Which is the Better Adjuvant during In-vitro Fertilization of Women with Recurrent Implantation Failure; Endometrial Scratching or HCG Intrauterine Perfusion

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Abstract

Objectives: To determine outcome of in-vitro fertilization (IVF) on using endometrial scratch (ES) or intrauterine perfusion with HCG (HCG-IUP) in women with recurrent implantation failure (RIF).

Patients & Methods: 93 women were randomly divided into: Control group received no intervention, Group ES were subjected to ES using an endometrial biopsy catheter during the luteal phase of the cycle preceding that of ET and Group HCG-IUP underwent intrauterine perfusion of 500 U of HCG just before embryo transfer (ET). The classic Testart slow freezing and rapid thawing protocol was applied for all women. Study outcomes included the implantation rate (IR), clinical pregnancy rate (CPR) and Frequency of pregnancy loss till 12th gestational week.

Results: Total IR was 38.7% and was 19.4%, 45.2% and 51.6% for control, ES and HCG-IUP groups, respectively. IR was significantly higher in ES and HCG-IUP groups in comparison to control group with non-significantly higher IR in HCG-IUP group. Total CPR was 63.9% and was 50%, 64.3% and 68.8% in study groups, respectively with non-significant inter-group difference. Total miscarriage rate was 30.4% and was 66.7% in control, 33.3% in ES and 18.8% in HCG-IUP with non-significantly higher rate in control group.

Conclusion: ES during the luteal phase of the cycle preceding ET or intrauterine perfusion with 500 U of HCG immediately before ET are efficient adjuvant to ovarian stimulation for women with RIF assigned for frozen ET. The reported non-significant differences between the IR with ES or HCG-IUP open the scale for physician preference and skill.

Keywords: Recurrent implantation failure, Endometrial scratching, HCG intrauterine perfusion, Implantation rate, Clinical pregnancy rate

Introduction

Successful pregnancy involves a synchronized, coordinated cross-talk between embryo capable of implanting, and endometrium enabling implantation. Endometrial implantation (EI) is an essential step for success of assisted reproductive techniques (ART).

Success of EI depends, in addition to the embryo quality and synchrony between embryo and endometrium, on endometrial receptivity (ER). Endometrial receptivity is defined as the period during which the endometrial epithelium acquires functional, but transient, ovarian steroid-dependent status supportive to blastocyst acceptance and implantation. Molecular signature of ER still remains understood, especially when evaluating the possible benefit of therapeutic interventions and implantation-related pathologies.

Recurrent implantation failure (RIF) refers to women who had three failed in vitro fertilization (IVF) attempts with good quality embryos. Such failure of embryo implantation can be attributed to multiple factors; uterine, male, or embryo factors, or the specific type of IVF protocol. Multiple trials and hypotheses were supposed to improve ER so as to improve ART outcome in women with RIF.

Administration of granulocyte colony stimulating factor at day of oocyte puncture or progesterone administration may increase chemical pregnancy and IR in patients with RIF. The freeze-all policy was found to significantly improve the ongoing pregnancy rate (OPR) and IR for women with RIF. Patients with multiple unexplained failed ET may benefit from pretreatment with combination of depot-leuprolide and letrozole for two months.
Hypothesis
Recurrent implantation failure as previously defined (6, 7, 9), had accused the endometrial factor as the responsible for occurrence of RIF. Thus, the current study tried to improve ER locally using either mechanical or hormonal factors supposing that stimulation of endometrium can improve its receptivity.

Objectives:
The current study aimed to determine the outcome of IVF on using endometrial scratch (ES) or intrauterine perfusion with HCG (HCG-IUP) in women with RIF.

Design
Prospective comparative study

Setting
ART units at Obstetrics departments in Benha and Tanta University Hospitals in conjunction with multiple private centers

Patients & Methods
The current study was started at June 2017 after approval of the study protocol by the Local Ethical Committee. The study intended to include women with RIF that was defined as the absence of implantation after three consecutive IVF cycles with transferring at least four good quality embryos in a minimum of three fresh or frozen cycles in a woman <40 years (6, 7, 9).

Patients' evaluation and randomization
All women fulfilling the target of the study were eligible for evaluation that included clinical examination and transvaginal ultrasonography (TVU), estimation of baseline serum levels of FSH, LH, E2 and prolactin and assurance of inclusion and exclusion criteria. Inclusion criteria included the presence of at least one patent tube and serum follicular stimulating hormone (FSH) level of ≤12 mIU/ml without any significant intrauterine or pelvic abnormalities as documented by pelvi-abdominal ultrasonography (US), hysteroscopy and/or laparoscopy. Exclusion criteria included woman’s age of >40 years, body mass index ≥35 kg/m², presence of manifestations of hyperandrogenemia, moderate to severe pelvic endometriosis, ovarian cyst or uterine lesions such as submucosal leiomyoma.

Women fulfilling the inclusion criteria were asked to choose a sealed dark envelop containing a card carrying the group label. These envelops were previously prepared by an assistant who was blinded about the significance of the labels. One-hundred and sixteen women were eligible for evaluation; 23 women were excluded for not fulfilling the inclusion criteria and 93 women were randomly divided into three groups: Control group included women who will not receive any intervention, Group ES included women who will be subjected to endometrial scratching (ES) and Group HCG-IUP included women who will undergo intrauterine HCG perfusion just before embryo transfer (ET) (Fig. 1). Patients’ enrolment data showed non-significant (p>0.05) difference between both groups (Table 1).
Table (1): Patients' enrolment data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ES</th>
<th>HCG-IUP</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.3±2.6</td>
<td>33.8±2.8</td>
<td>34.1±2.4</td>
<td>0.052</td>
</tr>
<tr>
<td>Body mass index (BMI) data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.5±4.8</td>
<td>85.2±3.9</td>
<td>85.2±5.7</td>
<td>0.279</td>
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<tr>
<td>Height (cm)</td>
<td>169.5±3.3</td>
<td>169.3±4.2</td>
<td>167.6±4.7</td>
<td>0.138</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>29.1±2.1</td>
<td>29.8±2</td>
<td>30.4±2.5</td>
<td>0.089</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.9±0.7</td>
<td>3.8±0.7</td>
<td>3.8±0.5</td>
<td>0.809</td>
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<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum FSH (mIU/ml)</td>
<td>7.71±1.12</td>
<td>7.71±1.17</td>
<td>8±0.94</td>
<td>0.457</td>
</tr>
<tr>
<td>Serum LH (mIU/ml)</td>
<td>4.41±1.46</td>
<td>4.64±1.69</td>
<td>4.45±1.5</td>
<td>0.816</td>
</tr>
<tr>
<td>Serum E2 (pg/ml)</td>
<td>46.28±125</td>
<td>45.21±875</td>
<td>43.56±25</td>
<td>0.622</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD; ES: Endometrial scratching; HCG-IUP: HCG intrauterine perfusion; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; P value indicates the significance of difference between the three groups; p>0.05 indicates non-significant difference between both groups.

Interventions

For all women, estradiol valerate (Progynova, 2 mg, Bayer Schering Pharma, UK) was started on day-2 of menses of replacement cycle as a daily dose of 2 mg with gradually increasing doses till endometrial thickness was 8 mm. TVU (Sonoline Prima 7.5 MHz, Siemens) was used for measuring endometrial thickness in the midsagittal plane from the outer edge of the endometrial/myometrial interface to the outer edge in the widest part of the endometrium. Intravaginal progesterone (Crinone 8%, progesterone vaginal gel, Merck serono, UK, once daily) was given for two days before ET and continued thereafter. On attendance to IVF unit, all women were positioned in lithotomy position, a vaginal speculum was applied, and cervix was identified and disinfected with betadine. Then, each woman undertook the assigned intervention according to the label on the card.

Endometrial scratching (Group ES)
The undertaken procedure for endometrial scratching was performed as previously described by Nastri et al. (12). An endometrial biopsy catheter was passed through the cervical canal up to the uterine fundus and the piston was drawn back to the end of the biopsy cannula until it self-locked, thus creating a negative pressure. Then, the catheter was moved back-and-forth within 2-4 cm and rotated over several ranges of 360° during 1–2 min. In case of blockage of the suction orifice, the catheter was replaced by another one. The obtained sample was discarded.

**Intrauterine HCG perfusion (Group HCG-IUP)**

Intrauterine HCG perfusion was conducted according to the procedure described by Mansour et al. (13). The HCG powder (Pregnyl, 5000 IU vial, Organon Pharmaceuticals, Roseland, NJ, USA) was dissolved using the supplied solvent (sodium chloride) to provide 1000 IU/ml and 0.5 ml was used for intrauterine perfusion using an intrauterine insemination catheter. The procedure was performed, while patient is in lithotomy position with full bladder, immediately before ET.

**Embryo transfer**

The classic Testart slow freezing and rapid thawing protocol (14, 15) was applied using a programmable freezer (Planer; Middlesex, UK) and embryo freezing and thawing kits (Irvine Scientific, Santa Ana, CA, USA) were used. After thawing, all embryos were transferred to culture in vitro for 2 days. Then, embryos were assessed for cell number and morphology and presence of cellular debris (16). On the third day, embryos in G1 and G2 grade were defined as good quality embryos and 1-3 embryos of good quality were transferred, while poorer grade embryos were discarded (17). In case of pregnancy, the progesterone treatment was continued up to pregnancy week 10 and women were follow-up till the 12th gestation week.

**Study outcomes**

- Primary outcome was the implantation rate defined as rate of women had positive chemical pregnancy that was diagnosed by measurement of β-human chorionic gonadotropin level on the 14th day after ET.
- Secondary outcomes included
  a. Clinical pregnancy rate defined detection of the rate of pregnancy sacs detected by TVU 2-weeks after assurance of chemical pregnancy in women had succeeded implantation.
  b. Pain rating of the ES procedure using a Visual Pain Scale (Likert) at 30-min, 60-min and one day after the procedure
  c. Frequency of pregnancy loss till 12th gestational week.

**Statistical analysis**

The obtained data are presented as numbers, percentages, mean±SD. Variance in parametric data of patients of studied groups was analyzed using One-way analysis of variance (one-way ANOVA test), non-parametric data were presented as numbers and were analyzed using Chi-square test with Yates correction. Statistical analyses were performed using Statistical analysis was conducted using the IBM SPSS (Version 23, 2015; IBM, South Wacker Drive, Chicago, USA) for Windows statistical package. P value <0.05 was considered statistically significant.

**Results**

Chemical pregnancy was detected in 36 women for total IR of 38.7%. Thirty women of intervention groups had positive chemical pregnancy for an IR of 48.4%;
14 women of ES (45.2%) and 16 women of HCG-IUP (51.6%) groups with non-significantly (p=0.611) higher IR among women of HCG-IUP group. On contrary, only 6 women (19.4%) of control group had positive chemical pregnancy with significantly lower IR in comparison to IR detected in ES (p=0.0297) and HCG-IUP (p=0.0079) groups (Table 2, Fig. 2).

Clinical pregnancy as assured by TVU examination was detected in 23 women of those had positive chemical pregnancy for a total CPR of 63.9%. Twenty women of intervention groups (66.7%) had clinical pregnancy, while only three women (50%) of control group had clinical pregnancy with non-significantly (p=0.438) higher CPR among women had intervention in comparison to control women. Among women had positive chemical pregnancy in ES group 9 women had clinical pregnancy (64.3%) and 11 women (68.8%) among those of HCG-IUP group with non-significantly (p=0.796) higher CPR among women of HCG-IUP group compared to those of ES group (Table 2, Fig. 3).

Unfortunately, 7 women of those had assured clinical pregnancy had miscarriage for a total miscarriage rate of 30.4%. Five women in intervention groups (25%) and two women in control group (66.7%) had miscarriage (25%) with non-significantly (p=0.144) higher incidence of miscarriage among control women and among women of ES group in comparison to HCG-IUP (p=0.436), (Table 2).

![Fig. (2): IR as judged by result of chemical pregnancy testing on 14-days after ET](image-url)
At 30-min after ES, 11 women had moderate pain sensation, 17 had mild and 3 patients had no pain sensation. At 60-min, pain was resolved spontaneously in 10 women and 13 women had no pain, and was lessened in 7 women, so 14 women had mild and only 4 women were still having moderate pain sensation. On 24-hr after the procedure, only 5 women were still had mild pain that resolved on the 2nd day after the procedure (Fig. 4).
Fig. (4): Distribution of patients of ES group according to severity of ES-induced pain sensation

Discussion

Recurrent implantation failure (RIF) refers to cases in which women have had three failed in vitro fertilization (IVF) attempts with good quality embryos \(^6,7,9\); this definition accused the endometrial factor as the responsible for occurrence of RIF. Thus, the current study tried to improve endometrial receptivity locally using either mechanical or hormonal factors supposing that stimulation of endometrium can improve its receptivity. In support of this suggestion, the implantation rate (IR) and clinical pregnancy rate (CPR) was increased with interventions by \(>2\)-fold (48.4% versus 19.4%) and about 1.33-folds (66.7% versus 50%), respectively in comparison to the corresponding rates in control women.

Concerning the primary outcome, for patients received ES, the obtained IR (45.6%) was significantly \((p=0.0297)\) higher compared to IR obtained in control women who received no intervention (19.4%), a finding that indicated the possibility of improving endometrial receptivity (ER) using mechanical endometrial injury. In line with this finding and assumption, multiple previous studies detected significant improvement of IR in women received ES either during diagnostic hysteroscopy for their first IVF trial \(^1,18,19\), during management of unexplained infertility \(^20\) or for women had RIF \(^21,22,23\).

Despite of the non-significant difference in CPR between women had ES and control women (64.3% versus 50%, respectively); CPR was increased by ES by about 30% of CPR reported in controls. The reported beneficial effect of ES on CPR and the reported figure coincided with that reported in previous literature \(^12,18,19,20,24\) and recently, Gricius et al. \(^25\) reported clinical pregnancy in 61.9% of patients had ES and in 50% of patients without ES.

In support of the application of ES for women with RIF undergoing IVF, Potdar et al. \(^21\) in systemic review found that in women with unexplained RIF inducing endometrial injury is 70% more likely to result in a clinical pregnancy as opposed to no treatment. Also, Siristatidis et al. \(^23\) documented that ES induced through office hysteroscopy in the preceding cycle in subfertile women with RIF.
improves live birth rates. Moreover, Lensen et al. (26) surveyed fertility care providers and found 92% recommend ES and 73% agreed that ES is beneficial in women with RIF undergoing IVF.

In trial to explain the mechanisms through which ES improves ER, Zhou et al. (27) reported significant difference in the endometrial expression profiles of 218 genes detected in the endometrial biopsy samples from clinical pregnant patients and non-pregnant patients. On the other hand, Karimzadeh et al. (28) attributed increased IR with local endometrial trauma to the release of chemical mediators such as histamine and growth factor. Other studies found ES triggers an influx of macrophages/Dendritic cells that, in turn, enhance endometrial expression of essential molecules that facilitate embryo and endometrium interaction (29) with increased local expression of proinflammatory cytokines especially tumor necrosis factor-α (30) (Gnainsky et al., 2010) which induced increased expression of macrophage inflammatory protein 1B (31) and both were positively correlated with clinical pregnancy outcome after endometrial biopsy treatment (30).

Intrauterine HCG perfusion also did favorably with significantly (p=0.0079) higher IR in comparison to control group with non-significantly (p=0.611) higher IR than ES. These data point to a possible role of local HCG perfusion on ER, which leads to increased endometrial ability for implantation by 2.7-folds higher than endometrium without intervention (51.6% versus 19.4%). Moreover, HCG-IUP allowed higher CPR (68.8%) in comparison to ES (64.3%) and control groups (50%), despite of the non-significant differences.

In line with these results, Craciunas et al. (32) performed searches for randomized controlled trials evaluating the effect of HGC-IUP around the time of ET for infertile women and reported that the pregnancy outcome for cleavage-stage ET using an HCG-IUP in a dose of ≥ 500 IU is promising. Thereafter, Craciunas et al. (33) re-performed the search and concluded that women undergoing cleavage-stage ET after HCG-IUP using ≥ 500 IU have an improved live birth rate. Huang et al. (34) reported biochemical and clinical PR of 49.02% and 48.05% after IU injection HCG before ET in women with three or more implantation failure.

Recently, Xie et al. (35) after systematic review and meta-analysis concluded that HCG-IUP is effective in improving CPR and live birth rate in women with two or more RIF and provide a potential therapeutic intervention for RIF and Gao et al. (36) after meta-analysis to evaluate whether HCG-IUP before embryo transfer can improve IVF-ET outcomes indicated that the procedure can improve IR, CPR, and ongoing pregnancy and live birth rates and HCG-IUP for infertile women with 500 IU within 15 minutes before ET can achieve optimal IVF-ET outcomes.

Multiple studies tried to explain the effect of HCG-IUP on implantation rate, HCG was found to regulate angiogenesis that required for implantation (37), directly regulate the response of endometrium to interleukin-1 and to amplify the cytokine-mediated effects on cell proliferation, migration, and the release of angiogenic factors (37) and regulate the differentiation of peripheral FOXP3+ Tregs (39). Recently, Shen & Qin (40) detected decreased endometrial expression of talin1 in normal pregnant mice on day 5, while in delayed implantation model expression of talin1 was found to be increasing from day 3 to day 5, but significantly decreased in both stromal and epithelial cells after activation of implantation or on HCG-IUP.

Clinically, Huang et al. (41) reported significantly decreased number of endometrial Treg in RIF women, but significantly increased on HCG-IUP with significantly increased CCL2 expression and mRNA levels and concluded that HCG-
IUP promote the recruitment of Tregs into endometrium by inducing chemokine CCL2.

**Conclusion:**
RIF is a disastrous condition for both the infertile family and physician. Controlled ovarian hyperstimulation only is not the appropriate therapeutic policy. ES during the luteal phase of the cycle preceding ET or intrauterine perfusion with 500 U of HCG immediately before ET are efficient adjuvant to ovarian stimulation for women with RIF assigned for frozen ET. The reported non-significant differences between the IR with ES or HCG-IUP open the scale for physician preference and skill. However, wider scale studies are mandatory for establishment of the obtained IR and CPR. Moreover, randomized controlled studies are needed to establish the dose-outcome relationship for HCG-IUP.

**References**


