

Embryo selection by morphokinetic evaluation using embryoscope

Abstract

Background: Mostly embryos were selected according to their morphological characteristics and this requires moving embryos outside the controlled environment of the incubator for microscopic examination. Such changes in the culture environment have potential deleterious effect on the embryos and it was found that Conventional embryo selection methods are commonly associated with relatively low clinical pregnancy rate of approximately 30% per transfer

Patients and methods: A randomized controlled double blind trial which was conducted on 773 couples during the duration started from May 2016 to July 2018. This study was conducted in multicenter, in Sunrise and in el Gezeera IVF center, Egypt.

All participants were randomized using automated web based randomization system ensuring allocation concealment into 2 groups: Group I included 456 women whose embryos were developed in a conventional incubator then assessed only by conventional morphologic criteria and group II which included 317 couples whose embryos were cultured in the embryoscope TMS and were evaluated using the multivariate morphokinetic model.

Results: In the present study, the chemical pregnancy rate and clinical pregnancy rate were better in embryoscope than in conventional group but with statistically insignificant difference with P value 0.093 and 0.108 respectively.

Conclusion: We concluded that there is an increase in reproductive results with the use of embryoscope TMS and a number of selection and deselection patterns based on embryo morphokinetics although this enhancement was not statistically significant, so we thought that a prerequisite for application of a morphokinetic pattern to deselect embryos with low possibility for implantation is the factor that could increase ICSI results rather than selecting the embryos with higher possibility for implantation and this might be the reason for better results with embryoscope but with no statistically significant results. Future research with a larger sample size is recommended in a multicentric study.

Keywords: embryoscope, ICSI, morphokinetic, embryos

Introduction

There have been many recent advances in the field of assisted reproductive technologies in the last decade.¹

Knowing that Cultivation followed by embryo selection is an important step in ICSI. Many researches were done to develop new culture media and incubators which enable us to grow better embryos (the blastocyst stage) trying to improve the pregnancy rate.²

Mostly embryos were selected according to the morphology which necessitates transfer of embryos from their incubators, Such changes in the culture environment have potential deleterious effect on the embryos and it was found that embryos selected according to traditional way had a relatively lower clinical pregnancy rate, thirty percent per transfer.¹

Time-lapse imaging (TLI) is an available technique that can be used. TLI systems permit continuous monitoring of embryo development without transfer from their incubator environment.

It is proposed that TLI systems might increase results of ICSI cycles as a result of the following benefits; decreased handling and exposure of embryos to suboptimal situations overcomes the risks of stress from temperature alterations, high oxygen exposures and pH

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alteration in culture medium and so enhances culture circumstances, Second, through serial imaging, more data about embryo development are reached, Moreover, TLI helps embryologists to evaluate the quality of embryos by monitoring timing of events and length of various intervals in embryo growth which is known as morphokinetic monitoring), which provides newer dimension to selecting and grading embryos.³

Time-lapse imaging has 3 systems that are currently available in practice which are (Primo Vision, EmbryoScope, and Eeva). All of them need the use of a digital inverted microscope which allows images of embryos at preset intervals that are incorporated to form videos. The EmbryoScope is a compact, self-contained incubator that contain a built-in camera which allows an individual culture set-up, in which culture dish has twelve unique wells, each holding twenty to twenty five μ l of medium.⁴

There is a lack of knowledge about the importance of using embryoscope in ICSI.

Our study focuses on the point of using the embryoscope to monitor embryos growth without the need to remove them outside their incubator media (which is done in the conventional incubators) thus avoiding such hazardous effects on the embryos and if this is going to improve the pregnancy rate in ICSI cycles or not.

Materials and methods

A randomized controlled double blind trial which was conducted on 773 couples at a private IVF unit (Sunrise IVF centre) during the duration started from May 2016 to July 2018. This study was conducted in multicenter, in Sunrise and in el Gezeera IVF center, Egypt.

An informed written consent was signed by all participants after explanation of the study design, benefits and possible outcome.

All participants were randomized using automated web based randomization system ensuring allocation concealment into 2 groups: Group I included 456 women whose embryos were developed in a conventional incubator then assessed only by conventional morphologic criteria and group II which included 317 couples whose embryos were cultured in the embryoScope TMS and were evaluated using the multivariate morphokinetic model.

All couples were indicated for the first or second trial of ICSI, with female age between 25 and 40 years old with $\text{FSH} < 10 \text{ mIU/mL}$, $\text{AMH} > 1$ and normal serum prolactin assessed during non stimulated cycle. Exclusion criteria included azospermic male, abnormal uterine cavity (assessed by hysteroscopy or hysterosalpingography), abnormal endocrinological measurement (as thyroid or adrenal gland disorders). Those with hydrosalpinx, ovarian cyst, hydrosalpinx or undergoing frozen embryo transfer were excluded from the study. Couples who decided to undergo PGD and those with one or two embryos (as all of them were transferred) were also excluded.

The participants were subjected to full history taking with special consideration to age, infertility duration, type and cause. Full medical history was obtained. Proper medical examination including general, abdominal and vaginal examination was achieved then ultrasound scanning was done for presence of 3 or more pre-antral follicles and ovarian cysts exclusion.

ICSI protocol and technique

All women were subjected to GnRH agonist or antagonist protocol as indicated. Controlled ovarian hyperstimulation was done using hMG and rFSH combination for stimulation of follicular growth.

Triggering of final oocyte maturation was done using 10,000 IU intramuscular injection of HCG (Pregnyl, Organon, the Netherlands) when at least 2 follicles reached a mean diameter of 18 mm then ovum pick-up (OPU) was done after 34-36 hours of triggering under guidance of transvaginal ultrasound. Maged Cycle was cancelled if less 3 mature follicles were detected, no oocyte were retrieved, failure of fertilization was found or if manifestation of early OHSS was observed.

Follicles were aspirated, and the oocytes were washed in Gamete Medium (Cook IVF). After washing, oocytes were cultured in fertilization medium (Cleavage Medium; CookIVF) at 5.5% CO_2 in air and 37°C for 4 hours before oocyte denudation. Oocyte denudation was performed by mechanical pipetting 40IU/mL of hyaluronidase in the same medium. Subsequently, ICSI was performed in a medium containing HEPES (Gamete Medium; Cook IVF) at 400 \times magnification using an Olympus IX7 microscope. Immediately after ICSI. The injected oocytes for TMS cycles were placed individually in pre equilibrated culture dishes (EmbryoSlide; Unisense Fertilitech A/S) under oil at 37°C and 5.5% CO_2 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% CO_2 in air. All embryos in both groups were incubated

at 37°C, 5.5% CO_2 , atmospheric O_2 concentration and were cultured individually until embryo transfer at day 3 (72 hours after ICSI) in Cleavage Medium (Cook IVF); from day 3 today 5, we used CCM Medium (Vitrolife).

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 UnisenseFertilTechA/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 UnisenseFertilTech A/S are likewise certified (certificate number: DGM-672).

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 conventional incubators, 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 nuclear, 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.⁵

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 multi nucleation. On day 3, they were defined as those with six or more cells and the previously mentioned fragmentation and symmetry features.⁶

Embryos considered to be viable on day 3 were those that were transferred or vitrified.⁷

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; Unisense Fertilitech A/S).

Embryo morphology and developmental events were annotated, including the precise timing of the observed cell divisions in the hours after ICSI. In TMS group, embryos were selected on day 3 and 5 by morphological features previously described.

The primary end point for this study was clinical pregnancy confirmed by the presence of gestational sacs with fetal heart beat detected by transvaginal ultrasound examination in week 6-8.

The purpose of the analysis was to assess whether the primary end point was affected by the incubation method, TMS versus conventional method.

For secondary outcomes, we analyzed fertilization rates, embryo development, implantation rates and chemical pregnancy (defined as having a serum β hCG level higher than 10IU/mL on day 14 after ICSI). Implantation rate was calculated by dividing the number of gestational sacs with fetal heartbeats detected by the number of embryos transferred.

Early pregnancy loss was considered when the β -hCG-positive pregnant cycles did not result in clinical pregnancy. The study was considered double-blind for the physician evaluating the primary effect and the statistician evaluating the results.

We started on the premise that the clinical pregnancy rate in our IVF program is about 50%, and our hypothesis was that usage of the TMS system 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 312 patients per arm, with 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 624 patients. We could increase the actual number included to 773 patients to backup the drop out cases.

Comparison of quantitative variables 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. $P<0.05$ was considered statistically significant.

This study was made using high O_2 tension, so the noticed improvements might not be got under different conditions.⁸

Results

During the study period, we identified 792 eligible couples. Of the 792 couples, 14 patients dropped out before randomization. After randomization, 5 patients dropped out before completing the cycle. Therefore 773 patients were finally included: 317 in the study group (TMS) and 456 in the control group.

In the current study, there was no statistically significant difference in both study groups regarding age and BMI (Table 1).

Also the results of the present study did not show statistically significant difference in both groups regarding the protocol used, E2 duration of stimulation (Table 2) (Table 3).

Table 1 Descriptive and cycle characteristics of the Patients in both groups

	Conventional incubator group (n=456)	Embryoscope group (n=317)	P value
Age (years)	31.52 \pm 3.71	30.94 \pm 2.52	0.221
BMI (kg/m ²)	28.7 \pm 3.61	29.3 \pm 2.7	0.16
Protocol used	Long protocol	293 /456 (64.25%)	181/317 (57.10)
	Antagonist protocol	163/456 (35.75%)	136/317 (42.90%)
E2 on day of HCG	2533 \pm 1874	2711 \pm 1528	0.108
P4 on day of HCG	0.59 \pm 0.64	0.62 \pm 0.41	0.317
Duration of stimulation (days)	13.8 \pm 2.3	12.6 \pm 4.42	0.116

Table 2 Embryo Development Characteristics in the TMS and Control Group

	Control group (1) (no.=456)	Embryoscope group (2) (no.=317)	P value	significance
Metaphase 2 Oocytes	7.55 \pm -5.82	8.21 \pm -4.3	0.116	NS
Fertilization Rate	78.43% \pm -15.7	75.2 % \pm -16.4	0.273	NS
EMBRYO fragmentation	7.3 \pm -1.57	6.8 \pm -0.54	0.088	NS
Embryo symmetry	1.66 \pm -0.54	1.7 \pm -0.46	0.115	NS
Rate of blastocyst formation	50.30%	55.70%	0.079	NS
Transferred embryos	2.84 \pm -0.77	2.7 \pm -0.5	0.205	NS
Day 3 transfer	288/456	169/317	0.38	NS
Day 5 transfer	168/456	148/317	0.19	NS

Table 3 The chemical and clinical pregnancy rates in both groups are shown in the following table

	Control group (1) (no.=456)	Embryoscope group (2) (no.=317)	P value	significance
Chemical pregnancy rate	59.42%	61.20%	0.093	NS
Clinical pregnancy rate	45.61%	48.58%	0.108	NS

Discussion

The first controlled randomized study to measure the enhancement in reproductive results after incubation and selection via the embryoScope time-lapse system was performed in 2014; they found that there was an increase in implantation and clinical pregnancy rates and decreases early miscarriage with application of the EmbryoScope.⁹

The assumed improvement in ICSI outcome is supposed to be due to as previously explained by Meseguer et al.¹³ well controlled and stable incubation environment, very slight treatment of embryos inside and outside their incubator, allowed more data about embryo growth for qualitative analysis of morphology and applying quantitative morphokinetic design for selection of embryos.⁹

Changes in temperature and pH circumstances in the incubator might hinder embryo development and quality.¹⁰

Through time lapse image acquisition we could decrease hazardous effects to the culture conditions and so to the development of embryos through combining the image acquisition and incubation in one system.^{11,12}

Many researches showed the predictive importance of morphokinetics.¹³⁻¹⁵ Based on morphokinetics, we could exclude embryos with less possibilities for implantation.

In a previous study made in 2012 revealed a relative increase in clinical pregnancy rate when regression model is used showed an increase of 15.7 percent.¹⁶

Moreover in a previous research made in 2014, showed a near results 16.9% per embryo transfer.⁹

In the present study, there was no statistically significant difference in embryoScope group in comparison to control conventional group regarding metaphase II oocyte, fertilization rate, and Embryo fragmentation, with P value 0.116, 0.273 and 0.088

Also in the previous study made in 2014, they noticed that morphokinetic and morphological categories are significantly correlated to implantation possibility.⁹

A recent research made by Kirkegaard et al.¹⁷ revealed that there was no significant difference in the morphokinetics of embryos which successfully was implanted and embryos which did not implant and they concluded that embryo selection by morphokinetics might not be generally applicable whoever embryo deselection using morphokinetics is more predictable, But they had a limited sample size.¹⁷

Moreover, in the current study there was no statistically significant difference in embryoScope group in comparison to conventional group regarding rate of blastocyst formation and transferred embryos with P value 0.079 and 0.205 respectively.

The drawback is that morphokinetic selection pattern is based upon special embryo cohort; women from one place, so it cannot be validated for all embryos as many factors could affect morphokinetics.¹⁸⁻²¹

In the present study, the chemical pregnancy rate and clinical pregnancy rate were better in embryoScope than in conventional group but with statistically insignificant difference with P value 0.093 and 0.108 respectively.

Limitation of the current study is the high cost of ICSI when using TMS incubators which costs more than conventional incubators.

Conclusion

We concluded that there is an increase in reproductive results with the use of embryoScope TMS and a number of selection and deselection patterns based on embryo morphokinetics although this enhancement was not statistically significant, so we thought that a prerequisite for application of a morphokinetic pattern to deselect embryos with low possibility for implantation is the factor that could increase ICSI results rather than selecting the embryos with higher possibility for implantation and this might be the reason for better results with embryoScope but with no statistically significant results. Future research with a larger sample size is recommended in a multicentric study.

Author contribution

The team of authors participated in this study in all of its aspects either in conception and design of the study, standardization to the patients, postoperative follow up and management, in analysis and interpretation of data and in the process of drafting the article or revising it for critically important intellectual content; and approval of the final 'to be published' version.

Disclosure statement

All authors declare that there are not any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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Conflicts of interest

Author has no any conflict of interest to declare.

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