

DNA damage/chromatin modification during cryopreservation does not increase in morphologically abnormal sperm

Satish Kumar Adiga, Ph.D.¹, Guruprasad Kalthur, Ph.D.¹, Dinesh Upadhya, M.Sc.¹, Pratap Kumar, M.D.¹, Rakesh Sharma, Ph.D.² and Ashok Agarwal, Ph.D., HCLD²

¹Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal-576 104, INDIA and
²Reproductive Research Center, Cleveland Clinic, Cleveland, OH, USA

Sperm cryopreservation has become an important component of assisted reproduction. During cryopreservation, sperm undergo dramatic changes in both intracellular and extracellular environment due to exposure to cryoprotectives, cooling, freezing, storage in liquid nitrogen, and thawing. The chemical and physical effects of these reagents/processes have a detrimental effect on chromatin, morphology, membrane integrity, and vitality of human spermatozoa.

The present study was undertaken to study the sperm DNA damage and chromatin modification induced during the process of freeze-thawing and to find out its association with sperm morphological abnormality (head abnormality). We used Single-cell gel electrophoresis (SCGE) or comet assay which has the ability to detect damage at the single cell level and acridine orange (AO) binding ability to evaluate the chromatin status of the sperm. In this study, semen samples from 25 males (sperm count >20 millions/ml) attending our center for infertility evaluation were included. After evaluating for sperm count, motility, vitality and morphology (WHO, 1999), the sperm DNA damage and chromatin status were measured by comet assay and AO binding. The semen samples were stored in liquid nitrogen using glycerol egg yolk citrate buffer. After one week, the extent of DNA damage and chromatin status was again evaluated in thawed samples. Although, our study showed a significant increase in the amount of DNA damage and chromatin denaturation ($p < 0.05$) after cryopreservation, we did not find any association between sperm morphology and freeze-thaw induced sperm DNA damage. These results demonstrate that the susceptibility of morphologically abnormal sperm to undergo DNA damage/chromatin modification is similar to morphologically normal sperm although cryopreservation process by itself alters the DNA integrity of the sperm.