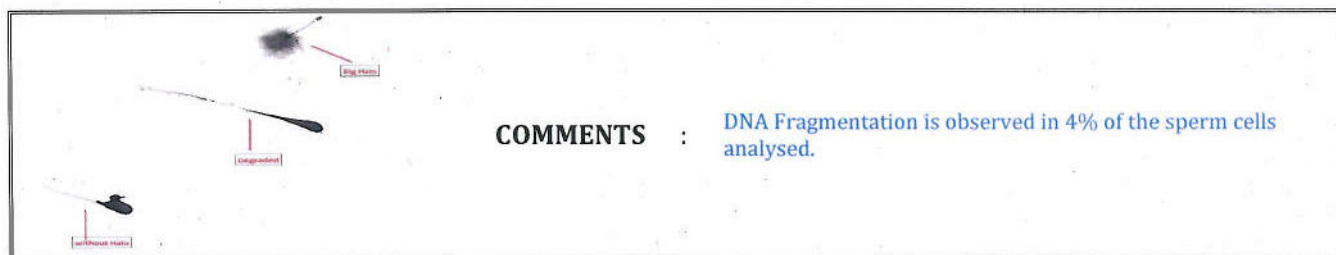


SPERM CHROMATIN DISPERSION ANALYSIS REPORT**

Specimen Description: Sample quality is optimum for the test.

RESULTS

	NO. OF TREATED SPERMS ANALYZED UNDER MICROSCOPE	NO. OF SPERM CELLS SHOWING HALO (Without DNA Fragmentation)	NO. OF SPERM CELLS NOT SHOWING HALO (Showing DNA Fragmentation)	SPERM DNA FRAGMENTATION (%)
CONTROL	300	294	6	2%
PATIENT	300	288	12	4%



- Couples with no known infertility problems were 7.0 times more likely to achieve a pregnancy/delivery if the DNA fragmentation index (DFI) was <30% using in-vivo fertilization.
- Infertile couples using IUI were 7.3 times more likely to achieve a pregnancy/delivery if their DFI was <30%.
- With routine IVF, infertile couples were approximately 2.0 times more likely to become pregnant if their DFI was <30%.*

*Evenson D, Wixon R. 2006. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. *Reprod Biomed Online* Apr;12(4):466-72, Hum Reprod, 19, 1401-8.

REAGENTS AND INSTRUMENTS

All fine chemicals and disposable lab wares:	Halotech DNA, Madrid, Spain
Microscope:	Carl Zeiss, Germany
Imaging system:	Metasystems

PRINCIPLE OF THE METHOD

The method is based on the sperm chromatin dispersion technique (Fernandez et al., J. Androl. 2003, Vol 24, p-59-66). Intact unfixed spermatozoa are immersed in inert agarose microgel on a pre-treated slide. An initial acid treatment denatures the DNA in those sperm cells with fragmented DNA. Following this, a lysis solution removes most of the nuclear proteins and when DNA breakage is not there, nucleoids with large halos of spreading DNA loops are produced emerging from a central core. In spermatozoa where DNA is fragmented, such a halo is either absent or is minimal.

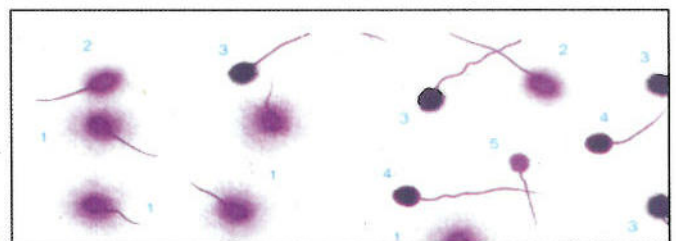
CLASSIFICATION OF SPERMATOZOA DONE IN THIS TEST AFTER TREATMENT

Spermatozoa without DNA fragmentation	Spermatozoa with DNA fragmentation
1. Spermatozoa with big halo: Those whose halo width is similar or higher than the minor diameter of the core.	1. The halo width is similar to or small than 1/3 of the minor diameter of the core.
2. Spermatozoa with medium sized halo: Those whose halo size is between large and very small halo.	2. Those which show no halo at all and present a core which is irregularly or weakly stained.

Without fragmentation

With fragmentation

1. Big halo 2. medium halo 3. Small halo 4. Without halo 5. Degraded



USES OF SDF ASSAY

1. To distinguish which couples are suitable for treatment by IUI. High SDF values have been shown to reduce the efficacy of intra-uterine insemination (IUI) from 16% to 4% (Bungum et al., 2004) or lower (Duran et al., 2002).
2. To assess the quality of semen samples or donors for suitability.
3. To assess the efficacy of medical interventions or treatment of infectious diseases. The percentage of spermatozoa with fragmented DNA is significantly higher in patients with *Chlamydia trachomatis* and *Mycoplasma* infections (Gallegos et al., 2008). Antibiotic therapy in these patients was demonstrated to significantly reduce SDF levels (Gallegos et al., 2008).
4. To provide answers to cases of unexplained infertility, ART failure or repeated abortions. High SDF levels have been shown to influence fertilization rate (Muriel et al., 2006a, Muriel et al., 2006b) and embryo quality (Velez de la Calle et al., 2008), leading to repeated pregnancy loss (Carrell et al., 2003) and low ART outcome (Henkel et al., 2004, Sakkas et al., 2004, Virro et al., 2004).

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