

Sperm DNA fragmentation testing a better tool for male fertility evaluation

Conventional semen analysis has been the cornerstone for evaluation of male fertility. But it exhibits a high degree of biological variability, inadequate and are only fair measures of fertility potential, ultimately not a good predictor of reproductive outcomes.

Over the last few decades, significant research has been done to evaluate at how the sperm DNA fragmentation can be used as a marker for male infertility. DNA fragmentation testing provide clinically relevant information, and predict reproductive outcomes, the improve the treatment strategies thus DNA fragmentation testing can serve as an adjunct to the semen analysis.

Also it will help ART specialists to adopt new advance sperm selection techniques like MACS and sperm sorting to circumvent DNA fragmentation..

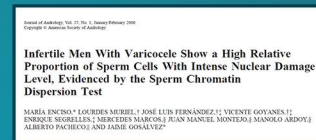


“Sperm DNA fragmentation has strong correlations with every fertility check point”

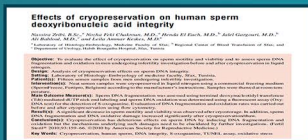
Paternal age



Varicocele



Sperm freezing



Pregnancy rate



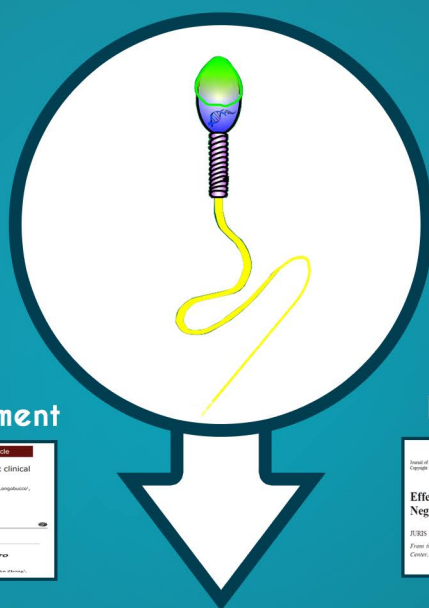
Sperm morphology



Embryo development



Leukocytospermia



SPERM DNA DAMAGE



Sperm Chroma Kit

Sperm chromatin dispersion test for assessing human sperm DNA fragmentation. The integrity of sperm DNA is being recognized as an important factor for successful reproductive outcomes. Sperm DNA fragmentation testing has gained importance as it offers a better diagnostic and prognostic potential than routine semen analysis. Several techniques exist to detect sperm DNA fragmentation, but Sperm chromatin dispersion (SCD) test, is a simple, reliable, and reproducible technique. Using SCD test DNA fragmentation can be accurately measured using conventional bright-field microscopy.

Kit contents:

- Pre coated slide 10 Nos
- Agarose 10 Nos
- Solution A
- Solution B
- Solution C
- Solution D

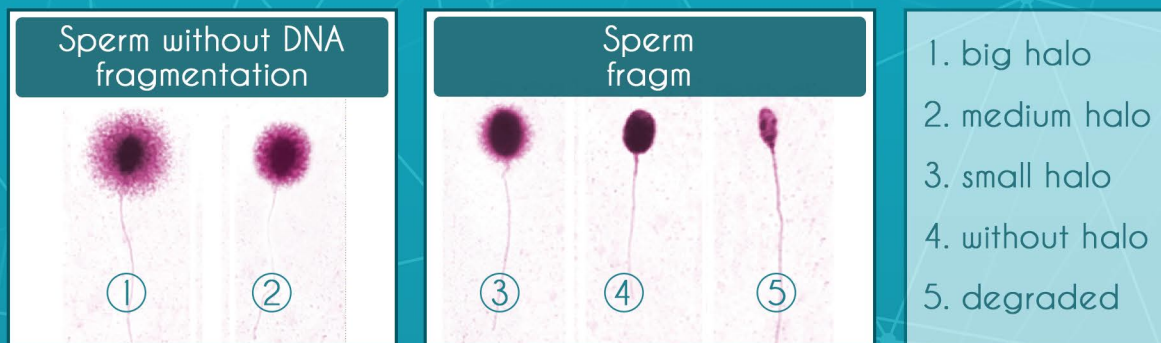
Equipment, Materials and reagents required but not provided in the kit

- Sperm chroma warmer 1
- Sperm chroma warmer 2
- Micro pipettes and micro tips
- Slide incubation trays
- Absolute Ethanol
- Distilled water
- Gloves

Procedure

- Assess the semen sample for its concentration. Dilute the semen sample with culture medium to 5-10M/ml.
- Melt the agarose at 90 °C by keeping it in the Sperm Chroma warmer 1 for 5 minutes
- Transfer the agarose to the sperm chroma warmer 2 that is maintained at 37°C and wait for 5 minutes.
- Add 25 micro-liters of the semen sample to agarose and mix well.
- Place the sperm cell suspension immediately onto the pre-treated slides and place the cover-slip. Avoid formation of air bubbles
- Leave the slide at 4°C for 5 minutes.

- g. After 5 minutes, remove the slide. Carefully remove the cover glass by sliding it off gently.
- h. Take care to maintain the slide in a horizontal position throughout the procedure
- i. Incubate the slides horizontally in Solution A for 7 minutes.
- j. After 7 minutes, incubate the slide horizontally in Lysis solution for 25 minutes.
(Note: Mix the lysis solution before use)
- k. Leave the slides in distilled water for 5 minutes
- l. Place the slide in 70% ethanol (2 minutes), followed by 90% ethanol(2 minutes) and finally in 100% ethanol(2 minutes). Leave to dry at room temperature
- m. Mix the Solution C and Solution D (1:1), and deposit a layer of stain, horizontally. Leave to stain for 15-20 minutes,
- n. Decant the stain and gently wash with distilled water and dry at room temperature.
- o. Visualize the slide under bright field microscope using 20X or 40X objective and grade the spermatozoa.



Sperm DNA fragmentation index calculation:

Classify at least 300 - 500 sperm cells as follows

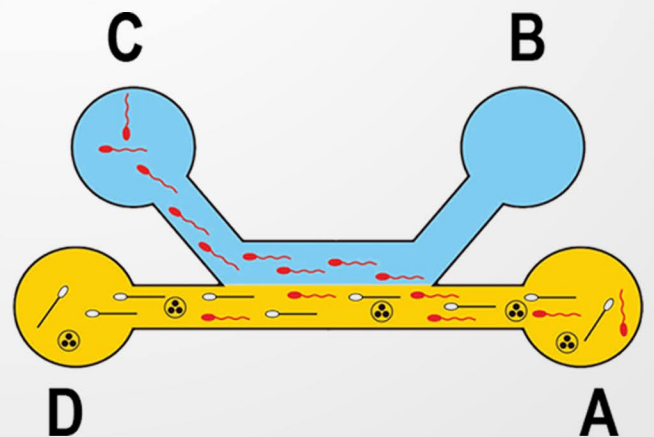
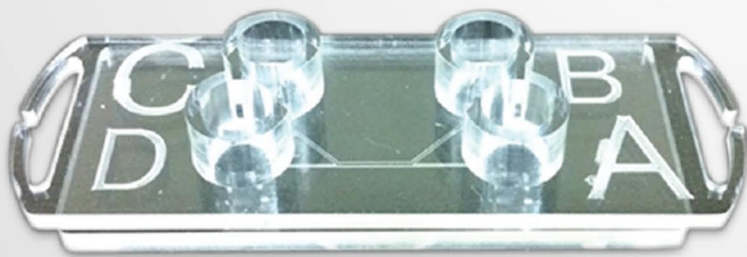
Sperm without DNA fragmentation	a. Big halo b. Medium halo
Sperm with DNA fragmentation	c. Small halo d. Without halo d. Without halo and degraded

$$\text{SDF(\%)} = 100 \times \frac{\text{No of sperm with fragmented DNA}}{\text{No of sperm counted}}$$



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