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Short title: Time-lapse morphokinetic assessment and competency of euploid blastocyst

Duration of blastulation may be associated with ongoing pregnancy rate in single euploid blastocyst transfer cycles

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Key message

Duration of blastulation as assessed by time-lapse morphokinetic analysis might be an independent predictor for ongoing pregnancy in single euploid blastocyst transfer cycles.

Author biography

Sezcan Mumusoglu is a specialist at the Department of Obstetrics and Gynaecology, Hacettepe University. He is now completing a clinical fellowship programme in the Division of Reproductive Endocrinology and Infertility. He completed the 'Introduction to Clinical Research Training (ICRT)' programme at Harvard Medical School in May 2016. His research interests are polycystic ovary syndrome and clinical IVF.

Abstract

Not all euploid embryos implant, necessitating additional tools to select viable blastocysts in preimplantation genetic screening cycles. In this retrospective cohort study, 129 consecutive patients who underwent 129 single euploid blastocyst transfers in cryopreserved embryo transfer cycles were included. All embryos were individually cultured in a time-lapse incubator from intracytoplasmic sperm injection up to trophoectoderm biopsy. Twenty-three time-lapse morphokinetic variables were tested among patients with (n = 68) or without (n = 61) ongoing pregnancy. All 23 time-lapse morphokinetic variables, apart from duration of blastulation (tB–tSB), were comparable between patients with or without ongoing pregnancy. Duration of blastulation was significantly shorter in patients with ongoing pregnancy (8.1 ± 3.2 versus 9.5 ± 3.4 h; P = 0.014); shorter duration of blastulation remained an independent predictor for ongoing pregnancy, when tested by logistic regression analysis (OR 0.81; 95% CI 0.70 to 0.93). One important limitation of this study, and a reason for caution, is the use of multiple comparisons, which can lead to differences at the 0.05 level simply by chance or random variation. Nonetheless, the study suggests that when more than one euploid blastocyst is available, priority might be given to those with a shorter duration of blastulation.

KEYWORDS: time-lapse morphokinetic, pre-implantation genetic testing, pre-implantation genetic screening, pre-implantation genetic diagnosis, duration of blastulation, embryo, blastocyst morphology

<A>Introduction

The goal of contemporary IVF is to maximize live birth rates with single embryo transfer, regardless of female age. To achieve this goal, selection of the most viable

embryo to transfer is of critical importance. Morphologic assessment of pre-implantation embryo development is subjective and might be misleading (Capalbo *et al*., 2014).

Time-lapse monitoring has recently been introduced into embryology practice by combining three basic elements: an incubator, an optical microscope and a software programme. This has been successful in permitting uninterrupted in-vitro culture conditions and assessing dynamic pre-implantation embryo development. With time-lapse assessment, morphological events closely correlated with embryo development and IVF outcome can be closely monitored (Castello *et al*., 2016). Time-lapse morphokinetic (TLM) assessment may be a valuable tool for predicting blastocyst formation (Motato *et al*., 2016) and to de-select embryos with a low probability of implantation (Liu *et al*., 2016). Time-lapse morphokinetic assessment may also be used to select the most competent embryo with highest implantation potential (Meseguer *et al*., 2011; Rubio *et al*., 2014). Although efforts have been made to predict the ploidy status of a pre-implantation embryo with TLM assessment, the predictive value is only low to modest (Campbell *et al*., 2013; Mumusoglu *et al*., 2017).

Preimplantation genetic screening (PGS) of 24 chromosomes with trophoectoderm biopsy enhances embryo selection. In the available three randomized controlled trials, good-prognosis patients were selected for inclusion, and embryo selection was compared with PGS versus standard morphological assessment. The studies clearly demonstrate improved ongoing pregnancy rate with PGS (Yang *et al*., 2012; Forman *et al*., 2013; Scott *et al*., 2013). Technological improvements in genetic platforms

used for PGS have further enhanced live birth rates along with a decrease in miscarriage rates (Brezina *et al*., 2016). Furthermore, carrying out PGS in addition to preimplantation genetic diagnosis (PGD) in couples with single gene disorders (Goldman *et al*., 2016) or translocations (Idowu *et al*., 2015) may enhance pregnancy outcome. Not all euploid embryos implant, however, owing to embryonic, endometrial factors, or both. Therefore, additional tools to select the most viable blastocyst among an euploid embryo cohort will further improve live birth rates in PGS cycles.

The aim of the present study was to analyse whether TLM variables differ among those euploid blastocysts that result in ongoing pregnancy or not after single euploid blastocyst transfer (SEBT).

<A>Material and methods

<B>Study design and participants

In this longitudinal retrospective cohort study, 129 consecutive patients undergoing 129 SEBT cycles at the Anatolia IVF Centre, Ankara, Turkey, between April 2015 and October 2016 were included. Only the chronologically first cycle was included for those patients who underwent multiple SEBT cycles. Of the included 129 patients, 114 underwent PGS for advanced maternal age (≥38 years). The remaining 15 patients underwent PGD for single gene disorders (n = 9) or chromosomal translocation of the either partner (n = 6); all 15 patients undergoing PGD also underwent PGS.

Protocols for ovarian stimulation and procedures carried out in the IVF laboratory have been previously described in detail (Mumusoglu *et al*., 2017)

<**B**>Time-lapse morphokinetic assessment, trophectoderm biopsy, genetic testing, blastocyst vitrification and warming

All embryos were individually cultured in a time-lapse incubator (Embryoscope; Vitrolife, Denmark) from intracytoplasmic sperm injection up to the stage of trophectoderm biopsy. Images were recorded with the use of the integrated microscope of the Embryoscope every 15 min from seven different focal planes. For this purpose, 15-mm intervals, 1280 Â ≈ 1024 pixels, three pixels per mm, monochrome, 8-bit, 0.5 s 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 variables included in our analysis are defined in **Supplementary Table 1**. All TLM variables studied and included in the presrent study were annotated as described by Ciray *et al*. (2014). The time points used in the present study for tSB and tB were as those recommended by Campbell *et al.* (2013). Duration of blastulation (tB–tSB) was calculated as the time period from initiation of blastulation (tSB) to full blastocyst formation (tB) as has been previously described (Campbell *et al*., 2013; Ciray *et al*., 2014).

Two expert embryologists prospectively annotated specific TLM variables on all the embryos from second polar body appearance up to blastocyst hatching with the assistance of an Embryoscope (Vitrolife, Denmark). Briefly, for the assessment of consistency between two expert embryologists (inter-observer variation) time-lapse

annotations for 15 TLM variables of 60 blastocysts from 24 patients were made. Intra-observer variation was also assessed (IYO). Both inter- and intra-observer variation were assessed by Bland–Altman plot of variability (Bland and Altman, 1986); briefly, if the mean of the differences of TLM variable clusters close to the mean of that TLM variable, it reflects high agreement. Kappa scores reflecting inter- and intra-observer agreement were evaluated by intra-class correlation coefficients. Overall, high concordance was noted for intra- (kappa score = 0.95) and inter-observer (kappa score = 0.91) agreement for annotation.

After trophoectoderm biopsy on day 5/6, blastocysts were vitrified for all patients. Laboratory procedures for trophectoderm biopsy, vitrification and warming of blastocysts, and methodology of genetic testing with the use of array comparative genomic hybridization (a-CGH) have been previously described by Mumusoglu *et al.* (2017). Trophoecetoderm biopsy was carried out on expanding, expanded, and hatched blastocysts 120–160 h after insemination (day 5 or 6). Briefly, all biopsy procedures were conducted on a heated stage in a dish prepared with three droplets of 6 ml G-MOPS-Plus buffered medium (Vitrolife) overlaid with pre-equilibrated mineral oil. On Day 5 or 6, a diode laser was used to make an opening of 10–15 µm in the zona pellucida and five to 10 trophectoderm cells were then aspirated into the trophectoderm biopsy pipette followed by laser-assisted removal of the target cells from the body of the embryo. After trophectoderm biopsy, blastocyst vitrification was carried out with the use of the Cryotop device and solutions (Kitazato Biopharma). The first equilibration was carried out in 7.5% ethylene glycol and 7.5% dimethylsulphoxide at room temperature for 12 min. Subsequently, blastocysts were transferred into 15% ethylene glycol, 15% dimethylsulphoxide, and 0.5 mol/l sucrose

for 1 min, and then placed on the film strip of the Cryotop in a single small drop. The excess solution was removed to leave just a thin layer around each embryo, and the Cryotop was submerged into liquid nitrogen, the strip was covered with the cap, and the sample was stored submerged in liquid nitrogen.

Morphologic assessment of blastocysts was made immediately before trophoectoderm biopsy and after incubation for 4–5 h after warming, immediately before embryo transfer according to Gardner staging (Gardner and Schoolcraft 1999). Blastocyst grading was categorized as excellent (3AA, 4AA, 5AA), good (3,4,5,6 AB or BA), average (3,4,5,6 BB or AC or CA) and poor (3,4,5,6 BC or CC). When more than one euploid embryo was available for warming, the one with the best morphological grading before vitrification was chosen for warming and SEBT.

<B>Preparation of endometrium and embryo transfer procedure

Single euploid blastocyst transfer was carried out in a cryopreserved embryo transfer cycle in all patients.

Artificial cycle with gonadotrophin releasing hormone agonist (GnRHa) suppression was used for preparation of endometrium in all patients. Briefly, GnRHa (Lucrin, Abbott, Istanbul, Turkey) was started on day 21 of the preceding cycle; oral contraceptive pre-treatment was used along with GnRHa in patients with irregular cycles. Oral oestrogen (Estrofem; Novo Nordisk, Istanbul, Turkey) was commenced on the second or third day of the menstrual cycle, with incremental dosing scheme from 2 mg/day to 6 mg/day. After 12–14 days of oestrogen use, transvaginal ultrasonographic examination was carried out to confirm that bi-layer endometrial

thickness was more than 7 mm during which vaginal progesterone gel (Crinone; Merck Sereno, Bedfordshire, UK) twice a day is commenced. Embryo transfer is scheduled on the 6th day of starting progesterone. Luteal support was continued up to 10th week of gestational age in conception cycles.

Clinical pregnancy was defined as visualization of gestational sac at transvaginal ultrasonographic examination. The main outcome measure was ongoing pregnancy, which was defined as pregnancy beyond 12 weeks of gestational age.

<**B**>Statistical analysis

Distribution characteristics of variables were visually assessed with the use of histograms, box plots, and Q-Q plots, and analysed with the use of Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous variables were expressed as mean ± SD or median and interquartile range, as appropriate. Comparisons were made with the use of independent-samples t-test or Mann–Whitney-U test according to distribution characteristics. Chi-squared and Fisher's exact tests were used to compare the categorical variables. Receiver operating characteristic curve (ROC) and Youden index were used to discriminate the predictive value of TLM variables and optimum cut-off points for ongoing pregnancy.

Logistic regression analysis was carried out to explore the independent variable(s) for ongoing pregnancy. Independent variables included in the model were based on our previous study (Mumusoglu *et al*., 2017) and the other available study (Kirkegaard *et al*., 2016), which included female age, body-mass index (BMI), antral follicle count, duration of infertility, number of previous IVF cycles, total FSH consumption,

ovarian stimulation protocol, oestradiol level on the day of triggering final oocyte maturation and number of retrieved oocytes. Odds ratios with 95% confidence intervals were calculated.

As multiple testing increases the risk of type-1 error, significant changes noted at the 0.05 level might be attributed to chance or random variation, and one should be cautious about the inferences, particularly those that are on the borderline of statistical significance at the nominated 5% level (Farland *et al*., 2016; Walters, 2016).

All statistical analyses were carried out with the use of the statistical package SPSS 22.0 (IBM Corp., USA).

The Institutional Review Board of Hacettepe University approved the study protocol on 14 June, 2016 (GO-16/422-43).

<A>Results

Of the 129 included patients, the mean female age, BMI and the number of oocytes retrieved were 36.9 ± 4.8 years, 25.0 ± 4.1 kg/m^2 and 9.8 ± 4.9, respectively.

Of the 129 SEBTs, 85 (65.9%) resulted in clinical pregnancy with 17 (20.0%) miscarriages. Hence, a total of 68 patients (52.7%) achieved an ongoing pregnancy.

The comparison of baseline demographic features, ovarian stimulation related factors and embryological data of the patients with or without ongoing pregnancy are

depicted in **Table 1**. All the features, apart from female age and BMI, were comparable between the two groups. The mean female age and BMI were significantly lower in patients with ongoing pregnancy (P = 0.013 and P = 0.036 respectively (**Table 1**).

Twenty-three TLM variables were compared among patients with or without ongoing pregnancy (**Table 2**). Of all the 23 TLM variables analysed, 22 were comparable between the two groups (**Table 2**). Significant difference, however, was observed in duration of blastulation (tB–tSB) among those euploid blastocysts that resulted in ongoing pregnancy (8.1 ± 3.2 versus 9.5 ± 3.4 h; P = 0.014).

The adjusted odds ratios for ongoing pregnancy based on the blastocyst morphology after warming, immediately before embryo transfer, are given in **Table 3**. In this comparison, when poor morphology was taken as the reference, blastocysts with good morphology had significantly higher ongoing pregnancy rate (adjusted OR= 5.1, 95% CI 1.1 to 23.4; P = 0.038). Overall, an increase in ongoing pregnancy rate was noted with transfer of better-quality blastocysts when tested by chi-square test linear-by-linear association (P = 0.036).

At logistic regression analysis, ongoing pregnancy was taken as the dependant variable and the following variables were taken as independent variables: female age, BMI (kg/m^2), duration of infertility (month), antral follicle count, number of previous cycles, ovarian stimulation protocol, total FSH consumption (IU), oestradiol level on the day of triggering (pg/ml), number of retrieved oocytes, duration of blastulation (tB–tSB) (h), genetic testing (PGS or PGD and PGS), day of embryo biopsy,

blastocyst morphology. Of the included variables, only duration of blastulation (OR 0.81, 95% CI 0.70 to 0.93; *P* = 0.004) was the significant independent predictor of ongoing pregnancy (**Table 4**). Within this model, overall, the blastocyst morphology was not a significant predictor for ongoing pregnancy. Euploid blastocysts with good and average morphology, however, had higher probability of achieving ongoing pregnancy compared with those with poor morphology (OR = 14.10, 95% CI 1.65 to 120.25; *P* = 0.016 and OR 7.1, 95% CI 1.04 to 48.17; *P* = 0.046, respectively).

When receiver operator characteristic curve analysis was conducted for duration of blastulation to predict ongoing pregnancy, the area under curve with 95% confidence interval was 0.61 (95% CI 0.51 to 0.70) (**Figure 1**). The optimum cut-off point for duration of blastulation was 6.26 h, with sensitivity and specificity of 82.0% and 64.7%, respectively. Furthermore, SEBT with duration of blastulation of 6.26 h or less was associated with significantly higher ongoing pregnancy (25/36 [69.4%]) compared with transfer of those with over 6.26 h (43/93 [46.2%]) (OR 2.64, 95% CI 1.17 to 6.0; *P* = 0.02).

<A>Discussion

In this study, after SEBT, we observed that duration of blastulation was an independent significant predictor for ongoing pregnancy. Duration of blastulation (tB–tSB) refers to calculated time period from start of blastulation to formation of a full blastocyst (Campbell *et al.*, 2013; Ciray *et al.*, 2014). Hence, shorter duration of blastulation among euploid embryos seems to be associated with higher ongoing pregnancy rate. The area under curve of duration of blastulation to predict ongoing pregnancy, however, was modest (0.61, 95% CI 0.51 to 0.70).

In our study, with univariate analysis, mean female age and BMI were significantly lower in patients who achieved ongoing pregnancy compared with those who did not. With logistic regression analysis, however, female age and BMI were no longer significant predictors of ongoing pregnancy. Our findings are in concordance with the previous studies reporting no affect of female ageing on pregnancy outcome in PGS cycles (Forman *et al*., 2012; Harton *et al*., 2013). Discordant to our findings, a recent study, however, reported a significant independent negative effect of BMI on live birth rate (Tremellen *et al*., 2016). In this study, evaluating 125 SEBT cycles, lean women (n = 70; BMI 18.5–24.9 kg/m^2) had a significantly lower miscarriage rate (14.2%) compared with overweight (n = 24; BMI 25–29.9 kg/m^2; 29.1%) and obese (n = 31; BMI ≥30 kg/m^2; 41.9%) women. Body mass index was also noted to be an independent contributor to live birth as tested by logistic regression analysis (Tremellen *et al*., 2016), which may support a non-genetic cause for obesity-related miscarriage in SEBT cycles. The reason for the discordant findings of the study by Tremellen *et al*. (2016) and ours might be that, although BMI is a continuous variable, categorical assessment by arbitrary thresholds as conducted by Tremellen *et al*. (2016) may decrease the power and therefore increase the risk of type 1 error (Altman and Royston, 2006). We should also note that the lack of significant effect of BMI on ongoing pregnancy outcome in our data set might be due to limited sample size.

Once euploid, the effect of blastocyst morphology on pregnancy rates in SEBT cycles is controversial. In the study by Capalbo *et al*., (2014), the effect of blastocyst morphology on ongoing implantation rate was assessed in 215 SEBT cycles, using the same blastocyst grading scheme as used in the present study). They reported similar

ongoing implantation rates after SEBT of excellent (*n* = 110 [49.1%]), good (*n* = 32 [59.4%]), average (*n* = 60 [43.3%]) and poor (*n* = 13 [53.8%]) morphology blastocysts. A recent study, however, refuted these findings and reported that blastocyst morphology was still an independent predictor of ongoing pregnancy in PGS cycles (Irani *et al*., 2017). In a total of 417 cryopreserved embryo transfer cycles, excellent quality embryos (*n* = 38 [84.2%]) yielded a statistically significantly higher ongoing pregnancy rate compared with poor quality (*n* = 106 [35.8%]) and average quality (*n* = 197 [55.8%]) embryos (adjusted OR 11.0, 95% CI 3.8 to 32.1 and adjusted OR 4.8, 95% CI 1.7 to 13.3, respectively); good-quality embryos (*n* = 76) were associated with a statistically significant higher ongoing pregnancy rate than poor-quality embryos (61.8% versus 35.8%; $P < 0.01$). The authors also reported that inner cell morphology was a better predictor of pregnancy outcome than trophoectoderm morphology or blastocoel expansion (Irani *et al*., 2017). Concordant with this study, we, in our series, reported an overall effect of blastocyst morphology on ongoing pregnancy as depicted by linear-by-linear association (**Table 3**). At logistic regression analysis, however, although there seemed to be a trend between higher-grade morphology and better ongoing pregnancy rate, it reached statistical significance as an independent predictor with good and average morphology. Lack of statistical significant association with excellent morphology might be due to limited sample size. Sub-classification according to blastocyst expansion, inner cell mass and trophoectoderm quality have not been conducted in the study owing to limited sample size and is a limitation of the current series.

Several studies have reported a beneficial effect of TLM assessment to select the most competent embryo to transfer compared with morphology alone (Meseguer *et al*.,

2012; Rubio *et al*., 2014; Adamson *et al*., 2016). Such a beneficial effect, however, was refuted by other studies (Kirkegaard *et al*., 2013; Park *et al*., 2015; Goodman *et al*., 2016). All these studies have been conducted in embryos that had not undergone PGS or PGD.

Data are lacking for the use of TLM to assess the competency of genetically tested euploid blastocysts (Yang *et al*., 2014; Kofinas *et al*., 2015). To the best of our knowledge, only two such studies have been published (Yang *et al*., 2014; Kofinas *et al*., 2015), one being an abstract (Kofinas *et al*., 2015). In the study by Yang *et al* (2014), 138 patients underwent PGS using array comparative genomic hybridization (Yang *et al*., 2014); sibling oocytes were randomized to culture in a time-lapse incubator (Group A; n = 582) versus conventional incubator (Group B; n = 581). One to two euploid blastocysts within the most predictive TLM variables as suggested by Meseguer *et al.* (2011) (Group A) or with the best morphological grade available (Group B) were selected for fresh transfer on day 6. The ongoing pregnancy rate was significantly higher in Group A (68.9%) compared with group B (40.5%). As a drawback, however, one cannot discriminate whether enhanced ongoing pregnancy rate in Group A compared with Group B is due to embryo selection or undisturbed culture condition. Of note, the authors reported a non-significant increase in clinical pregnancy and implantation rate with the transfer of euploid blastocysts with early initiation of blastulation (n = 27 patients; tIB <96.1 h) compared with those with delayed initiation of blastulation (n = 18; tIB ≥96.1 h) in Group A. Only 45 patients were included in total, making the results vulnerable to type II error.

In the abstract by Kofinas *et al.* (2015), 28 euploid embryos with known outcomes were included; TLM variables were standardized to syngamy as intracytoplasmic sperm injection was not routinely conducted. Time to full blastocyst formation was reported to be significantly shorter in pregnant patients; when receiver operator characteristic analysis was conducted for ongoing pregnancy, the area under the curve was 0.73, with 80.8 as the best discriminatory cut-off point. This small-scale abstract has not been published.

Although only three studies have been published, including ours, it seems that transfer of faster developing euploid blastocysts might be resulting in higher pregnancy rates. With our sample size, mean difference ± standard deviation of duration of blastulation noted in the current study and type 1 error of 0.05, our analysis had 69.4% power. Obviously, further larger-scaled studies are warranted to delineate such an association, if present, and optimize which TLM variables should be used for this purpose. As in-vitro culture conditions may affect morphokinetic development, each embryology laboratory should define their own cut-off points for such TLM variables.

With clustered data analysis, we recently reported that TLM assessment is not a good predictor of the ploidy status of pre-implantation embryos (Mumusoglu *et al.*, 2017). Time-lapse morphokinetic analysis is an advantage in PGS and PGD cycles permitting the timing of blastocyst biopsy. Making annotations prospectively for all available embryos, however, might be time-consuming. An alternative in PGS and PGD cycles might be make the annotations retrospectively for the euploid embryos only. Such a strategy would not only decrease the workload, but along with

morphology, duration of blastulation, may permit the selection of the more competent euploid blastocyst to transfer when there is more than one euploid embryo.

In the present study, we report on and make inferences based on a large number of statistical tests at the 0.05 significance level. As multiple comparisons increase the risk of type-1 error, and significant changes noted with such analyses might be attributed to chance or random variation, the inferences, particularly those that are on the borderline of statistical significance at the nominated 5% level, should be viewed with considerable caution (Farland *et al*., 2016; Walters, 2016). Limited sample size and lack of automated system for annotation are limitations of the current study. High kappa scores for intra- and inter-observer agreement, however, were reassuring.

In conclusion, shorter duration of blastulation, along with morphology, might be used as an adjunct to give priority and select the more competent euploid blastocyst in SEBT cycles.

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Declaration

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Figure 1: Receiver operator characteristics curve of duration of blastulation (tB–tSB) to predict ongoing pregnancy. Area under curve (95% CI 0.61; 0.51 to 0.70). ROC, receiver operator characterstic.

Table 1. Comparison of baseline demographic features, ovarian stimulation factors and embryological data of patients with or without ongoing pregnancy.[a]

	With ongoing pregnancy (n = 68)	*Without ongoing pregnancy (n = 61)*
Female age, years	35.9 ± 5.3	38.0 ± 4.0[b]
Female body mass index, kg/m^2	24.3 ± 3.7	25.8 ± 4.4[c]
Duration of infertility, month	56.3 ± 52.1	52.2 ± 49.8
Antral follicle count[d]	11.0 (6; 15)	11.0 (7; 14)
Number of previous cycles[d]	2.0 (0; 3)	2.0 (0; 3)
Ovarian stimulation protocol		
Long GnRH agonist protocol n (%)	39 (57.4)	31 (50.8)
GnRH antagonist protocol n (%)	29 (42.6)	30 (49.2)
Total FSH consumption, IU	2635.8 ± 1351.0	2599.4 ± 1190.4
Oestradiol level on the day of triggering, pg/ml	2458.3 ± 1606.6	2691.2 ± 1917.3
Number of retrieved oocytes	9.9 ± 5.6	10.4 ± 5.4
Number of embryos with two-pronuclei	6.6 ± 3.7	6.9 ± 4.0
Blastulation rate, %	54.0 ± 21.2	56.6 ± 22.2
Genetic testing		
PGS, n (%)	57 (83.8)	57 (93.4)
PGS and PGD, n (%)	11 (16.4)	4 (6.6)

[a]Values are given as mean ± SD or median (25th to 75th percentiles), unless stated otherwise.
[b]$P = 0.013$.
[c]$P = 0.036$ (all other comparisons are not statistically significant).
[d]Not normally distributed.
GnRH, gonadotrophin-releasing hormone; PGD, preimplantation genetic diagnosis; PGS, preimplantation genetics screening.

Table 2. Comparison of time-lapse morphokinetic variables of patients with or without ongoing pregnancy.

*TLM variable (*n*)*	*With ongoing pregnancy (*n = *68)*	*Without ongoing pregnancy (*n = *61)*
tPB2 (129)	4.0 ± 2.9	3.9 ± 2.6
tPNa (129)	8.7 ±1.6	8.3 ± 1.4
tPNf (129)	24.2 ± 2.9	24.1 ± 2.9
t2 (129)	26.6 ± 3.0	26.4 ± 3.0
t3 (112)	36.7 ± 4.0	36.5 ± 4.6
t4 (125)	38.3 ± 3.7	38.4 ± 4.4
t5 (114)	48.8 ± 6.3	48.9 ± 7.2
t6 (115)	51.8 ± 5.8	51.0 ± 6.7
t7 (114)	53.0 ± 6.4	54.0 ± 7.4
t8 (113)	55.6 ± 7.2	57.8 ± 8.2
t9 (125)	67.5 ± 9.7	69.5 ± 11.8
tM (129)	91.9 ± 7.8	91.9 ± 11.2
tSB (129)	99.5 ± 8.0	99.2 ± 10.1
tB (129)	107.6 ± 8.4	108.8 ± 9.9
tEB (111)	113.1 ± 8.0	113.7 ± 8.1
CC2[a] (112)	9.7 ± 3.2	10.5 ± 2.4
CC3[a] (100)	12.1 ± 3.7	12.0 ± 5.3
S2[a] (110)	2.1 ± 2.8	1.6 ± 2.4
S3 (109)	7.4 ± 6.4	9.4 ± 7.8
t9 to t2 (125)	40.9 ± 8.5	43.4 ± 9.7
t5 to t2 (114)	22.1 ± 5.4	22.8 ± 6.3
CC3/CC2a (102)	1.5 ± 1.1	1.4 ± 1.3
Duration of blastulation (129)	8.1 ± 3.2	9.5 ± 3.4[b]

tPB2: appearance of second polar body; tPNa: appearance of pronuclei (2PN); tPNf: both pronuclei faded/syngamy; t2, t3, t4, t5, t6, t7, t8 and t9 is the time (hour) between intracytoplasmic injection and two, three, four, five, six, seven, eight, and greater than nine-cell stage, respectively. tM: time from insemination to formation of a morula; tSB: time from insemination to start of blastulation; tB: time from insemination to formation of a full blastocyst; tEB: time from insemination to expanded blastocyst; CC2: the length of second cell cycle (t3–t2); CC3 the length of third cell cycle (t5–t3); S2 is from three to four (t4–t3); S3 is the synchrony in division five to eight cells (t8–t5); duration of blastulation is from start of blastulation to formation of a full blastocyst (tB–tSB).

[a]Not normally distributed.

[b]$P = 0.014$; all other comparisons are not statistically significant.
TLM, time-lapse morphokinetic.

Table 3. Blastocyst morphology[a] and ongoing pregnancy rates.

Blastocyst morphology	*With ongoing pregnancy (n = 66)*	*Without ongoing pregnancy (n = 57)*	*Adjusted OR 95% CI*[b]	P-*value*
Excellent, n (%)	4 (66.7)	2 (33.3)	5.3 (0.6 to 46.0)	NS
Good, n (%)	19 (65.5)	10 (34.5)	5.1 (1.1 to 23.4)	0.038
Average, n (%)	40 (51.9)	37 (48.1)	2.8 (0.7 to11.7)	NS
Poor, n (%)	3 (27.3)	8 (72.7)	1[c]	
Linear-by-linear association				0.036[d]

[a]Morphology could not be assessed in six collapsed blastocysts and hence these were excluded from the present analysis. The transfer of those six collapsed euploid blastocysts resulted in two ongoing pregnancies.
[b]Reference category.
[c]chi-square test.
[d]Odds ratio adjusted for female age and body mass index.
NS, not statistically significant.

Table 4. Logistic regression analysis to predict ongoing pregnancy.

Variable	*Odds ratio*	*95% CI*	P-*value*
Female age, years	0.913	0.816 to 1.022	0.113
Body mass index, kg/m^2	0.887	0.783 to 1.005	0.060
Duration of infertility, months	1.005	0.995 to 1.014	0.252
Antral follicle count	0.977	0.880 to 1.085	0.667
Number of previous cycles	1.007	0.815 to 1.244	0.948
Ovarian stimulation protocol	0.413	0.148 to 1.153	0.091
Total FSH consumption, IU	1.000	1.000 to 1.001	0.643
Oestradiol level on the day of triggering, pg/ml	0.971	0.935 to 1.009	0.132
Number of retrieved oocytes	1.081	0.909 to 1.285	0.378
Duration of blastulation (tB–tSB)	0.806	0.696 to 0.933	0.004
Genetic testing (PGS or PGD and PGS)	0.384	0.750 to 1.978	0.252
Day of embryo biopsy (day 5/6)	2.308	0.605 to 8.797	0.221
Blastocyst morphology			0.096
Excellent	15.344	0.951 to 247.483	0.054
Good	14.103	1.654 to 120.248	0.016
Average	7.074	1.039 to 48.172	0.046
Poor	1[a]		

[a]Reference category.
PGD, preimplantation genetic diagnosis; PGS, preimplantation genetic screening.

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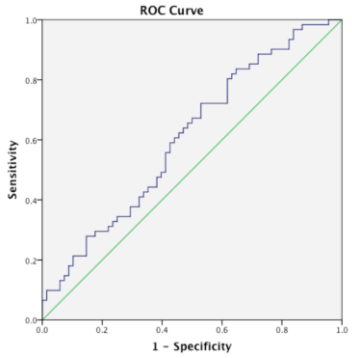

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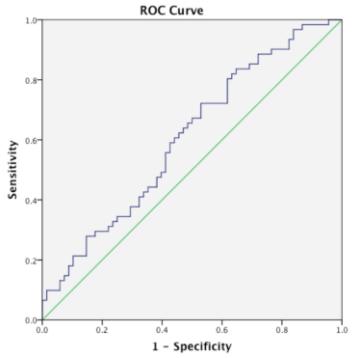

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