European Society of Human Reproduction and Embryology

Andrology Pre-congress course

Controversies in the Clinical Management of the Infertile Male

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Contents

Sunday, 25 June 2000 -Pavillion 41

Course 3: Andrology
"Controversies in the Clinical Management
of the Infertile Male"

Course Co-ordinators: L. Fraser (UK)
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Course description

This course is intended to provide discussion and debate on four areas of controversy concerning clinical management of the infertile male. At the end of each set of presentations there will be general discussion, the aim being to reach a consensus view which could be disseminated to all individuals working in this area.

Programme

I. Is clinical examination important in the management of the infertile male in the ICSI era?
09.00–09.30: Clinical examination is of minimal relevance - P Devroey (B)
09.30–10.00: Clinical examination is of great relevance - A Jequier (AUS)
10.00-10.30: Discussion
10.30-11.00: Coffee break

II. Should spermatids be used for ICSI?
11.00-11.30: Yes – J. Tesarik (F)
11.30-12.00: No – B. Jegou (F)
12.00-12.30: Discussion
12.30-13.30: Lunch break

III. Is genetic screening important?
13.30-14.00: Relevance of genetic screening to male infertility –
D. Meschede (D)
14.00-14.30: Results of ESHRE questionnaire on genetic screening in
infertility units - J. Kremer (NL)
14.30-15.00: Discussion
15.00-15.30: Coffee break

IV. Is infectious screening important?
15.30-16.00: Relevance of infectious screening to male infertility
– J.P. Wolf (F)
16.00-16.30: Results of ESHRE questionnaire on infectious screening in
infertility units – S. Hamamah (F)
16.30-17.00: Discussion

Clinical examination is of minimal relevance

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For sure the easiest way of doing medicine is to ask for a full battery of diagnostic tests. Needless to say that this is a very expensive approach. Moreover one has to question if knowing the diagnosis i.e. maturation arrest in case of extreme oligo-astheno-terato-zoosperma will help the patient in his endeavour to become pregnant.

This debate in pro and con condition is to a certain extent artificial. Since a debate is a public discussion between friends I will defend the thesis that clinical examination is of minimal relevance.

I still remember in a recent past infertility patients with longstanding infertility came at the outpatient clinic with books as bricks of reports and letters. After all these examinations the couples were still childless.

It seems illogical to examine all males of infertile couples. There is a need to define a distinct strategy taking into account the cost and the benefit. Furthermore, simplification is important. And thirdly only physical, hormonal examinations, which have a rational, should be performed. Fourthly, the history of patients will guide the choice for the test needed. Additional information is obtained by sperm analysis.

If sperm analysis is normal according WHO criteria (1987, 1992 en 1999), one can question which additional tests have to be done. If semen analysis is abnormal according to WHO criteria one have to interpret these results. The first remark is that the criteria used by WHO are arbitrarily chosen criteria. Those criteria are not evidence based. As demonstrated the mean sperm concentration in the fertile group varies from 11 to 241 x 106 (Barratt, HR 1995). The proposed 20 000 000 x 106 / ml sperm concentration is in contradiction with the 11 x 106 million. Furthermore individual variation can immensely vary from 2 to 40 x 106 ml spermatozoa.

Do the results of semen analysis predict future fertility? One thousand eighty nine couples were analysed. No Significant influence of any semen characteristic on the cumulative probability of conception has been observed (Polansky, FS 1988).

Will physical examination add any value? Already a decade ago it was clearly stated that routine examination of the male partner is of no prognostic value (Dunphy, FS 1989).

The reasons of discernible causes of testicular disease in oligospermia (n:110) were unknown (67%), maldescent (24%), torsion (2%), trauma (5%) and mumps orchitis (2%) (Jecquier, BJU 1993). One has to realise that only history taking would have indicated the diagnosis. Moreover if you have the correct diagnosis no treatment is available. It seems impossible to treat unknown causes of testicular disease, to treat mumps orchitis and maldescent testis. It is not only impossible to treat the testicular condition, also the oligospermic condition is almost untreatable. Evidence based approach did not show significant improvement of pregnancy rates (ESHRE Capri Workshop, HR 1996).

Is male subfertility a sign of any medical pathology. It has been previously published that in oligospermic patients the frequency of medical illnesses is 0.8% (10/1233). Only 6 out of the 1233 males had a testicular tumor (0.5%). The remaining 4 were cerebral and spinal cord tumors. One can accept that in oligospermic males a testicular examination is performed knowing that the frequency of the testicular pathology is 0.5% (Jarow, UClNA 1994).

To detect extra testicular pathologies extensive evaluations including radiological and neurological ones are needed. Cost benefit analysis does not seem to advocate routine extensive examinations of infertile males. There is no pathognomic finding on history, physical examination, semen analysis or hormone profile that identifies all patients with significant medical pathology (Honig, FS 1994).

In many hospitals today physical examination of the infertile male is performed to detect the presence of a varicocoele. The expressed opinions on the role of varicocoele and its subsequent treatment is debatable:

1) A varicocoele does not exert a detrimental effect on fertility (Uehling, IJF 1968).

2) Improved fertility after varicocoele correction: fact or fiction? (Vermeulen, FS 1989).

3) There are patients with infertility whose problem does seem to be resolved by varicocoele ligation (Jecquier, Baillière, 1997).

4) No statistical significant improvement in semen and pregnancy rates after ligation of the internal spermatic vein is found (Nilsson, 1979).

In a randomised controlled trial it has been clearly demonstrated that occlusion of the vena spermatica does not improve the pregnancy rate. In the non-treated male the pregnancy rate was 27% (13/48) and in the treated males the pregnancy rate was 25% (12/47) (Nieschlag, HR 1995). There is no evidence that occlusion of the vena spermatica increases the pregnancy rates.

In the presence of azoospermia, semen analysis will indicate its obstructive and non-obstructive origin. In azoospermic males further investigation is mandatory.

In non-obstructive azoospermia clinical examination and testicular biopsy optionally with intracytoplasmic sperm injection are indicated. A karyotype and testing for Yq deletions are mandatory.

In obstructive azoospermia clinical examination is mandatory. Vasal and epididymal obstruction has to be searched for. The detection of a bilateral congenital absence of the vas deferens is important for subsequent therapies. Testing for cystic fibrosis is mandatory.

In conclusion, routine examination of the male partner is of no prognostic value. Semen analysis does not predict future fertility. No statistically significant improvement in semen and pregnancy rates are observed after ligation of the spermatic vein. There is no known way to reverse primary testicular disease.

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Table 1. Treatment of varicocoele: counselling as effective as occlusion of the vena spermatica

	Patients	Pregnancies	%
Counselling	48	13	27
Varicocelectomy	47	12	25

Nieschlag HR 1995

Table 2.   Do the results of semen analysis predict future fertility?

1098 couples were analysed

No significant influence of any semen characteristics on the cumulative probability of conception

Polansky FS 1988

Table 3. Clomiphene citrate versus placebo for male infertility

	Patients	Pregnancies	%
Treated	145	26	17
Controls	137	20	14

ESHRE Capri Workshop HR 1996

Table 4. Medical illnesses 10/1233 (0.8%)

Testis tumor	5
Leydig cell tumor	1
Prolactionoma	1
Pituitary tumor	1
Craniopharyngioma	1
Spinal cord tumor	1

Jarow UClNA 1994

Table 5. Clinical value of semen morphology characteristics

	Fertile (n:42)	Infertile (n:124)
Sperm concentration	51/106 ml	24/106 ml
Range	11 – 241	10 – 337
WHO (1992)		
Morphology (1992)		
Normal	30%	20%
Range	7 – 58	0 – 62

Barratt HR 1995

Table 6. Discernible causes of testicular disease in oligospermia (n:110)

Unknown	74 (67%)
Maldescent	26 (24%)
Torsion	2 (2%)
Trauma	6 (5%)
Mumps orchitis	2 (2%)

Jecquier BrJU 1993

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The clinical examination is of great relevance in the management of male infertility in the era of ICSI

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Introduction

For many years, the definition of male infertility has been based upon the results of a semen analysis: patients have been said to be either fertile or infertile depending upon whether the different variables examined during the semen analysis were within or were outside certain numerical constraints.

It must be remembered however that firstly a semen analysis is a very difficult laboratory procedure to carry out and secondly the validity of the analysis is fraught with a wide variety of artefacts that may be induced both during and after the collection of the semen sample.

Changes in the results of a semen analysis are, with some notable exceptions, entirely non-specific and give the clinician no idea of the type of infertility present nor of its aetiology. Thus before a semen analysis is of value clinically it must have some additive clinical input in the form of a clinical history and examination. It is therefore clear that the diagnosis of infertility from a semen analysis alone is insufficient to even diagnose the presence or absence of male infertility let alone to define its cause. A semen analysis, free from all the various forms of collection artefact, in fact only indicates the probability of infertility and does not indicate for sure that a given patient is fertile or sterile (Eliasson, 1971).

It is commonly said that it is very difficult to find the cause of male infertility and its treatment is only satisfactorily resolved by the use of assisted conception. In reality, the diagnosis of many forms of infertility in the male is often very easy provided a careful clinical history is taken and a skilled clinical examination is carried out. Unfortunately today, the majority of male infertility is treated by gynaecologists who have frequently had little or no training in the examination of the genitalia in the male: in these circumstances, assisted reproduction is valuable in that it makes a virtue out of a necessity.

Unfortunately today too much of the decision-making in the management of male infertility is left to the scientists who have, of course, had no training in any aspect of medicine. This sad trend is preventing much needed work being carried out on the aetiology of male infertility, much of which can be gleaned from the taking of a careful clinical history.

It now may be of value to examine the types of male infertility that may be seen in an infertility clinic.

Primary Testicular Disease

Probably the most common cause of infertility among men is a condition that I like to call primary testicular disease. In women, anovulatory infertility is frequently caused by some form of abnormal interaction involving the pituitary and the ovary. However in the male, almost all

abnormalities in gamete production are the result of a primary disorder of the testis itself. This can be the result of damage to the testis by many different factors or may even be caused by abnormalities of the paracrine system within the testis. The changes in the testes in primary testicular disease are often focal making the diagnosis of this condition by testicular biopsy very difficult. The diagnosis of primary testicular disease can be made on clinical examination of the patient and may be associated with a rise in the serum ESH level (Jequier et al, 1986). However, normal levels of FSH in serum do not exclude the presence of this condition. The more severe is this disorder and the greater the volume of testicular tissue that is abnormal, the more abnormal will be the semen analysis. It must also be remembered that this condition is frequently progressive and thus what may be oligozoospermia at one point in time may be azoospermia six months later. It is important that this diagnosis is made and if necessary sperm cryopreservation should be carried out.

At present all forms of primary testicular disease respond poorly to treatment and where it is severe will indeed need the application of assisted reproduction. This diagnosis will also avoid the prolonged use of ineffectual treatment regimes.

Ductal obstruction as a cause of in fertility in the male

This is also a common cause of male infertility and may be found in around 10% of all men attending the clinic. Obstructive lesions may involve the rete testis (Guerin et al, 1981), the epididymis at various points along its length, the vasa deferentia and the ejaculatory ducts. On clinical examination, no abnormality of the epididymis will be palpable whereas when the obstruction is within the epididymis itself (and where spermatogenesis is itself normal) the proximal portion of the epididymis will be distended and thus the site of the obstruction is identifiable clinically. The palpation of a distended upper epididymis is known as a positive Bayle's sign after the French andrologist who described it (Bayle 1952). However when the vas or the ejaculatory ducts are obstructed the whole epididymis is distended.
It is a common practice for clinicians to collect sperm from all azoospermic patients by means of a testicular biopsy. As has been clearly pointed out in the past (Schlegel et al.1997), testicular biopsy can seriously damage the testis and such sperm collections should only be carried out where no other means of sperm retrieval is feasible e.g. in an azoospermic man with primary testicular disease. We have demonstrated that similar testicular damage can result from minor trauma to the testis in the ram. The use of testicular tissue from men with simple anejaculatory failure is to be condemned.

Vasal obstruction

The most common cause of vasal obstruction presenting in an infertility clinic is unwanted vasectomy. In my clinic, vasectomy related infertility amounts to nearly 10% of my total workload. Other causes of vasal obstruction include surgical strictures, infective problems and congenital absence of the vas. Some vasal strictures respond well to surgery and assisted reproduction is only required in conditions such as congenital absence of the vasa deferentia and where the vasal strictures are either long or multiple.

Ejaculatoiy duct anomalies

Ejaculatory duct obstruction is a less common condition than obstruction in other sites of the genital tract and is frequently the result of the formation of a Mullerian duct cyst. This diagnosis may be made by palpation of an epididymis that is distended along its whole length and on rectal examination, enlarged seminal vesicles are sometimes felt above the prostate. The presence of a

Mullerian duct cyst can be palpated at the apex of the median lobe. The diagnosis is confirmed by trans-rectal ultrasound. These disorders are usually best treated by trans-urethral resection but should the ejaculatory duct obstruction be congenital when long segments of the vas are absent, then the use of vasal wash-out for sperm collection is recommended.

Other disorders of the ejaculatory ducts include ectopic siting of the ducts and atresia of the ducts themselves.

Erectile and ejaculatory failure

This problem may also present in an infertility clinic and both these problems can be organic or psychogenic. If there is an organic basis to this problem, it can be treated by the use of penile vibrators or even by the use of electro-ejaculation. Ejaculatory failure is almost the rule among men with spinal cord injury and can certainly be treated by vasal wash-out and assisted reproduction.

Serious disease presenting in an infertility clinic

It is of course possible that serious disease the most obvious of which is testicular cancer that occurs among young men can present in an infertility. As this condition may occur among men with a history of testicular maldescent and with primary testicular disease, all men with an abnormal semen analyses must undergo a clinical examination at their first clinic visit. As the ages of the patients attending the infertility clinics seems to be rising, more attention needs to be paid to the possible presence of hereditary prostate cancer which may be occasionally seem in men in their mid-forties. However it is also possible for any disorder to present in the clinic and this must be born in mind when examining any patients with infertility.

Conclusion

There is in my opinion, no place for the routine application of assisted reproduction, especially intra-cyto-plasmic sperm injection (ICSI), to every male patient with infertility. The most important reason is that never again will any clinician be aware of the aetiology of the patient's disorder and no effort can thus be made to ensure any form of prevention. It is too trite to mention that there are now being described many genetic causes of infertility about which both the patients as well as the clinician must be aware. Lack of clinical evaluation will cause ICSI to be carried out on semen collection artefacts and serious disease will go undiagnosed. Careful clinical evaluation is essential to the proper management of male infertility: short cuts to "infertility treatment" through the use of ICSI are unacceptable in the present day management of infertility in the male.

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Should spermatids be used for assisted reproduction? Yes.

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LEARNING OBJECTIVES

1. Spermatid definition and staging
2. Clinical indications for the use of spermatids for assisted reproduction
   a) Postmeiotic maturation arrest
   b) Meiotic maturation arrest (use of cultured spermatids after transmeiotic in-vitro differentiation
3. Success rates of assisted reproduction with spermatids
4. Main causes of failure of assisted reproduction with spermatids
5. Improvement of spermatid quality by in-vitro culture
6. Responses to main concerns about the use of spermatids for assisted reproduction
   a) Indication
   b) Success rate
   c) Safety
7. Conclusion

1. SPERMATID DEFINITION AND STAGING

The term spermatid is used for immature germ cells in the period between the completion of meiosis and the spontaneous release from the union with the Sertoli cells (spermiation). During this period, spermatids undergo a series of morphological, molecular and functional transformations (spermiogenesis) and develop from the least differentiated forms (round spermatids) through intermediate forms (elongating spermatids) to the most advanced forms (elongated spermatids) of which the latest stages cannot be distinguished from spermatozoa by a simple microscopic observation. Because of this morphological variety, the scientific value of any report on assisted reproduction with spermatids is dependent on the exact definition of the spermatid developmental stages that have been used in the attempt. Several staging schemes have been proposed in the literature. However, that originally suggested by de Kretser and Kerr (1) and modfied for the application with the means of observation currently used for micromanipulation-assisted fertilization (2) appears to be the most convenient for this purpose and is now being used by most workers in the field. Briefly, round spermatids before the appearance of any signs of elongation are denoted as Sa stage, Sb and Sc is used for spermatids at intermediate stages of elongation, and Sd denotes late elongated spermatids. It is to be emphasized that most germ cells with the morphological appearance of spermatozoa that are identified in testicular biopsy samples are in fact Sd elongated spermatids because they have not undergone the physiological process of spermation but were separated artificially from Sertoli

cells during the sample handling. However, the distinction between Sd spermatids and spermatozoa can only be made with certainty when a spermatid is still attached to a Sertoli cell when examined under inverted microscope and manipulation with a microneedle is necessary to separate both cells from each other.

2. **CLINICAL INDICATIONS FOR THE USE OF SPERMATIDS IN ASSISTED REPRODUCTION**

a) **Postmeiotic maturation arrest**

Spermatogenesis can be arrested by a pathological process in the testis at any stage, but premeiotic and meiotic blocks represent the most frequent situations (3). However, postmeiotic arrest of spermatogenesis does occur in some patients, and this was the original indication for the use of spermatids in assisted reproduction, leading to the first pregnancies and births after fertilization with round (4) and elongated (5) spermatids.

b) **Meiotic maturation arrest**

Recent work with testicular biopsy samples from men with obstructive azoospermia (relatively intact spermatogenesis) has shown that, under appropriate conditions and in the presence of high concentrations of FSH (>25 IU/l) and testosterone (1 mM), a subpopulation of germ cells undergo a very rapid (1-2 days) meiotic and postmeiotic differentiation during in-vitro culture (6). When the same methodology was applied to 5 men with maturation arrest at the primary spermatocyte stage, the in-vivo arrested spermatogenesis was resumed in 2 of them and a few postmeiotic germ cells, including morphologically atypical elongated spermatids in one case, were detected after 2 days of culture (7). In both cases, oocytes microinjected with the in-vitro developed spermatids were fertilized and underwent early cleavage divisions, and a twin pregnancy, resulting in the birth of normal babies, was established in one of them (7).

3. **SUCCESS RATES OF ASSISTED REPRODUCTION WITH SPERMATIDS**

Fertilization, pregnancy and birth rates after round spermatid injection (ROSI) and elongated spermatid injection (ELSI) into oocytes is largely dependent on the stage of the injected spermatids, and on the severity of testicular failure. Global success rates of the technique have been reviewed recently (8). Briefly, fertilization, pregnancy and birth rates are higher for ELSI than for ROSI. The only viable pregnancies reported in the literature with the use of round spermatids freshly obtained from the ejaculate or from the testicular biopsy were achieved in cases in which at least a few elongated spermatids could be detected previously in the patient´s history. The first pregnancies and births in cases in which the patient had never shown more advanced cells than round spermatids were reported recently with the use of spermatid in-vitro culture, resulting in a limited development of round spermatids into elongated ones which were subsequently injected into oocytes (7, 9). However, the number of cases is still too small to definitively evaluate the clinical potential of this new technique. Even with the use of in-vitro culture, the chance of establishing a pregnancy does not exceed 5-10% (depending on the female age) which, however, still compares favourable with success rates with the use of freshly recovered spermatids in this category of patients. Moreover, the chance of assisted reproduction with in-vitro cultured spermatids can be predicted by the in-vitro differentiation of the cultured cells, viable pregnancies being reported only in those cases in which at least some changes characteristic of spermiogenesis had been achieved during the culture period (7, 9).

4. MAIN CAUSES OF FAILURE OF ASSISTED REPRODUCTION WITH SPERMATIDS

In the rabbit model, success rates of assisted reproduction with spermatids were shown to be correlated to spermatid maturity, more advanced stages of spermatid development performing better than the early stages (10). On the other hand, relatively high implantation rates were reported in the mouse model even with the use of developmetally younger cells, the secondary spermatocytes (11). Unlike the animal models, spermatids to be used for assisted reproduction treatment in human sterility originate from a diseased testis, and this background conditions their developmental fate. So, it has been shown that a great proportion of both meiotic and postmeiotic germ cells from men with complete maturation arrest at the round spermatid stage are actually apoptotic as revealed by the demonstration of fragmented DNA in their nuclei (12). The frequency of apoptotic germ cells in these cases was markedly higher as compared to cases in which the maturation arrest was incomplete and some spermatids entered the elongation phase (12). These observations may explain the observed differences in the success rate of spermatid conception reported in the literature and, more concretely, they help understand the particularly low success rates in cases of complete maturation arrest at the round spermatid stage in which the risk of inadvertently injecting an apoptotic spermatid is very high. Such inadvertent injection may lead to fertilization failure if the oocyte-activating factors within the injected spermatid have been destroyed by the apoptotic process (13). Even if fertilization does occur, however, embryos resulting from fertilization with apoptotic spermatids are at a great risk to arrest their development during the preimplantation period, especially at the time of embryonic gene activation at the 4-8-cell stage when the apoptosis-related DNA damage can become manifest as a failure of expression of developmentally important genes (13).

5. IMPROVEMENT OF SPERMATID QUALITY BY IN-VITRO CULTURE

In addition to making it possible to overcome the in-vivo meiotic block and to obtain a few spermatids for assisted reproduction in some patients with maturation arrest at the primary spermatocyte stage (7), in-vitro culture can bring about further benefits. First of all, cultured spermatids undergo additional nuclear and cytoplasmic maturational changes which may be beneficial for their reproductive capacity (14). Moreover, the frequency of apoptotic germ cells is significantly lower in cultured aliquots of testicular biopsy samples as compared to freshly recovered aliquots originating from the same samples (15). The achievement of the first births in cases of complete maturation arrest at the round spermatid stage (7, 9) may be at least partly explained by this mechanism.

6. RESPONSES TO MAIN CONCERNS ABOUT THE USE OF SPERMATIDS FOR ASSISTED REPRODUCTION

a) Indication

Some years ago, the clinical interest in the use of spermatids for assisted reproduction was a debated issue since several workers claimed the inexistence of postmeiotic maturation arrest. However, recent work by several independent groups shows clearly that spermatogenesis can indeed be arrested both at the round spermatid stage and in the course of spermatid elongation process even though these types of maturation arrest are less frequent as compared to maturation arrest at meiotic stages, namely at the primary spermatocyte stage (reviewed in 16). Moreover, the use of in-vitro differentiated spermatids is the desired endpoint of culture techniques aimed at overcoming the in-vivo maturation arrest at earlier stages of spermatogenesis. A lack of therapeutic indications is thus certainly not an argument against further development of spermatid conception techniques.

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b) Success rate

The success rate of assisted reproduction with spermatids still remains low, especially when only round spermatids are available. The predictable chance of pregnancy with the use of round spermatids is well below 10% even if the technique is to be applied to couples in whom the female partner is young and without any reproductive pathology. However, low efficiency of a technique is not a reason for its definitive rejection. Patients have to be given complete information about the technique proposed, including the expected chance of success, and it is up to them to decide about its eventual application. In the case of spermatid conception, this decision will be conditioned by the value that the couple attaches to the possibility of having progeny of their own genetic constitution as well as by geographical, cultural, legal and administrative factors which affect the availability of alternative solutions, such as the use of donor sperm or adoption.

c) Safety

Most concerns about the use of spermatids for assisted reproduction were related to the risk of chromosomal abnormalities of the resulting embryos, apoptotic DNA damage in developmentally blocked spermatids, incompleteness of genomic imprinting and the risk of transmititng abnormal mitochondrial genomes.
The risk of chromosomal abnormalities is mainly associated with the use of in-vitro cultured spermatids that have undergone the final phase of their meiotic divisions in culture. The use of intra-oocyte injection of primary spermatocytes into metaphase II oocytes in order to achieve haploidization was indeed burdened by a high frequency of chromosomal errors, namely premature sister chromatid segregation (17). In contrast, preliminary obervations on human spermatids resulting from in-vitro transmeiotic differentiation did not reveal any dramatic increase in the frequency of aneuploidy, and the two babies born after the clinical application of this technique had normal karyotypes (9). The risk of choosing a spermatid with apoptotic DNA damage for assisted reproduction is decreased by the use of in-vitro culture, leading to selection of non-apoptotic cells (15). Results of recent animal studies showing the normality of genomic imprinting in spermatids (18), the lack of the risk of transmitting abnormal mitochondrial genomes via spermatids (19) and the absence of long-term effects of by-passing spermiogenesis on several future generations (20) are reassuring for the applications of this technique to humans.

7. CONCLUSIONS

Male germ cell development may be arrested by different kinds of testiculopathy at any stage of spermatogenesis including spermiogenesis. The use of spermatids at different stages of postmeiotic differentiation for assisted reproduction in cases of spermiogenesis arrest has been reported by different centres. In addition, in-vitro developed spermatids have been used by one centre in cases of meiotic maturation arrest at the primary spermatocyte stage. Success rates of assisted reproduction with spermatids are higher when elongating or elongated spermatids (developing in vivo or resulting from in-vitro culture) are available. In contrast, success rates are low when round spermatids are used, mainly because of a high risk of inadvertent use of spermatids carrying apoptosis-related DNA damage. Some improvement has been achieved by the use of in-vitro cultured spermatids for assisted reproduction instead of freshly recovered ones. Both clinical data and animal experimental studies are reassuring as to the safety of assisted reproduction with spermatids.

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Relevance of genetic screening to male infertility

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INTRODUCTION

The borderland between andrology and human genetics has evolved into one of the most dynamic areas of research in reproductive medicine. It is now well documented that a substantial fraction of the male infertility caseload is genetically-based. Clear concepts for the integration of this knowledge into routine clinical practice are pivotal. This paper reviews the most pertinent data and attempts to give a broad outline of appropriate genetic screening for the infertile male.

LEARNING OBJECTIVES

The reader of this syllabus should be able to answer the following questions:
* What are the main indications for and benefits of karyotyping in male infertility?
* Does sperm chromosome analysis have a place in routine andrological practice?
* Which infertile men should be tested for CFTR gene mutations?
* How common are Y microdeletions and how are they passed on to offspring?
* Should infertile men be screened for androgen receptor mutations or polymorphisms?
* Why should the andrologist document the general medical and family history of his infertile patients?

KARYOTYPING - FOR WHOM AND TO WHAT END?

Every azoospermic man should be karyotyped. 14% of infertile men with azoospermic ejaculates have abnormal somatic chromosomes, most commonly 47,XXY [1]. The increased prevalence of aberrant karyotypes is probably limited to men with non-obstructive azoospermia, but this was demonstrated in only a single and yet unconfirmed study.

Oligozoospermic men are also at increased risk ($\approx 5\%$) to carry a chromosomal aberration [1]. Structural anomalies such as translocations and inversions predominate. The lower the sperm count, the higher the likelihood for an abnormal karyotype. For the individual oligozoospermic patient the presence or absence of a chromosomal abnormality cannot be predicted from any clinical, endocrine or semen parameter. Thus, only the most liberal use of karyotyping will uncover all chromosomal aberrations among men with oligozoospermia. Economic restrictions may dictate to perform a chromosome analysis only for those men with severe oligozoospermia, e.g. when the sperm concentration is below 5 million / ml.

Every man enrolled for intracytoplasmic sperm injection (ICSI) should have a chromosome analysis. The likelihood of finding a abnormal karyotype in a patient from this highly selected cohort is 4.5%, as the cumulated data from 10 studies involving almost 5000 patients indicate.

What are the benefits of demonstrating a chromosomal anomaly in an infertile man?
* (i) The basic etiology of the fertility problem may be clarified, as in all cases of 47,XXY. The etiologic significance of some numerical (e.g. 47,XYY) and structural aberrations (e.g. small

inversions) is less clear.

- (ii) A balanced parental chromosomal abnormality may give rise to pregnancies with an unbalanced karyotype. With the exception of certain gonosomal aberrations, most chromosomal imbalances will result in an abortion or stillbirth or, if compatible with viability, in malformations and mental retardation. It should also be noted that a structural chromosome anomaly is often more easily identified in the parental lymphocytes than in chorionic villus or amniotic fluid cells. This argues against a policy where parental karyotyping is skipped in favour of a routine prenatal chromosome test.
- (iii) Invasive prenatal diagnosis through chorionic villus sampling or amniocentesis should be recommended for all pregnancies fathered by a man with an abnormal karyotype.
- (iv) An abnormal karyotype in one of the partners may be associated with lower fertilization, implantation, and clinical pregnancy rates in assisted reproduction. However, this has not been a universal finding.
- (v) Many structural chromosome abnormalities are familial. The detection of an abnormal karyotype in an infertile man can be the starting point for a family study.

SPERM CHROMOSOME ANALYSIS - MOSTLY A RESEARCH TOOL

Human sperm chromosomes can be analysed after decondensation in hamster or mouse oocytes. Fluorescence in situ hybridization (FISH) became popular as a technically less demanding approach to sperm cell cytogenetics [2]. While FISH allows for the analysis of large numbers of germ cells, it does not provide a full karyotype.

Even though sperm chromosome analysis has its merits in the scientific study of male infertility, its utility for routine patient management is limited. It can be employed to estimate the proportion of chromosomally unbalanced sperm in men with abnormal somatic karyotypes such as translocations or 47,XXY mosaicism.. Notably however, a correlation between sperm chromosome studies and treatment outcome has not been demonstrated so far. Even a low chromosomal abnormality rate in sperm does not obviate the need to recommend invasive prenatal diagnosis for any pregnancy fathered by a patient with an abnormal lymphocyte karyotype.

Similar considerations apply to infertile men with regular somatic karyotypes. While several studies have shown an increased rate of sperm chromosome abnormalities as compared to fertile controls [2], the prognostic value of these findings is unclear. The rates of fertilization, clinical pregnancies, pregnancy losses and neonatal malformations were similar when men with elevated or normal frequencies of sperm chromosome anomalies were compared. Also, infertile men who had fathered a child with an abnormal karyotype by means of ICSI did not have a higher rate of sperm aneuploidy than other infertile patients [2].

Human spermatozoa with certain head shape abnormalities probably carry more structural chromosome abnormalities than morphologically normal germ cells. It is therefore prudent to avoid the usage of sperm with abnormal head morphology for ICSI. Several patients whose ejaculated spermatozoa were predominantly macrocephalic or round-headed had extremely elevated rates of sperm chromosome aberrations. Such monomorphic germ cell defects appear as a veritable indication for sperm karyotyping or FISH. If a very high chromosomal abnormality rate is confirmed, such patients may be advised against ICSI.

SCREENING FOR MUTATIONS IN THE CYSTIC FIBROSIS GENE - A MUST IN CBAVD

Mutations in the CFTR gene are the molecular basis of cystic fibrosis (CF). It is now well established that congenital bilateral absence of the vas deferens (CBAVD) is a minor genital variant of CF. More than 80% of men with CBAVD carry mutations in the CFTR gene [3]. There

are clinically similar conditions which are also associated with these mutations: congenital unilateral absence of the vas deferens (mutations in 38%), bilateral ejaculatory duct obstruction (mutations in 86%), and epididymal obstructive azoospermia (mutations in 32%) [3].

Every infertile man presenting with any of these disorders should be screened for CFTR mutations. His offspring could be affected with full-blown cystic fibrosis if the spouse also carries a mutation. Given a heterozygote frequency of 4-5% in Caucasian populations, this is not a very unlikely event. If both partners carry a CFTR mutation, conventional prenatal diagnosis or preimplantation genetic diagnosis can be considered.

The CFTR genotype is not predictive of ICSI success or failure. Fertilization and pregnancy rates were similar when CBAVD patients with or without CFTR mutations underwent MESA / ICSI or TESE / ICSI.

Male infertility without obstructive azoospermia is not an indication for CFTR genotyping. While one study claimed an increased prevalence of such mutations in men with oligo-, astheno- or teratozoospermia, this has not been confirmed by others.

SCREENING FOR Y MICRODELETIONS - A VALUABLE SCREENING TEST IN MEN WITH AZOOSPERMIA AND SEVERE OLIGOZOOSPERMIA

Submicroscopic deletions in at least three distinct regions ('azoospermia factors' AZFa, AZFb, AZFc) on the long arm of the Y chromosome can cause male infertility [4]. The prevalence of these microdeletions in men with otherwise unexplained azoospermia or oligozoospermia is 12.6% and 5.6%, respectively (data collated from 20 independent studies).

The detection of a Y microdeletion in an infertile man clarifies the etiology of his fertility problem. As assisted reproduction technologies now enable such patients to father children, the heritability of these genetic defects is a point to consider. All reports published so far demonstrate obligate father-to-son transmission of Y microdeletions. Thus, all sons of men carrying Y microdeletions will most likely have a fertility problem similar to that of their fathers. This prospect does not deter most couples where the male carries a Y microdeletion from going ahead with infertility treatment. Of 28 such couples 79% proceeded with ICSI, while only 7% chose donor insemination and 14% refrained from any form of treatment.

MUTATIONS AND POLYMORPHISMS IN THE ANDROGEN RECEPTOR GENE - NOT A MAJOR ISSUE IN INFERTILE MEN

Hormone-receptor binding studies performed in the 1980s suggested a high prevalence of androgen receptor dysfunction among infertile, but normally virilized men. However, the direct analysis of the gene encoding the androgen receptor has shown that mutations are very rare in such patients.

Exon 1 of the androgen receptor gene harbours a polymorphic CAG triplett repeat sequence. The number of contingent CAG tripletts ranges from 9 to 36 in the normal population. Expansion of the repeat sequence beyond a size of 37 CAGs causes a neurological disorder called X-linked spinobulbar muscular atrophy or Kennedy disease. Many of the affected subjects develop secondary spermatogenic failure.

Based on this observation it was hypothesized that CAG repeat lengths in the upper normal range might selectively compromize spermatogenesis without leading to neurological dysfunction. A statistically significant difference in CAG repeat size between infertile men and fertile controls was demonstrated in at least three studies. It is notable that none of the infertile men enrolled in these series had a CAG repeat tract longer than 36 units, i.e. in the clearly abnormal range. This notwithstanding, there is now considerable evidence for a slight difference in the distribution of CAG repeat tract lengths between infertile subjects and controls. The biological and clinical significance of this is obscure. At the moment, neither particular concern about Kennedy disease

in the offspring of infertile men nor a routine molecular analysis of the androgen receptor gene in men without overt clinical signs of androgen insensitivity appears justified.

PEDIGREE ANALYSIS - AN EFFECTIVE WAY OF GENETIC SCREENING

Non-geneticists tend to underestimate the value of a skilfully performed pedigree analysis. It entails the documentation of potentially heritable disorders in the counsellees and their relatives. In the author's experience, this is the most effective tool to uncover genetic risks for offspring of infertile parents-to-be. A study involving more than 600 couples enrolled for ICSI treatment showed that potentially heritable non-reproductive disorders can be identified in 2% of them, a rate significantly higher than in fertile controls. Rehearsing the medical history of the patients' close relatives revealed even more ‚significant genetic risk factors' (SGRF), defined as a risk of at least 0.5% for a severe and handicapping congenital disorder in offspring.
It is therefore good clinical practice to question men who desire infertility treatment about their general health and about congenital and other potentially heritable diseases in their families. Formal genetic counselling should ensue for those who have a heritable disorder or a positive family history.

GENETIC COUNSELLING FOR EVERY INFERTILE MAN?

Should every infertile man undergo routine genetic screening and counselling? The institution of such a policy would place a substantial economic and logistic burden on already strained health care systems. Therefore, evidence-based guidelines for a cost-effective, but still efficient genetic workup of the infertile male are needed. The outlines of a workable concept are now beginning to emerge. As reviewed above, some recommendations for genetic screening of infertile males can already now be made with good confidence, but further studies are needed.
It is noteworthy that a major fraction of infertile patients heading for assisted reproduction do not desire to undergo pre-treatment genetic counselling [5]. This is even true for couples with an easily identifyable genetic risk factor such as advanced maternal age or CBAVD in the male. Such reluctance may have other motives than simple lack of interest. In the author's experience, some couples are seriously concerned that the identification of a genetic risk factor could lead to their exclusion from the treatment program. Other patients argue that even in case of carriership for a genetic anomaly, they would still go ahead with treatment. In fact, this policy has been adopted by most couples where the male has a Y microdeletion or an abnormal karyotype. Furthermore, even given the prospect of mental retardation or another major genetic illness in their future child, many infertile couples would not consider terminating a pregnancy [5]. Many women hold on to this policy once they are pregnant through ICSI. If they have free choice between invasive and non-invasive prenatal diagnosis, they strongly prefer the latter [6]. In one study, even the majority of couples with a karyotypic abnormality in the male declined prenatal chromosome tests. Such decisions taken by infertile patients should be met with great respect, as disregarding them would be an assault on the patients' reproductive autonomy. This notwithstanding, the physician in charge of an infertile man has to ensure that significant genetic risk factors are identified and adequately communicated to the patient. Only this allows for a fully informed decision of the couple to either go ahead with or abstain from infertility treatment. While many couples choose the former option, a significant minority stops infertility treatment once a genetic risk factor is identified. One should also be aware of the unpleasant medicolegal implications of an inadvertent pregnancy outcome that in hindsight turns out as potentially avoidable. Furthermore, genetic screening prior to treatment for male infertility may enable patients to feel more comfortable with electing non-invasive prenatal tests, as many of them apparently prefer. We observed that women who underwent a genetic workup prior to ICSI

had a significantly lower rate of chorionic villus biopsies and amniocenteses than those who had not made use of this offer [6].

Even the most comprehensive pre-treatment genetic screening cannot safeguard against chromosomal anomalies or monogenic defects that arise de novo in the affected children. This needs emphasis as for example most unbalanced chromosomal aberrations that have been observed in ICSI children were of this de novo type, i.e. they did not originate from a predisposing parental abnormality.

SUGGESTED FURTHER READING

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Results of the ESHRE questionnaire on genetic screening in male infertility

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LEARNING OBJECTIVES

1. To know the possibilities and limitations of ESHRE-net to perform on-line questionnaires for ESHRE-members.
2. To know the differences between the genetic screening of ICSI patients in different countries.

INTRODUCTION

Since the introduction of ICSI, clinical geneticists entered the field of reproductive medicine more and more. Severe male subfertility, the primary indication for ICSI, may be associated with genetic defects, such as microdeletions on the Y chromosome, chromosomal abnormalities and mutations in the cystic fibrosis gene. Moreover, it is expected that a larger part of severe male subfertility has a genetic origin.

The knowledge that ICSI may cause transmission of genetic defects to the offspring, makes it necessary to counsel the patients thoroughly. Many fertility centres already have a structured way to screen and counsel the patients before ICSI. However, there is in Europe no uniform way of screening and counselling ICSI patients, which may be due to cultural differences or to the fact that reproductive genetics is a young and rapidly changing field, largely based on very recent literature.

Because of these observations and reflections, the organisers of this course thought that it would be interesting to make an inventory of the different ways of screening and counselling of ICSI patients in Europe. We planned to do this inventory by an on-line questionnaire on ESHRE-net, the rapidly growing web-site of our society.

METHODS

A questionnaire with 16 multiple-choice questions about genetic screening and counselling of ICSI-patients was developed. The questions were uploaded into the section of the Special Interest Group (SIG) Andrology of ESHRE-net. To invite the members to participate, a hyperlink was made on the homepage of ESHRE-net and an email was sent by the webmaster to about 1000 ESHRE-members of whom the email-address was known at the Central Office.

The answers automatically were sent to the author by e-mail. Subsequently, the answers were put in an Excel-spreadsheet using the copy and paste function of the software.

The questionnaire was launched on January 9th and was closed on April 9th. Because initially the number of responses was disappointing, a classic written questionnaire was sent to all ESHRE-members by snail-mail in February 2000.

The questionnaire consists of a short introduction, 16 multiple-choice questions, a post-button and an acknowledgement.

ESHRE-questionnaire on genetics and ICSI.

This on-line ESHRE-questionnaire focuses on the genetic screening of ICSI patients.
We would be very honoured if you want to answer the questions. It will take only a few minutes of your precious time. Thank you in advance! The results will be presented in Bologna at the pre-congress course of the SIG Andrology.

1. MICRODELETIONS: Do you test ICSI-men with severe oligo- or azoospermia for microdeletions on the Y chromosome? *(If no, go to question 5)*
- ❑ *Yes, testing is obligatory*
- ❑ *Yes, testing is voluntary*
- ❑ *No*
- ❑ *I do not know*

2. MICRODELETIONS: How many STS's do you use in your test?
- ❑ *< 5*
- ❑ *5-10*
- ❑ *10*
- ❑ *I do not know*

3. MICRODELETIONS: Do you test the father, brother or uncle of the patient, if the man has a microdeletion?
- ❑ *Yes*
- ❑ *No*
- ❑ *I do not know*

4. MICRODELETIONS: Would you perform ICSI if the man has a microdeletion?
- ❑ *Yes*
- ❑ *Yes, but only in combination with PGD*
- ❑ *No*
- ❑ *I do not know*

5. CHROMOSOME ANALYSIS: Do you test ICSI-men with severe oligo- or azoospermia for chromosomal abnormalities? *(If no, go to question 7)*
- ❑ *Yes, testing is obligatory*
- ❑ *Yes, testing is voluntary*
- ❑ *No*
- ❑ *I do not know*

6. CHROMOSOME ANALYSIS: Do you test the women of ICSI-men for chromosomal abnormalities?
- ❑ *Yes*
- ❑ *No*
- ❑ *I do not know*

7. CHROMOSOME ANALYSIS: Would you perform ICSI if the man has a chromosomal abnormality with potential consequences for the offspring?
- ❑ *Yes*
- ❑ *Yes, but only in combination with PGD*
- ❑ *No*
- ❑ *I do not know*

8. CYSTIC FIBROSIS: Do you test ICSI-men for mutations in the cystic fibrosis gene?

- ❏ *Yes, all ICSI-men with a severe oligo- or azoospermia*
- ❏ *Yes, only men with congenital absence of the vas*
- ❏ *No*
- ❏ *I do not know*

9. CYSTIC FIBROSIS: Would you perform ICSI if the man and woman are both CF-carriers?

- ❏ *Yes*
- ❏ *Yes, but only in combination with PGD*
- ❏ *No*
- ❏ *I do not know*

10. FAMILY HISTORY: Do you take an extensive family history before ICSI?

- ❏ *Yes, orally*
- ❏ *Yes, in writing*
- ❏ *Yes, both orally and in writing*
- ❏ *No*
- ❏ *I do not know*

11. GENETIC COUNSELLING: Do you offer ICSI couples genetic counselling?

- ❏ *Yes, all ICSI couples*
- ❏ *Yes, only if genetic abnormalities have been found*
- ❏ *No*
- ❏ *I do not know*

12. FOLLOW UP: Do you perform chromosomal analysis of the ICSI offspring?

- ❏ *Yes, prenatally*
- ❏ *Yes, postnatally*
- ❏ *Yes, pre- or postnatally*
- ❏ *No*
- ❏ *I do not know*

13. FOLLOW UP: Do you perform advanced ultrasound examination at 20 weeks in ICSI pregnancies?

- ❏ *Yes*
- ❏ *No*
- ❏ *I do not know*

14. FOLLOW UP: Do you have a structured procedure for the follow-up of the ICSI-children?

- ❏ *Yes, physical examination(s)*
- ❏ *Yes, questionnaire(s)*
- ❏ *Yes, both physical examination(s) and questionnaire(s)*
- ❏ *No*
- ❏ *I do not know*

15. GENERAL QUESTION: How many ICSI cycles are started in your centre per year?

- ❏ *< 100*
- ❏ *100-200*
- ❏ *200-500*
- ❏ *500*
- ❏ *I do not know*

16. GENERAL QUESTION: In which country do you work?

THAT'S IT !!! You can post your answers by clicking on this button.
Thank you very much for your kind collaboration. I am curious to see the final results! The results will be presented in Bologna at the pre-congress course of the SIG Andrology, and will be published on ESHRE-net afterwards.
Jan Kremer, Nijmegen, The Netherlands

RESULTS

The on-line questionnaire worked well. Filling in the electronic form appeared to be easy and sending the answers by e-mail gave no problems. It was easy to copy the answers in the spreadsheet. Despite the high number of visitors of the online questionnaire (537 hits till April 9th 2000), the number of reactions was disappointing: only 46 ESHRE-members filled in the form and posted the answers: 20 within one week and 32 within one month and 46 within 3 months.
In contrast, the number of reactions on the classic written questionnaire sent by snail-mail was rather high: 177 reactions came to the author by fax or snail-mail.
The 223 reactions (46 by email (21%) and 177 by fax or snail-mail (79%) came from 42 different countries: 165 reactions (74%) came from 25 European countries [Austria (2), Belgium (14), Croatia (1) Czech Republic (3), Denmark (7), Finland (6), France (20), Germany (18), Greece (4), Hungary (1), Iceland (1), Ireland (1), Italy (10), Lithuania (1), Netherlands (16), Norway (5), Portugal (4), Russia (4), Slovenia (1), Spain (11), Sweden (10), Switzerland (15), Turkey (1), UK (7) and Ukraine (2)] and 58 reactions (26%) came from 17 non-European countries [Argentina (1), Australia (13), Brazil (2), Canada (2), Chile (1), Columbia (2), India (2), Israel (1), Jordan (1), Lebanon (3), New Zealand (1), South Africa (1), Taiwan (3), Thailand (2), Uruguay (1), USA (19) and Venezuela (1)].
Most respondents do test ICSI-men for microdeletions (25% obligatory testing, 41% voluntary testing, 34% no testing). Most respondents used 5-10 STS's (13% less than 5, 30% 5-10, 16% more than 10 and 41% unknown). If the patient has a microdeletion, most respondents do not test male family members (24% testing, 65% no testing and 11% unknown). The majority of respondents would perform ICSI if the man has a microdeletion (82% yes, 11% only in combination with PGD, 4% no and 4% unknown).
Almost all respondents perform a chromosomal analysis before ICSI (54% obligatory testing, 37% voluntary testing and 9% no testing). Most respondents do not test the female partners of ICSI men (28% testing, 70% no testing and 2% unknown). The majority of respondents would perform ICSI if the man has a chromosomal abnormality with potential consequences for the offspring (30% yes, 30% only in combination with PGD, 30% no and 10% unknown).
Many respondents test for mutations in the cystic fibrosis gene (30% all ICSI men, 50% only men with congenital absence of the vas, 17% no testing, 3% unknown). If both partners are CF carriers most respondents would perform ICSI in combination with PGD (15% yes, 43% only in combination with PGD, 34% no, 8% unknown).
The majority of respondents take an extensive family history before ICSI (42% orally, 7% in writing, 34 both orally and in writing, 16% no family history and 1% unknown). Almost all respondents offer genetic counselling before ICSI (27% to all ICSI couples, 69% only if genetic abnormalities have been found, 3% no genetic counselling and 1% unknown).
Most respondents do not offer chromosomal analysis of the ICSI offspring (25% prenatally, 2% postnatally, 12% prenatally or postnatally, 57% no chromosomal analysis and 4% unknown). A high number of respondents offer advanced ultrasound examination at 20 weeks (75% yes, 18% no and 7% unknown). Most respondents have no structured procedure of the follow-up of ICSI children (5% physical examination, 17% questionnaire, 17% both examination and

questionnaire, 57% no follow-up procedure and 4% unknown). The number of cycles started in the centres of the respondents was <100 (23%), 100-200 (30%), 200-500 (34%), >500 (11%) or unknown (2%).

CONCLUSION

The on-line questionnaire on ESHRE-net appeared to be a promising method to perform questionnaires among ESHRE-members. On this moment the disappointing number of responses is the main problem. Apparently ESHRE-members are not used to this new medium and have still more affinity to classic methods of communication like fax and snail-mail. Since it has to be expected that ESHRE-net will grow to the most important medium of our society, this problem will be only temporary. More members will be on-line and the number of responses to cyber-questionnaires will be much higher.
The results of this questionnaire show that there are many differences between the way of screening and counselling of ICSI patients in the different countries. Most respondents perform a family history and test for microdeletions, chromosomal abnormalities and cystic fibrosis. Most respondents would offer ICSI if the man has a microdeletion. Less agreement exists in the case of chromosomal abnormalities and CF-carriers. Many respondents perform advanced ultrasound examination in ICSI-pregnancies, but only a minority offers chromosomal analysis of the ICSI offspring. The fact that most respondents have no structured follow-up procedure of ICSI-children is quite disappointing.

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The chronic inflammatory syndrome of male genital tract, the bacterial infection and sperm fertilizating ability

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1 - THE CHRONIC INFLAMMATORY SYNDROME OF MALE GENITAL TRACT

1.1 - Leukocytospermia

Leukocytosperma is defined by the presence of white blood cells (leukocytes and lymphocytes) in the seminal plasma. They are usually evaluated during the sperm analysis as a pourcentage of the sperm cells concentration. The normal value of leukocytospermia is not known. Normally, as it traduces the presence of an abnormal process, leukocytospermia is always pathologic. In normal sperm, it should be nil. The threshold above which leukocytospermia is supposed to be dangerous is 10^6 leucocytes/ml according to WHO. However men with much higher leukocytospermia can be fertile (Tomlinson et al., 1993; Wolff, 1995). Therefore it is clear that this threshold is of no significance. To define the threshold under which a leukocytospermic man is always fertile, the seminal level of leukocytes should be correlated with the results of gamete fusion tests or to IVF results. This has never been done.
The presence of polymorphogranulocytes in the semen is the first and most important sign of the acute inflammatory syndrome. In the chronic inflammatory syndrome there are more monocytes / macrophages. Hence, there is a need for a precise diagnosis.
But the cell count is difficult since it is performed on sperm smears. Smears are never homogeneous, the analysis is time consuming and results may vary acccording to the observer. The accuracy of evaluation can be improved by the use of monoclonal antibodies but the analysis of smears remains difficult for the reasons cited above.

1.2 - Origin and frequency of the genital tractus inflammatory syndrome (GTIS)

In most cases the GTIS results from a bacterial urogenital infection which frequently becomes chronic. In patients suffering from GTIS, urinary pains or disconfort during ejaculation, traducing the presence of an infection are frequent. Furthermore, the diagnosis of chronic prostatitis is overall difficult to establish and the number of cases is probably underestimated (Comhaire et al., 1980 ; Nickel, 1998 ; Weidner et al., 1999). Notably, the spermoculture can be negative despite the presence of a chronic bacterial prostatitis.

GTIS may also be consecutive to a viral infection, varicocele or tobacco comsumption (Zini et al., 1999).

1.3 – Origin of reactive oxygen species (ROS)

The increase in the level of ROS above the physiological level is related to the presence of leukocytes and to the excess residual cytoplasm in the midpiece of spermatozoa.
A positive correlation has been shown (r=0,704 ; P<0,001) between ROS and IL-8 produced by leukocytes (de Lamirande, 1998). Iwasahi and Gagnon reported similar results (Iwasahi et Gagnon, 1992) and showed a linear correlation between ROS production and the concentration of leukocytes in the semen. Aitken et al. who already reported a highly significant correlation (n=0.8) between ROS and leukocytes level (Aitken et al, 1992) confirmed this result and underlined the increase of ROS production during the sperm selection centrifugation (Zalata et al., 1995).
ROS can also come from the cytoplasm of spermatozoa and especially from excess residual cytoplasm in the midpiece (Aitken et al, 1992). This is the reason why oligoasthenospermic semen which present a lot of abnormal spermatozoa have also impaired sperm function. Indeed they have a higher ROS concentration than men with normal sperm parameters (Zalata et al., 1995).

1.4 – Physiological action of ROS

The reactive oxygen metabolites in the form of both superoxide anion and hydrogen peroxide are key mediators of sperm capacitation (Aitken et al., 1998a). Superoxide anion is responsible for the activation of hyperactivated motility (de Lamirande et al., 1993) while hydrogen peroxide appears to be a significant factor in controlling the tyrosine phosphorylation events associated with sperm capacitation (Aitken et al., 1998). Tyrosine phosphorylation is an important factor in the attainement of a capacitated state that sensitizes human spermatozoa to the calcium transients generated by progesterone.
ROS have also a role in induction of sperm chromatin compaction, that is thought to be of physiological importance in protecting the sperm genome from oxidative attack. One of the functions of the spermatozoa ROS generating system may be to create the peroxide species that will be used by sperm phospholipid glutathion peroxidase to effect the thiol oxidation stage of DNA compaction. Thus at low level of oxidative stress, phospholipid glutathion peroxidase is tought to reduce peroxides arriving at the sperm nucleus thereby oxidizing protamine thiols while protecting the sperm DNA from oxidative attack (Aitken et al., 1998).

1.5 – Deleterious effects of ROS: lipid peroxidation

ROS are extremely reactive since they have free electrons on the upper level of the oygen atoms. The superoxyde anion O^{2-} producted by the aerobic metabolism increases the production of more ROS and notably of hydrogen peroxyde and hydroxyl radical (OH). All ROS are active on lipids, nucleic acid and proteins (Storey et al. 1997 ; Aitken et al., 1998b). They induce on the polyunsaturated fatty acid a cascade of lipid peroxydation increasing thereby the level of ROS. They induce breaks on the DNA strands, especially at the level of mitochondria. They inhibit or modify some enzyme activity such as that of topoisomerase which can not bind anymore DNA strand during replication.

2 – IN VITRO EFFECTS OF ROS ON SPERM FUNCTIONS

2.1 – «Terroristes or good samaritains» (Aitken et al., 1995a)

At low level ROS do have a physiological action. Some authors like Tomlinson even suggested that leukocytes could have also a benefical effect on the sperm morphology by phagocytosis of

abnormal spermatozoa. (Tomlinson et al., 1993). This would seem an unlikely function for feminal phagocytes since this process of abnormal sperm phagocytosis has never been described. Furthermore, there would not appear to be sufficient phagocytic capacity within the normal male reproductive tract to make a significant impact on the morphology of normal sperm (Aitken et al., 1995a). On the contrary each time ROS are in excess, the lipid peroxydation cascade occurs and impairs the sperm function ability.

2.2 - Effect on sperm morphology

Alteration of the plasma membrane do not modify sperm head morphology since the nucleus shape keeps it intact. On the contrary flagella membrane alteration leads to a rolling-up of the flagella and consequently to sperm motility impairement.

2.3 – Motility

Experimental data showed that leukocytes impair sperm movement. Withdrawing of leukocytes from a sperm suspension using microbead covered with anti CD45 antibody improves sperm functional quality (Aitken et al., 1995b). When purified leukocytes are added to sperm suspension a decrease of sperm motility is observed (Aitken et al, 1995b). Hydrogen peroxide induces a progressive loss of sperm motility and a complete stop of the flagellar beats probably by a direct action on the axonem (de Lamirande and Gagnon, 1992).

2.5 – Acrosomal reaction

Hydrogen peroxide inhibits also the acrosome reaction as it has been shown in vitro by a study of the induction of ROS by a xanthine-xanthine oxydase on spermatozoa (Griveau et al., 1995).

2.6 – Fusiogenic ability

In a study using the zona free hamster egg penetration assay (HEPA) with 217 men from infertile couples Berger et al. showed a negative correlation between the leukocytes concentration and the fusiogenic ability of sperm. A ratio granulocyte over spermatozoa above 1 % was associated with an 8 fold increased risk of gamete fusion failure (Berger et al, 1982). Similarly, preincubation of sperm with leukocytes impairs the HEPA (Vogelpoel et al., 1991). For higher ROS concentrations sperm motility decreases as well as sperm fusiogenic ability evaluated by the HEPA and sperm movement parameters analyzed by CASA (Computer Aid Sperm Analysis) (Aitken et al., 1998b).

2.7 – Breaks of DNA strand

Aitken et al. (1998b) have studied sperm functions and DNA integrity in sperm suspensions incubated for 3 hours with increasing concentration of ROS. The number of DNA breaks increases significantly with the ROS level (P<0.001). Twigg et al., reported the iatrogenic DNA damage induced in human spermatozoa during sperm preparation especially by swim-up from a washed pellet (Twigg et al., 1998). These DNA alterations could lead to non-disjunctions, embryonic development arrests and/or early pregnancy terminations.

2.4 – Necrospermia

Since the inflammatory syndrome increases the number of DNA strand breakages, it induces apoptotic phenomenoms leading to necrospermia. The decrease in the percentage of living spermatozoa is a very early and sensitive phenomenon linked to the inflammatory syndrome.

3 – OXYDATIVE STRESS AND IN VIVO SPERM FERTILIZING ACTIVITY

3.1 – Origin of leukocytes

The hematotesticular barrier prevents leukocytes from getting into the seminiferous tubules except in case of orchititis. On the other hand, the leukocytes get easily into the epididymis through its mucosa (Wolff, 1995). As a consequence, any inflammatory syndrome inside the genital tract can result in spermatozoa exposure to leukocytes within the epididymis during the 12 days they migrate from the head to the cauda. During this time laps they can be subjected to dramatic injuries. Furthermore, these lesions occur when meiosis is completed and DNA breaks can no longer be repared. Hence, most of spermatozoa alterations occur prior to ejaculation.

3.2 – Antioxidative mechanisms

a - non enzymatic exogenous compounds

The first defence against the oxidative stress is the reduction of oxygen pressure. In the rabbit, oxygen pressure is very low in the uterus and tubes during fertilization. The second defence is composed by antioxidant compounds present in the seminal fluid : ascorbic acid, glutathion, a tocophérol. Polyamines (spermine, spermidine) which inhibit lipid peroxidation, transferrine and lactoferrine, which bind metallic ions involved in ROS production, are also present in the semen. It has been shown that the antioxidant capacity of seminal plasma of fertile men is higher than that of men whose spermatozoa show a reduced motility (Lewis et al., 1995). In uterine and tubal secretions there is taurine and hypotaurine which neutralize the hydroxyl radical and the cytotoxic aldehyde.

b - Endogeneous enzymatic activity

The superoxydes dismutases (SODs) and the complex glutathion peroxydase / glutathion reductase are the major enzymes of antioxidant defence in most cells. They constitute complementary systems. SODs transform the superoxide anion (O^{2-}) in hydrogen peroxyde (H_2O_2) less agressive. H_2O_2 is then eliminated by the complex glutathion peroxydase / glutathion reductase and by catalase.

3.3 –Expression of the inflammatory syndrome according to the balance between the oxidative stress and the antioxidant system

It is known that the threshold of 10^6 leukocytes per ml does not fit with a particular level of oxidative stress. It has been demonstrated that men with higher leukocytospermia can be fertile (Tomlinson et al., 1993). On the contrary, patients may be infertile with a low leukocytospermia. This has been suggested by a study showing that antiinflammatory drugs can restore the fecundity of patients with GTIS (Kulski et al., 1999).
Logically every thing depends on the balance between the oxidative stress and the defence mechanisms. A high level of leukocytospermia could be balanced by an efficient antioxidant system. As far as this later is powerfull enough the patient remains fertile. But the most

important factor of inflammation is its frequent chronic evolution. As effective as they could be, the defences can be overwelmed after years of oxidative stress. In spite of variable level of leukocytospermia the infertility could then arise. In this regard, should a leukocytospermic man be fertile does not mean that he will remain fertile. It is particularly interesting to underline that more than 50 % of the patients presenting an inflammatory syndrome had previously an urogenital infection or a positive sperm culture.

4 – OXIDATIVE STRESS AND IVF

4.1 – Leukocytospermia and IVF

Several studies have shown a negative correlation between the seminal leukocyte concentration and the fertilization rate during IVF (Talbert et al, 1987 ; Aitken et al., 1991 ; Krausz et al, 1994 ; Sukcharoen et al. 1995). Talbert et al showed that the most important seminal parameters in predicting a bad fertilization rate are a low motility and a high concentration of leukocytes (Talbert et al, 1987). Similarly Sukcharoen et al pointed out that the sperm morphology and the leukocytospermia are the most predictive factors of sperm in vitro fertilizing ability (Sukcharoen et al., 1995).

4.2 - Evaluation of the oxydative stress

Despite of a large body of in vitro data showing the deleterious effects of the inflammatory syndrome, the evaluation of the oxidative stress is not performed on a regular basis whereas it appears to be as important as the bacterial infection. Several reasons could explain this : it is difficult to count the leukocytes as already mentionned and moreover it has never been proven that the treatment of the inflammatory syndrome would improve sperm cell quality and thereby patient fertility.

4.3 – Addition of antioxidant compounds in the culture medium

Aitken et al., showed that preparative centrifugations increase sperm oxidative stress. Addition of antioxidant compounds should therefore protect spermatozoa but this point is still debatable (Geva et al., 1996). Parinaud et al. reported an improvment of sperm motility by glutathion supplementation of media (Parinaud et al., 1997), but in other studies, supplementation of preparation media with ascorbate and a-tocopherol, either singly or in combination, did not improve sperm motility (Donnelly et al., 1999) nor supplementation of media with glutathion and albumine (Aboura et al., 2000). However, most of sperm cell lesions probably occur in the genital tractus before ejaculation takes place.

5 – RANDOMIZED THERAPEUTIC TRIAL

5.1 – Antibiotics and sperm quality

All the antibiotics available have been tested for their teratogenicity. Thus, it has been verified that they do not alter the spermogram. However by their mechanism of action, on the cell membrane or DNA replication, they can be deleterious on semen quality. However, biologists are interested not only in sperm parameters but also in sperm fertilizing ability. In this regard the only test that would provide informations about the effects of antibiotics on sperm fusiogenic ability would either be the HEPA or the IVF itself. As far as it is known, none of these studies are currently performed before a drug is accepted for clinical use.

5.2 – Clearance delay

For this reason it appears appropriate to respect a clearance delay between any antibiotic treatment and ART. As spermatogenesis and sperm migration through the genital tractus takes 3 months, one should respect such a period of time before assessing sperm quality, the impact of a treatment, or involving a patient in ART. Indeed a sperm that have been in contact with either a bacterial infection, a leukocytospermia, a fever, an antibiotic treatment should not be used since spermatozoa fertilising ability could be altered.

5.3 – Therapeutic trials

It has never been shown that the treatment of the inflammatory syndrome improves sperm fertilizing ability. Some therapeutic trials have been performed. Yanushpolsly thought that antibiotics are not beneficial to the resolution of leukocytospermia since there is a high rate of spontaneous resolution (Yanushpolsky et al., 1995). However in this study semen analysis were performed 4 weeks after randomization which is notably too early. Lenzi et al., performed a placebo-controlled double-blind cross-over trial of glutathion therapy (Lenzi et al., 1993). In this study there were only 20 patients involved in the trial with a too short period of follow up (2 month). This study focused on sperm parameters and not male fertility. Kessopoulou et al., showed that vitamin E administration to infertile males resulted in the improvement of the zona binding test results. There was no data on the couple fertility (Kessopoulou et al., 1995). The same remarks can be formulated about the study of Yamamoto et al., who reported that antibiotic and increased frequency of ejaculation improved the resolution rate of leukocytospermia (Yamamoto et al., 1995). Many other therapeutic trial were performed but none to our knownledge demonstrate an improvement of in vivo and/or in vitro patient fertility. There is an obvious need for such a demonstration.

6 – CONCLUSIONS

The question of the threshold of leukocytospermia is of no relevance. It is obvious that the problem reside in the balance between the oxidative stress and the defence mechanisms (Sharma et al., 1999). Furthemore the presence of leukocytes within the male genital tract witnesses for the presence of a chemotatic factor (Solito et al., 1998). Indeed there is no inflammatory syndrome without reason. Hence, the inflammatory syndrome is always abnormal and presents a risk for the sperm quality. But if the acute syndrome represents a risk, the chronic syndrome is obviously deleterious since it may last for months or years.
The chronical inflammatory syndrome of the genital tract is responsible of the impairment of sperm functional ability and result in infertility. Its evaluation is difficult to perform and usually it is not done prior to inclusion of patients in ART. Yet this evaluation is essential since its treatment could improve the gamete quality. Furthermore, it should be kept in mind that it is not possible to have an embryo of a better quality than that of the gametes which are involved in its conception.

6.1 – Evaluation of the chronic inflammatory syndrome

It is mandatory to evaluate systematically the chronical inflammatory syndrome as well as the bacterial status of the genital tract. There is a need for a more efficient technique of evaluation that could be routinely used. We are currently trying to use the flow cytometry which presents several advantages : a large number of cells are evaluated (20 000), it is quick, there is a perfect identification of the leukocytes by monoclonal antibodies. It is also highly reproductible.
The antioxidant defence of the patient has to be evaluated by an assay of gluthation, catalase and

SOD (Sharma et al., 1999). The balance between the oxidative stress and the seminal defence could then be carefully evaluated. It could provide an indication on whether the inflammation is implicated or not in the sperm status and patient infertility. Once the implication of the oxidative stress is defined, the treatment can be discussed.

6.2 – Treatment of bacterial infections

Most inflammatory syndromes comes from bacterial urogenital infections. They are usually treated until the disappearance of the symptoms. However, the risk of a chronic asymptomatic evolution is important, which justify certainely a systematic evaluation of the sperm bacterial status 3 months after an infection. Such a study will be done soon. But it should be kept in mind that a negative spermoculture does not mean that there is no infection.

6.3 – Randomised therapeutic trial

The first problem to solve concerning the chronical inflammatory syndrome is its description and its most significant factors. It is difficult to establish a correlation between the syndrome itself and the sperm impairment. It is even more difficult to know whether its treatment is susceptible to improve the sperm quality. This is the reason why a prospective randomised study is mandatory. It can be run involving only patients involved in an assisted reproductive program since in this situation the results of the ART would be a strong criteria of evaluation of the sperm fertilizing ability. Such a study has to be multicentric, but must firstly rely on an efficient and standartised method of evaluation of leukocytospermia.

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Worldwide practice of infectious screening of ART centers for infertile couples : ESHRE questionnaire.

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In the absence of specific legislation or consensual recommendations in the worldwide regarding the routine screening of infectious transmissibility risk (HIV, HCV, HBV, HSV, …), before intra-couple intrauterine insemination (IUI), in vitro fertilization (IVF), or intra-cytoplasmic sperm injection (ICSI), an inquiry about the practice of ART centers concerning the infectious screening management has been performed.

1500 questionnaires were sent via ESHRE, and 155 answers were received. The results of such inquiry coming from 52 countries are the subject of this paper.

The ART centers were asked to specify their practice regarding serodifferent couples for hepatitis C and HIV. The answers were analysed in order to find a difference of acceptance attitude following the positivity of the plasmatic viral load. In case of acceptance of the ART request, the biological laboratory procedures for ART were evaluated: special protective measures, specific cryopreservation policy, and estimation of laboratory equipment status for treating such viral risks.

 A preliminary analyse of the European results concerning 79 centers, shows that the majority of the European ART centers accept to treat serodifferent couples for hepatitis C (65%), whatever the viral load. Whereas 78% of them refuse the request in case of HIV positivity. Only 12 european centers assume the ART procedures for HIV serodifferent couples. The discrepancy between the acceptance criterias concerning hepatitis C and HIV, seems to be linked with legal aspects in different countries.

A detailed analyse of the worldwide results will be reported and compared with European results.

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