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Original Article

Comparing the effect of sequential embryo transfer versus double blastocyst embryo transfer on pregnancy outcomes in intracytoplasmic sperm injection (ICSI) cycles in patients with repeated implantation failure: A randomized controlled trial

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ABSTRACT

Objective: Recurrent implantation failure (RIF) is the main challenge in assisted reproductive technology (ART) practice. Sequential embryo transfer, in which both, cleavage-stage embryo on day 3 and blastocyst on day 5, are sequentially transferred in the same cycle, has been suggested for increasing embryo implantation in RIF patients. The aim of the present study was to compare the effect of sequential embryo transfer versus double blastocyst embryo transfer on pregnancy outcomes in intracytoplasmic sperm injection (ICSI)/frozen embryo transfer (FET) cycles in RIF patients.

Materials and methods: This prospective study was enrolled 224 RIF patients undergoing ICSI/FET cycles and randomly divided to sequential and control groups. In sequential group, embryo transfer was conducted on day 3 (cleavage stage) and day 5 (blastocyst stage). In control group, two top-quality blastocysts were transferred on day 5.

Results: Two hundred and two couples accomplished the trial, and their data were analyzed. Results demonstrated that sequential embryo transfer on day 3 and day 5 compared to double blastocyst transfer on day 5 significantly increased implantation rate, clinical pregnancy rate and ongoing pregnancy rate in RIF patients (p-value = 0.0142, p-value = 0.0154, p-value = 0.0201, respectively). However, there were no significant differences in terms of chemical pregnancy rate, multiple pregnancy rate, miscarriage rate and ectopic pregnancy rate in the studied groups.

Conclusion: Sequential embryo transfer is associated with improved pregnancy outcomes in RIF patients. Further prospective studies with larger sample sizes are required to validate these results.

Introduction

Recurrent implantation failure (RIF) is a situation that is only applicable to patients experiencing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles [1]. RIF commonly refers to the failure in achieving clinical pregnancy after transferring of at least four good quality embryos during at least three fresh or frozen embryo transfer (FET) cycles in women under 40 years old [1]. Due to variations in definitions for RIF, there is limited data that precisely represents the incidence or prevalence of it [2]. Embryo implantation is an intricate physiological process. When an embryo reaches the blastocyst stage, it migrates to the uterine cavity and invades to the endometrial luminal surface, initiating implantation. Synchronized communication between a receptive endometrium and functional embryo is indispensable for successful implantation [3]. Among these two factors, the endometrium is considered to be more critical, because compromised endometrial factors are responsible for two thirds of implantation failures [3]. Numerous factors cause failure of these processes and subsequently

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lead to embryo implantation failure. Because of the variety of these potential risk factors [4], there have been no evidence-based therapeutic solution for treating embryo implantation failure yet.

Embryo transfer (ET) at the blastocyst stage has been suggested to improve the clinical outcomes in RIF's patients [1]. ET at the blastocyst stage may enable selection of high quality viable embryos because of embryonic genome activation and timely provide better physiological synchronization between the endometrium and the embryo stage [5]. However, extended embryo culture to the blastocyst stage may increases the risk of cycle cancellation. Studies have also shown blastocyst-stage transfer may result in an epigenetic alterations and higher chance of monozygotic twins [6,7]. Therefore, some studies recommended ET at the cleavage stage [8]. Nevertheless, it has demonstrated that clinical pregnancy rates after ET at the cleavage stage are significantly lower compared with the blastocyst stage, which might be possibly attributable to the use of morphological criteria on Day 3 [9]. Sequential ET is suggested to benefit from both cleavage- and blastocyst-stage embryos and prevent transfer cancellation risk [10]. Sequential embryo transfer is defined as the two-step transfer of embryos on different days within the same embryo transfer cycle [10]. The effectiveness of sequential transfer is still controversial and restricted data have been published in this regard. Although number of studies have claimed benefit effects of sequential embryo transfer compared to conventional embryo transfer [11,12], some studies found no significant differences in pregnancy rate between single and sequential embryo transfers [13,14].

The aim of the present study was to compare the effect of sequential embryo transfer versus double blastocyst embryo transfer on pregnancy outcomes in ICSI/FET cycles in RIF patients.

Materials and methods

Ethical consideration

The present study was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The trial was registered at the Iranian Registry of Clinical Trials (IRCT20160722029027N10). Written informed consent was obtained from all the participants.

Study population

RIF patients who had a history of failure to achieve pregnancy after three or more embryo transfers with high-quality embryos undergoing ICSI/FET cycles were evaluated for their eligibility to enter the study based on the following inclusion/exclusion criteria:

The inclusion criteria included infertile couples planned for their first ICSI/FET cycle with women younger than 40 years old having body mass index (BMI) under 30 kg/m^2 and normal serum follicle-stimulating hormone (FSH) levels (FSH < 12 IU/L) on day 2 or 3 of menstrual cycle.

The exclusion criteria were presence of any uterine lesions such as fibroids and endometrial polyps, hydrosalpinx, adenomyosis, intrauterine adhesions, endometriosis, uterine congenital anomalies, hormonal disruptions, inflammatory and autoimmune diseases, polycystic ovary syndrome (PCOS) and ovarian hyperstimulation syndrome (OHSS). In addition, participants with severe male factor of their spouses and chromosomal abnormalities were excluded.

Study design

The present study was carried out in the IVF center of Taleghani hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Patients, physicians, outcome assessor, and statistician were all kept blinded during the study. Patients were accidently allocated to one of two groups including sequential group or control group. Randomization was performed using computer-generated simple random tables in a 1:1 ratio.

Controlled ovarian hyperstimulation protocol

Controlled ovarian hyperstimulation was performed using GnRH antagonist protocol. Briefly, women underwent gonadotropin stimulation using follitropin alfa/lutropin alfa (Pergoveris®, Merck Serono, Germany) at a dose of 150–300 IU/day beginning from day 2–3 of the menstrual cycle. Gonadotropin dose was selected based on several factors such as age, BMI, and adjusted, if necessary, after 5–6 days according to the follicular development monitored by transvaginal ultrasonography. GnRH antagonist (0.25 mg/day; Cetrotide®, Merck Serono, Germany) commenced when the leading follicle reached the size of 14 mm in diameter and continued until the day of the ovulation induction. The ovulation was triggered by the administration of 250 μg recombinant human chorionic gonadotropin (hCG) (Ovitrelle®, 250 μg/0.5 ml, Merck Serono, Germany) when two or more follicles were >17 mm in diameter.

Oocyte retrieval, denudation and fertilization

Ovum pick-up (OPU) was conducted transvaginal 36 h after hCG injection. Cumulus cell–oocyte complexes (COCs) were retrieved and washed in MOPS-buffered medium (G-MOPS™ PLUS, Vitrolife Co., Sweden). Oocyte denudation was performed 2 h post-retrieval utilizing hyaluronidase (HYASE-10×™, Vitrolife Co., Sweden) followed by mechanical dissection. ICSI was conducted on all mature metaphase II oocytes 3–4 h after OPU. Afterwards, the injected oocytes were transferred to embryo culture medium (SAGE 1-Step™, Cooper Surgical Co., USA) until day 3. The embryo culture was conducted in an incubator with humidified atmosphere and 6% CO2. Top-quality cleavage stage embryos were selected for vitrification using commercial media (Kitazato BioPharma Co., Shizuoka, Japan) based on the manufacturer's protocol.

Embryo quality evaluation and key performance indicators (KPIs)

Day 3 embryo quality was evaluated according to the previous literature [15]. Briefly, the parameters including the number and symmetry of blastomeres, percent of fragmentation, presence of multinuclear blastomeres, presence of intracytoplasmic and extracytoplasmic morphological abnormalities were assessed in the embryos on day 3 of culture.

Top-quality cleavage stage embryos were determined as those with 8–10 symmetric blastomeres on day 3, <15% fragmentation, absence of multinucleation, absence of intracytoplasmic and extracytoplasmic abnormalities. Otherwise, the embryos were considered as low-quality embryos.

Blastocyst grading was done based on the Gardner score [16] as following: Expansion score 0 = no cavity, score 1 = blastocoel cavity less than half volume of the embryo, score 2 = blastocoel cavity more than half volume of the embryo, score 3 = cavity completely filling the embryo, score 4 = cavity larger than the embryo and thinning zona, score 5 = hatching blastocyst; for inner cell mass (ICM), Grade A = formed by many tightly packed cells, Grade B = several loosely packed cells, Grade C = few cells; for trophectoderm (TE), Grade A = many cells forming a cohesive layer, Grade B = few cells and loose layer, Grade C = very few large cells. Top-quality blastocysts were defined as blastocysts with expansion grades 4–5 or 2–3, and ICM and TE with AA, AB or BA classifications.

KPIs including fertilization rate, day 3 embryo development rate and blastocyst development rate were measured as following [17]:

Fertilization rate = number of normally fertilized oocytes (with 2 pronuclei and 2 polar bodies) per number of MII oocytes injected × 100

Day 3 embryo development rate = Number of 8-cell embryos on day 3 per number of normally fertilized oocytes × 100

FET cycle

The embryos were warmed in the commercial media (Kitazato BioPharma Co., Shizuoka, Japan) based on the manufacturer's protocol. After warming procedure, the embryos were located in embryo culture medium (SAGE 1-Step™, CooperSurgical Co., USA) and incubated at 37 °C in 6% CO2 until embryo transfer (ET) procedure. The warmed embryos were considered survived if they had 50% or more viable blastomeres with no evidence of degenerated morphology.

Post warming embryo survival rate = number of warmed embryos per number of survived embryos × 100

In sequential group, embryo transfer was conducted on day 3 and day 5. A top-quality embryo was transferred on day 3, then the remaining top-quality embryos were cultured (SAGE 1-Step™, CooperSurgical Co., USA) until day 5 and only one top-quality blastocyst was transferred. In control group, two top-quality blastocysts were transferred on day 5. ET was conducted using an embryo transfer catheter (Cook, USA) by an expert gynecologist under the guidance of ultrasound, based on the guideline provided by American Society for Reproductive Medicine (ASRM).

Endometrial preparation was conducted using 6 mg/d orally estradiol valerate (Aburaihan Co., Tehran, Iran) from the second (or third) day of the menstrual cycle for 14 days plus progesterone (400 mg, suppository, BID; Cyclogest, Actavis, England, UK) 5 days before ET until the 12th week of pregnancy.

Clinical outcome assessment

Chemical pregnancy rate was determined by number of pregnancies diagnosed by positive serum β-hCG (b-hCG > 50 mIU/ml) after two weeks from the day of ET per number of FET cycles × 100.

Clinical pregnancy rate was calculated by the number pregnancies with heartbeat of one or more confirmed by ultrasound after six weeks from the day of ET per number of FET cycle × 100.

The implantation rate was calculated from the number of observed gestational sacs by ultrasonography after six weeks from the day of ET per number of transferred embryos × 100.

Ongoing pregnancy rate was determined by the number pregnancies with heartbeat of one or more confirmed by ultrasound at >12 weeks gestational age per number of FET cycles × 100.

Multiple pregnancy rate was determined by the number of conceptions with more than one fetus in ultrasonography after six weeks from the day of ET per number of clinical pregnancies × 100.

Ectopic pregnancy rate was determined by the number of ectopic pregnancies confirmed with sonography or laparoscopy after six weeks from the day of ET per number of clinical pregnancies × 100.

Miscarriage rate was calculated by the number of pregnancy losses prior to 12 weeks' gestation per number of clinical pregnancies × 100.

Statistical analysis

The results were shown as mean ± SD. Statistical analysis was carried out using GraphPad Prism (GraphPad Software, USA). Student's t-test, Exact test and Chi-squared test were used for comparing the study groups. The $p < 0.05$ was considered as statistically significant.

Results

A total of 277 RIF patients were eligible to participate in this study from which 224 couples fulfilled the inclusion criteria and 202 couples accomplished the trial and their data were analyzed (Fig. 1). Table 1 represents the basic characteristic of the patients undergoing ICSI/FET cycles. There were no significant differences between the two groups in terms of mean age, body mass index (BMI), serum level of day 3 FSH, serum level of Anti-Mullerian hormone (AMH), duration of infertility, type of infertility (primary or secondary), history of abortion, history of ectopic pregnancy, number of previous ICSI cycles, number of previous ET, sperm count, AND sperm total motility.

Table 2 represents the characteristics of the studied patients during ovarian stimulation and ICSI/FET cycles including total dose of administered gonadotropin, duration of gonadotropin administration, mean number of oocytes retrieved, mean number of metaphase-II (MII) oocytes, fertilization rate, day 3 embryo development rate, post warming survival rate, duration of FET cycle and endometrial thickness on ET day. Based on the obtained data, there were no significant differences in these characteristics between the patients who were undergone ICSI/FET cycles in the studied groups. The results illustrated that the calculated KPIs including fertilization and day 3 embryo development rates were all in acceptable range according to the Vienna consensus [17].

Clinical outcomes in the studied groups

Overall comparison of clinical outcomes between the two study groups is presented in Table 3. The implantation rate, clinical pregnancy rate and ongoing pregnancy rate were significantly higher in the sequential group compared to control group (p-value = 0.0142, p-value = 0.0154, p-value = 0.0201, respectively). However, there were no significant differences in terms of chemical pregnancy rate, multiple pregnancy rate, miscarriage rate and ectopic pregnancy rate in the studied groups. Chemical pregnancy rate was 52.94% (54/102) for sequential group and 43% (43/100) for control group. Implantation rate was 24.51% for sequential group and 14.50% for control group. Clinical pregnancy rate was 42.15% (43/102) for sequential group and 26% (26/100) for control group. Ongoing pregnancy rate was 33.33% (34/102) for sequential group and 21% (21/100) for control group. Multiple pregnancy rate was 16.25% (7/43) for sequential group and 11.54% (3/26) for control group. Miscarriage rate was 18.60% (8/43) for sequential group and 15.38% (4/26) for control group. Ectopic pregnancy rate was 2.32% (1/43) for sequential group and 3.84% (1/26) for control group (Table 3).

Discussion

The present study demonstrated that sequential embryo transfer on day 3 (cleavage stage) and day 5 (blastocyst stage) compared to double blastocyst transfer on day 5 significantly increased implantation rate, clinical pregnancy rate and ongoing pregnancy rate in RIF patients. However, there were no significant differences in terms of chemical pregnancy rate, multiple pregnancy rate, miscarriage rate and ectopic pregnancy rate in the studied groups.

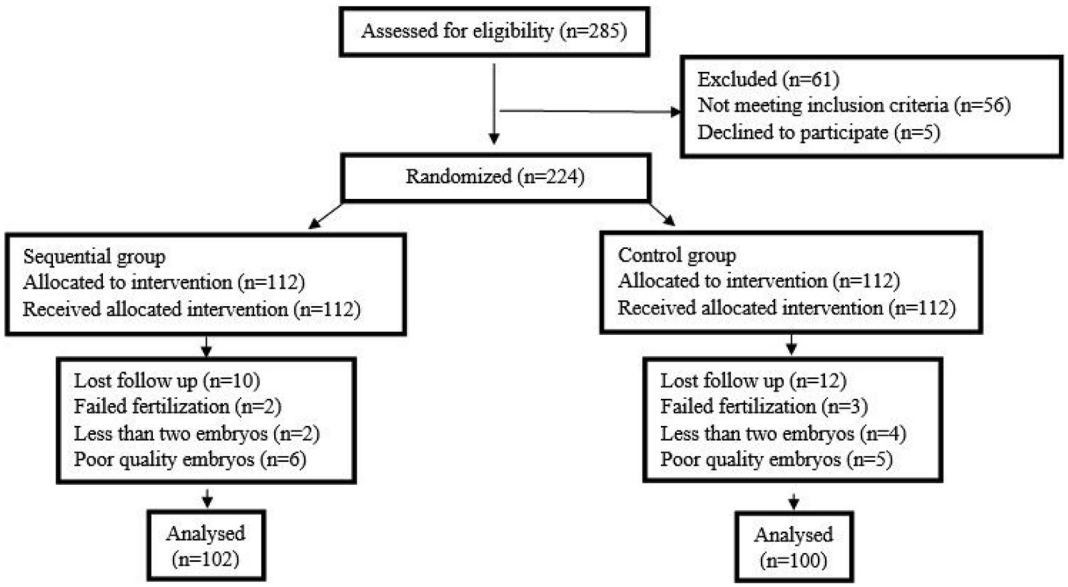

Fig. 1. Consort flow diagram.

Table 1
Demographic and clinical characteristics of the studied groups.

Group	Sequential	Control	p-value
Characteristics			
Number of patients	102	100	
Age (year)	33.92 ± 0.4794	34.90 ± 0.5192	0.1674
BMI	26.93 ± 0.2366	26.47 ± 0.2350	0.1681
FSH (Day 3) (mIU/ml)	5.967 ± 0.2074	6.457 ± 0.2169	0.1042
AMH (ng/ml)	3.366 ± 0.2733	3.436 ± 0.2594	0.8539
Duration of infertility (year)	7.049 ± 0.3634	8.030 ± 0.3465	0.0523
Type of infertility (primary) (%)	64.70	64	0.9166
Type of infertility (secondary) (%)	35.29	36	0.9166
History of abortion (%)	41.17	38	0.6672
History of ectopic pregnancy (%)	12.74	8	0.3574
Number of previous ICSI Cycles	2.784 ± 0.1187	2.520 ± 0.1087	0.1025
Number of previous ET	4.549 ± 0.1605	4.520 ± 0.1624	0.8990
Sperm (count/ml)	55.93 ± 2.818	48.79 ± 2.711	0.0694
Sperm total motility (%)	40.76 ± 1.693	37.18 ± 1.521	0.1172

AMH: Anti-Mullerian hormone; BMI: Body mass index; ET: Embryo transfer; FSH: Follicle stimulating hormone; ICSI: Intracytoplasmic sperm injection.

Table 3
Pregnancy outcomes of the studied groups.

Group	Sequential	Control	p-value
Parameter			
Chemical pregnancy rate	52.94% (54/102)	43% (43/100)	0.1589
Implantation rate	24.51%	14.50%	0.0142*
Clinical pregnancy rate	42.15% (43/102)	26% (26/100)	0.0154*
Ongoing pregnancy rate	33.33% (34/102)	21% (21/100)	0.0201*
Multiple pregnancy rate	16.25% (7/43)	11.54% (3/26)	0.5942
Miscarriage rate	18.60% (8/43)	15.38% (4/26)	0.7370
Ectopic pregnancy rate	2.32% (1/43)	3.84% (1/26)	0.7201

*Significant.

Transfer of the embryo from the laboratory to the uterus is one of the most crucial steps during ART cycles. Conventionally, embryos were transferred at cleavage-stage on day 3; however, over the past decade there has been a move to transferring day 5 blastocysts [18]. Transfer at the blastocyst stage reflects a physiologically correct time of natural implantation and increases synchrony between the embryo development and endometrium [18]. Current evidence reinforces the use of blastocyst transfers in ART practice. It has been shown that the clinical pregnancy and live birth rates were higher in women undergoing blastocyst transfers compared to those receiving cleavage-stage transfers [9,19]. However, prolonging culture to the blastocyst stage might decrease the quantity of viable embryos accessible for embryo transfer or cryopreservation. Therefore, to evade cycle cancelation sequential embryo transfer, in which both, cleavage-stage embryo on day 3 and

Table 2
Controlled ovarian hyperstimulation/intracytoplasmic sperm injection outcomes of the studied groups.

Group	Sequential (n = 102) Mean ± SD	Control (n = 100) Mean ± SD	p-value
Characteristics			
Total dose of gonadotropin (IU)	2983 ± 100.1	3053 ± 95.21	0.6174
Duration of gonadotropin administration (day)	9.388 ± 0.1449	9.700 ± 0.1573	0.1462
Mean number of oocytes retrieved	9.882 ± 0.5572	10.92 ± 0.6424	0.2232
Number of MII oocyte	7.902 ± 0.4589	9.110 ± 0.5561	0.0948
Fertilization rate (%)	85.58 ± 1.667	87.96 ± 1.459	0.1422
Day 3 embryo development rate (%)	75.14 ± 3.995	80.52 ± 2.330	0.1243
Top-quality day 3 embryo (%)	70.59 ± 2.224	75.60 ± 2.373	0.0625
Post warming survival rate (%)	89.70 ± 1.763	92.35 ± 1.606	0.1346
Top-quality blastocyst (%)	64.88 ± 2.171	69.94 ± 2.318	0.0565
Duration of FET cycle (day)	11.77 ± 0.1379	11.58 ± 0.1451	0.3321
Endometrial thickness on ET day (mm)	8.520 ± 0.1279	8.885 ± 0.1527	0.0676

ET: Embryo transfer; FET: Frozen embryo transfer; MII: Metaphase-II.

blastocyst on day 5, are sequentially transferred in the same cycle, has been suggested [11]. Study on sequential embryo transfer is limited. Earlier published data had indicated improved pregnancy rate following sequential embryo transfer [20]. However, later some researchers found no positive effect [14,21]. Herein, the obtained results verified that sequential embryo transfer compared to double blastocyst transfer significantly increased implantation rate, clinical pregnancy rate and ongoing pregnancy rate in RIF patients. In agreement with these results, Ismail Madkour et al. showed that sequential embryo transfer significantly increased pregnancy outcomes compared to day 3 embryo transfer in RIF patients [22]. In addition, Torky et al. disclosed that clinical pregnancy and live birth rates were significantly higher in the RIF patients receiving sequential embryo transfer compared to those undergoing double blastocyst transfer [23]. In contrast, Tehraninejad et al. revealed that sequential embryo transfer did not improve clinical pregnancy rate compared to blastocyst transfer on day 5 [13]. One explanation for these heterogeneous results could be due to the variations in definitions for RIF and inclusion criteria in all the above-mentioned studies.

Some studies have indicated that two-thirds of embryo implantation failures are due to an absence of endometrial receptivity [13]. The endometrium is receptive to the embryo for a specific period recognized as the window of implantation (WOI) [5]. It has been proposed that the WOI is not a persistent adjustable in all women and the designation of its movement is of vital importance, especially for RIF patients [24]. Some researchers reported that displacement of the WOI during the mid-luteal phase happens in 25–30% of patients with RIF [25,26]. Moreover, some cases may have different length and position in the menstrual cycle even in the same individual but through different periods [5,24]. Therefore, the sequential embryo transfer may overcome these problems with choosing the right moment for embryo transfer. Indeed, the sequential transfer may increase the chance of hitting the WOI which is only open for 2–4 days [27]. In addition, it has been suggested that in sequential transfer the earlier transferred cleavage embryo may modulate the immune response and generate a better endometrial environment for the second transfer [10]. Early pregnancy establishment requires a transient modulation of innate and adaptive maternal immunity for tolerating the embryo and supporting embryo development and implantation. Emerging evidence shows that the embryo starts to crosstalk locally with the uterus and immune cells in the uterus [28–30]. It seems that at the embryo–maternal interface, the pre-hatching embryo modulates the local uterine immunological milieu for induction of a state of immune tolerance essential for embryo survival and pregnancy establishment. Therefore, in sequential embryo transfer, it is possible that the D3 embryo starts to generate immune tolerance in the uterus and provides a better endometrial environment for the D5 embryo.

Multiple pregnancies are associated with maternal mortality and neonatal complications, such as preterm birth, low birth weight, hemorrhage, and respiratory problems [31]. Clearly there is direct link between multiple pregnancies and the number of embryos transferred [31]. Therefore, the main approach to prevent multiple pregnancies is to restrict the maximum number of embryos in a single embryo transfer [32]. As expected, the rate of multiple pregnancies is statistically lower after single embryo transfer (0%–3%) compared to double embryo transfer (24%–65%) [33]. Herein, although in both groups two embryos were transferred, the multiple pregnancy rate was 16.25% for sequential group and 11.54% for control group, and there was no significant difference between two groups. A possible explanation for low rate of multiple pregnancy rate despite double embryo transfer may be due to the disruption of endometrial receptivity in RIF patients. In this regard, the Practice Committees of the Society for Assisted Reproductive Technology and the ASRM recognized the enhanced implantation rates and called for reconsidering the practice of transferring multiple embryos [34]. However, concern remains regarding the risk of multiple pregnancies associated with sequential embryo transfer.

The main limitation of this study was the failure to evaluate live birth rate. In addition, there was a limiting number of patients, these hypotheses need prospective confirmation in a largescale prospective randomized trial studies with sufficient sample size.

Conclusion

By the way of conclusion, in women with RIF, the sequential transfer of cleavage- and blastocyst-stage embryos offered a better chance of implantation than conventional embryo transfer. Sequential embryo transfer could be considered as a suitable approach for improving IVF/ICSI outcomes in RIF patients.

Ethics approval and consent to participate

The present study was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The trial was registered at the Iranian Registry of Clinical Trials (IRCT20160722029027N10). Written informed consent was obtained from all the participants.

Authors' contributions

SS, SH, ZR conceptualization; methodology; ZR, HH collected, analyzed, and interpreted the patients' data. HZ, HH drafting the manuscript; ZR, HH revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from IVF center.

Code availability

Not applicable.

Declaration of competing interest

The authors declare that there are no competing interests related to the subject matter or materials discussed in this article.

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References

[1] Bashiri A, Halper KI, Orvieto R. Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. Reprod Biol Endocrinol 2018;16:121. https://doi.org/10.1186/s12958-018-0414-2.

[2] Hosseinirad H, Novin MG, Hosseini S, Nazarian H, Amidi F, Paktinat S, et al. Effect of 1,25(OH)2-vitamin D3 on expression and phosphorylation of progesterone receptor in cultured endometrial stromal cells of patients with repeated implantation failure. Acta Histochem 2020;122:151489. https://doi.org/10.1016/j.acthis.2019.151489.
[3] Liu C, Yao W, Yao J, Li L, Yang L, Zhang H, et al. Endometrial extracellular vesicles from women with recurrent implantation failure attenuate the growth and invasion of embryos. Fertil Steril 2020;114:416–25. https://doi.org/10.1016/j.fertnstert.2020.04.005.
[4] Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. Reprod Biomed Online 2014;28:14–38. https://doi.org/10.1016/j.rbmo.2013.08.011.
[5] Enciso M, Aizpurua J, Rodríguez-Estrada B, Jurado I, Ferrández-Rives M, Rodríguez E, et al. The precise determination of the window of implantation significantly improves ART outcomes. Sci Rep 2021;11:13420. https://doi.org/10.1038/s41598-021-92955-w.
[6] Sotiroska V, Petanovski Z, Dimitrov G, Hadji-Lega M, Shushleski D, Saltirovski S, et al. The day of embryo transfer affects delivery rate, birth weights, female-to-male ratio, and monozygotic twin rate. Taiwan J Obstet Gynecol 2015;54:716–21. https://doi.org/10.1016/j.tjog.2015.06.011.
[7] White CR, Denomme MM, Tekpetey FR, Feyles V, Power SGA, Mann MRW. High frequency of imprinted methylation errors in human preimplantation embryos. Sci Rep 2015;5:17311. https://doi.org/10.1038/srep17311.
[8] Yang L, Cai S, Zhang S, Kong X, Gu Y, Lu C, et al. Single embryo transfer by Day 3 time-lapse selection versus Day 5 conventional morphological selection: a randomized, open-label, non-inferiority trial. Hum Reprod 2018;33:869–76. https://doi.org/10.1093/humrep/dey047.
[9] 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. https://doi.org/10.1002/14651858.CD002118.pub5.
[10] Zhang J, Wang C, Zhang H, Zhou Y. Sequential cleavage and blastocyst embryo transfer and IVF outcomes: a systematic review. Reprod Biol Endocrinol 2021;19:1–9. https://doi.org/10.1186/s12958-021-00824-y.
[11] Torky H, Ahmad A, Hussein A, El-Desouky E-S, Aly R, Ragab M, et al. Comparing sequential vs day 3 vs day 5 embryo transfers in cases with recurrent implantation failure: randomized controlled trial. JBRA Assist Reprod 2021;25. https://doi.org/10.5935/1518-0557.20200083.
[12] Ismail Madkour WA, Noah B, Zaheer H, Al-Bahr A, Abdelhamid AMS, Shaeer M, et al. Does sequential embryo transfer improve pregnancy rate in patients with repeated implantation failure? A randomized control study. Middle East Fertil Soc J 2015;20:255–61. https://doi.org/10.1016/j.mefs.2015.04.002.
[13] Tehraninejad ES, Raisi E, Bakhtiyari Ghaleh F, Hossein Rashidi B, Aziminekoo E, Kalantari V, et al. The sequential embryo transfer compared to blastocyst embryo transfer in in vitro fertilization (IVF) cycle in patients with the three repeated consecutive IVF. A randomized controlled trial. Gynecol Endocrinol 2019:1–5. https://doi.org/10.1080/09513590.2019.1613639.
[14] Al-Hasani S, van der Ven H, Diedrich K, Reinecke A, Hartje H, Krebs D. Der Einfluß aufeinanderfolgender Embryotransfers auf die Schwangerschaft nach In-vitro-Fertilisation. Geburtshilfe Frauenheilkd 1990;50:640–3. https://doi.org/10.1055/s-2008-1026516.
[15] Balaban B, Brison D, Calderon G, Catt J, Conaghan J, Cowan L, et al. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26:1270–83. https://doi.org/10.1093/humrep/der037.
[16] Wirleitner B, Schuff M, Stecher A, Murtinger M, Vanderzwalmen P. Pregnancy and birth outcomes following fresh or vitrified embryo transfer according to blastocyst morphology and expansion stage, and culturing strategy for delayed development. Hum Reprod 2016;31:1685–95. https://doi.org/10.1093/humrep/dew127.
[17] Special E, Group I, Scientists A. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. Reprod Biomed Online 2017;35:494–510. https://doi.org/10.1016/j.rbmo.2017.06.015.
[18] Glujovsky D, Farquhar C. Cleavage-stage or blastocyst transfer: what are the benefits and harms? Fertil Steril 2016;106:244–50. https://doi.org/10.1016/j.fertnstert.2016.06.029.
[19] Eftekhar M, Mohammadi B, Tabibnejad N, Mortazavi Lahijani M. Frozen–-thawed cleavage stage versus blastocyst stage embryo transfer in high responder patients. Zygote 2020;28:511–5. https://doi.org/10.1017/S0967199420000428.
[20] Abramovici H, Dirnfeld M, Weisman Z, Sorokin Y, Lissak A, Rofe A, et al. Pregnancies following the interval double-transfer technique in an in vitro fertilization-embryo transfer program. J In Vitro Fert Embryo Transf 1988;5:175–6. https://doi.org/10.1007/BF01131183.
[21] Ashkenazi J, Yoeli R, Orvieto R, Shalev J, Ben-Rafael Z, Bar-Hava I. Double (consecutive) transfer of early embryos and blastocysts: aims and results. Fertil Steril 2000;74:936–40. https://doi.org/10.1016/S0015-0282(00)01549-1.
[22] Ismail Madkour WA, Noah B, Zaheer H, Al-Bahr A, Abdelhamid AMS, Shaeer M, et al. Does sequential embryo transfer improve pregnancy rate in patients with repeated implantation failure? A randomized control study. Middle East Fertil Soc J 2015;20:255–61. https://doi.org/10.1016/j.mefs.2015.04.002.
[23] Torky HA, Ahmad A, Hussein A, El-Desouky ES, Aly R, Ragab M, et al. Comparing sequential vs day 3 vs day 5 embryo transfers in cases with recurrent implantation failure: randomized controlled trial. J Bras Reprod Assist 2021;25:185–92. https://doi.org/10.5935/1518-0557.20200083.
[24] Stamenov GS, Parvanov DA, Chaushev TA. Successful pregnancy following mixed double embryo transfer in a patient with variable window of implantation. Case Rep Obstet Gynecol 2018;2018:1–4. https://doi.org/10.1155/2018/1687583.
[25] Gomaa H, Casper RF, Esfandiari N, Bentov Y. Non-synchronized endometrium and its correction in non-ovulatory cryopreserved embryo transfer cycles. Reprod Biomed Online 2015;30:378–84. https://doi.org/10.1016/j.rbmo.2014.12.005.
[26] Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M, Carranza F, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril 2013;100:818–24. https://doi.org/10.1016/j.fertnstert.2013.05.004.
[27] Haouzi D, Entezami F, Torre A, Innocenti C, Antoine Y, Mauries C, et al. Customized frozen embryo transfer after identification of the receptivity window with a transcriptomic approach improves the implantation and live birth rates in patients with repeated implantation failure. Reprod Sci 2021;28:69–78. https://doi.org/10.1007/s43032-020-00252-0.
[28] Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol 2017;17:469–82. https://doi.org/10.1038/nri.2017.64.
[29] Schumacher A, Sharkey DJ, Robertson SA, Zenclussen AC. Immune cells at the fetomaternal interface: how the microenvironment modulates immune cells to foster fetal development. J Immunol 2018;201:325–34. https://doi.org/10.4049/jimmunol.1800058.
[30] Walker CG, Meier S, Littlejohn MD, Lehnert K, Roche JR, Mitchell MD. Modulation of the maternal immune system by the pre-implantation embryo. BMC Genom 2010;11. https://doi.org/10.1186/1471-2164-11-474.
[31] Monteleone PAA, Peregrino PFM, Baracat EC, Serafini PC. Transfer of 2 embryos using a double-embryo transfer protocol versus 2 sequential single-embryo transfers: the impact on multiple pregnancy. Reprod Sci 2018;25:1501–8. https://doi.org/10.1177/1933719118756750.
[32] Tiitinen A. Single embryo transfer: why and how to identify the embryo with the best developmental potential. Best Pract Res Clin Endocrinol Metabol 2019;33:77–88. https://doi.org/10.1016/j.beem.2019.04.001.
[33] Freeman MR, Hinds MS, Howard KG, Howard JM, Hill GA. Guidance for elective single-embryo transfer should be applied to frozen embryo transfer cycles. J Assist Reprod Genet 2019;36:939–46. https://doi.org/10.1007/s10815-019-01433-w.
[34] Kim HH, Matevossian K. Are two better than one? Two sequential transfers of a single embryo may be better than a double-embryo transfer. Fertil Steril 2020;114:267–8. https://doi.org/10.1016/j.fertnstert.2020.04.064.