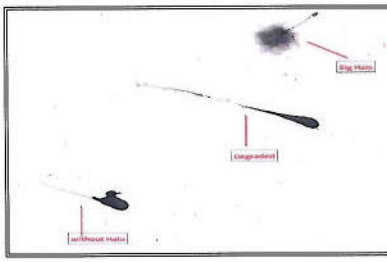
SPERM CHROMATIN DISPERSION ANALYSISREPORT**

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%



COMMENTS : DNA Fragmentation is observed in 4% of the sperm cells analysed.

- 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

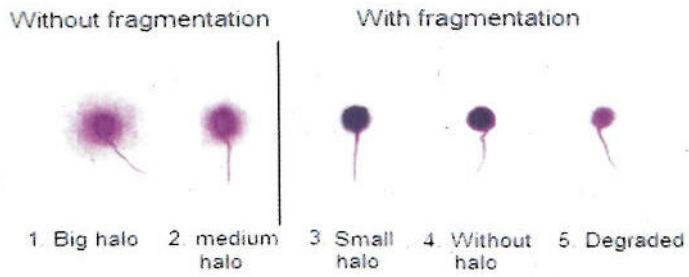
All fine chemicals and disposable lab wares:	Halotech DNA, Madrid, Spain
Microscope:	Carl Zeiss, Germany
Imaging system:	Metasystems

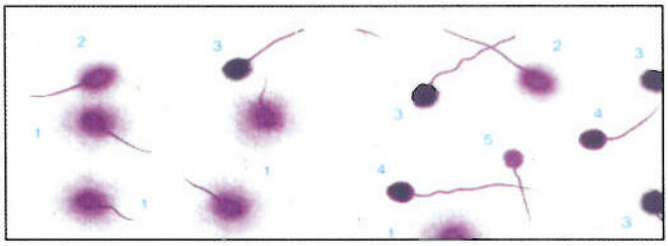
PRINCIPLE OF THE METHOD

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 that the minor diameter of the core. 2. Spermatozoa with medium sized halo: Those whose halo size is between large and very small halo.	1. The halo width is similar to or small than 1/3 of the minor diameter of the core. 2. Those which show no halo at all and present a core which is irregularly or weakly stained.





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)

BIBLIOGRAPHY

1. BUNGUM, M., HUMAIDAN, P., SPANO, M., JEPSON, K., BUNGUM, L. & GIWERCMAN, A. 2004. The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. Hum Reprod, 19, 1401-8.
2. CARRELL, D. T., LIU, L., PETERSON, C. M., JONES, K. P., HATASAKA, H. H., ERICKSON, L. & CAMPBELL, B. 2003. Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Arch Androl, 49, 49-55.
3. DURAN, E. H., MORSHEDI, M., TAYLOR, S. & OEHNINGER, S. 2002. Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod, 17, 3122-8.
4. GALLEGOS, G., RAMOS, B., SANTISO, R., GOYANES, V., GOSÁLVEZ, J. & FERNÁNDEZ, J. L. 2008. Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma. Fertil Steril, 90, 328-34.

5. HENKEL, R., HAJIMOHAMMAD, M., STALF, T., HOOGENDIJK, C., MEHNERT, C., MENKVELD, R., GIPS, H., SCHILL, W. B. & KRUGER, T. F. 2004. Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil Steril, 81, 965-72.
6. MURIEL, L., GARRIDO, N., FERNÁNDEZ, J. L., REMOHI, J., PELLICER, A., DE LOS SANTOS, M. J. & MESEGUER, M. 2006a. Value of the sperm deoxyribonucleic acid fragmentation level, as measured by the sperm chromatin dispersion test, in the outcome of in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril, 85, 371-83.
7. SAKKAS, D., D'ARCY, Y., PERCIVAL, G., SINCLAIR, L., AFNAN, M. & SHARIF, K. 2004. Use of the egg-share model to investigate the paternal influence on fertilization and embryo development after in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril, 82, 74-9.
8. VÉLEZ DE LA CALLE, J. F., MULLER, A., WALSCHAERTS, M., CLAVERE, J. L., JIMÉNEZ, C., WITTEMER, C. & THONNEAU, P. 2008. Sperm deoxyribonucleic acid fragmentation as assessed by the sperm chromatin dispersion test in assisted reproductive technology programs: results of a large prospective multicenter study. Fertil Steril, 90, 1792-9.
9. VIRRO, M. R., LARSON-OOK, K. L. & EVENSON, D. P. 2004. Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril, 81, 1289-95.

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