

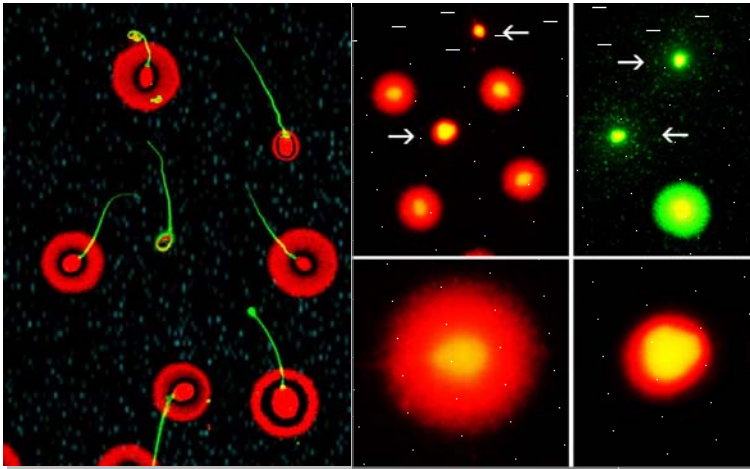
DYNAMICS OF SPERM DNA FRAGMENTATION IN FRESH AND FROZEN HUMAN SEMEN

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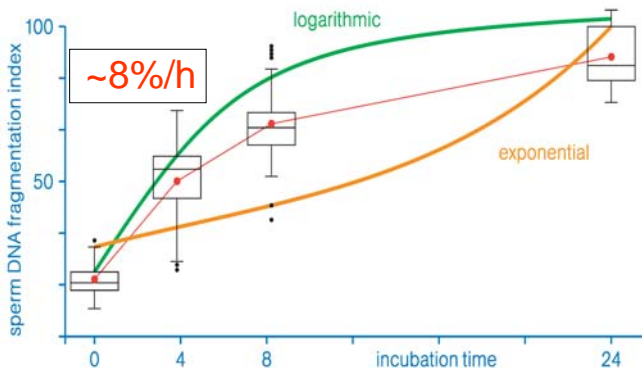
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Sperm DNA fragmentation (SDF) must be considered as a dynamic process, i.e. SDF change through time after ejaculation. To analyse the incidence of this phenomenon on sperm quality, the dynamics of SDF was assessed in semen samples before and after freezing. Fifteen donors were included in the analysis and from the same ejaculate three aliquots were obtained. One of them was kept fresh in the seminal plasma and two were frozen-thawed. After thawing one aliquot was maintained in the freezing media and the other capacitated. For analyzing SDF all samples were processed with Halosperm (Halotech DNA SL, Madrid, Spain). Sperm from different aliquots were incubated during a period of 72 hours at 37°C and the rate of SDF at different incubation times (from 0 to 72h) were scored. Results show that the dynamic behaviour of the SDF before and after freezing are different and, in general, after thawing selection of a specific sperm subpopulation, which show less variance in the distribution of SDF values than in fresh samples, was observed. These results indicate that both the management and specially frozen thawing selection of human sperm, for use in assisted reproduction techniques, must be completed quickly in order to minimize the DNA damage. Theoretically, this practice should result in an improvement in pregnancy rates.

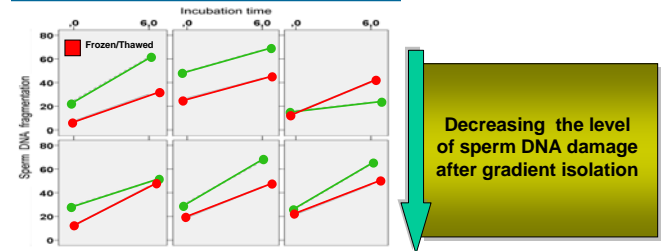
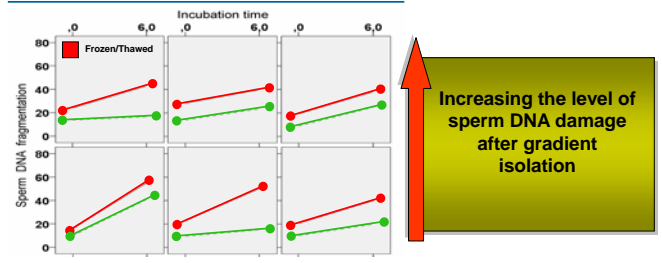
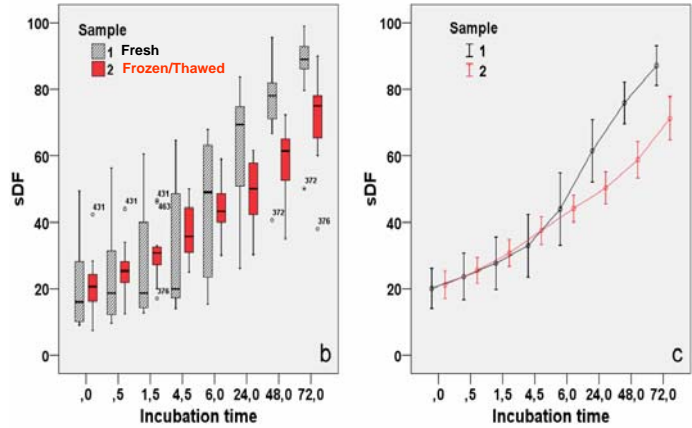


Semen samples processed with the SCD test (Halosperm) for the visualization of DNA fragmentation showed basically two different sperm populations. Those nuclei that remained compact or displayed very small halos of chromatin dispersion correspond to spermatozoa harbouring fragmented DNA. Those spermatozoa displaying a huge halo of chromatin dispersion correspond to unfragmented DNA. The absence of halo is a "hidden reality" (image in green fluorescence).



Box-and-whisker plots for the sperm DNA fragmentation index observed at different incubation times (37°C) for cryopreserved/thawed spermatozoa and the rate for sperm DNA fragmentation observed at each interval. Average sperm DNA fragmentation observed at different incubation times (Red line) and adjustments for logarithmic (green line), linear or exponential (orange line) functions. The rate of sperm DNA fragmentation observed during the first 4h of the experiment is on the order of 8%/hour.

EXPERIMENT: Sperm DNA fragmentation assessed in fresh samples (Grey) and cryopreservation and density gradient isolation (Red). Same donors.



First panel: Graphic representation for the rate of SDF in fresh samples and frozen/thawed samples and gradient density isolation. Second panel: Two time intervals were used for comparison (from 0 to 6 hours). Note that after sperm recovery the level of sperm DNA damage may improve with respect to the baseline or be of worst quality.

CONCLUSIONS

- 1) Sperm DNA fragmentation is a dynamic process
- 2) Sperm DNA longevity is different for each individual
- 3) The rate of sperm DNA damage after thawing assessed during the first 4 hours of incubation at 37°C, is of the order of 8%/h.
- 4) Sperm recovery after frozen/thawing using density gradient does not select for the best level of sperm DNA damage.

Gosálvez J, Cortés-Gutiérrez E, López-Fernández C, Fernández JL, Caballero P, Núñez R. Sperm DNA fragmentation dynamics in fertile donors. *Fertility and Sterility* (2009, in press)

Fernández JL, Vélez de la Calle JF, Tamayo M, Cajigal D, Agarwal A, Gosálvez J. Sperm DNA integrity and male infertility: current perspectives. *Arch Med Sci* (2009, in press)

Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, Lafrombois ME, & De Jonge C. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion (SCD) test. *Fertility and Sterility* 84: 833-42 (2005)