Reproductive BioMedicine Online (2014) **29**, 411–416

www.sciencedirect.com
www.rbmonline.com

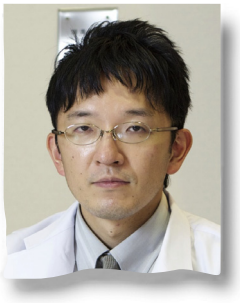
ARTICLE

Women's age and embryo developmental speed accurately predict clinical pregnancy after single vitrified-warmed blastocyst transfer

Keiichi Kato [a,b,*], Satoshi Ueno [a], Akiko Yabuuchi [a], Kazuo Uchiyama [a], Takashi Okuno [a], Tamotsu Kobayashi [a], Tomoya Segawa [c], Shokichi Teramoto [c]

[a] *Kato Ladies Clinic, 7-20-3, Nishi-shinjuku, Shinjuku, Tokyo 160-0023, Japan;* [b] *Department of Obsterics and Gynecology, Kanazawa University Graduate School of Medicine, 13-1, Takaramachi, Kanazawa, Ishikawa 920-8641, Japan;* [c] *Shinbashi Yume Clinic, 2-5-1, Shinbashi, Minatoku, Tokyo 105-0004, Japan*

* Corresponding author. *E-mail address*: k-kato@towako.net (K Kato).



Dr. Keiichi Kato is general director at the Kato Ladies Clinic in Tokyo, Japan. Dr. Kato earned his MD from the University of Kanazawa. His main research interests are minimal ovarian stimulation, natural-cycle IVF, and single-embryo transfer.

Abstract The aim of this study was to establish a simple, objective blastocyst grading system using women's age and embryo developmental speed to predict clinical pregnancy after single vitrified-warmed blastocyst transfer. A 6-year retrospective cohort study was conducted in a private infertility centre. A total of 7341 single vitrified-armed blastocyst transfer cycles were included, divided into those carried out between 2006 and 2011 (6046 cycles) and 2012 (1295 cycles). Clinical pregnancy rate, ongoing pregnancy rate and delivery rates were stratified by women's age (<35, 35–37, 38–39, 40–41, 42–45 years) and time to blastocyst expansion (<120, 120–129, 130–139, 140–149, >149 h) as embryo developmental speed. In all the age groups, clinical pregnancy rate, ongoing pregnancy rate and delivery rates decreased as the embryo developmental speed decreased ($P < 0.0001$). A simple five-grade score based on women's age and embryo developmental speed was determined by actual clinical pregnancy rates observed in the 2006–2011 cohort. Subsequently, the novel grading score was validated in the 2012 cohort (1295 cycles), finding an excellent association. In conclusion, we established a novel blastocyst grading system using women's age and embryo developmental speed as objective parameters.

http://dx.doi.org/10.1016/j.rbmo.2014.06.007
1472-6483/

KEYWORDS: blastocyst culture, in-vitro fertilization, minimal ovarian stimulation, outcome prediction, single embryo transfer

Introduction

Single blastocyst transfer is increasingly used during IVF treatment to prevent multiple conceptions. When several embryos are available after prolonged embryo culture, a thorough evaluation of each blastocyst is needed to identify those with the highest implantation potential. Since its inception, the blastocyst grading system developed by Gardner and Schoolcraft (1999), based on the degree of blastocyst expansion and the morphology of the inner cell mass and trophectoderm cells, was widely used in various institutions around the world. Subsequently, the association between these morphological parameters of the blastocyst and pregnancy rates has been evaluated in many studies (Gardner et al., 2000; Hill et al., 2013; Matsuura et al., 2010). The morphological evaluation of blastocysts, however, is inherently subjective, greatly observer-dependent, and the debate on which morphological parameters have the highest prognostic value is still inconclusive. Therefore, existing blastocyst grading systems need to be further improved by incorporating more objective and reproducible variables.

The developmental speed of the embryo is an important variable to consider while ascertaining its quality. For cleavage-stage embryos, it has been reported that the implantation rate is higher for embryos that cleave earlier (Lee et al., 2012). Similarly, studies involving blastocyst transfer procedures have examined differences in implantation rates between blastocysts developing up until day 5 compared with those developing up until day 6 (Liebermann and Tucker, 2006; Stehlik et al., 2005; Sunkara et al., 2010). These studies, however, did not provide details on the degree of expansion of the transferred blastocysts, and their results varied considerably. In a previous study by our group (Okimura et al., 2009), it was observed that the greater the degree of expansion of the blastocyst, the higher its implantation potential. Therefore, by examining those blastocysts with a uniform degree of expansion, the association between embryo developmental speed to blastocyst and the success rates may be examined more accurately. The aim of the present study was to analyse success rates after single vitrified-warmed blastocyst transfer (SVBT) according to women's age and embryo developmental speed, and to establish a novel blastocyst grading system that could help to predict outcome after SVBT.

Materials and methods

Patients and study design

This retrospective study was reviewed and approved by the independent Institutional Review Board of Kato Ladies Clinic, Tokyo (IRB approval number: 13–20, approved 12 September 2013). Written informed consent was also obtained from all patients undergoing IVF treatment at our centre, and the signed document also informed patients that de-indentified data could be used for retrospective analysis. A total of 5948 patients undergoing 7341 SVBT cycles who fulfilled the following inclusion criteria were included in this retrospective analysis: women's age 45 years or less; women undergoing SVBT with a 175–184 μm sized blastocyst showing a uniform degree of expansion; and availability of follow-up data on pregnancy outcome. Cycles resulting in monozygotic twin pregnancies and ectopic pregnancies were excluded. At our centre, the blastocyst transfer strategy was routinely indicated in cases of tubal factor infertility (e.g. tubal obstruction, hydrosalpinx or a history of extrauterine pregnancy), and after previous failed cycles with single cleavage-stage embryo transfers, and constituted a proportion of treatments carried out in our centre (Kato et al., 2012).

In the first part of the analysis, clinical pregnancy rates (CPR) were analysed in five subgroups that were divided before vitrification, according to five women's age (≤34, 35–37, 38–39, 40–41, and 42–45 years) and five blastocyst growth rate categories (≤119, 120–129, 130–139, 140–149, and ≥150 h). A simple grading system (grades A to E corresponded to a CPR of ≥54, 44–54, 34–43, 24–34 and <24%, respectively) was established based on actual clinical pregnancy rates observed in the first half of the cohort (6046 cycles carried out between 2006 and 2011). Subsequently, the predictive value of the above grading system was evaluated in the second half of the cohort (1295 cycles carried out during 2012). Additionally, ongoing pregnancy and delivery rates for grade were also calculated in each cohort.

Minimal ovarian stimulation, oocyte retrieval and fertilization procedures

All patients underwent a clomiphene-based minimal ovarian stimulation protocol or drug-free natural cycle IVF treatment (Kato et al., 2012; Teramoto and Kato, 2007). Oocyte retrieval was conducted without any anaesthesia, using a fine 21-22 G needle (Kitazato, Japan). Follicular flushing was not used during oocyte retrieval. Conventional insemination was carried out about 3 h after retrieval, ICSI was carried out 5 h after retrieval. P1/cleavage stage medium (Irvine Scientific, USA) or human tubal fluid (HTF; Irvine Scientific, USA) with 10% serum substitute supplement (SSS; Irvine Scientific, USA) was used as the culture medium after insemination.

Embryo culture, blastocyst monitoring and vitrification

Fertilization assessment was carried out 16–20 h after insemination. Normally, fertilized zygotes with two pronuclei were cultured individually in a drop of 20 μL of Quinn's Advantage Protein Plus cleavage medium (SAGE, USA) from days 1–3. The embryos were transferred to Quinn's Advantage Protein Plus blastocyst medium (SAGE, USA) on day 3 and cultured until day 5 to 7. All embryos were cultured at 37°C under the gas phase of 5% O2, 5% CO2 and 90% N2, with 100% humidity in water jacket small multigas incubators or dry desktop incubators (Astec, Japan).