

No benefit of culturing embryos in a closed system compared with a conventional incubator in terms of number of good quality embryos: results from an RCT

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STUDY QUESTION: Does culture in a closed system result in an increased number of good quality embryos (GQE) on Day 2 compared with culture in a conventional system?

SUMMARY ANSWER: Culture in a closed system up to 2 days after microinjection results in similar embryo development and morphological quality compared with culture in a conventional incubation system.

WHAT IS KNOWN ALREADY: Time-lapse imaging (TLI) incubators are rapidly being introduced into IVF laboratories worldwide, despite the lack of large prospective randomized trials demonstrating improvement in embryo development or pregnancy rates.

STUDY DESIGN, SIZE, DURATION: A randomized controlled trial including 364 patients (365 cycles) was conducted between May 2010 and February 2014. After oocyte collection, randomization was carried out and all of a patients' oocytes were allocated to culture in either a conventional incubator or a closed incubator system in proportion 1:2 until embryo transfer on Day 2. A total of 1979 oocytes were injected and cultured in the closed system, and 1000 in the standard incubator. The primary end-point was the number of GQE in the two groups.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: In total, 364 patients undergoing their first IVF cycle using ICSI, where at least one oocyte was retrieved, were randomized in a university hospital setting. Two hundred and forty patients were randomized for culture in a closed system and 124 patients for culture in the conventional incubator system (control group). Embryo assessments and final morphological scoring before transfer and cryopreservation were carried out at the same time points for embryos cultured in the conventional incubator and in the closed system.

MAIN RESULTS AND THE ROLE OF CHANCE: There was no significant difference in the mean \pm SD number of GQEs between groups: 2.41 ± 2.27 for the closed system group and 2.19 ± 1.82 for the control group ($P = 0.34$, difference 0.23, 95% confidence interval 0.69; -0.24). No significant differences were found in the number of 4-cell embryos, implantation-, pregnancy- or ongoing pregnancy rates. A significantly higher miscarriage rate was found in the TLI group compared with the control group (33.3 and 10.2%, $P = 0.01$).

LIMITATIONS, REASONS FOR CAUTION: Culture media, temperature and gas levels were similar in the open and closed incubator systems, but different culture dishes were used. Culturing embryos for longer time period (to the blastocyst stage) may give different results. Only ICSI patients were included, which may limit the generalizability of the results. Finally, the number of GQEs on Day 2 was used as a surrogate outcome for live birth.

WIDER IMPLICATIONS OF THE FINDINGS: The results are consistent with other, smaller randomized trials showing no difference in embryo quality when comparing culture in a conventional incubator with that of a closed TLI incubator system.

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Introduction

A number of developments with the aim to improve embryo culture have taken place in the assisted reproductive technology (ART) laboratory over the years, for example, the transition to more complex culture media (Gardner and Lane, 1997; Summers and Biggers, 2003; Lane and Gardner, 2007; Mantikou *et al.*, 2013), prolongation of culture from 2–3 to 5–6 days (Marek *et al.*, 1999; Nilsson *et al.*, 2005; Papanikolaou *et al.*, 2006; Glujovsky *et al.*, 2012), and culture at reduced oxygen tension (Waldenström *et al.*, 2008; Kovacic *et al.*, 2010; Bontekoe *et al.*, 2012; Kirkegaard *et al.*, 2013a). The ART sector is rapidly moving forward and many new techniques are being introduced without proper validation of the safety or potential benefits (Vajta *et al.*, 2010; Harper *et al.*, 2012).

Most procedures in the ART laboratory are still performed manually, but new, more automated technologies are now being developed (Meseguer *et al.*, 2012a). Recently, a new type of system for culturing human embryos has been introduced; the time-lapse imaging (TLI) system, where assessment of key events during embryo development can be performed without removing the embryos from the incubator. Although TLI systems can be more or less closed, the possible advantage of these systems is minimization of environmental fluctuations in temperature, pH and humidity, which could impair embryo quality (Fujiwara *et al.*, 2007; Zhang *et al.*, 2010).

When assessing embryos only during short intervals outside the incubator, as in conventional culture systems, important morphological and development events which could have an impact on implantation might be missed. TLI overcomes some of these problems by gaining information of embryo development through continuous image recordings. Several retrospective observational studies using this new methodology have indicated a number of early timing variables, mainly concerning the duration and synchrony of the first cell cycles that may predict blastocyst formation (Wong *et al.*, 2010; Cruz *et al.*, 2012; Dal Canto *et al.*, 2012; Chamayou *et al.*, 2013; Conaghan *et al.*, 2013; Kirkegaard *et al.*, 2013b), implantation (Meseguer *et al.*, 2011; Chamayou *et al.*, 2013) and pregnancy (Lemmen *et al.*, 2008; Meseguer *et al.*, 2012b). However, Kirkegaard *et al.* (2013b) showed, in a prospective cohort study, that although blastocyst formation could be predicted by a few time-lapse variables, there was no difference in the timing between implanted and non-implanted blastocysts. Only a few small studies have looked at the possible advantages of culturing in a TLI system *per se*. Nakahara *et al.* (2010) showed in a prospective study of 292 oocytes from 84 patients that the fertilization rate and number of good quality embryos (GQE) were similar when compared with culture in a standard incubator. Cruz *et al.* (2011), in an oocyte donation programme including 60 patients and 478 oocytes, found no differences in blastocyst and ongoing pregnancy rates for embryos cultured in a conventional incubator versus a TLI incubator, and Kirkegaard *et al.* (2012), in a small randomized controlled trial (RCT) (59 patients), found similar embryo development rates between the two systems, as well as similar pregnancy and implantation rates.

The aim of this RCT was to analyse in a large population whether culture of human embryos in a closed system with TLI is superior

to culture in a standard incubator in terms of number of GQEs on Day 2.

Materials and Methods

Clinical setting/patient group

An RCT was conducted at Reproductive Medicine, Sahlgrenska University Hospital, Gothenburg. Patients were recruited between May 2010 and February 2014. Patients were eligible if they were ≤ 40 years of age, undergoing their first IVF cycle using ICSI and at least one oocyte was retrieved. Patients undergoing egg donation were excluded.

In total, 364 patients were included and randomized. Only one cycle per patient was included. Randomization was carried out by the embryologist after oocyte retrieval by a web-based randomization programme and all the patients' oocytes were allocated to culture in either a conventional incubator or in a closed system, in proportion 1:2. The patients as well as the treating physician and the person performing the statistical analyses were blinded to which type of procedure was used until the outcome of transfer (pregnant versus not pregnant) was known. Embryologists were not possible to blind.

The study was approved by the Ethical Committee of the University of Gothenburg (Dnr: 666-09) and all patients signed an informed consent.

Stimulation, oocyte retrieval and ICSI

Ovarian stimulation was performed using down-regulation with a GnRH agonist (Suprecur, Sanofi, Paris, France) in a long protocol, followed by stimulation with recombinant FSH (Gonal-F, Merck Serono, Darmstadt, Germany, or Puregon, MSD, NJ, USA), or urinary-derived gonadotrophins (Menopur, Ferring, Copenhagen, Denmark). In a few cases ($n = 28$), a GnRH antagonist (Orgalutran, MSD) was used in a short protocol. Follicular development was monitored by serum estradiol levels and vaginal sonography. When two or more follicles reached ≥ 18 mm diameter, hCG (Pregnyl 5000 or 10 000 IU, MSD or Ovitrelle 6500 IU, Merck Serono) was administered. Oocyte retrieval was scheduled 36 ± 2 h after hCG injection. Crinone gel (Merck Serono) or progesterone MIC by vaginal route was given as luteal support after embryo transfer.

The oocyte–cumulus complexes were collected using transvaginal sonographically guided puncture, rinsed in MOPS (Vitrolife, Gothenburg, Sweden) and placed in a culture dish (Falcon, VWR, NJ, USA) containing G-IVF medium (Vitrolife).

The oocyte–cumulus complexes were denuded using hyaluronidase (Vitrolife). ICSI was performed within 5 h of oocyte retrieval on mature (metaphase II: MII) oocytes in pre-equilibrated culture dishes (Falcon) with droplets of Gamete (Vitrolife) with an overlay of 6 ml mineral oil (Ovoil, Vitrolife) under an inverted microscope.

Standard culture system

For the control (standard incubator) group, the oocytes were rinsed in G-I media directly after the ICSI procedure and then transferred to pre-equilibrated culture dishes (Falcon), with 20 μ l droplets of G-I media (Vitrolife) under mineral oil (Ovoil). The oocytes were cultured in a standard incubator at 37°C, 6% CO₂ and atmospheric O₂ concentration until embryo transfer on Day 2. pH and temperature were monitored on a weekly basis for the standard incubator, while CO₂ was monitored less frequently.

For the control group, the embryos were taken out of the standard incubator at 16–18 h after injection in order to check for fertilization, at 25–27 h post-ICSI for early cleavage screening, and finally at 43–45 h post-ICSI for assessment of quality prior to transfer and cryopreservation. Scoring was performed using an Olympus inverted microscope with a 20 × 1.5 Hoffman Modulation contrast objective.

Closed culture system

The EmbryoScope™ (Unisense Fertilitech, Århus, Denmark) is an incubator with a built-in microscope with a Leica 20 × 0.40 LWD Hoffman Modulation contrast objective. It is capable of acquiring images for up to 72 oocytes/embryos simultaneously. During image acquisition, the embryos are illuminated with low intensity red light at 635 nm for <0.5 s per image. For this study, images were acquired every 20 min, at seven focal planes.

For the study group (closed system), the oocytes were washed in G1 media after injection, transferred with a Cook Flexipet (Cook, Limerick, Ireland) to EmbryoSlides prepared as described below, and cultured in the EmbryoScope™ until time of transfer. The embryos in the EmbryoScope™ were incubated under the same culture conditions as the control group, i.e. at 37°C, 6% CO₂ and atmospheric O₂ concentration. CO₂ and temperature were monitored on a weekly basis for the EmbryoScope™.

According to the manufacturer's recommendations, EmbryoSlides (Unisense, Fertilitech) were prepared in the afternoon the day before oocyte retrieval with 25 µl culture medium (G1). The EmbryoSlides were covered with a 1.2 ml layer of oil (Ovoil) and pre-equilibrated in a standard incubator. In the study group, embryos were not removed from the EmbryoScope™ during assessments.

Embryo selection and transfer

At least two embryologists were involved in assessing embryo quality. For embryos cultured in the EmbryoScope™ as well as for embryos cultured in a conventional incubator, morphological assessment and selection for transfer were made at the same time points using the same criteria.

Additional information available from time-lapse sequences was not used for embryo assessment or selection.

Fertilized oocytes were scored for pronuclei on Day 1 at 16–18 h after ICSI and for early cleavage at 25–27 h after ICSI.

On Day 2, at 43–45 h post-ICSI, embryos were graded according to blastomere number, blastomere size and degree of fragmentation. An embryo was defined as a GQE on Day 2 when having 4–6 blastomeres and <20% fragmentation, with no multinucleation. When choosing embryos for transfer with otherwise equal quality on Day 2, early cleavage and the presence of nuclei in the cells were also taken into account. If no GQEs were available, embryos with an increased fragmentation rate could be transferred but were not cryopreserved.

One embryo (in a few cases two embryos, $n = 12$) of good quality or in some cycles of less good quality ($n = 27$) was transferred on Day 2 and super-numerary GQEs were frozen on the same day using a slow freezing protocol (Cook Medical, Ireland).

End-points

The primary end-point was the number of GQEs. Secondary end-points were fertilization rate, number of 4-cell embryos on Day 2, implantation, pregnancy, miscarriage and ongoing pregnancy rates. Ongoing pregnancy rate was defined as the presence of a gestational sac with fetal heartbeat ≥ 8 weeks.

Sample size determination and statistical analysis

The study was a superiority trial. The sample size was based on the primary outcome of the study; the number of GQEs. With an average number of GQEs of 3.9 and an SD of 3.1 (Lundin and Bergh, 2007), a total of 357 patients

were needed to show an increase with 1.0 GQEs in the intervention group (α -value 0.05, power 80%) if the randomization into the study versus control groups is performed 2:1, i.e. 238 patients in the intervention group and 119 patients in the control group.

The patients were randomized by a web-based computer program. Stratification was performed by minimizing for age and mean number of aspirated oocytes (Pocock, 1983). For descriptive statistics, continuous variables are presented as mean \pm SD and ranges. Categorical variables are presented as n (%).

For comparison between the groups, Fisher's exact test was used for dichotomous variables and the Mann–Whitney U -test was used for continuous variables. For main variables, 95% confidence intervals (CI) were presented for differences in estimates. A P -value of <0.05 was considered significant. In order to select independent predictors of the dependent variables ongoing pregnancy rate and miscarriage rate, univariable logistic regression analysis was first performed for each of the baseline variables. Variables with $P < 0.25$ were then entered into a stepwise multiple logistic regression analysis.

Statistical analyses were performed using SAS software version 9.3 (SAS Institute, Inc., NC, USA), and SPSS software version 22, 2013 (SPSS, Chicago, IL, USA).

Results

A flow-chart of patients included in the study is shown in Fig. 1.

In total, 364 patients (365 cycles) were randomized between May 2010 and February 2014. Their oocytes were allocated to culture in either the TLI incubator (241 cycles, 2280 oocytes) or a standard incubator (124 cycles, 1180 oocytes). Analysis was performed by intention-to-treat (Fig. 1), but excluding one cycle prior to analysis, due to this patient having been randomized twice.

Patient demographics are presented in Table I. No significant differences were found between the two groups.

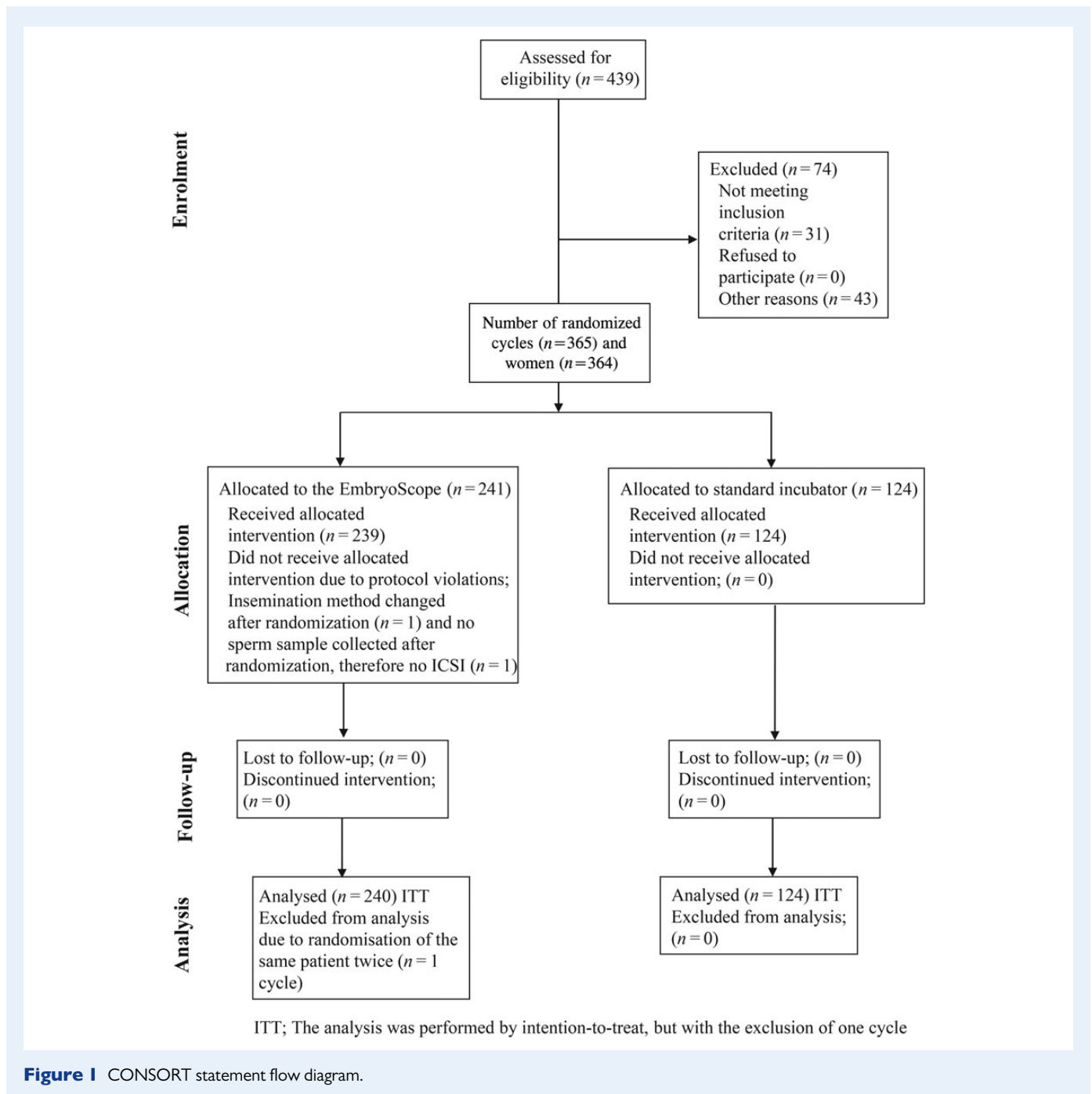
A total of 1979 oocytes were injected and cultured in the EmbryoScope™, and 1000 in the standard incubator. No significant difference was found between culture in the EmbryoScope™ and standard incubator regarding the number of GQEs on Day 2 (2.41 ± 2.27 for the EmbryoScope™ group and 2.19 ± 1.82 for the standard incubator group; $P = 0.34$, difference 0.23, 95% CI 0.69; -0.24), nor for any other embryo variables (Table II).

The pregnancy rate per randomized woman was 30.0% in the EmbryoScope™ and 31.5% in the standard incubator ($P = 0.87$) and per embryo transfer 33.5 and 34.2%, respectively ($P = 0.99$). The ongoing pregnancy rate was 20.0% in the EmbryoScope™ and 28.2% in the standard incubator ($P = 0.10$) per randomized cycle and 22.3 and 30.7% ($P = 0.13$) per embryo transfer, respectively (Table III). The miscarriage rate was 33.3% in the EmbryoScope group and 10.2% in the control group ($P = 0.011$).

In the stepwise multiple logistic regression analysis, the baseline variables 'smoking' [adjusted odds ratio (OR) 0.329; 95% CI 0.112–0.967, adjusted $P = 0.035$] and 'number of embryos transferred' (adjusted OR 3.351; 95% CI 1.447–7.759, adjusted $P = 0.0036$) were independently correlated to the variable ongoing pregnancy.

For the variable miscarriage, only the baseline variable 'group' (adjusted OR 4.367; 95% CI 1.393–13.699, adjusted $P = 0.0075$) was independently correlated to miscarriage.

For comparison, a proportion of transferred embryos ($n = 146$) was scored in both the EmbryoScope and in the Olympus microscope. We found that 134 (91.8%) were scored equally in both systems. Ten



embryos were scored as GQE in the EmbryoScope™ but not in the Olympus microscope, and two as GQE in the Olympus microscope but not in the EmbryoScope™. In four embryos, the number of cells differed by maximum one cell, in three embryos, the cell size symmetry was not equally scored and in five embryos, the percentage of fragmentation differed. The outcome of this comparison did not influence which embryo was selected for transfer.

Discussion

There are two key questions when comparing the culture of human embryos in a closed TLI system with a conventional incubator: (i) Is

the closed culture system superior to the conventional incubator concerning embryo development? (ii) Is the TLI system, when using new embryo variables identified from the TLI system, superior to conventional embryo morphology when selecting embryos for transfer? In the present study, we have addressed only the first question.

The main finding was that no significant difference between the two groups was found in the number of GQEs on Day 2. Neither were any significant differences in the number of 4-cell embryos, implantation-, pregnancy- or ongoing pregnancy rates detected, while the miscarriage rate was significantly higher in the TLI group.

The main results are in agreement with a recent RCT by Kirkegaard *et al.* (2012), where a closed incubator system was compared with a

Table I Baseline characteristics of patients for the two groups.

	EmbryoScope (n = 240)	Control (n = 124)	P-value
Age, years	31.8 ± 4.3 (21.4–39.7)	31.8 ± 4.1 (22.3–39.7)	0.90
BMI, kg/m ²	24.4 ± 3.9 (16.8–36.1)	24.3 ± 4.0 (16.5–34.0)	0.70
No. of smokers	30 (12.5)	11 (8.9)	0.39
No. of chew (oral tobacco) users	4 (1.7)	5 (4.0)	0.31
Cause of infertility, male factor	239 (99.6)	123 (99.2)	1.00
Cause of infertility, female factor	56 (23.3)	24 (19.4)	0.46
Duration of infertility, years	2.77 ± 1.5 (1.0–11.0)	2.79 ± 1.7 (1.0–12.0)	0.34
Pregnancies in previous relation	44 (18.3)	23 (18.5)	1.00
Pregnancies present relation	28 (11.7)	7 (5.6)	0.09
Miscarriages in previous relation	11 (4.6)	8 (6.5)	0.60
Miscarriages in present relation	20 (8.3)	5 (4.0)	0.18
Parous, previous relation	15 (6.3)	8 (6.5)	
Parous, present relation	0	0	

For categorical variables, n (%) is presented. For continuous variables, mean (SD) and range is presented. For comparison between the groups, Fisher's exact test was used for dichotomous variables and the Mann–Whitney *U*-test was used for continuous variables.

Table II Comparison of embryology data for the two groups.

	EmbryoScope (n = 240)	Control (n = 124)	P-value	Difference (95% confidence interval)
No. of oocytes retrieved per patient	9.50 ± 5.5 (1–32)	9.52 ± 4.5 (1–23)	0.47	
No. of injected (metaphase II) oocytes	8.25 ± 4.8 (0–27)	8.06 ± 4.0 (1–20)	0.69	
No. of fertilized (2 pronuclei) oocytes	4.70 ± 3.2 (0–21)	4.73 ± 3.1 (0–15)	0.92	
No. of 4-cell embryos Day 2	2.61 ± 2.2 (0–17)	2.65 ± 2.1 (0–10)	0.82	
No. of good quality embryos	2.41 ± 2.3 (0–16)	2.19 ± 1.8 (0–8)	0.34	0.227 (0.690; –0.236)
No. of frozen embryos	1.58 ± 2.3 (0–16)	1.30 ± 1.8 (0–8)	0.33	
No. of ET per randomized woman	215 (89.6)	114 (91.9)	0.47	
No. of transferred embryos, per ET	1.04 ± 0.2 (1–2)	1.03 ± 0.2 (1–2)	0.71	
No. of SET, per ET	206 (95.8)	111 (97.4)	0.71	
Reason for no ET (no. of cycles)				
OHSS, freezing of all embryos	13	3		
Failed fertilization	3	2		
Failed cleavage	9	4		

Continuous variables are presented as mean ± SD and ranges. Categorical variables are presented as n (%). For comparison between the groups, Fisher's exact test was used for dichotomous variables and the Mann–Whitney *U*-test was used for continuous variables.

ET, embryo transfer; SET, single embryo transfer; OHSS, ovarian hyperstimulation.

standard system. In that study, 676 oocytes from 59 patients were randomized between the two systems. The primary outcome was the number of 4-cell embryos on Day 2. No difference in the number of 4-cell embryos on Day 2, number of 7- to 8-cells on Day 3 or proportion of blastocysts on Day 5 was found. In addition, no differences in clinical pregnancy rates or implantation were found. In another controlled cohort study by Cruz et al. (2011), 478 oocytes from 60 egg donation cycles were randomly allocated to the two different incubator systems. No significant differences in the rate of GQEs, calculated as the proportion of blastocysts per cultured embryo, the number of transferred and frozen embryos or the pregnancy rate, were found between the

closed TLI system and the traditional culture system. It is important to acknowledge however, that in both these studies—like in our own study—the additional information provided by TLI was not used for selecting embryos for transfer, and only static images were used for assessment. Also, both these studies were powered for the evaluation of embryo quality, and not for pregnancy or live birth.

In the present study, the miscarriage rate was significantly higher in the EmbryoScope™ group. This is a worrying observation, although this finding has to be treated with caution since the study was not powered to detect differences in miscarriage rate. If the finding is true, one possible reason might be that the scoring of embryos based on traditional

Table III Comparison of implantation and pregnancy rates.

	EmbryoScope (n = 240)	Control (n = 124)	P-value	Difference (95% CI)
Implantation rate (%)	63/226 (27.9)	37/117 (31.6)	0.32	
No. of pregnancies per randomized woman (%)	72/240 (30.0)	39/124 (31.5)	0.87	-1.5 (-12.1; 9.2)
No. of pregnancies per ET (%)	72/215 (33.5)	39/114 (34.2)	0.99	
Biochemical pregnancies	9	2		
No. of deliveries/ongoing pregnancies per randomized woman (%)	48/240 (20.0)	35/124 (28.2)	0.10	-8.2 (-18.2; 1.8)
No. of deliveries/ongoing pregnancies per ET (%)	48/215 (22.3)	35/114 (30.7)	0.13	
No. of miscarriages (%)	24/72 (33.3)	4/39 (10.2)	0.011	23.1 (3.6; 41.4)

For categorical variables, n (%) is presented. For comparison between the groups, Fisher's exact test was used for dichotomous variables.

morphological criteria is more difficult in the EmbryoScope™ compared with a high resolution inverted microscope and thus affect selection of embryos for transfer negatively. In fact, we did experience some limitations when using the EmbryoScope™ for monitoring. The images on the monitor were not as sharp and clear as when visualized in the standard inverted microscope, and the focusing levels were limited. Also, embryos in the EmbryoScope™ tended to, despite placing them in the centre of a microwell, migrate to one side of the well, thus scoring them may be challenging at times. We also found it more challenging scoring nuclei in the EmbryoScope™ compared with the standard inverted microscope. It is important to emphasize that in this study, we used the traditional scoring of embryos in both systems. In the EmbryoScope system, we evaluated only a single picture. The full potential of the TLI to see additional morphological features was thus not utilized. However, it was found in the subgroup analysis that morphology evaluation correlated well between the EmbryoScope and the standard inverted microscope (91.8%).

In the present study, the failure to observe any beneficial effects of the closed culture system could be explained by the short incubation time (2 days) before assessment and transfer, and extending the culture time to 5 days might have given results indicating a benefit of a closed system. However, in many countries/clinics, the most common practice is still Day 2 transfer and it is important to determine if any potential benefit from investment into these new culture systems can be attained. Initial studies by Kirkegaard *et al.* (2012) and Cruz *et al.* (2011) did not find culturing embryos to Day 5 using TLI systems to be superior to standard culture regarding proportion of blastocysts or pregnancy outcome.

Until now, the aim of most published TLI studies has been to find timing variables for selecting embryos with a high potential for blastocyst development, implantation and pregnancy, while less attention has been given to comparisons between the different culture systems *per se*. Meseguer *et al.* (2012b) retrospectively analysed 7305 treatments from both TLI and non-TLI culture. They found that embryos cultured in the closed TLI system as well as being selected by a hierarchical grading system had a significantly increased clinical pregnancy rate compared with embryos cultured in standard incubators and selected by conventional morphology only. In a recent RCT by Rubio *et al.* (2014), analysing 843 couples, a significantly increased ongoing pregnancy rate was found when the TLI system was compared with a standard incubator system.

In this study, only good prognosis patients (≥ 6 MII oocytes, no recurrent miscarriages, no endometriosis) or donor recipients (with young donors) were included, the embryos were cultured to Day 3 or 5, and the embryos from the TLI system were selected not only by morphology but also by the hierarchical classification described by Meseguer *et al.* (2011). These factors may at least partially explain the difference in results compared with our study. In the RCT by Rubio *et al.* (2014), similar mean number of blastomeres, similar mean rate of embryo symmetry and a significantly higher mean embryo fragmentation rate on Day 3 were found in the TLI system, compared with the standard incubator system. In total, a slightly but significantly higher number of optimal embryos on Day 3 (46.2 versus 43.1%) was obtained in the TLI group.

Certainly, culture in the EmbryoScope™ provides a more stable environment for the embryos in terms of minimal fluctuations in pH, humidity and temperature. In addition, during image acquisition in the EmbryoScope™, the embryos are illuminated with long wavelength light and are subjected to lower light intensities (low intensity red light, 635 nm) than for embryos that are evaluated using a standard microscope. In an animal model, it was demonstrated that light in the range of 445–500 nm appears to be detrimental to blastocyst development (Oh *et al.*, 2007). In a standard IVF microscope, ~15% of light is <550 nm (Meseguer *et al.*, 2011). Furthermore, it has been shown that, for embryos in culture for 3 days, the total light exposure time in the EmbryoScope™ was 57 s compared with 167 s for an IVF treatment, using a standard microscope (Ottosen *et al.*, 2007; Meseguer *et al.*, 2011). These data would suggest a potential benefit when using a TLI system for longer incubation times.

It is clear that when scoring embryos at limited time points, important morphological events might be missed. Several such morphological events have been identified by TLI and suggested to be of predictive value for IVF success. In a study by Rubio *et al.* (2012) of 1659 transferred embryos, it was shown that embryos with a so-called direct cleavage from 2 to 3 cells (i.e. with a 2-cell stage shorter than 5 h) resulted in a significantly lower implantation rate compared with embryos with a 2-cell stage longer than 5 h (1.2 versus 20.2%). Hlinka *et al.* (2012) also found that out of 18 embryos showing a direct cleavage to 3 cells, none developed into blastocyst. Further, in both studies, it was noticed that these 'extra' cells could fuse at a later time, i.e. the embryos would appear to have cleaved in a normal, synchronized manner. Such anomalies would thus only be possible to detect in a TLI system. In a

recent study, retrospectively analysing 651 embryos using TLI, Wirka et al. (2014) identified four groups of atypical embryo behaviours, involving syngamy and cleavage patterns, that resulted in decreased embryo development.

Despite the lack of improvement of embryo development and pregnancy rate found in this present and other studies, the TLI systems may be of advantage from other perspectives. Important for the laboratory are the logistic reasons; since the whole developmental process is documented, important events can be analysed retrospectively at any time before selection for transfer. It also allows for more accurate analysis concerning timing and for the possibility of deselection of embryos with atypical cleavages. In combination with the use of single culture media and short insemination with sperm (1–4 h), the oocytes can be transferred to the TLI system on the day of oocyte retrieval, and be kept there until the time of transfer.

Only ICSI patients were included in this study due to the possibility to record the time of fertilization precisely (i.e. time of sperm injection), and because embryos can be kept in the EmbryoScope™ continuously from Day 0, in contrast to IVF embryos which have to be removed from the incubator for denudation at some stage post-fertilization.

The strengths of the present study are that it is an RCT in an area where few RCTs have been published, and that it is blinded to the patients, the physicians and the statistician. In addition, the randomization is performed per patient, instead of per cycles or oocytes, with concealed allocation using a web-based RCT program. Only ICSI patients were included, which could be seen both as a strength (more homogenous) and as a limitation (less generalizability).

The main limitations are having number of GQEs on Day 2 (a surrogate outcome to live birth) as the primary outcome and that the embryos have been cultured in different types of culture dishes.

Apart from the culture dishes and the open versus closed system, the culture conditions were similar regarding oxygen tension, culture medium, temperature and pH.

In conclusion, this large RCT comparing embryo development and morphology between embryos cultured in a closed TLI incubator with those cultured in a standard incubator showed no significant difference in the number of GQEs, implantation- or pregnancy rates, while a significantly higher miscarriage rate was found in the TLI system group. Further prospective and well-designed trials are needed to see if these new culture systems can identify predictive variables for pregnancy and live birth which are of additional importance to conventional morphology assessment when selecting embryos for transfer.

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Authors' roles

K.L. and C.B. designed the study. H.P. and U.S. performed the laboratory work together with the laboratory staff at Reproductive Medicine, Sahlgrenska University Hospital. A.T.-K. was in charge of patients' information and consent. All authors have taken part in the writing, reviewing and approval of the final version of the manuscript.

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Conflict of interest

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