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ARTICLE

The relationship between sperm head retardance using polarized light microscopy and clinical outcomes

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Abstract In human sperm head, birefringence can be seen under polarized light resulting from highly ordered structures within the acrosome and nucleus. Selecting sperm with partial head birefringence improves success of clinical pregnancies in patients with severe male factor infertility. The aim of this study was to establish a range of retardance in sperm heads using polarized light microscopy to select an optimum sperm for intracytoplasmic sperm injection (ICSI). Sperm heads of 63 couples undergoing ICSI in women aged 38 years or younger were imaged at the time of ICSI and later analysed for retardance blinded to embryo and cycle outcomes. Sperm head retardance was similar irrespective of whether fertilization occurred. Quality of embryos on day 3 and day 5 were higher when sperm were selected with head retardance ranging from 0.56 nm or greater to 0.91 nm or less. Selection of sperm with head retardance ranging from 0.56 nm or greater to 0.91 nm or less was associated with higher clinical pregnancy rates (OR 3.74 95% CI 1.43 to 9.77). Optimum sperm for selection at the time of ICSI was with head retardance within the range 0.56 nm or greater to 0.91 nm or less.

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Introduction

Polarized light enables structures with molecular order to be observed (birefringence) when a single ray of polarized light is refracted into two polarized rays travelling at different speeds. The difference between these phases is otherwise referred to as retardance (Gianaroli et al., 2010; Oldenbourg, 1996; Oldenbourg and Mei, 1995). Although polarized light microscopy (PLM) has been used to visualize biological structures, sperm has not been widely imaged. Improvements to

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resolution, control of annotation and its computer software, PLM now allows visualization of the condensed chromatin in sperm (Oldenbourg, 1996, 2010; Oldenbourg and Mei, 1995).

It has been shown with transmission electron microscopy that sperm heads exhibit birefringence based on highly ordered structures within the acrosome and nucleus (Baccetti, 2004). The head of sperm are birefringent owing to anisotropic structures within the nuclear and acrosomal regions. The birefringence within the sperm nucleus is created by molecular order within nucleoprotein filaments oriented longitudinally. Similarly, the acrosome has birefringence caused by protein filaments oriented longitudinally (Baccetti, 2004; Gianaroli et al., 2008). The use of PLM has enabled DNA damage to be positively correlated with increased sperm head retardance (Damasceno-Vieira et al., 2008).

A study to distinguish clinical outcomes dependent on using PLM on sperm before intracytoplasmic sperm injection (ICSI) found no significant differences between fertilization and cleavage rates when using PLM (Gianaroli et al., 2008). Good-quality embryos, however, were significantly higher on day 3 in the group that used PLM to assess the sperm for injection, as well as implantation and ongoing pregnancy rates. Selection of sperm with birefringence may indicate a normal sperm structure as well as DNA integrity (Crippa et al., 2009; Gianaroli et al., 2008; Magli et al., 2012; Peterson et al., 2011).

A further study has suggested that birefringence in a sperm head can be evaluated as total or partial. The difference between the two depends on the completion of the acrosome reaction (Gianaroli et al., 2008). Partial birefringence is characteristic of the completion of the acrosome reaction. The selection of sperm with partial birefringence increases the potential of selecting a sperm with DNA integrity, and increasing clinical pregnancies of patients with severe male factor infertility (Gianaroli et al., 2008; Magli et al., 2012). With the use of the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling assay, it has been shown that sperm with total head birefringence present with significantly higher proportions of DNA fragmentation compared with sperm with partial head birefringence (Peterson et al., 2011). These studies suggest that a possible optimum range of retardance exists that reflects partial birefringence and reduce DNA fragmentation of single sperm. No studies have specifically defined the relationship between birefringence of sperm head and protoplasmic structures.

To our knowledge, no published data have quantified sperm head retardance in humans. Therefore, the aim of this study was to determine a range of retardance in human sperm heads using polarized light microscopy (PLM) that might enable the selection of the optimum sperm for intracytoplasmic sperm injection (ICSI).

Materials and methods

Patients and study design

63 fresh cycles were included that took place between August 2010 and January 2013. During this time, methodologies for ICSI remained unchanged within the laboratory. Inclusion criteria were women 38 years or younger undergoing ICSI, who had between five and 15 mature oocytes collected, blastocyst culture and elective single embryo transfer on day 5. Patients were excluded if they had had over three previous fresh cycles with no success, used donor gametes, used cryopreserved gametes or blastomere biopsy.

Sperm that were assessed as having normal morphology according to the World Health Organization (WHO, 2010) were selected for ICSI. Before injection, each sperm head was imaged using PLM. Images were saved. Birefringence of individual sperm heads were later analysed blinded to embryo and cycle outcomes. Retardance of PLM was compared with fertilization rates 18 h after ICSI, cleavage rates and quality at 66 h after ICSI on day 3, blastulation and utilization rates (i.e. number of embryos either transferred fresh or frozen for later use) at 113–121 h after ICSI on day 5 and clinical pregnancy rates. Clinical pregnancy was defined as the presence of a gestational sac (with or without a fetal heart) as per ANZARD, The Australia and New Zealand Assisted Reproduction Database (Macaldowie et al., 2013). International Review Board approval for the study was obtained from the IVF Australia Human Research Ethics Committee (approved 23 August, 2010).

Assisted reproductive technology procedure

Ovarian stimulation was carried out using Puregon FSH (Merck Sharp and Dohme, South Granville, Australia) or Gonal F (Merck Serono, Frenchs Forest, Australia) at a dosage between 150 and 300 IU per day. Monitoring of stimulation was achieved with regular blood tests and ultrasounds. Ultrasound-guided oocyte collection under sedation was scheduled 36 h after trigger injection of choriogonadotropin alfa (Ovidrel 250 μg, Merck Serono, Frenchs Forest, Australia).

All day 0 culture dishes containing COOK cleavage medium and COOK culture oil (COOK Medical, 2013, Brisbane, Australia) were prepared the day before ultrasound-guided oocyte collection and equilibrated overnight in 6% carbon dioxide humidified gas atmosphere at 37.0°C.

Once oocytes were collected, they were taken to the laboratory where they were denuded of their cumulus and coronal cells. Oocytes were assessed for maturity. Metaphase I and Metaphase II oocytes were kept separate and transferred into the culture dish.

Timing of ICSI occurred 39–41 h after trigger injection to allow for optimum oocyte maturity and fertilization (Kilani et al., 2009) within a glass-bottomed tissue culture dish (Flurodish, World Precision Instruments, Florida, USA). Each mature oocyte (maximum 8) was placed into a 5 μL droplet of warmed COOK gamete buffer along with two droplets of 7% polyvinylpyrrolidone (PVP) (Sage-Cooper Surgical, Connecticut, USA), one droplet containing an aliquot of the prepared sperm. The droplets were then covered with warmed COOK culture oil. The ICSI dish was prepared and placed on 37°C warm plate for 20 min before gametes were transferred into and ICSI occured to ensure all media were pre-warmed.

Sperm preparation

Sperm was prepared by placing 2.0 ml of each patient's semen sample over a discontinuous gradient separated using 40% and 80% Puresperm (Nidacon, Molndal, Sweden). The

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