P-221 Prospective randomized sibling study on gamete preparation, insemination and subsequent culture of human oocytes in a time-lapse system using media systems with and without antioxidants

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Study question: Does the addition of antioxidants for gamete preparation, insemination and embryo culture lead to differences in embryo development and clinical outcome

Summary answer: Using an antioxidant-containing media system for sperm preparation, insemination and embryo culture imparts significantly higher good-quality blastocyst rates and improved clinical outcome in elderly patients.

What is known already: A previous study showed that adding combined antioxidants for sequential embryo culture in conventional incubators (interrupted culture) improves embryo viability and clinical outcome, especially for elderly patients. Here we investigated the combined effect of three antioxidants Acetyl-L-Carnitine (10 µM), N-Acetyl-L-Cysteine (10 µM), and α-Lipoic Acid (5 µM) during sperm preparation, insemination, and time-lapse culture in a single step medium on human embryo development and clinical outcome.

Study design, size, duration: Prospective randomized single center study including 143 couples for IVF/ICSI between August 2018 and December 2019. Inclusion required at least eight cumulus-oocyte-complexes (COCs) after retrieval. Cycles involving PGT, split IVF/ICSI, and surgically retrieved sperm were excluded. Immediately after retrieval oocytes were randomly distributed to a study or control media system with or without antioxidants (Vitrolife). Similarly, ejaculates were split and prepared with and without antioxidants.

Participants/materials, setting, methods: Sibling oocytes were inseminated in the respective group with accordingly prepared sperm. Single step embryo culture was conducted in medium with (Gx-TL) and without (G-TL) antioxidants in the EmbryoScope+. Embryo quality and clinical outcome were assessed in relation to maternal age (<35/>35 years). Good-quality embryos on day 3 were defined as 8- to 10-cells with even cells and low fragmentation; good-quality blastocysts as >3BB. Clinical outcome was assessed after single vitrified blastocyst transfer (SVBT).

Main results and the role of chance: From 143 participants (female age, 34.7±3.2 years), a total of 2424 COCs were collected; 1180 COCs/916 metaphase-II (MII) oocytes were allocated to Gx-TL media and 1244 COCs/981 MII oocytes to G-TL media. Age-related analysis in Gx-TL compared with G-TL in relation to allocated MII oocytes revealed a trend for higher fertilization rates in Gx-TL for both age groups (<35: 72.1% vs. 66.9%; >35: 70.7% vs. 64.9%, P<0.1). Good-quality day 3 embryo development/MII oocytes was higher, albeit not significant, in the elderly patients in Gx-TL (<35: 35.9% vs. 34.4%; >35: 31.1% vs. 27.9%). Overall day 5/6 blastocyst rate was similar for both media (<35: 48.2% vs. 49.9%; >35: 42.3% vs. 39.5%). Day 5/6 GQB rate was comparable for younger patients (<35: 23.8% for Gx-TL vs. 26.0% for G-TL) but significantly higher in Gx-TL in elderly patients (>35: 20.7% vs. 14.4%; P<0.05). A total of 200 SVBT were performed; 99 in the Gx-TL- and 101 in the G-TL-arm. We

noted almost similar implantation and ongoing pregnancy rates between Gx-TL vs G-TL in the younger (<35) age group (50.0% vs. 55.4%; 50.0% vs. 55.6%) but higher albeit not significant rates for Gx-TL in older (>35) patients (44.1% vs. 33.3%; 44.1% vs. 33.3%).

Limitations, reasons for caution: In almost 95% of the cycles, oocytes were inseminated by ICSI; thus results may not equally apply for cycles with IVF. The use of a closed time-lapse system may have prevented from some environmental oxidative stress. Therefore results may come out different with a similar study using standard incubation.

Wider implications of the findings: Supplementation of antioxidants to media for gamete isolation and preparation, as well as subsequent single step time-lapse culture may improve GQE/B rates and clinical outcomes in certain age groups, plausibly through the reduction of oxidative stress. Further studies in selected sub-groups (severe OAT syndrome / testicular cases) may be indicated.

Trial registration number: UMIN000034482