THE EFFECT OF FRAME-BY-FRAME MONITORING OF EMBRYOS ON OBSTETRIC AND PERINATAL OUTCOMES IN SINGLETON PREGNANCY IN ASSISTED REPRODUCTIVE TECHNOLOGY PROGRAMS

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Abstract*. The process of morphological examination of embryos is one of the most important selection methods, according to the results of which an assessment is made of a whole group of indicators, such as the number of blastomeres, the proportion of fragmentation, the severity of compactification, 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. An effective method of embryo selection is currently in high demand in this field, since it is a method of selecting embryos with the highest implantation potential. When analyzing many retrospective and prospective studies, the advantage and absence of differences in comparison with the traditional morphological assessment of embryo quality are emphasized, Almost all publications devoted to time-lapse microscopy focused on determining the time of specific events of embryo division and subsequent use of this information to create algorithms to help select an embryo for transfer, but there is insufficient data on obstetric and perinatal outcomes.*

Keywords*: assisted reproductive technologies, infertility, elective blastocyst transfer, time-lapse microscopy.*

The process of morphological study of embryos is one of the most important selection methods, the results of which are used to 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. An effective method of embryo selection is currently in high demand in the field, as it is a way to select embryos that have the highest potential for implantation. The method of continuous video surveillance allows the specialist to obtain a long and detailed chronicle of the development process of each individual embryo. During 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. In this regard, the introduction of time-lapse technology has allowed embryologists to equip themselves with an effective tool for the selection of promising embryos.

The purpose of the study is to reassess the safety of noninvasive monitoring by evaluating obstetric and perinatal outcomes of pregnancies obtained from embryos conceived in a time-lapse incubator compared with standard incubators.

Research methods. The study included 760 patients, which served as the platform for this study. Obstetric and perinatal data on newborns conceived using TDM (study group) or without

TDM (control group) were collected during a randomized controlled trial conducted at JSC Medical Company IDK (Samara, Russia) from 2016 to 2021.

In the present study, data related to labor and obstetric perinatal aspects were obtained from medical records and reports sent by referral centers, as well as from telephone or email interviews with patients. The first and main source of information was the patient. If the patient mentioned any abnormalities during pregnancy, childbirth, or the newborn, we increased the frequency of interactions to obtain accurate information and request copies of medical reports. Inclusion criteria for the study: IVF program and ICSI program; cycles using your own oocytes; embryo transfer on the fifth day of cultivation; endometrial thickness 8 mm or more on the day of embryo transfer. Exclusion criteria: cycles using donor oocytes; cycles with transfer of a thawed embryo; transfer of embryos on the third day of cultivation; embryo transfer on the third day; endometrial thickness less than 8 mm on the day of embryo transfer.

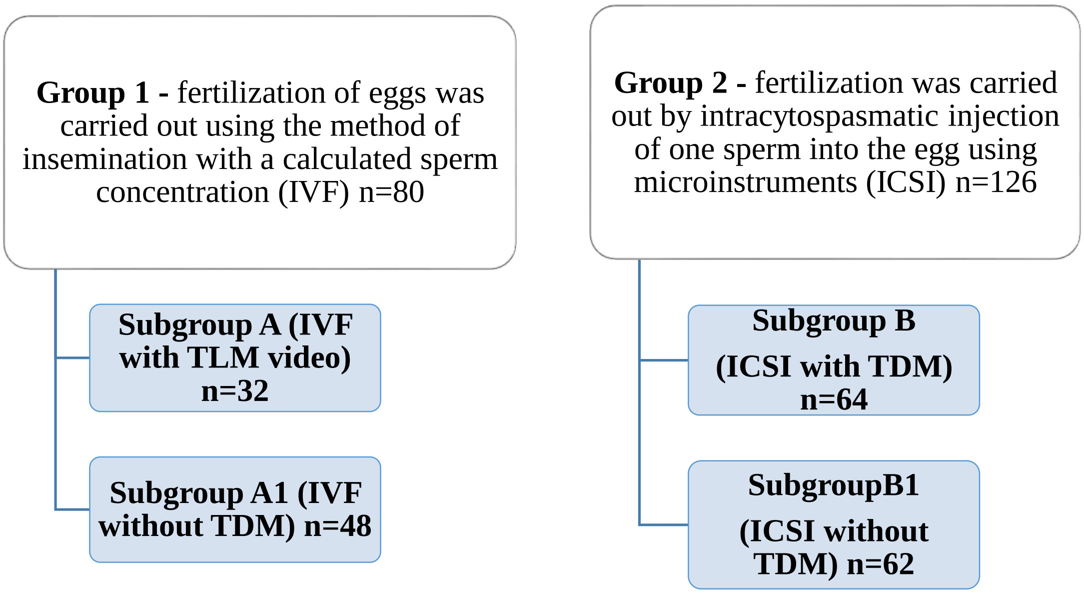

Figure 1. Study design

2 groups of patients were identified, whose embryos and their development indicators were analyzed: group 1 - IVF (in which fertilization of eggs was carried out by the method of insemination with a calculated concentration of sperm) and group 2 - ICSI (fertilization was carried out by the method of intracytoplasmic injection of one sperm into the egg using micro instruments).

Each of the groups was divided into subgroups, which differed in that the embryos were cultured and selected for transfer either using a standard technique or a video surveillance system was used (Fig. 1). The parameters characterizing the development of embryos were studied in patients of each group studied. The work used human embryos, 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 on the Protection of Human Rights and the Dignity of Human Beings Using Achievements of Biology and Medicine, 1997]. Permission to use embryos in the study was obtained from the Bioethics Committee at Samara State Medical University (extract from protocol No. 116 dated October 3, 2018).

Research results

The share of the IVF program was: 33.3% (subgroup A) and 43.6% (subgroup A1), the share of the ICSI program was 6.6% (subgroup B) and 56.3% (subgroup B1), respectively (p > 0, 05). The average age of women in subgroups A and B and in groups A1 and B1 was 31.70 ± 0.24 and 31.82 ± 0.20 years, respectively ($p > 0.05$). In both groups, the minimum age of patients was 23 years, the maximum age was 45 years.

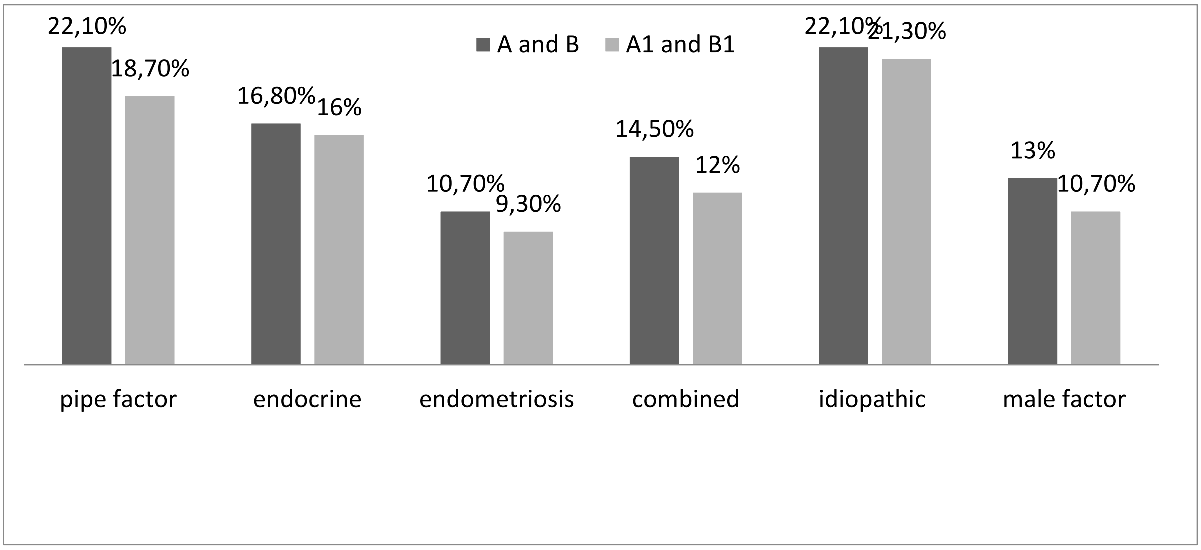

Figure 2. Structure of infertility in the studied groups

In both groups, the majority of women were under 35 years of age – 73.8% of patients in the main group and 70.9% of patients in the control group ($p > 0.05$). In subgroups A, B -c, the use of video monitoring and the proportion of women with primary infertility was 61.8%, in subgroups A1, B1 - 53.3%, secondary infertility was in 38.2% and 44% of women, respectively ($p > 0.05$).

The most common cause of infertility in the subgroups with and without TDM was tubal factor (22.10% and 18.7%, respectively) ($p > 0.05$) and idiopathic factor (22.10% and 21.30%, respectively) ($p > 0.05$). There were also no statistically significant differences between the subgroups in terms of the duration of infertility (subgroup A - 5.56 ± 1.01 years and in subgroup A1 - 5.43 ± 0.17, in subgroups B and B1 - 6.66 ± 1.34 years and 6 .4±1.38) respectively ($p > 0.05$). Thus, there were no significant differences in the main characteristics of the patients, including both age aspects and the structure of infertility. The parameters characterizing the development of embryos were studied in patients of each group studied. In group 1 of patients with the IVF program, data obtained both from video monitoring of embryo development (subgroup A) and standard methods (subgroup A1) were compared, the results of which are shown in Table 1.

In a comparative analysis of the data, the average values of the following indicators were studied: fertilization (%), cleavage (%), growth to blastocyst (%), freezing (%), average number of embryo transfers, hCG (+) (%), positive ultrasound results (%), CHI (%) and multiple birth rate. As follows from the comparative data in the table above, in the IVF group the indicators do not have a significant difference. Thus, no statistically significant differences were found between fertilization rates (%) and they were 74.1±13.9% and 76.7±14.3% in groups A and A1, respectively. A minimal difference was revealed between the crushing indicators (%): in subgroup A this parameter was higher - 97.3±3.2%. A similar picture was established when comparing the rate of growth to blastocyst (%), its average values in the A1 subgroup relative to the subgroup with video surveillance in the A subgroup were higher - 22.3 ± 5.7%. The average freezing index (%) between the studied subgroups also had statistically significant differences: in subgroup A1 they were lower than the results obtained during video control - subgroup Ai amounted to 32.1 ± 5.8%. The average number of embryos per transfer in subgroups A and A1 was 1.03±0.18 and 1.15±0.36, respectively. In this case, the average number of attempts was 1.72±0.46 and 2.23±0.47, respectively.

It should be noted that hCG and ultrasound indicators in subgroup A have minimal differences, which indicates the high quality of embryos that are cultured and selected for transfer using video surveillance technology. The rate of multiple pregnancy in subgroup A1 is almost 2 times higher than in subgroup A with video surveillance. This also confirms the sufficient level of biological competencies of the developing embryos.

Table 1

Comparative characteristics of development indicators of embryos obtained in the IVF program

	IVF with video n=32	IVF without video n=48	IVF General n=80
Average age of patients	32,6±2,8	33,4±3,4	33,1±3,2
Average number of attempts	1,72±0,46	2,23±0,47***	2,03±0,53
Average number of years of infertility	5,56±1,01	5,63±1,16	5,6±1,1
Average dose of FGS per stimulation	1457,1±269,7	1573,8±328,4	1527,1±309,8
Wed. number of obtained oocytes MII	6,5±1,48	5,69±1,19**	6,01±1,36
Wed embr. transfer	1,03±0,18	1,15±0,36*	1,1±0,3
Multiple pregnancy rate	4.77	8,22***	6,15

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

table 2

Comparative characteristics of development indicators of embryos obtained in the ICSI program

	ICSI with video n=64	ICSI without video n=62	ICSI Total n=126
Average age of patients	34,6±7^	34,9±7,2	34,8±7,1^
Average number of attempts	2,2±0,6^^^	2±0,44*^^	2,1±0,53
Average number of years of infertility	6,66±1,34^^^	6,4±1,38^^^	6,53±1,36^^^
Average dose of FGS per stimulation	1503,2±264,7	1645,9±361,9**	1573,4±323,1

Wed. number of oocytes MII	6,33±1,16	5,31±0,97***	5,83±1,18
avg.embr.transfer	1,28±0,45^^	1,37±0,49^^	1,33±0,47^^^
Multiple pregnancy rate	0±0^^^	20±3,77***^^^	9,84±10,38

Note: *-p<0.05, **-p<0.01, ***-p<0.001 statistical significance in relation to the ICSI group with video. ^-p<0.05, ^^-p<0.01, ^^^-p<0.001 statistical significance in relation to the corresponding indicators of groups with IVF

When comparing the data in the studied subgroups ICSI-B, B1, we see that the indicators do not have a significant difference. There were no statistically significant differences between fertilization rates (%) and they were 71.5 ± 16.5% and 74.4 ± 13.8% in subgroups B and B1, respectively. A minimal difference was detected between the crushing indicators (%) and in the B1 subgroup the indicator was higher - 98.2±6.5%. A similar picture was established when comparing the rate of growth to the blastocyst (%), the average indicators of the B1 subgroup were higher - 24.3 ± 4.9% (Table 2).

The mean freezing index values (%) also had statistically significant differences. The average number of embryos per transfer in subgroups B and B1 was 1.28±0.45 and 1.37±0.49, respectively. In this case, the average number of attempts was 2.2±0.6 and 2±0.44, respectively.
It should be noted that the difference between hCG and ultrasound indices in subgroup B is smaller. This indicates the high potential of embryos for implantation, which were selected using video surveillance technology. The lack of difference between CNB and CI indicates that all embryos that gave birth to pregnancy were implanted.

Moreover, the average number of embryos per transfer in this group is slightly lower than in subgroup B1, i.e. on average, fewer embryos were needed to achieve pregnancy.

Noteworthy is the extremely high rate of multiple pregnancy in subgroup B1 (ICSI without video). This is a risk group, since obstetric risks and the risks of having premature and low birth weight babies in this group are extremely high. It is necessary to pay attention to this group and carry out a stricter selection of embryos for transfer, while simultaneously reducing the number of transferred embryos.

There are a number of works in the scientific literature [3; 4], in which a higher proportion of cases of excellent quality embryo transfers was found in the group using video technology.

In most publications [12; 14] considered the assessment of 10-12 morphodynamic indicators of embryo division, which makes it possible to demonstrate the advantage of time-lapse technology. We can definitely state that this method of assessing embryos has no negative impact on their development, since all studies revealed fairly high results in the level of pregnancy development.

Thus, we can conclude that continuous video monitoring of embryos in stable cultivation conditions is more informative in terms of assessing their development in comparison with static observations. This makes it possible to increase the efficiency of identifying embryos that have the highest potential for implantation.

The results obtained allow us to conclude that the method of continuous cultivation of embryos with video monitoring of their development reduces the negative impact of external factors and increases the proportion of high-quality embryos. The ability to obtain images of embryos at different stages of their development allows the use of additional computer technologies to rank embryos, which improves the quality of their selection.

Currently, the system for autodetection of the morphodynamic profile of human embryos in vitro is being tested and additional data is being marked. Supplementing morphodynamic profiles with the results of pregnancy transfers and outcomes will make it possible to create data sets for training a decision support system. Currently, there is intensive development of non-invasive time-lapse technology at the intersection of medicine and information technology. The accumulated factual material will make it possible to create algorithms for training artificial intelligence and its use for selecting the most competent embryo for implantation. This will allow us to achieve higher rates of pregnancy and childbirth. Data corresponding to pregnancy, birth and newborn outcomes are shown in Table 3. There were no differences in pregnancy and birth outcomes between the two subgroups A:B and A1:B1. . There were no significant differences in birth defects, only one minor malformation, and one perinatal death in the non-TDM group. Neonatal data were not available in two cases (TDM) and four cases (no TDM). When pregnancies were analyzed, there were no differences between the two groups in the incidence of obstetric problems, including bleeding in the second and third trimester 4 (3.6%) TDM versus 5 (5.3%) in the control group and pregnancy-induced hypertension 6 (5, 4%) in the main group versus 5 (5.3%) in the control group (Table 2). Early and late miscarriages were common in the A subgroup (0.9%) versus 3.2% in the A1 subgroup. Delivery at term increases almost 2 times in subgroup A (39.3% versus 24.5%). Multiple pregnancy is higher in subgroup A1 (8.5% versus 3.6%).

There were no statistical differences in neonatal outcomes, Apgar score at 5 minutes was 9.5 (95% CI 9.2–9.9) (TLS) vs 9.4 (95% CI 9.2–9.7) (SI) . Minor malformations were found in one newborn in group A1 (1.1%), antenatal fetal death in 2 cases in subgroup A.

Table 3.

Obstetric and perinatal outcomes in groups with and without video monitoring.

	A and B subgroups n=112	A1 and B1 subgroups n=94	Fisher's exact test
Outcome of birth:			
No information	8 (7,1%)	6 (6,4%)	0,214
No pregnancy	50 (44,6%)	51 (54,3%)	0,0436
Early and late miscarriages	1 (0,9%)	4 (4,3%)	0,116
Late miscarriages	1 (0,9%)	3 (3,2%)	0,206
Premature birth (<37 weeks)	8 (7,1%)	7 (7,4%)	0,2101
Delivery on time	44 (39,3%)	23 (24,5%)	0,0093
All births	52 (46,4%)	30 (31,9%)	0,0122
Obstetric complications:			
multiple births:	4 (3,6%)	8 (8,5%)	0,0786
bleeding in the 2nd and 3rd trimesters	4 (3,6%)	5 (5,3%)	0,2211
Pregnancy-induced hypertension	6 (5,4%)	5 (5,3%)	0,2426
C-section	21 (18,8%)	18 (19,1%)	0,141

Perinatal outcomes			
Male newborns	32 (28,6%)	13 (13,8%)	0,0051
Female newborns	22 (19,6%)	17 (18,1%)	0,1364
Birth weight over 2800	43 (39,3%)	20 (0%)	0
Low birth weight (<2500 g)	9 (8%)	10 (0%)	0,0036
Apgar score at 5 min	9.5 (9.2–9.9)	9.4 (9.2–9.7)	
Developmental defects	0 (0%)	1 (1,1%)	0,4563
Antenatal mortality	2 (2,1%)	0 (0%)	0,207
Intrapartum mortality	0 (0%)	0 (0%)	1
Postnatal mortality	1 (0,9%)	0 (0%)	0,5437

To avoid bias and accurately study the effect of TDM on obstetric and perinatal outcomes, we analyzed two in the studied subgroups and we did not find significant differences between the two subgroups. The incidence of pregnancy-induced hypertension was similar in both groups, and the incidence of other pregnancy disorders was comparable, including bleeding in the second and third trimesters. Gestational age at delivery and the incidence of preterm birth were similar in the TDM and non-TDM groups.

We found no difference in the incidence of congenital malformations between the TDM and non-TDM groups, and the incidence of perinatal mortality was very low in both groups. In summary, among pregnancies achieved with TDM, no clinically significant increase in obstetric or perinatal risks was found, suggesting that this technology does not have any deleterious effects on embryo development. Our study, however, has some limitations. Our findings are based on retrospective data that were obtained in part through medical questionnaires and telephone calls, as this was the only way to collect information on the entire cohort of births from pregnancies with and without TDM of which we were notified. Early pregnancy loss was significantly lower in the TDM group.

In summary, among pregnancies achieved with TDM, no clinically significant increase in obstetric or perinatal risks was found, suggesting that this technology does not have any deleterious effects on embryo development. Our data on higher quality embryo transfer using time-lapse microscopy coincide with the data of other researchers [5, 9]. The lack of differences between the two groups in clinical characteristics (age, infertility factors, duration of infertility) suggests that a large proportion of cycles with transfer of excellent quality embryos in the main group may be due to the lack of influence of environmental factors during cultivation in a time-lapse microscope. TLM technology significantly reduces manual contact with culture dishes and embryos, leaving embryos in optimal growth conditions [10]. A large number of studies over the past 20 years have established a strong connection between the quality of the embryos transferred and the likelihood of pregnancy. The higher the morphological assessment of the quality of the embryo, the higher the likelihood of clinical pregnancy [16, 17,]. According to the internal instructions approved by JSC Medical Company IDK, the woman’s age, the quality of the embryos and the serial number of the IVF and ICSI program are the criteria for choosing the number of transferred embryos. In patients of late reproductive age, in the absence of pregnancy in previous IVF and ICSI programs and/or when receiving embryos of satisfactory quality, transfer of two embryos is usually discussed to increase the likelihood of pregnancy. However, our study also included patients with an unfavorable prognosis for pregnancy and childbirth. The higher proportion of excellent quality embryos transferred, combined with a higher proportion of cycles with embryo cryopreservation, suggests that there is a positive trend towards improved embryo quality using TLM. In our work, we did not analyze the cumulative indicator of clinical pregnancy (the occurrence of pregnancy per one cycle of stimulation - after the transfer of a "fresh" embryo and, in the absence of pregnancy, the subsequent transfer of a thawed embryo). However, a large proportion of cycles with cryopreservation of embryos in the main group may increase this indicator in the future. It is now quite difficult to predict future achievements of time-lapse microscopy, but there is no doubt that this technology will continue to be used and developed [10].

Conclusion. The study did not reveal statistically significant differences in clinical pregnancy rates, delivery rates, and early pregnancy loss rates between the TLM group and the traditional embryo culture group. In the nonelective single embryo transfer group, TLM had a 10% higher clinical pregnancy rate compared with the control group ($p = 0.03$). The birth rate did not differ between the TLM group and the conventional culture group depending on the type of embryo transfer.

Thus, our studies indicate that the use of time-lapse microscopy can increase the effectiveness of IVF and ICSI programs. Continuous monitoring at short intervals provides more information about embryo development than standard daily assessment. These advantages will allow the clinician to recommend the transfer of a single embryo to a married couple, without significantly reducing the chance of pregnancy and childbirth, but eliminating the risk of multiple pregnancies.

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