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ARTICLE

Traceability of human sperm samples by direct tagging with polysilicon microbarcodes

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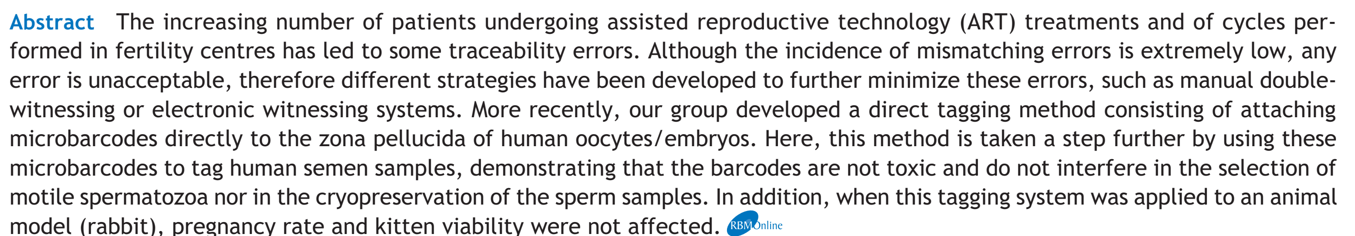
Sergi Novo obtained his PhD in Cell Biology from the Universitat Autònoma de Barcelona in 2013. His PhD was focused on developing a system to track oocytes and embryos during assisted reproductive treatments. He recently started working in a human assisted reproduction centre.



Abstract The increasing number of patients undergoing assisted reproductive technology (ART) treatments and of cycles performed in fertility centres has led to some traceability errors. Although the incidence of mismatching errors is extremely low, any error is unacceptable, therefore different strategies have been developed to further minimize these errors, such as manual double-witnessing or electronic witnessing systems. More recently, our group developed a direct tagging method consisting of attaching microbarcodes directly to the zona pellucida of human oocytes/embryos. Here, this method is taken a step further by using these microbarcodes to tag human semen samples, demonstrating that the barcodes are not toxic and do not interfere in the selection of motile spermatozoa nor in the cryopreservation of the sperm samples. In addition, when this tagging system was applied to an animal model (rabbit), pregnancy rate and kitten viability were not affected.

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Introduction

The growing number of patients who resort to assisted reproductive techniques (Centers for Disease Control and Prevention, A. S. for R. M, 2013; Ferraretti et al., 2013; Macaldowie et al., 2013) makes it indispensable to set up a reliable traceability control of the samples derived from the practice of these techniques. Despite the fact that the risk of sample mismatching errors (mix-ups) is extraordinarily small, several mix-ups have been reported in fertility clinics worldwide (Alvarez and Tweed, 2007; Bender, 2006; Devlin, 2009; Parry, 2011; Spriggs, 2003). To prevent such mix-ups, several strategies have been developed in the context of human assisted reproduction technologies (ART). One of them is the manual double-witnessing protocol used in most ART laboratory procedures, first recommended by the Human Fertilization and Embryology Authority in 2003 (Brison et al., 2004), and later by the European Society of Human Reproduction and Embryology (Magli et al., 2008). However, the effectiveness of the manual double-witnessing has been questioned because of the risk of involuntary automaticity (Toft and Mascie-Taylor, 2005). Consequently, a new generation of double-witnessing systems were generated, the electronic witnessing systems, which allow the automation of the process of sample identification during the laboratory process (Adams and Carthey, 2006). These systems are based on the labelling of all labware used for each particular case with barcode adhesive stickers (Matcher™, IMT, UK) or radio frequency identification adhesive labels (IVF Witness™, Research Instruments, UK), which can be identified by special readers connected to a computer.

An important limitation of these current systems is that the label is linked to the container and not directly to the sample. Therefore, the possibility of misidentification persists, as gametes and embryos are moved from one container to another several times during the course of an ART cycle. For this reason, our group proposed a direct gamete/embryo tagging system in which the tag and the sample would move together throughout the whole ART process. This system, initially developed for oocytes and embryos, is based on the attachment of polysilicon microbarcodes (barcodes, from now on) to the outer surface of the zona pellucida by means of their biofunctionalization with the wheat germ agglutinin lectin (Novo et al., 2013; Penon et al., 2012). The application of this system is simple, safe, highly efficient and allows the identification of human oocytes and embryos during the various steps of an ART cycle (Novo et al., 2014). However, due to the small size and very high numbers of sperm cells in a semen sample, the system cannot be applied to sperm cells using the same approach.

After searching the literature for all the reported mix-up errors, approximately two-thirds were found to be related to sperm misidentification; therefore it was decided to adapt the direct tagging system for semen samples, using non-biofunctionalized barcodes. In this case, the aim is to label the whole sample, instead of each individual sperm cell. The validation of this new approach was focused on two goals: to rule out any potential detrimental effect of barcodes on sperm viability, and to test the effectiveness of the tagging system during the laboratory procedures typically conducted in a human ART cycle. Moreover, any possible effect of barcodes on the ability of spermatozoa to fertilize was assessed in an animal model by artificially inseminating female rabbits with tagged rabbit spermatozoa.

Materials and methods

The use of human samples, animal care and protocols employed in this study were approved by the Ethics Committee on Animal and Human Research of the Universitat Autònoma de Barcelona and by the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya (protocol numbers 2115, approved on 24/05/2013 and 2299, approved on 13/12/2013).

Human sperm samples

Eight donors from 24 to 34 years old were recruited after a public call for semen donation for this specific study. Informed consent was obtained from all of them. Semen samples were obtained by masturbation after 3 to 5 days of sexual abstinence and ejaculated into a clean sterile container (Deltalab, Spain). Samples were processed within 60 min after ejaculation. Upon arrival, containers were placed over a hotplate (37°C) to allow seminal liquefaction. Afterward, a routine semen analysis (volume, pH, concentration, motility and morphology) was performed according to the World Health Organization's *Laboratory Manual for the Examination and Processing of Human Semen* (WHO, 2010).

Barcode fabrication and design

Barcodes were fabricated on 4-inch p-type (100) silicon wafers through silicon microtechnologies used for microelectromechanical systems fabrication, as previously described in detail (Novo et al., 2011).

Barcodes are designed as two-dimensional polysilicon microparticles 10 μm in length, 6 μm in width and with a thickness of 1 μm. They have a start reading marker and carry 8 bits of binary codification (two rows of 4 rectangular bits), which can be easily converted into a decimal number (**Figure 1a**) and associated with a specific donor (**Figure 1b–i**). The presence of 8 bits allows 256 different possible combinations (decimal numbers 0 to 255). However, the fabrication of barcodes with more bits or with other shapes and dimensions is available and could exponentially increase the number of possible combinations.

Sample tagging

Sample tagging containers were prepared in advance, by placing 120,000 barcodes of a specific codification, diluted in absolute ethanol, into each sterile container. Once ethanol was evaporated, containers were stored at room temperature from several days to up to 3 months.

For sample tagging, liquefied seminal samples were transferred to tagging containers and homogenously mixed with the barcodes by pipetting up and down (**Figure 2**).

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