Annals of Infertility & Reproductive Endocrinology

Case Report
Published: 24 Sep, 2018

Effects of Early Embryo Cleavage on Embryo Quality and Pregnancy Outcome

Seda Karabulut[1,2], Elif Yilmaz[3], Oya Korkmaz[1,2] and Ilknur Keskin[1,2]*

[1]*Department of Histology and Embryology, Istanbul Medipol University, International School of Medicine, Turkey*

[2]*Department of Histology and Embryology, Istanbul Medipol University, School of Medicine, REMER (Regenerative and Restorative Medicine Research Center), Turkey*

[3]*Medistate Hospital, IVF Center, Turkey*

Abstract

Objective: The aim of this study was to determine the embryo development rate, embryo quality and cleavage status of the Early Cleaved (EC) and NEC (No Early Cleavage) embryos and to analyze early embryo cleavage as a parameter for embryo selection process after Intracytoplasmic Sperm Injection (ICSI).

Materials and Methods: A total of 184 patients were included in the study. All embryos were checked for early cleavage at 25 hr to 27 hrs post ICSI. Embryo quality and embryo cleavage rates of the EC and NEC embryos were compared. For pregnancy assessment the patients were divided into two groups according to the presence of early cleaved embryos that were transferred. In the first group, all the embryos transferred were EC embryos and in the second group at least one of the embryos was NEC embryos.

Results: Normal embryo development rate (normal blastomere number at the time of embryo transfer) was found significantly higher in the EC embryos than in the NEC embryos (82.9% and 65.6% respectively) (p: 0.001). Embryo qualities were higher in the EC group. Pregnancy rates were significantly higher in the EC group (46.5% and 37.5% respectively).

Conclusion: EC embryos develop more normally, have better quality and have higher pregnancy rates when transferred hence should be used as an important parameter for embryo selection after ICSI.

Keywords: Embryo selection; Icsi; Multiple pregnancy; Embryo quality

OPEN ACCESS

Correspondence:
Seda Karabulut, Department of Histology and Embryology, Istanbul Medipol University, School of Medicine, Istanbul, Turkey, Tel: 005322739864; E-mail: seda2410@yahoo.com

Received Date: *22 Aug 2018*
Accepted Date: *20 Sep 2018*
Published Date: *24 Sep 2018*

Citation:
Karabulut S, Yilmaz E, Korkmaz O, Keskin I. Effects of Early Embryo Cleavage on Embryo Quality and Pregnancy Outcome. Ann Infert Rep Endocrin. 2018; 1(2): 1010.

Introduction

A useful and easy method to increase pregnancy and implantation rates by selecting the transferred embryo which has higher potential is crucial in assisted reproductive techniques [1-3]. The identification of most viable and most competent embryos is important for Intracytoplasmic Sperm Injection technique (ICSI). Many scoring and grading criteria have been developed for selecting the best embryos for transfer. Oocyte morphology, size, pronucleus morphology, blastomere number and size, fragmentation rate, zona pellucida morphology and blastocyst morphology are the most used selection criteria for transfer [4-6]. Authors have shown that Early Cleavage (EC) of an embryo, defined as the first mitotic division happening 25 hr to 27 hrs after microinjection, is a strong indicator of competence of the developing embryo and embryo viability [5,7,8-20]. Many studies have reported EC is strongly correlated with embryo morphology [14-17], development rate to the blastocyst stage [18], chromosome abnormalities [19], embryo viability [8,12,20], implantation rate [15,21], and abortion rate [10]. Within the fertilized oocytes 5% to 38% of them were shown to cleave early [6,9].

The aim of this study was to determine the embryo development rate, embryo quality and cleavage status of the Early Cleaved (EC) and NEC (No Early Cleavage) embryos and to analyze early embryo cleavage as a parameter for embryo selection process after Intracytoplasmic Sperm Injection (ICSI).

Material and Methods

A total of 84 patients were included in the study. All embryos were checked for early cleavage

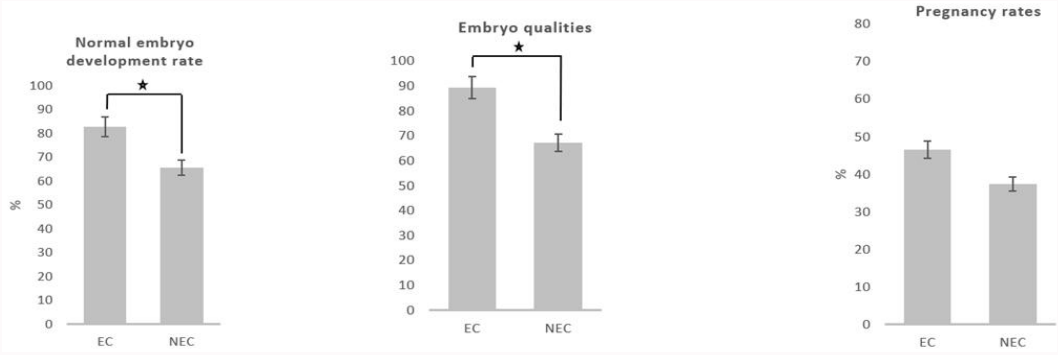

Figure 1: Normal embryo development rate, embryo qualities and pregnancy rates in Early Cleaved (EC) and non-cleaved embryos.

at 25 hr to 27 hrs post ICSI. Embryo quality and embryo cleavage rates of the EC and NEC embryos were compared. For pregnancy assessment the patients were divided into two groups according to the presence of early cleaved embryos that were transferred. In the first group, all the embryos transferred was EC embryos and in the second group none of the embryos transferred were EC or a mixture of EC and NEC embryos.

Ovarian stimulation and ICSI procedure

Ovulation induction was performed using a short or long Gonadotrophin-Releasing Hormone (GnRH) analogue suppression protocol or a GnRH antagonist protocol using human menopausal gonadotrophins or recombinant Follicle-Stimulating Hormone (FSH). Oocyte-Cumulus Complexes (OCC) was recovered 36 hrs after the administration 10000 IU of human chorionic gonadotrophin. Denuding procedure were performed using enzymatic (Hyase 10X Vitrolife, Sweden) and mechanical techniques at the same time. The nuclear maturation of the oocytes was assessed with an inverted microscope (Nikon, Japan). Metaphase II oocytes with a polar body and metaphase I oocytes that extrude their polar body within 4 hrs after oocyte pick-up, were injected with a motile sperm. Micro-injection was performed as described previously by Van Steirteghem et al. [25].

Assessment of fertilization and embryo development

Further culture of injected oocytes was performed in 25 µl microdrops of culture medium (G-IVF, Vitrolife) under light paraffin oil (OVOIL, Vitrolife). Presence of fertilizations was checked 16 to 18 hours after OPU and described as fertilized if Two Pronuclei (2PN) and two polar bodies were observed in the ooplasm. Fertilization rates were calculated by dividing the number of fertilized oocytes with the total number of mature oocytes obtained. Fertilized oocytes were checked for embryonic development on day 2 and 3. Embryo development rates were calculated by dividing the number of embryos developed with the total number of mature oocytes. The cleavage status and the quality of the embryos were assessed by an inverted microscope (X40) according to the criteria of Staessen et al. [26]. A and B grade embryos were grouped as top quality embryos whereas grade C and D embryos were grouped as poor quality. Top quality embryo rates were calculated by dividing the number of top quality embryos with the total number of embryos. Mean rates for all the parameters were calculated by averaging rates calculated for each couple.

Embryo transfer and pregnancy assessment

Embryo transfers were performed by the same clinician by the guidance of ultrasonography on day 3. Wallace (Smiths, Kent, UK) catheter were used for embryo transfer. A β-hCG value of ≥ 15 mIU/mL twelve days after the embryo transfer indicated a positive pregnancy.

Statistics

The data were analysed using SPSS (Statistical Package for Social Sciences) software (SPSS Inc., Chicago, IL, USA) for Windows 10.0. We used Shapiro-Wilk's test to evaluate the distribution of the data. Mann-Whitney U-test was used for abnormally distributed variables and Student's-t-test for normally distributed variables. Chi-square test was used to compare the pregnancy rates between the groups. For all analyses, statistical significance was assessed at $p<0.05$.

Results

Normal embryo development rate (normal blastomere number at the time of embryo transfer) were found significantly higher in the EC embryos than in the NEC embryos (82.9% and 65.6% respectively) (p=0.001). Embryo qualities were better (top quality embryo rate) in the EC embryos (89.3% and 67.2% resp) (p=0.0042). Pregnancy rates were found significantly higher in the first group than the second group (46.5% and 37.5% respectively) (p=0.04) (Figure 1).

Discussion

Many parameters may influence the outcome of ICSI but embryo selection is one of the most important factors that affect the outcome. Many parameters have been used to make this decision, including pronuclear morphology, blastomere morphology, blastocyst grading and choosing good most compotent and most viable embryos to increase implantation, pregnancy, and live birth rates is of great importance.

The use of early cleaved embryos to select the best embryos was first reported by Edwards et al. [27]. Until that time several studies have confirmed that EC is a strong indicator of embryo competency for selecting viable embryos [7-11,13,17,28,29]. Early cleavage time interval used in this study was 25 hr to 27 hrs after microinjection, which is the most frequently used time interval in most of the studies [23,30]. Many studies report the importance of EC to be used as embryo selection criteria before transfer and hence to help to avoid multiple pregnancies which is an important problem of assisted reproduction techniques. Many groups have reported an association with the quality of embryos and with rates of pregnancy and ongoing pregnancy when EC embryos were selected for embryo transfer [12,13,16,17,20,29]. Our findings confirm this study with an increased embryo quality in EC embryos than non EC embryos and

higher pregnancy rates when all the embryos that were transferred were EC embryos. We also confirm the study of Lee et al. [31] who reported that embryo quality was higher in early cleavage embryos than in non-early cleavage embryos. Also, Lee et al. [31] showed that early-cleavage embryos have a higher rate of normal development which is in accordance with our data. We have observed a higher rate of normal embryo development according to the NEC group in the EC group. In this regard, our results are similar to those of Lee et al. [31] results.

To increase pregnancy rates, implantation rates and to decrease multiple pregnancy rates, we conclude that early cleavage parameter should also be used to select best embryos for transfer. Further studies are needed to evaluate the behavior of most compotent embryos to decrease the number of embryos transferred and to reduce the risk of multiple pregnancies.

References

1. Steer C, Mills C, Tan S, Campbell S, Edwards RG. The cumulative embryo score: A predictive embryo scoring technique to select the optimal number of embryos to transfer in in-vitro fertilization and embryo transfer program. Hum Reprod. 1992;7(1):117-9.
2. Tan SL, Betts J, Mason B, Edwards RJ, Cambell S, Royston P, et al. Cumulative conception and live birth rates after in-vitro fertilization. Lancet. 1992;339(8806):1390-4.
3. Jones HW. Twins or more. Fertil Steril. 1995;63(4):701-2.
4. Borini A, Lagalla C, Cattoli M, Sereni E, Sciajno R, Flamigni C, et al. Predictive factors for embryo implantation potential. Reprod Biomed Online. 2005;10(5):653-68.
5. Ebner T, Moser M, Sommergruber M, Tews G. Selection based on morphological assessment of oocytes and embryos at different stages of preimplantation development. Hum Reprod Update. 2003;9(3):251-62.
6. Dennis SJ, Thomas MA, Williams DB, Robins JC. Embryo morphology score on day 3 is predictive of implantation and live birth rates. J Assist Reprod Genet. 2006;23(4):171-5.
7. Bos-Mikich A, Mattos AL, Ferrari AN. Early cleavage of human embryos: an effective method for predicting successful IVF/ICSI outcome. Hum Reprod. 2001;16(12):2658-61.
8. Salumets A, Hyden-Granskog C, Makinen S, Suikkari AM, Tiitinen A, Tuuri T. Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures. Hum Reprod. 2003;18(4):821-5.
9. Windt ML, Kruger TF, Coetzee K, Lombard CJ. Comparative analysis of pregnancy rates after the transfer of early dividing embryos versus slower dividing embryos. Hum Reprod. 2004;19(5):1155-62.
10. Van Montfoort AP, Dumoulin JC, Kester AD, Evers JL. Early cleavage is a valuable addition to existing embryo selection parameters: a study using single embryo transfers. Hum Reprod. 2004;19(9):2103-8.
11. Ciray HN, Ulug U, Bahceci M. Transfer of early-cleaved embryos increases implantation rate in patients undergoing ovarian stimulation and ICSI-embryo transfer. Reprod Biomed Online. 2004;8(2):219-23.
12. Shoukir Y, Campana A, Farley T, Sakkas D. Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: A novel indicator of embryo quality and viability. Hum Reprod. 1997;12(7):1531-6.
13. Sakkas D, Shoukir Y, Chardonnens D, Bianchi PG, Campana A. Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. Hum Reprod. 1998;13(1):182-7.
14. Ciray HN, Karagenc L, Ulug U, Bener F, Bahceci M. Use of both early cleavage and day 2 mononucleation to predict embryos with high implantation potential in intracytoplasmic sperm injection cycles. Fertil Steril. 2005;84(5):1411-6.
15. Ciray HN, Karagenc L, Ulug U, Bener F, Bahceci M. Early cleavage morphology affects the quality and implantation potential of day 3 embryos. Fertil Steril. 2006;85(2):358-65.
16. Ciray N. Even early cleavage and day 2 embryo score. Reprod Biomed Online. 2007;14(5):666.
17. Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod. 2001;16(12):2652-7.
18. Neuber E, Rinaudo P, Trimarchi JR, Sakkas D. Sequential assessment of individually cultured human embryos as an indicator of subsequent good quality blastocyst development. Hum Reprod. 2003;18(6):1307-12.
19. Hardarson T, Hanson C, Sjogren A, Lundin K. Human embryos with unevenly sized blastomeres have lower pregnancy and implantation rates: Indications for aneuploidy and multinucleation. Hum Reprod. 2001;16(2):313-8.
20. Shoukir Y, Chardonnens D, Campana A, Bischof P, Sakkas D. The rate of development and time of transfer play different roles in influencing the viability of human blastocysts. Hum Reprod. 1998;13(3):676-81.
21. Rienzi L, Ubaldi F, Iacobelli M, Romano S, Minasi MG, Ferrero S, et al. Significance of morphological attributes of the early embryo. Reprod Biomed Online. 2005;10(5):669-81.
22. Emiliani S, Fasano G, Vandamme B, Vannin AS, Verdoodt M, Biramane J, et al. Impact of the assessment of early cleavage in a single embryo transfer policy. Reprod BioMed Online. 2006;13(2):255-60.
23. Giorgetti C, Hans E, Terriou P, Salzmann J, Barry B, Chabert-Orsini V, et al. Early cleavage: An additional predictor of high implantation rate following elective single embryo transfer. Reprod BioMed Online. 2007;14:85-91.
24. Wharf E, Dimitrakopoulos A, Khalaf Y, Pickering S. Early embryo development is an indicator of implantation potential. Reprod BioMed Online. 2004;8(2):212-8.
25. Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, et al. High fertilization and implantation rates after intracytoplasmic sperm injection. Hum Reprod. 1993;8(7):1061-6.
26. Criteria of Staessen. 1989
27. Edwards RG, Fishel SB, Cohen J, Fehilly CB, Purdy JM, Slater JM, et al. Factors influencing the success of in vitro fertilization for alleviating human infertility. J In Vitro Fert Embryo Transf. 1984;1(1):3-23.
28. Terriou P, Giorgetti C, Hans E, Salzmann J, Charles O, Cignetti L, et al. Relationship between even early cleavage and day 2 embryo score and assessment of their predictive value for pregnancy. Reprod Biomed Online. 2007;14(3):294-9.
29. Fenwick J, Platteau P, Murdoch AP, Herbert M. Time from insemination to first cleavage predicts developmental competence of human preimplantation embryos in vitro. Hum Reprod. 2002;17(2):407-12.
30. Ardoy M, Calder_on G, Arroyo G, Cuadros J, Figueroa M, Herrer R, et al. ASEBIR criteria for the morphological evaluation of human oocytes, early embryos and blastocysts. ASEBIR clinical embryology papers 2008. Legal deposit M 6826-210. 2014.
31. Lee MJ, Lee RK, Lin MH, Hwu YM. Cleavage speed and implantation potential of early-cleavage embryos in IVF or ICSI cycles. J Assist Reprod Genet. 2012;29(8):745-50.