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DECREASE OF DEVELPMENTAL COMPETENCE AND INCREASE OF ABNORMAL SPINDLE OF GROWTH-RETARDED EMBRYO AFTER VITRIFICATION

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Objective: To assess characteristics of growth-retarded embryos, the developmental competence and the spindle shape of vitrified-warmed blastocysts were assessed using well-grown and growth-retarded blastocysts. **Design**: Human retrospective study.

Materials and methods: This study was approved by a local IRB of IVF Namba Clinic. In experiment 1, we compared the implantation rates of vitrified-warmed embryos which developed to the blastocyst stage on Day 5 after insemination (normally developing embryo) and on Day 6 (growth-retarded embryo). Five-hundred and forty-four patients who underwent IVF and single embryo transfer between January and December 2011 were included in the analysis. In experiment 2, vitrified surplus human blastocysts (n = 47) were donated for research from consenting couples completed IVF treatment. Vitrified blastocysts were fixed 18 h post-warming. Blastocyst immunostaining was performed using a mouse anti- α -tubulin Alexa 488[®] (Invitrogen, MD, USA) to visualize microtubules and Hoechst33342 (Dojindo, Osaka, Japan) to visualize DNA. Confocal image analysis of the blastocyst was accomplished by capturing a z-series stack of 0.5 µm thick sections encompassing the entire blastocyst. A spindle except for a spindle with fusiform poles and with chromosomes aligned at the equator was classified as abnormal. Data was compared by student's t-test.

Results: In experiment 1, the implantation rate of growth-retarded embryo (44 %, n = 179) was significantly lower (P < 0.05) than that of normally developing embryo (54%, n = 365). There was no difference in the abortion rate. In experiment 2, the incidence of abnormal spindle in growth-retarded embryo (58%, n = 133) was significantly higher (P < 0.01) than that in normally developing embryo (31%, n = 97).

Conclusion: In growth-retarded embryos, the incidence of abnormal spindle shape increased and the implantation competence decreased following vitrification compared with normally developing embryos. **SUPPORT**: None