UDC 612.64.57(575.1)
DOI: https://doi.org/10.17721/1728.2748.2026.104.11-18

Maftuna ISLAMBEKOVA, PhD Student
ORCID ID: 009-008-6071-9612
e-mail: maftunaivf@gmail.com
National University of Uzbekistan, Tashkent, Republic of Uzbekistan

Zulaykho MAMATOVA, PhD (Biol.)
ORCID ID: 000-0002-7503-6070
e-mail: mamatovazulaykho2121@gmail.com
National University of Uzbekistan, Tashkent, Republic of Uzbekistan
Reproductive Medical Center, Tashkent, Republic of Uzbekistan

REDUCTION OF OBSTETRIC AND PERINATAL RISKS IN IVF: THE STRATEGY OF SINGLE BLASTOCYST-STAGE EMBRYO TRANSFER

Background. ***Determining the optimal day for embryo transfer in in vitro fertilization (IVF) is a key factor influencing reproductive outcomes. Transferring a single embryo at the blastocyst stage (day 5) provides better synchronization with the endometrial receptivity window and allows for selection of embryos with higher developmental potential. This strategy is associated with improved implantation rates and a reduced risk of multiple gestations. The aim of this study was to compare clinical outcomes of embryo transfers performed on day 3 (cleavage stage) versus day 5 (blastocyst stage) in IVF cycles, focusing on implantation efficiency and pregnancy outcomes.***

Methods. ***A retrospective cohort study was conducted involving 576 women who underwent IVF treatment between 2022 and 2024 at a major reproductive center in Uzbekistan. Patients were grouped based on the timing of embryo transfer: Group 1: cleavage-stage embryo transfers (day 3) (n = 200); Group2: blastocyst-stage embryo transfers (day 5) (n = 376). Trends in transfer practices, number of embryos transferred, implantation rates, and pregnancy outcomes were analyzed.***

Results. ***Between 2022 and 2024, the proportion of blastocyst-stage transfers increased significantly from 65 % to 96 %, while cleavage-stage transfers declined to 4 %. The average implantation rate for day 5 transfers (64.2 %) was significantly higher than for day 3 transfers (51.2 %). Simultaneously, the proportion of elective single embryo transfers (eSET) rose from 30 % to 62 %, contributing to a reduction in multiple pregnancy rates. This information is based on retrospective analysis of clinical records from our IVF center.***

Conclusions. ***The shift toward blastocyst-stage embryo transfer and eSET in Uzbekistan reflects a broader alignment with international IVF standards. These changes are associated with improved implantation outcomes and reduced perinatal risks, supporting a more personalized and evidence-based approach to reproductive care.***

Keywords: ***embryo transfer, blastocyst-stage embryo, cleavage-stage embryo, embryo implantation, eSET, assisted reproductive technology (ART), embryo selection.***

Background

In assisted reproductive technology (ART) programs, fertilization of oocytes is carried out ex vivo under strictly controlled laboratory conditions, allowing close observation of the early stages of embryonic development in vitro. The resulting zygotes undergo successive cleavage divisions, forming embryos that vary in their degree of morphological and functional maturity. At this stage, the embryo represents an early form of human development characterized by active cellular division, differentiation, and compaction processes.

Embryo transfer into the uterine cavity may be performed at different stages of preimplantation development, most commonly at the cleavage stage or the blastocyst stage. The cleavage stage typically occurs 2–3 days after oocyte retrieval, when the embryo consists of approximately 4–8 blastomeres. At this point, several morphological parameters are evaluated, including blastomere symmetry, degree of cytoplasmic fragmentation, and presence of multinucleation – all serving as prognostic indicators of embryo viability.

The blastocyst stage, in turn, is reached on day 5–6 post-fertilization and represents a more differentiated structure comprising the inner cell mass (ICM), which gives rise to the embryo proper, and the trophectoderm, which forms extraembryonic tissues. Numerous studies have shown that blastocyst-stage embryo transfer is associated with higher implantation and clinical pregnancy rates, as it allows an additional level of natural selection for developmentally competent embryos and promotes synchronization between embryonic development and endometrial receptivity.

Thus, the selection of the optimal timing for embryo transfer – whether at the cleavage or blastocyst stage – remains a crucial factor in the individualization of ART protocols and has a direct impact on the effectiveness of infertility treatment, implantation success, and perinatal outcomes.

Until recently, the standard practice in in vitro fertilization (IVF) programs involved the transfer of embryos at the early cleavage stage, typically on the second or third day after fertilization. This approach was primarily dictated by the limited ability to sustain prolonged embryo culture under laboratory conditions and the need to minimize the influence of exogenous environmental factors on embryonic development. However, with the advancement of embryology techniques – including the improvement of culture media formulations, optimization of gas compositions within incubators, and the introduction of sequential media systems – it has become feasible to culture embryos in vitro until the blastocyst stage (day 5-6 post-fertilization).

Contemporary trends in assisted reproductive technologies indicate a progressive shift toward the transfer of embryos at the more advanced blastocyst stage. This practice is based on the concept that only morphologically and functionally competent embryos are capable of surviving and developing to the blastocyst stage in vitro. In this sense, extended culture serves as a form of natural selection, allowing the identification of the most viable embryos with the highest implantation potential.

A growing body of research suggests that blastocyst-stage transfer may increase the likelihood of clinical pregnancy and live birth compared to cleavage-stage transfer. The advantages of this approach are attributed not only to the greater physiological maturity of the embryo but also to improved synchronization between embryonic development and the endometrial implantation window. Collectively, these factors have led to the increasing adoption of blastocyst transfer as a preferred strategy in clinical practice, particularly among patients with multiple high-quality embryos available for selection.

Over the past decade there has been a steady shift in practice to transfer of embryos on day 5 or 6 when the embryos are 'blastocysts'. With the introduction of a variety of commercial preparations of sequential media in the late 1990s, the ART service sector witnessed an explosion of worldwide interest in blastocyst culture, with most clinics conducting research into its application in their own setting. As a result, a substantial volume of publications followed. These included conflicting trials and debates about the merits and drawbacks of extended culture. A lack of strong consensus about the best practice for blastocyst culture has not been aided by the fact that many of the trials were not prospectively randomised or were underpowered. The need for an evidence-based approach using meta-analysis of small trials was, therefore, required to assist in deciphering the overall effect of blastocyst culture to help identify participant subsets and practices that might best benefit from this approach (Glujovsky et al., 2022). Blastocyst culture is not novel; indeed, the very first report of an IVF pregnancy was from a transferred blastocyst (Edwards, & Brody, 1995).

Despite this, cleavage stage transfer was adopted as standard global practice early in the history of IVF for two reasons: the low developmental rate of embryos cultured past this stage; and the observation that unlike other primates, human embryos have an unusual propensity to survive when replaced prematurely into the uterus (Marston et al., 1977).

However, as knowledge of embryo metabolic requirements expanded, so did the range of more advanced culture media and co-culture techniques (Van Blerkom, 1993). One important finding was that the in vitro environment in which a cleavage stage embryo grows best is different from that for a blastocyst. This led to the development of stage-specific (sequential) media, notably the G1/G2 system introduced by Gardner et al. (1998). Under this protocol, embryos are transferred on day 3 from a low-glucose, amino-acid-restricted medium to one with higher glucose concentrations and a broader amino acid profile (Gardner et al., 1996).

At this stage, the embryo undergoes cell compaction and genomic activation so that the embryo is no longer under the control of transcripts and RNA messages of maternal origin (Braude et al., 1988).

With the application of stage-specific media, there have been reports of blastocyst development rates as high as 60 % to 65 % (Schoolcraft, & Gardner, 2001).

Interestingly, with the development of time-lapse systems stage-specific media is no longer considered essential.

There are two central arguments why blastocyst culture has possible advantages over traditional cleavage stage transfer. Firstly, it is considered to be physiologically premature to expose early-stage embryos to the uterine environment, particularly one that has been subjected to superovulation and thus high levels of oestrogen (Valbuena et al., 2001).

In vivo, embryos travel through the fallopian tubes and do not reach the uterus before the morula (16 cell compacted) stage (Croxatto et al., 1972), which equates to at least day 4 of in vitro culture. The uterus provides a different nutritional environment from the oviduct; therefore it is postulated that the uterine environment may cause stress on the embryo, if transferred at the cleavage stage (Baart et al., 2006) and result in reduced implantation potential (Gardner et al., 1996)**.**There is also evidence of a significant reduction in uterine pulsatility at the time when blastocysts are transferred and therefore less chance that embryos can be expelled (Fanchin et al., 2001)**.**

The second argument for blastocyst transfer is the reported higher implantation potential compared with cleavage stage embryos. As a consequence of self selection, it is postulated that only the most viable embryos are expected to develop into blastocysts. It is widely acknowledged that the morphological criteria used for selection of the best embryos on day 2 to 3 are limited (Steer et al., 1992). Many published studies that debate the correlation of morphological features with pregnancy rates can be found in the literature (Sjoblom et al., 2006). It is now understood that a disturbingly large proportion of morphologically normal day 3 embryos are chromosomally abnormal or mosaic, thus contributing to the 80 % to 90 % rate of implantation failure post-transfer that is observed in cleavage stage protocols. While the transfer of day 5 blastocysts cannot ensure the absence of chromosomal abnormality demonstrated that, et least in women older than 36 years, the incidence can be reduced from 59 % in day 3 embryos to 35 % in day 5 blastocysts.

Arguments against blastocyst culture are largely related to this process of self selection. Women undergoing blastocyst culture are expected to have a higher incidence of cycle cancellation due to failed embryo development (Marek et al., 1999) and of having fewer embryos cryopreserved (frozen). Overall utilisation rates have previously been described as the total number of embryos transferred plus the embryos thawed divided by the number of fertilised eggs. While this approach presents information about the comparative number of pregnancy opportunities that each treatment approach can provide a couple, it does not take into account the implantation potential for fresh and thawed embryos, and cumulative live birth rate is the only outcome that can assess this. An alternative efficacy formula was developed in the Schoolcraft & Gardner (2001) study that does take this into account. Using the formula (mean number of embryos transferred multiplied by implantation rate) plus (mean number of embryos cryopreserved multiplied by implantation rate) minus (1 minus cancellation rate), this group of researchers was able to demonstrate a 19 % greater efficiency in blastocyst culture compared with cleavage stage transfers. Disappointingly, such a utilisation and efficiency analysis is not possible in the majority of RCTs due to the lack of reporting of frozen-thawed cycle outcomes within a reasonable time frame for trials. A simpler proposal to both of these approaches is to report the live birth rates for both fresh and frozen-thawed cycles following a single oocyte retrieval in women randomised to either cleavage stage or blastocyst stage transfers.

The aim of this study is to conduct a comparative analysis of IVF clinical outcomes following embryo transfer on day 3 versus day 5 in the context of Uzbekistan, with a focus on embryonic developmental stage. Furthermore, the study seeks to analyze the evolution of IVF practices in Uzbekistan between 2022 and 2024 aimed at improving outcomes through the implementation of single embryo transfer strategies, and to assess their impact on reducing the incidence of multiple pregnancies.

Methods

In our study, we conducted a retrospective analysis of in vitro fertilization (IVF) protocols performed at one of the major reproductive centers in Uzbekistan over the period from 2022 to 2024. The sample included 576 women of reproductive age (mean age: 31.5 years) who underwent infertility treatment via IVF.

1. Controlled Ovarian Stimulation (COS)

All patients underwent controlled ovarian stimulation to induce the development of multiple follicles within a single cycle. Recombinant follicle-stimulating hormone (r-FSH) and/or human menopausal gonadotropin (hMG) were used. Follicular growth was monitored by serial transvaginal ultrasonography and serum estradiol (E2) measurements. To prevent premature luteinizing hormone (LH) surges, gonadotropin-releasing hormone (GnRH) antagonists or agonists were administered.

2. Ovulation Trigger

Final oocyte maturation was triggered using either human chorionic gonadotropin (hCG) or a GnRH agonist, depending on the individual risk of ovarian hyperstimulation syndrome (OHSS). Transvaginal follicular aspiration was performed 34–36 hours after trigger administration.

3. Oocyte Retrieval

Ovarian puncture was performed under ultrasound guidance using an aspiration needle under intravenous sedation. Retrieved follicles were immediately transferred to the embryology laboratory.

4. Oocyte Maturity Assessment

Oocytes were evaluated under an inverted microscope to determine meiotic status. Only mature oocytes at the metaphase II (MII) stage, identified by the presence of the first polar body, were used for further procedures.

5. Semen Preparation

Semen samples were processed using density gradient centrifugation and/or swim-up techniques. In cases of azoospermia, spermatozoa were surgically retrieved via PESA, TESA, or micro-TESE, followed by microscopic processing.

6. Fertilization

Fertilization was performed using one of two methods based on clinical indications:

- Conventional IVF: Co-incubation of mature oocytes with processed spermatozoa for 16–18 hours.
- Intracytoplasmic Sperm Injection (ICSI): Used in cases of male factor infertility or previous IVF failure.

7. Embryo Culture

Fertilized zygotes were cultured in specialized media under controlled conditions (5 % O_2, 6 % CO_2, 37 °C). Embryos were cultured either until the cleavage stage (Day 3) or to the blastocyst stage (Day 5 or 6), depending on embryo quality, quantity, and cycle strategy. Both Day 3 and blastocyst-stage embryos were used in this study.

8. Embryo Morphological Assessment

Daily morphological assessments were performed. Cleavage-stage embryos were evaluated based on blastomere number, symmetry, fragmentation degree, and presence of multinucleation. Blastocysts were graded using the Gardner scale, assessing degree of expansion, inner cell mass (ICM) quality, and trophectoderm appearance.

9. Vitrification

Surplus embryos not transferred were vitrified using modern rapid freezing protocols in liquid nitrogen at -196 °C with high concentrations of cryoprotectants (e.g., DMSO, ethylene glycol). Thawed embryos were used in subsequent cryocycles.

10. Embryo Transfer

Embryo transfer (ET) was performed under ultrasound guidance using a soft catheter without anesthesia. Both fresh and thawed embryos were used. The transfer day (Day 3 or Day 5) was determined based on embryo development stage and quality. The number of embryos transferred varied; however, elective single embryo transfer (eSET) was preferred, particularly in cases with high-quality blastocysts.

11. Luteal Phase Support

Following ET, all patients received luteal support with progesterone (administered vaginally, orally, or intramuscularly) until pregnancy was confirmed and continued until 10-12 weeks of gestation in successful cases.

12. Pregnancy Confirmation

Pregnancy was confirmed by serum β-hCG levels measured 9-12 days post-transfer. A follow-up transvaginal ultrasound was performed 5-7 days later to confirm intrauterine gestation.

13. Comparison Groups

To evaluate the impact of embryo developmental stage on IVF outcomes, all patients were retrospectively divided into two groups:

Group 1: Embryo transfer on Day 3 (cleavage stage) (n = 200)

Group 2: Embryo transfer on Day 5 (blastocyst stage) (n = 376)

Additionally, we analyzed the dynamics of embryo transfer practices over the study period, reflecting the gradual adoption of international recommendations aimed at reducing multiple pregnancy rates.

All primary data from 2022 to 2024 were systematized in Microsoft Excel (Microsoft Corp., USA). Preliminary data processing, percentage calculations, and graphical illustrations were performed in Excel.

Comparative analysis of categorical variables was carried out using the chi-square (χ^2) test of independence. This method was applied to evaluate:

- implantation rates of embryos transferred at the cleavage stage (Day 3) and the blastocyst stage (Day 5);
- rates of singleton and multiple pregnancies among couples undergoing IVF;
- trends in the frequency of single, double, and multiple embryo transfers from 2022 to 2024;
- distribution of embryo transfers by developmental stage (Day 3 vs. Day 5) over the study period.

The results of χ^2 analyses are reported with test statistics, degrees of freedom (df), and p-values. A p-value < 0.05 was considered statistically significant.

A retrospective analysis of embryo transfers that was performed at the clinic "*Center for Reproductive Medicine" (the first IVF clinic in Uzbekistan)* from 2022 to 2024 demonstrated a clear trend toward an increased proportion of blastocyst-stage transfers, with a concurrent decrease in Day 3 transfers.

This retrospective study was based exclusively on the analysis of previously collected, fully anonymized reproductive data. No direct interaction with patients occurred, and no clinical interventions, treatments, or medications were administered as part of the research process. In accordance with international ethical standards – including the Declaration of Helsinki, the International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines, and the Council for International Organizations of Medical Sciences (CIOMS) ethical framework – studies using de-identified data and posing no risk to participants are exempt from formal ethical review.

Furthermore, national regulations stipulate that research involving anonymized medical information does not require informed consent, provided that the data cannot be traced back to individual patients. Given that all datasets in this study were irreversibly de-identified prior to analysis, the study met all criteria for exemption from ethical approval and informed consent. The research consisted solely of a structured statistical and comparative evaluation of anonymized clinical records, without any influence on patient management or health outcomes.

Results

In the initial stage of clinical practice (prior to the analyzed period), In 2019, the majority of transfers (63 %) were conducted at the cleavage stage, with blastocyst-stage transfers accounting for only 37 %. However, from 2022 onward, there was a notable shift in clinical practice. By 2022, the proportion of blastocyst transfers exceeded 50 % for the first time, reaching 65 %, and continued to rise in subsequent years – 95 % in 2023 and 96 % in 2024. Consequently, by 2024, Day 3 transfers were nearly phased out, representing only 4 % of all embryo transfers (Fig. 1).

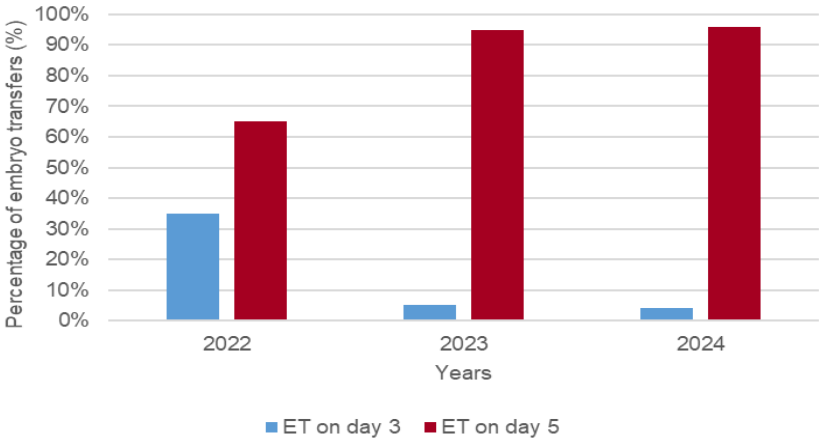

Fig. 1. Frequency of Day 5 (blastocyst) and Day 3 (cleavage stage) embryo transfers

A chi-square test confirmed that this change was statistically significant ($\chi^2 = 92.27$, df = 2, $p < 0.001$), indicating a consistent and robust adoption of blastocyst transfer in clinical practice.

The data obtained indicate significant progress in embryo culture and selection practices at our clinic, fully aligned with global trends in assisted reproductive technologies (ART). The shift toward preferential transfer of blastocyst-stage embryos is driven by multiple factors affecting both the clinical efficacy and safety of IVF programs.

Firstly, extended culture to Day 5 enables more stringent morphological and functional selection, as only embryos with high developmental potential progress to the blastocyst stage. This increases the likelihood of implantation and clinical pregnancy. Secondly, blastocyst transfer offers better synchronization with the endometrial receptivity window, further enhancing cycle efficiency.

Numerous international studies have demonstrated that blastocyst transfer is associated with higher live birth rates using fewer embryos, making it possible to safely implement the strategy of elective single embryo transfer (eSET), thereby reducing the risk of multiple pregnancies and related complications. Accordingly, the blastocyst-stage transfer strategy is now recognized as the "gold standard" in advanced reproductive care systems (ESHRE, ASRM).

Thus, the transformation of clinical practice in favor of blastocyst transfer reflects not only adherence to international recommendations but also the advancement of our clinic's technical and clinical capabilities, aimed at increasing the likelihood of healthy pregnancies and favorable patient outcomes.

A retrospective analysis was conducted on the distribution of the number of embryos transferred in IVF programs at our clinic over the period from 2022 to 2024. The results demonstrate a clear positive trend toward an increased rate of elective single embryo transfers (eSET), accompanied by a corresponding decrease in multiple embryo transfers.

In the initial stage of clinical practice (prior to the analyzed period), in 2019, eSET was performed in only 15 % of cases, whereas the transfer of two embryos occurred in 70 % of cycles. Transfers of three embryos were recorded in 14 % of cases, and four-embryo transfers in 2 %. Beginning in 2022, a consistent increase in eSET was observed: 29 % in 2022, 47 % in 2023, and 62 % in 2024.

Accordingly, by 2024, the proportion of double embryo transfers decreased to 36 %, while transfers of three or more embryos had nearly disappeared from clinical practice – 2 % and 0 %, respectively (Fig. 2).

A chi-square test confirmed that the observed changes in the distribution of embryo transfer strategies were statistically significant across the three-year period ($\chi^2 = 49.06$, df = 6, $p < 0.0001$). These results highlight the progressive adaptation of clinical protocols in Uzbekistan to globally recognized standards of reproductive medicine.

The obtained results confirm a deliberate shift in our clinic toward modern standards in assisted reproductive technologies (ART), prioritizing single embryo transfers (eSET). This aligns with trends observed in countries with advanced reproductive medicine systems – such as Sweden, Belgium, Japan, and Canada - where the proportion of eSET exceeds 70-80 %.

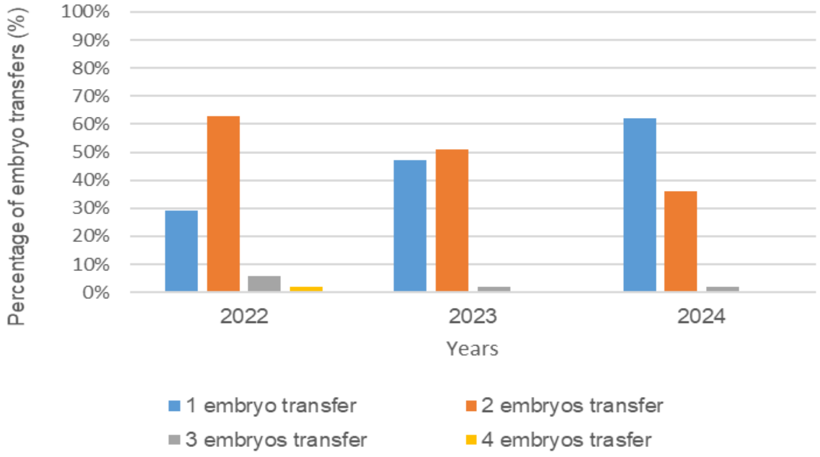

Fig. 2. Trends in the frequency of single, double, and multiple embryo transfers from 2022 to 2024

Numerous studies have demonstrated that single embryo transfer significantly reduces the risk of multiple pregnancy and its associated complications, including preterm birth, intrauterine growth restriction, preeclampsia, and other perinatal pathologies. At the same time, advancements in embryo culture to the blastocyst stage, preimplantation genetic testing, and cryopreservation techniques enable high pregnancy and live birth rates to be achieved even with the transfer of a single embryo.

International clinical guidelines – issued by organizations such as the European Society of Human Reproduction and Embryology (ESHRE), the American Society for Reproductive Medicine (ASRM), and the World Health Organization (WHO) – strongly recommend minimizing the number of embryos transferred, particularly in younger patients and those with a favorable prognosis.

In our clinic, the gradual transition from double and triple embryo transfers to eSET has been accompanied by a reduction in the incidence of multiple pregnancies (as detailed in the corresponding section), without compromising IVF success rates. This indicates a high level of clinical expertise, robust embryological support, and adherence to international protocols.

Moreover, the observed decrease in multiple embryo transfers reflects increasing patient confidence in the eSET strategy, as well as improved patient counseling and shared decision-making between physicians and patients.

This transformation in clinical practice highlights a broader shift toward individualized approaches in reproductive medicine, with an emphasis on patient safety and the reduction of multiple pregnancy risks. The increase in single embryo transfers is typically correlated with the growing use of blastocyst-stage embryo culture, which allows for more precise selection of embryos with the highest implantation potential. Furthermore, the adoption of eSET strategies mirrors global standards and recommendations aimed at improving perinatal outcomes and reducing obstetric and neonatal risks.

The clinical pregnancy rate was estimated based on transvaginal ultrasound confirmation of a gestational sac. In 2022, 2023, and 2024, the clinical pregnancy rates were 32%, 38%, and 41%, respectively, corresponding to 61, 73, and 79 clinical pregnancies per year. Multiple and singleton pregnancies were determined according to the number of gestational sacs visualized on ultrasound examination. Specifically, the presence of two or more gestational sacs was defined as a multiple pregnancy, whereas a single gestational sac corresponded to a singleton pregnancy. Based on these data, the proportions of multiple and singleton pregnancies were calculated for each year (Fig. 3).

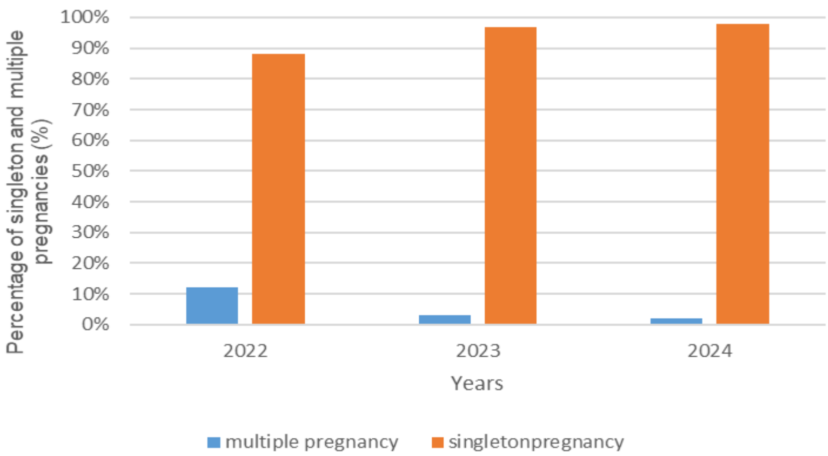

Fig. 3. Rates of singleton and multiple pregnancies among couples undergoing IVF from 2022 to 2024

An analysis of pregnancy outcomes at our clinic over the period from 2022 to 2024 revealed a marked decline in the incidence of multiple pregnancies, accompanied by a steady increase in singleton pregnancies. In 2019, multiple pregnancies accounted for 45 % of all observed pregnancies, while singleton pregnancies comprised 55 %. However, in subsequent years, the proportion of multiple pregnancies showed a consistent decrease: 12 % in 2022, 3 % in 2023, and reaching a minimum of just 1 % in 2024. Accordingly, the rate of singleton pregnancies steadily increased, reaching 99 % in 2024.

A chi-square test confirmed that these changes were statistically significant ($\chi^2 = 5.84$, df = 2, $p = 0.054$). Despite the lack of statistical significance the consistent and marked reduction in multiple pregnancy rates may reflect clinically meaningful improvements associated with the adoption of modern embryo transfer strategies. This trend reflects the increasing implementation of single embryo transfer strategies and adherence to international guidelines aimed at minimizing the risks associated with multiple gestations.

While nationwide data in Uzbekistan indicate a general upward trend in multiple pregnancy rates - likely related to the widespread adoption of assisted reproductive technologies (ART), delayed childbearing, and other socio-demographic factors - the data obtained from our clinic demonstrate the opposite pattern.

The sharp and sustained reduction in multiple pregnancies within our practice may be attributed to several key factors:

1. Changes in ART protocols: In recent years, there has been a shift toward elective single embryo transfer (eSET) in IVF cycles, aimed at minimizing complications associated with multiple gestations.

2. Stricter clinical standards: Our clinic has actively implemented policies to reduce the incidence of multiple pregnancies due to their higher maternal and fetal risks, in alignment with international recommendations (e.g., ASRM, ESHRE).

Thus, despite the national increase in multiple births, our clinic's strategy has been directed toward minimizing such outcomes, as reflected in the presented statistics. This trend reflects a deliberate and evidence-based clinical approach focused on improving the safety of pregnancy and childbirth for both the mother and the offspring.

These findings underscore the progressive development of clinical embryology practices and the optimization of embryo transfer protocols, balancing treatment efficacy with patient safety.

An analysis of embryo implantation dynamics over the 2022–2024 period shows a consistent and clinically significant advantage of blastocyst-stage (day 5) transfers compared to cleavage-stage (day 3) transfers. Throughout the analyzed years, implantation rates for blastocyst transfers remained consistently higher-rising from 53 % in 2022 to 75 % in 2024. In contrast, implantation rates for day - 3 embryos fluctuated between 45 % and 60 %, while blastocyst transfers reached peak values in 2022 and 2024, at 78 % and 75 %, respectively (Fig. 4).

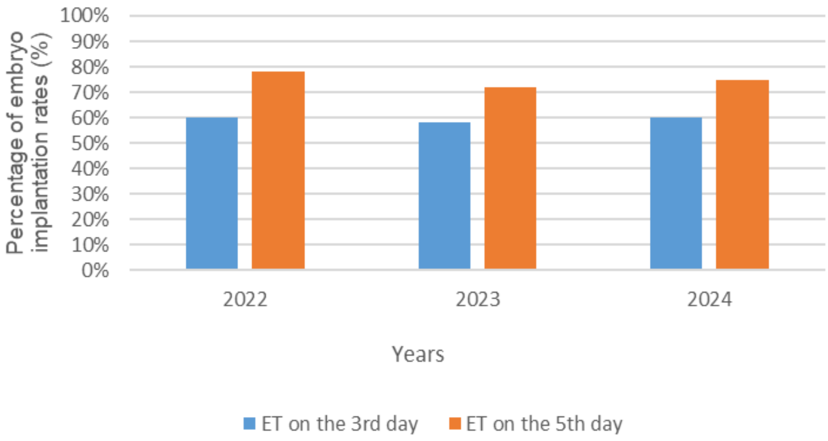

Fig. 4. Implantation rates of embryos transferred et the cleavage stage (Day 3) and blastocyst stage (Day 5)

In total, over the period 2022-2024, a total of 376 embryo transfer cycles were performed at the blastocyst stage (day 5), resulting in 283 confirmed implantations, corresponding to an implantation rate of 75 %. During the same period, 200 embryo transfers were carried out at the cleavage stage (day 3), with 119 successful implantations, yielding an implantation rate of 59.5 %. The difference in implantation rates between day-5 and day-3 transfers was statistically significant, as confirmed by a chi-square test ($\chi^2 = 16.72$; df = 1; $p < 0.0001$).

This difference in clinical outcomes between the two embryo transfer stages can be explained by several critical factors. First, extended culture to the blastocyst stage allows for a more refined selection of embryos, as some early cleavage-stage defects may only become apparent at later stages. Second, blastocysts represent a more developmentally advanced form, which more closely aligns with the natural window of endometrial receptivity, thereby improving the chances of successful implantation. Finally, recent advancements in culture protocols and in vitro media have enhanced the viability of embryos cultured to later developmental stages.

The systematic increase in the proportion of blastocyst transfers - from 37 % in 2019 to 96 % in 2024 - reflects the growing clinical recognition of their superior implantation potential. The observed effect demonstrates not merely a more frequent use of blastocyst-stage transfers but a statistically proven higher implantation rate, confirming their clinical advantage in assisted reproductive technologies.

These findings support the clinical rationale for blastocyst-stage transfer as a strategy to enhance implantation outcomes and improve the overall success of assisted reproductive technologies (ART).

Discussion and conclusions

The present study provides a comprehensive evaluation of embryo culture and transfer practices in IVF programs at our center between 2022 and 2024, demonstrating a substantial evolution in both clinical and embryological methodologies. During this three-year period, the proportion of blastocyst-stage (Day 5) transfers increased markedly from 65% in 2022 to 96 % in 2024, while cleavage-stage (Day 3) transfers decreased to 4 % ($\chi^2 = 92.27$, df = 2, $p < 0.001$). This shift reflects the effective implementation of advanced embryo culture protocols and precise selection criteria, enabling the preferential transfer of embryos with the highest developmental potential.

Concurrently, the rate of elective single embryo transfer (eSET) increased from 29% in 2022 to 62 % in 2024 ($\chi^2 = 49.06$, df = 6, $p < 0.0001$), accompanied by the near-complete discontinuation of transfers involving three or more embryos. This transition was associated with a substantial reduction in the incidence of multiple pregnancies, which declined from 12% to 1 % over the study period ($\chi^2 = 5.84$, df = 2, $p < 0.054$), while singleton pregnancies reached 99 %.

Analysis of implantation outcomes further corroborated the clinical advantage of blastocyst-stage transfers. Implantation rates for Day 5 embryos were 75% (283/376), significantly higher than the 59.5 % (119/200) observed for Day 3 embryos (($\chi^2 = 16.72$; df = 1; $p < 0.0001$). These findings indicate that extended culture facilitates more refined selection based on embryonic developmental competence, thereby enhancing implantation potential while minimizing the need for multiple embryo transfers.

In conclusion, the observed transformation in clinical practice – characterized by the widespread adoption of blastocyst-stage transfers and eSET - demonstrates measurable improvements in IVF outcomes. The results not only align with international standards in assisted reproductive technologies but also reflect tangible advancements in laboratory performance and patient-centered reproductive care within Uzbekistan. Collectively, these data provide an evidence-based framework for the optimization of embryo transfer protocols in clinical IVF practice.

Authors' contributions: Maftuna Islambekova – contributed to study design, data collection, statistical analysis, and manuscript drafting; Zulaykho Mamatova contributed to study supervision, data interpretation, and critical revision of the manuscript. All authors approved the final version of the manuscript.

Acknowledgements. The authors thank the First IVF Clinic of Uzbekistan – the Center for Reproductive Medicine – for providing the data.

Sources of funding. This study was carried out within the framework of the primary activities of National University of Uzbekistan, Tashkent, Republic of Uzbekistan, with no external funding involved.

References

Baart, E. B., Martini, E., van den Berg, I., Macklon, N. S., Galjaard, R. J., Fauser, B. C., & Martini, E. (2006). Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. *Human Reproduction*, 21(1), 223–233. https://doi.org/10.1093/humrep/dei291

Braude, P., Bolton, V., & Moore, S. (1988). Human gene expression first occurs between the four and eight-cell stages of preimplantation development. *Nature*, 332, 459–461. https://doi.org/10.1038/332459a0

Croxatto, H. B., Fuentaealba, B., Diaz, S., Pastene, L., & Tatum, H. J. (1972). A simple non-surgical technique to obtain unimplanted eggs from human uteri. *American Journal of Obstetrics and Gynecology*, 112(5), 662–668. https://doi.org/10.1016/0002-9378(72)90792-2

Edwards, R. G., & Brody, S. A. (1995). History and ethics of assisted human conception. In *Principles and practice of assisted human reproduction* (pp. 17–47). WB Saunders.

Fanchin, R., Ayoubi, J. M., Righini, C., Olivennes, F., Schönauer, L. M., & Frydman, R. (2001). Uterine contractility decreases at the time of blastocyst transfers. *Human Reproduction*, 16(6), 1115–1119. https://doi.org/10.1093/humrep/16.6.1115

Gardner, D. K., Lane, M., Calderon, I., & Leeton, J. (1996). Environment of the preimplantation human embryo in vivo: Metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cell. *Fertility and Sterility*, 65(2), 349–353. https://doi.org/10.1016/S0015-0282(16)58097-2

Gardner, D. K., Vella, P., Lane, M., Wagley, L., Schlenker, T., & Schoolcraft, W. B. (1998). Culture and transfer of human blastocyts increases implantation rates and reduces the need for multiple embryo transfers. *Fertility and Sterility*, 69(1), 84–88. https://doi.org/10.1016/S0015-0282(02)04576-4

Glujovsky, D., Quinteiro Retamar, A. M., Alvarez Sedo, C. R., Ciapponi, A., Cornelisse, S., & Blake, D. (2022). Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology. *Cochrane Database of Systematic Reviews*, 2022(5), CD002118. https://doi.org/10.1002/14651858.CD002118.pub6

Marek, D., Langley, M., Gardner, D. K., Confer, N., Doody, K. M., & Doody, K. J. (1999). Introduction of blastocyst culture and transfer for all patients in an in vitro fertilization program. *Fertility and Sterility*, 72(6), 1035–1040. https://doi.org/10.1016/S0015-0282(99)00414-0

Marston, J. H., Penn, R., & Sivelle, P. C. (1977). Successful autotransfer of tubal eggs in the rhesus monkey (Macaca mulatta). *Journal of Reproduction and Fertility*, 49, 175–176. https://doi.org/10.1530/jrf.0.0490175

Schoolcraft, W. B., & Gardner, D. K. (2001). Blastocyst versus day 2 or 3 transfer. *Seminars in Reproductive Medicine*, 19, 259–268. https://doi.org/10.1055/s-2001-18045

Sjoblom, P., Menzes, J., Cummins, L., & Mathiyalagan, B. (2006). Prediction of embryo developmental potential and pregnancy based on early-stage morphological characteristics. *Fertility and Sterility*, 86, 848–861. https://doi.org/10.1016/j.fertnstert.2006.03.040

Steer, C. V., Mills, C. L., Tan, S. L., Campbell, S., & Edwards, R. G. (1992). The cumulative embryo score: A predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. *Human Reproduction*, 7(1), 117–119. https://doi.org/10.1093/oxfordjournals.humrep.a137542

Valbuena, D., Martin, J., de Pablo, J., Remohi, J., Pellicer, A., & Simon, C. (2001). Increasing levels of estradiol are deleterious to embryonic implantation because they affect the embryo. *Fertility and Sterility*, 76(5), 962–968. https://doi.org/10.1016/S0015-0282(01)02844-3

Van Blerkom, J. (1993). Development of human embryos to the hatched blastocyst stage in the presence or absence of a monolayer of Vero cells. *Human Reproduction*, 8, 1525–1539. https://doi.org/10.1093/oxfordjournals.humrep.a138293

Отримано редакцією журналу / Received: 10.12.25
Прорецензовано / Revised: 22.02.26
Схвалено до друку / Accepted: 03.03.26
Опубліковано / Published: 27.04.26

Мафтуна ІСЛАМБЕКОВА, асп.
ORCID ID: 009-008-6071-9612
e-mail: maftunaivf@gmail.com
Національний університет Узбекистану, Ташкент, Республіка Узбекистан

Зулайхо МАМАТОВА, канд. біол. наук
ORCID ID: 000-0002-7503-6070
e-mail: mamatovazulaykho2121@gmail.com
Національний університет Узбекистану, Ташкент, Республіка Узбекистан
Центр репродуктивної медицини, Ташкент, Республіка Узбекистан

ЗНИЖЕННЯ АКУШЕРСЬКИХ ТА ПЕРИНАТАЛЬНИХ РИЗИКІВ ПРИ ЕКСТРАКОРПОРАЛЬНОМУ ЗАПЛІДНЕННІ: СТРАТЕГІЯ ПЕРЕНЕСЕННЯ ЕМБРІОНІВ НА СТАДІЇ ОДНІЄЇ БЛАСТОЦИСТИ

Вступ. ***Визначення оптимального дня для перенесення ембріона при екстракорпоральному заплідненні (ЕКЗ) є ключовим фактором, що впливає на репродуктивні результати. Перенесення одного ембріона на стадії бластоцисти (5-й день) забезпечує кращу***

синхронізацію з вікном імплантаційної сприйнятливості ендометрія і дає змогу відбирати ембріони з вищим потенціалом розвитку. Ця стратегія асоціюється з підвищенням частоти імплантації та зниженням ризику багатоплідної вагітності. Метою дослідження було порівняти клінічні результати перенесення ембріонів на 3-й (стадія дроблення) і 5-й день (стадія бластоцисти) у циклах ЕКЗ з акцентом на ефективність імплантації та результати вагітності.

М е т о д и . *Проведено ретроспективне когортне дослідження за участю 576 жінок, які проходили лікування методом ЕКЗ у період із 2022 по 2024 рік у великому центрі репродуктивної медицини в Узбекистані. Пацієнток розподілили на дві групи залежно від дня перенесення ембріона: група 1 – перенесення ембріонів на стадії дроблення (3-й день) (n = 200); група 2 – перенесення ембріонів на стадії бластоцисти (5-й день) (n = 376). Проаналізовано тенденції у практиці перенесення, кількість переносимих ембріонів, частоту імплантації та результати вагітності.*

Р е з у л ь т а т и . *У період із 2022 по 2024 рік частка перенесень на стадії бластоцисти значно зросла з 65 до 96 %, тоді як перенесення на стадії дроблення зменшилися до 4 %. Середня частота імплантації при перенесенні на 5-й день (75 %) була достовірно вищою, ніж при перенесенні на 3-й день (59,5 %). Одночасно частка елективного перенесення одного ембріона (eSET) зросла з 29% до 62 %, що сприяло зниженню частоти багатоплідних вагітностей.*

В и с н о в к и . *Перехід до перенесення ембріонів на стадії бластоцисти та впровадження стратегії eSET в Узбекистані відображає загальне наближення до міжнародних стандартів ЕКЗ. Ці зміни пов'язані з поліпшенням показників імплантації та зменшенням перинатальних ризиків, що підтримує більш персоналізований і доказовий підхід до репродуктивного лікування.*

К л ю ч о в і с л о в а : *перенесення ембріона, ембріон на стадії бластоцисти, ембріон на стадії дроблення, імплантація ембріона, eSET, допоміжні репродуктивні технології (ДРТ), відбір ембріонів.*

Автори заявляють про відсутність конфлікту інтересів. Спонсори не брали участі в розробці дослідження; зборі, аналізі чи інтерпретації даних; написанні рукопису або прийнятті рішення про публікацію результатів.

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.