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Efficacy of sequential embryo transfer in improving pregnancy rate in women with repeated unexplained implantation failure

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection . **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** No Fund Collection

Aim: In women with repeated IVF-failure, pregnancy rate was compared between sequential embryo transfer (ET: ET on day 3 and 5) vs. day 3 ET.

Patients and Methods: A randomized controlled clinical trial was conducted from September 2019 till September 2020 on 80 women with repeated IVF-failure: sequential ET (n=40) vs. day 3 ET (n=40). Pregnancy outcomes were compared between the two groups.

Results: Sequential ET, compared with day 3 ET, showed higher chemical, clinical, and ongoing pregnancy, i.e., with sequential ET showing 37%, 35%, and 32.5%, respectively; and with day 3 ET showing 17.5%, 15%, and 12.5%, respectively.

Conclusion: For patients with repeated IVF-failures, sequential ET improved the chemical, clinical, and ongoing pregnancy rates as long as good quality embryos were available.

Keywords: Sequential embryo transfer; Improving pregnancy rate; Repeated unexplained implantation failure

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INTRODUCTION

Considering the current success rate of IVF treatments and the mean number of embryos transferred in each cycle, it is recommended by Simon and Laufer to define RIF as failure of implantation in at least three consecutive IVF attempts, in which 1–2 embryos of high-grade quality are transferred in each cycle [1]. Repeated IVF–embryo transfer failures may occur for a variety of reasons typically include reduced endometrial receptivity secondary to uterine cavity anomalies, thin endometrium, abnormal changes in adhesion molecules expression and embryonic developmental abnormalities, such as low embryo quality due to a poor culture environment and genetic factors [2]. There is a specific time-frame, called "Window of implantation" (WOI). In this period, lasting approximately two days, a 6–8 day human embryo has a chance to be attached into the surface endometrial layer, composed of epithelial cells and to be implanted into the stromal cell layer [3]. Displacement of the WOI during the midluteal phase occurs in at least 25% of RIF's patients. Some authors report even higher incidence of more than 30% out-of-phase endometrium in patients with implantation failures. Changing the time of embryo transfer is a reasonable solution in these cases [4]. Blastocyst transfer is an approach aiming to improve the IVF/ET success rate in repeated IVF/ET failures. However, the results of this approach depend on the number of fertilized oocytes and the quality of the fertilized embryos. Ledee-Bataille et al. reported similar implantation rate of day 3 embryos *vs.* day 5 embryos in prospective randomized study. Thus, sequential transfer approach has the advantage of blastocyst transfer without exposing the whole cycle to the risk of cancelation [5].

AIM OF THE WORK

The aim of this study is to compare the pregnancy rate between two groups of women with repeated unexplained implantation failure, one group underwent sequential embryo transfer on day 3 and day 5 and the other one underwent embryo transfer on day 3.

PATIENTS AND METHODS

This randomized controlled study was conducted at Ain Shams University Maternity Hospital (IVF unit) on 80 women planned to have embryo transfer who met the

inclusion and exclusion criteria from September 2019 till September 2020. This study was approved by ethical committee of Ain Shams University. For all women, explanation of the study procedures was done. Informed written consent was obtained. Inclusion criteria included women aged 20-40 years old, with recurrent implantation failure; defined as failure to achieve clinical pregnancy after three *in vitro* fertilization and embryo transfer (IVF-ET) cycles with good quality embryos transferred-, normal karyotyping, normal endometrium checked by transvaginal ultrasound (TVUS) and hysteroscopy-, absence of hydrosalpinx checked by hysterosalpingogram (HSG), normal thrombophilia screening as anti-thrombin (previously called anti-thrombin III), protein C and S, lupus anticoagulant, factor V Leiden, prothrombin gene mutation, anti-β-2-glycoprotein-1 antibodies, anti-cardiolipin antibodies and availability of > 4 embryos on post-fertilization check for a good potential chance to have 3 good quality embryos available for transfer. Women with first ICSI trial, body mass index (BMI) >30 kg/m^2, major uterine abnormalities and/or pathologies, known genetic disorders and/or previous or potential poor/high responders by ovarian reserve tests were excluded.

Methods

All women were subjected to full history taking that included duration, causes of infertility and previous ICSI trials as regard number, places and dates of previous ICSI trials, protocol of induction of ovulation, dose of HMG, number and quality of oocytes, number and quality of embryos, day of transfer and causes of previous failures. Also, physical examination and investigations were done as autoantibodies (ANA and ANCA), lupus anticoagulant, and hormonal profile (FSH, LH, E2, TSH, serum prolactin, AMH at day 2-5 of the cycle). TVUS was done to determine antral follicular count (AFC), HSG and hysteroscopy. The study group was selected with regard to inclusion and exclusion criteria. Patients were randomized into two groups: Group A (n=40): women underwent sequential embryo transfer at day 3 and day 5. Group B (n=40): women underwent embryo transfer at day 3 only. The patients were randomized to either (Group A) or (Group B) based on tables with random numbers assigned to each group. The random numbers were generated by online Random number Generator computer program. Women who participated in this study followed a flexible antagonist protocol. Ovulation stimulation was started using r-FSH, (Gonapure®, Minapharm, Egypt) 150 IU S.C/I.M., on 2nd day of menstrual cycle. The daily individualized (rFSH) dose (Gonapure 150 IU) ranged between 150 and 450 IU, according to BMI, age, AMH, previous history of induction if available and the expected ovarian response. The dose was adjusted according to patient's response which was assessed by transvaginal ultrasound folliculometry which was done on day 6 and after that it was done day after day till leading follicle size reaches 18 mm or more. HMG (Menopur®, Ferring, Egypt) 75 IU S.C. was started on 5th day of menstrual cycle. 75 - 375 IU of human menopausal gonadotropin (Menopure 75 IU) were administered daily to all patients, with individual adjustments according to ovarian response as measured by serial ultrasound scans and serum E2 levels from day 6-8 of gonadotrophin stimulation. GnRH antagonist, Cetrotide (Cetrorelix®, MerckSerono, Egypt) 0.25 mg S.C. was added when a leading follicle of 14 mm was reached by follow up folliculometry and continued daily till the day of trigger. Human chorionic Gonadotrophin (HCG) (Choriomon®, IBSA, Egypt) 10000 IU I.M. was administered for final oocyte maturation when at least three leading follicles reach 17 – 18 mm in diameter and ovum retrieval was planned at 34 - 36 h. E2 and serum progesterone were assessed at the day of trigger before administration of HCG. Oocyte retrieval was performed under general anesthesia 34 - 36 hours following hCG administration using vaginal ultrasound guided single lumen needle. Intracytoplasmic sperm injection (ICSI) was performed 4 h after ovum retrieval, and the oocytes were checked for fertilization 16–18 h later. Normal fertilization was indicated by the appearance of two pronuclei. Once post-fertilization check confirmed availability of > 4 embryos, patients were randomized to one of the 2 groups. Embryos were cultured in commercial sequential IVF medium and checked on day 3. Good-quality embryos were defined as embryos containing >8 cells on day 3 with grade 1 (uniform blastomeres with no fragmentation) and grade 2 (blastomeres size will be slightly uneven with <20% fragmentation) embryos were transferred. Embryo transfer was done using Medison x6 ultrasound device and Labotect embryo transfer catheter (with inner catheter shaft and outer guiding cannula). The procedure begun with the patient was advised to fill her bladder to a comfortable status to allow excellent visualization of the endometrium on ultrasound and straightening of the uterocevical angle without causing any discomfort to the patient. The procedure was performed without analgesia or anesthesia under sterile conditions. The patient was placed in a dorsal lithotomy position with legs supported on a stirrup and draped with sterile towels. The cervix was visualized by placing a Cusco's speculum and cleaned with cotton swab soaked in saline solution aiming to clear the excess mucus of the cervix and reduce bacterial contamination to some extent. Direct embryo transfer technique (preload) was used under trans abdominal ultrasound guidance, where the catheter already loaded with embryos was passed through the cervix into the uterus. The tip of ET catheter was placed 1.5-2 cm below the fundus and the embryos were ejected as slowly as possible under US guidance, and the inner catheter was rotated and drawn out as gently as possible maintaining pressure on the plunger, then catheter was checked to confirm transfer of all embryos. After the procedure, the woman remained in the recovery room resting on her back for 15 minutes then she could resume normal activities. Second embryo transfer was done like first one but with caution. Number of total embryos transferred was restricted to 4 in both groups using embryos grade 1 or grade 2 only. In group B, embryos transfer were carried out on day 3 while in group A (sequential D3/D5) two good-quality embryos were transferred on day 3, then the remaining good-quality embryos were placed in

blastocyst culture medium (Quinn's Advantage Blastocyst Medium; SAGE) and cultured until day 5 and two good-quality blastocysts were transferred. On Day 5 transfer, the quality of embryos was assessed and only two good quality blastocysts were transferred in Group A. If on Day 5 there were no good quality blastocysts available to be transferred, we cancelled day 5 transfer and all of these patients were added to Group B. Luteal phase support was done with administration of progesterone (Prontogest®, amp, IBSA, Egypt) 100 mg once daily by intramuscular injection, started from the day of ovum retrieval and continued for 12 weeks of gestation if pregnancy was achieved. Pregnancy test –qualitative beta-HCG was done 2 weeks post the procedure. Chemical pregnancy was confirmed by positive serum B-HCG after 14 days of embryo transfer (Day 3). Clinical pregnancy was diagnosed by visualization of fetal heart pulsation 2 weeks after positive beta-HCG by trans-vaginal US. Trans-vaginal ultrasound was performed till 12 weeks to exclude miscarriage.

Sample Size Justification

The required sample size has been calculated using the G*Power Software (Universität Düsseldorf, Germany). The primary outcome measure was ongoing pregnancy i.e. the patients' proportion that their pregnancy continued beyond 12 weeks. Secondary outcome measures were clinical pregnancy rate i.e. the patients' proportion with viable fetal heart beats (FHB) (Beta HCG and US), implantation rate i.e. the proportion of gestational sacs seen on the ultrasound divided by the total number of embryos transferred, Biochemical pregnancy i.e. detection of hCG in blood or urine without subsequent clinical signs of pregnancy and early pregnancy loss i.e. the patients' proportion with their pregnancy failed to develop before 12 weeks of gestation.

Statistical Methods

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. So, the p-value was considered significant as the following: P > 0.05: Non significant, P < 0.05: Significant, P < 0.01: Highly significant.

RESULTS

Tab. 1. shows the comparisons between sequential ET group and D3 ET group regarding age, BMI, infertility duration, no. of failed ICSI trials, FSH, LH, TSH, prolactin, AMH and progesterone. The previous table shows that there was no statistically significant difference found between two groups regarding Age, BMI, and infertility duration, number of failed ICSI trials, FSH, LH, TSH, prolactin, AMH and progesterone. The previous table shows that there was no statistically significant difference found between two groups regarding No. of Oocytes', No. of fertilized oocytes and No. of Embryos (**Tab. 2.**). The previous table shows that number of chemical pregnancy, clinical pregnancy and on-going pregnancy was significantly higher in sequential ET group than D3 ET group (**Tab. 3.**).

DISCUSSION

Regarding basal descriptive data; statistical analysis of current results showed that there was no significant difference between sequential ET and D3 ET group regarding age, BMI, infertility duration, number of failed ICSI trials, number of oocytes, number of fertilized oocytes, number of embryos, FSH, LH, TSH, serum prolactin, AMH and progesterone with p= 0.135, 0.076, 0.113, 0.108, 0.103, 0.149, 0.736, 0.817, 0.806, 0.888, 0.976, 0.587 and 0.703. Regarding IVF-ET outcomes; statistical analysis of current results showed that number of chemical pregnancy, clinical pregnancy and on-going

Tab. 1. Comparisons between sequential ET group and D3 ET group regarding age, BMI, infertility duration, no. of failed ICSI trials, FSH, LH, TSH, prolactin, AMH and progesterone.

		Sequential ET group	D3 ET group	Test value•	P-value	Sig.
Age	Mean ± SD	33.65 ± 1.69	32.95 ± 2.40	-1.511	0.135	NS
	Range	31 – 37	29 – 37			
BMI	Mean ± SD	25.63 ± 2.19	26.48 ± 2.04	1.796	0.076	NS
	Range	21 – 29	23 – 31			
Infertility Duration	Mean ± SD	7.26 ± 0.79	6.92 ± 1.07	-1.602•	0.113	NS
	Range	6 – 9	4 – 8			
No. of failed ICSI trials	Median(IQR)	4 (4 - 5)	4 (3 - 5)	-1.607‡	0.108	NS
	Range	3 – 6	3 – 6			
FSH	Mean ± SD	7.41 ± 0.91	7.36 ± 0.93	-0.232•	0.817	NS
	Range	6 – 9.5	5.9 – 9.4			
LH	Median(IQR)	5.45 (3.9 - 7.55)	5.35 (3.75 - 7.30)	-0.245‡	0.806	NS
	Range	7 – 10.4	6 – 10.5			
TSH	Mean ± SD	2.08 ± 0.99	2.05 ± 0.98	-0.142•	0.888	NS
	Range	0.4 – 4.42	0.3 – 4.41			
Prolactin	Mean ± SD	14.57 ± 15.04	14.46 ± 15.05	-0.030	0.976	NS
	Range	4.88 – 84.6	4.8 – 84.5			
AMH	Mean ± SD	2.83 ± 0.71	2.68 ± 1.56	-0.545	0.587	NS
	Range	1.25 – 4.23	0.15 – 6.99			
Progesterone	Mean ± SD	1.23 ± 0.16	1.22 ± 0.19	-0.383	0.703	NS
	Range	0.4 – 4.42	0.3 – 4.41			

Tab. 2. Comparison between sequential ET group and D3 ET group regarding number of ocytes, number of fertilized ocytes and number of embryos.

		Sequential ET group	D3 ET group	Test value•	P-value	Sig.
		No. = 40	No. = 40			
No. of Ocytes	Mean ± SD	10.72 ± 1.84	11.60 ± 2.81	1.648	0.103	NS
	Range	7 – 14	6 – 16			
No. of fertilized oocytes	Mean ± SD	6.18 ± 1.78	6.78 ± 1.90	1.456	0.149	NS
	Range	3 – 8	3 – 10			
No. of Embryos	Mean ± SD	3.98 ± 0.86	3.90 ± 1.10	-0.339	0.736	NS
	Range	3 – 6	2 – 6			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
•: Independent t-test

Tab. 3. Comparison between sequential ET group and D3 ET group regarding chemical pregnancy, clinical pregnancy and ongoing pregnancy.

		Sequential ET group		D3 ET group		Test value*	P-value	Sig.
		No.	%	No.	%			
Chemical pregnancy	No	25	62.5%	33	82.5%	4.013	0.045	S
	Yes	15	37.5%	7	17.5%			
Clinical pregnancy	No	26	65.0%	34	85.0%	4.267	0.039	S
	Yes	14	35.0%	6	15.0%			
Ongoing pregnancy	No	27	67.5%	35	87.5%	4.588	0.032	S
	Yes	13	32.5%	5	12.5%			

pregnancy were significantly higher in sequential ET group than D3 ET group with p= 0.045, 0.039, and 0.032. Ashkenazi et al (2000) disagreed with current study and stated that no differences were detected among the three groups included in his study in either pregnancy or implantation rates (pregnancy: 36.8%, 41.4%, and 37.4%, respectively; implantation: 14.6%, 19.9%, and 19.8%, respectively). The double (consecutive) transfer of early embryos and blastocyst(s) didn't offer any advantage over the traditional early transfer [6]. Phillips et al., agreed with current study and stated that the consecutive transfer of day 3 embryos and blastocysts can prevent the total loss of a cycle when embryos fail to develop to the blastocyst stage in culture and thereby provide additional pregnancies [7]. Goto et al., was in line with current study and stated that taking advantage of both day-2 ET and blastocyst transfer, two-step ET may be an effective option for ET in patients who have an insufficient number of embryos [8]. Machtinger et al. corresponded with current study and stated that sequential transfer of embryos may be indicated for women with repeated IVF cycles, but the number of embryos transferred must be limited in order to prevent multifetal gestations [9]. Almog et al., agreed with current study and stated that patients with multiple consecutive IVF/ET failures, treated with the interval double transfer approach had significantly improved cycle success rates compared with regular day 2 or 3 embryo transfer protocol [10]. Fang et al., results showed that the clinical pregnancy rate of the D2/D3 group was higher than that of the D3 group (48.5% *vs.* 22.4%, P = 0.006) while the clinical pregnancy rates of the D3/D5 and D5 groups were not significantly different (50.9% *vs.* 45.8%) [11]. Dalal et al., agreed with current results, where their results showed that the clinical pregnancy rate of the D3/D5 group was significantly higher than that of the D3 group (48.5% *vs.* 29.3%), whereas the clinical pregnancy rates of the D3/D5 and D5 groups were not significantly different (48.5% *vs.* 53.0%) [12]. Madkour et al., corresponded with current study and stated that in patients with repeated implantation failures, treatment with the sequential embryo transfer approach had significantly improved pregnancy outcomes compared to regular day 3 transfers. Clinical pregnancy rate (per embryo transfer) was significantly higher in sequential ET group (37. 8%) compared to that in day 3 group (21.9%) (P value <0.05). Also, implantation rate (per embryos transferred) was significantly higher in sequential ET group (17.1%) compared to that in control group (10.5%) (P value <0.01) [13]. Nadkarni et al., was in line with current study and stated that sequential transfer is a very good efficacious approach in ART cycles if extended media are available as it gave maximum pregnancy rate and implantation rate. However it is associated with multiple pregnancies [14]. Hamdy and Deif agreed with current study and stated that sequential transfer on day 3 and day 5 in patients with adequate number of retrieved oocytes is associated with a higher embryo implantation and clinical pregnancy rates and is advocated for women having an acceptable number of embryos of good quality. Equally implantation and clinical pregnancy rates were highly significant in the sequential group than at day 3 or at day 5 groups of embryo transfer [15]. Tehraninejad et al., was against current study and stated that it seems that the double ET does not increase the chance of pregnancy rate compared to blastocyst ET on day 5 in the patients with the three repeated IVF-ET failures in cases where no less than 3 good-quality embryos are available. In this controlled trial, women scheduled for IVF/ET with the three repeated consecutive IVF failures were randomized to either sequential transfer of embryos on day 2 and on day 5 after ovum pick-up (group 1, n = 60) or blastocyst ET on day 5 (group 2, n = 60) as a control group. Chemical and clinical pregnancy rate was similar in the sequential ET group (40%) compared to the day 5 of ET group (38.3%) (P value = .85) [16]. Finally, Kaya et al., disagreed

with current results and stated that although sequential transfer seems to be an effective option in certain patient populations, sequential cleavage-stage transfer (D2/D3) or cleavage stage and blastocyst transfer (D3/D5) does not improve clinical pregnancy rates compared with double cleavage-stage embryo transfer. There was no significant difference in clinical pregnancy rates between the double group and the D2/D3 group (P=0.204) or the D3/D5 group (P=0.188). The D3/D5 group had significantly higher clinical pregnancy rates compared with the D2/D3 group (P=0.025) [17].

CONCLUSION

For patients with repeated IVF–embryo transfer failures, sequential transfer on day 3 and day 5 may improve the chemical, clinical and on-going pregnancy rates as long as good-quality embryos are available.

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