A randomized clinical trial of endometrial perfusion with granulocyte colony-stimulating factor in in vitro fertilization cycles: impact on endometrial thickness and clinical pregnancy rates

David H. Barad, M.D., a,b Yao Yu, Ph.D., a,b Vitaly A. Kushnir, M.D., a,b Aya Shohat-Tal, Ph.D., a,b Emanuela Lazzaroni, M.S., a,b Ho-Joon Lee, Ph.D., a,b and Norbert Gleicher, M.D. a,b

a The Center for Human Reproduction (CHR), and b Foundation for Reproductive Medicine, New York, New York

Objective: To investigate whether granulocyte colony-stimulating factor (G-CSF) affects endometrial thickness, implantation rates, and clinical pregnancy rates in routine, unselected IVF cycles.

Design: Registered, individually randomized, two-group, parallel double-blinded placebo-controlled clinical trial.

Setting: Academically affiliated private clinical and research center.

Patient(s): 141 consecutive, unselected, consenting women with no history of renal disease, sickle cell disease, or malignancy who were undergoing IVF.

Intervention(s): Sealed, numbered, opaque envelopes assigned 73 patients to receive G-CSF (Filgrastim, Amgen, 300 μg/1.0 mL) and 68 to receive placebo (saline).

Main Outcome Measure(s): Endometrial thickness, clinical pregnancy, and embryo implantation rates.

Result(s): The mean age for the whole study group was 39.59 ± 5.56 years (G-CSF: 39.79 ± 5.13 years; placebo: 39.38 ± 6.03 years). Endometrial thickness statistically significantly increased over the 5-day observation period for the whole group by approximately 1.36 mm. The increase in the G-CSF group was not statistically significantly different from the control group. Statistical models looking at treatment effects on clinical pregnancy and implantation rates demonstrated no effect of G-CSF treatment. There were no adverse events for either treatment group.

Conclusion(s): In normal IVF patients, G-CSF does not affect endometrial thickness, implantation rates, or clinical pregnancy rates. Because these results were obtained in an older patient population, they may not necessarily apply to younger women.

Clinical Trial Registration Number: NCT01202656. (Fertil Steril 2014;101:710–5. ©2014 by American Society for Reproductive Medicine.)

Key Words: Endometrial thickness, G-CSF, granulocyte colony-stimulating factor, in vitro fertilization, pregnancy rates, randomized controlled trial
Clinical pregnancy rates after embryo transfer increase with increasing endometrial thickness (1–6). A large number of studies have defined minimal thickness at approximately 7 mm (7–12). Most in vitro fertilization (IVF) patients reach this minimal thickness with routine protocols. Only a small number of IVF patients fail to achieve normal endometrial thickness with routine treatments, and no established treatments exist for such patients. Sildenafil citrate (Viagra) treatment has been recommended (13), but response is not consistent. There is evidence of increased endometrial thickness among postmenopausal women using antihypertensive beta-blockers such as atenolol (Tenormin) (14, 15), but no formal studies have been reported before embryo transfer to improve endometrial thickness. Even when given vasodilators, many women fail to reach minimal endometrial thickness and demonstrate low pregnancy chances (7–12). Thus, women who fail to achieve minimal endometrial development often do not undergo embryo transfer. A new therapeutic approach to improve endometrial thickness in such patients would be very desirable.

Recent reports from our center have suggested that intrauterine perfusion with granulocyte colony-stimulating factor (G-CSF) may be effective in women who are otherwise resistant to treatment (16, 17). Granulocyte colony-stimulating factor is a cytokine that stimulates neutrophilic granulocyte proliferation and differentiation. It is routinely and safely used in the treatment of neutropenia during cancer chemotherapy (18, 19). A U.S. patent (US 2009/0226397 A1, September 10, 2009) has claimed a benefit from G–CSF treatment in cases of implantation failure and repeated pregnancy loss, suggesting that G-CSF can affect the endometrium.

Human decidua is now thought to some degree to control trophoblast invasion via secretion of cytokines (20–23). Colony-stimulating factors (CSF) are a family of proteins (GM-CSF, CSF-1, G-CSF, and IL-3) that stimulate cellular proliferation and the induction of terminal differentiation of hemopoietic progenitor cells. CSF-1 has been detected in first-trimester placental tissue and in endometrial glands (24–27). The osteopetrotic mouse, which represents a natural knockout of the CSF-1 gene, demonstrates a low rate of fetal implantation and poor fetal viability (28). Local injury to the endometrium, which may lead to local release of cytokines including CSF, has been claimed to improve rates of embryo implantations, clinical pregnancies, and live births in assisted reproduction (29). CSF-1 may also have a role in establishing early endometriotic lesions, suggesting that CSF may well promote growth of endometrial tissue (30).

In a double-blinded, randomized, controlled trial of 68 women with recurrent miscarriage (four sequential pregnancy losses) 29 (82.9%) out of 35 treated with G-CSF delivered healthy infants, whereas patients receiving placebo delivered only in 16 (48.5%) out of 33 cases (P < 0.0061, odds ratio [OR] 5.1; 95% confidence interval [CI] 1.5–18.4) (31).

Our center for over 10 years has been treating a small number of patients with implantation failure with G-CSF, off label and under appropriate written informed consent. More recently, we reported successful treatment via intrauterine perfusions with G-CSF of patients with thin endometrium who were resistant to other routine treatments (16, 17). In a case series, we described 21 consecutive infertile women with endometria of <7 mm on day of human chorionic gonadotropin (hCG) administration in their first IVF cycles who were treated with 300 μg/1.0 mL of G-CSF (Nupogen, filgrastim; Amgen) administered by intrauterine catheter by slow infusion before noon. Endometrial thickness increased in these patients by 2.9 ± 2.0 mm and did not vary between conception and nonconception cycles. A 19.1% ongoing clinical pregnancy rate was observed, excluding one ectopic pregnancy (16).

Given this endometrial proliferation and subsequent unexpectedly high pregnancy rates in this adversely selected patient population, the question arose as to whether G-CSF perfusion may not also improve implantation and pregnancy rates in women with normal endometrial thickness. Our randomized controlled trial was designed to answer these questions. We describe the results of a double-blinded, randomized, controlled trial of intrauterine infusion of G-CSF compared with normal saline (as placebo) on endometrial thickness, implantation, and pregnancy rates among unselected IVF patients with normal endometrial thickness who were undergoing IVF.

MATERIALS AND METHODS

This study was approved by the center’s institutional review board before initiation of the trial in 2010. All patients presenting for embryos transfer between October 3, 2010, and January 1, 2013, were offered participation in the trial. Women with renal disease, sickle cell disease, or a history of malignancy were considered ineligible for medical reasons. Presuming an implantation rate of 10% and anticipating a 10% increase to 20% with treatment, about 200 embryos transferred in each study arm would be needed for 80% power and alpha of 0.05. A total of 419 eligible patients were offered participation: 141 patients consented to participate, and 278 declined. The principal reasons for refusal to participate were “lack of interest” and technical difficulties in presenting in timely fashion for the treatment to the center (a large portion of our center’s patients are long-distance patients who only come to the center for retrieval and transfer). No consenting patient was excluded from participation for medical reasons.

Randomization was performed after the patients had consented to participate. A computer-generated randomization table was used with separate randomization blocks for patients undergoing IVF and frozen embryo transfer (FET) in a first study cycle. Individual randomization cards were sealed in numbered opaque envelopes that were only accessible to the single staff member who administered the randomization table and prepared the study materials. Treatment assignment was blinded to patients, physicians, and nursing staff.

The flow chart for disposition of all patients is demonstrated in Figure 1. Of 141 participating patients, 129 underwent IVF, and 12 underwent FET in their first study cycle. Randomization resulted in 73 patients (67 IVF, 6 FET)
assigned to G-CSF and 68 patients (62 IVF, 6 FET) to placebo in a first study cycle. All patients entering a second treatment cycle were offered participation in a crossover trial. Thirty-five patients agreed to participate in such a crossover cycle: 16 (14 IVF, 2 FET) placebo to G-CSF, and 19 (18 IVF, 1 FET) G-CSF to placebo. The patients who did not crossover either had conceived in their first study cycle or chose not to have another assisted reproduction cycle.

The G-CSF used was Nupogen (300 μg/1.0 mL, Filgrastim; Amgen). One mL of G-CSF or placebo (normal saline) was administered on the morning of hCG administration before noon by slow transcervical intrauterine infusion, similar in technique to an intrauterine insemination. Endometrial thickness was assessed by routine vaginal ultrasound before infusion and again 5 days later at the time of embryo transfer.

Pregnancy outcomes were assessed based on positive pregnancy test (chemical pregnancy) and clinical pregnancy (gestational sac and fetal heart on ultrasound examination). Implantation rates were determined by the number of gestational sacs at least 28 days after embryo transfer based on the total number of embryos transferred per group.

Embryo quality was assessed using standard day-3 and day-5 embryo grading. Day-3 embryos with eight cells and <15% fragmentation were considered good. In day-5 embryos, expanded blastocysts with at least grade B trophectoderm and inner cell mass were considered good.

RESULTS

Baseline characteristics and outcomes are listed in Table 1 for both the first and second (crossover) cycles of treatment. The mean age for the 141 patients undergoing their first study cycle was 39.59 ± 5.56 years (G-CSF 39.79 ± 5.13, and placebo 39.38 ± 6.03; not statistically significant [NS]). The mean baseline follicle-stimulating hormone (FSH) level was 8.47 ± 3.94 (G-CSF 8.64 ± 3.92, and placebo 8.27 ± 4.01). Mean baseline antimüllerian hormone (AMH) level was 2.03 ± 2.26 (G-CSF 2.07 ± 2.17, and placebo 1.99 ± 2.38). None of the baseline characteristics were statistically significantly different between the two groups.

The overall endometrial thickness statistically significantly increased over the 5-day observation period for the whole group by approximately 1.36 mm (95% CI, 0.98, 1.74; P < .0001; paired t-test) in the first study cycle; however, it did not statistically significantly increase over the second study cycle 0.27 mm (95% CI, −0.44, 0.98; P = .45).

We fit a general linear model (GLM) looking at the change in endometrial thickness from the day of infusion until the
day of transfer to examine the effect of treatment adjusted for age (Fig. 2). The increase, while nominally greater in the G-CSF group, was not statistically significantly different. Subdividing the initial endometrial thickness into four levels (endometrium ≤ 8.23 mm, 8.24–10.35, 10.36–12.47, and ≥ 12.48 mm) also revealed no differences between treatment and placebo. Only six patients had a pretreatment endometrial thickness of less than 7 mm.

Forty-eight patients had a positive hCG concentration after their embryo transfer (24 in each group). Thirty-seven patients established a clinical pregnancy in the first cycle of treatment: G-CSF 18 (24.7%) of 73, and placebo 19 (27.9%) of 68. Among these 37 clinical pregnancies, there were four spontaneous pregnancy losses (1 G-CSF; 3 placebo).

Fifty-eight patients who did not become pregnant did not choose to have another IVF cycle and were dropouts from the second cycle (30 G-CSF; 28 placebo). Thirty-five patients completed a crossover treatment cycle (16 G-CSF; 19 placebo). For the 35 patients, the mean of washout time was 95 days. Clinical pregnancy was established in 15 patients: G-CSF 8 (50%) of 16, and placebo 7 (36.8%) of 19. Four of these established pregnancies miscarried, 2 in each group. There was no statistically significant difference in the overall pregnancy rate between the first and second study cycles ($P = .14$, chi-square).

A logistic regression model (Table 2) looking at treatment effects on ongoing pregnancy rates in the first treatment cycle adjusted for patient age and endometrial thickness at day of first infusion demonstrated no effect of G-CSF on clinical pregnancy rates ($OR = 1.03$; 95% CI, 0.46, 2.264; $P = .95$). As expected, patient age was a marginally statistically significant negative predictor for ongoing pregnancy with an odds ratio of 0.936 (95% CI, 0.87, 1.01; $P = .07$). No adverse events occurred in either treatment group.

---

**TABLE 1**

Characteristics (baseline and outcome) of study patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>G-CSF</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>First cycle (N = 141)</td>
<td>n = 73</td>
<td>n = 68</td>
</tr>
<tr>
<td>Endometrium (day of hCG trigger)</td>
<td>10.23 (± 2.01)</td>
<td>10.37 (± 2.22)</td>
</tr>
<tr>
<td>Endometrium (day of embryo transfer)</td>
<td>11.69 (± 2.50)</td>
<td>11.63 (± 2.14)</td>
</tr>
<tr>
<td>Change in endometrial thickness</td>
<td>1.45 (± 2.22)</td>
<td>1.25 (± 2.28)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>39.79 (± 5.13)</td>
<td>39.38 (± 6.03)</td>
</tr>
<tr>
<td>Baseline FSH</td>
<td>8.64 (± 3.92)</td>
<td>8.27 (± 4.01)</td>
</tr>
<tr>
<td>Baseline AMH</td>
<td>2.07 (± 2.17)</td>
<td>1.99 (± 2.38)</td>
</tr>
<tr>
<td>Oocytes retrieval (patient)</td>
<td>8.88 (± 7.22)</td>
<td>8.56 (± 5.83)</td>
</tr>
<tr>
<td>Pregnancy (hCG+) cycle 1</td>
<td>18/73 (24.65%)</td>
<td>19/68 (27.94%)</td>
</tr>
<tr>
<td>SAB</td>
<td>1/18 (5.6%)</td>
<td>3/19 (15.8%)</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>22/176 (12.5%)</td>
<td>28/168 (16.7%)</td>
</tr>
<tr>
<td>Clinical pregnancy (per hCG+)</td>
<td>17/18 (94.4%)</td>
<td>16/19 (84.2%)</td>
</tr>
<tr>
<td>Good quality embryos</td>
<td>72/158 (45.57%)</td>
<td>55/140 (39.29%)</td>
</tr>
<tr>
<td>Second cycle (N = 35)*</td>
<td>n = 19</td>
<td>n = 16</td>
</tr>
<tr>
<td>Endometrium (day of hCG trigger)</td>
<td>9.96 (± 2.27)</td>
<td>11.10 (± 1.93)</td>
</tr>
<tr>
<td>Endometrium (day of embryo transfer)</td>
<td>10.32 (± 2.15)</td>
<td>11.40 (± 1.17)</td>
</tr>
<tr>
<td>Change in endometrial thickness</td>
<td>0.36 (± 2.13)</td>
<td>0.16 (± 2.01)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>40.09 (± 5.20)</td>
<td>39.63 (± 3.81)</td>
</tr>
<tr>
<td>Baseline FSH</td>
<td>9.26 (± 3.73)</td>
<td>8.87 (± 4.14)</td>
</tr>
<tr>
<td>Baseline AMH</td>
<td>1.16 (± 0.93)</td>
<td>1.50 (± 2.69)</td>
</tr>
<tr>
<td>Oocytes retrieval (patient)</td>
<td>7.08 (± 6.38)</td>
<td>10.18 (± 6.46)</td>
</tr>
<tr>
<td>Pregnancy (hCG+) cycle 2</td>
<td>7/19 (36.84%)</td>
<td>10/16 (62.5%)</td>
</tr>
<tr>
<td>SAB</td>
<td>2/7 (28.57%)</td>
<td>2/10 (20%)</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>7/51 (13.7%)</td>
<td>11/48 (22.9%)</td>
</tr>
<tr>
<td>Clinical pregnancy (per hCG+)</td>
<td>5/7 (70.43%)</td>
<td>6/10 (60%)</td>
</tr>
<tr>
<td>Good quality embryos</td>
<td>14/45 (31.11%)</td>
<td>21/48 (43.75%)</td>
</tr>
<tr>
<td>Overall implantation rate</td>
<td>33/224 (14.73%)</td>
<td>35/219 (15.98%)</td>
</tr>
</tbody>
</table>

Note: AMH = antimullerian hormone; FSH = follicle-stimulating hormone; G-CSF = granulocyte colony-stimulating factor; hCG = human chorionic gonadotropin; SAB = spontaneous abortion.

* The second cycle was a cross-over cycle. The 16 patients originally allocated to placebo received G-CSF in the second cycle and the 19 received placebo.


---

**FIGURE 2**

Change in endometrial thickness in mm from day of hCG until day of transfer: C G-CSF; + placebo. Mean change solid line (G-CSF); dashed line (placebo).

A two-period crossover study analysis was conducted using the data collected from 141 patients using GLM and linear mixed effect models (LME). Responses were the change of endometrial thickness and clinical pregnancy. Analysis of the crossover trial, while adjusting for patient’s age, demonstrated no effect of G-CSF on either the change in endometrial thickness or on clinical pregnancy. We, however, did observe a marginally greater increase in endometrial thickness in first study cycles compared with the second cycles in the G-CSF group (estimated difference 1.21; \( P = .31 \)).

In total, 443 embryos were transferred in this study leading to an implantation rate of 33 (14.73%) of 224 with G-CSF and 35 (15.98%) of 219 with placebo. The implantation rate was not statistically different (\( P = .72 \)). The implantation rate for embryos in fresh IVF cycles of 61 (15.25%) of 400 was also not statistically significantly greater than that for cryopreserved embryos 7 (16.28%) of 43 (\( P = .88 \), chi-square).

We compared the embryo quality between the two treatment groups for 141 patients in their first cycles only. There were nominally more good quality embryos in the G-CSF group [72/158 (45.57%)] compared with placebo [55/140 (39.29%)], but the difference was not statistically significant (\( df = 174, t = 1.03; P = .31 \)). The odds ratio for higher embryo quality among the G-CSF treated group was 1.44 (95% CI, 0.71, 2.92; \( P = .31 \)).

**DISCUSSION**

We previously reported successful endometrial expansion to at least minimal thickness of 7 mm after uterine perfusion with G-CSF in a small group of women with thin endometrium resistant to standard treatments, who all expanded their endometrium and were able to proceed to embryo transfer and conceive (17). In a larger follow-up study, 21 patients with endometria <7 mm on the day of hCG administration were treated with G-CSF, resulting in significant endometrial expansion and a 19.1% ongoing pregnancy rate (16). Based on these observations, we launched this clinical trial to see whether G-CSF infusion could also improve IVF outcomes in random women undergoing IVF who had normal endometrial thickness, hypothesizing that G-CSF might in addition to its proliferative effects on endometrium also beneficially affect implantation. Such a hypothesis is also supported by published reports suggesting improved pregnancy survival after G-CSF treatments in habitual aborters and in women with presumed implantation failure (31, 32).

In this randomized clinical trial we were unable to detect a significant treatment effect of intrauterine perfusions with G-CSF on endometrial proliferation, implantation, or pregnancy rates. This study was designed to detect a 20% increase in implantation rate, which clearly did not occur. The number of participants in this trial was not sufficient to detect a smaller increase; though any increase in implantation rate attributable to treatment might be important, it was not practical to recruit the numbers of patients needed to detect smaller effects.

The results of this study, therefore, suggest that intrauterine perfusion of the endometrial cavity with G-CSF in women with normal endometrial proliferation does not offer any clinical benefit. Such a conclusion, however, still has to be viewed with caution: our center’s patients are to an unusual degree adversely selected. This is partially demonstrated by the relatively advanced age of the study population of approximately 40.0 years (39.58 ± 5.40 years), and relatively high FSH and relatively low AMH levels. In addition, over 80% of women who present to our center for a first IVF cycle have previously failed IVF treatments elsewhere, often multiple times at multiple centers. Thus, our investigated patient population does not represent a typical average patient population going through IVF cycles, even though the observed pregnancy rate of 32.6% in first cycles is quite good, considering their adverse selection. Also notable are the observed implantation rates in the study and placebo groups, both around 15%.

We cannot preclude the possibility that G-CSF effects in a less adversely selected and especially younger patient population may be more favorable. Our noted nominal advantages in favor of G-CSF-treated patients could point toward such a possibility. More likely, however, G-CSF loses its effectiveness in the presence of a normally proliferating endometrium, or at least does so when infused into the endometrial cavity. When treating habitual aborters and presumed implantation failure, others have administered G-CSF systemically by subcutaneous injection (31, 32). One, therefore, could further speculate that the systemic effects of G-CSF may differ from local effects, though such an explanation also appears unlikely, especially as G-CSF has demonstrated its effectiveness via local application in women with thin endometria resistant to standard treatments (16, 17).

In summary, this clinical trial failed to demonstrate a beneficial effect from intrauterine perfusion with G-CSF on IVF outcomes. This conclusion, however, may not be applicable to all patients, especially women younger than those investigated in our study.

**REFERENCES**


