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[Intervention Protocol]

Endometrial injection of embryo culture supernatant for subfertile women in assisted reproduction

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ABSTRACT

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

To evaluate the effectiveness and safety of endometrial injection of embryo culture supernatant before embryo transfer for improving rates of live birth or ongoing pregnancy in women undergoing ART.

BACKGROUND

The average age of women who gave birth to their first child has risen to almost 29 years of age, according to a recent Eurostat report. In Mediteranean countries, including Italy, Spain, Malta and Greece, the average age of women who gave birth to their first child is more than one year higher than in Europe overall (Eurostat 2015). In Spain the proportion of women who give birth to their first child at the age of between 30 to 39 is 59.4%, whereas in Greece it reaches 51.9%. Similarly, the mean age of mothers has increased from 2000 to 2014 for all birth orders, with age at first birth having the largest increase, up from 24.9 years in 2000 to 26.3 years in 2014 (CDC 2016). This rise of mean maternal age at primigravidity has led to a significant increase in subfertility as the likelihood of live birth gradually decreases with advancing age (Leridon 2004).

Assisted reproduction is the standard of care for subfertile couples who wish to conceive. It is estimated that the number of births worldwide as a result of in vitro fertilisation (IVF) has already exceeded five million births. However, pregnancy rates after IVF have remained unchanged during the last 10 years, at around 30% for cases undergoing intracytoplasmic sperm injection (ICSI) (Van Steirteghem 1993; Kuczyński 2001; Ben Rhouma 2003; Motteram 2015; European IVF-monitoring Consortium (EIM) 2017). In this context, current research focuses on enhanced understanding of cellular and molecular pathways involved in the process of implantation and the potential for targeted interventions to improve implantation rates.

Successful implantation is a result of a complex sequence of physiological events that must be synchronised in order for the zygote to travel through the salpinx and reach the endometrial cavity at a specific time (implantation window). This synchronisation necessitates a timely cross-talk between the zygote and the endometrium

(Lopata 1996).

Description of the condition

Despite substantial advances in assisted reproduction during the last decade, live birth rates have reached a plateau that seems impossible to overcome (European IVF-monitoring Consortium (EIM) 2017). Advancing maternal age at primigravidity increases the proportion of couples who need assisted reproduction. Bearing in mind the significant impact of subfertility on a couple's quality of life, every effort should be made to increase their chance of live birth. Many interventions have been investigated to overcome this situation, but with conflicting results and no firm consensus (Carney 2012; Siristatidis 2018; Lensen 2018). It is essential to find new evidence that could improve reproductive outcomes and this should be the aim of future clinical practice and research.

Despite efforts to improve understanding of human implantation in order to achieve a balance between regulation and dysregulation of endometrial function, and to facilitate the transfer of high-quality embryos in subfertile women undergoing assisted reproduction, implantation rates remain limited. Various local and systemic therapies have not succeeded in improving the success rate of IVF treatment (Siristatidis 2016; Bontekoe 2014; Farquhar 2015).

Description of the intervention

The injection of embryo culture supernatant in the endometrial cavity can be undertaken at various time intervals before embryo transfer. Embryos are put in a separate dish with fresh media, and then the supernatant (culture medium) is aspirated with an embryo transfer catheter. The supernatant is collected and can be used in a fresh cycle with day 3 embryos or blastocysts. Otherwise it can be cryopreserved and used in a cycle with vitrified/thawed embryos (Goto 2007).

The intervention is a straightforward, feasible technique that is easily accomplished before embryo transfer. The procedure involves placement of an IVF catheter in the endometrial cavity, which is loaded with the embryo culture supernatant aspirated from the dish that contained the embryos (after aspiration of the embryos themselves). The medium is then released close to the fundus of the uterine cavity.

How the intervention might work

Studies suggest the human embryo secretes various factors during its growth and prior to implantation, which seem to contribute to cross-talk with the maternal tissues, thus modulating endometrial receptivity. Specifically, researchers suggest that the human pre-implanted embryo produces immunosuppressive factors and vascular endothelial growth factor (VEGF) (which is essential for neoangiogenesis in the implantation site), human leukocyte antigen G (HLA-G), interleukins (including IL-1 and IL-8), leukemia inhibitory factor (LIF), monocyte chemoattractant protein 1 (MCP-1) and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) which modulate the implantation potential (Sheth 1991; Dinarello 1994; Giudice 1995; Tazuke 1996; Krüssel 2000; Spandorfer 2000; Caballero-Campo 2002; Desai 2006). Moreover, it downregulates the human mucin gene 1 (MUC-1) which naturally creates a barrier to the endometrial-embryo attachment (Meseguer 2001). At the same time, the endometrium regulates oestrogen, progesterone and other receptors in an effort to improve the endometrial receptivity for the process of implantation (Tazuke 1996; Tehraninejad 2012).

The rationale for the intervention is that it provides an altered and optimum endometrial environment through the secretion of embryonic factors, such as integrins, leukaemia inhibitory factor and other factors, which are considered to facilitate implantation. It is proposed that injection of the supernatant into the endometrial cavity prior to embryo transfer will stimulate the endometrium and provide better conditions for implantation to take place. An increased implantation rate will subsequently increase rates of clinical pregnancy and live birth.

Why it is important to do this review

Injection of embryo culture supernatant prior to embryo transfer is a promising procedure, but there is uncertainty as to its effectiveness and safety. There is an emerging need to summarise current evidence and provide a clear view on the effectiveness of this practice in order to encourage or disprove its clinical application. To date, no systematic reviews have investigated this field. In this Cochrane Review we will summarise the relevant evidence and identify any gaps or limitations in our current understanding. We will achieve this by assessing the methodological quality of existing and ongoing trials, which may encourage the conduct of more studies on this topic.

OBJECTIVES

To evaluate the effectiveness and safety of endometrial injection of embryo culture supernatant before embryo transfer for improving rates of live birth or ongoing pregnancy in women undergoing ART.

METHODS

Criteria for considering studies for this review

Types of studies

Published and unpublished randomised controlled trials (RCTs) that assess the effectiveness and safety of endometrial injection of embryo culture supernatant before embryo transfer in women undergoing IVF/ICSI. We will exclude pseudo-randomised and crossover trials. We will not apply limitations in terms of country of origin and language.

Types of participants

Women and couples undergoing IVF/ICSI cycles (both fresh and frozen). We will exclude oocyte donation cycles.

Types of interventions

Endometrial injection of embryo culture supernatant before embryo transfer, during an artificial reproductive cycle with IVF or ICSI, versus any other intervention or no intervention (usual care). In case of the existence of data describing a sham or placebo-type intervention, we will perform a stratified comparison as described in the 'Data synthesis' section.

Types of outcome measures

Primary outcomes

Effectiveness

- Live birth or (in studies not reporting live birth) ongoing pregnancy per woman/couple randomised:
  - live birth is defined as the delivery of a live foetus after 20 completed weeks of gestational age;
  - ongoing pregnancy is defined as the presence of a foetal heart on ultrasound scan after 12 weeks of gestation per woman/couple randomised;
  - cumulative live birth will also be reported, if data are available.
- Adverse events
  - miscarriage rates per woman/couple randomised. Miscarriage is defined as the loss of pregnancy before 20 completed weeks of gestational age.

Secondary outcomes

Effectiveness

- Clinical pregnancy rate per woman/couple randomised:
  - clinical pregnancy is defined as the presence of a foetal heart on ultrasound scan at seven weeks of gestation.
- Adverse events per woman/couple randomised: multiple and ectopic pregnancy rates, foetal growth restriction, preterm delivery (< 37 weeks of gestation) and foetal abnormality rate (chromosomal, congenital and anatomical).

Search methods for identification of studies

We will search for published and unpublished RCTs that assess the impact of endometrial injection of embryo culture supernatant before the embryo transfer, during an artificial reproductive cycle with IVF or ICSI, in consultation with the Cochrane Gynaecology and Fertility Group's Information Specialist.

Electronic searches

We will search the following databases from inception to present: the Cochrane Gynaecology and Fertility Group Specialised Register (Appendix 1), the Cochrane Central Register of Controlled Trials (CENTRAL CRSO) (Appendix 2), MEDLINE (Appendix 3), Embase (Appendix 4), and CINAHL Plus (Appendix 5). All searches will be carried out without any language or date restriction.

We will combine the MEDLINE search with the Cochrane highly sensitive search strategy for identifying RCTs that appears in the *Cochrane Handbook of Systematic Reviews of Interventions* (Chapter 6, 6.4.11; Lefebvre 2011). We will combine the Embase and CINAHL searches with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) www.sign.ac.uk/methodology/filters.html#random.

We will search the World Health Organization International Clincal Trials Registry Platform (http://apps.who.int/trialsearch/Default.aspx) and the ClinicalTrials.gov registry for ongoing and registered trials (Appendix 6). We will also search OpenGrey (www.opengrey.eu/) for grey literature. We will consult experienced clinicians for any ongoing or existing studies.

Searching other resources

We will examine the references lists of all studies (included and excluded) and relevant reviews in order to identify further relevant articles.

Data collection and analysis

We will enter data into Review Manager 5 (RevMan 5) (RevMan 2014). We will conduct statistical analysis in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Selection of studies

Two review authors will independently screen the titles and abstracts of the publications identified by the literature search strategy. They will exclude studies that do not meet the inclusion criteria and will retrieve the full-text of remaining publications. Three review authors will evaluate these independently to identify RCTs eligible for inclusion. Any potential disagreements related to study eligibility will be resolved by discussion with the first review author (CSS). We will list all studies excluded after full-text assessment in the 'Characteristics of excluded studies' tables, and will outline the study selection process in a PRISMA flow chart.

Data extraction and management

Two review authors will independently extract study characteristics and outcome data from the included studies using a pre-designed data extraction form. We will seek detailed information on participants, interventions, comparators, outcomes, study design, funding sources and declarations of interest for the primary investigators. For studies with multiple publications, we will use the main RCT report as the reference and we will supplement it with additional data from the secondary publications.

With the aim of retrieving additional data or methodological details where necessary, we will contact authors of the included studies. We will contact the authors via e-mail, including a reminder if needed (a second e-mail 15 days after the first communication, if we receive no reply or insufficient data). We will resolve any potential disagreements through consensus involving the first review author or the statistician/methodologist review author. One review author will import data into RevMan 5, and a second review author will validate the imported values against the data extraction form.

Assessment of risk of bias in included studies

Two review authors will independently assess risk of bias in the included studies using the Cochrane 'Risk of bias' assessment tool for selection, performance, detection, attrition, reporting and other bias (Higgins 2011). We will resolve any disagreements through discussion with review author CSS. We will explicitly report our judgement of risk of bias in the 'Risk of bias' table in the 'Characteristics of included studies' section with relevant information supporting our assessment. If necessary, we will group multiple outcomes.

In case we cannot make reliable judgements for a whole study, we will examine the risk of bias due to lack of blinding and missing data separately for different outcomes. Regarding blinding of medical personnel and patients, we expect to come across a considerable degree of bias owing to the nature of the intervention when compared to no intervention. In this case we will proceed on meticulous evaluation of the methods employed in each study to the extent of the presented material but also in request for further specifications.

We will also investigate the possibility of selective reporting through comparison of the protocol outcomes (if data are available) with the published study outcomes or the outcomes listed in the methods section with the reported results.

Measures of treatment effect

All defined outcomes will be binary (dichotomous), and we will use the numbers of events in the control and intervention groups of each study to calculate Mantel-Haenszel odds ratios (ORs). We will use Peto ORs for outcomes with low event rates, when needed, as described in the 'Data synthesis' section. We will reverse the direction of effect of individual studies, if required, to ensure consistency across trials. We will present 95% confidence intervals for the ORs.

Unit of analysis issues

We expect that all studies will have the woman (or couple) as the unit of randomisation. When data are not reported per woman (or couple), for example data reported 'per cycle', then we will make every effort to extract the data from the text or retrieve them through correspondence with the study authors, or both. If we are unsuccessful, we will summarise data to the furthest possible detail in narrative and in additional tables as necessary.

We will count multiple live births (twins, triplets) as a single live birth event.

Dealing with missing data

We will evaluate included studies to determine whether missing data are randomly distributed. Where data are missing, we will contact trial authors to retrieve as much information as possible. Where this is unobtainable, we will undertake imputation of individual values for our primary effectiveness outcome live birth/ongoing pregnancy only: live birth/ongoing pregnancy will be assumed not to have occurred in participants not reporting this. For other outcomes, we will analyse the available data.

Assessment of heterogeneity

We will initially consider whether the clinical and methodological characteristics of the included studies are consistent enough to provide a clinically meaningful results through data pooling in a meta-analysis.

We will assess statistical heterogeneity by the measure of the I^2 statistic. An I^2 statistic value of 30% to 60% will be considered as moderate heterogeneity, and a value of 60% to 90% as substantial heterogeneity across studies (Higgins 2011). In case of substantial heterogeneity with important clinical impact for a specific outcome, we will explore possible explanations through subgroup and sensitivity analyses (where data are available) and will consider not pooling the data.

Assessment of reporting biases

Through a thorough search for published and unpublished data, we aim to minimise the potential impact of publication bias and other reporting biases. We will use a funnel plot to explore publication bias when the number of included RCTs in the same analysis is at least 10.

Data synthesis

All outcomes are dichotomous (binary), and we will combine the data from similar RCTs using a fixed-effect Mantel-Haenszel model. We will report the pooled odds ratios with a 95% confidence interval. Where events are rare, and if all relevant criteria are fulfilled, we will consider the Peto method for pooling the data. An increase in the odds of the outcome will be displayed graphically in the meta-analyses to the right of the centre-line, and a decrease in the odds of an outcome to the left of the centre-line.

We will carry out pooled analyses for the following comparisons: women/couples randomised receiving endometrial injection of embryo culture supernatant before the embryo transfer, during an artificial reproductive cycle with IVF or ICSI, versus women/couples randomised receiving either:

- any other intervention during an artificial reproductive cycle with IVF or ICSI; or
- usual care (no additional intervention) during an artificial reproductive cycle with IVF or ICSI.

Subgroup analysis and investigation of heterogeneity

Clinical heterogeneity, as the diversity in interventions, may affect the results. Differences in the cross-talk between endometrium and embryo could affect the outcome of the endometrium culture injection at different times before embryo transfer. In addition, the quality of the embryo on day 3 or 5 may be different; the blastocyst is considered to be more viable with higher rates of successful implantation and the number of previous ART cycles reflects the potential of each woman to conceive (more unsuccessful attempts reduce the possibilities). Where there is substantial heterogeneity (I^2 statistic value > 60%), we plan to determine effects for the primary outcomes within the following subgroups, if data are sufficient for any meaningful analyses:

- age of the woman (< 37 years, 38 to 41 years, > 42 years);
- day of embryo transfer (3 or 5);
- type of cycle (frozen or fresh);
- time of endometrium culture injection before embryo transfer.

Sensitivity analysis

We will conduct sensitivity analyses for the primary outcomes in order 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:

- we restricted eligibility to studies without high risk of bias (studies at low risk of bias with respect to randomisation methods and not at high risk of bias in any domain);
- we had adopted a random-effects model;
- we have considered publication type (abstract vs. full text);
- miscarriage had been analysed per pregnancy rather than per woman;
- the primary outcome was live birth alone (not including ongoing pregnancy).

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

We will prepare a 'Summary of findings' table using the browser-based version of GRADEpro (GRADEpro GDT 2015). This table evaluates the overall quality of the body of evidence for the main review comparison (endometrial injection of embryo culture supernatant versus: a. no intervention; and b. any other intervention) on the primary and most important secondary outcomes (live birth/ongoing pregnancy, miscarriage, clinical pregnancy and adverse events) using GRADE criteria on study limitations, consistency of effect, imprecision, indirectness and publication bias. We will justify, document and incorporate judgements about evidence quality into reporting of results for each outcome. Two review authors (ES and DV) will independently assess the quality of the evidence, and will resolve any disagreements by consulting a third review author (CSS). We may prepare secondary 'Summary of findings' tables for other review comparisons.

We plan to extract study data, format our comparisons in data tables and prepare a 'Summary of findings' table before writing the results and conclusions of our review.

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* *Indicates the major publication for the study*

APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility Specialised Register search strategy

From inception to present
Procite platform
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 responders" or "poor responder" or "poor prognostic patients" or "controlled ovarian hyperstimulation" or "controlled ovarian stimulation" or "COH" 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 "ovarian stimulation" or "ovarian stimulation controlled ovarian stimulation" or "ovulation induction" or "ovulation stimulation" or "superovulation" or "superovulation induction" or "ovarian hyperstimulation"
AND
Keywords CONTAINS "uterine cavity injection" or "intrauterine flushing" or "Intrauterine injection" or "intrauterine instillation" or "flushing media" or "Flushing-Outcome" or "stimulation of endometrium embryo transfer" or "endometrial preparation" or "endometrial priming" or "endometrial receptivity" or "endometrial stimulation" or "embryo culture supernatant" or Title CONTAINS "uterine cavity injection" or "intrauterine flushing" or "Intrauterine injection" or "intrauterine instillation" or "flushing media" or "Flushing-Outcome" or "stimulation of endometrium embryo transfer" or "endometrial preparation" or "endometrial priming" or "endometrial receptivity" or "endometrial stimulation" or "embryo culture supernatant"

Appendix 2. CENTRAL (CRSO) search strategy

From inception to present
Web platform
#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 embryo*: TI,AB,KY
#5 (vitro fertili?ation):TI,AB,KY
#6 ivf:TI,AB,KY
#7 icsi:TI,AB,KY
#8 (intracytoplasmic sperm injection*):TI,AB,KY
#9 blastocyst*:TI,AB,KY
#10 infertil* or subfertil*:TI,AB,KY
#11 assisted reproducti*:TI,AB,KY
#12 poor responder*:TI,AB,KY
#13 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12
#14 (inject* adj5 culture*):TI,AB,KY
#15 (inject* adj5 medi*):TI,AB,KY
#16 (supernatant adj5 embryo*):TI,AB,KY
#17 (supernatant adj5 blastocyst*):TI,AB,KY
#18 (supernatant adj5 endometri*):TI,AB,KY
#19 (supernatant adj5 uter*):TI,AB,KY
#20 (endometri* adj5 inject*):TI,AB,KY
#21 (flush* adj5 endometri*):TI,AB,KY
#22 (flush* adj5 uter*):TI,AB,KY
#23 (culture* adj5 uter*):TI,AB,KY
#24 (culture* adj5 endometri*):TI,AB,KY
#25 (inject* adj5 uter*):TI,AB,KY
#26 (stimulat* adj2 endomet*):TI,AB,KY

#27 (culture* adj5 supernatant*):TI,AB,KY
#28 (flush* adj5 supernatant*):TI,AB,KY
#29 (transfer* adj5 supernatant):TI,AB,KY
#30 (inject* adj5 supernatant):TI,AB,KY
#31 (intrauter* adj5 inject*):TI,AB,KY
#32 (intrauter* adj5 flush*):TI,AB,KY
#33 (intrauter* adj5 supernatant*):TI,AB,KY
#34 (intrauter* adj5 culture):TI,AB,KY
#35 (instillation adj5 culture):TI,AB,KY
#36 (instillation adj5 uter*):TI,AB,KY
#37 MESH DESCRIPTOR Embryo Culture Techniques EXPLODE ALL TREES
#38 #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37
#39 #13 AND #38

Appendix 3. MEDLINE search strategy

From inception to present
Ovid platform
1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/
2 embryo transfer$.tw.
3 vitro fertili?ation.tw.
4 ivf-et.tw.
5 icsi.tw.
6 intracytoplasmic sperm injection$.tw.
7 (blastocyst adj2 transfer$).tw.
8 ivf.tw.
9 or/1-8
10 (inject* adj5 culture*).tw.
11 (inject* adj5 medi*).tw.
12 (supernatant adj5 embryo*).tw.
13 (supernatant adj5 blastocyst*).tw.
14 (supernatant adj5 endometri*).tw.
15 (supernatant adj5 uter*).tw.
16 (endometri* adj5 inject*).tw.
17 (flush* adj5 endometri*).tw.
18 (flush* adj5 uter*).tw.
19 (culture* adj5 uter*).tw.
20 (culture* adj5 endometri*).tw.
21 (inject* adj5 uter*).tw.
22 exp Embryo Culture Techniques/
23 (stimulat* adj2 endomet*).tw.
24 (culture* adj5 supernatant*).tw.
25 (flush* adj5 supernatant*).tw.
26 (transfer* adj5 supernatant).tw.
27 (inject* adj5 supernatant).tw.
28 (intrauter* adj5 inject*).tw.
29 (intrauter* adj5 flush*).tw.
30 (intrauter* adj5 supernatant*).tw.
31 (intrauter* adj5 culture).tw.
32 (instillation adj5 culture).tw.
33 (instillation adj5 uter*).tw.

34 or/10-33
35 randomized controlled trial.pt.
36 controlled clinical trial.pt.
37 randomized.ab.
38 randomised.ab.
39 placebo.tw.
40 clinical trials as topic.sh.
41 randomly.ab.
42 trial.ti.
43 (crossover or cross-over or cross over).tw.
44 or/35-43
45 exp animals/ not humans.sh.
46 44 not 45
47 9 and 34 and 46

Appendix 4. Embase search strategy

From inception to present
Ovid platform
1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/
2 embryo$ transfer$.tw.
3 in vitro fertili?ation.tw.
4 icsi.tw.
5 intracytoplasmic sperm injection$.tw.
6 (blastocyst adj2 transfer$).tw.
7 ivf.tw.
8 assisted reproduct$.tw.
9 ovulation induc$.tw.
10 superovulat$.tw.
11 COH.tw.
12 infertil$.tw.
13 subfertil$.tw.
14 (ovari$ adj2 induction).tw.
15 exp infertility therapy/
16 exp ovulation induction/
17 exp ovary hyperstimulation/
18 (ovar$ adj2 hyperstimulation).tw.
19 (ovar$ adj2 stimulat$).tw.
20 or/1-19
21 exp embryo culture/ and (uter* or endometri*).tw.
22 (inject* adj5 culture*).tw.
23 (inject* adj5 medi*).tw.
24 (supernatant adj5 embryo*).tw.
25 (supernatant adj5 blastocyst*).tw.
26 (supernatant adj5 endometri*).tw.
27 (supernatant adj5 uter*).tw.
28 (endometri* adj5 inject*).tw.
29 (flush* adj5 endometri*).tw.
30 (flush* adj5 uter*).tw.
31 (culture* adj5 uter*).tw.
32 (culture* adj5 endometri*).tw.
33 (inject* adj5 uter*).tw.

34 (stimulat* adj2 endomet*).tw.
35 (culture* adj5 supernatant*).tw.
36 (flush* adj5 supernatant*).tw.
37 (transfer* adj5 supernatant).tw.
38 (inject* adj5 supernatant).tw.
39 (intrauter* adj5 inject*).tw.
40 (intrauter* adj5 flush*).tw.
41 (intrauter* adj5 supernatant*).tw.
42 (intrauter* adj5 culture).tw.
43 (instillation adj5 culture).tw.
44 (instillation adj5 uter*).tw.
45 or/21-44
46 20 and 45
47 Clinical Trial/
48 Randomized Controlled Trial/
49 exp randomization/
50 Single Blind Procedure/
51 Double Blind Procedure/
52 Crossover Procedure/
53 Placebo/
54 Randomi?ed controlled trial$.tw.
55 Rct.tw.
56 random allocation.tw.
57 randomly.tw.
58 randomly allocated.tw.
59 allocated randomly.tw.
60 (allocated adj2 random).tw.
61 Single blind$.tw.
62 Double blind$.tw.
63 ((treble or triple) adj blind$).tw.
64 placebo$.tw.
65 prospective study/
66 or/47-65
67 case study/
68 case report.tw.
69 abstract report/ or letter/
70 or/67-69
71 66 not 70
72 (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)
73 71 not 72
74 46 and 73

Appendix 5. CINAHL Plus search strategy

From inception to present
EBSCO platform

#	Query
S30	S10 AND S29
S29	S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28
S28	TX(instillation N5 uter*)
S27	TX(instillation N5 culture)
S26	TX(intrauter* N5 culture)
S25	TX(intrauter* N5 inject*)
S24	TX(inject* N5 supernatant)
S23	TX(transfer* N5 supernatant)
S22	TX(culture* N5 supernatant*)
S21	TX(stimulat* N2 endomet*)
S20	TX(inject* N5 uter*)
S19	TX(culture* N5 endometri*)
S18	TX(culture* N5 uter*)
S17	TX(flush* N5 uter*)
S16	TX(flush* N5 endometri*)
S15	TX(endometri* N5 inject*)
S14	TX(supernatant N5 endometri*)
S13	TX (supernatant N5 embryo*)
S12	TX (inject* N5 medi*)
S11	TX (inject* N5 culture*)
S10	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9
S9	(MM "Embryo Transfer")
S8	TX blastocyst* N3 transfer*

(*Continued*)

S7	TX embryo* N3 transfer*
S6	TX ovar* N3 hyperstimulat*
S5	TX ovari* N3 stimulat*
S4	TX IVF or TX ICSI
S3	(MM "Fertilization in Vitro")
S2	TX vitro fertilization
S1	TX vitro fertilisation

Appendix 6. World Health Organization International Trials Registry Platform and ClinicalTrials.gov registry

From inception to present
Keywords or Title CONTAINS
"IVF" or "ART" and "endometrial injection" or "embryo culture supernatant"

CONTRIBUTIONS OF AUTHORS

CSS designed and drafted the protocol and is the guarantor of the review.

ES and DV contributed to the design and drafting of the protocol.

All authors critically reviewed the manuscript for content, and approved the final version for publication.

DECLARATIONS OF INTEREST

CSS, ES and DV have no conflicts of interest to disclose.

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- None, Other.