

# Oocyte Cryopreservation

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## Morphologic status and angulation of meiotic spindle in vitrification-warmed oocytes of donor bank: effects on the ICSI outcomes

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### Study question

Do the morphology and angulation of meiotic spindle correlate with the fertilization outcomes after ICSI in vitrification-warmed oocytes of donor bank?

### Summary answer

The correlation exists between the spindle morphology and fertilization outcomes, while it does not show between the spindle angulation and fertilization outcomes in vitrification-warmed oocytes.

### What is known already

The meiotic spindle is crucial for the fertilization process, including completion of meiosis, second polar body extrusion, formation of pronuclei, and the following mitosis. Since the structure of spindle is dynamic depending on the timeline of oocyte, temperature, and physical stress, characteristics of spindle in the vitrification-warmed oocytes may show different phenomenon with that in the fresh oocytes.

### Study design, size, and duration

Between June and September of 2017, five hundred and twenty-five oocytes from donor bank were included in the analysis. A total of 37 egg donors were involved: mean age  $24.7 \pm 3.5$  years; body mass index (BMI)  $19.7 \pm 1.8$  kg/m<sup>2</sup>; anti-mullerian hormone (AMH) value  $7.0 \pm 4.2$  ng/mL.

### Participants/materials, setting, methods

All the metaphase II (MII) oocytes were cryopreserved by vitrification (Cryotech<sup>®</sup>, Japan) at the bank. Once being matched to the appropriate recipients, the oocytes were warmed and cultured in standard conditions for at least 2.5 hours following with image taken for spindle (Oosight<sup>®</sup>, Hamilton Thorne). The spindle presence, morphology, and angle to polar body (PB) were measured. The correlation between the outcomes of ICSI and post-warmed spindle status were estimated.

### Main results and the role of chance

The overall survival rate of vitrification-warmed oocytes was 92.4% (485/525), and two-pronuclei (2PN) rate was 75.1%.

The morphology of post-warmed spindle was classified into normal, translucent, no-visible, and telophase; the 2PN rate in each group was as below (p-value was compared to the normal group): normal = 79.4%, translucent = 73.2% (p=0.30), no-visible = 48.6% (p<0.05), and telophase = 37.5% (p<0.05). Of the 2PN rates in oocytes with different angulation, no correlation was found: 0–30° = 79.8%; 31-60° = 76.1%; 61-90° = 82.1%; 91-120° = 80.0%; >120° = 90.0%.

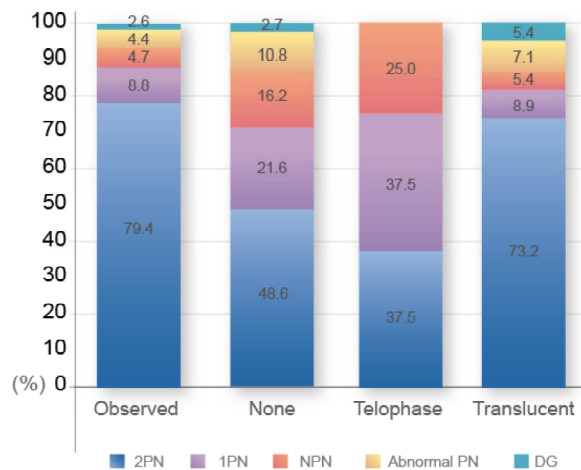
### Limitations, reason for caution

Individual biases in manipulation of spindle microscopy cannot be completely avoided.

### Wider implications of the findings

Unlike the biological representativeness of spindle angulation in fresh oocytes, the correlation between the spindle angulation and fertilization outcome was not observed in vitrification-warmed oocytes. The displacement of spindle in vitrification-warmed oocytes could be caused by the physical effects during the freezing-thawing procedures.

**Spindle Morphology and Following Fertilization Outcomes**



**Spindle Angulation and Following Fertilization Outcomes**

