Sperm injection at the para-polar body site in piezo-ICSI improves the survival rate of treated metaaphase II oocytes.

Yoshikazu Nagao; Shiori Ashibe; Yoku Kato; Rui Miyamoto; Borjigin Sarentonglaga

University Farm, Faculty of Agriculture, Utsunomiya University, Tochigi, Japan

Conventional and piezo-intracytoplasmic sperm injection (ICSI) are generally performed at the 3 o'clock position of a metaphase II (MII) oocyte when the first polar body (PB) is positioned at either 6 or 12 o'clock in order to avoid damage to the meiotic spindle. The choice of this injection site is based on the assumption that the meiotic spindle is located near the PB. However, it is possible that the ooplasmic cell membrane might be damaged in piezo-ICSI during drilling of the zona pellucida with piezo pulses; such damage might result in a reduction in the rate of successful development to the blastocyst stage. Here, we designed a new piezo-ICSI method in which the PB is set at either the 2 or 4 o'clock position. In this new method, zona drilling and sperm injection is performed through the para-PB site, which is the widest position in the perivitelline space. We examined how piezo pulses during the drilling of the zona pellucida affected the ooplasmic cell membrane and subsequent embryonic development.

We performed three experiments. In the first experiment, we investigated the influence of the distance between the cell membrane and edge of the micropipette when drilling the zona pellucida: this was performed by varying the position of the micropipette. After drilling the zona pellucida, we added piezo pulses for 0.5 sec with an intensity 1 or 12. In the second experiment, we examined the effect of the position of the injection site on the integrity of the meiotic spindle in MII oocytes by performing a sham injection of a conventional piezo group and para-PB piezo group. In a third experiment, we evaluated the effect of the injection site position in bovine ICSI on the survival of the oocytes; we also investigated competence to develop to the blastocyst stage and chromosomal integrity in blastocysts. We performed ICSI with the PB of the MII oocyte in either the 6 or 12 o'clock position (conventional piezo group), or 2 or 4 o'clock position (para-PB piezo group).

In experiment 1, the survival rate of the oocytes decreased as the distance between the micropipette and ooplasmic cell membrane became smaller. An analysis of cytoplasmic levels of free calcium ions (Ca2+) showed that these increased in oocytes as the distance between the micropipette and ooplasmic cell membrane was reduced. In experiment 2, there were no significant differences in meiotic spindle integrity. Even if the spindle was on the side, it was not damaged when displaced by the stretching cell membrane under pressure of the micropipette. In

experiment 3, the survival rate in the para-PB piezo group (90.1%) was significantly higher than that in the conventional piezo group (75.0%; $P < 0.05$). There were no significant differences in the rate of developing to the blastocyst stage of the surviving oocytes or the proportion of blastocysts showing chromosome damage.

In conclusion, the present study showed that our new piezo-ICSI method (para-PB site piezo-ICSI) is an improvement on current ICSI technology for animal and human reproduction.