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Assessment Methods for Human Embryos to Enhance Reproductive Potential

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Abstract Some clinics have invested in cutting–edge technology that allows for continuous video monitoring of embryo development. The approach is based on the employment of unique time–lapse technology, in which each embryo is photographed at a predetermined frequency beginning with fertilization and continuing throughout the incubation phase [7,8,9]. Optimizing the in vitro fertilization program with a unique approach to using time–lapse technology or video monitoring of embryo development. From 2016 to 2019, patient recruitment was carried out on the basis of Closed Joint Stock Company IDC (information diagnostic company) Medical Corporation (Samara, Russia). The Samara State Medical University's Bioethics Committee approved using human embryos in scientific research. Higher hCG (+)/CPR (clinical pregnancy rate) results were obtained in the study groups using video surveillance, and the difference between these indicators is minimal, indicating a high quality of embryos 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). Time–lapse technology is considerably more critical in the older reproductive age group (36+ years). The difference in hCG(+)/CPR levels of 34.7±8.1%/30.5±4.6% is slight in the video surveillance group. Analyzing the morphokinetic characteristics of embryo pre–implantation development allows for selecting competent embryos for transfer into the uterine cavity and cryopreservation.

Keywords Assisted reproductive technologies, Infertility, Elective blastocyst transfer, Time–lapse microscopy, Morphokinetics

1. Introduction

With the advent of new assisted reproductive technologies into clinical practice, the likelihood of having children in situations of previously incurable kinds of infertility in marriage has dramatically increased. Assistive technology has advanced over time, and medicine and embryology are now much beyond what was thought in the twentieth century [1]. Competency evaluation of cultured embryos is in high demand since it is a method for selecting an embryo with the best chances of implantation [1]. Some clinics offer modern equipment for continuous embryo growth monitoring, allowing you to watch and analyze the synchronization and rate of development without opening the incubator and removing the embryos outside. The approach is based on the employment of unique time–lapse technology, in which each embryo is photographed at a predetermined frequency beginning with fertilization and continuing throughout the incubation phase. Yet, this is not the sole advantage of time–lapse technology [7,8,9]. During IVF cycles, the embryologist frequently works with many embryos. These embryos differ in that some correspond to the recommended developmental criteria and are, therefore, the most hopeful for implantation in the uterine cavity, while others are less promising and do not correspond to modern views about embryo development. With the introduction of technology for the continuous monitoring of embryos, the embryologist now has access to the most thorough video chronicle of each individual embryo's early growth. The embryo goes through a number of major events (development stages) during its development, and the time it takes to go from one stage to the next is a crucial signal in determining its quality and implantation potential–development kinetics. Time–lapse technology has added a new tool to the embryologist's toolbox, allowing for better selection of the most promising embryos and therefore increasing the probability of conception.

The objective is to optimize the in vitro fertilization program with a differentiated approach to the use of time–lapse technology or video surveillance of embryo development, which enables the automatic formation of the morphodynamic profile of a human embryo based on video recording of the cultivation of a human embryo to the

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blastocyst stage.

2. Materials and Methods

Between 2016 and 2019, patients for the study were recruited at CJSC IDC Medical Corporation (Samara, Russia) throughout the period of 2016 to 2019. The study utilized human embryos per international ethical and legal norms for treating human embryos [Article 18 of the 1997 Council of Europe Convention for the Protection of Human Rights and the Dignity of the Human Being in the Application of Biology and Medicine]. The Bioethics Committee of the Samara State Medical University approved the use of human embryos in scientific study (statement from protocol No. 116 dated October 3, 2018). Participation in the study was authorized by the signed informed consent of all patients. Exclusion criteria included any problems or difficulties necessitating the elimination of embryo transfer (ET) throughout the trial cycle. Patients enrolled in ART programs were subjected to an anamnesis, gynecological examination, laboratory and instrumental investigations. CJSC IDC Medical Corporation implemented ART programs in compliance with the recognised norms of medical care. Under the direction of a stereomicroscope, gametes and embryos were detected (Nicon, Japan). Australia's COOK incubators were utilized for incubation at 5% O2.

Using Time–lapse technology, more than 100 cycles were studied. EmbryoVisor, an incubator with a built–in video camera, is the embodiment of the embryo development video surveillance system (Russia). From 1 to 5–6 days of development, embryos were grown in specific WOW dishes (Vitrolife, Sweden) using the universal medium Continius Single Culture (Irvine Scientific, USA). With this technique, there were no precise criteria for selecting patients for culture. The system is accessible online directly. To evaluate the development of embryos from day 1 to day 5–6 of in vitro cultivation, the time of the first cleavages, the time interval between the first and second cleavages, the nature of cleavage (morphokinetics), and the time of blastocyst formation were considered. All of the aforementioned characteristics served as predictors of embryo selection for transfer. The criteria for elective transfer of one embryo on the fifth day (5eSET) were the presence of more than two embryos of exceptional quality, the patient's age between 18 and 35 years old, and the lack of any prior IVF attempts. The criteria for selective single embryo transfer (5SET) were the presence of a scar on the uterine wall as a result of previous surgical operations and other clinical circumstances. In the assisted reproductive technologies (ART) laboratory of the Clinical Hospital CJSC IDC "Medical Company" (Mother and Child group of companies, Samara, Russia), the collecting, labeling, and preparation of visual information on cultured human embryos were performed. Markup information and graphic material have been uploaded to the SberCloud cluster. On the Christofari supercomputer of the SberCloud cluster, the convolutional neural network used to solve the multi–class classification issue is implemented. To standardize the description of the development of human embryos cultured in vitro, "Morphodynamic profile of a human embryo" was introduced. It consists of a set of morphokinetic states detected by us and positioned on the time scale based on the moment of their registration. All time thresholds (points) are presented in chronological order relative to fertilization. The acquired information was processed using Microsoft Excel 2007 and STATISTICA 6. *For unrelated ranges*, the significance of differences in numeric indicators was examined using the *Wilcoxon test*; *the Fisher–Irwin exact test* was utilized for qualitative values. At $p<0.05$, differences between groups were deemed statistically significant; Spearman's non–parametric rank correlation approach was used for correlation analysis.

3. Results and Discussion

For non–invasive monitoring of the pre–implantation development of human embryos in the ART laboratory, a multi–gas incubator with a reduced oxygen concentration (5%) and EmbryoVisor (Westtrade, Russia) video surveillance system were utilized. To standardize the description of the development of human embryos cultured in vitro, the idea of "Morphodynamic profile of a human embryo" was introduced in collaboration with the inventors of the EmbryoVisor system. It consists of a set of morphokinetic states detected by us and positioned on the time scale based on the moment of their registration. All time thresholds (points) are presented in chronological order relative to fertilization. Determination of the morphodynamic profile enables the ranking of developing embryos in order to identify the most promising embryo for implantation for transfer into the uterine cavity, as well as embryos for eventual cryopreservation (second stage embryos). On the basis of the identified markers, time–lapse images of human embryo cultivation cycles available to laboratories are marked according to the approved algorithm for preparing data sets for training a neural network, and data is uploaded for subsequent training of a neural network designed for automated recognition of the morphokinetic state of a human embryo cultivated to the blastocyst stage.

The following characteristics were incorporated in the profile's formation:

- time of production of pronuclei PN;
- time of disappearance of pronuclei;
- time of formation of 2, 3, 4, 5, 6, 7, and 8 blastomeres);
- time of the beginning of embryo compaction;
- time of full compaction of the embryo;
- time of the beginning of embryo cavitation;
- time of formation of a complete blastocyst;
- time of formation of expanded (enlarged) blastocyst;
- time of blastocyst hatching.

The following additional parameters are utilized to evaluate development dynamics:

- proper fertilization (number of observed pronuclei: 1, 2, 3 or more);
- the degree of embryo fragmentation (in percent);
- the presence of multinucleation (Figure 3);
- heterogeneity of the cytoplasm – the presence of endoplasmic reticulum EPR;
- heterogeneity of the cytoplasm (inclusions);
- vacuolization of embryonic cells;
- abnormalities in the shape of the embryo;
- the presence of reverse crushing;
- uniformity of blastomeres during crushing.

Furthermore, the markup system has been updated to meet the criteria imposed by the use of neural network technologies. The EmbryoVisor incubator software's standard marker configuration mechanism has added more marker groups. Optionally, the researcher creating the cycle markup can place numerous markers on the pronuclear section. We have selected and categorized 612 cycles of human embryo cultivation based on the information we have on the cultivation of more than 2,000 human embryos (a total of 13,367,420 frames). In addition to the states in the morphodynamic profile, the researchers highlighted the principal focus plane (the most informative). In addition, logical constraints were set during employee selection, eliminating plainly unfit candidates. These methods enabled a reduction in the number of frames sent for neural network training to 1,124,937. As part of the generation of the training sample, candidates that spent an abnormal amount of time at any of the developmental stages and embryos with a small number of stages passed were eliminated. In the first stage, the position of the embryo in the microwell is determined using a machine learning model based on the neural network architecture "Faster R–CNN".

This method permits the determination of the effective region of the image for further classification and the verification of the embryo's real presence in the image. The personnel distribution according to the classification parameters is shown in Table 1.

Table 1. Distribution of training sample frames

Marker ID	Stage ID	Decoding	Number
1	PrePN	From the beginning of cultivation to the appearance of pronucleus	3,603
11	PN2	Frames with 2PN embryos	92,869
12	PN1	Frames with 1PN embryos	17,209
13	PN3	Frames with 3 or more PN embryos	8,092
14	PostPN	Frames from the disappearance of the pronucleus to the first crushing	42,690
21	CL2	Two–cell stage	105,216
22	CL3	Three–cell stage	30,346
23	CL4	Four–cell stage	103,747
24	CL5	Five–cell stage	37,831
25	CL6	Six–cell stage	75,817
26	CL8	Eight–cell stage	164,692
27	CLR	Frames after marking reversible crushing	20,340
41	CMST	From the beginning of compactization to the formation of morules	118,133
42	CMFL	Stage from the formation of morule to the beginning of cavitation	95,138
43	CAST	Stage from the beginning of cavitation to complete blastocysts	96,390
44	BLFL	Complete blastocysts	44,136
45	BLEXP	Expanded blastocysts	12,980
46	BLHAT	Blastocysts hatching	9,131
99	LF	Last finishing mark frames	46,575

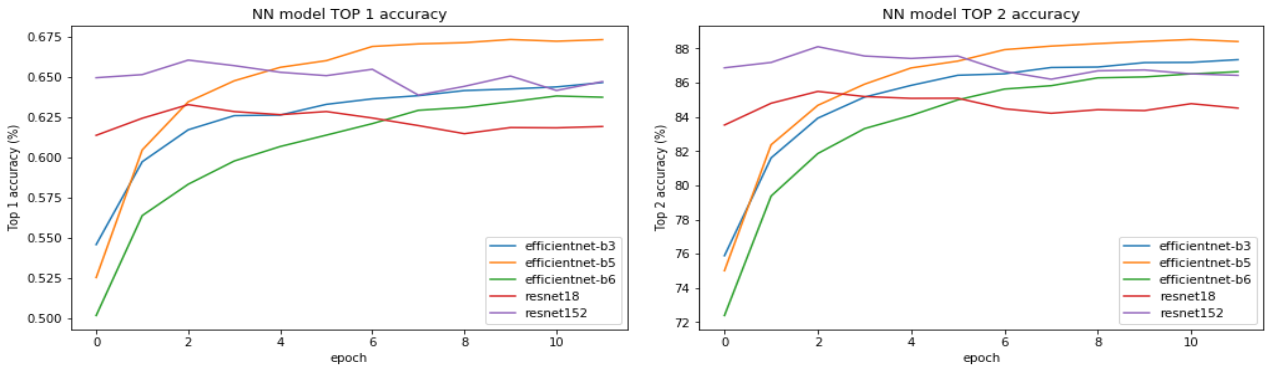

Figure 1. Comparison of models with different architectures

The part of the image containing the embryo is categorized and its current status is determined during the second stage. Comparing five architectures–ResNet18, ResNet152, EfficientNet–b3, EfficientNet–b5, and EfficientNet–b6 led to the selection of the ideal neural network design for this job. After examining the data, the EfficientNet–b5 architecture was selected to determine the current embryonic condition with the most incredible precision (Fig. 1).

At the third stage, the pronuclei and cells discovered in the image analysis are segmented (machine learning models based on the Faster R–CNN neural network architecture). Segmentation is conducted if the studied image is classified as an embryo with pronuclei or an embryo with 2–8 cells. The possibility of fixing the major morphodynamic events and their evaluation permits a more thorough approach to the evaluation of developing embryos, allowing for their ranking and the selection of the embryo with the most significant potential for transfer. The acquired results permit the development of a subsystem for automated recognition of the morphokinetic states of a human embryo and assessment of its implantation potential.

As part of the study, embryo development data obtained by IVF and ICSI procedures were evaluated using time–lapse technology. The control groups consisted of two parallel groups in which embryos were created by a similar method of fertilization and standard culturing. These are the key indices of embryo growth and their comparative comparison between groups with and without video surveillance.

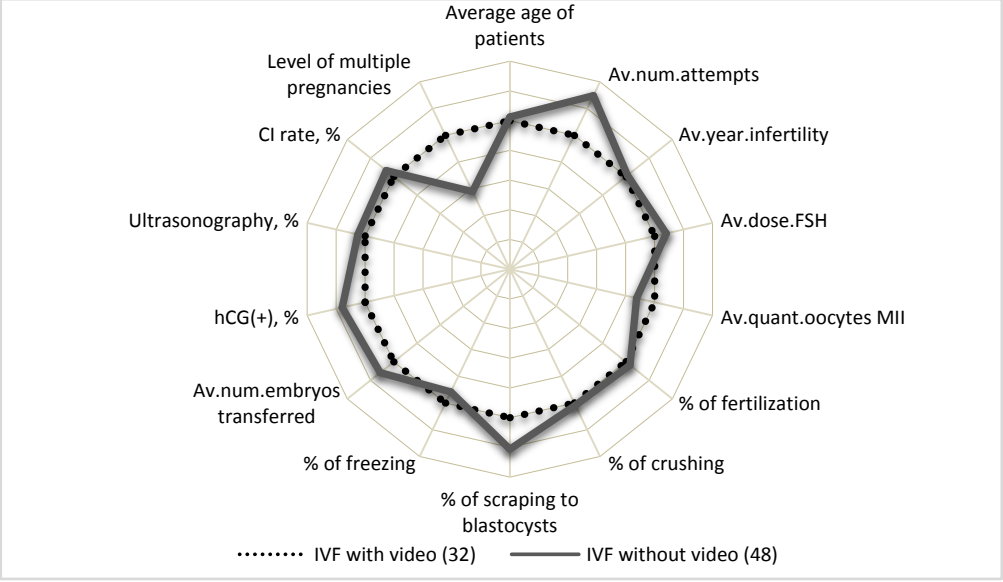

Figure 2. Comparative characteristics of the developmental indicators of embryos obtained in the IVF program

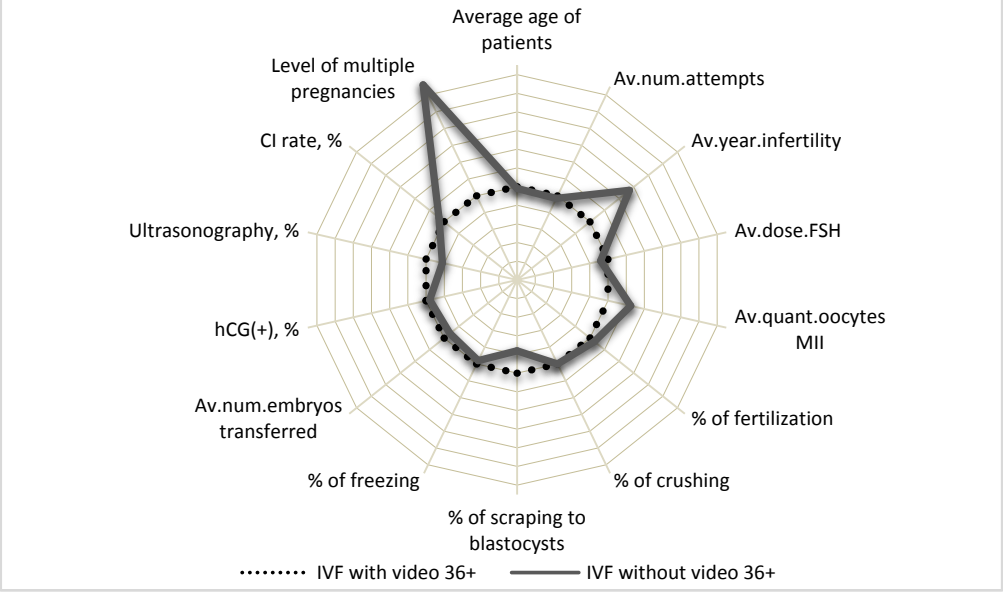

Figure 3. Comparative characteristics of the developmental indicators of embryos obtained in the IVF program in the age group over 36 years

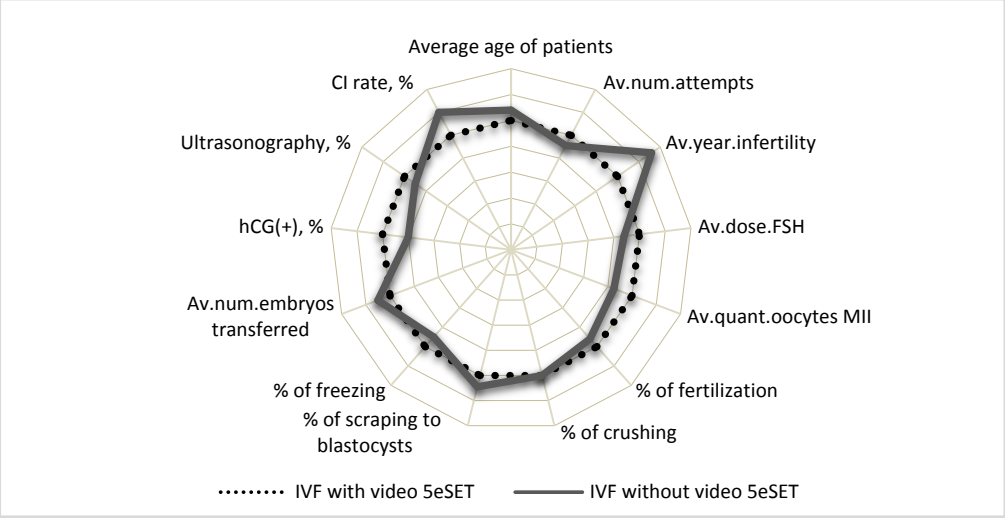

Figure 4. Comparative characteristics of the developmental indicators of embryos obtained in the IVF program and the transfer of one best embryo

Comparing the data within this group reveals that the indicators do not differ significantly. It should be highlighted, however, that the difference between the hCG and ultrasound levels in the IVF group (with video) is low, indicating the excellent quality of the embryos cultivated and selected for transfer utilizing video surveillance technology. The nearly twofold more significant rate of multiple births supports this hypothesis (Fig. 2).

When comparing the data within the age group, we find that the IVF group with video has greater blastocyst growth rates. This shows that the method of continuous video surveillance and culture has a good effect on oocytes in elderly individuals, whose oocytes are most susceptible to environmental variations (light, temperature changes, CO2 levels, pH). The little discrepancy between the clinical signs of hCG+ and CPR demonstrates the high quality of the grown embryos selected for transfer utilizing video surveillance technology. The rate of multiple pregnancies, which is more than twice as high, supports this hypothesis. Cleavage, Freeze, hCG+, CPR, and CI rate indices demonstrate the advantage of using video monitoring to identify the first–best embryo. This is presumably due to a steady environment and reduced stress during embryo cultivation (no fluctuations in temperature, pH). In the ICSI group, transferring one embryo (SET–single embryo transfer) and transferring one best embryo (eSET–elective single embryo transfer) on day 5 was associated with greater freezing, hCG+, ultrasonography, and CI rates compared to the general group (Fig.4).

Comparative characteristics of the development indicators of embryos obtained using ICSI with the transfer of the best embryo. Clearly demonstrate the benefits of embryo culture in a system with video surveillance. The lack of negative external influences during cultivation, the examination of morphokinetics, and the more objective selection of embryos for transfer contribute not only to the production of the most competent embryos, but also to the achievement of more significant CPR and CI clinical indicators. In the group using TLM, the pregnancy rate was high regardless of the type of embryo transfer (in subgroups 5eSET–70±8.5% and 5SET–38.2±4.9%), whereas, in the group using the traditional method of embryo cultivation and selection, the birth rate was 45% higher in the elective embryo transfer subgroup (55.6±6.7% in subgroups 5eSET and 36.9±6.1% in subgroups 5SET).

On the basis of the obtained data, the following conclusions can be drawn:

- the auto–detection of the morphodynamic profile of human embryos in vitro will provide the compilation of data sets for training the decision support system in the future to achieve higher pregnancy and birth rates;
- its culturing in an incubator under video observation permits the development of embryos with more significant implantation potential. In the study groups using video surveillance, hCG (+)/CPR results were higher, and the difference between these indicators was minimal, indicating a high quality of embryos 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);
- the significance of time–lapse technology is considerably more pronounced in the elder reproductive age group (36+ years). The difference between hCG(+)/CPR levels of 34.7±8.1% and 30.5±4.6% in the group with video surveillance is minor. This is likely due to the increased susceptibility of these patients' oocytes and embryos to harmful environmental conditions and stress, which is mitigated during cultivation in an incubator equipped with a video surveillance system;
- the highest rates of hCG (+)/CPR/CI (70±8.5% / 59.9±5.7% / 50.1±8.2%) were observed in the group of elective single embryo transfer on day 5 (5eSET) with the use of video surveillance technology, indicating that

these embryos were highly competent.

4. Conclusions

The video surveillance technology for embryo growth makes it possible to eliminate the effect of the human factor and raise the objectivity of evaluating the structure of embryos, thereby enhancing their selection and decreasing the rates of multiple births.

On the basis of the studies undertaken, the fundamental principles of using time–lapse technology have been analyzed and studied. Analyzing the morphokinetic parameters of embryos' pre–implantation development enables the selection of competent embryos for transfer into the uterus and cryopreservation. The objective selection of the most implantable embryo enables pregnancy, preventing ineffective transfers and shortening the time to conception. Single embryo transfer prevents the birth of premature and underweight infants (during the development of twins and triplets) and minimizes the chance of birth traumas. Such an approach does not necessitate additional public spending for nursing preterm and underweight infants.

Consequently, within the study context, the necessity and efficacy of non–invasive time–lapse technology for optimizing assisted reproductive technology programs have been demonstrated.

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