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Reduction of Multiple Pregnancies through the Implementation of Elective Single Embryo Transfer (eSET) in IVF Programs

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Abstract The conducted research complies with the ethical principles of the World Medical Association (Declaration of Helsinki) concerning research involving human subjects. This retrospective study did not involve any experiments, interventions, or manipulations that could affect human health. The analysis was based exclusively on previously collected clinical data and statistical processing, without disclosure of patients' personal information. The choice of embryo transfer day in in vitro fertilization (IVF) is a critical determinant of treatment success. This study analyzed clinical outcomes associated with day 3 (cleavage stage) and day 5 (blastocyst stage) embryo transfers in Uzbekistan, a country where IVF practices have undergone substantial transformation between 2023 and the first three months of 2025. A retrospective analysis was performed on 180 women undergoing IVF treatment at one of Uzbekistan's leading reproductive centers: 60 patients during the first three months of 2023, 60 patients during the first three months of 2024, and 60 patients during the first three months of 2025. The study evaluated changes in embryo transfer timing, the number of embryos transferred, and implantation rates. The proportion of blastocyst transfers increased from 35% in the first three months of 2021 to 96% in early 2025, while cleavage-stage transfers declined from 65% to 4%. Implantation rates for day 5 transfers consistently exceeded those for day 3 transfers (75.0% vs. 59.3% on average). At the same time, the rate of elective single embryo transfer (eSET) rose from 24% in 2023 to 62% in 2025, thereby reducing the risk of multiple pregnancies. These findings highlight a significant transformation in IVF practice in Uzbekistan, aligning with international guidelines that prioritize blastocyst transfer and eSET. Such changes have led to improved implantation outcomes and reduced perinatal risks, reflecting a progressive transition toward more personalized and safer reproductive technologies.

Keywords IVF, Blastocyst transfer, Cleavage-stage embryo, Embryo implantation, eSET, Uzbekistan, Assisted reproductive technology (ART), Embryo selection, Perinatal outcomes

1. Introduction

The optimal timing of embryo transfer (day 3 cleavage stage vs day 5 blastocyst stage) remains a pivotal clinical decision in IVF, with direct implications for implantation, clinical pregnancy, multiple pregnancy rates, and perinatal outcomes. Aggregate evidence from systematic reviews and meta-analyses indicates that blastocyst transfer is associated with higher clinical pregnancy and live birth rates per transfer, whereas effects on cumulative live birth rate (CLBR) are contingent on cycle cancellations, cryopreservation strategy, and patient case-mix [1,2,3,4].

Mechanistically, the advantages of day-5 transfer are attributed to natural selection during extended culture (only the most developmentally competent embryos reach the blastocyst stage), improved morphologic and morphokinetic information for embryo selection, and potentially better embryo–endometrium synchrony within the implantation window; these factors underpin the rationale for elective single embryo transfer (eSET) when sufficient high-quality embryos are available, reducing multiple pregnancy risk without compromising cumulative outcomes [5,6,7,8,9,10].

However, extended culture has limitations: a non-negligible fraction of embryos may arrest before blastulation (reported up to 30–50% in some series), notably among patients with diminished ovarian reserve or poor oocyte quality, increasing fresh-cycle transfer cancellations and affecting CLBR and cost-effectiveness; thus, cleavage-stage transfer remains clinically justified in such subgroups [11,12,13].

Perinatal safety data are mixed: population studies and meta-analyses report modest increases in preterm birth and

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birth-weight differences after blastocyst transfer in certain contexts, but when controlling for fresh vs frozen transfer and other confounders these differences often attenuate; therefore, rigorous adjustment for clinical and laboratory variables is required when assessing perinatal risk [14,15,16,17].

Technological variables (culture media, incubator systems, vitrification protocols, embryology laboratory expertise) significantly influence D5 outcomes — high-quality laboratory practice amplifies the benefits of blastocyst transfer. Adjunctive embryo selection tools (time-lapse imaging, PGT-A) may further refine eSET strategies, but their routine role and net impact on CLBR and long-term offspring health require continued evaluation [18,19,20,21].

Finally, guideline recommendations (ASRM, ESHRE) generally endorse eSET in appropriate patients, yet national adoption must reflect local demographics, resource availability, and economic considerations. In countries with developing IVF programs (including Uzbekistan), local CLBR and perinatal outcome data are essential to adapt international best practices to the national context [2,4,22].

D5 transfer typically improves per-transfer pregnancy outcomes and facilitates safe implementation of eSET; nonetheless, individualized decision-making — accounting for embryo yield, patient prognosis, laboratory capacity, and regional factors — is required [22].

2. Materials and Methods

This retrospective study analyzed in vitro fertilization (IVF) protocols performed at one of the leading reproductive centers in Uzbekistan between 2023 and 2025 (first three months of each year were included). The study population consisted of women of reproductive age undergoing infertility treatment via IVF. The parameters evaluated included embryo transfer timing (day 3 or day 5), number of embryos transferred, frequency of elective single embryo transfer (eSET), and implantation rates.

1. Controlled Ovarian Stimulation (COS)

Patients underwent ovarian stimulation to promote multifollicular growth within a single cycle. Recombinant follicle-stimulating hormone (r-FSH) and/or human menopausal gonadotropin (hMG) were administered. Follicular dynamics were tracked via serial transvaginal ultrasound scans and serum estradiol (E2) assays. To avoid premature luteinizing hormone (LH) peaks, gonadotropin-releasing hormone (GnRH) agonists or antagonists were introduced.

2. Ovulation Induction

Final oocyte maturation was triggered using either human chorionic gonadotropin (hCG) or a GnRH agonist, depending on the assessed risk of ovarian hyperstimulation syndrome (OHSS). Oocyte retrieval was performed 34–36 hours later.

3. Oocyte Retrieval

Follicular aspiration was carried out under transvaginal ultrasound guidance using a specialized aspiration needle and intravenous sedation. Collected oocytes were immediately transported to the embryology laboratory.

4. Oocyte Maturity Evaluation

Each oocyte was examined under an inverted microscope to determine its nuclear status. Only mature oocytes at the metaphase II (MII) stage, identified by the presence of the first polar body, were selected for insemination.

5. Semen Processing

Semen samples were prepared by density gradient centrifugation and/or swim-up. In azoospermic cases, spermatozoa were surgically obtained (PESA, TESA, or micro-TESE) and subsequently processed.

6. Fertilization Procedures

Depending on clinical indications, two fertilization approaches were applied:

- *Conventional IVF*: co-incubation of mature oocytes with spermatozoa for 16–18 hours;
- *Intracytoplasmic Sperm Injection (ICSI)*: performed in cases of male infertility or previous IVF failure.

7. Embryo Culture

Zygotes were maintained in specialized sequential media under controlled culture conditions (5% O_2, 6% CO_2, 37 ℃). Embryos were cultured either up to the cleavage stage (Day 3) or extended to the blastocyst stage (Day 5–6), depending on their developmental potential and the clinical plan.

8. Embryo Evaluation

Embryos were examined daily. Cleavage-stage embryos were graded according to cell number, blastomere symmetry, degree of fragmentation, and multinucleation. Blastocyst grading followed Gardner's criteria (expansion, inner cell mass, trophectoderm).

9. Vitrification

Surplus viable embryos not transferred were cryopreserved using vitrification in liquid nitrogen (–196 ℃) with cryoprotectants (dimethyl sulfoxide, ethylene glycol). These embryos were later used in frozen-thawed cycles.

10. Embryo Transfer (ET**)**

ET was carried out under ultrasound guidance using a soft catheter. Both fresh and vitrified-thawed embryos were transferred. The transfer stage (Day 3 or Day 5) was determined by embryo quality and number. Elective single embryo transfer (eSET) was prioritized when high-quality blastocysts were available.

11. Luteal Phase Support

After ET, progesterone supplementation (oral, vaginal, or intramuscular) was prescribed until pregnancy confirmation and continued up to 10–12 weeks of gestation if successful.

12. Pregnancy Assessment

Serum β-hCG testing was performed 9–12 days after ET. If positive, transvaginal ultrasound was conducted 5–7 days later to confirm intrauterine pregnancy.

13. Study Groups

Participants were retrospectively classified into two groups according to the stage of embryo transfer:

- Group 1*:* cleavage-stage transfers (Day 3);
- Group 2*:* blastocyst transfers (Day 5).

Additionally, temporal trends in embryo transfer practice were evaluated, reflecting the gradual shift toward international recommendations and the promotion of eSET to minimize multiple pregnancy rates.

3. Results and Discussion

A retrospective analysis of embryo transfers performed in our clinic between 2022 and 2025 demonstrated a clear trend toward an increasing proportion of blastocyst-stage transfers, accompanied by a corresponding decline in Day 3 transfers.

In 2022, the majority of embryo transfers were carried out at the cleavage stage (35%), while blastocyst-stage transfers accounted for 65%. By 2023, a substantial shift was observed, with blastocyst transfers rising to 95%**,** reflecting a decisive move toward later-stage transfer in clinical practice. This upward trend continued in subsequent years, reaching 96% in 2024 and 97% in 2025. As a result, Day 3 transfers progressively decreased, representing only 3% of all embryo transfers by 2025.

Taken together, these results demonstrate a near-complete transition from cleavage-stage to blastocyst-stage transfer between 2022 and 2025, in line with global trends aimed at enhancing IVF efficiency and improving reproductive outcomes. **(Figure 1).**

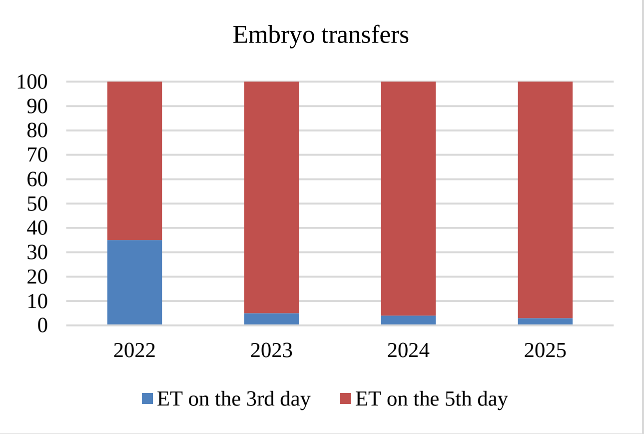

Figure 1. Frequency of Day 5 (blastocyst) and Day 3 (cleavage stage) embryo transfers

The findings of our study highlight substantial progress in embryo culture and selection practices at our clinic, fully consistent with international developments in assisted reproductive technologies (ART). The growing preference for blastocyst-stage transfer is determined by several factors that directly influence both the safety and clinical efficacy of IVF programs.

First, extended embryo culture to Day 5 allows for stricter morphological and functional selection, as only embryos with the highest developmental competence are able to reach the blastocyst stage. This improves implantation rates and clinical pregnancy outcomes. Second, blastocyst transfer ensures superior synchronization with the endometrial implantation window, further enhancing overall cycle efficiency.

A large body of international evidence confirms that blastocyst transfer is associated with higher live birth rates while requiring fewer embryos per transfer. This makes the elective single embryo transfer (eSET) strategy feasible and safe, significantly reducing the risk of multiple gestations and their associated complications. Consequently, blastocyst -stage transfer is now regarded as the “gold standard” in modern reproductive practice (ESHRE, ASRM).

Thus, the transformation of clinical practice toward routine blastocyst transfer reflects not only compliance with international guidelines but also the continuous advancement of our clinic’s laboratory and clinical capabilities, ultimately aimed at maximizing the likelihood of healthy pregnancies and favorable patient outcomes.

In addition, a retrospective evaluation of embryo transfer practices in IVF programs carried out at our clinic between 2022 and 2025 demonstrated a steady positive trend toward a higher proportion of elective single embryo transfers (eSET), alongside a progressive reduction in multiple embryo transfers.

Analysis of embryo transfer patterns in IVF programs between 2022 and 2025 demonstrated a marked shift in clinical practice, reflecting the progressive adoption of elective single embryo transfer (eSET).

In 2022, the proportion of eSET accounted for 29% of all cycles, while double embryo transfers represented 63%. Transfers of three embryos were observed in 6% of cases, and four embryos in 2%. By 2023, the share of eSET had risen to 47%, whereas three-embryo transfers declined to 2%, and four-embryo transfers were no longer performed (0%).

In 2024, eSET rates continued to increase, reaching 62%, while double embryo transfers decreased to 36%. Transfers involving three and four embryos were nearly eliminated from clinical practice, comprising only 2% and 0%, respectively.

By 2025, this trend had become even more pronounced: eSET was performed in 75% of cases, while double embryo transfers accounted for the remaining 25%. Transfers of three or more embryos were completely absent (0%).Overall, during the period 2022–2025, a steady transition toward predominant use of eSET was observed, fully consistent with international standards aimed at enhancing the safety of assisted reproductive technologies and reducing the incidence of multiple pregnancies **(Figure 2)**.

The findings of our analysis demonstrate a consistent shift in clinical practice toward modern standards of assisted reproductive technologies (ART), with an emphasis on elective single embryo transfer (eSET). Such a strategy corresponds to the global experience of leading reproductive medicine centers, including those in Sweden, Belgium, Japan, and Canada, where the share of eSET has already reached 70–80%.

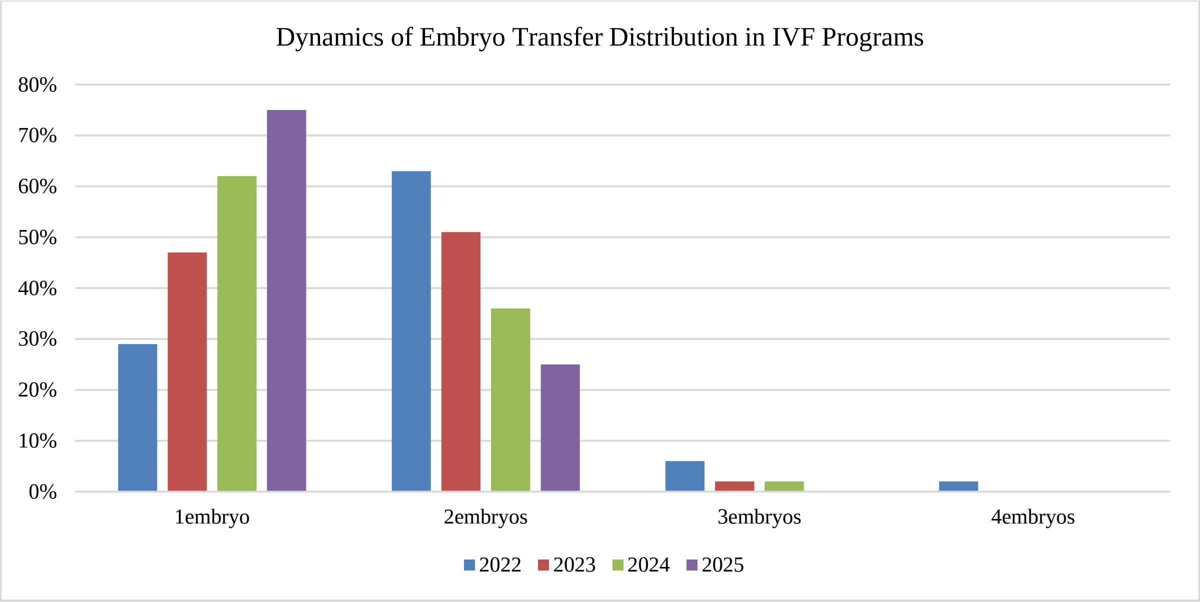

Figure 2. Trends in the frequency of single, double, and multiple embryo transfers from 2022 to 2025

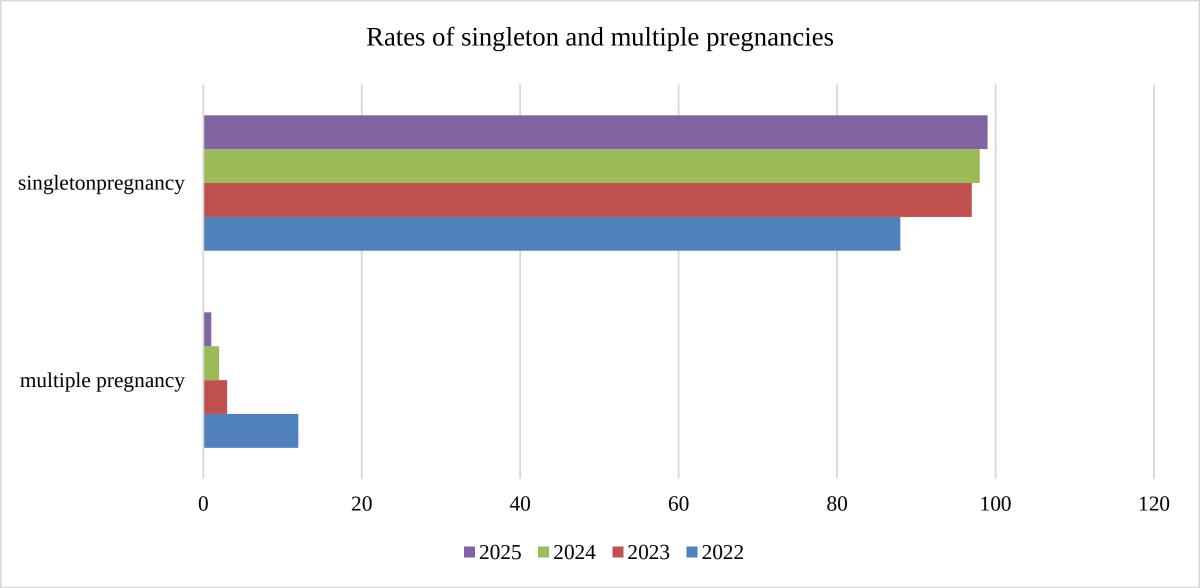

Figure 3. Rates of singleton and multiple pregnancies among couples undergoing IVF from 2022 to 2025

Evidence from numerous international studies confirms that transferring a single embryo substantially decreases the probability of multiple pregnancy and the associated risks, such as prematurity, impaired fetal growth, preeclampsia, and other adverse perinatal outcomes. At the same time, technological improvements—including extended culture of embryos to the blastocyst stage, the application of preimplantation genetic testing, and highly effective cryopreservation protocols—make it possible to maintain high pregnancy and live birth rates while transferring only one embryo.

Guidelines developed by professional 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) emphasize the importance of limiting the number of embryos transferred, particularly in younger patients with a favorable prognosis.

In our center, the gradual reduction in double and triple embryo transfers and the wider use of eSET were associated with a decline in the rate of multiple pregnancies (data presented in the corresponding section). Importantly, these changes did not negatively affect the overall efficiency of IVF programs, reflecting the high professional level of medical staff, reliable embryological support, and adherence to international recommendations.

In addition, the observed dynamics point to growing trust among patients in the eSET approach, supported by improved counseling practices and shared decision-making between physicians and patients.

Overall, the ongoing transformation of clinical strategy in our clinic is consistent with the global paradigm shift in ART, where priority is given to individualized treatment, patient safety, and the prevention of multiple pregnancies. The growing proportion of eSET corresponds with increased application of blastocyst-stage culture, which enables more accurate embryo selection with the highest implantation potential. Thus, the implementation of eSET strategies in our practice aligns with international standards aimed at improving perinatal health and minimizing obstetric and neonatal complications **(Figure 3).**

An analysis of pregnancy outcomes in our clinic over the period from 2022 to 2025 demonstrated a pronounced decline in the incidence of multiple pregnancies, accompanied by a steady increase in singleton pregnancies. In 2022, multiple pregnancies were observed in 12% of cases, while singleton pregnancies accounted for 88%. By 2023, the proportion of multiple pregnancies decreased to 3%, with singleton pregnancies reaching 97%. In 2024, multiple pregnancies were further reduced to 2%, while singleton pregnancies increased to 98%. Finally, in 2025, the rate of multiple pregnancies reached its lowest value of 1%, with singleton pregnancies comprising 99% of all clinical outcomes. These results clearly illustrate the effectiveness of strategies aimed at minimizing multiple gestations and promoting favorable perinatal outcomes.

Although national statistics in Uzbekistan demonstrate a steady rise in the rate of multiple pregnancies—primarily driven by the increasing use of assisted reproductive technologies (ART), delayed maternal age, and other socio-demographic shifts—the data from our clinic reveal a contrasting pattern.

The marked and consistent decline in multiple gestations observed in our practice can be explained by several crucial factors:

1. Adaptation of ART strategies. In recent years, elective single embryo transfer (eSET) has become more widely implemented in IVF cycles, significantly reducing the risks associated with multiple gestations.
2. Enhanced clinical regulations. Our clinic has deliberately adopted stricter policies to lower the frequency of multiple pregnancies due to their increased maternal and perinatal risks, fully in line with international guidelines (ASRM, ESHRE).

Hence, while multiple pregnancy rates continue to rise nationwide, our institutional approach has been specifically designed to minimize such outcomes, as evidenced by the presented clinical data. This trend reflects a carefully structured, evidence-based strategy aimed at improving maternal and neonatal safety.

Overall, these results highlight the continuous advancement of clinical embryology in our setting and the refinement of embryo transfer protocols, ensuring both treatment efficacy and patient well-being.

An analysis of embryo implantation rates over the period from 2022 to 2025 revealed a consistent and statistically significant advantage of blastocyst-stage (day 5) transfers compared to cleavage-stage (day 3) transfers.

Throughout the study period, implantation outcomes following blastocyst transfer remained consistently superior, despite some variation in annual results. In 2022, the implantation rate reached its peak at 78%, largely attributable to the transfer of multiple embryos within a single cycle. In subsequent years, a gradual decline was observed, with implantation rates decreasing to 75% in 2023, 72% in 2024, and 70% in 2025. Nevertheless, these values remained higher than those achieved with day-3 embryo transfers.

In contrast, implantation rates for cleavage-stage embryos during the same period were more stable, ranging narrowly between 60% and 63%. Thus, even with the modest reduction in blastocyst implantation rates by 2025, their outcomes continued to markedly surpass those of day-3 transfers, underscoring the clinical advantage of blastocyst-stage transfer as a preferred strategy **(Figure 4).**

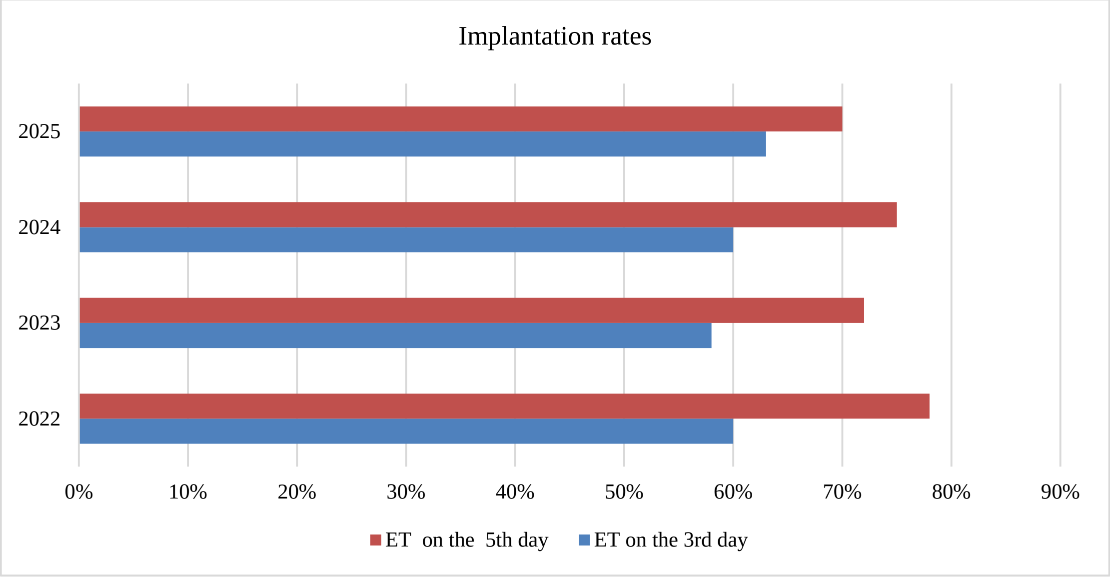

Figure 4. Implantation rates of embryos transferred at the cleavage stage (Day 3) and blastocyst stage (Day 5)

The observed differences in clinical outcomes between Day 3 and Day 5 embryo transfers can be attributed to several fundamental factors.

Firstly, extended in vitro culture to the blastocyst stage allows for a more stringent selection process, as developmental abnormalities that are not evident at the cleavage stage may manifest later, enabling the transfer of embryos with superior developmental potential.

Secondly, blastocysts represent a more advanced stage of embryonic development and are therefore more closely synchronized with the natural window of endometrial receptivity, which substantially increases the likelihood of implantation.

Finally, recent technological improvements—including optimized culture conditions, refined media formulations, and advanced incubator systems—have enhanced the viability of embryos maintained in culture until the blastocyst stage, thereby contributing to the overall success of IVF programs.

4. Conclusions

The results of this study highlight substantial progress in embryo culture and transfer strategies within assisted reproductive technology (ART) programs. The progressive decline in Day 3 cleavage-stage transfers and the transition toward Day 5 blastocyst-stage transfers reflect the integration of contemporary clinical standards aimed at improving both the efficacy and safety of IVF procedures.

Culturing embryos to the blastocyst stage enables more precise embryo selection, as only those with high developmental competence progress to this stage, which strongly correlates with both morphological and genetic quality. The transfer of such embryos significantly enhances implantation and pregnancy rates, while reducing the need for multiple transfers and minimizing the risk of multiple gestations—particularly when combined with elective single embryo transfer (eSET) strategies.

International recommendations (ESHRE, ASRM, Istanbul Consensus) emphasize the major advantages of blastocyst-stage transfer, including:

- improved cycle efficiency through enhanced embryo selection [Alpha Scientists in].
- better synchronization of the endometrial implantation window with embryonic development.
- the possibility of performing preimplantation genetic testing (PGT).
- reduction in the number of failed cycles.

The reduction of Day 3 transfers to only 4% by 2024 reflects the maturity of the laboratory environment, advancements in culture conditions, and optimized embryological logistics. This transformation underscores the trend toward individualized treatment, minimizing reproductive risks, and achieving consistent clinical outcomes even with single embryo transfer.

In summary, the adoption of blastocyst-stage transfer not only mirrors global trends in ART but also serves as a reliable marker of embryological quality within a given center. The presented findings demonstrate our clinic's strong alignment with international standards and its contribution to the optimization of both laboratory processes and clinical outcomes in infertility treatment.

REFERENCES

[1] Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev. 2016; (6): CD002118.

[2] Blake DA, Farquhar C, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. Cochrane Database Syst Rev. 2007; (4): CD002118.

[3] Martins WP, Nastri CO, Rienzi L, et al. Blastocyst vs cleavage -stage embryo transfer: systematic review and meta-analysis of reproductive outcomes. Ultrasound Obstet Gynecol. 2017; 49(5): 583–591.

[4] Kühlmann S, de Leeuw R, Tjon-Kon-Fat RI, et al. Blastocyst transfer versus cleavage stage embryo transfer: a systematic review and meta-analysis. Hum Reprod Open. 2021; 2021(4): hoab031.

[5] Gardner DK, Schoolcraft WB. In-vitro culture and transfer of human blastocysts: a practical approach. Hum Reprod Update. 1999; 5(5): 512–520.

[6] Pandian Z, Templeton A, Serour G, Bhattacharya S. Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database Syst Rev. 2015; (7): CD003416.

[7] Papanikolaou EG, Kolibianakis EM, Camus M, et al. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos. Hum Reprod. 2008; 23(1): 91–96.

[8] European Society of Human Reproduction and Embryology (ESHRE). ESHRE guideline: number of embryos to transfer during IVF/ICSI. Hum Reprod. 2024; 39(4): 647–680.

[9] ASRM Practice Committee. Guidance on the limits to the number of embryos to transfer: a committee opinion. Fertil Steril. 2021; 116(5): 1264–1272.

[10] Min JK, Hwang K, McLernon DJ, et al. Elective single embryo transfer following in vitro fertilization: systematic review. Hum Reprod Update. 2010; 16(5): 457–467.

[11] Fragouli E, Alfarawati S, Spath K, et al. Altered patterns of gene expression in human blastocysts from women of advanced maternal age. Hum Reprod. 2019; 34(2): 217–229.

[12] Racowsky C, Stern JE, Gibbons WE, Behr B, Pomeroy KO, Biggers JD. National collection of embryo morphology data into SART: associations among day-3 cell number, fragmentation and live birth rate. Fertil Steril. 2011; 95(7): 1985–1989.

[13] Xiao J, Rafique S, Gardner DK. When only one embryo is available, is it better to transfer at day 3? Reprod Biomed Online. 2019; 38(4): 421–428.

[14] Chambers GM, Hoang VP, Sullivan EA, et al. Risk of preterm birth after blastocyst embryo transfer: population-based study.

Fertil Steril. 2015; 103(4): 909–916.

[15] Dar S, Lazer T, Bider D, et al. Perinatal outcomes after blastocyst vs cleavage-stage transfer: a population analysis. Fertil Steril. 2014; 102(2): 372–378.

[16] Li Z, Wang YA, Ledger W, et al. Clinical outcomes following cryopreservation of blastocysts: comparison of vitrification and slow-freezing in a population study. Hum Reprod. 2014; 29(3): 667–674.

[17] Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Evidence of impaired endometrial receptivity after ovarian stimulation for IVF: randomized trial comparing fresh and frozen-thawed embryo transfer. Fertil Steril. 2011; 96(2): 344–348.

[18] Simopoulou M, Swennen G, Pantos K, et al. Considerations regarding embryo culture conditions and extended embryo culture. Reprod Biomed Online. 2018; 36(4): 405–419.

[19] Zou H, Wang S, Xiong F, et al. Blastocyst quality and reproductive and perinatal outcomes: a systematic review. Hum Reprod. 2023; 38(12): 2391–2406.

[20] Bourdon M, et al. The freeze-all strategy after IVF: indications and outcomes. Reprod Biomed Online. 2021; 43(1): 1–12.

[21] Viotti M, Gallo F, Paffoni A, et al. Preimplantation genetic testing for aneuploidy: current evidence and controversies. Genes (Basel). 2020; 11(6): 602.

[22] Marconi N, Källén K, Wang YA, et al. Perinatal outcomes in singleton live births after blastocyst-stage embryo transfer: retrospective analysis of large register data. Hum Reprod. 2023; 38(8): 1716–1725.