A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for in vitro fertilization

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Objective: To evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium on ongoing implantation rate (OIR).
Design: Multicenter, randomized, placebo-controlled, double-blinded prospective design.
Setting: Fourteen Scandinavian fertility clinics.
Patient(s): A total of 1,332 women with indication for in vitro fertilization or intracytoplasmic sperm injection; 1,149 received embryo transfer (GM-CSF: n = 564; control: n = 585).
Intervention(s): Oocytes were fertilized, and embryos cultured and transferred in control medium or test medium containing 2 ng/mL GM-CSF.
Main Outcome Measure(s): OIR at gestational week 7, with follow-up at week 12 and birth.
Result(s): At week 7, OIRs were 23.5% (GM-CSF), and 20.0% (control) (odds ratio [OR] 1.26, 95% confidence interval [CI] 0.91–1.75). At week 12, OIRs were 23.0% (GM-CSF) and 18.7% (control) (OR 1.35, 95% CI 1.06–1.72), and live birth rates were 28.9% and 24.1%, respectively (OR 1.35, 95% CI 1.03–1.78). The effect of GM-CSF was influenced by the human serum albumin concentration in the medium. Birth weight and abnormality incidence were similar in both groups. Exploratory analyses showed that GM-CSF increased OIR in women with previous miscarriage, especially in women with more than one miscarriage.
Conclusion(s): Addition of GM-CSF to embryo culture medium elicits a significant increase in survival of transferred embryos to week 12 and live birth. Our results are consistent with a protective effect of GM-CSF on culture-induced embryo stress. GM-CSF may be particularly efficacious in women with previous miscarriage.
Clinical Trial Registration Number: NCT00565747. (Fertil Steril® 2013;99:1600–9. ©2013 by American Society for Reproductive Medicine.)
Key Words: Embryo development, GM-CSF, in vitro fertilization, miscarriage, perinatal outcome

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In the female reproductive tract the embryo is naturally exposed to cytokines and growth factors, which play a physiologic role in regulation of normal blastocyst development, facilitate embryo implantation, and subsequently ensure optimal development of the fetus and placenta (1–3). Granulocyte-macrophage colony-stimulating factor

(GM-CSF, also referred to as CSF2) is a multifunctional cytokine identified in mice as essential for normal blastocyst development and subsequent fetal viability and health (4, 5). In mice, epithelial cells lining the female reproductive tract synthesize and secrete GM-CSF under regulation by ovarian steroid hormones, and expression is further induced in early pregnancy by factors in seminal fluid (6, 7). Blastocyst development is impaired in GM-CSF–null mutant mice, and is linked to altered placental structure, decreased fetal size, increased fetal loss in late gestation, and mortality during early postnatal life (4, 5, 8, 9). Studies in mouse embryos show that culture with GM-CSF alleviates many of the developmental differences associated with culture, with normalized rates of apoptosis and suppressed expression of heat shock proteins and stress response genes (5, 9).

In women, GM-CSF is synthesized in epithelial cells lining the oviduct and uterus (10, 11), consistent with findings in rodents and other mammalian species including sheep, cattle, and pigs (7, 12–14). GM-CSF synthesis is highest during the secretory phase of the menstrual cycle in women, corresponding to the time of conception and embryo implantation (11, 15).

In vitro conditions for human embryo culture are generally considered to be suboptimal, and embryo development is often arrested or delayed, with high rates of abnormal cell division and cell death (16). Only 25% of initiated fresh cycles in the U.K. in 2009 resulted in a live baby (17). This is linked with concerns regarding the increased incidence of perinatal mortality, low birth weight, preterm birth, and birth defects in singleton IVF babies (18). Supplementation of culture media with cytokines and growth factors known to be present in the reproductive tract has been shown to promote human embryo growth and development (3, 19–21). In vitro culture of human embryos in the presence of GM-CSF results in accelerated embryo development, an increase in the proportion of early cleavage embryos that develop to the blastocyst stage, and an increase in the number of viable inner cell mass cells with less apoptosis (21, 22).

A murine study showed no effect of 2 ng/mL GM-CSF on rates of mosaicism/aneuploidy (23), and we showed no difference in chromosomal constitution of embryos developed from donated human oocytes after in vitro culture with 2 ng/mL GM-CSF from fertilization to day 3 compared with the control (24). In the present study we evaluated the hypothesis that addition of 2 ng/mL GM-CSF to embryo culture medium would increase the ongoing implantation rate at gestational week 7 by >25%.

METHODS

Study Design and Participants

The study was a multicenter, randomized, placebo-controlled, double-blinded prospective design (ClinicalTrials.gov identifier: NCT00565747). In total, 1,925 Danish and Swedish patients with indication for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) were assessed for eligibility between November 2007 and August 2010 (the last baby was born in March 2011) at eleven Danish and three Swedish fertility clinics. Of these, 1,332 participants predominantly of Danish or Swedish nationality were included, and informed written consents were obtained before their participation (Fig. 1). The study was approved by regional Ethics Committees and was in accordance with the principles of Helsinki Declaration II. Patients were eligible for inclusion if they were aged 25–39 years, had a regular menstrual cycle of 21–35 days, were treated with a standard GnRH agonist or antagonist protocol, and had three or more follicles with a diameter of $\geq$14 mm on the day of hCG administration, including a leading follicle of $\geq$17 mm. Exclusion criteria were previous participation in the study, use of assisted hatching, use of nonejaculated sperm, medical conditions or genetic disorders prohibiting IVF/ICSI or interfering with interpretation of results, use of investigational drugs within 30 days before oocyte retrieval, severe chronic disease of relevance for reproduction, and oocyte donation. No significant differences were found in baseline characteristics between the two groups. (Table 1 and Supplemental Table 1 [available online at www.fertstert.org]).

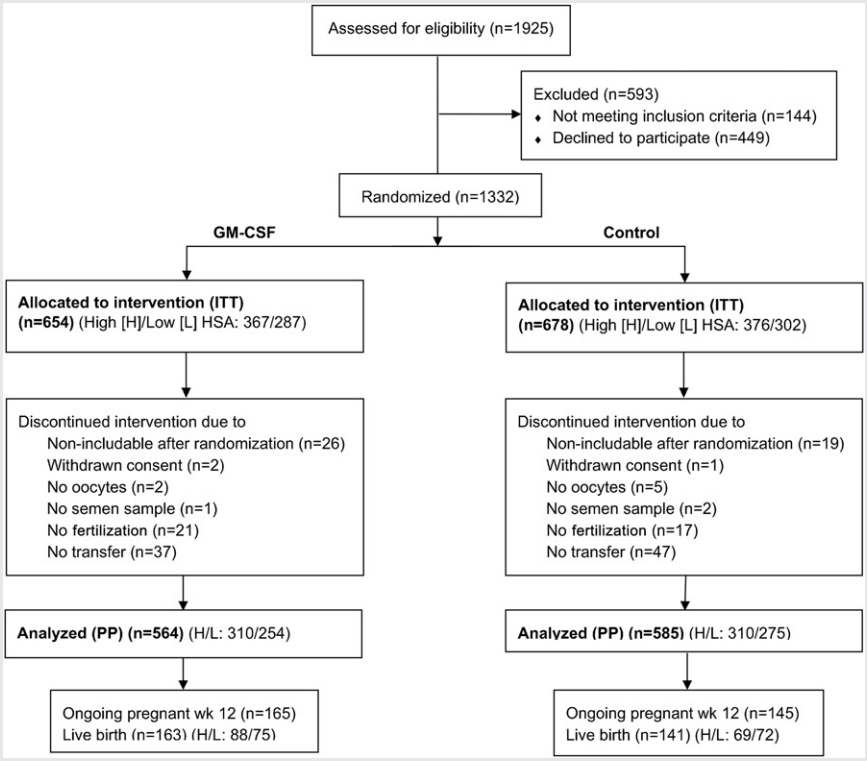
Randomization and Blinding

Randomization (blocks of four) was computer-generated individually for each clinic to maintain balance between the treatment groups at each site. To allow preparation of culture medium, the randomization was performed the day before oocyte aspiration. Participants and investigators were blinded to treatment allocation. Study media were packaged unidentifiably and labeled only with randomization number. For each new patient recruited, the lowest available randomization number was used.

Study Procedures

Hormonal stimulation and oocyte retrieval was undertaken according to the standard procedures of each clinic. Fertilization, embryo culture, and transfer was performed with the use of EmbryoAssist without cytokine (control) or formulated with 2 ng/mL GM-CSF (test) produced by ORIGIO. The concentration of 2 ng/mL GM-CSF was chosen based on murine data (5, 21, 25) and our human safety study on aneuploidy (24). Yeast-derived recombinant human GM-CSF (Leukine) licensed for human use was obtained from Genzyme. The concentration of human serum albumin (HSA) in test and control media was 2 mg/mL for the first 620 includable subjects with embryo transfer, and 5 mg/mL for the last 529. This increase in HSA concentration was enacted owing to suboptimal performance of the control medium revealed at the interim analysis, and an HSA concentration of 5 mg/mL was chosen because that concentration is commonly used in culture media. Insemination was performed by conventional IVF or ICSI 3 $\pm$ 1 hours after oocyte retrieval. Embryo evaluation was performed at fixed time points of 20, 27, 44, and 68 $\pm$ 1 hours after fertilization. On day 3, one or two embryos were transferred in an aliquot of the respective culture medium according to standard procedures. Serum hCG was measured 16–18 days after oocyte retrieval, and if positive, an ultrasound scan of the uterus was performed at gestational weeks 7 and 12. Data regarding pregnancy outcome were retrieved via the individual's civil registration number, and were collected as registry data from the Danish National Board of Health (93% recovery rate) supplemented with follow-up questionnaires completed and returned by each couple.

FIGURE 1



Flow chart of participant enrollment. HSA = human serum albumin; ITT = intention to treat; PP = per protocol.

Ziebe. GM-CSF in human embryo culture medium. Fertil Steril 2013.

Primary and Secondary End Points, and Follow-Up

The primary end point was ongoing implantation rate at gestational week 7, as determined by ultrasound. Secondary end points were top-quality embryos (defined as 4–5 cells at 44 hours and ≥7 cells at 68 hours after insemination, with ≤20% fragmentation and equally sized blastomeres [<25% difference] without multinucleation) and normally developed day 3 embryos (≥6 cells with ≤20% fragmentation at 68 hours). Follow-up included ongoing implantation rate week 12, live birth rate, and perinatal outcome.

Sample Size and Statistical Analyses

The study employed a two-stage adaptive design (26). The final calculated sample size (n = 1,300) was based on the hypothesis of a 25% increase in ongoing implantation rate at gestational week 7 and adjusted on the basis of the results of an interim analysis performed after inclusion of 301 patients with embryo transfer. Only the sample size was adjusted after interim analysis; the hypothesis and other study parameters were not changed. Analyses of the primary end point and corresponding positive pregnancy outcome or pregnancy loss to the time of live birth were performed with the use of a logistic regression model (SAS software version 9.2) with treatment and center as main factors, and covariates including age, body mass index (BMI), smoking, indication for treatment, IVF versus ICSI, previous number of IVF/ICSI treatments, previous pregnancies, previous miscarriages, oocytes, transferable embryos in current treatment cycle, and the HSA regime (low/high, where applicable). Odds ratios (ORs) were adjusted for the same set of covariates. The secondary end points were analyzed with the use of a multiplicative Poisson model (log-linear) with the same set of covariates. Fisher exact test was applied for analyses of baseline characteristics, and perinatal parameters. A P value of $<.05$ was considered to be significant.

RESULTS

In total 1,332 women were randomized to have their oocytes fertilized and embryos cultured and transferred in control medium or test medium containing GM-CSF. Of these, 1,149 includable women received embryo transfer (control: n = 585; GM-CSF: n = 564; Fig. 1). The average numbers of embryos transferred were 1.51 (control) and 1.49 (GM-CSF).

Full Study Cohort—Primary and Secondary End Points

Overall, ongoing implantation rates at gestational week 7 were 23.5% (GM-CSF) and 20.0% (control), a relative difference of 17.8% (P=.17; OR 1.26, 95% confidence interval [CI] 0.91–1.75; Table 1). Because suboptimal performance of the control medium was evident at interim analysis, the concentration of HSA was increased from 2 mg/mL to 5 mg/mL in

TABLE 1

Outcomes according to treatment group—full study cohort.

	GM-CSF			Control				*P* value (OR [95% CI])		
	Overall	Low HSA	High HSA	Overall	Low HSA	High HSA	Overall, relative difference (%)	Overall	Low HSA	High HSA
Intention to treat										
Patient age (y)	32.2 ± 3.7			32.4 ± 3.8						
Patient BMI (kg/m^2)	24.2 ± 4.2			24.3 ± 4.1						
No. of randomized cycles	654	367	287	678	376	302				
Per protocol										
No. of transfer cycles	564	310	254	585	310	275				
No. of transferred embryos	838	468	370	882	480	402				
Mean no. of embryos transferred	1.49	1.51	1.46	1.51	1.55	1.46				
Normally developed embryos, day 3 [n (% of fertilized)]	1,376 (42.0)	743 (40.0)	633 (44.6)	1,541 (43.9)	708 (38.9)	833 (49.3)		.55 (0.98 [0.91–1.05])		
Top-quality embryos [n (% of fertilized)]	511 (15.6)	284 (15.3)	227 (16.0)	590 (16.8)	266 (14.6)	324 (19.2)		.56 (0.97 [0.86–1.09])		
Ongoing implantation rate, wk 7 [($n_{embryos}$ (% of transferred embryos)]	197 (23.5)	112 (23.9)	85 (23.0)	176 (20.0)	86 (17.9)	90 (22.4)	17.8	.17 (1.26 [0.91–1.75])	.02 (1.48 [1.07–2.06])	.71 (1.07 [0.75–1.52])
Ongoing implantation rate, wk 12 [$n_{embryos}$ (% of transferred embryos)]	193[a] (23.0)	110 (23.5)	83 (22.4)	165[a] (18.7)	80 (16.7)	85 (21.1)	23.1	.02 (1.35 [1.06–1.72])	.007 (1.58 [1.13–2.21])	.53 (1.12 [0.79–1.60])
Live birth rate [n_{births} (% of transfer cycles)]	163 (28.9)	88 (28.4)	75 (29.5)	141 (24.1)	69 (22.3)	72 (26.2)	19.9	.03 (1.35 [1.03–1.78])	.05 (1.46 [1.00–2.12])	.28 (1.24 [0.84–1.85])
Follow-up on pregnancies (per protocol)										
Positive hCG [n (% of transfer cycles)]	214 (37.9)			218 (37.3)				.46		
Early pregnancy loss (≤wk 12) [n (% of women with positive hCG)]	49 (22.9)			73 (33.5)				.02		
Biochemical pregnancy[b]	29 (13.6)			44 (20.2)				.07		
Ectopic pregnancy	4 (1.9)			2 (0.9)				.45		
Miscarriage (loss wk 7–12)	16 (7.5)			27 (12.4)				.11		
Late pregnancy loss (>wk 12) [n (% of women with positive hCG)]										
Miscarriage/elective termination due to malformations	2 (0.9)			4 (1.8)				.69		
Follow-up on births [n (% of children born)]										
Deliveries	163			141						
Live-born children	194			164						
Stillborn children	0 (0)			0 (0)				–		
Perinatal death of children[c]	1 (0.5)			1 (0.6)				1.00		
Children born with abnormalities/ malfunctions[d]	11 (5.7)			8 (4.9)				.82		
Sex								.67		
Girls	88 (45.4)			79 (48.2)						
Boys	106 (54.6)			85 (51.8)						

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TABLE 1

Continued.

	GM-CSF			Control				*P* value (OR [95% CI])		
	Overall	Low HSA	High HSA	Overall	Low HSA	High HSA	Overall, relative difference (%)	Overall	Low HSA	High HSA
Singletons										
No. of children born	133			118						
Gestational age at birth (wk)	39.3 ± 2.9			39.6 ± 2.2				.62		
Deliveries [n (% of singletons)]								.53		
≥wk 37+0	120 (90.2)			110 (93.2)						
≥wk 32+0 and <wk 37+0	9 (6.7)			7 (5.9)						
<wk 32+0	4 (3.0)			1 (0.8)						
Birth weight (g)	3,344 ± 701			3,417 ± 597				.56		
Appropriateness of birth weight for gestational age [n (% of singletons)]								.08		
Appropriate	124 (93.2)			109 (92.4)						
Small	8 (6.0)			3 (2.5)						
Large	1 (0.8)			5 (4.2)						
Missing	0 (0)			1 (0.8)						
Twins										
No. of children born	58			46						
Gestational age at birth (wk)	36.4 ± 1.9			35.8 ± 2.7				.62		
Birth weight (g)	2,541 ± 532			2,403 ± 612				.14		
Triplets[e]										
No. of children	3			0						
Gestational age at birth (weeks)	35.1									
Birth weight (g)	2,085 ± 88									

Note: Plus-minus values are mean ± SD. Analyses of ongoing implantation rates and live births were performed with the use of a logistic regression model with treatment and center as main factors, and covariates including age, BMI, smoking, indication for treatment, IVF vs. ICSI, previous number of IVF/ICSI treatments, previous pregnancies, previous miscarriages, number of oocytes, transferrable embryos in current treatment cycle, and HSA regime (low/high, where applicable). Normally developed embryos and top-quality embryos were analyzed with the use of a multiplicative Poisson model (log-linear) with the same set of covariates. ORs were also adjusted for these covariates. Analyses of pregnancy loss were performed with the use of a logistic regression model. Fisher exact test was applied for analyses of perinatal parameters. Birth data from 93% of the participating individuals were validated by the Danish National Board of Health. BMI = body mass index; CI = confidence interval; GM-CSF = granulocyte-macrophage colony-stimulating factor; HSA = human serum albumin; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; OR = odds ratio.

[a] The number of embryos implanted (ongoing) as dizygotic twins was 2 × 27 for the GM-CSF group and 2 × 20 for the control group. Monozygotic twins: 2 × 4 (GM-CSF) and 2 × 3 (control). These numbers include one GM-CSF triplet pregnancy resulting from two transferred embryos.

[b] Positive hCG but no ultrasound verification of an intrauterine gestational sac.

[c] Defined as death within the first week of life.

[d] Diagnosed within 1 week after birth. There were no additional malformations in the intention-to-treat population.

[e] After transfer of two embryos.

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both control and GM-CSF test media after embryo transfer in 620 women, just over halfway through the treatment cohort. This alteration increased the ongoing implantation rate at gestational week 7 for the control group from 17.9% to 22.4%, but did not affect performance of the GM-CSF medium (23.9% and 23.0% in low and high HSA medium, respectively; Table 1). A test of interaction revealed a *P* value of $<.01$ justifying separate consideration of the odds ratios for data from the two HSA concentrations (Table 1).

A negative effect of age was seen on ongoing implantation rate at gestational week 7, with an OR for age per year of 0.94 (95% CI 0.91–0.98; $P=.001$), whereas there was no detectable effect of BMI. The variation in ongoing implantation rate at gestational week 7 between the 14 centers was large, with ongoing implantation rates at gestational week 7 varying from 13.4%–35.2% at the 11 largest centers contributing with >30 patients.

There was no difference either in the percentage of normally developed day 3 embryos (42.0% [GM-CSF] vs. 43.9% [control]; $P=.55$) or in the percentage of top-quality embryos (15.6% [GM-CSF] vs. 16.8% [control]; $P=.56$; Table 1).

Follow-Up at Gestational Week 12 and Live Birth

For gestational week 12, the ongoing implantation rates was 23.0% (GM-CSF) and 18.7% (control; $P=.02$; OR 1.35, 95% CI 1.06–1.72; Table 1) in the per-protocol population. When comparing only the subset of women who received medium with the low HSA concentration (2 mg/mL) in test or control medium, the ongoing implantation rate at gestational week 12 for the GM-CSF group was 23.5% vs. 16.7% for the control group ($P=.007$), whereas in high HSA concentration (5 mg/mL) it was 22.4% (GM-CSF) vs. 21.1% (control) ($P=.53$). Similar results were seen for the intention-to-treat population (data not shown).

Overall, the GM-CSF group had a significantly higher ongoing clinical pregnancy rate at gestational week 12 compared with the control group (29.3% vs. 24.8%, respectively; $P=.04$; OR 1.32, 95% CI 1.01–1.74; Fig. 2). Again, this was largely attributable to a beneficial effect of adding GM-CSF to the control medium containing a low concentration of HSA. The number of multiple pregnancies was not significantly different between the treatment groups. The total rate of early pregnancy loss (≤gestational week 12) was significantly higher in the control group than in the GM-CSF group (33.5% vs. 22.9%, respectively; $P=.02$). Live birth rates were 28.9% (GM-CSF) and 24.1% (control; $P=.03$; OR 1.35, 95% CI 1.03–1.78; Table 1). There were no differences in birth weight, gestational age at birth, or appropriateness of birth weight for gestational age (as defined by Marsál et al. [27]) between the two groups (Table 1). All reported adverse events were standard cases and occurred at the expected frequency and without any difference between test and control groups.

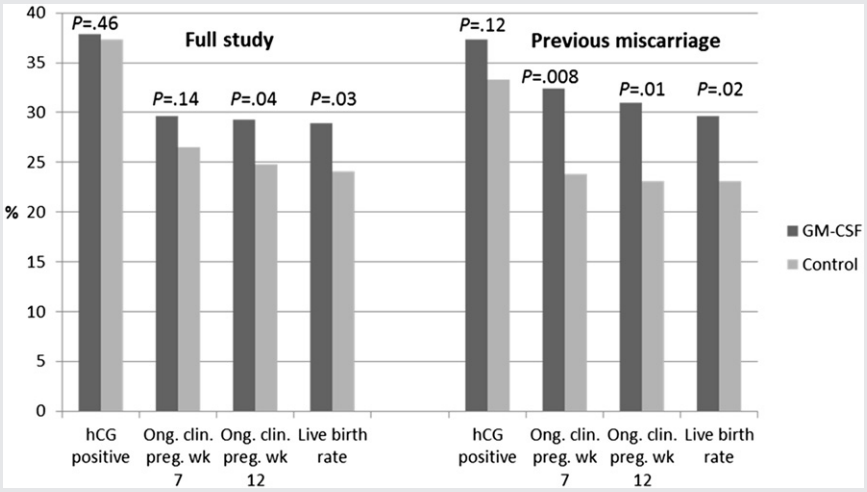
Subgroup of Previous Miscarriage Patients

Exploratory investigation revealed an interaction between treatment outcome and the incidence of previous miscarriage. In a subgroup of women who had previously experienced at least one miscarriage (spontaneous abortion; n = 289 patients with embryo transfer) the ongoing implantation rate at gestational week 7 for the patients receiving control treatment was not significantly different than that in the control group for women without prior miscarriage (17.0% vs. 21.2%; $P=.17$). For the women with previous miscarriage, addition of GM-CSF had a highly significant effect on ongoing implantation rate at gestational week 7 (24.5% [GM-CSF] vs. 17.0% [control], a relative difference of 44.8%; OR 2.33, 95% CI 1.39–3.92; $P=.001$). At gestational week 12, ongoing implantation rates were 23.2% (GM-CSF) and 16.5% (control; OR 2.17, 95% CI 1.29–3.65; $P=.003$; Table 2). This effect may be more pronounced in women with more than one previous miscarriages (Table 2). There was no evidence of a relationship between effect of GM-CSF and age. Data from the two HSA concentration periods is shown in Supplemental Table 2 (available online at www.fertstert.org). For the full study cohort, there were no differences in the secondary end points between the GM-CSF and the control groups. Within this subgroup, the number of pregnancies not progressing beyond biochemical detection was significantly lower in the GM-CSF group compared with the control group ($P=.03$), whereas there was no difference in ectopic pregnancies or early miscarriages (Table 2). The live birth rates were 29.6% (GM-CSF) and 23.1% (control; $P=.02$; OR 2.01, 95% CI 1.11–3.65; Table 2 and Fig. 2). There were no significant differences in birth weight or gestational age at birth (Table 2). The primary end point was also compared in other subgroups (according to age, BMI, indication for treatment, smoking, alcohol consumption, and previous IVF/ICSI cycles, pregnancies, and births), and no statistically significant effect of GM-CSF was seen (data not shown).

DISCUSSION

This large clinical study was undertaken to evaluate the potential benefit of GM-CSF addition to human IVF embryo culture medium. Although addition of GM-CSF did not elicit a 25% increase in the ongoing implantation rate at gestational week 7 in the full study cohort, a significant improvement in ongoing implantation rate was seen at gestational week 12 and in live birth rate. This finding is consistent with preclinical studies showing that GM-CSF exerts beneficial effects on developmental and implantation competence in mouse and human embryos (5, 21, 25). However, in the full study cohort, these differences were associated with an interaction between GM-CSF and the concentration of HSA in culture medium, such that the benefit of adding GM-CSF was only apparent in culture medium containing the lower concentration of 2 mg/mL HSA. An increase in HSA concentration to 5 mg/mL elevated ongoing implantation and live birth rates in the control group but did not alter the outcomes in the GM-CSF group. This concurs with a study reporting that the beneficial effect of GM-CSF on mouse embryo development is most evident under suboptimal conditions elicited by removal of serum albumin (28). A nonspecific "protein effect" by GM-CSF is unlikely, because a 1,000-fold excess of HSA is present in the media. It can reasonably be inferred from these observations that GM-CSF provides greater protection from culture-induced stress under suboptimal culture conditions.

FIGURE 2



Number of hCG-positive women, number of ongoing clinically pregnant women (weeks 7 and 12), and live birth rate in percentage of women with embryo transfer in the GM-CSF group (*darker bars*) and the control group (*lighter bars*) for the full study cohort (*left*) and the subgroup of women with previous miscarriage (*right*). Analyses were performed with the use of a logistic regression model with treatment and center as main factors, and covariates including age, body mass index, smoking, indication for treatment, in vitro fertilization (IVF) versus intracytoplasmic sperm injection (ICSI), previous number of IVF/ICSI treatments, previous pregnancies, previous miscarriages, number of oocytes, number of transferrable embryos in the current treatment cycle, and the HSA regime (*low/high*).

Ziebe. GM-CSF in human embryo culture medium. Fertil Steril 2013.

Interestingly, an exploratory subgroup analysis indicated that in women who had previously experienced at least one miscarriage, GM-CSF had a highly significant effect on ongoing implantation rate. It is not clear whether embryos of women with prior miscarriage exhibit poorer in vitro development, or why else they might exhibit elevated sensitivity to exogenous GM-CSF manifesting as improved implantation potential. Clearly, this exploratory analysis should be replicated in a well powered study, ideally where it is possible to distinguish unexplained miscarriage from those attributable to aneuploidy. It has been shown that patients with recurrent miscarriage exhibit a reduced level of GM-CSF in peripheral blood during pregnancy (29), suggesting that these patients may have impaired capacity to synthesize this cytokine, but whether a similar deficiency occurs in the reproductive tract awaits further investigation. We reason that for some individuals, failure of embryos to achieve full developmental competence during the early preimplantation phase is linked with progression to postimplantation embryo loss, and that supplementing culture medium with GM-CSF may increase developmental competence and thus resilience to later loss. This interpretation is consistent with studies in mouse embryos showing that GM-CSF reduces culture-induced expression of heat shock proteins linked with cellular stress and susceptibility to apoptosis (9). In patients with a previous miscarriage, there may be greater propensity for embryos to exhibit stress, and the presence of GM-CSF during oocyte fertilization, embryo culture, and transfer may be of relatively greater benefit for embryo acquisition of developmental competence and capacity to implant.

In the full study cohort there was also evidence of a lower incidence of early pregnancy loss in the GM-CSF group compared with the control group. This finding is in line with data from mouse experiments, where embryo culture in the presence of GM-CSF resulted in a substantial increase in progression of implanted embryos to term after transfer (25) and a GM-CSF–null mutation caused an elevated rate of fetal loss (4). Also, a bovine study showed that exposure to GM-CSF during IVF reduced later pregnancy loss (30).

An alternative explanation for diminished fetal loss after transfer of embryos cultured with GM-CSF involves potential effects of GM-CSF on the endometrium of women during the implantation period. It is notable that in the present study embryos were transferred to the uterus in the randomized culture medium (GM-CSF or control). GM-CSF is identified as a key regulator of the immune response in the uterine endometrium, targeting local dendritic cells to increase their recruitment, activation status, and capacity to present antigen to T cells (31). Because maternal dendritic cells are required to induce expansion of the uterine regulatory T-cell pool required to mediate immune tolerance of the embryo at implantation (32), exogenous GM-CSF could feasibly therefore contribute to achieving good quality implantation and robust placental development. Another member of the CSF family of cytokines, G-CSF (also referred to as CSF3), is indicated in a randomized trial of 68 patients to have possible efficacy in treatment of unexplained recurrent miscarriage (33). Like GM-CSF, G-CSF can act to regulate dendritic cells and promote immune tolerance (34). However it is important to note that GM-CSF and G-CSF have different receptors and induce different effects in target cells (35), so they may well act via distinct pathways to influence pregnancy outcome.

Regarding embryo quality, as judged against predefined classification, we found no direct association between

TABLE 2

Outcomes according to treatment group—previous miscarriage patients.

	GM-CSF	Control	Difference (relative, %)	*P* value (OR [95% CI])
Intention to treat				
No. of randomized cycles	161	169		
Per protocol				
No. of transfer cycles	142	147		
No. of transferred embryos	220	230		
Mean no. of embryos transferred	1.55	1.56		
Normally developed embryos, day 3 [n (% of fertilized)]	317 (39.2)	346 (40.9)		.71 (1.03 [0.88–1.20])
Top-quality embryos [n (% of fertilized)]	97 (12.0)	117 (13.8)		.73 (0.96 [0.72–1.25])
Ongoing implantation rate, wk 7 [$n_{embryos}$ (% of transferred embryos)]	54 (24.5)	39 (17.0)	44.8	.001 (2.33 [1.39–3.92])
1 previous miscarriage	38 (22.2)	32 (17.7)		.29 (1.33 [0.79–2.25])
>1 previous miscarriage	15 (34.1)	7 (14.3)		.03 (3.10 [1.13–8.56])
Ongoing implantation rate, wk 12 [$n_{embryos}$ (% of transferred embryos)]	51 (23.2)	38 (16.5)	40.3	.003 (2.17 [1.29–3.65])
1 previous miscarriage	36 (21.1)	31 (17.1)		.35 (1.29 [0.76–2.20])
>1 previous miscarriage	14 (31.8)	7 (14.3)		.04 (2.80 [1.01–7.77])
Live birth rate [n_{births} (% of transfer cycles)]	42 (29.6)	34 (23.1)	27.9	.02 (2.01 [1.11–3.65])
Follow-up on pregnancies (per protocol)				
Positive hCG [n (% of transfer cycles)]	53 (37.3)	49 (33.3)		.12
Early pregnancy loss (≤wk 12) [n (% of women with positive hCG)]	9 (17.0)	15 (30.6)		.11
Biochemical pregnancy[a]	2 (3.8)	10 (20.4)		.03
Ectopic pregnancy	1 (1.9)	0 (0)		1.00
Miscarriage (loss wk 7–12)	6 (11.3)	5 (10.2)		1.00
Late pregnancy loss (>wk 12) [n (% of women with positive hCG)]				
Elective termination due to malformations	2 (3.8)	0 (0)		.50
Follow-up on births				
No. of deliveries	42	34		
No. of live-born children	49	40		
Singletons				
No. of children born	35	28		
Gestational age at birth (wk)	38.4 ± 4.7	39.5 ± 2.3		.64
Birth weight (g)	3,275 ± 936	3,297 ± 587		.54
Median birth weight (g)	3,420	3,402		
Twins				
No. of children born	14	12		
Gestational age at birth (wk)	35.6 ± 2.6	35.8 ± 1.7		.94
Birth weight (g)	2,479 ± 575	2,492 ± 618		.74
Median birth weight (g)	2,512	2,465		

Note: Plus-minus values are mean ± SD. Analyses of ongoing implantation and live birth rates were performed with the use of a logistic regression model with treatment and center as main factors, and covariates including age, BMI, smoking, indication for treatment, IVF vs. ICSI, previous number of IVF/ICSI treatments, previous pregnancies, number of oocytes, transferable embryos in current treatment cycle, and HSA regime (low/high). Normally developed embryos and top-quality embryos were analyzed with the use of a multiplicative Poisson model (log-linear) with the same set of covariates. ORs were also adjusted for these covariates. Analyses of pregnancy loss were performed with the use of a logistic regression model. Fisher exact test was applied for analyses of perinatal parameters. Abbreviations as in Table 1.

[a] Positive hCG but no ultrasound verification of an intrauterine gestational sac.

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morphologic parameters in precompacted embryos and ongoing implantation/clinical pregnancy rate, despite common assumptions of such an association (36). This is perhaps unsurprising, given that embryos were evaluated at the precompaction stage, which is arguably less selective for embryo quality than longer-term culture to blastocyst stage (37). The data on embryo morphology do not conflict with earlier observations, where beneficial effects of GM-CSF on embryo viability, incidence of apoptosis, expression of stress response genes, and glucose uptake were all demonstrated at the later blastocyst stage of development (5, 9).

The variation in ongoing implantation rate at gestational week 7 among the 14 centers was large. However, a funnel plot showed that between-center variation for all but one center could be explained by differences in the distribution of demographic factors combined with simple sampling variation. Although this might be considered to be a limitation of the current study, we and others acknowledge the critical importance of establishing safety and efficacy of new IVF innovations in the "real world" clinical scenario, which necessarily must account for considerable variation in the practices and success rates of different clinics (38).

An important outcome of the present study is the demonstration of no overt adverse effects of GM-CSF inclusion in culture medium on stillbirth, perinatal death, or abnormalities, which provides a measure of confidence in the safety of this using this cytokine in human IVF clinical settings. Additionally, there was no detectable change in birth weight of singleton or twin pregnancies compared with control media. This contrasts with mouse studies where offspring born after embryo culture with GM-CSF achieved a higher birth weight than embryos cultured without cytokine and similar to embryos conceived in vivo (25). However, on the basis of existing information on effects of IVF on perinatal outcomes (39, 40), it is noteworthy that information from much larger populations of several thousand neonates would be required to determine conclusively whether exposure to GM-CSF in the preimplantation period has any such effect.

In conclusion, we have demonstrated a modest effect of GM-CSF on ongoing implantation rate and live birth rates in unselected women undergoing IVF treatment compared with a control medium. This positive effect was primarily evident in a culture medium containing 2 mg/mL HSA and diminished when the concentration of HSA was increased to 5 mg/mL. Exploratory analyses showed that GM-CSF increased ongoing implantation, clinical pregnancy, and live birth rates in a subgroup of women who had experienced previous miscarriage. However, this interpretation must be confirmed in a well powered and properly randomized study on this specific group of patients. Further studies will be needed to more fully define the mechanism of action through which GM-CSF may improve implantation and pregnancy rate in this particular subgroup. Finally, we acknowledge the importance of ongoing evaluation and reporting of the effects of GM-CSF in additional large cohort studies to more firmly quantify its clinical efficacy as well as to more fully determine whether any effects on perinatal outcome become evident.

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SUPPLEMENTAL TABLE 1

Baseline characteristics (intention to treat).

	GM-CSF	Control
n	654	678
Age (y)	32.2 ± 3.7	32.4 ± 3.8
BMI (kg/m^2)	24.2 ± 4.2	24.3 ± 4.1
Cause of infertility[a] [n (%)]		
Male factor	319 (49)	300 (44)
Unexplained	190 (29)	207 (31)
Tubal disease	111 (17)	127 (19)
PCO	24 (4)	23 (3)
Endometriosis	54 (8)	59 (9)
Uterine factor	8 (1)	4 (<1)
Other	24 (4)	36 (5)
Previous miscarriage [n (%)]	161 (25)	169 (25)
Previous IVF/ICSI [n (%)]	367 (56)	373 (55)
Smoking [n (%)]	89 (14)	82 (12)
Fertilization procedure [n (%)]		
IVF	323 (49)	342 (50)
ICSI	278 (43)	259 (39)
Both	49 (7)	67 (10)
Unknown	4 (<1)	10 (1)
No. of oocytes retrieved	9.6 ± 4.6	9.6 ± 4.9
No. of fertilized oocytes (2 pronuclei)	5.4 ± 3.5	5.6 ± 3.5

Note: Plus-minus values are mean ± SD. None of the covariates presented were significantly imbalanced following randomization. BMI = body mass index; GM-CSF = granulocyte-macrophage colony-stimulating factor; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; PCO = polycystic ovaries.

[a] More than one cause of infertility may have been selected for each individual/couple.

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SUPPLEMENTAL TABLE 2

Outcomes according to treatment group and human serum albumin (HSA) concentration—previous miscarriage patients.

	GM-CSF			Control				*P* (OR [95% CI])		
	Overall	Low HSA	High HSA	Overall	Low HSA	High HSA	Overall rel. diff. (%)	Overall	Low HSA	High HSA
Intention to treat										
No. of randomized cycles	161	86	75	169	98	71				
Per protocol										
No. of transfer cycles	142	77	65	147	82	65				
No. of transferred embryos	220	121	99	230	132	98				
Mean no. of embryos transferred	1.55	1.57	1.52	1.56	1.61	1.51				
Normally developed embryos, day 3 [n (% of fertilized)]	317 (39.2)	162 (34.9)	155 (44.9)	346 (40.9)	173 (39.5)	173 (42.4)		.71 (1.03 [0.88–1.20])		
Top-quality embryos [n (% of fertilized)]	97 (12.0)	52 (11.2)	45 (13.0)	117 (13.8)	52 (11.9)	65 (15.9)		.73 (0.96 [0.72–1.25])		
Ongoing implantation rate, wk 7 [$n_{embryos}$ (% of transferred embryos)]	54 (24.5)	33 (27.3)	21 (21.2)	39 (17.0)	23 (17.4)	16 (16.3)	44.8	.001 (2.33 [1.39–3.92])	.005 (2.58 [1.33–4.99])	.09 (2.00 [0.90–4.46])
Ongoing implantation rate, wk 12 [$n_{embryos}$ (% of transferred embryos)]	51 (23.2)	31 (25.6)	20 (20.2)	38 (16.5)	22 (16.7)	16 (16.3)	40.3	.003 (2.17 [1.29–3.65])	.009 (2.42 [1.24–4.70])	.13 (1.85 [0.83–4.12])
Live birth rate [n_{births} (% of transfer cycles)]	42 (29.6)	25 (32.5)	17 (26.2)	34 (23.1)	19 (23.2)	15 (23.1)	27.9	.02 (2.01 [1.11–3.65])	.04 (2.27 [1.05–4.93])	.26 (1.69 [0.68–4.19])

Note: Analyses of ongoing implantation rate and live birth were performed with the use of a logistic regression model with treatment and center as main factors, and covariates including age, BMI, smoking, indication for treatment, IVF vs. ICSI, previous number of IVF/ICSI treatments, previous pregnancies, number of oocytes, transferable embryos in current treatment cycle, and HSA regime (low/high, where applicable). Normally developed embryos and top-quality embryos were analyzed with the use of a multiplicative Poisson model (log-linear) with the same set of covariates. ORs were also adjusted for these covariates. Abbreviations as in Supplemental Table 1.

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