FRAGMENTATION TEST
Sperm Chromatin Structure Assay, Scsa

A method of examining of the genetic material (DNA) in the sperm.

About Fragmentation test

Fragmentation test provides only information of the percentage of sperm with fragmented DNA (the separation or breaking of DNA strands into pieces) and assess the degree of DNA damage (Pic. 1).

The sperm chromatin structure assay (SCSA) is a diagnostic test that indicates the degree of DNA fragmentation. If a large percentage of a man’s sperm (greater than 30%) have damaged chromatin (the complex of DNA), his chances of impregnating a partner are significantly reduced, and if he does impregnate his partner, she faces an increased risk of miscarriage.

Chromatin integrity evaluated by sperm chromatin structure assay are extremely important flow cytometry (a laser-based technology to analyze the characteristics of cells). Prior to flow cytometric sorting, semen is labeled with a fluorescent dye.

Through a specific SCSA-software (SCSA-Soft) a scatter plot is created, showing the ratio of green and red sperm. The percentage of red sperm is called DNA fragmentation index (DFI) - refers to the percentage of DNA strand breaks in the total sperm. The sperm with the most intensive green colour refers to the percentage of sperm with nuclear immaturity. Pic. 2 shows a typical SCSA cytogram of a sperm samples with low % DFI.

Sperm DNA fragmentation analysis is currently recommended by some authorities in specific situations:

- unexplained infertility
- persistent infertility after treatment of the female partner
- recurrent miscarriage
- exposure to reproductive toxins
- male cancer before and after treatment
- abnormal semen analysis
- male age > 50 years

The usefulness of the method is first of all relating to in vivo fertility (spontaneous pregnancy and intrauterine insemination (IUI)) and in particular in the many couples diagnosed with unexplained infertility.

The SCSA is gaining popularity in specialist andrology laboratories and appears to provide useful information on the probability of continued embryonic development and the establishment of pregnancy after fertilization.

As mentioned above, fragmentation test provides only information of the percentage of sperm with fragmented DNA and assess the degree of DNA damage. For this reason, it is superseded by magnetic-activated cell sorting (MACS). MACS was designed to selectively remove defective although morphologically indistinguishable cells from sperm preparations. It appears to be a safe and efficient method to select functional sperm. This technique may improve pregnancy rates when used to complement standard sperm selection methods in ART.

Success or failure factors
Oxidative stress (OS) plays an essential role in male infertility aetiology by affecting sperm quality, function, and also the integrity of sperm DNA. The assessment of oxidative stress in semen may be an important tool to improve the evaluation of sperm reproductive capacity. OS can also be linked with sperm DNA damage which may result in poor embryo development, miscarriage, and infertility.

Usually, most clinicians do not test infertile man for OS presence because available tests are expensive or difficult to perform as compared to a routine semen analysis. As antioxidant supplementation may improve sperm DNA integrity and pregnancy outcome, there is a clinical need to perform assays to identify OS in order to put these results in relationship with sperm DNA fragmentation.

In addition, OS in semen may be secondary to a lot of other exogenous sources such as environmental pollution by heavy metals and lifestyle factors such as obesity, smoking, and alcohol abuse. A healthy lifestyle and good eating habits (antioxidant rich foods such as cranberries, dark chocolate and green tea) can reduce sperm DNA fragmentation.

OS is related to sperm DNA fragmentation and high concentrations of round cells. The evaluation of OS and sperm DNA damage would make a solid contribution to standard seminal analysis profile and become diagnostic tools for evaluation of male infertility especially of idiopathic origin which must be taken into consideration during andrological workup.

**Complications**

Complication of this method is that the test irreversibly damages the sperm, after analysis they cannot be used for fertilisation purposes.

**Prognosis**

Several studies show that spermatozoa with DNA fragmentation are able to fertilize an oocyte, but are related to abnormal quality embryo, block in the blastocyst development, and lower pregnancy rates either natural or using IUI, IVF, or ICSI (intracytoplasmic sperm injection) procedures. Therefore DNA damage in sperm has associated with poor pregnancy outcome but is not necessarily associated with poor fertilization.

The advantage of SCSA is the objectivity of the test as well as the high reproducibility. A disadvantage is that the test irreversibly damages spermatozoa; after analysis they cannot be used for fertilisation purposes.

Studies indicate that DNA fragmentation measured by SCSA is not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intra cytoplasmic sperm injection, but could be related to spontaneous abortion rates.

There is not enough evidence that sperm DNA fragmentation testing will help predict the success of IVF or ICSI procedures. But, the SCSA has become an important tool for assessing semen quality in the human andrology laboratory as well as in the context of assisted reproductive techniques (ARTs) used for treating infertile couples.
Visualisation of sperm morphology using MSOME/IMSI and DNA fragmentation assessment in selected spermatozoa.

Normal spermatozoa showing absence of vacuoles (a) and absence of DNA damage in the same spermatozoa (b). Two highly abnormal spermatozoa (c) and presence of DNA fragmentation in both cases (d). Those spermatozoa showing presence of vacuolization (e) and lack of DNA fragmentation after the sperm the SCD-Oligo-Halo sperm test (f).

Green fluorescence is from native DNA, whereas red fluorescence is from fragmented DNA.

Sources

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