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THE USE OF ELECTRONIC HEALTH TOOLS FROM THE VERY BEGINNING OF LIFE - TIME-LAPSE EMBRYO MONITORING

PRIMENA ELEKTRONSKOG ZDRAVSTVA OD SAMOG POČETKA ŽIVOTA – KONTINUIRANI VIDEO MONITORING EMBRIONA

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Summary

Introduction. In addition to the already widespread use of electronic health services in monitoring the health status of patients, one should also refer to the possibility of using electronic health tools from the very beginning of life by monitoring growth and development of the embryo. In this way, the concept of electronic health would expand to cover the whole human life, from its very beginning to the end. **Monitoring the embryo development.** The efforts to improve in vitro fertilization success rate have been accompanied by the introduction of various procedures, including the evaluation of the embryo quality. A detailed monitoring of the kinetics of embryo development is achieved by laboratory techniques that provide continuous insight into the embryo development through applying time-lapse monitoring system. **Embryo quality evaluation programs based on time-lapse monitoring.** Special bioinformatic programs have been developed for automatic analysis of images obtained by time-lapse monitoring which allow a quantitative evaluation of the key moments in the development of the embryo and its morphology. **Implementation and limitations of time-lapse embryo monitoring.** The majority of authorities in the field of in vitro fertilization consider the use of time-lapse monitoring as a great advancement in the in vitro fertilization technology, obviously leading to a higher success rate. **Conclusion.** The systems for time-lapse monitoring of embryos represent powerful tools which help clinicians involved in in vitro fertilization and embryologists to select the best embryos, with the aim of improving the in vitro fertilization success rate. Despite all the advantages, these systems also have some shortcomings and limitations.

Key words: Time-Lapse Imaging; Fertilization in Vitro; Reproductive Techniques, Assisted; Embryonic Development; Embryo Transfer; Patient Safety

Sažetak

Uvod. Pored već široke upotrebe elektronskih zdravstvenih usluga za praćenje zdravstvenog stanja pacijenata, treba pomenuti i mogućnost upotrebe alata za elektronsko zdravstvo od samog početka života odnosno praćenja formiranja i razvoja embriona. Na ovaj se način pojam elektronskog zdravstva proširio na čitav ljudski život, od njegovog početka do kraja. **Nadzor razvoja embriona.** Napor da se poveća stopa uspeha oplodnje *in vitro* praćen je uvođenjem različitih postupaka, među kojima je i procena kvaliteta embriona. Detaljan nadzor kinetike razvoja embriona postiže se laboratorijskim tehnikama kojima se omogućava kontinuirani uvid u razvoj embriona primenom sistema kontinuiranog video-monitoringa. **Programi za evaluaciju kvaliteta embriona zasnovani na kontinuiranom video-nadzoru.** Kreirani su posebni bioinformatički programi za automatsku analizu slika dobijenih kontinuiranim video-nadzorom koji omogućavaju kvantitativnu procenu ključnih trenutaka u razvoju embriona i njegove morfologije. **Implementacija i ograničenja kontinuiranog video monitoringa embriona.** Većina eksperata u oblasti *in vitro* oplodnje smatra da je primena sistema kontinuiranog video-monitoringa veliki napredak tehnologije u ovoj oblasti koja dovodi do većeg stepena uspeha postupka. **Zaključak.** Sistemi za video-monitoring embriona predstavljaju moćne alate koji pomažu kliničarima koji sprovode vantelesnu oplodnju i embriolozima da odaberu najbolje embrione, sa ciljem da se poveća stopa uspeha vantelesne oplodnje. Uprkos svim prednostima, ovi sistemi imaju i neke nedostatke i ograničenja.

Ključne reči: time-lapse imidžing; in vitro fertilizacija; asistiranje reproduktivne tehnike; embrionalni razvoj; embriotransfer; bezbednost pacijenta

Introduction

Electronic health (e-health) is an “emerging field in the intersection of medical informatics, public health and business, referring to health services and information delivered or enhanced through the Internet and related technologies” [1]. In 2015, the European Commission proposed a definition of e-

health as the application of information-communication technologies (ICTs) to meet the needs of citizens, patients, health service providers, and creators of health policy [2].

The use of ICTs in e-health is aimed at:

– Advancement and improvement of prevention, diagnostics, treatment, and disease management;

Abbreviations

E-health	– electronic health
ICTs	– information-communication technologies
IVF	– in vitro fertilization
ICSI	– intracytoplasmic sperm injection

- Improvement of the accessibility to health services and enhancement of their quality by improving the efficiency of the health sector;

- Exchange of information between patients and health service providers, hospitals, health workers;

- Use of telemedicine and portable equipment for monitoring health conditions and other kinds of information.

The aim of this article is to give a short review of the use of e-health in monitoring the growth and development of the embryo with the emphasis on the time-lapse method, as a comprehensive tool of e-health.

Infertility is a serious health problem that affects a constantly increasing number of people all over the world [3]. After the birth of the first “test-tube” baby in 1978, in vitro fertilization (IVF) has become a widely practiced technique, appearing as a crucial solution to all those couples who went through all the procedures of infertility treatment [4]. It is estimated that globally more than 8.5 million children were born through IVF [5]. This procedure has become increasingly widespread in solving the infertility problem of many couples. Although a constant increase in its success rate is evident, it still remains relatively low. A strategy to increase its efficiency, which is still present in some countries and centers, is transfer of a larger number of embryos. However, this strategy increases the risk of multiple pregnancy, which may cause a number of harmful effects on both mother and child(ren). That is why, elective transfer of a single embryo is an imperative, which cannot be realized without proper selection and evaluation of its quality. Embryos can be selected by applying different methods – non-invasive or invasive. In non-invasive methods the embryos are selected based on their morphology or using techniques based on the analysis of their molecular components – the levels of proteomes and metabolomes. Detailed monitoring of the kinetics of embryo development is achieved by using techniques of different time-lapse monitoring systems [6].

Monitoring the embryo development

The development of human embryo is a dynamic process which begins after the union of the sperm and the ovum, forming a zygote. The zygote continues its further development through a series of mitotic cleavage divisions, forming blastomeres every 12 to 24 hours. Thus, on day 3, the embryo has 8 cells, on day 4 a morula is formed, and on day 5 the embryo forms a blastocyst [7]. Classical monitoring of this dynamic embryo development means static observations performed from time to time by an embryologist. Such an approach has two major shortcomings [8]:

1. Static observations are performed only in certain time moments during the day, which means an inevitable loss of valuable information and disturbance of optimal conditions for embryo cultivation;

2. This approach allows only individual, sequential interpretations based on subjective evaluations, and as such it shows large inter-observer errors and moderate intra-observer errors.

Standard microscopic observation of the embryo mans its evaluation once a day. According to the consensus given by the Special Interest Group of the European Association for Human Reproduction and Embryology and Alpha Scientists in Reproductive Medicine, the checking is carried out in the following time intervals [9]:

- Fertilization is checked 17 hours post insemination and syngamy after 23 hours

- Stage of early cleavage is checked 26 hours post intracytoplasmic sperm injection (ICSI) and 28 hours after classical IVF procedure

- On day 2, embryo is assessed 44 hours post insemination

- On day 3, embryo is assessed 68 hours post insemination

- On day 4, embryo is assessed 92 hours post insemination

- On day 5, embryo is assessed 116 hours post insemination.

More frequent controls are not acceptable, due to the disturbance of conditions in the cultivation medium, such as the stability of the pH, temperature, and humidity, which are of paramount importance for good embryo development.

The use of a system consisting of a camera, microscope, incubator, and a computer provides a situation in which embryos can be continuously monitored during their cultivation without disturbing the optimal conditions. Depending on the system used, embryos are photographed every 5 to 20 minutes. Such an approach represents the basis for a continuous monitoring of embryos, the so-called time-lapse monitoring.

Time-lapse monitoring system provides digital images of the embryo in the given time intervals. The obtained digital images are processed using a special software, resulting in time-lapse sequences of embryo development. Besides, time-lapse systems can also provide computer-assisted evaluation of morphokinetic parameters, thus enabling a semi-quantitative evaluation of embryo quality [10]. In this way, by applying appropriate software algorithms, it is possible to achieve theoretically most precise embryo selection, whose most important advantages are that they are non-invasive and non-subjective [11].

The time-lapse monitoring shows the time dimension changes as a variable, which is very important for assessment of the embryo quality and selection of the best ones for transfer. In the classical approach, the time dimension is considered as a discretionary variable, whereas in the time-lapse monitoring time dimension is transformed into a continuous variable, which corresponds more to the reality.

The time-lapse monitoring system provides about 1000 images of the embryo during the five-day development in the incubator, whereas classical monitoring gives evaluation of the embryo development only in 2 to 4 static moments. The use of time-lapse monitoring allows a detailed insight into division of the embryo cells and other morphological embryo events, such as the beginning of compaction and formation of the blastocyst cavity, along with some other phenomena, like disappearance of multinuclearity and fragmentations [12].

The above facts clearly show the advantages of the time-lapse monitoring over the conventional method of embryo observation. Comparative characteristics of the conventional and time-lapse monitoring are summarized in **Table 1**.

Generally, there are two types of systems for continuous embryo monitoring: 1. the existing incubator is additionally equipped with a microscope and a camera (Eeva system; PrimoVision system), and 2. an integrated incubator system with microscope + camera (EmbryoScope). Both system types use digital inverted microscope which takes images of the embryos in predefined time intervals, which allows creation of a video record of the events in the period of 72 or 120 hours. PrimoVision and Eeva systems are incorporated into the existing incubators, while EmbryoScope is a compact incubator with the built-in microscope/camera system. All three systems use different light sources. Also, they differ in the way the embryos enter the imaging field. EmbryoScope and PrimoVision use bright field technology, whereas Eeva system uses dark field technology. Bright field technology allows evaluation of the kinetic parameters of the embryo growth and development, whereas dark field provides an excellent evaluation of the kinetics and a weaker evaluation of the embryo morphology. The systems also differ in the way of

embryo cultivation. PrimoVision and Eeva culture dishes are suited for group cultivation, whereas EmbryoScope uses individual cultivation (EmbryoSlide). Group cultivation in the Eeva system uses special dishes for 12 embryos and in the PrimoVision system the dishes have space for 9 to 16 embryos sharing 50 - 120 μ l of the medium. According to some authors, group cultivation is favorable, since it has demonstrated that it improves embryo development. Individual cultivation in the EmbryoScope system is performed in dishes with a space for 12 embryos, each of which contains 20 - 25 μ l of medium.

Technical and clinical characteristics of the particular time-lapse monitoring systems are presented in **Table 2** and **Table 3**.

Evaluation programs based on time-lapse monitoring

The main point in using time-lapse monitoring in IVF is objective evaluation of embryo quality. The limiting factors of automatic embryo development monitoring are associated with image quality, morphological differences in different stages of embryonic development, position and transparency of the embryo. Special bioinformatic programs have been created for automatic analysis of images obtained by time-lapse monitoring which allow quantitative evaluation of the key moments in the development of the embryo and its morphology. Initially, it was recommended that each IVF institution should design its own algorithm based on the locally obtained data by time-lapse monitoring. Still, such an approach did not appear acceptable, since the number of institutions had a small number of patients to provide valid evaluation algorithms [13].

PrimoVision software has been developed specifically for PrimoVision time-lapse monitoring. It

Table 1. Comparison of conventional and time-lapse monitoring of embryo development in IVF

Tabela 1. Poređenje konvencionalnog monitoringa i kontinuiranog video-monitoringa embriona tokom postupka vantelesne oplodnje

Conventional monitoring/ <i>Konvenconalni monitoring</i>	Time-lapse monitoring/ <i>Kontinuirani video-monitoring</i>
The embryologist removes the embryos from the incubator at defined time intervals and examines them under a microscope/ <i>Embriolog u određenim vremenskim intervalima vadi embrione iz inkubatora i stavlja ih pod mikroskop radi procene</i>	The microscope/camera system takes images of embryos continuously at predefined time intervals <i>Sistem mikroskop-kamera kontinuirano snima embrione</i>
After completing the observation the embryos are returned to the incubator <i>Nakon završene procene vraća ih u inkubator</i>	The microscope/camera system is placed in the incubator or the integrated incubator/microscope/camera system is used/ <i>Sistem mikroskop-kamera se postavlja u inkubator ili se koristi jedinstveni sistem inkubator-mikroskop-kamera</i>
Every manipulation changes parameters in the incubator <i>Manipulacija inkubatorom menja parametre u inkubatoru</i>	Predefined parameters are constantly maintained in the incubator <i>Konstantno održavanje zadatih parametara u inkubatoru</i>
Disturbance of the optimal environment for embryo development <i>Narušavanje optimalnog okruženja za razvoj embriona</i>	Stable environment for embryo development <i>Stabilno okruženje za razvoj embriona</i>
Subjective evaluation of embryo development <i>Subjektivna procena razvoja embriona</i>	Objective evaluation of embryo development <i>Objektivnija procena razvoja embriona</i>

Table 2. Technical characteristics of the particular time-lapse monitoring systems**Tabela 2.** Tehničke karakteristike pojedinih sistema kontinuiranog video-monitoringa embriona

Technical characteristic <i>Tehničke karakteristike</i>	EmbryoScope <i>EmbryoScope</i>	PrimoVision <i>PrimoVision</i>	Eeva <i>Eeva</i>
Integrated incubator/ <i>Integrirani inkubator</i>	Yes/Da	No/Ne	No/Ne
Optics/ <i>Optika</i>	Bright field/ <i>Svetlo polje</i>	Bright field/ <i>Svetlo polje</i>	Dark field/ <i>Tamno polje</i>
Image frequency/ <i>Frekvencija slika</i>	10 min/ <i>10 minuta</i>	10 min/ <i>10 minuta</i>	5 min/ <i>5 minuta</i>
Focal plane/ <i>Žižna ravan</i>	7	1	1
Patient capacity/ <i>Kapacitet pacijenata</i>	6/system/ <i>6/sistem</i>	1/camera/ <i>1/kamera</i>	1/camera/ <i>1/kamera</i>
Number of embryos/patients <i>Broj embriona/pacijenata</i>	12 individual cultures <i>12 individualnih kultura</i>	16 group cultures <i>16 grupnih kultura</i>	12 group cultures <i>12 grupnih kultura</i>

gives insight into embryonic events and enables distant monitoring and consultations among fertility specialists and embryologists. Smart tool helps to measure blastomere fragmentation and symmetry. It possesses predefined work profiles which allow easy and fast acquisition of data about the kinetics of the embryo development and its morphology, offering different ways of comparison of particular embryos, which facilitates taking the final decision regarding their future – transfer, cryopreservation or rejection [14].

The Eeva system developed the EevaTest software which, based on embryo development, categorizes embryos into two categories of their potential: high and low.

EmbryoScope, using the EmbryoViewer software, enables formation of one or more models in concordance with specific clinical criteria that compare the monitored embryos using the Compare & Select program.

The software used in the EmbryoScope system gives the so-called KIDScore D3 for embryos on day 3 and KIDScore D5 for embryos on day 5. This is a morphokinetic score for each embryo, from 1 to 5, and in this way it measures the relative evaluation potential for implantation of each particular embryo. The tested model was validated through clinical practice [15].

Implementation and limitations of time-lapse embryo monitoring

The majority of authorities in the field of IVF consider time-lapse monitoring as a great advancement in the IVF technology, leading obviously to a higher success rate. Despite of this, there are opponents to this method [16]. Still, most of the arguments are on the side of those who support the use of time-lapse monitoring, since this technology offers more than the mere algorithms for kinetics of embryo development. The application of time-lapse monitoring can also detect numerous morphological events which cannot be noticed by conventional evaluation. Besides, it allows obtaining valuable data without taking embryos out of the stable environment of incubator and does not disturb their physiological development. This technology offers the possibility of selection of the best embryo for transfer since it gives an insight into its morphological and morpho-kinetic parameters.

The application of time-lapse monitoring results in:

1. Lower rate of early pregnancy losses
2. Higher implantation rate
3. Shorter time leading to IVF success, measured by live births.

Despite all of the advantages offered by time-lapse monitoring, it also has some shortcomings, such as the impossibility of embryo rotation. This prevents the possibility of observing all the em-

Table 3. Clinical characteristics of the particular time-lapse monitoring systems**Tabela 3.** Kliničke karakteristike pojedinih sistema kontinuiranog video-monitoringa embriona

Clinical characteristic <i>Kliničke karakteristike</i>	EmbryoScope <i>EmbryoScope</i>	PrimoVision <i>PrimoVision</i>	Eeva <i>Eeva</i>
Automatic diagnostics/ <i>Automatska dijagnostika</i>	No/Ne	No/Ne	Yes/Da
Need for operator/ <i>Potreba za operaterom</i>	Yes/Da	Yes/Da	No/Ne
Time needed for analysis <i>Vreme potrebno za analizu</i>	Yes/Da	Yes/Da	Automatic analysis <i>Automatska analiza</i>
Algorithm selection <i>Algoritam selekcije</i>	Defined by user <i>Korisnik definiše</i>	Defined by user <i>Korisnik definiše</i>	Yes/Da
Prediction of obtaining blastocyte on day 3 <i>Predikcija dobijanja blastociste na nivou 3. dana</i>	No/Ne	No/Ne	Yes/Da
Prediction of blastocyte implementation on day 3 <i>Predikcija implantacije blastociste na nivou 3. dana</i>	Yes/Da	No/Ne	No/Ne

bryo's tridimensional aspects, as they are visible only in one plane. Besides, the exposure of the embryos to continuous microscopic observations is also undesirable. Namely, depending on the type of time-lapse monitoring system, the embryos are (by mechanical movement) placed under the microscope at a predefined frequency (EmbryoScope system) or are cultivated in the dishes with common medium, so that rotational movements of the embryo dishes are avoided (systems PrimoVision and Eeva). Also, one should not forget the possible negative effects of light during the monitoring [17–19].

Conclusion

The time-lapse embryo monitoring systems represent powerful tools which help clinicians involved in "in vitro" fertilization and embryologists to select the best embryos, with the aim of enhancing the success rate of in vitro fertilization. Despite of all the advantages, these systems also have some shortcomings and limitations. It is expected that further technological development will certainly contribute to a more direct communication between the human life in its very conception and clinicians engaged in its coming into being.

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