

# The challenges of assessing the quality of spermatozoa

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In humans, the traditional endpoints used to assess semen quality are the volume and viscosity of semen as well as the number, motility, and morphology of spermatozoa (1). Although it is certainly intuitively logical to expect that these endpoints should serve as effective predictors of fertility, several decades of experience have demonstrated that it is only when these parameters reach extremely low values that any predictive meaning can be ascribed to them. Fertility can come about with very low sperm numbers (2) or spermatozoa having low motility or poor morphology (1). With the advent of intracytoplasmic sperm injection, single sperm that are immotile or have an abnormal appearance have been used to fertilize an oocyte. Over the past few years a series of tools designed to ascertain the quality of the genetic material in spermatozoa, from both humans and animal models, has been developed. Nevertheless, there is a dearth of studies in which the consequences of occupational and environmental exposures have been assessed using these tools. Furthermore, there is still no validated test that can be used to determine the quality of the chromatin of a single sperm before its delivery to the oocyte.

Assessing chromatin quality in spermatozoa presents challenges that are greater than for somatic cells. This is because of the more than sixfold compaction that sperm chromatin undergoes during spermiogenesis, the replacement of most histones with the very basic protamine, and the lack of an apparent organization of sperm chromatin into chromosomes. However, there is increasing awareness that it is critical to obtain an accurate assessment of sperm chromatin quality to assess fertility potential, the possibility of abnormal embryonic, and/or fetal development and consequences to future generations. A growing body of literature describes a wide range of tests designed to assess various aspects of chromatin structure and function in spermatozoa. Many of these tests first were developed for spermatozoa from animal models and are being adapted for human sperm.

Measures of chromatin quality can be broadly divided into four categories. The first assesses whether the condensed sperm chromatin can undergo proper decondensation; this can be examined either *in vitro* or monitored *in vivo* within a zygote (3, 4). The second covers a large group of tests designed to establish whether the actual chromatin structure has been modified by the xenobiotics or ambient conditions.

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These range from determining whether there is the correct number of chromosomes, using fluorescent *in situ* hybridization (FISH) (5), to determining the integrity of DNA stands. The latter primarily monitors the presence and extent of DNA single or double-stranded breaks and/or crosslinks, using the Comet assay, TUNEL assay, alkaline elution, or SCSA (sperm chromatin structure assay, acridine orange assay) (6–9). The third group encompasses assays designed to establish the functional aspect of sperm chromatin; these include both DNA template replication and *de novo* RNA synthesis assays (7). For the detection of alterations in specific transcripts, quantitative RT-PCR assays have been developed (8, 10). Finally, the monitoring of several aspects of the sperm nuclear epigenome has received attention lately. These specific components include the presence of protamines and the extent of disulfide crosslinks, the presence of histones (11), and the components of the nuclear matrix (12), the status of methylation of specific sites as well as the entire genome (13), and the functional significance of the presence of various RNAs including microRNAs (14, 15).

It is clear that not all of these sperm chromatin quality tests are necessary or will continue to be used, that additional new tests will be developed, and that one or a few tests will emerge as the new “gold standard.” However, there is little doubt that assessing semen and the quality of sperm chromatin has entered a new era: one in which the focus has changed from the number of sperm, their motility and appearance to one where the quality of the genetic material that is delivered to the oocyte is central.

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