MALE SUBFERTILITY

Evolution and current concepts

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Progress in male subfertility

a process of dispelling the myths

Until recently, male infertility has carried a poor prognosis for treatment, and the majority of couples with a significant male factor needed to consider donor insemination to achieve a pregnancy. In-vitro fertilization (IVF) raised the potential of generating embryos *in vitro*. However, although occasional pregnancies could be achieved after IVF and embryo transfer the early techniques were found to be of limited benefit in males with severe oligozoospermia, asthenozoospermia, antispermatozoal antibodies in the semen or 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 or the number of abnormal semen parameters. Consequently, pregnancy rates for IVF and embryo transfer remained low.

Subsequently, when the gamete intra-Fallopian transfer (GIFT) technique was introduced, new hopes were raised because the method was considered to have the benefit of being closer to the natural process of fertilization and would therefore be a more successful procedure for all non-tubal forms of infertility. However, GIFT proved to have major limitations for male factor infertility, but proved to be more effective than IVF and embryo transfer for many other categories of infertility. A modified protocol, involving the transfer of greater numbers of spermatozoa, enabled reasonable pregnancy rates to be achieved in case of moderate oligozoospermia, but it was shown subsequently that the pregnancy wastage rate was higher possibly because of polyspermic fertilizations and the inability to control the quality of embryos available *in vivo*.

The problem of managing the male factor in IVF is made even more difficult because of diagnostic limitations particularly with respect to the reliance on semen analysis, the complex and often multifactonal nature of infertility, the findings that some relatively "normozoospermic" men fail to achieve IVF and generally poor level of understanding of the aetiological nature of male infertility.

Significant improvements are now occurring as a result of determined efforts focused on understanding the limitations of IVF procedures. This process has involved the reconsideration of virtually every aspect of sperm function and the fertilization process. Accordingly, many traditional "myths" have been dispelled enabling useful and sometimes dramatic progress in managing male infertility.

Examples of a more enlightened viewpoint include:

(I) there **is** important information to be gained from the clinical examination of the male partner;

(II) where clinical assessment is undertaken, the finding of varicocoeles **may indeed** be relevant;

(III) it **is indeed** useful to biopsy the testes of azoospermic men despite the finding of raised follicle-stimulating hormone (FSH) concetrations;

(IV) semen analysis does **not** provide an accurate assessment of male fertility potential;

(V) morphologically abnormal spermatozoa are **not necessarily** genetically abnormal;

(VI) sperm-mucus interaction evaluation, eg by postcoital testing **can** be useful;

(VII) the female partner **must still** be fully investigated even if the male is severely oligozoospermic or azoospermic;

(VIII) there is **no** clear "bottom line" for sperm numbers to achieve fertilization *in vitro* (or even *in vivo* in the case of intrauterine insemination);

(IX) spermatozoa, which can achieve normal fertilization when microinsemination is applied, are **not necessarily** those that are motile, morphologically normal spermatozoa or "mature", i.e. those spermatozoa collected more distal from the testes along the male genital tract; and

(X) azoospermia resulting from Klinefelters or supposed Sertoli cell-only syndromes **may** now sometimes be offered treatment with the germ cell of the husband.

The road to 1998

Clinical andrology

Until recently, and despite constant appeals to the contrary, infertility treatment has often been undertaken by doctors who saw almost no value in direct consultation with the male partner, let alone conducting physical examination. The male was represented by his semen analysis report. Occasionally, men whose semen analysis showed azoospermia or severe oligozoospermia were referred for further assessment by urologist. The appraisal included a clinical assessment to check testicular volumes (comparative with Prader orhidometer), to identify dilatation and distension of the epididymides as a sign of distal obstruction, to detect the presence of varicocoele(s) and to undertake the palpation of the vasa and a rectal examination to determine the pathology of the seminal vesicles and prostate gland. Occasionally, Mullerian duct cysts may be detected clinically. Investigations included a chromosomal analysis to exclude Klinefelters syndrome (considered untreatable), and serum gonadotrophin and testosterone estimations to detect hypogonadotrophic (treatable) and hypergonadotrophic states (reflecting spermatogenic failure; considered untreatable). Further investigations of men with normal testicular volumes included a scrotal exploration with vasography (for an evaluation of the distal genital structures and genital tract patency), along with a testicular biopsy for a histological evaluation. This could reveal a range of findings, from germinal aplasia (Sertoli cell-only syndrome),through maturational arrest to hypospermatogenesis, all conditions with largely unexplained causes, although maldescent of testes, specific infections, trauma (e.g. unrelieved haematomas from "petrol tank slap" during motorcycle accidents), chemotherapy and irradiation are sometimes identified. Occasionally cystoscopic evaluations are useful to examine the ejaculatory ducts, prostate, prostatic utricles and seminal vesicle cysts. The identification of post-viral orchitis, or past infection through sexually transmitted disease, can often explain disturbances in semen quality by lesions left after inflammation.

More recently, the clinical examination of the male has been incorporated into the combined management of couples within dedicate clinics offering a comprehensive evaluation and treatment service. The prevalence of different male factor categories can be presented according to the treatment potential, and it is estimated that 12% of case are untreatable and sterile, 13% are potentially treatable and 75% have untreatable subfertility. The comprehensive approach has evolved from the set-up required for IVF procedures, and may often incorporate an ultrasound assessment of the genito-urinary tract of the male. This has led to a more accurate volumetric assessment of the testes, greater specificity of varicocoelae detection

and grading by duplex or even triplex scanning systems incorporating colour Doppler, and the improved detection of intrapelvic features by intracavitary scanning.

A genetic basis for the subfertility of some individuals is now also being increasingly recognized and advanced clinics incorporate genetic screening (eg to detect the integrity of the azoospermia factor; AZF genes DAZ and RBMI) as a routine for cases with severe oligozoospermia or azoospermia. The findings of gene deletions and chromosomal anomalies underlying male infertility requires high level genetic counselling in order that couples can make informed decisions regarding their future treatment options.

PIVET Practice: *In the genetic screening clinic at PIVET we have reported a number of chromosomal anomalies as well as DAZ deletions and cystic fibrosis gene deletion (in some men with congenital absence of the vas) among cases preparing for ICSI. Following counselling most couples will still pursue IVF-ICSI despite the risk of transmission of similar genetic disorders to offspring, but a few patients have withdrawn from the program, choosing donor insemination or discontinuing treatment altogether.*

Laboratory investigations

The laboratory investigations of the male partner of an infertile couple has seen many advances over the years. Nevertheless, the real value of the laboratory tests in helping make an accurate diagnosis and prognosis is often not apparent. It is particularly disappointing to see basic and accepted tests being challenged constantly and found wanting, and so it might be more informative to examine here the reasons for this and to learn some salutary lessons, rather than simply cataloguing those tests which have become available, most of which do not have high predictive value in real clinical situations.

The purpose of majority of tests applied to the man under investigation is to assess his potential fertility, and the most common test is semen analysis. While it is regarded as an important investigation, semen analysis has been shown repeatedly to have limited value in predicting the chance of conception (although there has been improvement with the recent introduction of more strict criteria in the assessment of sperm morphology). An examination of the history and rationale of this test shows that the expectations placed upon it may be too demanding, as illustrated below.

Introduction and rationale

For any test of male fertility to be of value, it must have some firm biological basis and be able to reasonably discriminate between nominally fertile and infertile groups. Unfortunately, semen analysis has been unable to satisfy this requirement with any degree of reliability. The examination of semen is a descriptive practice, enabling man with no spermatozoa present in their ejaculate to be identified. However, the value of the test as a predictor of fertility for men with spermatozoa in their semen has always been doubt. Early attempts to develop a threshold of sperm concentration which demarcated the fertile and infertile men looked at respective populations and arrived at a figure of 60×10^6 /ml as the lowest sperm concentration compatible with fertility. However, further analysis of a larger population showed that there was no difference in the frequency distribution of sperm counts between fertile and infertile men except at concentrations $< 20 \times 10^6$ spermatozoa/ml; even then, 5% of fertile men had sperm concentrations below this value compared with 16% of infertile men. Nevertheless, a threshold of 20×10^6 spermatozoa/ml is commonly used as a limit of normality, even though Macleod (1971) concluded some 20 years later that there is "good reason to believe that a reasonable chance of pregnancy is present at lower count levels if the wife has passed the usual tests and if the sperm motility is good". The notion of the test being a good discriminator between fertile and infertile men was therefore flawed from the outset.

Variability in the results

For a test to be useful, some degree of reproducibility must be seen so that the repeat investigation of a patient will not give conflict results. Technical variability has been addressed for semen analysis by the recommendation of standardized protocols and internal quality control procedures. Nevertheless, a large degree of inter-laboratory variation in the performance of semen analysis can be demonstrated with an external quality assessment programme. Biological variability can also compound the problem of test reproducibility, and this is clearly seen for semen analyses where samples from the same man can be

oligozoospermic or normozoospermic on different occasions. This then brings into question what the test can really be expected to achieve. It is apparent that it is only the ejaculate which is able to be assessed and therefore only a limited aspect of the potential of spermatozoa to successfully meet and fertilize an oocyte. Repeat tests are required to build up a composite picture of mans overall fertility.

General principles

Being so far down the road in development and application of numerous sophisticated tests in the investigation of male fertility, it seems trite to consider the reasons why the basic investigations have not satisfied our requirements. Yet it is important to identify these problems so that we can maximize the true potential of the newer tests and avoid making some elementary mistakes.

Any new tests must have a definite rationale. However, we must be very clear about the particular aspect of male fertility which is being assessed so that unfair expectations of the tests are not made. This may help with establishment of a panel of tests which complement each other and assist in building an overall picture. Nevertheless, each test must satisfy the rigours required of any investigative technique before being applied in general clinical practice. This means that attention must be given to the validity of the test in terms of the reproducibility (by virtue of the technical variations in the laboratory and biological variation between samples), its ability to successfully identify men with reduced fertility (but at the same time not having an unacceptably high rate of false identification) and its robustness to prove useful in the hands of different laboratories.

PIVET Practice: *Following an extensive experience with a range of sperm function tests (including hamster oocyte penetration, hemi-zona binding assays, the hypo-osmolar swelling test, computer- assisted sperm motility assessment, creatine kinase levels in semen and various acrosome reaction systems, at Pivet reliance is now placed on two systems only:*

(I) rigid application of semen analysis according to WHO 1992 (evaluated by quality assurance);and

(II) routine application of the ARIC test (acrosome reaction to ionophore challenge).

Available treatments

A standardised investigation of the couple is now recommended. Unfortunately the past management of the males in isolated urological consultations has been associated with very few successes. In fact, the separation of male and female partners, as found in many unenlightened clinics, has failed to recognise the multifactorial basis of most cases of infertility, and simply led to a delay in the comprehensive management of the couple. It also caused an often inaccurate focusing on one or other individual as "causing" the infertility problem, with associated anguish and often withdrawal from considering further management when treatment trials of the male failed to achieve pregnancy or donor insemination was suggested. Of further interest, busy donor insemination clinics discovered that a significant portion of their referred cases required additional treatment of the female partner (e.g. ovarian stimulation, pelvic surgery, intrauterine insemination preparations and even IVF or related procedures) to achieve pregnancy with high-grade donor spermatozoa.

In the evaluation of males for specific treatments, clinicians attempt to delineate whether the problem relates to genital tract obstruction or diminished sperm production. The former may sometimes be corrected by specific surgery (e.g. vasovasostomy and vasoepididymostomy, and preferably by micro-surgical techniques and opening blocked ejaculatory ducts by transurethral resection). The question of varicocoele corrections remains controversial, possibly because treatment trials have relied on clinical evaluations only, and conventional surgical techniques have high failure of recurrence rates. We would like to see data utilising high-resolution duplex or triplex scanning with either the laparoscopic resectioning of testicular veins or the venous embolisation technique performed within a dedicated infertility clinic setting to decide on the value of the procedure. The most effective treatment of sperm antibodies is still unclear. Nevertheless, the use of steroid therapy to help reduce the level of antibodies or the stripping of antibodies from the spermatozoa seem effective strategies. However, the removal of the ipsilateral testis in cases of unilateral non-correctable obstruction with high antisperm, antibody levels seems a controversial and drastic approach, and we feel it to be entirely unnecessary given the recent advances in IVF techniques.

A wide range of options have been described in the treatment of male subfertility. For non-obstructive cases, the gonadotrophin treatment of hypogonadotrophic hypogonadism is highly effective even when sperm concentrations achieved are low, although there are few such cases. The majority of cases have unexplained (normogonadotrophic oligozoospermia) causes, and no treatment has withstood investigation within randomised controlled studies. Treatment have included gonadotrophin therapy by either follicle-stimulating hormone of luteinizing hormone alone or in combination (human menopausal gonadotrophin), androgen therapy, indomethacin, clomiphene and tamoxifen. In our experience with these treatments ranging over 20 years, only prolonged treatment by tamoxifen has seemingly produced a few cases of improved sperm profiles and function. Growth hormone has recently been suggested of potential benefit, but early unpublished results are not encouraging. The use of anti-oxidant vitamins (C and E) along with zinc is widely prescribed for theoretical reasons but is of unproved value. Cases of hypergonatrophic oligozoospermia have generally been regarded as beyond specific therapeutic consideration.

Achievements and challenges for 1998

As we approach the next century, it is worth reflecting on what has been achieved to date and identifying the challenges of tomorrow.

Investigation of male partner

The clinical assessment of the male partner should become more extensive, using revealing procedures such as ultrasound and fine-needle biopsy of the testes. Such techniques are likely to play an increasing role in assessment of the men with azoospermia and severe oligozoospermia. In particular, the use of rectal ultrasound may well prove helpful in identifying the causes of treatable non-symptomatic deep pelvic infection. Nevertheless, the extensive clinical evaluation of the moderately oligozoospermic or normozoospermic man is at present unlikely to reveal any underlying cause of subfertility in the majority of cases, and remains a constant challenge.

The laboratory role in the diagnosis of male subfertility is becoming clearly divided into that of service and research as experience and the availability of facilities increase. The provision of even the most fundamental of tests, such as semen analysis and sperm antibody detection, can now be performed using external quality assessment schemes to ensure the reliability of results. Attention to the relatively uninspiring, and often humbling, world of quality assurance is of prime importance at a time when laboratory accreditation and formal proficiency testing are becoming widespread. It is essential that a thorough evaluation of current and new diagnostic tests under routine conditions is performed before they are introduced into the clinical laboratory. At present, this is being undertaken with ARIC test in terms of quantifying the assay variability and biological variability. It is hoped that promising tests such as the measurement of sperm creatinine kinase will follow. The continued progress of laboratories engaged in high-quality meticulous research is important in the development of new diagnostic tests. However, the transition from a promising to a proven testis a step to be undertaken with great care and following rigorous scrutiny.

Workup of the female partner

From the introductory comments, it can be seen that modern infertility management requires managing both partners together. Indeed it is wise to establish a protocol of workup which which does not initially focus upon the perceived or "obvious" cause of infertility. Among couples reffered for infertility treatment where severe oligozoospermia or azoospermia has already been identified as "the cause" of the problem, further investigations will reveal significant female findings in approximately 30% of cases. In pre-IVF days, many infertility specialists discovered that the successful treatment of mild to moderately severe male factor infertility was achieved by paying attention to the female partner (e.g. revealing and treating her endometriosis, ensuring adequate ovulation and improving cervical mucus qualities). It is more imperative in the current era of severe male factor disorders to investigate the female partner fully. For all treatments involve her active and informed participation whether in an insemination or IUI program, or as a preliminary to IVF where it is imperative to have control over all pelvic pathologies to safely optimise the chances of IVF success.

Treatment of male subfertility, 1998

Specialized sperm preparation techniques, particularly using pentoxifylline; PTX for enhancement, now enable many couples to be treated using their own gametes.

PIVET Practice: *PTX was first used within the IVF programme at PIVET in 1986 ie. Before the advent of alternative treatment strategies such as oocyte micromanipulation. Over the years it has continued to be useful in aiding fertilization in selected IVF cases, with a beneficial affects also being seen in certain cases also being treated by IUI. In both instances the ERIC tests appears to be invaluable in identifying suitable patients. The stimulation of spermatozoa by PTX should remain a therapeutic option in the treatment of couples with a spermatozoa by PTX should remain a therapeutic option in the treatment of couples with a male factor present. As an adjunct to IVF it has the advantage of being simpler and less costly to perform compared with micromanipulation. However its use should be restricted to selected cases and the merits over and above those of invasive procedures such as ICSI should be discussed with the individual patients. The use of PTF for IUI in selected cases gives an alternative therapeutic strategy to those patients not wishing or unable to undertake IVF.*

Intrauterine insemination treatment usually sufficient for cases of moderate sperm disorder, whilst severe cases will progress to IVF. Very severe cases will require additional microinsemination techniques (ie intracytoplasmic sperm injection; ICSI) to generate embryos. This is now also possible for azoospermic men, where spermatozoa can be collected from the epididymis (microsurgical epididymal sperm aspiration; MESA or percutaneous epididymal sperm aspiration; PESA);or testicular biopsy samples. In fact, current developments indicate that small numbers of testicular spermatozoa can be collected from men with maturational arrest and even cases of germinal aplasia (so called Sertoli cell only syndrome) and Klinefelters syndrome, with high-quality embryos generated following intracytoplasmatic sperm injection. In addition a case of necrospermia was successfully treated at PIVET by testicular sperm aspiration (motile sperm recovered) with ICSI. These results have even been achieved using cryopreserved testicular spermatozoa, immotile spermatozoa, and also spermatids. Using these procedures, pregnancies can now be achived in female partner of hypergonadotrophic men, considered impossible until now. In addition, the ICSI technique has been successful in the treatment of cases with extreme ASAB levels, not amenable to a sperm washing/extraction technique and for cases globozoospermia, previously an untreatable group.

PIVET Practice: *Several modifications of the ICSI technique have been applied at PIVET with improved and sometimes spectacular results. These include the homogenisation of testicular biopsy samples, immobilisation of spermatozoa by stroking the tail, extended culture of freshly collected testicular sperm samples for up to one week with peak motility gained on day 5 (not suitable for epididymal or cryopreserved spermatozoa), and the use of PTX to stimulate motility in specimens with entirely immotile spermatozoa at the time of recovery. We have now reported a number of successful pregnancies where minimal testicular spermatozoa were recovered, as few as 9 in one case.*

The recovery of spermatozoa from cases of azoospermia at PIVET reveals 100% successful recovery from all cases of obstructive azoospermia (includes post vasectomy, post inflammatory and congenital absence of the vas) whilst in non-obstructive cases spermatozoa were recovered in 75% overall (including cases of germinal arrest, so-called germinal aplasia; 50% recovery, post-inflammatory, unexplained spermatogenic arrest, spinal-injured males, cryptorchidism and chromosomal abnormalities; 50% recovery).

Given these frontier developments in IVF and microinsemination procedures, we have now begun to change our work-up of the male where severe sperm disorders or azoospermia exist. Such cases still undergo conventional clinical work-up and investigations, as outlined previously. In addition at scrotal exploration, epididymal and testicular sperm samples are teased into culture medium by the semenologist prior to fixing in Bouins solution for histological evaluation. This process considerably enhances treatment options for the couples and provides a back-up situation for surgery in the event of the failure of anastomotic procedures. For those not interested in corrective surgery, testicular biopsy samples with spermatozoa recovery can be taken by simple needling under local anesthesia. Whatever the recovery techniques, such cryopreserved specimens can be utilized in IVF using ICSI technique in an ordered schedule which does not rely on trying to undertake procedures on the husband and wife on the same day. Clearly these new developments extend the andrology side of infertility management extensively, creating a larger range of treatment options and reducing the demand for donor semen.

Future challenges

While we have come a long way in our ability to identify and treat subfertile couples with a male factor present, there is still much to learn. Numerous papers presented at meetings and to journals concerned with reproductive medicine attest to an intensive current interest in all aspects of the male factor. These include our basic understanding of spermatogenesis, the events which enable spermatozoa and oocytes to interact successfully, the short-term benefits and long-term limitations of new techniques such as ICSI, the relative merits of different treatment strategies along with the choice of the most appropriate for individual patients, and possible ways of improving embryo quality and on-going pregnancy rates.

PIVET Practice: *For the present, at PIVET the aforementioned advanced strategies are applied routinely. There is a strong reliance on the ARIC test as a predictor for fertilization potential and where the result is poor (<10%), it is repeated after PF enhancement to determine if improvement can be shown for clinical application. Thereafter, depending upon other clinical criteria, couples may be offered IUI or IVF. A range of sperm preparation techniques have been developed over 20 years of experience ofor application in specific situations and are still applied in the primary management of many cases. However, where predictive tests indicate persistent poor ARIC score, there is severe oligozoospermia (<1 million progressively motile spermatozoa/ml) or azoospermia cases requiring epididymal or testicular sperm recovery, or other specific situations such a necrospermia, globozoospermia or the spinal injured male, then IVF with ICSI is applied using prolonged in vitro sperm culture for testicular samples. So too is IVF with ICSI applied if previous IVF procedures disply a low rate of fertilization (<50% of oocytes) regardless of the preliminary investigatory tests which are all limited in their predictive value.*