

European Society of Human Reproduction and Embryology



COURSE 2

**ART for male infertility: looking beyond the
sperm**

**Special Interest Group Andrology
European Academy of Andrology**

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**Course 3 - Pre-congress course organized by the
Special Interest Group in Andrology and the European Academy of Andrology**

“ART for male infertility: looking beyond the sperm”

Course co-ordinators: ESHRE: H Tournaye (B) , L Björndahl (S)

EAA: A Giwercman (S), A. Lenzi(I)

Course description: This course aims at giving an overview of important aspects in clinical andrology for health care providers working in the field of the assisted reproduction

09.00 - 09.30: The man – a patient or just a sperm source? - **A. Jequier(AUS)**

09.30 - 09.45: Discussion

09.45 - 10.15: What every clinician need to know about basic semen analysis –
L. Björndahl (S)

10.15 - 10.30: Discussion

10.30 - 11.00: Coffee break

11.00 - 11.30: Should we bother about male accessory gland infection? –
G. Dohle (NL)

11.30 - 11.45: Discussion

11.45 - 12.15: Male infertility and androgen deficiency – is there a link? –
M. Zitzmann (D)

12.15 - 12.30: Discussion

12.30 - 13.30: Lunch

13.30 - 14.00: New sperm function tests and ART: evidence based results –
M. Bungum (DK)

14.00 - 14.15: Discussion

14.15 - 14.45: Transmission of genetic based male infertility from father to son by
ART: evaluation of risks - **U. Kvist (S)**

14.45 - 15.00: Discussion

15.00 - 15.30: Coffee break

15.30 - 16.00: Cancer and male reproductive function - **F. Dondero (I)**

16.00 - 16.15: Discussion

16.15 - 16.45: Erectile and sexual dysfunction: what should the ART specialist
know? - **E. Jannini (I)**

16.45 - 17.00: Discussion

17.00 - 17.30: When to embark for ART (all speakers)

17.30 - 18.30: Business meeting of the SIG Andrology

The Management of the Infertile Male: The Limitations of a Semen Analysis and the Importance of the Clinical Assessment.

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Abstract

The object of this presentation is to demonstrate the limitations of the use of a semen analysis in the treatment of infertility in the male as well as to demonstrate the importance of the clinical evaluation in the treatment of this problem.

Introduction

For most clinicians involved in the management of infertility, it is infertility in the male that causes them the most problems. Firstly, much less is known about the causation of infertility in the male than is the case in the female thus making the generation of a clinical diagnosis so much more difficult. The second problem is that the changes that may occur in the semen from an infertile male, gives little clue to the underlying cause of the infertility: indeed the changes that occur are often non-specific. In this lecture, the limitations of a semen analysis in the management of male infertility will be discussed and the importance of a careful clinical assessment will be emphasised.

The limitations of a semen analysis and its use in the management of the infertile male patient.

The very first person to suggest that a semen analysis was important in the evaluation of an infertile couple appears to have been a Philadelphian surgeon by the name of Edward Martin in the early part of the last century (cited by Jequier, 1991). By the fifties, John MacLeod had elevated the analysis of semen to the level of a science (MacLeod 1956) and he also stressed the importance of a semen analysis in the evaluation of all infertile couples.

However in or around 1980, it became clear that many of the variables within a semen analysis that would be helpful in indicating the presence of male infertility were not being identified by many clinical pathology laboratories and that gross errors in counting sperm were common (Jequier & Ukombe, 1983). Thus what information that could be obtained from the performance of a semen analysis was not being sought. The British Andrology Society then began a series of workshops to rectify this problem culminating in the special training of laboratory personnel and the development of stringent methods of quality assurance. Today semen analysis is carried out reasonably well and one must now question the whole role of semen analysis in the management of male infertility. Despite the care and the precision with which many laboratories carry out a semen analysis today, the diagnostic value of a semen analysis is however extremely limited. Firstly the changes that occur in the semen of men with infertility are almost always non-specific and give the clinician very little idea of the cause of a patient's infertility. There are only a few causes of infertility (and globozoospermia is a good example) where the

cause of the infertility can be determined solely from a semen analysis. In the vast majority of patients with infertility, no clue as to the cause of the infertility can be obtained from the examination of a sample of semen.

There are other reasons why a semen analysis is only of limited value in the determination of infertility in the male. Over time, it has become clear that the relationship between infertility and changes in the variables in a semen analysis is not a simple one. Reduced sperm numbers can be found in men of known fertility (Rehan, Sobrero & Fertig 1975) and infertility can be present among men with high sperm numbers in their ejaculate. Pregnancy can also occur occasionally among men who only have a few thousand sperm in their ejaculate (Thomson, Lincoln & Mortimer, 1993). Although it is clear that the higher the sperm count, the better the pregnancy rate, only when the sperm count is at the extremes can a semen analysis reasonably predict the absence or the occurrence of conception.

Even sperm function tests although they may correlate better with fertility than the variables within a semen analysis, still have problems when it comes to predicting fertility and of course still give the clinician little idea as to the cause of the abnormal function of the sperm and thus of the infertility itself.

It has been known for some time that the presence of pathology in the female can influence the potential fertility of a man with a 'normal' sperm count. It is clear that among women with endometriosis undergoing donor insemination, larger numbers of sperm are needed to achieve conception than is the case among women who do not have endometriosis (Hammond, Jordan & Sloan, 1986). Thus if the fertility of a given sperm count can be determined by the presence or absence of a female problem, then how can an accurate and highly reproducible sperm count possibly assist the clinician in either the determination of the infertility or in defining its cause?

Time is also an important factor in infertility. The longer that a patient is infertile, the less likely is that patient to conceive spontaneously (Cooke et al. 1981). However there is no way in which one can apply quality assurance to the 'trying time' to pregnancy.

It must also be remembered that semen is not a homogeneous fluid. Indeed, the components that make up seminal fluid do not mix until they are either in the vagina or in the pot used to collect the semen for analysis. As sperm can be found in human cervical mucus less than two minutes after ejaculation, this means that these sperm cannot have been involved in the coagulation process. It is thus entirely possible that, as these sperm have had a 'head start' on the other sperm trapped in the coagulum, it is these sperm that are responsible for fertilisation. Thus of all the sperm that we see in a semen analysis pot, only a few may be representative of those that achieve fertilisation. It has also been pointed out that the semen used in a semen analysis has also been in contact with light, with a high oxygen tension and with a reduction in temperature (Bjorndahl & Qvist 2003). Thus to use semen analyses for the assessment of fertility may not be appropriate. It is therefore no surprise that a semen analyses correlates so poorly with fertility and it is also clear that a even a highly reproducible semen analysis will add little to ones understanding of male infertility and can offer almost no information as to its cause.

However, the most important question to be asked is whether the abandonment of the generation of highly reproducible semen analyses and of quality assurance in relation to semen analysis will lead to the poor quality investigations that we saw some 20-30 years ago. This is indeed unlikely as the need for well-performed semen analysis in relation to

IVF is now in demand. Although highly reproducible semen analyses may still be needed for research and in particular for toxicological studies, they are for the clinician nothing more than screening tests and it is difficult for the clinician to understand why such accuracy is needed in relation to this particular investigation.

The importance of diagnosis in the clinical management of infertility in the male

Infertility is a symptom, not a diagnosis. An abnormal semen analysis is itself not a diagnosis: it is a physical sign. The changes that may be seen in the semen from infertile men are usually entirely non-specific and with a few major exceptions (globozoospermia is a good example), give the clinician no clue as to the cause of the male infertility.

Without a diagnosis of the cause of infertility, no measures to prevent any such problem can be instituted nor can any rational treatment be given.

A semen analysis may indicate the presence of infertility but very rarely provides the cause of the abnormal sperm count. For example, a reduced sperm count may be the result of disordered ejaculation, of reduced sperm production, of a partial or unilateral ductal obstruction or be simply due to an artefact that occurred during the collection of the semen sample. Thus little diagnostic precision can be obtained from the examination of a semen sample.

Why is a diagnosis so important in the management of male infertility?

One problem that faces those involved in the management of male infertility is that so much less is known about the causes of infertility in the male than is the case in the female. However, unless some serious attempts are made to understand the pathophysiology underlying any reduction in sperm count in the male, this situation will continue. The persistent emphasis on the sperm count together with the lack of attention to the clinical aspects of male infertility is the cause of this problem. The late Professor Dame Sheila Sherlock used to teach us that 'you never make a diagnosis unless you think of it first': it is only a careful history and clinical examination that will help you to think of many possible diagnoses.

The routine collection of sperm from the testis from an azoospermic man without a clear pre or peri-operative diagnosis (as appears to be frequently the case in many clinics today) and the consequent failure to diagnose conditions such as congenital absence of the vas, could lead to the birth of a baby with cystic fibrosis – and also major medico-legal consequences. Failure to identify micro-deletions of the Y chromosome may also give rise to problems in the future (Kent-First et al. 1996)

Without a diagnosis, the treatment of the patient may involve the application of unnecessary IVF. If IVF, applied in such circumstances, leads to serious morbidity or even the death of the female partner when a correct diagnosis and the application of an alternative form of treatment would have been successful in achieving a natural conception, both a tragedy as well as serious medico-legal problems could have been avoided. The fact that death is rare following IVF (Venn et al., 2001) does not mean that IVF can be used with impunity by all infertility clinicians treating male infertility. To the many clinicians who say that IVF/ICSI is still the only way to treat male infertility, I would suggest that they look a bit harder – at their male patients.

Is the training in the management of male infertility adequate?

One of the major problems in the management of infertility in the male is that today, due to the widespread use of IVF, the treatment is so frequently carried out by Gynaecologists, many of whom have had little or no training in Urology and who therefore have a poor understanding of the anatomy and physiology of the genital tract and who have even less understanding of the pathology that may be seen in the lower urinary tract. The importance of Urological experience among Gynaecologists has been suggested in the past (Cummins & Jequier, 1997) but it is now stated that this should be a mandatory part of the training given to infertility clinicians. A plea is made to include basic urology as a formal part of the training of all Gynaecologists involved in the treatment of male infertility and thus by inference by all clinicians involved in this type of work. At the present time, it is suggested that Gynaecologists are simply taking on work for which they have been inadequately trained.

Where are the Urologists in the management of male infertility?

In many parts of the world, with the USA being the notable exception, Urologists seem to have abrogated almost all responsibility for the management of male infertility. Whether this disinterest relates to the demands of prostate cancer in an aging population or it is because the technique of IVF and ICSI that is so frequently used to treat male infertility and is basically a gynaecological procedure is unclear. Due to the apparent lack of interest in male infertility by the Urologists, the infertile male patient goes into treatment often without ever having been examined by a clinician. Frequently no investigations other than a semen analysis have been carried out. Due to the narrow base of training of specialists today, few Gynaecologists have had any training in Urology or even general surgery and are thus unable to carry out many of the investigations such as cystoscopy and vasography that are so useful in the clinical diagnosis of the cause of infertility in men.

Conclusions

There is no doubt that IVF and in particular IVF/ICSI have revolutionised the management of infertility in the male. However, it is my contention that overall the infertile male patient is being badly managed. Far too much attention is being given to the semen analysis, which, for the most part, only provides information about a series of changes that are largely non-specific and give the clinician little idea of the cause of the infertility in an infertile male patient. Due to an inadequate or even absent clinical assessment, these patients are at times, being propelled into complex and expensive treatment that may be unnecessary. Better training is needed for the Gynaecologists in this side of infertility should be encouraged and the Urologists should be encouraged to regain an interest in the management of male infertility.

References

- Bjorndahl L & Qvist U. (2003) Sequence of ejaculation affects the spermatozoon as a carrier and its message. *Reproductive Biomedicine Online* **7**, 43-47.
- Cooke ID, Suleiman RA, Lenton EA & Parsons RJ (1981) Fertility and infertility statistics: their importance and application. *Clinical Obstetrics & Gynaecology* **8**, 531-548.
- Cummins JM & Jequier AM (1994) Treating male infertility needs more clinical andrology, not less. *Human Reproduction* **12**, 1214-1219.
- Hammond MG, Jordan S & Sloan CS (1986) Factors affecting pregnancy rates in a donor insemination programme using frozen semen. *American Journal of Obstetrics & Gynecology* **55**, 480-485.
- Jequier AM & Ukombe EB (1983) Errors in the performance of a semen analysis. *British Journal of Urology* **55**, 434-436.
- Jequier AM, Ansell ID & Holmes SC (1985) Congenital absence of the vasa deferentia presenting with infertility. *Journal of Andrology* **6**, 15-19.
- Jequier AM (1991) Edward Martin (1859-1938) The founding father of modern clinical andrology. *International Journal of Andrology* **14**, 1-10.
- Jequier AM (2005) Is quality assurance in semen analysis still really necessary? A clinician's viewpoint. *Human Reproduction* **20**, 2039-2042.
- Kent-First MG, Kol S, Muellem A, Ofir R, Manor D, Blazer S, First N & Itskovitz-Eldor J (1996) The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Molecular Human Reproduction* **2**, 943-950.
- MacLeod J (1956) Human semen. *Fertility & Sterility* **7**, 368-386.
- Rehan N, Sobrero AJ & Fertig JW (1975) The semen of fertile men: statistical analysis of 1300 men. *Fertility & Sterility* **26**, 492-502.
- Thomson JA, Lincoln PJ & Mortimer D. (1993) Paternity by a seemingly infertile vasectomised man. *British Medical Journal* **307**, 299-300.
- Venn A, Hemminki E, Watson L, Bruinisma F & Healy D. (2001) Mortality in a cohort of IVF patients. *Human Reproduction* **16**, 2691-2696.

What every clinician need to know about basic semen analysis

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Learning objectives

The participant should be able to explain

- interpretation of semen analysis in relation to
 - the man and his clinical problem
 - the sample collection
 - the laboratory service
- the importance of standardization in laboratory andrology
- the basic principles of semen analysis
 - unique properties of laboratory andrology
 - the importance of
 - assessing sufficient numbers of sperm
 - standardized temperature for motility assessment
 - using improved Neubauer chambers and positive displacement pipettes for sperm concentration assessment
 - standardized staining methods and assessment criteria for morphology investigation
- the basics of Quality Control, Quality Assessment and Quality Improvement
- definitions of normal values and reference values, and related problems in semen analysis

Introduction

It may seem presumptuous to state that clinicians might benefit from knowing more about modern laboratory andrology. However, the lesson from a large number of training courses for laboratory staff world-wide is that the laboratories often experience a resistance from the clinical side, when it comes to changes in techniques and in the interpretation of results.

A number of initiatives have been taken to improve the standards in laboratory andrology. Early warning signals inspired the development of the World Health Organization's recommendations (WHO 1999), and further detailed laboratory manuals have also been developed (e.g. Kvist & Björndahl 2002) to enhance these efforts and to establish good laboratory practice globally. Also training courses have been developed and implemented, using controllable methods (Björndahl et al 2002).

Principles of basic semen analysis

There are many factors that influence the final results of semen analysis. Some of these factors are unique for semen analysis; others occur also in investigations of blood and other body fluids. In contrast to a blood sample the semen sample is highly heterogeneous at ejaculation, and – if the whole ejaculate is collected – a coagulum is formed, including the entire ejaculate; this coagulum usually disappears within 10-15 minutes. However, the well mixed liquefied semen containing sperm, prostatic fluid and seminal vesicular fluid in the laboratory pot may not at all represent physiology in vivo (cf. Björndahl & Kvist 2003).

It is common knowledge that the abstinence time influences the quantity of volume and sperm. However, also the quality and quantity of sexual arousal preceding the sample collection does influence the final composition of the ejaculate (Pund et al 2004). This is not surprising taking into consideration that the accessory gland secretion and the sperm transport in the male is not a continuous process but a function controlled by the autonomous nerve system. Thus the time between ejaculations may actually be less important than the quality and quantity of nerve activity immediately preceding the ejaculation. Still, there is a relation between abstinence time and different parameters in semen analysis and it is thus important to at least record the abstinence time to give a better base for the clinical interpretation of data.

Another factor that may influence the quality of the semen sample is whether the sample is collected in the home of the patient or in a room close to the laboratory. For the patient, the former is often easier and much less stressful, but in practical terms it is often difficult to transport the sample to the laboratory with sufficient protection from e.g. cold shock (which makes sperm immotile) and without considerable delay before examination can begin. Poor motility in a sample assessed more than one hour ejaculation and perhaps after exposure to temperatures at +15°C or below, can be due to these circumstances rather than a real dysfunction of the man or his sperm.

A general feature in laboratory science is to decrease the influence of random factors. When cells are counted or classified, it is essential that sufficient numbers of cells are assessed. When low numbers of cells are assessed the likelihood of random errors to distort the results is much greater. For instance, when 100 cells are investigated the random error can be estimated to $\pm 20\%$, while assessment of 400 cells reduces the random errors to $\pm 10\%$. Thus, irrespective of the competence of the analyst, assessments of low numbers of cells allow a substantial influence of random errors.

Techniques and equipment

Sperm motility declines after ejaculation – faster for some men and slower for other. For the interpretation of results it is therefore important to state how long time after sample collection the motility assessment was done. Also the temperature at which the motility assessment is done will affect the results – in general more rapidly motile sperm at 37°C than at lower temperatures. The reason for recommending analyses to be done at 37°C is because it is much more difficult (and expensive) to standardize the temperature to “room temperature” (which without control easily can vary between 18 and 28°C). Furthermore, since also the well mixed semen sample can be very heterogeneous, the recommendation is to assess two separate aliquots and compare the results before accepting the count.

Sperm concentration is best assessed in a chamber with known and easily controllable depth (volume). Shallow chambers (10-20 μm deep) with removable cover slip have the disadvantage that even minor errors in cover slip application will affect the assessed volume considerably. Shallow chambers which are filled with capillary force do not fill uniformly, and generally give lower values for sperm concentration. Therefore the 100 μm deep chambers originally developed for assessment of blood cells are recommended, particularly one type called improved Neubauer chamber, because the pattern of areas is very adequate for sperm counting purposes. Another crucial piece of equipment for sperm counting is the positive-displacement pipette – a pipette with a piston in the tip – to measure an exact volume of semen to dilute for sperm concentration assessment. Ordinary air-displacement pipettes are made for liquids with viscosity similar to that of water – the volume obtained in the pipette tip will decrease with higher viscosity. Since the viscosity of fresh semen is very variable, but always higher than that of water, only positive-displacement pipettes will aspirate correct volume for fresh semen samples. Also for sperm counts there is a recommendation for

duplicate assessments, but mainly to detect and avoid errors due to poor sampling from the diluted sperm suspension and problems with the loading of the counting chamber.

A highly controversial topic is the issue with sperm morphology assessment (cf Mortimer & Menkveld 2001). In general there is a consensus that poor sperm morphology is a negative sign in relation to fertility. The controversy is about the criteria used for assessment. The first morphology assessment schemes were based on microscopic observations on sperm from semen. Here, certain types of morphological abnormalities were defined, and sperm not fulfilling the criteria of these abnormalities were classified as normal. It was also assumed that head abnormalities were more important than other abnormalities, and in general sperm were only classified as normal or abnormal with no information where abnormalities occurred. Later the so called strict criteria (Tygerberg strict criteria) were developed. This scheme is based on observations of sperm morphology among sperm which had passed through cervical mucus and also on sperm which could bind to zona pellucida. In opposition to the former scheme, the strict criteria are an attempt to define the morphology of sperm with fertilizing ability. When assessing sperm morphology according to the “strict criteria”, sperm that do not comply with the definition of “normal” morphology are by definition abnormal. Furthermore, WHO recommendations (1999) are to use the strict criteria and to record possible abnormalities in four different categories in each abnormal sperm.

Basic semen analysis techniques require light microscopy. For fresh semen analysis (motility, concentration) phase contrast optics (20-40X objective) is essential to be able to see the sperm at all, but for morphology and vitality assessment bright field optics (100X objective) to enable sufficient resolution and focal depth. For sperm morphology it is necessary to stain the sperm. What is seen in the microscope depends on slide preparation and fixation (shrinkage of structures) and which stain is used (different organelles are stained very differently with different techniques). The most common technique is the modified Papanicolaou staining – modified from the technique used for cervical smears. However, if a laboratory uses the general cytology Papanicolaou technique the sperm on their slides will be very poorly stained and their assessments will not be consistent with those from laboratories using the proper technique.

Sperm vitality assessment is mainly of interest when there are very few motile sperm. The question is if the immotile sperm are dead or alive (e.g. immotile cilia syndrome). Fairly simple methods exist where dead sperm take up certain stains, while live sperm (with intact cell membrane) will not be stained. Unfortunately the method presently recommended by the WHO appears to kill a proportion of the sperm (Björndahl et al 2004). A better method is available in the NAFA-ESHRE Manual (Kvist & Björndahl, 2002) and in the next edition of the WHO guidelines the recommended method is likely to be corrected.

Training

All laboratory staff doing basic semen analysis require proper training. The Special Interest Group in Andrology (SIGA) of ESHRE has developed and implemented a standardized 4-day course introducing laboratory staff as well as laboratory directors to the recommended techniques and equipment. After such an introduction further in-house training is essential; training that could be integrated with Internal Quality Control. The ESHRE-SIGA courses on basic semen analysis is available in several countries or regions (mainly in the local language), and further dissemination to other regions is on-going.

Quality Control, Quality Assurance and Quality Improvement

The systematic structure and documentation of quality in laboratory services in clinical settings has developed rapidly the last twenty years. Early the focus was on Quality Control (QC) – the assessment if the services match the quality goals set for the service. Soon the wider concept of Quality Assurance (QA) was embraced – including all measures taken by a service in order to be able to deliver and maintain a certain level of quality in the service. However, in a competitive world it is important not only to maintain a level, but to always improve the performance; the meaning of Quality Improvement (QI). QI is based on QA and QC.

It is essential for a laboratory to run Internal Quality Control to ascertain that the results are consistent over time and between different analysts. Participation in an External Quality Assessment program provides a tool for the laboratory to know if its performance is comparable with other laboratories. Collaboration in international External QA schemes is also important for a meaningful application of scientifically evaluated method improvements; to start assessing samples for e.g. a specific morphological feature is useless unless the laboratory uses the same staining technique and assessment criteria as the publishing laboratory. Thus, an absolute prerequisite for meaningful External QA and result transfers between laboratories is that the methods used are standardized.

Normal values and reference values

In the history of clinical laboratory science the so called normal values had a central position. The term “normal” actually refers to the “normal distribution” or “Gaussian distribution” according to which 95% of results from healthy individuals would be within the “normal limits” and the mathematically defined standard deviation could be used to define the 95% interval. The problem with this concept is that also results from individuals with a certain disorder or disease may occur within these limits – the overlap between healthy and not healthy can be considerable. Therefore, the concept of “reference values” has been introduced. A reference population can be a healthy population or a reference population with a certain disorder, and the “reference range” is still supposed to contain 95% of the results from this reference population, but it doesn’t require the normal or Gaussian distribution.

Establishing reference values for semen analysis results is linked with specific problems. In common clinical chemistry, all tests are related to the one and only individual involved. In infertility investigations, there are two individuals involved, but still we would like to have indices for infertility when interpreting semen analysis results. This means that the results from a number of men with very good semen analysis results can be classified as indicative of poor fertility.

Another aspect that creates difficulties is the present lack of standardization of techniques and absence of External QA. With standardized techniques and ongoing External QA both results and reference values will be more easily transferable. The establishment of reference values could even be done in collaboration between centres using the same techniques and verifying the performance with External QA.

When discussing the establishment new reference values, it is also important to consider that the reference values and quality requirements can vary with different use for the results.

Different use of results from semen analysis

When reference values are implemented, different reference populations (as well as modified techniques) should be used for different applications. The most common field is of course the infertility investigation. Here the most appropriate reference ranges should help the clinician to decide which men could benefit from further clinical investigation and which couples might directly continue to Artificial Reproductive Treatment. In the infertility treatment situation, other reference values would help the embryologist to choose the best treatment option on that day. For post vasectomy samples extended examination of semen centrifugation pellets is required in order to ensure that azoospermia actually has been achieved. In the group of men obtaining endocrine substitution therapy due to pituitary or hypothalamic deficiency, low but increasing sperm numbers call for other modifications of sperm counting techniques and related QC methods.

Conclusions

For the informed clinician, it is essential that the semen analysis report does not only contain a few odd numbers indicating the sperm concentration, proportion motile, normal and live sperm. Information about completeness of sample collection, abstinence time, time of collection and time when assessment began, total semen volume and total number of sperm in the ejaculate gives the clinician a better base to evaluate the sperm concentration data. Furthermore, information on the numbers of sperm the laboratory routinely base their assessments on, temperature for motility analysis, staining procedures for morphology and vitality assessments, and that Internal Quality Control and External Quality Assessment is implemented, is also important to enable the clinician to interpret the results of semen analysis properly.

Annotated references

- Björndahl L, Barratt CL, Fraser LR, Kvist U, Mortimer D (2002) ESHRE basic semen analysis courses 1995-1999: immediate beneficial effects of standardized training. *Hum Reprod* 17:1299-1305. *Evaluation of ESHRE SIGA courses in basic semen analysis.*
- Björndahl L, Kvist U (2003) Sequence of ejaculation affects the spermatozoon as a carrier and its message. *Reprod Biomed Online*. 7:440-8. *Review discussing the physiology of ejaculation in relation to semen analysis.*
- Björndahl L, Söderlund I, Johansson S, Mohammadi M, Pourian MR, Kvist U (2004) Why the WHO recommendations for eosin-nigrosin staining techniques for human sperm vitality assessment must change. *J Androl*. 25:671-8. *A review of sperm vitality methods, including problems with techniques in the present WHO guidelines.*
- Kvist U, Björndahl L (eds) (2002) *Manual on Basic Semen Analysis*. ESHRE Monographs (2). Oxford, United Kingdom: Oxford University Press. *This practical manual was developed by the Nordic Association for Andrology and ESHRE SIGA based on recommendations in the WHO guidelines. It is also a practical basis for the ESHRE SIGA courses in basic semen analysis.*
- Mortimer D, Menkveld R (2001) Sperm morphology assessment--historical perspectives and current opinions. *J Androl*. 22:192-205. *An important bench mark publication regarding human sperm morphology and the development of different assessment criteria.*
- Mortimer, D. (1994) *Practical Laboratory Andrology*. Oxford University Press, New York, USA. *This comprehensive textbook gives a thorough background to Laboratory Andrology as well as practical advice for the implementation of and routine use of any basic analysis*
- Pound N, Javed MH, Ruberto C, Shaikh MA, Del Valle AP (2002) Duration of sexual arousal predicts semen parameters for masturbatory ejaculates. *Physiol Behav*. 76:685-9. *Example of a recent paper on effects of duration on sexual arousal on semen parameters.*
- World Health Organization (WHO) (1999) *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*. Cambridge, United Kingdom: Cambridge University Press. *This is the basis for all laboratory andrology – with aim to achieve global standardization and increased quality.*

Should We Bother About Male Accessory Gland Infection?

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Learning objectives

By the end of this chapter, readers will be able to:

- Understand the relationship between male accessory gland infection and male infertility
- Initiate a diagnostic process for male accessory gland infection
- Initiate appropriate treatment for male accessory gland infection

Summary

Colonization of the male genital tract is probably common and usually self-limiting. The male accessory sex glands often harbour microorganisms, like ureoplasma and chlamydia trachomatis, which may colonize the urogenital tract without obvious signs of infection.

Occasionally, these microorganisms and other bacteria cause symptomatic urethritis, prostatovesiculitis and epididymitis with deterioration of the semen quality and leucocytospermia.

A history of urogenital infection occurs in 1.6- 10.3% of men attending fertility clinics.

Infection may have a detrimental effect on sperm quality by reducing forward motility and possibly affecting the number of morphological normal spermatozoa. In addition, infections may be the source of autoantibodies against spermatozoa, found in about 8% of the infertile male population. Leucocytes may produce large amounts of reactive oxygen species and cytokines, directly effecting sperm motility and fertilizing capacity.

In contrast to the situation in women, there is no clear evidence that male accessory gland infections can result in epididymal blockage or vassal obstruction, with the exception of genital tuberculosis. Although chlamydia trachomatis is a well-documented source of urethritis and prostatitis, the infection does not seem to cause obstruction of the reproductive tract in men, as it does in women. If male urogenital infection causes obstruction it is most likely located at the level of the ejaculatory ducts. Chronic prostatitis may cause scarring of the prostatic and ejaculatory ducts, resulting in low seminal volume and low fructose. These men present with severe oligozoospermia or azoospermia, normal size testis and normal gonadotrophins. Chronic epididymitis can result in progressive deterioration of semen quality due to loss of epididymal function.

Introduction

Infections of the male urogenital tract are potentially correctable causes of male infertility. Urethritis, epididymitis and prostatitis have been mentioned as male accessory gland infection (MAGI) by the WHO. MAGI are linked to infertility without strong proof of causality or efficacy of their treatment (Weidner et al., 1999). The most common aetiological causes of bacterial prostatitis are gram-negative pathogens, predominantly strains of escherichia coli. The role of gram-positive bacteria in bacterial prostatitis is controversial. Whereas enterococci may cause bacterial prostatitis and associated recurrent urinary tract infection, the significance of chlamydia trachomatis and ureoplasma urealyticum in MAGI is unclear. A recent cross-sectional study suggests that sperm quality declines with age only in subfertile men with MAGI (Rolf et al., 2002).

Bacteria and infertility

There are several reasons why there is doubt about MAGI influencing male infertility:

- Most pathogenic bacteria isolated from patients with MAGI show no impact on sperm parameters in *in vitro* studies. A direct effect of bacteria on spermatozoa seems unlikely.
- Semen is characterized by low bacteria counts and asymptomatic men may have positive cultures
- No clear correlation is found with the number of leucocytes in the semen and MAGI
- Urethral contamination is usually present in semen cultures
- 80% of prostatitis patients have no bacteria in their ejaculate
- The contact time between the infectious agent and the spermatozoa is relatively short in patients with MAGI, except for men with epididymitis.

It seems therefore unlikely that bacteria, viruses, chlamydia or mycoplasma have a direct effect on spermatozoa and cause impaired male fertility.

In contrast to the situation in woman not much is known about the consequences of acute and chronic chlamydial infections in men and their impact on semen quality. It is postulated that up to 50% of men infected with chlamydia are asymptomatic and in those with symptoms, the most common presentation is urethritis. Studies also suggest that most upper genital tract infections in young men, including epididymitis, are often attributable to chlamydia.

Epididymitis is thought to be important because fertility might be affected due to inflammation, obstruction and functional impairment, especially where both epididymi are affected. As well as creating a physical blockage to the movement of sperm, chlamydia may also cause epithelial damage that reduces spermatogenesis, induces immunological responses that destroy or hinder sperm, and reduces the female partner's fertility. It can be concluded that chlamydia trachomatis is a frequent pathogen in male genital inflammation since the microorganisms are rarely present in healthy men. There are, however, no conclusive studies showing that men infected with chlamydia trachomatis are less fertile than uninfected men. Male genital chlamydial infection is mainly a threat to the female genital organs.

Leucocytospermia

According to WHO classification, $> 1 \times 10^6$ WBC per mL is defined as leucocytospermia. The clinical significance of an increased concentration of white blood cells (WBC) in the ejaculate is under debate: leucocytospermia is often found in men without obvious signs of urogenital infection and with negative cultures. Most studies show no significant association between white blood cell count and microorganisms in semen: in more than fifty percent of men with leucocytospermia semen cultures showed negative. There seems to be agreement that only an increased number of leukocytes is indicative for MAGI. Leukocytes, especially neutrophilic granulocytes can produce large amounts of reactive oxygen species and cytokines, which may influence sperm function. The great majority of leukocytes are neutrophilic granulocytes and can be detected the peroxidase staining reaction.

Although most authors consider leucocytospermia a sign of inflammation, they are not necessarily associated with bacterial or viral infections: metabolites from the urine may enter the prostate due to reflux during voiding with insufficient pelvic floor relaxation and cause a chemical prostatitis. Also, autoimmune diseases of the prostate may cause leucocytospermia. No clear relation exists between leucocytospermia and conception, both natural as with ART.

Reactive oxygen species

Impairment of sperm function can be caused by the formation of an excess of reactive oxygen species (ROS). The deleterious effect of ROS on semen quality has been documented and reviewed (Depuydt et al., 1996). There are several reasons why spermatozoa are more vulnerable to ROS than other cells:

- The sperm membrane contains a high level of polyunsaturated fatty acids, which are extremely susceptible to peroxidation. Peroxidative damage may result in a loss of membrane functions and may lead to a reduction in fertilizing ability, motility and viability.
- In contrast to other cells, spermatozoa have a very limited system to repair damaged structures as a consequence of their small amount of cytoplasm and an inactive, highly condensed chromatin.
- Spermatozoa are equipped with a poor defence system against ROS, as catalase is fully absent, and glutathione peroxidase and superoxide dismutase are present in relatively low amounts.
- Mitochondria are particularly vulnerable to oxidative stress: ROS probably influences sperm motility through mitochondrial damage with depletion of ATP. Since ROS produced in the prostate or seminal vesicles have only a limited exposure time to spermatozoa they are probably not very harmful. Also seminal plasma contains large amounts of antioxidants that prevent sperm damage. Within the epididymis, however, exposure time is much longer and the amount of scavengers is limited.

Cytokines

Cytokines are polypeptide mediators involved in the communication network of cells of the immune system. Various cytokines are involved in inflammation and may influence sperm function. The prostate seems to be the main site of origin of interleukin-6 and 8 in the seminal plasma. Although it is accepted that cytokines, especially IL-6, must play an important role in the male accessory gland inflammatory process, elevated cytokine levels do not seem to depend on the number of leukocytes in the ejaculate. There is still considerable lack of knowledge about the normal values of cytokines in semen and their influence on sperm parameters. No effect of interleukins concentration was found on progressive sperm motility and morphology. It has been suggested that some interleukins may protect spermatozoa against ROS, especially interleukin-8. Cytokines are mainly used for the diagnosis of MAGI.

Inflammatory-associated obstruction

Chronic infection of the prostate can result in scarring and obstruction of the ejaculatory ducts (EDOs). Usually, these obstructions are incomplete, causing poor sperm quality with low seminal volume (Dohle et al., 2003). This diagnosis can be suspected in men with severe oligozoospermia or azoospermia, normal physical findings and normal serum follicle-stimulating hormone (FSH). Other features that suggest obstruction are epididymal congestion, enlarged seminal vesicles and cystic lesions of the epididymis and prostate. Acquired ductal obstructions account for most of the cases of obstructive azoospermia. Infections of the ductal system and surgery of the scrotum and the groin are the most important causes of this type of obstructions. Ductal infections used to be caused by ascending urinary tract infections, gonorrhoea and tuberculosis. chlamydia trachomatis has been discussed as an infective agent, potentially causing blocks of the ductal system without actual evidence.

Epididymal blocks are often asymmetrical and usually found in the corpus and the tail. A history of epididymitis or urethritis is often found in these men. In men with progressive deterioration of semen quality and in men with low semen volume signs of an EDO can be found on trans-rectal ultrasound of the prostate. The ultrasonographic diagnosis of EDO is based upon dilatation of seminal vesicles or abnormalities such as midline prostatic cysts and calcifications in the region of the ejaculatory ducts. Unfortunately, not all patients with EDO have dilated seminal vesicles and, conversely, not all patients with dilated seminal vesicles have EDO.

Treatment

Symptomatic improvement is often accomplished with appropriate antimicrobial treatment of men with MAGI. Antibiotics may also effect ROS production and white blood cell count in semen (Vicari et al, 2000). However, controversial results on the effect of antimicrobial therapy on semen quality have been published (Purvis et al., 1995). antibiotic treatment often only eradicates microorganisms and has no clear positive effect on inflammatory alterations and ROS production by leucocytes. Also it will not alter functional deficits and anatomical dysfunctions caused by the inflammation process.

Therapy is usually started with a 2-week regimen of fluoroquinolone or tetracycline in case of chlamydia. Treatment is also effective gonococcal urethritis. Vicari (2000) found that epididymal infections were associated with more severe semen abnormalities and higher amounts of ROS as compared to men with urethritis and prostatitis. Only non-significant effect of antibiotics on sperm parameters were found after 3 months of antibiotic treatment. The effect on WBC counts and ROS production was mainly seen in men with prostatitis and very limited in men with epididymitis. No clear effect on pregnancy rates were found. MAGI often reoccurs due to insufficient treatment protocols.

Art and infection

Bacteriospermia is a common observation in ejaculates used for IVF: in about 35% bacteria can be cultured. Contradictory results have been published on the influence of bacteria on the results of IVF and ICSI. Most studies failed to demonstrate any effect on fertilization; in some studies the number of pregnancies were reduced if the ejaculate contained significant amounts of bacteria. The role of chlamydia infection of the semen has been investigated in several studies and no influence could be demonstrated on the outcome of IVF. The absence of any effect of bacteria on IVF procedures can be explained by the use of antibiotics in the culture media used and the sperm washing procedure, which showed effective in clearing most bacteria. Still, guidelines for ART advise first to treat MAGI before starting an IVF procedure.

Conclusion

No clear evidence exists for the potential negative effect of MAGI on male fertility. Semen quality can be reduced mainly by the production of ROS from leucocytes, especially in the epididymis. Exposure time of ROS in the epididymis is long enough to cause permanent damage to the spermatozoa and thus impair spontaneous conception rates. Some bacteria may cause a chronic infection of the prostate and the epididymis, resulting in (partial) obstruction of the ejaculatory ducts with low seminal volume and oligozoospermia. Treatment can eradicate bacteria in the accessory glands, but leucocytes and ROS production may continue to be produced and infertility will persist.

References

1. Purvis K, Christiansen E (1993) Infection of the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. *Int J Androl* 16: 1-13.
2. Weidner W, Krause W, Ludwig M. (1999) Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. *Hum Reprod Update* 5: 421-432.
3. Depuydt CE, Bosmans E, Zalata A, Schoonjans F, Comhaire FH. (1996) The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. *J Androl* 17: 699-707.

4. Everaert K, Mahmoud A, Depuydt C, Maeyaert M, Comhaire F. (2003) Chronic prostatitis and male accessory gland infection--is there an impact on male infertility (diagnosis and therapy)? *Andrologia*. 35(5):325-30.
5. Dohle GR. (2003) Inflammatory-associated obstructions of the male reproductive tract. *Andrologia*. 3;35(5):321-4.
6. Vicari E. (2000) Effectiveness and limits of antimicrobial treatments on seminal leucocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. *Hum Reprod* 15;12:2536-44.

Male infertility and androgen deficiency – is there a link?

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Learning Objectives

- Testosterone is able and necessary to qualitatively initiate, maintain and re-initiate spermatogenesis and formation of spermatozoa.
- Testosterone acts synergistically to FSH, which is also a prerequisite for spermatogenesis.
- Under physiological circumstances, only the combination of testosterone and FSH yields quantitatively and qualitatively normal spermatozoa.
- Testosterone acts indirectly via somatic testicular cells on spermatogenesis. These are most likely Sertoli cells.
- The testicular effects of testosterone are thus paracrine.
- These effects are partly mediated by its metabolization products estradiol and dihydrotestosterone.
- Testosterone and FSH cooperate during regulation of spermatogenesis but act via different pathways.

Lecture summary

Basic features of intratesticular testosterone

Testosterone (T) production and spermatogenesis are the two primary functions of the testis in man. Normal testicular function is dependent on the intratesticular activity of the pituitary gonadotropins, LH and FSH. LH stimulates Leydig cells to produce T within the testis. Intratesticular T (ITT) is an absolute prerequisite for normal spermatogenesis. FSH is also vital for normal testicular function and is necessary for quantitatively normal spermatogenesis in man. Specifically, FSH is thought to play an important role early in spermatogenesis during spermatogonial maturation as well as late in the process during spermiation. The relative roles of intratesticular androgens and FSH are not fully understood in man. Control of the intratesticular hormonal environment is in large part regulated through negative feedback of T at the level of the hypothalamus and the pituitary. Exogenous T has been shown to

dramatically suppress gonadotropin release when administered at supraphysiological as well as physiological doses. Administration of T alone has been shown to reduce sperm production in the majority of men. Gonadotropin withdrawal has also been shown to dramatically reduce ITT, which, in turn, decreases sperm production. However, suppression of spermatogenesis is not uniform, and why some men are nonresponders is not clear. Possibilities include incomplete gonadotropin suppression, particularly with regard to FSH as well as inconsistencies in ITT suppression.

The clinical human model of hypogonadotropic hypogonadism

In male hypogonadotropic hypogonadism testosterone therapy is sufficient for maturation and maintenance of secondary sex characteristics. For stimulation of spermatogenesis administration of gonadotropins is necessary. If pulsatile gonadotropin-releasing hormone (GnRH) is not indicated or desired, human chorionic gonadotropin (hCG) is used as the source of luteinizing hormone (LH) activity to stimulate testosterone secretion by Leydig cells, whereas human menopausal gonadotropin (hMG) is used as the source of folliclestimulating hormone (FSH). More recently, recombinant gonadotropins have also been used clinically. Several animal studies have investigated the relative contributions of both gonadotropins for induction and maintenance of spermatogenesis. Therefore FSH and LH/testosterone in combination are required to maintain spermatogenesis to full extent.

Thus, idiopathic hypogonadotropic hypogonadism (IHH) is an important disease model in the human male. Men suffering from this disorder classically display a number of clinical symptoms including absent pubertal development, lack of secondary sexual characteristics and infertility. Microadenomas with functional significance (producing hormone or leading to hypopituitarism) exclude the diagnosis of IHH. However, radiologic anomalies of the pituitary gland without functional significance is occasionally seen in IHH as well as in a small proportion of normal healthy adults, which is confirmed by a nearly uniform response to physiological regimens of exogenous GnRH-replacement therapy.

A small subset of patients with this disorder presents with a partial form of GnRH deficiency as assessed by some degree of testicular growth despite hypogonadal testosterone levels. In contrast to most other causes of male infertility, IHH is typically curable in many patients. Indeed, long-term GnRH therapy or gonadotropins successfully induce spermatogenesis in the majority of these patients. However, 20–30% of IHH with the most severe form of GnRH deficiency remain azoospermic. This latter observation has attracted the attention of many clinicians and researchers to focus their attention on the management of IHH and the role of testosterone.

Many of the IHH patients have a testis that resembles the prepubertal testis. Precocious puberty can be induced in immature monkeys following various hormonal regimens, and in these experiments it was demonstrated that there was an increased proliferation of Sertoli cells accompanied by germ cell proliferation, a process regulated by both FSH and LH, thus testosterone.

Testicular histology in relation to testosterone action

While testicular biopsy was accepted as a diagnostic tool for the assessment of infertility in the 1970s, the evolution of assisted reproduction techniques (i.e. IVF and ICSI), has popularized the usage of testicular biopsies during the course of treatment of infertile men. Additionally, testicular biopsies have been used in previous studies to rule out Sertoli cell-only syndrome prior to starting therapy in IHH men as well as to monitor the efficacy in inducing spermatogenesis. These histological studies revealed that most IHH men had prepubertal testes. However, these

studies included few subjects and focused mostly on seminiferous tubule diameter, basement membrane thickness and the ratio of germ cells to Sertoli cells.

While a great deal is known about the histology of the normal adult testes, less is known about prepubertal testes, and there is an even greater paucity of information regarding the histology of IHH testes. The normal adult human testis is composed of seminiferous tubules resting on a tunica propria containing a number of myoid cell layers separated from the seminiferous epithelium by a zone of connective tissue and a basement membrane. The seminiferous epithelium contains the nonproliferating fully mature Sertoli cells and the proliferating and differentiating generations of germ cells, from spermatogonia to fully mature spermatozoa. The interstitium has clusters of Leydig cells in the angular intervals between the seminiferous tubules. In the prepubertal testis, only immature Sertoli cells and type A spermatogonia are present. The prevailing view is that among the type A spermatogonia resides a stem cell population and that these stem cells must be stimulated directly, or indirectly via Sertoli cells, in order for spermatogenesis to be initiated. It is well established that normal spermatogenic activity in healthy men is due to the interaction of Sertoli cell-produced growth factors and germ cells with the support of testosterone produced by the Leydig cells.

Male hypogonadism in after testicular sperm extraction

For men with azoospermia, retrieval of spermatozoa from the testes for assisted reproduction using intracytoplasmic sperm injection (ICSI) offers an opportunity for fertility despite limited sperm production. Failure to extract spermatozoa may occur in up to 57 % of TESE attempts especially in cases of non-obstructive azoospermia. Multiple testicular biopsies can result in the loss of significant amounts of testicular tissue and can interrupt the testicular blood supply underneath the tunica albuginea with risks of testicular devascularization and subsequent atrophy of the testis.

Operation-induced vascular damage can lead to a decrease of Leydig cell function causing a decrease of serum testosterone levels: consequent androgen deficiency may have serious long-term health consequences. A decrease in serum testosterone levels following non-microsurgical TESE in patients with azoospermia for up to one year after the procedure was described: in patients with non-obstructive azoospermia in whom no spermatozoa were found following a micro-epididymal sperm aspiration and a simple testicular biopsy, *de novo* androgen deficiency occurred in 16 %, indicating that, long term hormonal follow up is recommended after TESE.

New sperm function tests and ART: evidence based results

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Learning Objectives

- To understand how human sperm chromatin is organized and get a brief overview of the causes of sperm DNA breaks
- To obtain an overview of the most frequently used sperm DNA integrity testing methods, with particular focus on the sperm chromatin structure assay (SCSA)
- To get the most recent knowledge regarding use of sperm chromatin integrity testing in clinical practice including decision considering use of ART

Lecture summary

Introduction

Since the introduction of assisted reproduction techniques (ART) there has been a continuous search for markers with the potential to predict a couple's chance of obtaining a pregnancy and to be used for improving the relatively low baby-take-home rates, which have been held stable (20-30%) during the last two decades (Andersen et al., 2005).

Traditionally, microscopic assessment of human spermatozoa (WHO, 1999) is used to evaluate semen quality before ART cycles. The parameters normally used as sperm concentration, motility and morphology are, however, poorly standardized (Giwerzman et al., 1999), subjective (Auger et al., 2000), and not powerful predictors of male fertility (Bonde et al., 1998; Guzick et al., 2001), and, therefore, better and complementary methods to predict male fertility have been searched for.

During recent years there has been an increased focus on the role of sperm chromatin integrity in relation to fertility. Although spermatozoa with defect DNA are shown to be able to fertilize an oocyte when they are injected directly into it (Twigg et al., 1998), normal sperm DNA integrity is essential for normal fertilisation, embryo and foetal development as well as for a normal postnatal health.

Recently, sperm DNA integrity tests have been recognized as an independent measure of sperm quality that may be of better prognostic value than standard sperm parameters, both for in vivo (Evenson et al, 1999; Spano et al., 2000) and for in vitro fertility (Larsson et al., 1999; Larson-Cook et al., 2003; Saleh et al., 2003; Bungum et al., 2004; Gandini et al, 2004; Virro et al., 2004, Bungum et al., submitted). Several assays have been developed to evaluate sperm DNA integrity. Currently, the most used tests are TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling) (Sailer et al., 1995) and SCSA (sperm chromatin structure assay) (Evenson et al., 1980).

It has also been shown that in some cases factors causing sperm DNA damage can be efficiently treated (Greco et al., 2005).

Although 30% of patients seeking fertility treatment have high rates of sperm DNA breaks (Bungum et al., 2004) and several sperm DNA integrity testing methods now are available, very few fertility clinics have implemented DNA integrity testing as a routine. In order to treat infertility more effectively, the newest knowledge in the field should be used in clinical practice. SCSA can be used in order to improve infertility diagnosis and treatment.

Sperm DNA organization and causes of sperm DNA damage

Human sperm chromatin differs from both human somatic cells and sperm cells in other mammals, in structure and composition. During spermiogenesis histones are replaced by protamines (Poccia 1986), organized into unique supercoiled doughnuts, called toroids (Ward and Coffey 1991). In contrast to other mammals in which sperm DNA is associated with only one protamine (P1), human spermatozoa have two types of protamine (P1 and P2). P2 has fewer thiol groups for disulphide bonding and this makes human sperm chromatin less stable than in other mammals (Jager 1990). In addition, in human spermatozoa (in contrast to sperms of mouse, bull and other species) about 15% of histones remain un-replaced by protamines that also lead to the less compact chromatin conformation.

Due to the condensed and insoluble nature of sperm chromatin, the genetic integrity of spermatozoa is normally well protected during the transport through the male and female reproductive tracts. However, infertile men were shown to have higher rates of sperm DNA damage than fertile men, characterized by both single and double DNA strand breaks (Evenson et al., 1999; Irvine et al., 2000). So far it is known that damage may arise from four potential sources (1) deficiencies in recombination during spermatogenesis (2) protamination disturbances; (3) abortive apoptosis and (4) oxidative stress (Sakkas et al., 1999; Agarwal et al., 2003; Erenpreiss et al., 2006), although the source(s) of sperm DNA damage in the male germ line have not yet been completely elucidated. It is known that diseases, fever, cigarette smoking and advanced age can also contribute to the sperm DNA damage (probably by inducing the oxidative stress)

Due to its significant role in both physiology and pathology of human reproduction, much focus has been paid on oxidative stress caused by reactive oxygen species (ROS). There is a growing evidence that seminal oxidative stress is involved in male infertility (Aitken et al. 1992; Lewis et al. 1995), and is one of the main source of sperm DNA strand breaks (Cummins et al. 1994; Twigg et al. 1998; Donnelly et al. 1999; Irvine et al. 2000; Saleh et al. 2003; Aitken and Sawyer 2003; Aitken et al. 2003a, 2003b). In addition, oxidative stress disturbs sperm functions, particularly motility, through the stimulation of a lipid peroxidation cascade in the plasma membrane (Aitken and Clarkson 1987; Aitken et al. 1998a, 1998b). Elevated ROS levels are reported in 25-80% of semen samples from men who consult infertility clinics (Griveau and Le Lannou, 1997; Pasqualotto et al., 2000). Leukocytes or abnormal spermatozoa present in the ejaculate are among the main sources of ROS in semen (Aitken et al. 1992). The potentially compromised spermatozoa can also be further damaged during their preparation for ART as repeated centrifugations are shown to cause iatrogenic damage to sperm chromatin structure (Aitken and Clarkson, 1988).

Assessment of sperm DNA damage

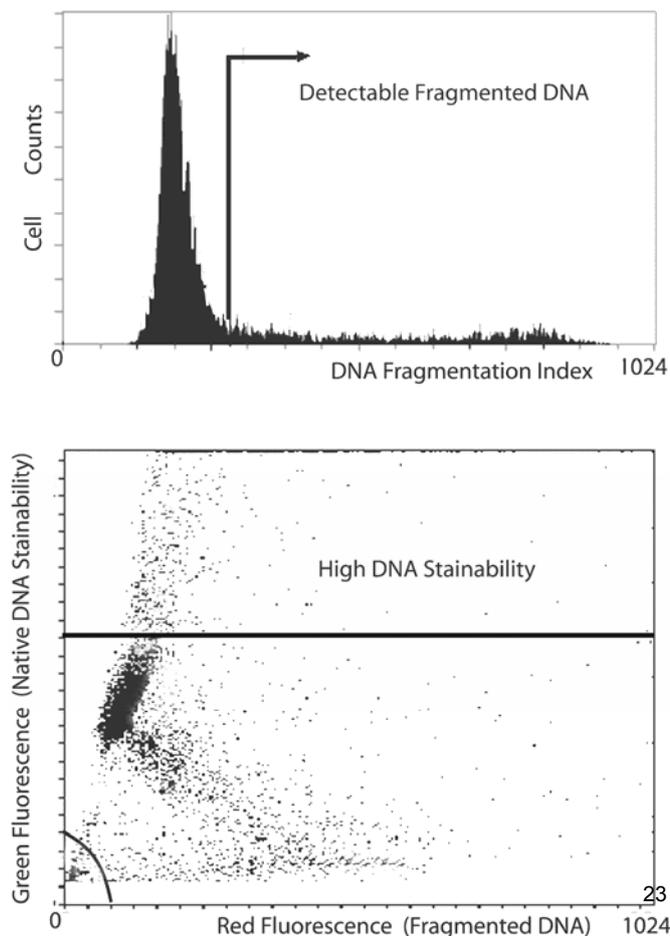
The two most commonly used tests for assessment of sperm DNA integrity are TUNEL and SCSA. They both label single or double stranded DNA breaks (although SCSA is an indirect test for sperm DNA breaks). Whilst TUNEL can be applied in both bright field, fluorescence microscopy, and by using flow cytometry, the SCSA is a flow cytometric method. The clear benefit by using flow

cytometry is the high amount of cells analyzed, 5-10 000 compared to 2-300 cells usually analyzed in bright field and fluorescence microscopy. SCSA is currently the method that has provided the most clear clinical cut-off levels seen in relation to predict fertility potential. Other tests for sperm chromatin structure assessment include Comet assay (Morris et al., 2002), Acridine Orange Test (AOT) (Tejada et al. 1984), Toluidine Blue Test (Erenpreiss et al., 2004) and the Sperm chromatin dispersion test (Fernandez et al., 2003). All these methods are, however, designed for the microscopic visualisation, therefore with limitations as considers numbers of cells being evaluated as previously explained.

Sperm chromatin structure assay (SCSA)

SCSA, first introduced by Evenson in 1980 is based on use of acridine orange, to evaluate the ratio of single- to double-stranded DNA following acid treatment causing denaturation of double stranded DNA in case of impairment of sperm chromatin structure (including DNA strand breaks and/or improper protamination). After short acid treatment, normal chromatin remains double stranded, abnormal - denaturates. Sperm chromatin damage is quantified by flow cytometric measurements of the metachromatic shift from green (native, double-stranded DNA) to red (denatured, single-stranded DNA) fluorescence and displayed as red vs. green fluorescence intensity cytogram patterns (Fig. 1). The extent of DNA denaturation is expressed as DNA fragmentation Index (DFI) (cells outside the main population), which is the ratio of red to total fluorescence intensity, i.e. the level of denatured DNA over the total DNA. Additionally, the fraction of high DNA stainable (HDS) cells is expressed, which are thought to represent immature spermatozoa with incomplete chromatin condensation.

Figure 1. Histo- and cytogram visualizing the results following SCSA. Results are expressed as DNA fragmentation index (DFI) and HDS (High DNA stainability).

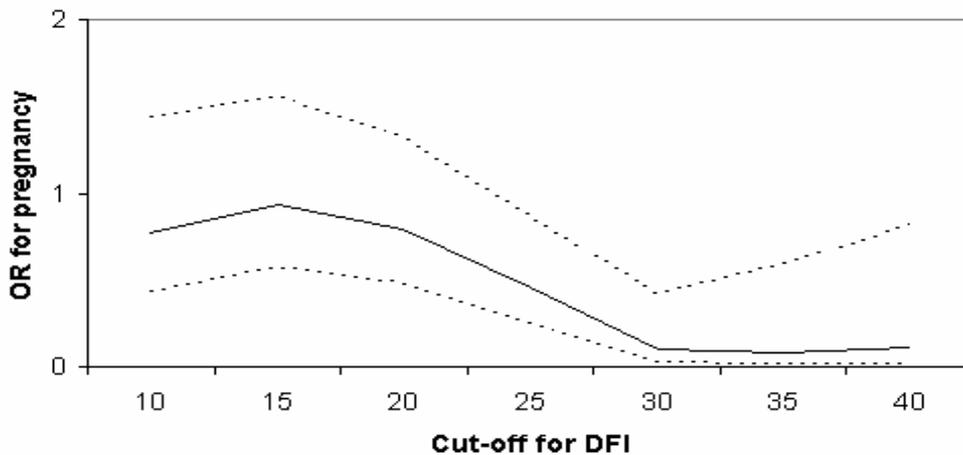


Sperm DNA damage and ART

Intrauterine insemination (IUI)

DFI can be used as an independent predictor of fertility in couples undergoing IUI (Bungum et al., 2004; Bungum et al., submitted) (Fig. 2). It has been shown that the chance of obtaining a pregnancy by IUI is extremely low when the proportion of sperm cells with DNA damage exceeds 30 % by means of SCSA (Saleh et al., 2003; Bungum et al., 2004; Bungum et al., submitted). Also by using TUNEL assay it has been demonstrated that when using semen samples with > 12 % sperm DNA fragmentation no pregnancies occur (Duran et al., 2002).

Figure 2. Odds ratio of IUI for biochemical pregnancy is significantly lower in the group with DFI > 30% than in those with DFI < 30%.



In vitro fertilization (IVF) and Intracytoplasmic sperm injection (ICSI)

The outcome of IVF and ICSI seen in relation to sperm DNA damage have been shown to be more controversial. Although some studies have reported that fertilisation rates (Lopes et al., 1998; Hammadeh et al., 1998), embryo quality (Tomlinson et al., 2001) and development (Seli et al., 2004) were negative correlated to DNA damage, others have not been able to show the same (Bungum et al., 2004; Bungum et al. submitted).

In regard to pregnancy and birth the first reports claimed that a sperm DNA fragmentation index (DFI) threshold of 27%, detected by SCSA, was necessary to obtain a successful pregnancy in IVF and ICSI (Larsson et al., 2000; Larsson-Cook et al., 2003). However, later these results could not be repeated by the same authors (Virro et al., 2004) or by the other research groups (Bungum et al., 2004; Gandini et al., 2004; Check et al., 2005; Bungum et al., submitted), demonstrating that successful pregnancies in IVF/ICSI cycles can even be obtained using semen samples with a high proportion of DNA damage. In the paper from 2004 (Bungum et al., 2004) we demonstrated that significantly higher clinical pregnancy rates (52.9 % vs. 22.2 %) and delivery rates (47.1 % vs. 22.2 %) were seen after ICSI compared with IVF when semen samples with high levels of sperm DNA damage were used. In this study, when DFI exceeded 27% the odds ratio for a positive reproductive outcome after ICSI compared with standard IVF was 8 for biochemical pregnancy, 4 for clinical pregnancy and 3 for delivery. This data is in agreement with an extended dataset of 1000 ART couples (Bungum et al., submitted) and other reports showing that sperm DNA damage is more predictive in IVF than in ICSI (Hammadeh et al., 2001; Host et al., 2000; Larsson-Cook et al., 2003).

Sperm DNA damage and early pregnancy loss

Convincing data on early pregnancy loss following ART as a possible consequence of sperm DNA damage is lacking. While some studies indicate a trend in increased human spontaneous abortions when the DFI is $\geq 30\%$ (Evenson et al., 1999; Carrell et al., 2003; Virro et al., 2004; Check et al., 2005) others have not been able to find the same risk of pregnancy loss (Bungum et al., submitted).

Sperm chromatin integrity testing used in clinical practice

Existing data points to a role of sperm DNA damage assessment in the routine infertility investigation (Agarwal and Allamaneni, 2005). Sperm DNA integrity testing can provide complementary information to the traditional sperm parameters on the quality of the spermatozoa. Some cases of unexplained or idiopathic infertility, when a traditional semen analysis falls into normal range and no evident female reproductive system pathologies can be explained by impairment of sperm DNA integrity. In addition, ICSI is the most efficient method to achieve pregnancy if DFI exceeds the level of 30%, despite the other sperm parameters (Bungum et al., 2004; Bungum et al., submitted). However, high DFI levels should be confirmed by a second test because there is a high (37%) chance to have DFI < 30% in the second test in these cases due to the DFI variability over the time (Erenpreiss et al., in press).

Antibiotics and antioxidant therapy

Apart from being a parameter that can be used for selection of the most proper ART technique, sperm DNA integrity assessment might help in disclosing some potentially treatable conditions. Male genital tract infection and inflammation have been associated to 8-35% of male infertility cases. Among the main sources of ROS in semen are leukocytes or abnormal spermatozoa present in the ejaculate (Aitken et al. 1992), which in turn can induce DNA strand breaks (Sakkas et al., 1999). Bacterial seminal infection can be treated with antibiotics, shown to improve sperm parameters by increasing antioxidant activity (Omu et al., 1998).

Antioxidant therapy has shown to be promising in decreasing sperm DNA fragmentation. After supplementation with an antioxidant, male factor patients significantly decreased their % DFI (Greco et al., 2005). Further studies, however, are needed in order to investigate whether antioxidant supplementation in men with high DFI can play a role in infertility treatment.

Summary

It is now evident that DNA damage influences the fertility outcome for natural conception and after ART procedures. DNA damage was reported to be associated with poor fertilization, impaired implantation and pregnancy rates, and an increased incidence of abortions. The precise nature of DNA breaks present in human spermatozoa, and origin of this damage is currently unknown.

Several techniques can be used to detect sperm DNA damage including TUNEL, Comet, and SCSA assays. SCSA currently is the only method that has provided the clearest clinically applicable cut-off levels. Our study (Bungum et al., submitted), based on about 1000 ART-cycles allows us to define SCSA as a valuable diagnostic tool in selecting the most appropriate procedure for an infertile couple undergoing ART. We therefore suggest that all men seeking infertility work-up and treatment should be tested with SCSA as a supplement to the standard semen analysis. In the future, some of the causes of sperm DNA integrity impairment may become treatable, and the use of ART in such subjects may become unnecessary.

References

1. Andersen A.N., Gianaroli L., Felberbaum R., de Mouzon J. and Nygren K.G. (2005) The European IVF-monitoring programme (EIM), European society of Human Reproduction and Embryology (ESHRE) Assisted reproductive technology in Europe, 2001. Results generated from European registers by ESHRE. The European IVF-monitoring programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). *Hum Reprod* 20,1158-1176.
2. Agarwal A. and Said T.M. (2003) Role of sperm chromatin abnormalities and DNA damage in male infertility. *Hum Reprod Update* 9, 331-345.
3. Ahmadi A. and Ng S.C. (1999) Fertilizing ability of DNA-damaged spermatozoa. *J Exp Zool* 284,696-704.
4. Aitken R.J., Baker M.A. and Sawyer D. (2003b) Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. *Reprod Biomed* 7:65–70.
5. Aitken R.J., Buckingham D.W., West K., Wu FC, Zikopoulos K and Richardson DW (1992) Contribution of leucocytes and spermatozoa to the generation of reactive oxygen species in the ejaculates of oligozoospermic patients and fertile donors. *J Reprod Fertil* 94, 451–462.
6. Aitken R.J. and Clarkson J.S. (1987) Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *J Reprod Fertil* 81, 459–469.
7. Aitken R.J., Gordon E., Harkiss D., Twigg J.P., Milne P., Jennings Z. and Irvine D.S. (1998a) Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol Reprod* 59:1037–1046.
8. Aitken R.J., Harkiss D., Knox W., Paterson M. and Irvine D.S. (1998b) A novel signal transduction cascade in capacitating human spermatozoa characterised by a redox-regulated, cAMP-mediated induction of tyrosine phosphorylation. *J Cell Sci* 111, 645–656.
9. Aitken RJ, Ryan AL, Curry BJ, Baker MA (2003a) Multiple forms of redox activity in populations of human spermatozoa. *Mol Hum Reprod* 9:645–661
10. Aitken R.J. and Sawyer D. (2003) The human spermatozoon—not waving but drowning. *Adv Exp Med Biol* 518, 85–98.
11. Auger J., Eustache F., Ducot B., et al. (2000) Intra- and inter-individual variability in human sperm concentration, motility and vitality during a workshop involving ten laboratories. *Hum Reprod* 15,2360-2368. Review.
12. Bonde J.P.E, Ernst E., Jensen T.K., et al. (1998) Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 1998; 352:1172-1177.
13. Bungum M., Humaidan P, Axmon A., Spano M., Bungum L., Erenpreiss J., and Giwercman A. (2006). Sperm DNA integrity assessment in prediction of outcome of assisted reproduction. Submitted.
14. Bungum M., Humaidan P., Spano M., Jepson K., Bungum L. and Giwercman A. (2004) The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. *Hum Reprod* 19, 1401-1408.
15. Carrell D.T., Liu L., Peterson C.M., Jones K.P., Hatasaka H.H., Erickson L., and Campcell B. (2003) Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. *Arch Androl* 49, 49–55.
16. Check J.H., Graziano V., Cohen R., Krotec J. and Check ML. (2005) Effect of an abnormal sperm chromatin structural assay (SCSA) on pregnancy outcome following (IVF) with (ICSI) in previous IVF failures, *Arch Androl* 5,121–124.
17. Cummins J.M., Jequier A.M. and Kan R. (1994) Molecular biology of human male infertility: links with ageing, mitochondrial genetics and oxidative stress? *Mol Reprod Dev* 37, 345–362.

18. Donnelly E.T., McCure N. and Lewis S.E. (1999) The effect of ascorbate and alpha-tocopherol supplementation in vitro on DNA integrity and hydrogen peroxide-induced DNA damage in human spermatozoa. *Mutagenesis* 14, 505–512.
19. Duran E.H., Morshedi M., Taylor S. and Oehninger S. (2002) Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. *Hum Reprod* 17, 3122-3128.
20. Erenpreiss J., Jepson K., Giwercman A., Tsarev I., Erenpreissa J. and Spano M. (2004) Toluidine blue cytometry test for sperm DNA conformation: comparison with the flow cytometric sperm chromatin structure and TUNEL assays. *Hum Reprod* 19, 2277-2282.
21. Erenpreiss J., Spano M, Erenpreissa J., Bungum M., and Giwercman A. (2006) Sperm chromatin structure and male fertility: biological and clinical aspects. *Asian J Androl* 8, 11-29. Review
22. Erenpreiss J. , Bungum M., Spano M, Elzanaty S., Orbidans J. and Giwercman A (2006) Intra-individual variation in Sperm Chromatin Structure Assay parameters in men from infertile couples: clinical implications. *Hum Reprod*, In Press
23. Evenson D.P., Jost L.K., Marshall D., Zinaman M.J., Clegg E., Purvis K., deAngelis P. and Claussen O.P. (1999) Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. *Hum Reprod* 14,1039-1049.
24. Evenson D.P., Darzynkiewicz Z. and Melamed M.R. (1980) Relation of mammalian sperm chromatin heterogeneity to fertility. *Science* 210, 1131–1133.
25. Gandini L., Lombardo F., Paoli D., Caruso F., Eleuteri P., Leter G., Ciriminna R., Culasso F., Dondero F., Lenzi A. and Spano M. (2004) Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. *Hum Reprod* 19:1409-1417.
26. Giwercman A., Spanò M., Lahdetie J., et al. (1999) Quality assurance of semen analysis in multicenter studies. *Asclepios Scand. J. Work Environ Health* 25,Suppl 1, 23-25.
27. Greco E., Romano S., Ferrero S., Baroni E., Minasi M.G., Ubaldi F., Rienzi L. and Tesarik J. (2005) ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment. *Hum Reprod* 20:2590-2594.
28. Guzick D.S., Overstreet J.W., Factor-Litvak P., Brazil C.K., Nakajima S.T., Coutifaris C., Carson S.A., Cisneros P., Steinkampf M.P., Hill J.A., Xu D. and Vogel D.L. (2001) Sperm morphology, motility and concentration in infertile and fertile men. *N Engl J Med* 345,1388-1393.
29. Griveau J.F. and Le Lannou D. (1997) Reactive oxygen species and human spermatozoa: physiology and pathology. *Int J Androl* 20, 61-69. Review.
30. Hammadeh M.E., Al-Hasani S., Gauss C., Rosenbaum P., Georg T., Diedrich K. and Schmidt W. (2001) Predictive value of chromatin decondensation in vitro on fertilization rate after intracytoplasmic sperm injection (ICSI). *Int J Androl* 24, 311-316.
31. Host E., Lindenberg S. and Smidt-Jensen S (2000) The role of DNA strand breaks in human spermatozoa used for IVF and ICSI, *Acta Obstet Gynecol Scand* 79, 559–563
32. Irvine D.S., Twigg J.P., Gordon E.L., Fulton N., Milne P.A. and Aitken R.J. (2001) DNA integrity in human spermatozoa: relationships with semen quality. *J Androl* 21, 33-44.
33. Jager S. (1990) Sperm nuclear stability and male infertility. *Arch Androl* 25, 253–259.
34. Larson K.L., De Jonge C.J., Barnes A.M., Jost L.K. and Evenson D.P. (2000) Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. *Hum Reprod* 15, 1717-1722.
35. Larson-Cook K.L., Brannian J.D., Hansen K.A., et al. (2003) Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. *Fertil Steril* 80,895-902.
36. Lewis S.E.M., Boyle P.M., McKinney K., Young I.S. and Thompson W. (1995) Total antioxidant capacity of seminal plasma is different in fertile and infertile men. *Fertil Steril* 64:868–870.

37. Lopes S., Sun J.G., Jurisicova A., Meriano J. and Casper RF. (1998) Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. *Fertil Steril* 69: 528-532
38. Morris I.D., Ilott S., Dixon L. and Brison D.R. (2002) The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (Comet assay) and its relationship to fertilization and embryo development. *Hum Reprod* 17, 990-998.
39. Omu A.E., al-Othman S., Mohamad A.S., al-Kaluwby N.M. and Fernandes S. (1998) Antibiotic therapy for seminal infection. Effect on antioxidant activity and T-helper cytokines. *J Reprod Med* 43, 857-864.
40. Pasqualotto F.F., Sharma R.K., Nelson D.R., Thomas A.J. and Agarwal A, (2000). Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril*.73, 459-464.
41. Poccia D. (1986) Remodeling of nucleoproteins during gametogenesis, fertilization, and early development. *Int Rev Cytol* 105, 1-65. Review.
42. Sailer B.L., Jost L.K. and Evenson D.P. (1995) Mammalian sperm DNA susceptibility to in situ denaturation associated with the presence of DNA strand breaks as measured by the terminal deoxynucleotidyl transferase assay. *J Androl* 16, 80-87.
43. Tejada, R.I., Mitchell J.C., Norman A., Marik J.J. and Friedman S. (1984) A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence, *Fertil Steril* 42, 87-91.
44. Sakkas D., Mariethoz E., Manicardi G., Bizzaro D., Bianchi P.G. and Bianchi U. (1999) Origin of DNA damage in ejaculated human spermatozoa. *Rev Reprod*, 4, 31-37.
45. Saleh R.A., Agarwal A., Nada E.A., El-Tonsy M.H., Sharma R.K. and Meyer A. (2003) Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility *Fertil Steril* 79,Suppl 3,1597-1605.
46. Seli E., Gardner D.K., Schoolcraft W.B., Moffatt O., Sakkas D. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. *Fertil Steril* 2004; 82: 378-383 .
47. Spanò M., Bonde J.P., Hjollund H.I. Kolstad H.A., Cordelli E . and Leter G. (2000) Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. *Fertil Steril* 73, 43-50.
48. Tomlinson M.J., Moffatt O., Manicardi G.C., Bizzaro D., Afnan M. and Sakkas D. (2001) Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. *Hum Reprod* 16, 2160-2165.
49. Twigg J.P., Irvine D.S. and Aitken R.J. (1998) Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. *Hum Reprod* 13, 1864-1871.
50. Virro M.R., Larson-Cook K.L. and Evenson D.P. (2004) Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. *Fertil Steril* 81, 1289-1295.
51. Ward W.S. and Coffey D.S. (1991) DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. *Biol Reprod* 44, 569-574
52. World Health Organization (1999) WHO Laboratory Manual for the Examination of Human Sperm and Sperm-Cervical Mucus Interaction Cambridge University Press, Cambridge.

Cancer and male reproductive function

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Introduction

Cancer in children and adults can be cured by surgery, chemo-, or radiotherapy in a high percentage of cases, improving significantly survival rates and increasing disease-free intervals. Therefore, long-term effects of tumor therapies must be taken under consideration when treating these patients. Oncological treatments cause gonadal dysfunction, including infertility, hypogonadism and sexual problems. Their negative effects depend on the therapeutic modalities, the substances and the doses and can be irreversible. Thus, emerges the importance of offering the opportunity to preserve progeny in oncological patients by cryopreservation of their sperm and also to prevent or treat promptly other andrological problems that they may develop.

Cancer, treatments and andrological problems

Fertility and sperm banking

The commonest malignancies within the reproductive age range are testicular cancer, Hodgkin's lymphoma and leukemia. Modern cancer therapies can achieve high cure rates. However, these treatments are aggressive and can impair gonadal function, especially at a time when family planning has not started or is not yet finished. Other side effects that may have a high impact on quality of life of these patients are androgen deficiency and sexual problems. Chemotherapy and radiotherapy adversely affect spermatogenesis. The extent of impairment cannot be predicted and testicular function recovery depends on therapeutic intervention itself, the doses administered, and the individual susceptibility of the patient. Indeed in some cases these patients following treating may become azoospermic. From the other hand, retroperitoneal lymphadenectomy accompanying orchidectomy in particular cases of testicular tumors can cause retrograde ejaculation.

Cryopreservation of semen represents a preventive therapeutic option in patients with malignancies in order to preserve their fertilizing capability. In fact, in case of non recovery of their reproductive function they can attempt fathering their own genetic children thanks to their frozen sperm samples and assisted reproduction technology (ART). Several years ago, given the poor recovery of cryopreserved gametes and the possibilities that intrauterine insemination (IUI) offered, perceiving paternity remained difficult. Today with the in vitro fertilization (IVF) and the intracytoplasmic sperm injection (ICSI), even the poorest samples in terms of concentration and motility can be used with acceptable rates of success. Patients should thus be offered the opportunity for sperm banking before treatment if their clinical condition permits.

In fact, various authors have reported a decline in semen quality before therapy for testicular cancer and Hodgkin's disease. In testicular cancer, it has been suggested that this impairment is related to the direct effect of tumour and the production of β hCG by some cancer histotypes, and in Hodgkin's disease to the presence of constitutional symptoms accompanying the disease (fever, weight loss, etc.) (Morrish et al., 1990; Viviani et al., 1991; Fossa et al., 2000; Botchan et al., 1997

a,b; Petersen et al., 1998; Tal et al., 2000; Rueffer et al., 2001). It is also necessary to take into account the general stress associated with tumoral illness (Meirow and Schenker, 1995).

We performed a study with the aim to examine the quality of semen in patients affected by testicular cancer (after orchidectomy) or Hodgkin's disease, in both cases before treatment (chemo- or radiotherapy). We evaluated 342 patients affected by TC (n=232) or HD (n=110) who cryobanked sperm before initiating chemo or radiotherapy. All TC patients were evaluated 1 month after orchidectomy. Semen samples were collected by masturbation into sterile plastic jars, after 2-7 days of abstinence, were allowed to liquefy for 60 minutes at 37°C and were then evaluated according to the World Health organization (WHO, 1992, 1999). Variables taken into consideration were: ejaculate volume (ml), sperm concentration ($n \times 10^6$), total sperm count ($n \times 10^6$), forward motility (%) and morphology (% of atypical forms).

The two groups of patients (TC/HD) were further subdivided by total sperm count, as an index of sperm testicular production, into two subgroups: A: $< 40 \times 10^6$ / ejaculate; B: $\geq 40 \times 10^6$ for evaluate and compare the quality of spermatogenesis.

In our TC patient group, after orchidectomy and before therapy, even after exclusion of the 10 patient showing azoospermia or cryptozoospermia, the mean of the semen parameters remained in the normal range and there was no deterioration of spermatogenesis as a consequence of disease progression.

In the HD patients group, after exclusion of the four patients with azoospermia or cryptozoospermia, we found that on average they showed both quantitatively and qualitatively normal spermatogenesis, with only 19.8% having a total sperm count $< 40 \times 10^6$ / ejaculate. As regards TC, patients with $< 40 \times 10^6$ / ejaculate represented the 35.5%. This may be explained by the fact that these patients, all young, were studied for semen quality very quickly after diagnosis and before the start of chemotherapy. In conclusion the semen quality observed in our TC and HD patient groups seems better than results reported in current literature (Gandini et al.,2003).

Low testosterone in adulthood

Treatment with chemotherapy and radiotherapy if particularly aggressive can result in raised LH and low testosterone levels. Thus, hypogonadism can occur requiring testosterone supplementation. Adult patients may complain of decreased libido, erectile dysfunction, and low energy. These patients tend to be less interested in sex and have less sexual activity.

In addition to effects on sexuality, men with low testosterone have evidence of more general impairment in quality of life concerning the physical, role, cognitive and social functioning. Low testosterone is also associated with osteoporosis, a marked increase in dyspnoea and lesser increases in complaint of pain, sleep disturbance, constipation and nausea. Moreover, patients with deficiency of this hormone have higher body mass index (BMI) and higher systolic and diastolic blood pressure, resulting in a higher cardiac risk.

Patients with mild androgen deficiency or androgen deficiency of recent onset may not note a decrease in facial or body hair growth, since relatively low levels of androgens are required to maintain sexual hair growth. With low-standing hypogonadism, the growth of facial hair will diminish, and the frequency of shaving may also decrease. Moreover, fine wrinkles may appear in the corners of the mouth and eyes.

Pubertal boys

A group which requires special attention are pubertal boys. Spermatogenesis is already occurring to some extent in the testes of boys at very early stages of pubertal development. For this reason, as previously said, cryopreservation of spermatozoa is feasible and recommended before the juvenile patients receive chemotherapy or radiotherapy. This preventive option has great importance since these boys may preserve their future fertility possibilities.

Ethical aspects are extremely important when gametes of young boys are being frozen. Cryopreservation of spermatozoa is applied in clinical practice, but there is no guarantee of restoring fertility, and that has to be explained to the family and to the adolescent patients. A young child cannot give his consent for the procedure and the procedure has to be carried out after parental consent. These boys also understand the importance of this preventive action and give their consent. Fertility is not the only crucial issue concerning young patients. Antitumoral therapies can within this age range too affect androgen production. Testosterone deficiency developing during puberty leads to poor secondary sexual development and eunuchoid skeletal proportions. The penis fails to enlarge, the testes remain small, and the scrotum does not develop the marked rugae characteristic of this period. The voice remains high-pitched and the muscle mass does not develop fully, resulting in less than normal strength and endurance. The lack of appropriate stimulation of sexual hair growth results in sparse axillary and pubic hair and absent or rare facial, chest, upper abdominal, and back hair. Although the androgen-mediated pubertal growth spurt will fail to take place, the epiphysial plates of the long bones will continue to grow under the influence of insulin-like growth factor-I and other growth factors. Thus, the long bones of the upper and lower extremities will grow out of proportion to the axial skeleton leading to eunuchoid habitus.

Genetic risks of using cryopreserved spermatozoa

There is much concern about whether an increased genetic risk exists for the offspring of patients suffering from malignancies and treated by chemo- or radiotherapy. First of all, there is the risk arising from the malignant disease itself and the following possibly mutagenic chemo- and radiotherapy. In addition, there is a potential risk of genetically determined disease in the offspring when using assisted fertilization techniques with cryopreserved spermatozoa.

As regards chemo- or radiotherapy there is evidence that these treatments are able to cause structural chromosomal anomalies in spermatozoa of oncological patients. In consequence their born children might have increased rate of anomalies. In order to prevent this event, the physician should suggest patients not to have children before a period of at least 6 months up to 2 years after the chemo- or radiotherapy has ended. From the other hand, there seems to be no increased genetic risk or increased risk for malformation that could result from the underlying oncological disease or the applied assisted fertilization technique with cryopreserved spermatozoa.

Conclusions

The good results obtained in the management of various tumors, such as testicular cancer, give doctors the aim not only to cure the oncological disease but also to consider carefully the long-term health of cured patients and tailor treatments to the level of risk posed by the individual tumor presentation in order to minimize gonadal damage. First of all, physicians and oncology specialists must recommend patients to cryopreserve their sperm before starting toxic therapies that can impair gonadal function. Counseling of patients in respect to fertility issues takes place at a point of time when the physical and psychological integrity of the patient is disturbed. Moreover, there is need for discussion of ethical questions arising from the fact that sperm banking and thus preservation of progenitive ability are set up for patients that may die. Especially in juvenile patients this becomes even harder, but these boys fully cooperate. In case of death cryodepots are destroyed as is contractually defined, but the use of semen samples of incurably ill patients remains a hard issue to face for the physician, the patient and the patient's partner. Notwithstanding, cryopreservation may contribute to the patient's personal stability in an acute and oppressive situation.

Another aspect involves the possibility that patients treated for tumors develop hormonal dysfunction and subsequent andrological problems. This can have a significant impact on the quality of life and contribute to other health problems. For this reason screening for hormonal

deficiencies should be considered as a routine part of patient management and in case of problems it is andrologists' task to assist patients.

References

- Agarwal A, Tolentino MV Jr, Sidhu RS, Ayzman I, Lee JC, Thomas AJ Jr, Shekarriz M. Effect of cryopreservation on semen quality in patients with testicular cancer. *Urology*. 1995 Sep;46(3):382-9.
- Anger JT, Gilbert BR, Goldstein M. Cryopreservation of sperm: indications, methods and results. *J Urol*. 2003 Oct;170:1079-84.
- Bahadur G. Fertility issues for cancer patients. *Mol Cell Endocrinol*. 2000 Nov 27;169(1-2):117-22. Review.
- Bahadur G., Ozturk O., Muneer A., Wafa R., Ashraf A., Jaman N., Patel S., Oyede A.W., Ralph D.J. Semen quality before and after gonadotoxic treatment. *Human Reproduction* 2005 20(3):774-781
- Botchan A, Hauser R, Yogev L, Gamzu R, Paz G, Lessing JB, Yavetz H. Testicular cancer and spermatogenesis. *Hum Reprod*. 1997 Apr;12(4):755-8.
- Dearnaley D, Huddart R, Horwich A. Regular review: Managing testicular cancer. *BMJ*. 2001 Jun 30;322(7302):1583-8. Review
- Dondero F, Lombardo F, Gandini L, Salacone P, Lenzi A.: «The effect of cryopreservation on sperm motility, sperm morphology and sperm function tests in fertile donors and neoplastic patients.» 4th Interational Conference European association of Tissue Banks. Monduzzi Ed, 1995, European Association of tissue Bank - Special Standards for Semen Criobanking - Aprile 1997
- Fossa SD, Abyholm T, Norman N, Jetne V. Post-treatment fertility in patients with testicular cancer. III. Influence of radiotherapy in seminoma patients. *Br J Urol*. 1986 Jun; 58(3): 315-9
- Fossa SD, Chen J, Schonfeld SJ, McGlynn KA, McMaster KA, Gail MH, Travis LB Risk of contralateral testicular cancer: a population-based study of 29515 U.S. men *J Natl Cancer Inst*, 2005;97:1056-66.
- Fossa SD, Ous S, Abyholm T, Norman N, Loeb M. Post-treatment fertility in patients with testicular cancer. II. Influence of cisplatin-based combination chemotherapy and of retroperitoneal surgery on hormone and sperm cell production. *Br J Urol*. 1985 Apr; 57(2): 210-4
- Fritz K, Weissbach L. Sperm parameters and ejaculation before and after operative treatment of patients with germ-cell testicular cancer.
- Gandini L, Lombardo F, Salacone P, Paoli D, Anselmo AP, Culasso F, Dondero F, Lenzi A.: Testicular cancer and Hodgkin's disease: evaluation of semen quality. *Hum Reprod*. 2003 Apr;18(4):796-801.
- Hansen PV, Trykker H, Svennekjaer IL, Hvolby J. Long-term recovery of spermatogenesis after radiotherapy in patients with testicular cancer. *Radioter Oncol*. 1990 Jun; 18(2):117-25
- Hendry WF, Stedronska J, Jones CR, Blackmore CA, Barrett A, Peckham MJ. Semen analysis in testicular cancer and Hodgkin's disease: pre- and post-treatment findings and implications for cryopreservation.
- Horwich A, Shipley J, Huddart R Testicular germ-cell cancer *Lancet* 2006;367:754-65.
- Howell SJ, Shalet SM.: Testicular function following chemotherapy. *Hum Reprod Update* 2001 Jul;7(4):363-369
- Huddart RA, Norman A, Moynihan C, Horwich A, Parker C, Nicholls E, Dearnaley DP Fertility, gonadal and sexual function in survivors of testicular cancer *British Journal of Cancer* 2005;(93):200-207.
- Laguna MP, Pizzocaro G, Klepp O, Algaba F, Kisbenedek L, Leiva O. EAU guidelines on testicular cancer. *Eur Urol* 2001 Aug;40(2):102-110.
- Lampe, H., Horwich, A., Norman, A. et al. (1997) Fertility after chemotherapy for testicular germ cell cancer. *J. Clin. Oncol*. 15:39-245.

Meirow D, Schenker JG. Cancer and male infertility. *Hum Reprod.* 1995 Aug;10(8):2017-22. Review.

Meseguer M, Molina N, García-Velasco J, Remohí J, Pellicer A, Garrido N. Sperm cryopreservation in oncological patients: a 14-year follow-up study. *Fertil. Steril.* 2006 Mar;85(3):641-5.

Morrish DW, Venner PM, Siy O, Barron G, Bhardwaj D, Outhet D. Mechanisms of endocrine dysfunction in patients with testicular cancer. *J Natl Cancer Inst.* 1990 Mar 7;82(5):412-8.

Panidis D, Rousso D, Stergiopoulos K, Papathanasiou K, Delkos D, Papaletsos M. The effect of testicular seminoma in semen quality. *Eur J Obstet Gynecol Reprod Biol.* 1999 Apr;83(2):219-22.

Park YS, Lee SH, Song SJ, Jun JH, Koong MK, Seo JT: Influence of motility on the outcome of in vitro fertilization/intracytoplasmic sperm injection with fresh vs. frozen testicular sperm from men with obstructive azoospermia. *Fertil Steril.* 2003 Sep;80(3):526-30.

Petersen PM, Giwercman A, Skakkebaek NE, Rorth M. Gonadal function in men with testicular cancer. *Semin Oncol.* 1998 Apr;25(2):224-33. Review.

Petersen PM, Skakkebaek NE, Giwercman A. Gonadal function in men with testicular cancer: biological and clinical aspects. *APMIS.* 1998 Jan;106(1):24-34; discussion 34-6. Review.

Schirren C., Laudahn G., Heinze I., Hartman E., Ritcher E., Correlation studies of morphological and biochemical parameters in human ejaculate in various andrological diagnoses. *Androl.* 1979;393.

Schrader M, Muller M, Straub B, Miller K. The impact of chemotherapy on male fertility: a survey of the biologic basis and clinical aspects. *Reprod Toxicol.* 2001 Nov-Dec;15(6):611-7. Review.

Sherman J.F.: Cryopreservation of Human Semen. In "Handbook of the Laboratory Diagnosis and Treatment of Infertility. B.A. Keel, B.W. Webster. CRC Press, Boca Raton, Ann Arbor, Boston, 1990.

Skakkebaek NE, Rajpert-De Meyts E, Jorgensen N, Carlsen E, Petersen PM, Giwercman A, Andersen AG, Jensen TK, Andersson AM, Muller J. Germ cell cancer and disorders of spermatogenesis: an environmental connection *APMIS.* 1998 Jan;106(1):3-11; discussion 12. Review.

Spanò M, Cordelli E, Leter G, Lombardo F, Lenzi A, Gandini L.: Nuclear chromatin variations in human spermatozoa undergoing swim-up and cryopreservation evaluated by the flow cytometric sperm chromatin structure assay. *Mol Hum Reprod.* 1999 Jan;5(1):29-37.

Tal R, Botchan A, Hauser R, Yogev L, Paz G, Yavetz H. Follow-up of sperm concentration and motility in patients with lymphoma. *Hum Reprod.* 2000 Sep;15(9):1985-8.

Erectile and sexual dysfunction: what should the ART specialist know?

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Learning objectives

The aims of this lecture are:

- 1) to discuss sexual dysfunction due to infertility and its treatment, by presenting data on the use of type-V phosphodiesterase inhibitors in male infertile patients undergoing infertile couple management procedures,
- 2) to discuss the relationship between sexual dysfunction and infertility,
- 3) to discuss the treatment of stress-induced infertility,
- 4) to discuss the relationship between stress and fertility.
- 5) to give ART specialists the necessary 'know how' regarding erectile and male sexual dysfunction.

Lecture summary

Involuntary childlessness is considered to be a chronic stressor for couples suffering from infertility. Stress itself may interfere with spermatogenesis and fertility rate. The long period of diagnostic and treatment procedures may also have a negative impact on the sex life of the infertile couple. These associations will be brought into the light in order to give ART specialists the necessary 'know how' regarding erectile and male sexual dysfunction.

With the assumption that infertility itself, semen collection (for analysis, IUI or IVF programs) and targeted intercourse for the post-coital test are all stressful ^{1 2}, we designed a pilot, uncontrolled study to evaluate differences in patient compliance during seminal harvesting by masturbation or sexual intercourse with the administration or non- of Sildenafil citrate.

1. Sexual dysfunction due to infertility and its treatment, by presenting data on the use of type-V phosphodiesterase inhibitors in male infertile patients undergoing infertile couple management procedure

In an open-label experimental design, we studied men, without sexual dysfunction, who were undergoing semen collection for intrauterine artificial insemination (IUI), and couples, undergoing planned intercourse for a post coital test (PCT) to study sperm cervical mucus interaction in cases of couple infertility. Semen parameters were: normozoospermia, associated oligo-asthenozoospermia (sperm concentration: $<20 \times 10^6/\text{mL}$; motility: $<50\%$) or oligo-astheno-teratozoospermia (OAT, atypical forms $>70\%$). All patients underwent the same procedure (semen collection for IUI or programmed intercourse before PCT) with and without the administration of Sildenafil (50 mg *p.o.*, 1 hour before the procedure repetition) with an interim period of 2 weeks to one month between the two analyses. Each patient thus acted as his own control. No therapies were used in either partner in the interim period. Base sexual performance was evaluated by IIEF multidimensional scale ³ and results were evaluated by both patient log (in which were recorded the presence of sexual stimulation, erection quality, success of sexual intercourse, and a global-efficacy question: “did the treatment improve your sexual capacity during semen collection or sexual intercourse?”), and a modified IIEF questionnaire exploring sexual response and the patient’s agreement to the therapy (ACUTE IIEF-15) ¹. The questions were referred to sexual intercourse during PCT or to masturbation during IUI. All questions were preceded by the phrase: “*during infertility treatment...*”, followed by questions similar to those of IIEF

2. Sexual dysfunction and infertility

In our infertility clinic, which analyses semen from 4-5,000 patients a year, we observe subjects who experience great difficulty or complete inability to ejaculate when a semen sample is required. All these patients declare themselves as without erectile problems in normal conditions. Furthermore, erectile disturbances are frequently described both in programmed intercourse during hormone-induced ovulation and when semen collection is required for use in an assisted reproduction program. Physician, patient, and partner all agree that this is due to the stress of the unnatural request for erection on demand. This kind of erectile dysfunction can significantly interfere with the success of such techniques.

In our group of sexually potent subjects, sexual dysfunctions were frequently present during diagnostic procedures in the absence of Sildenafil, as demonstrated by the ACUTE IIEF-15 and patient log. Total erectile dysfunction was found in 7 subjects during masturbation for semen collection before IUI and in 5 during coitus for PCT. Partial impotence, with ejaculation during tumescence or incomplete erection, was reported by 11 IUI patients during masturbation and 3 PCT patients during sexual intercourse. It should be noted that the higher prevalence of transitory erectile dysfunction with respect to common clinical experience is probably due to the particular attention paid to sexual symptoms during this study.

After Sildenafil treatment, no important adverse events were reported. All patients answered the global-efficacy question positively and reported improved erectile capacity (mean without Sildenafil: 1.9 ± 0.9 vs. with Sildenafil: 2.5 ± 1.1 , $p < 0.05$). No changes were observed in responses to questions on petting (2.2 ± 0.0 vs. 2.2 ± 0.1), ejaculation difficulties (1.9 ± 0.2 vs. 2.1 ± 0.4 , $p = 0.1$), or orgasm (2.0 ± 0.0 vs.

2.0±0.0). Significant improvements were found after Sildenafil therapy in responses to questions on the ability to obtain a full erection (mean: 1.8±0.4 vs. 2.1±0.6, $p<0.05$), confidence in obtaining and maintaining an erection (1.8±0.4 vs. 2.4±0.4, $p<0.001$), and, interestingly, sexual desire (mean: 1.9±0.4 vs. 2.4±0.6, $p<0.05$). The increase in sexual desire may possibly be due to the temporary increase in testosterone levels after sexual activity^{4, 5, 6}, although such an increase has previously been found in chronic but not acute treatments. The possibility of inducing a psychological addiction was ruled out at the 6-month follow-up, when none of the patients reported chronic or sporadic Sildenafil re-use.

Infertility may be due to sexual problems of the female (loss of libido, vaginismus, with or without heavy dyspareunia) or male (erectile dysfunction, loss of libido and ejaculation disorders). These symptoms may account for more than 5% of all infertility⁷. Conversely, infertility itself can induce sexual problems. The psychological response of men to perceived infertility can be substantial (Table 1), and may result in sexual dysfunction⁸. In fact, there is a strong link between a man's perceptions of his own masculinity and virility and his ability to impregnate women. The stress induced by the intrusive nature of fertility diagnosis and therapy has been shown to result in a high frequency of several sexual dysfunctions: loss of libido, premature or retarded ejaculation, erectile dysfunction and reduced sexual activity⁹. A third of couples undergoing infertility diagnosis and therapy report that their sexual relationship was negatively affected by their treatment¹⁰. In particular, male fears of lost potency due to infertility may be exacerbated by and during scheduled sexual intercourse¹¹: in 10% of patients libido is diminished by such regimentation¹².

3. Treatment of stress-induced infertility

Stress can be considered as both an event (a distressing external circumstance) and a response (the disturbance of internal homeostasis). A reduction in fertility is a common strategic response to negative environmental stressors in mammals and lower animals¹³. The negative relationship between various stress conditions and female fertility has been broadly described. In particular, psychological stress (high levels of depression or anxiety) is related to a lower pregnancy rate and a lower *in vitro* fertilization (IVF) success rate¹⁴. In fact, differences in stress conditions may significantly influence the outcome of IVF and intracytoplasmic sperm injection (ICSI) procedures. In a recent retrospective study on 207 subjects, women who became pregnant during IVF/ICSI showed lower levels of depression than those who did not¹⁵. This is a vicious circle: stressed couples are less fertile, while infertile couples give higher scores on depression and anxiety scales^{16 17}.

The rheological seminal parameters of the males undergoing semen collection for IUI showed no changes between samples taken before and after Sildenafil administration. An increase was observed in sperm concentration ($p=0.1$) and total number of spermatozoa ejaculated ($p=0.1$); due to the low number of observations, these increases were not statistically significant. A similar, not statistically significant decrease in non-linear progressive motile sperm ($p=0.4$) was observed, while a significant increase was observed in linear progressive motility ($p=0.005$). Atypical forms and white cell numbers were unchanged. The effect of Sildenafil treatment in the 12 couples undergoing PCT was evident and also statistically significant. In fact, Sildenafil administration before the second PCT attempt significantly increased the number of spermatozoa observed by microscope field (250x) in the cervical mucus ($p<0.05$) as well as the total number of spermatozoa with linear progressive motility ($p<0.05$). It is worth noting that two pregnancies were obtained in this group after Sildenafil administration.

Our findings cannot be explained by a direct effect of Sildenafil on seminal production: a recent study vs. placebo demonstrated that the drug (100 mg) did not have *in vivo* effects on sperm physiology or seminal parameters in normal males¹⁸. Furthermore, another type V phosphodiesterase inhibitor,

Tadalafil, administered chronically to 421 men with or without impotence at doses of 10 and 20 mg for 6 months, had no adverse effects on spermatogenesis or reproductive hormones¹⁹. As we did not measure testosterone levels before and after Sildenafil, we cannot correlate these results to androgens, but in any case there is unlikely to be a correlation.

Our hypothesis is that Sildenafil is able to reduce stress levels, thus inducing a more complete ejaculation and a subsequent increased number of good quality spermatozoa in the semen. The importance of the effect of stress was also demonstrated when comparing masturbation in the presence or absence of visual sexual stimulation, the presence of which resulted in recovery of spermatozoa of greater fertilizing potential in both normo- and cryptozoospermic men²⁰. In a study of 500 males involved in an IVF program, 8% showed a decrease in sperm count, motility, and fertility indices between the pretreatment sample and the sample subsequently obtained for fertilization²¹. The use of Sildenafil was proposed in cases of extremely difficult semen collection in IVF programs²².

4. Stress and fertility

There have been many psychological and psychoanalytical theories attempting to explain stress-induced male infertility, which may cause a man to become defensive against the feeling of dependency that accompanies attempts at procreation². However, the effect of stress on human semen quality is less clear than with female fertility. It may act directly on the seminiferous epithelium²³ or indirectly by depressing testosterone production and/or increasing prolactin levels²⁴. Other Authors have found that stressful conditions (workplace, family, and others) may adversely affect semen quality^{25 26 27}, leading in some cases to transitory azoospermia or oligozoospermia. Semen specimens from men experiencing chronic or acute stress may show defects in sperm morphology and vitality and a greater frequency of morphological defects when compared with non-stressed men. However, other Authors have reported that stress has no effect on male reproductive functions²⁸. These discrepancies may be explained by differences in evaluation of seminal parameters and particularly by the definition and experimental standardization of stressful conditions. This has been demonstrated by Fenster *et al.*, who found that stress at work and other life events was not related to differences in semen quality, while the recent death of a close family member was associated with a reduction in straight-line velocity and percentage of linear progressive motile sperm and, marginally, with a morphometric change in the sperm head²⁹. These findings suggest that the fecundity of men experiencing a major stressful event might be temporarily diminished.

A particular form of stress is that of infertile couples and subjects undergoing assisted reproductive technologies. In these conditions, a detrimental effect of stress on sperm count, motility, and morphology has been observed by Harrison *et al.*²¹ and denied by others³⁰. We previously demonstrated a relationship between alexithymia, extroversion, neuroticism, psychoticism, coping style towards stressors, and seminal parameters in 132 males on their first seminal fluid examination³¹. Difficulties in feeling or expressing emotions may exert a negative effect on seminal parameters.

5. The ART specialists and the necessary ‘know how’ regarding erectile and male sexual dysfunction

Infertility has a profound impact on both marital and individual psychological functioning³². The heterogeneity of psychological responses characterizes infertile couples³³. Women and men may respond differently to the stress of infertility and some gender-dependent differences have been demonstrated. For women, pregnancy and motherhood are developmental milestones, highly emphasized in almost all cultures. For this reason female infertility is frequently a heavy stressor. In the

Stressful Life Events Scale, from a list of 87 items, infertility is ranked as one of the most negative stressful situations – akin to the death of one’s child or spouse³⁴, normally thought to be the most stressful life experience³⁵. As men become more involved in infertility diagnosis and treatment, they too experience the psychological stress associated with infertility, even though they may suppress feelings concerning infertility better than women³⁶, demonstrating a lower response in various indices of emotional disturbance³⁷. However, men experience greater distress when the cause of fertility failure is a male factor³⁸. In fact, a diagnosis of male infertility may be more stressful for both partners than a diagnosis of infertility related to the female partner³⁹. Infertile men have lower self-esteem, higher anxiety and show more somatic symptoms than fertile subjects^{40 41}

Although infertility can be a stressor *per se*, its treatment can also be distressing. Wright and colleagues conclude in their literature review that infertile patients are on average more psychosocially distressed during their evaluation or treatment for infertility than control subjects⁴². The ESHRE monograph on Guidelines for counseling in infertility recently suggested that great attention should be paid to the psychological aspects of infertile couples, and especially of infertile men⁴³.

The impact of the diagnosis and subsequent treatment of infertility, in addition to the social pressure to become parents experienced by many infertile couples may have a marked effect upon their psychological functioning. This can lead to sexual disturbances, which may be cured with a short-acting, type V phosphodiesterase inhibitor such as Sildenafil.

Finally, on the ground of the above-mentioned considerations the ART specialist must be able to handle the possible male sexual dysfunctions that can be cause or effects of infertility and its treatment. At least the IIEF inventory should be always considered in the diagnosis as well as in the follow-up of the infertile patient⁴⁴.

¹ Jannini E.A., Lombardo F., Salacone P., Gandini L., Lenzi A. (2003) Treatment of sexual dysfunctions secondary to male infertility with sildenafil citrate. *Fertil. Steril.*, 81, 705-707.

² Lenzi A., Lombardo F., Salacone P., Gandini L., Jannini E.A. (2003) Stress, sexual dysfunctions, and male infertility. *J. Endocrinol. Invest.*, 26 (suppl. n. 3), 72-76.

³ Rosen R.C., Riley A., Wagner G., Osterloh I.H., Kirkpatrick J., Mishra A. (1997) The international index for erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. *Urology* 49, 822-830.

⁴ Fabbri A., Jannini E.A., Gnessi L., Moretti C., Ulisse S., Bonifacio V., Frajese G., Isidori A. (1988) Evidence for low bioactive LH in male impotence: possible relationship with altered GnRH pulsatility. *J. Clin. Endocrinol. Metab.*, 67, 867-875.

⁵ Jannini E.A., Screponi E., Carosa E., Pepe M., Lo Giudice F., Trimarchi F., Benvenga S. (1999) Lack of sexual activity from erectile dysfunction is associated with a reversible reduction in serum testosterone. *Int. J. Androl.*, 22, 385-392.

⁶ Carosa E., Benvenga S., Trimarchi F., Lenzi A., Pepe M., Simonelli C., Jannini E.A. (2002) Lack of sexual activity for erectile dysfunction causes a reversible reduction of LH bioavailability. *Int. J. Impotence Res.*, 14, 93-99.

-
- ⁷ Rantala M.L., Koskimies A.I. (1998) Sexual behavior of infertile couples. *Int. J. Fertil.*, 33, 6-30.
- ⁸ Irvine S.C.E. (1996) Male infertility and its effect on male sexuality. *Sexual Marital. Ther.*, 11, 73-280.
- ⁹ Benazon N., Wright J., Sabourin S. (1992) Stress, sexual satisfaction, and marital adjustment in infertile couples. *J. Sex Marital. Ther.*, 18, 273-284.
- ¹⁰ Collins A., Freeman E.W., Boxer A.S., Tureck R. (1992) Perception of infertility and treatment stress in females as compared with males entering in vitro fertilization treatment. *Fertil. Steril.*, 57, 350-356.
- ¹¹ Nachtigall R.D., Becker G., Wozny M. The effects of gender-specific diagnosis on men's and women's response to infertility. *Fertil. Steril.* 57: 113-121, 1992.
- ¹² Sigg C. (1994) Sexuality and sterility. *Ther Umsch.* 51, 115-119.
- ¹³ Levy A., Grauer E., Ben-Nathan D., de Kloet R.E. (Eds.) (1998) *New frontiers in stress research.* Harwood Acad. Pub., Amsterdam.
- ¹⁴ Thiering P., Beaurepaire J., Jones M., Saunders D., Tennant C. (1993) Mood as predictor of treatment outcome after in vitro fertilization/embryo transfer technology (IVF/ET). *J. Psychosom. Res.*, 37, 481-491.
- ¹⁵ Verjaak C.M., Smeenk J.M.J., Eugster A., van Minnen A., Kremer J.A.M. (2001) Stress and marital satisfaction among women before and after their first cycle of in vitro fertilization and intracytoplasmic sperm injection. *Fertil. Steril.*, 76, 525-531.
- ¹⁶ Newton C.R., Sherrard W., Glavac I. (1999) The Fertility Problem Inventory: measuring perceived infertility-related stress. *Fertil. Steril.*, 72: 54-62.
- ¹⁷ Wischmann T., Stammer H., Scherg H., Gerhard I., Verres R. (2001) Psychosocial characteristics of infertile couples: a study by the 'Heidelberg Fertility Consultation Service'. *Human Reprod.* 16, 1753-1761.
- ¹⁸ Aversa A., Mazzilli F., Rossi T., Delfino M., Isidori A.M., Fabbri A. (2000) Effects of Sildenafil (Viagra) administration on seminal parameters and post-ejaculatory refractory time in normal males. *Hum. Reprod.* 15, 131-135.
- ¹⁹ Hellstrom W. J. G., Overstreet J.W., Yu A., Saikali K., Shen W., Beasley C. M., Watkins V. S. (2003): Tadalafil has no detrimental effect on human spermatogenesis or reproductive hormones. *J. Urol.*, 170, 887-891.
- ²⁰ Yamamoto Y., Sofikitis N., Mio Y., Miyagawa I. (2000) Influence of sexual stimulation on sperm parameters in semen samples collected via masturbation from normozoospermic men participating in an assisted reproduction program. *Andrologia*, 32, 131-138.

-
- ²¹ Harrison K.L., Callan V.J., Hennessey J.F. (1987) Stress and semen quality in an in vitro fertilization program. *Fertil. Steril.*, 48, 633-636.
- ²² Tur-Kapsa I., Segal S., Moffa F., Massobrio M., Meltzer S. (1999) Viagra for temporary erectile dysfunction during treatments with assisted reproductive technologies. *Hum. Reprod.*, 14, 1783-1784.
- ²³ MacLeod J. (1978) The significance of deviations in human sperm morphology. *Adv. Exp. Biol. Med.*, 10, 481-494.
- ²⁴ Kreuz L.E., Rose R.M., Jennings R. (1972) Suppression of plasma testosterone levels and psychological stress. *Arch Gen. Psychiat.*, 26, 479-482.
- ²⁵ Hubert W., Hellhammer D.H., Freishem C.W. (1985) Psychobiological profiles in infertile men. *J. Psychosom. Res.* 29: 161-165.
- ²⁶ Giblin P.T., Polant M.L., Moghissi K.S., Ager J.W., Olsen J.M. (1988) Effects of stress and characteristic adaptability on semen quality in healthy men. *Fertil. Steril.*, 49, 127-132.
- ²⁷ Gerhard I., Lenhard K., Eggert-Kruse W., Runnebaum B. (1992) Clinical data which influence semen variables in infertile men. *Hum. Reprod.*, 7 830-837.
- ²⁸ Poland M.L., Giblin P.T., Ager J.W., Moghissi K.S. (1986) Effects of stress on semen quality in semen donors. *Int. J. Fertil.*, 3, 229-231.
- ²⁹ Fenster L., Katz D.F., Wyrobek A.J. (1997) Effects of psychological stress on human semen quality. *J. Androl*, 18, 194-202.
- ³⁰ Pellicer A., Ruiz M. (1989) Fertilization in vitro of human oocytes by spermatozoa collected in different stressful situations. *Human Reprod.*, 4, 817-820.
- ³¹ Morelli G., De Gennaro L., Ferrara M., Lenzi A. (2000) Psychosocial factors and male seminal parameters. *Biol. Psychol.*, 53, 1-11.
- ³² Lombardo F, Morelli G, De Gennaro L. (1999) Male infertility: psychological and social factors. In: Facchinetti F., Nijs P., Richter (*Eds.*), *European Psychosomatic Obstetrics and Gynaecology*. Editeam, Bologna, pp. 57-60.
- ³³ Freeman E.W., Boxer A.S., Rickels K., Tureck R., Mastroianni L. (1985) Psychological evaluation and support in a program of in vitro fertilization and embryo transfer. *Fertil. Steril.*, 43, 48-53.
- ³⁴ Dohrenwend B.S., Dohrenwend B.P. (1981) *Stressful life events: their nature and effect*. Wiley, New York.
- ³⁵ Freeman E.W., Boxer A.S., Rickels K., Tureck R., Mastroianni L. (1985) Psychological evaluation and support in a program of in vitro fertilization and embryo transfer. *Fertil. Steril.*, 43, 48-53.

-
- ³⁶ Dhillon R., Cumming C.E., Cumming D.C. (2000) Psychological well-being and coping patterns in infertile men. *Fertil. Steril.*, 74, 702-706.
- ³⁷ Berg B.J., Wilson J.F., Weingartner P.J. (1991) Psychological sequelae of infertility treatment: the role of gender and sex-role identification. *Soc. Sci. Med.*, 33, 1071-1080.
- ³⁸ Slade P., Raval H., Buck P., Lieberman B.E. (1992) A 3-year follow-up of emotional, marital, and sexual functioning in couples who were infertile. *J. Reprod. Inf. Psychol.*, 10, 233-243.
- ³⁹ Mikulincer M., Horesh N., Levy-Shiff R., Manovich R., Shalev J. (1998) The contribution of adult attachment style to the adjustment to infertility. *Br. J. Med. Psychol.*, 71, 265-280.
- ⁴⁰ Kedem P., Mikulincer M., Nathanson Y.E. (1990) Psychological aspects of male infertility. *Br. J. Med. Psychol.*, 63, 73-80.
- ⁴² Wright J., Allard M., Lecours S., Sabourin S. (1989) Psychosocial distress and infertility: a review of controlled research. *Int. J. Fertil.*, 34, 126-142.
- ⁴³ Boivin J., Kentenich H. (2002) Guidelines for counseling in infertility. ESHRE Monograph, Oxford press, Oxford.
- ⁴⁴ Corona G., Jannini E.A., Maggi M. (2005) Inventories for male and female sexual dysfunctions. *Int. J. Impot. Res.*, Nov 3; [Epub ahead of print]