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Free communication 5:

Vitrification and warming human blastocyst by use of a cryoloop, using laser to artificially induce blastocyst collapse prior to vitrification

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Introduction. Even though transfer of blastocyst in many IVF programs seems promising with regard to pregnancy rates, it has so far not proven to be a system that can completely replace transfer of early cleavage stage embryos. The main concern is that cryopreservation procedures have been clinically less successful as compared to freezing of day 2 or day 3 embryos. However, with the introduction of vitrification of human blastocysts, interestingly very high clinical pregnancy rates have been reported by Mukaida et al. ((1)). Based on this evidence and extensive clinical use in Japan we have now introduced this technique to our clinical IVF program.

We have furthermore tested a new technique to artificially collapse the blastocysts prior to vitrification using laser instead of piercing the blastocyst with a needle as described by Mukaida et al. ((1)).

Methods. The method used at Fertilitetcentrum is based on Mukaida et al.'s ((1)), with minor modifications. Both vitrification and warming solutions were provided by Vitrolife AB. The base medium was Gamete using Ficoll 70 and Sucrose to provide the solutions with different osmolarity. In addition, both DMSO and ethylene glycol were added on the same day that the blastocysts were vitrified.

Blastocysts deemed of sufficient quality are scored on the day of vitrification. Each of the blastocysts that were to be vitrified were placed in separate, numbered microdrops (20 µl) and kept in a 5% CO_2 incubator until the vitrification procedure.

Artificial blastocyst collapse: Approximately 15 min prior to the vitrification procedure, it is necessary to accomplish a blastocoel collapse. This was performed by the use of a laser (Fertilase). Briefly, the ZP is breached by laser, using a maximum of 5 ms. In an expanded blastocyst, the breach can be confirmed by a small protrusion of the trophectoderm. In this study, all blastocysts were collapsed, irrespective of their expansion status.

Results. The preclinical results presented here originated from a period of 3 months (August through October 2005). In total, 28 blastocysts were vitrified and warmed. Of those, 25 were recovered (89%). Of the 25 recovered, 23 (92%) expanded after warming and would have been transferred if applicable. An 82% survival rate was therefore accomplished per vitrified blastocyst.

The clinical results are from a 6-month period (January through June 2006). A total of 50 blastocysts were rewarmed in 40 cycles resulting in 34 transfers (82% SET). The results from these 34 transfers were: 22 chemical (65%) and 15 (44%) clinical pregnancies (ongoing). The implantation rate was 17/34 (50%).

Conclusions. The main finding from this study is that the use of laser for artificial collapse gives comparable results as compared to published data on mechanical collapse. The blastocyst survival rate is very good and, more importantly, the first 34 transfers have shown a favorable clinical pregnancy rate. These results show that vitrification is a powerful tool for cryopreservation of blastocysts.

Ref: Mukaida T, Nakamura S, Tomiyama T, Wada S, Oka C, Kasai M et al. Vitrification of human blastocysts using cryoloops: clinical outcome of 223 cycles. Hum. REprod 2003; 18,384-91