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EFFICACY OF USING CULTURE MEDIA IN ASSISTED REPRODUCTIVE TECHNOLOGY PROGRAMS

One of the steps to successfully solve the problem of human infertility is the development and improvement of culture media used for the cultivation of embryos in ART programs. In the past, significant progress has been made in this direction – single-step and two-step culture media have been developed. Both media are widely used in practice to improve the efficiency of individual ART results. The aim of this work was to determine the effectiveness in usage of commercial culture media (Continuous Single Culture Irvine and Origio Sequential Cleav/Blast) in IVF and ICSI programs. A comparative analysis of their impact on embryo quality, fertilization and pregnancy outcomes in IVF and ICSI programs at the Embryology Laboratory of LLP "Centre ECO" in 2017-2021 was conducted. 2650 programs took place, of which 760 programs accounted for IVF and 1890 programs for ICSI. The results of the analysis revealed no significant differences in the use of the above-mentioned media to obtain good quality blastocysts in the IVF program. The usage of the studied culture media showed a higher level of obtaining blastocysts of optimal quality in the ICSI programs when cultured in single-step medium compared to sequential medium. The difference between the two relative values was statistically significant (t = 8.08; p0.001). Comparative analysis of the results of embryo cultivation in different nutrient media allow us to conclude that both single-step and sequential culture media, equally contribute to the quality development of blastocysts and demonstrate positive clinical results. Program efficacy based on the factor of ART program ending in pregnancy using CSC Irvine nutrient media was 46.44% for IVF, and 41.42% for ICSI. Culturing embryos in Origio Sequential Cleav/Blast medium in IVF programs showed pregnancy outcomes at 49.51%, in ICSI programs – 42.85%.

Key words: infertility, embryo, culture/nutrient medium, IVF, ICSI.

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Көмекші репродуктивті технологиялар бағдарламаларында қоректік орталарды қолданудың тиімділігі

Ерлі-зайыптылардың бедеулігінің мәселесін шешудегі табысты кезеңдердің бірі болып көмекші репродуктивті технологиялар (КРТ) бағдарламалары аясында эмбриондарды культивирлеуде пайдаланылатын қоректік орталарды әзірлеу және жетілдіру болып табылады. Соңғы онжылдықтарда бұл бағытта айтарлықтай прогресс орын алды – бір сатылы және екі сатылы қоректік орталар әзірленді. Сәтті өткен жеке КРТ нәтижелерінің деңгейін арттыру үшін тәжірибеде екі қоректік орта да қолданылады. Бұл жұмыстың мақсаты IVF және ICSI бағдарламаларында коммерциялық қоректік орталарды (бір сатылы орта Continuous Single Culture Irvine және екі сатылы орта Origio Sequential Cleav/Blast) қолданудың тиімділігін анықтау болды. ЖШС "ЭКО ОРТАЛЫҒЫ" эмбриология зертханасының базасы аясында 2017-2021 жылдар аралығындағы IVF және ICSI бағдарламаларында бұл қоректік орталардың эмбриондардың сапасына, ұрықтандыру нәтижелеріне, жүктіліктің басталуына әсері жайлы салыстырмалы талдау жүргізілді. Барлығы 2650 бағдарлама жүргізілді, олардың ішінде 760 бағдарлама IVF-тың үлесі, ал 1890 бағдарламасы ICSI-дің үлесі болды. Талдау нәтижелері IVF бағдарламасында жақсы сапалы бластоцисталарды алу үшін аталған қоректік орталарды қолдануы статистикалық маңызды айырмашылықтарды көрсеткен жоқ. ICSI бағдарламасында зерттелген қоректік орталарды қолдануы оңтайлы сапалы эмбриондарды алуда бір сатылы ортаны қолдану екі сатылы ортаны қолдануға қарағанда айтарлықтай жоғары деңгейді көрсетті. Осы екі салыстырмалы шаманың айырмашылығы статистикалық маңызды болды (t = 8,14; p < 0,001). Эмбриондарды

қоректік орталарды өсірудің нәтижелерін салыстырмалы талдауда келесі қорытынды жасауға мүмкіндік береді: бір сатылы CSC Irvine және екі сатылы Origio Sequential Cleav/Blast қоректік орталар бластоцисталардың сапалы дамуына бірдей деңгейде әсер етті және оң клиникалық нәтижелер көрсетті. Жүктіліктің басталуы критерийі бойынша IVF жүргізгенде CSC Irvine қоректік ортасын қолданған бағдарламалардың нәтижелілігі 46,44%, ал ICSI жүргізгенде 41,42% құрады. Жүктіліктің басталуы критерийі бойынша эмбриондарды Origio Sequential Cleav/Blast қоректік ортасында өсіргенде IVF бағдарламасының нәтижелілігі 49,51%, ал ICSI бағдарламалардың нәтижелілігі 42,85% құрады.

Түйін сөздер: бедеулік, бластоцист, қоректік орта, IVF, ICSI.

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Результативность использования культуральных сред в программах вспомогательных репродуктивных технологий

Одним из этапов успешного решения проблемы бесплодия супружеских пар является разработка и совершенствование питательных сред, применяемых для культивирования эмбрионов в программах ВРТ. За последние десятилетия был достигнут значительный прогресс в этом направлении – были разработаны одноступенчатые и двуступенчатые питательные среды. Для повышения уровня успешности индивидуальных результатов ВРТ на практике широко используются обе среды. Целью данной работы явилось определение результативности использования коммерческих культуральных сред (одноступенчатая среда Continuous Single Culture Irvine и последовательная среда Origio Sequential Cleav/Blast) в программах ЭКО и ИКСИ. Проведен сравнительный анализ их влияния на качество эмбрионов, результаты оплодотворения и наступления беременности в программах ЭКО и ИКСИ на базе лаборатории эмбриологии ТОО «Центр ЭКО» за период с 2017 по 2021 год. Всего было проведено 2650 программ, из которых 760 программ приходятся на долю ЭКО, а 1890 программ – на долю ИКСИ. Результаты анализа не выявили статистически значимых различий при использовании указанных питательных сред для получения бластоцист хорошего качества в программе ЭКО. Использование изучаемых культурульных сред в программе ИКСИ показала более высокий уровень получения бластоцист оптимального качества при культивировании в одноступенчатой среде по сравнению с культивированием в двуступенчатой среде. Разность двух относительных величин статистически достоверна ($t = 8,14$; $p < 0,001$). Сравнительный анализ результатов культивирования эмбрионов в питательных средах: CSC Irvine и Origio Sequential Cleav/Blast позволяют сделать вывод о том, что обе питательные среды, как одноступенчатая так и последовательная в равной степени способствуют качественному развитию бластоцист и демонстрируют положительные клинические результаты. Результативность программ по критерию наступления беременности с использованием питательной среды CSC Irvine составила при проведении ЭКО 46,44%, при ИКСИ – 41,42%. Культивирование эмбрионов в питательной среде Origio Sequential Cleav/Blast в программах ЭКО показало результативность по критерию наступления беременности на уровне 49,51%, в программах ИКСИ – 42,85%.

Ключевые слова: бесплодие, бластоциста, питательная среда, ЭКО, ИКСИ.

Abbreviations

ART – assisted reproductive technologies; IVF – *in vitro* fertilization; ICSI – intracytoplasmic sperm injection; TVOR – transvaginal oocyte retrieval.

Introduction

In modern society, infertility is an urgent medical and social problem. According to WHO, the number of couples who face the problem of infertility ranges from 8 to 29%. In European countries, almost 10% of married couples are infertile, in America this value reaches 8-15%, in Canada – 17%, in Australia – 15.4% [1, 2]. The rate of infertile marriages in Russia appears at 17.5%, and in Kazakhstan – 15% and it does not tend to decrease [3, 4, 5].

Various female and male factors may be involved in the etiology of infertility, so it is extremely difficult to calculate the overall rate of infertility for the entire population. The team of authors of the Scientific Center for Obstetrics, Gynecology and Perinatology named after V.I. Kulakov has discovered that the rate of infertility caused by disorders of the female reproductive system is 42.6-65.3%, appearance of

infertility due to the fertility issues of both spouses made up 27.7-48.4%, but in some cases the cause of infertility remains unclear. In respect to these global trends, there remains a need to improve the health of the reproductive system and assisted reproductive technologies around the world, and in Kazakhstan in particular [6]. Regarding this, the urgent task of modern biological medicine is to improve the methods of ART [6, 7, 8].

The regulation of any ART program obligates to cultivate embryos in artificial conditions, which are as close to natural ones as possible, for the first 5-7 days after fertilization. The success of the cultivation stage depends primarily on the quality and quantity of eggs and semen samples. At the same time, the quality, pH, temperature, etc. of the media are of significant importance [9, 10]. Therefore, culture (nutrient) media are a main part of the cultivation system. They perform the crucial task of minimizing stress on gametes and embryos by creating an optimal *in vitro* environment, ensuring the correct balance of ions, energy substrates and other components [11, 12].

An important achievement in reproductive technology is the development of two-step nutrient media. Composition of two-step media is very similar. The embryo is cultured in the first-step medium until day 2 after fertilization, and then cultivated in the second-step medium from day 3 to day 5 of development. It was believed that this method of embryo cultivation finely imitated natural changing conditions of the environment [11, 13, 14].

Later, single-step culture media, which were suitable for both early and late stages of embryo development, were invented. The advantage of such nutrient media is that there is less manipulation involving embryos, thereby minimizing environmental stress and the possibility of damage [10, 11].

Currently, technologies of *in vitro* fertilization, culturing of embryos and embryo transfer are in wide demand worldwide. Scientists all over the world, together with IVF laboratories, have been experimenting with different conditions while cultivating human embryos, modifying culture media, setting incubators with the use of different methods of gas supply and pH control for many years. All of these aspects are very important in the selection of good quality embryos, their transfer and the further course of pregnancy, since the goal of every IVF center is to improve its efficiency [14, 15, 16].

The purpose of this work was to study the effectiveness of the use a single-step nutrient medium CSC Irvine and a two-step Origio Sequential Cleav/Blast medium in IVF and ICSI programs used in the laboratory of embryology LLP “Centre ECO”, Almaty.

Materials and methods

Embryos were cultured in Labotect (Germany) and ESCO Miri plate incubators up to 5-6 days using Continuous Single Culture media (Irvine Scientific, USA) and Origio Sequential Cleav/Blast (Origio, Denmark). Embryos in CSC Irvine medium were cultured from day 1 to day 5, without changing the medium. Embryos cultured in Origio Sequential Cleav/Blast medium needed a medium change on day 2 of development (stage 4-8 blastomeres). The evaluation of fertilization and cleavage was carried out on days 1, 3, 5 and 6 (in the case of the presence of embryos up to 6 days).

Features of normal fertilization (after 16-18 hours after fertilization) during the evaluation process include the presence of 2 pronuclei and 2 polar bodies. Normal fertilization was brought into account. Embryos with abnormal fertilization (3 pronuclei, no fertilization, etc.) were excluded from further cultivation [6].

Evaluation on the 3rd day after fertilization included the degree of embryo cleavage. During this step the number and morphology of blastomeres were assessed: equality, sphericity, as well as presence of fragmentation, multinucleation, and vacuoles. Non-developing embryos, as well as embryos with >50% fragmentation and multinucleation were excluded from further cultivation. In some cases, embryos were transferred on the 3rd day in agreement with the treating doctor [6].

On day 5 (after 115-117 hours after fertilization), the embryos completed the compactization processes and reached the blastocyst stage. *The Gardner et al and Scolarshifts* classification was used to evaluate the quality of blastocysts of the 5th day of development [6].

Obtaining human oocytes. An important step in the IVF program is to retrieve mature preovulatory oocytes capable of fertilization *in-vitro*. Stimulation of superovulation is performed with medications prescribed by the treating doctor. Then, oocytes are harvested by transvaginal puncture of follicles, after which the obtained oocytes are placed in an incubator for further fertilization. In most cases, about 9-10 oocytes are obtained per cycle, but the number of oocytes obtained may vary depending on the response to hormonal stimulation. In 90% of cases, the oocytes obtained this way will be mature.

In some cases, an oocyte can be retrieved during a so-called natural cycle where only 1-2 oocytes develop and may be obtained [17].

Obtaining and processing of human semen. Sperm in more than 90% of all cases is obtained from the patient's partner or from donor ejaculate. After collection, sperm is processed in the laboratory to preserve and isolate spermatozoa with high motility and normal morphology. Spermatozoa can also be extracted surgically from the seminal ducts, epididymis or testicles. In some cases spermatozoa are stored frozen until fertilization [17].

Fertilization of oocytes using the standard IVF method.

Fertilization by IVF is performed after 2-4 hours. After TVOR and includes several steps.

1. Preparation of embryological protocol, checking the anamnesis and data on previous IVF/ICSI programs.

2. Preparation of working place.

3. The processed sperm is placed in a thermoblock on the work surface. The name of the patient and her spouse tagged on the test tube, in the sperm preparation protocol and in the embryological protocol, are carefully checked by double check (with the help of colleagues).

4. Add the required concentration of spermatozoa to a four-well plate or central well fertilization dish prepared in advance (100,000 spermatozoa per medium volume up to 1 ml or 10,000 spermatozoa per oocyte). Evaluate the concentration of spermatozoa in the dish using an inverted microscope.

5. Then, using a roller dispenser, transfer the oocytes to the sperm wells, maximum 5 oocytes per well. Then place the plate or the dish with the central well in the incubator.

6. Fertilization data is recorded in the embryological protocol, indicating the time of fertilization procedure, number of fertilized oocytes and the number of the incubator in which the cells were placed.

7. Denudation and fertilization evaluation procedure is performed 16-18 hours after fertilization [6, 17].

Fertilization of human oocytes by ICSI.

The ICSI procedure is a modern, high-tech method that is performed under a microscope using glass microinstruments. As well as the IVF method, it is performed 2-4 hours after the TVOR.

1. Preparation of embryological protocol, checking the anamnesis and data on previous IVF/ICSI programs.

2. Preparation of working place.

3. The processed sperm is placed in a thermoblock on the work surface. The name of the patient and her spouse tagged on the test tube, in the sperm preparation protocol and in the embryological protocol, are carefully checked by double check (with the help of colleagues).

4. Oocyte preparation. The oocytes retrieved during the puncture are surrounded by a large number of cumulus cells, which are necessary for the development of oocytes inside the follicles. Before the ICSI procedure, the cumulus is carefully removed with the help of enzyme hyaluronidase/cumulase. Only after cumulus removal the maturity of the egg can be evaluated and the fertilization can be carried out.

5. For fertilization by ICSI, a single mature sperm is selected based on its motility and morphological structure. In the case of appearance of only a single motile sperm after all the manipulations (processing of spermatozoa), other sperm preparation methods are used.

6. Preparation of the selected sperm. The spermatozoon is immobilized by cutting off the tail with a needle.

7. The immobilized sperm is aspirated into a glass needle. The oocyte is fixed in the desired position with a microsuction cup (holding), then a micro-needle with a sperm contained in it is pierced through the oocyte shell, and the contents of the egg are partially aspirated. The sperm is then carefully implanted into the cytoplasm of the oocyte.

8. The fertilized oocyte is placed in a special medium for further cultivation [6, 17].

Results

Obtaining mature oocytes at the MII stage capable of fertilization *in vitro* is an important aspect in ART programs. There are various classifications describing the structural features of female gametes. An accurate evaluation of oocyte quality and degree of maturity can be established only after removal of the oocyte-cumulus complex when performing fertilization by ICSI. In case of standard IVF fertilization such an assessment is not performed. The quality and maturity of the oocytes in the standard IVF program can only be evaluated the next day during the evaluation of fertilization [18].

This study presents the results of an analysis of embryo culturing in IVF and ICSI programs, using single-step and sequential media from 2017 to 2021. During this time, a total of 2650 ART programs were conducted at the laboratory of embryology.

The average age of women taking part in research is 33,2±1,8 years and that of men is 34,4±1,6 years. Human embryos were evaluated on days 1, 3, and 5 after fertilization. General characteristics of human oocytes and blastocysts obtained in the 2017-2021 IVF/ICSI programs are shown in Tables 1, 2, 3.

Table 1 – Characteristics of oocytes and fertilization results in IVF programs

Material characteristics	IVF	
	n	%
Total of immature oocytes (GV, MI)	356	6,00
Normally fertilized oocytes (2PN, 2PB)	4289	72,27
Abnormally fertilized oocytes (3PN etc.)	322	5,43
Absence of fertilization (0-0; 0-1)	659	11,11
Oocytes degenerated after fertilization	308	5,19
Total amount of oocytes retrieved	5934	100
Note: PN – pronucleus; PB – polar body; N – absolute number; % – frequency of occurance; Gv (germinal vesicle)– immature oocyte, no polar body; MI (metaphase I) – immature oocyte, no polar body.		

A total of 5934 oocytes (760 programs) were retrieved for IVF programs over the entire study period. All obtained oocytes were fertilized by standard IVF method. Based on the data in Table 1, we can observe that 356 oocytes (6,00%) were found to be immature (GV, MI stages) out of the total number of oocytes. Normal fertilization, namely the presence of 2 pronuclei and 2 polar bodies, was observed in 4289 oocytes, which represent 72,27% of the whole amount of oocytes. Abnormal fertilization (3pn – triploid, 4pn – tetraploid, etc.) was observed in 322 oocytes (5,43%). In 659 oocytes (11,11%) there was no fertilization observed, 308 oocytes (5,19%) degenerated after the procedure of fertilization.

Table 2 – Characteristics of fertilized oocytes in the ICSI programs

Material characteristics	ICSI	
	n	%
Total of mature oocytes	13372	100
Normally fertilized oocytes (2PN, 2PB)	10845	81,10
Abnormally fertilized oocytes (3PN etc.)	259	1,94
Absence of fertilization (0-0; 0-1)	1904	14,24
Oocytes degenerated after fertilization	364	2,72
Note: PN – pronucleus; PB – polar body; N – absolute number; % – frequency of occurance;		

As it is shown in the Table 2, a total of 19540 oocytes were obtained for ICSI programs in 2017-2021. After the denudation process, 13372 oocytes were mature (MII stage), which represent 68,43% of the total number of cells obtained in 1890 programs. The number of immature oocytes (GV, MI) was 6168 oocytes, or 31,57%. Normal fertilization after ICSI was observed in 10845 oocytes (81,10%). The number of abnormally fertilized oocytes in ICSI programs made up 259 oocytes (1,94%). This value is significantly lower compared to the results of IVF programs, where the rate of abnormal fertilization

was represented by 5,43%. This can be explained by the fact that during the ICSI fertilization, a single sperm is selected and implanted directly into the oocyte, while during the IVF fertilization, oocytes are placed in a culture medium with a certain concentration of sperm, where random fertilization occurs. In this case, a single oocyte can be fertilized by 2 or more spermatozoa, due to which abnormal fertilization occurs. No fertilization was observed in 1904 oocytes (14,24%). The number of oocytes degenerated after fertilization procedures made up 364 (2,72%).

Table 3 – Characteristics of blastocysts obtained in IVF/ICSI programs

Material characteristics	IVF		ICSI	
	n	%	n	%
Blastocysts of good quality	2119	78,34	6947	85,00
Poor quality blastocysts	586	21,66	1228	15,00
Total blastocysts	2705	100	8175	100
Note: N – absolute number; % – frequency of occurance;				

As demonstrated in Table 3, a total of 2705 blastocysts were obtained during the entire period in the IVF programs on the 5th and 6th days of development. Of these, 2119 embryos (78,34%) were of good quality, suitable for transfer and cryopreservation. The number of poor quality blastocysts made up 586 (21,66%).

In the ICSI programs performed, the total number of blastocysts was 8175, of which good quality blastocysts were 6947 (85,00%) and poor quality blastocysts unsuitable for transfer or cryopreservation were 1228, or 15,00%.

Table 4 shows the results of using CSC Irvine and Origio Sequential Cleav/Blast nutrient media in IVF and ICSI programs.

During the IVF programs, a total of 2292 blastocysts cultured in CSC Irvine single-step nutrient medium were obtained. Of these, 1795 blastocysts (78.32%) were of good quality and were suitable for transfer or cryopreservation. The remaining 497 blastocysts (21.68%) were blastocysts of poor quality, not suitable for transfer or cryopreservation. The number of blastocysts in the two-step Origio Sequential Cleav/Blast nutrient medium was 413, almost 5.5 times fewer than blastocysts cultured in CSC Irvine nutrient medium. This difference in the total amount of blastocysts cultivated in Cleav/Blast compared to the total of blastocysts cultivated in Irvine can be explained by the fact that the number of programs using single-stage CSC Irvine medium in embryology laboratories increased each year. In the two-stage medium, 324 (78.50%) of the total number of blastocysts were blastocysts of good quality and 89 (21.50%) were blastocysts of poor quality.

Table 4 – Results of using CSC Irvine and Origio Sequential Cleav/Blast nutrient media in IVF/ICSI programs depending on the quality of blastocysts

Material character-ristics	IVF				t	ICSI				t, p
	CSC Irvine		Origio Sequential Cleav/Blast			CSC Irvine		Origio Sequential Cleav/ Blast		
	n	%	n	%		n	%	n	%	
Total blastocysts	2292	100	413	100		6562	100	1613	100	
Blastocysts of good quality	1795	78,32	324	78,50	0,08	5712	87,00	1235	76,57	8,14 p < 0,001
Poor quality blastocysts	497	21,68	89	21,50	0,04	850	13,00	378	23,43	4,23 p < 0,001
Note: N – absolute number; % – frequency of occurance; t – Student's t-test; p – value.										

Thus, there were no statistically significant differences in the use of the above-mentioned nutrient media to obtain good quality blastocysts in the IVF program.

In the ICSI programs as well as in the IVF programs, there is a difference in the number of blastocysts obtained after cultivating the cells in different media. The number of blastocysts cultured in Origio Sequential Cleav/Blast nutrient medium was 4.1 times lower than the number of blastocysts cultured in CSC Irvine nutrient medium. There is a total of 6562 blastocysts obtained using CSC Irvine nutrient medium, of which 5712 (87.00%) were blastocysts of good and excellent quality, 850 (13.00%) were poor quality blastocysts. Using Origio Sequential Cleav/Blast nutrient medium, a total of 1613 blastocysts were obtained, of which 1235 (76.57%) were good quality blastocysts and 378 (23.43%) were low quality blastocysts. Statistical analysis of the data showed a high level of good quality blastocysts when cultured in single-step CSC Irvine medium compared to the number of those cultured in two-step Origio Sequential Cleav/Blast medium. The difference between the two relative values is statistically significant ($t = 8.14$; $p<0.001$). According to that, there was also statistically significant difference in the occurrence of low quality blastocysts ($t = 4.23$; $p<0.001$). The overall efficacy of IVF/ICSI programs is shown in Table 5.

Table 5 – Clinical effectiveness of IVF and ICSI programs

Characteristic	IVF				t	ICSI				t
	CSC Irvine		Origio Sequential Cleav/Blast			CSC Irvine		Origio Sequential Cleav/Blast		
	n	%	n	%		n	%	n	%	
Embryo transfer	435	100	103	100		961	100	133	100	
Pregnancy	202	46,44	51	49,51	0,39	398	41,42	57	42,85	0,20
Negative result (absence of pregnancy)	233	53,56	52	50,49	0,40	563	58,58	76	57,15	0,23
Note: N – absolute number; % – frequency of occurance; t – Student's t-test.										

A total of 760 IVF programs were performed in the laboratory in 2017-2021, as mentioned above. Of these, 613 programs were performed using CSC Irvine nutrient media. As shown in Table 5, a pregnancy occurred in 202 cases out of the 435 transfers performed, which made up 46.44%. Negative results were observed in 233 patient cases (53.56%). It is to note that in the remaining 178 cases (29.03%), a delayed transfer was performed where the patients' embryos were cryopreserved and the transfer was performed in the following cycles. Delayed transfer was performed due to the risk of ovarian hyperstimulation, insufficient endometrial quality for transfer, and when planning surgical interventions. From 147 programs with Origio Sequential Cleav/Blast medium, the embryos transfer took place in 103 patient cases (70.06%), the programs ended up in pregnancies in 51 cases (49.51%), patients faced negative results in 52 cases (50.49%). Delayed transfer was performed in 44 programs, which made up 29.93% of the total number of IVF programs.

During this period, 1890 ICSI programs were performed. Of these, 1620 programs were performed using the CSC Irvine medium. 961 (59.32%) programs ended with embryo transfer to the uterine cavity, in which 398 cases (41.42%) resulted in pregnancy, while in 563 cases (58.58%) there was a negative result (absence of pregnancy). It is to note that there were 659 programs with delayed transfer, representing 40.68% of the total number of programs conducted using the CSC Irvine environment. Of the 270 programs using the Origio Sequential Cleav/Blast medium, only 133 (49.26%) embryo transfers were performed, the remaining 137 programs (50.74%) ended in delayed transfers. The transfers resulted in clinical pregnancies in 57 cases (42.86%) and absence of pregnancies in the remaining 76 patients (57.14%).

During the introduction of ART, the culture medium composition, main advantages and disadvantages of media have been studied [6, 13, 19]. At the same time, there are only few studies in the scientific field devoted to a comparative analysis

of the efficacy of specific commercial media in practical usage [20, 21].

There is no single opinion on the cultivation methods, since some authors more likely believe in necessity of sequential culture media, known as the "back to nature" approach, in embryo cultivation, as the embryo needs different concentrations of nutrients at different stages of its development [13, 21, 22]. The others stick to the opinion that a sufficient concentration of nutrients in modern nutrient media can provide all the necessary needs of the embryo during its development. Also, the advantages of single-step media include minimizing the manipulation of the embryos [19, 23, 24]. Consequently, there is no unified view among clinical embryologists as to which approach is more optimal, and both are still widely used in practice to improve the success of individual ART programs [13, 25].

Conclusion

A comparative analysis of the results of embryo cultivation in different culture media – CSC Irvine (one-step) and Origio Sequential Cleav/Blast (sequential) – suggests that both media equally contribute to optimal blastocyst development and demonstrate positive clinical results. According to the pregnancy outcome criterion, the success rate of IVF programs using CSC Irvine nutrient medium made up 46.44%, in ICSI programs – 41.42%. Efficacy of IVF programs while culturing embryos in Origio Sequential Cleav/Blast nutrient medium resulted in 49.51%, that of ICSI programs resulted in 42.85%. Referring to the fact that the rate of pregnancy in natural conditions makes up 25-30%, the pregnancy rates obtained under the study can be considered quite high.

It is important to note that there appeared the tendency to switch to the use of one-step nutrient media in our case. This may be explained by a number of advantages in using single-step media, namely, a reduction in the total volume of nutrient media used, a reduction in the number of manipulations over the embryos, which reduces the risk of embryo damage and, consequently, increases the success rate of ART programs.

Conflict of Interest

All authors have read and are familiar with the content of the article and have no conflict of interest.

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