Peertechz

CLINICAL GROUP

JOURNAL OF Gynecological Research and Obstetrics

ISSN: 2581-5288 DOI: https://dx.doi.org/10.17352/jgro

Konstantinos A Economou*

Consultant Clinical Embryologist, "Embryogenesis" Assisted Reproduction Unit, Greece

Received: 26 December, 2018
Accepted: 30 December, 2018
Published: 31 December, 2018

***Corresponding author:** Dr. Konstantinos A Economou, "Embryogenesis" Assisted, Reproduction Unit, 49 Kifissias Avenue and German School of Athens Street, 151 23, Maroussi, Athens, Greece, Tel: +30 6977245904; E-mail: keconomou@gmail.com

Keywords: Pronuclear transfer; Spindle transfer; Polar body transfer; Intracytoplasmic sperm injection; Embryo fragmentation; Embryonic arrest; *In Vitro Fertilization*

https://www.peertechz.com

Check for updates

Opinion Article

Human zygote reconstruction by spindle, polar body or pronuclear transfer to treat repeated embryo fragmentation or embryo developmental arrest: The future is now

Summary

A common and very difficult issue to overcome as a clinical embryologist during an IVF cycle is the event of severe embryonic fragmentation or, more rarely, full developmental arrest. The current approach to bypass these phenomena is oocyte donation, but this raises several ethical concerns from the couple's side, since the offspring will bear only 50% of the biological characteristics of the parents. Apart from spindle and polar body transfer, the emerging technique in order to overcome these obstacles is called Pronuclear Transfer (PNT). PNT is safest to perform with improved results than spindle or polar body transfer. In PNT, the two parental pronuclei are being transferred into a donor's enucleated zygote and after a fusion process has been achieved, the newly constructed embryo may result into a newborn bearing both the paternal and maternal biological characteristics. This process opens a whole new horizon in assisted reproduction and will probably replace the traditional oocyte donation in the form we know it today.

Abbreviations

IVF: *In Vitro Fertilization*; ICSI: Intracytoplasmic Sperm Injection; IVM: *In Vitro* Maturation; PGD: Preimplantation Genetic Diagnosis; PGS: Preimplantation Genetic Screening; HVJ-E: Hemagglutinating Virus of Japan Envelope

Introduction

Since the beginning of the IVF era in 1978 with the birth of Louise Brown, improving pregnancy rates in IVF has become the Holy Grail chase of every assisted reproduction clinic. Although major steps have taken place towards this direction and many procedures employed in the embryology laboratory such as ICSI, IVM, PGD, PGS, oocyte and embryo vitrification etc, contribute towards this direction, there is still a major issue that cannot be treated. The cases were severe embryonic fragmentation or developmental arrest impairs embryonic development and prohibits blastocyst formation, implantation and live birth. Although embryonic "lifting" by early fragmental aspiration may improve the outcome [1], this solution is quite invasive and possibly hazardous for the developing embryo, while it cannot assist in the case of complete embryonic arrest.

It is believed that a major role in embryonic fragmentation and/or embryonic arrest is being played by apoptotic granulosa cells [2]. The ideal scenario in order to overcome these problems would be to develop a method that somehow replaces the maternal cytoplasm with a fresh and more potent cytoplasm that will contribute in a smooth embryonic development, blastulation and implantation of the developing embryo.

In order to achieve this goal some attempts have been made either by oocyte spindle transfer [3], or by polar body transfer [4,5]. Despite originally developed to treat mitochondrial disease issues, these methods may also treat severe cytoplasmic fragmentation or developmental arrest cases. The most commonly used method though, is the pronuclear transfer (PNT) [6,7].

In pronuclear transfer the end product included in the zygote, i.e. the two pronuclei are being transferred into enucleated donor oocytes and after a fusion process, the reconstructed zygote is being transferred to the patient. An embryo free of cytoplasmic fragments or developmental arrest arising most of the times after PNT is giving rise to the new baby.

Discussion

The present article emphasizes the techniques aim to

Citation: Economou KA (2018) Human zygote reconstruction by spindle, polar body or pronuclear transfer to treat repeated embryo fragmentation or embryo developmental arrest: the future is now. J Gynecol Res Obstet 4(3): 049-051. DOI: http://dx.doi.org/10.17352/jgro.000059

overcome the excess cellular fragmentation or developmental arrest in an IVF cycle. Although spindle transfer and polar body transfer appear as valid solutions, pronuclear transfer is a rapidly increasing method in such circumstances. All three methods aim at replacing the maternal cytoplasm with a donor cytoplasm and thus for all three methods the use of donated ova is of paramount importance; But not in the traditional manner we know today.

In spindle transfer [3], the oocytes to be manipulated are being treated with Cytochalasin B in order to disrupt microfilaments and increase plasma membrane flexibility before manipulation. The spindle is gently aspirated into the micromanipulation tool and next transferred into the perivitelline space of an enucleated donor ovum cytoplast. Membrane fusion between the patient spindle and the donor cytoplast is taking place by electrofusion [3]. After a successful fusion of the spindle, the reconstituted oocyte is being injected by ICSI with a single spermatozoon. The new zygote produced after ICSI bears the maternal and paternal chromosomes and the donor's cytoplast and is able to develop into a normal blastocyst.

The main disadvantage of the method though is the fact that by micromanipulating the attract in order to transfer it to the donor cytoplast it is quite possible to disturb the chromosomal alignment on the spindle and thus to introduce a man-derived chromosomal abnormality to the reconstructed zygote.

Polar body nuclear transfer (PBNT) is another alternative to treat these cases [4,s5]. In the PBNT the first polar body (PB1) that contains the maternal chromosomal constitute is being removed by polar body biopsy. The spindle of the donor oocyte is being removed as described above by micromanipulation and the maternal PB1 is being transferred into the perivitelline space of the donor cytoplast. The fusion of the PB1 and oolemma membranes in this case takes place through HVJ-E mediation [4]. The reconstructed oocyte is again injected by ICSI and the newly formed zygote is ready to conclude meiosis II and proceed with further embryonic development.

As previously, the technique of polar body transfer bears a significant disadvantage: it is very common that PB1 is fragmented in the perivitelline space of the maternal ovum. So, it is possible when biopsing the PB1 to leave some of the fragments behind and transfer only a fraction of the PB1 into the donor perivitelline space. The fragments left behind usually contain chromosomes. It is obvious that in this way there is a great risk to artificially create aneuploidies, mainly monosomies, in the reconstructed zygote.

The most robust technique rapidly growing worldwide for the treatment of mitochondrial diseases, excessive and repeated embryo fragmentation and embryonic developmental arrest, is the pronuclear transfer (PNT). In PNT both the maternal and donor oocytes are initially injected through ICSI with the paternal sperm. After 18 hours post ICSI the ova are examined for normal fertilization and those exhibiting two pronuclei are being used for the technique. Employing cytoskeletal inhibitors such as Nocodazole and Latrunculin A [7], an enucleation procedure of both parental pronuclei is taking place. The same is happening in the donor zygote as well. In the final step the parental pronuclei are transferred into the donor enucleated zygote and the fusion of the membranes is again HVJ-E mediated. The reconstituted zygote is ready for further development and the blastocyst produced is ready for therapeutic embryo transfer to the patient.

The PNT technique is the safest and more advantageous procedure compared with either the spindle or PB1 transfers because:

1. It encompasses less micromanipulation and thus is easiest and safest for the zygotes.

2. There is no danger to artificially and exogenously create chromosomal abnormalities, since the pronuclei are separately packaged in large clearly visible formations and

3. Fertilization has already been achieved and so the procedure takes place only on fertilized ova and not on all the oocytes yielded by the couple, thus is more time and cost-effective.

Conclusion

A few years ago, reconstitution of a human zygote for therapeutic purposes would seem as a science-fiction film script. We live though in the era that assisted reproduction technologies advance in a manic pace. Every clinical embryologist worldwide has come across the unfortunate event of severe and repeated embryonic fragmentation or even worse a complete developmental arrest. Since these phenomena are known to be associated with maternal apoptotic granulosa cells, the only solution had been oocyte donation. There are so many patients though who raise ethical concerns against this approach, since the maternal 50% of the couple's biological contribution to the offspring is lost. Three new approaches of zygote reconstruction have been used to overcome these concerns: the spindle transfer, the polar body transfer and the pronuclear transfer.

Among the three, pronuclear transfer is the most frequently used as it is the most safe, time and resource effective. Parental pronuclei are separately packaged in large clearly visible formations that can be easily and quickly manipulated without the risk of exogenously-introduced chromosomal abnormalities into the new construct.

The clinical embryologist has now a very powerful weapon in his arsenal to overcome excessive and insisting embryonic fragmentation or the detrimental embryonic arrest that could be so devastating, destroying the IVF cycle of a couple. What in the past could be a dream, has become a reality through the pronuclear transfer procedure. So we can loudly say that the future is now!

Citation: Economou KA (2018) Human zygote reconstruction by spindle, polar body or pronuclear transfer to treat repeated embryo fragmentation or embryo developmental arrest: the future is now. J Gynecol Res Obstet 4(3): 049-051. DOI: http://dx.doi.org/10.17352/jgro.000059

References

1. Kim SG, Kim YY, Park JY, Kwak SJ, Yoo CS, et al. (2018) Early fragment removal on *in vitro* fertilization day 2 significantly improves the subsequent development and clinical outcomes of fragmented human embryos. Clin Exp Reprod Med 45: 122-128. Link: https://goo.gl/BkWPWK

2. Bencomo E, Pérez R, Arteaga MF, Acosta E, Pena O, et al. (2006) Apoptosis of cultured granulose-lutein cells is reduced by insulin-like growth factor I and may correlate with embryo fragmentation and pregnancy rate. Fertil Steril 85: 474-480. Link: https://goo.gl/T2Y72L

3. Zhang J, Liu H, Luo S, Lu Z, Chávez-Badiola A, et al. (2017) Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. Reprod Biomed Online 34: 361-368. Link: https://goo.gl/a6yeHd

4. Zhang SP, Lu CF, Gong F, Xie PY, Hu L, et al. (2017) Polar body transfer restores the developmental potential of oocytes to blastocyst stage in a case of repeated embryo fragmentation. J Assist Reprod Genet 34: 563-571. Link: https://goo.gl/gnF8zX

5. Ma H, O'Neil RC, Gutierrez NM, Hariharan M, Zhang ZZ, et al. (2017) Functional Human Oocytes Generated by Transfer of Polar Body Genomes. Cell Stem Cell 20: 112-119. Link: https://goo.gl/abXAJd

6. Zhang J, Zhuang G, Zeng Y, Grifo J, Acosta C, et al. (2016) Pregnancy derived from human zygote pronuclear transfer in a patient who had arrested embryos after IVF. Reprod Biomed Online 33: 529-533. Link: https://goo.gl/FajQXE

7. Hyslop LA, Blakeley P, Craven L, Richardson J, Fogarty NME, et al. (2016) Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. Nature 534: 383-386. Link: https://goo.gl/BVxzPs

Citation: Economou KA (2018) Human zygote reconstruction by spindle, polar body or pronuclear transfer to treat repeated embryo fragmentation or embryo developmental arrest: the future is now. J Gynecol Res Obstet 4(3): 049-051. DOI: http://dx.doi.org/10.17352/jgro.000059