

Growth retardation in human blastocysts decreases their implantation potential possibly due to an increase of the incidence of abnormal spindles

Shu Hashimoto, Ami Amo, Keijiro Ito, Yoshiharu Nakaoka, Yoshiharu Morimoto

IVF Namba Clinic

Abstract

Objectives: There are conflicting data on whether the human embryo growth rate affects the outcome of vitrified–warmed blastocyst transfer. To assess the potential of growth-retarded embryos, the implantation potential and the spindle shape of vitrified–warmed blastocysts were assessed among normally developing and growth-retarded blastocysts.

Materials & methods: This was a retrospective cohort study including 878 single vitrified–warmed blastocyst transfers between 9 January 2010 and 10 July 2012, and an experimental study using 108 vitrified–warmed blastocysts donated to research.

This study was approved by the local Institutional Review Board of IVF Namba Clinic. In a clinical study, we compared the implantation rates of vitrified–warmed embryos that developed to the blastocyst stage on day 5 after insemination (normally developing embryos) with those that required culture to day 6 (growth-retarded embryo). Eight hundred seventy-eight patients who underwent *in vitro* fertilization and single blastocyst transfer were included in Experiment 1. In Experiment 2, vitrified surplus human blastocysts ($n = 108$) were donated for research from consenting couples who had completed their

infertility treatment. These were fixed for 18 h post-thawing. Blastocysts were immunostained with an anti- α -tubulin antibody to visualize microtubules, an anti- γ -tubulin antibody to image centrosomes and Hoechst 33342 or DAPI to visualize DNA. Confocal image analysis was accomplished by capturing a z-series stack of 0.5- μ m-thick optical sections encompassing the entire blastocyst. Only spindles with fusiform poles and with chromosomes aligned at the equator were classified as normal. Data were compared using the Mann–Whitney nonparametric *U* test.

Results: In Experiment 1, the implantation rate of growth-retarded embryos (47%, $n = 270$) was significantly lower ($P < 0.05$) than that of normally developing embryos (57%, $n = 608$). In Experiment 2, a total of 533 spindles were analyzed in both day 5 and day 6 blastocysts. The incidence of abnormal spindles in growth-retarded embryos (47%, $n = 274$) was significantly higher ($P < 0.01$) than in normally developing embryos (30%, $n = 259$).

The incidence of abnormal spindle morphology increased and the implantation competence decreased following vitrification in growth-retarded compared with normally developing embryos.

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Key words: blastocyst formation, growth retardation, embryo transfer, pregnancy rate, spindle