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ARTICLE

Effects of in-vitro or in-vivo matured ooplasm and spindle-chromosome complex on the development of spindle-transferred oocytes

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Abstract To study the effects of in-vitro matured ooplasm and spindle-chromosome complex (SCC) on the development of spindle-transferred oocytes, reciprocal spindle transfer was conducted between in-vivo and in-vitro matured oocytes. The reconstructed oocytes were divided into four groups according to their different ooplasm sources and SCC, artificially activated and cultured to the blastocyst stage. Oocyte survival, activation and embryo development after spindle transfer manipulation were compared between groups. Survival, activation, and cleavage rates of reconstructed oocytes after spindle transfer manipulation did not differ significantly among the four groups. The eight-cell stage embryo formation rates on day 3 and the blastocyst formation rate on day 6 were not significantly different between the in-vitro and in-vivo matured SCC groups when they were transplanted into in-vivo matured ooplasm. The rate of eight-cell stage embryo formation with in-vitro matured ooplasm was significantly lower ($P < 0.05$) than that of embryos with in-vivo matured ooplasm, and none of the embryos developed to the blastocyst stage. Therefore, SCC matured *in vitro* effectively supported the in-vitro development of reconstructed oocytes. Ooplasm matured *in vitro*, however, could not support the development of reconstructed oocytes, and may not be an appropriate source of ooplasm donation for spindle transfer.

KEYWORDS: in-vitro maturation, mtDNA, oocytes, parthenogenetic activation, spindle transfer, spindle-chromosome complex

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Introduction

Mature human oocyte contains between 19,000 and 25,000 mitochondria based on electron microscopic morphometry, and the estimated mtDNA copies per oocyte range from 90,000 and about 150,000, according to measurements of the mitochondrial gene ATPase 6 (Van Blerkom, 2004, 2008). In contrast, sperm cells only contain between 10 and 700 copies of mtDNA (Hecht et al., 1984; Shitara et al., 2000), and sperm mtDNA is eliminated quickly after fertilization by ubiquitination followed by selective digestion by endonucleases (Sutovsky et al., 1999, 2003). Therefore, mtDNA is transmitted entirely through the maternal line via the mitochondria contained in the ooplasm.

Since the first report of diseases caused by mtDNA mutations in 1988 (Wallace et al., 1988), more than 150 mtDNA mutations associated with serious human disorders have been identified. These disorders include myopathies, neurodegenerative diseases, diabetes, cancer, and infertility (Solano et al., 2001). The transfer of the nuclear genome into an enucleated oocyte containing normal mitochondria is probably the first choice to effectively prevent the transmission of mtDNA disorders (Tachibana et al., 2009). Although prenatal genetic diagnosis can select embryos with a reduced mutation load, variation between blastomeres in single embryos limits the effectiveness of such screening.

Spindle transfer, essentially a modified cloning technique, transfers the meiotic spindle and attached chromosomes (spindle-chromosome complex, SCC) from one mature oocyte to another to select for a cytoplasm or mtDNA background. This technology has been used to generate both cattle and mice after subsequent fertilization (Bai et al., 2006; Bao et al., 2003; Wakayama et al., 2004; Wang et al., 2001), and has generated live monkeys (Macaca mulatta) after sperm injection (Tachibana et al., 2009). Similar techniques, such as germinal vesicle transfer and pronuclear transfer, have also been used to prevent the transmission of mtDNA from one generation to the next (Craven et al., 2010; Cummins, 1998; Fulka, 2004; Takeuchi et al., 2001; Trounson, 2001). Potential problems could arise, however, as the transferred germinal vesicle or pronuclear transfer is still obviously surrounded by mitochondria, which will also be carried over into the donor ooplasm. As pronuclear transfer also involves the destruction of a zygote, usage may be restricted because of ethical and moral considerations.

Recent studies using donated human in-vivo matured oocytes for spindle transfer after ovarian hyperstimulation treatment are promising. Tachibana et al. (2009, 2013) demonstrated the feasibility and outcomes of spindle transfer with human oocytes donated by healthy volunteers on the basis of their prior studies in a monkey model. They recruited seven volunteers who underwent ovarian stimulation. A total of 106 in-vivo matured metaphase II (MII) oocytes were retrieved in their study (Tachibana et al., 2013). Although these pioneering works are encouraging, more studies with human oocytes are needed to further optimize spindle transfer protocols and ensure that these procedures are safe. Donated human oocytes matured *in vivo* are difficult to acquire, hindering the further development of spindle transfer techniques and its possible use in preventing inherited mtDNA diseases.

Immature oocytes retrieved from infertile women undergoing IVF treatment are commonly used for research purposes. Most of these oocytes mature *in vitro* after 24 h of in-vitro culture. These in-vitro matured oocytes from infertile patients could therefore be a potential cost-effective source of ooplasm or karyoplasts for spindle transfer. If the cytoplasm, spindle apparatus of in-vitro matured oocytes could support the development of spindle transfer embryos, the exogenous hormone treatment of donor or recipient patients is unnecessary. Such treatments are costly and can cause severe health problems. It is unclear, however, whether in-vitro matured human ooplasm or karyoplasts are capable of supporting the development of reconstructed oocytes.

To study the effects of in-vitro matured oocytes (ooplasm and karyoplast, respectively) on spindle transfer efficiency, reciprocal spindle transfer between in-vivo matured and in-vitro matured human oocytes was conducted, and the reconstructed oocytes were parthenogenetically activated rather than fertilized with donor sperm to avoid the generation of human embryos. The in-vitro development of parthenogenetic embryos was examined to evaluate the developmental potential of reconstructed oocytes with differently sourced ooplasm and SCC.

Materials and methods

Oocyte donation, experimental design and ethical approval

Patients undergoing intracytoplasmic sperm injection were routinely asked to donate their immature oocytes for research at the Reproductive Medicine Center, First Affiliated Hospital, Sun Yat-sen University. More than 95% of them agreed to donate their immature oocytes for research and provided written informed consent before treatment. Therefore, in-vitro matured oocytes were available almost every day for various studies in our centre, including the present study of spindle transfer.

Mature oocytes were donated by azoospermic couples who had been diagnosed with severe spermatogenic failure before IVF treatment. The couples were fully informed and aware that they were at high risk of sperm failing to inseminate their oocytes. Between 2009 and 2014, six such couples were diagnosed with severe Spermatogenic Failure. After biopsy on both sides of the testis, and an extensive search under the microscope, one of the couples decided to cryopreserve their oocytes for possible future use. The other five couples chose to undergo intrauterine insemination with donated sperm in an attempt to achieve pregnancy (the women were young and did not have any infertility factors) and donated all of their oocytes for spindle transfer research. Written informed consent was obtained before spindle transfer. All of the patients followed a protocol using gonadotrophin-releasing hormone agonist and Gonal-F (Gonal-F; Merck Serono, The Netherlands) for ovarian stimulation (Khoudja et al., 2013). Oocyte retrieval was carried out 34–36 h after the administration of 10,000 IU HCG (Ovidrel; Merck Serono, The Netherlands). Oocytes lacking a polar body were considered immature (germinal vesicle and metaphase I oocytes) after stripping for intracytoplasmic sperm injection (ICSI) on the day of oocyte retrieval (day 0). Only the oocytes remaining at the metaphase I stage were used for in-vitro maturation.