Novel treatment for sperm activation improves embryo development in mouse intracytoplasmic sperm injection (ICSI)

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Introduction

- We have developed a novel ex-vivo sperm treatment designed to improve sperm function by modulating sperm metabolism.
- Step-wise activation of metabolic pathways improves sperm motility phenotype compared to standard-of-care sperm preparation media in samples from men seeking treatment for infertility and in healthy donors.
- In mouse models of assisted reproduction, activating sperm with the Ohana sperm treatment increased sperm hyperactivation, fertilization, embryo development and live birth rates compared to control conditions.

Experimental rationale

- Results from mouse in-vitro fertilization (IVF) studies showed that the Ohana sperm treatment led to improved embryo development from two-cell embryo to blastocyst, indicating impact beyond fertilization (Figure 1; Figure 2).
- A mouse ICSI study was designed to evaluate the effects of the Ohana sperm treatment compared to control sperm preparation on embryo development independent of fertilization.
- Most mouse ICSI is done using piezo-actuated micromanipulation to improve oocyte survival of injection; however, this approach has been shown to artificially activate oocytes.
- We developed a mouse ICSI procedure that more closely replicates how ICSI is done with human gametes, using a standard ICSI micropipette without piezo-actuated micromanipulation.
- For each experiment, epididymal sperm from one male C57Bl/6J mouse was processed in parallel using control conditions or Ohana activation then injected in to stripped oocytes pooled from multiple superovulated female CD1 mice. Injected oocytes were cultured overnight, the resulting two-cell embryos counted and cultured to blastocyst. The number of two-cell embryos that developed to blastocyst at Day 4 was quantified for control vs treatment.

Figure 1. Ex-vivo sperm activation inspired by a sperm's journey in the female reproductive tract[1,2]

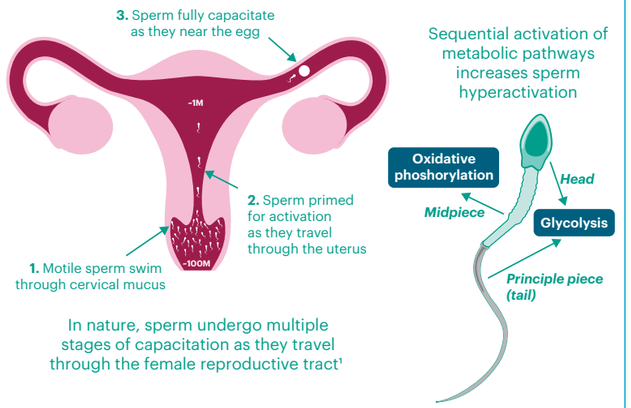
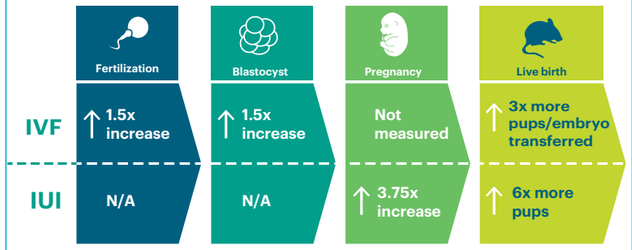


Figure 2. Ex-vivo optimization of sperm metabolism improves sperm function in mouse IUI and IVF, from fertilization through live birth



IUI, intra-uterine insemination; IVF, in-vitro fertilization; N/A, not applicable

Methods

Sperm collection and activation

- One 12 week-old male C57Bl/6J mouse was sacrificed and cauda epididymides isolated. Sperm was collected by making 3–4 cuts across and placing one into a 2mL Eppendorf tube with complete TYH buffer and the other into a tube with Ohana treatment medium 1 and incubating at 37˚C and 5%CO_2 for 10 minutes to allow motile sperm to swim out of the tissue.
- The epididymides were removed from the tubes and discarded, and the tubes centrifuged for 5 minutes at 2000RPM to pellet sperm cells. The supernatant was aspirated and discarded, the sperm cells were washed once then resuspended up to 1ml volume in the same buffer as used for the swim out and incubated for 40 minutes at 37˚C and 5%CO_2.
- 1mL of THY buffer or Ohana treatment medium 2 was added to the tube and incubated 10 minutes at 37˚C and 5%CO_2. Samples were centrifuged for 5 minutes at 1500 RPM to pellet the sperm, and 1.7mL supernatant was aspirated and discarded, leaving sperm in 300 µl volume, ready for insemination.

Isolation and preparation of oocytes

- Four to six 8 week-old female CD1 mice were induced to superovulate by intraperitoneal injection of 5IU pregnant mare serum gonadotropin (PMSG), then 5IU human chorionic gonadotropin (hCG) 48h later.
- Females were sacrificed and oviducts dissected to collect the cumulus-oocyte complexes (COCs) 12–13 hours after hCG trigger. Isolated COCs were incubated in 300µg/ml hyaluronidase for 30 seconds, then washed in CZB-HEPES to remove hyaluronidase and dissociate the cumulus cells.
- Stripped oocytes were washed with KSOM, then placed in 100µL drops of KSOM overlayed with oil and incubated at 37˚C and 5%CO_2 until the ICSI procedure.

ICSI

- Sperm tails were removed by repeated aspiration through 25G needle into a 1mL syringe 20–30 times, then sperm heads were immediately placed into a 20µL drop of 3% (w/v) polyvinylpyrrolidone (PVP). Sperm heads were aspirated into an ICSI micropipette with inner diameter 5µM, 30˚ angle with spiked tip.
- Two oocytes were transferred to a 3µL drop of CZB-HEPES on the ICSI plate. A single oocyte was positioned on the holding pipette so that the polar body is in either the 6 or 12 o'clock position, and the injection pipette containing the sperm head placed at the 3 o'clock position relative to the oocyte. The sperm head was advanced to the tip of the pipette and the injection pipette slowly advanced through the zona pellucida until near the cortex at the opposite side of the egg, into the oolemma. A small amount of oolemma was aspirated into the injection pipette, then the sperm head was deposited into the egg and the injection pipette gently withdrawn.
- The ICSI procedure was performed using a Narashige Takanome Micromanipulator System on up to 10 eggs at a time. Injected oocytes were kept for 15 minutes at room temperature, then washed in KSOM and incubated overnight at 37˚C and 5%CO_2. The next day, resulting two-cell embryos were counted for each sperm condition and the embryos transferred to fresh KSOM drops for culture to blastocyst.

Results

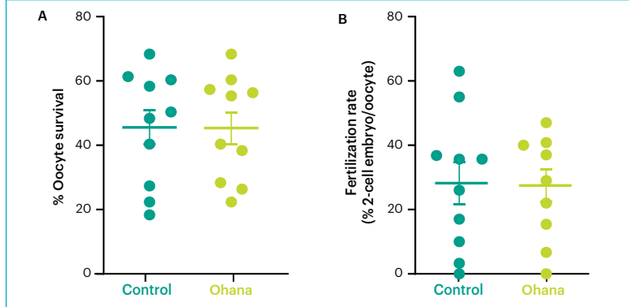
Oocyte survival and fertilization rates

- There was no significant difference in oocyte survival and fertilization between control and treatment groups (Figure 3).
- Out of 531 total oocytes injected with control activated sperm, 242 (46%) survived (mean ± SEM 45.2±5.6%, n=10). Out of 521 oocytes injected with Ohana activated sperm, 235 (45%) survived (mean ± SEM 45.0±5.1%, n=10).
- Fertilization rate was assessed by evaluating the number of two-cell embryos per oocyte 18 hours after ICSI procedure. Of the 242 oocytes that survived injection with control sperm, 59 (24%) formed two-cell embryos compared to 60 out of 235 (26%) for Ohana activated sperm (mean ± SEM 28.2±6.7% for control vs. 27.5±5.0% for Ohana, n=10).

Blastocyst development from 2-cell embryo

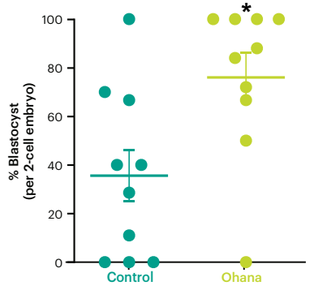
- There is substantial variation in blastocyst development from two-cell embryo with mouse ICSI, ranging from ~30 to 70% when performed without piezo-actuated micromanipulation[3, 4] and ~45 to 80% with piezo-actuated micromanipulation[3, 5, 6]
- In this set of experiments, 42% (25 of 59; mean ± SEM 35.6±11.0%) of two-cell embryos generated with control sperm developed to blastocyst, a rate within the range of published reports. With Ohana activated sperm, 87% (52 of 60) two-cell embryos developed to blastocyst (mean ± SEM 76.1±10.0%, p<0.005 by paired t-test), a two-fold increase relative to control.

Figure 3. No significant difference between rates of oocytes (A) survival and (B) fertilization with control and Ohana sperm activation



N=10 experiments; 1,052 oocytes injected overall

Figure 4. Significant increase in blastocyst development from two-cell embryo with Ohana sperm activation compared to control



*p<0.005 by paired t-test; N=10 experiments' 1,052 oocytes injected overall

Conclusions

Ohana's ex-vivo sperm treatment improves sperm function in embryo development beyond fertilization.

- **It is well understood that ICSI prevents failed fertilization, but does not improve other outcomes such as embryo development, implantation, pregnancy, or birth.[7] Activating sperm using the Ohana treatment significantly increased the probability a two-cell embryo developed into a blastocyst compared to control conditions in a mouse ICSI study.**
- **These results confirm earlier observations that the Ohana sperm treatment impacts beyond fertilization in mouse IVF and indicate that the Ohana sperm treatment may improve sperm-mediated oocyte activation and early embryo development.**
- **Additional experiments to evaluate live birth rate after trans-cervical embryo transfer of day 4 blastocysts following ICSI are in progress.**
- **These data showing that the Ohana sperm treatment improves early embryo development in mice in both conventional IVF and ICSI-IVF suggest that the Ohana treatment for human sperm may also improve embryo development for patients undergoing IVF with ICSI.**
- **The Ohana sperm treatment is currently being tested in a clinical study evaluating fertilization and embryo development in conventional IVF (NCT04142112).**

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