19. Assisted reproduction for male factor infertility

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Until recently, male infertility has carried a poor prognosis for treatment and the majority of couples with a significant male factor would have to consider donor insemination in order to achieve a pregnancy. *In vitro* fertilization (IVF) raised the potential of generating embryos *in vitro*, but, although occasional pregnancies could be achieved after IVF and embryo transfer (ET)[1], the early techniques were found to be of limited benefit in males with severe oligospermia, asthenospermia, antispermatozoal antibodies (ASABs) in the semen, or with abnormal sperm morphology. It was found to be difficult to achieve fertilization and the degree of difficulty was directly proportional to the severity of the semen disorder[2,3] or the number of abnormal semen parameters. Consequently pregnancy rates for IVF-ET remained low.

Subsequently, when the GIFT (gamete intrafallopian transfer) technique[4] was introduced, new hopes were raised as the method was considered to have the benefit of being closer to the natural process of fertilization and would therefore be superior for all non-tubal forms of infertility. However, although GIFT proved to be more effective than IVF-ET for many categories of infertility[5], it proved to have major limitations for male factor infertility. A modified protocol[6] involving the transfer of greater sperm numbers enabled reasonable pregnancy rates to be achieved in cases of moderate oligospermia but it was subsequently shown that the pregnancy wastage rate was higher[7,8], possibly due to polyspermic fertilizations and the inability to control the quality of embryos available *in vivo*.

The problem of managing the male factor by IVF is made even more difficult because of diagnostic limitations, particularly with respect to the reliance on semen analysis, the complex and often multifactorial nature of infertility, the finding that some relatively 'normospermic' men fail to achieve *in vitro* fertilization and the generally poor state-of-the-art in understanding the aetiological nature of male infertility. This chapter will not dwell on semen analysis parameters, which are dealt with comprehensively in another chapter, but will focus on recent clinical and laboratory developments which are beginning to have a dramatic impact on the prognosis for male factor infertility. These will be discussed under three headings – evaluation, *in vitro* techniques and clinical procedures.

EVALUATION

Semen analysis

Male factor infertility may include normospermic and polyspermic males whose spermatozoa lack the capacity for fertilization. However, the majority of cases are identified by obvious semen abnormalities, e.g. oligospermia, asthenospermia or teratospermia. Sometimes genital tract inflammation may be identified by the presence of raised numbers of polymorphonuclear leukocytes. Some laboratories have included automated semen analysis[9] and the zona-free hamster penetration (HOP) test[10,11] but standardization is difficult and the clinical value of both techniques remains uncertain. Most laboratories are familiar with the World Health Organization (WHO) laboratory manual which was significantly upgraded in 1987[12] and which describes the standard conditions for collection of the semen sample, its delivery and the standardization of laboratory procedures. The WHO standards indicate that a normal sample will contain at least 20×10^6 spermatozoa/ml with at least 50% exhibiting good to excellent forward progressive movement within 60 minutes after collection. We have adopted a simple approach to

the categorization of semen abnormalities; one which takes into account variations in number, motility and morphology:

- moderate oligospermia ($< 10 \times 10^6$ motile sperm/ml);
- severe oligospermia ($< 5 \times 10^6$ motile sperm/ml);
- very severe oligospermia ($< 1 \times 10^6$ motile sperm/ml); and
- abnormal morphology (< 50% of normal forms).

The above categories appear to have some relevance in that *in vitro* fertilization results do bear a direct relationship to the degrees of oligospermia. Normospermic fertilization rates *in vitro* are usually around 70% of suitable grade preovulatory oocytes. The rates are lower for oligospermia, being around 50% for moderate, 20–25% for severe; and 10% or lower for very severe grades. Abnormal morphology *per se* does not appear to influence *in vitro* results unless it causes the number of available motile sperm with normal head morphology to fall within the oligospermic ranges. However, experienced embryologists within IVF laboratories are usually less concerned with *numbers* than with the *proportion* of sperm displaying progressive motility (< 30% signifies difficulty regardless of the original sperm numbers or other semen parameters (see Figure 1) and the *grading* of that motility (even very low numbers of sperm with high-graded motility (+++) can be expected to achieve satisfactory rates of *in vitro* fertilization). It is in the evaluation of these latter parameters that automated semen analysis may prove objectively beneficial.

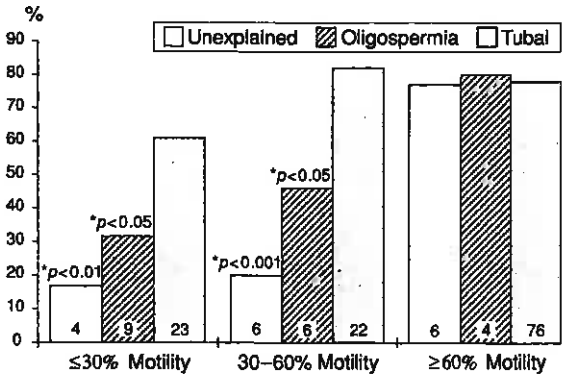

Figure 1 Data reported in Helsinki, 1984[3] indicating that asthenospermia is a relevant predictor of *in vitro* fertilization even if the total motile sperm numbers fall in the normal range. This was most marked for cases of unexplained infertility, implying that a proportion of these have abnormal sperm function. The statistics analyse oocyte fertilization rates (number of cases noted) for the respective infertility subgroup compared with the normospermic tubal group

Antisperm antibodies in semen

It is useful to screen for antisperm antibodies (ASABs) routinely in couples being assessed for infertility (e.g. semen, female serum and cervical mucus are suggested). An alternative but less satisfactory approach is to screen semen and female serum on the basis of a poor *in vitro* fertilization rate or failed fertilization. In the routine assessment of infertile males at PIVET Medical Centre, ASABs in semen are detected by the indirect immunobead test (Bio-Rad), with categorization of antibodies into the appropriate immunoglobulin class (IgA, IgG, IgM). The presence of both IgA and IgG together in semen has been correlated significantly with negative postcoital tests and reduced fertilization rates *in vitro*[13]. IgM is only occasionally found in semen (5/1534; 0.03%) and does not appear to affect fertilization. The mixed agglutination reaction (MAR) test may be a less expensive screening aid for ASABs.

Structural sperm examination

Semen samples from cases of asthenospermia and unexplained failed fertilization may be evaluated further. Zamboni[14] has revealed a range of ultrastructural abnormalities of the spermatozoon and has shown the electron microscope to have an invaluable role in such cases. In particular, certain axonemal defects are of relevance when considering motility enhancement and micromanipulation treatments. Some cases displaying head defects with nuclear abnormalities should be excluded from assisted reproduction. However, these studies are enormously time consuming and extremely demanding of specialized skills; hence individual cases should be screened and selected with due respect to the relevance of the information likely to be obtained.

Sperm function studies

Acrosome reaction to ionophore challenge

One test of sperm function developed at PIVET, which is rapidly proving clinically relevant, examines the ability of the sperm to undergo an acrosome reaction following challenge with the calcium ionophore A23187. This test has become known as the ARIC (acrosome reaction to ionophore challenge) test[15].

Before mammalian spermatozoa can fertilize, they need to capacitate and complete the acrosome reaction, but the role of the acrosome in human fertility is obscure. In a randomized controlled study of men categorized on the basis of previous IVF experience as fertile ($n = 53$) or infertile ($n = 26$), their semen samples were prepared under capacitating conditions to give high (> 80%) concentrations of motile cells and divided into two aliquots. Respective samples were challenged with the Ca^{2+} ionophore A23187 (10 µmol/l) or with vehicle (dilute dimethyl sulphoxide). Acrosome reactions were scored by epifluorescence microscopy on ethanol permabilized cells using fluoroscein isothiocyanate (FITC)-conjugated *Pisum sativum* lectin (Figure 2). The acrosome reaction following ionophore challenge (ARIC) was defined as the percentage *difference* between spontaneous (control) and ionophore-induced rates. From Figure 3 it can be seen that the ARIC results showed a significant demarcation between fertile (ARIC = 30%) and infertile (ARIC = 12%) men ($p < 0.001$) by Kruskal-Wallis ANOVA). Furthermore, multiple regression and principal factor analysis indicate that the ARIC is more closely related to fertility than any other single semen parameter and the rate of spontaneous acrosome reaction bears no relationship at all (Figure 4).

Thus only a subset of capacitated spermatozoa at any time is capable of undergoing an acrosome reaction in response to Ca^{2+} influx and it appears that one major cause of human fertilization failure relates to a defective acrosomal reaction. The nature of the defect remains obscure. One possibility is that infertile sperm have defective or inadequate phospholipase A_2, but a more likely explanation is that these spermatozoa suffer membrane damage through lipid peroxidation as a result of radical oxygen species formation.

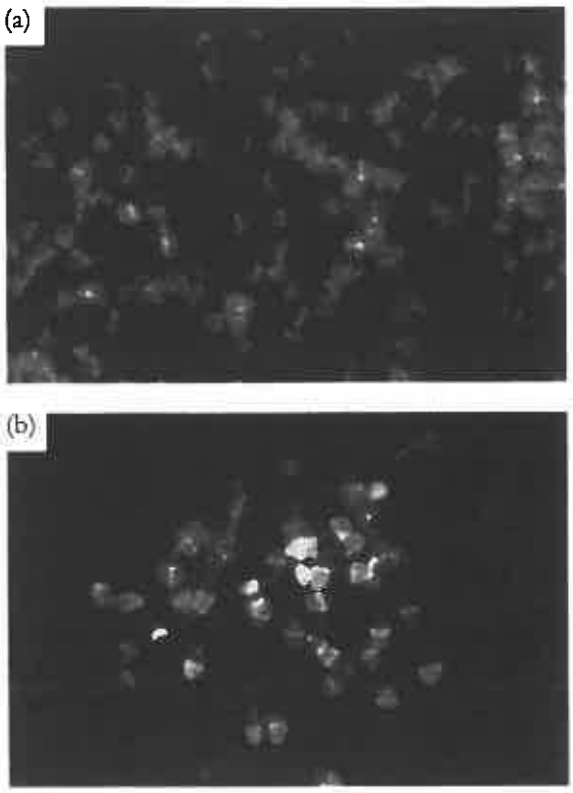

Figure 2 Fluoroscein isothiocyanate staining of acrosomes examined under fluorescent microscopy. Unreacted sperm display epifluorescence of the entire acrosomal cap, whereas fully reacted sperm fluoresce only in the region of the equatorial segment. Following challenge with the calcium ionophore A23187, semen sample (a) displays a high proportion of reacted spermatozoa but only a small proportion have reacted in sample (b). ARIC scores are the difference between spontaneous and induced reactions

Zona binding and IVF of 'test' oocytes

On occasions it may prove difficult to determine if the cause of fertilization failure is attributable to defects of the oocytes or the spermatozoa. Crossed insemination studies *in vitro* can provide the answer, i.e. testing the husband's sperm against donated oocytes (e.g. supernumerary from a GIFT programme) and testing the wife's oocytes with donor sperm during a split fertilization study in IVF. Such testing poses ethical problems concerning the fate of embryos derived from cross-

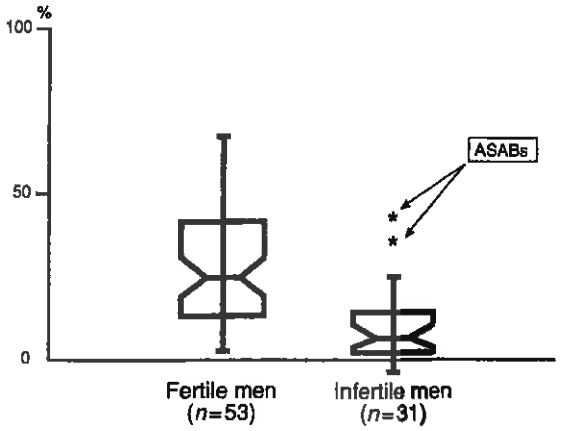

Figure 3 Tukey box and whisker plots of induced acrosome reaction (ARIC) scores for two groups of men whose fertility was judged from their previous *in vitro* fertilization experience (infertile = failed fertilization as well as abnormal semen parameters). The notches in the boxes indicate the 95% confidence intervals and show the reduced ARIC scores to be significantly diagnostic of infertility. All the infertile men had ARIC scores under 25% except for two whose infertility was due to antispermatozoal antibodies (ASABs)

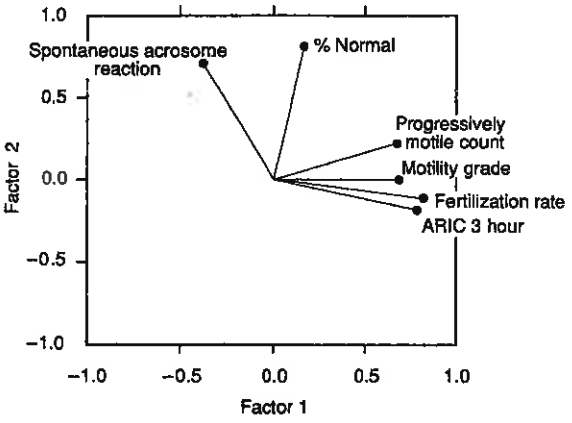

Figure 4 Principal factor analysis of various semen parameters and both the spontaneous and induced acrosome reaction rates in a correlation study with oocyte fertilization rates *in vitro*. The closest correlation was with the ARIC score following a short (3 hour) incubation and there was no relationship with the proportion of spontaneously reacted acrosomes. There was only a very slight correlation between sperm morphology and *in vitro* fertilization

insemination as well as being 'wasteful' of oocytes which might otherwise have been fertilized and cryopreserved for the patient or donated to another infertile couple. A more readily acceptable laboratory test involves the evaluation of sperm binding capacity to the zona pellucida of discarded oocytes. These can have the zonae bisected to compare the binding of control and test spermatozoa against the separate segments in the hemi-zona assay[16]; or by using separate fluochromes (FITC and TRITC; tetramethyl-rhodamine B isothiocyanate) to differentiate test and control sperm on undissected eggs[17]. The human sperm–zona pellucida binding test can be applied to salt-stored supernumerary oocytes or even those oocytes which failed to fertilize after insemination in a treatment attempt, and may predict the ability of test sperm to fertilize human oocytes *in vitro*. However, the results appear to be influenced by factors related to the quality and state of activation of the test oocyte which may limit its useful applicability.

IN VITRO TECHNIQUES

Modifications of sperm preparation and sperm–oocyte culture

Considerable discussion has emerged recently concerning the influence of reactive oxygen species on sperm function[18]. Oxidant generation is a part of normal human metabolism and, when present in excess, oxidants can cause tissue injury[19]. The reactive oxygen species comprise the free hydroxyl radical (which can stimulate the lipid peroxidase chain reaction forming lipid hydroperoxides), free oxygen radicals (normally converted by the enzyme superoxide dismutase to hydrogen peroxide) and non-radical hydrogen peroxide. Normally, the latter is rapidly removed by the cellular enzymes catalase and glutathione peroxidase in a reaction which is selenium dependent. Apart from the normal enzymatic removal mechanisms, a number of compounds, both natural (e.g. α-tocopherol) and synthetic (e.g. propyl gallate) have antioxidant properties.

Reactive oxygen species are usually present in excess in the semen of oligo/asthenospermic men and alternative approaches to the conventional overlay sperm preparation technique are currently

being explored to reduce their presence. These include passage of the semen through a discontinuous gradient of high density media such as Percoll or Nycodenz[20]. However, centrifugation may also act to concentrate the superoxides with the separated sperm or, in any event, further damage affected spermatozoa. Sedimentation techniques avoiding or limiting centrifugation may be preferred[21,22].

Irrespective of the sperm preparation technique employed, the final number of motile spermatozoa available may be too few to achieve appropriate numbers around the oocytes in fertilization tubes. Therefore culture in microdroplets (25–100 µl) under paraffin oil is usually preferred and a further advance appears to be the technique of culturing in very small volumes (5–10 µl) within straws[23].

Antisperm antibodies in semen

Men with both IgA and IgG antibodies in their semen are asked to produce their ejaculate directly into 5 ml of culture medium for IVF preparation and the sample jar is passed from the collection room to the adjacent laboratory through a double hatchway. The ejaculate is immediately pipetted through a small bore glass pipette to break up the coagulum (without awaiting spontaneous liquefaction) and is then centrifuged. This treatment presumably helps to separate the sperm from the antibody-laden seminal plasma before the antibodies have a chance to bind to the sperm and is particularly beneficial in oligospermic cases as shown in Table 1.

Table 1 Fertilization rates of oocytes from patients with IgA and IgG antispermatozoal antibodies present in their semen. The ejaculates were collected in to culture medium for immediate treatment or allowed to liquefy normally without medium

	Couples	*Fertilization*	*Rate (%)*
Normospermic			
into medium	6	36/52	69
no medium	8	26/45	58
Oligospermic			
into medium	12	22/83	27
no medium	8	5/43	12*

$p = 0.05$

Although it is not strictly a male factor cause of infertility, the presence of ASABs in the female circulation requires some modification in management. The chance of fertilization in GIFT appears to be reduced[4], hence IVF is preferred. Fertilization is achieved by substituting antibody-free donor serum for maternal serum in the fertilization culture medium[24]. Once embryos have been generated they are not affected by ASABs *in vitro* or *in vivo* and implantation rates are normal for the transfer method applied (uterine or tubal). Thereafter, pregnancy and pregnancy outcomes are also normal, in keeping with general IVF results.

Sperm stimulation

At PIVET the methylxanthine-derived phosphodiesterase inhibitor pentoxifylline (PF; Hoescht Australia Ltd) is used *in vitro* and is known to elevate intracellular levels of cyclic adenosine 3'5' monophosphate[25] (Figure 5) with the effect of boosting the number of motile sperm available for insemination. In a preliminary report[26] PF was shown to increase the incidence of pregnancy in cases with previous fertilization failure. The earlier protocol has now been modified to reduce the PF exposure–insemination interval with consistently improved results. Briefly, the protocol involved washing the sperm once in culture medium, harvesting motile sperm by sedimentation, then incubating with PF for 30 minutes followed by a

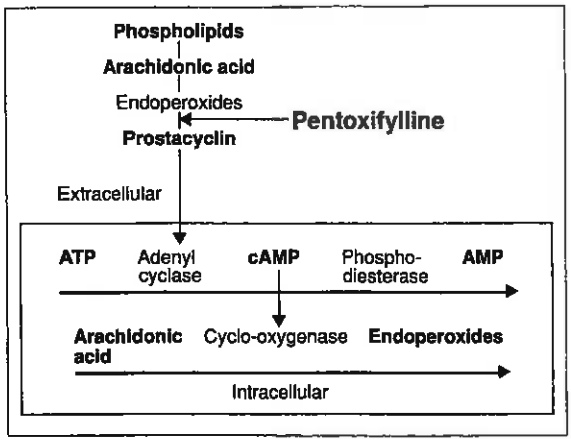

Figure 5 Schematic diagram depicting known actions of pentoxifylline which is thought to raise intracellular levels of cAMP (after Müller[25])

second wash just prior to insemination. The results from a reported study[27] are summarized in Table 2.

The modified PF protocol gave a significant improvement in fertilization rates compared with untreated sperm and significantly fewer patients experienced fertilization failure (PFI 12/31 failures; PF2 3/30: $p < 0.02$). These benefits are highlighted in Figure 6. Overall 16 pregnancies ensued and four were from semen samples in the very severe oligospermic category, one being as low as 0.2×10^6/ml. Our further experience confirms these benefits and a total of 37 healthy infants have been delivered to March, 1991. The pattern of pregnancy wastage is not different from that occurring in the IVF programme generally[28], indicating that pentoxifylline treatment of sperm in cases of oligo/asthenospermia appears to be a safe and valuable treatment option in IVF-related procedures.

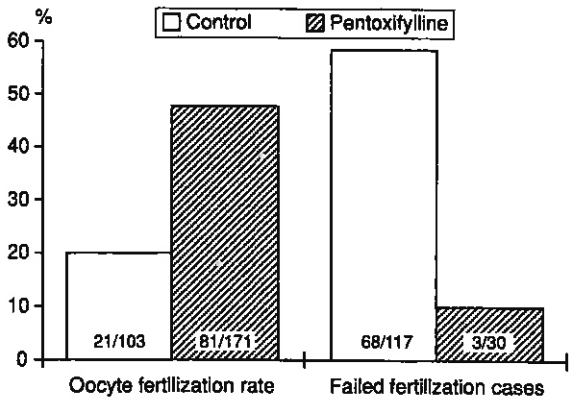

Figure 6 Improved oocyte fertilization rate ($p < 0.001$) and diminished chance of failed fertilization ($p < 0.02$) from preliminary *in vitro* exposure of spermatozoa to pentoxifylline

Epididymal sperm collections

Epididymal sperm collected from males with congenital absence of the vas can fertilize oocytes and the resulting embryos can implant successfully with resulting healthy live offspring[29]. A modified Percoll sperm preparation technique was described by Asch's colleagues[30] in association with such cases but its relevance is uncertain at this stage. At PIVET high epididymal sperm collections have been performed from cases of epididymal duct obstructions as well as congenital absence of the vas. Sperm samples are collected from well

Table 2 PIVET data to November 1989 showing fertilization rates and pregnancies achieved in 61 couples whose infertility was due to a significant male factor, mostly oligoasthenospermia, $n = 30$ cases with defined male factor. Two pentoxifylline (PF) protocols were studied (PF1 original; PF2 modified). Pronuclear oocytes (by PROST) or cleaved embryos (by TEST) were transferred to the Fallopian tubes. Motile sperm count: $3.37 \pm 3.86 \times 10^6$/ml (mean ± SD). (Reproduced with kind permission of *Fertility and Sterility*)

	Protocol 1		*Protocol 2*	
	n	%	*n*	%
Fertilization rates				
control	33/91	36.3	21/103	20.4
pentoxifylline	52/154	33.8	81/171	47.4*
Embryos transferred				
control	21		10	
pentoxifylline	34 (8)†		64 (18)†	
Pregnancy rates				
per collection	7/19	36.8	9/27	33.3
per transfer	7/31	22.6	9/30	30.0

*Pentoxifylline vs. control, $p < 0.001$; †number of patients with only pentoxifylline-generated embryos transferred

proximal to the obstruction by aspiration into a fine cannula and syringe containing human tubal fluid medium (HTFM)[31] at 37°C. Centrifugation is avoided, relying on a sedimentation technique to harvest motile spermatozoa. This technique has been compared with passage through a discontinuous Percoll gradient and the former method appears superior. There also appears to be a benefit in exposing such epididymal sperm preparations to 1% pentoxifylline for 30 minutes prior to oocyte insemination. We have reported a successful pregnancy achieved using sperm from the epididymal caput[32] and Table 3 documents a wider experience at PIVET. This shows that current techniques enable a fertilization rate of 20% of oocytes and those embryos which are generated have a normal implantation rate.

Micromanipulation

A range of micromanipulation techniques have been described including microinjection[33], zona drilling[34], zona opening[35] and zona cutting or partial zona dissection (PZD)[36] and occasional pregnancies have been reported. At PIVET we have explored the latter three techniques in animal and human studies, including chromosomal analysis of resulting embryos (Edirisinghe *et al.*, submitted). The methodology involves cumulus removal with 50 IU/ml hyaluronidase in the presence of trypsin inhibitor. Zona drilling involves the application of acid medium (pH 2.5) directly to the zona using a glass microinjection pipette attached to a Picospritzer instrument and zona opening uses glass hooks. A small series of micromanipulation cases were undertaken at PIVET prior to withdrawal of ethical approval when a restructured ethics committee became concerned that overcoming the zona barrier might enable genetically abnormal offspring to arise. From nine cases of severe oligospermia, five pregnancies were achieved following micromanipulation but each involved the additional transfer of at least one control embryo. At this stage the role of the techniques remains uncertain, although Cohen (see Chapter 12) has continued to achieve pregnancies from the partial zona drilling technique when embryo transfers are combined with antibiotic and corticosteroid administration to the women. More recently, subzonal microinjection is providing encouraging results[37].

Table 3 The results of sperm aspiration and IVF in obstructive azoospermia from 11 cases completing 15 attempts. Epididymal sperm numbers ranges from 0.1 to 17.5 x 10^6 (mean ± SD = 2.96 ± 4.17 x 10^6)

	n	%
Fertilization outcome		
failed sperm collection	3/15	20
failed fertilization	6/15	40
fertilization cases	6/15	40
fertilization rate of oocytes	20/113	18
Pregnancies		
rate per attempt	2/15	13
rate per patient treated	2/11	18
rate per patient transferred	2/6	33

Split fertilization

The option of fertilization of a proportion of the available oocytes should be considered by all couples undergoing IVF who are in the severe oligospermic category. As can be seen in Table 4, this has the effect of minimizing the chance of failed fertilization as well as providing important diagnostic information if oocytes are sufficient in number and randomized for insemination. As discussed previously, the utilization and/or disposal of resultant embryos may pose important ethical considerations. The routine use of pentoxifylline is already impacting by reducing the requirement for donor sperm back-up.

Reinsemination

Although reinsemination of oocytes which fail to demonstrate clear pronuclei at 16–20 hours is widely practised, it has limited benefits. It appears that oocytes, which do show pronuclei or division the following day, do so mostly as a consequence of the initial insemination and the delay in fertilization may represent a functional disorder of the spermatozoa with a slower development of the acrosome reaction. Maturational delays of the oocyte may also contribute. Over a 10-year period of routinely reinseminating 'unfertilized' oocytes at

Table 4 Results of 'split fertilization' IVF procedures in 50 cases over a 2-year period 1987–88 at PIVET showing a significant benefit from randomly dividing retrieved oocytes into two fertilization groups when there is severe male factor infertility. A total of 369 oocytes were retrieved, giving a mean number of 7.4 oocytes per case

	n	%
Fertilization rate		
husband	43/182	23.6% of oocytes
donor	122/187	65.2% of oocytes*
Fertilization failure		
husband but not donor	22/50	44.0% of cases
both husband and donor	4/50	8.0% of cases

*$p < 0.001$

PIVET, three pregnancies are thought to have arisen from the second insemination procedure, but each resulted in a blighted ovum pregnancy. This finding accords with other reports[38] and questions the value of the exercise. However, it is difficult to cease as an occasional benefit cannot be denied to the couple and they invariably seize the option when it is presented. For severe oligospermic cases, this generally means the collection of a further fresh semen sample and husbands should be counselled accordingly.

CLINICAL PROCEDURES

The experience from IVF-ET and GIFT indicated that, apart from the problem of harvesting adequate numbers of spermatozoa, there is a functional defect in the spermatozoa in male factor cases and the severity of this dysfunction is proportional to the degree of oligospermia. The procedures of PROST (pronuclear stage tubal transfer) and TEST (tubal embryo stage transfer) were developed to combine the benefits of IVF with the advantage of tubal transfer. Those benefits include the use of enhanced fertilization techniques, the ability to carry out morphological assessment of pronuclear oocytes and embryos and the ability to select only fertilized eggs or embryos for transfer. The advantage of tubal transfer was the expectation of higher pregnancy rates reflected by the GIFT experience[4].

Pronuclear stage tubal transfer

The procedure is indicated for male factor infertility, circulating ASABs in the female partner and failed GIFT cases. At PIVET ovarian stimulation is by one of two schedules: clomiphene citrate/human menopausal gonadotrophin (hMG) with human chorionic gonadotrophin (hCG) trigger given on the 6th day of consecutive oestradiol (E_2) rise; or Lucrin/hMG with hCG given on the 7th day of E_2 rise. Oocyte recovery is performed 36 hours later by the transvaginal ultrasound-directed method using the PIVET–Cook double lumen aspiration/flushing needle (William Cook Australia)[39]. Husbands produce their semen 2 hours after and oocytes are inseminated 4–6 hours after recovery and further cultured in HTFM + 10% deactivated maternal/donor serum (dMS). The coronal coat is dissected free for the pronuclear check at 16–20 hours and, to avoid high-order multiple pregnancies, a maximum of three 2-cell pronuclear oocytes (selected according to initial oocyte grading) are transferred in 25 µl HTFM + 20% dMS 4 cm into one Fallopian tube via a Teflon catheter at laparoscopy. During the luteal phase, Proluton 50 mg is given by intramuscular injection (i.m.i.) on days 0, 1, 2, 3 and 4; and hCG 1000 IU is given i.m.i. on days 4, 7, 10 and 13, dated from oocyte recovery. This regimen of luteal support therapy has been adopted routinely following the demonstration of significant benefits[40,41].

Tubal embryo stage transfer

This procedure is similar to PROST but involves the transfer of cleaving embryos 1 or 2 days later when selective morphological criteria can be applied on the resulting embryos. It is, therefore, indicated for the more severe cases of male factor infertility, post-cryopreservation embryo transfers, ovum donation and IVF surrogacy. Ovarian stimulation, oocyte recovery and laboratory procedures are the same as PROST; however, embryos are cultured through to the 4- and 8-cell stages. Again, a maximum of three embryos is transferred into one Fallopian tube either at laparoscopy or by the transcervical route (TC-TEST)[42] in 25 µl HTFM + 20% dMS via a Teflon catheter. Luteal phase therapy is the same as for PROST.

A significant overall benefit of tubal transfer techniques was demonstrated when implantation and pregnancy rates were compared with those following conventional IVF-ET[43]. Therefore, at PIVET the policy during 1988 and 1989 has been to treat male factor cases by a tubal transfer procedure if there is no underlying tubal factor complicating the case. In effect this has meant that a small proportion of moderate cases of male factor (motile sperm count 5–10 x 10^6/ml) have been treated by GIFT and severe cases (< 5 x 10^6/ml) have been treated by either PROST or TEST. Cases were designated as male factor if such was the principal diagnosis following comprehensive investigations according to a described protocol[44], even if significant female factors were demonstrable. Conversely, a small proportion of the non-male factor category contained men with mild and moderate sperm disorders if these were considered to provide a minor contribution to the couple's infertility disorder. The period includes certain research trials and, during 1988 only, some patients considered to have a poor prognosis (15%) had more than three embryos transferred. A policy of strict adherence to the limit of three oocytes or embryos has been observed from the beginning of 1989 following a number of high-order multiple pregnancies in the latter half of 1988, including a quadruplet pregnancy from TC-TEST and a quintuplet pregnancy from PROST.

The results of this policy covering the 2-year period 1988 and 1989 are shown in Figures 7, 8 and 9. The pregnancies include seven cases from men categorized as very severe oligospermia (< 1 x 10^6 motile sperm/ml), one case having as few as 0.2 x 10^6 motile sperm/ml, and two pregnancies following very high epididymal sperm collection in cases of obstructive azoospermia. It can be seen that the highest pregnancy rates (41 and 42%) were achieved in the GIFT programme and these were not different in the male and non-male groups (Figure 7). However, only a small proportion of selected male factor cases (moderate degree only, first attempt) were included and, for the reasons outlined in the introduction, GIFT was avoided as a main option for significant male factor cases. Of greater interest are the three important observations:

(1) The failed fertilization rate per patient (identified as the difference between transfer and collection pregnancy rates in Figure 7) was highest in the PROST/TEST group (39/189; 20.6%) where most of the identifiable male factor cases were included. However, the rate was only marginally higher than the non-male factor cases treated in the same programme (24/189: 12.7%; not significant). This reflects the benefits of improved laboratory techniques including the use of pentoxifylline as discussed above.

(2) The pregnancy rate per transfer was not different for male factor cases treated by IVF-ET or PROST/TEST. This finding challenges the question of a benefit for the two-stage procedures. Previously we have shown a significantly higher pregnancy rate for the tubal transfer procedures compared with IVF-ET[43] but the lack of difference in this series possibly relates to an increasing proportion of patients undergoing Lucrin/hMG stimulation which may be improving postovulatory endometrial receptivity. However, the implantation rate (identifiable pregnancy sacs arising per oocytes transferred) was significantly higher in the PROST/TEST programme than with IVF-ET (Figure 8; $p = 0.05$ for the respective male factor and non-male groups having uterine or tubal transfers; $p = 0.002$ for the total cases having uterine or tubal transfers). These differences relate to some technical or physiological factor, as a similar number of embryos were transferred in each programme

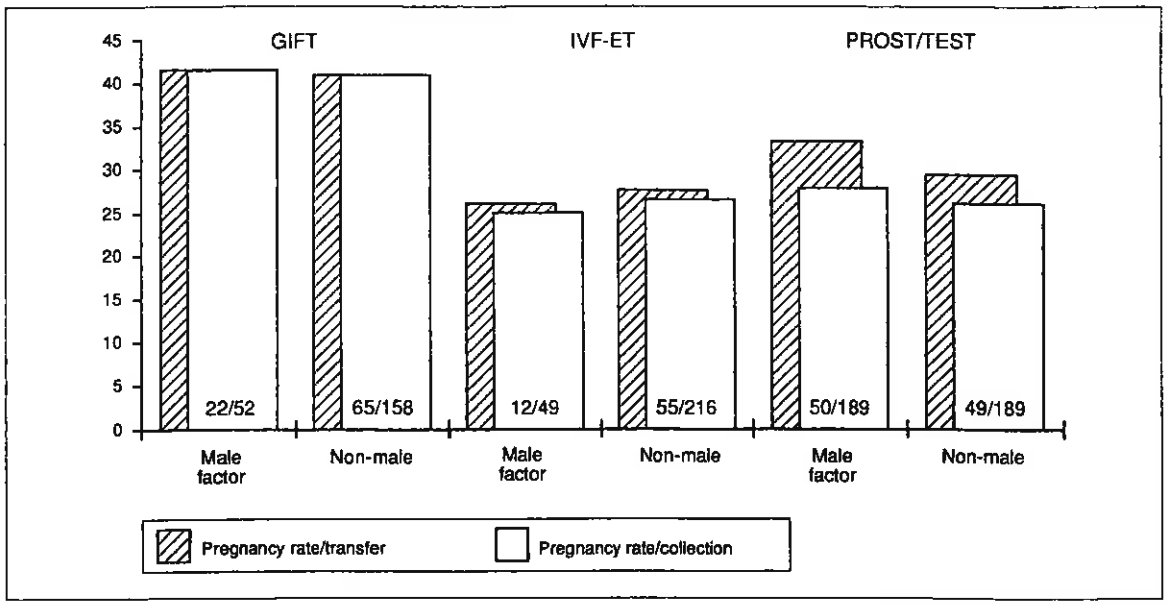

Figure 7 Complete data for 2-year period at PIVET (1988–89) showing pregnancy rates for those couples reaching the stage of oocyte retrieval and those having a transfer procedure (differences between the two being due to fertilization failures). The majority of male factor cases were treated in the PROST or TEST programmes

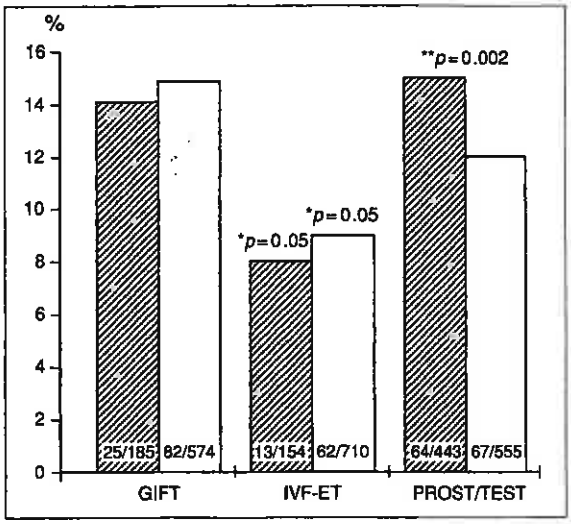

Figure 8 Implantation rates (number of gestational sacs detected on early pregnancy scan per total number of embryos transferred) for all IVF-related procedures during 1988–89. Reduced implantation rates are shown for respective male (hatched bars) and non-male (empty bars) categories having uterine transfers ($p < 0.05$). The combined categories show a more significant difference ($p < 0.002$)

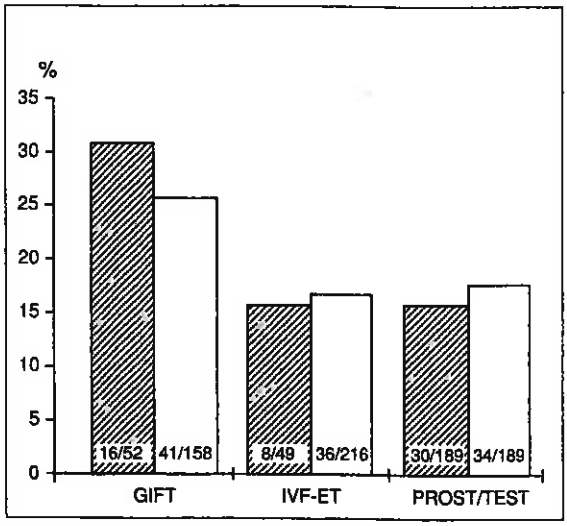

Figure 9 Live birth rates (signifying one or more surviving infants) for all IVF-related procedures during 1988–89 for male factor (hatched bars) and non-male (empty bars) factors

(mean numbers 3.0 and 3.4, respectively; no significant difference). Among possible causes for the higher implantation rates with tubal transfer may be a more secure placement in the tubal method, some special benefit conferred to the early developing embryo by a substance within the tubal lumen, or simply protection from a relatively hostile uterine lumen during the early postovulatory phase. Whatever the cause, the results continue to imply that the optimum chance of pregnancy arises following a tubal transfer procedure, although this consideration may be of greater relevance the fewer the number of embryos transferred.

(3) Pregnancies achieved after the transfer of embryos generated from men with severe male factor disorder have a similar outcome to those generated from non-male causes of infertility and the results are similar for both IVF-ET and PROST/TEST programmes (Figure 9). Concerns by some that IVF now enables fertilization and pregnancies by spermatozoa which do not have the capacity to achieve this naturally, and which might have been a protection mechanism against genetic abnormalities, are beginning to be allayed. At least the outcomes with respect to pregnancy wastage[28] appear no different from those seen in subfertile populations generally (with or without gamete manipulation) and congenital abnormalities identified after IVF and related procedures[45,46] are not increased in the male factor group.

With respect to the treatment programme selected, the pregnancy outcome after PROST and TEST is similar to other IVF procedures. In particular, ectopic pregnancy rates are not increased above IVF-ET (4–8%), unless embryos are transferred to defective Fallopian tubes when rates of 20–40% have been reported[45,47]. Our current policy is to avoid tubal transfers in all women with known tubal disorders, those who have had previous ectopics and those who have had any reconstructive tubal surgery, even if the laparoscopic appearance of the tube is near normal. However, as discussed earlier, the GIFT procedure appears generally unsuitable in male factor cases because the chance of fertilization of any of the three oocytes may be too low in severe cases; there is not the opportunity for selection of fertilized oocytes or cleaving embryos; and there is insufficient information derived from the treatment cycle upon which to base suitable counselling should pregnancy not ensue. Furthermore, some reports cited earlier indicate a higher pregnancy wastage among GIFT cases for male factor infertility.

CONCLUSIONS

Improved techniques in IVF are increasing the options for the management of male factor infertility. In particular, improved sperm separation methodology, sperm motility enhancement and micromanipulation can improve the chance of fertilization. Transfer of embryos into the Fallopian tubes by PROST or TEST has been our preferred technique to maximize the chance of pregnancy, but this option should probably be considered in context with other aspects of the case, including the informed choice of the couple. Further improvements are anticipated with improved diagnostic methods for evaluation of sperm function and related research, e.g. eliciting the acrosome reaction *in vitro*.

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