Acıbadem Univ. Sağlık Bilim. Derg. January, February, March 2026
https://doi.org/10.31067/acusaglik.1861882

ORIGINAL ARTICLE / ARAŞTIRMA YAZISI Obstetrics and Gynaecology/Kadın Hastalıkları ve Doğum

Obstetric and Neonatal Outcomes of Twin Pregnancies Following Double Embryo Transfer on Day 3 versus Day 5

Ahmet Kurt[1], Ayşe Gizem Yıldız[2], Dilara Sarikaya Kurt[3], Recep Taha Ağaoğlu[4], Ayberk Çakır[5], İnci Kahyaoğlu[2]

[1] Gemlik State Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye

[2] Etlik City Hospital, Department of Obstetrics and Gynecology, Ankara, Türkiye

[3] Mudanya State Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye

[4] Etlik City Hospital, Department of Perinatology, Ankara, Türkiye

[5] Acıbadem University Atakent Hospital, Department of Obstetrics and Gynecology, Istanbul, Türkiye

Ahmet Kurt
0000-0001-8402-5675

Ayşe Gizem Yıldız
0009-0001-6332-6724

Dilara Sarikaya Kurt
0000-0002-1492-0305

Recep Taha Ağaoğlu
0000-0002-6570-1006

Ayberk Çakır
0000-0001-5749-4556

İnci Kahyaoğlu
0000-0002-2283-9128

Correspondence: Ahmet Kurt
Gemlik State Hospital, Department of Obstetrics and Gynecology, Bursa, Türkiye
Phone: +90 505 396 49 41
E-mail: mflkurt@gmail.com

Received: 13.01.2026
Accepted: 23.01.2026

ABSTRACT

Background: Multiple pregnancies following assisted reproductive technologies (ART) remain a major contributor to perinatal morbidity and mortality. While the impact of embryo transfer day —cleavage-stage (day 3) versus blastocyst-stage (day 5)— has been extensively studied in singleton pregnancies, data focusing specifically on twin pregnancies resulting from double embryo transfer (DET) are limited. This study aimed to compare obstetric and neonatal outcomes of twin pregnancies following DET on day 3 (D3) versus day 5 (D5).

Methods: This retrospective single-centre cohort study included fresh ART cycles performed between 2010-2022. A total of 47 liveborn twin pregnancies following DET were analysed (D3: n = 28; D5: n = 19). Maternal demographic and clinical characteristics were recorded. Primary outcomes included gestational age at birth, birthweight, and composite adverse perinatal outcome (CAPO). Secondary outcomes included neonatal morbidities such as NICU admission, respiratory distress syndrome, sepsis, and neonatal mortality.

Results: Maternal baseline characteristics were comparable between groups. Gestational age at birth and birthweight did not differ significantly between D3 and D5 transfers ($p > 0.05$). The incidence of CAPO was similar in both groups (35.7% vs 39.5%, p=0.711). Rates of NICU admission, Apgar score ≤ 7 at 5 minutes, respiratory distress syndrome, and neonatal mortality were also comparable.

Conclusions: The day of double embryo transfer (D3 vs D5) was not associated with obstetric or neonatal outcomes in twin pregnancies. These findings suggest that limiting embryo number and appropriate patient selection are more critical than transfer day in reducing risks related to multiple pregnancies.

Keywords: Double embryo transfer; Twin pregnancy; Transfer day; Neonatal outcomes.

ÖZET

Giriş: Yardımcı üreme teknolojileri (ART) sonrası çoğul gebelikler, perinatal morbidite ve mortalitenin önemli bir nedeni olmaya devam etmektedir. Tekil gebeliklerde embriyo transfer gününün —bölünme aşaması (3. gün) ile blastosist aşaması (5. gün)— etkisi kapsamlı bir şekilde incelenmiş olsa da, iki embriyo transferi (DET) sonucu oluşan ikiz gebeliklere odaklanan veriler sınırlıdır. Bu çalışma, 3. gün (D3) ve 5. gün (D5) DET sonrası ikiz gebeliklerin obstetrik ve neonatal sonuçlarını karşılaştırmayı amaçlamıştır.

Yöntemler: Bu retrospektif tek merkezli kohort çalışması, 2010-2022 yılları arasında gerçekleştirilen taze ART döngülerini içermektedir. DET sonrası toplam 47 canlı doğan ikiz gebelik analiz edilmiştir (D3: n = 28; D5: n = 19). Birincil sonuçlar arasında doğumdaki gebelik yaşı, doğum ağırlığı ve bileşik perinatal olumsuz sonuçlar (CAPO) yer almıştır. İkincil sonuçlar arasında NICU yatışı, solunum sıkıntısı sendromu, sepsis ve yenidoğan mortalitesi gibi yenidoğan morbiditeleri yer almıştır.

Bulgular: Maternal bazal parametreler, gruplar arasında karşılaştırılabilir düzeydeydi. Doğumdaki gebelik yaşı ve doğum ağırlığı, D3 ve D5 transferleri arasında anlamlı bir fark göstermedi ($p > 0{,}05$). CAPO insidansı her iki grupta da benzerdi (35,7% vs 39,5%, p=0,711). NICU yatış oranları, 5 dakikada Apgar skoru ≤ 7, Respiratuvar Distres sendromu ve yenidoğan mortalitesi oranları da benzerdi.

Sonuçlar: İkiz gebeliklerde çift embriyo transferinin yapıldığı gün (D3 ve D5) obstetrik veya yenidoğan sonuçları ile ilişkili değildi. Bu bulgular, çoklu gebeliklerle ilişkili riskleri azaltmada embriyo sayısını sınırlamak ve uygun hasta seçimi yapmanın transfer gününden daha önemli olduğunu göstermektedir.

Anahtar kelimeler: İki embriyo transferi; İkiz gebelik; Transfer günü; Yenidoğan sonuçları.

Infertility is a public health issue with economic, demographic, psychological and medical consequences. According to data from the World Health Organisation (WHO), it affects approximately 10–15% of couples of reproductive age worldwide (1). Along with the increasing incidence of infertility over the years, the rise in the use of assisted reproductive technologies (ART) has increased implantation/pregnancy rates as well as the incidence of multiple pregnancies. This is consistently associated with adverse maternal and perinatal outcomes, such as preterm labour, low birth weight, and admission to the neonatal intensive care unit (NICU), compared to singleton pregnancies (2–4). These risks have led to the development of embryo transfer strategies aimed at minimising multiple pregnancies without compromising live birth rates (5).

Elective single embryo transfer (eSET) is known to maintain pregnancy rates in patients with a favourable prognosis while significantly reducing the risk of multiple pregnancies (6–8). Guidelines support eSET in suitable candidates (9); however, in certain clinical circumstances and depending on national regulations, double embryo transfer (DET) continues to be performed. In this context, the day of transfer has emerged as another potentially important variable. In clinical practice, there is a growing tendency to prefer blastocyst stage (day 5, D5) transfer over cleavage stage (day 3, D3) transfer (10). It is thought that reaching day 5 helps in selecting chromosomally healthy embryos (11,12). However, the evidence is heterogeneous and may depend on patient selection and laboratory performance. Most comparative studies between D3 and D5 transfers have focused on singleton outcomes or mixed cohorts without separating multiple pregnancies after DET. Consequently, data specific to multiple pregnancies after DET remain limited, particularly regarding obstetric and neonatal outcomes such as gestational age at delivery, birth weight, Apgar scores, NICU admission, and composite perinatal outcomes (6,7). Clarifying whether the transfer day (D3 and D5) is associated with these clinically meaningful outcomes in twins could inform counselling and policy decisions regarding embryo transfer strategies when DET results in multiple pregnancies. Therefore, we aimed to compare obstetric and neonatal outcomes in twin pregnancies resulting from double embryo transfer between days 3 and 5 in ART cycles.

Materials and Methods

This retrospective, single-centre cohort study selected ART cycles in which double embryo transfer (DET) was performed on day 3 (cleavage, D3) or day 5 (blastocyst, D5) at the Assisted Reproductive Treatment Centre (ART Centre) of Etlik Zübeyde Hanım Training and Research Hospital between 2010 and 2022. Data from a total of 1121 cycles meeting the inclusion criteria were retrospectively reviewed from files and the computer system. The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Training and Research Hospital prior to commencement (Decision no: 22.09.2022-12-14) and was conducted in accordance with the principles of the Declaration of Helsinki.

Study Population and Eligibility

Treatment processes and embryo transfer numbers at our centre were conducted in accordance with the Turkish Ministry of Health regulations (13). In the classification of pregnancy status, β-hCG >10 IU/L positivity was considered pregnancy; cases with only β-hCG positivity and no ultrasound findings were considered biochemical pregnancies, while clinical pregnancy was accepted according to the ESHRE (European Society of Human Reproduction and Embryology) definition as confirmation of an intrauterine gestational sac by ultrasound or histopathological evidence (14); Live birth was defined as the recording of a baby as alive according to WHO criteria, demonstrating respiration, heartbeat, umbilical cord pulsation, or voluntary muscle movement after delivery (15). Neonatal mortality was defined as death occurring during the neonatal period among liveborn infants and was assessed and reported at the neonate level based on hospital neonatal records.

Twin pregnancies were confirmed ultrasonographically according to standard definitions, and neonatal outcomes were assessed at the newborn level in all twins born alive from appropriate pregnancies. Chorionicity was determined by ultrasonographic examination during the first trimester based on standard criteria. Monochorionicity was diagnosed in the presence of a single placental mass and with the “T-sign” or absence of the “lambda (twin peak) sign,” whereas dichorionicity was defined by the visualization of two separate placentas or the presence of the “lambda sign.” Cases with uncertain chorionicity or confirmed monochorionicity were excluded from the analysis.

Inclusion Criteria

The study included women aged 18–45 years who underwent double embryo transfer (DET) during fresh ART cycles conducted at Etlik Zübeyde Hanım Training and Research Hospital between 2010 and 2022, with the transfer day determined as day 3 (D3) or day 5 (D5), and cases with complete maternal data were followed up until delivery and perinatal outcomes, in which twin pregnancy was confirmed ultrasonographically after DET.

Exclusion Criteria

The following were excluded from the study: patients under 18 or over 45 years of age, frozen/thawed (FET) cycles, transfer strategies other than double embryo transfer (DET) (single embryo or ≥3 embryos), transfers performed on days other than day 3 (D3) and day 5 (D5), and cycles that did not result in ultrasound-confirmed twin pregnancies (singleton pregnancies, triplets and above, or pregnancies that became singleton after selective reduction) were excluded. Pregnancies that did not reach clinical viability or live birth (biochemical, ectopic, anembryonic; spontaneous miscarriage in multiple pregnancies) and cases lost to follow-up or with incomplete perinatal records were also excluded. Additionally, pregnancies that were initially triplets/quadruplets but were reduced to twins through selective or spontaneous reduction, as well as monochorionic twin cases, were excluded from the study.

Parameters and Analyses

The exposure variable of the study was the transfer day, and DETs resulting in twin pregnancies were divided into two groups: D3 (cleavage) and D5 (blastocyst). The demographic and clinical characteristics, baseline ultrasound and laboratory parameters, and pregnancy outcomes of the patients were obtained from the records.

The primary outcomes were gestational age at birth (weeks), birthweight (grams) and composite adverse perinatal outcome (CAPO). CAPO evaluated at the neonate level and defined as the presence of at least one of the following: admission to NICU, 5-minute Apgar ≤7, need for invasive mechanical ventilation, or neonatal death. Each CAPO component was also examined individually. One- and five-minute Apgar scores were reported descriptively; only 5-minute Apgar <7 contributed to CAPO.

Secondary outcomes included neonatal morbidities: oxygen requirement, need for invasive mechanical ventilation (non-invasive support reported descriptively), respiratory distress syndrome (RDS), phototherapy, surfactant use, total parenteral nutrition (TPN), culture-proven sepsis, intraventricular haemorrhage (IVH), necrotising enterocolitis (NEC), retinopathy of prematurity (ROP), and neonatal death.

IVF/ET Protocol

At our hospital, controlled ovarian stimulation within the scope of assisted reproductive techniques is individualised according to the patient's clinical characteristics and expected ovarian response. For this purpose, the GnRH agonist long protocol, GnRH antagonist protocol, or microdose flare-up agonist protocol is applied. The GnRH agonist long protocol is usually preferred in young, responsive cases with normal ovarian reserve, while the GnRH antagonist protocol is preferred in cases of polycystic ovary syndrome (PCOS) and in patients at high risk of ovarian hyperstimulation syndrome (OHSS) due to its shorter treatment duration and reduced risk of OHSS. The antagonist protocol is also considered appropriate for cases with low ovarian reserve (DOR).

In ovarian stimulation, recombinant FSH [e.g. Gonal-f (Merck Serono) or Puregon (Organon)] is used alone or in combination with human menopausal gonadotropin (hMG) [e.g. Menogon (Ferring) or Merional (IBSA)]. The starting dose is planned to be 150–450 IU daily, taking into account age, body mass index (BMI) and antral follicle count (AFC), and is adjusted during the stimulation process according to the clinical response. For pituitary suppression, leuprolide acetate [Lucrin (Abbott)] is used in the agonist long protocol, cetrorelix acetate [Cetrotide (Merck Serono)] in the antagonist protocol, and a low-dose GnRH agonist is preferred in the microdose flare-up protocol.

From the 5th day of stimulation, patients are monitored by transvaginal ultrasound; serum LH, progesterone, and oestradiol (E2) levels are also assessed, and the gonadotropin dose is titrated according to the current response. When at least three follicles with an average diameter ≥18 mm are observed, recombinant hCG 250 µg [Ovitrelle, Merck Serono] is administered for oocyte maturation. Approximately 36 hours after the hCG injection, oocyte retrieval (OPU) is performed under transvaginal ultrasound guidance, and the retrieved oocytes are fertilised by intracytoplasmic sperm injection (ICSI).

Embryo transfer is performed on day 3 (D3) or day 5 (D5) after oocyte retrieval, guided by transabdominal ultrasound and with a full bladder. All patients receive luteal phase support starting on the day of OPU and continuing until the β-hCG test. For luteal support, 100 mg of intramuscular progesterone [Progestan, Koçak] or 8% vaginal progesterone gel [Crinone, Merck Serono] is used daily. In β-hCG positive cases, luteal support is continued until the 10th–12th week of pregnancy. β-hCG measurement is performed 14 days after OPU (~11th day for D3 transfer, ~9th day for D5 transfer).

The decision regarding embryo transfer on day 3 or day 5 was made according to routine clinical practice at our centre, based on embryo developmental characteristics, the number and quality of embryos available, previous ART history, and clinician judgement. No randomization protocol was applied, and the transfer day reflected individualized, routine clinical decision-making.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation or median (min–max), and categorical variables were presented as numbers and percentages. Comparisons between groups were made using the Student's t-test or Mann–Whitney U test for continuous variables and the Chi-square or Fisher's exact test for categorical variables. A p value < 0.05 was considered statistically significant.

Results

A total of 1,121 ART cycles in which double embryo transfer (DET) was performed were reviewed retrospectively. Pregnancy was achieved in 459 cycles. Of these, 4 were ectopic and 62 were biochemical pregnancies, yielding 393 clinical pregnancies. Among clinical pregnancies, 323 were singleton and 70 were multiple gestations. Fourteen multiple gestations were excluded owing to insufficient follow-up. Of the remaining 70, 10 ended in spontaneous abortion and 4 were lost to follow-up, leaving 56 multiple gestations with accessible outcome data. Among these, 2 were anembryonic twin pregnancies and 5 involved intrauterine demise of one twin. Three triplet pregnancies were excluded (one spontaneous termination, one post-reduction singleton, and one anembryonic gestation). The final analysis comprised 47 liveborn twin pregnancies (D3: n=28; D5: n=19). **Figure 1** and **Figure 2** depict the selection flow.

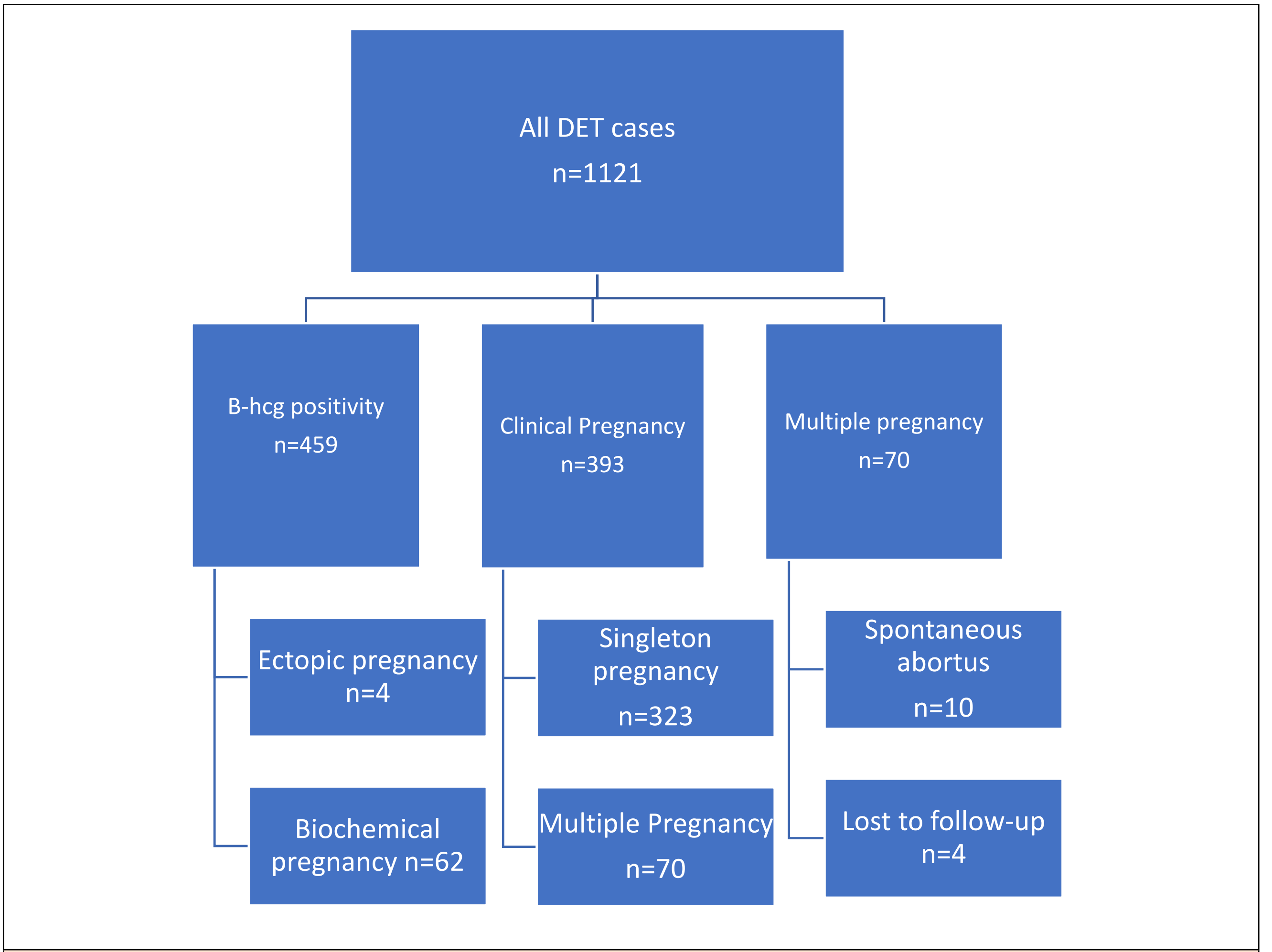

Figure 1: Flowchart of the study population and pregnancy outcomes following double embryo transfer (DET).
The chart illustrates the distribution of all DET cases (n=1121), showing β-hCG positivity, clinical pregnancies, and subsequent categorization into singleton and multiple pregnancies, with exclusion of biochemical, ectopic, and lost-to-follow-up cases.

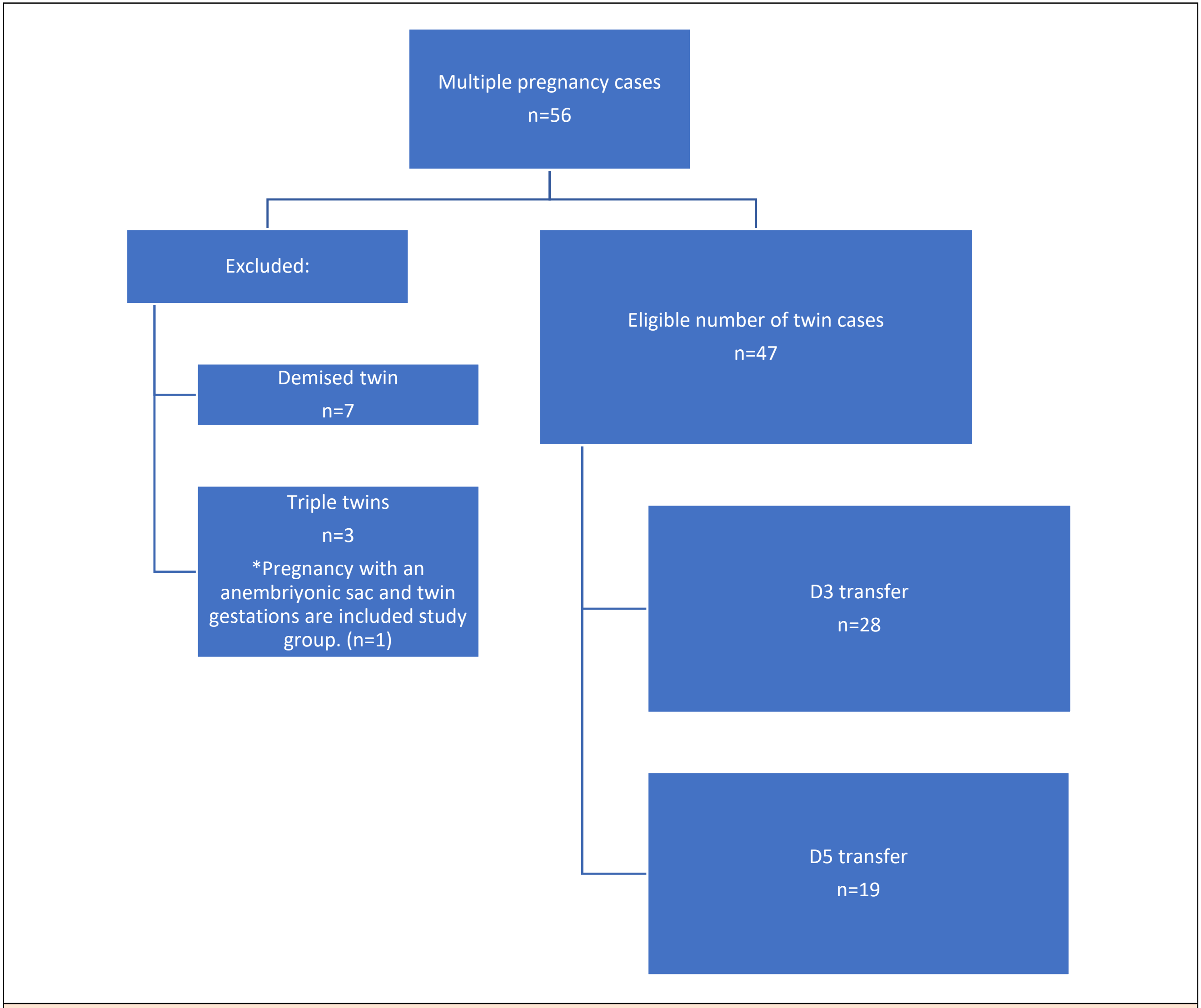

Figure 1: Selection process of twin pregnancies included in the final analysis.
The diagram shows exclusion of cases with a demised twin or triple gestation, resulting in 47 eligible twin pregnancies divided into day 3 (D3, n=28) and day 5 (D5, n=19) transfer groups.

The participants' demographic, clinical, and laboratory characteristics are shown in **Table 1**. There were no significant differences between the D3 and D5 groups in age, gravida, parity, abortus, BMI, or duration of infertility ($p > 0.05$ for all). The distribution of infertility etiologies (male factor, tubal factor, diminished ovarian reserve, ovulatory dysfunction, unexplained infertility) was likewise comparable ($p > 0.05$). Among ultrasound and laboratory measures, estradiol (E2) levels were lower in the D5 group compared with the D3 group ($p = 0.010$), whereas AFC, AMH, FSH, and LH showed no between-group differences.

The neonatal outcomes of the groups are presented in **Table 2.** All parameters, including CAPO, were analysed at the neonate level. Gestational age at birth and birth weight did not differ significantly between D3 and D5. Apgar scores at 1 and 5 minutes and the proportion with 5-minute Apgar ≤7 were similar in the two groups ($p > 0.05$ for all). Rates of NICU admission, oxygen requirement, invasive mechanical ventilation, and surfactant use were comparable, as were the occurrences of respiratory distress syndrome (RDS), sepsis, intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), and neonatal mortality ($p > 0.05$ for all). The incidence of the composite adverse perinatal outcome (CAPO) was also not different between groups (35.7% vs 39.5% for D3 and D5, respectively; $p = 0.711$).

Table 1. Comparison of Demographic, Clinical, and Laboratory Parameters Between Patients Who Underwent Day 3 and Day 5 Embryo Transfer Following IVF.

Variables	D3 Transfer (n:28)	D5 Transfer (n:19)	p-value
Demographic Characteristics			
Age (years)	32.6 ± 4.3	34.1 ± 3.9	0.246[a]
Gravida	1 (0-1)	1 (0-2)	0.431[b]
Parity	0 (0-0)	0 (0-0)	0.607[b]
Abortus	0 (0-1)	0 (0-1)	0.407[b]
BMI (kg/m^2)	26.67 ± 4.97	26.73 ± 3.28	0.961[a]
Duration of Infertility (months)	46 (36-90)	72 (42-96)	0.307[b]
Cause of Infertility			
Male factor (%)	8 (28.6%)	3 (15.8%)	0.485[c]
Tubal factor (%)	2 (7.1%)	1 (5.3%)	>0.99[c]
Diminished ovarian reserve (%)	12 (42.9%)	6 (31.6%)	0.435[c]
Ovulatory dysfunction (%)	23 (82.1%)	13 (68.4%)	0.276[c]
Unexplained infertility (%)	11 (39.3%)	9 (47.4%)	0.582[c]
Ultrasound and Laboratory Parameters			
Antral Follicle Count	14.0 ±8.8	13,9 ±10.2	0.994[a]
AMH (ng/mL)	2.10 (0.98-5.77)	1.79 (0.91-4.45)	0,735[b]
FSH (mIU/ml)	7,27 ±2,38	7,65 ±3,68	0.675[a]
LH (IU/L)	5,07 ±2,06	5,47 ±2,59	0.559[a]
E2 (pg/ml)	52,67 ± 18,80	39,14 ±13,98	**0.010[a]**

[a] Independent samples t-test was used for comparisons between groups. Data are presented as mean ± standard deviation.
[b] The Mann–Whitney U test was used for comparisons between groups. Data are presented as median (interquartile range).
[c] Categorical variables were compared using the chi-square or Fisher's exact test, as appropriate. Results are shown as n (%).
Abbreviations: IVF, in vitro fertilization; BMI, body mass index; AMH, anti-Mullerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol.

Table 2. Comparison of Neonatal Outcomes Between Day 3 and Day 5 Embryo Transfer Groups in Twin Pregnancies.

Variables	D3 Transfer (n=56)	D5 Transfer (n=38)	p-value
Gestational age at birth (weeks)	35 (32-36)	35 (34-36)	0.586[a]
Birth weight (g)	2238 (1688-2395)	2170 (1750-2485)	0.820[a]
1st minute Apgar score	9 (8-9)	9 (7-9)	0.516[b]
5th minute Apgar score	10 (9-10)	10 (8-10)	0.535[b]
5th minute Apgar Score ≤7 (n,%)	9 (16.1%)	7 (18.4%)	0.766[b]
CAPO, n (%)	20 (35.7%)	15 (39.5%)	0.711[b]
NICU admission, n (%)	20 (35.7%)	13 (34.2)	0.881[b]
Secondary neonatal outcomes			
Oxygen requirement, n (%)	15 (26.8%)	13 (34.2%)	0.440[b]
Invasive mechanical ventilation requirement, n (%)	8 (14.3%)	3 (7.9%)	0.516[b]
RDS, n (%)	6 (10.7%)	4 (10.5%)	>0.99[b]
Phototherapy requirement, n (%)	7 (12.5%)	4 (10.5%)	>0.99[b]
Surfactant requirement, n (%)	3 (5.4%)	3 (7.9%)	0.683[b]
TPN requirement, n (%)	5 (8.9%)	5 (13.2%)	0.519[b]
Sepsis, n (%)	3 (5.4%)	1 (2.6%)	0.645[b]
IVH, n (%)	1 (1.8%)	1 (2.6%)	>0.99[b]
NEC, n (%)	-	2 (5.3%)	0.161[b]
ROP, n (%)	-	2 (5.3%)	0.161[b]
Neonatal death, n (%)	4 (7.1%)	2 (5.3%)	>0.99[b]

[a] The Mann–Whitney U test was used for comparisons between groups. Data are presented as median (interquartile range).
[b] Categorical variables were compared using the chi-square or Fisher's exact test, as appropriate. Results are shown as n (%).
Abbreviations: NICU, neonatal intensive care unit; RDS, respiratory distress syndrome; TPN, total parenteral nutrition; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; CAPO, composite adverse perinatal outcome.

Discussion

This study evaluated the relationship between the day of transfer (D3 vs D5) and the main obstetric and neonatal outcomes in twin pregnancies resulting from double embryo transfer (DET). Our findings show no significant difference between the D3 and D5 groups in terms of gestational age at birth, birth weight, Apgar scores, NICU admission, and other neonatal morbidities. Although maternal baseline parameters were generally similar, the E2 level was found to be lower in the D5 group.

Previous studies have shown that maternal age, ovarian reserve, and embryo quality, as well as local regulations and guidelines, are decisive factors in determining the number of embryos. The literature emphasises that DET increases clinical pregnancy rates (CPR) but also raises multiple pregnancy rates (MPR) (16). In our series, the clinical multiple pregnancy rate was 70/393 = 17.8% (rate within clinical pregnancies), and when cases with unknown outcome information and abortions were excluded, the multiple live birth rate (LBR) was 47/1121 = 4.2%. These rates indicate a lower multiple pregnancy burden compared to some series where DET was applied; we believe this may be due to differences in patient selection, age distribution, and centre protocols.

In a randomised controlled trial comparing sequential SET with DET in cases with two or more implantation failures, similar results were reported in terms of biochemical/clinical pregnancy, early pregnancy loss, multiple pregnancy, and implantation rates (17). This finding supports the use of DET in selected cases with ≥2 failed IVF cycles in our clinic and our lower multiple pregnancy rates compared to those reported in the literature. Recent studies have reported that double embryo transfer, particularly with good-quality blastocysts, may increase the risk of clinical pregnancy loss (CPL), even if it results in a singleton birth (12,18). This observation suggests that the potential disadvantages of multiple blastocyst transfers should be considered alongside their possible advantages; it has been suggested that in cases leading to singleton births after DET, the early loss of an embryo may affect the uterine environment and the course of the remaining embryo. In the context of transfer day, the literature shows that the blastocyst policy does not increase cumulative live births, but it does increase the live birth rate after fresh transfer, reduces cumulative pregnancy loss, and decreases the average number of transfers required to achieve a live birth (1.55 vs 1.82; $p<0.001$) (19). These findings suggest that D5 transfers may improve treatment efficiency, leading to success with fewer embryo transfers; thus, they may indirectly contribute to reducing multiple pregnancies by limiting the number of embryos transferred. However, it should be noted that patient selection in these studies favoured younger patients with a good prognosis. Indeed, although it has been suggested in the literature that D5 transfer may reduce multiple pregnancy rates (due to better embryo selection), a significant proportion of twin pregnancies were observed in both the D5 and D3 groups in our cohort. This study supports the view, emphasised by Cornelisse et al. (20), that the most effective way to reduce multiple pregnancies is to limit the number of embryos (SET) and strictly adhere to patient selection criteria (21). Furthermore, there are studies reporting that D5 does not have a clear advantage over D3 in patients undergoing SET (22). Although the overall outcomes of ART pregnancies are favourable, multiple pregnancy itself increases the risk of preterm birth, low birth weight, placental abnormalities, ectopic pregnancy, and pregnancy-related hypertensive disorders. Although our study did not find a difference between D3 and D5 in terms of gestational age and birth weight, this issue is debated in the literature: Many studies have reported similar obstetric/neonatal outcomes regardless of transfer day (23,24); on the other hand, there are also publications reporting differences according to transfer day, particularly in terms of preterm labour (<37 weeks) and reduced birth weight (25,26). For example, a large cohort study from Canada (n=12,712 singleton births) showed that blastocyst transfer may increase the risk of preterm birth (27). However, considering that these studies focused on singleton pregnancies and that multiple pregnancies already carry a high baseline risk, it should be noted that the findings in our twin cohort may naturally differ from this literature. We did not observe any significant differences between twins born on D3 and D5 across a wide range of outcomes, including 1- and 5-minute Apgar scores, NICU admission, RDS, phototherapy, surfactant, sepsis, IVH, NEC, ROP, and neonatal mortality; this suggests that the transfer day is not a determinant of neonatal outcomes in multiple pregnancies. The fact that blood parameters were largely similar in both groups supports the homogeneity of the groups. The lower E2 level detected in the D5 group is consistent with findings in the literature indicating a tendency for lower E2 levels in D5 transfers and a possible decrease in implantation/clinical pregnancy rates, particularly at high E2 levels (28).

The strengths of this study include reducing confusion related to chorionicity by excluding monochorionic twins and pregnancies that were reduced to twins, evaluating results at the newborn level, and providing detailed reporting of centre protocols. To our knowledge, this study, which is the first to directly compare the transfer day (D3 vs D5) in twin pregnancies resulting from DET, provides an innovative and original contribution to the literature and adds value to clinical decision-making processes.

This study has several limitations that should be acknowledged. First, its retrospective, single-centre design and possibility of selection bias. Second, the relatively small sample size – particularly in the D5 group – reflects the low incidence of eligible liveborn dichorionic twin pregnancies after strict exclusion criteria and may increase the risk of type II error, especially for rare neonatal outcomes such as necrotizing enterocolitis, retinopathy of prematurity, and neonatal death. Accordingly, non-significant findings should be interpreted as exploratory rather than confirmatory. In addition, the choice of embryo transfer day is a multifactorial clinical decision influenced by hormonal parameters, embryo development and quality, laboratory conditions, and clinician judgement, none of which could be fully controlled for in this retrospective analysis, resulting in potential residual confounding. Furthermore, the extended study period encompasses substantial advancements in assisted reproductive technologies, embryology laboratory practices, and embryo culture systems, which may have introduced temporal variability that could not be fully accounted for. Additionally, improvements in neonatal intensive care practices, supportive technologies, and clinical protocols over the study period may have influenced neonatal morbidity and survival outcomes and could not be fully adjusted for in this retrospective analysis. Accordingly, the findings of this study should be interpreted as preliminary and hypothesis-generating rather than as definitive evidence.

Finally, neonatal outcomes were limited to the early postnatal period, and long-term neonatal, childhood, and neurodevelopmental outcomes could not be evaluated. Future multicentre, prospective studies with larger sample sizes and long-term follow-up are warranted to validate and extend these findings. In summary, the transfer day (D3 vs D5) does not appear to be associated with primary neonatal outcomes in twin pregnancies following DET. The most effective strategy for reducing multiple pregnancies, consistent with the literature, is embryo number limitation (SET) and careful patient selection (9,29,30). These findings support the importance of focusing on embryo numbers rather than transfer days in clinical counselling.

Conclusion

In conclusion, our study differs from many studies in the literature in that it directly evaluates twin pregnancies obtained after double embryo transfer (DET) according to the transfer day. Our findings reveal that the embryo transfer day (D3 or D5) is not a determining factor in neonatal outcomes in twin pregnancies. This original contribution emphasises once again that the main strategy for reducing perinatal risks in multiple pregnancies is the correct placement of the number of embryos and careful patient selection rather than the day of embryo transfer. However, the results need to be confirmed by multicentre, prospective studies that also include subgroup analyses.

Acknowledgements

The authors would like to express their gratitude to the medical staff and research team at Etlik Zübeyde Hanım Women's Health Training and Research Hospital for their valuable support in data collection and study coordination. We also appreciate the efforts of all participants who contributed to this research.

Authors' contributions

A.K: Manuscript writing, Methodology; A.G.Y.: Manuscript writing, Literature research; D.S.K.: Manuscript editing, Data curation, Conceptualization; R.T.A.: Data analysis, Validation; A.Ç: Investigation, Resources; İ.K: Supervision, Protocol management.

Funding

This research received no specific grant from any funding agency.

Data availability

Due to hospital policies, patient data and study materials cannot be shared. However, the data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (Decision no: 22.09.2022-12-14). Written informed consent was obtained from all participants.

Consent for publication: Not applicable.

Conflict of Interest: The authors declare no conflict of interests.

References

1. Infertility Prevalence Estimates, 1990-2021. World Health Organisation. April 2023. https://www.who.int/publications/i/item/978920068315
2. Thurin A, Hausken J, Hillensjö T, Jablonowska B, Pinborg A, Strandell A, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. N Engl J Med. 2004 Dec 2;351(23):2392–402.
3. Wang Y, Shi H, Chen L, Zheng D, Long X, Zhang Y, et al. Absolute Risk of Adverse Obstetric Outcomes Among Twin Pregnancies After In Vitro Fertilization by Maternal Age. JAMA Netw Open. 2021 Sep 1;4(9):e2123634.
4. Lang M, Zhou M, Lei R, Li W. Comparison of pregnancy outcomes between IVF-ET pregnancies and spontaneous pregnancies in women of advanced maternal age. J Matern Fetal Neonatal Med. 2023 Dec;36(1):2183761.
5. Rodriguez-Wallberg KA, Palomares AR, Nilsson HP, Oberg AS, Lundberg F. Obstetric and Perinatal Outcomes of Singleton Births Following Single- vs Double-Embryo Transfer in Sweden. JAMA Pediatr. 2023 Feb 1;177(2):149–59.
6. Stillman RJ, Richter KS, Banks NK, Graham JR. Elective single embryo transfer: a 6-year progressive implementation of 784 single blastocyst transfers and the influence of payment method on patient choice. Fertil Steril. 2009 Dec;92(6):1895–906.
7. Mullin CM, Fino ME, Talebian S, Krey LC, Licciardi F, Grifo JA. Comparison of pregnancy outcomes in elective single blastocyst transfer versus double blastocyst transfer stratified by age. Fertil Steril. 2010 Apr;93(6):1837–43.
8. Mersereau J, Stanhiser J, Coddington C, Jones T, Luke B, Brown MB. Patient and cycle characteristics predicting high pregnancy rates with single-embryo transfer: an analysis of the Society for Assisted Reproductive Technology outcomes between 2004 and 2013. Fertil Steril. 2017 Nov;108(5):750–6.
9. Practice Committee of the American Society for Reproductive Medicine and the Practice Committee for the Society for Assisted Reproductive Technologies. Electronic address: ASRM@asrm.org. Guidance on the limits to the number of embryos to transfer: a committee opinion. Fertil Steril. 2021 Sep;116(3):651-4.
10. Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Electronic address: asrm@asrm.org. Blastocyst culture and transfer in clinically assisted reproduction: a committee opinion. Fertil Steril. 2018 Dec;110(7):1246–52.
11. Zech NH, Lejeune B, Puissant F, Vanderzwalmen S, Zech H, Vanderzwalmen P. Prospective evaluation of the optimal time for selecting a single embryo for transfer: day 3 versus day 5. Fertil Steril. 2007 Jul;88(1):244–6.
12. Samaher Alfaraj FA, Alzaher F, Alshwaiaer S, Ahmed A. Pregnancy outcome of day 3 versus day 5 embryo transfer: A retrospective analysis. Asian Pacific Journal of Reproduction, 2017, 6(2): 89-92.
13. Regulation on Assisted Reproductive Treatment Practices and Assisted Reproductive Treatment Centers, published by the Ministry of Health of the Republic of Turkey in the Official Gazette dated March 6, 2010, issue no. 27513.
14. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. Hum Reprod. 2017 Sep 1;32(9):1786–801.
15. International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Volume 2, Instruction Manual, World Health Organization, Geneva, 1993
16. Dietrich JE, Cáceres Valcárcel I, Capp E, Strowitzki T, Germeyer A. High multiple pregnancy rates after double embryo transfers in human: a retrospective cohort study. Reprod Fertil. 2025 Apr 1;6(2):e240078.
17. Hatamizadeh N, Eftekhar M, Aminimajomerd Z, Moeinaddini S. Comparison of double blastocyst transfer versus sequential transfer on pregnancy outcomes in individuals with frozen embryo transfer and a history of recurrent implantation failure: An RCT. Int J Reprod Biomed. 2025 Apr;23(4):313–22.
18. Wang Y, Wan Q, Lu X, Li L, Wang H, Chen L, et al. The transfer of double morphologically good Day 5 blastocysts increases the risk of clinical pregnancy loss in singleton pregnancies following frozen-thawed embryo transfer. Front Endocrinol (Lausanne). 2025;16:1508014.
19. Cornelisse S, Fleischer K, van der Westerlaken L, de Bruin JP, Vergouw C, Koks C, et al. Cumulative live birth rate of a blastocyst versus cleavage stage embryo transfer policy during in vitro fertilisation in women with a good prognosis: multicentre randomised controlled trial. BMJ. 2024 Sep 16;386:e080133.
20. Cornelisse S, Ramos L, Arends B, Brink-van der Vlugt JJ, de Bruin JP, Curfs MH, et al. Comparing the cumulative live birth rate of cleavage-stage versus blastocyst-stage embryo transfers between IVF cycles: a study protocol for a multicentre randomised controlled superiority trial (the ToF trial). BMJ Open. 2021 Jan 13;11(1):e042395.
21. Moustafa MK, Sheded SA, El Aziz Mousta MA. Elective single embryo transfer versus double embryo transfer in assisted reproduction. Reprod Biomed Online. 2008 Jul;17(1):82–7.
22. Coskun S, Hollanders J, Al-Hassan S, Al-Sufyan H, Al-Mayman H, Jaroudi K. Day 5 versus day 3 embryo transfer: a controlled randomized trial. Hum Reprod. 2000 Sep;15(9):1947–52.
23. Wang N, Zhao X, Ma M, Zhu Q, Wang Y. Effect of Day 3 and Day 5/6 Embryo Quality on the Reproductive Outcomes in the Single Vitrified Embryo Transfer Cycles. Front Endocrinol (Lausanne). 2021;12:641623.
24. Garbhini PG, Suardika A, Anantasika A, Adnyana IBP, Darmayasa IM, Tondohusodo N, et al. Day-3 vs. Day-5 fresh embryo transfer. JBRA Assist Reprod. 2023 Jun 22;27(2):163–8.
25. Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Blastocyst versus cleavage stage transfer in in vitro fertilization: differences in neonatal outcome? Fertil Steril. 2010 Oct;94(5):1680–3.
26. Kalra SK, Ratcliffe SJ, Barnhart KT, Coutifaris C. Extended embryo culture and an increased risk of preterm delivery. Obstet Gynecol. 2012 Jul;120(1):69–75.

27. Dar S, Librach CL, Gunby J, Bissonnette F, Cowan L, IVF Directors Group of Canadian Fertility and Andrology Society. Increased risk of preterm birth in singleton pregnancies after blastocyst versus Day 3 embryo transfer: Canadian ART Register (CARTR) analysis. Hum Reprod. 2013 Apr;28(4):924–8.
28. Li Q, Ruan L, Zhu L, Yang Z, Zhu M, Luo Y. Elevated estradiol levels in frozen embryo transfer have different effects on pregnancy outcomes depending on the stage of transferred embryos. Sci Rep. 2022 Apr 4;12(1):5592.
29. McLernon DJ, Harrild K, Bergh C, Davies MJ, de Neubourg D, Dumoulin JCM, et al. Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials. BMJ. 2010 Dec 21;341:c6945.
30. Kamath MS, Mascarenhas M, Kirubakaran R, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database Syst Rev. 2020 Aug 21;8(8):CD003416.