O-122 ICSI in a box: development of a successful automated sperm injection robot with external supervision and minimal manual intervention.

N. Costa-Borges[1], G. Giralt[2], E. Albó[2], A. Alvarez[3], J. Ramos[3], I. Hernandez[3], M. Luis[2], G. Calderón[1], S. Munne[2]

[1]Embryotools S.L., Research and Development, Barcelona, Spain ; [2]Overture, Research and Development, Barcelona, Spain ; [3]Overture, Research and Development, Madrid, Spain

Study question: Is it possible to automate the way sperm is injected in an oocyte and improve ICSI consistency between embryologists?

Summary answer: The developed ICSI robot demonstrated a high degree of consistency and operator skill independence, allowing human supervision and external control, but minimal manual intervention.

What is known already: ICSI is a clinical procedure that is currently performed worldwide in most IVF centers and its use will only increase with more utilization of egg freezing. Since its implementation, the technique has been conducted manually by highly skilled embryologists. However, success rates can vary significantly depending on the experience of the operator. We leverage our experience in robotics, AI algorithms and embryology to develop an automated ICSI robot that requires minimal manual intervention with the aim to standardize the consistency of the procedure and, ultimately, improve overall results maintaining embryologist oversight.

Study design, size, duration: The ICSI robot was developed to have supervised automated control on critical steps of the injection procedure, including injection pipette advancement, zona pellucida and oolemma penetration with piezo-pulses, and pipette removal after injection. Manual intervention is required only for immobilization and capture of spermatozoa with a joystick gamepad and to release the sperm in the ooplasm, without the need for micromanipulation skills. In parallel, piezo-ICSI was performed in a conventional micromanipulation station as a control.

Participants/materials, setting, methods: Hamster and mouse oocytes were collected from superovulated females. For testing the efficiency of the automated system, hamster oocytes were injected with human donor sperm, as historically used in manual ICSI training programs, and survival rates evaluated after overnight culture. Mouse oocytes were injected with mouse sperm heads and subsequently cultured *in vitro* for five days. Blastocysts obtained were vitrified and embryo transfers are ongoing to evaluate term developmental rates.

Main results and the role of chance: The technical components of the ICSI robot were engineered to integrate AI algorithms, optics, cell microinjectors and mechatronics. AI algorithms were developed to identify the morphological structures of MII oocytes, including the zona pellucida, perivitelline space and polar body, both in the hamster and mouse models. The system detects and analyzes both the pipette and the oocyte and chooses the best area and plane for injection, allowing automated control of the subsequent injection steps. Using the hamster oocyte-human sperm model, a survival rate of 91% (n=110) was

achieved with the robot, which was statistically similar (p=0.335) to the results obtained in the controls injected manually (96%, n=28). The average time spent in each injection cycle, which includes scanning of the oocyte and injection pipette, and injection of the sperm into the oocyte, was approximately two minutes per ICSI operation. This time was comparable to the time required by highly experienced operators with manual piezo-injection. In the mouse, 91% (n=53) of the oocytes injected survived the procedure, of which, 92% developed to two-cells and 87% to the blastocyst stage. No statistical differences were found when compared these efficiencies with manual controls (n=40, 98%, 97% and 92%, respectively).

Limitations, reasons for caution: The developed ICSI robot has shown highly consistent results, independently of operator skills, both in hamster and mouse oocytes. However, additional validations should be performed to enlarge the sample size of injected oocytes and to evaluate the efficiency of the system in other oocyte species, including translational studies to humans.

Wider implications of the findings: The combination of multidisciplinary teams allows the development of automated processes that can reduce variability in certain IVF procedures, while supervised and assisted by experienced embryologists. It is expected that other laboratory procedures can be automated in the field of assisted reproductive treatments in a near future.

Trial registration number: N/A