**The Efficacy of Endometrial Injury during Frozen-Thawed Embryo Transfer Cycles in Patients with Repeated Implantation Failure- A Case Series**

**Abstract**

**Background and Objective:** Repeated implantation failure (RIF) is still remain an ongoing challenge. RIF may be contributed to the embryo or the endometrium, which any abnormalities of each two may result implantation failure. The aim of present study was to report the effect of endometrial injury (EI) during frozen embryo transfer (FET) on pregnancy outcome in RIF patients.

**Material and methods:** In this retrospective case series, since 2018 to 2020, 20 patients with a history of RIF who underwent EI during FET cycle were evaluated.

**Results:** Chemical, clinical and ongoing pregnancy and live birth was achieved in 8 (40%), 7 (35%), 6 (30%) and 6 (30%) patients, respectively.

**Conclusion:** The present study shows that using EI in FET cycle is a feasible, safe and efficient method in improving clinical outcomes. More researches are needed to find out the real effect of EI during FET in RIF patients.

**Key words:** Embryo transfer, Cryopreservation, Case report, Repeated implantation failure, Endometrium

**Introduction**

Although, in-vitro fertilization (IVF) has experienced considerable progress regarding the rate of implantation and live birth, unexplained repeated implantation failure (RIF) is still an ongoing challenge in this era.

RIF has been defined variously, but widely accepted definition describes RIF as three embryo transfer failures that one or two high-quality embryos has been transferred in each cycle(1)

A successful implantation requires a good quality embryos and a receptive endometrium, which any abnormalities of each two results may result implantation failure. Aiming to improve endometrial receptivity, several studies have shown that using EI during luteal phase before IVF/intra-cytoplasmic sperm injection (ICSI) leads to higher rate of implantation and clinical pregnancy (2-4). EI has shown beneficial effects on pregnancy rate when used prior to FET cycles in patients with RIF(5). To the best of our knowledge, there is no study on EI during FET cycle.

In the present retrospective case series, twenty patients with RIF who had undergone endometrial injury during FET cycle are described.

**Methods**

In this retrospective case series, we report a series of patients with a history of RIF who underwent endometrial injury during FET cycle from 2018 to 2020 at Mehr medical institute.

The participants in this study were recruited from patients having at least 3 failed IVF cycles. Those included were: one or more good and top quality embryos, age≤40 years and basal FSH less than 10 ng /ml. Patients with non-transferable embryos and type C endometrial pattern were excluded from the study.

Data were extracted from the patients´ records. Informed consent was not required in this study due to that the study was retrospective and patients´ information was not recorded for the objective of the study. The study was approved by Research Ethics committee of Guilan university of medical sciences.

**Endometrial preparation for FET**

All participants were undergone ultrasonography on the second day of the FET cycle. Estradiol 4 mg/day were started on day two and continued until detecting an endometrial thickness of 6-7 mm and three-line pattern in vaginal ultrasonography, under a very light sedation, hysteroscope guide was introduced into the uterus and four points in the fundus underwent endometrial injury using scissors.

The speculum was passed through the vagina to view the cervix. We used a hard hysteroscope with a diameter of 6.5 mm for hysteroscopy. An electronic pump was used for the insertion of distending medium to provide an intrauterine pressure of nearly 45 mm Hg. To make four local 2 mm depth and width injuries, we inserted the scissors through a 4 mm hysterescopic channel. The lesions were made on posterior and anterior endometrium 10-15 mm away from fundus.

 Once, an endometrial thickness equal to or more than 8 mm, estradiol was continued at 6 mg/day and 400 mg suppository plus 100 mg intramuscular progesterone was added for 3 to 5 days based on day 3 or 5 embryos. The tip of catheter (Labotect Gmbh, Germany) was placed at 1-1.5 cm from the fundus and a maximum of 3 embryos were transferred transvaginally. The luteal phase support was performed with 400 mg suppository plus 100 mg intramuscular progesterone. Clinical pregnancy was defined as the presence of gestational sac with cardiac activity under transvaginal examination. The ongoing pregnancy was defined as positive cardiac activity after 16 weeks of gestation. Clinical and ongoing pregnancy and live birth was considered as primary outcome.

**Results**

Descriptive statistical analysis (mean, standard deviation, median, maximum, and minimum) was performed using SPSS program.

Twenty cases entered the study, with a mean age and number of previous failed implantation of 36±3.6 (range 28-39) and 4.9±1.3, respectively. The basal characteristics of the participants are represented in table I. All couples had a history of high-quality embryo transfer in previous cycles. Long protocol was used to induce ovarian stimulation in all cases. Patients´ cycle characteristics are presented in table II. Chemical, clinical and ongoing pregnancy and live birth was achieved in 8 (40%), 7 (35%), 6 (30%) and 6 (30%) patients, respectively (Table III).

**Discussion**

In this case series, reporting 6 live births in 20 patients, we demonstrated that endometrial injury offers a good chance of pregnancy in patients with a history of RIF.

Implantation is a multi-staged process in which embryo attaches the endometrium and starts invading deeper layers of uterus. During the implantation window, the crosstalk between embryo and endometrium leads to the attachment and invasion of embryo into endometrium. Endometrial receptivity can be evaluated by endometrial stromal decidualization and the development of pinopods and microvilli on uterine luminal epithelial surface. On molecular level, these changes are made via adjustments in the transcription and expression of certain cytokines, growth factors, and adhesive molecules.

Studies indicated that endometrial injury may increase endometrial receptivity by regulating the expression of genes related to implantation (6, 7). LEI can be performed using scratching, biopsy, or hysteroscopy in the luteal phase of cycle prior ovarian stimulation for IVF and has been shown to improve implantation in RIF patients (8). Three mechanisms has been described for these improvements: endometrial decidualization (9), cytokines and growth factors secretion (10), and synchronization between endometrium and embryo development (11).

Contradictory results have been reported based on the time and manner of endometrial injury. Despite that some studies have shown increased rate of pregnancy in cycles with LEI, others have not observed any significant difference regarding pregnancy outcomes between patients with and without receiving LEI. A two-fold increase in implantation rate following 2-4 local injuries by a biopsy catheter in various days of the spontaneous menstrual cycle prior IVF-ET treatment in RIF patients was indicated (12). In a prospective controlled trial, it was indicated that taking two small samples from anterior and posterior uterus walls endometrium, using a Novak curette, on oocyte retrieval day, has negative impacts on endometrial receptivity and implantation (13). In a study on patients with a failed IVF/ICSI, endometrial injury increased the live birth rate by 4.6% in the second IVF/ICSI. Endometrial scratching was performed using an endometrial biopsy catheter in the menstrual period before the start of ovarian stimulation (14).

A retrospective cohort study on RIF patients suggested that soft curettage of the endometrium twice, prior to FET cycle, positively affects pregnancy rates (15). Furthermore, a randomized control trial indicated that applying EI with a Piplle catheter on the third day of the menstrual cycle prior to FET in patients with two or more failures of implantation may enhance clinical outcomes (5).

It is clear that the timing and number of endometrial biopsies, also the degree of EI, may influence the endometrial receptivity and pregnancy outcomes. The key difference of our study compared with previous studies in this era is in the time of performing EI. Previous literature mainly studied the efficacy of EI in luteal phase prior to FET cycle, whereas our study performed EI during FET cycle when an ENT of 6 or 7 mm was detected on ultrasound. Another difference of our study is in terms of the method of performing EI, which was through using scissors in four areas of the fundus.

The rate of live birth following EI was 30% which demonstrates the efficacy of this method in patients with more than 3 failed IVF-ET cycles. The present study shows that using EI in FET cycle is a feasible, safe and efficient method in improving clinical outcomes. More researches are needed to find out the real effect of EI during FET in RIF patients.

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Table 1- Basal characteristics of cases

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Patients** | **Age** | **Primary infertility** | **Secondary****infertility** | **Number of previous failed implantation** | **FSH** | **AMH** |
| **1** | 39 |  |  | 3 | 6.5 | 0.8 |
| **2** | 35 |  | 6 | 7 | 4.2 | 4.3 |
| **3** | 33 | 6 |  | 3 | 4.4 | 2.8 |
| **4** | 38 | 8 |  | 3 | 9.1 | 1.2 |
| **5** | 39 |  | 3 | 4 | 8.4 | 0.5 |
| **6** | 39 | 22 |  | 4 | 4.7 | 0.6 |
| **7** | 38 | 18 |  | 7 | 5.6 | 4.2 |
| **8** | 30 | 13 |  | 6 | 3.8 | 2 |
| **9** | 32 | 9 |  | 4 | 8.2 | 0.8 |
| **10** | 36 | 7 |  | 4 | 4.2 | 7.82 |
| **11** | 31 |  | 2 | 4 | 3 | 4.3 |
| **12** | 32 |  | 5 | 5 | 3.5 | 1.2 |
| **13** | 39 | 15 |  | 6 | 3.6 | 4.1 |
| **14** | 39 | 6 |  | 5 | 4.8 | 2.4 |
| **15** | 39 | 8  |  | 7 | 3.2 | 2.9 |
| **16** | 38 |  | 4 | 5 | 6.2 | 1.1 |
| **17** | 38 |  | 13 | 5 | 3.8 | 4.3 |
| **18** | 28 | 10 |  | 4 | 8.7 | 1.3 |
| **19** | 39 | 15 |  | 6 | 5.4 | 1.3 |
| **20** | 38 | 6 |  | 6 | 4.2 | 1.6 |

FSH: Follicle Stimulating Hormone

AMH: Anti mullerian hormone

Table 2- The cycle characteristics of cases

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patients** | **Dose of ovarian stimulation** | **Sperm concentration**  | **Sperm morphology** | **Sperm** **motility** | **2PN/****metaphase II** | **Endometrial thickness** | **Number of top and good quality transferred embryos** |
| **1** | 3825 | 34 | 61 | 5 | 1.25 | 7 | 3 |
| **2** | 2475 | 45 | 40 | 13 | 0.92 | 9.8 | 2 |
| **3** | 3600 | 35 | 9 | 4 | 0.77 | 8.2 | 3 |
| **4** | 2850 | 29 | 63 | 17 | 1.17 | 6.8 | 1 |
| **5** | 4050 | 53 | 73 | 17 | 0.33 | 8 | 1 |
| **6** | 5325 | 66 | 81 | 19 | 0.33 | 8.1 | 1 |
| **7** | 1200 | 40 | 68 | 14 | 0.38 | 8 | 3 |
| **8** | 3000 | 61 | 72 | 18 | 0.71 | 8.23 | 1 |
| **9** | 4275 | 61 | 85 | 20 | 1.00 | 12 | 2 |
| **10** | 1350 | 40 | 75 | 17 | 0.79 | 8 | 2 |
| **11** | 1125 | 42 | 70 | 17 | 0.71 | 7.9 | 3 |
| **12** | 2700 | 58 | 78 | 18 | 0.70 | 8.2 | 2 |
| **13** | 1200 | 37 | 71 | 18 | 1.08 | 7 | 3 |
| **14** | 2550 | 42 | 74 | 17 | 1.10 | 8 | 3 |
| **15** | 2025 | 40 | 70 | 7 | 0.64 | 8 | 2 |
| **16** | 2100 | 66 | 72 | 18 | 1.00 | 9.3 | 3 |
| **17** | 1800 | 37 | 65 | 10 | 0.50 | 9.6 | 2 |
| **18** | 3525 | 26 | 75 | 13 | 0.60 | 8 | 3 |
| **19** | 2550 | 18 | 72 | 18 | 0.80 | 6.8 | 2 |
| **20** | 1950 | 29 | 70 | 15 | 0.50 | 10 | 3 |

2PN: Two-pronuclei

Table 3- the pregnancy outcome of cases

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patients** | **Chemical pregnancy** | **Clinical** **pregnancy**  | **Ongoing pregnancy** | **Live birth** | **Implantation rate** |
| **1** | n | n | n |  |  |
| **2** | n | n | n |  |  |
| **3** | p | p | p | p | 0.67 |
| **4** | n | n | n |  |  |
| **5** | n | n | n |  |  |
| **6** | n | n | n |  |  |
| **7** | n | n | n |  |  |
| **8** | p | n | n |  |  |
| **9** | p | p | p | p | 0.67 |
| **10** | n | n | n |  |  |
| **11** | n | n | n |  |  |
| **12** | n | n | n |  |  |
| **13** | n | n | n |  |  |
| **14** | p | p | p | p | 0.60 |
| **15** | p | p | p | p | 0.80 |
| **16** | p | p | p | p | 0.60 |
| **17** | p | p | n |  | 0.50 |
| **18** | n | n | n |  |  |
| **19** | n | n | n |  |  |
| **20** | p | p | p | p | 0.25 |