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OUTCOME OF CONSECUTIVE EMBRYOS TRANSFER IN REPEATED IMPLANTATION FAILURE CASES

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ABSTRACT

Introduction: The majority of intracytoplasmic sperm injection (ICSI) cycles lead to the transfer of embryos to the uterus, but most do not implant. Thus, the main cause of failure in IVF is implantation failure**.** Repeated failure in vitro-fertilization treatment is frustrating to patient and doctors, patient with good quality embryo pose a special therapeutic challenge (consecutive embryo transfer). **Objectives:** To examine whether consecutive transfer of embryos on day 3 and on day 5 improves ICSI success rates in patients with multiple consecutive implantation failures. **Study design:** This is a randomized controlled clinical trial include 100 patients randomly distributed into two groups: **Group A** (The study Group) consists of 50 patient subjected to Consecutive (double) embryo transfer at day 3 and at day 5. **Group B** (The control group): consists of 50 patient subjected to embryo transfer at day 5. **Results:** There was statistical non-significant difference of age, cause of infertility, number of previous trials, oocyte retrieved, and embryos available, multiple pregnancy. There was statistical significant difference of pregnancy rate and clinical pregnancy rate in two groups 54% to 34%. **Conclusion:** For patients with repeated ICSI-embryo transfer failures, sequential transfer on day 3 and day 5 may improve the clinical pregnancy rate in cases of repeated implantation failure as long as good-quality embryos are available.

KEYWORD: recurrent failure-embryos transfer-ICSI.

INTRODUCTION

Recurrent implantation failure (RIF) is a clinical entity which refers to a situation when implantation has repeatedly failed to reach a stage recognizable by pelvic Ultrasonography.[1] There is as yet no universally accepted definition for RIF, despite many publications on this topic.[2] The latter condition merely refers to the failure to achieve a pregnancy after several ICSI attempts, a common cause being poor response to ovarian stimulation, suboptimal embryo quality, advanced maternal age and uterine factors are also relatively common causes for recurrent ICSI failure.[3]

In a few cases, even when good-quality embryos are repeatedly transferred over a number of cycles, many do not become pregnant. This is termed recurrent implantation failure (RIF) and it has emerged over recent years as a challenging and highly controversial issue in modern IVF/ICSI practice.[4] Recurrent implantation failure (RIF) is defined as the lack of any pregnancy in three consecutive In Vitro Fertilization/ Intra Cytoplasmic Sperm Injections- Embryo Transfer (IVF/ICSI-ET) cycles or good-quality embryo transfers.[4] Probable underlying etiologies for RIF are aneuploidy of embryos, uterine cavity abnormalities, diminished endometrial response, and insufficiencies in transfer techniques. These factors result in decreased pregnancy rates even at successful IVF centers, and RIF remains a problem, of both economical and psychological aspects for couples.[5]

Loutradis *et al*. demonstrated increased treatment success with an interval double transfer technique in women who had more than three failed treatment cycles with transfer of good embryos. The variability in the endometrial maturation process and increasing the receptivity "window" were cited by those authors as the main reasons for performing an interval double transfer cycle.[6] Loutradis et al. have reported an improvement of IVF/ET outcome in patient with repeated IVF/ET who underwent endometrial biopsy prior to the recent cycle. Their explanation for such improvement was that local injury to the endometrium inducing production of cytokines which enhance implantation.[6]

OBJECTIVES

To examine whether consecutive transfer of embryos on day 3 and on day 5 improves ICSI success rates in patients with multiple consecutive implantation failures.

PATIENTS AND METHODS

This is a randomized controlled clinical trial conducted at International Islamic Center for Population Studies and Research (IICPSR) Al Azhar University in assisted reproductive unit in the period from June 2014 until March 2017. The 100 patients randomly distributed into two groups (Group A & B. using enveloped sealed).

Group A (The study Group): consists of 50 patient subjected to consecutive (double) embryo transfer at day 3 and at day 5.

Group B (The control group): consists of 50 patient subjected to embryo transfer at day 5.

All patients subjected to Inclusion criteria; Age <40 year, Repeated implantation failure despite good quality of embryos, Good quality embryos on day 3 suitable for blastocyst transfer and No Uterine anomalies. All cases subjected to ovarian stimulation through long, short or antagonist protocol according to age, patient response and hormonal profile. Monitoring by transvaginal ultrasound until >3 follicles measuring >18mm then injection of 10000 IU of human chorionic gonadotropin.

Ovum pick up

34 to 36 hours after injection of human chorionic gonadotropin (HCG) collected oocyte in culture media (global total). Intracytoplasmic sperm injection (ICSI) was performed within 4 h after Ovum pick up (OPU), and the oocyte were checked for fertilization 16– 18 hrs. Later. Normal fertilization was indicated by the appearance of two pronuclei. Once post-fertilization check confirmed availability of enough number of embryos, patients were randomized to one of the 2 groups. Embryos were cultured in culture media (global total). Embryos were checked on day 3, Good-quality embryos were defined as embryos containing more than 6 cells on day 3 with grade 1 (uniform blastomeres with no fragmentation) and grade 2 (blastomeres size was slightly uneven with<20% fragmentation) embryos were being transferred.

Selection and embryos transfer

Number of embryos to be transferred was restricted to total 3 whether in single or in sequential transfer using embryos grade 1 or grade 2 only. In group 2, embryo transfer was carried out on day 5 while in group 1 (sequential D3/D5) two good-quality embryos were transferred on day 3, then the remaining good-quality embryos were placed in blastocyst culture medium and cultured until day 5 and good-quality blastocyst was transferred. At the time of embryo transfer the patients was randomized to embryo transfer on day 5 or consecutive embryo transfer on day 3 and day 5 using enveloped sealed, Luteal phase support with Progesterone 400 microgram vaginal ovules twice daily.

Outcome

Pregnancy considered to have occurred if serum β-human chorionic gonadotropin (β-hCG) concentration 14 days after ET was ≥50 U/L. Clinical pregnancies were defined by the observation of a gestational sac with or without a fetal heartbeat on ultrasound evaluation 4 weeks after ET.

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (SPSS) version 20, the comparison between two groups with qualitative data was done by using *Chi-square test*. The comparison between two independent groups with quantitative data and parametric distribution was done by using *Independent t-test*. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value < 0.05: Significant

RESULT

Table 1: Comparison of mean and SD for age and other variables among studied groups (n=100).

Total number of cases (n=100).				
Characteristics	**Minimum**	**Maximum**	**Mean**	**Std. Deviation (SD)**
Age/ year	21	40	31.11	± 5.164
Previous cycle, N	2	7	3.36	±0.73
Retrieved oocyte	4	33	9.16	± 4.18
Fertilized oocyte	3	13	6.55	± 2.3
Embryos available	3	13	5.49	± 2.13
No. of transferred embryos in Day 5	2	3	2.76	± 0.43

The age of the patients ranged from 21 to 40 years with mean and SD of 31.11±5.164 and the mean and SD of the Previous cycle 3.36± 0.73. The No. of retrieved oocyte ranged from 4 – 33 with mean and SD of 9.16 ±4.18. Regarding the No. of fertilized oocyte ranged from 3 -13 with mean and SD of 6.5±2.4. Regarding the No. of transferred embryos in day 5 ranged from 2-3 with mean and SD of 2.76 ±0.43.

Table 2: Comparison of mean and SD for age and other important variables between case and control groups (n=50 for each group).

Characteristics	Case (n=50)		Control (n=50)	
	Mean	Std. Deviation (SD)	Mean	Std. Deviation (SD)
Age/ year	31.46	± 5.65	30.68	± 4.7
Previous cycle, n	3.38	± 0.7	3.34	± 0.77
Retrieved oocyte	10.96	± 4.7	7.36	± 2.55
Fertilized oocyte	7.72	± 2.18	5.38	± 1.8
Embryos available	6.90	± 1.7	4.02	± 1.15
Numb of transferred embryos in day 5	2.68	± 0.47	2.84	± 0.37
No. of transferred embryos in day 3	2.28	± 0.45	-	-

The mean and SD of age in case group is 31.68± 5.65 and the mean and SD of the Previous cycle 3.38± 0.7, the mean and SD of retrieved oocyte is 10.96 ±4.7 and the mean and SD of the transferred embryos in day 3 is 2.28± 0.45. The mean and SD of age in control group is 30.64± 4.7 and the mean and SD of the Previous cycle 3.34± 0.77, the mean and SD of retrieved oocyte is 7.36±2.55 and the mean and SD of the transferred embryos in day 5 is 2.84± 0.37.

Table 3: Frequency of age group, result of pregnancy test, US and No. of gestational sacs in case and control groups (n=50 for each group).

Characteristics	Case (n=50)		Control (n=50)	
	No.	%	No.	%
Age group/ year				
20-24 y	3	6%	8	16%
25-29 y	19	38%	15	30%
30-34 y	16	32%	13	26%
35-40 y	12	24%	14	28%
Pregnancy test				
Positive	28	56%	18	36%
Negative	22	44%	32	64%
Clinical pregnancy				
Positive US	27	54%	17	34%
Negative US	23	46%	33	66%
No. of gestational sac				
Single	22	44%	13	26%
Multiple	5	10%	4	8%
No. of gestational sac	23	46%	33	66%

The frequency of age in case and control group/ years, most of studied groups fall between the age of 25-35 years, about the result of pregnancy test was positive in (56%, 36%) in case and control group respectively. Also result of clinical pregnancy which documented by ultrasound was positive in (54%, 34%) in case and control group respectively.

Table 4: Distribution of βHCG and ultrasound among all studied groups (n=100).

Output Result	βHCG			Ultrasound		
	No (n=100)	%	P value	No (n=100)	%	P value
Positive	46	46%	0.045	44	44%	0.044
Negative	54	54%		56	56%	

Regarding the result of **β**HCG among total studied groups 46% had positive result and 54% had negative result, but regarding the result of clinical pregnancy documented by ultrasound 44% had positive result and 54% had negative result, p value is significant (< 0.05).

Table 5: Sequential and Conventional transfer of embryos in studied patients with multiple consecutive implantation failures (n=50 for each group).

Outcome result	No. of previous trial	Cases (n=50)		Control (n=50)		P value
		No.	%	No.	%	
Positive β-hCG	< 4 times	14	28%	17	34%	0.003
	≥ 4 times	14	28 %	1	2%	
Positive ultrasound	< 4 times	14	28%	16	32%	0.003
	≥ 4 times	13	26%	1	2%	

There was statistically significant association was found between Positive β-hCG and number of previous trials in case and control group (P=0.003).There was statistically significant association was found between Positive ultrasound and number of previous trials in case and control group (P=0.003).

Table 6: Comparison of βHCG and ultrasound among case study and case control groups (n=50 for each group).

Outcome of result	Case study (n=50)		Case control (n=50)		P value
	No.	%	No.	%	
βHCG negative					
• Positive	28	56%	18	36%	0.045
• Negative	22	44 %	32	64%	
Clinical pregnancy (US)					
• Positive	27	54%	17	34%	0.044
• Negative	23	46%	33	66%	

Regarding comparison of the result of **β**HCG among the studied and control groups, in case study group 56% had positive result and 44% had negative result in comparison to 36% and 64% in case control group. P value is significant (< 0.05). But regarding comparison the result of clinical pregnancy which documented by ultrasound 54% had positive result and 46% had negative result in comparison to 34% and 66% in case control group. p value is significant (< 0.05).

DISCUSSION

Our results show that this approach improves success in ICSI/ET treatment cycles. Both groups of patients have comparable ages and similar characteristics of the stimulation cycle. Moreover, there was no significant difference in the number of embryos replaced nor was there a difference in the numbers of previous cycle failures. The findings of a pregnancy rate of 56% and a clinical pregnancy rate of 54% in our study group are significant compared to 34% in control group.

Our findings are in agreement to study who report a 50% pregnancy rate and a 38% clinical pregnancy rate,[6] also another study reported similar results for sequential transfer cycles in a similar group of patients. In their study, pregnancy rates of 30.3% and 17.1% were reported for double transfer vs.day3 transfer cycles.[7] In similar results Comparing consecutive on day 2 or 3 and day 5 to control group on day 2 or 3 Pregnancy as defined above (β-hCG level >10 m IU/Ml) was documented in 44.6% of the study group women and in 24.2% of the controls (p =0.001).[8] Clinical pregnancy was documented in 38.5% and 19.6% in the study and control groups, respectively (p =0.001). Live birth rate was 29.2% and 15.1% in the study and control groups, respectively (p=0.001).A retrospective matched case–control study was conducted and the outcomes of 213 patients with a history of repeated IVF–embryo transfer failure were analyzed, of which 33 women underwent sequential embryo transfer on day 2 and day 3 (D2/D3 group), 66 women on day 3 and day 5 (D3/D5group), 85 women underwent day-3 embryo transfer only (D3 control group) and 29 women underwent day-5 embryo transfer only (D5 control group) ,The results showed that the clinical pregnancy rate of the D2/D3 group was higher than that of the D3 group (48.5% versus 22.4%, P = 0.006) while the clinical pregnancy rates of the D3/D5 and D5 groups were not significantly different (50.9% versus 45.8%). Day-2 and day-3 sequential embryo transfer may improve the clinical outcomes for patients with repeated IVF–embryo transfer failures.[9] In another study consecutive on day 2 or 3 and day 5 to control group on day 2 or 3 pregnancy rate and clinical pregnancy rate (per embryo transfer) were significantly higher in sequential ET group (43.2% and 37.8% respectively) compared to that in day 3 group (27.4% and 21.9% respectively) (X2 = 4.04 and 4.4, P value <0.04 and <0.03 respectively), Also, implantation rate (per embryos transferred) was significantly higher in sequential ET group (17.1%) compared to that in day 3 group (10.5%).Similarly Ongoing pregnancy (per embryo transfer) was, also, significantly higher in sequential ET group (33. 8%) compared to that in day 3group (19.2%).[10] Comparing the increase in ICSI success after the interval double transfer technique cannot be attributed to an increase in the number of embryos replaced. We hypothesize that the reinserting of the catheter may affect the endometrial cavity by inducing factors, which may enhance implantation.

However, others have found a significantly higher implantation rate for embryos transferred at the blastocyst stage than that for embryos transferred on day 3.[11] Cancellation of the entire treatment cycle can happen due to failure of the embryos to develop to blastocyst stage with unfavorable emotional and economic consequences. Thus, interval double transfer approach has the advantage of blastocyst transfer without exposing the all cycle to the risk of cancellation. However the fact is that the double transfer approach enhances implantation is not doubtful according to our findings. The accurate mechanism for these findings needs to be further investigated.[12] It is important to note that this technique is suitable for patients having an adequate amount of good quality embryos to be replaced on both days of transfer and thus not suitable for poor responders it has been shown that embryos can induce an increase in endometrial receptivity.[13] Therefore, during sequential transfer, the embryo transferred on day 3 is co-cultured with the endometrium, which may improve the embryonic development potential and induce an increase in endometrial receptivity, thereby facilitating implantation of the sequentially transferred embryo.[14] Also, mechanical stimulation of the endometrium has been reported to increase the pregnancy rate in patients with repeated IVF–embryo transfer failures.[15]

Prospective studies blastocyst transfer was shown to significantly improve the implantation and live birth rates in patients with repeated ICSI–embryo transfer failures; however, it is possible that no blastocyst forms during blastocyst culture so that no embryo can be transferred.[17,16] Day-3 and day-5 transfer ensures transfer, on day 3, thereby reducing the effect of the high risk of cancellation of blastocyst transfer. Concern remains regarding the risk of multiple pregnancy associated with sequential embryo transfer due to the high number of embryos transferred. In this study The D3/D5 group had a higher number of transferred embryos than the D5 group, but the incidence of multiple pregnancies was not different. Also others suggested that the second transfer procedure might have a deleterious influence, possibly related to infection or trauma, on the implantation of embryos transferred 2 days earlier.[18] However another study found no significant differences in pregnancy rates with and without immediately repeated transfers.[19]

CONCLUSION

In conclusion, for patients with repeated ICSI–embryo transfer failures, sequential transfer on day 3 and day 5 may improve the clinical pregnancy rate in cases of repeated implantation failure as long as good-quality embryos are available.

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REFERENCES

1. K.W. Sharif, S Ghunaim, management of 273 cases of recurrent implantation failure: result of combined evidence based protocol, Reproductive Biomedicine, 2010; 21: 373-380.
2. Das M and Holzer HE, Recurrent implantation failure: gamete and embryo factors. Fertil. Steril, 2012; 97: 1021-1027.
3. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G and Gianaroli L., ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum. Reprod, 2011; 26: 1616-1624.
4. El-Toukhy T and Taranissi M., Towards better quality research in recurrent implantation failure: standardizing its definition is the first step. Reprod. Biomed. Online, 2006; 12: 383–385.
5. Margalioth EJ, Ben-Chetrit A, Gal M and Eldar-Geva T., Investigation and treatment of repeated implantation failure following IVF-ET. Hum Reprod, 2006; 21(12): 3036-3043.
6. Loutradis D, Drakakis P, Dallianidis K, Bletsa SR, Milingos S, Doumplis N, et al. A double embryo transfer on days 2 and 4 or 5 improves pregnancy outcome in patients with good embryos but repeated failures in IVF or ICSI. ClinExpObstet Gynecol, 2004; 31: 63-6.
7. Machtinger R, Dor J, Margolin, M, Levron, J, Baum, M, Ferber, B, Shulman A, Bider D, Seidman DS., Sequential transfer of day 3 embryos and blastocysts after previous IVF failures despite adequate ovarian response. Reprod. Biomed. Online, 2006; 13: 376–379.
8. Almog B, Levin I, Wagman I, Kapustiansky R, Schwartz T, Mey-Raz N, Amit A, Azem F., Interval double transfer improves treatment success in patients with repeated IVF/ET failures. J. Assist. Reprod. Genet, 2008; 25: 353–357.
9. Cong Fang, Rui Huang, Ting-Ting Li, Lei Jia, Li-Lin Li, Xiao-Yan LiangDay-2 and day-3 sequential transfer improves pregnancy rate in patients with repeated IVF–embryo transfer failure: a retrospectivecase–control study Reproductive BioMedicine Online, 2013; 26: 30-35.
10. Wael A. Ismail Madkour Bassel Noah, HenaZaheer, Awatif Al-Bahr, Amr M.S. Abdelhamid Mahmoud Shaeer, Ashraf Moawad. Does sequential embryo transfer improve pregnancy rate in patients with repeated implantation failure? a randomized control study. Middle East Fertility Society Journal, 2015; 20: 255-261.
11. Karaki RZ, Samarraie SS, Younis NA, Lahloub TM, Ibrahim MH., Blastocyst culture and transfer: a step toward improved in vitro fertilization outcome. FertilSteril, 2002; 77: 114-8.
12. Papanikolaou E, Efstratios M. Kolibianakis E, Tournaye H, Venetis C, Fatemi H., Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. Hum Reprod, 2008; 23: 91-99.

13. Wakuda K, Takakura K, Nakanishi K, Kita N, Shi H, Hirose M, et al. Embryo-dependent induction of uterine receptivity in the Mouse endometrium. J ReprodFertil, 1999; 115: 315-24.
14. Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I., Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilisation. Fertil. Steril, 2003; 79: 1317–1322.
15. Zhou L, Li, R, Wang, R, Huang, H.X, Zhong K., Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. Fertil. Steril, 2008; 89: 1166–1176.
16. Guerif F, Lemseffer M, Bidault R, Gasnier O, Saussereau M, Cadoret Single Day 2 embryo versus blastocysts-stage transfer: a prospective study integrating fresh and frozen embryo transfers. Hum Reprod, 2009; 24: 1051– 1058.
17. Levitas, E, Lunenfeld, E, Har-Vardi, I, Albotiano, S, Sonin, Y, Hackmon-Ram, R, Potashnik, G., Blastocyst–stage embryo transfer in patients who failed to conceive in three or more day 2–3 embryo transfer cycles: a prospective, randomized study. Fertil. Steril, 2004; 81: 567–571.
18. 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: 936–940.
19. Tur-Kaspa I, Yuval Y, Bider D et al. Difficult or repeated sequential embryo transfers do not adversely affect in-vitro fertilization pregnancy rates or outcome. Human Reproduction, 1998; 13: 2452-2455.