Future Prospects in Reproductive Andrology

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Summary

The introduction of ICSI, the endrocrinological aspects of the ageing male, new treatments for ejaculatory dysfunction and new insights into the molecular basis of primary testicular failure have renewed interest in clinical andrology. There is an urgent need to define and organise the subspeciality of clinical andrology. Apart from urology and gynaecology, clinical andrology nowadays overlaps with many other disciplines, eg. Endocrinology, microsurgery, paediatrics, spermatology, histopathology, oncology, embryology, and genetics. In the 21st century all these elements will generate new insights and treatment strategies.This paper tries to highlight some important new developments in the field of reproductive andrology. Of course only time will tell how the andrological landscape will look in the next century.

Clinical andrology: a challenge for the 21st century?

In many countries, clinical andrology has had a long tradition. In the beginning, mainly endocrinologists were active in the field of andrology, because they were among the first to be initiated into the secrets of the hypothalamo-pituitary-gonadal axis. Many, often ill defined, treatments were used in order to influence this hormonal axis in the hope of improving a man's fertility potential. However, in the year 2000, clinical andrology has become highly influenced by both urologists and gynaecologists. The former have been involved in the surgical aspects and, as andrology increasing became something to be carried out under a microscope by bringing spermatozoa closer to oocytes, gynaecologists too became important players in the field.

"The speciality of andrology is confined to the study and treatment of disorders of reproductive function — the management of the male infertility" says Jequier (1990). Nieschlag's definition, however, is broader: "The central topics of andrology are infertility, hypogonadism, male contraception, erectile dysfunction and male senescence" (Nieschlag, 1997). The integration of all these subspecialities into one specific training of a single specialist will be one of the greatest challenges of the next century. This issue has given rise a lot of discussion in recent years (Jequier and Cummins, 1997; Tournaye 1997 and 1998). But in the meantime clinical andrology is still the subject of a political discussion in most countries. Who is best placed to take care of the (infertile) male?

It is certain, however, that within the 21st century, clinical andrology will become an established subspeciality with a multidisciplinary profile. Within this new speciality, new developments will arise. The start has already been given: the treatment of erectile dysfunction and the treatment of non-obstructive azoospermia are cases in point. Medical male contraception is in the pipeline but more developments will follow. Only time can tell what the andrological landscape will look like in the decades to come.

From genetic screening to genetic diagnosis

The development of molecular biology is sure to have an important influence on clinical andrology. Tiepolo and Zufardi describe in 1976 a small number of patients with

deletions at the distal end of their Y-chromosome (Tiepolo and Zufardi, 1976). Since then the hunt for genes encoding for spermatogenesis has been started. In recent years, the AZF- regions on the Y-chromosome have been explored. These genes have an important signal function in the genetic network that supports spermatogenesis (Vogt, 1997). At present Y-chromosome deletion screening has become part of the basic investigation for men with a Y-deletion are informed that they have a genetic background causing their problem and that there is a possibility of passing this gene defect to their male offspring whenever intracytoplasmic sperm-injection is successful. However, in years to come this genetic screening will be replaced by genetic diagnosis. When the genetic network encoding for spermatogenesis has been completely understood, more and more genotypes will be correlated to specific phenotypes (expressions at testicular level). We may therefore expect that genetic tests will be not only inform us about the aetiology of the testicular dysfunction, but will also provide guidelines for further treatment. The type of AZF-deletion on the long arm of the Y-chromosome already provides an indication of the chances of recovering testicular sperm in men with non-obstructive azoospermia (Brandell et al, 1998). Other genetic tests related to spermatogenesis will follow and performing a genetic test will become highly automated and will involve many genes. Currently, gene-chips are available that can analyse the gene expression of about 40,000 human genes and thousands of polymorphisms. In the 21st century automated gene analysis will become an important step in the work-up of a man with unexplained spermatogenic dysfunction.This computerised analysis will not have diagnostic value but will also influence our treatment strategies.

Beyond Van Leeuwenhoeck

Semen analysis by light microscopy is one of the cornerstones in the andrological work-up. For more than 10 years, computerised semen analysers have been used. These so-called CASA systems (computer assisted semen analysis) calculate the density of spermatozoa and analyse motility characteristics. However, these CASA systems have so far been unable to replace manual light-microscopic semen analysis, mainly because a computer has a limited discriminative power to distinguish spermatozoa from other cells, e.g. debris of the same dimensions. The same is true with the more recently introduced automated systems for analysing sperm morphology.

Yet more and more semen samples of poor quality are used for assisted reproduction. In follow-up studies on offspring after ICSI, it has been shown that sex chromosome aneuploidy is a major adverse outcome effect. In the near future, automated systems may be developed to enable us to analyse the aneuploidy rate of a semen sample using specific probes, e.g. probes for the X-and the Y-chromosome. By assessing to aneuploidy rate of a semen sample, ICSI-candidates may be better counselled with a view to future treatment. Maybe one day it will even become possible to filter out aneuploidic spermatozoa from oligozoospermic semen samples used for ICSI. The assessment of the genetic fitness of a semen sample or the genetic upgrading of a semen sample may one day become important steps in andrological work-up and treatment strategies.

The testicular stem cell

Spermatogenesis in the adult male is a continuous process. Spermatogonia have the capacity to renew themselves and to initiate meiosis followed by a process of differentiation. Only a small proportion of spermatogonia, the so-called stem cells, have the potential to renew themselves.

Some years ago, American researchers showed that spermatogenesis can be re-initiated after transplanting these testicular stem cells (Brinster and Zimmerman, 1994). Spermatogonia from pre-pubertal mice were transplanted into the seminiferous tubules of adult mice with Sertoli-cell only tubules after cytotoxic treatment. Using a genetic marker, Brinster and Zimmerman (1994) proved that the adult recipient mice produced spermatozoa derived from the pre-pubertal donor mice. They also showed that the recipient mice could reproduce in-vivo after transplantation. These experiments were also performed using stem cells that had been frozen and thawed (Avarbock et al, 1996). Furthermore, they

performed xenogeneous transplantation: rat testicular stem cells were successfully transplantated into the empty tubuli seminiferi of immunodeficient mice. These experiments, once extrapolated to a human model, may have important applications. When an adult man undergoes a sterilising cytostatic treatment, spermatozoa can be frozen in order to circumvent sterility after his treatment. However, no such prevention is possible before puberty since no active spermatogenesis is present. Here, testicular tissue containing stem cells may be thawed at adult age and be reintroduced into the empty seminiferous tubules (autologeous transplantation).

Other applications may be even more futuristic: Short (1998) states that in the future it may become possible to develop human stem cells to maturity in surrogate animals. So far, preliminary experiments have not been successful. Transplantation of the stem cells from hamsters, rabbits and dogs has failed to initiate spermatogenesis in recipient mice, probably because the phylogenetic gap between these species is too wide. However, the phylogenetic gap between great apes and humans is smaller than that between rats and mice, a model in which xenotransplantation worked!

Spermatozoa from the lab

For many years, in-vitro culture of human oocytes has been performed with variable success. Yet it is a given fact that in the 21st century controlled ovarian hyperstimulation will become obsolete. Wu and co-workers have reported successful in-vitro maturation of primordial follicles obtained from the follicular fluid of patients undergoing in-vitro fertilisation (Wu et al, 1998). Although others have not yet reproduced this work, in-vitro maturation of human oocytes will become a reality.

Whether in-vitro maturation of spermatozoa will become a reality remains very questionable. Spermatogenesis is not a cell-autonomous process characterised by a strong interaction between the differentiating stem cells and the Sertoli cells. At present, the in-vitro culture of spermatogenetic cells is limited to an in-vitro culture of mainly postmeiotic stages in patients with a normal spermatogenesis (Tesarik et al, 1998). Transmeiotic culture has been reported in a few patients (Tesarik et al, 1999), but again it has not been possible to reproduce this work so far. In animal models, only a small part of the whole spermatogenetic process can be induced to take place under in-vitro conditions. Testicular cells entering the spermatocyte stage be taken through meiosis up to the spermatid stage (Rassoulsadegan et al, 1993) but these spermatids show a high aneuploidy rate and spermiogenesis is not observed. As long as the specific interactions between Sertoli cells and spermatogenetic cells and the necessary co-factors remain unknown, complete in-vitro spermatogenesis will remain unattainable.

Instant semen

Adult men undergoing cytostatic treatment can bank their semen. In order to stop all biological and biochemical processes, spermatozoa are stored at a temperature of -196° celsius. In order to prevent cryodamage, the spermatozoa are incubated with cryoprotectants such as glycerol. Banking semen is expensive and difficult: need for liquid nitrogen, limited transportability of sample, risk of viral transmission in the liquid phase. Recently Yanachimachi's group reported the successful lyophilisation (freeze-drying) of mouse spermatozoa (Wakayama and Yanagimachi., 1998) During lyophilisation, cells are put in a state of anhydrobiosis by sublimation of ice. In these mouse experiments spermatozoa were incubated in a culture medium containing 10 % foetal bovine serum. After freeze-drying, the spermatozoa were put in a vacuum-sealed ampoule. These ampoules were stored and transported for several months at temperatures of 4 degrees up to 30 degrees Celsius. After rehydratation by adding saline, the sperm heads were isolated and introduced into mature oocytes by means of intracytoplasmic sperm injection. Fertilisation in-vitro was very successful and 14 out of the 46 embryos transferred into pseudo-pregnant foster mothers implanted. This technique represents a major breakthrough for the trade in transgenic mouse strains. Whether we will able to store human sperm in our kitchen next to the lyophilised coffee remain unclear. Only time will tell.

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