in group I the husbands' sperms were unable to invade cervical mucus, either their wives' or a donor's, despite a seminal analysis which was reasonably adequate. The shaking phenomenon was clearly seen to trap the sperms in the cervical mucus when previously they had been swimming vigorously in the seminal plasma. In 1 case (a vasectomy reversal patient - see 3.4) a few sperms were found moving slowly in the central part of the mucus, and in another 2 couples the wife's mucus was of poor quality and resisted invasion by donor sperms. In 2 couples in group II, the husbands sperms did not penetrate the wife's mucus effectively. The results of the crossed hostility test indicated that in 1 couple this was due to poor sperm motility, and in another couple this was due to poor quality mucus. Similarly, in group III, poor mucus penetration was observed in 3 couples, and on testing it was found that this

was due to poor sperm motility in 1 couple and poor quality mucus in the other 2 couples. The shaking phenomenon was not seen for any couple in groups II and III. Adequate sperm penetration was always seen when sperms were set against donor mucus.

Husband's Antibody Status	Number of Couples	Cervical Hostility			
		Husband + Wife	Husband + Donor	Donor + Wife	Donor + Donor
GAT + SIT positive	22	21	21	2	0
IFT positive	10	2	1	1	0
Negative	12	3	1	2	0

TABLE 15 Results of Crossed Hostility Testing in 44 Infertile Couples related to Antibody Results in the Husbands

As a result of the poor correlation between IFT and the other ASA tests, and the lack of correlation with impaired sperm penetration of cervical mucus, IFT was discontinued.

3.1.4 Semen GAT and MAR (IgG) (see 2.3.4) (Hendry & Stedronska, 1980)

Reproducible results were found in 172 (97%) of 178 patients who were tested more than once (Table 16)

Result of Repeat MAR Test	RESULT OF FIRST MAR TEST			
	Negative	Doubtful	Positive	Strongly Positive
Negative	136	13	3	
Doubtful	7	1	2	
Positive		1	1	
Strongly Positive			1	13

TABLE 16 Comparison between results of first and second MAR (IgG) test in 178 patients

The results of the MAR tests related to the results of serum gelatin agglutination tests are shown in Table 17: there was a very good overall correlation (χ^2 = 178; $p < 0.001$), taking the positive and strongly positive together and comparing them with the combined doubtful and negative results. Twenty-nine (85%) of 34 patients with antisperm antibodies had a positive MAR test, whilst negative results were obtained with 174 (97%) of 179 patients without antibodies. In contrast, spontaneous sperm clumping was commonly observed irrespective of the serum sperm agglutination test results.

Serum Antisperm Antibody Test	RESULT OF MAR TEST			
	Negative	Doubtful	Positive	Strongly Positive
Negative (179 patients)	151	23	2	3
Positive (34 patients)	3	2	3	26

TABLE 17 Results of MAR (IgG) tests related to results of serum GAT

The relation between the MAR test results and serum GAT titre is shown in Table 18.

Both patients with strongly positive MAR tests and serum titres of antisperm antibodies of less than 32 impregnated their wives without further treatment. The other three patients with serum antibody titres below this level had negative or doubtful MAR tests. Twenty-seven (93%) of 29 patients with antisperm antibody titres of more than 32 had positive MAR tests. Three patients had strongly positive MAR tests without serum sperm agglutinins; one had had reversal of vasectomy, one had ulcerative colitis and one had dermatitis herpetiformis. Four patients with positive serum antisperm antibody tests had negative MAR tests; good sperm penetration of cervical mucus was noted in three cases on sperm-cervical mucus contact testing. The other patient had a history of dysuria followed by the development of angulation of the penis, resembling that seen in Peyronie's disease. Two additional patients had transiently negative MAR tests following high dose prednisolone treatment, which subsequently reverted to positive.

Serum Spermagglutination Titre	RESULT OF MAR TEST			
	Negative	Doubtful	Positive	Strongly Positive
Negative			2	3
1/4	1			1*
1/8		1		
1/16	1			1*
1/32				3
1/64	1			3
1/128				7
1/256	1			9
1/512			1	3
⩾ 1/1028				1

TABLE 18 Results of MAR (IgG) test related to serum GAT in 37 patients with positive results in one or other test. ** Produced pregnancy without treatment.

3.1.5 Serum TAT and MAR (IgG) (see 2.3.5) (Stedronska & Hendry, 1985)

The overall results are shown in Table 19. The test was not possible in 69 patients (9.5%) due to low motility (14 cases), low count (25 cases), or azoospermia (30 cases). In 484 patients with sperm counts that would have been passed as normal by our usual criteria (greater than 20 million spermatozoa per millilitre, with 40% motility within 3 hours of production), the presence of IgG antisperm antibodies was detected by MAR testing in 48 (10%). Likewise, such antibodies were detected in 18 (23%) of 78 patients with low sperm motility (less than 40%) and 19 (15%) of 128 with low sperm counts (less than 20 million per millilitre). Overall, IgG antisperm antibodies were detected in 85 (12%) and excluded in 566 (78.5%) of the 720 male partners of these infertile marriages.

Average sperm count	Number of patients	MAR (IgG) Test		
		Positive	Negative	Not possible
Normal	484	48 (10%)	436	
Low motility (< 40%)	78	18 (23%)	46	14 (18%)
Low count (< 20 m/ml)	128	19 (15%)	84	25 (19.5%)
Azoospermia	30	–	–	30
Total	720	85 (12%)	566 (78.5%)	69 (9.5%)

TABLE 19 MAR (IgG) test on 1279 Semen Samples in 720 Male Partners of Infertile Marriages (1980 to 1981)

The results of the serum TAT in 85 men with positive or doubtful MAR test results are shown in Table 20. In all cases with serum TAT titres of 32 or more, the MAR test was positive or strongly positive ; all doubtful results were obtained with TAT titres of 16 or less and, hence were of doubtful clinical significance.

Serum TAT titre	MAR (IgG) TEST			
	Negative	Doubtful	Positive	Strongly positive
Not done		2	5	2
Negative		2	6	
4		1	1	
8		1	3	3
16		2	4	5
32				4
64			1	13
128			1	13
256				8
512			1	4
⩾ 1024				3
Total		8	22	55

TABLE 20 Serum TAT titres in 85 Men with Positive MAR (IgG) tests (1980 to 1981)

The overall correlation between serum TAT and MAR test results is shown in Table 21. Negative serum TAT was found in 94 patients with negative or doubtful MAR tests. Statistical analysis (Table 22) confirmed a highly significant correlation between serum antisperm antibodies (TAT) result and MAR test (taking negative/doubtful together and comparing with positive/strongly positive: $X_2 = 127$; $p < 0.001$).

Serum TAT titre	MAR (IgG) TEST				
	Not possible	Negative	Doubtful	Positive	Strongly positive
Not done	34	448	27	5	2
Negative	28	86	8	7	
4	1		1	1	
8	1		1	3	3
16	2		2	4	5
32	2				4
64	1			1	13
128				1	13
256					8
512				1	4
> 1024					3
Total	69	534	39	23	55

TABLE 21 Serum TAT Titres in 720 men who had routine MAR (IgG) tests (1980 to 1981)

Serum TAT	MAR (IgG) TEST	
	Negative/ Doubtful	Positive/ Strongly positive
Negative	94	7
Positive	4	64

TABLE 22 Serum TAT and MAR (IgG) Results (1980 to 1981)

As a result of these studies, MAR (IgG) testing was done as a routine therafter on all semen samples from male partners of infertile marriages referred to the author.

3.1.6 Serum and seminal plasma TAT, MAR (IgG & IGA (see 2.3.6) (Hendry et al, 1982a)

The MAR (IgA) test was applicable to all except one of the semen samples suitable for IgG testing, although the reaction with IgA was slower and less easy to read than the IgG reaction; furthermore, the spermatozoa tended to lose motility about 10 minutes after addition of the anti-IgA, and so the reading had to be completed by this time. In the one exceptional case, the spermatozoa were caught in large clumps, and a satisfactory conclusion could not be reached in the IgA test. The results of the MAR (IgA) test appeared to be reasonably consistent (Table 23).

FIRST MAR IgA TEST	REPEAT MAR IgA TEST	
	0/±	++/+++
0/±	17	2
++/+++	2	13

TABLE 23 Correlation between results of first and second MAR (IgA) test in 34 subfertile men

In 34 patients who had two or more tests, the first and second results were comparable in 30 instances (taking

negative and doubtful together and comparing them with positive and strongly positive, we found χ^2 = 22.61, p <0.001). The MAR (IgA) test were negative or doubtful in 42 (44%) of 94 samples with positive or strongly positive MAR tests for IgG (Table 24).

MAR (IgG)	MAR (IgA)	
	0/±	++/+++
0/±	8	2
++/+++	42	52

TABLE 24 Correlation between results of MAR tests for IgG and IgA in 104 samples from subfertile men

The results of the MAR (IgA) tests are shown correlated with the presence of seminal plasma antibodies, defined by TAT in Table 25a and by GAT in Table 25b.

SEMINAL PLASMA TAT	MAR IgA TEST 0/±	++/+++
Negative	20	3
Positive (titre ⩾4)	8	18

TABLE 25a Results of MAR (IgA) tests related to seminal plasma TAT of 49 patients

SEMINAL PLASMA GAT	MAR IgA TEST 0/±	++/+++
Negative	18	8
Positive (titre ⩾4)	9	16

TABLE 25b Results of MAR (IgA) tests related to seminal plasma GAT in 51 patients

There was a good correlation with the results of both tests, which was more significant with TAT (χ^2 = 15.732, P<0.001) than with GAT (χ^2 = 5.649, 0.02>P 0.01). This was largely due to the increase in the number of patients with positive MAR (IgA) tests who were found to have seminal plasma antibodies with the more sensitive TAT test.

The 20 patients who had sperm-cervical mucus penetration tests done are summarized in Tables 26a,b. It may be seen that good penetration of cervical mucus was only seen in the absence of antisperm antibodies from the seminal plasma and with a negative or dubious MAR (IgA) test. In contrast, good and poor penetration of mucus was seen with positive serum antibody tests and with positive MAR (IgG) tests. However, in three cases with dubious MAR (IgA) tests, penetration of cervical mucus was poor, and two of these patients had antisperm antibodies in seminal plasma. Overall, there was evidently a strong association between the results of the MAR (IgA) test and the ability of spermatozoa to penetrate cercical mucus. The probability of results shown in Table 26b having been obtained by chance is

calculated to be 1 in 277 by Fisher's exact method for 2 x 2 tables. On the other hand, there is evidently no correlation between the results of MAR (IgG) test and cervical mucus penetration.

SPERM/CERVICAL MUCUS PENETRATION* Husband	Donor	NUMBER OF PATIENTS	SERUM ANTIBODY TITRES GAT	SIT	TAT	MAR Test IgG 0 / ±	++ / +++
1-	2+	15	32 - 512	0 - 128	16 - 2048		15
2±	2+	5	16 - 128	0 - 8	8 - 128		5

TABLE 26a Results of SCMC tests comparing husbands' and fertile donors' spermatozoa, related to antisperm antibodies in serum and results of the MAR (IgG) test

SPERM/CERVICAL MUCUS PENETRATION* Husband	Donor	NUMBER OF PATIENTS	SEMINAL PLASMA ANTIBODIES (+ TITRES) GAT Positive	TAT Positive	MAR Test IgA 0 / ±	++ / +++
1-	2+	15	6/12 (4-16)	9/10 (4-256)		12
			2/3 (8-16)	2/3 (8-64)	3	
2±	2+	5	0/4	0/5	5	

TABLE 26b Results of SCMC tests comparing husbands' and fertile donors' spermatozoa related to antisperm antibodies in seminal plasma and results of the MAR (IgA) test

Unfortunately, the special serum (Avgh) containing

anti-D that was both IgA and IgG was in short supply, and this test was ultimately discontinued.

3.1.7 IBT (IgG and IgA) and MAR (IgG) (see 2.3.7) Rajah et al, 1992

Both MAR and IBT tests were possible in 103 of 109 patients; in the remainder azoospermia, severe oligozoospermia or asthenozoospermia prevented completion of the test. There was a highly significant correlation between MAR (IgG) and IBT(IgG) (Table 27) (χ^2 = 74.7; p <0.001). There was also a highly significant correlation between serum TAT and IBT (IgG) (χ^2 = 17.6; p <0.001). However, there were 5 men who had positive serum tests for ASA (mostly producing head-to-head agglutination) which were not associated with detectable surface bound antibody on their spermatozoa as shown by either MAR or IBT.

MAR (IgG)	IBT (IgG)		
	Negative	Positive	
Negative	73	2	75
Positive	4	24	28
	77	26	103

TABLE 27 Correlation between MAR (IgG) and IBT (IgG)

There was highly significant correlation between IBT (IgA) and seminal plasma TAT (χ^2 = 7.8; p<0.01) although there were 5 men who had positive IBT and negative TAT (Table 28). The correlation between IBT (IgG) and seminal plasma TAT was less good although it still reached statistical significance

(χ^2 = 4.2; p<0.05). There was no significant correlation between MAR (IgG) and seminal plasma TAT (χ^2 = 2.9; p 0.05).

The MAR test took 3 minutes to do, compared to the IBT which took 30 minutes on average. Reagents for MAR (IgG) cost approximately one-third of the cost of reagents for IBT (IgG and IgA).

Seminal Plasma TAT	IBT (IgA)		
	Negative	Positive	
Negative	7	5	12
Positive	1	13	14
	8	18	26

TABLE 28 Correlation between seminal plasma TAT and IBT (IgA)

3.1.8 Serum TAT and ELISA (see 2.3.8) (Stedronska-Clarke et al, 1987)

The optical density reading generated by the company positive control serum ranged from 0.90 to 0.98 OD with a negative control background of 0.04 to 0.10. Our patient sera known to be positive by TAT testing gave low-level reactivity (0.1-0.14) with positive (above background) readings usually requiring a 1/16 concentration. The titre given by ELISA was lower than that given by TAT even when the same criteria were used to define the end-point. These data are summarized in Table 29. We found that a number of TAT-negative control sera that had been provided by healthy men of normal fertility and seminal analysis nevertheless

gave a positive result in the ELISA. These data are summarized in Table 30. If one assumes that TAT to be the "gold standard" for detection of antisperm antibodies, then the ELISA used in this study gave a false-negative detection rate of 5/8 (63%) and a false-positive rate of 3/4 (75%).

Serum	TAT	ELISA
1	32	16
2	64	32
3	512	0,16
4	2048	16,32
5	2048	16,128

TABLE 29 Comparison of ASA titre by ELISA and TAT methods

TAT	ELISA	
	Positive	Negative
Positive	3	5
Negative	3	1

Table 30 Positive and Negative Test Results: TAT vs ELISA

As a result of this small study, no futher use was made of ELISA testing, and TAT continued to be our "gold standard".

3.2 MEDICAL TREATMENT

3.2.1 Comparison of effectiveness and side effects

The results described below were obtained in sequential studies done over 12 years. Long term low dose prednisolone (5mg TDS for 6-12 months) produced few side effects apart from some weight gain and a tendency to moon face. Although semen quality often improved strikingly, few pregnancies resulted. The intermittent high dose regimen described by Shulman (1976) appeared to offer better results, and indeed some spectacular successes were obtained; unfortunately one patient then developed aseptic necrosis of the hips and this treatment was abandoned. The moderate dose intermittent regimen was developed and introduced at Barts. in collaboration with Prof. Michael Besser. Side effects were few and transient, and results were as good as those obtained with Shulman's regime. It was therefore submitted to double blind prospective controlled trial comparing its effectiveness with placebo.

3.2.2 Continuous low-dose prednisolone (see 2.4.2) (Hendry et al, 1979

Group 1 (Patients with oligozoospermia) - Average sperm-counts and antisperm antibody titres before treatment are shown in table 31(a), and these may be compared with the results during long-term prednisolone treatment (table 31(b). Sperm counts rose to consistently normal levels in 10 (67%) of the 15 patients, and serum antisperm antibody levels declined, but never fell to less than 32. Two of the wives became pregnant (antibody titres 1024 and 128)

and 2 further wives became pregnant with AIH and sperm washing. In 1 patient with high titres of antibodies

(1024), the sperm count, which was less than 1 million per ml before treatment, rose to near normal levels on long term prednisolone, and then declined to less than 1 million per ml again when prednisolone was stopped (fig. 11).

GAT Titre	Average Initial Sperm Counts (millions/ml)			
	<5	6-10	11-20	>20
32	1			
64			1	
*128	(3)	(1)	2(1)	
256		1		
512	1			
≥1024	3			
Total	**8**	**2**	**5**	

TABLE 31(a) Average sperm counts and GAT titres in 15 oligozoospermic men before treatment with prednisolone (figures in brackets not assayed >128).

GAT Titre	Average Sperm Counts on Treatment (millions/ml)			
	<5	6-10	11-20	>20
32	1			2
64				2
*128	(1)	(2)	1	2(1)
256				1
512				1
≥1024				1
Total	**2**	**2**	**1**	**10**

TABLE 31(b) Average sperm counts and GAT tites in 15 oligozoospermic men during treatment (Figures in brackets not assayed >128).

Group 2 (Patients with normal sperm counts) - Sperm counts were well maintained in this group, and pronounced variation in seminal analysis in 6 cases became consistently normal. Antisperm antibody titres declined slightly but never dropped below 32. Two pregnancies were produced (antibody titres both 1 in 64); one further pregnancy occurred after AIH with sperm washing.

Histological examination of the 3 testicular biopsy specimens showed that spermatogenesis was proceeding satisfactorily in most of the tubules, although local areas of atrophy were also present (mean scores 6.7 to 8.45 out of 10). However, in 1 case there were localised lymphocytic and plasma-cell infiltrates adjacent to and extending into seminiferous tubules, which showed pronounced reduction of spermatogenesis; this patient had a very high GAT titre of 1024 (for details see section 3.5).

3.2.3 Cyclical high-dose methylprednisolone (MP) (see 2.4.3) (Hendry et al, 1981)

Twenty-eight patients completed three or more courses of MP, 11 received two courses, and 6 had only one course. Fourteen wives (31%) became pregnant in the cycle following treatment of the husband: 2 after one course, 6 after the second course, and 6 after the third or subsequent courses. Eleven pregnancies occurred after treatment was given from days 21 to 28, and 3 after treatment was given to 10 men from days 1 to 7 or later. One miscarriage occurred.

Sperm counts following treatment are shown in Table 32; counts of 20 million per millilitre or above were obtained by 39 (87%) of the 45 men, compared with 29 (64%) before treatment.

	AVERAGE SPERM COUNTS (millions per ml)				
	0 - 10	11 - 20	21 - 40	>41	Unknown
Before treatment	14	4	4	25	
After treatment	5		9	30	1

TABLE 32 Distribution of average sperm counts of 45 patients before and after treatment with Methylprednisolone

The GAT results before and after treatment are shown in Fig. 12: there is no evidence that the titres fell more profoundly in men whose wives became pregnant, compared with those who did not. The SIT titres in 33 men tested in one laboratory (CHW) before and after treatment are shown in Fig. 13; production of pregnancy appeared to be associated with a fall in sperm immobilization titres to zero or near zero; however, a similar fall was obtained in many men whose wives did not become pregnant. Seminal plasma antibody testing was introduced in the latter part of this study, and it is difficult to draw firm conclusions as yet; however, serial testing showed that the steroid treatment caused the antisperm antibodies to disappear from seminal plasma in six patients, two of whose wives became pregnant (Table 33).

	Seminal plasma antibodies present (GAT)	
	Before treatment	After treatment
Wife pregnant	4 / 7	2 / 7
Wife not pregnant	16 / 24	12 / 25

TABLE 33 Relationship between presence or absence of seminal plasma antibodies (by GAT) before and after treatment, and occurrence of pregnancy in the spouse.

An example of serial sperm counts, MAR test results, and antibody levels in serum and seminal plasma is shown in Table 34 in one patient who received MP from days 1 to 7 in three successive cycles. The rise in sperm counts and fall in serum GAT and SIT titres that accompanied MP therapy can be observed; the MAR test for IgA became negative as the seminal plasma antibodies disappeared; however, the MAR test for IgG stayed positive.

TREATMENT	DATE	SPERM COUNT		MAR TEST		ANTISPERM ANTIBODIES			
						SERUM		SEMINAL PLASMA	
		m/ml	% motility	IgG	IgA	GAT	SIT	GAT	SIT
	30/10/79	4	25	+++					
	29/11/79	11	25						
	8/1/80	3	50	+++		128	8	16	-
M.P.* ①									
	14/3/80	54	40	+++	+	256	8	8	-
M.P. ②									
	10/4/80	66	40	+++	-	32	1	-	-
M.P. ③									
	20/5/80	145	40	+++	-	16	-	-	-

TABLE 34 Serial counts, MAR test and ASA titres following three courses of MP given in successive months from day 1 to day 7 of the wife's menstrual cycle.

Table 35 shows serial sperm counts and MAR test (IgG) results for a period of 3 weeks before and after the third course of MP in a man whose initial serum GAT titre was 256 and had fallen to 16 prior to this treatment. The MAR test became negative on the third post treatment day at which time good sperm penetration of donor cervical mucus was observed.

TIME IN RELATION TO 7 - DAY COURSE OF M.P.*	SPERM COUNT m/ml	% Motility	MAR (IgG)
Before	16	40	+++
After:			
Day 1	171	50	+
3	43	50	-
7	33	50	+
9	24	40	+
15	36	50	+
20	65	50	+

TABLE 35 Serial sperm counts and MAR (IgG) test results for a period of 3 weeks before and after a 7 day course of MP.

Altogether 54 patients were treated with MP (including 9 patients described previously who had one course of MP that was not synchronized with the wife's cycle (Hendry et al, 1979)). Three (6%) experienced such severe side effects that treatment was discontinued; one had marked dyspepsia, and treatment was suspended until he had had a course of cimetidine; he subsequently tolerated two further courses of MP, taking one tablet of cimetidine 1 hour before each dose of MP. One patient had a small hematemesis, and no further treatment was given. One patient developed transient pain in the hips lasting for a few days and refused further treatment. In addition, 14 (26%) other patients experienced less severe side effects: transient pain in the hips (3), dyspepsia (1), headaches (1), flashing lights (2), tinitus (1), aggressive behaviour usually directed against the wife (3), or marked blotchy face and acne (3). In general, men

who were fit and worked in physically demanding occupations suffered fewer side effects and responded less well than men who were unfit and had sedentary occupations.

One year after treatment one patient developed bilateral aseptic necrosis of the hips. This treatment was therefore abandoned, and this complication was documented in the world literature (Hendry, 1982).

3.2.4 Cyclical moderate dose prednisolone (see 2.4.4) (Hendry et al, 1986)

Of the 76 men treated, 25 (33%) reported that their partners became pregnant during a treatment cycle. A life-table analysis of monthly conceptions is shown in Table 36; it takes into account 20 patients who left the study before completion of nine courses of treatment and 2 men who had not completed treatment at the time of analysis. The cumulative pregnancy rate is compared with that of the normal population and that of a group of unselected subfertile men in Figure 14. There is a linear relationship between cycles of therapy and cumulative pregnancy rate. One woman became pregnant at 12 months, but no further pregnancies were recorded after additional treatment therafter.

We compared the successful group and the remainder. The age ranges of the two groups were similar. There was a preponderance of successful patients among those who had been trying to produce a pregnancy for less than 2 years (fig. 15). Serum and seminal plasma TAT titres before and after treatment are shown in Figures 16,17, respectively; there were no significant differences between successful and unsuccessful patients in either absolute levels or change in

antibody titres. The results of seminal analysis before and after treatment were available for 50 men: there were no

Number of Cycles	Number of Patients	Number of Pregnancies	Lost to Follow up	Pregnancy Rate/Month	Cumulative Probability of Pregnancy
1	76	4		0.054	0.054
2	72	4		0.057	0.108
3	68	5	3	0.078	0.178
4	60	2	3	0.034	0.206
5	55	0	1		0.206
6	54	4	9	0.084	0.273
7	41	2	2	0.051	0.310
8	37	1	4	0.029	0.330
9	32	2		0.065	0.374

TABLE 36 Cumulative pregnancy rate

significant differences in sperm counts or motility before treatment or in sperm counts after treatment. However, sperm motility after treatment was significantly better in the successful group, compared with the unsuccessful group ($\chi^2 = 7.9$; $P < 0.02$). Abnormalities of ovulation required treatment in 11 (44%) of 25 women who eventually conceived, compared with 18 (35%) of 51 who did not.

No major complications occurred with this regimen. However, 40 patients (53%) reported transient side effects, including folliculitis (18), weight gain (10), headache (10), sweats or flushes (8), dyspepsia (7), musculoskeletal pain (7), and/or mood changes (10). There was no difference in the incidence of side effects between successfully and unsuccessfully treated patients.

Since this regimen appeared to be equally as effective as the high dose MP regimen (3.2.3) but better tolerated and free of major complications, it was submitted to double blind prospective controlled trial against placebo.

3.2.5 Controlled clinical trial of cyclical moderate dose prednisolone versus placebo (see 2.4.5) (Hendry et al, 1990a)

Twenty seven patients completed the full 18 months of treatment or the partner conceived and treatment was stopped. Six completed only 9 months of treatment and then declined to continue (4 prednisolone, 2 placebo). Four had to stop treatment owing to the severity of side-effects (1 prednisolone, 3 placebo), and 6 dropped out of the trial after taking fewer than nine courses of treatment (3 prednisolone, 3 placebo).

Nine pregnancies occurred during prednisolone treatment (1 on the higher dose), 1 during placebo treatment, and 1 was produced by a man allocated placebo but who had not started treatment. Analysis of results by treatment allocated with no exclusions gave significantly better results with prednisolone (pregnancies 9 of 33 allocated prednisolone versus 2 of 27 allocated placebo; $p < 0.05$, Fisher's exact test). Analysis by treatment received also showed a significant benefit from prednisolone (pregnancies in 9 of 29 who received prednisolone versus 1 of 20 who received placebo; $p < 0.05$, Fisher's exact test). The results are plotted against time in Fig. 18. Three pregnancies occurred among 16 couples who had tried for less than 3 years, and 8 among the 27 who had tried for 3 or more years.

There was no significant effects of prednisolone or placebo on seminal analysis (see table 37). There was no consistent change in levels of antibody to sperm in serum on placebo or prednisolone. In contrast, there was a significant fall in antibody levels in seminal plasma after prednisolone treatment ($p<0.05$, Wilcoxon's signed rank sum test), though not after placebo (Fig. 19). In most of the men who produced pregnancies seminal plasma antibody disappeared.

Seminal analysis	SPERM COUNT (m/ml)			MOTILITY (%)		
	0–10	11–20	20+	⩽10	20–30	40+
Before	7	9	26	7	15	19
Placebo	1	11	11	5	6	11
Prednisolone	2	9	17	2	9	17

TABLE 37 Distribution of seminal analyses before and after prednisolone and placebo treatment

Mild side-effects, including acne, weight gain, dyspepsia, and irritability, were experienced by 18 (60%) of 30 patients taking prednisolone and by 5 (19%) of 26 taking placebo.

Serious side-effects necessitating withdrawal from the trial occurred in 3 patients taking placebo; there was 1 case each of tachycardia with hypertension, irritability, and indigestion, and diminished libido with impotence. Only 1 patient taking prednisolone had side-effects necessitating withdrawal-glaucoma with migraine and irritability. In general, however, the treatment was well tolerated; irritability caused the most trouble, leading to strain in the marital relationship in some couples.

3.3 SURGICAL TREATMENT OF INFERTILE MALES

3.3.1 Morbidity and complications

Virtually all the patients described in this section were operated on by the author and followed up personally as far as possible. With modern anaesthetic techniques post operative discomfort is minimised and most go home the same

day. Complications were rare, largely due to obsessional care about haemostasis within the scrotum. Wound healing was usually completed within 7 days, and the use of subcutaneous closure of the dartos layer and subcuticular skin closure with Vicryl meant that there were no stitches to be removed. Wound infection was exceptionally rare.

3.3.2 Unilateral testicular obstruction - diagnosis (see 2.5.2) (Hendry et al, 1982a)

The details of the sites of obstruction, sperm counts, antisperm antibody results and treatments for the 32 patients are given in Table 38.

Clinical findings

The past medical history gave useful and relevant information in 27 cases (84%), but the findings at operation were not always as expected from the history. In 7 patients who had had a hernia repair, the obstruction was found in the groin in 5, where the vas was caught in fibrosis or had been divided; however, in one case epididymal obstruction was found and the vas was patent, while in another case there was no evidence of obstruction on exploration. In another example of post-operative occlusion, the vas had been divided just inside the internal inguinal ring at laparotomy in childhood. Four patients had had large parts of their epididymes excised during previous removal of epididymal cysts. Four others had suffered severe injuries - one in a fall-astride rupture of urethra, one having stood on a mine, both being complicated by recurrent urinary infections; the third patient had damaged one testicle in a building site injury, and the fourth during army exercises. Six patients admitted to previous venereal disease, 2 with

epididymitis. One had had a strange tropical infection, possibly sand-fly fever. Three had had epididymovasostomies in the past.

In 3 cases with no relevant past history, useful observations were made on clinical examination. Unilateral absence of the vas was found in 2 cases. The tail of the epididymis in one man was so distended that he had noticed it himself and pointed it out. Similar distension was noted in several other patients. Unilateral obstruction was an unsuspected finding at scrotal exploration, done at the time of testicular biopsy, in 2 cases with inexplicably low sperm counts.

Seminal analysis

The average pre-treatment sperm counts were more than 20 million per ml in 15, between 10 and 20 million per ml in 2, and less than 10 million per ml in 15 cases. Significant abnormalities were present in the contralateral, non-obstructed testes in 5 of the patients with counts of less than 10 million per ml - 3 had had orchiopexies and 2 had large varicoceles; correction of the obstruction produced normal sperm counts and pregnancies in the wives of 3 of these men. In 7 of these severely oligozoospermic cases the cause of the low sperm count was unclear since spermatogenesis in the unobstructed testis was normal.

PATIENTS	SITE OF OBSTRUCTION	SPERM COUNT M/ML	ANTISPERM ANTIBODIES TAT TITRE	MODE OF AGGLUTINATION
1	Hernia repair	38	128	HH
2	Epididymis-tail	1	128	HH
3	Previous ep-vas	8	128	HH
4	Epididymis & vas	4	64	HH
5*	Epididymis-tail	3	16	HH
6*	Epididymis-tail	4	16	HH
7*	Epispadias repair	97	16	HH
8	Epidiymis-tail	18	2048	HH/TT
9	Absent vas	38	512	HH/TT
10	Hernia repair	46	512	HH/TT
11	Epididymis-tail	30	512	HH/TT
12	Epididymis-tail	47	512	HH/TT
13	Previous ep-vas	2	265	HH/TT
14	Ruptured urethra	28	128	HH/TT
15	Ejaculatory duct	<1	64	HH/TT
16	Epididymis-tail	1	64	HH/TT
17	Previous ep-vas	89	64	HH/TT
18	Previous ep-cyst	22	64	HH/TT
19	Ejaculatory duct	23	64	HH/TT
20	Hernia repair	105	32	HH/TT
21	Hernia repair	15	8	HH/TT
22*	Previous laparotomy	4	512	TT
23	Hernia repair	1	64	TT
24	Absent vas	35	64	TT
25	Previous ep-cyst	29	32	TT
26	Previous ep-cyst	44	16	TT
27	Epididymis-tail	29	0	--
28	Epididymis-tail	2	0	--
29*	Epididymis-tail	<1	0	--
30*	Epididymis-tail	2	0	--
31	Epididymis-head	0.8	0	--
32	Previous ep-cyst	1.5	0	--

* Wives became pregnant

TABLE 38 Details of 32 patients with unilateral testicular obstruction

Antisperm antibodies

Twenty six (81%) of the 32 patients had antisperm antibodies detectable in serum - the titres are shown in Table 38. Twenty-one (81%) of the 26 men with antibodies showed evidence of HH agglutination in pure or mixed form. This incidence in unilaterally obstructed males is significantly higher than that found in 162 bilaterally obstructed (vasectomised) males ($\chi^2 = 6.16$; $P<0.02$) and both groups showed evidence of HH agglutination highly significantly more often than did the 160 naturally subfertile males ($P<0.001$, see Table 39).

Group	Number of Patients	Serum TAT: Number (and per cent) with HH or Mixed Agglutination
Unilaterally obstructed males	26	21 (81%)
Bilaterally obstructed (vasectomised) males	162	89 (55%)
Spontaneously Infertile males	160	38 (24%)

TABLE 39 Differing incidence of HH or mixed agglutination in subfertile males according to presence or absence of testicular obstruction

In retrospective analysis of the 160 unselected naturally infertile males (Table 40) definite evidence of obstruction (surgically proven, unilateral or bilateral) was

found highly significantly more often in men with evidence of HH agglutination in pure or mixed form (37%) than in those with TT agglutination (10%) (χ^2 = 15.53; $P<0.001$). When all cases with a past history of hernia repair, testicular operation or injury, epididymo-orchitis or other significant infection, or with epididymal cysts on examination - any of whom might have had obstruction - are included, the incidence of definite or possible obstruction is still significantly higher in those with HH or mixed agglutination (50%) than in those with TT agglutination (32%) (χ^2 = 4.077; $P<0.05$).

Four patients with pure HH agglutination on TAT had evidence of other autoimmune diseases with antibodies that may have been cross-reacting with the spermatozoa. One had diabetes with islet cell antibodies, one had thyrotoxicosis with thyroid microsomal and thyroglobulin antibodies, and one had an unusual painless parotid swelling with gastric parietal cell antibodies. The other patient, with thyroid microsomal antibodies, underwent scrotal exploration - there was no evidence of obstruction, but testicular biopsy showed focal round cell infiltration of seminiferous tubules.

Overall, it appeared that a cause for the antisperm antibodies could be defined in 47% of the 38 patients with HH agglutination, whereas the cause remained obscure in 90% of those with TT agglutination. In 160 naturally infertile males with antisperm antibodies, unilateral testicular obstruction was demonstrated surgically in 12 cases (7.5%), but could have been present in a further 32 (20%).

Mode of Agglutination	Definite Testicular Obstruction		Possible Testicular Obstruction			
				Previous		
	Bilateral	Unilateral	Epididymal Cyst	Hernia Repair	Epididymo Orchitis	Injury, Operation, Infection
HH/mixed (38 cases)	7	7 (37%)		1	2	2
TT/TT-TT (122 cases)	7	5 (10%)	2	6	10	9

TABLE 40 Incidence of definite and possible testicular obstruction in 160 spontaneously subfertile males with ASA related to mode of agglutination in TAT

3.3.3 Unilateral testicular obstruction - results of treatment (see 2.5.3) (Hendry, 1986)

The commonest site for unilateral obstruction was the tail of the epididymis, which usually followed previous genital or urinary infection. The findings at scrotal exploration were clear cut and easy to recognise (Fig. 20). The next most common group was vasal blocks usually due to previous groin surgery, but sometimes post infective (Fig. 21). The sites of obstruction and probable causes so far as could be ascertained are shown in Table 41.

	Post-infective	Surgery/ injury	Congenital	Cause unknown
Upper epididymis		5		6
Tail of epididymis	25	6		9
Vas deferens	8	12	4	
Ejaculatory duct	1	2	1	1
Total	**34**	**25**	**5**	**16**

TABLE 41 Sites and probable causes of unilateral obstruction in 80 subfertile males

The surgical methods of reconstruction used are listed in Table 42. In 10 patients with irreparable blocks the obstructed testis was removed and replaced with a prosthesis, after full discussion with the patient and his female partner.

Site	Surgical treatment			
	Ep-vas	Vas-vas	Orchidectomy	None
Upper epididymis	5			6
Tail of epididymis	34		1	5
Vas diferens		9	9	6
Ejaculatory duct				5
Total	**39**	**9**	**10**	**22**

TABLE 42 Surgical treatment in 80 subfertile males with unilateral testicular obstruction

Seminal analysis, serum FSH and testicular biopsies

The average pre-treatment sperm counts are shown in Table 43. Forty patients (50%) presented with severe oligozoospermia (<5 million/ml) and in 22 (27.5%) the initial sperm count was less than 1 million/ml. The serum FSH levels were normal in all except one of these patients and testicular biopsies showed adequate spermatogenesis (Johnsen score-count 8.0 or more) in the obstructed testis in all patients except for the one with the elevated serum FSH, whose score-count was only 5.9. Biopsy of the contralateral testis was done in only two cases and both were normal at 9.1 and 9.2.

Moderate oligozoospermia was seen in 19 patients and testicular biopsies showed adequate spermatogenesis in all except two, who had Johnsen counts of 7.8 and 5.9. Normal sperm counts of 20 million/ml or more were recorded in only 21 patients, all of whom had normal testicular biopsies.

Serum TAT	N	Initial sperm count M/ml		
		⩽5	6–20	>20
0	19	13	5	1
4	3	1	1	1
8				
16	8	5	1	2
32	12	5	3	4
64	11	6	1	4
128	6	3	2	1
256	4	1	2	1
512	6	3		3
⩽1024	11	3	4	4
Total	80	40	19	21

TABLE 43 Distribution of average initial sperm counts (million/ml) related to serum ASA titres

Antisperm antibodies

The serum TAT results are also shown in Table 43. It may be seen that there is no correlation between antisperm antibody titres and average initial sperm counts. Altogether, 61 patients (76%) had antisperm antibodies, amongst whom 17 (21%) had very high titres of 512 or more. The mode of agglutination was recorded in 54 patients, amongst whom head-to-head (H-H) agglutination was observed in pure or mixed form in 36 (66%). The relationship between serum and seminal plasma antibody titres is shown in Fig. 22: 22 of 34 patients studied (65%) had antibodies in seminal plasma, often in very high titres.

Response to treatment

Analysis of the pregnancies produced by the 60 patients with adequate follow-up is shown in Tables 44 a,b,c. Table 44a shows that the highest success rate was observed amongst those starting with the lowest sperm count:

12 of 30 (40%) with initial sperm counts ≤ 5 million/ml, including eight who started with counts <1 million/ml. Vaso-vasostomy appeared to be the most satisfactory surgical procedure, although it should be noted that four pregnancies were produced with prednisolone treatment even though the testicular obstruction was unrelieved. Most of the pregnancies produced were associated with improvement in the sperm output after treatment (Table 44b). The pregnancy rate was not influenced by the level of antisperm antibodies until very high titres were reached (≥1024) (Table 44c), however, it should be remembered that many of these patients received prednisolone treatment for the antisperm antibodies.

Surgical treatment	Initial sperm count M/ml			Totals
	≤5	6–20	>20	
Ep-vas	7/18	1/10	2/5	10/33 (30%)
Vas-vas	2/5		1/1	3/6 (50%)
Orchidectomy	1/2	0/2	1/6	2/10 (20%)
None (steroids)	2/5	1/2	1/4	4/11 (36%)
Totals	**12/30 (40%)**	**2/14 (14%)**	**5/16 (31%)**	**19/60 (32%)**

TABLE 44a Pregnancies produced/number treated in 60 patients with adequate follow-up related to initial sperm counts and surgical treatment

Post treatment sperm count M/ml	Initial sperm count M/ml		
	⩽5	6–20	>20
Not known	2/2	1/1	1/1
⩽5	2/13	0/1	0/1
6–20	6/10	0/4	0/1
>20	2/5	1/8	4/13

TABLE 44b Pre-treatment and post-treatment average sperm count results

Post treatment sperm count M/ml	Serum TAT		
	⩽32	64–516	⩾1024
Not known	4/4	–	–
⩽5	1/9	1/6	–
6–20	2/6	3/4	1/5
>20	3/11	4/10	0/5
Totals	10/30 (30%)	8/20 (40%)	1/10 (10%)

TABLE 44c Post-treatment sperm counts and serum ASA titres (many patients received prednisolone therapy)

Orchiectomy

In 10 patients with high titres of antisperm antibodies and incorrectable obstruction or unilateral absence of the vas, the obstructed testis was removed and replaced with a prosthesis. Histological examination of

these testes revealed focal mononuclear cell infiltrates and sperm granulomas in the epididymis, and sometimes amongst the seminiferous tubules (see Section 3.5).

After orchiectomy, striking falls were observed in serum and seminal plasma ASA titres which continued to fall with subsequent prednisolone therapy (Figs. 23 & 24). Two men subsequently produced pregnancies.

3.3.4 Bilateral testicular obstruction - incidence of ASA (see 2.5.4) (Hendry et al, 1983)

Many of the results of this study were extended and are described in more detail in the next section. However, two statistically significant observations were made in this study which were subsequently shown to be important. The patients are shown grouped according to the site of obstruction in Fig. 25. This is related to their domicile in Table 45. It may be seen

Site of Obstruction	Number of Patients	Number (%) Coming from Abroad
Empty epididymes	25	9 (36%)
Caput epididymis	48	6 (12.5%)
Cauda epididymis	34	19 (56%)
Absent vas		
- bilateral	29	8 (28%)
- unilateral	8	2 (25%)
Blocked vas	23	12 (52%)
Ejaculatory duct	1	
Total	**168**	**56 (33%)**

TABLE 45 Site of bilateral testicular obstruction related to domicile

that over half (56%) of the patients with blocks at the cauda epididymis came from abroad, whereas most (87.5%) of those with spermatozoa confined to the caput epididymis lived in the British Isles. This difference is highly significant (χ^2 = 17.67; P<0.001) and will be considered further in the next section.

Serum antisperm antibodies

The number of patients in each category with positive serum antisperm antibody tests is shown in Table 46.

Group	Number of Cases	Serum TAT Positive (%)
Empty epididymes (impaired spermatogenesis)	22	3 (13.6)
Bilateral absent vasa	29	6 (20.7)
Caput epididymis	48	14 (29.2)
	99	23 (23.2)
Empty epididymes (normal spermatogenesis)	3	3
Unilateral absent vas	8	4 (50)
Cauda epididymis	34	18 (52.9)
Vasal block	23	10 (43.5)
Ejaculatory duct	1	
	69	35 (50.7)

TABLE 46 Incidence of serum ASA related to site of obstruction in men with bilateral testicular obstruction

Statistical analysis showed that ASA occurred significantly more often with blocks at the cauda epididymis (52.9%) than with those at the caput epididymis (29.2%) (χ^2 = 4.7; P<0.05) and also significantly more often than in men with congenital bilateral absent vasa (20.7%) (χ^2 = 6.9, P <0.01).

3.3.5 Bilateral testicular obstruction - results of surgery and ASA (see Section 2.5.5) (Hendry et al, 1990b)

The operative findings in 370 cases are shown in Fig. 26. Epididymovasostomy gave good results with postinfective caudal blocks (patency 52%, pregnancy 38%), while postinfective vasal blocks were better corrected by total anatomical reconstruction (patency 73%. pregnancy 27%) than by transvasovasostomy (patency 9%, no pregnancies). Poor results were obtained with capital blocks (patency 12%, pregnancy 3%), in which substantial lipid accumulation was demonstrated in the ductuli efferentes; three-quarters of these patients had sinusitis, bronchitis or bronchiectasis (Young's syndrome). There is circumstantial evidence to suggest that this syndrome may be a late complication of mercury intoxication in childhood (Pink Disease).

The incidence of ASA in the various groups is shown in Table 47. This confirmed that ASA occurred significantly more often with caudal than with capital epididymal blocks, and with blocked compared to absent vasa. Eighty-two (77.4%) of 106 patients with capital epididymal blocks had chronic sinusitis, bronchitis or bronchiectasis (Young's syndrome) whereas only 9 (8.5%) had a past history of genital infection. In contrast, a history of epididymitis, urethritis or smallpox was given by 38 (54.3%) of 70 with

caudal epididymal blocks, and only 1 had chronic bronchitis (different from those with capital blocks, χ^2 = 87.2, p <0.001). The most common cause of vasal blocks was previous genital or urinary infection (16 cases) and there was a history of tuberculosis in 7, of which 3 were active (significantly different from capital blocks (χ^2 = 69.8, p <0.001; no significant difference from caudal epididymal blocks). It can be concluded from the data on Table 47 that postinflammatory blocks are most likely to be associated with development of ASA.

Site of problem	Number	Serum GAT or TAT positive (%)
Epididymis		
Caput	106	29 (27.4)
Cauda	69	36 (52.2)
Vas deferens		
Absent – bilateral	67	11 (16.4)
– unilateral	19	6 (31.6)
Blocked	40	19 (47.5)
Ejaculatory duct	14	1 (7.1)
Totals	369	112 (30.4)

TABLE 47 Incidence of serum ASA in 369 men with obstructive azoospermia related to site of obstruction

The relationship between presence or absence of ASA and production of pregnancy by 66 men whose obstruction was corrected (as evidenced by postoperative sperm counts of 10 million per ml or more) is shown in Table 48. It can be seen that pregnancy is significantly more likely to occur if the man has not produced ASA (by Fishers Exact Test p<0.05).

Pregnancy produced	Serum GAT or TAT Positive	Negative	
Yes	11	20	31
No	22	13	35
	33	33	66

TABLE 48 Relationship between serum ASA and production of pregnancy after correction of obstructive azoospermia (sperm counts ≥ 10 million per ml)

Unfortunately, the groups most suitable for surgical correction (postinflammatory caudal epididymal and vasal blocks) are those most likely to be associated with ASA production.

3.3.6 Genital tract injuries in childhood (see 2.5.6) (Parkhouse & Hendry, 1991)

The 17 patients with unilateral testicular obstruction had sperm counts ranging from 1 to 89 x 10^6/ ml, with 11 having counts below 20 x 10^6/ ml. All had serum antibodies to spermatozoa, with titres 1:128 in 12 cases (70%). The 13 patients with bilateral testicular obstruction all had azoospermia, but only 6 (46%) had serum antibodies and none of the 6 had antibody titres >128. Antibody titres of the patients with bilateral obstruction were compared with the antibody titres of patients with unilateral obstruction (Fig. 27) and the difference between the 2 groups was significant at the 1% level (Wilcoxon's rank sum test).

Following corrective surgery and prednisolone treatment for the antibodies, where appropriate, 5/17 patients with unilateral blocks and 1/11 with bilateral blocks successfully produced pregnancies.

3.4.1 Vasectomy reversal: laboratory results (see 2.6.1) (Royle et al, 1981; Parslow et al, 1983; Hendry et al 1983b)

The interval between operation and follow-up was over one year in all 130 patients. Antisperm antibody results have been analyzed for all patients, but occurrence of pregnancies in their partners has been studied only in those who were actively trying to produce a pregnancy.

The results of TAT and GAT in serum coincided within 2 titres in 222 (92%) of 242 tests. In 20, the TAT was 2 or more titres higher than GAT: in 17 of these cases, there was a significant element of head-to-head agglutination, which is only detected by TAT testing. Since head-to-head agglutination is fairly common after vasectomy, though rare in naturally subfertile males, the results of the TAT have been used in this analysis.

The antisperm antibody results in serum and seminal plasma before vasectomy reversal are shown in Table 49. A positive result was obtained in the sera of 95 (79%) of 121 men tested. Seminal plasma antibodies were found in only 8 (9.5%) of 85 men tested, all of whom had serum titres of 512 or more. Overall, 41 (34%) of 121 men tested had serum titres of 512 or more. The corresponding figures 3 months or more after reversal are also shown in Table 49: 78 patients had seminal plasma tested, of whom 23 (29.5%) had antibodies, which appeared at serum titres of 64 and higher.

Serum TAT Titre	Number of Patients	Seminal Plasma TAT Titre						
		No. tested	0	4	8	16	32	≥64
Not done	9	4	4					
0	26	15	15					
4	5	2	2					
8	5	4	4					
16	4	2	2					
32	7	7	7					
64	11	9	9					
128	6	3	3					
256	16	11	11					
512	23	15	12	1	1	1		
≥1024	18	13	8	2	1	1	1	
				3	2	2	1	
Total	130	85	77	8/85 (9.5%)				
Not done	15	12	11			1		
0	8	5	5					
4	2	2	2					
8	6	5	5					
16	5	5	5					
32	4	4	4					
64	12	10	9		1			
128	11	10	5		3		1	1
256	10	8	4		2	1		1
512	9	7	2	1	2	1	1	
≥1024	12	10	3	1	1	4		1
				2	9	7	2	3
Total	130	78	55	23/78 (29.5%)				

Before Reversal

After Reversal

TABLE 49 Semen and seminal plasma TAT titres in 130 men (a) before and (b) 3 months after vasectomy reversal

These results are shown graphically in Fig. 28, and compared with the findings in 146 unselected spontaneously infertile males studied at the same time.

3.4.2 Fertility after vasectomy reversal (see 2.6.1) (Parslow et al, 1983; Royle et al, 1981)

Ninety patients were operated upon by Mr. Michael Royle in Brighton, and 40 were done at St. Bartholomew's Hospital in London. The results of follow up sperm counts and pregnancies in the partners of those wanting children are shown in Table 50(a). Excluded are those lost to follow up. The pregnancy rates for the 2 hospitals were similar at 44% and 45%.

	Total no.	Information available	SPERM COUNT			? PREGNANCY	
			0	< 20	⩾ 20 m/ml	Trying	Success
Brighton	90	69	4	24	41	63	28 (44%)
Barts	40	35	3	9	23	29	13 (45%)

TABLE 50(a) Sperm counts and incidence of pregnancies (a) related to hospital

There was no benefit from per-operative steroids. Pregnancy rates of 52% and 53% were achieved by those men with preoperative serum titres in the range 0 to 16 and 32 to 256, respectively (Table 50b). Significantly fewer pregnancies (25%) were produced once the serum titre reached 512 or more (Table 51) ($\chi^2 = 5.88$; $p<0.02$).

Serum TAT titre	Total no.	Information available	SPERM COUNT			? PREGNANCY	
			0	< 20	⩾ 20 m/ml	Trying	Success
⩽ 16	40	32	5	7	20	29	15 (52%)
32–256	40	36	1	14	21	32	17 (53%)
⩾ 512	41	31	2	9	20	28	7 (25%)

TABLE 50(b) Related to serum TAT titres

Serum TAT titre	Pregnancy	
	No	Yes
0 – 256	29	32
512 – 1024+	21	7

TABLE 51 Serum TAT titres and pregnancy rates

The number of patients with seminal plasma antibodies is relatively small, but it can be seen from Table 52 that the pregnancy rate remained at 44% with seminal plasma antibody titres of up to 16. Only one pregnancy was produced by the 4 men with titres of 32 or more.

Seminal plasma TAT titre	Total no.	Information available	SPERM COUNT			? PREGNANCY	
			0	< 20	⩾ 20 m/ml	Trying	Success
0	55	51	3	15	33	45	20 (44%)
4	2	2		1	1	2	1
8	9	8	1	2	5	7	3 (44%)
16	7	7		4	3	7	3
⩾ 32	5	5	1	2	2	4	1 (25%)

TABLE 52 Sperm counts and incidence of pregnancies related to seminal plasma TAT titres

A few patients exhibited an unusual course, and 2 are worthy of brief description. The first (Fig. 29) had no detectable antibodies before or immediately after reversal without steroids, and a good sperm count was obtained: however, antisperm antibodies then appeared in both serum and seminal plasma, and as the titre rose, so the sperm count fell, to nearly zero, and temporary and incomplete correction of this fall was obtained with a 6-month course of prednisolone (5mg three times daily). The second patient (Fig. 30) started with a serum titre of 512 and a seminal plasma titre of 8: after reversal without steroids, a good count was obtained, the serum antibodies dropped, the seminal plasma antibodies disappeared, and the wife twice became pregnant.

3.4.3 Findings in failed vasectomy reversal (see 2.6.2) (Royle & Hendry, 1985)

The findings in 23 patients who were azoospermic prior to reconstructive surgery are shown in Table 53(a). In the first category were 12 patients in whom the anastomosis of the vasa had become blocked. Following repeat vaso-vasostomy by the technique described, spermatozoa were restored to the ejaculate in 10 patients: the wife of a further patient became pregnant before a sperm count had been done. Of eight patients followed up for 6 months or more, five (62%) impregnated their female partners.

In the second category (patients with epididymal blocks which were treated by epididymo-vasostomy), three of four patients regained satisfactory sperm counts and two wives became pregnant.

In the third group, comprising patients with very high antisperm antibody titres of 1024 or more, appropriate surgery and prednisolone treatment led to reasonable sperm counts in six patients (⩾20 m/ml in 3), but none of the wives became pregnant.

The overall pregnancy rate for this group was 37%.

Category	Treatment	No.	Follow-up sperm counts			Pregnancies*
			0	<20	⩾20	
1 Blocked vas	Redo vas-vas	12	1	3	7	5/8 (62%)
2 Epididymal block	Ep-vas	4	1		3	2/4 (50%)
3 Antisperm antibodies (⩾1024)	Surgery + prednisolone	7	1	3	3	0/7

TABLE 53 (a) Results obtained by reoperation after previous failed attempt at vasectomy reversal

Nine men with persistently poor sperm counts following previous vasectomy reversal were explored and the results are shown in Table 53(b). A blockage was found in the vas on one side, although at least partial patency had obviously been restored on the other side. The cases fell into the same categories as before, except that in eight of the nine cases only one side remained blocked. Corrective surgery resulted in improved sperm counts and one pregnancy has been obtained. In this particular case the patient had a rather poor testicle on one side which had been successfully reconnected, but was putting out less than 1 million spermatozoa per ml. Following reconnection of the contralateral normal testicle, the sperm count returned to normal and his wife has recently been delivered of a normal baby.

Category	Treatment	No.	Follow-up sperm counts		
			0	<20	⩾20
1 Blocked vas	Redo vas-vas	5		1	4 *
2 Epididymal block	Ep-vas	2		2	
3 Antisperm antibodies (⩾1024)	Surgery + prednisolone	2		1	
4 Testicular failure	None	½			

TABLE 53(b) In 9 oligozoospermic patients

3.5 AUTO-IMMUNE ORCHITIS IN SUBFERTILE MALES (see 2.7) (Hendry et al, 1979; Hendry, 1987; Hendry et al, 1990b)

3.5.1 Histological findings

Focal infiltration of lymphocytes and plasma-cells extending into the inside of a seminiferous tubule (Fig. 31) (i.e. beyond the blood-testis barrier) in a man with severe oligozoospermia and high serum titre of ASA (>1024) was first noticed by Dr. R.C.B. Pugh, and reported and illustrated in Hendry et al (1979). In discussion, the possibility was raised that this might be a manifestation of autoimmune orchitis. Subsequent observations indicated that this finding was very rare, extremely patchy in distribution, and sometimes seen in men without ASA. It could not, therefore, be regarded as diagnostic of autoimmune orchitis. Furthermore, clinical observation indicated that severely oligozoospermic or even azoospermic men with high titres of ASA (≥512) could respond well to prednisolone with normalisation of sperm concentration without this change on testicular biopsy (see below 3.5.2).

Studies of the testis and epididymis in 10 men with unilateral uncorrectable obstruction or absence of the vas showed patchy mononuclear cell infiltration of epididymal tubules (Fig. 32) and in one case, prominent infiltration of rete testis (Fig. 33). These changes were recorded by Dr. M.C. Parkinson and illustrated in Hendry et al (1990b), with the comment that they may support a diagnosis of autoimmune orchitis, although attention was drawn in discussion to their very patchy distribution. They had, however, not been recorded before 1979 as evidence of autoimmune orchitis occurring spontaneously in man.

3.5.2 Response to treatment (see 2.7)

Four azoospermic and 3 severely oligozoospermic men showed marked improvement in sperm concentration whilst receiving prednisolone 5mg T.D.S. The data shown in Fig. 11 were from Hendry et al (1979) and were used to illustrate the marked beneficial effect of steroid therapy. The best examples are shown in Figs. 34 and 35, men with no demonstrable mononuclear cell infiltrate on testicular biopsies, but a pronounced response to prednisolone therapy leading to production of 2 pregnancies. This response to therapy was taken as presumptive evidence of autoimmune orchitis (Hendry et al, 1990b).

CHAPTER 4: DISCUSSION

4.1 LABORATORY METHODS

4.1.1 Which tests to use?

The first and most important question regarding ASA in male subfertility was "do they matter?" In September 1976, at the end of the first year of routine serum testing for ASA in subfertile males at CHW, we found over one-third positive for IFT, and around 10% positive for agglutinating and immobilising antibodies by GAT and SIT. Clearly the first priority was to see which tests correlated with each other, and it quickly became apparent (Table 13) that there was a very good agreement between GAT and SIT, if a serum titre of 32 or more by GAT was accepted as significant, as suggested by Rumke et al (1974) (see Table 4 p 41). Husted (1975) had found a similar correlation. On the other hand, there was no relationship whatsoever with the results of IFT testing (Table 14). However, this finding by itself was not sufficent to condemn the latter tests, although such a high proportion of men with immunological infertility seemed unlikely. Another test was needed to put the antibody test against, preferably a test showing a fundamental defect in the processes known to be necessary for fertilisation. Failure of sperm penetration of cervical mucus was not a new phenomenon - "cervical hostility" had been recognised for many years, and, as the name implied, was generally taken to indicate that the mucus was "rejecting" the man's spermatozoa. Condom treatment was often recommended, to keep the spermatozoa away from the mucus for 6 months, to allow the "hostility" to subside. Results had needless to say, been poor and unpredictable. Kremer and Jager (1976) drew attention to the fact that autoantibodies in the man,

as well as isoantibodies in the woman, led to failure of penetration of the mucus. Once this fact had dawned on us, it was possible to show by crossed hostility testing that the spermatozoa from most men with ASA could penetrate neither mucus from their female partner nor that from a donor, whereas their partner's mucus accepted donor sperm readily. Clearly the concept of "cervical hostility" was outdated and in need of revision, but equally importantly, this test gave us the opportunity to find out which ASA tests were associated with significant impairment of sperm function in cervical mucus, and which were not. It quickly became apparent that ~~semen~~ serum GAT and SIT gave results that correlated well, though the correlation was not perfect.

The association between the presence of ASA in the male and failure of sperm penetration of cervical mucus in the female, which was known to impair fertiity (Fjallbrant 1968b - see Table 6 p 43), clearly needed further study. Accordingly, a symposium on Immunological Infertility was arranged and held at the Institute of Urology on 11th February 1978. The proceedings were subsequently published as a book entitled "Spermatozoa, Antibodies and Infertility" (Cohen & Hendry, 1978). Many new facts were learned from the 11 invited speakers and some insight was gained into the complex problems of reproductive immunology. Rumke (1978) provided a clear description of the autoantigenicity of spermatozoa, and drew attention to some differences between spontaneously infertile and vasectomised men, pointing out that seminal plasma antibodies are rarely seen in the latter population. Shulman (1978) provided a critical evaluation of the available tests and drew attention to some important

principles on diagnostic application of ASA tests, including advice that at least 2 different test methods should be used in each patient; that undiluted semen should never be used, that known positive and negative controls should always be included, and that there should be no hesitation in discarding test results if the donor semen was poor, or did not behave as expected with control sera. Kremer et al (1978) developed the hypothesis that ASA present on the spermatozoa or in cervical mucus caused cross linking between the spermatozoa and receptors on the glycoprotein micelles in the cervical mucus, leading to characteristic non-progressive shaking or jerking movement of the spermatozoa. Furthermore, they indicated that this phenomenon was only observed when the spermatozoa were coated with IgA, either by itself or in combination with IgG, but not with sperm coated with IgG alone. The presence of IgA on the sperm correlated with the presence of seminal plasma ASA as shown by TAT; Rumke (1978) proposed that this autoantibody was probably locally produced in the genital tract. Hjort et al (1978) had used $F(ab)_2$ fragments to block the immobilising effect of some but not all sera. Finally, Shulman (1978) reviewed the results of treatment up to that time, and speculated on possible future developments. These, and other valuable contributions on spermatozoal biology and the properties of cervical mucus, provided the scientific foundations for the work that was to be done in our departments in the next 12 years. In particular the fundamental importance of measuring ASA in seminal plasma as well as serum was recognised, coupled with the significance of the class of antibody bound to the

spermatozoa.

4.1.2 Agglutination and immobilisation tests

Statistical confirmation of the relevance of TAT in the evaluation of male subfertility came from the careful studies of Hargreave (1982, 1983) and Hargreave et al (1980, 1984) (supervisor of this thesis) who measured serum and seminal plasma TAT titres in 645 men attending the infertility clinic and compared them with 151 men requesting vasectomy, who all claimed fatherhood of 2 or more children, and at the time of testing had at least one child under the age of 2 years.

TAT Titre	Fertile Men*		Infertile Men*	
	Serum	Seminal PLasma	Serum	Seminal Plasma
0	137	52	518	310
4	2		2	5
8	8		25	22
16	3		19	4
32	1		20	14
64			15	10
128			10	6
256			13	3
512			9	1
1024			4	1
≥2048			11	2
Total	**151**	**52**	**645**	**378**

*(p<0.01 by Wilcoxon Rank Sum Test)
(Hargreave, 1983)

TABLE 54 Comparison of TAT titres in serum and seminal plasma between fertile and infertile men (from Hargreave 1983)

It may be seen from Table 54 that no serum titres over 32, and no positive seminal plasma titres were seen amongst the fertile men. The serum TAT results provided confirmation of a serum titre of 32 as a reasonable lower working limit of significance as suggested by Rumke et al (1974), and the seminal plasma TAT results confirmed the fundamental importance of testing both serum and seminal plasma. Hargreave (1982) found that SIT was only positive in association with agglutinating activity, and found both tests positive in 7.5% of infertile husbands - remarkably similar to our own findings (Table 13).

Further confirmation of the validity of GAT, TAT and SIT was provided by the WHO's Task Force on Immunological Methods In Fertility Regulation (Hjort and Griffin, 1985). Semen samples from 26 fertile and 82 infertile males were collected and distributed to 18 investigating laboratories, where sera were tested by a variety of tests including GAT, TAT, SIT, sperm cytotoxicity (SCT), passive haemagglutination (PHA) and immunobead binding (Bronson et al, 1985), and various ELISA methods (Mettler et al, 1985). GAT and TAT gave 7 and 12% positive results respectively in infertile males, and negative results for fertile males from all except one laboratory. SIT correlated well with TAT and GAT in all except one laboratory. Indirect immunobead testing (IBT) correlated well with GAT, TAT and SIT, but SCT and PHA did not; however, IBT gave the highest incidence of ASA (21% positive for sperm reactive IgG) in the infertile male group, and may have included some false positive results. Shulman et al (1985) also reported good overall correlation between indirect IBT and GAT, whilst noting that

a number of sera were IBT positive with low and probably insignificant GAT titres. Returning to the WHO study, ELISA testing gave a high frequency of positive results in all groups, including fertile controls (Mettler et al, 1985).

Winnett and Suominen (1982) compared 8 techniques of ASA testing, including TAT, GAT, SIT, IFT, and others, and concluded by recommending TAT. Parslow (1985) in her PhD Thesis, showed that both GAT and TAT produce reproduceable results (within 1 or 2 dilutions) when repeated with the same control sera and fresh semen from the same donor on many different occasions, and with a battery of sera using different donor spermatozoa. TAT was more sensitive, particularly with head-to-head (H-H) agglutination. However, no test is perfect: Jager et al (1987) reported finding serum TAT titres between 16 and 128, and seminal plasma titres between 128 and 2048 in a fertile man whose baby shared his haplotype on tissue typing. Although IgA and IgG were detected by MAR and IBT on almost all motile spermatozoa, only 40% showed the shaking phenomenon on SCMC testing, the remainder showing excellent penetration of cervical mucus. This rare man appeared to be the exception who proved the rule that the results of ASA testing should always be checked by sperm-cervical mucus penetration testing before recommending treatment.

4.1.3 Mode of Agglutination

TAT is more sensitive than GAT, since the small clumps produced by head-to-head (H-H) agglutination can be seen under the microscope, but are not visible macroscopically; the large clumps produced by tail-to-tail (T-T) agglutination, on the other hand, are easily seen by both

techniques. This was reflected in the results obtained with our vasectomy reversal men (see 3.4.1, page 138), when GAT and TAT coincided within 2 titres in 92%, although in 20 TAT was 2 or more titres higher than GAT, and in 17 of these there was prominent H-H agglutination. The significance of the mode of agglutination remains obscure. Friberg (1974a) developed TAT, and recognised that H-H agglutination was most common in women, whereas T-T agglutination was more common in men (Friberg, 1874b). Fractionation and absorption investigations indicated that H-H agglutination was caused by IgM antibodies, whereas T-T agglutination was usually caused by IgG antibodies in serum (Friberg, 1974c) and IgA antibodies in seminal plasma (Friberg, 1974d). T-T agglutinating antibodies were found in seminal plasma with serum titres ≥ 64, but none of the men with pure H-H agglutinating antibodies had antibodies in the seminal fluid (Friberg, 1974e). These conclusions were all subsequently confirmed by Friberg (1980a), although it was noted that the total immunoglobulin concentration in serum and seminal plasma were similar in fertile men and in infertile men with H-H or T-T sperm agglutinating antibodies (Friberg, 1980b). Post-coital tests in the female partners were poor if the man had moderate or high titres of T-T agglutinating antibodies, but were normal if the man had H-H agglutinating antibodies, or low titres of T-T agglutinins (Friberg, 1981). Spermagglutination in the ejaculate was much more marked with T-T agglutinating antibodies (Friberg and Tilly-Friberg, 1977). In azoospermic men, the development of ASA only occurred if there was complete spermatogenesis (i.e. did not occur with Sertoli-cell-only syndrome or

maturation arrest at or before spermatid level) (Friberg and Kjessler, 1975). Friberg and Fritjofsson (1979) described 10 infertile men with ASA and a history of inguinal herniorrhaphy - exploration showed occlusion of the vas deferens at the site of hernia repair in 5, and 3 others had small spermatoceles. Only 1 of these 8 men had H-H agglutinating antibodies. The findings are at variance with our own observations, which indicated that H-H or mixed agglutination was found significantly more often with unilateral (81%) or bilateral (55%) testicular obstruction than in spontaneously infertile males of whom only 24% showed such agglutination (Tables 38 and 39, pages 124). Girgis et al, 1979 studied 50 cases of azoospermia, 32 of whom had testicular obstruction: 13 (40%) had ASA, of whom 9 (70%) had H-H or mixed agglutination - results more in accordance with our own observations.

4.1.4 Direct MAR and IBT

The mixed antiglobulin reaction was first applied to spermatozoa by Coombs et al (1973) as an indirect test to define the class of antibody in semen and seminal plasma from infertile men with ASA, and compare them with normal and vasectomised men. Sera were found to be positive for IgG, but not for IgA, in most of the infertile and many of the vasectomised men, but in only 1 of the normal men. Although there was some difficulty in reading the results, definite evidence of IgA class of ASA was found in 6 of the 7 samples of seminal plasma from the infertile men, but not in the normal controls, even in the man with ASA in serum. A direct test was possible in 1 infertile man, the only one from whom a fresh semen sample could be obtained, and both

IgG and IgA antibody were found adhering to his spermatozoa; similar tests from a normal man were negative. With remarkable foresight, the authors concluded that the infertility in these men might have been due to locally produced IgA antibody, rather than through transmission of IgA from serum. A direct erythrocyte-platelet antiglobulin reaction had been originally described by Coombs et al (1956), and Jager et al (1978) modified this test for use applied directly to fresh semen, so that it could be incorporated into routine seminal analysis as a screening test for IgG on the spermatozoa. There was excellent correlation with the presence of circulating ASA and with impairment of sperm penetration of cervical mucus. Our studies at CHW (see 3.1.4 and 3.1.5) amply confirmed these observations. This test, applicable to over 90% of semen samples produced by the male partners of infertile marriages, demonstrated surface-bound IgG on the spermatozoa of 12%, and excluded the presence of ASA in almost 80%. It was, however, very sensitive, and gave strongly positive resutls even with low serum titres of circulating antibody shown by GAT or TAT, in men who were subsequently shown to be fertile (see Table 18. p100). It was therefore just a screening test, and it was emphasised that serum and seminal plasma titres of ASA should also be defined if the MAR was positive, and the importance of checking serum TAT in patients in whom MAR was technically impossible was emphasised (e.g. in men with azoospermia or severe oligozoospermia). The MAR is now included as a routine with seminal analysis in most up-to-date laboratories.

As an alternative, many laboratories use direct IBT with washed patients' spermatozoa to screen for ASA. This test is also exquisitely sensitive (Adeghe et al, 1986), and it is not surprising that low or moderate levels of sperm surface-bound ASA detected by this technique have no intrinsic prognostic value for subsequent fertility (Barrett et al, 1992). Clearly this is just a screening test, and full evaluation of a particular patients' ASA status should include measurement of serum and seminal plasma unbound ASA by TAT.

Jager et al (1980) also used the MAR with cells coated with IgG or IgA (obtained from colostrum) to define the class of antibody bound to the spermatozoa of infertile men with ASA. The results showed that the percentage of motile spermatozoa with IgA bound to their surface showed no direct relation to the unbound ASA activity in serum and seminal plasma, but was roughly proportional to the percentage of spermatozoa exhibiting the shaking phenomenon on SCMC testing. Subsequently Jager et al (1981) showed that the Fc part of the antibody became attached to receptors in the cervical mucus. We used Group O Rh positive RBC's sensitised with serum containing anti-D which was partly IgA although mainly IgG to study the same question, and reached largely the same conclusions as Jager et al (1980).

Successful sperm penetration of cervical mucus was not seen when MAR (IgA) was positive or strongly positive, although it was seen in patients with significant serum titres of ASA (though negative for seminal plasma ASA) who had negative or doubtful MAR (IgA) (see Tables 26 a & b,p106). ASA after vasectomy reversal are always a difficult problem

(see below section 4.4), but it is germaine to mention here that Meinertz et al (1990) used MAR to define the percentage of spermatozoa with IgG or IgA bound to the sperm membrane and found that the conception rate fell from 86% in a subgroup with a pure IgG response, to 43% when IgA was present on the sperm as well, to 22% when 100% of sperm were covered with IgA, and ultimately to zero when there was IgA on all the sperm and a strong immune response as shown by a serum TAT titre of 256 or more. Meinertz et al (1991) used a similar MAR technique to show that motile spermatozoa emerging from the epididymis in men undergoing vasectomy reversal had both transuded and locally produced ASA bound to their surface, and that follow up semen samples 4-12 months later showed identical results. In this regard, Patrizio et al (1992) have shown by indirect IBT studies that ASA binding was the same using either sperm emerging from the testes of men with absent vasa or normally ejaculated sperm, and hence concluded that sperm acquired the surface antigens detected by IBT within the testes, prior to transit through the epididymis. Before this, it had been thought that ASA mostly entered the semen from the prostate at the time of ejaculation, conclusions derived from split-ejaculate studies of the total immunoglobulin content of whole semen (Rumke, 1974). This question has important therapeutic implications, which will be considered in more detail in the next section.

As an alternative to red blood cells, latex particles coated with immunoglobulin have been used for MAR (Comhaire et al, 1988), and satisfactory sensitivity and specificity was observed by McClure et al (1989).

The MAR test differs from the IBT in that the indicator red blood cells are covered with incomplete antibody (mostly IgG) which are mixed with unwashed spermatozoa, and the reaction is completed by the addition of antihuman Ig; if there is antibody on the spermatozoa, mixed agglutinates are formed which are easily recognised. The immunobeads, on the other hand, are coated with class specific antihuman Ig, which would react non-specifically with unbound Ig in seminal plasma; it is therefore necessary to wash the spermatozoa thoroughly before carrying out this test. The latter test is, as a result, more time consuming and laborious. However, there is an excellent correlation between MAR and IBT (IgG), and since the former test is cheaper and quicker it is probably preferable for routine screening.

A positive MAR-test result necessitates definition of the class of antibody present on the spermatozoa since this determines the effect of the ASA on sperm penetration of cervical mucus and hence the effect on fertility. The IBT (IgA) correlated well with seminal plasma antibody measured by TAT, although there were some patients in whom all the antibody was bound to the spermatozoa, leaving no unbound Ig to be detected by TAT. The IBT (IgA) thus adds further useful information in defining not only the class of antibody, but also what percentage of the spermatozoa are affected. There is evidence that once this exceeds 50%, there is likely to be impairment of cervical mucus penetration (Bronson et al, 1984). Over 80%, a significant decline in ovum fertilization is probable on attempting in vitro fertilization (see 4.5.1).

IBT have also been used extensively as an indirect test for ASA in serum and seminal plasma, even though Bronson et al (1984) showed that residual ASA in seminal plasma detected by this technique were not representative of the cell-bound Ig present on the spermatozoa. Some strange results have been obtained with class specific IBT used indirectly. Thus, Matson et al (1988, 1989) found that poor post coital tests, and impaired fertility were only found when both IgG and IgA ASA were present, but there was no impairment with either class of antibody alone. These results are so contrary to those of Kremer et al (see 4.1.6) and of our studies (see above) that false positive results were probably a problem caused by use of undiluted serum, and failure to define the dilution at which the effect disappeared. All in all, the indirect IBT test seems to give less than satisfactory results that correlate poorly with those reported from elsewhere.

A specific radiolabelled antiglobulin test, using live spermatozoa as the substrate has been used by Haas et al, (1980, 1981, 1982, 1983) to quantify the amount and class of antibody on spermatozoa, and a similar double-antibody technique was used by Parslow et al (1985) to compare the class of antibody on spontaneously infertile men and those following vasectomy reversal (see below, section 4.4).

4.1.5 IFT and ELISA

Our experience with IFT and ELISA showed poor correlation with GAT and TAT respectively (see 3.1.2 and 3.1.8), probably because the former tests use fixed spermatozoa, thus revealing subsurface antigens which have little relevance to impairment of fertility and hence to

clinical practice. Similar conclusions were reached by Mettler et al (1985) (see above).

Many of the problems associated with ASA work in human infertility have resulted from the plethora of tests that have sprung up, each investigator designing a new method of quantifying or defining the antibody, and few taking the trouble to compare the results with those obtained by GAT and TAT, old fashioned and non-specific, perhaps, but none-the-less tests that have stood the test of time, and as indicated in this section, have been shown again and again to be reliable and reproducible. When combined with MAR or IBT to define the presence and class of bound antibody, they provide a reliable laboratory base on which to base clinical work in subfertile men.

4.1.6 PCT and SCMC testing

The post coital test provides an insight into the behaviour of spermatozoa in the female, and was never found to be normal in the partners of our 50 men with significant serum titres of antibody (section 3.1.2). Kremer et al (1978b) studied 32 infertile couples with 'unexplained' poor post coital tests and found ASA in 30: in 25 the antibodies were in the male, in 5 in the female partner. The PCT has a marked prognostic value, the time to conception being inversely related to the number of motile spermseen: thus, Hull et al (1982) showed a fivefold greater chance of conception associated with a positive compared to a negative PCT; after 2 years the cumulative conception rates were 84% and 16% respectively. Subsequently, Glazener and Hull (1987) showed that amongst many causes of poor PCT, ASA in semen showed by TAT or MAR was highly significantly

associated with failure of sperm to invade despite colonisation of clefts in the mucus.

Of course, definition of the cause of the poor PCT requires SCMC testing, using donor sperm and donor mucus to define the partner with the problem. We preferred to define the depth of sperm penetration in our crossed hostility test by the technique originally described by Miller and Kurzrok in 1932, rather than define the percentage of sperm showing the shaking phenomenon in the SCMC test described by Kremer and Jager (1976), but the information derived from the test was the same. Care must, however, be taken to ensure that poor sperm motility or forward progression does not give false positive results: Menge and Beitner (1989) carried out multivariate analysis in 754 couples and showed significant independant effects of sperm concentration, motility, forward progression and ASA on sperm-cervical mucus penetration. Nonetheless, Franken et al (1988) found the SCMC test a reliable monitor of treatment, and found that improvement in SCMC characteristics correlated well with ultimate production of pregnancy.

Sperm-cervical mucus contact thus forms the point of interaction between male and female partners where the effects of ASA in either partner become manifest, and without significant reaction at this point, it is very unlikely that there is a significant immunological aspect to the couples infertility. This observation was fundamental to the initiation and development of medical treatment that was used in subfertile males in the ensuing 15 years, to be described in the next section.

4.2 MEDICAL TREATMENT

4.2.1 Case selection and pretreatment work-up

Subfertile males were selected for medical treatment on the basis of positive ASA tests in serum and/or seminal plasma at titres generally accepted as significant, with clearly demonstrated evidence of impaired sperm function on SCMC testing, as compared to normal control spermatozoa. An abnormal PCT was an essential prerequisite. It was realised that the ASA could be associated with moderate or severe impairment of sperm concentration and motility (also recognised by Rumke, 1981), which could improve or even normalise with treatment. In practice, it was difficult to know when oligozoospermia could be due to unilateral testicular obstruction with associated ASA, and when it was due to a primary effect of the ASA. If there were reasonable grounds for suspecting obstruction or if the man was azoospermic, the scrotum was explored before starting medical treatment. Otherwise, medical treatment proceeded so long as there were some spermatozoa in the ejaculate (obviously a positive MAR was expected if it was technically possible to do the test). Care was taken to make sure that the female partner had patent tubes, and was ovulating normally; failure of donor sperm to penetrate her mucus adequately on x-hostility test was always investigated and treated by gynaecological colleagues. Standard pretreatment medical checks are listed in section 2.4.1.

4.2.2 Which medical regimen?

In man, production of circulating antibodies is usually only slightly affected by corticosteroid administration: however, manifestations of cell-mediated

immunity can be suppressed by prolonged treatment (Claman, 1972). The success of the long term low dose prednisolone in improving the sperm counts but with relatively few pregnancies in the female partners may reflect its greater effectiveness in suppressing the cell mediated component of the antibody-dependant reaction - perhaps clearing the blockage to sperm flow, but leaving the antibody bound to the spermatozoa. This concept is developed further in Section 4.5.

Intermittent high-dose methylprednisolone (MP) was recommended by Shulman (1976) as a more effective method of reducing circulating antibody titres. A significant decrease in IgG had been observed in 86%, and IgA in 43%, of 17 normal adults 2-4 weeks after MP 96 mg/day for 5 days (Butler and Rossen, 1973). Timing of treatment was therefore important, which lead Shulman to recommend that the MP should be given to the man from day 21-28 of the female partner's menstrual cycle. A practical problem was that it was entirely possible that the man's wife could be pregnant at the time, possibly after a preceding course of treatment; this lead us to change the regimen to give the drug from day 1-7 of alternate cycles. There was no evidence of reduction in pregnancy rate as a result (see 3.2.3). The results of this study arecompared with those reported from elsewhere, and

Author (and year)	Corticosteroid (daily dose)	Pregnancies/ Patients Treated		Side Effects Major	Minor
		No	%	%	%
Hendry et al (1981)	Methylpred. (96mg)	14/45	31	6	26
De Almeida & Jouannet (1981)	Dexamethasone (2mg)	3/14	21	0	Some
Hargreave & Elton (1982)	Methylpred. (96mg)	5/13	38	? 1	63
	Betamethasone (2mg)	6/7	85		
Shulman & Shulman (1982)	Methylpred (96mg)	31/71	44	2	16
Hendry et al (1986)	Prednisolone (40/80mg)	25/76	33	0	53
Alexander et al (1983)	Prednisolone (60mg)	7/19	37	0	Some
	Control	3/25	12	0	Some
Fredricsson (1988)	Prednisolone (48/96mg)	6/16	38	0	Some

TABLE 55 Reported results of intermittent corticosteroid therapy for male infertility due to ASA

with the results of the cyclical moderate dose regimen (see 3.2.4) in Table 55. It may be seen that approximately one-third of the female partners became pregnant irrespective of the exact drug and dosage used (with the exception of Hargreave's betamethasone regime, where numbers are too small to draw valid conclusions.

The problem was the side-effects. The 31 year old man whose results are shown in Table 33 as a classic example of falling ASA titres in serum, disappearance of ASA from seminal plasma coincident with disappearance of IgA from his spermatozoa, along with a spectacular improvement in sperm concentration and motility, occurring with 3 courses of MP, failed to impregnate his wife and developed bilateral aseptic necrosis of the hip joints one year later. This required bilateral hip replacement and lead to litigation on the grounds that he was not warned of this possible side

effect. The prednisolone regime was therefore modified with the help and guidance of Prof. M. Besser at St. Bartholomew's Hospital. The cyclical moderate dose regimen (see 3.2.4) which was developed in its place proved to be just as effective and mercifully free of serious side effects. Ultimately it was submitted to double blind controlled clinical trial, when it was shown to be significantly more effective than placebo (see 3.2.5). Interestingly, 3 patients taking placebo, and only 1 taking prednisolone, had to be withdrawn from the trial because of side effects - an indication of the worry, tension and anxiety surrounding treatment of this sort. All patients were given a copy of the Information Sheet reproduced in Section 2.4.1, and advised not to proceed unless they were prepared to face up to the possibility of serious side-effects. The large proportion that opted to proceed is evidence of the great motivation of couples in search of a solution to their infertility problem.

Serial observations showed a significant fall in ASA levels in seminal plasma with prednisolone treatment (figure 19, p 120) indicating that this was the probable mode of action of the drug. This was also observed by Fredricsson (1988). Nonetheless, it was not possible in any of the 3 studies to distinguish from the antibody titres alone which men would succeed in producing a pregnancy from those that would not, although success appeared to be associated with a fall in SIT to zero (figure 13), with disappearance of seminal plasma ASA (see 3.2.5), and sperm motility after treatment was significantly better in the successful compared to the unsuccessful (see 3.2.4). It is possible to

envision a dynamic struggle between sperm pushing forward under their own motility, being held back by the seminal plasma ASA, and treatment perhaps altering the balance in favour of the former.

Three other controlled trials of prednisolone have been reported, which failed to show benefit for the active treatment. However in all these trials the active drug was only given for 3 cycles, not long enough to distinguish any effect from that of the placebo (de Almeida et al, 1985; Haas and Manganiello, 1987; Bals-Pratsch et al, 1992). Indeed, there was no difference at 3 months in our own study (see Fig. 18, p120).

An alternative immunosuppressive regimen using cyclosporin A for 6 months with a dose of 5 to 10 mg/Kg/day was used by Bouloux et al (1986) at St. Bartholomew's Hospital. Seminal plasma and serum ASA fell in 3 of 9 subjects on treatment and 3 successful pregnancies occurred. However, conceptions were unrelated to falls in ASA titres. Treatment was carefully monitored and well tolerated by all patients.

4.2.3 Other treatment options

Artificial insemination and sperm washing

Kremer et al (1978c) recommended intrauterine AIH to get beyond the barrier created by cervical mucus, and they obtained three pregnancies in 15 women whose husbands had spermagglutinin titres of 32 or more. Artifical insemination can be combined with sperm washing, using either phosphate-buffered saline (Halim et al, 1974) or sterile 4% human serum albumin (Shulman et al 1978). We treated 30 couples with this technique for 1-13 (average

4-5) cycles with production of only three pregnancies. Analysis showed that in all three cases where the wife became pregnant, the husband had previously received steroid therapy, and no other pregnancies occurred. There are good grounds for doubting whether antibodies (especially IgA) can be removed from spermatozoa by simply washing them (Adeghe 1987), although Boettcher et al (1982) reported a pregnancy following AIH using sperm ejaculated into fresh Tyrode's tissue culture medium and immediately washed and separated. A similar approach has been studied by Lenzi et al (1988) who found no reduction in surface bound ASA after washing and swim-up. Nevertheless, there are continuing reports of success with carefully timed intrauterine AIH with washed spermatozoa from men with ASA with pregnancy rates from 21% (Galle et al, 1990) to 73% (Windt et al, 1989).

Increasingly ingenious methods of separation of antibody-free spermatozoa from the rest are being studied, including supplementing the medium with 10-50% fetal cord serum, which reduced a positive direct MAR to negative, only to return to positive in media without the FCS (Hinting et al, 1989), to immunobinding to polystyrene Petri dishes coated with antihuman Ig, thus increasing the number of motile antibody-free spermatozoa (Jevlin et al, 1989) to magnetic separation with supermagnetic polymer microspheres coated with anti Ig (Dynabeads) (Foresta et al, 1990). Passage of diluted ejaculate through a column of Sephadex G.200 dextran beads produced physical separation of antibody-free from antibody-laden sperm and resulted in good sperm velocity and improved sperm function as measured by hamster egg penetration testing (Kiser et al, 1987).

However, the question of ASA and their effects on sperm egg fusion, and the place of in vitro fertilization as a treatment option will be considered separately in Section 4.6.

Antibiotics

Prostatitis was noted in 37% of 43 men with antisperm antibodies by Fjallbrant and Obrant (1968). We found that 55% of 31 patients with antibodies had positive semen cultures, compared to only 15% of 32 unselected patients without such antibodies (see 3.1.2, p 93). Fjallbrant and Nilsson (1977) reported eight cases with more than 20 white blood cells per HPF in expressed prostatic secretion, who were treated with long-term antibiotics: in five cases, the antibody titres dropped, cervical mucus penetration improved and the wives became pregnant. In our experience with 290 patients, however, pregnancies occurred with equal frequency with or without positive semen cultures, irrespective of whether the organisms were treated with antibiotics or not (unpublished observations). Furthermore, electron microscopic studies in 57 patients with large numbers of cells in the ejaculate showed that in 60% the leucocytes contained spermatozoa, and in only 28% were bacteria seen within the cells (Hughes et al, 1981). Nevertheless, prostatitis can be associated with antisperm antibodies, and a positive culture in expressed prostatic secreations should probably be treated by long-term low-dose antibiotics before and during prednisolone therapy. In the author's experience, however, this is not often necessary.

4.3 SURGICAL TREATMENT

4.3.1 Immunological response to testicular obstruction

In their first large study of infertile men, Rumke and Hellinga (1959) noted that occlusion in the vas deferens or epididymis lead to formation of ASA more frequently than was observed in the others (see Tables 9 & 10, p52). This was attributed to post obstructive extravasation of spermatozoa into the interstitium, lymphatics and even the capillaries as a result of the occlusion - a finding that was subsequently confirmed experimentally by vasectomy in rams and boars (Ball and Setchell in 1983), and clinically in man, by finding spermatozoa in an abdominal lymph node one year after vasectomy (Ball et al, 1982). Although patchy local obstructive changes are commonly observed in the epididymis at autopsy, especially in the ductuli efferentes (Ball and Mitchinson, 1984) relatively few and certainly not more than half (Rumke, 1972) develop ASA, suggesting that there is normally a degree of local tolerance to extravasated spermatozoa. Complete obstruction to the outflow, however, seems to produce antigenic overload with stimulation of ASA production in some (though not all) individuals (Rumke, 1968). Rumke (1965) reviewed the past histories of 64 patients with ASA, and found possibly relevant information such as previous genital infection, surgery or injury in just over half: our findings were similar in 40% of 50 men with significant titres of ASA (see 3.1.2, p 93). On physical examination, Rumke (1965) found abnormalities suggesting that one or both efferent ducts were obstructed in about half: we found definite obstruction in 16% and possible obstruction in a further 20% of 160

unselected spontaneously infertile males (Table 39, p25).

Rumke and Titus (1970) studied the association between testicular obstruction and ASA formation by subcutaneous injection of sperm, unilateral vasectomy and vasoligation in rats. A brisk ASA response was dependant upon the injection of adequate numbers of sperm, and was observed more often after vasectomy than vasoligation, presumably because more sperm were extravasated. Antibody titres fell significantly after removal of the obstructed testis in all animals. Kessler et al (1985) used inbred DBA/IJ mice, which are known to be high antibody formers: unilateral vasectomy consistently induced an ASA response and significantly reduced fertility, whereas sham operation or unilateral orchiectomy did not.

Bilateral testicular obstruction inevitably causes sterility as a result of the ensuing azoospermia, and the individual may or may not form ASA depending on his immunological responsiveness and the cause of the blockage (see below). With unilateral obstruction, on the other hand, sperm output may be maintained from the unobstructed testis and impairment of fertiity will depend on whether ASA are formed or not. This was graphically illustrated by our study of 30 men who had genital tract injuries in childhood: ASA titres were significantly higher in those with unilateral obstruction (Fig. 27, p137) suggesting that there are probably a number of men with unilateral obstruction who retain their fertiity because they did not produce ASA. These and other factors need to be considered in more detail if the true significance of testicular obstruction with ASA formation in male subfertility, and the obverse, that is,

the relevance of ASA on the results of surgical treatment of unilateral and bilateral testicular blockage, are to be understood more clearly.

4.3.2 Unilateral testicular obstruction

It is difficult to diagnose testicular obstruction, especially if only one side is blocked. There are usually no local symptoms and the findings on examination may be indefinite. The first study (3.3.2) was therefore directed towards defining diagnostic indications for scrotal exploration since there is no satisfactory non-invasive test for testicular obstruction. The clinical history is often helpful - previous epididymitis or a hernia repair in infancy or childhood should alert suspicion. On examination, characteristic distension of the tail of the epididymis on the affected side, with a little tenderness, may be the only clue. Strangely, some testicular asymmetry may present, and the obstructed testis is often the larger. Studies into the reasons for this are continuing, but it remains a baffling observation to the present day. It is important, however, as the obstructed testis may have perfectly normal spermatogenesis while the contralateral, unobstructed one may have quite severe atrophic changes. The finding of ASA on routine testing always raises the possibility of obstruction, especially if H-H agglutination is reported. Once again, the reason for this association remains obscure (see 4.1.3, above) but the information serves as a useful diagnostic pointer.

In the second, larger study (3.3.3) it was noted with some surprise that half of the patients with unilateral obstruction had severe oligozoospermia despite apparently

normal spermatogenesis as shown by testicular biopsy in almost all the obstructed testes. Three-quarters had ASA, often in high titres, but there was no obvious correlation between the sperm concentration and the ASA titre (Table 43, p130). Many (65%) had ASA in seminal plasma as well as serum, often in very high titre (see Fig. 22, p130) indicating that these men belonged firmly in the grouping of spontaneously infertile as opposed to vasectomy reversal men few of whom have seminal plasma antibody even after reversal (see fig. 28, p140). This difference is discussed in more detail below (4.4).

Confirmation of the diagnosis of unilateral obstruction and surgical correction required exploration of the scrotum. The diagnosis was usually fairly clear, but deciding what to do was not always so obvious. If there was a block at the tail of the epididymis, epididymo-vasostomy was straightforward, and has a patency rate of around 50% (see below). In men with severe oligozoospermia the results were excellent: 7 of 18 subsequently impregnanted their wives, and overall 10 of 33 in this group were successful. Reconnection of a normal testis in a man with a low sperm output clearly does good. With vasal blocks, often the results of stripping out the vas with the hernia sac during repair in infancy or childhood, primary repair may be impossible. Should the surgeon attempt to graft the remnant of the vas onto the other side (transvasovasostomy) thus endangering the patency of the normal vas and risking production of azoospermia, or should such a testis be removed? In practice, it was found best to take biopsies and sew up, then discuss the situation with the patient and

wife, and return for definitive surgery another day. With severe or moderate oligozoospermia and normal biopsies, clearly nothing was to be lost by attempting reconstruction. With normal sperm concentration and high ASA titres, on the other hand, removal of the obstructed testis makes sense, and the scrotum is by then sufficiently well healed to receive a prosthesis in its place.

Following orchidectomy profound falls in antibody titre occurred, a phenomenon that was first described in man by Bandhauer in 1966. The fall was particularly marked in seminal plasma (see figs. 23 & 24, p132). Detailed study of these men, and their testes are continuing. Should surgical correction or orchidectomy precede prednisolone therapy? This question is considered elsewhere (4.2.1), and in general it seems to make sense to correct any anatomical abnormality first, before subjecting the patient to a prolonged course of medical treatment that cannot be repeated if it fails.

It is remarkable how often the diagnosis of unilateral testicular obstruction is missed, or, if it is thought of it is dismissed as unimportant. "Only one cylinder is needed to fire on" is a remark commonly heard, even from medical colleagues. Authorities as eminent as Bedford (1976) and Silber (1986) have concluded that "unilateral blockage does not interfere with fertility". There is now ample evidence to indicate that sometimes it does, and when it does, it represents a real chance for therapeutic improvement in a man's fertility. Fortunately, the impact of disease affecting one testicle on the function of the contralateral organ is now receiving increasing attention(Tarter & Kogan, 1988).

4.3.3 Bilateral testicular obstruction (excluding vasectomy reversal)

The most striking finding in the early analysis of patients with obstructive azoospermia (see 3.3.4) confirmed by the later figures (3.3.5) was the marked and significant differences in the incidence of ASA in the different groups, depending on the site and aetiology of the blocks. The lowest incidence was recorded with congenital absence of the vasa - only 16% had ASA in the final analysis. This low incidence has recently been confirmed by Patrizio et al (1989), who found only 11% positive, compared to 71% in men with failed vasectomy reversal. These findings are, however, much lower than those previously recorded by Amelar et al (1975), who found significant titres of ASA in 18 (62%) of 29 men with absent vasa. Certainly, our studies showed that serum ASA occurred significantly less often with congenital absence of the vasa than with postinflammatory blocks in the vas or at the tail of the epididymis, roughly half of whom developed ASA. This is not surprising, since the inflammatory process that lead to the block would probably have altered the local mononuclear cell population, and set the scene for immunological response to the sperm. Although Hargreave et al (1984) found no increase in the overall incidence of ASA in men attending a clinic for sexually transmitted diseases compared to the normal population, serum ASA developed in male beagle dogs injected with Brucella canis: sperm agglutinins, chiefly IgA, were found in seminal plasma of chronically infected but not normal control dogs (George & Carmichael, 1984). Testicular atrophy eventually developed after more than 4 months

associated with development of cutaneous delayed-type hypersensitivity reactions when tested with soluble canine testicular extracts (see section 4.4). We have observed development of high titres of ASA in a man after an attack of non-specific urethritis, apparently immunised at the time of the acute inflammation (Shahmanesh et al, 1986, see vol. 2). If a block developed during the healing phase, ASA production would naturally be perpetuated.

The incidence of ASA in men with blocks in the caput epididymis was significantly less, at 27%. Many of these men had co-existing sinusitis, bronchitis or bronchiectasis, a syndrome first described by Young in 1970. Histopathologically, the obstruction appears to be due to failure of spermatozoa to flow through the ductuli efferentes (which have a ciliated epithelium similar to the nasal and respiratory passages). The onset is gradual (Jequier et al, 1983) and there is little extravasation of spermatozoa in the interstitium. Rare in men born since 1955, when mercury containing teething powders were withdrawn, it seems that this peculiarly English (and Australian) condition may be a late manifestation of Pink Disease (mercury intoxication in childhood) (Hendry et al, 1990b).

The results of epididymo-vasostomy were best in the post inflammatory caudal blocks, similar to those obtained with vasovasostomy for postinfective vasal blocks (see 3.3.5). There was however, a considerable shortfall between patency and pregnancy rates, which may also be observed in other reported series (Table 56). The high incidence of ASA in these patients,

AUTHOR	YEAR	NUMBER OF CASES	PATENCY (%)	PREGNANCY (%)
Dubin & Amelar	1984	69 46*	20 39	10 13
Jequier	1985	24	12.5	4
Fogdestam et al	1986	41*	85	37
Schoysman & Bedford	1986	565	-	18
Lee	1987	97 158*	31 37	12 20
Thomas	1987	50*	66	42
Silber	1989b			
Corpus		139*	78	56
Caput		51*	73	31

TABLE 56 Recent results of epididymo-vasostomy (* = microsurgical technique)

and the observed statistically significant reduction in pregnancy rate with which the presence of ASA is associated (Table 48, p137), indicates that the immunological sequelae of the block may be responsible for some of the failures of surgical treatment. Nevertheless, Phadke and Padukone pointed out as long ago as 1964 that pregnancies can be produced after surgical correction by successful epididymo-vasostomies even in the presence of circulating ASA. Our results support this observation, though statistically pregnancy is less likely with positive serum ASA, and there may be a case for adjuvant prednisolone treatment in cases where patency has been achieved but pregnancy does not occur.

4.3.4 Vasectomy reversal

Patients presenting for vasectomy reversal appeared to offer a golden opportunity to study the effects of ASA on spermatozoa, since most were previously fertile, and it was known that 60-80% had ASA following the vasectomy (Ansbacher, 1973: Hellema and Rumke, 1978: Rose and Lucas, 1979). With the help of Mr. M.G. Royle in Brighton, we quickly recruited 130 such cases, and studied their ASA before and after the reversal procedure, and followed their sperm counts and subsequent fertiity. It was known that many would be fertile, from the work of Silber (1977, 1978), but the effects of ASA were poorly understoood. Ansbacher (1977) showed that ASA levels persisted after vasectomy, rose transiently after the reversal procedure, and then fell to preanastomosis levels by 1 year. Sullivan and Howe (1977) found ASA in the serum of 48% of men whose wives were pregnant, and 94% of those whose women were not pregnant. We found ASA in the sera of 79%, and in seminal plasma of 9.5% before, and 29.5% after the reversal procedure. This was still considerably fewer, for each serum titre, than we were finding in spontaneously infertile men (fig. 28, p140). Overall, 44% impregnated their wives, and we could show no statistically significant fall off in fertility until very high serum titres ($\geq$512) were reached, and even then 25% were apparently fertile. Furthermore, a small number of men appeared to be fertile with seminal plasma ASA titres as high as 16. These results were quite different from those we were used to seeing in spontaneously infertile males.

Linnet et al (1981) published results of a similar study at the same time as our first publication (Royle et

al, 1981) and showed a highly significant association between presence or absence of seminal plasma antibody and fertility. This was clearly at variance with our 1 year follow up results published by Parslow et al (1983), mentioned above, and we speculated in discussion whether the difference might be due to the class of antibody. This was the subject of Dr. Parslow's PhD Thesis, which was successfully presented to the University of London in 1985, and reported by Parslow et al (1985). Using a sophisticated radio-labelled double antibody technique, the class of antibody on spermatozoa from vasectomy reversal and spontaneously infertile men was defined and quantified, and it was clearly shown that failure of penetration of cervical mucus was dependant on the class of antibody, IgA being much more powerful in this effect than IgG. Most spontaneously infertile men had the former, and most vasectomy reversal men had the latter. The confirmation of these conclusions in a much larger series published in 1990 by Meinertz and colleagues (including Linnet) has been described in section 4.1.4 (page154), and provides satisfying reassurance of the results of our earlier studies.

AUTHOR	YEAR	NUMBER OF CASES	PATENCY (%)	PREGNANCY (%)
LEE & McLAUGHLIN	1980	41 26*	90 96	46 54
FALLON et al	1981	36	74	57
FITZPATRICK	1978	14	90	64
MIDDLETON et al	1987	73	81	49
AMELAR & DUBIN	1979	26	88	53
KESSLER & FREIHA	1981	83	92	45
URQUHART-HAY	1981	50	84	52
COS et al	1983	87*	75	46
SOONAWALLA & LAL	1984	194 339*	81 89	44 63
BECKER et al	1991	1012*	86	52
SILBER	1989a	282*+	91	81
MEINERTZ et al	1990	145	90	53

* microsurgical technique

\+ excluding 44 patients with no sperm in vas fluid

TABLE 57 Some recent results of vasectomy reversal

Our surgical results with vasectomy reversal are rather similar to those described from elsewhere (Table 57) but failures continue to occur from time to time. Further collaboration with Mr. M.G. Royle in Brighton allowed us to define 3 main causes of failure: (i) fibrosis at the site of anastomosis requiring redo vaso-vasostomy, which gave very satisfactory results, as previously described by Silber (1977); (ii) failure of flow due to epididymal tubule blow out, presumably due to back pressure, also described by Silber (1979), and readily corrected by epididymo-vasostomy; and (iii) very high ASA response ($\geq$1024) for whom further surgery and adjuvant prednisolone therapy had little or nothing to offer. We commented in 1985 that this group continued to present a significant therapeutic challenge, and this remains the position, although persisting unilateral obstruction should be excluded by surgical exploration in men with a less than perfect result.

The present day success rate of vasectomy reversal has recently been reported by Belker et al (1991), the results of 1469 microsurgical procedures. The pregnancy rates were closely related to the interval between vasectomy and reversal, falling from 76% if the interval was less than 3 years, to 53% between 3 and 8 years, to 44% between 9 and 14 years, to 30% after more than 15 years. The results can be quoted to patients asking for advice on whether to proceed with reversal, and they can be fine-tuned with the results of the preoperative serum ASA results, giving a rather less good prognosis if the titre is 512 or more. However, it should be remembered that antibody levels can fall after the reversal (fig. 30), or even appear when there were no ASA

before, apparently provoked by the procedure itself (fig. 29 p142). Even under the worst circumstances, however, this procedure carries a success rate that is as good as that achieved by modern high technology methods such as in vitro fertilisation or gamete intrafallopian transfer, and the surgeon should not hesitate to reconstruct the vasa if this is what the couple wish.

4.4 AUTOIMMUNE ORCHITIS

The patchy mononuclear cell infiltrate observed in a few testicular biopsies (see 3.5.1 p145), and the marked improvement in sperm output obtained with long term low dose prednisolone in severely oligozoospermic or even azoospermic men with very high ASA titres (see 3.5.2 p146), raised the possibility of autoimmune orchitis. This condition has been much studied and well documented in different species of experimental animals (an excellent review by Kosuda and Bigazzi in 1987 runs to 249 references), but it has not been identified as a disease entity occurring spontaneously in man (Hjort, 1977), although Mancini et al (1965) provoked immune responses in men awaiting castration for prostatic carcinoma by injection of human testicular material. Patchy lesions consisting of congestion, oedema and sloughing of germinal cells were seen in the testis, along with positive skin tests indicating delayed hypersensitivity; circulating antibodies were detected by complement fixation and other tests. Although the results of animal experiments vary from species to species, and from breed to breed it does appear that both circulating antibody and delayed hypersensitivity are needed to produce this lesion (Brown et al, 1967), which is usually provoked by injection of either testicular

homogenate or spermatozoal antigens with complete Freund's adjuvant (see 1.4.3, p36).

Spermatozoal antigens are usually hidden by the blood-testis barrier (Johnson, 1973), but modern immunological theory suggests that this is supplemented by a degree of local immune tolerance provided by suppressor T lymphocytes which line the male genital tract (El Demiry et al, 1985). Evidence in favour of this hypothesis is two fold. First, spontaneous occurrence of immune orchitis has been observed in mice after neonatal thymectomy, which presumably removed the source of the suppressor cells; the incidence and severity of the orchitis was increased by unilateral vasectomy (Taguchi and Nishizuka, 1981). Secondly, pretreatment with testicular antigen in incomplete Freund's adjuvant in guinea pigs markedly reduced the incidence and severity of immune orchitis when the animals were subsequently challenged with antigen in complete Freund's adjuvant. The pretreatment rendered T lymphocytes temporarily specifically unresponsive as judged by in vitro lymphocyte proliferative response, whereas ASA formation was not affected. Cyclophosphamide pretreatment abolished the protective effect suggesting that the tolerance was mediated by the CY-sensitive suppressor T-cells (Hojo and Hiramine, 1982). Thus "physiologic" autoimmunity can become "pernicious" if the local tolerance mechanisms are disturbed (Cohen, 1984). Further evidence for this is the spontaneous occurrence of autoimmune orchitis in certain breeds of animals, including dark mink (Tung et al, 1984), beagles (Fritz et al, 1976) and tail-less mice (Dooher et al, 1981). Examples have also been seen occurring spontaneously in

related dogs used for breeding which became sterile (Allen & Longstaffe, 1982; Allen & Patel, 1982). In all of these examples, the histological findings were similar to those described in experimental immune orchitis (see 1.4.3, p 36 , that is focal mononuclear cell infiltration around and inside seminiferous tubules where spermatogenesis was diminished or absent. Ultimately the animals became sterile with aspermatogenesis.

Although these changes have been observed very rarely in our subfertile men with severe oligozoospermia or azoospermia and very high ASA titres (see Figs. 31-33, p145) the changes have been very focal, and altogether absent in some men who responded well to prednisolone therapy (for example, see Figure 35, p146). Indeed, a recent project set up to study the nature of these mononuclear cells has made no progress at all due to the paucity of the number of cells available to study (Prof. Isaacson, personal communication). There is, however, another mechanism of hypersensitivity that might be producing subtle changes with the passage of time in these testes in the absence of cellular infiltration. Although sperm production appears to continue normally in man (Bagshaw et al, 1980) marked depression of spermatogenesis has been noted after vasectomy in guineapigs, even if it was only done on one side (Alexander, 1973). The histological and skin-test changes were similar to those seen in autoimmune orchitis provoked by injection of spermatozoa and complete Freund's adjuvant. Bigazzi et al (1976) showed that vasectomised rabbits with high levels of ASA developed an orchitis with granular deposits of IgG and C3 in the basement membrane of the seminiferous tubules

associated with thickening of the basement membrane, accumulation of macrophages and polymorphs, and destruction of the basal lamina, Sertoli and spermatogenic cells. Formation of sperm antigen-antibody complexes at the basement membrane was presumed to be the underlying pathological process. Heavy granular deposits of IgG and C3 were also found in the basal lamina of the seminiferous tubules of dogs (Allen and Patel, 1982) and dark mink with autoimmune orchits (Tung et al, 1984). Similar depositis of IgG and C3 have been observed in testicular biopsies from subfertile men (Salomon et al, 1982) resembling the immune deposits seen in glomerulonephritis (Salomon and Hedinger, 1982). In one case these changes appeared to have followed previous epididymectomy, and were associated with mild atrophic changes in both the affected and the contralateral testes. In a clearly illustrated review of the histological and immunoelectron microscopic changes Salomon and Hedinger (1987) staged the changes, relating the immunological and morphological changes, and concluded that this provided evidence of immune complex orchitis as a disease entity in man. Similar changes have been described by Lehman et al (1987), and by Harrison et al (1981), who proposed that this might be the mechanism of contralateral damage observed after testicular torsion. We also observed evidence of degenerative changes, which we called sympathetic orchiopathia, after experimental testicular injury in rats (Wallace et al, 1982). However, from our point of view this proposed mechanism of testicular damage must remain speculative at present in the absence of any positive pathological evidence.

The first description of the response to corticosteroids of this condition in man was provided by Bassili and El-Alfi in 1970. Thirteen agoozpermic and 5 oligozoospermic men had positive lymphoid blastoid transformation tests in response to seminal antigens, 11 controls were negative. The patients were given prednisolone 40mg per day for 2 weeks, then the dose was gradually reduced over the next 9 weeks. Four showed a good response, all starting with sperm counts of less than 1 million per ml, and one reached 40 million per ml, when his wife became pregnant. This response is very similar to that shown by our patients (see section 3.5.2) and suggests that we are observing the same phenomenon. It is important, clinically, to think of this condition which can be recognised by the co-existence of high serum ASA titres, empty epididymes on surgical exploration and reasonably normal testicular biopsies (with or without focal mononuclear cell infiltrate). It is obviously important not to damage the epididymis or vas in such cases, by unnecessary epididymovasostomy, and it is the author's practice <u>not</u> to do vasography in such cases until the testicular histology and ASA results are available. Certainly there seems to be a condition resembling autoimmune orchitis which occurs spontaneously in man, but it remains difficult to characterise, and it may be that it will turn out to be due more to a combination of high circulating antibodies with toxic-complex (type 2 or 3) autoimmune reaction than to classical cell mediated (type 4) reaction that we have been seeking rather vainly up to the present time. Studies are continuing in this area both in

azoospermic and severely oligozoospermic men with high ASA titres and no evidence of obstruction (primary autoimmune orchitis) and in men with similar changes secondary to unilateral or bilateral testicular obstruction.

4.5 IN VITRO FERTILISATION

4.5.1 The effects of ASA on sperm-egg fusion

Soon after the development of the zona-free hamster egg system for assessment of fertilizing capacity of human or other species of spermatozoa by Yanagimachi et al (1976), reports began to appear indicating that treatment of human sperm with antisera containing ASA caused a significant decrease in ova penetrated compared to normal sera. Since Fab preparation of antisera exhibited a similar inhibutory effect, it was concluded that the blocking effect was due to the antibodies (Menge & Black, 1979). Tzartos (1979) showed that the ASA inhibited passage through the zona pellucida, as well as sperm-egg fusion in zona-free hamster eggs and proposed that the ASA were blocking or masking specific receptors on the sperm surface. These observations were quickly confirmed by many other workers, mostly using the human sperm- zona free hamster egg preparation (Alexander, 1984; Brannen-Brock & Hall, 1985; Menge et al, 1984) but also with boar sperm (Smith et al, 1983) and with a sea urchin sperm-egg preparation (Saling et al, 1982). Some workers noted variability in the effect of ASA, however, and Bronson et al (1981, 1989) even noted enhancement of sperm-egg penetration on some occasions. This observation was confirmed in a carefully conducted study by Aitken et al (1988), in which we were able to collaborate by providing sera and seminal plasma from known ASA-positive patients:

the antibodies could either suppress or stimulate sperm-egg fusion, the proportion showing stimulation being higher in the vasectomy reversal compared to the infertile population.

Monoclonal antibody studies showed that only a small number of sperm-specific antibodies caused significant inhibition of fertilization in mice apparently by blocking the acrosome reaction and sperm binding to the zona pellucida (Saling & Lakoski, 1985; Lee et al, 1986). Monoclonal antibodies that cross reacted with human sperm similarly showed inhibition of penetration of zona-free hamster eggs (O'Rand & Irons, 1984) and human oocytes (Moore et al, 1987). The latter observation was obviously important, as some variation in the effects of monoclonal antibodies on sperm function had been shown depending on whether zona-free hamster eggs or homologous eggs were used for study (Primakoff & Hyatt, 1986).

A problem with interpretation of the results of these studies arose with the plethora of methods used to measure the presence or absence of ASA, exactly the same problem that has bedevilled fertility work in general. In reviewing the studies summarised above, those of Menge et al (1984) and Aitken et al (1988), using standard agglutination and immobilisation tests, both showed that the effects of ASA on sperm-egg fusion as assessed by the zona-free hamster egg penetration test were very variable but generally inhibitory. Observations on human sperm-egg fusion done at the time of in vitro fertilisation have given rather confusing results. Clarke et al (1985) showed that ASA in men, especially of IgA class reduced the proportion of eggs fertilised if more than 80% of sperm were affected, as shown

by direct IBT. Junk et al (1986) and Matson et al (1988) using an indirect IBT concluded that both IgG and IgA were needed to produce significant reduction in fertilization, whereas either class alone did not produce any effect. In fact, these differences are probably technical, and Clarke et al (1988) certainly showed a highly significant reduction in fertilisation of supernumerary human oocytes when human sperm were treated with sera from women with ASA - an effect that could be reduced by immunoabsorption of the IgG fraction of the serum. Similar conclusions were formed by Kamada et al (1985) and Tsukui et al (1986). Tsukui (1988) showed with electron microscopy studies that the number of acrosome reacted sperm was significantly reduced by treatment with ASA positive serum. Mandelbaum et al (1987), on the other hand, used an indirect IBT for IgA, IgG and IgM on serum, seminal plasma, and follicular fluid from 40 couples undergoing in vitro fertilisation and concluded that ASA in men had no effect on fertilisation, whereas they did in women. Once again technical problems with the indirect IBT, perhaps due to its sensitivity, appeared to cause confusion. Be that as it may, the concensus obtained from reviewing the studies summarised above appears to be that ASA can interfere to some extent with sperm-egg fusion, but that the effect is variable and by no means absolute, and that once the egg is fertilised there is little evidence that subsequent development is adversely affected.

4.5.2 Results of IVF in patients with ASA

The first reports of pregnancies occurring successfully after in vitro fertilisation of ova in couples with a significant ASA problem appeared in 1984-1986.

Yovich et al (1984) described fertilisation of 15 of 20 ova from all of 5 women with ASA shown by GAT and SIT, 2 of whom became pregnant. Naaktegeboren et al (1985) described successful in vitro fertilisation from a man with ASA after methyl prednisolone treatment had failed. Devroey et al (1986) described a man with a serum titre of 64 on TAT, whose partner had persistently poor PCT's; 6 eggs fertilised, 3 were returned by laparoscopic zygote intrafallopian transfer and twin pregnancy resulted. Fertilization rate is generally poor when sperm quality is poor (Van Vem et al, 1985), but the proportion can be improved when the man has ASA by increasing the number of motile sperm added to the ovum (Hamilton et al, 1989), washing the sperm in Earle's solution (Hinting et al, 1989) collecting the sperm in medium with 10% serum and using a swim-up procedure to improve the number of motile sperm (Van der Merwe et al, 1990; Elder et al, 1990). Nevertheless, fertilization does seem to depend on the percentage of sperm covered with antibody, and success is uncommon when more than 70% of sperm are affected (de Almeida et al, 1989). Sperm binding to the zona pellucida is reduced in the majority of unsuccessful attempts; however, if fertilisation does occur, pregnancy appears to proceed normally.

This conclusion coincides with our experience at St. Bartholomew's Hospital where work is in progress looking at the effects of ASA in subfertile men on the results of in vitro fertilisation (Rajah et al, unpublished observations).

Egg fertilisation and pregnancies after in vitro fertilisation have been studied, comparing 11 couples (group 1) where the man had antibodies to spermatozoa with 20

couples (Group 2) where the man had no such antibodies. In Group 1, 21 (45%) of 47 eggs fertilised, compared to 93 (73%) of 128 eggs in Group 2. In Group 1, 5 women had 3 fertilised eggs transferred and 2 (40%) became pregnant. In Group 2, 18 women had 3 fertilised eggs transferred and there were 6 pregnancies (33%).

Antibodies to spermatozoa thus appeared to reduce the egg fertilisation rate, but not the pregnancy rate once ovum fertilisation had occurred. These results provided some support for the view that IVF offers an additional treatment option to the infertile couple where the man has ASA (Barlow, 1988; Alexander, 1990).

4.6 CONCLUSIONS (Hendry, 1992 b)

Spermatozoa are clearly highly antigenic to the man who produces them, probably because spermatogenesis does not commence until puberty when the immune system is mature, and since their chromosomal make-up is totally altered during meiosis. Normally, they are hidden by the blood-testis barrier (Johnson, 1973), probably supplemented by a degree of local immune tolerance (el Demiry et al, 1985). ASA may arise as a primary local phenomenon, perhaps initiated by genital inflammation such as non-specific urethritis (Shahmanesh et al, 1986), and it is known that certain individuals with particular genotypes are especially susceptible (Law et al, 1979). Alternatively, they may be secondary provoked by obstruction to the testicular outflow tracts: the spermatozoa are directed to the lymphatics leaving the testis (Ball and Setchell, 1983) which drain to the abdominal lymph nodes (Ball et al, 1982), where systemic antibody production is stimulated. If both testes are

blocked, for example after vasectomy, the man will be azoospermic and the diagnosis obvious. Analysis of large numbers of vasectomised men indicates that the strength of the antibody response to this particular stimulus depends on the individual's immunological responsiveness (Rose & Lucas, 1979). If, however, only one testis is obstructed, the sperm output from the other testis may be normal but fertility can be impaired by antibody production which is sustained by the spermatozoa reabsorbed from the blocked testis (Kessler et al, 1985) - this diagnosis may be much more elusive. It is important to distinguish between primary and secondary causes of antibody production for 2 reasons: first because the class of antibody produced may be significantly different, and secondly because surgical treatment may be necessary in men with testicular obstruction.

ASA are most readily detected bound to the patient's own spermatozoa, using as a marker either sensitised red blood cells in the direct MAR test or, after washing thoroughly to get rid of unbound immunoglobulin in seminal plasma, by direct IBT. Whilst the former test has the advantage of being quick, reliable and easy to read, it is generally only useful for detection of IgG, whereas with class specific IB, the class of antibody on the spermatozoa can be defined. The MAR test is thus an excellent screening test, but positive results should be studied further with IB tests. The amount of unbound antibody should be measured in serum and seminal plasma by TAT - this has replaced the older macroscopic GAT. A number of new tests have been developed based on ELISA, IFT or radioimmuno-assay using

fixed spermatozoa as substrate - the results correlate poorly with those obtained by the classical agglutination tests and with impairment of fertility, probably due to exposure of subsurface antigens during fixation - these tests are best avoided in clinical practice.

ASA interfere with fertility in a number of ways. First, they cause impairment of spermatozoal penetration of cervical mucus; it appears that the Fc part of the antibody becomes attached to receptors in the mucus (Jager et al, 1981) - this is reflected in poor post-coital tests (PCT), and may be seen on SCMC testing. This may be mistaken for "cervical hostility" unless the test is also done with donor spermatozoa and donor cervical mucus as a crossed hostility test. This phenomonon is produced by IgA on the spermatozoa, but not by IgG. This is why primary locally produced ASA (mostly IgA) is more harmful than secondary, systemic ASA which is mainly IgG. Thus for a given serum titre of ASA, a spontaneously infertile male will probably have a much higher seminal plasma titre than a man after vasectomy reversal. Fertility after vasectomy reversal may be normal if the ASA titres are low and the class purely IgG, but if becomes progressively impaired as serum titres increase and the percentage of spermatozoa affected by IgA rises (Meinertz et al, 1990).

ASA may interfere with sperm motility and with sperm-egg fusion and the presence of antibody should always be sought in patients with asthenozoospermia, especially if in vitro fertilisation (IVF) is contemplated. Furthermore, oligozoospermia or even azoospermia may be observed in men with very high titres of antibody, apparently due to a form

of cell-mediated or immune-complex immune orchitis. It follows that serum and seminal plasma should be checked for ASA by TAT in all patients in whom direct MAR or IBT may be impossible due to insufficient numbers of active spermatozoa.

Once the presence of ASA has been detected in a subfertile male, and titres defined, the testicles should be examined carefully to see if there is evidence of obstruction: a history of epididymitis or of hernia repair in childhood may provide a clue, and indicate which side might be affected. In cases of doubt, the testicles should be explored, the epididymes examined with magnification to search for distended tubules, and vasography may be done to exclude vasal or ejaculatory duct obstruction; a testicular biopsy is usually taken and spermatogenesis assessed by an objective score system. If obstruction is present it should be corrected by epididymo- or vaso-vasostomy as required. In some cases with irreparable lesions - for example, absence of a great length of vas - the obstructed testicle may be best removed and replaced with a prosthesis. This may be followed by a profound fall in antibody production, especially noticeable by a drop in seminal plasma TAT titre.

Once the anatomical situation has been defined, and corrected surgically if necessary, medical treatment can follow. After careful medical work up, and after ensuring that the female partner has been fully investigated and any co-existing defects corrected, the couple should be given the opportunity to choose between steroid therapy for the male, or going directly for IVF or gamete intrafallopian transfer (GIFT). The advantages, disadvantages, possible

side effects and costs are outlined and the couple encouraged to take time to make their choice and take further advice if they wish to. Of course, the sperm quality, especially motility, may make it easier to choose - if poor, steroid therapy may well improve it; if good, they may prefer to go straight for an assisted fertilisation technique; of these IVF is usually preferable in the first instance, to see whether the spermatozoa are capable of fertilising the ova. If not, then adjuvant medical therapy is clearly required.

In selecting the most appropriate drug treatment, the physician should take into account the sperm count and motility and the patients' general health. If the sperm concentration is normal, cyclical prednisolone is the treatment of choice, in a dose of 20mg twice daily given with meals to the man from the first to the tenth day of the female partners cycle, then 5mg daily on days 11 and 12. Alcohol is expressly forbidden during these days. After 9 cycles, one-third of the female partners can expect to become pregnant, significantly more than were produced by men taking placebo in the double-blind crossover trial. Sperm motility usually improves markedly and supplementary IVF or GIFT may be recommended if pregnancy does not occur spontaneously. In men with high antibody titres and severe oligozoospermia or even azoospermia, in whom obstruction has been excluded by exploration, considerable improvement in sperm output can be obtained with prednisolone 5mg thrice daily for 6 months - possibly due to suppression of the immune orchitis. In men who cannot take prednisolone, cyclosporin provides an alternative drug which has been

shown to be effective in this setting (Bouloux et al, 1986).

Side effects with prednisolone in the dose regimens outlined above are generally mild and transient. However, all patients must be warned about side effects to be expected, and complications that can occur with prednisolone, and the usual precautions are observed. Two instances of aseptic necrosis of hip have been reported, both using methylprednisolone 96mg daily for 7 days - effectively three times the dose recommended above (Shulman & Shulman, 1982; Hendry, 1982 b). This regimen has now been abandoned, and aseptic necrosis has not been seen or reported since then.

Antibodies to spermatozoa can be found in 8-12% of the male partners of infertile marriages but are relatively uncommon in the female partners (Kremer et al, 1978). The presence of these antibodies should not be missed, since their presence may alert the surgeon to the existence of surgically correctable obstruction, and since effective medical therapy gives the physician an opportunity to produce a real improvement in the man's reproductive potential. Furthermore, enhancement of spermatozoal performance by treating the antibodies will greatly improve the gynaecologist's chances of success with assisted reproduction techniques.

A

THESIS ENTITLED

"AUTO ANTIBODIES TO

SPERMATOZOA IN SUBFERTILE HUMAN MALES"

(Volume 2 of 2 volumes)

presented by

William Forbes Hendry

MB, ChM (with commendation)

FRCS (Edinburgh and England)

For the degree of

Doctor of Medicine

in the

University of Glasgow

Department of Urology,

St. Bartholomew's Hospital,

London, EC1A 2BE.

April 1992

FIGURE 1: GAT showing macroscopically visible flocculation.

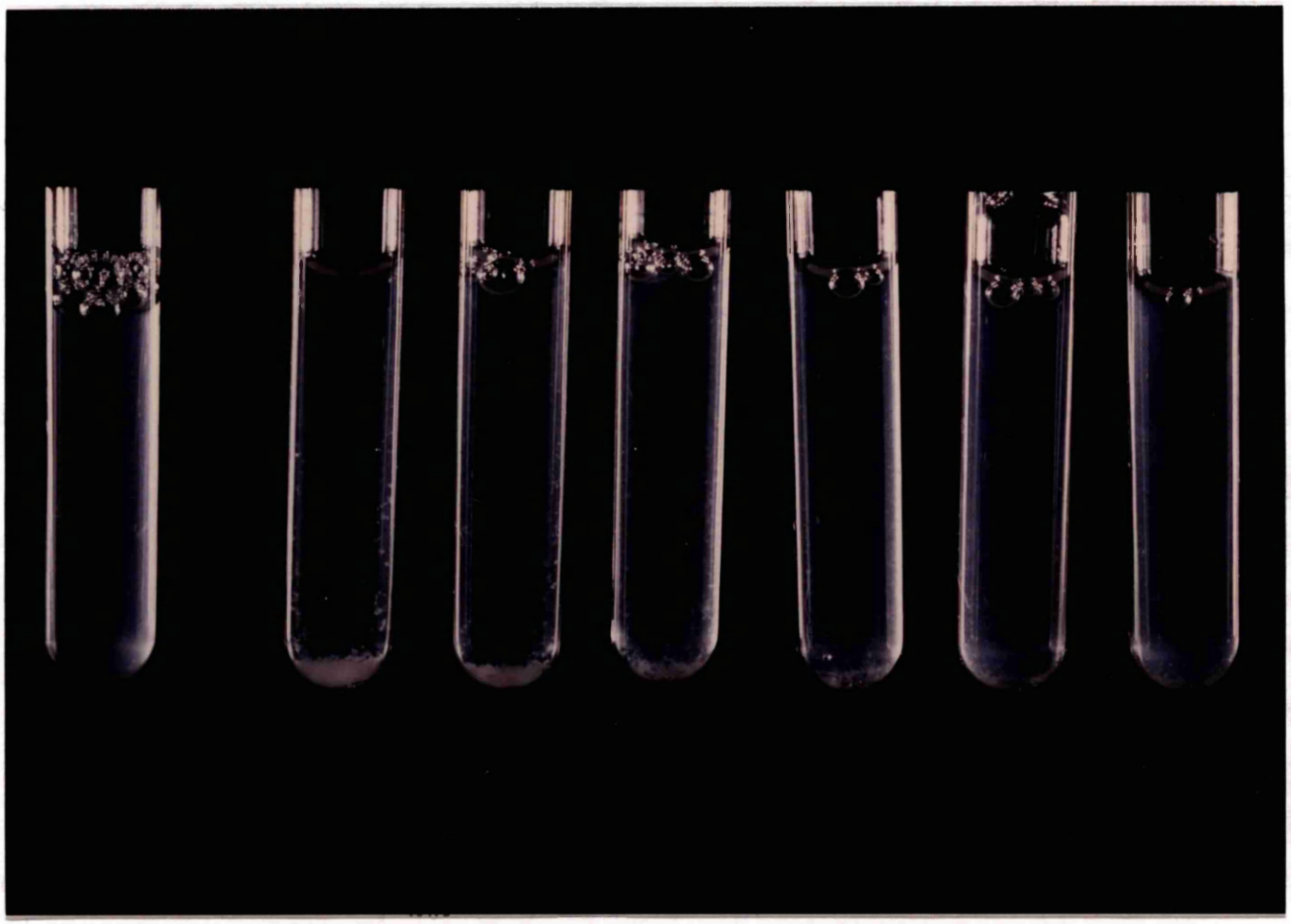


FIGURE 2: TAT - equipment

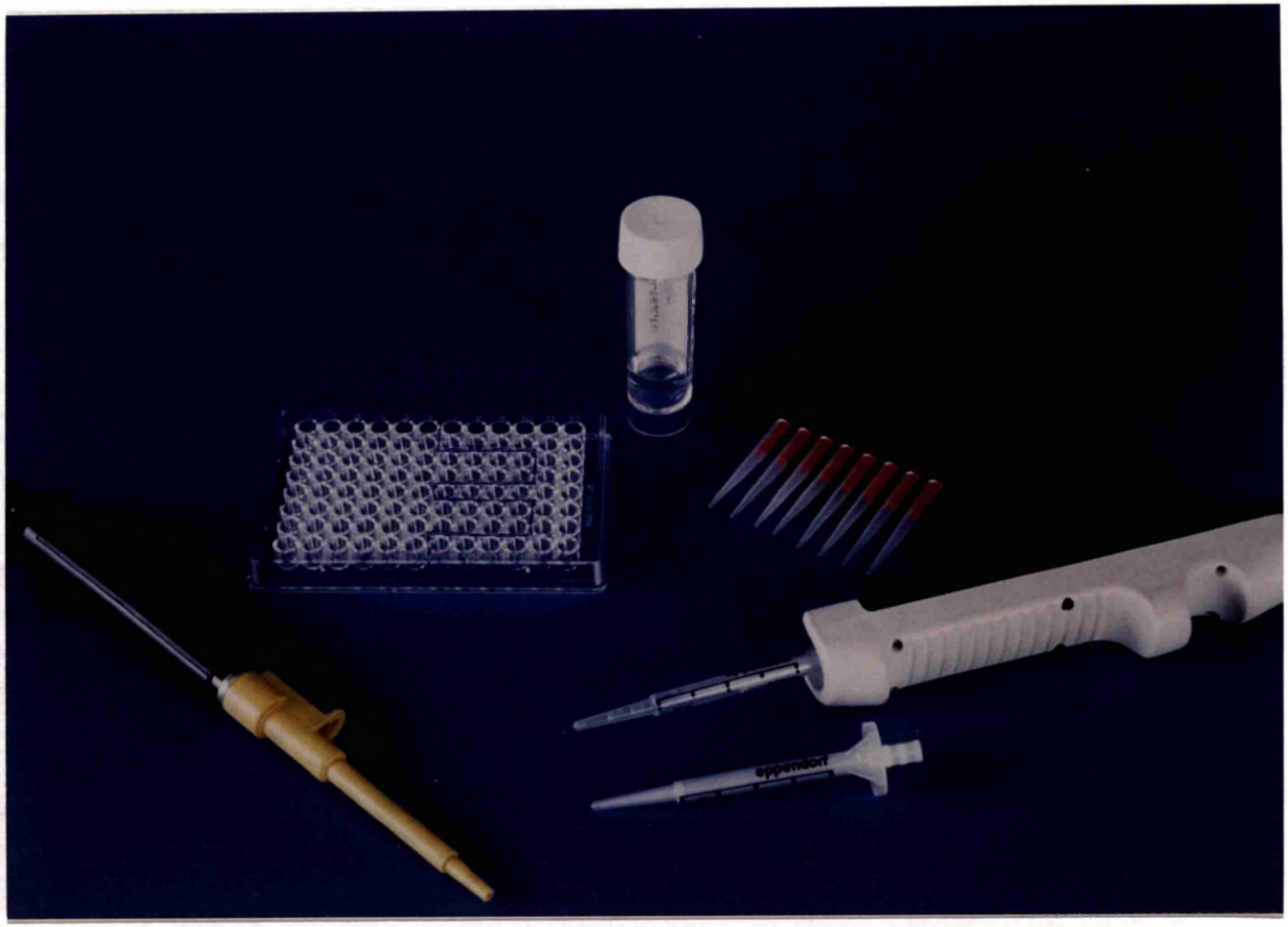


FIGURE 3: TAT - swim-up procedure to obtain motile sperm preparation.

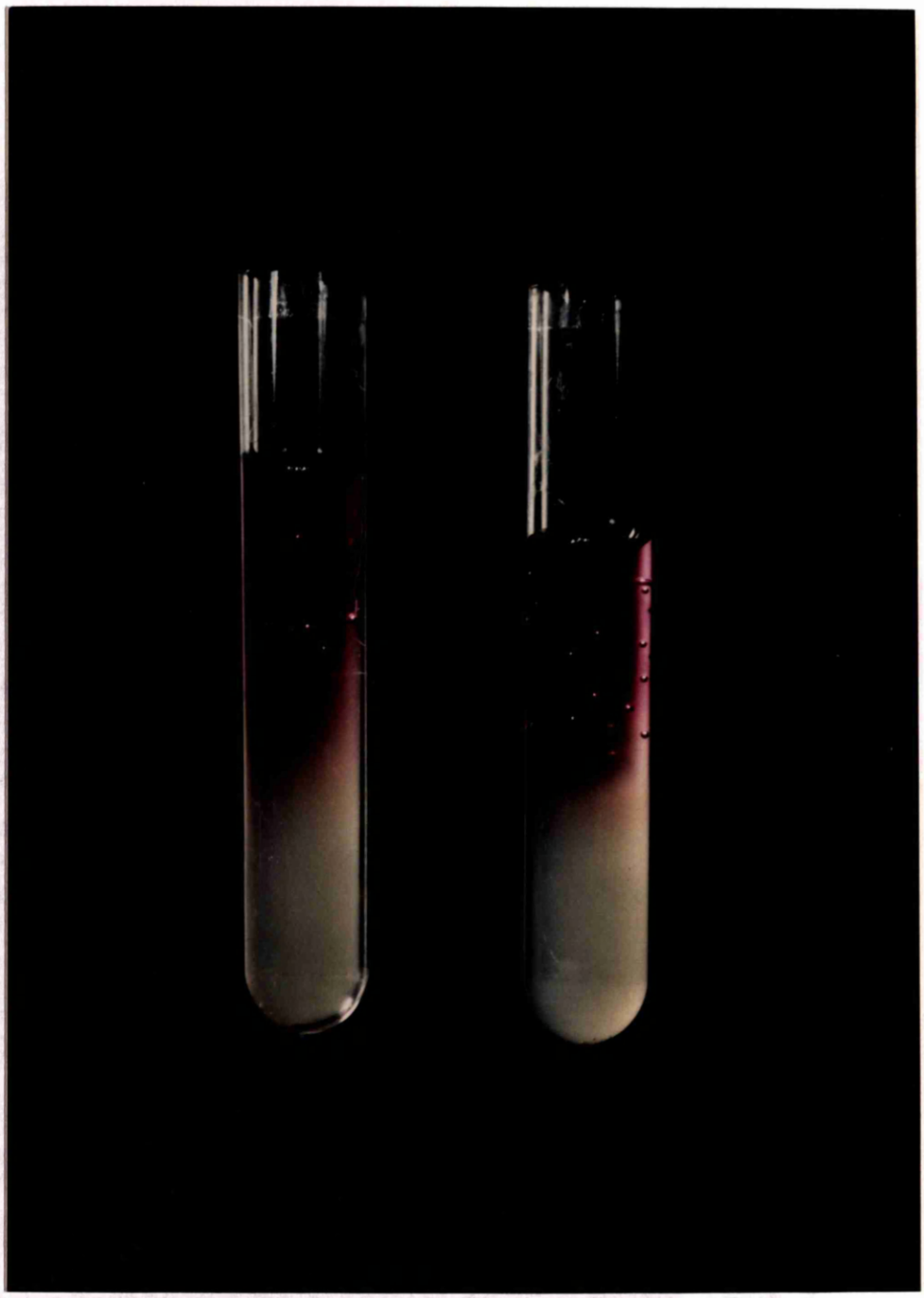


FIGURE 4: TAT - mode of agglutination: HH on left, TT on right.

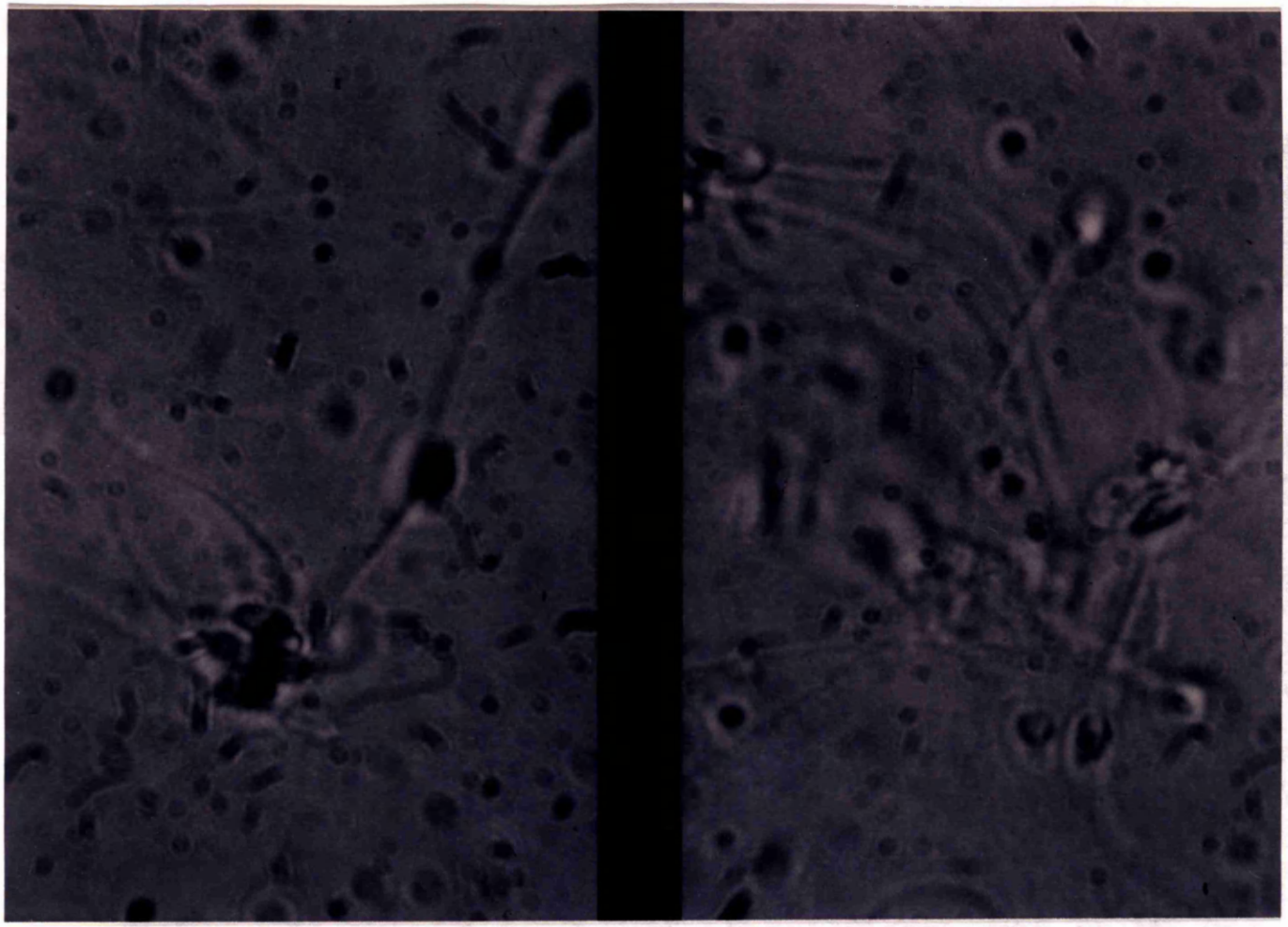


FIGURE 5: TAT - inverted microscope.

FIGURE 6: MAR - clumps of erythrocytes with attached spermatozoa.

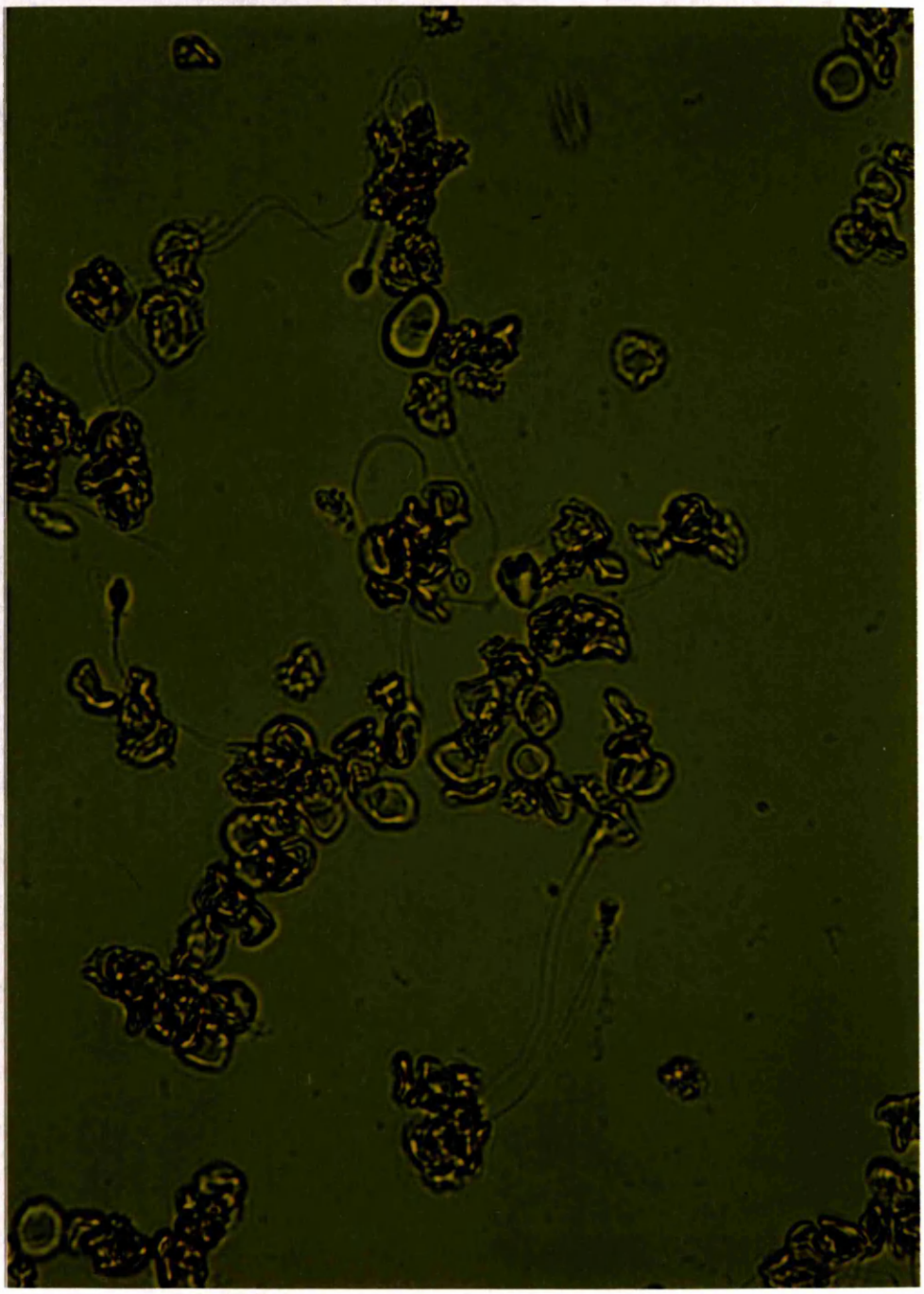


FIGURE 7: IBT - spermatozoa with attached IB.

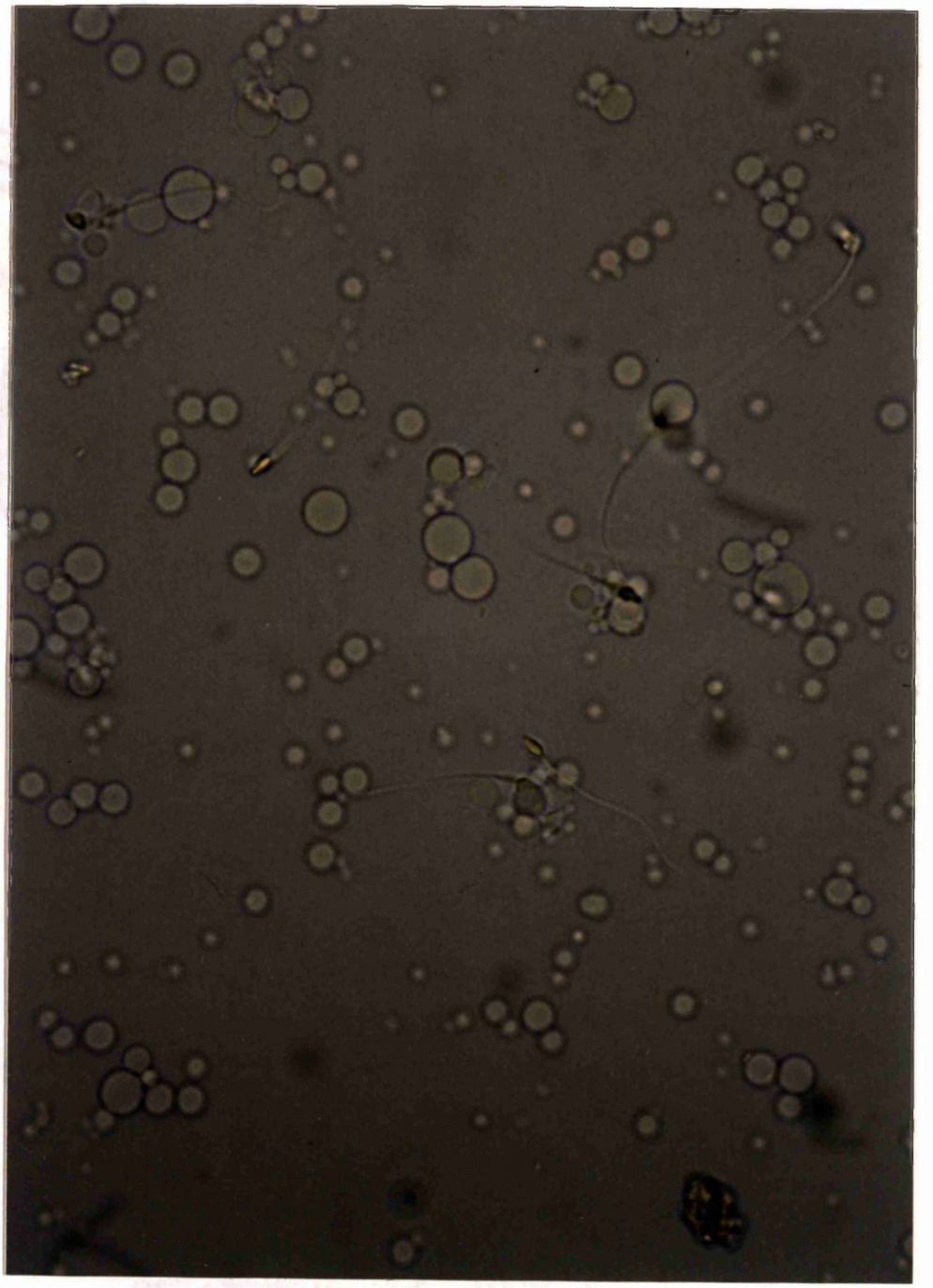


FIGURE 8: Epididymo-vasostomy.

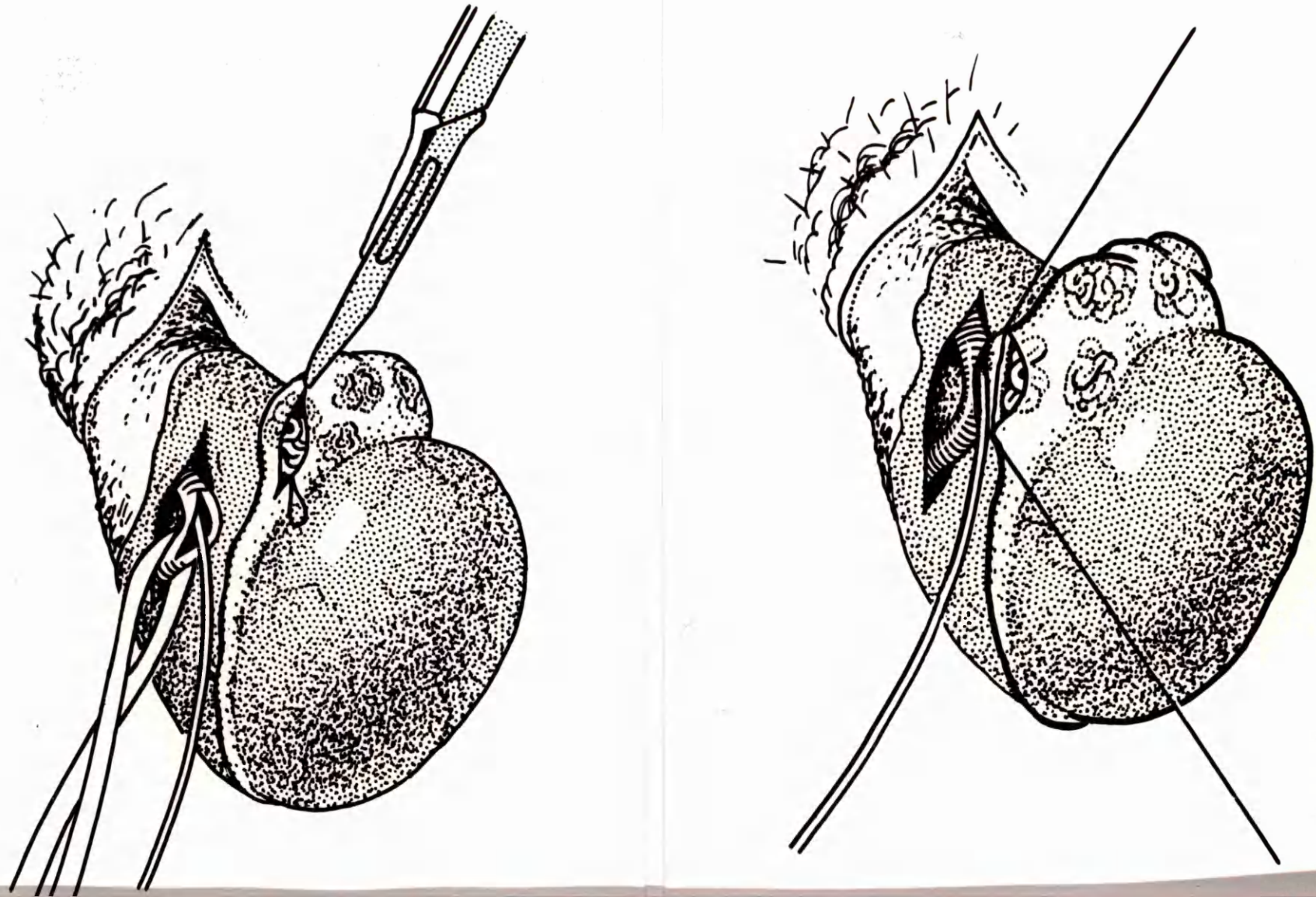


FIGURE 9: Vaso-vasostomy.

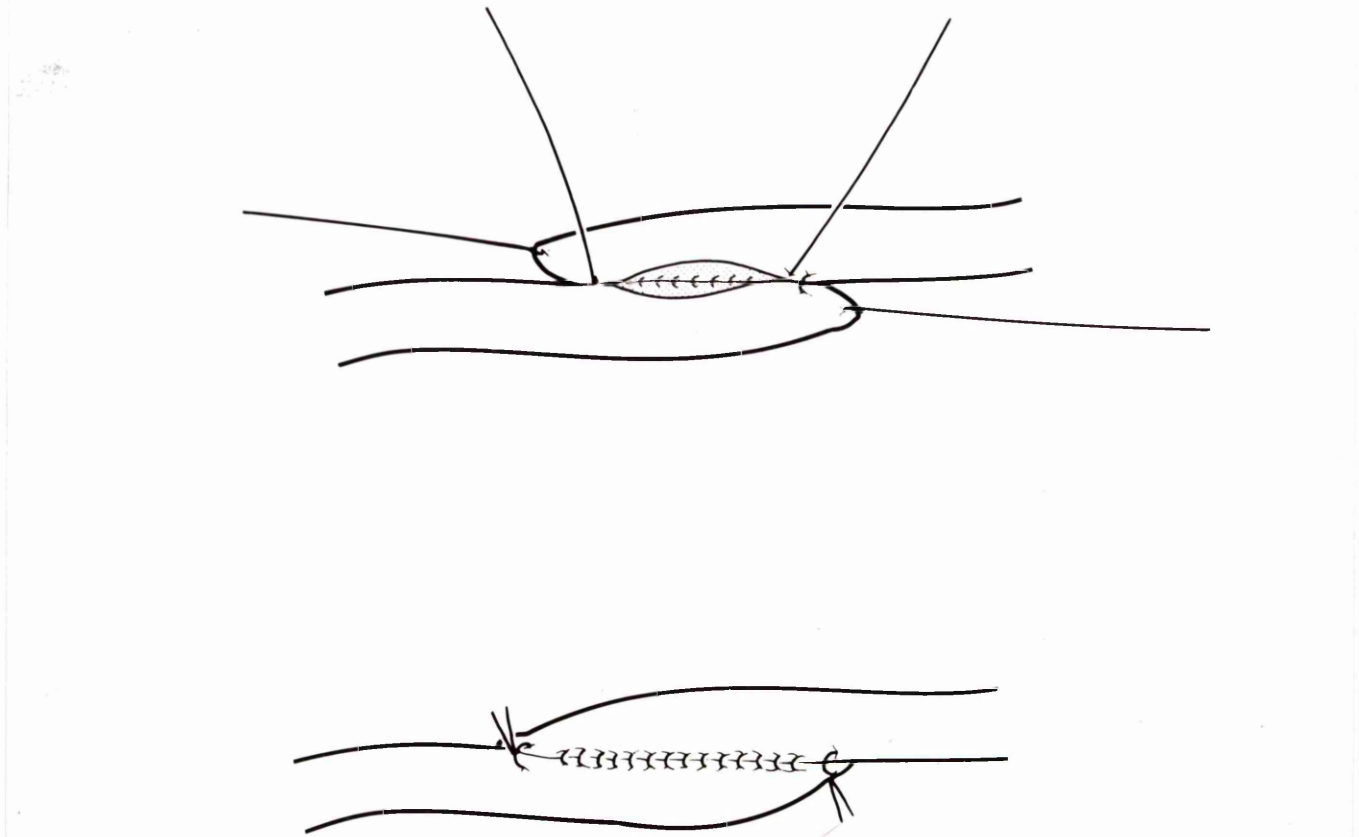


FIGURE 10: Transvaso-vasostomy.

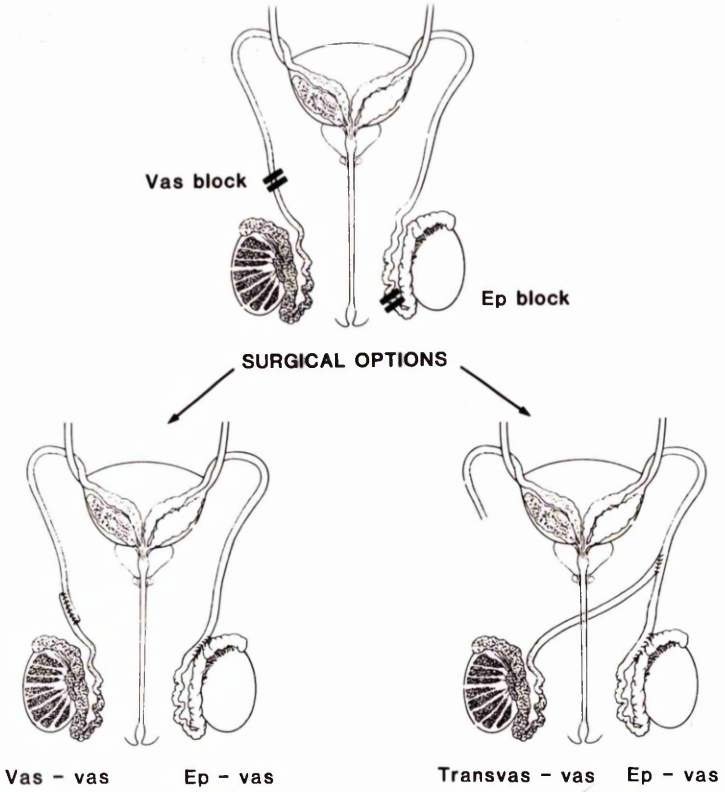


FIGURE 11: Improvement in sperm count with long term low dose prednisolone.

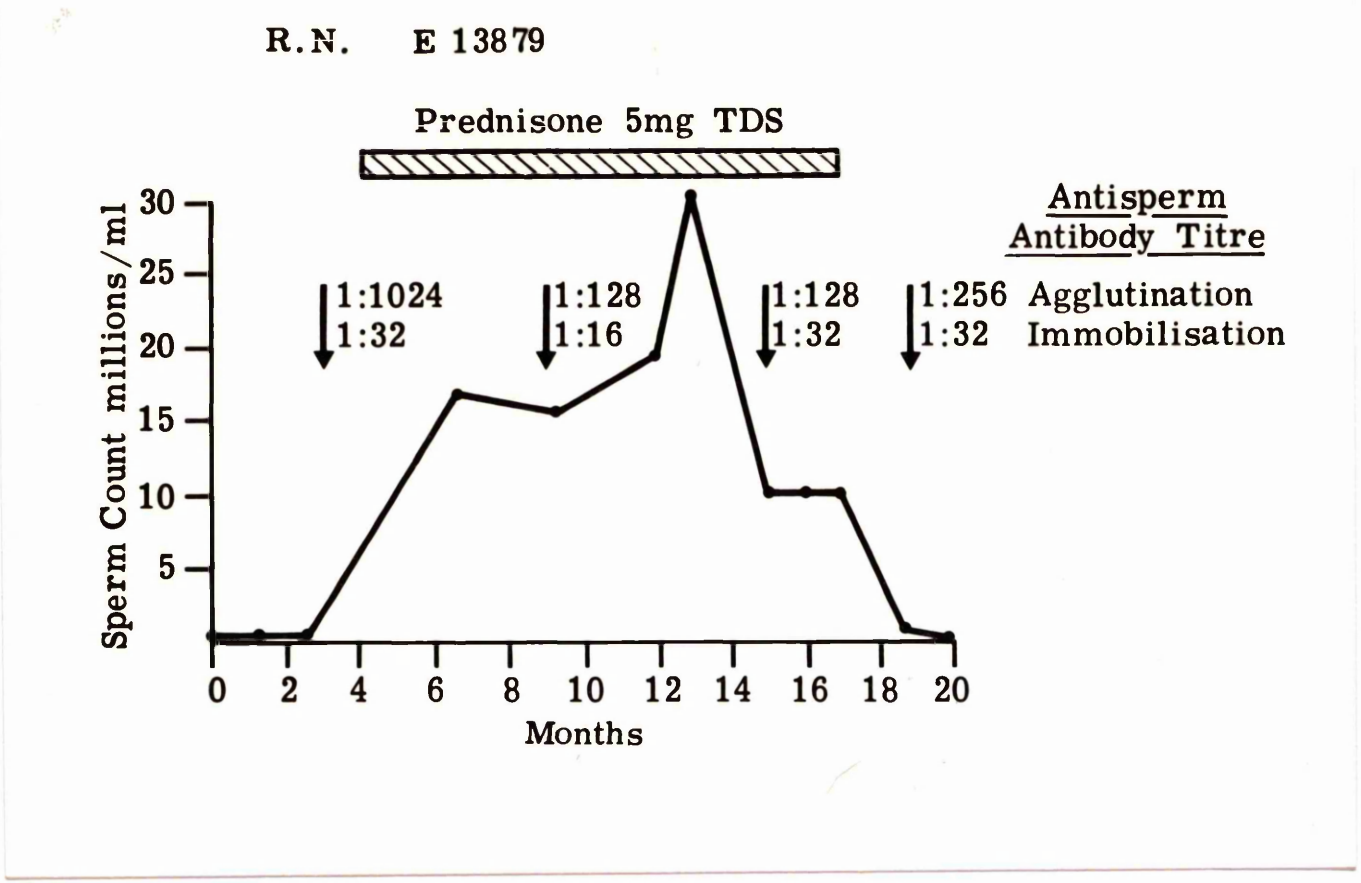


FIGURE 12: Serum GAT titres before and after MP.

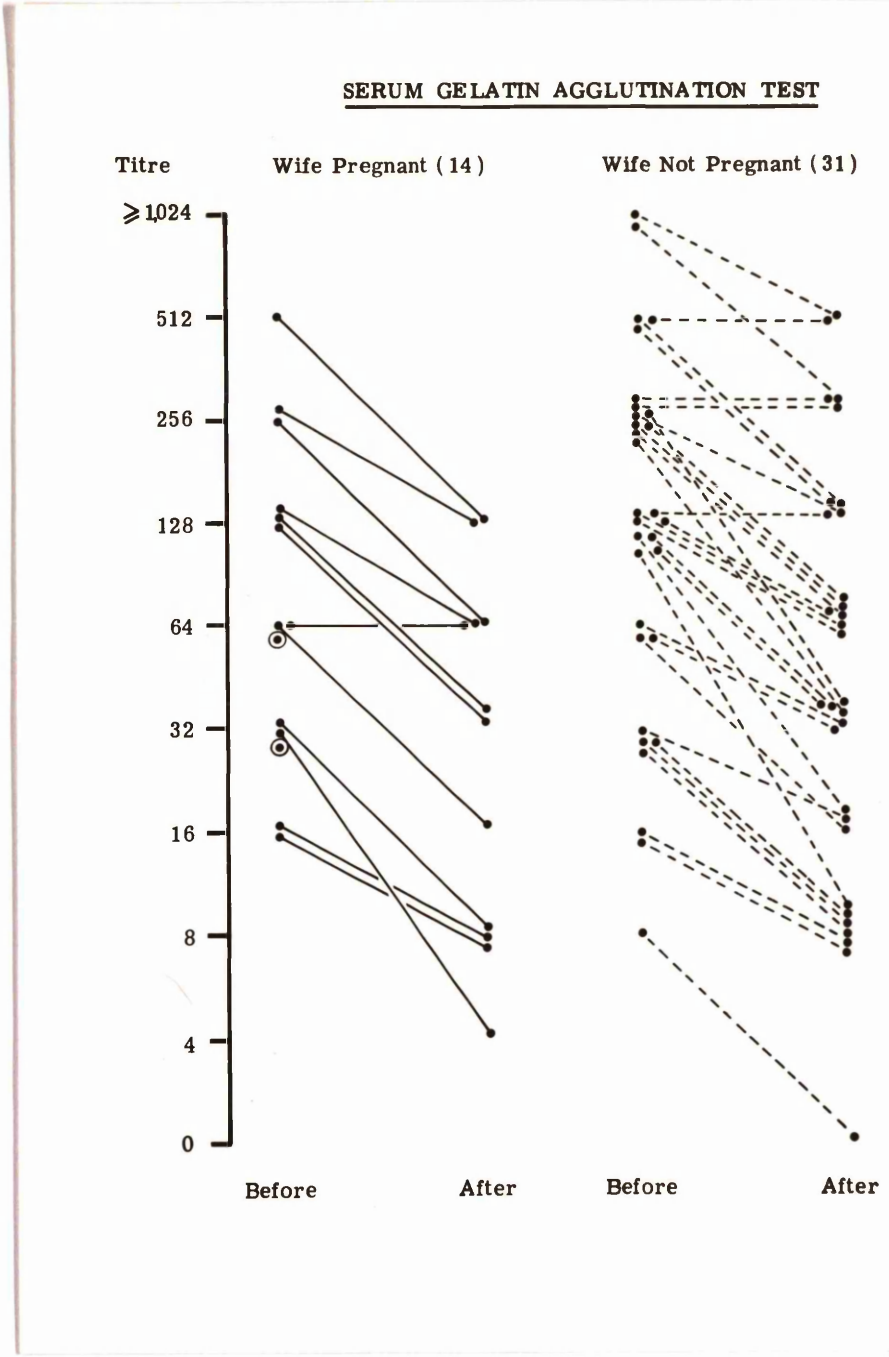


FIGURE 13: Serum SIT titres before and after MP.

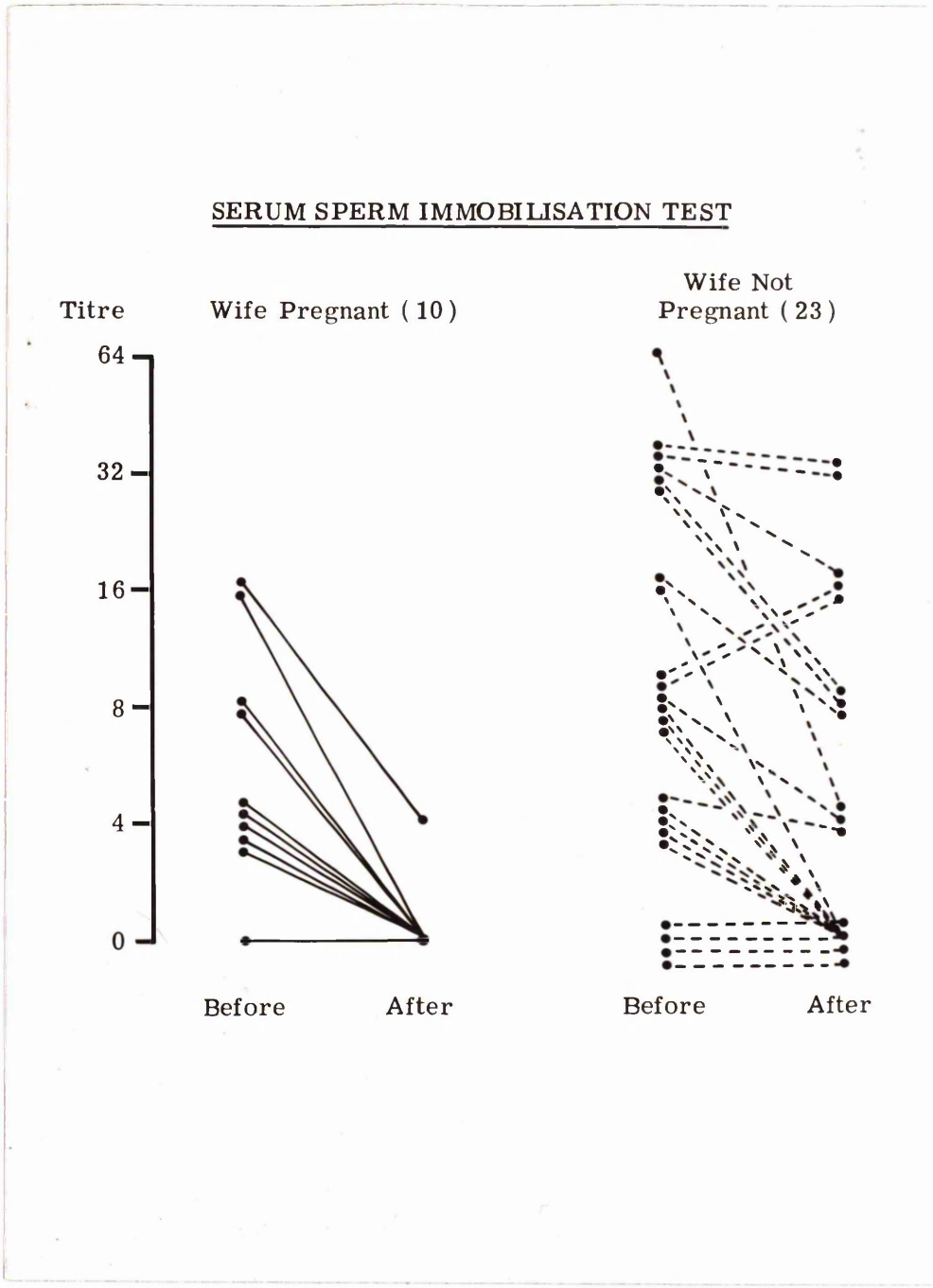


FIGURE 14: Cumulative pregnancy rate for subfertile men with ASA treated with cyclical moderate dose prednisolone, compared with the normal population (Cooke et al, 1981) and an unselected group of infertile men (CHW 1971-1978).

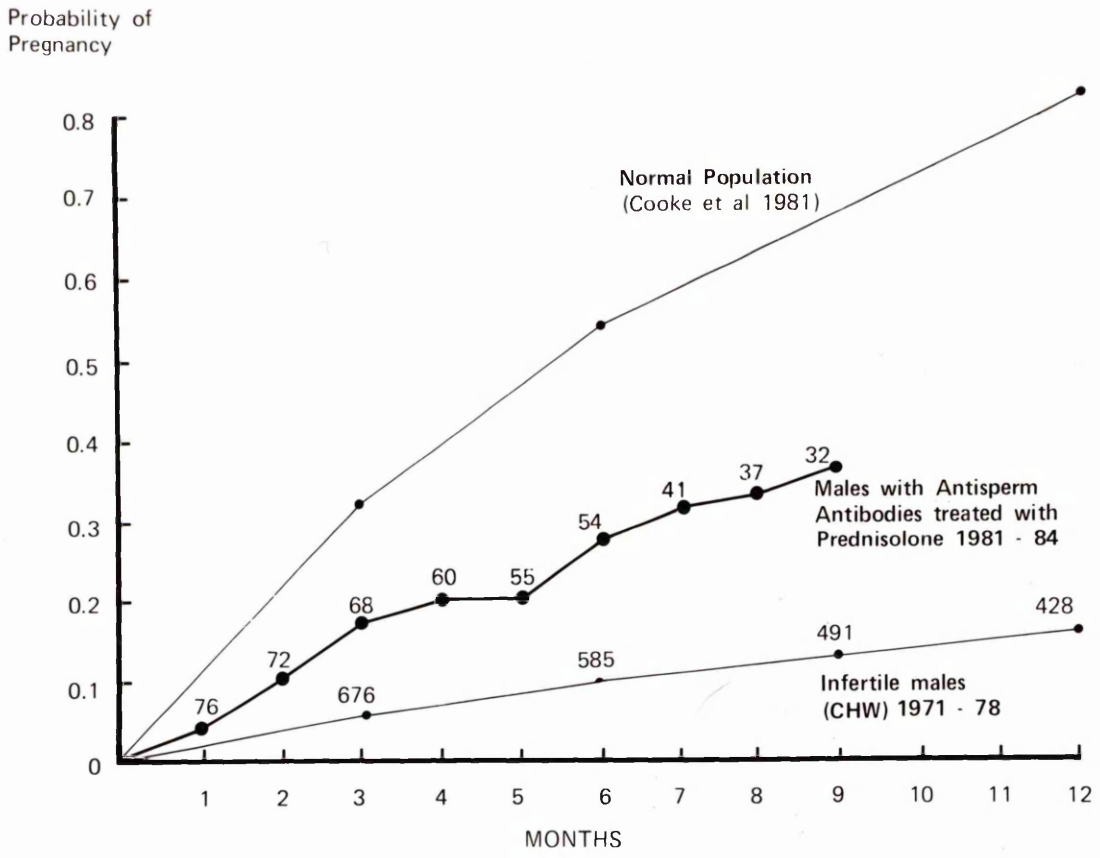


FIGURE 15: Years of infertility before prednisolone therapy, comparing those who successfully produced pregnancies with those who did not.

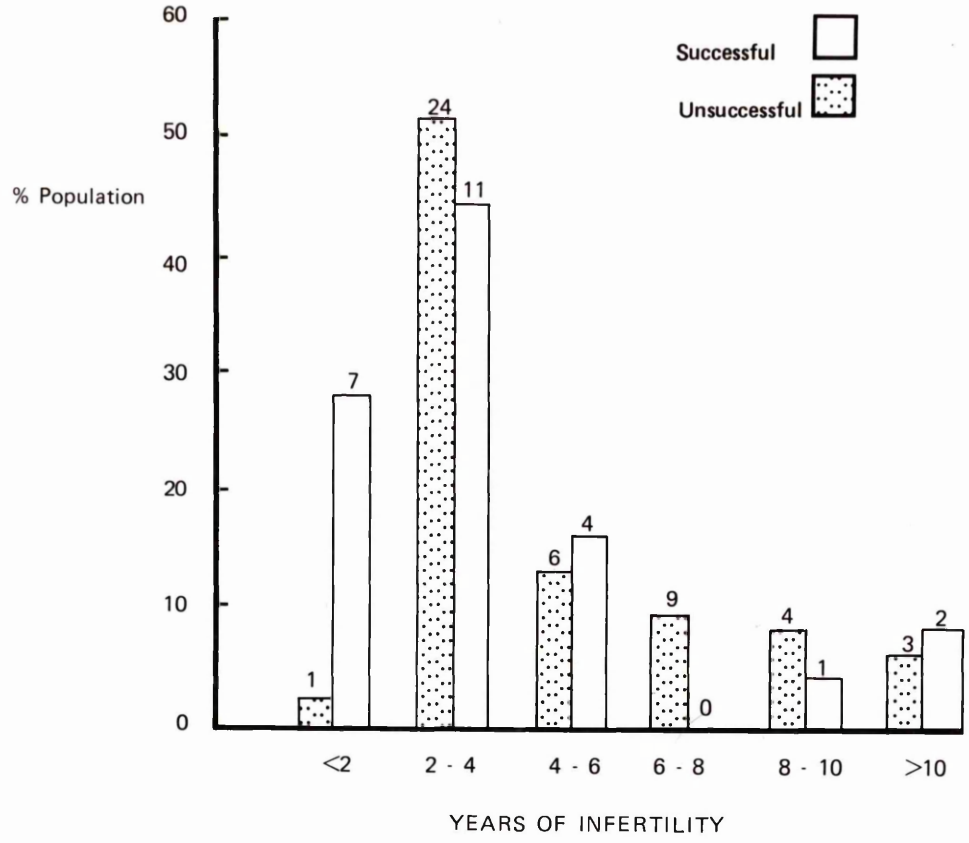


FIGURE 16: Serum TAT titres before and after moderate dose prednisolone treatment.

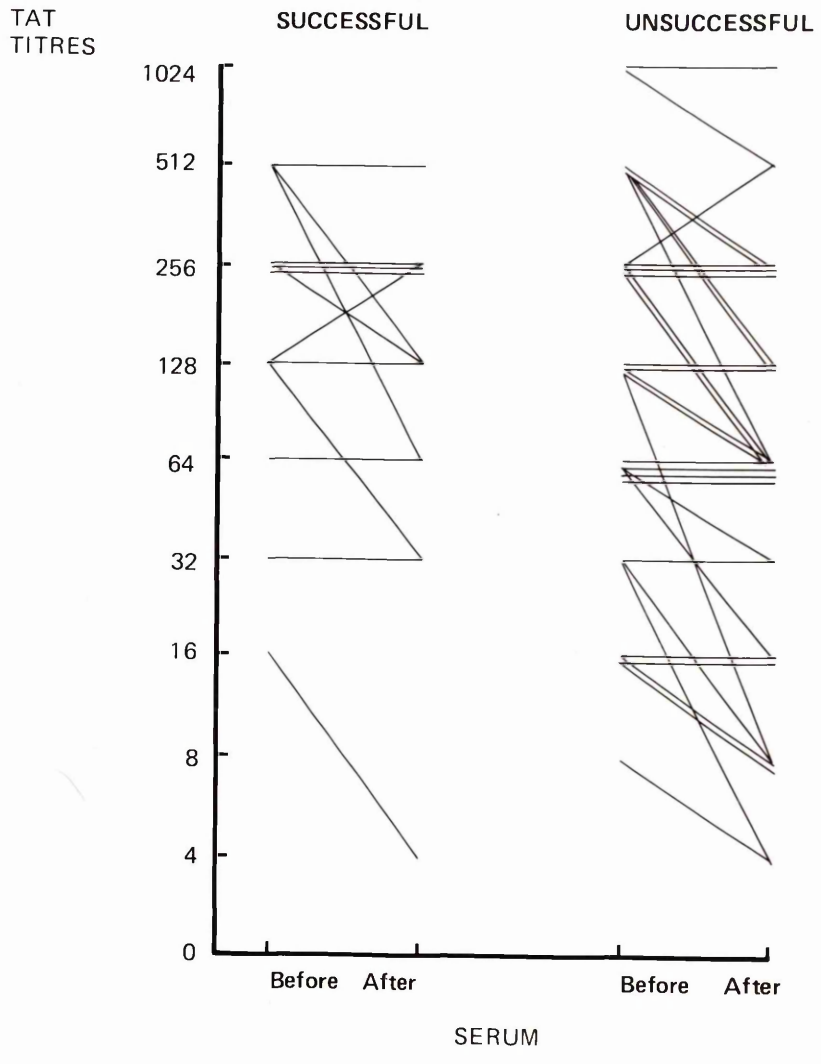


FIGURE 17: Seminal plasma TAT titres before and after moderate dose prednisolone treatment.

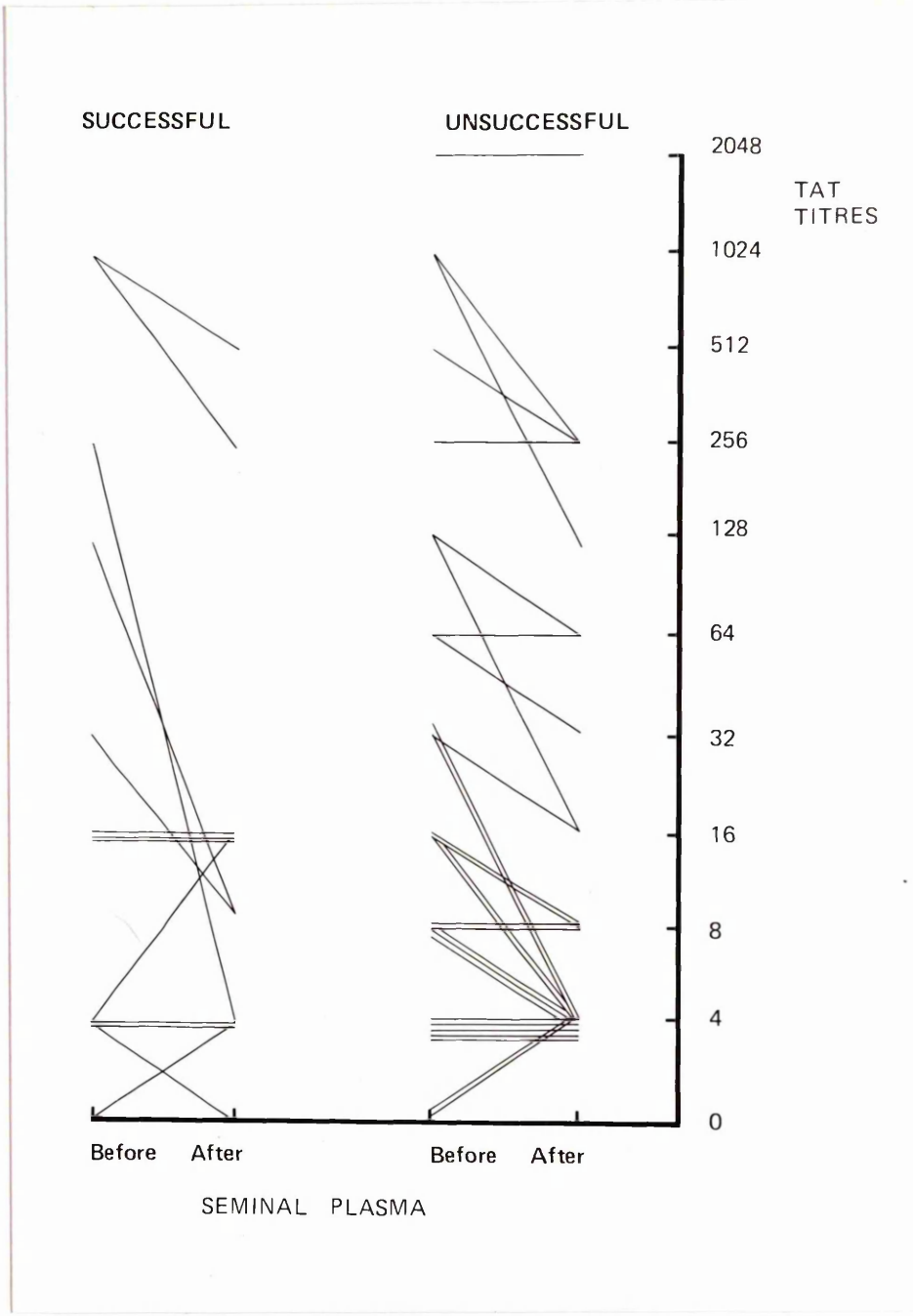


FIGURE 18: Percentage of patients receiving prednisolone and placebo who produced pregnancies.

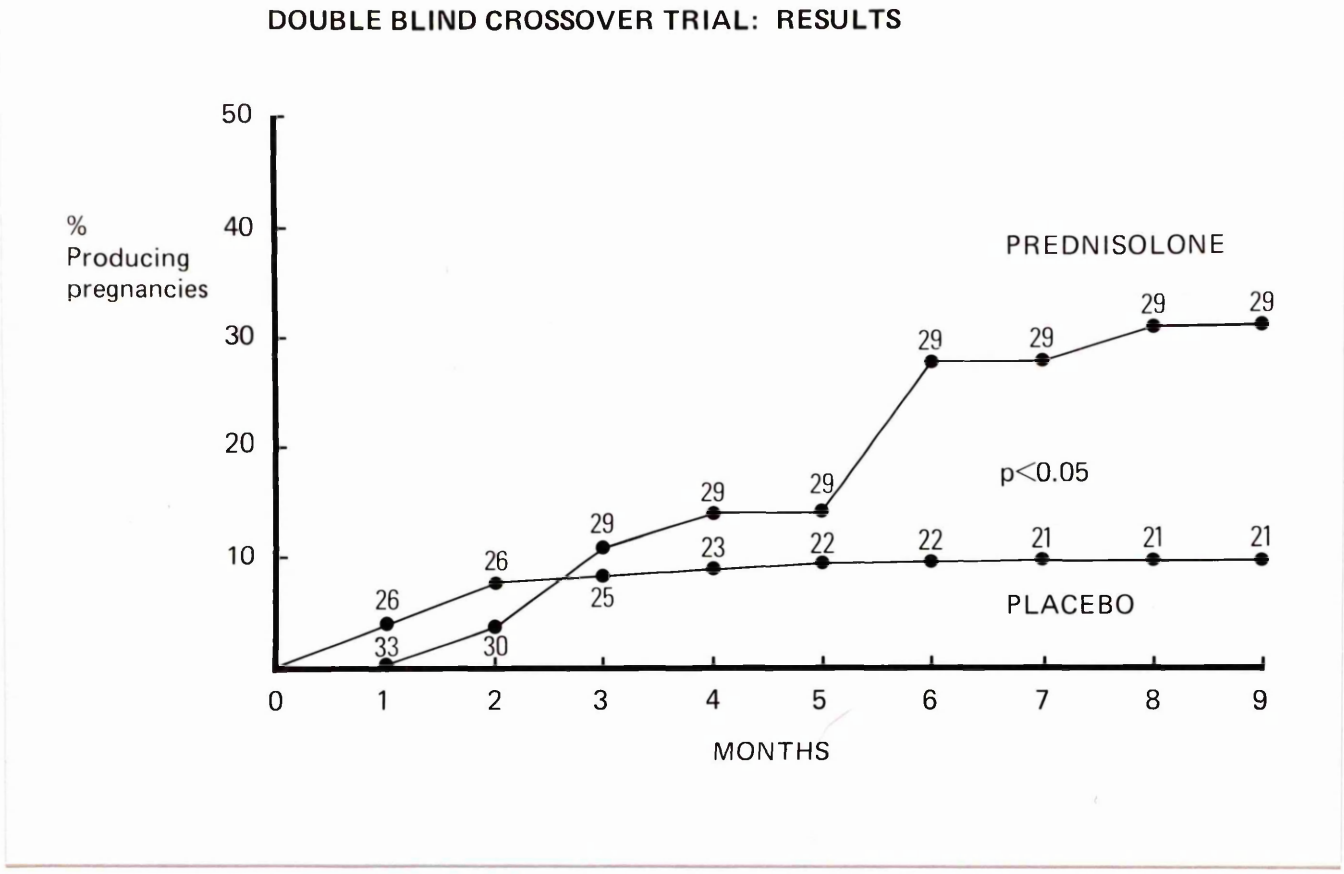


FIGURE 19: Seminal plasma TAT titres for patients receiving prednisolone and placebo.

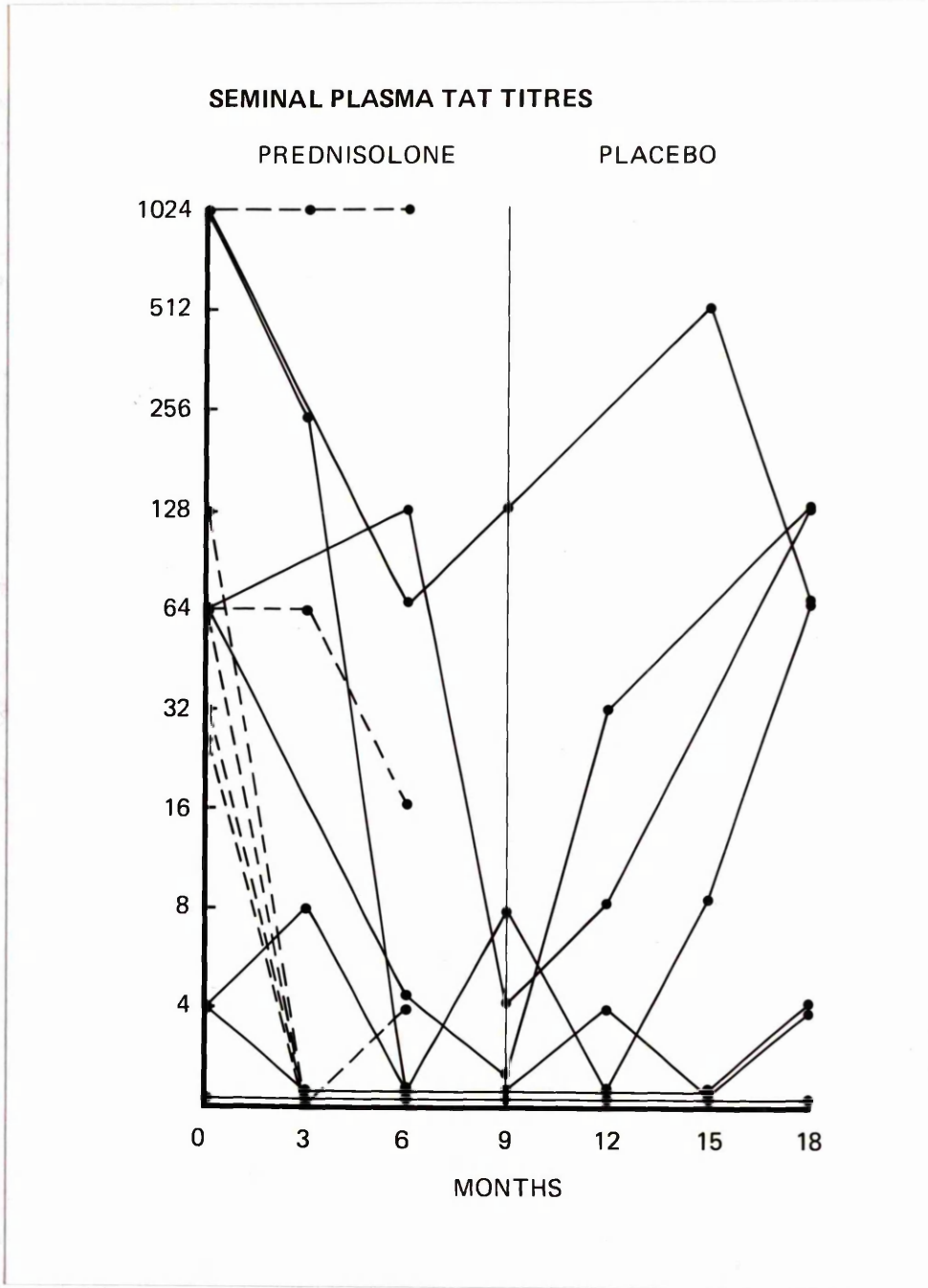


FIGURE 20: Caudal epididymal block affecting the right testicle: note the distension of the epididymis compared to the normal appearance on the left side (the right vas is held in tissue forceps).

FIGURE 21: Vasal obstruction following hernia repair in childhood.

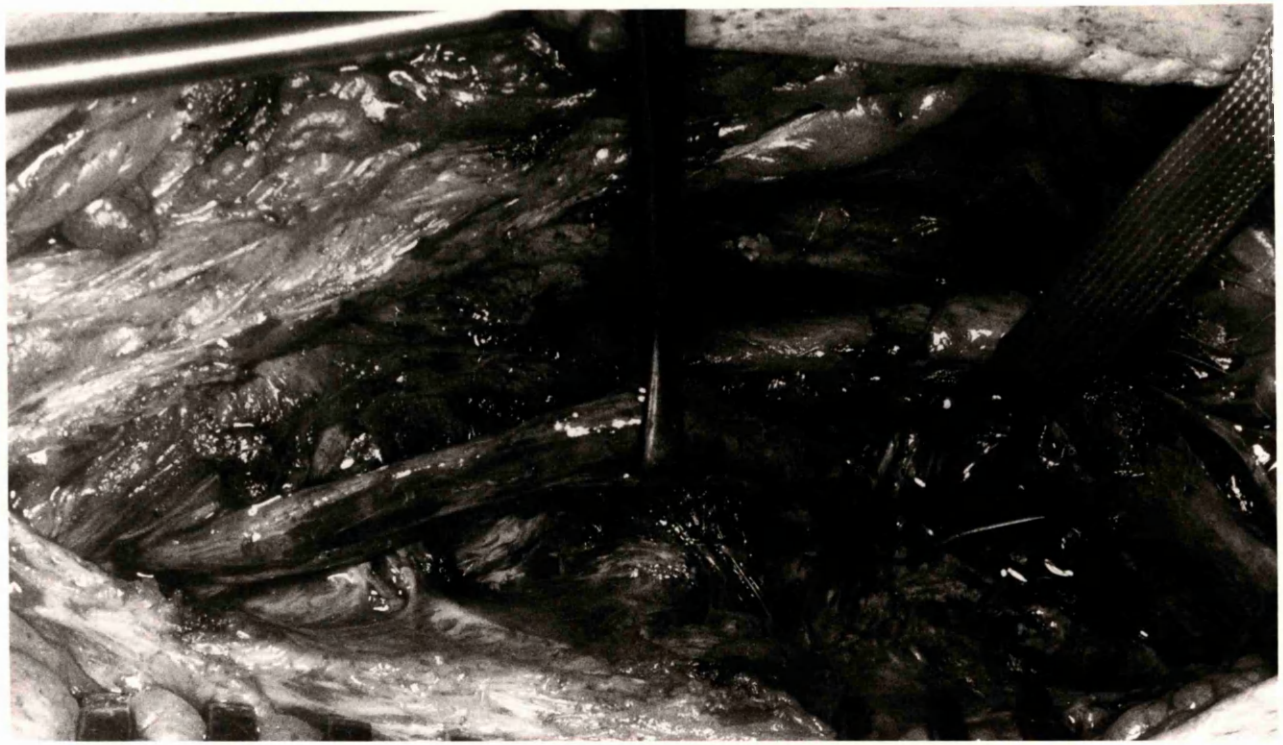


FIGURE 22: Serum and seminal plasma TAT titres in 34 patients with unilateral testicular obstruction: note that most had seminal plasma ASA, often in high titres. Those represented by open circles produced pregnancies after treatment.

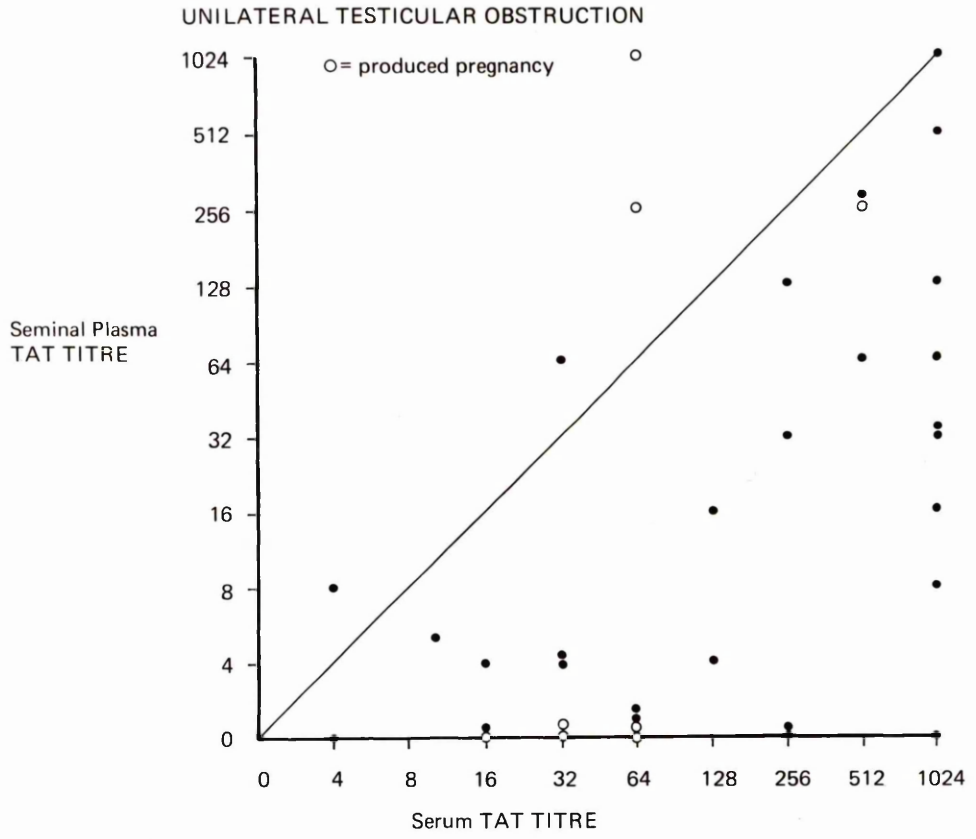


FIGURE 23: ASA titres before and 6 months after removal of the obstructed testis, and on subsequent prednisolone therapy. Pregnancies produced by those represented by open circles - serum TAT titres.

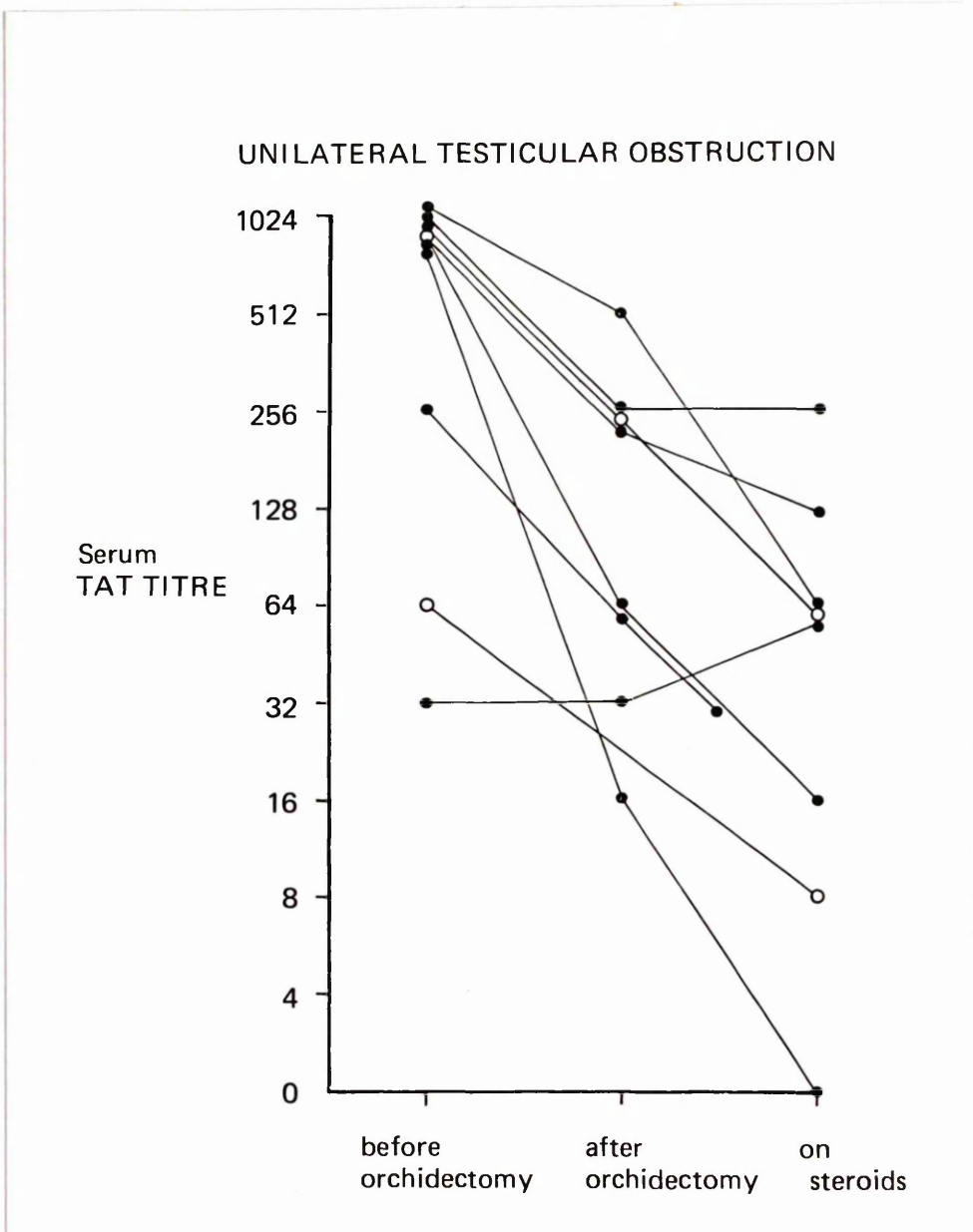


FIGURE 24: ASA titres before and 6 months after removal of the obstructed testis, and on subsequent prednisolone therapy. Pregnancies produced by those represented by open circles - seminal plasma TAT titres.

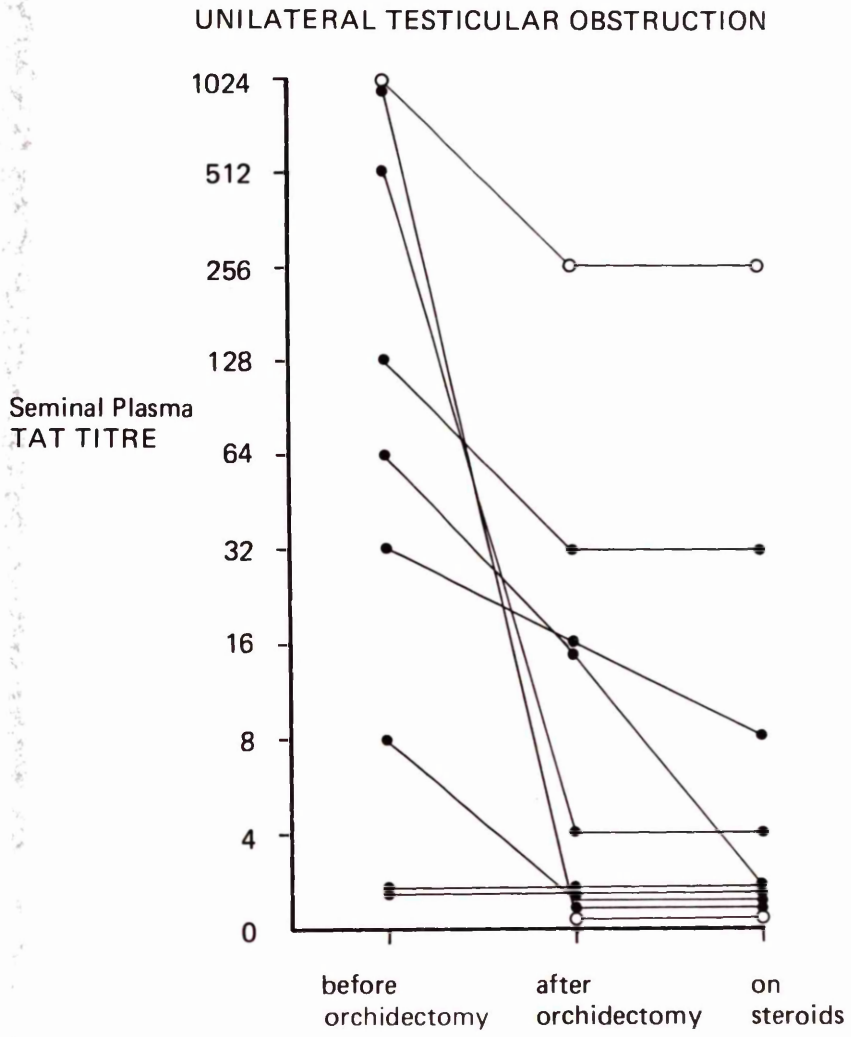


FIGURE 25: Sites of obstruction in 168 azoospermic males.

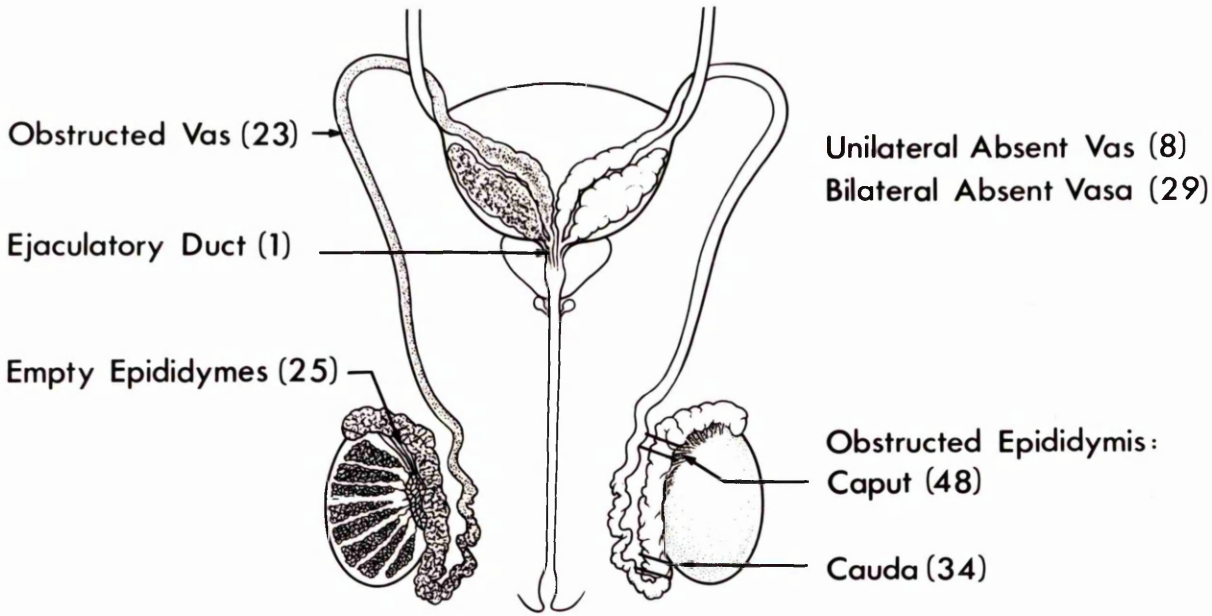
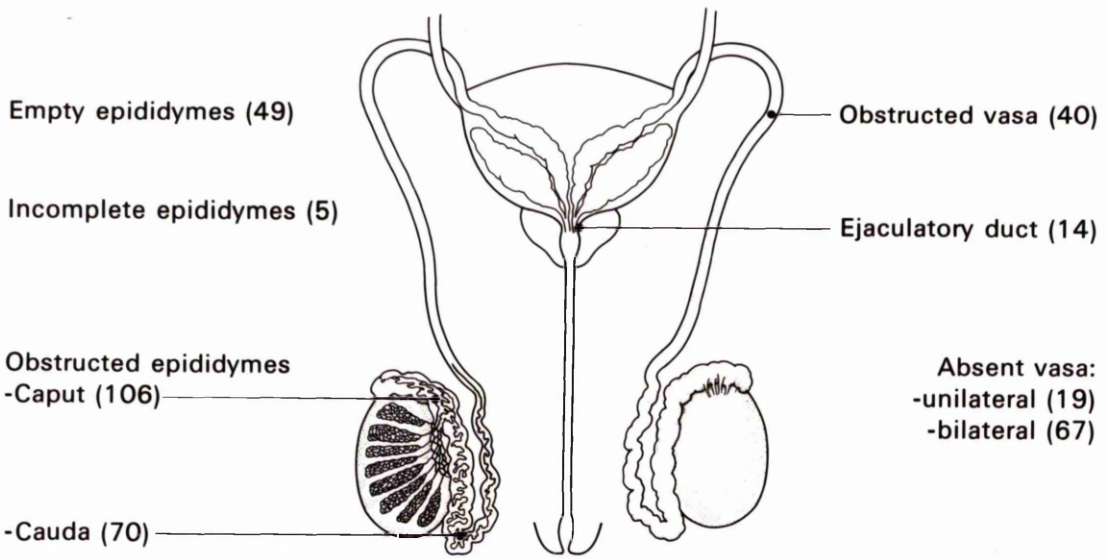


FIGURE 26: Sites of obstruction in 370 azoospermic males.



Sites of obstruction in 370 azoospermic males

FIGURE 27: Serum TAT titres in men with unilateral and bilateral testicular obstruction following inguinal or pelvic surgery in childhood.

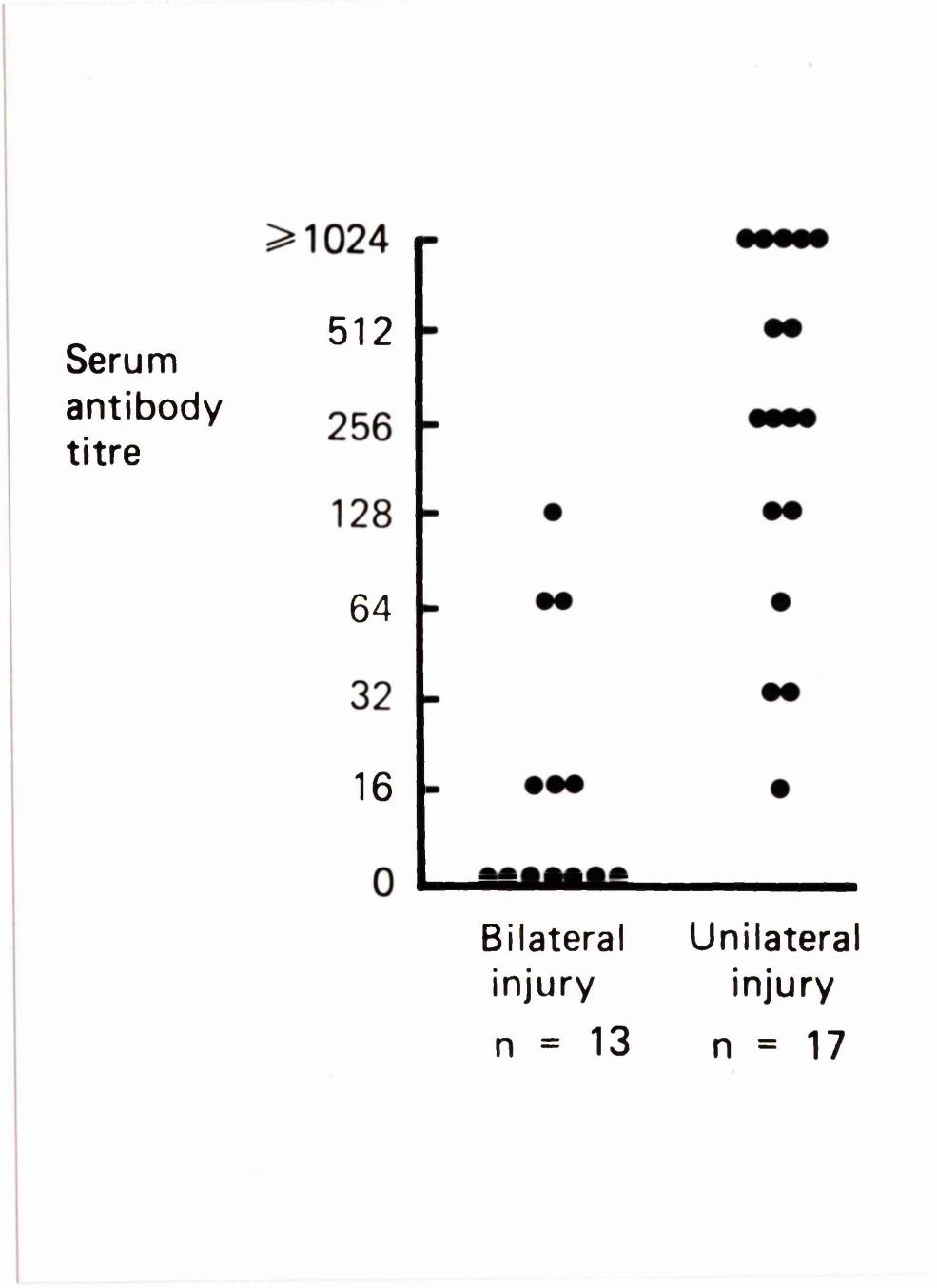


FIGURE 28: Percentage of spontaneously infertile males, and vasectomised men before and after reversal, with seminal plasma ASA related to serum TAT titres.

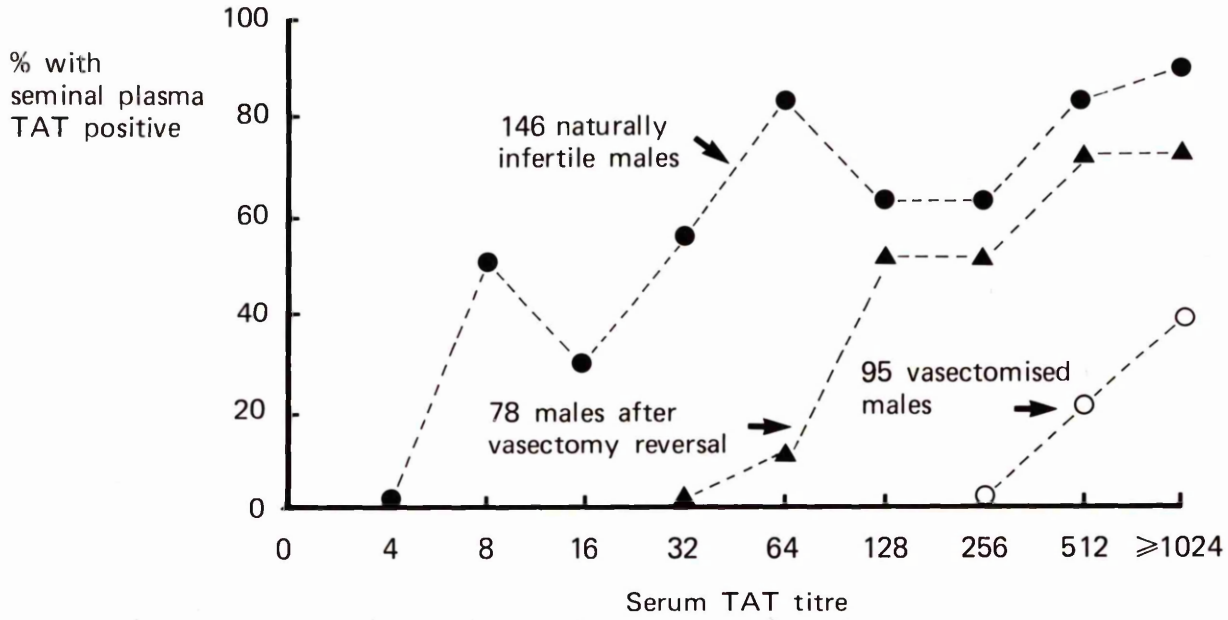


FIGURE 29: Sperm counts and TAT titres in serum and seminal plasma before and after vasectomy reversal: note that ASA were absent before reversal and that an initially good sperm count fell as the titre of antibody rose.

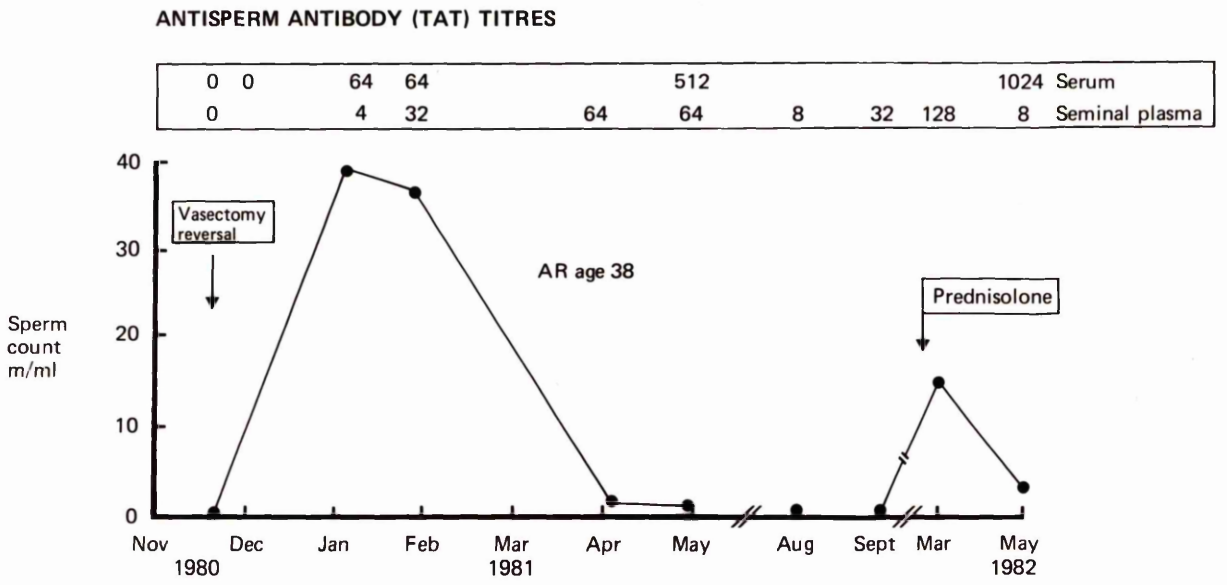


FIGURE 30: Sperm counts and TAT titres in serum and seminal plasma before and after reversal. The ASA titres fell profoundly after reversal and the wife became pregnant twice.

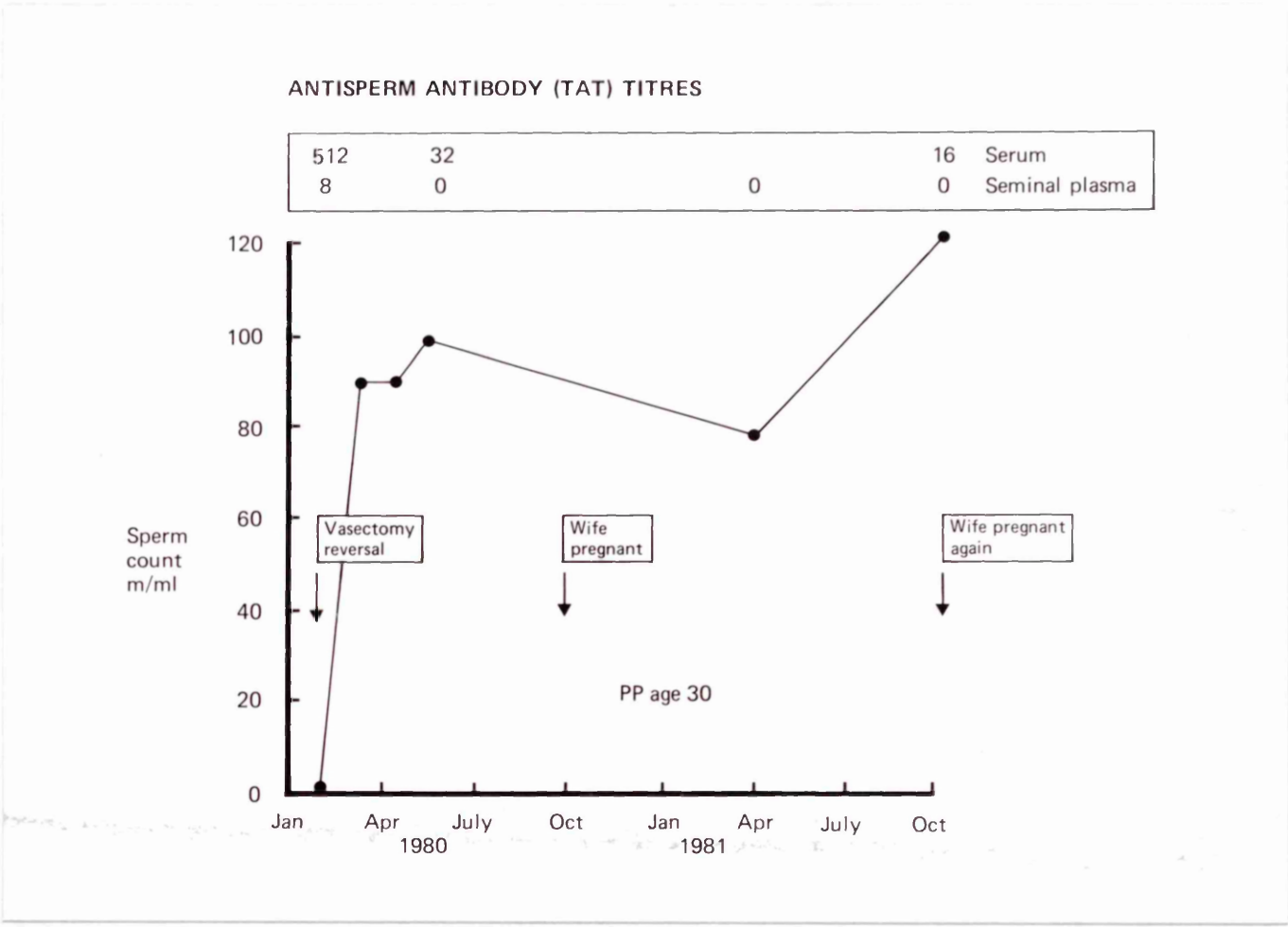


FIGURE 31: Testicular biopsy from a man with severe oligozoospermia and high serum titres (1024) ASA: focal infiltration of lymphocytes and plasma cells extending into inside of seminiferous tubules (H&E).

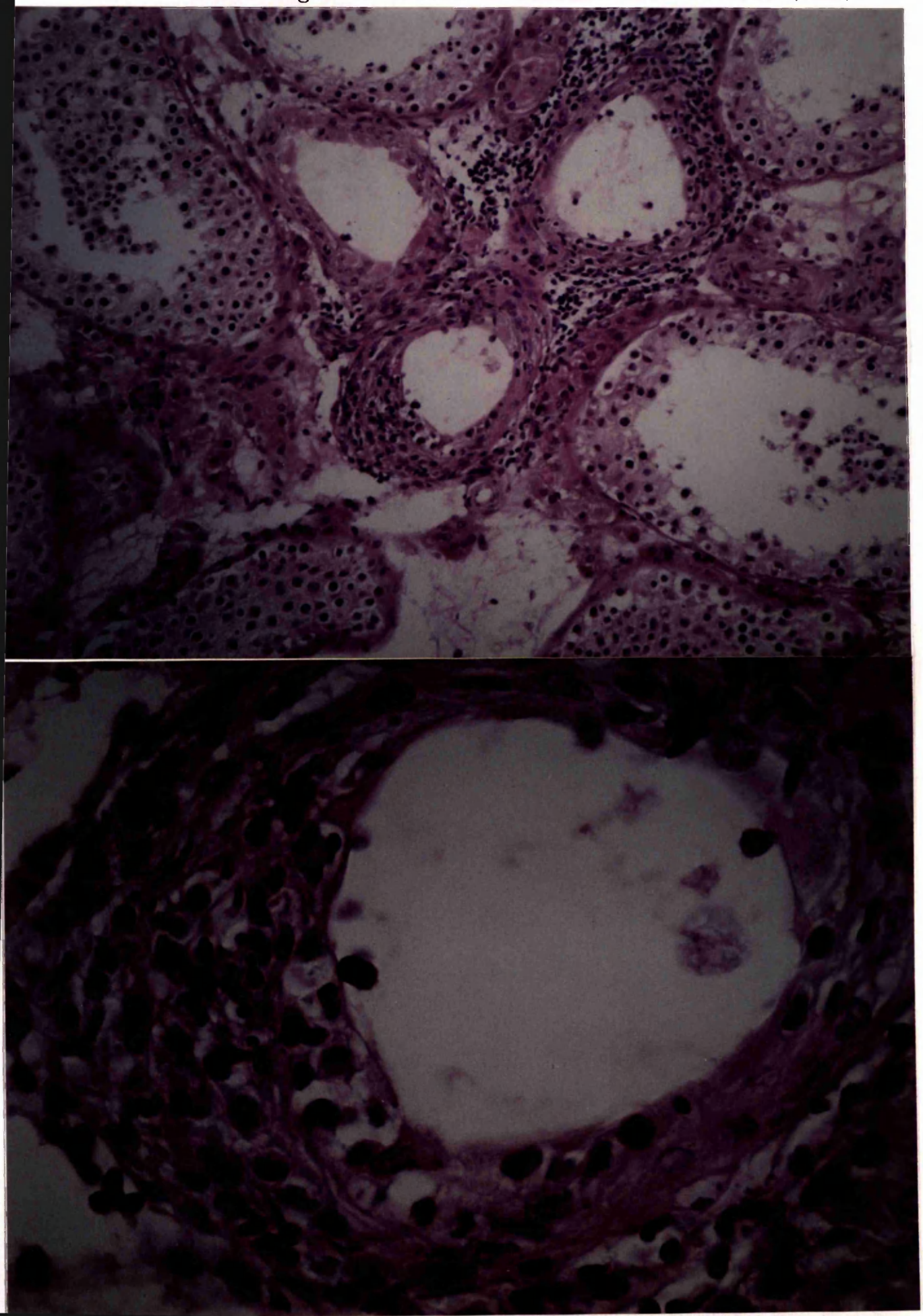


FIGURE 32: Epididymis with mononuclear cell infiltrate (H&E).

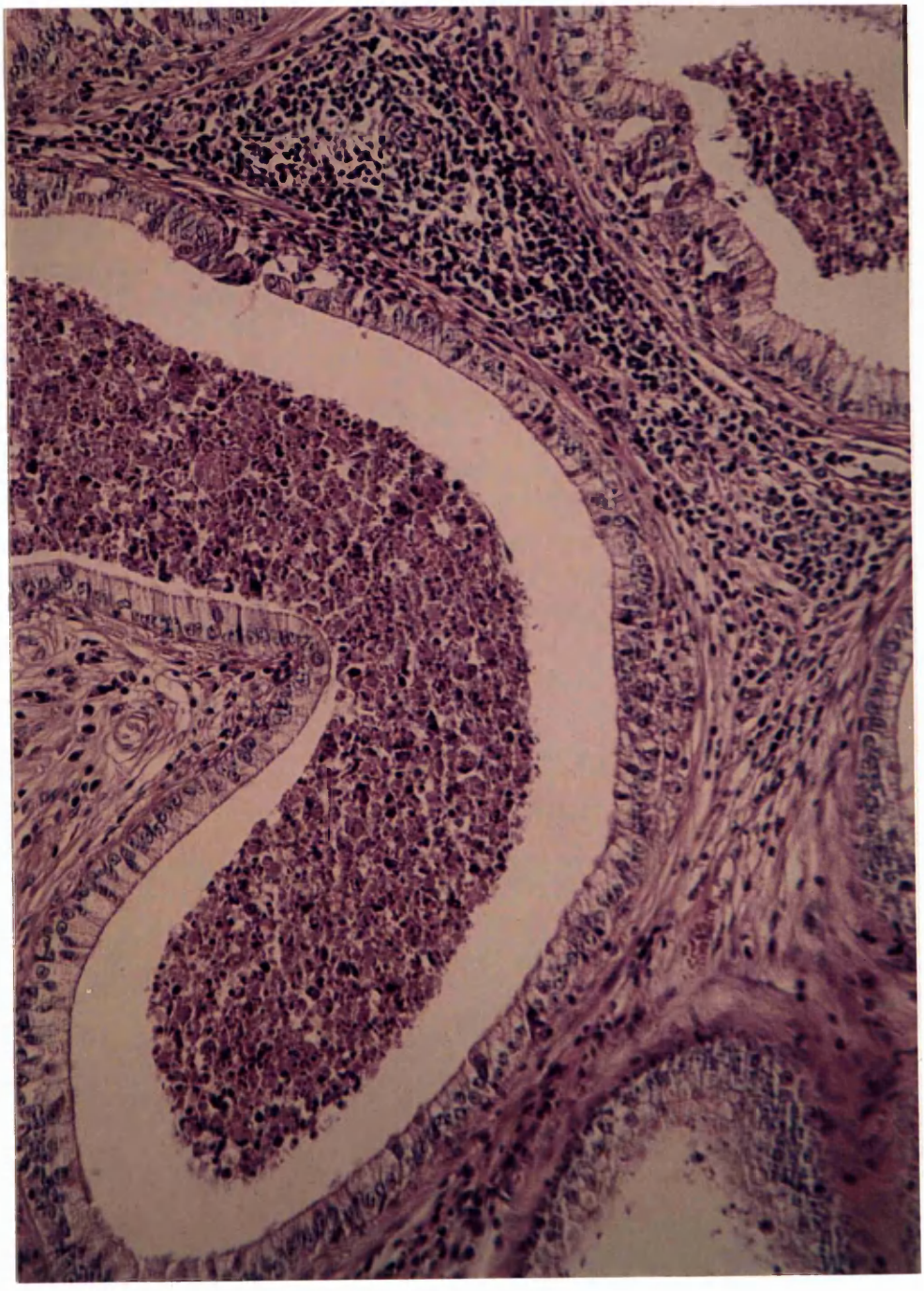


FIGURE 33: Rete testis in patient with high vasal block, empty epididymis and high titres of ASA (1024) (H&E).

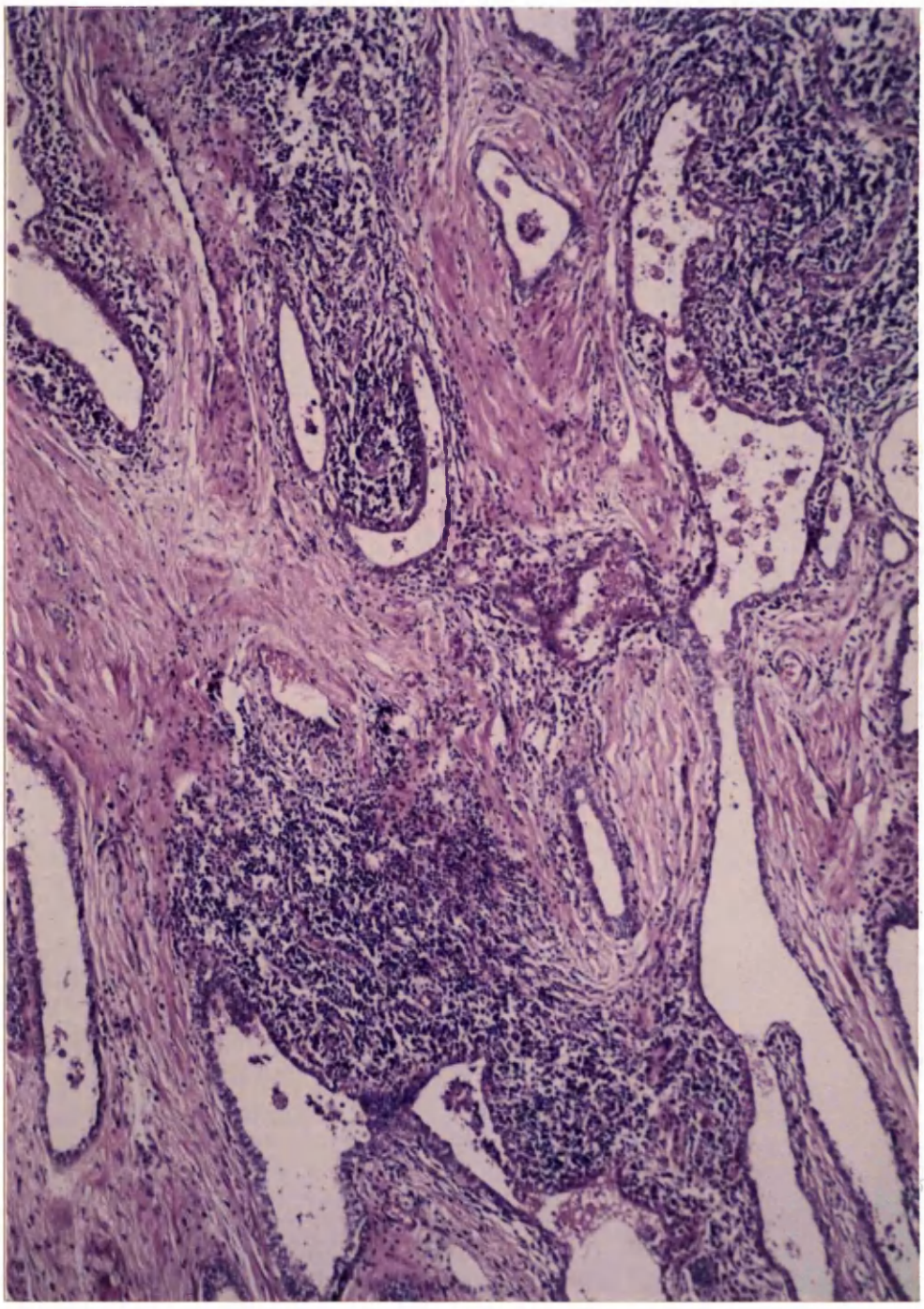


FIGURE 34: Sperm count and TAT titres before and during long term, low dose prednisolone treatment (Johnsen counts shown).

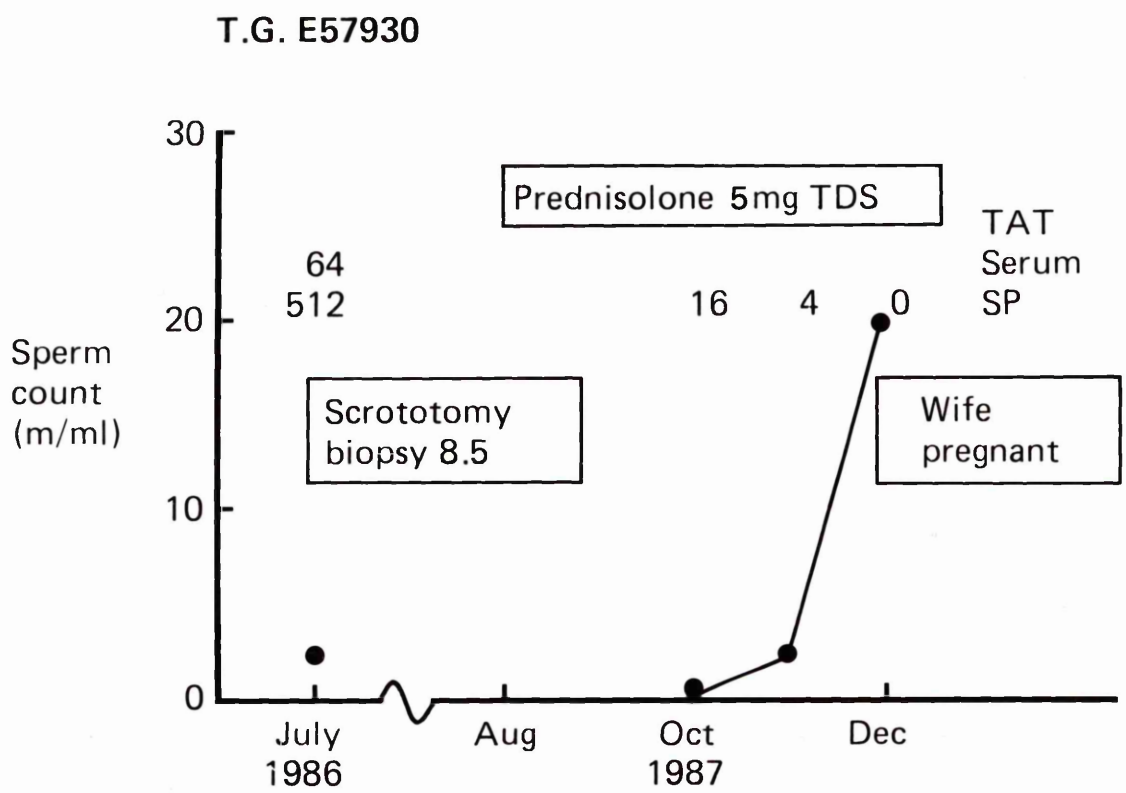
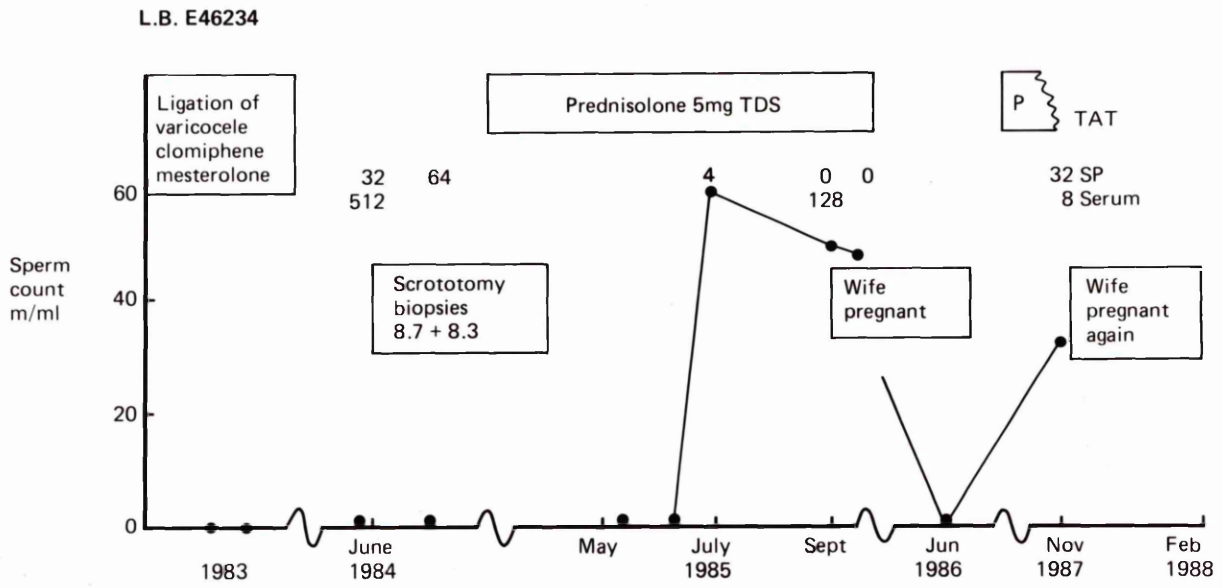


FIGURE 35: Sperm counts and TAT titres in a man with empty epididymes and good spermatogenesis (Johnsen counts shown) but no demonstrable mononuclear cell infiltrate on biopsy, before during and after long term low dose prednisolone therapy.



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