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Monograph

Sperm Chromatin Structure Assay (SCSA)

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Introduction

The Sperm Chromatin Structure Assay (SCSA) is a demonstrative procedure utilized to distinguish strange sperm with critical DNA discontinuity (Ohsu, 2021). Initially presented by Evenson in 1980, this examine uses stream cytometry to recognize the weakness of sperm DNA to corrosive actuated denaturation DNA in situ (Evenson and Donald, 2016). The SCSA evaluates the degree of sperm DNA discontinuity brought about by both inborn and outward factors and measures the level of fracture as the DNA Fracture File (DFI). The utilizations of SCSA include the assessment of male barrenness and subfertility, toxicology studies, and appraisal of research facility semen test quality.

The sperm chromatin structure measure (SCSA) is a creative demonstrative device equipped for distinguishing sperm tests described by an elevated degree of DNA fracture, which alludes to little breaks in the chromosomes of the sperm. Tests displaying a high level of divided DNA (>30%) have been related with an almost four-overlay decline in term pregnancies and a multiplying of unsuccessful labors. Regardless of whether a male has a palatable sperm count, great motility, and typical morphology, a serious level of fracture might in any case be noticed, which could add to fruitfulness challenges in a couple. The usage of SCSA might be suggested for explicit patients in view of their conceptive history. In any case, it is critical to take note of that the SCSA doesn't gauge the sperm's capacity to prepare an egg.

The SCSA really recognizes typical sperm and those with DNA discontinuity. This testing strategy utilizes specific colors, complex instrumentation, for example, a stream cytometer, and a laser bar to measure the level of sperm with flawless chromatin (DNA) versus those with divided chromatin.

Principle

SCSA is broadly utilized as a symptomatic device to identify sperm tests showing a serious level of DNA fracture and an absence of trade among histone and protamine proteins in sperm cores (Evenson and Donald, 2013). In SCSA, sperm irregularity is characterized as an expanded defenselessness of sperm DNA to denaturation prompted by intensity or corrosive therapy (Evenson and Donald, 2013). In principle, a completely experienced and solid sperm core, which is rich in disulfide bonds (S), ought to have its DNA protected in a twofold abandoned structure (Bungum et al., 2011). Low pH treatment uncovered flawed sperm DNA at destinations of harm. Acridine orange (AO) staining is then performed, where AO atoms intercalate into twofold abandoned DNA in flawless sperm, while total of AO particles happens in single-abandoned DNA of blemished sperm (Evenson et al., 2002; Bungum et al., 2011). During stream cytometry examination utilizing blue light, unblemished sperm radiate green fluorescence (showing local DNA), while blemished sperm transmit red fluorescence (demonstrating harmed DNA) (Evenson and Donald, 2016; Evenson et al., 2002; Ajina et al., 2017). The signs are then dissected utilizing programming to inspect both sperm DNA fracture (sDF) and abnormal chromatin structure.

Causes For Sperm DNA Damage

The integrity of sperm DNA is closely linked to the transfer of paternal DNA into the oocyte during fertilization. The causes of sperm DNA damage can be categorized into intrinsic and extrinsic factors. Intrinsic factors are related to various pathophysiological events that occur during spermatogenesis, while extrinsic factors are a result of exposure to endogenous sources of DNA breaks after birth.

Intrinsic factors

- **Abnormality in recombination and chromatin restructuring:** Abnormal recombination and chromatin restructuring can contribute to sperm DNA abnormalities. During spermatogenesis, there may be abnormal crossing-over of chromatid segments between homologous chromosomes, which involves programmed DNA double-stranded breaks mediated by specific nucleases (Bannister et al., 2004). To prevent undesired changes, a DNA damage checkpoint is activated, leading to the suspension of meiosis progression when DNA damage is detected (Page et al., 1997). The incorrect activation or inactivation of this checkpoint is suspected to be a cause of fragmented DNA in ejaculated spermatozoa. However, this hypothesis lacks direct confirmation in humans at present (Oleszcuzuk et al., 2016). During spermiogenesis, DNA double-strand breaks are introduced to relieve torsional stress and allow the substitution of nucleosome histone cores with transitional proteins. Disruptions to these processes can negatively impact the chromosomal integrity of sperm (Marcon and Boissonneault, 2004; Laberge and Boissonneault, 2005).

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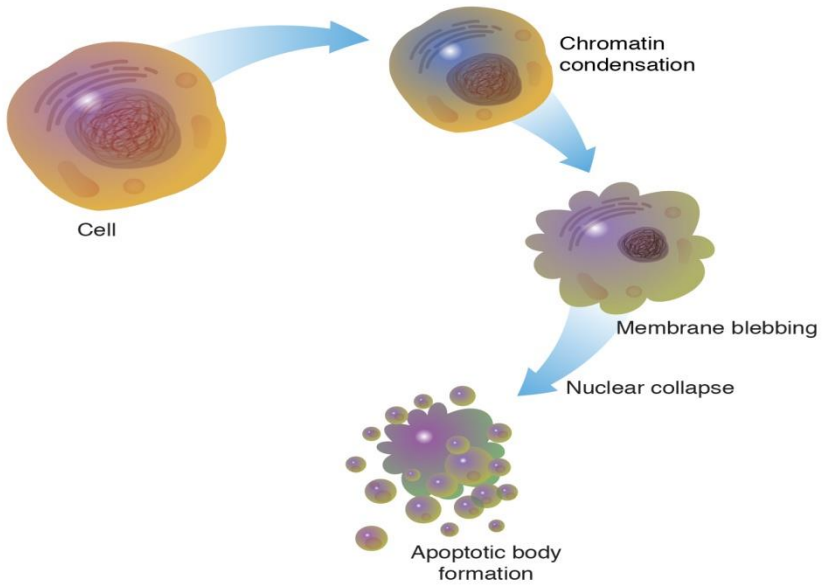
- ***Abortive apoptosis:*** Apoptosis is a programmed cell death process that eliminates abnormal cells and prevents their excessive proliferation. Inefficient activation of apoptosis can result in the overpopulation of germ cells or the survival of defective germ cells, leading to sperm DNA damage (Oleszcuzuk et al., 2016; Sakkas, 1999).



Dig: Image of Apoptosis

Reference: https://www.genome.gov/genetics-glossary/apoptosis

- **Oxidative Stress:** Oxidative stress refers to the imbalance between reactive oxygen species (ROS) activity and the presence of endogenous antioxidant agents (Sakkas, 1999). Elevated levels of ROS can lead to DNA damage, including both single-stranded and double-stranded breaks, which are commonly observed in the sperm of infertile men (Agarwal et al., 2003; Aitken and Krausz, 2001; Delamirande, 1997).

Extrinsic factors

- **Age:** Although males produce sperm throughout their adulthood, older age is associated with increased number of DNA double-stranded breaks and decreased frequency of sperm apoptosis (Oleszcuzuk *et al.,* 2016). Such observation is implicative of deterioration of sperm selection, quality, and integrity (Ahmed *et al.,* 2012).
- **Heat stress:** High temperatures cause adverse effects to sperm DNA and male fertility. Excessive heat is related to impaired sperm chromatin integrity (Ahmed *et al.,* 2012) and testis overheating is associated with reduced fertility potential (Thonneau *et al.,* 1998).
- **Smoking:** Toxins in common tobaccos may increase the prevalence of fragmented DNA (Evenson *et al.,* 2002). Smoking is associated with significantly escalated levels of seminal ROS and oxidative stress

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(Saleh *et al.,* 2002) . Increased ROS activity leads to apoptosis and increased fragmentation of DNA (Sakkas, 1999).

Procedure

The genetic integrity of sperm plays a crucial role in successful fertilization and subsequent embryo development leading to a viable pregnancy. Conventional semen analysis, which focuses on external characteristics such as sperm concentration, motility, and morphology, has limitations in its ability to provide comprehensive diagnostic and prognostic information. In contrast, a Sperm DNA Fragmentation test offers valuable insights into the genetic quality of sperm, aiding in the assessment of male infertility and guiding treatment decisions. The Sperm Chromatin Structure Assay (SCSA®) Test is an advanced diagnostic tool specifically designed for male infertility assessment. This test accurately and rapidly identifies individuals who are less likely to achieve a full-term pregnancy, whether through natural conception or assisted reproductive technologies.

The SCSA® test primarily measures two factors: %DFI (DNA Fragmentation Index) and %HDS (High DNA Stainability). The %DFI indicates the percentage of sperm with DNA fragmentation, while the %HDS represents the percentage of sperm with abnormal nuclear proteins and chromatin structure, potentially hindering successful fertilization. The SCSA test has only one protocol, described here in Basic Protocol . Two methods for analyzing the data are shown in Basic Protocol. Support Protocols are provided for preparation and shipping of samples, selection and use of reference samples, and instrument set-up.

Applications

Diagnosis of male infertility or subfertility

The SCSA can be performed to assess the sperm abnormality, it is a valid instrument to determine male infertility or subfertility. Although the causes and events that actuate sperm DNA damage and fragmentation are not yet fathomed, Sperm DNA fragmentation has been shown to be closely correlated with fertility and subfertility in not only humans, but also bulls, boars, and stallions. Such finding asserts the DFI determined by SCSA to be a strong independent predictor of in vivo pregnancy and a clinically useful technique.(Oleszczuzuk *et al.,* 2016, Saleh *et al.,* 2002 , Spano *et al.,* 2010 , Bungum , 2004 and Evanson 1994).

Currently, 25% DFI is the established clinical threshold in classifying males into statistical probability of: 1) increased time for natural pregnancy, 2) lower chance of Intrauterine insemination (IUI) success, 3) more miscarriage, or 4) infertility. High HDS values are in positive correlation to pregnancy failures.In such cases, other assisted reproductive technologies (ART) may be performed, including intracytoplasmic sperm injection (ICSI) (for sperm sample with DFI>25%) or testicular sperm extraction (TESE) (for sperm sample with DFI>50%) (Evanson and Donald 2013).

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Evaluation of cool-stored semen

SCSA is also performed to assess the quality of laboratory sperm samples that have been stored for at least 24 hours. Semen samples that have been stored at appropriate conditions will have essentially no change, while greater change in DNA quality indicates an improper handling. (Love and Charles , 2005)

Advantages

SCSA has numerous advantages when compared to other sperm DNA fragmentation (sDF) assays [TUNEL assay, COMET assay, and Sperm Chromatin Dispersion (SCD)], which include:

- The SCSA offers advantages in terms of time and cost efficiency compared to other existing sperm fragmentation protocols. It allows for the analysis of 5000-10000 spermatozoa in less than 5 minutes, this rapid analysis capability surpasses the efficiency of alternative protocols.
- The SCSA offers higher objectivity and accuracy compared to conventional sperm analysis methods. Traditional approaches for assessing infertility or subfertility rely on parameters such as sperm count, morphology, and motility. However, there have been instances where pregnancies failed despite these parameters falling within the normal range. In contrast, the SCSA utilizes machine-guided measurements of DNA Fragmentation Index (DFI) and High DNA Stainability (HDS) values, which are determined objectively without subjective human-eye evaluation. This automated process leads to higher precision, with coefficient of variation (CV) testing showing values of 1-3%. By eliminating the subjectivity of human interpretation, the SCSA enhances the objectivity and accuracy of sperm analysis, providing more reliable information for assessing male fertility or subfertility.
- The SCSA offers higher repeatability compared to traditional semen analysis parameters such as sperm count, morphology, and motility. These parameters can vary within a short period of time, leading to less repeatable results. In contrast, the SCSA demonstrates a high level of repeatability in clinical settings, with values ranging from 0.98 to 0.99.

Limitations

- The association between DNA Fragmentation Index (DFI) and reproductive outcomes has shown poor correlation based on findings from several meta-analyses,
- The threshold for DNA Fragmentation Index (DFI) and High DNA Stainability (HDS) in the SCSA test has been established based on the vulnerability of sperm DNA to acid-induced denaturation, rather than a direct measurement of sperm DNA integrity.

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