Original Research

Pregnancy success rate at recurrent implantation failure patients after hysteroscopic endometrial injury: preliminary study

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Summary

Purpose of Investigation: To assess better implantation and pregnancy rates following endometrial injury in the presence of previous implantation failure, through a retrospective study in a tertiary University Clinic. The aim of this study is to assess improved embryo implantation after hysteroscopic fundal endometrial injury in repeated implantation failure in patients. *Materials and Methods:* Twenty-nine women who had recurrent implantation failure participated in the study. They had three or more implantation failures with good quality embryo. Anti-Mullerian hormone (AMH) levels, age, antral follicle count, body mass index, and FSH levels were collected. Frozen/thawed embryos (FTE) were transferred in all subjects. Before embryo transfer the authors performed hysteroscopic endometrial injury by micro cold knife. *Results:* Median age was 38.62 ± 2.47 years in the study. AMH levels changed between 0.35 and 3.8 ng/ml. The pregnancy rate following hysteroscopy: 17 (58,6%) were pregnant and 12 of 29 subjects did not become pregnant. The women who became pregnant following hysteroscopy were younger and had a higher antral follicle count than the non-pregnant women ($p = 0.030$ and $p = 0.0017$ respectively; $p < 0.05$). *Conclusion:* The endometrial injury before frozen-thawed embryo transfer had a positive effect on patients who had previous recurrent implantation failure.

Key words: Recurrent implantation failure; In-vitro fertilization; Infertility; Local endometrial injury; Clinical pregnancy rate.

Introduction

Infertility has been historically a growing problem in humankind and it may be related to be part of the various medical problems that have increased up to 50% since 1955 in the world [1] and nearly 10-15% of couples who are already suffering from it [2]. Perfect implantation and higher pregnancy rates require the development of high-quality embryos and good endometrial receptivity. Even with better in-vitro fertilization techniques and improving embryo culture conditions, implantation is the still limiting step in the in-vitro fertilization/embryo transfer (IVF-ET) process [3].

Endometrial micro-environmental changes are the most important for implantation of the developing embryo. In humans endometrial receptive period is mid-secretory 19-23 days of cycle, which called as window of implantation (WOI). Implantation is very complicated interaction between the embryo and endometrium. Thin endometrium has poor receptivity and, immunologic factors adversely affect implantation of embryo. In recent years it has been shown that endometrial receptivity regulated by signaling molecules, prostaglandins, growth factors, cytokines, chemokine, leukemia integrin, and cadherin [3-6].

Pregnancy rates following IVF-ET procedure are determined by the woman's age, the indication for IVF, uterine pathology, the treatment protocol employed, ovarian reserve, immunological factors, number of embryos trans-ferred, number of prepared embryos, embryo quality, embryo transfer technique, sperm quality, and luteal phase support. However, from the previously described factors, both reduced endometrial receptivity and low embryo quality are the most important ones [7].

Though a clear definition remains to be established, in general terms, repeated implantation failure (RIF) depicts the clinical situation in which a woman fails to achieve pregnancy after the transfer of good quality embryos [8, 9]. In patients with repeated IVF treatment failure, blastocyst transfer is also often advised. This recommendation is based on studies that have found higher implantation rate for women who underwent blastocyst transfer compared to those in whom embryos were transferred on day 2 or 3 [10-12]. The second step is the evaluation of endometrial cavity by hysteroscopy.

A systematic review of the literature and meta-analysis of seven studies published until 2012 showed that pregnancy rate in women who did undergo local endometrial injury (LEI) was 71% higher compared to pregnancy rates in women with no intervention. In respect of data presented in the ESHRE PGD Consortium of 2009, the implantation rates were determined to be lower than 23.9% in this category [13].

Endometrial injury is an intentional mechanical trauma to the endometrium by biopsy or curettage. The hypothesis

of this intervention emerged from rodent experiments more than a century ago. Uterine trauma in rodents induced decidualization and that observed under the influence of progesterone when the endometrium was ready for implantation [14]. Also, injury-induced local inflammatory reaction could favor implantation [15]. Natural killer (NK) cells which are reduced during controlled ovarian stimulation, endometrial injury may increase them within the endometrium [16, 17]. Small limited endometrial injury could be successfully generated with pipelle biopsy or hysteroscopy [17].

Impaired endometrial receptivity may be one of underlying factors for the unexplained infertility. The present authors believe that endometrial injury facilitate the preparation of receptive endometrium [18]. The aim of this study is to show improved embryo implantation and pregnancy rates after hysteroscopic fundal endometrial injury.

Materials and Methods

Medical records of infertile couples that had good quality embryo and IVF failure were retrospectively reviewed. Patients who had more than three unsuccessful IVF attempt and anti-Mullerian hormone (AMH) level less than 0.35 and greater than 4 ng/ml were excluded. The authors took into consideration patients FSH levels, BMI, antral follicular count (AFC), and their effect on pregnancy.

Controlled ovarian stimulation protocol was begun on the second day of menstruation. Patients underwent ovarian stimulation using protocols with combination of GnRH agonist/GnRH antagonist and recombinant FSH/hMG. The authors used recombinant FSH 150-450 IU/day, hMG 75-150 IU/day and GnRH antagonist 0.125 mg/day in the ovarian stimulation.

After oocyte fertilization by intracytoplasmic sperm injection (ICSI) procedure, embryos were cultured in a blast assist extended culture media. Embryo quality was assessed by an embryologist at days 2 and 3 after fertilization. The authors preferred to freeze embryos at five days of culture in the blastocyst stage, because they planned hysteroscopic surgery for endometrial injury. They performed endometrial injury by office hysteroscopy under intravenous sedation by propofol in the operation room.

Endometrial injury technique included a hysteroscope (2.9 mm 30-degree optic) between the seventh and tenth days of menstrual cycle before embryo transfer preparation. Endometrial injury was performed with three cuttings of 0.5 cm on the front endometrial wall, 1 cm below the endometrial fundus level. A cold endoscopic micro-knife was also used and no energy modality was used.

Endometrial preparation in the FTE cycle, included hormone replacement therapy that was the standard endometrial preparation protocol for FTE cycles with GnRH agonist daily: 10 u a day it begun in the previous cycle and oral estrogen (ethinyl estradiol) for 20 days. Ethinyl estradiol 1 mg was used in the first week once a day, in the second week twice a day, and in the last week three times a day. When endometrial thickness was more than > 7.0 mm at transvaginal sonography, patients began to apply vaginal progesterone 8% twice a day and an addition oral estrogen three times a day. Embryo transfer was made by a Wallace catheter under transvaginal ultrasound guidance.

After embryo transfer, patients received luteal phase support by 8% of vaginal progesterone gel daily. Serum hCG level was measured 12 days after embryo transfer and ultrasound was performed when the hCG level more than 1500 mIU/ml). Clinical pregnancy diagnosis was done by visualization of gestational sac by ultrasound pregnancy.

The Acibadem Mehmet Ali Aydınlar University Atakent Hospital Ethics Committee approved the study. All patients gave written informed consent about stimulation protocol, freeze-thaw procedure, and information texts for hysteroscopic surgery, and informed all complications about surgery and anesthesia.

Statistical analysis was performed by Number Cruncher Statistical System 2007 (NCSS). Mean and standard deviation for each continuous variable and frequency were calculated in both pregnant and non-pregnant (control) groups. Distribution of each variable determined by Kolmogorov-Smirnov Test. The Mann-Whitney U test and Student's *t*-test was used to compare independent samples depending on the data distribution. Odds ratios and their 95% confidence intervals (CIs) were calculated. *P* value < 0.05 was considered statistically significant.

Results

The study was performed at Acibadem Mehmet Ali Aydınlar University Atakent Hospital ART and IVF Unit between 2015-2016 with 29 women who fulfilled the inclusion criteria and history of implantation failure. Median age was 38.62 ± 2.47 years in the study. AMH levels changed between 0.35 and 3.8 ng/ml. The pregnancy rate following hysteroscopy included 17 (58.6%) that were pregnant and 12 of 29 subjects that did not achieve pregnancy. Comparison of age, AMH, basal FSH, BMI, and AFC of the two groups is shown in Figure 1. The women who became pregnant following hysteroscopy were younger than the non-pregnant ($p = 0.030$; $p < 0.05$) (Figure 1). The level of AMH was borderline in the pregnant women after hysteroscopy according to the non-pregnant women and it was not statistically significant ($p = 0.0568$). (Figure 2). Although FSH level was lower in pregnant than in non-pregnant women, this difference was statistically not significant ($p = 0.58$) (Figure 3). BMI (Figure 4) of both group was not statistically significant ($p = 0.58$). AFC was higher in the pregnant group (Figure 5)

Discussion

The present study showed that injury to the endometrium with frozen thawed embryo transfer had a positive effect on patients who had previous recurrent implantation failure. Several studies have shown that LEI can improve implantation rates in unexplained repeated implantation failure patients [19, 20]. The present study supported the facilitative effect of LEI= patients who underwent hysteroscopy after recurrent implantation failure.

Endometrial receptivity is provided by membrane projections by hairy-like cell microvilli, that are called 'pinapodes'. Supporting induction of endometrial decidualization by injury to the endometrium increases the probability of embryo implantation and mimics the same effect as pinapods [21, 22].

Various kinds of cytokines and growth factors, such as leukemia inhibitory factor, tumor necrosis factor-α, inter-
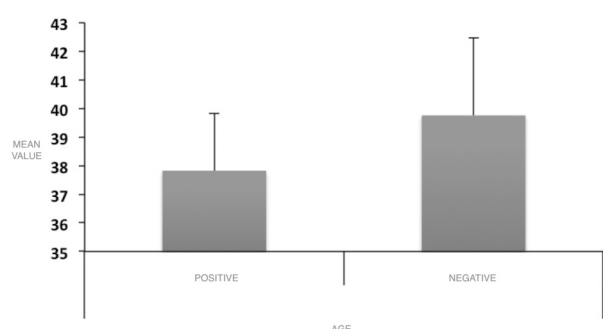
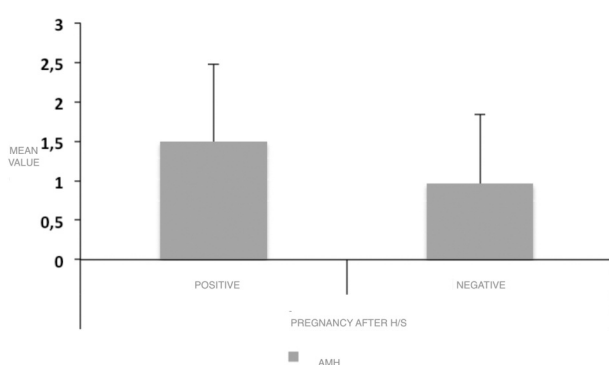Figure 1. — Age distribution of pregnant and non-pregnant groups after hysteroscopy.

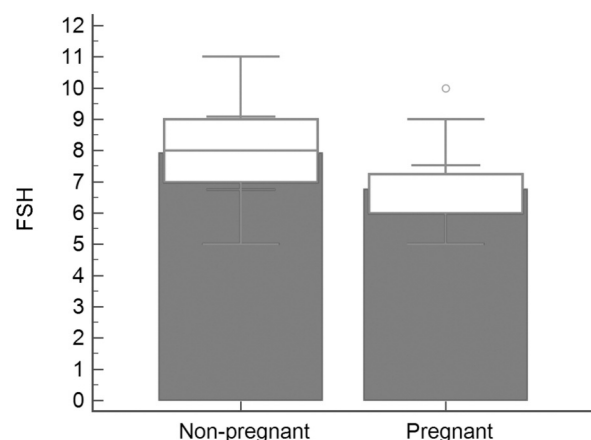Figure 2. — AMH levels of pregnant and non-pregnant women pregnancy after hysteroscopy.

Figure 3. — FSH level and pregnancy rate.

Figure 4. — BMI and pregnancy rate.

Figure 5. — AFC and pregnancy rate.

leukin-15, and macrophage inflammatory protein 1B (MIP-1B) are induced by the injury and they have a positive effect on implantation. Results supported that injury induced an inflammatory response from endometrium with IL-6, IL-8, IL-12, IL-13, and monocyte chemotactic protein-1(MCP-1), INF- γ, VEGF, and in long-term effect monocytes recruit the injury site, thus they improve pregnancy rate [23, 24]. With t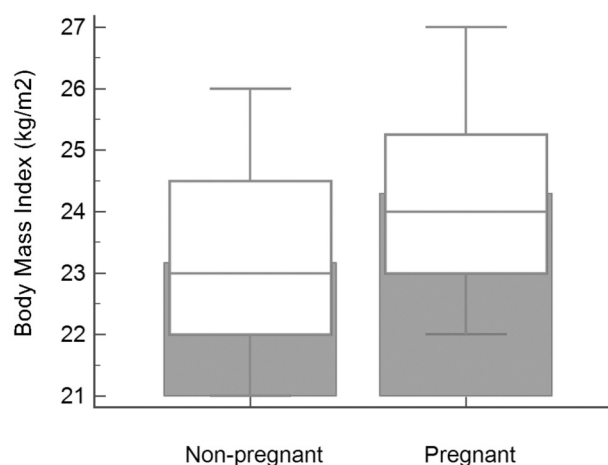hese mediators the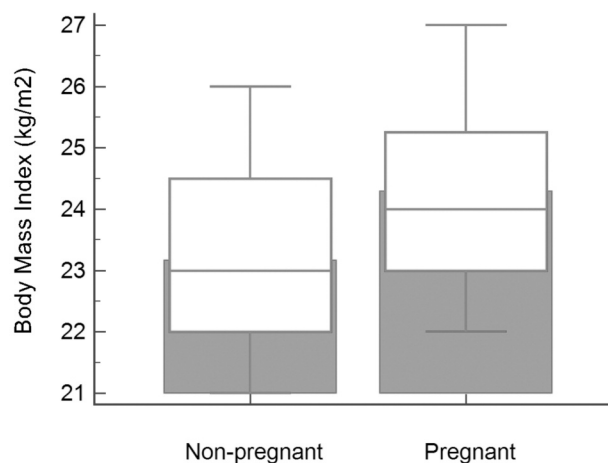re is an interaction between embryo and endometrium. Another study showed that secondary inflammatory reaction was important for implantation. Moreover decidual NK cells have a potential angiogenetic effect which induces vascular growth [25]. Remodeling of the endometrial layer is related to matrix metalloproteinases and their natural inhibitors. Matrix metalloproteinases and their natural inhibitors play an important role in the remodeling of endometrial tissue. [26] Additionally adhesion molecules play a critical role in implantation failure [27].

An animal study showed that endometrial injury increases progenitor cells which provoke endometrial regeneration and proliferation [28, 29]. Several other studies have shown that endometrial injury may be an effective method to improve the pregnancy rates. Some authors used different techniques for the injury of endometrium such as Pipelle canula, Novac, and hysterescopy [30]. Further studies need to

clarify which technique is better. The present authors only used hysteroscopy because it is simple and non-traumatic.

The present authors suggest that injury of endometrium may improve endometrial receptivity in woman that have unexplained infertility. The limitation of this study is retrospective design. More studies are needed to investigate the selection criteria and optimal surgical technique for local endometrial injury. This prospective study is ongoing to assess new sonographic and histopathologic parameters which are important endometrial receptivities.

Acknowledgment

References

[1] Volgsten H., Skoog Svanberg A., Ekselius L., Lundkvist O., Sundström Poromaa I.: "Risk factors for psychiatric disorders in infertile women and men undergoing in vitro fertilization treatment". *Fertil. Steril.*, 2010, *93*, 1088.

[2] Oliva A., Spira A., Multigner L.: "Contribution of environmental factors to the risk of male infertility". *Hum. Reprod.*, 2001, *16*, 1768.

[3] Dekel N., Gnainsky Y., Granot I., Racicot K., and Mor G.: "The role of inflammation for a successful implantation". *Am. J. Reprod. Immunol.*, 2014, *72*, 41.

[4] Dunne C., Taylor B.: "Does endometrial injury improve implantation of frozen thawed embryos?" *Arch. Gynecol. Obstet.*, 2014, *290*, 575

[5] Almog B., Shalom-Paz E., Dufort D., Tulandi T..: "Promoting implantation by local injury to the endometrium". *Fertil. Steril.*, 2010, *94*, 2026.

[6] Galliano. D., Bellver,C.Díaz-García J., Simón,andA.Pellicer C..: "Art and uterine pathology: how relevant is the maternal side for implantation?". *Hum. Reprod. Update*, 2015, *21*, 13.

[7] Laufer N., Simon A.: "Recurrent implantation failure: current update and clinical approach to an ongoing challenge". *Fertil. Steril.*, 2012, *97*, 1019.

[8] Polanski L.T., Baumgarten M.N., Quenby S., Brosen J., Campbell B.K., Raine-Fenning N.J.: "What exactly do we mean by recurrent implantation failure? A systematic review and opinion". *Reprod. Biomed. Online*, 2014, *28*, 409.

[9] Vlachadis N., Vrachnis N., Economou E., Siristidis C.: "Zooming in on the definition of 'recurrent implantation failure'". *Reprod. Biomed. Online*, 2014, *29*, 144.

[10] Cruz J.R., Dubey A.K., Patel J., Peak D., Hartog B., Gindoff P.R.: "Is blastocyst transfer useful as an alternative treatment for patients with multiple in vitro fertilization failures?" *Fertil. Steril.*, 1999, *72*, 218.

[11] Guerif F., Bidault R., Gasnier O., Couet ML., Gervereau O., Lansac J., *et al.*: "Efficacy of blastocyst transfer after implantation failure". *Reprod. Biomed. Online*, 2004, *9*, 630.

[12] Levitas E., Lunenfeld E., Har-Vardi I., Albotiano S., Sonin Y., Hackmon-Ram R., *et al.*: "Blastocyst stage embryo transfer in patients who failed to conceive in three or more day 2–3 embryo transfer cycles: a prospective randomized study". *Fertil. Steril.*, 2004, *81*, 567.

[13] Moutou C., Goossens V., Coonen E., De Rycke M., Kokkali G., Renwick P., *et al.*: "ESHRE PGD Consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010". *Hum. Reprod.*, 2014, *29*, 880.

[14] Loeb J.: "On the chemical character of the process of fertilization and its bearing upon the theory of life phenomena". *Science,* 1907, *26*, 425.

[15] Levin D., Hasson J., Cohen A., Or Y., Ata B., Barzilay L., *et al.*: "The effect of endometrial injury on implantation and clinical pregnancy rates". *Gynecol. Endocrinol.*, 2017, *33*, 779.

[16] Siristatidis C., Vrachnis N., Vogiatzi P., Chrelias C., Retamar AQ., Bettocchi S., *et al.*: "Potential pathophysiological mechanisms of the beneficial role of endometrial injury in in vitro fertilization outcome". *Reprod. Sci.,* 2014, *21*, 955.

[17] Junovich G., Mayer Y., Azpiroz A., Daher S., Iglesias A., Zylverstein C., *et al.*: "Ovarian stimulation affects the levels of regulatory endometrial NK cells and angiogenic cytokine VEGF". *Am. J. Reprod. Immunol.*, 2011, *65*, 146.

[18] Liang Y., Han J., Jia C., Ma Y., Lan Y., Li Y., Wang S.: "Effect of Endometrial Injury on Secretion of Endometrial Cytokines and IVF Outcomes in Women with Unexplained Subfertility". *Mediators of Inflammation: Hindawi Publishing Corporation Mediators of Inflammation,* 2015, *2015*, Article ID 757184

[19] Reljic M., Knez J., Kovač V, Kovačic B.: "Endometrial injury, the quality of embryos, and blastocyst transfer are the most important prognostic factors for in vitro fertilization success after previous repeated unsuccessful attempts". *J. Assist. Reprod. Genet.*, 2017, *34*, 775

[20] Kanazawa E., Nahashima A., Yonemoto K., Otsuka M., Yoshioka N., Kuramoto T., *et al.*: "Injury to the endometrium prior to the frozen-thawed embryo transfer cycle improves pregnancy rates in patients with repeated implantation failure". *J. Obstet. Gynaecol. Res.,* 2017, *43*, 128.

[21] Nikas G.: "Endometrial receptivity: Changes in cell-surface morphology". *Semin. Reprod. Med.*, 2000, *18*, 229.

[22] Garris D.R.: "Cytomorphometric analysis and surface ultrastructure of developing decidua". *Tissue Cell*, 2003, *35*, 233.

[23] Gnainsky Y., Granot I., Aldo PB., Barasht A., Or Y., Schechtman E.: "Local injury of the endometrium induces an inflammatory response that promotes suc- cessful implantation". *Fertil. Steril.*, 2010, *94*, 20.

[24] Sherer D.M., Abulafia O.: "Angiogenesis during implantation, and placental and early embryonic development". *Placenta*, 2001, 22, 1.

[25] Dekel N., Gnailsky Y., Granot I., and Mor G.: "Inflammation and implantation". *Am. J.Reprod. Immunol.*, 2010, *63*, 17.

[26] Marbaix E., Kokorine I., Moulin P., Donnez J., Eeckhouty Y., Courtoy PJ.: "Menstrual breakdown of human endometrium can be mimicked in vitro and is selectively and reversibly blocked by inhibitors of matrix metalloproteinases". *Proc. Natl. Acad. Sci. U S A.,* 1996, *93*, 9120.

[27] Donaghay M., Lessey BA.: "Uterine receptivity: alterations associated with benign gynecological disease". *Sem. Reprod. Med.*, 2007, *25*, 461.

[28] Hyodo S., Matsubara K., Kameda K., Matsubara Y.: "Endometrial injury increases side population cells in the uterine endometrium: a decidive role of estrogen". *Tohoku J. Exp. Med.*, 2011, *224*, 47.

[29] Chan R., Schwab K., Gargett C.: "Clonoenicity of human endometrial epithelial and stromal cells". *Biol. Reprod.*, 2004, *70*, 1738.

[30] Potdar N., Gelbaya T., Nardo LG.: "Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis". *Reprod. Biomed. Online*, 2012, *25*, 561.

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