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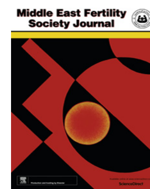

Original Article

Short abstinence: A potential strategy for the improvement of sperm quality

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ABSTRACT

Objective: To determine the effect of short (4 h) and long (4 days) abstinence periods on sperm quality based on functional and biochemical parameters in a population of normozoospermic men.

Methods: Two semen samples were collected in succession from potentially fertile, normozoospermic men (n = 100) after an abstinence period of 4 days and 4 h respectively. The mean values of semen volume, pH, viscosity, sperm concentration, percentage of total and progressively motile sperm, sperm kinematics/velocity, normal morphology, acrosome status, DNA fragmentation, intracellular superoxide ($O_2^{-\bullet}$) levels and seminal antioxidant status were compared between the two abstinence duration groups.

Results: A significant increase in total and progressive motility and velocity parameter values were observed after short abstinence compared with long abstinence periods. Sperm DNA fragmentation and intracellular $O_2^{-\bullet}$ levels were not significantly different between the two abstinence periods. Despite the decrease in semen volume, sperm concentration and total sperm number after short abstinence periods, all mean values of the conventional semen parameters remained above the lower reference limits as reported by the WHO.

Conclusion: The data from this most comprehensive study of its kind challenges the generally accepted guidelines of the prolonged abstinence periods since the results show that 4 h of sexual abstinence yielded significantly better sperm samples from a functional point of view. Although this study was performed on normozoospermic men, future studies with infertile men might yield similar findings that could lead to employing short abstinence as a strategy to improve the outcome of ART and fertility preservation.

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1. Introduction

The World Health Organization (WHO) guidelines for semen analysis provide a standard approach for the prognosis of fertility and diagnosis of infertility in men. These guidelines have been universally adopted by most human andrology laboratories during the last three decades [1,2]. Therefore, standardized semen analysis, according to the WHO, remains the initial screening and cornerstone for the evaluation of male fertility.

Individual variations in semen parameters are influenced by several factors that can be predictable and can be controlled for. Apart from various environmental and genetic factors [3,4], sexual abstinence has evidence of an association with sperm quality [5–7]. According to the prescribed guidelines of the WHO, subjects must remain abstinent for a minimum period of 48 h, but not longer than seven days before collecting a sample for a standard semen analysis [2]. More constricted abstinence intervals of three to four days have also been suggested by the Nordic Association for Andrology (NAFA) and the European Society of Human Reproduction and Embryology (ESHRE) [8]. However, the basis for these recommendations remains uncertain and is not supported by sufficient scientific evidence.

During the last half century, several studies have sought to determine the optimal time frame for ejaculatory abstinence, however the results are often found to be contradictory. In general, these studies assessed a wide range of abstinence duration cutoffs (≥1–18 days). Prolonged sexual abstinence has generally been reported to increase semen volume, sperm concentration and the total sperm count [5,7,9,10]. However, the overall quality of spermatozoa has shown to be influenced by the efficiency of epididymal storage and the transit rate of spermatozoa, which is apparently dependent on the frequency of ejaculation [11,12].

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Progressive motility [6] as well as the percentage of motile spermatozoa [5,13,14] were found to decrease substantially with increased abstinence duration, while no significant differences were noticed in other studies [7,15,16]. Only a few studies are available on the impact of abstinence time on advanced sperm functional parameters such as intracellular Reactive Oxygen Species (ROS) production and DNA integrity; while their findings are apparently inconsistent. Shortening the abstinence time resulted in a significant decrease in sperm DNA fragmentation [5,16,17] and ROS levels [18], whereas other studies reported no significant differences [7,19,20].

Chronobiological studies showed that various changes occur in the body every 4 h [14]. Despite this, no comprehensive study investigated the effect of a short abstinence period lasting 4 h on conventional semen parameters, in addition to various existing sperm functional parameters such as acrosome reaction, sperm ROS and DNA fragmentation, and seminal plasma antioxidant capacity. The typical time after which semen samples should be collected for standardized analysis remains unclear and needs to be further investigated.

This study was to determine and compare the effect of short abstinence (4 h) and recommended abstinence (4 days – designated as "long") periods on sperm quality based on functional and biochemical parameters in a population of normozoospermic men.

2. Materials and methods

Before the commencement of the study, ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences at Stellenbosch University. Informed written consent was obtained from all subjects and the study was performed in accordance with the Declaration of Helsinki [21]. Freshly ejaculated semen samples, with scheduled periods of abstinence, were collected from potentially fertile, healthy males (20–30 years old), taking part in the sperm donor program at the Stellenbosch University Reproductive Research Group. All samples were collected according to the WHO guidelines [2] by means of masturbation, in a private room adjoining the andrology laboratory. The first sample was collected at 8:00 am after exactly 4 days of abstinence. The second sample was collected from the same donor after 4 h subsequent to the first collection. One hundred sets of samples were included in this study on the basis of the following inclusion criteria for the first sample: sample volume $\geq$1.5 mL, sperm concentration $\geq 15 \times 10^6$/mL and total sperm motility $\geq$40% [2].

Samples were delivered to the laboratory within an average of ten minutes and analysed immediately after liquefaction. Semen volume was measured by weighing of the sample container, considering the density of semen to be 1 g/mL [22]. Semen pH was assessed by pH-indicator paper (Merck Millipore, Darmstadt, Germany).

Viscosity was assessed, according to filling time of a single chamber in a disposable 8 chamber 20-μm depth slide (SC 20-01-08-B; Leja® Products B. V., Nieuw-Vennep, the Netherlands). The results were quantified according to Rijnders et al. [23] and expressed in the unit Centipoise (Pc).

The sperm concentration and motility/kinematic parameters were determined with Computer Aided Sperm Analysis (CASA) (Sperm Class Analyzer version 5.4 - SCA®, Microptic, S.L., Barcelona, Spain) with a disposable eight-cell chamber Leja slide. The following SCA® settings were used; heating stage set at 37 °C; green filter; pH1 condenser; positive phase contrast observation setting; brightness ± 400; contrast ± 100; objective, 10×; eyepiece, 10×; capture, 50 images per second. The system analysed the following motility parameters: Fast progressive motility (Type A), slow progressive motility (Type B), non-progressive motility (Type C), Immotile (Type D), in addition to a number of kinematic parameters including curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), Wobble (WOB), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF).

For sperm morphology assessment, an air dried smear of each semen sample was fixed and stained with SpermBlue stain (SpermBlue®, Microptic, S.L., Barcelona, Spain) according to the manufacturer's guidelines [24]. Stained spermatozoa were evaluated by Computer Aided Sperm Morphology Analysis (CASMA) using the SCA® system (blue filter, 100× oil immersion objective, 10× eyepiece, brightness ± 435, contrast = 100). A total of 100 spermatozoa from randomly selected microscopic fields of the slide were analysed according to WHO guidelines [2].

Sperm viability was estimated by means of a dye exclusion technique [2], using the membrane-impermeant Eosin-Nigrosin stain (Sigma-Aldrich, St Louis, MO, USA). From each sample, 100 spermatozoa were evaluated in duplicate using the counter module of the SCA® system (positive phase contrast observation setting; a 20× objective lens; a 10× eyepiece and a blue filter). The number of pink-stained (non-viable) spermatozoa and those unstained (viable) were counted in various randomly selected fields. Results were expressed as percentage of viable spermatozoa.

Acrosome reacted spermatozoa were measured according to Goss et al. [25], using Fluorescein isothiocyanate-labelled Pisum sativum agglutinin (FITC-PSA) (Dako, North America, Inc.) The slide was visualized with fluorescent microscopy (Nikon Corporation, Tokyo, Japan); with a green fluorescein filter at ×1000 magnification and oil immersion at 510–560 nm. 200 spermatozoa were counted in various randomly selected fields. Results were expressed as percentage acrosome intact (bright and uniformly green fluorescing acrosome region) and acrosome reacted spermatozoa (no fluorescence or dull green fluorescing acrosomes).

Sperm DNA fragmentation was determined by using a terminal deoxynucleotidyl transferase–mediated fluorescein-TUNEL assay with an APO-DIRECT™ kit (BD Biosciences Pharmingen, San Diego, CA, USA) as described by Sharma et al. [26]. Spermatozoa within the PI/RNase solution were analysed by flow cytometry (Becton Dickinson FACSCalibur analyser, Life Technologies, San Jose, CA, USA). The green (FITC-dUTP) fluorescence intensity was measured in the FL-1 channel (530/30 nm bandpass filter), whereas the red (PI) fluorescence was set to be measured in the FL-2 channel (585/42 nm bandpass filter). The output data were imported and analysed using Flowjo© V10 software (FlowJo, Ashland, Or, USA). Results were expressed as percentage of DNA fragmented spermatozoa.

Intracellular superoxide ($O_2^{-\bullet}$) was quantified according to Goss et al. [25] using dihydroethidium (DHE) as a probe. The fluorescence signals of the labelled spermatozoa were assessed by means of flow cytometry, and DHE emissions were detected in the FL-2 channel. Results were expressed as median DHE fluorescence intensity (MFI).

Thiobarbituric acid reactive substances (TBARS) levels in seminal plasma were measured, as described by Jentzsch et al. [27], by means of spectrophotometric methods using a SPECTRA-max PLUS-384 spectrophotometer with SoftMax® Pro 4.8 software (Molecular Devices Corporation, Labotec Industrial Technologies, Cape Town, South Africa) for data acquisition and analysis. Molar extinction coefficient (1.54×10^5/M/cm) was used to calculate concentrations. Values were expressed in μmol/L.

Catalase activity was measured according to Aebi [28]. The rate of decomposition of H_2O_2 was measured spectrophotometrically (SPECTRAmaxPLUS-384, Molecular Devices, San Francisco, CA, USA) at 240 nm. Data were expressed as units/mL (U/mL).

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