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Improved embryonic development and utilization rates with EmbryoScope: a within-subject comparison versus a benchtop incubator

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Research Article

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Summary

The objective of this study was to investigate whether, in consecutive intracytoplasmic sperm injection (ICSI) cycles, embryonic development in an incubator with a time-lapse imaging (TLI) system is better than the previous one obtained in a benchtop incubator (G-185) with similar cultivation characteristics. The study was of a retrospective within-subject design, in which each cycle served as its own control. Data were obtained via the chart review of patients undergoing ICSI in a private university-affiliated *in vitro* fertilization (IVF) centre who fulfilled the following criteria: second ICSI attempt in which embryos were cultured in a TLI incubator system (TLI group, $n = 71$), preceded by a first ICSI attempt in which embryos were cultured in a benchtop incubator (Control group, $n = 71$). Embryonic development up to the fifth day of development, oocyte utilization rate (OUR; transferred embryos plus frozen embryos per total number of retrieved oocytes) and embryo utilization rate (EUR; transferred embryos plus frozen embryos per normally fertilized oocyte) were compared between the groups. There were significant differences in the day 2 non-cleavage rate, day 5 embryo rate, blastocyst development rate, frozen blastocyst rate, OUR, and EUR, in favour of the TLI group. Embryonic development, frozen blastocyst rate, OUR and EUR in the second ICSI cycle were significantly improved when the culture was performed in the EmbryoScope, compared with those rates obtained with culture in a G-185 in the first ICSI cycle of the same patients. The results may also lead to higher cumulative pregnancy outcomes following embryo thawing and transfer.

Introduction

Infertility, defined by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) as 'a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce, either as an individual or with his/her partner', is estimated to affect between 8–12% of reproductive-aged couples worldwide (Vander Borght and Wyns, 2018).

Depending on the type of infertility, different treatments can be used, including assisted reproductive techniques (ART) [i.e. *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI)], which are processes in which embryos are generated *in vitro*, cultivated in an incubator and replaced into the uterus.

Usually, embryos are removed from the incubator for assessment of morphology under an inverted microscope. Although morphology has been used almost exclusively for embryo selection over the past 3 decades, its subjectivity is undeniable and the embryo assessment lacks validation (Sakkas and Gardner, 2013).

As a result of the constant pursuit for improvement in embryo culture, the time-lapse imaging (TLI) system, which allows a non-invasive continuous assessment of embryo morphokinetic parameters in a closed culture system, has been developed. The main promise is that a TLI system would improve embryo development by reducing oscillations in pH, humidity and temperature (Park *et al*., 2015). Conversely, the system also has disadvantages, such as exposure to (i) distinct light for longer periods for embryo assessment, (ii) heat generation due to motion, (iii) magnetic fields, and (iv) lubricants. Other disadvantages include the significant expenditures on equipment and materials, and the availability of laboratory space (Chen *et al*., 2017).

Some studies have compared embryonic development in closed systems with TLI versus conventional incubators. Rubio *et al.* (2014) observed significantly increased ongoing pregnancy

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rates and decreased miscarriage rates with TLI compared with conventional incubators. Recently, Barberet *et al.* (2018) found significantly higher day 2 high-quality embryo rates and frozen embryo rates with TLI compared with a benchtop incubator. Some studies, however, failed to demonstrate any significant difference amongst the outcomes obtained with the TLI and conventional incubator (Nakahara *et al.*, 2010; Cruz *et al.*, 2011; Kirkegaard *et al.*, 2012; Park *et al.*, 2015).

Four of these aforementioned studies were randomized clinical trials, and two provided controversial conclusions (Rubio *et al.*, 2014; Park *et al.*, 2015). Notwithstanding the fact that these studies presented conflicting results, one has to bear in mind that only one of them compared TLI with benchtop incubators (Barberet *et al.*, 2018), reporting that they are more alike in terms of culture conditions than standard incubators; namely, a dried atmosphere and small culture chamber volume. The remaining studies compared TLI with conventional incubators. To our knowledge, it has never been investigated whether embryonic development can be improved within subject by changing from a benchtop incubator in the first intracytoplasmic sperm injection (ICSI) cycle to the EmbryoScope, a TLI incubator, in the following ICSI cycle.

The objective of this study was to investigate whether, in consecutive intracytoplasmic sperm injection (ICSI) cycles, embryonic development in the EmbryoScope is better than the previous one obtained in a benchtop incubator.

Materials and methods

Patients and experimental design

This study was of a retrospective repeated-measures within-subject design, in which each cycle served as its own control. Data were obtained via the chart review of patients undergoing ICSI in a private university-affiliated IVF centre who fulfilled the following criteria: second ICSI attempt in which embryos were cultured in a TLI incubator system (TLI group, $n = 71$), preceded by a first ICSI attempt in which embryos were cultured in a benchtop incubator (Control group, $n = 71$). To avoid bias, we only allowed a 1-year increment in female age between the first and second cycles for patients who did not become pregnant during the first cycle. ICSI cycles were performed between January 2018 and September 2020. Embryonic development up to the fifth day of development was compared between the groups. The main outcome measure was blastocyst development rate.

The exclusion criteria were as follows: female patients undergoing ICSI for fertility preservation, freeze-all cycles, ICSI cycles with vitrified/thawed or donated oocytes, surgical sperm retrieval, cryopreserved sperm, vitrified/thawed embryo transfer, or preimplantation genetic testing. All patients signed written informed consent forms, with agreements to share the outcomes of their own cycles for research purposes, and the study was approved by the local institutional review board.

Controlled ovarian stimulation and laboratory procedures

Recombinant follicle-stimulating hormone (r-FSH, Gonal-F®, Serono, Geneva, Switzerland; or Rekovelle®, Ferring, Saint-Prex, Switzerland) and gonadotropin-releasing hormone (GnRH) antagonist (cetrorelix acetate, Cetrotide® Merck KGaA, Serono, Geneva, Switzerland) were used for controlled ovarian stimulation. To avoid bias, we only included patients in which the same r-FSH and starting dose (300 IU) were administered in both ICSI cycles.

Recombinant human chorionic gonadotropin (r-hCG, 250 µg, Ovidrel®, Merck KGaA, Geneva, Switzerland) was administered to trigger the final follicular maturation upon the observation of adequate follicular growth and serum estradiol levels. Oocyte retrieval was performed 35 h later.

No changes were allowed regarding the types of drugs used for controlled ovarian stimulation and trigger in both cycles.

Semen analysis and preparation

Semen samples were collected in the laboratory by masturbation and were analyzed according to the World Health Organization guidelines (WHO, 2010). Sperm samples were prepared using a two-layered density gradient centrifugation technique (50% and 90% Isolate, Irvine Scientific, Santa Ana, CA, USA).

Intracytoplasmic sperm injection

ICSI was performed according to Palermo *et al.* (1992) 4 h after oocyte retrieval. Oocytes in metaphase II were selected for ICSI, even if they were immature at the time of retrieval. Sperm was selected at ×400 magnification using an inverted Nikon Eclipse TE 300 microscope, and injected into the oocytes in a microinjection dish prepared with buffered medium (Global w/HEPES, LifeGlobal, Guilford, USA) covered with paraffin oil (Paraffin oil P.G., LifeGlobal), on an inverted microscope heated stage (37.0 ± 0.5°C).

Embryo culture

Media refresh was not performed in any of the systems. In the TLI group, injected oocytes were individually cultured in a 16-well culture dish (EmbryoSlide, Unisense Fertilitech, Aarhus, Denmark) in 22.5 µl of continuous single culture media (Global® total®, LifeGlobal), overlaid with 1.8 ml of mineral oil (Paraffin oil P.G.) in a TL-monitored incubator (EmbryoScope+, Unisense Fertilitech, Aarhus, Denmark) set at 37°C with an atmosphere of 5.5% O_2 and 7.0% CO_2 until day 5 of embryo development.

In the Control group, injected oocytes were cultured in 30 µl drops of culture medium (Global® total®) and covered with the same paraffin oil in a benchtop incubator (G-185 Incubator, K-Systems, Birkeroed, Denmark) with an atmosphere of 5.1% O_2 and 7.1% CO_2 at 37°C, until day 5 of embryo development. The embryos were removed from the benchtop incubator for morphological assessments on days 1, 2, 3 and 5 of development.

On day 1 of development, normal, abnormal or no fertilization was recorded for each embryo. The cleavage rate is the number of embryos with ≥ two cells on day 2 of development per the number of normally fertilized oocytes. On day 5 of development, successful blastulation was recorded. The day 5 embryo rate is the number of embryos that reached the fifth day of development, irrespective of blastomere number or blastulation status, without arresting, per normally fertilized oocytes. Blastocyst development was evaluated considering embryos that reached the stage of full blastocyst onwards on the fifth day of development. Blastocyst development rate was calculated as the number of blastocysts per normally fertilized oocytes.

Clinical follow-up

Embryo transfer was performed on day 5 of embryo development and one or two embryos were transferred per patient, depending on female age and embryo quality.

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Women with a positive beta-human chorionic gonadotropin (β-hCG) pregnancy test performed 10 days post embryo transfer underwent a transvaginal ultrasound scan 2 weeks later. Clinical pregnancy was confirmed upon detection of a fetal heartbeat. Clinical pregnancy rate was calculated per embryo transfer. Implantation rate was calculated per transferred embryos. Miscarriage was defined as clinical pregnancy loss before 20 weeks.

Data analysis and statistics

The post hoc achieved power was 82.6%, considering the sample size, effect size obtained for blastocyst development rate and 5% significance level. The study power was calculated using G*Power 3.1.7 (Franz Faul, Universität Kiel, Germany).

Embryonic development (fertilization rate, cleavage rate, and blastocyst development rate) was compared between the groups using generalized linear models (GzLM), followed by Bonferroni post hoc. The analysis was not adjusted for any variables as no significant differences were found in the demographic information or ovarian response between the groups. Oocyte utilization rate (OUR; calculated as transferred embryos plus frozen embryos per total number of retrieved oocytes) and embryo utilization rate (EUR; calculated as transferred embryos plus frozen embryos per normally fertilized oocytes) were also compared between the groups using the same analysis. The results are expressed as means ± standard error (SE), with 95% confidence interval (CI), odds ratios (OR) and *P*-values. A P-value < 0.05 was considered statistically significant. Data analysis was conducted using SPSS Statistics 21 software (IBM, New York, NY, USA).

Results

The demographic data, characteristics of controlled ovarian stimulation (COS) and semen quality for both groups are shown in Table 1. There were no significant differences between the groups in maternal age, response to COS and number of injected oocytes. Paternal age and semen characteristics were also similar between the groups.

The results from GzLM analysis are shown in Table 2. There were significant differences in day 2 non-cleavage rates (OR: 0.285, CI: 0.234–0.347), day 5 embryo rates (OR: 1.385, CI: 1.331–1.442), blastocyst development rates (OR: 1.358, CI: 1.267–1.456), frozen blastocyst rates (OR: 1.163, CI: 1.085–1.248), OURs (OR: 1.232, CI: 1.155–1.314), and EURs (OR: 1.269, CI: 1.202–1.341), all in favour of the TLI group.

Embryo transfers were performed in 63/71 cycles (88.7%) in the Control group and in 65/71 cycles (91.5%) in the TLI group ($P = 0.573$). The mean number of transferred embryos were the same between the Control and TLI groups (1.8 ± 0.3, $P = 0.986$). Similar results were observed between the Control and TLI groups for pregnancy rate [19/63 (30.2%) vs. 20/65 (30.8%), respectively; $P = 0.940$], implantation rate (24.6 ± 40.0% vs. 26.1 ± 41.6%, respectively; $P = 0.830$), and miscarriage rate [4/19 (21.1%) vs. 3/20 (15.0%), respectively; $P = 0.622$].

Discussion

The objective of the present study was to compare embryonic development in a closed culture system (EmbryoScope) and a benchtop incubator (G-185) in consecutive within-subject ICSI cycles. Our results demonstrated that embryonic development from the cleavage stage to blastocyst development is significantly improved with the EmbryoScope, which in turn led to significantly higher rates of frozen blastocysts, OUR and EUR, compared with G-185. Our results are consistent with those from a recent study that showed higher rates of cleavage-stage top-quality embryos and blastocyst development, and higher numbers of frozen embryos with TLI compared with G-185 (Barberet *et al.*, 2018).

Despite the fact that the comparison of clinical outcomes was beyond the scope of this study for obvious reasons, no significant differences were observed between the two systems. Nevertheless, as more blastocysts were frozen after TLI culture, one could hypothesize that after the transfer of thawed embryos, cumulative pregnancy outcomes may have also been higher in this group compared with the G-185 group.

Findings from the present study supported previous evidence that the frequent opening of the incubator door and handling of culture dishes outside the incubator for morphological assessment entail stressful conditions to the embryos, which can result in compromised development (Fujiwara *et al.*, 2007; Zhang *et al.*, 2010), irrespective of the embryologist's experience. The observed improvements in embryonic development and utilization rates may be explained by the incubation conditions and minimal handling. It is well known that the main advantage of closed culture systems is their rigorously controlled and stable microenvironments, which avoid oscillations in temperature and pH. The gas supply in the EmbryoScope is constantly purified by filters, and the gas concentrations and temperature show minimal fluctuations after the door is opened and faster recovery of the microenvironment stability compared with other incubators (Meseguer *et al.*, 2011). As a result, there is no need to remove embryos from the incubator for morphological assessment. as the EmbryoScope integrates incubation and image acquisition into one system, the culture is not disturbed from post-ICSI to the very moment of embryo transfer.

Another possible explanation for improved embryonic development is the fact that a specific dish (EmbryoSlide) is used in the EmbryoScope. This dish contains 16 wells that are distributed in a way that allows culture medium communication between two sets of eight embryos. It could be suggested that embryos of the same set provide support for each other, therefore enhancing their development potential. In fact, it has been previously demonstrated that embryos cultivated in groups showed higher blastocyst development rates, possibly due to the production and secretion of factors, primarily the platelet-activating factor that prevents apoptosis (O'Neill, 1998) and stimulates blastomere cleavage (O'Neill, 1997), therefore improving embryo development and viability (Ebner *et al.*, 2010).

Despite the similar chamber volume and atmosphere found between the G-185 and EmbryoScope, this study comes with limitations: (i) the fact that we only included patients in which the same r-FSH and starting dose were administered in both ICSI cycles, FSH dose adjustments depended on patients' responses to stimulation, and therefore could have changed from the first cycle to the second. Nevertheless, mean total FSH doses did not differ significantly between the groups; (ii) we used different culture dishes in each system; nevertheless, embryo quality seems to be unrelated to any of those dishes (Wu *et al.*, 2016); (iii) it is not possible to confirm how much of embryonic improvement was due to the culture conditions; (iv) the study design is not ideal for the comparison of clinical outcomes, and also underpowered to do so; and (vi) the regression-to-the-mean phenomenon, which refers to the idea that rare or extreme events are likely to be followed by more typical ones and, over time, outcomes regress to the average

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Table 1. Comparison of demographics, characteristics of COS and semen quality in Control and TLI groups

Variables	Control group	TLI group	*P*-value
Maternal age (years old)	36.9 ± 4.2	37.8 ± 3.7	0.165
BMI	24.6 ± 4.0	24.2 ± 3.9	0.567
FSH dose	2742.6 ± 615.3	2773.5 ± 514.3	0.779
Estradiol	2323.3 ± 2219.9	2408.1 ± 2615.1	0.882
Follicles	13.7 ± 10.5	13.7 ± 10.3	0.961
Retrieved oocytes (*n*)	9.9 ± 7.9	10.5 ± 8.3	0.687
Mature oocytes (*n*)	6.7 ± 5.5	7.2 ± 6.2	0.639
Mature oocyte rate (%)	68.4 ± 18.4	69.6 ± 21.3	0.719
Injected oocytes (*n*)	7.3 ± 4.6	8.0 ± 6.1	0.422
Paternal age (years old)	39.8 ± 6.1	40.8 ± 5.7	0.331
Semen volume	1.8 ± 1.5	1.8 ± 0.5	0.917
Sperm concentration ($\times 10^6$/ml)	21.4 ± 31.8	20.2 ± 25.0	0.788
Total sperm count ($\times 10^6$)	40.5 ± 44.2	39.2 ± 39.9	0.892
Sperm motility (%)	40.7 ± 20.3	40.5 ± 22.5	0.953
Progressive sperm motility (A+B) (%)	35.7 ± 19.1	32.1 ± 21.8	0.263
Normal forms (%)	2.0 ± 1.2	1.8 ± 1.1	0.305

Note: Values are means ± standard deviation (SD), unless otherwise noted. BMI: body mass index; COS: controlled ovarian stimulation; FSH: follicle-stimulating hormone; TLI: time-lapse imaging.

Table 2. Comparison of embryonic development between Control and TLI groups using GzLM followed by Bonferroni post hoc test

Variables	Control group ($n = 71$)	TLI group ($n = 71$)	*P*-value
Normal fertilization (%)	74.8 ± 2.7 (69.6–80.1)	77.4 ± 2.7 (72.2–82.6)	0.499
Abnormal fertilization (%)	6.2 ± 1.5 (3.1–9.2)	6.8 ± 1.5 (3.8–9.8)	0.767
Non-fertilization (%)	16.8 ± 2.1 (12.7–20.8)	11.9 ± 2.1 (7.8–15.9)	0.098
Oocyte degeneration post injection (%)	2.2 ± 1.3 (0.22–4.7)	3.9 ± 1.3 (1.4–6.3)	0.352
Day-2 non-cleavage (%)	3.8 ± 0.2 (3.3–4.3)	1.1 ± 0.1 (0.9–1.3)	<0.001
Cleavage (%)	85.3 ± 1.2 (83.0–87.7)	84.2 ± 1.3 (81.7–86.8)	0.521
Day-5 embryos (%)	62.4 ± 1.0 (60.5–64.3)	86.4 ± 1.1 (84.2–88.6)	<0.001
Blastocyst development (%)	40.9 ± 1.1 (38.8–43.1)	55.6 ± 1.3 (53.1–58.1)	<0.001
Frozen blastocyst (%)	31.8 ± 0.8 (30.3–33.3)	37.0 ± 0.9 (35.2–38.9)	<0.001
OUR	40.7 ± 1.0 (38.8–42.7)	50.2 ± 1.1 (48.0–52.4)	<0.001
EUR	52.4 ± 1.1 (50.3–54.7)	66.6 ± 1.2 (64.3–68.9)	<0.001

Note: Values are means ± standard error (95% confidence interval). EUR: embryo utilization rate; GzLM: generalized linear models; OUR: oocyte utilization rate; TLI: time-lapse imaging.

or mean, could have been responsible for the improved embryonic development in the second ICSI cycle. If this was true, however, we should also have seen improvements in the clinical outcomes.

The results of the present study, in addition to those previously published, suggest that the TLI system results in better embryonic development compared with the benchtop incubator. Despite the insufficient evidence to support TLI superiority over conventional embryo incubation (Chen *et al.*, 2017), well designed clinical trials, considering specific groups of patients, different types of TLI systems, and the applicability of available morphokinetic algorithms, are still required for the elucidation of TLI effectiveness.

In conclusion, embryonic development, frozen blastocyst rate, OUR and EUR in the second ICSI cycle were significantly improved when culture was performed in the EmbryoScope, compared with those rates obtained with a culture in the G-185 during the first ICSI cycle of the same patients. Even though the clinical outcomes were similar between the groups, the results may also lead to higher cumulative pregnancy outcomes following embryo thawing and transfer.

Declarations of interest. None.

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