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Comparison of embryo development of ICSI and IVF derived embryos from modified natural cycles according to time-lapse technology

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Abstract

Background: to compare the effect of intracytoplasmic sperm injection (ICSI) and conventional fertilization (IVF) on the development of embryos derived from modified natural cycles, in order to find the most effective fertilization method.

Methods: Seventy-two embryos were evaluated, of which 43 resulted from intracytoplasmic sperm injection (ICSI) and 29 from conventional fertilization (IVF). The evaluation was performed according to the KIDScore Day 5 algorithm, but also according to the Gardner and Schoolcraft evaluation system. The quality of each embryo was evaluated and categorized according to their quality on the 5th day of their development, the presence of multiple nuclei at the 2 and 4 cell stage (MN2, MN4 respectively), the direct cleavage of blastomeres (DC) and the reverse cleavage of blastomeres (RC). Additionally the time points of each cellular event was recorded.

Results: the fertilization method is independent of the qualitative classification of embryos, the appearance of multiple nuclei, the direct cleavage and the reverse cleavage of blastomeres. The time points of cellular events were similar between the two fertilization methods.

Conclusions: No significant difference was observed in the development of ICSI and IVF derived embryos from modified natural cycles. Therefore, neither of the two fertilization methods is considered more appropriate for the embryonic development in modified natural cycles. It is suggested to evaluate both the medical history and the gametes quality of the couple in order to choose the appropriate fertilization method. It is also recommended to use both methods of embryos evaluation in combination.

Key words: IVF, time-lapse, modified natural cycle, KIDScore Day5

Introduction

Time-lapse monitoring (TLM) of human embryos during IVF treatment has emerged as a promising, noninvasive tool which allows the accurate measurement of numerous morphokinetic variables, proposed to be used in models that aimed at predicting blastocyst formation, aneuploidy status, and the chance of implantation. Time-lapse variables, however, are also affected by a number of external factors such as the insemination technique (standard IVF vs. intracytoplasmic sperm injection [ICSI])[1,2].

Results of many studies on the effect of fertilization method on embryonic development differ, as there are studies that report a negative effect of either ICSI[3-6] or IVF (7) in embryo development and others that result in non-correlation of fertilization method with embryonic development[8].

The aim of the present study was to determine how standard IVF vs. ICSI fertilization affected morphokinetic parameters (Multinucleation at the 2 and/or 4 cell stage [MN-2, MN-4], Direct cleavage [DC], Reverse cleavage [RC]), during prolonged embryo culture in modified natural cycles using time-lapse technology.

Materials and Methods

Study design

This study was performed at Akeso – Embryoart Fertility Center from July 2019 to September 2020. A total of 72 intracytoplasmic sperm injection (ICSI) treatment cycles and 29 in-vitro fertilization (IVF) treatment cycles were included in this study. Infertility causes were age (6.98% ICSI vs 13.79% IVF), poor ovarian response (30.23% ICSI vs 24,14% IVF), tubal factor infertility (Fallopian Tube Obstruction) (30.23% ICSI vs 37.93% IVF) and unexplained infertility (32.56% ICSI vs 24.14% IVF). ThE protocol was approved by the Scientific Board and Bioethics Committee of Alexandra Hospital [Approval Number: 21/03/2017).

The modified natural cycle protocol included an ultrasound on day 9, then ultrasound observation of the ovaries and endometrium, every 2nd day of the cycle. A GnRH antagonist (Cetrotide EMD – Serono, Canada) and gonadotrophin (Gonal - f – Serono, Germany) were administered until ovulation induction day. After the administration of recombinant chorionic gonadotropin oocyte retrieval was performed. In male factor cases, ICSI was applied[9]. Cryopreservation was performed on day 5 embryos.

Oocyte retrieval and fertilization method

Oocyte cumulus complexes were cultured in Universal IVF Medium (Medical Origio GmbH, Berlin, Germany) at 37 °C under 5.5% CO2 and 5.0% O2. IVF and ICSI procedure were performed in Universal IVF Medium.

Embryo culture and time-lapse recording

Following fertilization, fertilized oocytes were individually placed and cultured in Sage Sequential Medium (SAGE 1 – Step, CooperSurgical). All embryos were cultured to the blastocyst stage in EmbryoScope+ time-lapse incubator (Vitrolife, Copenhagen, Denmark) with the culture conditions of 37 °C, 5.5% CO2, 5.0% O2 . Images of each embryo were obtained every 10 min. The precise timing of completing each fertilization procedure was recorded individually.

Morphology assessment was performed by using the Gardner grading system and the KIDScore Day5 algorithm. According to the Gardner grading system: Grade I blastocysts were ≥ 3 AA, AB or BA. Grade II blastocysts were < 3 or BB, AC or CA and Grade III embryos were those with small blastocoel cavity (grade 1 or 2) and BC, CB or CC ICM and TE quality. KIDScore Day5 algorithm considers the morphology and the morphokinetic traits of an embryo. The higher the score, the greater the statistical chance of implantation[10]. Blastocysts were separated into 3 categories: Grade I blastocysts were rated as 7,6 –

9,9, Grade II blastocysts were rated as 5,6 – 7,5 and Grade III blastocysts were rated as 1 – 5,5.

Statistical analysis

Quantitative variables (tPNf, t2 – t8, tSB, tB, tEXP**)** were analyzed using the Kolmogorov – Smirnov test and categorical variables were analyzed using the *Fisher's exact test*. All statistical analyses were performed using STATA statistical software analysis at the 5% significance level.

Results

Correlation between fertilization method and qualitative classification of embryos according to the Gardner and Schoolcraft evaluation system

The percentage of MNC-ICSI derived embryos in each category was: Grade I: 51.16%, Grade II: 18.60% and Grade III: 30.23%. Respectively, the percentages for the MNC-IVF group were as follows: Grade I: 58.62%, Grade II: 20.69% and Grade III: 20.69% (Table 1). There was no significant difference in the distribution of embryos in the 3 quality categories between the two fertilization methods. The results are not statistically significant (p-value = 0.666).

Correlation between fertilization method and qualitative classification of the embryos according to the KIDScore Day5 algorithm

The embryos were divided into the above 3 quality categories according to the score given by the algorithm. The percentage of MNC-ICSI derived embryos was: Grade I category 34.88%, Grade II category 27.91% and Grade III category 37.21%. Respectively, as for the MNC-IVF study group we observed the following classification: Grade I 37.93%, Grade II 27.59% and Grade III 34.48% (Table 2). Our results are non-statistically significant (p-value = 0.960).

Correlation between fertilization method and multiple nuclei at the 2 and/or 4 cell stage

Evaluation was performed at Day 1 (26 - 28 ± 1 hours after fertilization), at Day 2 (44 ± 1 hours after fertilization) and at Day 3 embryos (68 ± 1 hours after fertilization). For ICSI derived embryos the occurrence of multiple nuclei in the 2-cell stage was 44.19% and 48.28% for IVF derived embryos (Table 3).

Table 1. Rates of MNC-ICSI and IVF derived embryos in each qualitative category according to Gardner and Schoolcraft evaluation system.

GARDNER AND SCHOOLCRAFT EVALUATION SYSTEM	ICSI	IVF	P-VALUE
Grade I	51,16%	58,62%	
Grade II	18,6%	20,69%	0,666
Grade III	30,23%	20,69%	

Table 2. Rates of MNC-ICSI and IVF derived embryos in each qualitative category according to KIDScore Day 5 algorithm.

KIDSCORE DAY 5 ALGORITHM	ICSI	IVF	P-VALUE
Grade I	34,88%	37,93%	
Grade II	27,91%	27,59%	0,960
Grade III	37,21%	34,48%	

A decrease in MN occurrence was observed during the transition from the 2-cell stage to the 4-cell stage. More specifically, for ICSI derived embryos MN-2 rate was 44.19% and MN-4 rate was 16.28%, ie a decrease of 27.91%. Respectively, in IVF derived embryos, MN-2 rate was 48.28% and MN-4 rate was 20.69%, ie a decrease of 27.59%. Although, IVF derived embryos showed slightly increased occurrence of multiple nuclei, our results (MN-2 p-value = 0,733, MN-4 p-value = 0,633) lead to the conclusion that there is no correlation between the fertilization method and the occurrence of MN embryos.

Correlation between fertilization method and direct cleavage (DC)

The rate of DC at this study was 20.93% in ICSI derived embryos and 27.59% in IVF derived embryos with a non-statistically significant result (p-value = 0.514) (Table 3). Therefore, the fertilization method does not appear to affect the appearance of this morphokinetic characteristic.

Correlation between fertilization method and reverse cleavage (RC)

Although in the present study the rate of reverse cleavage was 23.18%, where 16.28% corresponded to ICSI derived embryos and 6.90% corresponded to IVF embryos derived embryos (Table 3). The result, however, is non-statistically significant (p-value = 0.238).

Correlation between fertilization method and realization of specific cellular events

We did not observe a significant difference between the values of the two fertilization methods. However, ICSI derived embryos performed the majority of their early cellular events slightly earlier. The results were not statistically significant.

Discussion

The purpose of our study was to evaluate the effect of fertilization method on embryo development from modified natural cycles. The EmbryoScope+ incubator performs a detailed observation of embryos and in combination with the KIDScore Day5 algorithm, it is possible to predict embryo implantation and to evaluate and correlate embryo quality with other cellular events (MN-2, MN-4, DC, RC) that have been argued to affect the embryonic development.

Previous studies have reported a negative effect of ICSI on embryonic development and a reduced blastocyst rate and/or poorer quality blastocysts[1,4-6]. However, those two methods show similar fertilization rate, while complete fertilization failure has been reported to be more frequent in cases where IVF was applied[7]. Recent studies suggest that the two fertilization methods show similar blastocyst rate and high blastocyst quality rate[8], which is consistent with our results. Additionally, our study concludes that early cell divisions occur slightly earlier in ICSI embryos, which was also reported from other studies[11].

The morphokinetic characteristic involving the presence of multiple nuclei in at least one blastomere at the 2 and/or 4 cell stage is called Mutlinucleation (MN-2, MN-4 respectively)[12]. This feature is associated with reduced implantation rate[13,14], increased rate of chromosomal abnormalities[15-17], increased

Table 3. Rates of specific morphokinetics characteristics in ICSI and IVF derived embryos.

CELLULAR EVENT	ICSI	IVF	P-VALUE
MN2	44,19%	48,28%	0,733
MN4	16,28%	20,69%	0,633
DC	20,93%	27,59%	0,514
RC	16,28%	6,9%	0,238

miscarriage rate[18] and lower blastocyst rate[19]. It is also associated with higher rate of unequal blastomeres, higher rate of fragmentation and lower rate of developing embryos[14]. Multiple nuclei rate ranges from 30 to 45%[20,21], which is in agreement with the results of this study, where multiple nuclei rate was 44.19% at 2 cell stage embryos derived from ICSI and 48.28% at IVF cases respectively. We observed a decreased MN rate during the transition from the 2 cell stage to the 4 cell stage, which is also in agreement with the results of previous studies[14,22,23]. Previous and recent studies do not correlate the fertilization method with the formation of MN[14,24]. We conclude that there is no correlation between the fertilization method and the occurrence of MN embryos

The division of a blastomere into 3 or more blastomeres or the division of one blastomere into two in less than 5 hours is called Direct cleavage (DC)[25]. It has been argued that its existence is associated with reduced blastocyst formation[26] and reduced implantation rate[25,27,28]. It was also argued that the later this occurs during embryo development, the smaller its impact is on blastocyst formation rate, implantation rate and aneuploidy rate[29]. The majority of studies suggest that DC is due to spindles with more than two poles, which lead to incorrect division patterns and possibly incorrect distribution of genetic material[25,29]. An increased occurrence of this characteristic has been observed during the first cell division in IVF derived embryos, while during the other cell divisions no significant difference is observed between the two fertilization methods[29]. The incidence rate of direct cleavage has been reported to range from 13.7%, 18% to 26.1%[25,29]. In our study, its incidence rate was 20.93% in ICSI derived embryos and 27.59% in IVF derived embryos. The results, however, were non-statistically significant (p-value = 0.514) and thus we conclude that the fertilization method does not seem to affect the occurrence of this morphokinetic characteristic. A slightly increased occurrence in IVF derived embryos was observed which is also supported in the literature[29] and therefore requires further study.

The third morphokinetic characteristic observed and correlated with embryo quality refers to the reunion of already divided blastomeres, called reverse cleavage, RC which is associated with embryos with reduced implantation rate[27], while its effect on blastocyst formation is unclear. There are studies that support no effect on blastocyst formation[20,21], in contrast to others that associate it with a reduced rate of either good quality blastocysts or overall blastocyst formation[30]. The causes are not clear, however, the disruption of distribution of E-cadherin in cell membranes, which is favored by changes in pH, temperature and osmolality[27] as well as fertilization with low sperm progressive motility have been strongly reported. According to the literature the incidence rate of reverse cleavage is 6.8%[1], and 27.4%[30], while in the present study was 23.18% (16.28% ICSI derived embryos and 6.90% IVF derived embryos p-value = 0.238), leading to the conclusion that there is no correlation between fertilization method and RC. However, its incidence rate in ICSI derived embryos shows a two-fold increase compared to IVF derived embryos. A previous study in agreement to this conclusion, supported that the increased incidence rate of reverse cleavage in ICSI derived embryos was attributed to low progressive sperm motility use[30]. However in another study a slightly increased incidence rate of RC was observed in IVF derived embryos and was thought to be due to cell membrane disorders, which are favored by changes in pH, temperature and osmolality[27]. Concluding, a further study of the correlation between fertilization method and RC is proposed.

Our results do not propose a more favorable fertilization method related to embryos derived from modified natural cycles. However, it is worth studying further the correlation between RC and fertilization

method and which factors cause this morphokinetic characteristic.

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Conflict of interest

None to declare

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