REVIEW

G-CSF and stem cell therapy for the treatment of refractory thin lining in assisted reproductive technology

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Abstract

Purpose The study aims to describe two promising therapeutic options for resistant “thin” endometrium in fertility treatment: granulocyte colony-stimulating factor (G-CSF) and stem cell therapy.

Methods A review of the scientific literature related to patients with thin endometrium undergoing fertility treatment.

Results Sufficient endometrial growth is fundamental for embryo implantation. Whether idiopathic or resulting from an underlying pathology, a thin endometrium of <7 mm is associated with lower probability of pregnancy; however, no specific thickness excludes the occurrence of pregnancy. We specifically reviewed two relatively new treatment options for resistant thin lining: intrauterine G-CSF and stem cell therapy. The majority of the reviewed trials showed a significant benefit for intrauterine G-CSF infusion in improving endometrial thickness and pregnancy rates. Early results of stem cell therapy trials seem promising.

Conclusions EMT <7 mm is linked to lower probability of pregnancy in assisted reproductive technology. Intrauterine G-CSF infusion appears to be a potentially successful treatment option for resistant cases, while stem cell therapy seems to be a promising new treatment modality in severely refractory cases.

Keywords Thin endometrium · Endometrial thickness · Pregnancy rates · G-CSF · Stem cell therapy

Background

Endometrial receptivity is a key step in the embryo implantation process [1–5]. During each menstrual cycle, and for a brief period of time, the endometrium represents the fertile “soil” for the implanting embryo [6]. Certain endometrial development appears to be crucial for adequate endometrial receptivity, and some reports proposed that a minimal endometrial thickness (EMT) of 6 mm is required to achieve implantation in assisted reproductive technologies (ARTs) [7]. However no cutoff appears to preclude implantation as successful pregnancies were documented at a much lower thickness (4 mm) [8–10]. In fact and up to this date, there is no consensus on a cutoff value of an EMT below which implantation rates decline in ART. Using receiver operating characteristic (ROC) area under the curve, a cutoff limit of endometrial thickness (on day of HCG trigger) above which implantation could be predicted was not detected by three reports, whereas two studies reported a threshold thickness of 8 mm [11–15]. A recent systematic review and meta-analysis of 10,724 cases showed that EMT as an independent variable is not predictive for the occurrence of pregnancy [16]. This meta-analysis, however, found that the most commonly reported cutoff of 7 mm occurred in only 2.4% of the cases (260/10,724), and it was associated with a significant drop in the probability of pregnancy [16]. In 2008, Senturk et al. reviewed thin endometrium and some of the available treatment modalities, and concluded that these were ineffective [17]. More recently, Lebovitz et al. concluded that the treatment of “thin” endometrium remains a challenge, with only minor improvements achieved with the currently available treatment

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modalities [18]. We have recently published a detailed review that aimed at defining thin endometrium in infertility patients and concluded that of the available treatment modalities, only vaginal sildenafil and intrauterine granulocyte colony-stimulating factor (G-CSF) were consistent in showing improvement in endometrial thickness and pregnancy rates of patients with thin endometrium, and that early stem cell results are promising [19]. Here, we provide a focused review on emerging and promising treatment options for refractory thin endometrium, namely, intrauterine G-CSF and stem cell therapy.

Materials and methods

We conducted an electronic review of the literature pertaining to thin endometrium, its pathophysiology, and treatment through January 2017. Abstracts, case reports, original, and review articles were considered. The Cochrane database, Pubmed, Medline registries, and other online sources were searched using the broad terms: thin endometrium, thin endometrium and IVF, thin endometrium and ART, and treatment of thin endometrium. The titles and abstracts were screened, and relevant articles were analyzed in detail to determine which studies could be included in the review. Furthermore, the references cited in relevant studies and review articles were hand searched to identify further relevant studies. Studies were considered eligible if they were conducted to assess or compare different treatment modalities of thin endometrium. Changes in mean endometrial thickness and pregnancy rates were the two principle summary measures, and were independently extracted from individual studies.

Results (summarized in Table 1)

G-CSF

G-CSF, a hematopoietic growth factor, was reported to have positive effects in non-hematopoietic cells, including the endometrium [35]. It was hypothesized that intrauterine G-CSF might have a direct role promoting endometrial growth after successful treatment of four patients [20]. A pilot trial showed that intrauterine G-CSF (given 6–12 h prior to HCG trigger) significantly improved endometrial thickness in patients with thin lining, resulting in an overall clinical pregnancy rate of 19.1% [21]. Lucena et al. successfully treated one patient, while Check and colleagues failed with another patient [22, 23]. Li et al. administered intrauterine G-CSF (100 µg) on the day of ovulation, or day of progesterone start, or day of HCG administration to 59 patients with thin endometrium (<7 mm) [24]. The cycle cancellation rate due to thin endometrium was significantly lower after treatment (69.39 vs. 48.75 vs. 17.5%;

$P < 0.05$ self-controlled vs. control vs. treatment groups, respectively), with a trend towards better implantation and clinical pregnancy rates [24]. Similarly Kunicki et al. administered intrauterine G-CSF (300 µg) 6–12 h prior to HCG trigger to patients with an EMT of 7 mm, resulting in significant improvement in EMT within 72 h and the pregnancy rate of 18.9% [25]. Recently, Barad et al. administered intrauterine G-CSF to patients undergoing IVF or FET, regardless of endometrial thickness. The study group received 300 µg/cm³ G-CSF on the day of HCG trigger without improvement in endometrial thickness [26]. In another prospective trial, Shah et al. administered intrauterine G-CSF to patients with thin lining (<8 mm) that were resistant to treatment with estradiol and vaginal sildenafil, and expanded the treatment to patients with repeated implantation failure who had an EMT >8 mm [27]. G-CSF (300 µg) was administered into the endometrial cavity after 10 days of oral estradiol and vaginal sildenafil to all 231 patients, resulting in a significant increase in EMT and clinical pregnancy rate of 38.07% [27]. In another trial, Eftekhari et al. compared intrauterine G-CSF therapy to direct embryo transfer in patients with thin lining (<7 mm) [28]. All patients received oral estradiol and vaginal sildenafil priming, and on either day 12 or 13, 34 patients accepted intrauterine G-CSF while another 34 refused. The cycle cancellation rate due to thin endometrium was similar between both groups (15.2%), and so was the increase in endometrial thickness and pregnancy rates. Interestingly, the EMT increased from 5.63 ± 0.78 to 7.91 ± 0.55 mm in the G-CSF group [28].

Xu et al. compared intrauterine G-CSF and G-CSF with endometrial scratch to no intervention [29]. The G-CSF was administered when the lead follicle measured 12 mm; the EMT increased significantly after treatment without a difference between G-CSF and G-CSF + scratch. The implantation and clinical pregnancy rates were significantly higher in both treatment subgroups [29]. Tehraninejad and colleagues treated 15 patients who previously had an IVF cycle canceled due to resistant thin endometrium (<6 mm) with intrauterine G-CSF (300 µg) on the day of egg retrieval [30]. The patients suffered from various infertility diagnoses. After intrauterine G-CSF treatment, the endometrial lining significantly increased in the cohort, with 3/12 (20%) patients subsequently conceiving [30]. Most recently, Lee and colleagues also demonstrated that intrauterine G-CSF (300 µg) on either trigger day or retrieval day significantly increased the EMT in patients with thin lining, resulting in pregnancy rate of 22.0% [31]. Interestingly, there was a trend for higher implantation, clinical pregnancy, and ongoing pregnancy rates when the G-CSF was instilled on the day of HCG trigger vs. day of egg retrieval [31].

Stem cell therapy

Emerging evidence supports that endometrial stem cells are present in both the basalis and functionalis layers of the human

Table 1 Studies evaluating G-CSF and stem cell therapy in treatment of thin lining

Author	Year	Study type	Diagnosis	Study vs. control group	Number of patients or cycles	Intervention	Mean EMT prior to intervention (mm)	Mean EMT post intervention (mm)	P value	Pregnancy rate	P value
Gleicher [20]	2011	Case series	POI, repeated IVF failure, AS		4 patients	Intrauterine G-CSF (300 µg) infusion 2–9 days before ET	5.0 ± 1.2	8.6 ± 1.1	N/A	100%	N/A
Gleicher [21]	2013	Prospective observational	DOR, male factor, PCOS		21 patients	Intrauterine G-CSF (300 µg) infusion 6–12 h before HCG trigger	6.4 ± 2.1	9.3 ± 2.1	<0.001	19%	N/A
Lucena [22]	2013	Case report	Thin endometrium in IVF cycles		1 patient	Intrauterine G-CSF (300 µg) infusion on day of oocyte retrieval	5.7	8.9	N/A	Full-term pregnancy	N/A
Check [23]	2014	Case report	Thin endometrium during IVF-ET		1 patient	Intrauterine G-CSF (30 million units)	5.0	5.0	N/A	No pregnancy	N/A
Li [24]	2014	Retrospective	Multiple	Study group	40 cycles	Intrauterine G-CSF (100 µg) infusion at varying times of the cycle	6.53 ± 0.65	6.75 ± 1.17	0.403	30.30%	N/S
				Self-controlled group	49 cycles	FET	N/A	N/A	N/A	20%	N/A
Kunicki [25]	2014	Prospective observational	N/A	Control group	80 cycles	FET	N/A	N/A	N/A	29.27%	N/A
Barad [26]	2014	RCT	All patients undergoing IVF or FET (regardless of EMT)	Study group first cycle	37 patients	Intrauterine G-CSF (100 µg) infusion 6–12 h before HCG trigger	6.74 ± 1.75	8.42 ± 1.73	<0.001	18.90%	N/A
				Control group first cycle	73 patients	Intrauterine G-CSF (300 µg) infusion on day of HCG trigger + IVF-ET	10.23 ± 2.01	11.68 ± 2.22	N/S	94.40%	N/S
				Study group second cycle	68 patients	IVF-ET	10.37 ± 2.14	11.62 ± 2.28	N/S	84.20%	N/S
				Control group second cycle	19 patients	Intrauterine G-CSF (300 µg) infusion on day of HCG trigger + IVF-ET	9.96 ± 2.27	10.32 ± 2.13	N/S	70.43%	N/S
Shah [27]	2014	Observational cohort	Thin endometrium <8 mm or repeated implantation failure	Study group	16 patients	IVF-ET	11.1 ± 1.93	11.26 ± 2.01	60%		N/A
Eftekhari [28]	2014	Non-randomized trial	Thin endometrium <7 mm	Study group	231 patients	Intrauterine G-CSF (300 µg) after 10 days of priming with oral estradiol and vaginal sildenafil	7.98 ± 1.3	10.97 ± 1.23	<0.0001	38.07%	N/A
Xu [29]	2015	Prospective controlled	Unresponsive thin endometrium	Study group	34 patients	Intrauterine G-CSF (300 µg) on cycle day 12 or 13	5.63 ± 0.78	7.91 ± 0.55	0.1	32.1%	0.1
				Control group	34 patients	FET	5.76 ± 0.86	8.23 ± 0.82	12%		0.038
				Study group	27 patients	Intrauterine G-CSF (300 µg) ± endometrial scratch when lead follicle = 12 mm	5.7 ± 0.71	8.4 ± 2.01	<0.001	48.10%	
Tehraninejad [30]	2015	Non-randomized trial	Thin endometrium <6 mm	Control group	52 patients	FET	6.5 ± 0.5	N/A	25%		N/A
Lee [31]	2016	Retrospective	Thin endometrium <8 mm	Study group	15 patients	Intrauterine G-CSF (300 µg) on day of oocyte retrieval	3.6 ± 0.98	7.12 ± 0.84	<0.001	20%	N/A
Nagori [32]	2011	Case report	AS	Study group	50 patients	Intrauterine G-CSF (300 µg) infusion on day of HCG trigger or egg retrieval	7.2 ± 0.6	8.5 ± 1.5	<0.001	22.0%	N/A
Singh [33]	2014	Prospective case series	AS	Study group	1 patient	Intrauterine endometrial angiogenic stem cells	3.6	7.1	N/A	Clinical IUP	N/A
Santamaria [34]	2016	Prospective	AS or endometrial atrophy	Study group	6 patients	Mononuclear stem cell implantation in sub-endometrial zone	1.38 ± 0.39	5.48 ± 1.14	0.002	N/A	N/A
				Control group	16 patients	BMDSC injection into spiral arterioles	4.3 m and 4.2	6.7 m and 5.7	0.004 m and 0.03	10/16	N/A

POI premature ovarian insufficiency, IVF in vitro fertilization, AS Asherman's syndrome, G-CSF granulocyte-colony stimulating factor, ET embryo transfer, DOR diminished ovarian reserve, PCOS polycystic ovarian syndrome, HCG human chorionic gonadotropin, IVF in vitro fertilization, IVF-ET in vitro fertilization-embryo transfer, FET frozen embryo transfer, BMDSC bone marrow-derived stem cells

endometrium, and it is thought that these stem cells play a role in regenerating the endometrial lining during each estrous cycle [36–39]. Cervello recently demonstrated that human endometrial adult stem cells are able to generate human endometrium after transplantation in NOD-SCID mice renal capsules [40]. Non-endometrial stem cells seem to have a role in endometrial regeneration as well; in fact, hematopoietic and non-hematopoietic bone marrow-derived stem cells (BMDSCs) are recruited to the endometrium in response to injury as shown by Taylor and colleagues [41]. These findings were further affirmed when hematopoietic progenitor cells were shown to be recruited to the uterine epithelium to play an important role in epithelial regeneration and, even more interestingly, when male origin BMDSCs were shown to be present in endometria of female bone marrow transplant recipients [42, 43]. In their elegant study, Cervello et al. also demonstrated that BMDSCs exert their regenerative effects in a paracrine fashion by ultimately stimulating endometrial side cell population [44]. In fact, uterine ischemia/reperfusion injury results in a twofold increase in bone marrow-derived stem cell recruitment to the endometrium, which is independent of G-CSF and only serves in uterine repair after injury rather than monthly cyclic regeneration of the endometrium [45]. The role of BMDSC in endometrial regeneration after injury was further validated in several murine models [46–48].

These findings triggered researchers to investigate the effects of bone marrow stem cells in the treatment of Asherman's syndrome and thin endometrium in a murine model. Female mice with AS were given BMDSCs via the tail veins and later bred after three estrous cycles [49]. Nine of 10 treated mice conceived compared to only 3/10 non-treated mice [49]. Similarly, Kilic et al. treated Asherman's syndrome in Wistar albino rats with either mesenchymal stem cells (MSCs) or oral estrogen or combined MSC and oral estrogen [50]. All treatment groups demonstrated improvement in the level of fibrosis when compared to control, which was mostly noted with combined MSC and estrogen treatment [50].

Zhao et al. further elucidated these regenerative capabilities of BMDSCs when rats with thin endometria were infused with BMDSCs and compared to endometria of normal rats [51, 52]. The treatment groups showed significant increase in EMT compared the control groups in both trials, respectively (325.35 ± 75.51 vs. 187.53 ± 34.38 μm ; $P < 0.05$) and (359.13 ± 49.70 vs. 187.53 ± 34.38 μm ; $P < 0.05$) [51, 52].

A recent comparative study delineated the role of various BM-derived cell subtypes in endometrial regeneration [53]. The investigators found that freshly isolated unfractionated BM cells, hematopoietic progenitor cells, endothelial progenitor cells (EPCs), mesenchymal stem cells, and in vitro cultured mouse Oct4⁺ BM-derived hypoplast-like stem cells supported endometrial regeneration after injury [53].

The first human application was in 2011, when a patient with AS and refractory thin endometrium (3.6 mm) was

treated with intrauterine autologous endometrial angiogenic stem cells [32]. The infusion was followed by high-dose estradiol valerate, aspirin (75 mg PO daily), and four cycles of cyclical estrogen and progesterone therapy until the EMT reached 7.1 mm. Three donor oocyte embryos were subsequently transferred resulting in a single viable intrauterine pregnancy [32]. Similar findings were noted in a case series of six patients with refractory AS who were treated with autologous mononuclear stem cells. The mean endometrial thickness improved from 1.38 ± 0.39 mm to 4.05 ± 1.4 , 5.46 ± 1.36 , and 5.48 ± 1.14 mm at 3, 6, and 9 months, respectively ($P < 0.05$) [33]. Cervello et al. investigated whether human bone marrow-derived stem cells would promote endometrial growth in rats with Asherman's syndrome [54]. The BMDSCs engulfed small endometrial vessels of damaged horns and resulting in proliferation of epithelial gland cells in a paracrine manner by upregulation of thrombospondin 1 and downregulation of insulin-like growth factor 1 [53].

In the most recent human trial, Santamaria and colleagues treated patients who had Asherman's syndrome and endometrial atrophy with autologous CD 133+ BMDSCs [34]. The infusion, administered into the spiral arterioles, increased the EMT from 4.3 to 6.7 mm, and from 4.2 to 5.7 mm in patients with AS and endometrial atrophy, respectively. The improvement in endometrial lining lasted up to 6 months, and subsequent conception attempts resulted in three spontaneous pregnancies (2, 4, and 19 months after treatment), and seven other pregnancies following 14 embryo transfers [34].

Discussion

The diagnosis of "thin endometrium" is a frustrating condition that occurs not infrequently in clinical practice. It is not exactly clear how thin endometrium lowers the probability of pregnancy, but it is hypothesized that the proximity of the embryo to the reactive oxygen species-rich basal layer could be detrimental for embryo development and implantation, or possibly that with thin lining, there is not enough soil to sustain the "seed" [55].

The main challenge for clinicians is determining a certain thickness below which they consider an endometrium thin, and how to treat it when it is refractory to conventional therapy. The most widely accepted measurement is 7 mm; however, this cutoff is not absolute, as pregnancies can occur at lower cutoffs [8–10]. Another challenge is the reliability of endometrial thickness measurement as a predictor for the occurrence of pregnancies in IVF cycles, as it seems to be only a good factor for the assessment of conception probability, which significantly drops below an EMT of 7 mm [55]. Other key factors such as endometrial pattern play a vital role in successful implantation as well, and may be as important as endometrial thickness [56]. Newly emerging diagnostic tools

such as endometrial receptivity assay (ERA) seem to be very accurate in determining the window of implantation. In one study, ERA determined that 75% of patients ($n = 13$) with an EMT ≤ 6 mm had a receptive endometrium according to the customized endometrial receptivity microarray test [57]. This technique however is invasive and costly, and these early results must be further validated with larger studies.

Over the years, several treatment options have been suggested and assessed as a treatment for refractory thin endometrium with or without Asherman's syndrome [19]. Extended estrogen therapy, luteal GnRH-a supplementation, low-dose HCG during endometrial preparation, tamoxifen citrate as an ovulation induction agent, long courses of pentoxifylline and tocopherol or tocopherol only, aspirin, acupuncture, and neuromuscular electric stimulation with biofeedback were all inconsistent in improving endometrial thickness [19]. Vaginal sildenafil improved the endometrial thickness in a significant percentage of patients with thin lining due to various diagnoses that had prior IVF and ICSI cycles, indicating that it could be a reasonable first-line treatment option [19].

Treatment with intrauterine G-CSF gained significant interest despite its cost, and the majority of trials showed promising positive effects in patients with thin endometrium and possibly those with repeated implantation failure [27]. In fact, only one case report and one other retrospective study in FET cycles failed to demonstrate a positive effect of G-CSF in improving EMT [23, 24]. On the other hand, there is no evidence that G-CSF administration is beneficial to all patients undergoing IVF or FET [26]. While the use of G-CSF seems promising in increasing EMT and possibly pregnancy rates, most studies suffer from small sample sizes, and different studies used different doses and time points when G-CSF was administered, making interpretation rather difficult. In addition, GCSF has been given by intrauterine infusion but there is no data on whether other routes of administration, such as the subcutaneous route, are comparable. Larger prospective, randomized, placebo-controlled, trials are therefore sorely needed.

Stem cell therapy appears to be the most promising treatment option for cases with Asherman's syndrome with refractory thin lining (<5 mm). Initial reports on intrauterine angiogenic endometrial stem cells showed improvement in endometrial thickness in patients with Asherman's syndrome or refractory thin endometrium [32, 33]. Several murine models with induced thin endometrium showed significant improvement back to normal after bone marrow mesenchymal stem cell administration [51, 52]. In a promising early human trial, patients with AS and refractory thin endometrium had significant improvement in their endometrial thickness that lasted up to 6 months after autologous bone marrow-derived stem cells were infused via the uterine arterioles, with excellent pregnancy rates [34]. These results present a valuable viable option to patients who fail other treatment options; however,

stem cell therapy remains a very invasive and expensive treatment option requiring bone marrow biopsy, cell sorting, and interventional radiology assistance for administration into the uterine arterioles.

In conclusion, a receptive endometrium plays a critical role in embryo implantation, and adequate endometrial growth is essential to this process. Poor endometrial development is linked to a lower probability of pregnancy, yet it is not the sole predictor of pregnancy occurrence. Of the several available treatment modalities, vaginal sildenafil appears to be a reasonable first-line option, whereas intrauterine G-CSF infusion before the HCG trigger could be a second-line treatment option, provided that large randomized studies evaluating outcomes, dosage, method, and timing of administration during the stimulation cycles are conducted. Stem cell therapy appears to be a very promising option for the most refractory cases; however, more research is warranted to evaluate the safety, effectiveness, and cost of this modality before it becomes integrated in the treatment of this frustrating condition.

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