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EFFECTIVENESS OF EMBRYO QUALITY WITH TIME LAPSE IMAGING FOR THE SCREENING OF EMBRYOS IN COMPARISION WITH CONVENTIONAL MORPHOLOGICAL ASSESSMENT UNDERGOING IVF/ICSI.

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ABSTRACT

Background and Aim: In human embryology, many in vitro fertilization (IVF) centers are rapidly being replaced by closed embryo incubation systems with time-lapse imaging. The timing of early mitotic events during preimplantation embryo development is important for subsequent embryogenesis in many mammalian species in Humans from the oocytes during the ovum pickup

The Aim of the study is to assess an embryo's ability to develop into a goodquality during the early-cleavage stage using time-lapse imaging in a closed inion system after 3rd-day transfer.

Methods: A total of 50 embryos had their oxygen consumption rates measured, and their detail of cleavage orientation and morpho kineticbehavior was observed. In total, -25embryos and 25 by using time-lapse imaging and normal embryos were assessed respectively. In total, 50 were examined by using, time-lapse imaging and normal embryology.This study assessed 50 embryos from single embryo transfers after ICSI cycles performed between April 2018 to April 2021

Conclusion: Individual embryos that can develop into good-quality blastocysts could be selected at day 3 of culture using these systems. Presently there is insufficient evidence to support that TLI is superior to conventional methods for human embryo incubation and selection.

KEYWORDS :

INTRODUCTION:

In recent years, there is rapid development in clinical practice, human-assisted reproduction (ART) which helps many couples in solving fertility issues in improving theefforts thathave been directed toward improving embryo selection. The identification of embryos with a higher capacity for implantation will reduce the number of embryos for transfer without reducing the chances of pregnancy in a cycle of assisted reproduction(Rubio I et al., 2014).Subfertility is a common problemthat is affecting 1 in 7 couples. In vitro fertilization / intracytoplasmic sperm injection (IVF/ICSI) is a fertility treatment often recommended to these couples.

To this end, different non-invasive embryo selection methods have been designed that provide information on how to distinguish embryos with better prognoses. (Scott L 2003) There are different methods of embryo gradation but they are all based on morphology, and evaluation of morphology under the microscope is subject to observer subjectivity Following IVF, human preimplantation embryosare traditionally scored for the quality at the zygote stage and then again at the 4cell to 8cell stage approximately 48–72 h later.(Brooks KE et al., 2019) Single embryo transfers (SETs) reduce the incidence of multiple pregnancies after IVF (Barberet Jet al., 2019,McLernon et al., 2010), but the selection of embryos will depend on the higher developmental potential is crucial to ensure high implantation and birth rates.Time-lapse systems (TLS or TLI = time-lapse imaging) like the EmbryoScope© may further improve embryo selection while maintaining stable culture conditions (Armstrong S, et al).

One of the noninvasive embryo evaluation methods to have come into the limelight in recent years by the use of time-lapse monitoring systems (TMS). The 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 in vitro (Aparicio B et .al 2013, Kirkegaard K et.al 2012, Herrero J et.al 2013).Inthe conventional group,all the embryos are cultured in our daily used incubators; embryo scores are evaluated on day 3 by the embryologist and in the time-lapse-auto,all the embryos are cultured in EmbryoScope®; embryo scores are evaluated on day 3 by the KID Score system.

To date, morphological characteristics are still the most common method for assessing embryo developmental potential. This method requires embryologiststo move the embryos outside the conventional incubator for microscopic examination at a specific time point once a day for 3-6 days after insemination to observe the morphology of the embryos and select the embryos. However, the assessments are relatively subjective and not comprehensive enough to observe embryonic development, thus important developmental events might be missed(Medicine AS et al.,2011). Although more developmental information can be obtained by removing the embryos multiple times

out of the incubator, prolonged exposure to the atmosphere changes the temperature, humidity, and PH value of the embryo culture media, which adversely affects the embryos.

The TLI system offers researchers the ability to explore the impact of other non-invasive parameters on outcomes, in the embryo as well as in the oocyte and zygote(Barberet J et al., 2019). A Time-lapse imaging system (TLS) is a newly developed non-invasive embryo quality assessment method. The optical system which is installed in the TLS, and images can be photographed and stored every 5-20 minutes, whencompared to the conventional incubator, the TLS provides stable culture conditions, and consistent observation of embryo development, thereby improving embryo quality and selection. (Chen M et al., 2020) Although the TLS has been routinely used in many IVF centers, it remains unclear if the TLS improves ART outcomes. Therefore, thisstudy was designed to compare the cumulative live birth rate of the TLS with conventional incubators. In this study, we will provide the comprehensive morphometric, morphologic, and morphokinetic description of embryos starting from the oocyte in a subset of SET. The primary objective of this study is to determine if the use of TLI or undisturbed culture in IVF/ICSI treatment results in a higher live birth rate when compared to current standard methods of embryo incubation and assessment by single embryo transfer such as pregnancy outcome, fertilization rate, cleavage rate, high-quality embryo rate, blastocyst formation rate, among these three groups.Single embryo transfers (SETs) reduce the incidence of multiple pregnancies after IVF, but the selection of embryos with higher developmental potential is crucial to ensure high implantation and birth rates.

MATERIALS AND METHODS

Study design:

It is an observational study conducted at MHRT hospital and research center.The anticipated enrolment is the patients enrolled for IVF and conducted from April 2020 to April 2021.A total of 36 patients were enrolled in this study for the two groups 18 in each were selected.In group A the participants will undergo embryo culture and selection in the TLS, and in group B,the participants will undergo embryo culture in the conventional incubators and embryo selection by the morphological characteristics TLI systems may be due to either a closed undisturbed culture system or the use of morphokinetic parameters for embryo selection or both. The participants sample consisted of patients' embryos with known implantation statuses, which can be transferred as a single day-3day transfer from a fresh IVF/ICSI cycle.

Ethical approval

The Written informed consent was obtained from all patients, stating consent towards research and/or methodological development. The project was approved by the ethics committee Formed by our Medical Health and Research Institute.

INCLUSION CRITERIA:

The inclusion criteria for patients were as follows; age 20–38 years, first or second intracytoplasmic sperm injection (ICSI) cycle, body mass index (BMI) of >18 and,the inclusion criteria are broad in keeping with the latest guidelines for IVF/ICSI treatment. Themale partner is at least 18 years of age at the time of consent and both partners give written informed consent. Normal uterine and tubal factor infertility.

EXCLUSION CRITERIA:

The exclusion criteria were a 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.

Embryo culture

Standard embryology

For the standard embryo culture group, oocytes after ICSI were washed in blastocyst medium containing 10 % HSA, and then transferred the pre-equilibrated culture dish, with 20 µl droplets of blastocyst medium containing 10 % HSA under light oil. The embryos were cultured until transfer in a standard incubator at 37 °C, 5 % CO2 and 90 % N2 for 3 days. The embryos were taken out of the incubator at 16–18 h post-injection for fertilization check, at 40–42 h post-ICSI for early cleavage evaluation, and at 64–66 h post-ICSI for quality assessment of transfer and cryopreservation. The time used for each check and assessment was recorded.

TLI embryology

For the TLI embryo culture group (EmbryoScope™), oocytes were washed in the same way as described abovefor the standard embryology group. We utilized in thisstudy the EmbryoScope™,a commercial, FDA-approved TLI system. It is made upof an incubator with a built-in microscope, which acquires images of cultured embryos continuously. In thisstudy, we acquired images every 10 min at seven focalplanes. Pre-equilibrated embryo culture dishes (EmbryoSlide™) were used in conjunctionwith the EmbryoScope™, which were prepared by following the manufacturer's instructions. Briefly, each well contained 25 µl of Blastocyst medium containing 10 %HAS, and the whole dish was covered with 1.5 ml of lightoil. After ICSI, individual oocytes were loaded to the centerof the well and cultured in the EmbryoScope™ at 37 °C,5 % CO2, %5 O2 and 90 % N2 atmosphere for 3 days untiltransfer. The images taken by the TLI were reviewed at16–18 h post-injection for fertilization check, at 40–42 hpost-ICSI for early cleavage evaluation, and at 64–66 hpost-ICSI for embryo quality assessment for embryo transfer and cryopreservation. The embryology staff time usedfor each check and assessment was recorded.

RESULTS

Primary outcomes of this study are to check for clinical pregnancy rate after 5 weeks of embryo transfer, gestational sacwith fetal heart beat present in the uterus. The results of this study will provide evidence for the efficacy and safety of time-lapse system compared with conventional incubators in patients.

On day 3, at 64–66 h post ICSI, embryos were scored according to blastomere numbers, size and amount of fragmentation. Embryos of Grade A (high quality) had ≥ 8 blastomeres with equal size, <10 % fragmentation or slightly unequal size and no fragmentation; Embryos of Grade B (fair quality) had ≥ 6 blastomeres with equal size, <25 % fragmentation or slightly unequal size and <10 % fragmentation; Embryos of Grade C (poor quality) had > 25 % fragmentation or blastomeres with severely unequal size. Examples for embryo grading criteria are demonstrated in Figure 1 and 2. Embryos of grade A and B were considered suitable for transfer or cryopreservation.All embryo transfers were carried out on day-3, within 2 h from the embryo quality assessment. Panels a and b (Grade A) demonstrate best quality embryos; Panels c and d depict intermediate grade embryos (Grade B); and Panels e and f show worst grade embryos (Grade C).

In a population of relative poor prognosis patients, embryos cultured in a TLI system and by standard embryology up to day-3, thus, demonstrated similar development and similar implantation as well as clinical pregnancy rates. The small number of investigated patients, however, does not preclude the possibility of atype 2 error. In other words, this study does not preclude the possibility that a larger patient populationmight demonstrate significant differences between TLIand standard embryology.

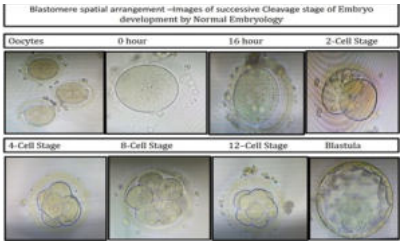

Figure 1: Blastomere spatial arrangement. Images of successive cleavages leading to formation ofembryo by normal Embryology

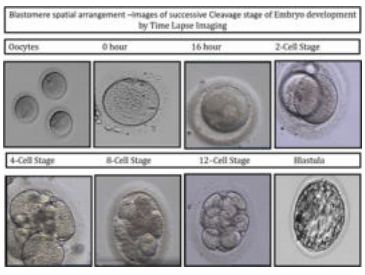

Figure 2: Blastomere spatial arrangement. Images of successive cleavages leading to formation of embryo by time lapse imaging

DISCUSSION

In vitro culture and selection of embryos with high developmental competence are a vital step for all assisted reproductive technologies (ART). Embryo selection methods are essential to increase the efficacy of ART, promote single embryo transfer, and reduce time from treatment start to live birth. Culturing of embryos in the optimal conditions is essential for a successful in vitro fertilisation (IVF) programme.Morphology evaluation remains the primary method of embryo assessment, in most IVF centers worldwide in spite of the numerous studies reporting relatively low pregnancy rates using this method.(Maheshwari A et. al 2014)The capacity to assess, rank and garding of embryos correctly for quality will allow for transfer of the potentially 'best' embryo first, thereby shortening the time to pregnancy, although not improving cumulative pregnancy and live birth rates.In this study, we focused on SETs from ICSI attempts, we studied the potential impact of a total of 36morphokinetic, morphometric and morphologic parameters on birth outcomes comparing Time lapse Imaging and conventional methods.

Time-lapse technology introduces the concept of stable culture conditions, in connection with the possibility of continuous viewing and documenting of the embryo throughout development as they happen rather than just evaluate snapshots of it. Even the highest morphological quality embryo can fail to implant, and there have been several reports of cases of top-quality blastocysts being aneuploid (Alfarawati S et al.,2011, Capalbo A et al.,2014). Still only one third of embryo transfers are successful. Therefore, improvements can still be implemented by the study including introduction of additional techniques such as PGS, TLI, or both combined.There has been research which agreed that clinical parameters are minimally affected by culture media type (Basile N et al.,2013), oxygen concentration (Kirkegaard K et al., 2013), stimulation protocol (Muñoz M et al.,2013), insemination method (Cruz M et al.,2013, Bodri D et al.,2013), and other confounding factors that may affect embryo kinetics. Even though the population size was relatively small, our analyses were based on homogeneous cycles, i.e. young women whose transferred embryos were found to be high-grade according to conventional morphology evaluation. In addition, our conclusions were established from a specific, highly selected population. We assessed day 2/3 transfers, our findings cannot be generalized to embryos cultured up to the blastocyst stage.

CONCLUSIONS

The full impact on clinical care needs to be explored, the technology could be useful for research and industry purposes as the steps of embryo development can be precisely standardized and hence Time lapse technology can revolutionize the quality control in lab.The time-lapse technology is evaluated for embryo selection which has proven to be safe, undisturbed, continuous embryo observation option that can aid embryo selection and could be used for research purposes. However, pregnancy and neonatal outcome data must be collected as well.

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