ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Time-lapse morphokinetic assessment has low to moderate ability to predict euploidy when patient– and ovarian stimulation–related factors are taken into account with the use of clustered data analysis

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Objective: To study whether time-lapse morphokinetic (TLM) assessment predicts ploidy status when patient– and ovarian stimulation–related factors are taken into account.
Design: Retrospective cohort study.
Setting: Private IVF clinic.
Patient(s): In total, 103 consecutive patients (415 blastocysts) were included. All embryos were individually cultured in a time-lapse incubator from intracytoplasmic sperm injection up to trophectoderm biopsy. Following trophectoderm biopsy on day 5 or 6, blastocysts were vitrified and 23 TLM parameters were analyzed.
Intervention(s): Correlations between patient– and ovarian stimulation–related factors and TLM parameters were tested in a multilevel mixed-effects linear regression model and assessed by means of intraclass correlation coefficient (ICC).
Main Outcome Measure(s): Predictive ability of TLM parameters for euploidy.
Result(s): The majority of TLM parameters had ICCs of 16%–47%. None of the patient– or ovarian stimulation–related factor had any systematic effect on any TLM parameter; however, body mass, total FSH dose, duration of infertility, number of previous cycles, antral follicle count, ovarian stimulation protocol, and E_2 on the trigger day had a significant impact on some TLM parameters. With the use of multilevel mixed-effects logistic regression analysis, of the ten TLM parameters that were initially noted to be significantly different among euploid and aneuploid blastocysts in the univariate analysis, only five remained significant. However, the areas under the receiver operating characteristic curves at regression analysis were low, ranging from 0.55 to 0.63.
Conclusion(s): Five TLM parameters, all related to timing of blastocyst development, have limited ability to predict euploidy when patient– and ovarian stimulation–related factors are taken into account. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Time-lapse, morphokinetic parameters, aneuploidy, preimplantation genetic testing, confounding, cluster analysis

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The contemporary goal of in vitro fertilization (IVF) is to maximize live birth rates with the use of single-embryo transfer. An objective assessment tool to evaluate embryo ploidy status and viability is of critical importance for selection of the best embryo to be transferred. Blastocyst-stage embryo transfer may enhance embryo selection (1), but embryo morphology, even at the blastocyst stage, might be misleading (2).

Aneuploidy is the main contributor to implantation failure (3) and increased risk of miscarriage (4) in IVF. Currently, blastocyst-stage embryo biopsy is the method of choice for assessment of the ploidy status (5). Despite the lack of any detrimental effect of trophectoderm biopsy on implantation rate (5), noninvasive assessment of ploidy status with high validity would be very useful.

Morphokinetic assessment of preimplantation embryo development has been a breakthrough in human embryology in the past decade. Sophisticated time-lapse incubators along with single-step medium permitted not only uninterrupted in vitro culture and embryo development but also provided continuous information about dynamic changes during the preimplantation period. There have been efforts to predict aneuploidy by means of various time-lapse morphokinetic (TLM) parameters, six studies reporting a significant association with some TLM parameters and the ploidy status (6–11), and four refuting any such association (12–15).

The main drawback of the available ten studies is that each embryo is treated as an individual, ignoring the fact that all of the embryos from the same patient may act in a similar fashion affected by patient–and ovarian stimulation–related factors (16). The aim of the present study was to evaluate the association between various TLM parameters and ploidy status at the blastocyst stage with the use of clustered data analysis.

MATERIAL AND METHODS

Study Design and Participants

In this retrospective cohort study, 103 consecutive patients undergoing 103 cycles of intracytoplasmic sperm injection (ICSI) and preimplantation genetic screening (PGS) at the Anatolia IVF and Women's Health Center, Ankara, from April 2015 to April 2016 were enrolled. Only one ICSI cycle per patient was included; for those patients who underwent multiple ICSI cycles during this time period, only the chronologically first cycle was included.

A total of 416 blastocysts were biopsied. No result, owing to amplification failure, was noted in six blastocysts (1.4%); of those six blastocysts, one lost viability at warming and was therefore excluded. Rebiopsy followed by revitrification was undertaken for the remaining five blastocysts. Thus, a total of 415 blastocysts were included in the current analysis.

The main indication for PGS was advanced maternal age (AMA; $\geq$38 years; n = 87). Because in our setting, we do PGS routinely along with preimplantation genetic diagnosis (PGD) for single-gene disorders and balanced translocations, 16 couples undergoing PGD for single-gene disorders (n = 5) and chromosomal translocations (n = 11) were also included. Because clustered data analysis was performed to overcome patient– and ovarian stimulation–related factors as confounding (16), patients with at least two blastocysts to be biopsied were included (17).

Protocols for ovarian stimulation, procedures performed in the IVF laboratory regarding trophectoderm biopsy, vitrification and warming process of blastocysts, and methodology of genetic testing with the use of array comparative genomic hybridization are presented in detail in Supplemental Appendix 1 (Supplemental Appendix 1, Supplemental Fig. 1, and Supplemental Tables 1 and 2 are available online at www.fertstert.org).

Time-lapse Imaging and Assessment

All embryos were individually cultured in a time-lapse incubator (Embryoscope; Vitrolife) from ICSI up to the stage of trophectoderm biopsy.

Images were recorded with the use of the integrated microscope of the Embryoscope every 15 minutes from seven different focal planes. For this purpose, 15-μm intervals, 1,280 Å $\approx$ 1,024 pixels, 3 pixels per mm, monochrome, 8-bit, 0.5 seconds per image, and single 1-W red light-emitting diode were used. A time point was automatically assigned to each image, reported as hours after time zero (t0); t0 was defined as the time of injecting the sperm into the oocyte. Various TLM parameters included in our analysis are defined in Supplemental Table 1.

All annotations were made in a prospective fashion by two experienced senior embryologists. Before the present study, high intra- (kappa score = 0.95) and interobserver (kappa score = 0.91) clinical agreement was noted between these two embryologists (data not presented).

Statistical Analysis

Distribution characteristics of variables were visually assessed with the use of histograms, box plots, and Q-Q plots and analyzed with the use of Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables were expressed as mean $\pm$ SD or median and interquartile range (IQR) as appropriate. Comparisons were made with the use of independent-samples *t* test or Mann-Whitney *U* test according to distribution characteristics.

Multilevel mixed-effects models account for the correlation among observations in the same cluster and give an estimate of this correlation. Because embryos generated from a patient do not provide independent information, multilevel models were used. Intraclass correlation coefficients (ICCs) were calculated from the specified models to delineate to what extent the variation in each TLM is explained by patient–and ovarian stimulation–related factors. In a multilevel random-effects model (level one: embryo; level two: patient), ICC corresponds to the correlation of measurements within the same individual as well as to the proportion of variance explained by the individual random effect.

Multilevel mixed-effects linear regression analysis was performed for all 23 TLM parameters to determine which had any significant effect on the ploidy status adjusted by