

Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope

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Objective: To determine whether incubation in the integrated EmbryoScope time-lapse monitoring system (TMS) and selection supported by the use of a multivariable morphokinetic model improve reproductive outcomes in comparison with incubation in a standard incubator (SI) embryo culture and selection based exclusively on morphology.

Design: Prospective, randomized, double-blinded, controlled study.

Setting: University-affiliated private in vitro fertilization (IVF) clinic.

Patient(s): Eight hundred forty-three infertile couples undergoing intracytoplasmic sperm injection (ICSI).

Intervention(s): No patient intervention; embryos cultured in SI with development evaluated only by morphology (control group) and embryos cultured in TMS with embryo selection was based on a multivariable model (study group).

Main Outcome Measure(s): Rates of embryo implantation, pregnancy, ongoing pregnancy (OPR), and early pregnancy loss.

Result(s): Analyzing per treated cycle, the ongoing pregnancy rate was statistically significantly increased 51.4% (95% CI, 46.7–56.0) for the TMS group compared with 41.7% (95% CI, 36.9–46.5) for the SI group. For pregnancy rate, differences were not statistically significant at 61.6% (95% CI, 56.9–66.0) versus 56.3% (95% CI, 51.4–61.0). The results per transfer were similar: statistically significant differences in ongoing pregnancy rate of 54.5% (95% CI, 49.6–59.2) versus 45.3% (95% CI, 40.3–50.4) and not statistically significant for pregnancy rate at 65.2% (95% CI, 60.6–69.8) versus 61.1% (95% CI, 56.2–66.1). Early pregnancy loss was statistically significantly decreased for the TMS group with 16.6% (95% CI, 12.6–21.4) versus 25.8% (95% CI, 20.6–31.9). The implantation rate was statistically significantly increased at 44.9% (95% CI, 41.4–48.4) versus 37.1% (95% CI, 33.6–40.7).

Conclusion(s): The strategy of culturing and selecting embryos in the integrated EmbryoScope time-lapse monitoring system improves reproductive outcomes.

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Key Words: Early pregnancy loss, embryo culture, embryo selection, implantation, ongoing pregnancy rate, time-lapse

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In recent years, clinical practice efforts have been directed toward improving embryo selection. The

identification of embryos with a higher capacity for implantation means we can reduce the number of embryos for

transfer without reducing the chances of pregnancy in a cycle of assisted reproduction. To this end, different noninvasive embryo selection methods have been designed that provide information on how to distinguish embryos with better prognosis (1). There are different methods of embryo gradation (2), but they are all based on morphology, and evaluation of morphology under microscope is subject to observer subjectivity (1). One of the noninvasive embryo evaluation methods to have come into the limelight in recent years is the time-lapse

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monitoring systems (TMS). Image capturing with time-lapse devices is a noninvasive method that offers the possibility of 24-hour monitoring of embryo development and of increasing the quantity and quality of information without disturbing the culture conditions (3–5). The system generates unique determinations that combine the morphologic assessment with the timing of embryonic cleavages, thus diminishing observer subjectivity (6). With an integrated TMS (EmbryoScope; UnisenseFertiliTech A/S, Aarhus, Denmark), the image acquisition unit is an integral part of the culture chamber, which facilitates the monitoring of embryos while they remain inside a controlled, stable culture environment. The safety of this system has been demonstrated (7). In 2011, Meseguer et al. (8) published a report on the development of a multivariable model in which, through a decision tree, the embryos were classified according to their implantation rate. This proposed a predictive algorithm based on a combination of morphokinetic exclusion and selection criteria that can be applied in real time to exclude morphologically normal embryos that display aberrant cleavage patterns.

Our previous retrospective cohort study published in 2012 showed a relative improvement in clinical pregnancy rate of more than 10% per embryo transferred by using the same algorithm and incubation conditions (9). Our present work is a prospective, randomized study to validate this multivariable model. We randomly selected a control group of patients whose embryos were cultured in standard incubators (SI) and were assessed only by conventional morphological criteria is compared with a study group in which the embryos were cultured in a tri-gas incubator with a built-in camera for the automatic acquisition of images at specific times and evaluation by the multivariable model. In this way, we could compare the results for both groups and verify an expected improvement in pregnancy rates of at least 10% in the study group compared with the controls. To our knowledge, ours is the first randomized clinical study available on this topic.

MATERIALS AND METHODS

Study Design and Participants

An experimental, prospective, triple-blind, double-center, randomized controlled trial was performed at two IVI clinics, one in Valencia and one in Bilbao. The study complies with the Spanish law governing assisted reproductive technologies (14/2006), and the institutional review board approved the study as did the institution's ethics committee (1009-C-088-IR). The study was registered with the Clinical Trial Web site (www.clinicaltrials.gov; registration number: NCT01549262).

In the study, 930 patients undergoing assisted reproduction were selected based on the inclusion-exclusion criteria we will describe, resulting in an embryo cohort from a group of patients that had autologous or oocyte donation (OD) cycles between February 2012 and July 2013. The oocyte recipients entered our OD program for one of the following diagnoses: failure to achieve pregnancy after at least three cycles of assisted reproduction techniques, genetic female or chromosomal disorders, or low response to controlled ovarian hyperstimulation.

The patients were randomly divided into a control group of patients whose embryos were developed in a conventional incubator, which were assessed only by conventional morphologic criteria, and a study group, in which embryos were cultured in the EmbryoScope TMS and were evaluated using the multivariate morphokinetic model.

The inclusion criteria for both autologous patients and recipients were as follows: age 20–38 years, first or second intracytoplasmic sperm injection (ICSI) cycle, body mass index (BMI) of >18 and <25 kg/m². The exclusion criteria were severe male factor (total motile sperm <1 million), hydrosalpinx, presenting uterine diseases after two-dimensional ultrasound evaluation, and/or three-dimension (if in doubt) or hysteroscopy (for acquired or congenital uterine abnormalities), endocrinopathies (thrombophilia), recurrent pregnancy losses, endometriosis, or patients receiving concomitant medication as a treatment for any other condition that might interfere with the results of the study. For autologous treatments, low-responder patients (fewer than six metaphase II (MII) per cycle) or those with a follicle-stimulating hormone (FSH) basal determination >12 or an antimüllerian hormone (AMH) concentration of <1.7 pmol/L (based in our own clinical experience) were also excluded. Each patient was enrolled in the study only once.

The time-lapse technology used is CE-certified (i.e., meets the health and safety requirements for equipment in the European Union), and in our study it was used for the purposes for which it was approved. The CE certificate (number: DGM-673) endorses the quality of the system from UnisenseFertiliTech A/S in terms of its manufacture and final inspection of the IVF incubators and accessories related to class II (including IVF incubators and the plates used for such incubators). The production, installation, and servicing of IVF incubators and accessories from UnisenseFertiliTech A/S are likewise certified (certificate number: DGM-672).

Randomization

Patients entering the trial were allocated to either TMS (study group) or SI (control group) using a computer-generated randomization table (obtained by SPSS software; IBM), which was handled by an embryologist at the laboratory in charge (A.G.) the day before the oocyte retrieval or oocyte donation. The study is considered double-blind because: [1] the gynecologist (evaluating the primary effect) did not know to which group the patients had been assigned, and [2] the statistician evaluating the results only knew the incubators by a binary code and not by type.

Ovarian Stimulation in Autologous Patients and Oocyte Donors

All donors were obtained from the IVI oocyte donation program. The detailed selection criteria for donors were as described by Garrido et al. (10). Briefly, all donors had normal menstrual cycles lasting 26 to 34 days, were aged 18–34 years (average 25.5 years), had a body mass index (BMI) of 18–25 kg/m², had received no endocrine treatment (including gonadotropins and oral contraception) for the 3 months

preceding the study, and had a normal uterus and ovaries at transvaginal ultrasound (no signs of polycystic ovary syndrome) (11).

The protocol for controlled ovarian stimulation was as described by Melo et al. (12), and both GnRH agonist and antagonist treatments were included. Human chorionic gonadotropin (hCG, Ovitrelle; Serono Laboratories) was administered subcutaneously when at least three leading follicles had reached a mean diameter of R18 mm. Transvaginal oocyte retrieval was scheduled 36 hours later. Donor oocytes were used in fresh cycle, but in some cases we used oocytes from our oocyte bank. The oocyte vitrification protocol has been previously described elsewhere and yielded comparable results to what has previously been demonstrated by our group (13). The protocol for endometrial preparation of recipients was as described by Meseguer et al. (14). After embryo transfer, all patients received luteal phase support every 12 hours, whereby autologous patients received a daily dose of 400 mg and oocyte recipients a daily dose of 800 mg of vaginal micronized progesterone (Progeffik; Effik).

Ovum Pick-up and ICSI

Follicles were aspirated, and the oocytes were washed in Gamete Medium (Cook IVF). After washing, oocytes were cultured in fertilization medium (Cleavage Medium; Cook IVF) at 5.5% CO₂ in air and 37°C for 4 hours before oocyte denudation. Oocyte denudation was performed by mechanical pipetting 40 IU/mL of hyaluronidase in the same medium. Subsequently, ICSI was performed in a medium containing HEPES (Gamete Medium; Cook IVF) at 400× magnification using an Olympus IX7 microscope. Immediately after ICSI, the injected oocytes for TMS cycles were placed individually in preequilibrated culture dishes (EmbryoSlide; Unisense Fertilitech A/S) under oil at 37°C and 5.5% CO₂ in air in a time-lapse incubator (EmbryoScope). Zygotes for the conventional incubator (Heraeus; HeraCell) cycles were placed in normal Petri dishes (Falcon) (drop culture) of culture media (Cleavage Medium; Cook IVF) under oil at 37°C and 5.5% CO₂ in air.

Embryo Culture

All embryos in both the SI and TMS were incubated at 37°C, 5.5% CO₂, atmospheric O₂ concentration and were cultured individually until embryo transfer at day 3 (72 hours after ICSI) in Cleavage Medium (Cook IVF); from day 3 to day 5, we used CCM Medium (Vitrolife). For SI, we prepared the culture dishes with three washing drops of 100 μL and six culture drops of 50 μL, covered by 7 mL of mineral oil. For the TMS, we placed 25 μL of culture media in each well of the EmbryoSlide, covered with 1.2 mL of mineral oil. From D3 to D5, a new EmbryoSlide were used. In the TMS, the imaging system uses low-intensity red light (635 nm) from a single light-emitting diode with short illumination bursts of 30 ms per image to minimize embryo exposure to light and to avoid emitting damaging short-wavelength light. Image stacks were acquired at five to seven equidistant focal planes every 15 or 20 minutes during embryo development inside the EmbryoScope.

Embryo Scoring and Selection

On the day of oocyte capture, all patients included in this study were assigned the day of embryo transfer (day 3 vs. day 5) based on previous medical criteria. Categorization by the embryologist was not considered for deciding the day of transfer. For embryos incubated in the SI, embryo morphology was evaluated at 48 and 72 hours after ICSI. Evaluated parameters included cell number, symmetry, and granularity as well as the type and percentage of fragmentation (fragment defined as an anuclear, membrane-bound extracellular cytoplasmic structure and calculating the percentage of the total volume of the embryo constituted by fragments), presence of multinucleated blastomeres, and degree of compaction as previously described elsewhere (11). According to the scoring methods, we selected the embryos from the SI for transfer on day 3. On day 2, optimal embryos were defined as those with four cells, less than 15% fragmentation, high or moderate symmetry, and no multinucleation. On day 3, they were defined as those with six or more cells and the previously mentioned fragmentation and symmetry features (15). Embryos considered to be viable on day 3 were those that were transferred or vitrified (16).

Embryo scoring and selection with TMS was performed by analysis of time-lapse images of each embryo on an external computer with software developed for time-lapse image analysis (EmbryoViewer workstation; UnisenseFertilitech A/S). Embryo morphology and developmental events were annotated, including the precise timing of the observed cell divisions in the hours after ICSI. The precise timing of cell division and developmental parameters, such as blastomere symmetry and multinucleation, were determined, deriving the following morphokinetic parameters: time of cleavage to two-blastomere embryo (t2), time of cleavage to three-blastomere embryo (t3), time of cleavage to four-blastomere embryo (t4), and time of cleavage to five-blastomere embryo (t5). Additionally, the duration of the second cell cycle (cc2)—that is, the duration of the two-blastomere embryo phase (t3 – t2)—and synchrony (s2) in divisions from a two-blastomere embryo to a four-blastomere embryo (t4 – t3) were calculated. We used the hierarchical classification of embryos described by Meseguer et al. (8); the model is represented graphically in Supplemental Figure 1 (available online).

Human blastocysts were scored on day 5 (120 hours) according to the expansion of blastocoel cavity and the number and integrity of both the inner cell mass and trophectoderm cells. We defined for comparison the following groups: group A, complete trophectoderm and high-cell number compact inner cell mass; group B, incomplete trophectoderm and several grouped cells; and group C, few cells in trophectoderm or inner cell mass (17). According to this grading system, we selected the embryos for transfer on day 5. Optimal blastocysts were those with trophectoderm cells and inner cell mass in A or B groups (14). In TMS group, embryos were selected on day 3 and 5 by using hierarchical classification on those embryos, which were defined as morphologically optimal. In other words, we used the initial morphology to discard those embryos with suboptimal morphology and then morphokinetics were applied. Embryo

morphokinetic categories were not used to decide the number of embryos for transfer or the day of transfer. To make a comparison between morphology and the time-lapse classification tree categories, as we previously published and compared, we defined and analyzed the morphology of the transferred embryos using the same and prior published morphology categories (8), a description of which is provided herein.

Category 1: The two pronuclear (2PN) embryo consists of two cells at 27 hours after insemination, four cells at day 2, and eight cells at day 3. The blastomere size is even at the two-, four-, and eight-cell stages, no multinucleation is observed at any time, and the fragmentation is less than 10%.

Category 2: The 2PN embryo consists of one to two cells at 27 hours, three to four cells at day 2, and six to eight cells at day 3. Only one mismatch is allowed: either one cell at 27 hours, three cells at day 2, or six to seven cells at day 3. Blastomeres are even sized at the two-, four-, and eight-cell stages, no multinucleation is observed at any time, and the fragmentation is less than 20%.

Category 3: The 2PN embryo consists of one to two cells at 27 hours, two to four cells at day 2, and six to eight cells or morula at day 3. The embryo can have asymmetric blastomeres, and multinucleation can be observed in maximally one blastomere at each stage. The degree of fragmentation is less than 20%.

Category 4: The 1PN or 2PN embryo consists of one to two cells at 27 hours, two to six cells at day 2, and four or more than eight cells or morula at day 3. The embryo can have asymmetric blastomeres and be multinucleated. The degree of fragmentation is less than 50%.

Category 5: The embryo consists of any number of cells at 27 hours, day 2, and day 3. Asymmetric blastomere size, multinucleation, and any degree of fragmentation is allowed. Atretic embryos and embryos with arrested development belong to this category.

Outcome Measures

The primary end point for this study was ongoing pregnancy confirmed by the presence of gestational sacs with fetal heartbeat detected by transvaginal ultrasound examination in week 12. The binary response variable of ongoing pregnancy was either “1” for the presence of gestational sacs with fetal heartbeat or “0” for the absence of gestational sacs with fetal heartbeat. The purpose of the analysis was to assess whether the primary end point was affected by the incubation method, TMS versus SI.

For secondary outcomes, we analyzed fertilization rates, embryo development (defined previously), implantation rates and pregnancy (defined as having a serum β -hCG level higher than 10 IU/mL on day 14 after ICSI), and early pregnancy loss. Pregnancy was defined as the detection of a positive β -hCG 2 weeks after embryo transfer. Implantation rate was

calculated by dividing the number of gestational sacs with fetal heartbeat detected by the number of embryos transferred. Early pregnancy loss was considered when the (β -hCG-positive) pregnant cycles did not result in an ongoing pregnancy.

Sample Size Calculation and Statistical Analysis

We started on the premise that the clinical pregnancy rate in our IVF program is about 50%, and our hypothesis was that the combination of embryo morphology and the morphokinetic algorithm along with better growing conditions (demonstrated by 7 and 9) would increase the chances of pregnancy by at least 10%. We used macro N2IPV! 2006.02.24 (Domenech, Granero, and Sesma) for the sample size and power determination of two independent proportions. The sample size required per group was 388 patients per arm, with an alpha risk of 5% and beta risk of 20%, which means a power of 80%. The calculation method followed a normal asymptotic approximation and a one-sided hypothesis. That is, we needed a total number of 776 patients (388 in the control group and 388 for the study group) to complete the study with the proposed hypothesis. We increased the number of patients recruited approximately 15% with the intention of overcoming the potential loss of patients.

Comparison of quantitative variables (Tables 1 and 2) between the TMS and control groups was done using Student's *t* test for independent samples when data were normally distributed (tested by Kolmogorov-Smirnov test). For comparing categorical data, Fisher's test was performed to compare proportions among the groups according to the TMS incubation and selection procedure performed. Yates's correction equation was used instead when the expected frequency was less than 5. $P < .05$ was considered statistically significant.

We calculated the positive pregnancy test rate and ongoing pregnancy rates in each group and the corresponding relative risk (RR) with 95% confidence intervals for all intended cycles (all initiated cycles enrolled in the study), all treated cycles, and all cycles with transfer (of one or more embryos). Furthermore, the early pregnancy loss was calculated on the cycles that led to a positive pregnancy test as the ones that did not result in an ongoing pregnancy. Implantation rate was calculated as the number of implanted embryos out of the transferred embryos. Differences between the two groups were tested using Fisher's exact test. $P < .05$ was considered statistically significant. Power determination of the main variable outcome (ongoing pregnancy rate) was performed to demonstrate the validity of the results by using the previously described SPSS macro.

To confirm and quantify the crude two group analysis, the implantation of all transferred embryos were fitted to a logistic regression with the following covariates: type of incubation (TMS or SI), class variable, two states; type of cycle (autologous or donation), class variable, two states; day of transfer (day 3 or 5), class variable, two states; and the age of the patient at the time of transfer, continuous variable, measured in years.

TABLE 1

Descriptive characteristics of the patient and IVF laboratory practice in the time-lapse and control groups.

	TMS group (n = 438)	Control group (n = 405)	P value
Age (y), patients and recipients	34.7 ± 2.7 (34.4–34.9)	34.6 ± 2.7 (34.4–34.9)	NS
Age (y), patients and donors	30.4 ± 5.5 (29.9–31.0)	30.0 ± 5.5 (29.5–30.5)	NS
BMI (kg/m ²)	23.2 ± 3.7 (22.6–23.7)	23.04 ± 2.8 (22.5–23.5)	NS
COS protocol			
Long GnRH agonist (%)	14.8 (65)	16.4 (66)	NS
Short GnRH antagonist (%)	85.2 (373)	83.6 (339)	NS
Total dose of FSH	1,781 ± 631 (1,709–1,853)	1,832 ± 603 (1,763–1,900)	NS
Total dose of hMG	1,127 ± 664 (1,035–1,219)	990 ± 538 (913–1,066)	NS
E ₂ on hCG day (pg/mL)	1,981 ± 943 (1,882–2,079)	1,964 ± 962 (1,860–2,067)	NS
P ₄ on hCG day (ng/mL)	0.76 ± 0.38 (0.72–0.80)	0.74 ± 0.35 (0.70–0.78)	NS
Days of stimulation	13.0 ± 3.45 (12.5–13.6)	13.2 ± 3.42 (12.6–13.8)	NS
Donor recipients (%)	47.8 (208) (43.2–52.6)	49.1 (200) (44.2–53.9)	NS
Metaphase II oocytes (n)	8.0 ± 2.7 (7.76–8.26)	8.1 ± 3.0 (7.8–8.3)	NS
Fertilization rate (%)	75.3 ± 16.5 (73.8–76.9)	74.0 ± 17.4 (72.3–75.7)	NS
Day 3 ET (% of total)	72.5 % (318) (68.3–76.7)	75.5 % (306) (71.3–79.7)	NS
Blastocyst ET (%)	27.5 (120) (23.3–31.7)	24.5 (99) (20.3–28.7)	

Note: Values are mean, and values in brackets are 95% confidence intervals or the total number of patients. BMI = body mass index; COS = controlled ovarian stimulation; E₂ = estradiol; ET = embryo transfer; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; hCG = human chorionic gonadotropin; hMG = human menopausal gonadotropin; NS = not statistically significant; P₄ = progesterone; TMS = time-lapse monitoring system.

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RESULTS

During the study period, we identified 930 eligible couples between February 2012 and July 2013. Of the 930 couples, 74 patients dropped out before randomization. After randomization, 13 patients dropped out before completing the cycle. Therefore, 843 patients were finally included: 438 in the study group (TMS) and 405 in the control group. The participant flow through the trial is displayed in [Supplemental Figure 2](#) (available online).

Descriptive Characteristics of the Patients, Donors, and Recipients and Preincubation Characteristics in the TMS and Control Group

Test results of the effectiveness of randomization based on patient, donor, and recipient characteristics are shown in [Table 1](#). There were no differences between the two groups studied for any of the patient, donor, or recipient parameters; both presented a similar age, body mass index, and ovarian stimulation parameters, such as gonadotropin dosage, number of oocytes donated, duration of stimulation, and final E₂ serum concentrations. The mean fertilization

rate was similar in both groups at approximately 74.5% per patient oocyte cohort; there was only one fertilization failure in the SI group. The number of oocytes obtained and number of MII oocytes did not differ between the two groups. Oocyte donors who used vitrified warmed oocytes were 120 (27.4%) and 112 (27.6%) in TMS and SI groups, respectively. The number of patients going through their first attempt in the clinic was 305 (69.6%) and 328 (81.0%) in TMS and SI groups, respectively ($P < .05$). Regarding the differences in the distribution of the patients between the two clinics, only a slightly increased proportion of day 5 transfers in IVI Bilbao were observed (31.6%) versus IVI Valencia (22.0%) ($P < .05$); the remaining clinical features compared were not statistically significantly different (data not shown).

Embryo Development Characteristics in the TMS and Control Group

Detailed results for the developmental characteristics are shown in [Table 2](#). The embryo fragmentation percentage was higher in the TMS group compared with SI (7.5% vs.

TABLE 2

Descriptive characteristics of the embryo development and fate in the time-lapse and control groups.

	TMS group (n = 2,638)	Control group (n = 2,427)	P value
Embryo fragmentation (%)	7.5 ± 0.1 (7.2–7.9)	6.9 ± 9.4 (6.5–7.1)	.006
No. of blastomeres	6.9 ± 2.3 (6.8–6.9)	6.9 ± 2.7 (6.8–7.0)	NS
Embryo symmetry	1.7 ± 0.5 (1.7–1.7)	1.7 ± 0.5 (1.7–1.7)	NS
Optimal embryos (day 3) (%)	46.2 (1,219) (44.3–48.1)	43.1 (1,046) (41.3–45.1)	.010
Blastocyst rate (%)	52.3 (576) (50.3–54.2)	50.5 (471) (48.5–52.5)	NS
Optimal blastocyst (day 5) (%)	20.9 (230) (19.4–22.4)	16.6 (155) (15.1–18.1)	.001
Transferred embryos	1.86 ± 0.37 (1.8–1.9)	1.86 ± 0.40 (1.8–1.9)	NS
Cryopreserved embryos	3.9 ± 2.2 (3.6–4.1)	3.6 ± 2.2 (3.4–3.9)	NS

Note: Values are mean, and values in brackets are 95% confidence interval or the total number of embryos.

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6.9%). There was a statistically significantly higher percentage of optimal embryos on day 3 in the TMS group (46.2% compared with 43.1% in the control group; $P=.01$). Likewise, there was a statistically higher percentage of optimal embryos on day 5 out of all embryos cultured to day 5 in the TMS group (20.9%) compared with the control group (16.6%) ($P=.001$).

Comparison between Morphology and Time-lapse Categories

The observed correlations between morphokinetic categories and embryo implantation in the transferred embryos cultured and selected in the TMS are presented in [Supplemental Table 1](#) (available online). The transferred embryos split into the previously described morphology categories and cultured in SI are described in [Supplemental Table 2](#) (available online).

Outcome Results

Taking into account all cycles, including those that did not result in an embryo transfer, the ongoing pregnancy rate (OPR) was significantly increased at 51.4% (95% CI, 46.7–56.0) ($P=.005$) for the TMS group compared with 41.7% (95% CI, 36.9–46.5) for the SI group ([Table 3](#)). For pregnancy rate (PR), differences were not statistically significant at 61.6% (95% CI, 56.9–66.0) versus 56.3% (95% CI, 51.4–61.0). Results per transfer were similar: statistically significant differences in OPR 54.5% (95% CI, 49.6–59.2) versus 45.3% (95% CI, 40.3–50.4) ($P=.01$) and not statistically significant for pregnancy rate at 65.2% (95% CI, 60.6–69.8) versus 61.1% (CI 95%, 56.2–66.1). Results per transfer for day 3 versus day 5 transfers are presented in [Supplemental Table 3](#) (available online). The early pregnancy loss rate was statistically significantly decreased ($P=.01$) for the TMS group with 16.6% (95% CI, 12.6–21.4) versus 25.8% (95% CI, 20.6–31.9). The implantation rate of the transferred embryos was statistically significantly increased at 44.9% (95% CI, 41.4–48.4) versus 37.1% (95% CI, 33.6–40.7) ($P=.02$). The increase in the clinical outcome was not statistically significantly different between clinics as analyzed by a

chi-square analysis ($P>.05$). We did not have any reports of ectopic pregnancy.

Logistic Regression Analysis

A logistic regression analysis was performed on OPR to account for the effect of the remaining confounding factors addressed in the [Materials and Methods](#) section.

The factors used in the logistic regression model were incubation type, day of transfer, oocyte source, and the egg age of the patient at the time of oocyte retrieval. The final modeling results are given in [Table 4](#). The analysis was based on 843 patients who either presented an ongoing pregnancy or failed. Of the four covariates, only incubation type and day of transfer had a statistically significant effect in the model. Taking into account these other covariates led to an odds ratio (OR) of the TMS group versus the control group of 1.36 (95% CI, 1.10–1.68).

DISCUSSION

Our present study represents the first controlled, randomized prospective study to quantify the improvement in reproductive outcome after incubation and selection using the EmbryoScope time-lapse system. The observed increase in implantation and ongoing pregnancy rates using the advantages of undisturbed embryo culture in a stable device such as the EmbryoScope time-lapse incubator in association with the use of the model of embryo selection developed by our group represents an important improvement in reproductive medicine. Incubation and selection in the studied TMS improves the ongoing pregnancy and implantation rate and reduces the early pregnancy loss.

The observed increase in clinical parameters may have multiple explanations, as previously described by Meseguer et al., such as: [1] strictly controlled and stable incubation conditions, [2] minimal handling of embryos inside and outside the incubator, [3] increased information on embryo development for qualitative evaluation of morphology, and [4] the use of quantitative morphokinetic parameters for selecting viable embryos.

TABLE 3

Outcome results per intention to treat, per cycle, per transfers and per embryo transferred.

Outcome	TMS group	Control group	RR	P value
All cycles with oocyte retrieval	438	405		
Pregnancy (% of all treated cycles)	61.6 (56.9–66.0)	56.3 (51.4–61.0)	1.09 (0.98–1.23)	.12
Ongoing pregnancy (% of all treated cycles)	51.4 (46.7–56.0)	41.7 (37.0–46.6)	1.23 (1.06–1.43)	.005
All transfers	415	373		
Pregnancy (% of all transfers)	65.3 (60.6–69.7)	61.1 (56.1–65.9)	1.07 (0.95–1.19)	.22
Ongoing pregnancy (% of all transfers)	54.5 (49.6–59.2)	45.3 (40.3–50.4)	1.20 (1.04–1.39)	.01
All pregnant cycles	271	228		
Early pregnancy loss (% of all pregnancies)	16.6 (12.6–21.4)	25.8 (20.6–31.9)	0.64 (0.45–0.91)	.01
All transferred embryos	775	699		
Implantation rate (% of all transferred embryos)	44.9 (41.4–48.4)	37.1 (33.6–40.7)	1.43 (1.05–1.39)	.02

Note: Results shown as proportion with 95% confidence limits in brackets, relative risk (RR) with 95% confidence limits in brackets and the corresponding P value (Fisher's exact test). Total number of cycles are also presented in brackets.

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TABLE 4

Logistic regression analysis of ongoing pregnancy after week 12 as affected by incubation type, day of transfer, oocyte source, and age of the patient.

Model effect	Values	OR (95% CI)	P value
Incubation	TMS versus SI	1.41 (1.06–1.86)	.015
Day of transfer	Day 5 versus day 3	1.53 (1.08–2.16)	.016
Oocyte source	Autologous versus donation	0.89 (0.60–1.30)	NS
Age	Years	Per year, 1.01 (0.98–1.05)	NS

Note: CI = confidence interval; OR = odds ratio; NS = not statistically significant; SI = standard incubator; TMS = time-lapse monitoring system.

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Strictly controlled and stable incubation conditions are important for embryo development because variations in temperature and pH may impair embryo development and quality (18). The safety of the TMS used in this study has been validated (7, 19). The TMS that was used purifies the gas in the chamber by constant recirculation through an active carbon filter, a HEPA filter, and a UV filter cartridge to effectively remove volatile organic compounds, contaminants, and particles from the airstream, which ensures a stable and reliable gas supply. The TMS shows minimal variation of temperature and gas concentrations when doors are opened as well as a fast recovery to optimal conditions compared with the larger and slower SI (9).

Time-lapse image acquisition can minimize disturbance to the culture environment and the embryo development by integrating the incubation and image acquisition into one system. In contrast, with SI embryo development has to be monitored by removing the embryos from the incubator (7, 20).

Several studies have demonstrated the predictive value of morphokinetics (8, 21–24). Our embryo quality assessment model, published in 2011, correlates embryo morphology evaluation and clinical results (8). The use of morphokinetic variables for embryo selection allowed us to reject embryos with a lower chance of implantation while distinguishing embryos with higher probabilities of implanting based on morphokinetic characteristics. The relative improvement of clinical pregnancy using the regression model was estimated to be 15.7% per embryo transfer, as published in the retrospective study of 8,414 cycles performed in 2012 (9). The results obtained in our present prospective study are in line with this estimation, which is 16.9% per embryo transfer.

The main limitation in this trial is that we did not know how much of this improvement was due to the culture conditions because the system does not allow us to differentiate. We performed the study conscientiously and assumed that the improvement was related to both culture conditions and the selection model, but the contribution of each was not enough to perform a randomized study to clarify that point.

Several publications have demonstrated the potential use of morphokinetics to improve selection by using early (22) and late development parameters (23, 24), and some of them were validated prospectively (25), which confirms the

potential improvement that can be provided by morphokinetics selection. In favor of the potential improvement that is provided by the use of morphokinetics are the results provided in Supplemental Tables 1 and 2. We observed that both morphokinetic and morphology categories are significantly related to implantation potential. In relation to the morphokinetic algorithm, the selection process clearly increased the proportion of transferred embryos corresponding to the A and more likely to the A+ category. The model significantly distinguished between embryos with different implantation potential, although the + category provided by cc2 overcomes the categorization of the s2 variable. A consequence could be the trading of place in the hierarchy between the variables s2 and cc2, leaving cc2 as the secondary and s2 as the tertiary variable after this analysis. In any case, we have been able to identify prospectively the real benefit that is provided by the use of our morphokinetic algorithm for embryo selection.

The trial had several secondary limitations. [1] The randomization were not perfectly performed as the patient distribution to the two groups would have been expected to be closer to a 50:50 ratio than the reported 51.9:48.1. The main reason for this deviation was limited patient requests for TMS culture. [2] The inclusion criteria were quite strict in favor of good-prognosis patients; in consequence, the potential value of the EmbryoScope system for improving clinical outcome might only be relevant to good-prognosis patients. [3] This randomized, controlled trial was performed using high O₂ tension, and is possible that the observed improvements may not be detected under different conditions if we also consider that culturing in low O₂ does appear to be beneficial, at least in extended-culture embryos (26).

As minor aspect that could be a potential confounder in our study is the disparity in the volumes of media used in the SI and TLM. We have also observed an inverse correlation between the percentage of embryo fragmentation and the proportion of optimal embryos between SI and TLM; in both variables, the differences were very small, and the increased percentage of embryo fragmentation did not finally affect the proportion of optimal embryos; the increased proportion in TMS may be due a higher proportion of embryos with an optimal number of blastomeres.

In a recently published study, Kirkegaard et al. (27) did not find any significant differences in the morphokinetics of embryos that implanted and those that failed to implant in their study population, and thus they suggested that morphokinetic selection criteria may not be universally applicable whereas deselection by morphokinetics seems more generally predictable. Although the study by Kirkegaard's group was prospective, it was based on a limited sample size, which was recognized by the investigators. Hence, a comparison of their study (n = 84) to our current one (n = 513) is not meaningful. The morphokinetic selection criteria published to date have been based on a specific embryo cohort (e.g., patients from one clinic/chain of clinics) and therefore may not be valid for all embryo cohorts, as several factors have been shown to impact morphokinetics (28–31).

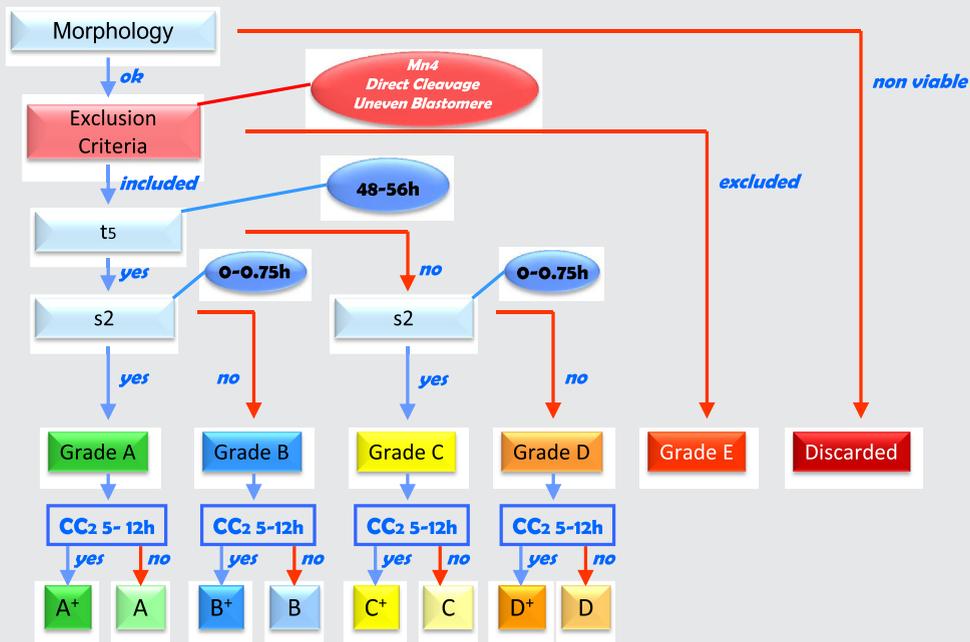
Our study prospectively demonstrated an improvement in reproductive outcomes by using the EmbryoScope TMS and a set of selection and deselection criteria based on embryo morphokinetics. The study is limited by the unknown effect of the more stable culture environment provided by the EmbryoScope incubator. However, better embryo development is an integral part of the concept of culture in this specific time-lapse device and hence a prerequisite for applying a morphokinetic algorithm to choose from morphologically optimal embryos that resulted in the improved outcomes.

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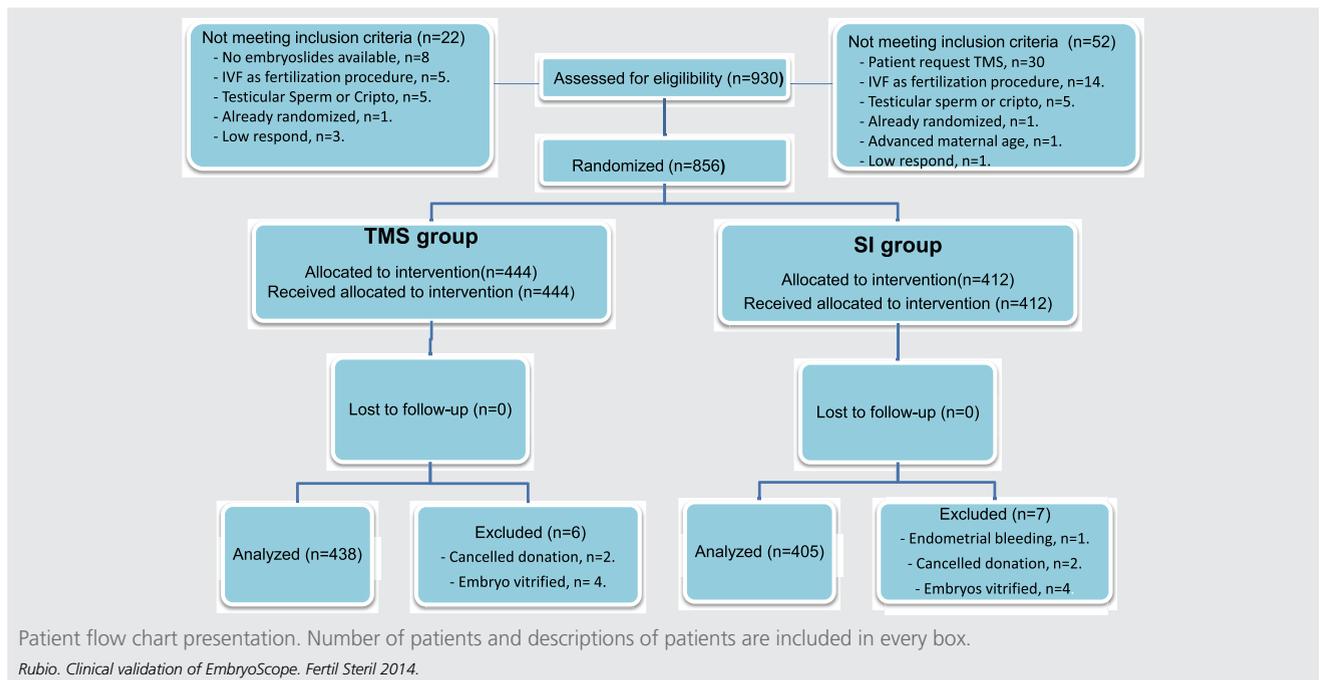
SUPPLEMENTAL FIGURE 1



Morphokinetic decision tree algorithm used for embryo selection. For the division to five cells, a shorthand notation of t5 is used; we defined the duration of the second cell cycle (cc2) as the time from division to a two-blastomere embryo until division to a three-blastomere embryo: $cc2 = t3 - t2$. We defined the second synchrony (s2) as the duration of the division transition from a two-blastomere embryo to a four-blastomere embryo: $s2 = t4 - t3$. We defined direct cleavage as those cases in which cc2 was lower than 5 hours. The time of all events is expressed as hours after ICSI microinjection.

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SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL TABLE 1

Implantation in the embryo categories of the hierarchical classification tree model applied in the time-lapse monitoring system, results are only referred to those embryos with known implantation (known implantation data embryos).

Embryo category	N total (n = 513)	N implanted (no.)	Implantation (%)	Embryo category	Implantation (%)
A+	122	74	60.6	A	52.9
A-	80	33	41.2		
B+	46	24	52.2	B	43.9
B-	36	12	33.3		
C+	67	30	44.8	C	39.5
C-	52	17	32.7		
D+	28	13	46.5	D	29.3
D-	30	4	13.3		
E	51	7	13.7	E	13.7

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SUPPLEMENTAL TABLE 2

Implantation in the embryo morphologic categories observed in the standard incubator, results are only referred to those embryos with known implantation (known implantation data embryos), $P = .007$.

Embryo morphology category	Implantation (%)
I (72)	38.8
II (224)	35.7
III (n = 146)	24.7
IV (27)	11.1
V (0)	—

Note: Values between brackets refer to the number of embryos in each morphology category.

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SUPPLEMENTAL TABLE 3

Outcome results depending on embryo transfer day done day 3 versus blastocyst stage (day 5).

	TMS group	Control group	P value
Day-3 transfers	301	284	
Transferred embryos per treatment	1.90 (1.86–1.94)	1.89 (1.84–1.93)	.694
Pregnancy	61.8 (56.3–67.3)	58.8 (53.1–64.5)	.460
Ongoing pregnancy	49.8 (44.1–55.4)	44.0 (38.2–49.8)	.159
Implantation rate (% of all transferred embryos)	40.7 (36.1–45.2)	34.3 (29.9–38.8)	.050
Early pregnancy loss (% of all pregnancies)	19.9 (36/187) (14.1–25.6)	25.1 (42/168) (18.5–31.6)	.151
Blastocyst transfer	114	90	
Transferred embryos per treatment	1.78 (1.69–1.86)	1.80 (1.72–1.88)	.701
Pregnancy (% of all transfers)	74.3 (66.2–82.3)	68.5 (58.9–78.1)	.363
Ongoing pregnancy (% of all transfers)	66.4 (57.7–75.1)	49.4 (39.1–59.7)	.015
Early pregnancy loss (% of all pregnancies)	10.7 (9/85) (4.1–17.2)	27.8 (17/62) (16.6–38.9)	.003
Implantation rate (% of all transferred embryos)	57.5 (49.5–65.3)	46.9 (37.8–60.0)	.082

Note: Results shown as proportion with 95% confidence limits in brackets, relative risk (RR) with 95% confidence limits in brackets and the corresponding P value (Fisher's exact test). Number of cycles are also presented between brackets. TMS = time-lapse monitoring system.

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