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ARTICLE

Influence of temperature and sperm preparation on the quality of spermatozoa

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Annelies Thijssen graduated in July 2012 with a Masters in biomedical sciences – clinical molecular sciences from the Transnational University Limburg, Diepenbeek, Belgium. She finished her master thesis on 'Methods for optimal sperm selection and preservation in the IVF laboratory' at the Genk Institute for Fertility Technology of the Ziekenhuis Oost-Limburg, Genk, Belgium. In September 2012, she started a PhD project 'Sperm banking in Belgium: medical, ethical and economical aspects', a collaboration between Hasselt University and the Ziekenhuis Oost-Limburg with Willem Ombelet as promoter.

Abstract This study investigated the effects of long-term (24 h) in-vitro sperm incubation at room temperature (RT; 23°C) versus testis temperature (35°C) on various sperm-quality parameters. Semen samples ($n = 41$) were prepared both by density-gradient centrifugation (DGC) and the swim-up technique in order to compare the influence of sperm preparation on sperm quality after incubation. Progressive motility and morphology were significantly higher after incubation at RT compared with 35°C ($P < 0.001$ and $P < 0.01$, respectively). The proportions of acrosome-reacted, apoptotic and dead spermatozoa were significantly lower in samples incubated for 24 h at RT compared with 35°C ($P < 0.001$, $P = 0.01$ and $P < 0.001$, respectively). The number of motile, morphologically normal, non-acrosome-reacted and nonapoptotic spermatozoa recovered after sperm preparation was significantly higher in DGC compared with swim-up samples ($P < 0.001$). However, spermatozoa prepared by swim-up showed better survival after incubation compared with DGC-prepared spermatozoa, especially when incubated at 35°C. In conclusion, this study indicates a significantly better and longer preservation of sperm quality when incubation is performed at RT. These findings may convince laboratories to change the routinely used sperm storage conditions in order to maximize the quality of the prepared sperm sample.

KEYWORDS: acrosome reaction, apoptosis, flow cytometry, sperm preparation, sperm quality, temperature

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Introduction

Sperm quality is a very important factor in the IVF laboratory since male infertility accounts for 20—30% of the infertility cases (ESHRE, 2012) and treatment options are mainly based on sperm-quality improvement techniques (Elder and Dale, 2011). The sperm-quality parameters routinely used in the IVF laboratory are concentration, motility, viability and morphology (Ombelet et al., 1997a; WHO, 2010). However, sperm-function assessments could additionally provide a valuable indication of sperm quality. Fertilization of an oocyte with an apoptotic spermatozoon has been shown to have detrimental effects on fertilization rate, implantation rate and embryo survival in assisted reproduction treatment (de Vantery Arrighi et al., 2009). Furthermore, occurrence of the acrosome reaction is essential to achieve fertilization in intrauterine insemination (Grunewald et al., 2006). Both of these sperm-function parameters can be easily determined by use of flow cytometry; however, this technique is not routinely available in the IVF laboratory.

The quality of the sperm sample is influenced by various laboratory factors, including: (i) use of different sperm preparation techniques (Boomsma et al., 2007; Chen and Bongso, 1999; Marchesi et al., 2010); (ii) temperature during sperm preparation (Franken et al., 2011; Otsuki et al., 2008); (iii) time interval from sperm preparation to IUI (Yavas and Selub, 2004); and (iv) temperature during long-term in-vitro incubation of prepared sperm samples (Aitken et al., 1996; Makler et al., 1981; Matsuura et al., 2010; Petrella et al., 2003). It is well known that the testis temperature is approximately 2—3°C below body temperature (Elder and Dale, 2011), as this is required for the production and maintenance of viable spermatozoa (Appell et al., 1977; Setchell, 1998). Despite the numerous articles published on the harmful effects of long-term in-vitro sperm incubation at body temperature, it is still current practice in most IVF laboratories to store prepared sperm samples at this unfavourable temperature prior to their use in assisted reproduction treatment (Matsuura et al., 2010).

Therefore, this study aimed to examine the effects of long-term (24 h) in-vitro sperm incubation at room temperature (RT; 23°C) versus testis temperature (35°C) on various sperm-quality parameters. In order to compare the influence of sperm preparation on sperm quality, native semen samples were split and prepared either by density-gradient centrifugation (DGC) or the swim-up technique. Conventional sperm-quality parameters such as concentration, progressive motility, normal morphology and viability (WHO, 2010) were taken into account. Additionally, sperm function was analysed by determining the proportions of spontaneously acrosome-reacted and apoptotic spermatozoa in the sample via flow cytometry.

Materials and methods

Collection of semen samples

Following a 2—7-day abstinence period, semen samples ($n = 41$) were obtained through masturbation from patients presenting at the fertility centre for an initial diagnostic semen analysis. Inclusion criteria were a sperm concentration of $\geq$15 million/ml and a motility of $\geq$32% progressively motile spermatozoa, according to World Health Organization (WHO) reference limits for normal semen samples (WHO, 2010). Routine sperm analysis and sperm preparation were initiated after liquefaction at RT (23°C) and within 1 h of production. Ethical approval of the study was granted by the ethics committee of Ziekenhuis Oost-Limburg (reference 13/055U, approved 31 May 2013).

Experimental design

Figure 1 gives a schematic overview of the experimental design. The native semen sample was split and one half of the sample was prepared by DGC while the other half was prepared by the swim-up technique. Aliquots of DGC and swim-up prepared sperm samples were then incubated for 24 h at RT or 35°C. Prior to incubation, samples were gassed with a gas mixture (6% CO_2, 5% O_2 and 89% N_2) for 90 s in order to maintain the pH of the incubation medium. Samples incubated at 35 ± 0.5°C were placed in a Labotect transport incubator (Cell-Trans 4016; Labor-Technik, Göttingen, Germany); while the RT samples were incubated on a bench in the laboratory (23 ± 1°C). Although RT samples were exposed to light during incubation, in contrast to the samples incubated at 35°C, this should not have an influence on sperm quality (Makler et al., 1980). For the final hour of the incubation period, the samples that were stored at RT were placed in the Labotect transport incubator in order to restore sample temperature to 35°C and obtain an equivalent motility count for both samples (Birks et al., 1994). Analysis of sperm-quality parameters, routine as well as flow cytometry measurements, was performed in duplicate on the native sample, after sperm preparation and after incubation.

Sperm preparation

Earle's balanced salt solution (EBSS; E3024; Sigma; Origio, The Netherlands) was supplemented with sodium pyruvate (S8636; Sigma; Origio), penicillin—streptomycin 100 × solution (Life Technologies, Invitrogen, Belgium) and 5% human serum albumin (HSA; Red Cross, Belgium) for use in sperm preparation (Ombelet, 1998). A three-layer gradient (90%, 70%, 40%) was prepared by diluting PureSperm 100 (Nidacon International; Origio) with supplemented EBSS (Chen and Bongso, 1999). The 90% gradient layer of 1.5 ml was layered

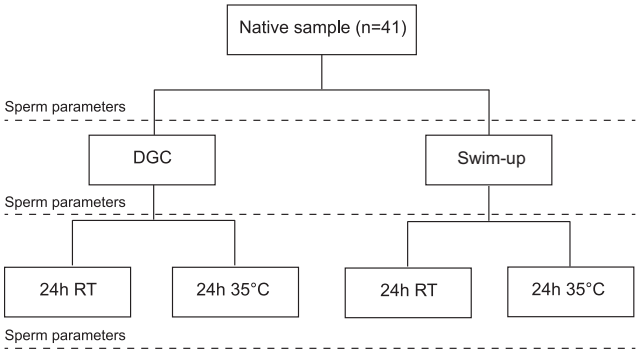

Figure 1 Schematic overview of the experimental design. DGC = density-gradient centrifugation; RT = room temperature (23°C).