Cochrane Library

Cochrane Database of Systematic Reviews

GM-CSF (granulocyte macrophage colony stimulating factor) supplementation in culture media for women undergoing assisted reproductive technology (ART) (Protocol)

Armstrong S, MacKenzie J, Woodward B, Pacey A, Farquhar C

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 reproductive technology (ART).
Cochrane Database of Systematic Reviews 2019, Issue 12. Art. No.: CD013497.
DOI: 10.1002/14651858.CD013497.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Protocol]

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

Sarah Armstrong[1], Jeanette MacKenzie[2], Bryan Woodward[3], Allan Pacey[1], Cindy Farquhar[4]

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

Contact address: Sarah Armstrong, Department of Oncology & Metabolism, Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Academic Unit of Reproductive and Developmental Medicine, Level 4, The Jessop Wing, Sheffield, S10 2SF, UK. sarahcarmstrong@yahoo.co.uk.

Editorial group: Cochrane Gynaecology and Fertility Group
Publication status and date: New, published in Issue 12, 2019.

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 reproductive technology (ART). *Cochrane Database of Systematic Reviews* 2019, Issue 12. Art. No.: CD013497. DOI: 10.1002/14651858.CD013497.

ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

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

BACKGROUND

Description of the condition

In 1978, Louise Brown was the first child born as a result of in vitro fertilisation (IVF). Since then, the world of assisted reproductive technologies (ART) has advanced at a very rapid pace (Chronopoulou 2015; Steptoe 1978). ART 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. ART 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 ART 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. Over the past decade, the success rates of IVF, measured as the live birth of a baby, have remained relatively static. In an attempt to improve the success rates, a series of medical and non-medical adjuncts to an IVF cycle have been developed. These are often referred to as 'add-ons' and are sometimes novel interventions or therapies that have shown some promise in initial studies, or they may have been around for many years, but have not yet been proven to be effective through randomised controlled trials (RCTs). Granulocyte macrophage colony stimulating factor (GM-CSF)-containing culture media is one such add-on being offered, often at an additional cost to the IVF cycle (Heneghan 2016).

GM-CSF is a growth factor and we understand that pregnancy is supported through secretion and modulation of cytokines and growth factors which play a vital role in cell division, growth, and differentiation (Richter 2008; Robertson 1994). Many different growth factors have been evaluated as supplements to human IVF culture media; however, GM-CSF-supplemented culture media is known to be one of the better studied supplements in RCTs. Culture media are available as both sequential preparations and single-step preparations in some parts of the world. There is currently insufficient evidence to recommend either sequential or single-step media as being superior for the culture of embryos to days five or six (Sfontouris 2016).

There are many different culture media available that do not contain GM-CSF. Modern culture media contain up to 80 components including nutrients, vitamins, and growth factors (Chronopoulou 2015; Dyrlund 2014; Morbeck 2014). Most companies disclose the components, but concentrations are rarely disclosed due to commercial competition (Biggers 2000). For this review, any culture media contain GM-CSF can be compared in an RCT against any culture media not containing GM-CSF. This review will address the efficacy and safety of GM-CSF-supplemented culture media when compared to culture media not containing GM-CSF. Live-birth and miscarriage are the primary outcomes.

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 that are 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). Among the CSF group, GM-CSF has been most widely studied and its extensive research on ART 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 (Baldwin 1992) needed in the immunomodulation of early pregnancy and embryogenesis (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 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), 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 revealed that those cultured in GM-CS-supplemented culture media had more viable inner cell mass 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 have revealed an improvement in the clinical preg-

nancy and live birth rates in those randomised to culture of their embryos in GM-CSF supplemented culture media (Mignini 2013; Sfontouris 2013; Tevkin 2014; Ziebe 2013). There were no major and minor birth abnormalities (Mignini 2013; Sfontouris 2013; Tevkin 2014).

Why it is important to do this review

GM-CSF-supplemented culture media is widely commercially available and is being offered to women undergoing ART worldwide. It is often considered an 'add-on' or supplementary therapy given alongside standard IVF in an attempt 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 offer certainty on what should be done in practice. Using GM-CSF can carry an additional cost to women undergoing IVF. Therefore, it is 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 containing GM-CSF, in women or couples undergoing ART.

METHODS

Criteria for considering studies for this review

Types of studies

We will include all published and unpublished RCTs. We will include cross-over studies for completeness, but pool only data from the first phase in meta-analyses because this design of study is not valid in the context of infertility trials (Vail 2003). We will exclude quasi and pseudo-randomised trials. There will be 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 will also be included.

Types of interventions

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).

Types of outcome measures

Primary outcomes

1. Live birth per woman randomised, defined as a live baby born after 20 weeks' gestation.
   a. Ongoing pregnancy, defined as clinical pregnancy of 12 or more weeks' gestation will be used as a surrogate for live birth in cases where studies do not report live birth.
2. Miscarriage per woman randomised. Where possible, the definition we will use is miscarriage of clinical pregnancy. Where a paper does not report miscarriage of clinical pregnancy, we will attempt to calculate it by subtracting clinical pregnancy per woman randomised from live birth per woman randomised.

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. It includes ectopic pregnancy. Note that multiple gestational sacs are 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. Still birth (defined as a baby born with no signs of life after 20 completed weeks of pregnancy).

Search methods for identification of studies

We will search for relevant studies without language or date restriction in consultation with the Cochrane Gynaecology and Fertility group Information Specialist.

Electronic searches

We will design search strategies for the following databases: the Gynaecology and Fertility Group Specialised Register of Controlled Trials, Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE Ovid, Embase Ovid, and CINAHL EBSCO. The MEDLINE search will be combined with the Cochrane highly sensitive search strategy for identifying randomised trials which appears in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2019; Section 4.3.1). 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).

See Appendix 1; Appendix 2; Appendix 3; Appendix 4; Appendix 5.

Other electronic sources of trials will include:

1. trial registers for ongoing and registered trials: ClinicalTrials.gov (www.clinicaltrials.gov) and the World Health Organization International Trials Registry Platform search portal (apps.who.int/trialsearch/Default.aspx). See Appendix 6.
2. DARE (Database of Abstracts of Reviews of Effects) on the Cochrane Library (onlinelibrary.wiley.com/o/cochrane/cochrane_cldare_articles_fs.htm). See Appendix 7.
3. Web of Knowledge (wokinfo.com). See Appendix 8.
4. OpenGrey (www.opengrey.eu/) for unpublished literature from Europe. See Appendix 9.
5. LILACS database (lilacs.bvsalud.org/en/). See Appendix 10.
6. PubMed and Google Scholar (for recent trials not yet indexed in the major databases). See Appendix 11 and Appendix 12.

Searching other resources

We will handsearch reference lists of included and excluded studies retrieved by the search.

Data collection and analysis

Selection of studies

Two review authors will independently assess eligibility of all studies identified by the search utilising Covidence (Covidence). We will retrieve the full-test publications of potentially eligible studies. We will screen the full texts to identify studies for inclusion and record reasons for exclusion in the 'Characteristics of excluded studies' table. We will resolve any disagreements by discussion or consultation with a third review author.

Data extraction and management

Two review authors will independently extract data on study characteristics and primary and secondary outcomes from eligible studies using a data extraction form designed and piloted by the review authors. We will include 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.

We will resolve any disagreements or discrepancies by discussion. Where studies have multiple publications, we will use the main trial report as the reference and obtain additional details from secondary papers which will appear as subreferences. We will correspond with study investigators for further information on study methods and results, as required. This correspondence will be documented in the 'Characteristics of included studies' table.

Assessment of risk of bias in included studies

Two review authors will independently assess the included studies for methodological quality and undertake data extraction according to the Cochrane 'Risk of bias' assessment tool (Higgins 2011). We will assess 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). We will present and describe all our judgements in the 'Risk of bias' table. We will resolve any disagreements by discussion.

Measures of treatment effect

We will summarise the effects and adverse events related to the intervention as odds ratios (ORs) using a fixed-effect model. We will present 95% confidence intervals (CIs) for all outcomes to evaluate the precision of the estimate. Will consider 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 will consider whether the rates of events in both the intervention and control arm reflect current practice. For example, major discrepancies in direction and magnitude of effect will be explored in the results section and reflected in our risk of bias assessment.

Unit of analysis issues

The denominator for all outcomes will be the number of women randomised. We will use no per cycle data.

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

Dealing with missing data

We will analyse the data on an intention-to-treat basis and attempt to obtain missing data from the primary investigators. We will assume that participants who drop 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 will make no other assumptions.

Assessment of heterogeneity

We will consider whether the clinical and methodological characteristics of the included studies are sufficiently similar for meta-analysis to provide a clinically meaningful summary. We will assess statistical heterogeneity using the I^2 statistic and consider an I^2 statistic greater than 50% to indicate substantial heterogeneity (Higgins 2019). When there is significant heterogeneity, we will undertake planned subgroup analyses to explore this is more detail.

Assessment of reporting biases

We will reduce the potential impact of publication and reporting bias by performing a comprehensive search for eligible studies and looking for duplication of data. We will 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 are 10 or more studies included in an analysis. When possible, we will use published protocols and prospective trial registration webpages 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 will perform meta-analyses, as appropriate, where data are available from multiple studies investigating the same treatment, and where the outcome has been measured in a standard way between the studies. We will use a fixed-effect model. We will undertake this meta-analysis according to methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* in the following comparisons (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

Where data are available, we will conduct subgroup analyses 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 two or three) versus studies replacing embryos at blastocyst stage (day five).

If we detect substantial heterogeneity, we will explore possible explanations in subgroup analyses. We will take any statistical heterogeneity into account when interpreting the results, especially if there is any variation in the direction of effect.

Sensitivity analysis

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

1. eligibility was restricted to studies without high risk of bias (low risk studies are defined 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 will prepare 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) using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness, and publication bias) (GRADEpro GDT). We will justify and document judgements about evidence quality (high, moderate, low, and very low) and incorporate this into reporting of the results for each outcome. The 'Summary of findings' table will compare GM-CSF (including G-CSF)-supplemented embryo culture media versus any other non-GM-CSF-supplemented embryo culture media (control).

ACKNOWLEDGEMENTS

We would like to thank Jasmine Lee for her contribution to the protocol draft.

We thank Rik van Eekelen, Demián Glujovsky, Debbie Blake, Mohamed Youssef, Eleonora Uphoff, and the editors who gave their time to peer review the protocol.

REFERENCES

Additional references

Agerholm 2010

Agerholm I, Loft A, Hald F, Lemmen JG, Munding B, Sorensen PD, et al. Culture of human oocytes with granulocyte-macrophage colony-stimulating factor has no effect on embryonic chromosomal constitution. *Reproductive Biomedicine Online* 2010;**20**(4):477-84.

Baldwin 1992

Baldwin GC. The biology of granulocyte-macrophage colony-stimulating factor: effects on hematopoietic and nonhematopoietic cells. *Developmental Biology* 1992;**151**(2):352-67. [0012-1606]

Biggers 2000

Biggers JD. Ethical issues and the commercialization of embryo culture media. *Reproductive Biomedicine Online* 2000;**1**:74-6.

Block 2003

Block J, Drost M, Monson RL, Rutledge JJ, Rivera RM, Paula-Lopes FF, et al. Use of insulin-like growth factor-I during embryo culture and treatment of recipients with gonadotropin-releasing hormone to increase pregnancy rates following the transfer of in vitro-produced embryos to heat-stressed, lactating cows. *Journal of Animal Science* 2003;**81**(6):1590-602.

Burgess 1977

Burgess AW, Wilson EMA, Metcalf D. Stimulation by human placental conditioned medium of hemopoietic colony formation by human marrow cells. Blood 1977; Vol. 49, issue 4:573-83.

Chronopoulou 2015

Chronopoulou E, Harper JC. IVF culture media: past, present and future. Human Reproduction Update 2015; Vol. 21, issue 1:39-55.

Coughlan 2014

Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. *Reproductive BioMedicine Online* 2014;**28**(1):14-38.

Covidence [Computer program]

Veritas Health Innovation. Covidence. Melbourne, Australia: Veritas Health Innovation.

Dyrlund 2014

Dyrlund TF, Kirkegaard K, Poulsen ET, Sanggaard KW, Hindkjaer JJ, Kjems J, et al. Unconditioned commercial embryo culture media contain a large variety of non-declared proteins: a comprehensive proteomics analysis. Human Reproduction (Oxford, England) 2014; Vol. 29, issue 11:2421-30.

Giacomini 1995

Giacomini G, Tabibzadeh SS, Satyaswaroop PG, Bonsi L, Vitale L, Bagnara GP, et al. Epithelial cells are the major source of biologically active granulocyte macrophage colony-stimulating factor in human endometrium. Human Reproduction (Oxford, England) 1995; Vol. 10, issue 12:3259-63.

GRADEpro GDT [Computer program]

McMaster University (developed by Evidence Prime). GRADEpro GDT. Hamilton (ON): McMaster University (developed by Evidence Prime).

Hambartsoumian 1998

Hambartsoumian E. Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. American Journal of Reproductive Immunology 1998; Vol. 39, issue 2:137-43.

Heneghan 2016

Heneghan C, Spencer EA, Bobrovitz N, Collins DR, Nunan D, Pluddemann A, et al. Lack of evidence for interventions offered in UK fertility centres. *BMJ* 2016;**355**:i6295.

Higgins 2011

Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Higgins 2019

Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook..

Jokhi 1994

Jokhi PP, King A, Loke YW. Production of granulocyte-macrophage colony-stimulating factor by human trophoblast cells and by decidual large granular lymphocytes. Human Reproduction (Oxford, England) 1994; Vol. 9, issue 9:1660-9.

Lazzari 2002

Lazzari G, Wrenzycki C, Herrmann D, Duchi R, Kruip T, Niemann H, et al. Cellular and molecular deviations in bovine in vitro-produced embryos are related to the large offspring syndrome. Biology of Reproduction 2002; Vol. 67, issue 3:767-75.

Lighten 1998

Lighten AD, Moore GE, Winston RM, Hardy K. Routine addition of human insulin-like growth factor-I ligand could benefit clinical in-vitro fertilization culture. Human Reproduction (Oxford, England) 1998; Vol. 13, issue 11:3144-50.

Lim 2006

Lim JJ, Lee DR, Song HS, Kim KS, Yoon TK, Gye MC, et al. Heparin-binding epidermal growth factor (HB-EGF) may improve embryonic development and implantation by increasing vitronectin receptor (integrin alphanubeta3) expression in peri-implantation mouse embryos. Journal of Assisted Reproduction and Genetics 2006; Vol. 23, issue 3:111-9.

Mignini 2013

Mignini Renzini M, Dal Canto M, Coticchio G, Comi R, Brigante C, Caliari I, et al. Clinical efficiency and perinatal outcome of ART cycles following embryo culture in the presence of GM-CSF in patients with miscarriage or early pregnancy loss history. Human Reproduction (Oxford, England) 2013; Vol. 28, issue Suppl 1:i139-49.

Morbeck 2014

Morbeck DE, Krisher RL, Herrick JR, Baumann NA, Matern D, Moyer T. Composition of commercial media used for human embryo culture. Fertility and Sterility 2014; Vol. 102, issue 3:759-766.e9. [0015-0282]

Rahmati 2015

Rahmati M, Petitbarat M, Dubanchet S, Bensussan A, Chaouat G, Ledee N. Colony stimulating factors 1, 2, 3 and early pregnancy steps: from bench to bedside. Journal of Reproductive Immunology 2015; Vol. 109:1-6. [0165-0378]

Richter 2008

Richter KS. The importance of growth factors for preimplantation embryo development and in-vitro culture. Current Opinion in Obstetrics & Gynecology 2008; Vol. 20, issue 3:292-304.

Robertson 1992

Robertson SA, Mayrhofer G, Seamark RF. Uterine epithelial cells synthesize granulocyte-macrophage colony-stimulating factor and interleukin-6 in pregnant and nonpregnant mice. Biology of Reproduction 1992; Vol. 46, issue 6:1069-79.

Robertson 1994

Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG. The role of cytokines in gestation. Critical Reviews in Immunology 1994; Vol. 14, issue 3-4:239-92.

Robertson 1999

Robertson SA, Roberts CT, Farr KL, Dunn AR, Seamark RF. Fertility impairment in granulocyte-macrophage colony-stimulating factor-deficient mice. Biology of Reproduction 1999; Vol. 60, issue 2:251-61.

Robertson 2007

Robertson SA. GM-CSF regulation of embryo development and pregnancy. Cytokine & Growth Factor Reviews 2007; Vol. 18, issue 3-4:287-98.

Roudebush 2004

Roudebush WE, Massey JB, Kort HI, Elsner CW, Toledo AA, Mitchell-Leef D, et al. Exposure of preimplantation embryos to platelet-activating factor increases birth rate. Journal of Assisted Reproduction and Genetics 2004; Vol. 21, issue 8:297-300.

Savion 2002

Savion S, Zeldich E, Orenstein H, Shepshelovich J, Torchinsky A, Carp H, et al. Cytokine expression in the uterus of mice with pregnancy loss: effect of maternal immunopotentiation with GM-CSF. Reproduction 2002; Vol. 123, issue 3:399-409.

Seymour 1997

Seymour JF, Lieschke GJ, Grail D, Quilici C, Hodgson G, Dunn AR. Mice lacking both granulocyte colony-stimulating factor (CSF) and granulocyte-macrophage CSF have impaired reproductive capacity, perturbed neonatal granulopoiesis, lung disease, amyloidosis, and reduced long-term survival. Blood 1997; Vol. 90, issue 8:3037-49.

Sfontouris 2013

Sfontouris I, Lainas G, Anagnostara K, Kolibianakis E, Lainas T. Effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on pregnancy rates in patients with multiple unsuccessful IVF attempts. *Human Reproduction (Oxford, England)* 2013;**28**(Suppl 1):160-162.

Sfontouris 2016

Sfontouris IA, Martins WP, Nastri CO, Viana IG, Navarro PA, Raine-Fenning N, et al. Blastocyst culture using single versus sequential media in clinical IVF: a systematic review and meta-analysis of randomized controlled trials. *Journal of Assisted Reproduction and Genetics* 2016;**33**(10):1261-72.

Siristatidis 2013

Siristatidis C, Vogiatzi P, Salamalekis G, Creatsa M, Vrachnis N, Glujovsky D, et al. Granulocyte macrophage colony stimulating factor supplementation in culture media for subfertile women undergoing assisted reproduction technologies: a systematic review. International Journal of Endocrinology 2013; Vol. 2013:704967.

Sjöblom 1992

Sjöblom C, Wikland M, Robertson SA. Granulocyte-macrophage-colony stimulating factor (GM-CSF) promotes inner cell mass blastomere viability in human pre-implantation embryos. Fertility and Sterility. Elsevier, 1992; Vol. 76, issue 3:S70. [0015-0282]

Sjöblom 1999

Sjöblom C, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor promotes human blastocyst development in vitro. Human Reproduction (Oxford, England) 1999; Vol. 14, issue 12:3069-76.

Sjöblom 2002

Sjöblom C, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor (GM-CSF) acts independently of the beta common subunit of the GM-CSF receptor to prevent inner cell mass apoptosis in human embryos. Biology of Reproduction 2002; Vol. 67, issue 6:1817-23.

Sjöblom 2005

Sjöblom C, Roberts CT, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. Endocrinology 2005; Vol. 146, issue 5:2142-53.

Spanos 2000

Spanos S, Becker DL, Winston RM, Hardy K. Anti-apoptotic action of insulin-like growth factor-I during human

preimplantation embryo development. Biology of Reproduction 2000; Vol. 63, issue 5:1413-20.

Steptoe 1978

Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* 1978;**2**:366.

Tevkin 2014

Tevkin S, Lokshin V, Shishimorova M, Polumiskov V. The frequency of clinical pregnancy and implantation rate after cultivation of embryos in a medium with granulocyte macrophage colony-stimulating factor (GM-CSF) in patients with preceding failed attempts of ART. Gynecological Endocrinology 2014; Vol. 30, issue Suppl 1:9-12.

Torry 2007

Torry DS, Leavenworth J, Chang M, Maheshwari V, Groesch K, Ball ER, et al. Angiogenesis in implantation. Journal of Assisted Reproduction and Genetics 2007; Vol. 24, issue 7:303-15.

Vail 2003

Vail A, Gardener E. Common statistical errors in the design and analysis of subfertility trials. *Human Reproduction (Oxford, England)* 2003;**18**:1000-4.

Vuorela 2000

Vuorela P, Carpen O, Tulppala M, Halmesmaki E. VEGF, its receptors and the tie receptors in recurrent miscarriage. Molecular Human Reproduction 2000; Vol. 6, issue 3:276-82.

Wegmann 1992

Wegmann TG, Guilbert LJ. Immune signalling at the maternal – fetal interface and trophoblast differentiation. *Developmental & Comparative Immunology* 1992;**16**(6):425-30. [0145-305X]

Young 2001

Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, et al. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nature Genetics 2001; Vol. 27, issue 2:153-4.

Yu 2012

Yu Y, Yan J, Li M, Yan L, Zhao Y, Lian Y, et al. Effects of combined epidermal growth factor, brain-derived neurotrophic factor and insulin-like growth factor-1 on human oocyte maturation and early fertilized and cloned embryo development. Human Reproduction (Oxford, England) 2012; Vol. 27, issue 7:2146-59.

Zhao 1994

Zhao Y, Chegini N. Human fallopian tube expresses granulocyte-macrophage colony stimulating factor (GM-CSF) and GM-CSF alpha and beta receptors and contain immunoreactive GM-CSF protein. Journal of Clinical Endocrinology and Metabolism 1994; Vol. 79, issue 2:662-5.

Ziebe 2013

Ziebe S, Loft A, Povlsen BB, Erb K, Agerholm I, Aasted M, et al. A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for in vitro fertilization. Fertility and Sterility 2013; Vol. 99, issue 6:1600-9.

APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility Group specialised register (CGFG) search strategy

PROCITE platform

From inception to date of search

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 Title CONTAINS "granulocyte colony-stimulating factor" or "granulocyte macrophage colony stimulating factor" or "GM-CSF" or "G-CSF" or "Embryogen"

Appendix 2. CENTRAL Register of Studies Online (CRSO) search strategy

Web platform

From inception to date of search

#1 MESH DESCRIPTOR Embryo Transfer EXPLODE ALL TREES

#2 MESH DESCRIPTOR Fertilization in Vitro EXPLODE ALL TREES

#3 MESH DESCRIPTOR Sperm Injections, Intracytoplasmic EXPLODE ALL TREES

#4 (vitro fertili?ation):TI,AB,KY

#5 (ivf or icsi):TI,AB,KY

#6 (intracytoplasmic sperm injection*):TI,AB,KY

#7 (assisted reproduct*):TI,AB,KY

#8 (ovulation induc*):TI,AB,KY

#9 superovulat*:TI,AB,KY

#10 (ovarian hyperstimulation):TI,AB,KY

#11 COH:TI,AB,KY

#12 infertil*:TI,AB,KY

#13 subfertil*:TI,AB,KY

#14 blastocyst*:TI,AB,KY

#15 embryo*:TI,AB,KY

#16 (recurrent adj3 miscarriage*):TI,AB,KY

#17 (recurrent adj3 abortion*):TI,AB,KY

#18 (recurrent adj3 implantation*):TI,AB,KY

#19 (implantation failure*):TI,AB,KY

#20 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19

#21 MESH DESCRIPTOR Colony-Stimulating Factors EXPLODE ALL TREES

#22 (CSF-2 or CSF-3):TI,AB,KY

#23 (Colony stimulating adj3 factor*):TI,AB,KY

#24 (granulocyte macrophag*):TI,AB,KY

#25 (GM CSF):TI,AB,KY

#26 gcsf:TI,AB,KY

#27 gmcsf:TI,AB,KY

#28 (g csf):TI,AB,KY

#29 (Embryogen or blastogen or Leucomax):TI,AB,KY

#30 #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29

#31 #20 AND #30

Appendix 3. MEDLINE search strategy

Ovid platform

From 1946 to date of search

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/

2 vitro fertili?ation.tw.

3 ivf-et.tw.

4 ivf.tw.

5 icsi.tw.

6 intracytoplasmic sperm injection$.tw.

7 assisted reproduct$.tw.

8 ovulation induc$.tw.

9 (ovari$ adj2 stimulat$).tw.

10 superovulat$.tw.

11 ovarian hyperstimulation.tw.

12 COH.tw.

13 infertil$.tw.

14 subfertil$.tw.

15 (ovari$ adj2 induction).tw.

16 blastocyst$.tw.

17 embryo$.tw.

18 (recurrent adj3 miscarriage$).tw.

19 (recurrent adj3 abortion$).tw.

20 (recurrent adj3 implantation$).tw.

21 implantation failure$.tw.

22 or/1-21

23 exp colony-stimulating factors/ or exp granulocyte-macrophage colony-stimulating factor/ or exp macrophage colony-stimulating factor/

24 (CSF-2 or CSF-3).tw.

25 (Colony stimulating adj3 factor$).tw.

26 granulocyte macrophag$.tw.

27 GM CSF.tw.

28 gcsf.tw.

29 gmcsf.tw.

30 g csf.tw.

31 (Embryogen or blastogen or Leucomax).tw.

32 or/23-31

33 22 and 32

34 randomized controlled trial.pt.

35 controlled clinical trial.pt.

36 randomized.ab.

37 randomised.ab.

38 placebo.tw.

39 clinical trials as topic.sh.

40 randomly.ab.

41 trial.ti.

42 (crossover or cross-over or cross over).tw.

43 or/34-42

44 exp animals/ not humans.sh.

45 43 not 44

46 33 and 45

Appendix 4. Embase search strategy

Ovid platform

From 1980 to date of search

1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/

2 vitro fertili?ation.tw.

3 icsi.tw.

4 intracytoplasmic sperm injection$.tw.

5 (ivf or et).tw.

6 assisted reproduct$.tw.

7 ovulation induc$.tw.

8 (ovari$ adj2 stimulat$).tw.

9 superovulat$.tw.

10 ovarian hyperstimulation.tw.

11 COH.tw.

12 infertil$.tw.

13 subfertil$.tw.

14 (ovari$ adj2 induction).tw.

15 blastocyst$.tw.

16 embryo$.tw.

17 (recurrent adj3 miscarriage$).tw.

18 (recurrent adj3 abortion$).tw.

19 (recurrent adj3 implantation$).tw.

20 implantation failure$.tw.

21 or/1-20

22 exp colony stimulating factor/

23 exp granulocyte macrophage colony stimulating factor/

24 exp colony stimulating factor 1/

25 (CSF-2 or CSF-3).tw.

26 (Colony stimulating adj3 factor$).tw.

27 granulocyte macrophag$.tw.

28 GM CSF.tw.

29 gcsf.tw.

30 gmcsf.tw.

31 g csf.tw.

32 (Embryogen or blastogen or Leucomax).tw.

33 or/22-32

34 Clinical Trial/

35 Randomized Controlled Trial/

36 exp randomization/

37 Single Blind Procedure/

38 Double Blind Procedure/

39 Crossover Procedure/

40 Placebo/

41 Randomi?ed controlled trial$.tw.

42 Rct.tw.

43 random allocation.tw.

44 randomly.tw.

45 randomly allocated.tw.

46 allocated randomly.tw.

47 (allocated adj2 random).tw.

48 Single blind$.tw.

49 Double blind$.tw.

50 ((treble or triple) adj blind$).tw.

51 placebo$.tw.

52 prospective study/

53 or/34-52

54 case study/

55 case report.tw.

56 abstract report/ or letter/

57 or/54-56

58 53 not 57

59 (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)

60 58 not 59

61 21 and 33 and 60

Appendix 5. CINAHL search strategy

Ebsco platform

From 1982 to date of search

#	Query
S45	S32 AND S44
S44	S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41 OR S42 OR S43
S43	TX allocat* random*
S42	(MH "Quantitative Studies")
S41	(MH "Placebos")
S40	TX placebo*
S39	TX random* allocat*
S38	(MH "Random Assignment")
S37	TX randomi* control* trial*
S36	TX ((singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX ((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl* n1 mask*))
S35	TX clinic* n1 trial*
S34	PT Clinical trial
S33	(MH "Clinical Trials+")
S32	S21 AND S31
S31	S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30
S30	TX (Embryogen or blastogen or Leucomax)
S29	TX g csf
S28	TX gmcsf
S27	TX gcsf
S26	TX GM CSF
S25	TX granulocyte macrophag*

(Continued)

S24	TX (Colony stimulating N3 factor*)
S23	TX (CSF-2 or CSF-3)
S22	(MM "Colony-Stimulating Factors+") OR (MM "Granulocyte Colony-Stimulating Factor") OR (MM "Granulocyte-Macrophage Colony-Stimulating Factor")
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
S20	TX implantation failure*
S19	TX recurrent N3 implantation*
S18	TX recurrent N3 abortion*
S17	TX recurrent N3 miscarriage*
S16	TX embryo*
S15	TX blastocyst*
S14	TX subfertil*
S13	TX infertil*
S12	TX COH
S11	TX ovarian hyperstimulation
S10	TX superovulat*
S9	TX ovulation induc*
S8	TX assisted reproduct*
S7	TX intracytoplasmic sperm injection*
S6	TX vitro fertili?ation
S5	(MM "Embryo Transfer")
S4	TX ovar* N3 hyperstimulat*
S3	TX ovari* N3 stimulat*
S2	TX IVF or TX ICSI
S1	(MM "Fertilization in Vitro")

Appendix 6. Clinicaltrials.gov and WHO portal for ongoing trials search strategy

Web platform

From inception to date of search

csf* and embryo*

csf* and ivf*

colony stimulating factor and embryo

colony stimulating factor and ivf

Appendix 7. DARE search strategy

Web platform

From inception to date of search

csf* and embryo*

csf* and ivf*

colony stimulating factor and embryo

colony stimulating factor and ivf

Appendix 8. The Web of Knowledge search strategy

Web platform

From inception to date of search

csf* and embryo*

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

Appendix 9. OpenGrey search strategy

Web platform

From inception to date of search

csf* and embryo*

csf* and ivf*

Appendix 10. LILACS database search strategy

Web platform

From inception to date of search

colony stimulating factor and embryo*

colony stimulating factor and ivf*

Appendix 11. PubMed search strategy

Web platform

(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

(csf AND embryo* OR ivf*)

(colony stimulating factor AND embryo*)

CONTRIBUTIONS OF AUTHORS

SA wrote the first draft of the protocol

All authors contributed to the edit.

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.