ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Analysis of the morphological dynamics of blastocysts after vitrification/warming: defining new predictive variables of implantation

Aila Coello, Ph.D., Marcos Meseguer, Ph.D., Arancha Galán, Ph.D., Lucia Alegre, Ph.D., José Remohí, M.D., and Ana Cobo, Ph.D.

Instituto Valenciano de Infertilidad, INCLIVA-Universidad de Valencia, Valencia, Spain

Objective: To describe the morphological dynamics of vitrified/warmed blastocysts and to identify quantitative morphological variables related to implantation. Subsequently, by using the most predictive parameters, to develop a hierarchical model by subdividing vitrified/warmed blastocysts into categories with different implantation potentials.
Design: Observational, retrospective, cohort study.
Setting: University-affiliated private IVF center.
Patient(s): The study included 429 vitrified/warmed blastocysts with known implantation data, which were evaluated by time-lapse imaging. Blastocysts were routinely placed in EmbryoScope (Vitrolife) immediately after warming until transfer.
Intervention(s): None.
Main Outcome Measure(s): Embryos were vitrified and warmed by the Cryotop method (KitazatoBiopharma). The studied variables included the initial and minimum thicknesses of zona pellucida (μm), the initial and maximum areas (μm^2), the area of inner cell mass (μm^2), expansion (whether the embryo reexpands or not after warming), and collapsing or contraction after warming. After defining the optimal ranges according to the consecutive quartiles with the highest probability of implantation, a logistic regression analysis was performed by combining the former variables and the blastocyst morphological classification criteria defined by the Spanish Association of Embryologists into A, B, C, or D categories.
Result(s): Reexpansion of vitrified/warmed blastocysts correlated strongly with implantation (44.6% for reexpanded vs. 6.5% for the blastocysts that did not reexpand after warming). Throughout the logistic regression analysis, the model identified the maximum blastocyst area, odds ratio (OR) = 0.41 (95% confidence interval [CI], 0.22–0.77), followed by the initial area, OR = 0.62 (95% CI, 0.35–1.08) as the most predictive variables related to implanting embryos. Blastocyst morphology was not considered relevant in our model. The hierarchical tree model subdivided embryos into four categories, A–D, with lowering expected implantation potentials (from 47.3% for A to 14.2% for D).
Conclusion(s): The analysis of warmed blastocysts by time-lapse imaging may provide objective quantitative markers for the blastocyst implantation potential. We propose a hierarchical model to classify vitrified/warmed blastocysts according to their implantation probability. The observed correlations and the proposed algorithm should be validated in a prospective trial to evaluate its efficacy. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Embryo vitrification, warming, time lapse, morphokinetics, blastocyst

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/18919-23830

Extending embryo culture and transfer in the blastocyst stage has proven a successful approach in assisted reproduction technology as it allows better embryo selection, which, in turn, results in higher implantation rates (1–4). This policy involves increased blastocyst vitrification because supernumerary embryos are routinely vitrified for their later use. Nowadays, a frequent strategy involves the cryopreservation of all viable blastocysts and their transfer in subsequent frozen cycles (5), which implies replacing embryos in a more physiologic environment and preventing the aggravation of ovarian hyperstimulation syndrome (6).

This significant increase in frozen blastocyst transfer cycles encourages the development of more accurate selection criteria for vitrified blastocysts. Traditionally, assessments of fresh blastocysts have been based on morphological appearance and on evaluating three parameters: degree of

Received February 5, 2017; revised July 23, 2017; accepted July 24, 2017.
A.C. has nothing to disclose. M.M. has nothing to disclose. A.G. has nothing to disclose. L.A. has nothing to disclose. J.R. has nothing to disclose. A.C. has nothing to disclose.
Supported by the Spanish Ministry of Economy and Competitiveness (PI14/00523) through an Instituto de Salud Carlos III program; and a grant from Fertility Innovation (Merck Serono).
Reprint requests: Marcos Meseguer, Ph.D., Instituto Valenciano de Infertilidad, Plaza de la Policía Local, 3, Valencia 46015, Spain (E-mail: marcos.meseguer@ivi.es).

Fertility and Sterility® Vol. ■, No. ■, ■ 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2017.07.1157

blastocoele expansion, trophectoderm (TE), and inner cell mass (ICM) (7). However, after the vitrification and warming procedures, blastocysts undergo multiple morphological changes that may make evaluating blastocyst quality difficult. First, they are dehydrated by the addition of cryoprotectants during cooling and then rehydrated by the removal of cryoprotectants during warming. Such blastocoele shrinkage and swelling can lead to cell damage and may affect either survival or morphological integrity (8, 9).

Blastocysts are often collapsed immediately after warming. Thus, a postwarmed culture provides the opportunity to evaluate vitrified/warmed blastocysts more accurately. Some investigators recommend assessing survival and quality within 2–4 hours after warming (8, 9). Indeed, the ability to reexpand within a few hours of warming has been reported as a strong indicator of blastocyst potential (10, 11). However, some blastocysts take longer to reexpand, and a single assessment would not be enough to score blastocyst quality. This increment in blastocoele size is sometimes difficult to assess because it is time dependent and may change during blastocoele collapse. In such cases, assessments of blastocysts should be postponed.

It is well-known that each observation involves exposure to the suboptimal conditions outside a controlled incubator environment, which may potentially affect treatment success (12). Hence the continuous monitoring of warmed blastocysts through time-lapse systems can provide us with valuable information about their implantation potential while they remain inside a controlled stable culture environment.

Although some studies have not successfully demonstrated the benefits of using time-lapse systems for embryo selection (13, 14), others have incorporated embryo morphokinetic parameters into potential selection models and have improved clinical outcomes (15, 16). The EmbryoScope system (Vitrolife) offers the chance to take measures of different structures of embryos through EmbryoViewer (Vitrolife) drawing tools. With this instrument, the parameters involved in the morphological dynamics of warmed blastocysts, such as the thickness of zona pellucida (ZP) or the blastocyst area, can be assessed during culture. The retrospective analysis of these parameters allows quantitative values to be established, which, in turn, may be used as predictors of implantation.

The aim of this study was to describe the morphological dynamics of vitrified/warmed blastocysts and then to identify new markers capable of predicting implantation. Subsequently, by using the most predictive parameters, we developed a hierarchical model by subdividing warmed blastocysts into categories with different implantation potentials.

MATERIALS AND METHODS

Study Design and Patient Population

This research project was conducted at the Instituto Valenciano de Infertilidad (IVI) in Valencia (Spain). The procedure and protocol for analyzing embryos were approved by an Institutional Review Board (IRB reference 1511-VLC-062-AC), which controls and approves database analyses and clinical IVF procedures for research at the IVI.

The present retrospective study included all the patients from our ovum donation program who had undergone warming cycles between November 2014 and December 2015 and whose vitrified/warmed blastocysts had been cultured in EmbryoScope. Only the blastocysts from the transfers in which all the transferred blastocysts did or did not implant were selected for the analysis. These embryos were defined as known implantation data (KID) (17). Of the 386 warming cycles with the 528 warmed blastocysts, 429 KID blastocysts from 335 transfers were analyzed. The cycles with a partial implantation were excluded from the study because it was not possible to determine which of the two transferred embryos was actually implanted.

Stimulation Protocol, IVF, Embryo Culture, and Cryopreservation Policies

All the vitrified/warmed blastocysts used in this study were obtained in stimulated oocyte retrieval donor cycles. The selection criteria for donors can be found in Garrido et al. (18), according to Spanish law. All the donors had normal menstrual cycles that lasted 26–34 days, had normal weight (body mass index [BMI] of 18–28 kg/m^2), and had not been on endocrine treatment (including gonadotropins and oral contraception) in the 3 months before the study. They had a normal uterus and normal ovaries according to transvaginal ultrasound examinations (no signs of polycystic ovary syndrome) and an antral follicle count of $>$20 on day 1 of gonadotropin administration.

Donors were stimulated by the controlled ovarian stimulation protocol, described by Munoz et al. (19), with GnRH antagonist treatment. GnRH agonist (Decapeptyl; Ipsen Pharma) was administered IM when the mean diameter of at least eight leading follicles reached 18 mm. Follicular aspiration was performed by vaginal ultrasonography 36 hours later. The retrieved oocytes were then cultured in fertilization medium (Cook) at 5% CO_2, 5% O_2, and 37°C before insemination by intracytoplasmic sperm injection. After 16–19 hours, fertilization was confirmed by the presence of two pronuclei, and zygotes were placed into individual droplets of 50 μL of Cook cleavage media for culture. On day 3, embryos were assessed under an inverted microscope and were transferred into individual droplets of CCM medium (Vitrolife) for culture to the blastocyst stage.

Blastocysts were scored according to the Asociación para el Estudio de la Biología de la Reproducción (ASEBIR) criteria, which are based on assessing the expansion degree, ICM, and TE appearance (20, 21). Cryopreservation policies depended on the morphological quality of the surplus embryos after fresh ET, or, in some cases, all good-quality embryos were cryopreserved. Blastocyst cryopreservation was performed on day 5 or 6. A summary of this classification tree is offered in Supplemental Figure 1. The type A, B, and C blastocysts were selected for either transfer or vitrification.

Embryo Vitrification Protocol

The Cryotop method used for blastocyst vitrification has been described elsewhere (22). Briefly, blastocysts were equilibrated