Human Reproduction vol.12 no.5 pp.875–883, 1997

DEBATE

Clinical andrology

Attitudes to clinical andrology: a time for change

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Since the advent of in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (Palermo *et al.*, 1992), it has now become possible to treat very successfully many different forms of infertility in the human male (Van Steirteghem *et al.*, 1993; Payne *et al.*, 1994). Even in the condition known as primary testicular disease which is probably the most common known cause of an abnormal sperm count in the male (Jequier and Holmes, 1993) and where the sperm numbers can fall to very low levels, conceptions can result using this technique. Thanks to ICSI, it is now also possible to achieve pregnancies from sterile azoospermic men using spermatozoa harvested directly from the testes themselves (Devroey *et al.*, 1995).

So successful has this technique become that it is now being used to treat all forms of male infertility with little attention being given to its aetiology; indeed in many clinics no clinical history is being taken from the male patients nor are they undergoing any form of clinical examination. Scant regard is thus being paid to either the cause of these disorders or to the possible genetic consequences that may result from the use of ICSI in overcoming infertility in the male (Vogt *et al.*, 1992; Cummins *et al.*, 1994).

It is the authors' view that the time has now come to reassess the important role of clinical andrology in the managment of infertility. We must remember that treatment, however effective, is not the sole objective in any section of medicine. Only by the identification of the aetiology of male infertility and by an in-depth understanding of its patho-physiology can any progress be made towards its prevention which is after all just as important as treatment. Only by very careful history-taking can possible causes of this disorder be identified and their presence confirmed by clinical examination. Let us now look at this problem in more detail and examine ways in which the situation can be changed for the benefit of all future infertile male patients.

Traditionally, male infertility is treated by either gynaecologists or urologists. Most gynaecologists have had little or no training in urology and often have a poor understanding of the many urological disorders that can cause or aggravate infertility in the male. They have usually had minimal experience in the examination of the male patient, let alone a male patient with disease of the genito–urinary tract. As a consequence, such clinicians may not be able to recognize disease processes even when they are present. They frequently have had little surgical experience in urology and thus cannot contribute either to any surgical alleviation of this problem or to the design of new procedures that might obviate the need for assisted conception.

This sad state of affairs has thus tended to allow male patients to be viewed simply as a semen analysis result and as a consequence these patients are reduced to little more than a number on a pathology report. Indeed, the oft used and quite appalling phrase 'male factor' infertility speaks volumes for the attitudes that prevail among the gynaecological clinicians regarding the infertile male patient. The inability of many gynaecologists to assess the male patients properly has also allowed the scientists to play a role in determining treatment for these men, a situtation that further separates the treatment of male infertility from the determination of its cause.

The urologists however, unlike the gynaecologists, do indeed have an understanding of the disorders of the male genital tract and can of course examine the infertile male patients with a great degree of competence. However, as with primary testicular disease, there is still no treatment other than assisted conception for the more severe forms of this disorder: many urologists have no access to such therapy nor do they take part in much of its application which, even when treating male infertility, largely involves treatment of the female patient by the gynaecologists and the scientists. The exclusion of the urologists from the practical application of IVF and ICSI encourages the persistant use of operations such as vaso-epididymostomy and vasectomy reversal when epididymal aspiration and *in vitro* fertilization with ICSI may be a much better and more sucessful treatment option than either of these surgical procedures (Silber *et al.*, 1995).

Many years ago, it was clearly demonstrated that infertility is the disorder of a couple not of a single individual (Steinberger and Rodriguez-Rigau, 1983). Thus the problem of infertility must of necessity involve both partners. The factors that influence fertility are frequently interactive and thus it is preferable that a single clinician is competent to evaluate the problem in that couple and not simply in one partner alone. For a gynaecologist to see the female patient and a urologist to see her male partner (a situation that indeed exists in many clinics) is thus a very unsatisfactory state of affairs as it makes the integration of treatment regimes very difficult indeed. For this reason, the clinicians who treat infertility should be able to diagnose disorders in both partners and to design treatment as indicated by this assessment.

Thus the next question to ask is how to achieve this ideal situation within an infertility clinic. The answer of course lies

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in the training of the clinicians treating infertile patients for whom clinical andrology must now become an important part of their curriculum.

Clinicians undertaking the treatment of infertility must therefore now undertake a wide area of training. The curriculum must involve a great deal of basic physiology, gynaecology and urology. These practitioners must be able to examine both male and female patients competently and recognize pathologies in either partner where they exist. The clinicians must also be taught semenology and of course all the practical aspects of reproductive technology.

The treatment of infertility must, in our view, no longer be the preserve of gynaecology. In many countries, including Australia, the subspeciality examinations in infertility and reproductive endocrinology appear to relate solely to the female patient. Scant attention seems to be given, at least in the published curricula of such examinations, for any aspect of male infertility except that which relates to the examination of semen and its manipulation within an IVF laboratory. This state of affairs is greatly in need of change. In our view, such a subspeciality examination needs to be removed from the over-riding influence of the Obstetric and Gynaecological Colleges if only to emphasize that infertility is a male as well as a female problem and also to facilitate the inclusion of the urologists into this sphere of therapy.

We would thus like to see the some changes made to the training of clinicians involved in the management of infertility. Firstly, all clinicians treating infertile couples should have training in both urology and gynaecology. Gynaecologists wishing to subspecialize in infertility should have a minimum of one year in full time urology and the urologists should spend the same length of time in gynaecology. This should be a standard requirement for anyone claiming to be a subspecialist in this area. Both urologists and gynaecologists should have training in the clinical as well as the laboratory aspects of reproductive technology, semen handling, semen analysis and sperm and embryo storage as well as the laboratory techniques involved in embryo manipulation. Infertility should now be a subject set aside from either urology or gynaecology thus allowing the two specialities to come together, at long last.

We believe that the next decade will be a very important time for clinical andrology: either we will come to understand the patho-physiology of the genital tract, or reproductive technology will become so successful that we will not bother to find out. If the former comes to pass there may be many ways in which we can prevent the disorders collectively known as male infertility and avoid the need for treatment altogether. If the latter occurs, then prevention will be forgotten and all infertility will be treated by assisted conception with all the problems that it may give future generations (Cummins and Jequier, 1994).

The choice between these two options by the infertile patients is likely to be obvious.

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Declining clinical andrology: fact or fiction?*

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The debate initiated by Jequier and Cummins (1997) provides an excellent basis for an evaluation of the current status of the clinical investigation of the aetiology of male infertility. In their paper, the authors express their concern about the negative impact of intracytoplasmic sperm injection (ICSI) on the clinical investigation of male infertility.

For years, male infertility has been an area of frustration. Despite the growing knowledge on spermatogenesis and sperm function thanks to advances in both cell biology and molecular biology, there has been hardly any substantial advance in the treatment of male infertility. Indeed, any ‘clinician attempting to deal with male infertility is constantly reminded of the lack of detailed understanding of the pathophysiology of defects in spermatogenesis and spermiogenesis which appear to underlie the disorders seen in the majority of the patients’ (Baker, 1993). For many years, this discrepancy between our basic knowledge of mechanisms leading to male infertility and their treatment has resulted in the dissemination of so-called empirical treatments (Belaïsch, 1993). Although still widely applied, most of these treatments are assumed to be ineffectual

*Partially based on an internet-published paper on http://ferti.net, where all commentaries on the present paper may be posted.

and it is only assisted reproductive technology (ART) which offers interesting perspectives for the treatment of unexplained male infertility (O'Donovan *et al.*, 1993). In recent years, most efforts in ART, a body of technologies emerging from co-operation between gynaecologists and reproductive scientists, have focused on the enhancement of the in-vitro fertilization (IVF) process. Although the basic physiology of normal fertilization still remains poorly understood, these efforts led ultimately to the first successes with ICSI (Palermo *et al.*, 1992).

Since the introduction of ICSI, the clinician dealing with the infertile male has access to a powerful tool for alleviating male infertility. By means of ICSI, fertilization and pregnancies can be obtained successfully with spermatozoa recovered or from an ejaculate, or from the epididymis either from the seminiferous tubules irrespective of whether spermatogenesis is normal or deficient and irrespective of whether the underlying pathophysiology is understood or not. ICSI is thus rapidly becoming a routine treatment for all reproductive clinicians dealing with male infertility. Nowadays this treatment is certainly not the exclusive province of gynaecologists alone.

But the magic of ICSI may also put a spell on the infertile patient who is less interested in the pathophysiology of his problem than in having his own child as quickly as possible. In the near future cost–benefit analysis and the utilitarianism often associated with 'evidence-based medicine' may also provide a reason for health authorities to support ICSI as a treatment of long-standing male infertility rather than to support basic research aimed at understanding its pathophysiology. Thus long-standing male infertility, although remaining an ill-defined condition, may become a trivial issue with a simple cost-effective approach bypassing any detailed diagnostic work, i.e. ICSI.

The risk of a disease as described by Jequier and Cummins (1997) may exist. Worse, it may eventually reach epidemic proportions, probably not because of the utilitarianism of ART clinicians but rather because of patients' attitudes towards their own infertility and because authorities are focusing on short-term health policies.

But what about the cure?

The ultimate desire of the couple experiencing infertility is the wife's pregnancy. Traditionally, the female has been considered the prime cause of barren marriages. It is for this reason that the gynaecologist is often the first fertility specialist to enter the field. Accurate assessment of the female factor is very important even in male subfertility. Indeed, male and female subfertility are known to be closely interrelated. Many couples where the male partner may be subfertile will probably never seek medical help because the female partner fully compensates for the 'male factor', resulting in no delay in conception. Conversely, a male partner may be judged to have a problem while in fact the couple's fertility status may be negatively influenced by a concurrent and even more important problem in the female. The diagnosis of male subfertility can obviously only be made after an appropriate assessment of the 'female factor'.

To cope with this particular situation, Jequier and Cummins suggest a change in attitude involving at least a joint clinical approach by both an urologist and a gynaecologist or, even better, a change in the training of the reproductive clinician dealing with male infertility, i.e. the reproductive andrologist. Neither their plea for a change nor their proposal is new (Jequier, 1990). Any gynaecologist or urologist involved in male infertility should acquire minimal theoretical and practical skills in investigating and treating disorders of the genito-urinary tract of either sex. Whether this change may prevent the 'decline' in clinical andrological investigation is questionable.

Indeed, proper training in urology or gynaecology or even both is certainly not a guarantee of good medical practice and many examples of the 'decline' in male clinical andrology are, in fact, just examples of poor medical practice. A recent letter in this journal (Canale and Caietti, 1996) illustrates that permanent re-training should be compulsory and may be more effective in preventing poor medical practice in clinical andrology than the fact of whether the clinician was originally trained as a gynaecologist, urologist, endocrinologist or dermatologist.

A training programme for a clinical reproductive andrologist should perhaps not be limited to a full or partial training in urology and gynaecology alone. Practical skills or theorethical understandings in the male infertility clinic may be unfamiliar in either of the two disciplines, e.g. electroejaculation, genetics. It may therefore be preferable to set out minimum standards for the practical and theoretical skills which a reproductive andrologist should have or acquire, including principles from reproductive endocrinology, clinical genetics, embryology and microsurgery. In Europe, the European Academy of Andrology is trying to set up such standards and to approve male infertility clinics with a broad multidisciplinary set-up for training in reproductive andrology (Nieschlag, 1996).

Secondly, we may ask whether the current standards for the evaluation of the male partner are sufficiently relevant to prevent a further decline in clinical andrology. Through a concerted action involving urologists, endocrinologists and gynaecologists, the World Health Organization (WHO) has set standards for the investigation of the infertile couple. For the male, these standards were first published as a supplement to *The International Journal of Andrology* (Comhaire *et al.*, 1987) and were later incorporated without substantial modifications in a WHO manual (Rowe *et al.*, 1993). This WHO standardized investigation is now becoming generally accepted as the gold standard for investigating the male partner (Comhaire, 1995).

The investigation consists mainly of history taking, physical examination, semen analysis, laboratory investigations and additional technical investigations. But will this investigation eventually provide an improvement in the understanding, the treatment and the prevention of male infertility?

History taking is an important key since 'it will contribute to the diagnosis in one fourth of cases' (Rowe *et al.*, 1993). Indeed, history taking may provide many keys to diagnostic categorization. History taking, as suggested in the manual, will provide many keys to 'damage-done-conditions', e.g. mumps orchitis, cryptorchidism, chemotherapy. Only few keys will refer to conditions that are amenable, e.g. sexually transmitted disease or vasectomy where microsurgery may be

of some help, delayed puberty which raises the possibility of hypogonadotrophic hypogonadism or a misuse of anabolic steroids leading to spermatogenic depression.

The physical and andrological examination, again, may also provide many keys to 'damage-done-conditions', e.g. small testicular volume, but may also provide some keys to 'conditions-under-debate', e.g. varicocele or male accessory gland infection (MAGI). Again, only a few keys may refer to amenable conditions, i.e. epididymal swelling which might be related to an obstruction, small and soft testes which may be related to hypogonadotropic hypogonadism, or scrotal swelling which might be indicative of testicular malignancy. If we look at the additional tests, again, many tests will refer only to non-manageable conditions. Endocrine testing including follicle stimulating hormone (FSH) and testosterone may be the key to the curable condition of hypogonadotrophic hypogonadism. Hyperprolactinaemia may be associated with sexual dysfunction. But a screening blood analysis, anti-chlamydia antibodies and urine analysis may be only weak indicators for manageable conditions. As for the other additional tests, their efficiency in diagnosing causes of male infertility has never been properly assessed. Additional investigations such as thermography or ultrasound combined with Doppler may again be keys to 'conditions-under-debate' such as varicocele. While the incidence of testicular cancer may be increasing and may be closely related to male infertility (Carlsen *et al.*, 1995), the WHO manual does not advocate any screening for testicular malignancies. Testicular biopsy is indicated only if both testicular volume and serum FSH concentrations are normal.

So far, it looks as if the 'WHO standardized investigation' may be a key to only a few amenable conditions which are currently not under debate: mainly infertility related to hypogonadotrophic hypogonadism, a rather infrequent problem with an incidence of ~1 in 3500 (Comhaire *et al.*, 1987), or obstructions of the male genito–urinary tract. The impact of the 'WHO standardized investigation' on the treatment of male infertility by methods with a proven benefit may therefore be rather limited. Its direct impact on understanding the pathophysiology of male infertility may be even more limited. Making a final diagnosis of 'idiopathic teratozoospermia' by rigorously applying the WHO's 'objective criteria' will probably not contribute to our understanding of the underlying pathophysiology.

Yet this standardized investigation has some merits and indirect benefits. It may allow a proper selection of patients who may benefit from specific, i.e. non-empirical treatments. For sure, any treatment or even cure which may obviate the need for ART is certainly more than welcome. The WHO categorization, although not helpful at all in providing any accurate prognosis for the infertile couple, may constitute a criterion to allow a better selection of infertile male subpopulations for further clinical or fundamental research into male infertility. Finally, the history-taking and clinical examination can easily establish a reassuring basis for subsequent counselling of the couple. One of the co-authors of the manual evaluated the impact of a WHO-like standardized investigation in more than 1000 couples attending a male infertility clinic and concluded that basically it did not improve the chance of fertility but mainly provided an opportunity for supportive counselling (Hargreave *et al.*,1986).

There is obviously an urgent need to redefine the standard investigation of male infertility since ICSI was introduced. ICSI has created many new opportunities to treat patients suffering from unexplained severe male infertility who at present cannot benefit from any specific or efficient treatment. But ICSI has also raised many new concerns (Cummins and Jequier, 1995; Cummins, 1997), many of which can be dealt with only by an accurate clinical work-up (Tournaye and Van Steirteghem, 1997). For this, new standards for andrological investigation should preferably originate from sound clinical evidence and should include updated tests based on actual insights into both the genetics and the epidemiology of male infertility. At present, investigations for deletions associated with cystic fibrosis (Jarvi *et al.*, 1995; van der Ven *et al.*, 1996) or spermatogenic failure (Kent-First *et al.*, 1996) may be more important in terms of prevention of infertility and understanding the pathophysiology of male infertility than screening for subclinical varicocoeles. Screening for testicular carcinoma-in-situ in subfertile men with a history of cryptorchidism may also be more important than screening for an ill-defined and debatable condition like subclinical MAGI. It is therefore questionable whether the 'WHO standardized investigation' still represents the 'gold standard' for investigation of the male partner in the era of ICSI.

In the next decade we should not only reorganize the training of the reproductive andrologist and be vigilant in keeping good clinical practice on the right track, but we must definitely set new standards for the clinical investigation of the infertile male. Only then can a clinician provide a satisfactory basis for further research into the pathophysiology and prevention of male infertility. Even the reproductive andrologist who has chosen to fight the good fight against the raiders of male fertility, may become impotent without a suitable weapon!

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Clinical andrology is important for treatment of male infertility with ICSI

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In-vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) has become the most important method of treatment for most forms of male infertility. Because of its high success rate the spectrum of indications has been continuously expanded from severe forms of male infertility to moderate or even mild reductions of sperm quality. Some infertility experts even suggest performing only ICSI for all forms of male infertility because of the reduced pregnancy rates with conservative and surgical treatment, intrauterine insemination (IUI) or IVF compared with ICSI. Therefore, in many IVF programmes male infertility patients are often referred to ICSI treatment very quickly, sometimes even without a sufficient diagnostic andrological work-up. This attitude creates two problems: firstly, with regard to the quality of medical practice (good medical practice), and secondly, the quality and progress of scientific knowledge (research into male infertility).

With regard to the first point, clinical evaluation of every male infertility patient's clinical history, physical examination, repeated semen analysis and, based on these findings, additional microbiological, endocrinological and immunological examinations, are necessary. We should never forget that the worst possible situation for a patient, and certainly also for the physician, is reduced male infertility due to a testicular malignancy which remains undetected. Furthermore, despite the limited success rate of conventional treatment, it is our own experience that in several cases sperm quality or spermatogenesis can be improved by antibiotic, anti-inflammatory or immunological treatments and, following this, some potential cases of testicular sperm extraction (TESE) can be treated with the less invasive form of treatment by ICSI with spermatozoa retrieved from the ejaculate.

Secondly, a complete careful evaluation of the male patient, including history (medical, social, environmental), physical examination and evaluation of semen quality or spermatogenesis (e.g. testicular biopsy) are important requirements for identification of the aetiology of male infertility. Furthermore, it creates a valuable clinical basis for further scientific approaches to discover the pathophysiology of male infertility which consequently may open new approaches regarding prevention and treatment.

In summary, quality standards regarding treatment of male infertility have to include an appropriate diagnostic evaluation of the male by a physician specifically and sufficiently trained in andrology and reproductive medicine. ICSI is not a substitute for the andrologist, on the contrary, ICSI requires an andrologist. ICSI has opened a new field in clinical practice and research regarding treatment of male infertility as well as improving our understanding of sperm function and spermatogenesis which unfortunately has been so poor in the past.

Molecular biology in the modern work-up of the infertile male: the time to recognize the need for andrologists

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In a few months the reproductive scientific community will be celebrating the 20th anniversary of the first in-vitro fertilization (IVF) baby and, at the last census, it was calculated that some 1 500 000 births have occurred worldwide through the use of various assisted reproduction techniques (Olivennes *et al.*, 1997). Taken as an isolated entity, this number is extremely satisfactory and it demonstrates that the establishment and practice of IVF and its associated technologies has been successful. Over the years, a number of breakthroughs have contributed to improve the overall success of these technologies. These include variations in protocols of ovulation induction, better in-vitro culture methods and more refined media, effective embryo cryopreservation and intracytoplasmic sperm injection (ICSI), to name just a few. Since the advent of ICSI, some 1500 births with close follow-up have been reported (Palermo *et al.*, 1996; Tournaye and Van Steirteghem 1997); reassuringly, the frequency of major and minor congenital abnormalities in children conceived through ICSI (2.6% of births) is within the range observed with standard IVF.

The wide use and availability of assisted reproductive techniques is in sharp contrast to our slow progress towards the understanding of the aetiology(ies) of many forms of infertility. This has been particularly true for male infertility. Recently, basic research utilizing the applications of modern molecular biology in a variety of mammalian and non-mammalian experimental models are starting to pay great dividends with regard to our understanding of spermatogenesis, spermiogenesis and sperm function. With the expansion of this knowledge base, there is little doubt that the andrologist of the 21st century will rely on the use of molecular biology to assess the infertile male, as well as to gain further insights on how a spermatozoon is made, how it functions in fertilization and whether any of these processes can be repaired when found to be defective. Moreover, these techniques may also form the basis of diagnostic tests for this poorly understood clinical problem.

The widespread use of ICSI, in addition to its success for treating infertility, may also be particularly advantageous for identifying human models in which to investigate genetic causes of male infertility. Moreover, patients that display severe forms of male infertility or that have unexplained failure at fertilization may possess genetic mutations that underlie their phenotype and, therefore, could be of great value for understanding the control of spermatogenesis and sperm function. In addition, it will be necessary to seek reassurance that the use of abnormal or dysfunctional gametes in assisted reproduction will not result in the generation of other pathological entities. What follows are some examples that clearly establish the importance of linking clinical medicine to basic research, utilizing the power of molecular genetics. The translation of observations at the laboratory bench to the clinic and *vice versa* will clearly have profound implications with regard to the future assessment and treatment of the infertile male, as well as for the development of diagnostics in the andrology laboratory. The study and treatment of male infertility clearly will benefit from the clinician and scientist working as a team.

One such example that may ultimately have profound implications with regard to our knowledge of spermatogenesis is the application of Y chromosome screening to assess deletions in the long arm, region 11.23. A wide variety of spermatogenic defects, ranging from the 'Sertoli cell only' phenotype to meiotic maturation arrest and severe hypospermatogenesis have been associated with deletions of variable regions of Yq in the azoospermia factor (AZF) locus. At present, the molecular identity of AZF is still unknown. Alterations in the YRRM 1 and 2 sequences (Y chromosome RNA recognition motif), AZFa, AZFb, AZFc, DAZ (deleted in azoospermia) have been reported to be associated with these aforementioned phenotypes and numerous sequence-tagged sites (STSs) have been isolated and are currently used to screen patients with azoospermia or idiopathic oligozoospermia (Ma *et al.*, 1993; Affara *et al.*, 1996; Reijo *et al.*, 1995, 1996; Vogt *et al.*, 1996). Approximately 15% of patients in these categories have been found to carry deletions. However, the majority of men with severe spermatogenic defects (85%) show an intact AZF, thus highlighting the possibility that either point mutations or other genetic defects might be responsible for the phenotype observed. Autosomal homologues of the *DAZ* gene have been identified on chromosome 3. This homologue is not identical to the *DAZ* gene on the Y chromosome, displaying only one sequence repeat of 24 amino acids (the *DAZ* on the Y has 7–16 sequence repeats), but possessing a similar putative RNA binding domain. Furthermore, the *DAZ* sequence on the Y chromosome is expressed specifically in the testis while the autosomal *DAZ* is also expressed in the ovary. The current hypothesis is that *DAZ* is a gene potentially encoding for an RNA-binding protein, and may be involved in the regulation of meiosis. This is certainly supported by the recent cloning and characterization of the *Drosophila* homologue of *DAZ* (named *boule*), which has been shown functionally in the fly to play a role in the regulation of meiosis during spermatogenesis (Eberhart *et al.*, 1996). This connection between infertility, genetics and the elucidation of regulatory elements governing normal spermatogenesis would not have been possible if it were not for the advent of molecular biology and molecular genetics. The practical use of this information can be found in the clinician–patient relationship and is important to the infertile couple for a number of reasons. Firstly, it will finally provide an explanation for the problem, and this alone can relieve some of the anxiety or existing feelings of guilt. Secondly, it may provide evidence that genetic transmission of male infertility to a male offspring might occur. Thirdly, it may help to avoid varicocele operations which, in these instances would not be able to improve the fertility status. Finally, it may help the couple to decide whether or not to embark on the entire cycle of assisted reproduction.

A list of genes whose deletion in the mouse generates defects in the male germ cell lineage has been recently summarized (Sassone-Corsi, 1997). It is becoming increasingly clear that the regulation of gene expression in germ cells follows defined rules, and molecules involved in the regulation of transcriptional activity function as checkpoints to determine the fate of the male germ cells. The use of transgenic and targeted gene disruption technologies is contributing greatly to our knowledge of regulatory events in spermatogenesis. For example, alterations in protamine synthesis has been achieved in transgenic mice by modifying the 3′ untranslated region of the *Prm1* gene and this results in premature nuclear condensation and arrest of spermatid differentiation (Lee *et al.*, 1995; Sassone-Corsi, 1997). Moreover, disruption of the gene encoding retinoic acid receptors (RAR-α) in mice results in high post-natal mortality and sterility in the male survivors with severe degeneration of the germinal epithelium. Further, animals lacking one of the retinoid receptors (RXR-β) expressed specifically in Sertoli cells are also sterile with a severe reduction in the number and motility of spermatozoa and a high number of spermatozoa with ultrastructural abnormalities. What is remarkable in these studies is that most of the genes indispensable for proper male germ cell differentiation are dispensable for oogenesis, supporting the concept that molecular regulators of meiosis may be sex-specific (Sassone-Corsi, 1997).

Another example where medicine and science, together, could ultimately contribute to the knowledge, diagnosis and treatment of specific forms of male infertility, is immotile Cilia

Syndrome (ICS, or Kartagener syndrome). This syndrome is characterized by chronic sinusitis, bronchiectasis, dextrocardia and male infertility secondary to sperm immotility. The absence or shortening of the inner dynein arms of the axoneme is the basis for sperm immotility and ciliary epithelial dysfunction. Of relevance to this discussion is the observation that immotile mutant strains of the flagellated algae, *Chlamydomonas*, have been found to have a genetic defect in the *p28* gene, which encodes the inner dynein arms. Recently, the human homologue of this gene has been cloned and linked to human chromosome 1, short arm, region 35.1 (Kastury *et al.*, 1997). Again, such approaches have evolved from the analysis of similar sorts of genetic defects that have been amenable to testing in other experimental models. It will soon be possible to determine whether males that have immotile spermatozoa or severe asthenozoospermia possess mutations in this particular gene. It is interesting to speculate that, in the future, patients displaying variations in the severity of Kartagener syndrome (e.g. with completely immotile spermatozoa or severe asthenozoospermia in the absence of respiratory problems) will be described genetically. The heterogeneity of the phenotypes in these instances could very well be similar to that seen in cystic fibrosis where a mild, variant form of the disease, is responsible for congenital absence of the vas deferens (Patrizio and Zielenski, 1996).

Some additional data concerning the morphology and function of the non-motor and motor proteins of the sperm flagellum is emanating from different laboratories. The flagellum is composed of a number of cytoskeletal elements, whose coordinate action is essential for proper motility. The fibrous sheath, present only in the sperm principal piece, is a cylinder of fibres which underlies the plasma membrane and envelops the outer dense fibres and the axoneme. It is believed that the fibrous sheath, although not considered to be a motor structure, may regulate the proper plane of bending of the sperm flagellum by restricting the sliding movements of the axoneme (Lindemann *et al.*, 1992; Si and Okuno, 1993). Recently, 34 patients have been reported to have dysplasia of the fibrous sheath and dynein deficiency (Chemes, 1997). Semen parameters included rigid, short and thick flagella, severe asthenozoospermia and necrozoospermia. Two of these patients were brothers, and only in seven of the patients was it possible to document chronic sinusitis/bronchitis. Ultrastructural examination of the spermatozoa from these patients revealed hypertrophy and hyperplasia of the fibrous sheath, as well as axonemal anomalies such as the lack of dynein arms and the lack of central doublets, strongly suggesting that this condition might represent a variant of ICS. In these 34 patients there were no spontaneous or conventional IVF pregnancies and fertilization via ICSI was used successfully in two cases. However, the majority of these patients had a variety of treatments over long periods of time (varicocelectomy, use of gonadotrophins, antioxidants, etc.), none of which changed their semen parameters. Moreover, some individuals with degeneration of the sperm axoneme have been described as having Usher's syndrome (Hunter *et al.*, 1988). These men have non-progressive congenital hearing impairment and vestibular deficit due to axonemal defects in otic ciliary axonemes. The basis of the instability of these axonemal structures is unknown at this time.

Another area of active research is aimed at uncovering molecules involved in the correct assembly of sperm components of the fibrous sheath. A number of polypeptides have been extracted from the fibrous sheath of mammalian sperm flagella. A major constituent of the mouse sperm fibrous sheath is a polypeptide called p82, a phosphoprotein localized exclusively to the principal piece of the flagellum. Molecular cloning of mouse p82 predicted that it encodes a germ cell-specific member of the A Kinase Anchoring Protein (AKAP) family, a family of proteins that anchors the RII subunit of protein kinase A to the cytoskeleton (Carrera *et al.*, 1994). The human homologue of the mouse AKAP82 exists in the human sperm fibrous sheath in both its precursor (M_r = 97 000; pro AKAP82) and mature (M_r = 82 000; AKAP82) forms; both forms are phosphorylated on tyrosine residues under conditions that support capacitation (Carrera *et al.*, 1996). The identity of this protein as an AKAP has profound implications with regard to its role in regulating motility, given the fact that sperm motility is regulated by cAMP and protein kinase A (Tash, 1989). It is of great interest that the mouse *AKAP* gene is located on the X chromosome (Moss *et al.*, 1996), since this represents the first example of an X-linked gene in which both transcription and translation is restricted to haploid male germ cells. The regulation of phosphorylation of this protein, as well as its function in regulating motility through its interaction with protein kinase A, will most likely tell us a lot about how sperm motility and capacitation are regulated. Such information might provide the andrologist with biochemical and molecular approaches to examine these specific aspects of sperm function as they relate to male infertility.

Centrosome restoration and microtubule-mediated motility are both required to complete fertilization, and an increasing number of sperm-associated proteins have been identified as responsible for the success of various post-fertilization events (Schatten 1994). Defects in proteins such as γ-tubulin (which nucleate microtubules), centrin (a calcium induced severing protein), pericentrin (a protein of the intermediate filaments), the nuclear mitotic apparatus (NUMA), to name a few, may soon be found to underlie the cause of specific forms of male infertility that cannot be resolved even using ICSI (Oakley and Oakely, 1989; Doxsey *et al.*, 1994; Simerly *et al.*, 1995; Navara *et al.*, 1996). There is evidence in cattle that sperm quality may affect the size of the sperm aster (i.e. smaller asters with disarrayed microtuble patterns in spermatozoa with qualitative deficiencies) and this, in turn, is reflected in low live birth rates (Navara *et al.*, 1996). Molecular assays for these proteins could soon be implemented in andrological work-ups of the infertile male (e.g. in-vitro decondensation of sperm nuclei and formation of microtubules after disulphide reduction and exposure to *Xenopus* egg-extracts). Theoretically, in males with this specific defect in sperm aster formation, ICSI should not be able to circumvent the post-fertilization developmental events unless normal centrosome microinjection therapy could be carried out; such therapy is completely speculative at this time. In this regard, it is interesting to note,

however, that heterologous centrosomal microinjection has led to microtubule nucleation in animal models (Schatten, 1994).

Although these represent just a few examples, they highlight the fact that the andrology laboratory and andrology work-ups in the future will include genetic testing, as well as the development of new diagnostics and tests of sperm function, many of which may utilize molecular biology. In order to insure cost-effectiveness of such testing programmes, one possibility would be to establish some laboratories (e.g. in larger institutions or in the private sector) as referral centres to offer genetic screening to the infertile couples. It will be also be advantageous to set-up 'DNA banks' for the purposes of depositing DNA from couples with unexplained infertility. As basic and clinical information becomes available, this source of DNA could then be utilized in retrospective studies in order to further understand the aetiology of specific forms of male infertility.

Andrology as a formal discipline

We conclude with a comment on the issue raised in the article of Jequier and Cummins (1997) on attitudes toward clinical andrology. There is no doubt that andrology has to be recognized as a discipline. However, this is a difficult task whose resolution involves a great deal of expertise in bio-politic, and solutions may be different depending on geographic location. Who can be considered an andrologist? Andrology is the study of the reproductive functions of the male, both in normal and pathological conditions.

The andrologist is a physician who wishes to specialize in the study, diagnosis and treatment of the male with regard to reproductive function. Since andrologists will likely be working in close collaboration with or within reproductive units, subspecialists in reproductive endocrinology and infertility, as well as urologists with a keen interest in male infertility, are probably best suited for being trained in andrology. However, it is really not important if the primary training is in gynaecology, urology, internal medicine or endocrinology (in fact, in Europe some fine andrologists have their primary training in dermatology). What is important is proper and recognized training in this particular subspecialty. The andrologist must have a solid knowledge of the human male reproductive system (anatomy, physiology, pathology, endocrinology, genetics, pharmacology, diagnostic techniques, medical and surgical therapy) and must have familiarity with laboratory techniques used to evaluate male reproductive function. Since the majority of patients who need andrological evaluations are attempting to start or add to a family, the training should also include clinical competence in female reproductive disorders and an understanding of assisted reproductive techniques.

How can andrology training be established? In agreement with the Jequier and Cummins article, the training for this subspecialty can be achieved through a rotation in urology specifically targeted at learning how to do a complete male genital examination, how to perform transrectal and scrotal ultrasounds, and how to perform epididymal or testicular surgery for diagnostic or reproductive purposes. The female component of this subspecialty can be taught with an equivalent rotation in gynaecological reproductive units. There will be grey areas. Who is going to do vasectomy and vasectomy reversal operations? What about varicocele surgeries? Is the evaluation and treatment of impotence to be carried out by andrologists or urologists? Hopefully this debate will open a forum for more constructive input, as well as a concrete plan of action. In Europe a step forward for the official recognition of andrology has been the creation of the European Academy of Andrology (EAA, 1996), a non-profit organization which is concerned with the qualification and training of andrologists as well as encouraging basic research in all fields of andrology, including male contraception.

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