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ARTICLE

Sperm head vacuoles are not affected by in-vitro conditions, as analysed by a system of sperm-microcapture channels

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After graduating in biology and physiology at the University of Innsbruck (Austria), Anton Neyer entered the field of IVF and clinical embryology in 2002 at the IVF Centers Prof. Zech, in Bregenz, Austria. In 2010, he became a senior clinical embryologist (ESHRE). His current research interests focus on andrology, embryo quality assessment and embryo culture techniques.

Abstract Since the introduction of the motile sperm organelle morphology examination, there has been increasing recognition of the fact that the presence of large nuclear vacuoles might have deleterious effects on embryo development. Nevertheless, one fundamental question still being debated is whether specific in-vitro conditions during the handling of semen have an impact on vacuole formation. This study's objective was to analyse whether incubation temperature (20, 37°C) or oxidative stress stimulates the formation of nuclear vacuoles. Furthermore, it examined whether vacuoles disappear in the presence of an acrosome reaction inducer. Therefore, a system of sperm-microcapture channels was developed to permit the observation of the same living spermatozoa over a period of 24 h. Neither incubation at 37°C nor induction of oxidative stress led to de-novo formation of nuclear vacuoles. Induction of the acrosome reaction using calcium ionophore A23587 did not lead to any modifications in the proportion of spermatozoa with vacuoles or to the disappearance of pre-existing vacuoles. According to these observations, it is concluded that nuclear vacuoles on the sperm head are already produced at earlier stages of sperm maturation and are not induced or modulated by routine laboratory environments.

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KEYWORDS: acrosome reaction, IMSI, MSOME, nuclear vacuoles, oxidative stress, spermatozoa

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Introduction

Numerous studies have demonstrated that the use of motile sperm organelle morphology examination (MSOME) to select spermatozoa with normal nuclear shapes prior to intracytoplasmic sperm injection (ICSI) improves IVF results in couples with previous failure of implantation (Bartoov et al., 2002, 2003; Junca et al., 2004; Berkovitz et al., 2006a; Hazout et al., 2006; Antinori et al., 2008; Nadalini et al., 2009; Cassuto et al., 2009; Takeuchi et al., 2009; Tasaka et al., 2009) and in patients with a high degree of DNA fragmentation (Berkovitz et al., 2005; Hazout et al., 2006).

It is increasingly recognized that large nuclear vacuoles (LNV) have a major negative impact on ICSI outcome in terms of blastocyst formation (Vanderzwalmen et al., 2008; Cassuto et al., 2009; Knez et al., 2011, 2012), ongoing pregnancies (Bartoov et al., 2002, 2003; Junca et al., 2004; Berkovitz et al., 2005), miscarriage rates (Bartoov et al., 2003; Berkovitz et al., 2006a,b; Antinori et al., 2008) and malformation in offspring (Berkovitz et al., 2007; Cassuto et al., 2011).

It is becoming more and more evident that the major alterations observed in spermatozoa carrying LNV are linked with chromatin condensation defects (Perdrix et al., 2010; Boitrelle et al., 2011; Cassuto et al., 2011; Franco et al., in press). Furthermore, it is assumed that aneuploidy and DNA fragmentation rates rise when vacuolization increases (Garolla et al., 2008; Perdrix et al., 2010; Franco et al., 2008; Hammoud et al., 2012; Oliveira et al., 2010; Berkovitz et al., 2005; Hazout et al., 2006). The presence of vacuoles on the sperm head might reflect nuclear weakness and/or molecular defects. It is suggested that these structures represent disorganization of sperm chromatin packaging, disaggregation of sperm chromatin fibres, abnormal chromatin remodelling during sperm maturation or persistence of histones and their possible epigenetic modifications, which, in turn, may render spermatozoa more vulnerable to DNA damage.

Although the genesis of vacuoles is not unequivocally clear, the use of intracytoplasmic morphologically selected sperm injection (IMSI) may be a helpful tool for the selection of spermatozoa. Several publications state that in-vivo formation of vacuoles occurs during spermiogenesis (Baccetti et al., 1989; Francavilla et al., 2001; Sardi-Segovia et al., 2010; Tanaka et al., 2012) and may be a direct indication of insufficient chromatin condensation (Boitrelle et al., 2011). Nevertheless, the fundamental question still remains whether specific in-vitro conditions during the preparation and the handling of semen promote the formation of LNV as a result of oxidative stress during IVF treatment.

Peer et al. (2007) and Schwarz et al. (in press) report a negative impact of temperature on the morphological integrity of sperm nuclei. It was therefore important for the present study to analyse and confirm whether the swim-up procedure, the selection of spermatozoa before ICSI or IMSI and, finally, the insemination of the oocytes are crucial steps that may impair the sperm head morphology, resulting from their prolonged exposure at 37°C.

Furthermore, during liquefaction, the semen sample is — according to its quality — exposed to round cells and leukocytes, which are potential sources of reactive oxygen species (ROS). According to Agarwal and Said (2003), the concentration of ROS positively correlates with the number of spermatozoa with amorphous heads, damaged acrosomes, mid-piece defects, cytoplasmic droplets and tail defects. Chenoweth (2007) as well as Tremellen and Tunc (2010) suggests that a variety of stressors, for example oxidative damage which is frequently observed in male infertility patients exhibiting the presence of high leukocyte concentrations, may produce crater defects in the form of deep vacuoles in mammals. Consequently, the second question of this study was whether vacuoles are produced under oxidative stress in the mature spermatozoa.

A more physiological theory has been put forward by Kacem et al. (2010). Using *Pisum sativum* agglutinin to assess the acrosome status, they postulate that sperm vacuoles, detected by MSOME, are mostly of acrosomal origin. The authors conclude that a large majority of normal, regularly shaped spermatozoa showing no vacuole have already undergone their acrosome reaction and should be selected for injection. Montjean et al. (2012) tested the effect of acrosome reaction inducers. After incubation for 90 min in either hyaluronic acid or follicular fluid, they observed a highly significant decrease in the presence of vacuoles. To confirm these observations, this study examined whether nuclear vacuoles disappear as a consequence of the acrosome reaction.

The fundamental question is whether the vacuoles observed during sperm selection are exclusively of in-vivo origin or whether one part emerges from a non-pathological in-vitro environment. The aim of this study was to ascertain whether it is feasible to observe the occurrence of nuclear vacuoles (LNV and small vacuoles) resulting from different incubation temperatures. This study also tested whether oxidative stress induces vacuole formation or whether, on the contrary, the acrosomal reaction process provokes their disappearance.

As far as is known, there is no applicable method that permits the observation of the same population of spermatozoa over a long period of time. This study therefore developed a system of sperm-microcapture channels, which permits the continuous monitoring of the same cohort of spermatozoa for a precise and accurate analysis of how individual spermatozoa respond to different conditions.

Materials and methods

Handling and preparation of semen samples

All specimens were collected by masturbation after an abstinence period of 48—72 h. Sperm concentration and motility were assessed according to WHO criteria (World Health Organization, 1999). The percentage of morphologically normal spermatozoa was analysed by MSOME (Vanderzwalmen et al., 2008; Oliveira et al., 2009). Only semen with more than 20% spermatozoa without LNV were included in this study.

The patients gave explicit consent for their excess spermatozoa which were not used for treatment to be isolated in the sperm-microcapture channels for 24 h to analyse

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