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[Intervention Review]

GM-CSF (granulocyte macrophage colony-stimulating factor) supplementation in culture media for women undergoing assisted reproduction

Sarah Armstrong¹, Jeanette MacKenzie², Bryan Woodward³, Allan Pacey¹, Cindy Farquhar⁴

¹Department of Oncology & Metabolism, Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Sheffield, UK. ²Fertility Plus, Women's Health, Auckland District Health Board, Auckland, New Zealand. ³IVF Consultancy Services, Leicester, UK. ⁴Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand

Contact: Sarah Armstrong, scarmstrong1@sheffield.ac.uk.**Editorial group:** Cochrane Gynaecology and Fertility Group.**Publication status and date:** New, published in Issue 7, 2020.**Citation:** Armstrong S, MacKenzie J, Woodward B, Pacey A, Farquhar C. GM-CSF (granulocyte macrophage colony-stimulating factor) supplementation in culture media for women undergoing assisted reproduction. *Cochrane Database of Systematic Reviews* 2020, Issue 7. Art. No.: CD013497. DOI: [10.1002/14651858.CD013497.pub2](https://doi.org/10.1002/14651858.CD013497.pub2).

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ABSTRACT

Background

GM-CSF (granulocyte macrophage colony-stimulating factor) is a growth factor that is used to supplement culture media in an effort to improve clinical outcomes for those undergoing assisted reproduction. It is worth noting that the use of GM-CSF-supplemented culture media often adds a further cost to the price of an in vitro fertilisation (IVF) cycle. The purpose of this review was to assess the available evidence from randomised controlled trials (RCTs) on the effectiveness and safety of GM-CSF-supplemented culture media.

Objectives

To assess the effectiveness and safety of GM-CSF-supplemented human embryo culture media versus culture media not supplemented with GM-CSF, in women or couples undergoing assisted reproduction.

Search methods

We used standard methodology recommended by Cochrane. We searched the Cochrane Gynaecology and Fertility Group Trials Register, CENTRAL, MEDLINE, Embase, CINAHL, LILACS, DARE, OpenGrey, PubMed, Google Scholar, and two trials registers on 15 October 2019, checked references of relevant papers and communicated with experts in the field.

Selection criteria

We included RCTs comparing GM-CSF (including G-CSF (granulocyte colony-stimulating factor))-supplemented embryo culture media versus any other non-GM-CSF-supplemented embryo culture media (control) in women undergoing assisted reproduction.

Data collection and analysis

We used standard methodological procedures recommended by Cochrane. The primary review outcomes were live birth and miscarriage rate. The secondary outcomes were clinical pregnancy, multiple gestation, preterm birth, birth defects, aneuploidy, and stillbirth rates. We assessed the quality of the evidence using GRADE methodology. We undertook one comparison, GM-CSF-supplemented culture media versus culture media not supplemented with GM-CSF, for those undergoing assisted reproduction.

Main results

We included five studies, the data for three of which (1532 participants) were meta-analysed. We are uncertain whether GM-CSF-supplemented culture media makes any difference to the live-birth rate when compared to using conventional culture media not supplemented with GM-CSF (odds ratio (OR) 1.19, 95% confidence interval (CI) 0.93 to 1.52, 2 RCTs, N = 1432, $I^2 = 69\%$, low-quality evidence). The evidence suggests that if the rate of live birth associated with conventional culture media not supplemented with GM-CSF was 22%, the rate with the use of GM-CSF-supplemented culture media would be between 21% and 30%.

We are uncertain whether GM-CSF-supplemented culture media makes any difference to the miscarriage rate when compared to using conventional culture media not supplemented with GM-CSF (OR 0.75, 95% CI 0.41 to 1.36, 2 RCTs, N = 1432, $I^2 = 0\%$, low-quality evidence). This evidence suggests that if the miscarriage rate associated with conventional culture media not supplemented with GM-CSF was 4%, the rate with the use of GM-CSF-supplemented culture media would be between 2% and 5%.

Furthermore, we are uncertain whether GM-CSF-supplemented culture media makes any difference to the following outcomes: clinical pregnancy (OR 1.16, 95% CI 0.93 to 1.45, 3 RCTs, N = 1532 women, $I^2 = 67\%$, low-quality evidence); multiple gestation (OR 1.24, 95% CI 0.73 to 2.10, 2 RCTs, N = 1432, $I^2 = 35\%$, very low-quality evidence); preterm birth (OR 1.20, 95% CI 0.70 to 2.04, 2 RCTs, N = 1432, $I^2 = 76\%$, very low-quality evidence); birth defects (OR 1.33, 95% CI 0.59 to 3.01, $I^2 = 0\%$, 2 RCTs, N = 1432, low-quality evidence); and aneuploidy (OR 0.34, 95% CI 0.03 to 3.26, $I^2 = 0\%$, 2 RCTs, N = 1432, low-quality evidence). We were unable to undertake analysis of stillbirth, as there were no events in either arm of the two studies that assessed this outcome.

Authors' conclusions

Due to the very low to low quality of the evidence, we cannot be certain whether GM-CSF is any more or less effective than culture media not supplemented with GM-CSF for clinical outcomes that reflect effectiveness and safety. It is important that independent information on the available evidence is made accessible to those considering using GM-CSF-supplemented culture media. The claims from marketing information that GM-CSF has a positive effect on pregnancy rates are not supported by the available evidence presented here; further well-designed, properly powered RCTs are needed to lend certainty to the evidence.

PLAIN LANGUAGE SUMMARY

Growth factor-supplemented culture media for women undergoing assisted reproduction

Review question

Does culture media containing the growth factor GM-CSF (granulocyte macrophage colony-stimulating factor) improve the chances of a pregnancy and live-born baby, and reduce the risk of miscarriage, twin or triplet pregnancy, premature birth, birth defects, genetic problems in the baby, and stillbirth?

Background

Assisted reproduction includes processes whereby a woman's eggs and a man's sperm are combined to achieve fertilisation outside of the body. Embryos are placed in a solution called culture medium to support the growing embryo until it can be replaced into the woman's uterus. Culture medium supplemented with GM-CSF is widely available in clinics and is often offered as an 'add-on' to an in vitro fertilisation (IVF) cycle in an effort to improve the success rates of treatment. Using GM-CSF-supplemented culture medium can make IVF more expensive.

Study characteristics

The evidence is current to October 2019. We obtained data from three randomised controlled trials (a type of study in which participants are randomly assigned to one of two or more treatment groups) of 1532 infertile women undergoing IVF or intracytoplasmic sperm injection (ICSI), a specialised form of IVF whereby the sperm is injected into the egg. We compared GM-CSF-supplemented culture media versus culture media not supplemented with GM-CSF for those undergoing assisted reproduction.

What the review found

Low-quality evidence reveals that we are uncertain whether GM-CSF-containing culture media makes any difference to the live-birth rate when compared to using culture media not containing GM-CSF. This suggests that if the rate of live birth associated with culture media not containing GM-CSF is 22%, the rate with the use of GM-CSF-containing culture media would be between 21% and 30%. Low-quality evidence also reveals that we are uncertain whether GM-CSF-containing culture media makes any difference to miscarriage when compared to using culture media not containing GM-CSF. This suggests that if the miscarriage rate associated with culture media not containing GM-CSF is 4%, the rate with the use of GM-CSF-containing culture media would be between 2% and 5%. Low-quality evidence for pregnancy, birth defects, and genetic problems with the baby, and very low-quality evidence for twin or triplet pregnancies, and premature birth, reveals that we are uncertain whether GM-CSF-containing culture media makes any difference to these outcomes when compared to culture media not containing GM-CSF. Two studies looked at stillbirth, but as no stillbirths occurred in either study, we were unable to analyse this outcome.

Overall conclusions

Due to the very low to low quality of the evidence, we cannot be certain whether GM-CSF is any more or less effective or harmful than culture media not supplemented with GM-CSF. It is important that independent information on the available evidence is made accessible to those considering using GM-CSF-supplemented culture media. In the meantime, more large studies are needed to increase the certainty of our conclusions.

SUMMARY OF FINDINGS

Summary of findings 1. GM-CSF-supplemented culture media compared to culture media not supplemented with GM-CSF for women undergoing assisted reproduction

GM-CSF-supplemented culture media compared to culture media not supplemented with GM-CSF for women undergoing assisted reproduction

Patient or population: women undergoing assisted reproduction

Setting: fertility clinics

Intervention: GM-CSF-supplemented culture media

Comparison: culture medium not supplemented with GM-CSF

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Nº of participants (studies)	Quality of the evidence (GRADE)
	Risk with culture media not supplemented with GM-CSF	Risk with GM-CSF-supplemented culture media			
Live birth or ongoing pregnancy	Study population		OR 1.19 (0.93 to 1.52)	1432 (2 RCTs)	⊕⊕⊕⊕ LOW ^{1 2}
	223 per 1000	254 per 1000 (210 to 303)			
Miscarriage	Study population		OR 0.75 (0.41 to 1.36)	1432 (2 RCTs)	⊕⊕⊕⊕ LOW ³
	36 per 1000	27 per 1000 (15 to 48)			
Clinical pregnancy	Study population		OR 1.16 (0.93 to 1.45)	1532 (3 RCTs)	⊕⊕⊕⊕ LOW ^{1 4}
	263 per 1000	293 per 1000 (250 to 342)			
Multiple gestation	Study population		OR 1.24 (0.73 to 2.10)	1432 (2 RCTs)	⊕⊕⊕⊕ VERY LOW ^{1 3}
	36 per 1000	44 per 1000 (26 to 72)			
Preterm birth	Study population		OR 1.20 (0.70 to 2.04)	1432 (2 RCTs)	⊕⊕⊕⊕ VERY LOW ^{1 3}
	36 per 1000	43 per 1000 (25 to 70)			
Birth defects	Study population		OR 1.33 (0.59 to 3.01)	1432 (2 RCTs)	⊕⊕⊕⊕ LOW ³

	14 per 1000	18 per 1000 (8 to 40)			
Aneuploidy	Study population		OR 0.34 (0.03 to 3.26)	1432 (2 RCTs)	⊕⊕⊕⊕ LOW ⁵
		3 per 1000	1 per 1000 (0 to 9)		
Stillbirth	Study population		-	1432 (2 RCTs)	-
	See comment ⁶	See comment ⁶			

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **OR:** odds ratio; **RCT:** randomised controlled trial

GRADE Working Group grades of evidence

High quality: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate quality: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low quality: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low quality: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

¹Downgraded once for inconsistency, as the included studies report differing directions of point estimates: one supports the intervention, and one does not support the intervention.

²Downgraded once for imprecision as broad confidence intervals and a low number of included studies, at least one of which is very small.

³Downgraded twice for imprecision as very broad confidence intervals and a low number of included studies.

⁴Downgraded once for risk of bias. One included study had an unclear risk of selection bias, performance bias, and detection bias due to limited information available from published abstract.

⁵Downgraded twice for imprecision as included studies had so few reported incidences of aneuploidy that the point estimate is not precise and has very broad confidence intervals.

⁶No stillbirths occurred in either arm of the included studies, therefore the result is inestimable.

BACKGROUND

Description of the condition

Assisted reproduction provides the opportunity to have a family for those unable to become pregnant spontaneously for a variety of reasons, including; infertility; those in single-sex relationships; single women; and those using surrogates. Assisted reproduction is often referred to as a 'cycle', reflecting its stepwise process. It involves a series of procedures from ovarian stimulation and oocyte collection, to mixing the gametes, culturing and assessing the quality of ensuing embryos, and replacing embryos into the uterus of the woman. The success of assisted reproduction is a culmination of all the elements of the cycle, and is in part due to the ability to culture human embryos in vitro using culture media capable of supporting the developing embryo. GM-CSF (granulocyte macrophage colony-stimulating factor)-supplemented culture media was developed in an effort to improve this particular part of the cycle, leading to better outcomes for those undergoing in vitro fertilisation (IVF).

GM-CSF-supplemented culture media can be described as an assisted reproduction 'add-on'. Add-ons are optional extras to an assisted reproduction cycle, which are sometimes novel interventions or therapies that have shown some promise in initial studies, or have been around for many years, but have not yet been proven to be effective through randomised controlled trials (RCTs). GM-CSF-supplemented culture media is one such add-on, often provided at an additional cost to the IVF cycle (Heneghan 2016).

For the purposes of this review, any culture media containing GM-CSF may be compared in a randomised controlled trial (RCT) against any culture media not containing GM-CSF. We addressed the efficacy and safety of GM-CSF-supplemented culture media when compared to culture media not containing GM-CSF. The primary outcomes were live birth and miscarriage.

Description of the intervention

GM-CSF (also known as colony-stimulating factor (CSF)-2) and granulocyte colony-stimulating factor (G-CSF or CSF-3) belong to the CSF family. They are a group of cytokines known for their role in haemopoietic cell proliferation, differentiation, and activation, as well as being an apoptosis suppressor (Rahmati 2015). Their involvement in reproduction was initially investigated in the 1970s in human placenta-conditioned media (Burgess 1977). Amongst the CSF group, GM-CSF is the most widely studied, and its extensive research on assisted reproduction has led to the development of new embryo culture media supplemented with human recombinant GM-CSF. EmbryoGen and BlastGen are examples of commercially available sequential culture media containing GM-CSF.

GM-CSF is a cytokine that is produced by the oestrogen-primed epithelial cells in the female reproductive tract (Robertson 1992). It is maximally expressed at the luminal and glandular epithelial cells of the endometrium in the secretory phase, and in the lining of the fallopian tube during the late proliferative and early mid-secretory phases of the menstrual cycle (Giacomini 1995; Zhao 1994). Later during implantation, GM-CSF is produced by the chorionic villi cells and the maternal decidua (Jokhi 1994). In response to local inflammatory stimuli, GM-CSF acts by stimulating and activating mature monocytes, granulocytes, macrophages,

and dendritic cells which promote chemotactic, phagocytic, and cytotoxic actions as well as antigen-presenting properties needed in the immunomodulation of early pregnancy and embryogenesis (Baldwin 1992; Robertson 2007).

How the intervention might work

The control of the immunological environment during early pregnancy involves a series of autocrine and paracrine signalling between the maternal fetal interface (Robertson 1994; Robertson 2007; Wegmann 1992). Several studies have suggested an association between recurrent pregnancy loss and infertility and the dysregulation of growth factors and cytokines (Hambartsoumian 1998; Torry 2007; Vuorela 2000). In studies of genetically GM-CSF-deficient mice, there was a reduced inner cell mass observed which resulted in delayed blastocyst formation, increased fetal resorption in late gestation, decreased fetal size, and greater postnatal mortality (Robertson 1999). Other murine studies have also supported that GM-CSF is crucial in optimal fetal growth and survival, as animal models lacking GM-CSF expression experience more pregnancy losses and impaired long-term survival of the newborn animals (Savion 2002; Seymour 1997).

The initial studies of growth factor supplementation of culture media are limited mostly to animal models, but have largely revealed improved blastocyst development rates, Lighten 1998; Sjöblom 1992; Sjöblom 1999; Spanos 2000; Yu 2012, and increased implantation and birth rates (Block 2003; Lim 2006; Roudebush 2004; Sjöblom 2005). The use of growth factor supplementation in human culture media has been limited, as it is costly to produce, and there are concerns about adverse effects (Richter 2008). Most growth factors are anti-apoptotic, that is they inhibit programmed cell death. If not controlled, adverse effects may occur, as apoptosis is a crucial phenomenon in embryogenesis. Inhibition of apoptosis may lead to abnormal embryo development such as the well-documented 'large offspring syndrome' that occurs in mice models (Lazzari 2002; Young 2001).

Early studies on human embryos have revealed that those cultured in GM-CSF-supplemented culture media had more viable inner cell masses and reduced apoptosis. This could potentially contribute to improved fetal viability (Sjöblom 1999; Sjöblom 2002). Supplementation of culture media with GM-CSF is reported to be safe for human embryos; there are no increases or changes in ploidy rates or embryonic chromosomes (Agerholm 2010). Furthermore, initial RCTs in women revealed an improvement in the clinical pregnancy and live-birth rates of those women randomised to culture of their embryos in GM-CSF-supplemented culture media (Mignini 2013; Sfountouris 2013; Tevkin 2014; Ziebe 2013). There were no major and minor birth abnormalities (Mignini 2013; Sfountouris 2013; Tevkin 2014).

Why it is important to do this review

GM-CSF-supplemented culture media is widely commercially available and is offered to women undergoing assisted reproduction worldwide. It is often considered an 'add-on', or supplementary therapy, given alongside standard IVF in an effort to improve success rates (Heneghan 2016). There is currently no up-to-date systematic review of RCTs on this topic, and the one published systematic review relied on non-randomised studies and studies where oocytes rather than women were randomised (Siristatidis 2013). The available RCTs were small with differing

results and did not provide certainty as to what should be done in practice. Use of GM-CSF can carry an additional cost to women undergoing IVF. It was therefore important to distil the available RCT evidence in a meaningful way to provide information on the effectiveness and safety of this intervention for women, clinicians, and embryologists, and regulatory and advisory bodies such as the Human Fertilisation and Embryology Authority (HFEA).

OBJECTIVES

To assess the effectiveness and safety of GM-CSF-supplemented human embryo culture media versus culture media not supplemented with GM-CSF, in women or couples undergoing assisted reproduction.

METHODS

Criteria for considering studies for this review

Types of studies

We included all published and unpublished RCTs. We included cross-over studies for completeness, but only pooled data from the first phase in meta-analyses because this study design is not valid in the context of infertility trials (Vail 2003). We excluded quasi- and pseudo-randomised trials. There was no limitation on language, publication date, or publication status.

Types of participants

Women undergoing IVF or intracytoplasmic sperm injection (ICSI) for any cause of infertility, using autologous or donor oocytes. Women undergoing IVF or ICSI with a background of recurrent miscarriage or recurrent implantation failure were also included.

Types of interventions

We included all studies that compared GM-CSF (including G-CSF)-supplemented embryo culture media versus any other non-GM-CSF-supplemented embryo culture media (control).

Types of outcome measures

Primary outcomes

1. Live birth per woman randomised, defined as a live baby born after 20 weeks' gestation. We used ongoing pregnancy, defined as clinical pregnancy of 12 or more weeks' gestation, as a surrogate for live birth in cases where studies did not report live birth.
2. Miscarriage per woman randomised. The definition used was miscarriage of clinical pregnancy.

Secondary outcomes

1. Clinical pregnancy per woman randomised, defined as presence on ultrasound scan of one or more gestational sacs, or definitive signs of clinical pregnancy. This included ectopic pregnancy. Note that multiple gestational sacs were counted as one clinical pregnancy.
2. Multiple gestation per woman randomised.
3. Preterm birth per woman randomised (defined as birth before 37 weeks' gestation).
4. Birth defects (defined as any structural anomaly present at birth that may interfere with function depending upon the organ or structure involved).

5. Aneuploidy (defined as any genetic disorder diagnosed during pregnancy or at the time of birth).
6. Stillbirth (defined as a baby born with no signs of life after 20 completed weeks of pregnancy).

Search methods for identification of studies

We searched for relevant studies with no language or date restriction in consultation with the Cochrane Gynaecology and Fertility Group Information Specialist.

Electronic searches

We designed search strategies for the following databases:

1. Cochrane Gynaecology and Fertility Group Specialised Register of Controlled Trials; ProCite platform, searched 15 October 2019 (Appendix 1);
2. Cochrane Central Register of Controlled Trials (CENTRAL); Ovid platform, searched 15 October 2019 (Issue 9; 2019) (Appendix 2);
3. MEDLINE; Ovid platform, searched from 1946 to 15 October 2019 (Appendix 3);
4. Embase; Ovid platform, searched from 1980 to 15 October 2019 (Appendix 4);
5. CINAHL (Cumulative Index to Nursing and Allied Health Literature); Ebsco platform, searched from 1961 to 15 October 2019 (Appendix 5);
6. LILACS (Latin American and Caribbean Health Science Information database) (lilacs.bvsalud.org/en/), Web platform, searched 15 October 2019 (Appendix 6).

The MEDLINE search was combined with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials in Section 4.3.1 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2019). The Embase and CINAHL search strategies are combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN; www.sign.ac.uk/methodology/filters.html#random).

Other electronic sources of trials (Web platforms, searched 15 October 2019) included:

1. trial registers for ongoing and registered trials: US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov) and the World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (apps.who.int/trialsearch/Default.aspx) (Appendix 7);
2. DARE (Database of Abstracts of Reviews of Effects) on the Cochrane Library (onlinelibrary.wiley.com/o/cochrane/cochrane_cldare_articles_fs.htm) (Appendix 8);
3. Web of Knowledge (wokinfo.com) (Appendix 9);
4. OpenGrey (www.opengrey.eu/) for unpublished literature from Europe (Appendix 10);
5. PubMed and Google Scholar (for recent trials not yet indexed in the major databases) (Appendix 11 and Appendix 12).

Searching other resources

We handsearched reference lists of included and excluded studies retrieved by the search, and communicated with experts in the field to inquire after any additional studies.

We did not perform a separate search for adverse effects of GM-CSF-supplemented culture media. We considered adverse effects described in the studies only.

Data collection and analysis

Selection of studies

All review authors independently undertook assessment of eligibility of all studies identified by the search using Covidence (Covidence). We retrieved the full-text publications of potentially eligible studies. Three review authors (SA, JM, and AP) screened the full texts to identify studies for inclusion, and recorded reasons for exclusion of the excluded studies in the 'Characteristics of excluded studies' table. Any disagreements were resolved by discussion or consultation with another review author.

Data extraction and management

Two review authors (SA and JM) independently extracted data on study characteristics and primary and secondary outcomes from the included studies using a data extraction form designed and piloted by the review authors. We included the following characteristics of included studies in the data extraction form:

1. methods;
2. participants;
3. interventions;
4. outcomes, including adverse events;
5. funding source for studies.

Any disagreements or discrepancies were resolved by discussion. Where there were multiple publications for a study, we used the main trial report as the reference and obtained additional details from secondary papers, which appear as subreferences. We corresponded with study investigators for further information on study methods and results as required. This correspondence is documented in the 'Characteristics of included studies' table and in [Appendix 13](#).

Assessment of risk of bias in included studies

Two review authors (SA and JM) independently assessed the included studies for methodological quality and undertook data extraction according to the Cochrane 'Risk of bias' assessment tool (Higgins 2011). We assessed selection bias (random sequence generation and allocation concealment), attrition bias (incomplete outcome data), reporting bias (selective reporting), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessors), and other biases (other problems that could put a trial at high risk of bias). Our judgements are presented and described in the 'Risk of bias' table in [Characteristics of included studies](#). Any disagreements were resolved by discussion.

Measures of treatment effect

We summarised the effects and adverse events related to the intervention as odds ratios (ORs) using a fixed-effect model. We presented 95% confidence intervals (CIs) for all outcomes to evaluate the precision of the estimate. We considered the clinical relevance of the results from the meta-analysis of each comparison, taking into account the precision of the estimate. When adding data from individual studies to comparisons, we considered whether the rates of events in both the intervention and control arm reflect

current practice. For example, we explored major discrepancies in direction and magnitude of effect in the [Results](#) section, and these are reflected in our 'Risk of bias' assessment.

Unit of analysis issues

The denominator for all outcomes was the number of women randomised. We did not use per-cycle data.

We counted multiple births (e.g. twins or triplets) as one live-birth event.

Dealing with missing data

We analysed the data on an intention-to-treat basis and attempted to obtain missing data from the primary investigators ([Appendix 13](#)). We assumed that participants who dropped out after randomisation (e.g. because of cycle cancellation), or who were lost to follow-up or withdrew, did not achieve clinical pregnancy or live birth. We made no other assumptions.

Assessment of heterogeneity

We considered whether the clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. We assessed statistical heterogeneity using the I^2 statistic, considering an I^2 statistic greater than 50% to indicate substantial heterogeneity (Higgins 2019). Where there was significant heterogeneity, we undertook planned subgroup analyses to explore this in more detail.

Assessment of reporting biases

We reduced the potential impact of publication and reporting bias by performing a comprehensive search for eligible studies and looking for duplication of data. We decided to construct a funnel plot to explore the possibility of small-study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies) if there were 10 or more studies included in an analysis. When possible, we used published protocols and prospective trial registration web pages for included studies to investigate selective reporting (i.e. comparisons of outcomes listed in the study protocol versus outcomes reported in papers).

Data synthesis

We performed meta-analyses where data were available from multiple studies investigating the same treatment, and the outcome was measured in a standard way between the studies. We used a fixed-effect model. We undertook meta-analysis according to the methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* for the following comparison (Higgins 2019).

1. Studies that include GM-CSF supplementation in human embryo culture media versus any other non-GM-CSF-supplemented human embryo culture media.

Subgroup analysis and investigation of heterogeneity

We conducted subgroup analyses for all outcomes when data were available to determine the separate effect between the following subgroups.

1. Studies including only women with recurrent implantation failure, defined as the failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles (Coughlan 2014), versus studies not including women with recurrent miscarriage.
2. Studies using single-step culture media versus studies using sequential culture media.
3. Studies including only women with donor oocytes versus studies using autologous oocytes.
4. Studies including only women with recurrent miscarriage (loss of three or more consecutive pregnancies before 20 weeks' gestation) versus studies not including women with recurrent miscarriage.
5. Studies replacing embryos at cleavage stage (day 2 or 3) versus studies replacing embryos at blastocyst stage (day 5).

Sensitivity analysis

We conducted sensitivity analyses for the primary outcomes to determine whether the conclusions were robust to arbitrary decisions made regarding eligibility and analysis. These analyses included consideration of whether the review conclusions would have differed if:

1. eligibility was restricted to studies without high risk of bias (we defined low risk of bias studies as those with low risk of bias in at least the following two domains: random sequence generation and allocation concealment);
2. a random-effects model had been adopted;
3. the summary effect measure was risk ratio rather than OR.

Overall quality of the body of evidence: 'Summary of findings' table

We prepared a 'Summary of findings' table to evaluate the overall quality of the body of evidence for the main review outcomes (live birth, miscarriage, clinical pregnancy, multiple gestation, preterm birth, birth defects, aneuploidy, stillbirth) using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness, and publication bias) (Summary of findings 1) (GRADEpro GDT). We justified and documented

judgements about the quality of the evidence (high, moderate, low, and very low) and incorporated this information into the reporting of the results for each outcome. The 'Summary of findings' table compared GM-CSF-supplemented embryo culture media versus any other non-GM-CSF-supplemented embryo culture media (control).

RESULTS

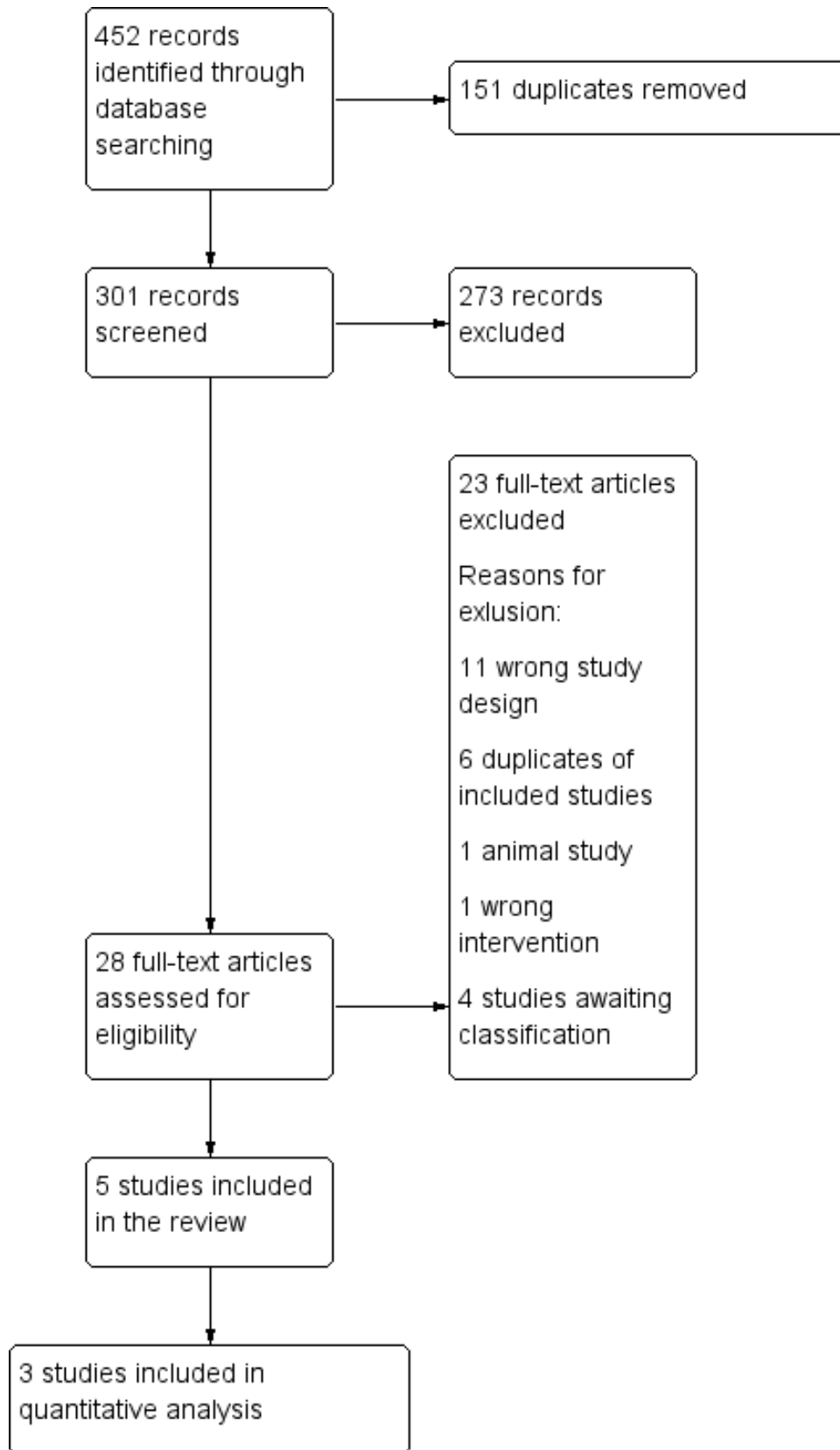
Description of studies

Results of the search

The following databases were systematically searched by Marian Showell, the Information Specialist at Cochrane Gynaecology and Fertility, on 15 October 2019: Cochrane Gynaecology and Fertility Specialised Register, CENTRAL, MEDLINE, Embase, and CINAHL. In addition, the 2019 European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) conference abstracts were handsearched by review author SA on 28 October 2019. In addition, a Google search using the terms 'GM-CSF, culture media, RCT, and live birth' was undertaken on 28 October 2019.

The search returned 452 records, 151 of which were duplicates. This left 301 titles and abstracts for screening, which was undertaken by all co-authors using the online software Covidence (Covidence). Each record was screened by two review authors at every stage. We considered 28 papers to be eligible for full-text screening. We excluded 23 full texts for the following reasons: 11 were the wrong study design; seven were trial registry information only, without data; three were duplicates of included studies; one was an animal study; and one was the wrong intervention. We considered five studies to be eligible for inclusion in the review (Rose 2020; Sbracia 2014; Zafardoust 2017; Zavvar 2016; Ziebe 2013), of which three could be used in meta-analysis (Rose 2020; Sbracia 2014; Ziebe 2013). The two studies that were not included in meta-analysis were conference abstracts, and the data could not be extracted reliably without further information from the study authors (Zafardoust 2017; Zavvar 2016); unfortunately we were unable to contact the authors of these studies to obtain the needed clarification. The PRISMA figure illustrates the flow of studies through the review (Figure 1).

Figure 1. PRISMA study flow diagram.



Included studies

Five studies were eligible for inclusion in the review. Two of these were conference abstracts that could not be included in meta-analysis because data could not be reliably extracted ([Zafardoust 2017](#); [Zavvar 2016](#)). The remaining three studies included two fully published and peer-reviewed papers, [Rose 2020](#); [Ziebe 2013](#), and one conference abstract ([Sbracia 2014](#)).

The largest study was undertaken in Europe; the trial was co-ordinated from the Netherlands, and participants were recruited from 14 fertility clinics in Sweden and Denmark ([Ziebe 2013](#)). [Ziebe 2013](#) included a total of 1332 participants, of whom 654 were randomised to the intervention arm and 678 were randomised to the control arm. The study was sponsored, co-ordinated, and authored by the worldwide market-leading manufacturer of GM-CSF-supplemented culture media. Women in the intervention arm had all of their embryos cultured in GM-CSF-supplemented culture medium at a concentration of 2 ng/mL from fertilisation through to embryo transfer. Women randomised to the control arm of the study had all of their embryos cultured in an IVF culture medium that did not contain GM-CSF from fertilisation through to embryo transfer. Both IVF and ICSI were undertaken, and a maximum of two embryos were transferred on day 3 in a fresh embryo transfer cycle. The inclusion criteria for the study were as follows: women aged 25 to 39 years, women who had a regular menstrual cycle of 21 to 35 days, women treated with a standard gonadotropin-releasing hormone (GnRH) agonist or antagonist protocol, and women with three or more follicles with a diameter of 14 mm on the day of human chorionic gonadotropin (hCG) administration, including a leading follicle of 17 mm. The exclusion criteria were: previous participation in the study; use of assisted hatching; use of non-ejaculated sperm; medical conditions or genetic disorders prohibiting IVF/ICSI or interfering with the interpretation of results; use of investigational drugs within 30 days before oocyte retrieval; severe chronic disease of relevance for reproduction; and oocyte donation.

[Rose 2020](#) was a smaller, single-centre study undertaken in a fertility clinic in Australia. A total of 100 women were randomised, 50 to the intervention arm and 50 to the control arm of the study. There were no dropouts. [Rose 2020](#) was a cross-over RCT, but the published data were from the first phase of the trial prior to cross-over. The study was sponsored by the worldwide market-leading manufacturer of GM-CSF-supplemented culture media. The same company also funded two co-authors of the study for statistical support. The women in this study underwent fresh embryo transfer following IVF or ICSI. The women in the intervention arm had all of their embryos cultured in GM-CSF-supplemented culture medium from fertilisation through to embryo transfer on day 5. The concentration of GM-CSF in the intervention culture medium was 2 ng/mL, and the medium was changed on day 3 following observation, scoring, and washing, to the next phase of sequential fresh culture medium with the same concentration of GM-CSF. The control culture medium did not contain GM-CSF, and similarly, day 3 embryos were observed, scored, washed and then transferred to a fresh sequential culture medium. All trial participants had a day 5 embryo transfer, apart from one woman in the control arm and two in the intervention arm who underwent day 3 embryo transfer. Participants underwent single-embryo transfer, except four women in the control arm and six women in the intervention arm, who underwent double-embryo transfer.

The inclusion criteria were: patients must have previously had consecutive transfer of two or more embryos without a positive pregnancy outcome OR have had a history of at least one previous pregnancy loss OR a previous history of poor embryo development (<20% of embryos developing on the time at day 3 or no blastocysts above grade 2 on day 5). Other additional inclusion parameters included a maternal age between 25 and 41 years, the use of a standard GnRH agonist or antagonist protocol, and three or more follicles of > 14 mm as seen by transvaginal ultrasound before the day of hCG administration. Exclusion criteria included: a need for surgical sperm retrieval (except in cases of previous vasectomy), the use of another investigational drug within 30 days of oocyte retrieval, and/or the presence of a severe chronic disease that could impact the IVF cycle or reproductive outcomes.

[Sbracia 2014](#) was another small, single-centre RCT, undertaken in a fertility clinic in Italy. The study was written as an abstract for an international conference. The authors reported that there was no funding for the study. A total of 100 women were randomised, 50 to the intervention arm and 50 to the control arm of the study. The women in the intervention arm had all of their embryos cultured in GM-CSF-supplemented culture media at a concentration of 2 ng/mL from fertilisation through to embryo transfer. Women in the control arm of the study had all of their embryos cultured in a medium not containing GM-CSF from fertilisation to embryo transfer. The brand name of the control culture medium was not disclosed in the paper. Fresh embryo transfer of up to a maximum of three embryos following ICSI was undertaken in all cycles in both the intervention and control arms of the study. The inclusion criteria were: women with recurrent implantation failure, three or more consecutive failed IVF cycles with a total of at least 8 good embryos replaced in the uterus, and women aged 40 or less. The exclusion criteria were: women aged over 40, chromosomal defects in the couple, metabolic diseases (diabetes, etc.), and other genetic diseases (thalassaemia, cystic fibrosis, etc.).

Both [Zafardoust 2017](#) and [Zavvar 2016](#) were eligible for inclusion, however data could not be reliably extracted for meta-analysis. [Zafardoust 2017](#) was a conference abstract which outlined that it was a single-centre RCT undertaken at a fertility clinic in Iran. The study included couples undergoing frozen embryo transfer following an ICSI cycle with their own gametes. Couples were randomised to either have their frozen embryos thawed and cultured in a test medium containing 2 ng/mL of GM-CSF, or a control medium not containing GM-CSF. Couples were eligible for inclusion in the study if the female partner was < 40 years old, had at least four good-quality embryos after thawing (grade A), and had not had more than one previous embryo transfer. Couples were excluded from entering the study if they needed ICSI cycles requiring pre-implantation genetic diagnosis, if the female partner had an anatomic disorder of the uterus, or one or more hydrosalpinges. The abstract outlines that 90 women were randomised, and 10 were excluded from the final analysis due to various reasons, however the original numbers of women randomised to each group are not disclosed. The outcome of interest reported by the study was clinical pregnancy, which is reported as two percentages, alongside a P value. However, it was not clear which percentage belonged to which arm of the study, therefore it was impossible to extract any meaningful data for meta-analysis. Review author SA attempted to contact two of the authors of this study by email on three separate occasions for clarification of these issues, but unfortunately no response was forthcoming.

[Zavvar 2016](#) was also a conference abstract, which outlined a single-centre RCT undertaken in a fertility clinic in Iran. [Zavvar 2016](#) sought to compare the outcomes of women undergoing ICSI who were randomised to receive an embryo culture medium containing 2 ng/mL GM-CSF or to a culture medium not containing GM-CSF. The inclusion criterion was women who produced only immature oocytes in spite of stimulation with gonadotropins. No exclusion criteria were described. The day of embryo transfer and length of time embryos were exposed to the intervention or control media were not described. The outcome of interest, the clinical pregnancy rate, was reported as percentages alongside a P value. However, it was not possible to identify which result was associated with which arm of the study, therefore we could not reliably include data from this study. Review author SA attempted to contact the authors of this study on two separate occasions by email, but unfortunately no response was received.

Excluded studies

We excluded 23 studies following full-text screening. Eleven studies were the wrong study design, and were excluded for the following reasons: [Agerholm 2010](#) was a phase I safety study and did not replace embryos; [Fawzy 2019](#), [Shapiro 2003](#), [Sjoblom 1998](#), [Sjoblom 1999](#), [Sjoblom 1999a](#), and [Sjoblom 2001](#) randomised oocytes opposed to women or couples; [Kinoshita 2019](#) was a retrospective study; [Min 2017](#) and [Sfontouris 2013](#) were observational studies; and [Siristatidis 2013](#) was a systematic review. [Scarpellini 2011](#) was excluded because it did not study the intervention we were interested in, and [Siqueira 2016](#) was excluded because it was an animal study. Six studies were duplicate references of included studies: [Rose 2020](#), [Sbracia 2014](#), [Zafardoust 2017](#), and [Zavvar 2016](#). Four studies are awaiting classification

because the nature of the study and whether women or oocytes was randomised was unclear ([ISRCTN94726536](#); [NCT01689428](#); [NCT01689454](#); [NCT02651285](#)).

Risk of bias in included studies

Allocation

We assessed the risk of selection bias for the three studies included in meta-analysis to be low ([Figure 2](#)) ([Rose 2020](#); [Sbracia 2014](#); [Ziebe 2013](#)), as random sequence generation was described in detail and considered to be adequate to achieve a truly random sequence. [Rose 2020](#) described how 50 cards with 'control' and 50 with 'BlastGen' written on them were placed in sealed envelopes by a person unrelated to the trial. They were shuffled several times, and the envelopes were then numbered and opened in consecutive order by the embryologist when an eligible participant was scheduled for egg retrieval. [Sbracia 2014](#) described how participants were randomised using a computer-generated number sequence; however, allocation concealment was not described, therefore we deemed this study to be at unclear risk. [Ziebe 2013](#) described how they used a computer-generated randomisation list in blocks of four for each individual clinic in order to maintain balance between the treatment groups at each site. Allocation concealment was described in detail and considered to be at low risk on the basis that each study site received a list of study-specific consecutive patient ID numbers and a corresponding number of identical-looking randomised bottles of test and control media that were individually labelled with the corresponding study-specific ID numbers. On site, the lowest number on the list was always allocated to any new patient recruited at the time of informed consent signature. Consequently, the clinician, embryologist, and participant were all blinded to the allocation.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias): All outcomes	Blinding of outcome assessment (detection bias): All outcomes	Incomplete outcome data (attrition bias): All outcomes	Selective reporting (reporting bias)	Other bias
Rose 2020	+	+	+	+	+	+	-
Sbracia 2014	+	?	?	?	+	+	-
Zafardoust 2017	?	?	?	?	-	-	-
Zavvar 2016	?	?	?	?	?	?	-
Ziebe 2013	+	+	+	+	-	+	-

Zafardoust 2017 and Zavvar 2016 could not be included in meta-analysis, but were considered to be at unclear risk of selection

bias because no description of randomisation or allocation concealment was provided.

Blinding

We considered [Rose 2020](#) and [Ziebe 2013](#) to be at low risk for performance and detection bias. [Rose 2020](#) described how clinicians, sonographers, statisticians, and participants were completely blinded to the intervention. [Ziebe 2013](#) described how participants and investigators, including clinicians and embryologists, were blinded to treatment allocation. Following email correspondence, we established that the clinicians performing the ultrasound scans were blinded to the treatment allocation at all times.

[Sbracia 2014](#) did not provide any description of blinding of participants, personnel, or outcome assessors, and was therefore assessed as at unclear risk of bias of performance and detection bias. We judged [Zafardoust 2017](#) and [Zavvar 2016](#) to be at unclear risk of performance and detection bias because there was no description of who, if anyone, was blinded.

Incomplete outcome data

We assessed [Rose 2020](#) and [Sbracia 2014](#) as at low risk of attrition bias. Both studies reported no dropouts. We considered [Ziebe 2013](#) to be at high risk of attrition bias because despite all dropouts being accounted for, the reasons given were not included within the predefined exclusion criteria. For example, no oocytes retrieved, no semen sample, no fertilisation, no embryo transfer, and "non-includable after randomisation" were given as reasons for exclusion after randomisation, however none of these were listed as exclusion criteria. We contacted the authors to obtain accurate intention-to-treat (ITT) data for both arms of the study, which they were able to provide.

We considered [Zafardoust 2017](#) to be at high risk of attrition bias because 10 women were not included in the final analysis, with no reasons provided. We considered this to be a high rate of attrition in a small study. Unfortunately we were unable to use data from this study as the data could not be reliably extracted. We considered [Zavvar 2016](#) to be at unclear risk of attrition bias as dropouts were not described. We could not include data from this study because it was unclear how many participants were included in the analysis.

Selective reporting

We rated [Rose 2020](#), [Sbracia 2014](#), and [Ziebe 2013](#) as being at low risk of reporting bias because the study authors confirmed via email correspondence that they had reported all outcomes as per their prospective clinical trials registrations (NCT02305420, NCT01718210, and NCT00565747, respectively).

We rated [Zafardoust 2017](#) as being at high risk of reporting bias. The available abstract did not report data on miscarriage, multiple pregnancy, and beta human chorionic gonadotropin (BHCG) levels, which are secondary outcomes noted on the prospective clinical trials register ([Zafardoust 2017](#)). We attempted to contact the study authors to establish if further trial data were available, but received no response. We rated [Zavvar 2016](#) as being at unclear risk of reporting bias. We had no access to a protocol or an online clinical trial registry.

Other potential sources of bias

We have been in extensive contact with the authors of [Ziebe 2013](#) via email to clarify various numbers from their published study. The co-authors of this study have been very forthcoming in answering all of our queries and have offered clear explanations of how various numbers are reached in their paper. However, we have assessed [Ziebe 2013](#) as at high risk of bias for this domain because the numbers published in the paper differ from those published in this review, that is we discovered through correspondence that some participants were inaccurately described as miscarriages opposed to biochemical pregnancy losses. We also asked for individual participant data in relation to those babies that suffered aneuploidy or birth defects, or both. On reviewing the data, we discovered that some women underwent termination of pregnancy in light of aneuploidy or birth defects, which had not been included in their aneuploidy or birth defect data. We also discovered that one baby had been classified as having a birth defects, when in fact it was reported as having immature lungs secondary to prematurity. In addition, the reporting of multiple pregnancies in the paper is very confusing. We clarified all of these issues through correspondence, which is summarised in [Appendix 13](#). One co-author of this review has written a letter, which has been published, outlining the concerns regarding the statistical analysis presented in the paper ([Farquhar 2015](#)). Examples of concerns include the adjustment of sample size and the increase of concentration of human serum albumin following interim analysis, and the reporting of 'ongoing implantation rate' as number of transferred embryos opposed to per woman.

Effects of interventions

See: [Summary of findings 1 GM-CSF-supplemented culture media compared to culture media not supplemented with GM-CSF for women undergoing assisted reproduction](#)

GM-CSF-supplemented culture media versus culture media not supplemented with GM-CSF for women undergoing assisted reproduction

A total of five studies undertook this comparison. Three of these studies (1532 participants) reported data that could be included in meta-analysis ([Rose 2020](#); [Sbracia 2014](#); [Ziebe 2013](#)).

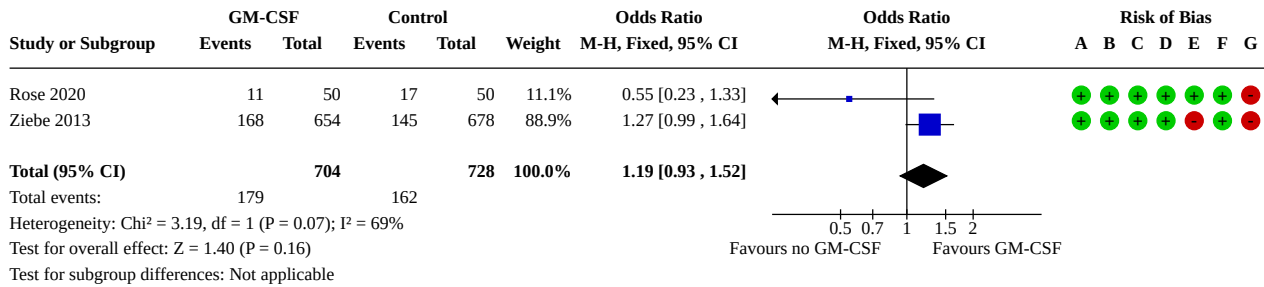
Primary outcomes

1.1 Live birth

Two studies (N = 1432) provided live-birth data ([Rose 2020](#); [Ziebe 2013](#)). We obtained ITT live-birth data following correspondence with the authors of [Ziebe 2013](#) (see [Appendix 13](#)). There were 179 live births reported amongst the 704 women randomised to the GM-CSF arm, and 162 live births amongst the 728 women randomised to the control arm. No studies reported ongoing pregnancy as a proxy to live birth.

We are uncertain whether GM-CSF-supplemented culture media makes any difference to the live-birth rate when compared to using conventional culture media not supplemented with GM-CSF (odds ratio (OR) 1.19, 95% confidence interval (CI) 0.93 to 1.52, 2 RCTs, N = 1432, $I^2 = 69%$, low-quality evidence) ([Analysis 1.1](#); [Figure 3](#)).

Figure 3. Forest plot of comparison: 1 GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, outcome: 1.1 Live birth.



Risk of bias legend

- (A) Random sequence generation (selection bias)
- (B) Allocation concealment (selection bias)
- (C) Blinding of participants and personnel (performance bias)
- (D) Blinding of outcome assessment (detection bias)
- (E) Incomplete outcome data (attrition bias)
- (F) Selective reporting (reporting bias)
- (G) Other bias

The evidence suggests that if the rate of live birth associated with conventional culture media not supplemented with GM-CSF was 22%, the rate with the use of GM-CSF-supplemented culture media would be between 21% and 30%.

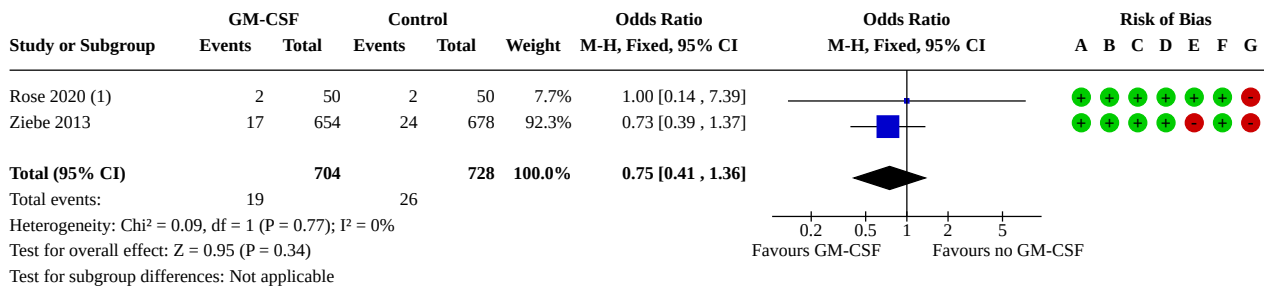
1.2 Miscarriage

Two RCTs (N = 1432) provided miscarriage data (Rose 2020; Ziebe 2013). The authors of both studies were able to clarify that the miscarriages were of clinical pregnancies. Based on correspondence, we were able to remove terminations of pregnancy that had been classified as miscarriage in these two studies (Appendix 13). Terminations of pregnancy as a result of

aneuploidy or birth defect are accounted for in Analysis 1.6 and Analysis 1.7. All miscarriages were first-trimester losses, apart from one in the control arm of Rose 2020, which was a midtrimester loss at 17 weeks' gestation. There were 19 miscarriages amongst the 704 women randomised to the GM-CSF arm, and 26 miscarriages amongst the 728 women randomised to the control arm.

It is unclear whether use of GM-CSF-supplemented culture media makes any difference to miscarriage rate when compared to conventional culture media not supplemented with GM-CSF (OR 0.75, 95% CI 0.41 to 1.36, 2 RCTs, N = 1432, I² = 0%, low-quality evidence) (Analysis 1.2; Figure 4).

Figure 4. Forest plot of comparison: 1 GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, outcome: 1.2 Miscarriage.



Footnotes

- (1) One miscarriage in the control arm was at 17 weeks gestation, the remainder <12 weeks.

Risk of bias legend

- (A) Random sequence generation (selection bias)
- (B) Allocation concealment (selection bias)
- (C) Blinding of participants and personnel (performance bias)
- (D) Blinding of outcome assessment (detection bias)
- (E) Incomplete outcome data (attrition bias)
- (F) Selective reporting (reporting bias)
- (G) Other bias

The evidence suggests that if the miscarriage rate associated with conventional culture media not supplemented with GM-CSF was 4%, the rate with the use of GM-CSF-supplemented culture media would be between 2% and 5%. It is worth noting that these figures are per woman randomised, hence the apparently very low miscarriage rates. They do not include miscarriages that occurred before the diagnosis of a clinical pregnancy on ultrasound scan, otherwise known as biochemical pregnancy losses.

Secondary outcomes

1.3 Clinical pregnancy

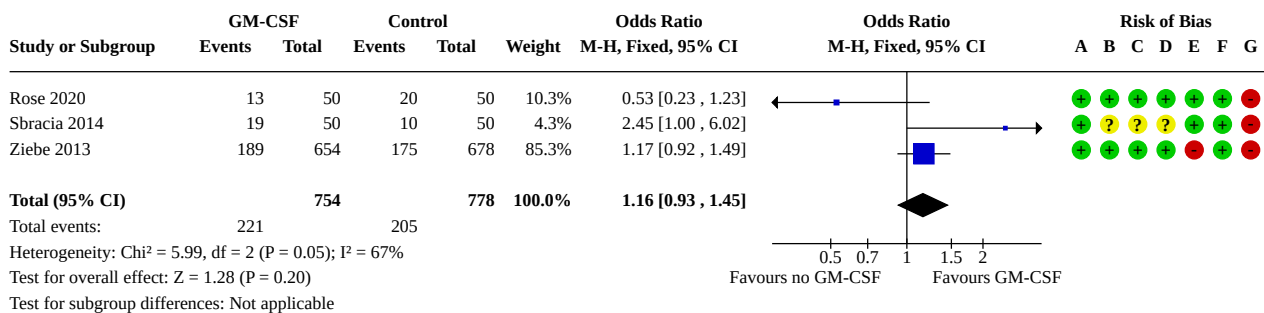
Three studies (N = 1532) reported clinical pregnancy rates (Rose 2020; Sbracia 2014; Ziebe 2013). Both Rose 2020 and Ziebe 2013 describe how an ultrasound scan was performed at seven weeks' gestation in order to diagnose clinical pregnancy. Information on the methods of Sbracia 2014 was limited, as the study is only available as a conference abstract, and we received no response to

our emails to the authors of the study. The authors of Sbracia 2014 describe pregnancy rate as their primary outcome, however there are no further details as to what stage pregnancy was diagnosed, and whether they were clinical pregnancies diagnosed with ultrasound. The authors report an "implantation rate", which we have taken to mean a biochemical pregnancy rate. Consequently, for the purposes of this review, we have assumed the pregnancy rate in Sbracia 2014 to be clinical.

There were 221 clinical pregnancies amongst the 754 women randomised to the GM-CSF arm, and 205 clinical pregnancies amongst the 778 women randomised to the control arm.

We are uncertain whether GM-CSF-supplemented culture media makes any difference to the clinical pregnancy rate when compared to using a conventional culture medium not supplemented with GM-CSF (OR 1.16, 95% CI 0.93 to 1.45, 3 RCTs, N = 1532 women, I² = 67%, low-quality evidence) (Analysis 1.3; Figure 5).

Figure 5. Forest plot of comparison: 1 GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, outcome: 1.3 Clinical pregnancy.



Risk of bias legend
 (A) Random sequence generation (selection bias)
 (B) Allocation concealment (selection bias)
 (C) Blinding of participants and personnel (performance bias)
 (D) Blinding of outcome assessment (detection bias)
 (E) Incomplete outcome data (attrition bias)
 (F) Selective reporting (reporting bias)
 (G) Other bias

The evidence suggests that if the clinical pregnancy rate associated with conventional culture media not supplemented with GM-CSF was 26%, the rate with the use of GM-CSF-supplemented culture media would be between 25% and 34%.

1.4 Multiple gestation

Two studies (N = 1432) reported multiple gestation rate (Rose 2020; Ziebe 2013). The multiple gestation rate was clarified following correspondence with authors of both studies (Appendix 13). The authors of Ziebe 2013 also detail the incidence of monozygotic and dizygotic twins, but in this review we did not differentiate between types of twins. The authors of Rose 2020 report single-embryo transfer as standard, but explain that four women in the control arm and six women in the intervention arm received double-embryo transfer (Appendix 13). The authors of Ziebe 2013 describe how a maximum of two embryos were replaced per woman with a mean embryo transfer rate of 1.51 for the control arm and 1.49 for the GM-CSF arm. There was one triplet pregnancy in the intervention

arm of the study by Ziebe 2013. The remaining multiple gestations reported here were all twins.

There were 31 women with a multiple gestation amongst the 704 women randomised to the GM-CSF arm, and 205 women with a multiple pregnancy amongst the 728 women randomised to the control arm.

We are uncertain whether GM-CSF-supplemented culture media makes any difference to the multiple pregnancy rate when compared to use of a conventional culture medium not supplemented with GM-CSF (OR 1.24, 95% CI 0.73 to 2.10, 2 RCTs, N = 1432, I² = 35%, very low-quality evidence) (Analysis 1.4).

The evidence suggests that if the multiple gestation rate associated with conventional culture media not supplemented with GM-CSF was 4%, the rate with the use of GM-CSF-supplemented culture media would be between 3% and 7%.

1.5 Preterm birth

Two studies (N = 1432) reported the preterm birth rate, defined as the birth of a baby (or babies in the case of multiple pregnancy) under 37 weeks' gestation, per woman randomised (Rose 2020; Ziebe 2013). Preterm birth was detailed in the published study by Ziebe 2013. For singletons, the preterm birth data were easily extractable. For women with multiple gestations, the authors of Ziebe 2013 report gestational age at birth with a standard deviation, therefore we clarified these data with the study authors to establish the number of preterm births (Appendix 13). We sought preterm birth data through correspondence with the authors of Rose 2020 (Appendix 13). We counted twins and triplets that were born before 37 weeks as one event for this outcome, as we undertook ITT analysis.

There were 30 women with a preterm birth amongst the 704 women randomised to the GM-CSF arm, and 26 women with a preterm birth amongst the 728 women randomised to the control arm.

We are uncertain whether GM-CSF-supplemented culture media makes any difference to the preterm birth rate when compared to using a conventional culture medium not supplemented by GM-CSF (OR 1.20, 95% CI 0.70 to 2.04, 2 RCTs, N = 1432, I² = 76%, very low-quality evidence) (Analysis 1.5).

The evidence suggests that if the preterm birth rate associated with conventional culture media not supplemented with GM-CSF was 4%, the rate with the use of GM-CSF-supplemented culture media would be between 3% and 7%.

1.6 Birth defects

The authors of two studies (N = 1432) were able to provide details on birth defects following correspondence (Appendix 13) (Rose 2020; Ziebe 2013). The authors of Rose 2020 explained that there was one baby with multiple birth defects, which was detected antenatally (this participant was classified as experiencing a miscarriage in the published study, but we have clarified that this was a termination of pregnancy, and it has therefore been removed from the miscarriage group in this review). The authors of Ziebe 2013 provided details on 22 infants who were born with defects (three participants underwent termination of pregnancy for birth defects). We did not count any infants as having both birth defects and aneuploidy, but rather divided them into one of the two groups. We are not aware of twins within the birth defects group, and have assumed all data to be per woman randomised.

Thirteen women had a baby with a birth defect amongst the 704 women randomised to the GM-CSF arm, and 10 women had a baby with a birth defect amongst the 728 women randomised to the control arm.

We are uncertain whether GM-CSF-supplemented culture media makes any difference to the rate of birth defects when compared to using a conventional culture medium not supplemented by GM-CSF (OR 1.33, 95% CI 0.59 to 3.01, I² = 0%, 2 RCTs, N = 1432, low-quality evidence) (Analysis 1.6).

The evidence suggests that if the birth defect rate associated with conventional culture media not supplemented with GM-CSF was 1%, the rate with the use of GM-CSF-supplemented culture media would be between 1% and 4%.

1.7 Aneuploidy

Two studies (N = 1432) provided data regarding aneuploidy after we inquired about this outcome (Rose 2020; Ziebe 2013). The authors of Rose 2020 described how one baby had a trisomy, which was detected antenatally, and Ziebe 2013 reported that one baby had a trisomy detected antenatally.

No women had a baby with aneuploidy amongst the 704 women randomised to the GM-CSF arm, and two women had a baby with aneuploidy amongst the 728 women randomised to the control arm. We are uncertain whether GM-CSF-supplemented culture media makes any difference to the rate of aneuploidy when compared to using a conventional culture medium not supplemented by GM-CSF (OR 0.34, 95% CI 0.03 to 3.26, I² = 0%, 2 RCTs, N = 1432, low-quality evidence) (Analysis 1.7).

The evidence suggests that if the aneuploidy rate associated with conventional culture media not supplemented with GM-CSF was 0.3%, the rate with the use of GM-CSF-supplemented culture media would be between 0% and 0.9%.

1.8 Stillbirth

Two studies (N = 1432) reported stillbirth (Rose 2020; Ziebe 2013). Following correspondence, the authors of Rose 2020 provided data on stillbirth that were not published (Appendix 13). There were no stillbirths reported in either arm of the study, hence the OR was not estimable (Analysis 1.8). The average rate of stillbirth ranges from approximately 4 per 1000 total births in high-income countries to approximately 28 per 1000 total births in low-income countries such as sub-Saharan Africa (Lawn 2016), therefore the stillbirth rate in this review is better than average for the high income countries.

Planned additional analyses

We did not need to undertake a funnel plot to explore the possibility of small-study effects as there were only three included studies in the quantitative analysis.

Subgroup analyses

1) Studies including only women with recurrent implantation failure. Two studies were defined as including women with "poor prognosis" as a result of previous recurrent implantation failure. The definition of recurrent implantation failure (the failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles (Coughlan 2014)) was met by only one of these studies (Sbracia 2014). When examining this study alone, the only outcome it informs is clinical pregnancy. The low-quality evidence suggests that GM-CSF-supplemented culture media may slightly improve pregnancy rates when compared to culture media not supplemented by GM-CSF (OR 2.45, 95% CI 1.00 to 6.02, 1 RCT, N = 100, low-quality evidence).

2) Single-step versus sequential culture media. A single-step culture medium supplemented with GM-CSF would involve culturing the embryos in one medium following fertilisation all the way through to blastocyst embryo replacement if required. The included study that cultured embryos through to blastocyst utilised a sequential culture medium supplemented with GM-CSF (Rose 2020), therefore we were unable to undertake this subgroup analysis. Correspondence with Cooper Surgical revealed that a single-step culture medium supplemented by GM-CSF is yet to

obtain its CE mark (certification mark that indicates conformity with health, safety, and environmental protection standards for products sold within the European Economic Area), and for this reason is not yet available in Europe from this company.

3) Donor versus autologous oocytes. No included studies utilised donor oocytes, so a subgroup analysis was not possible.

4) Studies including only women with recurrent miscarriage. No included studies involved only women who had experienced recurrent miscarriage, so a subgroup analysis was not possible.

5) Studies replacing embryos at cleavage stage versus blastocyst stage. We know that [Rose 2020](#) was the only study that definitely replaced all embryos at day 5, thereby satisfying the criterion of blastocyst stage transfer. The authors of [Ziebe 2013](#) describe how they undertook all day 3 embryo transfers, which classifies this study as cleavage stage transfer. The authors of [Sbracia 2014](#) did not describe whether they undertook cleavage stage or blastocyst stage transfer. However, they do report using EmbryoGen as the intervention culture media, which is a culture medium licensed to culture embryos to day 3, therefore we have assumed for the sake of subgroup analysis that [Sbracia 2014](#) is classified as a cleavage stage transfer study.

Two studies, one cleavage stage transfer, [Ziebe 2013](#), and one blastocyst stage transfer, [Rose 2020](#), reported on the outcome of live birth. The subgroup analysis for both cleavage stage and blastocyst stage transfer did not alter the finding from the pooled meta-analysis. In other words, for both subgroup analyses and the main pooled meta-analysis, we are uncertain whether GM-CSF-supplemented culture media makes any difference to the live-birth rate when compared to using conventional culture media not supplemented with GM-CSF. The quality of the evidence of the subgroup analyses is low given that only one study informs each analysis.

The same two studies that reported on live birth also reported on miscarriage ([Rose 2020](#); [Ziebe 2013](#)), one cleavage and one blastocyst stage transfer. The subgroup analyses did not change the outcome of the main meta-analysis. In other words, we are uncertain whether GM-CSF-supplemented culture media makes any difference to the miscarriage rate when compared to using conventional culture media not supplemented with GM-CSF. The quality of the evidence of the subgroup analyses is low given that only one study informs each analysis.

The outcome clinical pregnancy is informed by three included studies. Two studies undertook cleavage stage transfers ([Sbracia 2014](#); [Ziebe 2013](#)), and one undertook blastocyst stage transfer ([Rose 2020](#)). The subgroup analyses did not alter the results of the main pooled meta-analysis. Low-quality evidence remains of uncertainty as to whether GM-CSF-supplemented culture media makes any difference to the clinical pregnancy rate when compared to using a conventional culture medium not supplemented with GM-CSF.

The outcome multiple gestation is informed by two included studies, one of which undertook cleavage stage transfers, [Ziebe 2013](#), and the other blastocyst stage transfers, [Rose 2020](#). The subgroup analyses did not alter the results of the main pooled meta-analysis. Low-quality evidence remains of uncertainty as to whether GM-CSF-supplemented culture media makes any

difference to the multiple gestation rate when compared to using a conventional culture medium not supplemented with GM-CSF. We downgraded the quality of the evidence from moderate for the main meta-analysis, to low, given that only one study informs each subgroup analysis.

Sensitivity analyses

We decided to undertake three sensitivity analyses for the primary outcomes to determine whether our conclusions were robust to arbitrary decisions made regarding the eligibility and analysis. The analyses were as follows.

1) If eligibility was restricted to studies without high risk of bias (studies at low risk of bias were defined as those with low risk of bias in at least the following two domains: random sequence generation and allocation concealment). For live birth and miscarriage, our two primary outcomes, both studies included in the meta-analysis were low risk according to our definition, therefore we did not need to undertake this sensitivity analysis.

2) If a random-effects model had been adopted. We applied the random-effects model to both of our primary outcomes, and it did not alter the conclusions of the review.

3) If the summary effect measure was risk ratio rather than OR. We altered the summary effect measure to risk ratio for both live birth and miscarriage, however it did not alter the conclusions of the review.

DISCUSSION

Summary of main results

Despite GM-CSF-supplemented culture media being commercially available for a number of years, there were very few RCTs from around the world with data that could be included in this review. The three trials included in meta-analysis, [Rose 2020](#); [Sbracia 2014](#); [Ziebe 2013](#), involved a total 1532 women, of whom the vast majority (1332 women) were from [Ziebe 2013](#), a trial designed, conducted, and written by Cooper Surgical, one of the global market leaders in culture media supplemented with GM-CSF. Having said this, there was transparency in communication with the authors of [Ziebe 2013](#); this review contains ITT data and further details on methods as a result of the authors' willingness to share information ([Appendix 13](#)).

For the primary and secondary outcomes assessed, including live birth, miscarriage, clinical pregnancy, multiple gestation, preterm birth, birth defects, and aneuploidy, due to very low- to low-quality evidence we cannot be certain whether GM-CSF is any more or less effective than culture media not supplemented with GM-CSF for clinical outcomes that reflect effectiveness and safety ([Summary of findings 1](#)). We were unable to undertake analysis of stillbirth, as there were no events in either study arm; however, the lack of events in either arm supports the hypothesis that there is no advantage or disadvantage to using culture media supplemented by GM-CSF versus culture media not supplemented by GM-CSF.

Overall completeness and applicability of evidence

The evidence for this review was dominated by one large, multicentre European RCT, which was designed and conducted by industry. However, despite concerns about the equipoise of the trial designers, co-ordinators, and data analysts, the study appears

to be well executed overall. There is without doubt a number of flaws in the design of the study, in particular the statistical analysis of the results presented in the paper (Farquhar 2015); however, we sought ITT data from the authors, which means the data are as transparent as possible (Appendix 13). The three studies included in the quantitative analysis have a number of similarities as described below, but most notable is the concentration of GM-CSF within the intervention culture media, which is the same across all studies. This possibly reflects the dominance of one particular company who supplied the intervention culture media for all of the included studies. On balance, the available data represent women or couples attending for assisted reproduction with their own gametes, on a single fresh embryo transfer cycle. Two hundred of the 1532 included women were considered to be 'poor prognosis', with recurrent implantation failure or a history of poor embryo development. Further studies including frozen cycles, donor oocytes, and cumulative embryo transfer data from one cycle are required to make the evidence more broadly applicable to the types of women or couples attending for assisted reproduction.

The studies included in the quantitative analysis were conducted in high-income countries, as defined by the World Bank (World Bank). Two included studies were undertaken in the upper-middle-income country of Iran (Zafardoust 2017; Zavvar 2016), however no data could be reliably extracted from these studies, therefore the data available for this review were based solely on those residing in high-income countries. There was a mixture of IVF and ICSI across the three studies included in quantitative analysis. All three studies undertook fresh embryo transfers, and no cumulative embryo transfer data were available from subsequent frozen embryo transfer cycles.

The intervention culture media contained the same concentration of GM-CSF in all three studies, which makes the intervention arm more homogenous in terms of what participants received than the control culture media, which were described in less detail. Rose 2020 describes a control of "standard embryo culture media", whilst the authors of Sbracia 2014 do not provide any information on the control medium. The authors of Ziebe 2013 describe using "EmbryoAssist without cytokine", a culture media manufactured by Cooper Surgical. The potential variation in the control culture media makes the result more generalisable to 'real-world' practice, where individual clinics use a variety of culture media 'as standard'.

The inclusion of studies with variations in day of embryo transfer, poor- and good-prognosis patients, variation in underlying medical conditions of participants, and numbers of embryo transferred helps make the results of this review generalisable.

The two studies that could not be included in the quantitative analysis, Zafardoust 2017; Zavvar 2016, were in some ways different to the studies included in the quantitative analysis. For example, Zafardoust 2017 utilised frozen embryos, and Zavvar 2016 included women in whom immature oocytes were retrieved in spite of stimulation with gonadotropins. Both studies included the same concentration of GM-CSF in the intervention culture media (2 ng/mL), which were supplied by Cooper Surgical, the supplier of all of the intervention culture media across all included studies. Both Zafardoust 2017 and Zavvar 2016 undertook ICSI. It may be possible to include data from these two studies in the future, if we are able to make contact with the authors to clarify issues regarding data and methods. Both studies reported that there was

no significant difference in the pregnancy rate between the GM-CSF and conventional culture media arms of their studies.

Quality of the evidence

Overall, the quality of the evidence using the GRADE approach is low or very low for all outcomes (Summary of findings 1). Live birth, clinical pregnancy, multiple gestation, and preterm birth were all downgraded once for inconsistency. There were differing point estimates in the included studies, with one supporting GM-CSF-supplemented culture media, and one supporting the control culture medium. The point estimates have broad confidence intervals, and in many cases the I^2 result is high, representing a high degree of heterogeneity between trials.

Live birth, miscarriage, multiple gestation, preterm birth, birth defects, and aneuploidy were all downgraded for imprecision. We downgraded live birth once and the remaining outcomes twice. These outcomes had point estimates with broad or very broad confidence intervals, from a low number of included studies, at least one of which was very small in terms of number of participants. We downgraded clinical pregnancy once for risk of bias, as all studies that inform this outcome have high risk of other bias, and one study was at unclear risk of selection, performance, and detection bias. The unclear risk of bias in this study was due mainly to a lack of information from the available published abstract.

We were unable to undertake meta-analysis of the results for stillbirth because there were no occurrences of stillbirth in either arm of the study in the one trial that reported this outcome.

Regarding risk of bias, both Rose 2020 and Ziebe 2013 were considered to be overall low risk of bias. Rose 2020 was low risk in all domains except for other bias, which was rated high risk because although the study appears to be well run, the sample size of 100 participants is small, and is unlikely to be powered to detect meaningful difference between the groups in terms of clinical outcomes. We considered Ziebe 2013 to be at low risk of selection, performance, detection, and reporting bias, but at high risk of attrition and other bias. Sbracia 2014 was rated as having high risk of other bias and unclear risk of selection, performance, and detection bias.

Potential biases in the review process

We aimed to identify all eligible studies for inclusion in this review, and contacted authors of all five included studies on many occasions in an effort to include as much information as possible. The authors of two studies were forthcoming with further study information, which helped us to acquire a full picture of the study outcomes, as well as providing information needed to assess and establish risk of bias (Appendix 13).

Agreements and disagreements with other studies or reviews

We found one published systematic review examining GM-CSF-supplemented culture media for women undergoing assisted reproduction (Siristatidis 2013). This review undertook a search of studies published between 1966 and 2012. Siristatidis 2013 included all study designs except case series and case reports. The primary outcome was live birth per woman/couple. Secondary outcomes were clinical pregnancy per woman/couple (defined as

evidence of fetal heart on ultrasound at seven weeks), miscarriage rate (defined as the number of miscarriages divided by the number of clinical pregnancies), fertilisation rate (rate of oocytes fertilised per oocytes retrieved), and laboratory parameters, such as progression of embryos to blastocyst stage, blastocyst performance and hatching, and chromosomal abnormalities of the embryos. The search yielded 152 records, 112 of which were discarded. Six of the remaining 41 studies were considered eligible for inclusion in the review, four of which were RCTs and two prospective observational studies. The review by [Siristatidis 2013](#) has one RCT in common with our review ([Ziebe 2013](#)). The other three RCTs included in [Siristatidis 2013](#) were excluded here because they randomised oocytes ([Shapiro 2003](#); [Sjoblom 2001](#)), or no embryos were replaced in women ([Agerholm 2010](#)).

The authors of [Siristatidis 2013](#) concluded that most of the included studies trend towards favouring the supplementation of culture media with GM-CSF in terms of good-quality embryos reaching the blastocyst stage, improved hatching initiation and number of cells in the blastocyst, and reduction in cell death. However, no statistically significant differences were found in implantation and pregnancy rates in all but one trial, which reported favourable outcomes in terms of implantation and live birth. The authors of [Siristatidis 2013](#) go on to propose properly conducted and adequately powered RCTs to further validate and extrapolate the current findings. The quality of included studies is deemed by the [Siristatidis 2013](#) authors to be "average".

Our review adds two further RCTs, and has the advantage of conducting a meta-analysis and undertaking a 'Risk of bias' assessment, as well as applying GRADE to the findings of the review. The fact that three of the included RCTs in [Siristatidis 2013](#) randomised oocytes or did not replace embryos into the women means that interpreting the data and applying it to real-world clinical situations is almost impossible, as the trial design is not adequate for phase III clinical trials assessing clinical outcomes.

We received email correspondence from a reader of the protocol for this review who expressed concerns that we were planning to include studies that used GM-CSF or G-CSF as the intervention medium ([Appendix 14](#)). The author expressed concerns about this decision as they felt those media were not homogenous enough to be included in one group. This review only found studies that included GM-CSF as the intervention medium and we explained that we would look into this issue for the re-run of the review in the coming years.

AUTHORS' CONCLUSIONS

Implications for practice

As is the case with most in vitro fertilisation (IVF) add-ons, granulocyte macrophage colony-stimulating factor (GM-CSF)-supplemented culture media is already widely in use in many fertility clinics across the world, well before the arrival of this systematic review of randomised trials. Despite GM-CSF being available commercially for a number of years, there are still only five randomised trials, only three of which have data to extract for meta-analysis, and two of which are extremely small and not adequately powered to detect a meaningful clinical differences between groups. It is also notable that two of the three studies included in quantitative analysis were sponsored by industry;

however, the risk of bias in these studies appears to be low ([Figure 2](#)).

The findings of this review reveal that overall there is very low- to low-quality evidence to suggest that GM-CSF supplemented culture media is no more or less effective or harmful than culture media not supplemented with GM-CSF for the following outcomes: live birth, miscarriage, clinical pregnancy, multiple gestation, preterm birth, birth defects, and aneuploidy. The evidence for stillbirth was also low quality, but we were unable to undertake meta-analysis as there were no events in either arm of the studies.

Given these findings, clinicians, embryologists and women/couples considering using GM-CSF-supplemented culture media during an assisted reproduction cycle should be aware that the available evidence neither supports, nor opposes its use. It would appear important that independent information on the available evidence is made accessible to those considering using GM-CSF-supplemented culture media, particularly in the face of strong marketing information. GM-CSF-supplemented culture media is marketed as being recommended for those who have experienced recurrent clinical and biochemical pregnancy loss; recurrent implantation failure; unexplained infertility; and advanced maternal age ([Cooper Surgical 2020a](#)). However, the available evidence from randomised controlled trials (RCTs) only includes two small studies (N = 200), which included women aged 40 or over, or women who had experienced recurrent miscarriage or implantation failure ([Rose 2020](#); [Sbracia 2014](#)). The claim that GM-CSF has a positive effect on implantation and pregnancy rates is simply not supported by the available evidence presented here ([Cooper Surgical 2020a](#)).

Implications for research

The question of whether GM-CSF-supplemented culture media has any advantage over culture media not supplemented by GM-CSF urgently requires further high-quality, independent, adequately powered RCTs to add to this systematic review. The ideal RCT would pre-register a protocol, recruit women/couples attending for assisted reproduction, of all ages and backgrounds, with all types of infertility, undergoing IVF or intracytoplasmic sperm injection (ICSI). It would be powered highly enough to be able to detect whether particular subgroups would benefit from this type of culture media, for example those with previous failed IVF attempts. Women, clinicians, embryologists, and those assessing outcomes would be blinded to the intervention. After randomisation, women would remain in the group to which they had been allocated for the sake of data analysis, regardless of whether they had received the allocated intervention, or whether they dropped out, in order to maintain the effects of randomisation. Data would be analysed using intention-to-treat, maintaining the denominator as all women who were randomised to that arm of the study, for all outcomes, including pregnancy, miscarriage, and live birth. The study would be registered on a trials registry beforehand, and the protocol published, including which statistical analyses were planned. The study would ideally be designed and conducted, and data analysed by independent researchers, who hold equipoise regarding the efficacy of GM-CSF-supplemented culture media.

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preimplantation embryo development. *Biology of Reproduction* 2000;**63**(5):1413-20.

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culture medium for in vitro fertilization. *Fertility and Sterility* 2013;**99**(6):1600-9.

* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES
Characteristics of included studies [ordered by study ID]

Rose 2020
Study characteristics

Methods	<p>Study design: single-centre randomised controlled trial (RCT). The study was cross-over in design, but only data from the first phase of the study, prior to cross-over, are included in the review.</p> <p>Country: Australia</p> <p>Setting: fertility clinic, Adelaide</p> <p>Sponsorship source: EmbryoGen/BlastGen media was supplied by ORIGIO, a Cooper Surgical company. A grant from Cooper Surgical also funded MLH and EJK for statistical support. Fertility SA and The Robinson Institute, University of Adelaide provided in kind support from staff and affiliates providing time and expertise.</p> <p>Trials registry number: NCT02305420 (prospectively registered)</p>
Participants	<p>Inclusion criteria: the inclusion criteria selected for a poor-prognosis patient population. Patients must have previously had consecutive transfer of 2 or more embryos without a positive pregnancy outcome OR have had a history of at least 1 previous pregnancy loss OR a previous history of poor embryo development (< 20% of embryos developing on the time at day 3 or no blastocysts above grade 2 on day 5). Other additional inclusion parameters included a maternal age between 25 and 41 years, the use of a standard gonadotrophin-releasing hormone (GnRH) agonist or antagonist protocol, and 3 or more follicles of > 14 mm as seen by transvaginal ultrasound before the day of human chorionic gonadotropin administration.</p> <p>Exclusion criteria: exclusion criteria included a need for surgical sperm retrieval (except in cases of previous vasectomy), the use of another investigational drug within 30 days of oocyte retrieval, and/or the presence of a severe chronic disease that could impact the in-vitro fertilisation (IVF) cycle or reproductive outcomes.</p> <p>Number of participants randomised to each arm of the study: 50 to intervention arm, 50 to control arm.</p> <p>Dropouts: none</p> <p>Group differences: participants were matched for age, body mass index (BMI), and smoking status. A greater number of participants in the intervention group underwent intracytoplasmic sperm injection (ICSI) opposed to IVF versus the control group (37/50 versus 29/50).</p> <p>Fresh or frozen cycle?: fresh cycle</p> <p>IVF or ICSI?: IVF and ICSI</p> <p>Length of time exposed to intervention medium: 5 days</p> <p>Trade name and concentration of granulocyte macrophage colony-stimulating factor (GM-CSF) in intervention medium: EmbryoGen days 0 to 3 followed by BlastGen days 3 to 5</p> <p>Trade name of control medium: "Standard embryo culture medium"</p> <p>Day of embryo transfer: day 5 (apart from 1 in the control group and 2 in the intervention group who transferred 1 embryo on day 3 due to delayed embryo development)</p>

Rose 2020 (Continued)

Number of embryos transferred: single-embryo transfer. "Couples who insisted on transferring two embryos, once embryo quality was known, were included in the study but were recorded as having a trial variation (four in the standard group and six in the intervention group)."

Interventions	<p>Intervention: GM-CSF-supplemented culture medium</p> <p>Control: Sydney IVF medium</p>
Outcomes	<ul style="list-style-type: none"> • Live-birth rate • Miscarriage rate • Clinical pregnancy rate • Multiple gestation rate • Preterm birth rate • Birth defect rate • Aneuploidy rate • Stillbirth rate
Notes	Email correspondence with all additional data outlined in Appendix 13 .

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"50 cards with control and 50 with BlastGen written on them were placed in opaque sealed envelopes by a person unrelated to the trial. They were shuffled several times and the envelopes were marked from 1-100 in consecutive order."
Allocation concealment (selection bias)	Low risk	"When the eligible patient was scheduled for an egg retrieval the embryologist took the next envelope by sequence and the random assignment was made."
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Clinicians, sonographers, statisticians, and patients were completely blinded"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Sonographers were blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All dropouts accounted for.
Selective reporting (reporting bias)	Low risk	"We reported all outcomes as per our prospective clinical trials registration (ClinicalTrials.gov Identifier: NCT02305420)."
Other bias	High risk	There is a potential conflict of interest regarding the funding and administration of this study, as it was granted by the manufacturer of the intervention culture medium. However, the study authors have been very forthcoming with information regarding the trial, and there has been transparency. We assessed the study as at high risk of other bias due to the small sample size of 100 participants.

Sbracia 2014
Study characteristics

Methods	<p>Study design: single-centre RCT</p> <p>Country: Italy</p> <p>Setting: fertility clinic</p> <p>Sponsorship source: no funding</p> <p>Trials registry number: NCT01718210 (prospectively registered)</p>
Participants	<p>Inclusion criteria: women with recurrent implantation failure, 3 or more consecutive failed IVF cycles with a total of at least 8 good embryos replaced in the uterus, women aged 40 or less</p> <p>Exclusion criteria: over 40 years of age, chromosomal defects in the couple, metabolic diseases (diabetes, etc.), other genetic diseases (thalassaemia, cystic fibrosis, etc.)</p> <p>Number of participants randomised to each arm of the study: 50 to the intervention arm and 50 to the control arm</p> <p>Dropouts: none</p> <p>Group differences: not disclosed</p> <p>Fresh or frozen cycle?: fresh cycle</p> <p>IVF or ICSI?: ICSI</p> <p>Length of time exposed to intervention medium: from fertilisation to embryo transfer</p> <p>Trade name and concentration of GM-CSF in intervention medium: EmbryoGen</p> <p>Trade name of control medium: not disclosed</p> <p>Day of embryo transfer: not disclosed</p> <p>Number of embryos transferred: a maximum of 3 embryos were transferred</p>
Interventions	<p>Intervention: GM-CSF-supplemented culture medium</p> <p>Control: standard IVF medium</p>
Outcomes	Clinical pregnancy rate
Notes	Emailed 29 October 2019 and 9 January 2020 for further information, but received no response

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Patients were randomised by a computer generated number sequence"
Allocation concealment (selection bias)	Unclear risk	Allocation concealment not described
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No description of who, if anyone, was blinded

Sbracia 2014 (Continued)

Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No description of blinding of outcome assessors
Incomplete outcome data (attrition bias) All outcomes	Low risk	Intention-to-treat data reported, and no dropouts described.
Selective reporting (reporting bias)	Low risk	The 2 outcomes reported in the study were outlined on the trials registry ClinicalTrials.gov Identifier NCT01718210.
Other bias	High risk	Small sample size of 100 participants

Zafardoust 2017
Study characteristics

Methods	<p>Study design: single-centre RCT</p> <p>Country: Iran</p> <p>Setting: fertility clinic</p> <p>Sponsorship source: infertility and recurrent abortion treatment centre of Avicenna</p> <p>Trials registry number: www.irct.ir/trial/11703 (registered during recruitment)</p>
Participants	<p>Inclusion criteria: couples undergoing treatment with their own gametes, women aged < 40 years old, women with at least 4 good-quality embryos after thawing (grade A), women who had not had more than 1 previous embryo transfer</p> <p>Exclusion criteria: cycles requiring pre-implantation genetic diagnosis, women with an anatomic disorder of the uterus or hydrosalpinx/hydrosalpinges</p> <p>Number of participants randomised to each arm of the study: number randomised to each arm before dropout was not disclosed</p> <p>Group differences: the average age between the 2 groups differed</p> <p>Fresh or frozen cycle?: frozen cycles</p> <p>IVF or ICSI?: ICSI</p> <p>Length of time exposed to intervention medium: from embryo thawing through to embryo transfer</p> <p>Trade name and concentration of GM-CSF in intervention medium: BlastGen 2 ng/mL</p> <p>Trade name of control medium: Vitrolife, USA</p> <p>Day of embryo transfer: not disclosed</p> <p>Number of embryos transferred: not disclosed</p>
Interventions	<p>Intervention: GM-CSF-supplemented culture medium</p> <p>Control: standard IVF medium</p>
Outcomes	Clinical pregnancy rate

Zafardoust 2017 (Continued)

Notes Emailed on 29 October 2019, 26 November 2019, and 9 January 2020 for further information, but received no response

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No description of how randomisation was undertaken
Allocation concealment (selection bias)	Unclear risk	No description of how allocation concealment was undertaken
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No description of who, if anyone, was blinded
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Blinding of outcome assessors not described
Incomplete outcome data (attrition bias) All outcomes	High risk	10 women were not included in the final analysis, which is a high rate of attrition.
Selective reporting (reporting bias)	High risk	No data on miscarriage, multiple pregnancy, and beta human chorionic gonadotrophin (BHCG) levels, which are secondary outcomes noted on trials register
Other bias	High risk	Small sample size of 90 participants

Zavvar 2016
Study characteristics

Methods	<p>Study design: single-centre RCT</p> <p>Country: Iran</p> <p>Setting: fertility clinic</p> <p>Sponsorship source: not disclosed</p> <p>Trials registry number: not disclosed</p>
Participants	<p>Inclusion criteria: women who produce only immature oocytes in spite of stimulation with gonadotropins</p> <p>Exclusion criteria: not disclosed</p> <p>Number of participants randomised to each arm of the study: 31 women were randomised to receive the intervention medium, and 45 were randomised to receive the control medium</p> <p>Group differences: not disclosed</p> <p>Fresh or frozen cycle?: not disclosed</p>

Zavvar 2016 (Continued)

IVF or ICSI?: ICSI

Length of time exposed to intervention medium: not disclosed

Trade name and concentration of GM-CSF in intervention medium: MediCult 2 ng/mL

Trade name of control medium: not disclosed

Day of embryo transfer: not disclosed

Number of embryos transferred: not disclosed

Interventions	Intervention: GM-CSF-supplemented culture medium Control: standard IVF medium
Outcomes	Clinical pregnancy rate
Notes	First author contacted by email for further information on 26 November 2019 and 9 January 2020, but no response received.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No description of how random sequence was generated
Allocation concealment (selection bias)	Unclear risk	No description of how allocation was concealed
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Blinding was not mentioned.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Blinding of outcome assessors was not described.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	We assessed the study as at unclear risk, as we do not have access to number of dropouts or indeed how many participants were included in the analysis.
Selective reporting (reporting bias)	Unclear risk	No access to a protocol or a trial registry entry
Other bias	High risk	Small sample size of 76 participants

Ziebe 2013
Study characteristics

Methods	Study design: multicentre RCT (14 different clinics) Country: study co-ordinated in the Netherlands. Participants were recruited from clinics in Sweden and Denmark.
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Ziebe 2013 (Continued)

Setting: fertility clinics

Sponsorship source: supported by ORIGIO, Maløv, Denmark

Trials registry number: NCT00565747 (prospectively registered)

Participants	<p>Inclusion criteria: women aged 25 to 39 years, women who had a regular menstrual cycle of 21 to 35 days, women treated with a standard gonadotrophin-releasing hormone(GnRH) agonist or antagonist protocol, women with 3 or more follicles with a diameter of ≥ 14 mm on the day of human chorionic gonadotrophin (hCG) administration, including a leading follicle of ≥ 17 mm</p> <p>Exclusion criteria: previous participation in the study, use of assisted hatching, use of non-ejaculated sperm, medical conditions or genetic disorders prohibiting in vitro fertilisation(IVF)/ intracytoplasmic sperm injection (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</p> <p>Number of participants randomised to each arm of the study: 654 women randomised to receive GM-CSF-supplemented culture medium, and 678 women randomised to the control culture medium</p> <p>Group differences: no significant between-group differences in baseline characteristics</p> <p>Fresh or frozen cycle?: fresh</p> <p>IVF or ICSI?: IVF and ICSI</p> <p>Length of time exposed to intervention medium: from fertilisation through to embryo transfer</p> <p>Trade name and concentration of GM-CSF in intervention medium: ORIGIO 2 ng/mL</p> <p>Trade name of control medium: EmbryoAssist</p> <p>Day of embryo transfer: day 3</p> <p>Maximum number of embryos transferred: 2</p>
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Interventions	<p>Intervention: GM-CSF-supplemented culture medium</p> <p>Control: standard IVF medium</p>
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Outcomes	<ul style="list-style-type: none"> • Live-birth rate • Miscarriage rate • Clinical pregnancy rate • Multiple gestation rate • Preterm birth rate • Birth defect rate • Aneuploidy rate • Stillbirth rate
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Notes	Email correspondence with all additional data outlined in Appendix 13 .
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Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Randomisation (blocks of four) was computer-generated individually for each clinic to maintain balance between the treatment groups at each site."
Allocation concealment (selection bias)	Low risk	Following email correspondence: "Randomization was performed by ORIGIO a/s, and based on a randomisation list per clinic generated automatically using www.randomization.com. Each study site received a list of study specific consecutive patient ID numbers (e.g. clinic 1: 01001, 01002, 01003, 01004 etc.)"

Ziebe 2013 (Continued)

and a corresponding number of identically looking randomized bottles of test and control media individually labelled with the corresponding study specific patient ID numbers. On site the lowest number on the list was always allocated to any new patient recruited, at the time of informed consent signature. Therefore, both the clinician, embryologist and patient, was blinded to the treatment allocation. The master randomisation list was held by ORIGIO a/s. All data analysis was performed externally by a Clinical Research Organization (CRO). During the interim analysis the statistician was blinded at all times to the media, which were presented as Medium A and Medium B. For the final statistical analyses, the codes for blinding were broken after database lock and patient classification."

Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Participants and investigators were blinded to treatment allocation. Study media were packaged unidentifiably and labelled only with the randomization number. For each new patient recruited, the lowest available randomization number was used."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Following email correspondence: "The clinicians performing the ultrasound scans were blinded to the treatment allocation at all times."
Incomplete outcome data (attrition bias) All outcomes	High risk	All dropouts accounted for, however the reasons given were not included in the predefined exclusion criteria.
Selective reporting (reporting bias)	Low risk	The authors confirmed through email correspondence that all outcomes were published.
Other bias	High risk	There is a potential conflict of interest regarding the funding and administration of this study as it was granted by the manufacturer of the intervention culture medium. However, the study authors have been very forthcoming with information regarding the trial, and there has been transparency. We assessed this trial as at high risk of other bias because the data provided in private email correspondence differ from the published data.

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Agerholm 2010	Wrong study design
Fawzy 2019	Wrong study design
Kinoshita 2019	Wrong study design
Min 2017	Wrong study design
Scarpellini 2011	Wrong intervention
Sfontouris 2013	Wrong study design
Shapiro 2003	Wrong study design
Siqueira 2016	Animal study

Study	Reason for exclusion
Siristatidis 2013	Wrong study design
Sjoblom 1998	Wrong study design
Sjoblom 1999	Wrong study design
Sjoblom 1999a	Wrong study design
Sjoblom 2001	Wrong study design

Characteristics of studies awaiting classification [ordered by study ID]

ISRCTN94726536

Methods	Prospectively randomised controlled study
Participants	500 women undergoing ICSI and embryo culture Inclusion criteria: <ul style="list-style-type: none"> Female patients with at least 1 previous unsuccessful in vitro fertilisation (IVF) attempt, in which a clinical pregnancy was not established (including biochemical pregnancies or miscarriages) Aged between 25 and 45 Exclusion criteria: <ul style="list-style-type: none"> Women older than 45 years Patients with male partners with testicular spermatozoa or severe oligoasthenoteratozoospermia (total sperm number less than 1,000,000 and sperm motility less than 5%)
Interventions	Participants are randomly allocated to 1 of 2 groups. Group 1: Participants receive ICSI treatments carried out using ISM1 for embryo culture and transfer. Group 2: Participants receive ICSI treatments carried out using EmbryoGen for embryo culture and transfer.
Outcomes	Primary outcome measure: Implantation rate (defined as the number of implanted embryos per number of transferred embryos) is measured 30 days after embryo transfer. Secondary outcome measure: Pregnancy rate (defined as the number of pregnancies per number of transferred embryos) is measured using ultrasound of gestational chamber 30 days after embryo transfer.
Notes	Country of recruitment: Italy Overall trial status 'completed' Publication status 'results overdue' Unable to determine if women or embryos were randomised Emailed contact author 29 October 2019, but received no response

NCT01689428

Methods	Prospective randomised controlled trial
Participants	<p>100 infertile couples with an indication for standard IVF or intracytoplasmic sperm injection. All couples had at least a previous miscarriage and/or biochemical pregnancy experience after assisted reproductive technology (ART) treatment.</p> <p>Inclusion criteria:</p> <ul style="list-style-type: none"> • At least 1 (maximum 3) miscarriage and/or biochemical pregnancy after ART treatment <p>Exclusion criteria:</p> <ul style="list-style-type: none"> • Frozen spermatozoa • Frozen oocytes • Women with polycystic ovaries (PCO) and polycystic ovarian syndrome (PCOS) • Women with more than 4 ART treatments
Interventions	<p>Intervention: embryo culture in EmbryoGen medium</p> <p>Control: embryo culture in ISM1 medium</p>
Outcomes	<p>Primary outcome measure:</p> <p>Ongoing pregnancy rate after 12 weeks of gestation [Time Frame: 12 weeks after conception]</p> <p>Secondary outcome measure:</p> <p>Ongoing implantation rate after 12 weeks of gestation [Time Frame: 12 weeks after conception]</p>
Notes	<p>Country of recruitment: Italy</p> <p>Unable to determine if women or oocytes were randomised</p>

NCT01689454

Methods	Described as both a prospective randomised controlled trial and an observational cohort study
Participants	<p>Infertile couple with an indication for standard IVF or intracytoplasmic sperm injection. All couples had previous implantation failures after ART treatment (minimum 2, maximum 4 embryo replacements).</p> <p>Inclusion criteria:</p> <ul style="list-style-type: none"> • Women experiencing at least 2 (maximum 4) failures of implantation after ART treatments (with elective embryo transfer) <p>Exclusion criteria:</p> <ul style="list-style-type: none"> • Frozen spermatozoa • Frozen oocytes • Women PCO and PCOS • Women with previous pregnancy, miscarriage, or biochemical pregnancy
Interventions	<p>Control: embryo culture in ISM1 medium</p> <p>Intervention: embryo culture in EmbryoGen medium</p>

NCT01689454 (Continued)

- | | |
|----------|---|
| Outcomes | <ul style="list-style-type: none"> • Ongoing pregnancy rate after 12 weeks of gestation • Ongoing implantation rate after 12 weeks of gestation |
|----------|---|

Notes	Unable to determine if RCT or cohort study, as methods unclear
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NCT02651285

Methods	Randomised parallel assignment trial
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Participants	180 women aged 20 to 28 years
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Inclusion criteria:

- Infertility
- Healthy condition
- Good ovarian reserve (anti-mullerian hormone (AMH) levels more than 1 µg/mL)

Exclusion criteria:

- Chromosomal defects in the couple
- Metabolic diseases (diabetes, etc.)
- Other genetic diseases (thalassaemia, cystic fibrosis, etc.)

Interventions	Intervention: granulocyte colony-stimulating factor (G-CSF) medium Control: medium without G-CSF
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Outcomes	Primary outcome measures: <ul style="list-style-type: none"> • Pregnancy rate [Time Frame: 12 months] The number of participants who become pregnant after in vitro fertilisation (IVF) where G-CSF medium is used divided for the number of participants treated • Implantation rate [Time Frame: 12 months] The number of embryos implanted after IVF where G-CSF medium is used divided for the number of embryos transferred Secondary outcome measures: <ul style="list-style-type: none"> • Number of blastocysts developed [Time Frame: 12 months]. The number of blastocysts obtained after IVF where G-CSF medium is used divided for the number of fertilised oocytes
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Notes	Emailed authors for more information 29 October 2019, 9 January 2020, and 28 January 2020, but received no reply.
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	Unsure of nature of study and whether oocytes or women were randomised
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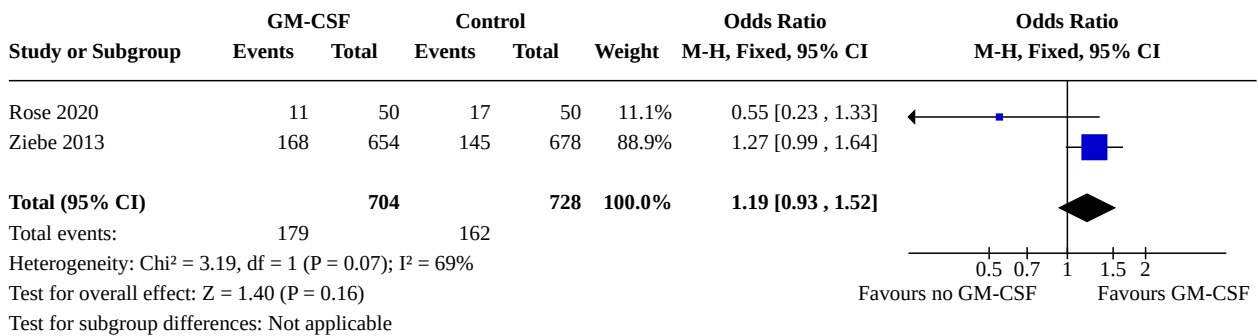
ICSI: intracytoplasmic sperm injection

DATA AND ANALYSES

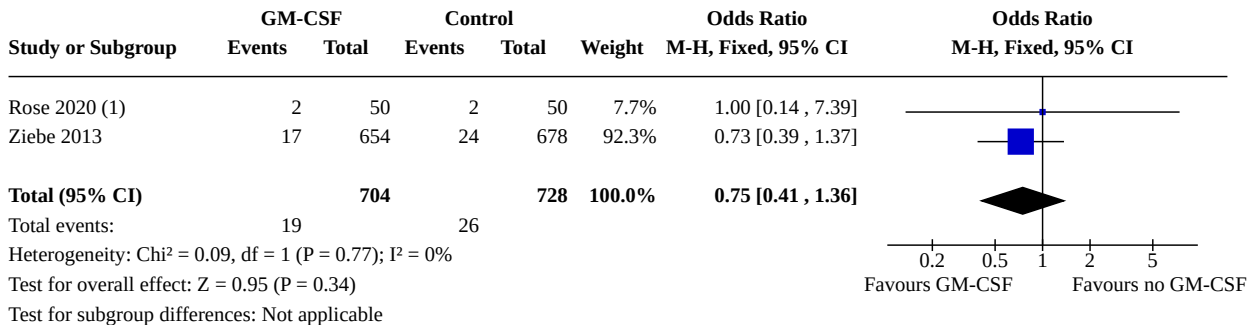
Comparison 1. GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Live birth	2	1432	Odds Ratio (M-H, Fixed, 95% CI)	1.19 [0.93, 1.52]
1.2 Miscarriage	2	1432	Odds Ratio (M-H, Fixed, 95% CI)	0.75 [0.41, 1.36]
1.3 Clinical pregnancy	3	1532	Odds Ratio (M-H, Fixed, 95% CI)	1.16 [0.93, 1.45]
1.4 Multiple gestation	2	1432	Odds Ratio (M-H, Fixed, 95% CI)	1.24 [0.73, 2.10]
1.5 Preterm birth	2	1432	Odds Ratio (M-H, Fixed, 95% CI)	1.20 [0.70, 2.04]
1.6 Birth defects	2	1432	Odds Ratio (M-H, Fixed, 95% CI)	1.33 [0.59, 3.01]
1.7 Aneuploidy	2	1432	Odds Ratio (M-H, Fixed, 95% CI)	0.34 [0.03, 3.26]
1.8 Stillbirth	2	1432	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable

Analysis 1.1. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 1: Live birth



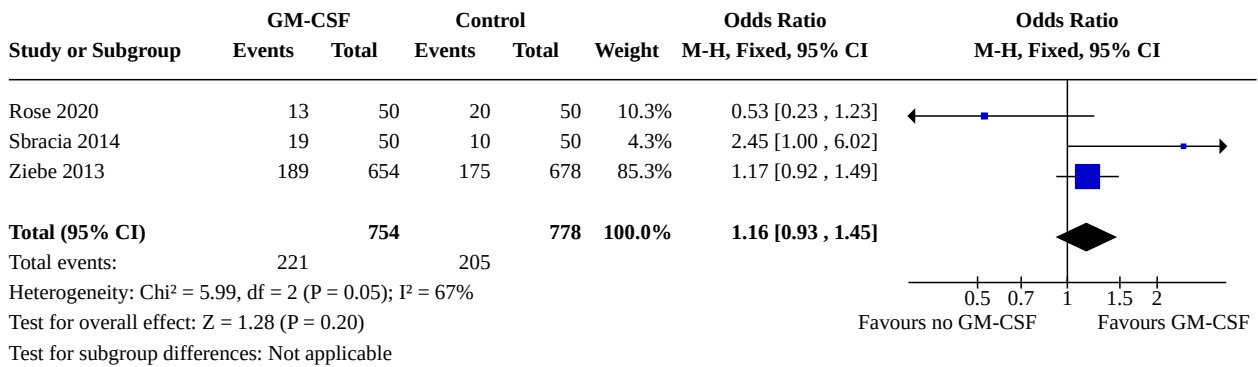
Analysis 1.2. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 2: Miscarriage



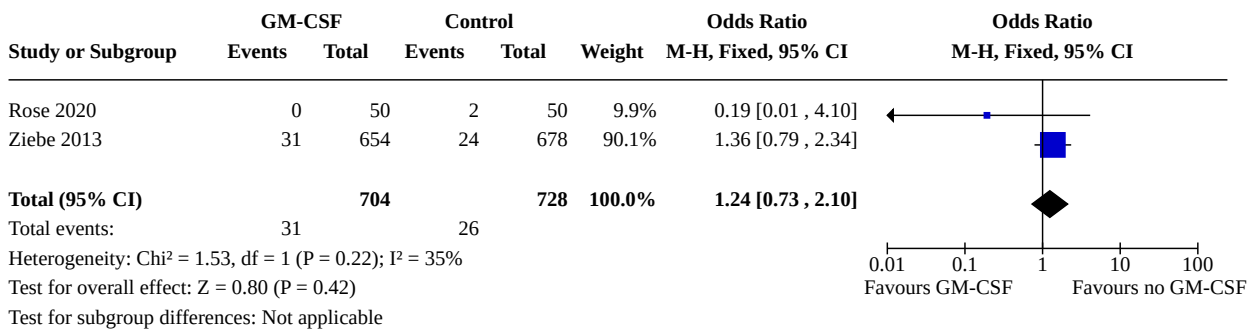
Footnotes

(1) One miscarriage in the control arm was at 17 weeks gestation, the remainder <12 weeks.

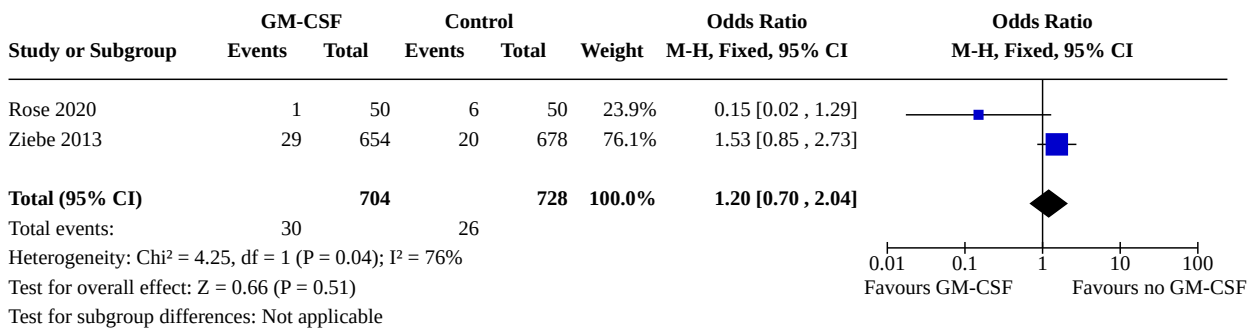
Analysis 1.3. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 3: Clinical pregnancy



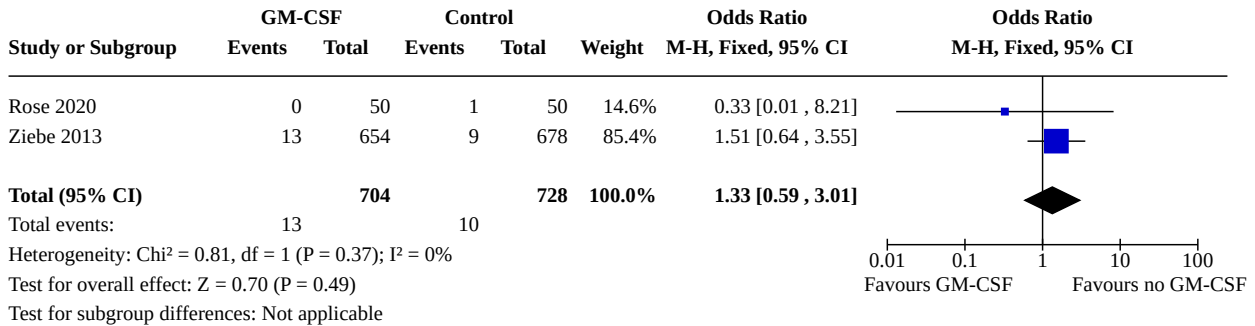
Analysis 1.4. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 4: Multiple gestation



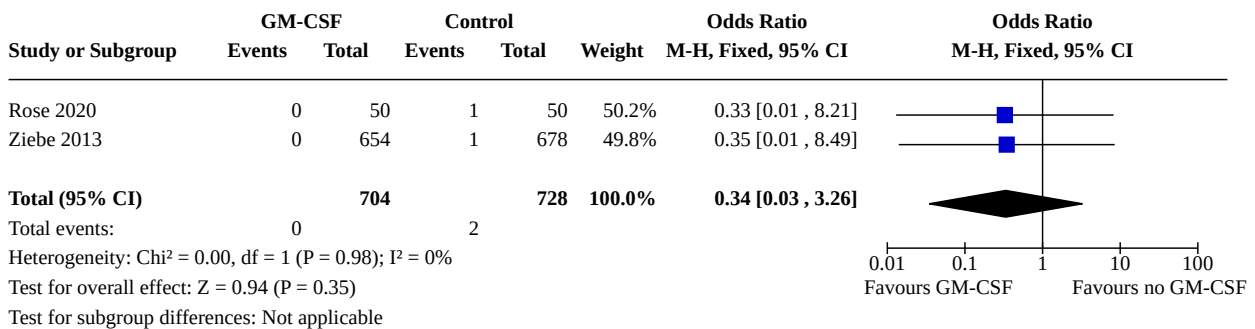
Analysis 1.5. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 5: Preterm birth



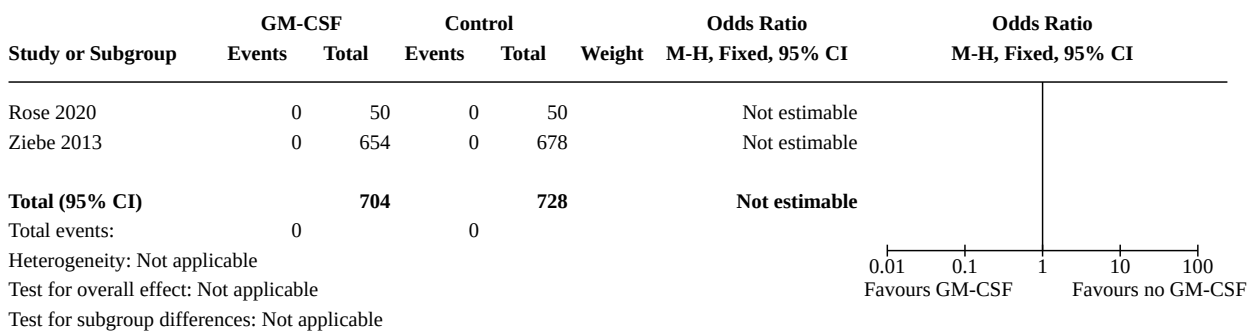
Analysis 1.6. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 6: Birth defects



Analysis 1.7. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 7: Aneuploidy



Analysis 1.8. Comparison 1: GM-CSF-supplemented culture medium versus culture medium not supplemented with GM-CSF, Outcome 8: Stillbirth



APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility Group Specialised Register search strategy

PROCITE platform

Searched 15 October 2019

Keywords CONTAINS "IVF" or "ICSI" or "ET" or "intracytoplasmic sperm injection techniques" or "intracytoplasmic sperm injection" or "in-vitro fertilisation " or "in vitro fertilization" or "Embryo Transfer" or "ovarian stimulation" or "ovarian stimulation controlled ovarian stimulation" or "ovulation induction" or "ovulation stimulation" or "superovulation" or "superovulation induction" or "ovarian hyperstimulation" or "poor prognostic patients" or "controlled ovarian hyperstimulation" or "controlled ovarian stimulation" or "COH" or "embryo culture" or "embryo culture media" or "blastocyst culture technique" or "blastocyst media" or "blastocyst" or "culture" or "Culture-Media" or "culture techniques" or Title CONTAINS "IVF" or "ICSI" or "ET" or "intracytoplasmic sperm injection techniques" or "intracytoplasmic sperm injection" or "in-vitro fertilisation " or "in vitro fertilization" or "Embryo Transfer" or "embryo culture" or "embryo culture media" or "blastocyst culture technique" or "blastocyst media" or "blastocyst" or "culture" or "Culture-Media" or "culture techniques"

AND

Keywords CONTAINS "granulocyte colony-stimulating factor" or "granulocyte macrophage colony stimulating factor" or "GM-CSF" or "G-CSF" or "Embryogen" or "Sage culture medium" or Title CONTAINS "granulocyte colony-stimulating factor" or "granulocyte macrophage colony stimulating factor" or "GM-CSF" or "G-CSF" or "Embryogen" or "Sage culture medium" (54 records)

Appendix 2. Cochrane Central Register of Controlled Trials (CENTRAL)

OVID platform

Searched 15 October 2019 (Issue 9, September 2019)

- 1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (2207)
- 2 vitro fertili?ation.tw. (2997)
- 3 ivf-et.tw. (583)
- 4 ivf.tw. (5555)
- 5 icsi.tw. (2657)
- 6 intracytoplasmic sperm injection\$.tw. (1133)
- 7 assisted reproduct\$.tw. (1459)
- 8 ovulation induc\$.tw. (1108)
- 9 (ovari\$ adj2 stimulat\$).tw. (2278)
- 10 superovulat\$.tw. (205)
- 11 ovarian hyperstimulation.tw. (1427)
- 12 COH.tw. (397)
- 13 infertil\$.tw. (6380)
- 14 subfertil\$.tw. (698)
- 15 (ovari\$ adj2 induction).tw. (61)
- 16 blastocyst\$.tw. (1240)
- 17 embryo\$.tw. (6590)
- 18 (recurrent adj3 miscarriage\$).tw. (268)
- 19 (recurrent adj3 abortion\$).tw. (231)
- 20 (recurrent adj3 implantation\$).tw. (180)
- 21 implantation failure\$.tw. (452)
- 22 or/1-21 (15673)
- 23 exp colony-stimulating factors/ or exp granulocyte-macrophage colony-stimulating factor/ or exp macrophage colony-stimulating factor/ (3300)
- 24 (CSF-2 or CSF-3).tw. (98)
- 25 (Colony stimulating adj3 factor\$).tw. (3135)
- 26 granulocyte macrophag\$.tw. (1092)
- 27 (Culture* adj5 growth factor*).tw. (40)
- 28 GM CSF.tw. (1674)
- 29 gcsf.tw. (262)
- 30 gmcsf.tw. (103)
- 31 g csf.tw. (2805)
- 32 (EmbryoGen or BlastGen or blastogen or Leucomax).tw. (24)
- 33 SAGE 1-step.tw. (4)
- 34 or/23-33 (7699)
- 35 22 and 34 (153)

Appendix 3. MEDLINE search strategy

Ovid platform

Searched from 1946 to 15 October 2019

GM-CSF (granulocyte macrophage colony-stimulating factor) supplementation in culture media for women undergoing assisted reproduction (Review)

40

- 1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (40326)
- 2 vitro fertili?ation.tw. (22322)
- 3 ivf-et.tw. (2249)
- 4 ivf.tw. (22695)
- 5 icsi.tw. (8019)
- 6 intracytoplasmic sperm injection\$.tw. (6901)
- 7 assisted reproduct\$.tw. (14151)
- 8 ovulation induc\$.tw. (4066)
- 9 (ovari\$ adj2 stimulat\$).tw. (6795)
- 10 superovulat\$.tw. (3348)
- 11 ovarian hyperstimulation.tw. (4929)
- 12 COH.tw. (1664)
- 13 infertil\$.tw. (58082)
- 14 subfertil\$.tw. (4898)
- 15 (ovari\$ adj2 induction).tw. (284)
- 16 blastocyst\$.tw. (21461)
- 17 embryo\$.tw. (341298)
- 18 (recurrent adj3 miscarriage\$).tw. (2188)
- 19 (recurrent adj3 abortion\$).tw. (2372)
- 20 (recurrent adj3 implantation\$).tw. (523)
- 21 implantation failure\$.tw. (1479)
- 22 or/1-21 (431419)
- 23 exp colony-stimulating factors/ or exp granulocyte-macrophage colony-stimulating factor/ or exp macrophage colony-stimulating factor/ (63409)
- 24 (CSF-2 or CSF-3).tw. (395)
- 25 (Colony stimulating adj3 factor\$).tw. (35409)
- 26 granulocyte macrophag\$.tw. (19010)
- 27 (Culture* adj5 growth factor*).tw. (5038)
- 28 GM CSF.tw. (18309)
- 29 gcsf.tw. (600)
- 30 gmcsf.tw. (472)
- 31 g csf.tw. (14257)
- 32 (EmbryoGen or BlastGen or blastogen or Leucomax).tw. (35)
- 33 SAGE 1-step.tw. (2)
- 34 or/23-33 (90883)
- 35 22 and 34 (1884)
- 36 randomized controlled trial.pt. (491373)
- 37 controlled clinical trial.pt. (93315)
- 38 randomized.ab. (457040)
- 39 randomised.ab. (91296)
- 40 placebo.tw. (206997)
- 41 clinical trials as topic.sh. (188692)
- 42 randomly.ab. (319649)
- 43 trial.ti. (206046)
- 44 (crossover or cross-over or cross over).tw. (81967)
- 45 or/36-44 (1306338)
- 46 exp animals/ not humans.sh. (4627546)
- 47 45 not 46 (1201919)
- 48 35 and 47 (48)

Appendix 4. Embase search strategy

Ovid platform

Searched from 1980 to 15 October 2019

- 1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (67279)
- 2 vitro fertili?ation.tw. (29584)
- 3 icsi.tw. (15791)
- 4 intracytoplasmic sperm injection\$.tw. (9347)
- 5 ivf.tw. (39523)
- 6 assisted reproduct\$.tw. (21962)
- 7 ovulation induc\$.tw. (5543)

GM-CSF (granulocyte macrophage colony-stimulating factor) supplementation in culture media for women undergoing assisted reproduction (Review)

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8 (ovari\$ adj2 stimulat\$.tw. (10800)
 9 superovulat\$.tw. (3777)
 10 ovarian hyperstimulation.tw. (7354)
 11 COH.tw. (2380)
 12 infertile.tw. (81510)
 13 subfertile.tw. (6767)
 14 (ovari\$ adj2 induction).tw. (330)
 15 blastocyst\$.tw. (28689)
 16 embryo\$.tw. (378546)
 17 (recurrent adj3 miscarriage\$.tw. (3806)
 18 (recurrent adj3 abortion\$.tw. (3314)
 19 (recurrent adj3 implantation\$.tw. (1112)
 20 implantation failure\$.tw. (3091)
 21 or/1-20 (500910)
 22 exp colony stimulating factor/ (5954)
 23 exp granulocyte macrophage colony stimulating factor/ (37514)
 24 exp colony stimulating factor 1/ (12734)
 25 (CSF-2 or CSF-3).tw. (635)
 26 (Colony stimulating adj3 factor\$.tw. (41992)
 27 granulocyte macrophag\$.tw. (21016)
 28 (Culture* adj5 growth factor*).tw. (5955)
 29 GM CSF.tw. (25621)
 30 gcsf.tw. (2478)
 31 gmcsf.tw. (1648)
 32 g csf.tw. (23305)
 33 SAGE 1-step.tw. (14)
 34 (EmbryoGen or blastGen or blastogen or Leucomax).tw. (420)
 35 or/22-34 (101247)
 36 Clinical Trial/ (954250)
 37 Randomized Controlled Trial/ (572677)
 38 exp randomization/ (84808)
 39 Single Blind Procedure/ (36997)
 40 Double Blind Procedure/ (164394)
 41 Crossover Procedure/ (61141)
 42 Placebo/ (330524)
 43 Randomized controlled trial\$.tw. (213916)
 44 Rct.tw. (34381)
 45 random allocation.tw. (1919)
 46 randomly.tw. (420773)
 47 randomly allocated.tw. (33522)
 48 allocated randomly.tw. (2487)
 49 (allocated adj2 random).tw. (810)
 50 Single blind\$.tw. (23587)
 51 Double blind\$.tw. (197107)
 52 ((treble or triple) adj blind\$.tw. (1025)
 53 placebo\$.tw. (293366)
 54 prospective study/ (557816)
 55 or/36-54 (2331046)
 56 case study/ (65015)
 57 case report.tw. (385105)
 58 abstract report/ or letter/ (1076178)
 59 or/56-58 (1516287)
 60 55 not 59 (2278531)
 61 (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.) (5836378)
 62 60 not 61 (2120418)
 63 21 and 35 and 62 (179)

Appendix 5. CINAHL search strategy

Ebsco platform

Searched from 1961 to 15 October 2019

S58 S34 AND S57 16
 S57 S56 NOT S55 598,106
 S56 S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41 OR S42 OR S43 OR S44 OR S45 OR S46 OR S47 OR S48 OR S49 625,300
 S55 S53 NOT S54 163,549
 S54 MH (human) 1,982,918
 S53 S50 OR S51 OR S52 185,486
 S52 TI (animal model*) 2,818
 S51 MH (animal studies) 107,579
 S50 MH animals+ 85,636
 S49 AB (cluster W3 RCT) 302
 S48 MH (crossover design) OR MH (comparative studies) 236,274
 S47 AB (control W5 group) 94,594
 S46 PT (randomized controlled trial) 86,203
 S45 MH (placebos) 11,435
 S44 MH (sample size) AND AB (assigned OR allocated OR control) 3,699
 S43 TI (trial) 95,545
 S42 AB (random*) 269,798
 S41 TI (randomised OR randomized) 93,431
 S40 MH cluster sample 3,898
 S39 MH pretest-posttest design 38,357
 S38 MH random assignment 55,470
 S37 MH single-blind studies 12,785
 S36 MH double-blind studies 42,502
 S35 MH randomized controlled trials 86,500
 S34 S21 AND S33 76
 S33 S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 8,753
 S32 TX SAGE 1-step 18
 S31 TX (Culture* N5 growth factor*) 607
 S30 TX (EmbryoGen or BlastGen or blastogen or Leucomax) 1
 S29 TX g csf 2,195
 S28 TX gmcsf 34
 S27 TX gcsf 96
 S26 TX GM CSF 824
 S25 TX granulocyte macrophag* 1,373
 S24 TX (Colony stimulating N3 factor*) 4,099
 S23 TX (CSF-2 or CSF-3) 2
 S22 (MM "Colony-Stimulating Factors+") OR (MM "Granulocyte Colony-Stimulating Factor") OR (MM "Granulocyte-Macrophage Colony-Stimulating Factor") 3,955
 S21 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S17 OR S18 OR S19 OR S20 32,225
 S20 TX implantation failure* 5,559
 S19 TX recurrent N3 implantation* 159
 S18 TX recurrent N3 abortion* 205
 S17 TX recurrent N3 miscarriage* 434
 S16 TX embryo* 22,469
 S15 TX blastocyst* 2,163
 S14 TX subfertil* 893
 S13 TX infertil* 16,457
 S12 TX COH 234
 S11 TX ovarian hyperstimulation 825
 S10 TX superovulat* 84
 S9 TX ovulation induc* 1,856
 S8 TX assisted reproduct* 4,780
 S7 TX intracytoplasmic sperm injection* 878
 S6 TX vitro fertili?ation 6,908
 S5 (MM "Embryo Transfer") 1,079
 S4 TX ovar* N3 hyperstimulat* 825
 S3 TX ovari* N3 stimulat* 978
 S2 TX IVF or TX ICSI 4,883
 S1 (MM "Fertilization in Vitro") 3,376

Appendix 6. LILACS search strategy

Web platform

Searched 15 October 2019

colony stimulating factor and embryo*

colony stimulating factor and ivf*

Appendix 7. ClinicalTrials.gov and WHO ICTRP search strategy

Web platform

Searched 15 October 2019

csf* and embryo*

csf* and ivf*

colony stimulating factor and embryo

colony stimulating factor and ivf

Appendix 8. DARE search strategy

Web platform

Searched 15 October 2019

csf* and embryo*

csf* and ivf*

colony stimulating factor and embryo

colony stimulating factor and ivf

Appendix 9. Web of Knowledge search strategy

Web platform

Searched 15 October 2019

csf* and embryo*

csf* and ivf*, limited by Web of Science Categories REPRODUCTIVE BIOLOGY, OBSTETRICS GYNECOLOGY and MEDICINE RESEARCH EXPERIMENTAL

Appendix 10. OpenGrey search strategy

Web platform

Searched 15 October 2019

csf* and embryo*

csf* and ivf*

Appendix 11. PubMed search strategy

Web platform

Searched 15 October 2019

(colony stimulating factor [title] OR csf [title] AND embryo* [Title/Abstract] OR ivf* [Title/Abstract] limited by clinical trials and randomised controlled trial

Appendix 12. Google Scholar search strategy

Web platform

Searched 15 October 2019

(csf AND embryo* OR ivf*)

(colony stimulating factor AND embryo*)

Appendix 13. Correspondence with authors of included studies

Correspondence with the authors of Ziebe 2013

Amendment #1 for Clinical Investigation Report, DK001 The effect of granulocyte-macrophage colony stimulation factor (GM-CSF) during in vitro culture of human embryos on subsequent implantation rates (DK001) Rep1.02.03.05

Issued by: Senior Clinical Coordinator, Bibi Munding Rasmussen Consultant, Sandra Maher. This confidential document is the property of Cooper Surgical. Permission has been granted by Cooper Surgical to publish this correspondence.

Background: The Cochrane organization is working on a review concerning GM-CSF containing culture media and are including the DK001 study, published in "Fertility and Sterility" (Ziebe et al. 2013). In relation to this they have approached Søren Ziebe, Professor in Clinical Embryology and principal investigator for the DK001 study, for information on the Intend-to treat (ITT) population and some clarifications.

Information requested and answers:

1) Please could you let us know the clinical pregnancy rate (ongoing implantation rate at 7 weeks) per woman randomised? i.e. twin or triplet pregnancy would be n=1.

Answer provided by e-mail 2019.Dec.06; Søren Ziebe (Rigshospitalet, DK) and Bibi Munding Rasmussen (Cooper Surgical - ORIGIO a/s): Based on the intend-to-treat (ITT) data analysis, the clinical outcome gestational week 7 per woman randomised was as follows:

1. Clinical pregnancy rate (CPR): GM-CSF: 28.9% (189/654) Control: 25.8% (175/678)

2. Ongoing CPR: GM-CSF: 26.5% (173/654) Control: 23.5% (159/678)

Further information: Please notice that the objective of the DK001 study was to evaluate effect of GM-CSF in embryo culture medium on ongoing implantation rate (OIR), which is the reason why the publication is focusing on OIR's and supported with data on ongoing clinical pregnancy rates gestational week 7 and 12. The primary endpoint OIR gestational week 7 was chosen based on previous studies indicating a protective effect of GM-CSF on culture-induced embryo stress. Ziebe et al. (2013) report data on the per-protocol (PP) population, which is the subset of ITT patients fulfilling inclusion/exclusion criteria, who had an embryo transfer Day 3 and did not violate the protocol in a way that could impair evaluation of the primary endpoint, ongoing OIR gestational week 7.

For a comparison of the ITT- and PP-population, we suggest presenting data per embryo transfer cycle (all transfer patients):

ITT-population:

3. Clinical pregnancy rate per transfer (CPR): GM-CSF: 32.2% (189/587) Control: 28.9% (175/605)

4. Ongoing CPR per transfer: GM-CSF: 29.5% (173/587) Control: 26.3% (159/605)

PP-population:

5. Clinical pregnancy rate per transfer (CPR): GM-CSF: 32.4% (183/564) Control: 29.2% (171/585)

6. Ongoing CPR per transfer: GM-CSF: 29.6% (167/564) Control: 26.5% (155/585)

Please refer to the enclosed Table A, for further information on clinical pregnancy data for both the ITT- and PP-population, calculated per embryo transfer cycle and including data on the primary outcome OIR.

References: 1a) and 1b)

1b) Please can you confirm whether the clinical pregnancies at 7 weeks were all scanned at this stage?

The scanning gestational week 7 was defined like this: week 7 plus 0-6 days; and day 0 calculated as "the day of oocyte aspiration" minus "14 days".

Out of total 364 ITT- patients with a clinical pregnancy (GM-CSF: 189; Control: 175), there were 37 patients where the ultrasound scanning gestational week 7 was performed either too early (GM-CSF: 9; Control: 4) or too late (GM-CSF: 12; Control: 12).

None of the patients were undergoing ultrasound scanning before gestational week 6, and the cases where the scanning was performed too late were all performed within gestational week 8-10. Moreover, there have been no overestimations of clinical pregnancy rate gestation week 7, as majority of deviations were due to scanning performed later than gestational week 7. This is further supported by the fact that the number of miscarriages observed gestational week 7 were similar in the two groups (GM-CSF: 14; Control: 17), while the miscarriages observed gestational week 12 were higher in the control group (GM-CSF: 3; Control: 10).

Please refer to the enclosed Table B, for an overview of deviations concerning the ultrasound scanning gestational week 7 for both the ITT- and PP-population.

Reference: 2a)

2) Please could you confirm how allocation was concealed? For example, who performed the randomisation? Was it done electronically or with envelopes? Who held the master randomisation list?

Answer provided by e-mail 2019.Dec.06; Søren Ziebe (Rigshospitalet, DK) and Bibi Munding Rasmussen (CooperSurgical - ORIGIO a/s): Randomization was performed by Cooper Surgical - ORIGIO a/s, and based on a randomisation list per clinic generated automatically using www.randomization.com. Each study site received a list of study specific consecutive patient ID numbers (e.g. clinic 1: 01001, 01002, 01003, 01004 etc.) and a corresponding number of identically looking randomized bottles of test and control media individually labelled with the corresponding study specific patient ID numbers. On site the lowest number on the list was always allocated to any new patient recruited, at the time of informed consent signature. Therefore, both the clinician, embryologist and patient, was blinded to the treatment allocation. The master randomisation list was held by ORIGIO a/s. All data analysis was performed externally by a Clinical Research Organization (CRO) and performed on blinded data. **CORRECTION:** During the interim analysis the statistician was blinded at all times to the media, which were presented as Medium A and Medium B. For the final statistical analyses, the codes for blinding were broken after database lock and patient classification.

Reference: 2c)

3) Please could you clarify whether those who performed the ultrasound scans were blinded to the interventions?

Answer provided by e-mail 2019.Dec.06; Søren Ziebe (Rigshospitalet, DK) and Bibi Munding Rasmussen (Cooper Surgical - ORIGIO a/s): Yes, also the clinicians performing the ultrasound scans were blinded to the treatment allocation at all times.

Reference: 2c)

4) Please clarify the number of twins in the intervention group. Under table 1 you write that there were 2x27 sets of twins for the intervention group. However, in table 1 you write that 58 twins were born which equals 29 sets of twins.

Answer provided by e-mail 2019.Dec.06; Søren Ziebe (Rigshospitalet, DK) and Bibi Munding Rasmussen (CooperSurgical – ORIGIO a/s): Apologize for the inconsistency in reporting. The correct numbers are as follows: GM-CSF: 58 twin births, corresponding to 29 sets of twins; Control: 50 twin births, corresponding to 25 sets of twins. **CORRECTION:** As stated under the answer for question 1), Ziebe et al. (2013) report data on the PP population, which is the subset of ITT patients fulfilling the inclusion/exclusion criteria, who had an embryo transfer Day 3 and did not violate the protocol in a way that could impair evaluation of the primary endpoint, ongoing implantation week 7.

Please notice, that the numbers stated below Table 1 in the publication by Ziebe et al. (2013) is number of embryos implanted (ongoing) as dizygotic twins (2x27 for GM-CSF group and 2x20 for the control group) and number of embryos implanted (ongoing) as monozygotic twins (2x4 for GM-CSF group and 2x3 for the control group). Therefore, these numbers are including the triplet pregnancy in the GM-CSF group resulting from transfer of two embryos, which in principal is due to implantation of 2 dizygotic twins and 2 monozygotic twins, although one of the twins is forming part of both twin groups. In other words, the difference in numbers reported by Ziebe et al. in Table 1 and below the table, is due to the fact that the table is presenting gestational age and birth weight according to whether the children born was a result of a singleton, twin or triplet live birth; while the ongoing implantation of twins stated below the table is counting the triplet pregnancy as 4 ongoing implantations in the GM-CSF group (dizygotic twins: 1x2; monozygotic twins: 1x2).

Please have an overview of dizygotic and monozygotic twins born after twin pregnancies: Number of twins born in the PP-population: GM-CSF: 58 twins, corresponding to 29 sets of twins (2x26 dizygotic twins, 2x3 monozygotic twins). Control: 46 twins, corresponding to 23 sets of twins (2x20 dizygotic twins, 2x3 monozygotic twins). Number of twins born in the ITT-population: GM-CSF: 60 twins, corresponding to 30 sets of twins (2x27 dizygotic twins, 2x3 monozygotic twins). Control: 48 twins, corresponding to 24 sets of twins (2x21 dizygotic twins, 2x3 monozygotic twins). When including the triplet pregnancy as 4 embryos implanted due to 1 set of dizygotic twins and 1 set of monozygotic twins, the number of embryos implanted (ongoing) as twins in the GM-CSF groups changes to 2x27 dizygotic twins, 2x4 monozygotic twins, as stated below Table 1 in the publication by Ziebe et al. (2013). Please refer to the enclosed Table A, for an overview of children born for both the ITT- and PP-population.

Reference: 3a), 3b) and 3c)

5) Please could you confirm that you have published all the outcomes you set out to assess?

Answer provided by e-mail 2019.Dec.06; Søren Ziebe (Rigshospitalet, DK) and Bibi Munding Rasmussen (CooperSurgical – ORIGIO a/s): Yes

Reference: 2c)

6) Number of live births.

ITT-population: 7. Live birth rate per transfer: GM-CSF: 28.6% (168/587) Control: 24.0% (145/605)

PP-population: 8. Live birth rate per transfer: GM-CSF: 28.9% (163/564) Control: 24.1% (141/585)

Please refer to the enclosed Table A, for further information on clinical outcome for both the ITT- and PP-population, calculated per embryo transfer cycle.

References: 1a), 1b) and 1c)

7) Number of stillbirths.

There were no stillbirths, neither in the GM-CSF nor in the control group. Please refer to the enclosed Table A, for further information on clinical outcome for both the ITT- and PP-population, calculated per embryo transfer cycle.

Reference: 1d)

8) Number of ectopic pregnancies.

ITT-population: 9. Ectopic pregnancies per transfer: GM-CSF: 0.7% (4/587) Control: 0.3% (2/605)

PP-population: 10. Ectopic pregnancies per transfer: GM-CSF: 0.7% (4/564) Control: 0.3% (2/585)

Please refer to the enclosed Table A, for further information on clinical outcome for both the ITT- and PP-population, calculated per embryo transfer cycle.

References: 1a) and 1b)

9a) Miscarriage of clinical preg (up to 20/40).

ITT-population: 11. Late pregnancy loss (>week 12) per transfer: GM-CSF: 0.3% (2/587) Control: 0.7% (4/605)

PP-population: 12. Late pregnancy loss (>week 12) per transfer: GM-CSF: 0.4% (2/564) Control: 0.7% (4/585)

Please refer to the enclosed Table A, for further information on clinical outcome for both the ITT- and PP-population, calculated per embryo transfer cycle.

References: 1a) and 1b)

9b) We are interested in miscarriage of clinic pregnancy, so this is miscarriages of those women who were pregnant at 7/40 following evidence of gestational sac on scan, which you kindly clarified was all of these women. GM-CSF 189 were pregnant at 7/40. Minus 168 live births = 21 pregnancy losses. We know there were 17 miscarriages in this group, plus 2 terminations of pregnancy. This makes 19 pregnancy losses. Can you please clarify the other 2 pregnancy losses. I'm guessing they weren't ectopics as these are generally picked up before 7 weeks' gestation? Control group 175 women were pregnant at 7/40. Minus 145 live birth= 30 pregnancy losses. We know there were 27 pregnancy losses, plus 4 termination of pregnancies. This totals 31 pregnancy losses. Please could you kindly clarify?

Please know that in order to be able to calculation live birth based on clinical pregnancies week 7 minus miscarriages and ectopic pregnancies, you need to account for 5 mistakes in how results of the scanning gestational week 7 were reported.

GM-CSF: 2 out of the 4 patients with an ectopic pregnancy were not accounted for as clinical pregnancy week 7. CONTROL: 3 out of the 27 patients with miscarriage week 7 were not accounted for as clinical pregnancy week 7. All 5 patients were forming part of both the ITT- and PP-population. As these mistakes are causing similar underestimations of clinical pregnancy and implantation rate week 7 in both groups, without affecting the endpoints or other outcome parameters of the study, we did not make a correction, Please have an overview of data, where these 5 patients have been accounted for:

	ITTGM-CSF	ITT Control	PP GM-CSF	PP Control
Clinical pregnancy week 7	191 (189+2)	178 (175+3)	185 (183+2)	174 (171+3)
Miscarriages <= week 12	17	27	16	27
Miscarriages/elective terminations > week 12	2	4	2	4
Ectopic pregnancies	4	2	4	2
Live birth	168	145	163	141

References: 1e

10) Multiple pregnancies

ITT-population:

13. Twin pregnancies per transfer: GM-CSF: 5.1% (30/587) Control: 4.0% (24/605)

14. Triplet pregnancies per transfer: GM-CSF: 0.2% (1/587) Control: 0.0% (0/605)

PP-population:

15. Twin pregnancies per transfer: GM-CSF: 5.1% (29/564) Control: 3.9% (23/585)

16. Triplets pregnancies per transfer: GM-CSF: 0.2% (1/564) Control: 0.0% (0/585)

Please refer to the enclosed Table A, for further information on clinical outcome for both the ITT- and PP-population, calculated per embryo transfer cycle.

Reference: 3a), 3b) and 3c)

11a) You mentioned terminations of pregnancy as a result of malformations. Please could you confirm whether these were isolated defects or trisomies? If at all possible we'd love a breakdown of reasons for termination. We are particularly interested in aneuploidy and birth defects.

The study was not powered to draw conclusions concerning malformations, but rates were similar without any trends in the two groups (GM-CSF or control). The number and types of miscarriage/elective termination due to malformations after gestational week 12 are list in the table next page.

11b) Your paper states that there were abnormalities detected within 1 week of birth in 11/654 in the intervention group and 8/678 in the control group. Please can we confirm whether these number contain the termination of pregnancy babies or not? If possible, please could we have a breakdown of the aneuploidies/abnormalities for all 11 and 8 babies in these groups?

Please know that the abnormalities reported as detected within 1 week of birth, does not include the termination of pregnancy babies:

GM-CSF	Control
Elective terminations >week 12	
1. fetus with acrania	1. dead fetus without further information
2. fetus with myelomeningocele: a developmental congenital disorder caused by incomplete closing of the neural tube	2. fetus with multiple malformations (trisomy for chromosome 13)
	3. fetus with hypocephalus and myelomeningocele

(Continued)

4. fetus due to risk of serious physical disorder due to injury or illness during prenatal development

Live births

1. fetus with hypospadias glandis/penis arcuatus	1. Preterm birth week 27. The child died due to immature lungs
2. fetus with hypospadias glandis	2. Hypospadias without specifications
3. fetus with retentio testis unilateralis	3. Mild hypospadias
4. fetus with ductus arteriosus persistens	4. Retentio testis unilateralis
5. fetus with stenosis arteriae pulmonalis congenita	5. Heart failure and chromosome deletion (Steno Fallots tetralogy)
6. fetus with defectus septi ventriculorum cordis/defectus septi atriorum cordis	6. Twins with defectus septi atriorum cordis and one of the twins also had ductus arteriosus persistens
7. fetus with defectus septi atriorum cordis/ductus arteriosus persistens	7. Palatoschisis unilateralis without specifications
8. fetus with hernia diaphragmatica congenita	8. Congenital malformation in the hip without specifications
9. fetus with luxatio coxae congenita unilateralis	
10. fetus with talipes equinovarus	
11. fetus with congenital malformation in one foot without specifications	

All cases were forming part of both the ITT- and PP-population.

12) Please can we check that all the twins and triplets delivered <37 week's gestation? Preterm birth is one of our outcomes and I can see that you've documented preterm birth for singletons, but we need to add the multiple gestation pregnancies too. Please can you confirm?

(N.B. twin and triplet numbers represent actual numbers of infants)

ITT population, singletons			PP population, singletons	
Gestational age	GM-CSF	CONTROL	GM-CSF	CONTROL
>=wk 37+0	124	112	120	110
>=wk 32+0 and <wk 37+0	9	8	9	7
<wk 32+0	4	1	4	1
TOTAL	137	121	133	118
ITT population, twins			PP population, twins	

(Continued)

>/=wk 37+0	30	26	30	24
>/=wk 32+0 and <wk 37+0	28	18	26	18
<wk 32+0	2	4	2	4
TOTAL	60	48	58	46
ITT population, triplets		PP population, triplets		
>/=wk 37+0				
>/=wk 32+0 and <wk 37+0	3		3	
<wk 32+0				
TOTAL	3	0	3	0

Reference: 2b)

Table A: Overview of clinical data for ITT- and PP-population

DK001	ITT		PP	
	GM-CSF	CONTROL	GM-CSF	CONTROL
#Patients randomized	654	678		
#Transfer cycles	587	605	564	585
#Embryos transferred	874	913	838	882
Embryos transferred (mean)	1.49	1.51	1.49	1.51
#Ongoing implantation w.7	204	181	197	176
Ongoing implantation w.7 (%)	23.3+/-1.4	19.8+/-1.3	23.5+/-1.5	20.0+/-1.3
#Ongoing implantation w.12	199	170	193	165
Ongoing implantation w.12 (%)	22.8+/-1.4	18.6+/-1.3	23.0+/-1.5	18.7+/-1.3
#Clinical Pregnancies w.7	189	175	183	171
Clinical Pregnancy rate w.7 (%)	32.2	28.9	32.4	29.2
#Ongoing clinical pregnancies w.7	173	159	167	155
Ongoing clinical pregnancy rate w.7 (%)	29.5+/-1.9	26.3+/-1.8	29.6+/-1.9	26.5+/-1.8
#Clinical pregnancies w.12	171	155	166	151
Clinical Pregnancy rate w.12 (%)	29.1	25.6	29.4	25.8

GM-CSF (granulocyte macrophage colony-stimulating factor) supplementation in culture media for women undergoing assisted reproduction (Review)

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(Continued)

#Ongoing clinical pregnancies w.12	170	149	165	145
Ongoing clinical pregnancy rate w.12 (%)	29.0+/-1.9	24.6+/-1.8	29.3+/-1.9	24.8+/-1.8
#live birth	168	145	163	141
Live birth rate (%)	28.6+/-1.9	24.0+/-1.7	28.9+/-1.9	24.1+/-1.8
#Stillborn children	0	0	0	0
#Liveborn children	200	169	194	164
#Singletons	137	121	133	118
#Twins	60	48	58	46
Dizygotic	54	42	52	40
Monozygotic	6	6	6	6
#Triplets*	3	0	3	0
#Pos. hCG	222	226	214	218
#Early pregnancy loss (<=w.12)	52	77	49	73
#Biochemical	31	48	29	44
#Ectopic	4	2	4	2
#Miscarriage (loss w.7-12)	17	27	16	27
#Miscarriage observed week 7	14	17	14	17
#Miscarriage observed week 12	3	10	2	10
#Late pregnancy loss (>w.12)	2	4	2	4
Miscarriage/elective termination due to malformations				

*Including 1 set of monozygotic twins

Table B: Overview of deviations concerning the ultrasound scanning gestational week 7 for ITT- and PP-population

DK001	ITT		PP	
	GM-CSF	CONTROL	GM-CSF	CONTROL
#Clinical pregnancies w.7	189	175	183	171
Time of ultrasound scanning:				

(Continued)

#Week 7 plus 0-6 days	168	159	164	155
Week 6	9	4	8	4
Weeks 8-10	12	12	11	12

References:

1. Statistically analysis report, DK001 TFL FINAL FULL STUDY 19 MAR2012
 - a) Table 30
 - b) Table 31
 - c) Table 34
 - d) Table 61
 - e) Note to File, Rep1.02.03.05
2. Clinical Investigation Report DK001, Rep1.02.03.05
 - a) Appendix D. 1
 - b) Appendix D. 10
 - c) Appendix J
3. Final derived data Excel DK001:
 - a) DK001_Outcome_Stage 1
 - b) DK001_Outcome_Stage 2
 - c) DK001_BIRTHS

Correspondence with the authors of Rose 2020

1) Please could you clarify how long the embryos were exposed to the intervention culture medium?

They were exposed to GM-CSF containing media for 5 days - Embryogen from Day 0-3 and Blastgen from Day 3-5

2) Please could you clarify on what day embryos were replaced?

3) Were they all Single embryo transfers? Or multiple?

All trial participants had a single fresh embryo transfer apart from one in the standard group and three in the EmbryoGen®/BlastGen™ group who did not achieve fertilisation and 1 participant in the standard group and two in the EmbryoGen®/BlastGen™ group who did not have an embryo of sufficient quality to transfer. All trial participants had a day 5 embryo transfer apart from one in the standard media group and two in the BlastGen group who transferred one embryo on day 3 due to delayed embryo development. Couples who insisted on transferring two embryos, once embryo quality was known, were included in the study but were recorded as having a trial variation (four in the standard group and six in the EmbryoGen®/BlastGen™ group).

4) Please could you share the inclusion and exclusion criteria? If you have a protocol this would be fantastic to see.

This will be further explained in the paper;

The inclusion criteria selected for a poor prognosis patient population. Patients must have previously had consecutive transfer of two or more embryos without a positive pregnancy outcome OR have had a history of at least 1 previous pregnancy loss OR a previous history of poor embryo development (<20% of embryos developing on the time at Day 3 or no blastocysts above grade 2 on Day 5). Other additional inclusion parameters included a maternal age between 25 and 41 years, the use of a standard GnRH agonist or antagonist protocol and three or more follicles of >14mm as seen by transvaginal ultrasound before the day of human Chorionic Gonadotrophin (hCG) administration. Exclusion criteria included a need for surgical sperm retrieval (except in cases of previous vasectomy), the use of another investigational

drug within 30 days of oocyte retrieval and/or the presence of a severe chronic disease that could impact the IVF cycle or reproductive outcomes.

5) Please could you clarify that it was exactly 50 participants in each group? Were there any drop outs?

See the flow chart in the paper

6) We are interested in miscarriage. Please can I clarify the following numbers with you to check we have it accurately. I note one late miscarriage in the control medium group. Please can you confirm if this was pre or post 20 weeks, or if it was a stillbirth? I note that the clinical pregnancy rate is possibly written up wrong in your paper (i.e. they have been written up in the wrong order perhaps?).

live birth GMCSF 11/50 Control medium 17/50

Clinical preg rate GMCSF 13/50 Control medium 20/50

Ongoing clinical pregnancy rate GMCSF 11/50 Control medium 18/50

Miscarriage of clinical preg GMCSF 2/50 Control medium 3/50

This is correct - the control group had higher pregnancy rates but this was not statistically significant. The late miscarriage was at 17 weeks.

7) Any information you can give us on how the randomisation sequence was generated, how allocation was concealed, who exactly was blinded, was the person performing ultrasound blinded? Did you report all outcomes you assessed?

50 cards with control and 50 with BlastGen™ written on them were placed in concealed envelopes by a person unrelated to the trial. They were shuffled several times and the envelopes were marked from 1-100 in consecutive order. When the eligible patient was scheduled for an egg retrieval the embryologist took the next envelope by sequence and the random assignment was made. Clinicians, sonographers and patients were completely blinded, embryologists were partly blinded. We report all outcomes as per our prospective clinical trials registration ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02305420) Identifier: NCT02305420). Patients were guaranteed to have BlastGen™ in 1 of 2 cycles (if they didn't conceive in their first). They were blind as to which cycle the BlastGen™ media was used.

8) Finally, we took this study from the abstract at ESHRE 2019. Have you published the full study elsewhere?

This paper was accepted for publication by RBM online on the 14th of January 2020 and should be available for advanced online publication in the next week or so.

Thank you so much for your thorough reply to my questions. I look forward to seeing the full paper in the coming weeks. I just want to clarify one thing. Is this a cross-over study? The results here, are they all from the first phase of the study or are some taken from the second phase?

You are correct that this is only the first phase, and not a crossover.

I spotted in table 2 that you mention 2 sets of twins in the control group. Please can I confirm this? Am I right in thinking there were no twins in the intervention arm? The other outcomes we are interested include preterm birth rate per woman randomised (<37 weeks' gestation), birth defects per woman randomised, aneuploidy per woman randomised, stillbirth per woman randomised (>20 weeks' gestation). Thank you for any further information you can provide us.

In terms of the additional information, although we did not report them in our trial I have looked through our records and pulled the data out. Answered below:

Yes two sets of twins, both in the standard medium group (none in BlastGen™ arm).

Preterm birth rate per woman randomised (<37 weeks' gestation)

- 9 cases of preterm birth (2 sets of twins), Standard = 8, BlastGen™ = 1

Birth defects per woman randomised

- One birth defect noted in the standard group (genetic). The child was born mosaic for a trisomy 18 and no obvious phenotype present as yet.

Aneuploidy per woman randomised

- No PGT was done as part of the trial.

Stillbirth per woman randomised (>20 weeks' gestation)

- One case of stillbirth (Standard Group) born at 21+3 weeks (termination, fetal abnormalities, we have no genetic results except the amino/harmony was normal). This would mean minus one miscarriage from the standard group.

Thank you for your reply. Just for clarity, we will classify these results as follows:

- **Aneuploidy** GM-CSF 0/50 Control 1/50 (mosaic T18)
- **Stillbirth** (we do not consider TOPs to be stillbirths, or miscarriages. However that particular baby will be counted as birth defect) GM-CSF 0/50 Control 0/50
- **Birth defect** GM-CSF 0/50 Control 1/50
- **Preterm birth** GM-CSF 1/50 Control 6/50
- **Multiple gestation** GM-CSF 0/50 Control 2/50

Yes that all looks correct and makes sense. Thanks for clarifying.

Appendix 14. Other correspondence

The following email was received regarding the protocol of this study. In light of this email, we will alter the protocol for future iterations of this review to remove studies that use 'GCSF' as an intervention opposed to GM-CSF. We did not find or include any studies that used G-CSF as an intervention.

Dear Sarah

I've just read with interest your protocol for the Cochrane Review on use of GM-CSF in IVF. I'm very pleased to see this is happening and would like to thank you for taking this on. It promises to be an important and valuable study and I'll look forward to seeing the outcome. However I have one major concern. The protocol states in the methods section: "We will include all studies that compare GM-CSF (including G-CSF)-supplemented embryo culture media versus any other non-GM-CSF-supplemented embryo culture media (control)." This is very worrying and if indeed this will be done, it threatens the validity and usefulness of the study.

GM-CSF and G-CSF are two entirely different cytokines, with no homology in their protein or DNA sequence, no relatedness in their tertiary structure, and only very limited relatedness in their biological function. Although their names are similar this is very misleading. Their synthesis in the female reproductive tract is regulated differently, and they act via different receptors in their respective target cells. While there is strong biological and preclinical rationale for evaluating GM-CSF in human embryo development, there is almost no rationale for G-CSF.

It does not make any sense at all to pool the clinical studies on these two cytokines!

I very much hope you will reconsider the protocol in light of this.

I would be very happy to provide further information on these cytokines, and/or discuss the points above by email or phone/zoom with you and/or Dr Farquhar if that helps.

Best wishes,

Sarah Robertson, PhD FAA

The University of Adelaide

HISTORY

Protocol first published: Issue 12, 2019

Review first published: Issue 7, 2020

CONTRIBUTIONS OF AUTHORS

SA wrote the first draft of the protocol and the review.

SA, AP, JM, and BW undertook screening of abstracts.

SA, JM, and AP undertook screening of full-text articles.

SA and JM undertook data extraction, 'Risk of bias' assessment, and application of GRADE.

All authors contributed to the edits of the full review.

DECLARATIONS OF INTEREST

SA: none.

JM: none.

BW: none.

AP: none.

CF: none.

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- Bath Clinical Society, UK

GBP 1000 was awarded to SA to go toward attendance at a conference to present the findings of this review.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We changed the phrase "assisted reproductive technology (ART)" to "assisted reproduction" in the review title. We feel this is easy to understand and avoids use of an acronym.

We included all outcomes in the 'Summary of findings' table.

INDEX TERMS

Medical Subject Headings (MeSH)

Abortion, Spontaneous [epidemiology]; Aneuploidy; Bias; Confidence Intervals; Congenital Abnormalities [epidemiology]; Culture Media [*chemistry]; Fertilization in Vitro [*methods]; Granulocyte-Macrophage Colony-Stimulating Factor [*therapeutic use]; Live Birth; Pregnancy Rate; Pregnancy, Multiple [statistics & numerical data]; Premature Birth [epidemiology]; Randomized Controlled Trials as Topic; Reproductive Techniques, Assisted

MeSH check words

Female; Humans; Pregnancy