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Methods for Assessing Human Embryos to Increase Reproductive Potential

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Abstract- The embryological stage of ART programs is one of the most important, since the assessment of the quality of oocytes, their fertilization and in vitro cultivation to the stage of preimplantation embryos largely determines its success. Morphological evaluation of embryos is the main method of embryo selection. Time-lapse microscopy is one of the modern methods of selecting a high-quality embryo for transfer. In the analysis of many retrospective and prospective studies, they emphasize the advantage and lack of differences compared to traditional morphological assessment of the quality of embryos Almost all publications devoted to time-lapse microscopy have focused on determining the timing of specific events of embryo division and then using this information to create algorithms that help to select embryo for transfer.

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Abstract- The embryological stage of ART programs is one of the most important, since the assessment of the quality of oocytes, their fertilization and in vitro cultivation to the stage of preimplantation embryos largely determines its success. Morphological evaluation of embryos is the main method of embryo selection. Time-lapse microscopy is one of the modern methods of selecting a high-quality embryo for transfer. In the analysis of many retrospective and prospective studies, they emphasize the advantage and lack of differences compared to traditional morphological assessment of the quality of embryos Almost all publications devoted to time-lapse microscopy have focused on determining the timing of specific events of embryo division and then using this information to create algorithms that help to select embryo for transfer.

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I. Introduction

The process of morphological study of embryos is one of the most important selection methods, the results of which evaluate a whole group of indicators, such as the number of blastomeres, the proportion of fragmentation, the severity of compaction, size and shape, as well as their correspondence to the stage of development, the formation of the blastocyst, the size of its cavity, the state of the internal cell mass with trophoblast [1, 2]. An efficient method of embryo selection is currently in high demand in this field, since it is a method of selecting embryos that have the highest potential for implantation [3]. The method of continuous video surveillance allows the specialist to obtain a long and detailed chronicle of the development process of each individual embryo. In the process of development, the embryo goes through several stages of development, and the duration of each stage also serves as a significant indicator of quality and potential, which is characterized as developmental kinetics [4]. In this regard, the introduction of time-lapse technology made it possible for embryologists to arm themselves with an effective tool for selecting promising embryos [5].

The aim of the study is to develop an algorithm for optimizing the in vitro fertilization program with a differentiated approach to the use of time-lapse technology or video surveillance of the development of embryos and artificial intelligence, which allow automatic formation of the morphodynamical profile of a human embryo based on video recording of the process of cultivating a human embryo to the blastocyst stage.

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II. Materials & Methods

The study was carried out on the basis of CJSC Medical Company IDK (Samara, Russia) in the period from 2016 to 2019. Human embryos were used in the work, the study of which was carried out in compliance with international ethical and legal standards for the treatment of human embryos [Art. 18. Council of Europe Convention for the Protection of Human Rights and Dignity of the Human Being in the Use of Biology and Medicine, 1997]. Permission for the use of embryos in research was obtained from the Committee on Bioethics at the Samara State Medical University (excerpt from protocol No. 116 dated October 3, 2018) [6, 7]. Written consent was obtained from the patients included in the study to participate in the study. Exclusion criteria from the study included all conditions in which it is necessary to cancel the ET embryo transfer procedure in a cycle. Examination of patients in the ART program included a comprehensive examination, including the study of anamnesis, gynecological examination, laboratory tests and instrumental studies. ART was performed in accordance with the accepted standards of medical care in CJSC IDK Medical Company. Gametes and embryos were identified under the control of a stereomicroscope (Nicon, Japan). For incubation at 5% O2, COOK incubators (Australia) were used.

Transvaginal ovarian puncture was carried out at 36-37 hours after the start of the ovulation trigger. The identification of oocyte-cumulus complexes in the follicular fluid was carried out using a Nikon stereomicroscope (Japan), after which they were removed with a sterile micropipette. The complexes were washed and cleaned from liquid and blood using a HEPES buffer solution (G-mops, Vitrolife, Sweden). After counting the oocytes, they were transferred to special cups with a central well (Nunc) containing G-IVF+ culture medium (Vitrolife, Sweden) for pre-incubation for 2-3 hours (conditions: CO2 - 6%, O2 - 5%, at a temperature of 37°. After incubation, mechanical and enzymatic removal of cumulus cells (denudation of oocytes) was carried out. In this case, the complexes were placed in a hyaluronidase solution for 30 seconds, followed by washing from enzymes in a buffer solution

by a mechanical method. 5–6-day old blastocysts were examined according to a system based on classification (D.K. Gardneretal., 1999) and the RAHR Guidelines "Evaluation of oocytes and embryos" (Russia, 2021) [8, 9]. Over 100 cycles were analyzed using TimeLapse technology. The video surveillance system for embryo development included an incubator with an installed video camera Embryovizor (Russia). Embryos were cultured in special WOW dishes (Vitrolife, Sweden) in a universal medium Continius Single Culture (Irvine Scientific, USA) from 1 to 5-6 days of cultivation. There were no specific criteria for selecting patients for culture using this system [10]. The system has direct on-line access. To assess the development of embryos from days 1 to 5-6 of in vitro cultivation, the time of the first cleavages, the time range between the first and second cleavages, as well as the nature of cleavage (morphokinetics), and the time of blastocyst formation were taken into account. All of the above criteria served as predictors of the selection of embryos for transfer [11].

The criteria for elective transfer [12] of one embryo on the 5th day (5eSET) were: the presence of more than 2 embryos of excellent quality, the patient's age is up to 35 years, and the absence of previous IVF attempts in history. The criteria for selective single embryo transfer (5SET) were: the presence of a scar on the uterus after previous surgical interventions and other clinical situations.

Obtaining and processing information about human embryos was carried out in the laboratory of assisted reproductive technologies (ART) of the IDK Clinical Hospital of CJSC IDK Medical Company (Mother and Child group of companies, Samara, Russia). Graphic data and markup information have been uploaded to the SberCloud cluster. A convolutional network of neurons designed to differentiate embryos based on multiclass division [13] was installed on the Christofari supercomputer of the SberCloud cluster. To standardize the description of the development of human embryos cultured in vitro, we introduced the concept of "Morphodynamic profile of a human embryo" [14]. It includes a set of morphokinetic states identified by us, located on the time scale in accordance with the moment of their registration. All time cutoffs (points) are given in chronological order relative to the moment of fertilization.

III. Results & Discussion

The cultivation of human embryos in vitro in the practice of embryological laboratories is currently a proven and standardized technique. The quality of media, consumables, technical capabilities of incubators make it possible to bring the conditions of growth and development of embryos in vitro as close as possible to natural conditions. Nevertheless, the problem of determining reliable predictors of the developing embryo, which has the highest chances of implantation, is extremely relevant. These aspects are especially significant in order to safely and effectively implement the strategy of transferring a single embryo into the uterine cavity to prevent the development of multiple pregnancies, the birth of premature and low birth weight babies. In this regard, the development of non-invasive technologies for ranking developing embryos in order to select them for transfer to the uterine cavity in a modern embryological laboratory is extremely in demand. A special term was also introduced – “morphokinetics”, which reflects the visual fixed state of the human embryo. Successive stages of the morphokinetic state constitute morphodynamics.

Monitoring the process of embryo development makes it possible to fix various stages of morphokinetic transformations, to establish the presence of cytoplasmic and extracytoplasmic structures - multinucleation, fragmentation, vacuolization, etc., and also to evaluate their contribution to the early development of embryos [15, 16]. At present, it is extremely important to identify the predictors of the development of a competent embryo, which determine its implantation potential [17]. Predictors in this case are prognostic parameters, the evaluation of which together serves as a method for differentiation and selection of embryos. For non-invasive monitoring of the pre-implantation development of human embryos, a multi-gas incubator with a reduced oxygen concentration (5%) with a video surveillance system Embryovizor (Westtrade, Russia) was used [18]. This equipment allows, without taking out a dish with developing embryos, to evaluate the first cell divisions, to determine the time intervals of embryo crushing, compaction and formation of blastocysts, and also detects intracellular changes [19].

During the analysis of video files, we can state signs of normal - 2PN2PB or abnormal fertilization (3PN) (Fig. 1, 2).

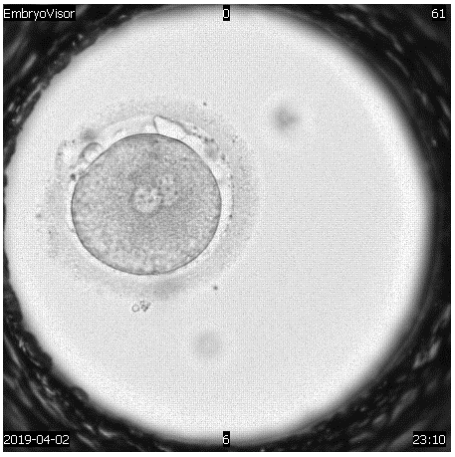

Figure 1: Human embryo of the 1st day of development at the 2PN2PB stage (zygote), magnification 200X.

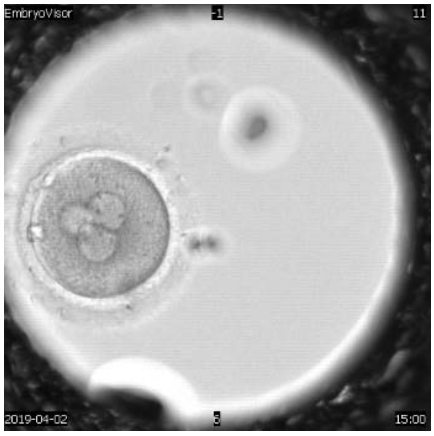

Figure 2: Human embryo of the 1st day of development at the 3PN2PB stage, magnification 200X.

The second feature is reverse crushing. If it is detected in the development of the embryo, this reduces its chances of implantation in the presence of others in which we have not recorded this feature (Fig. 3). The presented series of images show a series of frames that show the dynamics of the development of this process. In the first case, the embryo, which began division from the three-cell stage, goes through the stage of reverse cleavage into the two-cell stage.

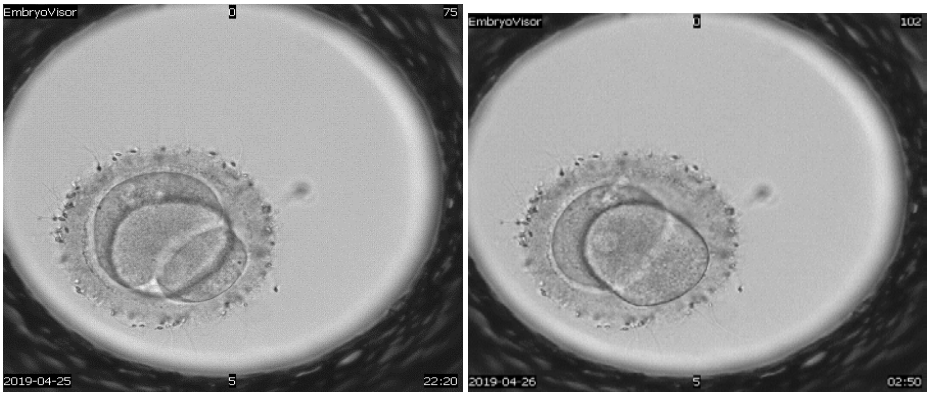

Figure 3: Reverse crushing of human embryos 3-2, magnification 200X.

In the second case, a normally divided embryo passes into a four-cell stage and returns to a two-cell stage (Fig. 4).

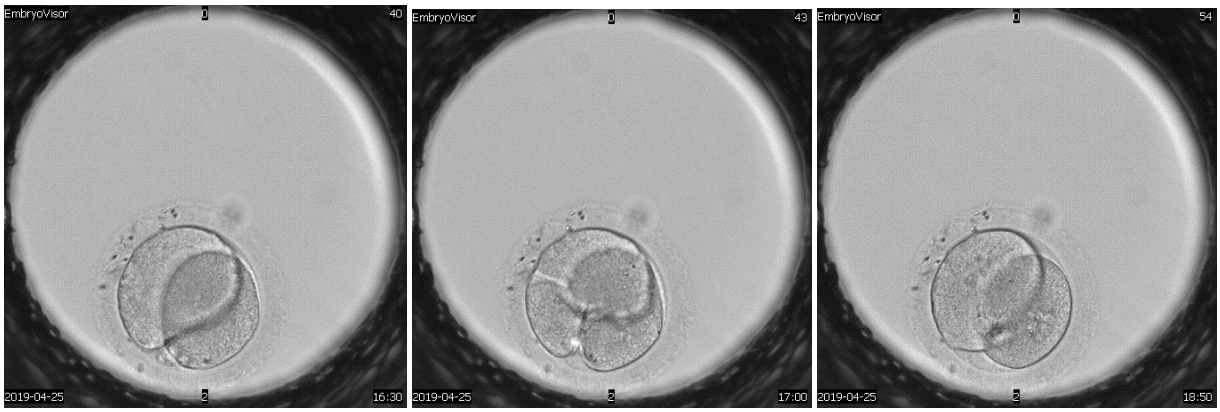

Figure 4: Reverse cleavage of 2-4-2 embryos, magnification 200X.

The appearance of multinucleation (several nuclei in developing blastomeres) is also a reason to exclude embryos for transfer and cryopreservation if other embryos of comparable quality are available.

That is, if there are embryos of a comparable organization, those that have reverse cleavage, multinucleation, play the role of a “reserve player” and

are subject to cryopreservation and transfer to the uterine cavity, if there are no other embryos.

Fragmentation - the appearance of nuclear-free fragments - is a variable and relative sign. Distinguish between small cell and cryocellular fragmentation. In accordance with our proposed classification for assessing the quality of developing embryos, the presence of fragmentation up to 10% is not a factor that reduces the competence of developing embryos. However, in the presence of a higher level of fragmentation, it can lead to its complete fragmentation or stop in development.

To standardize the description of the development of human embryos cultured in vitro, together with the developers of the Embryovizor system, the concept of "Morphodynamic profile of a human embryo" was introduced. It includes a set of morphokinetic states identified by us, located on the time scale in accordance with the moment of their registration. All time cutoffs (points) are given in chronological order relative to the moment of fertilization.

Table 2: Comparative characteristics of the indicators of the development of embryos obtained in the IVF program

	IVF with Video n=32	IVF without Video n=48	IVF total n=80
Average age of patients	32,6±2,8	33,4±3,4	33,1±3,2
Average attempts	1,72±0,46	2,23±0,47***	2,03±0,53
Average years of infertility	5,56±1,01	5,63±1,16	5,6±1,1
Average dose of FGS	1457,1±269,7	1573,8±328,4	1527,1±309,8
Medium MII	6,5±1,48	5,69±1,19**	6,01±1,36
% fertilization	74,1±13,9	76,7±14,3	75,6±14,1
% crushing	95,7±2,9	97,3±3,2**	96,7±3,2
% growth of doblastocyst	18,4±4,2	22,3±5,7***	20,7±5,4
Freeze %	35±6,9	32,1±5,8*	33,3±6,4
average embryonic tolerance	1,03±0,18	1,15±0,36*	1,1±0,3
HCG (+),%	36,7±6	42,5±7,4***	40,2±7,5
Ultrasound, %	34,3±7,1	36±6,7	35,4±6,9
CI, %	36,9±8,9	39,3±7,3	38,3±8
Multiple pregnancy rate	8,22±1,72	4,77±0,86***	6,15±2,12

*Note: *-p<0.05, **-p<0.01, ***-p<0.001 statistical significance in relation to IVF group with video.*

The tables below show the main data on embryo development indicators and their analysis in a comparative aspect in groups with and without video monitoring.

When comparing the data in this group, we see that the indicators do not have a significant difference. However, it should be noted that the hCG and ultrasound values in the IVF group (with video) have a minimal difference, which indicates the high quality of the embryos that are cultured and selected for transfer using video surveillance technology. The multiple pregnancy rate, which is almost 2 times higher, confirms this conclusion.

When comparing the data in this group, we see that the indicators do not have a significant difference. It should be noted that the difference between hCG and ultrasound in the ICSI group (with video) is smaller, which indicates the high quality of the embryos that are cultured and selected for transfer using video surveillance technology. the lack of difference between CNB and CI indicates that all the embryos that gave birth were implanted. Moreover, the average number of embryos per transfer in this group is slightly lower than in the ICSI group (no video). The multiple pregnancy rate in the ICSI group (no video) is extremely high. This is a risk group, since obstetric risks and the risks of giving birth to premature and low birth weight children in this

group are extremely high. Attention should be paid to this group and a more rigorous selection of embryos for transfer should be carried out, while at the same time reducing the number of transferred embryos.

When comparing the older age group in this group, we see that the rates of growth to the blastocyst in the IVF group with video have higher values. this suggests that the continuous video surveillance and culture system has a positive and no negative effect on oocytes in older patients, whose oocytes are most sensitive to environmental fluctuations (light, temperature changes, CO2 levels, pH). Clinical indicators: hCG +, CNB have a minimal difference, which indicates the high quality of the embryos that are cultured and selected for transfer using video surveillance technology. The multiple pregnancy rate, which is more than 2 times higher, confirms this conclusion. Cleavage, Freeze, HCG+, CNB, Chi indicators show the advantage of selecting 1 best embryo using video surveillance. Most likely, this is due to stable conditions and reduced stress during the cultivation of embryos (no fluctuations in temperature, pH). In the ICSI group, when taking one embryo (SET - single embryo transfer) and transferring one best embryo (eSET - elective single embryo transfer) on day 5, compared with the general sample, higher rates of freezing, hCG+, ultrasound and CI were found.

These data strongly demonstrate the advantage of culturing embryos in a system with video surveillance. The absence of negative influence of external factors during cultivation, analysis of morphokinetics and more objective selection of embryos for transfer contribute not only to the formation of the most competent embryos, but also allow to achieve higher clinical indicators of CNB and CI [20].

In the study group, where TML was used, an increased probability of pregnancy was established, regardless of the option of embryo transfer: 5eSET - 70±8.5% and 5SET - 38.2±4.9%. In the study group, where the traditional method of cultivation and selection of the embryo was used, the pregnancy rate was 45% higher in the sample in which the elective transfer was performed: 5eSET - 55.6±6.7% and 5SET - 36.9±6.1%.

IV. Conclusion

According to numerous publications, knowledge of the characteristics of the morphokinetics of a developing embryo makes it possible in some cases to predict its future fate. For example, the presence of direct division of the zygote into three blastomeres is an unfavorable marker and indicates a high level of aneuploidy of such embryos, while reverse division indicates a possible violation of cytokinesis. A short interval between the second and third cleavage is a prognostically favorable sign in the development of the embryo and most often demonstrates a high level of growth to the blastocyst stage. The fixed features of the morphodynamic profile are factors in the ranking of embryos and their selection for transfer and cryopreservation.

Based on the data obtained, we can draw the following conclusions:

1. Cultivation in an incubator with video surveillance allows the formation of embryos with a higher competence for implantation. In the study groups using video surveillance, higher results of hCG (+) / CNB were obtained and the difference between these indicators is minimal, which indicates a high quality of embryos that are selected for transfer (IVF 36.7 ± 6% / 34.3 ± 7.1 % with video surveillance and 42.5±7.4%/36±6.7% without video surveillance ICSI 30.1±6.6%/24.1±5% with video surveillance and 35±6.6%/ 25.3±4.9% without video surveillance).
2. In the group of older reproductive age (36+ years), the time lapse technology demonstrates even higher significance. The difference between hCG(+)/CNB values 34.7±8.1%/30.5±4.6% is minimal in the group with video surveillance. Most likely, this fact is associated with the high sensitivity of the oocytes and embryos of these patients to adverse environmental factors and stress, the implementation of which is reduced during cultivation in an incubator with a video surveillance system.
3. The fact of the highest rates of hCG (+) / CNB / CI 70 ± 8.5% / 59.9 ± 5.7% / 50.1 ± 8.2% in the group of elective single embryo transfer on day 5 (5eSET) using video surveillance technology indicates the high competence of these embryos.
4. Video surveillance technology for the development of embryos can reduce the influence of the human factor and increase the objectivity of assessing the structure of embryos, improving their selection, reducing the rates of multiple pregnancy.

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