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ARTICLE

Easy sperm processing technique allowing exclusive accumulation and later usage of DNA-strandbreak-free spermatozoa

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Thomas Ebner, PhD, graduated with honours from the Paris Lodron University of Salzburg, Austria in 1992. After completing his doctorate and post-doctoral theses, he became a university lecturer in Salzburg. He has published more than 80 papers as first and co-author. Research interests include non-invasive IVF selection processes, laser application, vitrification and culture media. He was certified as a senior clinical embryologist in 2008. Currently he is scientific director of the European School of ART in Linz.

Abstract Sperm DNA fragmentation is increased in poor-quality semen samples and correlates with failed fertilization, impaired preimplantation development and reduced pregnancy outcome. Common sperm preparation techniques may reduce the percentage of strandbreak-positive spermatozoa, but, to date, there is no reliable approach to exclusively accumulate strandbreak-free spermatozoa. To analyse the efficiency of special sperm selection chambers (Zech-selectors made of glass or polyethylene) in terms of strandbreak reduction, 39 subfertile men were recruited and three probes (native, density gradient and Zech-selector) were used to check for strand breaks using the sperm chromatin dispersion test. The mean percentage of affected spermatozoa in the ejaculate was 15.8 ± 7.8% (range 5.0–42.1%). Density gradient did not significantly improve the quality of spermatozoa selected (14.2 ± 7.0%). However, glass chambers completely removed 90% spermatozoa showing strand breaks and polyethylene chambers removed 76%. Both types of Zech-selectors were equivalent in their efficiency, significantly reduced DNA damage ($P < 0.001$) and, with respect to this, performed better than density gradient centrifugation ($P < 0.001$). As far as is known, this is the first report on a sperm preparation technique concentrating spermatozoa unaffected in terms of DNA damage. The special chambers most probably select for sperm motility and/or maturity.

KEYWORDS: density gradient centrifugation, sperm chromatin dispersion test, sperm motility, strand breaks, Zech-selector

Introduction

A relatively high number of patients fail to achieve pregnancy despite the obvious absence of a male or female factor of infertility. It is likely that many of these couples actually present with a genomic male factor, including meiotic alterations, aneuploidy or sperm DNA damage (Sakkas and Alvarez, 2010). In particular, sperm DNA fragmentation

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is increased in poor-quality semen samples and correlates with failed fertilization, impaired preimplantation development and reduced pregnancy outcome (Borini et al., 2006; Carrell et al., 2003; Duran et al., 2002; Evenson et al., 1999; Seli et al., 2004; Velez de la Calle et al., 2008; Zini et al., 2008).

Several tests have been established for the analysis of sperm DNA fragmentation. Amongst others, TdT-mediated-dUTP nick-end labelling (TUNEL) (Gorczyca et al., 1993), comet assay (Enciso et al., 2009; Hughes et al., 1996) and sperm chromatin structure assay (SCSA) (Evenson et al., 2002) as well as the sperm chromatin dispersion (SCD) test (Fernandez et al., 2003) are the approaches most commonly used in IVF laboratories. These assays can be subdivided into two categories, those directly detecting DNA damage (e.g. TUNEL) and those measuring DNA fragmentation after a rather mild denaturation process (e.g. SCSA, SCD). Although direct proof of strand breaks would be appreciated, all of the above mentioned tests unveil certain limitations (Bungum et al., 2004; Neguescu et al., 1998; Sakkas and Alvarez, 2010).

One important aspect with respect to sperm DNA fragmentation is the question whether breaks are single- or double-stranded since single-stranded defects are probably easier to repair as compared with double-stranded DNA breaks (Sakkas and Alvarez, 2010). In this respect it should be kept in mind that the processing of spermatozoa could also cause an apparent increase in DNA strand breaks (Dalzell et al., 2004; Donnelly et al., 2001; Gosálvez et al., 2009; Twigg et al., 1998); thus, the sperm processing technique applied for removing DNA-damaged spermatozoa is of utmost importance (Sakkas and Alvarez, 2010).

Common sperm preparation techniques may reduce the percentage of strandbreak-positive spermatozoa (Ahmad et al., 2007; Jackson et al., 2010; Marchesi et al., 2010), but, to date, there is no reliable approach to completely filter out spermatozoa with strand breaks from an ejaculate.

This study was started in order to test the efficiency of a rather new sperm processing technique (Zech-selector) with respect to the reduction of spermatozoa with DNA damage.

Materials and methods

During the study period, 39 patients with known male subfertility who presented at the study centre's andrology laboratory for a second analysis of their ejaculate were recruited. The mean age of the men was 37.7 ± 6.5 years. An abstinence time of 3—5 days was recommended.

All ejaculates were processed and analysed strictly according to the World Health Organization (WHO) manual (1999). Half of men suffered from isolated teratozoospermia (51%). A smaller percentage had isolated astheno- (8%) or oligozoospermia (8%). However, the remaining 33% of patients showed a drop in more than one sperm parameter, including five cases of oligoasthenoteratozoospermia (OAT).

The study was approved by the Institutional Review Board of the Landes- Frauen- und Kinderklinik, Linz, Austria, and the patient received verbal information about the nature of the study. Participation in the study allowed the patients to receive the DNA analysis free of charge.

After control of liquefaction, the ejaculate was processed immediately in order to avoid excessive contact between seminal plasma and spermatozoa which could have altered chromatin packaging, thus possibly interfering with DNA staining.

It was planned to make three analyses of DNA fragmentation per patient. After sterile masturbation, a small volume (about 25 μl) of raw semen was kept in order to have a reference value (sample 1). The rest of the ejaculate was split into two unequal parts in order to treat them differently.

The first volume (1—2 ml) was processed using routine density gradient centrifugation technique (sample 2). In detail, semen was placed on the top of two layers (40% and 80%) of GM501 Gradient (Gynemed, Lensahn, Germany). After layering the sample was centrifuged at 180*g* for 20 min. Subsequently, both layers containing silane-coated colloidal silica were carefully removed and the pellet resuspended in BM1 medium (NMS Bio-Medical, Praroman, Switzerland). In order to reduce additional manipulation of the spermatozoa, only one centrifugation step was performed at 180*g* for 10 min. Finally, the purified sperm sample was incubated at 37°C for approximately half an hour, to allow for swim-up, and then strandbreak measurement was performed.

In parallel, a patented (European patent number 1,432,787) sperm selecting chamber (Zech-selector, AssTIC Medizintechnik GmbH, Leutsch, Austria) made of glass or polyethylene was filled with 1—3 ml of ejaculate (sample 3). These chambers accumulate an adequate number of motile spermatozoa without exposure to centrifugation stress (Ebner et al., 2003). In principle, both devices consist of two concentric wells which are overlaid by a U-ring and a cover glass (**Figures 1** and **2**) and progressive motile spermatozoa migrate from the ejaculate in the outer well to concentrate in the medium-filled inner well by using a capillary bridge created by the overlaying U-ring. After 1 h, a 25 μl sperm sample was taken from the central chamber and referred to further analysis.

Patients whose ejaculate had to be processed for more than 1 h (e.g. due to delayed liquefaction) were excluded from the study for the sake of homogeneity of the study group. Thus, it could be guaranteed that all three samples (neat semen, density gradient and sperm selecting chamber) were analysed within 1 h (including time for liquefaction), in other words prolonged contact with seminal plasma was avoided.

If the volume of ejaculate was large (>5 ml), both types of chambers (glass and polyethylene) were used giving four values in these patients.

It has to be clarified that the only limitation with the present sperm preparation technique is that patients diagnosed with OAT were processed slightly differently. Since sperm count and progressive motility were reduced in these ejaculates, the number of spermatozoa migrating to the medium-filled inner well was reduced. Therefore, careful removal of the inner volume (in order not to cause contamination) and subsequent concentration of the motile spermatozoa by single-step centrifugation (10 min at 180*g*) was performed.

It should be kept in mind that filling of the chambers can be tricky. It is important that a minimum volume of 2 ml is used for the polyethylene chamber and at least 3 ml should