ORIGINAL RESEARCH ARTICLE

Comparison between implantation rate of two and three embryo transfers at cleavage- and blastocyst-stage: A three-year retrospective single-center cohort study from a developing country

DOI: 10.29063/ajrh2023/v27i4.8

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Abstract

In sub-Saharan Africa, multiple embryo transfer is common among fertility clinics for various reasons including endometrial receptivity, number of viable embryos, patient choice, and clinic policy. The study was based on a retrospective analysis of data obtained at the Medical Art Center, Lagos, Nigeria, from January 2018 to December 2020. 518 out of 576 cycles met the inclusion criteria for this study. Implantation was confirmed using β-hCG test results. Our study revealed no significant difference between the total implantation rate of 2-ET and 3-ET. However, there was a significantly higher implantation rate in the 2-ET fresh blastocyst transfer when compared with the 3-ET. 3-ET does not have an implantation rate advantage over 2-ET. Hence, 2-ET is safer to employ when compared to 3-ET for patients in developing countries who cannot afford aneuploidy test for single embryo transfer. *(Afr J Reprod Health 2023; 27 [4]: 73-76).*

Keywords: Embryo transfer, in-vitro fertilization, embryology, sub-Saharan Africa

Résumé

En Afrique subsaharienne, le transfert d'embryons multiples est courant dans les cliniques de fertilité pour diverses raisons, notamment la réceptivité de l'endomètre, le nombre d'embryons viables, le choix du patient et la politique de la clinique. L'étude était basée sur une analyse rétrospective des données obtenues au Medical Art Center, Lagos, Nigeria, de janvier 2018 à décembre 2020. 518 cycles sur 576 répondaient aux critères d'inclusion de cette étude. L'implantation a été confirmée à l'aide des résultats du test β-hCG. Notre étude n'a révélé aucune différence significative entre le taux d'implantation total de 2-ET et de 3-ET. Cependant, il y avait un taux d'implantation significativement plus élevé dans le transfert de blastocyste frais 2-ET par rapport au 3-ET. 3-ET n'a pas d'avantage de taux d'implantation sur 2-ET. Par conséquent, le 2-ET est plus sûr à utiliser que le 3-ET pour les patients des pays en développement qui ne peuvent pas se permettre un test d'aneuploïdie pour le transfert d'un seul embryon. *(Afr J Reprod Health 2023; 27 [4]: 73-76).*

Mots-clés: Transfert embryonnaire, fécondation in vitro, embryologie, Afrique subsaharienne

Introduction

Embryo transfer (ET) refers to the process of delivering embryo(s) fertilized in the *in vitro* fertilization (IVF) laboratory to the uterus of an intending mother or gestational carrier for implantation to occur. ET is the last step of an IVF cycle. The process was first established in Nigeria in 1984 and the steps involved have previously been reported in reputable peer-reviewed journals[1,2]. Although a relatively simple procedure, embryo transfer is of vital importance as skills are required of the clinical embryologist to select embryos based on their morphological qualities depending on the developmental stage of the embryos. The clinician is also expected to be well-skilled in delicately delivering the embryo(s) to the uterus.

Double embryo transfer (2-ET) is the most widely used ET method in fertility clinics, although elective Single Embryo Transfer (eSET) is fast becoming popular in recent times with the advent of Preimplantation Genetic Testing (PGT) for aneuploid embryos. However, in a developing country like Nigeria, it is difficult to convince patients to accept an eSET because of the financial

implications that come with paying out-of-pocket for PGT or undergoing a second embryo transfer in the case of an initially failed IVF cycle. Due to this financial burden and the country's poor economic indices, some patients specifically ask for more than two embryos to be transferred. Moreover, multiple gestations are considered a blessing in Western Africa. It is also widely assumed that transferring a high number of embryos increases the probability of pregnancy[3]. Some practitioners thus, prey on these patients' mindsets to agree to a three-embryo transfer (3-ET) and use this as their baseline for transfer as a means to boost the clinic's live birth rate (LBR).

This study was carried out to evaluate the difference between the implantation rate from 2-ET and 3-ET over 3 years. We selected these two multiple embryo transfer methods as they are sub-Saharan Africa's most widely used methods. From our findings, this is also the first study in Africa to explicitly compare the implantation rate of these two methods.

Methods

Procedure for controlled ovarian stimulation, intracytoplasmic sperm injection (ICSI), embryo vitrification, freeze-thaw, and transfer

The protocols for controlled ovarian stimulation and Intracytoplasmic Sperm Injection (ICSI) were as described in our previous works[4,5]. All oocytes were inseminated using the ICSI procedure. Fertilized oocytes were cultured using Vitrolife sequential media (G1 and G2) with a mineral oil overlay (Vitrolife Ovoil). The procedure for vitrification and freeze-thawing of embryos was also described in our published work[6]. ET was only done for patients with endometrial lining thickness ≥7mm. Only embryos with <50% fragmentation and ≥ 4 blastomeres were selected for cleavage-stage ET, while only embryos with visible inner cell mass and trophectoderm were selected for blastocyst-stage ET. ET was carried out after placing embryos in Vitrolife EmbryoGlue media and loaded using a Labotect Embryo Transfer catheter. ET was done with transabdominal ultrasound guidance in all cases. Implantation was determined two weeks post-ET using serum β-hCG levels checked two days apart. The outcome was considered positive when higher than 50 mIU/ml.

Data collection

The study was based on a retrospective analysis of data obtained at the Medical Art Center, Institute of Reproductive Medicine, Lagos, Nigeria, from January 2018 to December 2020. 518 out of 576 cycles met the inclusion criteria for this study: cycles ending with two or three transferred embryos. In a couple of cases, frozen and fresh embryos were transferred simultaneously. We exempted these cases from the study. Also, 12 cycles that had cleavage-stage freeze-thawed embryos (eight 2-ET and four 3-ET) were exempted from the study as this group is negligible. Thus, there were 504 cycles included in this study.

Statistical analysis

Data collected were inputted into a Microsoft Excel spreadsheet. The table was also constructed using Microsoft Excel. Data was exported from Microsoft Excel to GraphPad Prism 8.0.2. and statistical analysis for the significance at $p < 0.05$ using Fischer's exact test for dichotomous variables was done.

Results

The results are summarized in Table (1) and explained below.

Cleavage-stage embryo transfer

There was no significant difference between 2-ET and 3-ET performed for cleavage-stage embryos. However, there was a higher implantation rate for the 3-ET, 32.79% against 23.86% for 2-ET. For 2-ET, n = 88 while for 3-ET, n = 61.

Blastocyst-stage embryo transfer (Total)

There was no significant difference between 2-ET (50.18%) and 3-ET (42.86%) of all blastocyst transfers.

Blastocyst-stage embryo transfer (Fresh)

2-ET had a significantly (p = 0.0075) higher implantation rate (62.71%) when compared with 3-ET (30%). One hundred eighteen cycles had 2-ET while 20 cycles had 3-ET.

Table 1: Implantation rate of 2-ET and 3-ET for cleavage-stage, blastocyst-sage (fresh, frozen, total) and Total embryo transfer

	2 ET	3 ET	P
CLEAVAGE	21/88 **(23.86%)**	20/61 **(32.79%)**	0.2652
BLASTOCYST	74/118 **(62.71%)**	6/20 **(30.00%)**	0.0075 *
FROZEN (BLASTOCYST)	62/153 **(40.52%)**	30/64 **(46.88%)**	0.4518
TOTAL (BLASTOCYST)	136/271 **(50.18%)**	36/84 **(42.86%)**	0.2621
TOTAL (ALL TRANSFERS)	157/359 **(43.73%)**	56/145 **(38.62%)**	0.3198

* Significantly different from 2-ET at $P < 0.05$

Blastocyst-stage frozen embryo transfer (FET)

153 FET cycles had 2-ET while 64 cycles got 3-ET. The implantation rate for 2-ET was 40.52% which was not significantly different from 3-ET (46.88%).

All embryo transfer

The total number of transfers included in this study is 504. 71% was 2-ET while 29% was 3-ET. 2-ET had a higher implantation rate (43.73%) in general when compared to the 3-ET (38.62%), but the difference is insignificant.

Discussion

Africa has average infertility of 10.1%, while some countries peak at 32%[7]. With a high proportion of infertility in Africa and its importance to fecundability, multiple gestations are a welcome condition for patients undergoing IVF cycles in sub-Saharan Africa. Furthermore, some practitioners tend to transfer more embryos with the probability of a higher pregnancy rate and, hopefully, a higher LBR than other clinics[3]. It is majorly a business-oriented cause rather than a patient-oriented reason. Unfortunately, there is a strong relation between multiple births and the number of transferred embryos[8]. Given the risks associated with multiple gestations, the recommended maximum number of embryos to be transferred is two; however, at patients' request (after outlining the risks involved) and in exceptional cases, we increase the maximum number of embryos transferred to three, which accounted for the small sample size of our fresh blastocyst 3-ET group.

Limiting the number of embryos transferred has been proposed worldwide to minimize the number of clinically aided multiple gestations[9]. In our study, we discovered that 3-ET produced a better implantation rate than 2-ET on cleavage-stage embryos. However, this difference is not significant. To our knowledge, this is the first study to compare these two multiple ET methods in Africa. The blastocyst stage of the 2-ET group had a significantly higher implantation rate when compared to 3-ET in the fresh transfer group, in contrast to no significant difference reported by Milki *et al.*[10], which could be a result of the large disparity between the sample size of the two groups in our study. It is noteworthy that the sample size for the 2-ET blastocyst group was greater than five-fold the 3-ET blastocyst group, this accounts for a larger variety that may have affected the result. A further comparison was made in the implantation rate between 2-ET and 3-ET for freeze-thawed embryo transfer. The result showed no significant difference. These embryos were also at the blastocyst stage when they were vitrified, thawed, and transferred. It is of note that the 3-ET, in this case, was lesser than half the number of the 2-ET. Afterwards, the blastocyst-stage ET were all grouped, irrespective of their state (fresh or frozen) and we compared the implantation rate of the 2-ET with the 3-ET subgroup. It resulted in an insignificant difference with a higher positive pregnancy result from the 2-ET group compared with the 3-ET group, which was also evident in the total embryo transfers. Our study shows no significant difference in implantation rate when either 2-ET or 3-ET was used for the day-3 transfer or day-5 freeze-thawed transfer.

Assisted Reproductive Technology is a known risk factor for multiple gestations from multiple ET[11], this risk is further increased by the degree of multiple gestations. 3-ET poses a higher health risk to both the carrier and the resulting fetuses if all three embryos are implanted as found by research carried out in China[12], Korea[13], Canada[14] and the

European Society of Human Reproduction and Embryology (ESHRE) Capri Workshop Group[11]. Some multiple gestational complications include preterm birth, preeclampsia, perinatal mortality, low birth weight, and maternal morbidity[11-14].

Ethical clearance

All research protocol was approved by the Institution Review Board.

Conclusion

3-ET does not have a positive pregnancy rate advantage over 2-ET. Hence, when eSET is not attainable, 2-ET should be considered except if the patient insists on 3-ET after being informed of the risks.

References

1. Ashiru O, Akinola L, Okanlawon A and Adegoke F. Reflections on the state of the art of human reproduction. African journal of medicine and medical sciences. 1993;22(3):1-6.
2. Ashiru O, Fagbohun C, Abisogun A, Dada M and Giwa-Osagie O. In vitro fertilization and embryo transfer of human oocytes in Lagos. Medipharm Med J. 1986;1:23-6.
3. Aldemir O, Ozelci R, Baser E, Kaplanoglu I, Dilbaz S, Dilbaz B and Tekin OM. Impact of transferring a poor quality embryo along with a good quality embryo on pregnancy outcomes in IVF/ICSI cycles: a retrospective study. Geburtshilfe und Frauenheilkunde. 2020;80(8):844.
4. Okeke C, Ailoje-Ibru K, Olukoya K, Ogbeche R, Adewusi A, Iloabachie E and Ashiru O. Successful pregnancy outcome after in vitro fertilisation following Pre-implantation Genetic Diagnosis/Polymerase Chain Reaction screening for single gene disorder (sickle cell anaemia) before embryo transfer: The clinical experience of an in vitro fertilisation clinic in Nigeria. Nigerian medical journal: journal of the Nigeria Medical Association. 2014;55(1):87.
5. Olukoya OY, Okeke CC, Ailoje-Ibru K, Ogbeche RO, Adewusi AJ and Ashiru OA. Multiple gestations/pregnancies from IVF process in a fertility center in Nigeria, 2009–2011: Implementing policy towards fewer (double and single) embryo transfer. Nigerian quarterly journal of hospital medicine. 2012;22(2):80-4.
6. Iloabachie E, Ashiru O, Osumah O and Oladimeji M. Outcomes of effective cryopreservation. A two-year assessment of survival rates of vitrified and warmed blastocysts. The experience of an IVF clinic in a developing country. Fertility and Sterility. 2018;110(4):e232.
7. Gerais AS, Rushwan H. Infertility in Africa. Population sciences (Cairo, Egypt). 1992;12:25-46. Epub 1992/07/01. PubMed PMID: 12319540.
8. Monteleone PAA, Peregrino PFM, Baracat EC and Serafini PC. Transfer of 2 Embryos Using a Double-Embryo Transfer Protocol Versus 2 Sequential Single-Embryo Transfers: The Impact on Multiple Pregnancy. Reproductive sciences (Thousand Oaks, Calif). 2018;25(10):1501-8. Epub 2018/03/22. doi: 10.1177/1933719118756750. PubMed PMID: 29558870.
9. Koshida S, Ono T, Tsuji S, Sato Y, Murakami T, Arima H and Takahashi K. Impact of the recommendation for embryo transfer limitation on multiple pregnancy: A population-based study in Japan. European journal of obstetrics, gynecology, and reproductive biology. 2019;237:113-6. Epub 2019/04/29. doi: 10.1016/j.ejogrb.2019.04.018. PubMed PMID: 31029969.
10. Milki AA, Fisch JD and Behr B. Two-blastocyst transfer has similar pregnancy rates and a decreased multiple gestation rate compared with three-blastocyst transfer. Fertil Steril. 1999;72(2):225-8. Epub 1999/08/10. doi: 10.1016/s0015-0282(99)00262-9. PubMed PMID: 10438984.
11. The ESHRE Capri Workshop Group. Multiple gestation pregnancy. Human Reproduction. 2000;15(8):1856-64. doi: 10.1093/humrep/15.8.1856.
12. Wei J, Wu QJ, Zhang TN, Shen ZQ, Liu H, Zheng DM, Cui H and Liu CX. Complications in multiple gestation pregnancy: A cross-sectional study of ten maternal-fetal medicine centers in China. Oncotarget. 2016;7(21):30797-803. Epub 2016/04/30. doi: 10.18632/oncotarget.9000. PubMed PMID: 27127170; PubMed Central PMCID: PMCPMC5058718.
13. Choi SH, Park YS, Shim KS, Choi YS, Chang JY, Hahn WH and Bae CW. Recent trends in the incidence of multiple births and its consequences on perinatal problems in Korea. Journal of Korean medical science. 2010;25(8):1191-6. Epub 2010/08/03. doi: 10.3346/jkms.2010.25.8.1191. PubMed PMID: 20676332; PubMed Central PMCID: PMCPMC2908790.
14. Bissonnette F, Cohen J, Collins J, Cowan L, Dale S, Dill S, Greene C, Gysler M, Hanck B, Hughes E, Leader A, McDonald S, Marrin M, Martin R, Min J, Mortimer D, Mortimer S, Smith J, Tsang B, van Vugt D and Yuzpe A. Incidence and complications of multiple gestation in Canada: proceedings of an expert meeting. Reproductive BioMedicine Online. 2007;14(6):773-90. doi: https://doi.org/10.1016/S1472-6483(10)60681-5.