Cryopreservation and sperm DNA integrity

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Abstract

Cryopreservation of sperm is an extremely important issue in the field of male infertility as freezing can have detrimental effects on a variety of sperm functions, some of them not accessible to the traditional semen quality analysis. In this study, chromatin structure variations in human spermatozoa in semen were studied with the sperm chromatin structure assay (SCSA), both before and after cryopreservation. Samples were divided into two aliquots: the first was analysed without further treatment, while the second was stored in liquid nitrogen at −196 °C using standard cryopreservation techniques. The fresh and thawed aliquots were also assessed by light and fluorescence microscopy (after Acridine Orange staining, AO), and computer-assisted semen analysis (CASA) of motility. Overall sperm quality was found to deteriorate after cryopreservation. When thawed spermatozoa were subjected to an extra swim-up round, a general improvement in nuclear maturity was seen in post-rise spermatozoa.

Abbreviations: ALH – Amplitude of Lateral Head displacement; AO – Acridine Orange; ART – Assisted Reproductive Technology; BCF – Beat Cross Frequency; CASA – Computer-Assisted Semen Analysis; DFI – Dna Fragmentation Index; FCM – Flow Cytometric; LIN – Linearity Index; SCSA – sperm chromatin structure assay; VEL – Curvilinear Velocity; WHO – World Health Organization

Introduction

Sperm DNA chromatin integrity appears to be important for correct spermatozoa functioning (Agarwal and Said 2003; Sakkas et al. 2003) and methods focusing on characterisation of sperm chromatin condensation and stability have received increasing attention (Perreault et al. 2003). One of the most interesting techniques today available is the flow cytometric (FCM) sperm chromatin structure assay (SCSA) (Evenson et al. 1980, 2002), which has demonstrated to be an independent predictor of fertility either in vivo (Evenson et al. 1999; Spanò et al. 2000) or in vitro (Larson-Cook et al. 2003; Saleh et al. 2003). The assessment of sperm DNA/chromatin quality in human semen samples, carried out using a variety of techniques, is becoming a relevant end-point as sperm DNA and chromatin abnormalities have been associated with failures in in vitro assisted...

Sperm cryopreservation may be of help to any assisted reproduction program, mainly, for example in the case of semen cryopreserved prior to chemo- or radiotherapy, which may induce azoospermia or teratogenicity, together with sperm aneuploidies and DNA fragmentation (Martin et al. 1997; Robbins et al. 1997; Morris 2002; Meistrich and Byrne 2002; Ståhl et al. 2004). It is well known that temperature variations (e.g. during cooling, freezing and thawing) can cause detrimental changes in sperm functions (i.e. motility) and structure (nucleus, membrane and mitochondria) that can impair its fertility capabilities (Spanò et al. 1999; Donnelly et al. 2001a, 2001b; Duru et al. 2001; Duty et al. 2002; Thompson-Cree et al. 2003; Isachenko et al. 2004), but the results are somehow conflicting as far as the extent of the induced cell damage and the influence of the sperm processing procedure employed.

Therefore, it would be interesting to assess nuclear chromatin damage induced by extreme temperature changes and express sperm quality in terms of cell-to-cell integrity using a variety of endpoints, thus helping the physician increase the chance of achieving successful fertilisation. In this study the FCM SCSA was used to evaluate the chromatin structure in fresh semen of normozoo- spermic subjects and to measure sperm nuclear integrity changes after cryopreservation. Results were compared with those of Acridine Orange (AO) staining on slide, of standard microscopy and sperm motility assessment by computer-assisted semen analysis (CASA). The same endpoints have also been applied to assess sperm quality of the post-rise thawed sperm undergoing the swim-up technique.

Materials and methods

Subjects

We studied 19 healthy subjects, aged 30–38 years, attending our Laboratory of Seminology and Immunology of Reproduction, Rome, Italy for pre-marriage checks. All samples were considered normal under World Health Organization guidelines (WHO 1999). None of the subjects had received medical treatment in the 3 months prior to the study. They were all advised on the study’s nature and purpose and signed a detailed consent form.

Semen quality analysis

Semen was collected by masturbation after 3–5 days of abstinence. Ejaculates were left to liquefy at 37 °C and examined within 1 h of collection. The parameters considered were ejaculate volume (ml), sperm concentration (n × 10⁶/ml), forward motility (%), percentage of atypical forms (sperm and head morphology). The evaluation was carried out under a light microscope under WHO criteria (WHO 1999). The same biologist performed all seminal fluid examinations in order to minimize variability. CASA of sperm motility was also performed. The CASA system (Cell Soft; Cryo Resources, New York, NY, USA) was equipped with a heated (35 °C) stage. The parameters assessed were curvilinear velocity (VEL, μm/s), linearity index (LIN), amplitude of lateral head displacement (ALH, μm), and beat cross frequency (BCF, Hz). The CASA analysis was performed under the following parameter settings: 25 frames to be analysed, 25 frames/s, 2 track points for calculation of motility, 8 track points for calculation of velocity, velocity range 10–150 μm/s, cell size range 4–20, 8 track points for ALH calculation, minimum velocity for ALH calculation 20 μm/s, minimum linearity for ALH calculation 3.5 μm. At least 300 cells were examined in each sample. Each sample was divided into two aliquots: (A) the first was evaluated without further processing; (B) the second was stored under standard cryopreservation techniques at −196 °C in liquid nitrogen. Finally, (C) an aliquot of the cryopreserved sample was subjected to a swim-up procedure after thawing. Each aliquot was analysed under the light microscope, by fluorescence microscopy after AO staining and by FCM SCSA.

Semen cryopreservation

After liquefaction, an aliquot from each sample was diluted (1:1) with freezing medium (test yolk buffer; Irvine Scientific, Santa Ana, CA, USA).