Case Report

Treatment of Repeated Implantation Failures with Sequential Embryo Transfer to Achieve a Successful Pregnancy: A Case Report

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Abstract

To report a case with multiple embryo transfers failure. A 30-year-old woman, married for 10 years, has undergone five ovulation stimulation cycles, and eight transfers cycles without achieving clinical pregnancy. A successful pregnancy was obtained after day 2 cleavage stage embryo combined with the day 5 blastocyst transfer, and a healthy male infant was delivered at 39^{+3} weeks.

Keywords: Repeated/recurrent implantation failure; Sequential/combined embryo transfer

1. Introduction

Repeated/recurrent implantation failure (RIF) refers to infertile patients who have undergone 3 or more cycles with transferring 1-2 high-quality embryos and fail to achieve implantation or clinical pregnancy. The incidence rate is around 10%-15% [1] and is regarded as a prominently tough issue in the assisted reproductive technology (ART). The embryonic and maternal factors are classified as the main reasons that contribute to RIF [2-3]. Specifically, on the maternal aspect, the unsatisfactory endometrial receptivity is considered to be one of the main causes of RIF. Hence, the improvement of endometrial receptivity and the promotion of the interaction

between embryo and endometrium are crucial at improving the pregnancy outcome of women with RIF. This article reports on a successful pregnancy and delivery of a patient with multiple transfer failures via using sequential embryo transfer.

2. Case Report

The patient is a healthy 30-year-old gravida 1 para 0 Chinese woman married for over 10 years. The left Fallopian tube resection and right Fallopian tube ligation were undergone due to the left ectopic pregnancy in 2011. The patient experienced five controlled ovarian stimulation cycles from 2011 to 2015, with eight transfer cycles invalidated to be pregnant (Table 1). The hysteroscopy showed no abnormalities when the second transfer failed. In addition, the couple has normal chromosomes.

Number of ovarian stimulation	Protocol	Number of oocytes obtained	Transferable embryos	Transferred embryos/blastocysts	Outcome
1	Agonist	4	2 (D3)	2 fresh embryos	Non-pregnancy
2	Agonist	12	6 (D3) 2 (D5)	3 fresh embryos 3 frozen embryos 2 blastocysts	Non-pregnancy
3	Mild stimulation	7	4 (D3)	2 frozen embryos 2 frozen embryos	Non-pregnancy
4	Mild stimulation	0	0	0	Non-pregnancy
5	Mild stimulation	3	2 (D3) 1 (D5)	2 frozen embryos 1 blastocyst	Non-pregnancy

Table 1: The overview of multiple transfers details.

2.1 Ovarian stimulation protocol

In early March 2016, Antagonist protocol began on day three of menstruation, Human menopausal gonadotropin (HMG Lizhu, Zhuhai, China) 150IU was injected daily for 7 days, Cycle monitoring was conducted every 1–2 days, 0.125mg GnRH antagonist cetrorelix (Cetrotide; Merck-Serono, Geneva, Switzerland) daily was added in the presence of follicle(s) with diameter >12mm or luteinizing hormone (LH) levers >10IU/L. When one dominant follicle reached a diameter >18mm, the final oocyte maturation was induced with 0.2mg gonadotropin-releasing hormone (GnRH) agonist triptorelin (Decapeptyl, Ipsen, Signes, France). Transvaginal ultrasound (TVS)-guided oocyte retrieval was performed 35 hs after oocyte triggering. All follicles of less than 12mm were put aside for the second stimulation in the luteal phase. One oocyte was subsequently collected for *in vitro* fertilization (IVF), followed by D3 embryo (with 8 cells grading 2) frozen using vitrification [4]. Embryo quality was determined according to the number and regularity of blastomeres and the degree of fragmentation [5]. Luteal phase ovarian stimulation started one day after oocyte retrieval with a combination of 100mg/d clomiphene (Fertilan; Codal-Synto Ltd, Limassol,

Cyprus) and 225IU/d HMG for 6 days. When the size of two dominant follicles >18mm, 5000IU human chorionic gonadotropin (HCG, Lizhu, Zhuhai, China) was injected for triggering final maturation. Oocyte retrieval was performed 36 h later. Six oocytes were harvested and fertilized via IVF. One D2 embryo (with 4 cells grading 2) was frozen, the other 3 embryos were continuing cultured for 4 days but failed to form blastocysts.

2.2 Endometrial preparation and embryo transfer

Hormone replacement therapy was used for endometrial preparation in May 2016. Oral estradiol valerate 4 mg/d (Progynova, Schering, Germany) was administrated from day three of the menstrual cycle for 6 days, then 6 mg/d for 5 days. When endometrial thickness was 10 mm, 20mg/d dydrogesterone and 90mg/d of micronized progesterone (Crinone, Merck Serono S.A., Switzerland) were added. One day 2 cleavage embryo (4 cells,grading 2) warming and the transfer was performed two days after progesterone administration, another D3 embryo (8 cells,grading 2) was thawed after 3 days of progesterone and cultured to blastocyst (Grading B5bb) for the transfer after 48 hours of culture. Blood HCG showed 636.7 IU/L on the 14th day after the first transfer. Intrauterine single pregnancy, fetal vascular pulsation was confirmed after 5 weeks of transfer. A baby boy was delivered by cesarean section at 39^{+3} weeks of gestation, weighing 3.36kg, 50cm of length, with Apgar score 10 points in one minute after birth.

3. Discussion

For RIF patients with morphologically "normal" endometrium and embryos, in addition to embryonic factors, more reasons may lie in the poor endometrial microenvironment. Therefore, how to improve the receptivity of the endometrium has become an important breakthrough [6-7]. Achieving the precise synchronization of the interaction between the endometrium and the embryo is crucial for embryo implantation. In addition to being regulated by estrogen and progesterone, a variety of signal molecules produced locally in the endometrium, such as prostaglandins, transforming growth factors, cytokines, chemokines, integrins and leukaemia inhibitory factors also participate in governing the establishment of endometrial receptivity, thus providing a necessary environment for the survival, implantation and embryonic development. Moreover, during embryo implantation, sufficient chemokines and inflammatory cytokines are required to regulate the invasive ability of the embryo at the maternal-fetal interface to ensure successful embryo implantation [8-9]. Studies have found that early embryo-derived growth factors, cytokines and signalling molecules may play a critical role in ameliorating endometrial receptivity, promoting the subsequent blastocyst implantation. The endometrium can be induced by embryos in the early stage to enter the state of "implantation window" [10], which may be related to the secretion of some growth factors, such as transforming growth factor, epidermal growth factor, granulocyte-macrophage stimulating factor etc. Thus, the endometrial receptivity, as matter of fact, could be induced by these factors and likewise, the embryonic development is promoted. Melnick et al compared the rate of blastocyst formation in the condition of embryos co-cultured and separately cultured with endometrium and embryos, and found that the embryo growth and blastocyst formation rate of the co-culture group were significantly more effective than those of the separately cultured group [11].

Further, Binder and colleagues demonstrated that co-culture of endometrium and embryos *in vitro* could improve endometrial receptivity, which is conducive to embryo growth and implantation [12]. The conditioned media of early embryos from human abnormal fertilized tri-pronuclear (3PN) zygotes co-cultured with endometrial epithelium was added to endometrial stromal cells, the expression of matrix metalloproteinase-3 (MMP-3) was significantly increased, which has been shown to promote implantation of blastocysts [13]. Hence, the accumulated evidence indicates that the early dialogue between the endometrium and the embryo is beneficial for the implantation. Basically, for IVF, embryos are cultured *in vitro* for 3 to 5 days, which is known as the difference between IVF and natural conception. Therefore, under the condition that the endometrium lacks the stimulation of embryo-derived factors, the endometrial receptivity may be affected, especially for some patients with the poor endometrial environment, which would further perturb embryo growth and the synchronization of the embryo and the endometrium, thereby reducing the chance of embryo implantation.

Conventionally, the developmental potential of embryos is not yet fully displayed when transferring at the cleavage stage. However, in the case of simple blastocyst transfer, due to the late embryonic stage, the endometrium is in the absence of the embryo-derived growth factors, cytokines and signal molecules produced by the early stage of embryos, which may not be favourable to those patients with unsatisfactory endometrial receptivity. At present, whether combined/sequential transfer can optimize the outcome of RIF has not yet been fully determined, there is still controversy. Fang et al. investigated the sequential transfer of embryos on the second and the third day respectively, which could greatly increase the RIF pregnancy rate to some extent [14]. However, Tehraninejad and colleagues provided data on the third and fifth day of combined blastocyst transfer and the results failed to suggest an improvement in RIF pregnancy [15].

4. Conclusion

The present study shows that the day 2 cleavage stage embryo combined with the day 5 blastocyst has beneficial effects on the patient with 8 cycles of transfer failure, with a successful pregnancy and delivery of a healthy infant. The quality of previously transferred embryos was excellent, most likely, failures were due to the unfavourable receptivity of the endometrium. Consequently, we speculate that the factors secreted by the early embryos may have an inducing effect on the endometrium, increasing its receptivity and creating favourable conditions for the subsequent blastocyst transfer. Double embryo transfer provides an alternative option for RIF patients. However, further research is essential to explore the effectiveness of day 2 and day 5 sequential transfer on patients with RIF.

Conflicts of Interest

The authors declare no conflict of interest.

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