

Successful treatment of unresponsive thin endometrium

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Objective: To assess whether inadequate, thin endometrium (<7 mm), after failure to expand with standard treatment options, will be responsive to cytokine treatment.

Design: Prospective cohort study of four patients.

Setting: Two independent IVF centers in New York City.

Patient(s): Four consecutive women undergoing IVF who, after standard endometrial preparation, still demonstrated highly inadequate endometrium.

Intervention(s): Transvaginal endometrial perfusion with granulocyte colony-stimulating factor (G-CSF).

Main Outcome Measure(s): Endometrial thickness on day of ET, with pregnancy as secondary endpoint.

Result(s): We report successful endometrial expansion to at least minimal thickness of 7 mm after uterine perfusion with G-CSF in four patients previously resistant to treatment with estrogen and vasodilators. All four patients therefore reached ET, and all four also conceived, although one pregnancy required termination because of intra-mural, corneal ectopic location. Endometrial expansion to minimal thickness occurred within approximately 48 hours from infusion.

Conclusion(s): Uterine perfusion with G-CSF represents a promising new tool for the currently mostly intractable problem of inadequate, thin endometrium. This treatment also deserves further investigation for its potential to improve implantation chances in association with IVF and, therefore, pregnancy rates. (*Fertil Steril*® 2011;95:2123.e13–e17. ©2011 by American Society for Reproductive Medicine.)

Key Words: Granulocyte colony-stimulating factor, in vitro fertilization (IVF), endometrial thickness, inadequate endometrium, implantation, pregnancy chance

Decidua exerts control over trophoblast invasion via secretion of cytokines (1–3). That local injury can induce endometrial decidualization and improve implantation has been known since 1907 (4). Barash et al. (5) applied planned endometrial injury to human IVF, increasing pregnancy rates. Massive release of cytokines and growth factors from injured endometrium has been suggested as an underlying process (6).

Granulocyte colony-stimulating factor (G-CSF) contributes to human reproductive success. It improves implantation (7–10; reference 8 refers to granulocyte-macrophage CSF, GM-CSF), appears essential for implantation (11), is a remedy for implantation failure (7), affects human decidual macrophages (12), ovulation (13), ovarian function in general (14), and granulosa cell function (GM-CSF) (15), improves ovarian stimulation in poor responders (16), is predictive of IVF outcome (17), is a biomarker for oocyte/embryos with implantation potential (18), reduces unexplained

repeated pregnancy loss (7, 19), plays a role in the genesis of early endometriotic lesions (20), and suppresses autoimmunity (21).

In animal models G-CSF promotes follicle development in newborn rats (22). Adding CSF to bovine embryo culture enhances development and post-transfer survival and reduces pregnancy loss (23). Using endometrial coculture, G-CSF >130 pg/mL was associated with significantly improved IVF pregnancy chance (9).

Administration of G-CSF does not seem to affect embryonic chromosomal constitution and therefore seems safe (24).

Granulocyte colony-stimulating factor thus demonstrates divergent roles in reproduction, having distinct effects on endometrium and implantation. A potentially growth-expanding effect on endometrium may be suspected from its role in establishing early endometriotic lesions (20). A direct growth-promoting effect on endometrial thickness has, however, never before been reported.

An unresolved IVF problem is the nonresponsive, thin endometrium. To maximize pregnancy rates, studies suggest a minimal endometrial thickness of 7.0 mm and preferably >9.0 mm (25, 26). Approximately 0.6%–0.8% of patients do not reach minimum thickness (27). Treatment regimens proposed (28) are widely considered inefficient (29).

Failing to improve endometrial thickness, options become limited: IVF cycles are cancelled; embryos are cryopreserved

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“all-freeze cycles”), hoping for better endometrium in future cycles; or transfers are done in the face of inadequate endometrium, accepting reduced pregnancy chances. Gestational carriers represent very costly and psychologically difficult last resorts. Effective treatments are therefore urgently needed.

CASE REPORT

On the basis of anecdotal reports and a US patent application (7), we sporadically used subcutaneously injected G-CSF (Neupogen, Fil-gastrim; Amgen Manufacturing, Thousand Oaks, CA), off label and with appropriate experimental informed consent, in suspected implantation failure.

Because diagnosis of implantation failure is always tentative, clinical effects of G-CSF in this indication are difficult to assess. In contrast, however, potential proliferative effects on endometrium can be sonographically documented.

In June 2010 an out-of-state patient (patient 1) presented before anticipated ET, despite best endometrial preparation (estradiol 2 mg twice daily per os and 1 mg three times daily per vagina; and sildenafil citrate vaginal suppositories, 25 mg four times daily), with severely inadequate endometrium (3–4 mm) and demonstrating fluid in the cavity (Fig. 1A).

Refusing all-freeze and cycle cancellation and insisting on other options, she was offered fluid aspiration but was advised of likely quick reaccumulation. Requesting aspiration, she pressed for more options, being unable to afford additional cycles or other trips to New York. She was then offered experimental, off-label treatment with G-CSF.

Because a Tomcat catheter was used for fluid aspiration, the treating physician decided to use the same catheter to administer G-CSF (30 MU [300 mcg/1 mL]) by slow intrauterine infusion, only switching attached syringes.

Remarkably quick endometrial improvement (Fig. 1B) established endometrial G-CSF perfusion as a standard approach. Among three subsequent cases, one (patient 3) was treated at a different IVF center. The four cases reported here thus had three treating physicians.

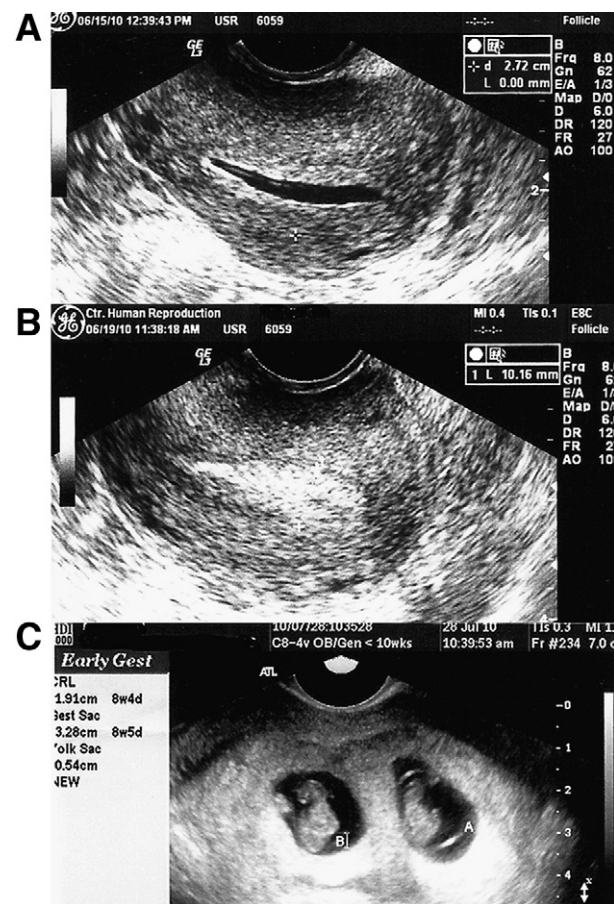
All four women presented with inadequate endometrial thickness only days before ET (Table 1) and were transferred maximally two embryos. Only patient 1 underwent endometrial fluid aspiration (approximately 1.0 mL of thick mucus): a Tomcat catheter, with attached 10-mL syringe, was introduced into the endometrial cavity under abdominal ultrasound control. Fluid (mucus) was aspirated, and the syringe was removed and replaced with a 1-mL microsyringe containing 30 MU (300 mcg/1 mL) of G-CSF, which was slowly emptied into the endometrial cavity, sonographically creating an endometrial fluid pocket, fully absorbed within 24 hours.

All four patients, at both centers, were informed that G-CSF to improve endometrial thickness was administered as experimental treatment, utilizing a US Food and Drug Administration–approved medication for a nonapproved clinical indication (“off label”). Because these treatments were not part of a study, they were not subject to approval by the centers’ institutional review boards. Patients, however, were subject to the centers’ informed consent process, covering all treatments involving off-label use of medications, including written informed consent. Written consent was also obtained for chart review and scientific publication of relevant data.

Granulocyte colony-stimulating factor treatment has since been approved by the institutional review board of the Center for Human Reproduction for two prospectively randomized, placebo-controlled

FIGURE 1

Ultrasound studies in patient 1. (A) Very thin endometrium (depending on area of measurement, 3.0–4.0 mm), surrounding an entirely fluid-filled endometrial cavity on day –5 before ET. (B) Same endometrium 4 days later, 1 day before ET (day –1). (C) Twin pregnancy at approximately 8 weeks, 3 days gestational age.



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studies (ClinicalTrials.gov ID #NCT01202643; ClinicalTrials.gov ID #NCT01202656).

Patients were between 33 and 45 years of age; three were in egg donor cycles. Table 1 summarizes patient characteristics and IVF cycle outcomes. All four patients reached a thickness of at least 7 mm and underwent ETs with one or two embryos.

Serial ultrasound observations in these patients suggest that endometrial expansion to 7.0 mm is reached within 48 hours from G-CSF infusion (Table 1 and Figs. 1 and 2).

All four patients conceived; the first patient with a twin pregnancy. Pregnancies in patients 1, 3, and 4 are normally progressing. Patient 2 experienced an intramural ectopic pregnancy (Fig. 2D), which failed methotrexate therapy and required surgical evacuation. This patient had a partial Asherman syndrome after a spontaneous pregnancy loss and dilation and curettage (Table 1).

DISCUSSION

We here report four IVF patients, in two infertility centers, with extraordinarily poor endometrial quality, despite maximal standard

TABLE 1**Patient characteristics.**

Patient	Age (y)	Diagnosis	Day of diagnosis	Day of perfusion	Endometrium before/after (mm)	Other procedures	Embryo transfer	Pregnancy	Outcome
1 ^a	34	POI	-5	-5	3-4/10.2	Fluid aspiration	Yes	Yes (twin)	Ongoing
2 ^b	45	Partial Asherman	-10	-9	4.8/7.3		Yes	Yes	Intramural ectopic
3 ^c	33	Repeated IVF failures	-7	-7	6.5/9.1		Yes	Yes	Ongoing
4 ^d	41	POI	-6	-2	Right: 4.3/8.1 Left: 6.2/8.3	Both horns perfused	Yes	Yes	Ongoing

Note: POI = primary ovarian insufficiency (aka premature ovarian failure, POF); FET = frozen embryo transfer.

^a Second egg donation cycle; two embryos transferred; fluid in cavity (thick mucus) was aspirated before endometrial perfusion on day -5 before ET. Patient is at time of this report in the 28th week of a normally progressing twin pregnancy (Fig. 1A-1C).

^b Second FET cycle (donor eggs); developed Asherman syndrome after dilation and curettage after miscarriage; before cycle, underwent hysteroscopic resection of adhesions, and endometrial lining was observed only "in patches." On ultrasound patchy endometrium confirmed with small fluid accumulation and areas of small calcifications (Fig. 2A). Fluid amount was deemed too small for aspiration, and fluid disappeared after G-CSF perfusion. A right ectopic intramural pregnancy was diagnosed, failed methotrexate treatment, and required surgical evacuation (Fig. 2A-2D).

^c Third ET (FET cycle), performed at second IVF center after patient had failed seven IVF cycles elsewhere, all described as reaching ET with inadequate endometrial thickness; Only one embryo transferred; the patient, at time of this report, is in her 23rd week of a normally progressing singleton pregnancy.

^d First egg donation cycle; severely bicornuate (almost double) uterus; both horns were perfused separately, each with half of the usual dose of medication. On a scheduled day-3 ET, 1 day after G-CSF perfusion, endometria of both uterine horns were still too thin (4.0 and 6.0 mm, right and left side, respectively). The next day, both horns had reached 8.3 mm, and separate single ETs into both horns were performed. At time of this report, the patient is in her eighth week of a normally progressing singleton pregnancy in her right uterine horn.

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therapy with estrogen and vasodilator therapy. All four without G-CSF perfusion in all likelihood would have experienced cycle cancellation or other unsatisfactory alternative treatments, reducing pregnancy chances and increasing costs as well as emotional distress.

These cases are reported ahead of two ongoing, prospectively randomized studies of G-CSF because of unexpectedly clear results in clinical circumstances without effective treatment options. This small G-CSF experience may therefore have immediate impact on cycle outcomes around the world and encourage prospectively controlled evaluations of G-CSF, as currently underway at our center.

Not only did all four patients reach ET, in itself a remarkable achievement, but all four patients also conceived. Because women with poor endometria are well recognized for lower-than-expected IVF pregnancy rates (29), this outcome is unexpected.

In contrast to other potential CSF effects, which require complex laboratory assessments, a proliferative effect on endometrium can be easily observed by measuring endometrial thickness. In this series, G-CSF, via endometrial perfusion, proved highly effective (Figs. 1 and 2). Indeed, G-CSF seems, even in women with extremely thin endometrium, able to induce minimal endometrial thickness of 7.0 mm within approximately 48 hours. It also may be effective in eliminating (and/or preventing recurrence of) endometrial fluid (mucus).

Patients who are resistant to endometrial expansion with standard treatments will not suddenly spontaneously expand. The improvements in endometrial thickness observed here are therefore, with great likelihood, attributable to G-CSF. Although a final judgment on the degree to which G-CSF affects endometrial thickness awaits prospectively randomized studies, this case series alone suggests that endometrial perfusion with G-CSF enhances endometrial thickness to a significant degree.

Granulocyte colony-stimulating factor was here offered off label, primarily to avoid cycle cancellations and/or all-freeze cycles. It was not administered with the goal of improving pregnancy chances. The observed pregnancy success, therefore, came unexpectedly. Considering the unfavorable pregnancy prognosis of these four patients, it is, however, difficult to overlook that all four also conceived.

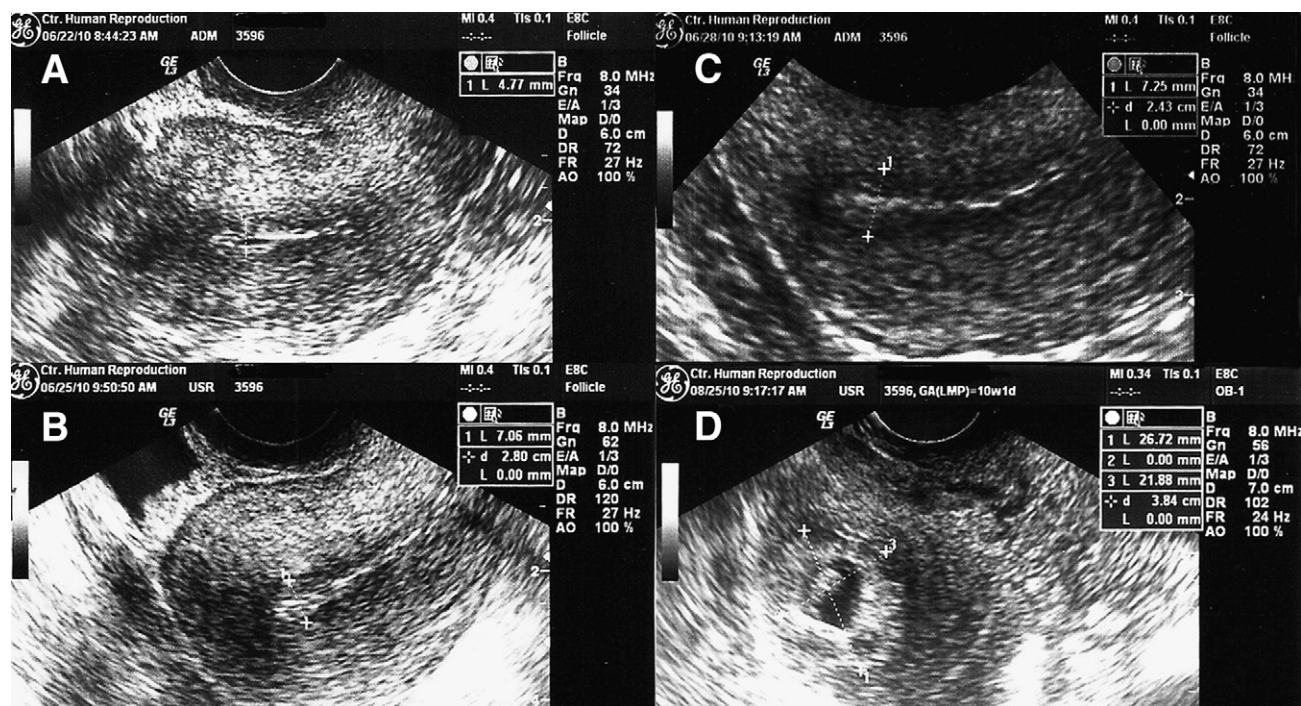
Although one pregnancy had to be terminated because of intramural location (Fig. 2D), it is hard to imagine that this was a consequence of G-CSF. Implantation just a few millimeters more proximally would likely have resulted in a normal pregnancy. The other three pregnancies are progressing normally: patient 1, with thin endometrium and mucous fluid in the endometrial cavity (twins); patient 3, with six prior ET failures in Italy, all accompanied by documented abnormally thin endometrial measurements; and patient 4, with extreme bicornuate uterus with one prior IVF failure with documented inadequate endometrial thickness in Austria.

We caution against over-interpreting these results. Although endometrial expansion in response to G-CSF perfusion, according to sonographic documentation, seems well documented, the pregnancy success observed here is unexpected and statistically potentially still spurious. The above-described animal and human data, suggesting improved implantation rates (7, 10, 23), may be indicative of improved pregnancy rates. Whether G-CSF indeed improves pregnancy chances remains, however, undetermined and subject to properly designed prospectively randomized clinical trials.

Two prospectively randomized studies at our center utilizing G-CSF, one investigating expansion of endometrial thickness and the other potential effects on routine IVF pregnancy rates, will offer first results in 2011.

FIGURE 2

Ultrasound studies in patient 2. (A) Thin endometrium (4.8 mm) and minor fluid accumulation within fundal area of endometrial cavity only on day -10 before ET. Also seen are small calcifications, primarily below endometrial basement membrane. Patient received G-CSF the next day (day -9). (B) Remarkable improvement within 48 hours from infusion (day -7), with endometrium reaching thickness of 7.1 mm in fundal area and absence of endometrial fluid. (C) Endometrium at 7.3 mm on day -4. (D) Right intramural corneal pregnancy after methotrexate failure and before surgical removal.



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