

Basic Test to Diagnose Male Infertility

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Abstract

Infertility is defined as inability to conceive after one year of unprotected intercourse. Male infertility is nearly half in out of 15% infertile couple. Oxidative stress is an important factor of this morbidity. DNA integrity in sperm is essential for accurate transmission of genetic information. Sperm DNA fragmentation Index (DFI) is an important parameter to get a conclusion of male infertility. On searching literature for review in Google Scholar, Pubmed and Cochrane library and after doing meta analysis, found there are four major types of tests for DNA fragmentation namely Sperm chromatin structure assay (SCSA) Comet, Tunel, Sperm Chromatin dispersion (SCD) assay. These tests have their own intrinsic merits and demerits. Therefore DFI is a valuable test to identify idiopathic infertility. It gives worth information to clinician as well as to the couple.

Keywords: Infertility; Sperm; DNA Fragmentation; Assisted Reproductive Technologies.

Introduction

Male infertility is quite common these days. It contributes almost 50% to total cases of infertility (Lguchi N, 2006). Morbidity of this disease is significant in Indian population (Dada et al, 2012). Oxidative stress is an important factor in this morbidity. DNA integrity in sperm is essential for accurate transmission of genetic information. Sperm DNA fragmentation Index (DFI) is an important parameter to get a conclusion of male infertility.

Benefits of DNA Fragmentation Index

- ❖ Routine semen analysis, in relation to fertility tells little.
- ❖ Sperm DNA damage testing is an early guard point to diagnose infertility.
- ❖ Before going for ICSI (intracytoplasmic sperm injection, an invasive procedure for fertile women), DFI of sperm gives valuable opinion.
- ❖ Sperm DNA fragmentation leads to impaired fertilization, slow early embryo development, chromosomal disorders in embryo, reduced implantation and abortions. Childhood cancers have also been associated with it.
- ❖ Detection of DNA fragmentation can be guide for IUI or IVF.

With the Help of DFI, customization of the treatment of infertility is possible.

Material

On searching literature for review in Google Scholar, Pubmed and Cochrane library I found thousands of publications on sperm DNA fragmentation index (DFI) in male infertility. Out of which I have selected recent 20 years papers. Meta-analyses showed that infertility test is significantly predictive of pregnancy success rate.

Result

There are four major types of tests for DNA fragmentation namely Sperm chromatin structure

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assay (SCSA) Comet, Tunel, Sperm Chromatin dispersion (SCD) assay. These tests have their own intrinsic merits and demerits.

Sperm Chromatin Structure Assay (SCSA)

This is a flow cytometric test that measures the susceptibility of sperm nuclear DNA to acid-induced denaturation. It measures only single stranded fragment. Test has the ability to measure large number of cells rapidly and identifies immature sperm with incomplete chromatin condensation (HDS) It is highly reproducible diagnostic and prognostic tool and requires expensive instrument, technical expertise.

TUNEL Assay

This test detects nicks (free end of DNA) and measures single and double stranded fragment. It is very sensitive assay (flow cytometric) and fast test, can be completed in three hours. It has high reproducibility with good precision. Paraformaldehyde fixation before permeabilization prevents the loss of small fragments of DNA. False positive results are possible from necrotic cells. It has many protocols, hence high interlaboratory variations exists.

Comet Assay

Sperms are embedded in agarose on glass slide followed by applying gel electrophoresis to evaluate DNA migration in comet tail. It measures both single and double stranded fragment. Only 5000 sperms can be assayed (few sperm, small sample). However, it is sensitive, repeatable and capable of detecting damage in every sperm.

Sperm Chromatin Dispersion Test

This is a simple, inexpensive and convenient form of assay. Unlike other tests it measures the absence of DNA damage rather than damaged sperm. Low density nucleotides are relatively faint.

Discussion

DNA fragmentation Index (DFI) is a percentage of damaged DNA. Some investigators suggested negative correlation between quality of Sperm DNA and IVF (Sun et al 1997) and ICSI (Lopes et al 1998). There is no correlation between normal fertilization and quality of Sperm DNA (Sakkas et al 1996, Morris et al 2002). Chohan et al found similar levels of DNA fragmentation identified by SCSA, TUNEL, and SCD.

These are sensitive tools for DNA fragmentation (Journal of Andrology 2006). SCSA is a gold standard but requires expensive instrumentation for optimal and unbiased analysis (Evenson et al 1999, 2002). No pregnancy is seen if the DFI is >27% (Larson-cook et al 2003). In TUNEL test clinical threshold is yet to be established. Comet assay is a simple, time consuming (Hughes et al 1999) and provides inconsistent results. SCD is a simple, fast, accurate and highly reproducible method (Fernandez et al, 2005).

Patients Presenting with the Following should have DNA Fragmentation Test

1. All idiopathic couples presenting for infertility;
2. Men older than 40 years, even if prior fertility;
3. Men with known exposure to toxicants
4. Men with pathologies like varicocele, tumor

Therefore DFI is a valuable test to identify idiopathic infertility. It gives worth information to clinician as well as to the couple. By DFI clinician are able to customize the treatment of infertile couple. Data also suggests that intracytoplasmic sperm injection (ICSI) may help to overcome the reduced pregnancy prognosis with high DFI over other ART (assisted reproductive technologies) or natural methods.

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