

# Detection of DNA damage in human spermatozoa with the Halosperm®Test (Fa. Gynemed)

## Establishment of a new diagnostic tool for routine application in a human genetic laboratory

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### Introduction

Approximately 15-20 % of males in the western industrial countries are affected by idiopathic infertility. Determining a distinct genetic cause is not possible in those cases and the conventional sperm parameters (motility, morphology, concentration) only offer an estimation of the male fertility potential. In fact, probably 10-15 % of males with normal semen parameters are sterile. The sperm DNA integrity plays a crucial role for the male fertility. During spermiogenesis a highly compact and complex structure of the chromatin is achieved by protamination of the DNA. The proper condensation of the chromatin permits the transfer of the male genetic information to the oocyte and onto the next generation. In addition, it has a protective function against DNA damage. Abnormalities of the DNA structure or DNA damage in terms of single and double strand breaks (DNA fragmentation) are suggested to negatively influence the male fertility. Based on different studies infertile men possess substantially more spermatozoa with fragmented DNA compared to fertile controls. As a potential risk factor for fertilisation failure, poor embryo development, reduced embryo implantation and recurrent miscarriages the percentage of DNA fragmentation (DNA fragmentation index, DFI) in an ejaculate is used as an additional and independent marker of sperm quality.

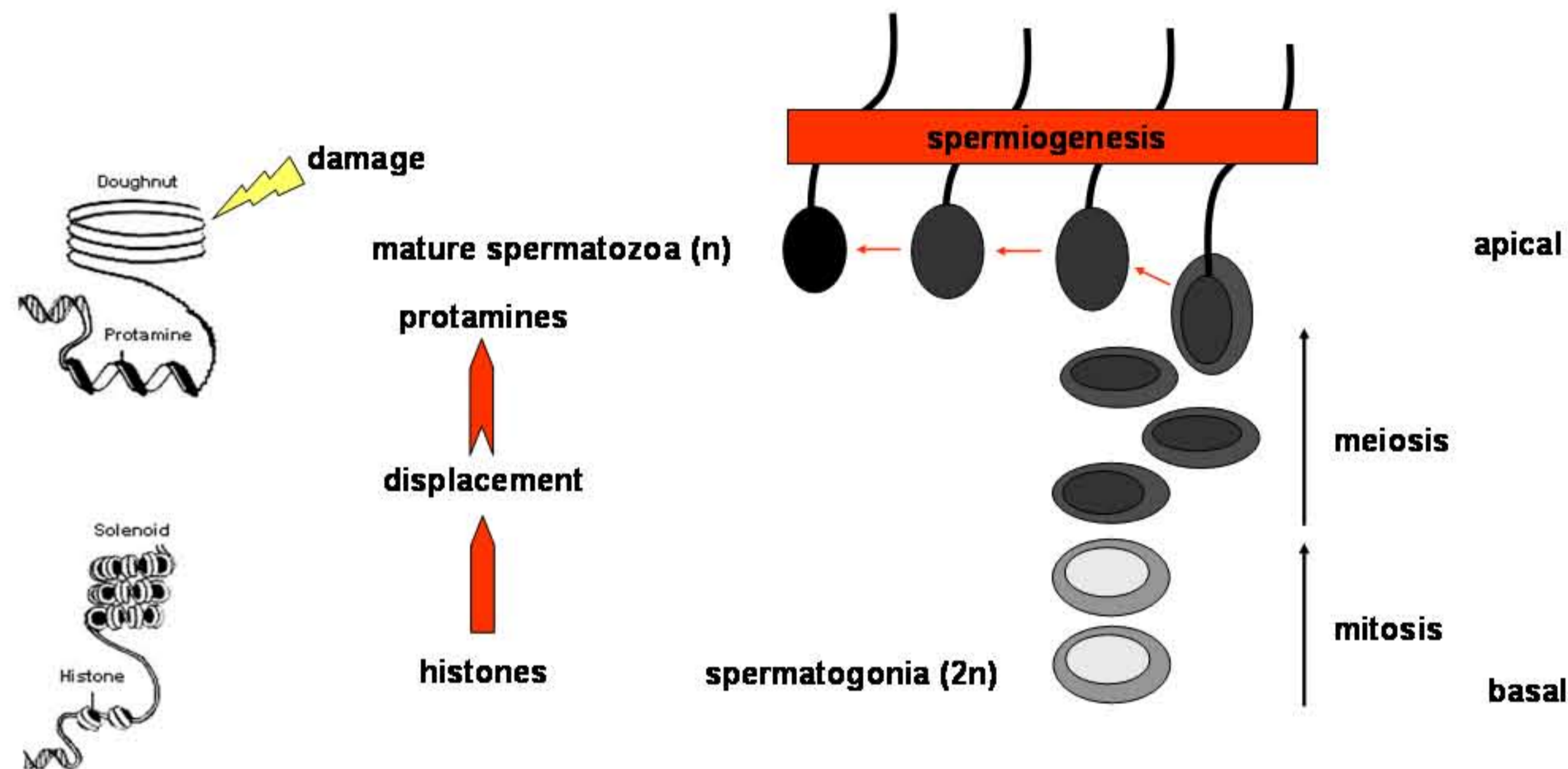


Figure 1: Schematic spermatogenesis in the testicular germinal epithelium.

Diploide (2n) spermatogonia emerge to haploide (n) spermatids by passing through mitosis and meiosis. During differentiation to mature spermatozoa 85 % of histones bound to the DNA are replaced by protamines leading to a nearly sixfold more condensed DNA (doughnut-loop model) compared to DNA in somatic cell metaphase chromosomes. Reasons for DNA damage may be failure during spermatogenesis which result in an incomplete chromatin packaging increasing the vulnerability of DNA to reactive oxygen species (ROS). ROS occur during oxygen stress situations, for example heat exposition of the testis, infections or ageing and can implicate DNA instability and strand breaks. Another cause may be apoptotic processes.

### Method

For detection of DNA fragmentation we use the commercially available Halosperm®Test (Fa. Gynemed) an improved Sperm Chromatin Dispersion (SCD) test. The method includes acid denaturation to generate single-stranded DNA segments from DNA breaks and deproteinization of nuclear proteins. The semen samples are diluted in buffer and can be processed directly or be stored in liquid nitrogen for a subsequent analysis. The preparation is stained with 4'-6-Diamidin-2'-phenylindol-dihydrochlorid (DAPI). Using fluorescence microscopy the percentage of DNA fragmentation is estimated by counting 500 spermatozoa for each sample. Simultaneously with each patient sample we process a semen sample with known DNA fragmentation on the same slide as an intern control. Before the routine application of the Halosperm®Test in our laboratory we trained the method by analysing 24 ejaculates.

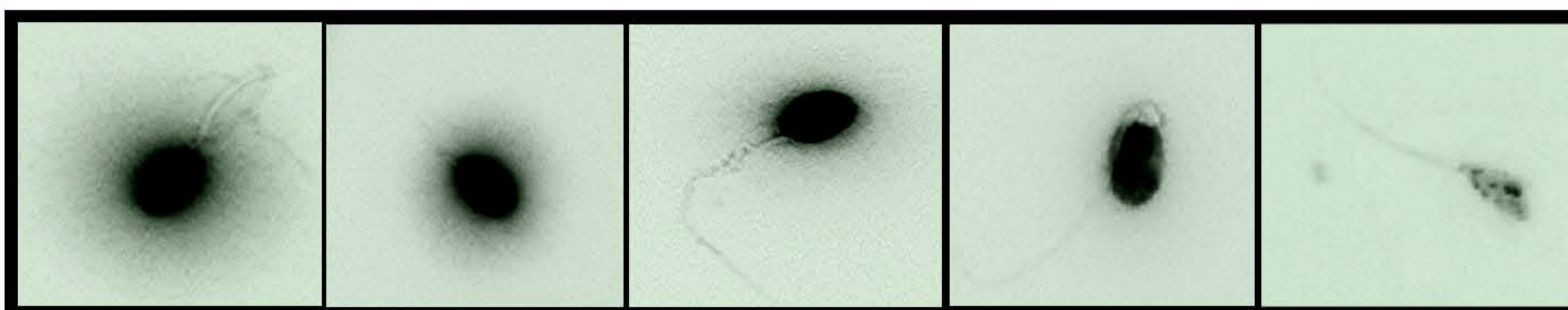


Figure 2: Five different levels of DNA fragmentation in human spermatozoa can be distinguished (DAPI images inverted).

Spermatozoa with intact DNA show big (A) or medium-sized (B) DNA loops (halos) dispersing from a central nuclear core. Sperms with fragmented DNA show a small halo (C) or exhibit no halo with a solid (D) or a faint staining (E) of the core.

The DNA fragmentation index (DFI, % of fragmented spermatozoa) is classified into three categories:

- DFI values from 0 – 15 % are consistent with normal male fertility.
- DFI values > 15 % – < 30 % correspond with a decreased male fertility implicating intrauterine insemination (IUI) as method for sterility treatment.
- DFI values ≥ 30 % are pathologic. Achieving pregnancy spontaneously is unlikely and assisted reproductive treatment (ART) including in-vitro-fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) is suggested.

### Prospect

The evaluation of damaged sperm DNA in combination with the conventional semen analysis may improve the diagnostic and prognostic approaches in the prediction of pregnancy for couples with male infertility. The Halosperm®Test could be an effective method to avoid unsuccessful intrauterine insemination (IUI) in couples with normal semen parameters but high DNA fragmentation. The prospect is to avoid hopeless therapies as well as to explain failed infertility treatment. Since May 2009 we have investigated 31 ejaculates of males in preparation for ART in cooperation with the Praxisklinik Sydow „Am Gendarmenmarkt“, Berlin. So far we observed the following trends: 1.) Good progressive motility of spermatozoa seems to correlate with a low DFI value. 2.) The DFI value increases with advanced paternal age.

Nevertheless, for a statistically significant conclusion analysis of more ejaculates will be required.

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