Reproductive BioMedicine Online (2014) 28, 401–404

www.sciencedirect.com
www.rbmonline.com

SHORT COMMUNICATION

# Aggregated chromosomes transfer in human oocytes

Junko Otsuki [a,*], Yasushi Nagai [a], Tadashi Sankai [b]

[a] *Nagai Clinic, 607-1 Kamihikona, Misato, Saitama 341-0004, Japan;* [b] *Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Japan*
[*] Corresponding author. *E-mail address:* otsuki.midori.junko@gmail.com (J Otsuki).

Junko Otsuki obtained her PhD in embryology from Ochanomizu University in 2007. As a laboratory director of Nagai Clinic, she has investigated quality control problems related to mineral oil and human serum albumin in IVF and has defined two types of cytoplasmic abnormalities in human oocytes. Recent work involving molecular and morphological aspects of meiosis has discovered a phase of chromosome aggregation that precedes chromosome separation and also a novel mechanism that may be responsible for the formation of hermaphrodite individuals. Junko has also investigated cytokinesis in mouse and human oocytes. At the ESHRE conference in 2007, she won the prize for the best paper on assisted reproduction laboratory research.

**Abstract** Germinal-vesicle (GV) transfer, spindle–chromosome complex transfer in metaphase-II oocytes and two pronuclei transfer have been evaluated as possible treatments for patients who have mitochondrial diseases. However, GV transfers often lead to heteroplasmy while the other two methods are frequently associated with aneuploidy. The present study used a new method based on the transfer of aggregated chromosomes, which occurs in human oocytes, before the metaphase spindle is established.


**KEYWORDS:** aggregated chromosomes, aneuploidy, heteroplasmy, microinjection, mitochondrial disease, nuclear transfer

## Introduction

In women, the incidence of oocyte aneuploidy rises steadily with increasing age, from an abnormality rate of about 1 in 500 at the youngest maternal ages to about 1 in 20 at age 45 (Hook, 1981). This has been attributed to an age-dependent reduction in the number of mitochondria and copies of mtDNA in oocytes (May-Panloup et al., 2007) and/or an age-dependent decline in mitochondrial function. Such mitochondrial changes may impair nuclear spindle activity and chromosome segregation during the extrusions of the first and second polar bodies, possibly due to lower energy production in the affected oocytes.

Furthermore, mutations of mtDNA cause genetic diseases that are maternally inherited through the cytoplasm. For patients who have mitochondrial diseases, nuclear transfer or cytoplasmic transfer was introduced to prevent transmission of mtDNA mutations from the carrier women to their offspring. At this stage, three methods for nuclear transfer have been proposed. These include germinal vesicle (GV), metaphase-II spindle–chromosome complex and pronuclear transfers using donor oocytes and embryos. However, the latter two procedures are performed after first polar body extrusion, where a high rate of chromosome desegregation occurs depending on the quality of oocytes. Moreover, it has been reported that following spindle–chromosome complex transfer, a significant proportion of zygotes (52%)

1472-6483/$ - see front matter 
http://dx.doi.org/10.1016/j.rbmo.2013.10.024

showed abnormal fertilization as determined by an irregular number of pronuclei (Tachibana et al., 2013). It is for this reason that it may be better to perform nuclear/chromosome transfer before chromosome segregation starts during first meiosis. Moreover, the problem with GV transfer is that mitochondria are densely accumulated around the germinal vesicle.

This study group tried to overcome the above problems by injecting oocytes with aggregated chromosomes (AC). A phase during which meiotic human chromosomes become aggregated or assembled is a feature of human oocytes that was confirmed in a previous study (Otsuki and Nagai, 2007). In human oocytes, this unique characteristic, which appears to be chromosome aggregation, is visible from the first or second polar body extrusions to metaphase-II spindle formation. The aggregation is also visible from GV breakdown to metaphase-I spindle formation (Otsuki and Nagai, 2007). Following the completion of nucleolar breakdown, chromosomes become assembled into a single aggregation that heralds the start of nuclear membrane breakdown (Otsuki and Nagai, 2007).

## Materials and methods

### GV-stage oocytes

GV-stage human oocytes that were retrieved unexpectedly during follicular aspirations for IVF and intracytoplasmic sperm injection (ICSI) procedures were donated by patients who provided informed consent. The study was conducted at the Nagai Clinic after approval was obtained from the Ethics Committee (reference number Hum-9, approved 24 July 2012). Presence of the GV stage was confirmed using an inverted microscope after partial removal of granulosa cells.

### Chromosome transfer

The GV-stage oocytes were cultured in HTF medium (InVitroCare, Japan) containing 1% human serum albumin (HSA; InVitroCare) in an incubator at 37°C with 5% $CO_2$ at most for 10 h until nucleolar breakdown and chromosome aggregation were observed. Then the oocytes at the AC phase were transferred to HTF HEPES medium (InVitroCare) containing 1% HSA and cytochalasin B (5 μg/ml; Sigma, Japan) in a glass-bottomed culture dish at 37°C immediately before the manipulation commenced to avoid oocyte membrane damage. The AC in an oocyte were extracted with ICSI injection pipettes, that had a tip internal diameter of 5–6 μm (MT-INJ30; Kitazato BioParma Co, Japan) or 6 μm (PINU06–25FT; Prime Tech, Japan) using a Piezo Micro Manipulator (PMM-150FU; Prime Tech) and an inverted microscope (Eclipse TE2000; Nikon). After the removal of the AC, the oocyte (oocyte A) was transferred to HTF medium (InVitroCare) containing 1% HSA and incubated at 37°C with 5% $CO_2$ for 1 h. After that, another oocyte (oocyte B) that attained the AC phase was placed into a drop of HTF HEPES medium containing 1% HSA and cytochalasin B (5 μg/ml) and the oocyte without AC (oocyte A) was placed in a different drop of HTF HEPES medium containing 1% HSA without cytochalasin B in the same culture dish. Subsequently, the AC were extracted from oocyte B and injected into oocyte A. Then the AC-transferred oocytes were cultured in HTF medium containing 1% HSA for 1 day. As a control, the same amount of cytoplasm was extracted from 10 oocytes of the AC phase in the same conditions and cultured for a day.

### Time-lapse observations

In order to observe cytoplasmic movements within a host oocyte that received AC transfer and to record the extrusion of the first polar body, time-lapse recordings were performed using a time-lapse monitoring system (Primo Vision, Vitrolife, Japan). The cytoplasmic images were captured every 5 min.

## Results

The AC were tightly condensed (**Figure 1**A, F) and hardly become fragmented. The AC could be clearly observed and enucleation was easily confirmed under differential interference contrast (DIC) optics: i.e. it was as clear as spindle observation under a polscope (**Figure 1**B, G). The AC were consistently extracted with a small amount of cytoplasm (**Figure 1**C, H). The chromosome clump could be injected with no difficulty into the oocyte in which AC were extracted in advance (**Figure 1**D, D′). Polar body extrusion was observed by microscopy on the next day, approximately 10 h after the karyoplast transfer (**Figure 1**E).

In total, AC were successfully removed in 52 out of 54 (96.3%) oocytes and 25 AC transfers were performed (**Table 1**). Successful injections of AC were performed in 13 out of 25 oocytes (52.0%). In 13 oocytes containing transferred AC, polar body extrusion was confirmed in six oocytes (46.2%) approximately 10 h after the karyoplast transfer. In addition, there were no polar body extrusions from 27 enucleated oocytes. In contrast, all control oocytes in which aspiration of the same volume of cytoplasm was performed extruded polar bodies (**Table 1**). All 27 patients who donated GV-stage oocytes for this study also contributed sibling metaphase-II oocytes that progressed to normal embryo development after IVF and/or ICSI procedures.

Time-lapse observations revealed the dynamic changes of the cytoskeleton (**Figure 1**I–P) and the release of the metaphase-II polar body (**Figure 1**Q). In addition, the formation and location of the spindle were confirmed using DIC microscopy (**Figure 1**R, S).

## Discussion

This study successfully extracted aggregated human chromosomes with only a minimal amount of ooplasm (**Figure 2**). This was probably a much smaller amount of ooplasm than in the previously reported three procedures. Since it has been reported very recently that there are no recipient mitochondria detected in embryonic stem cells derived from spindle–chromosome complex-transferred oocytes (Tachibana et al., 2013), the transfer of AC could be more promising in avoiding the concern about heteroplasmy by limiting the carry over of mtDNA. As the AC in human