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Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies (Review)

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Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

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ABSTRACT

Background

In order to overcome the low effectiveness of assisted reproductive technologies (ART) and the high incidence of multiple births, metabolomics is proposed as a non-invasive method to assess oocyte quality, embryo viability, and endometrial receptivity, and facilitate a targeted subfertility treatment.

Objectives

To evaluate the effectiveness and safety of metabolomic assessment of oocyte quality, embryo viability, and endometrial receptivity for improving live birth or ongoing pregnancy rates in women undergoing ART, compared to conventional methods of assessment.

Search methods

We searched the Cochrane Gynaecology and Fertility Group Trials Register, CENTRAL, MEDLINE, Embase, CINAHL and two trial registers (February 2018). We also examined the reference lists of primary studies and review articles, citation lists of relevant publications, and abstracts of major scientific meetings.

Selection criteria

Randomised controlled trials (RCTs) on metabolomic assessment of oocyte quality, embryo viability, and endometrial receptivity in women undergoing ART.

Data collection and analysis

Pairs of review authors independently assessed trial eligibility and risk of bias, and extracted the data. The primary outcomes were rates of live birth or ongoing pregnancy (composite outcome) and miscarriage. Secondary outcomes were clinical pregnancy, multiple and ectopic pregnancy, cycle cancellation, and foetal abnormalities. We combined data to calculate odds ratios (ORs) for dichotomous data and 95% confidence intervals (CIs). Statistical heterogeneity was assessed using the I^2 statistic. We assessed the overall quality of the evidence for the main comparisons using GRADE methods.

Main results

We included four trials with a total of 924 women, with a mean age of 33 years. All assessed the role of metabolomic investigation of embryo viability. We found no RCTs that addressed the metabolomic assessment of oocyte quality or endometrial receptivity.

We found low-quality evidence of little or no difference between metabolomic and non-metabolomic assessment of embryos for rates of live birth or ongoing pregnancy (OR 1.02, 95% CI 0.77 to 1.35, $I^2 = 0\%$; four RCTs; N = 924), live birth alone (OR 0.99, 95% CI 0.69 to 1.44, $I^2 = 0\%$; three RCTs; N = 597), or miscarriage (OR 1.18, 95% CI 0.77 to 1.82; $I^2 = 0\%$; three RCTs; N = 869). A sensitivity analysis excluding studies at high risk of bias did not change the interpretation of the results for live birth or ongoing pregnancy (OR 0.90, 95% CI 0.66 to 1.25, $I^2 = 0\%$; two RCTs; N = 744). Our findings suggested that if the rate of live birth or ongoing pregnancy was 36% in the non-metabolomic group, it would be between 32% and 45% with the use of metabolomics.

We found low-quality evidence of little or no difference between groups in rates of clinical pregnancy (OR 1.11, 95% CI 0.85 to 1.45; $I^2 = 44\%$; four trials; N = 924) or multiple pregnancy (OR 1.50, 95% CI 0.70 to 3.19; $I^2 = 0\%$; two RCTs, N = 180). Rates of cycle cancellation were higher in the metabolomics group (OR 1.78, 95% CI 1.18 to 2.69; $I^2 = 51\%$; two RCTs; N = 744, low quality evidence). There was very low-quality evidence of little or no difference between groups in rates of ectopic pregnancy rates (OR 3.00, 95% CI 0.12 to 74.07; one RCT; N = 417), and foetal abnormality (no events; one RCT; N = 125). Data were lacking on other adverse effects. A sensitivity analysis excluding studies at high risk of bias did not change the interpretation of the results for clinical pregnancy (OR 1.03, 95% CI 0.76 to 1.38; $I^2 = 40\%$; two RCTs; N = 744).

The overall quality of the evidence ranged from very low to low. Limitations included serious risk of bias (associated with poor reporting of methods, attrition bias, selective reporting, and other biases), imprecision, and inconsistency across trials.

Authors' conclusions

According to current trials in women undergoing ART, there is no evidence to show that metabolomic assessment of embryos before implantation has any meaningful effect on rates of live birth, ongoing pregnancy, miscarriage, multiple pregnancy, ectopic pregnancy or foetal abnormalities. The existing evidence varied from very low to low-quality. Data on other adverse events were sparse, so we could not reach conclusions on these. At the moment, there is no evidence to support or refute the use of this technique for subfertile women undergoing ART. Robust evidence is needed from further RCTs, which study the effects on live birth and miscarriage rates for the metabolomic assessment of embryo viability. Well designed and executed trials are also needed to study the effects on oocyte quality and endometrial receptivity, since none are currently available.

PLAIN LANGUAGE SUMMARY

Metabolomics for improving pregnancy outcomes

Review question

Cochrane researchers reviewed the evidence about the effectiveness of metabolomics as an evaluation tool to improve the rates of ongoing pregnancy, live birth, and miscarriage in women who were undergoing assisted reproductive technology (ART).

Background

Metabolomics is the scientific study of the chemical 'fingerprints' that biological cells, tissues, or organs produce after various cellular processes. They have been proposed as a powerful non-traumatic method to assess the quality of oocytes, viability of embryos, and receptivity of the endometrium in subfertile women undergoing ART. The final aim of their use is to overcome the high incidence of multiple births and to enhance the performance of ART. However, evidence on their use remains contradictory. Therefore, it was important to evaluate the current evidence on the effectiveness of metabolomics versus conventional techniques (such as the assessment by morphology only) in providing sufficient information on the adequacy of the physiology and function of embryos, oocytes and endometrium, to facilitate targeted subfertility treatments.

Study characteristics

We found four randomised controlled trials, with a total of 924 women, that compared metabolomic profile assessment with morphology assessment of embryos. The women were an average age of 33 years old. All studies were conducted between 2011 and 2013; length of follow-up was not specified in any of them. The evidence is current to 26 February 2018.

Study funding sources

One study was supported by an unconditional grant from a biotechnology company (Molecular Biometrics Inc.). The very low conditional superiority for the primary outcome and premature termination of the trial were potentially associated with the funder's interest in the results. One study received funding from a national health organisation, but the equipment was provided by Molecular Biometrics Inc., one was self-funded, while the source of funding was not stated in the fourth study.

Key results

We found low-quality evidence of no meaningful difference between the intervention and control groups in rates of live birth, ongoing pregnancy, miscarriage, or clinical pregnancy, and multiple pregnancy. We found very low-quality evidence of no meaningful difference between the groups for ectopic pregnancy, and low-quality evidence that cancellation rates were higher in the intervention group. Our findings suggest that if the rate of live birth or ongoing pregnancy was 36% in the non-metabolomic group, it would be between 32% and 45% with the use of metabolomics. Data were lacking on other adverse effects. No properly designed studies reported metabolomic assessment of oocyte quality or endometrium receptivity.

Quality of evidence

The overall quality of evidence ranged from low to very low. Limitations included serious risk of bias (associated with poor reporting of methods, attrition bias, selective reporting and other bias), imprecision, and inconsistency across trials.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [\[Explanation\]](#)

Metabolomics compared to non-metabolomics for embryo quality assessment						
Patient or population: Women undergoing IVF/ICSI cycles Setting: Assisted reproduction units from Greece, Sweden and Germany Intervention: Metabolomic assessment of embryos Comparison: Non-metabolomic assessment						
Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with non-metabolomic assessment	Risk with metabolomic assessment				
Live birth rate (Delivery of a live fetus after 20 completed weeks of gestation)	296 per 1000	294 per 1000 (225 to 377)	OR 0.99 (0.69 to 1.44)	597 (3 RCTs)	⊕⊕○○ LOW ^{1,2}	
Live birth rate or ongoing pregnancy (Delivery of a live fetus after 20 completed weeks of gestation or the presence of a fetal heart on ultrasound scan after 12 weeks)	353 per 1,000	353 per 1,000 (293 to 418)	OR 1.02 (0.77 to 1.35)	924 (4 RCTs)	⊕⊕○○ LOW ^{1,2}	
Miscarriage (Loss of pregnancy before 20 completed weeks of gestation)	107 per 1,000	127 per 1,000 (87 to 181)	OR 1.18 (0.77 to 1.82)	869 (3 RCTs)	⊕⊕○○ LOW ^{1,2}	

Clinical pregnancy (Presence of a fetal heart on ultrasound scan at seven weeks of gestation)	421 per 1,000	446 per 1,000 (382 to 515)	OR 1.11 (0.85 to 1.45)	924 (4 RCTs)	⊕⊕○○ LOW ^{1,2}
Cancellation (Exclusion of a patient after recruitment)	129 per 1,000	209 per 1,000 (149 to 286)	OR 1.78 (1.18 to 2.69)	744 (2 RCTs)	⊕⊕○○ LOW ^{1,3}
Multiple pregnancy (presence of more than 1 gestational sac)	184 per 1,000	256 per 1,000 (138 to 422)	OR 1.50 (0.70 to 3.19)	180 (2 RCT)	⊕⊕○○ LOW ^{1,2}
Ectopic pregnancy (a pregnancy in which the fetus develops outside the uterus)	Not estimable - only one event reported		OR 3.00 (0.12 to 74.07)	417 (1 RCT)	⊕○○○ VERY LOW ¹⁴
Foetal abnormalities (structural or functional anomalies of the foetus)	Not estimable - no events reported		not estimable	125 (1 RCT)	⊕○○○ VERY LOW ¹⁴

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

CI: Confidence interval; OR: Odds ratio;

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

- ¹ Downgraded one level for serious risk of bias including poor reporting of methods, attrition bias, selective reporting, and other biases. Sensitivity analysis by risk of bias did not appreciably change the estimates.
- ² Downgraded one level for serious imprecision: wide confidence interval was compatible with benefit in either arm, or no difference between the groups
- ³ Downgraded one level for moderate heterogeneity across trials ($I^2 = 51\%$)
- ⁴ Downgraded two levels for very serious imprecision, only one or no events

BACKGROUND

Description of the condition

Subfertility is defined as the inability of a couple of reproductive age to conceive spontaneously after 12 months of regular unprotected sexual intercourse. An estimated 15% of couples are affected by subfertility, with different underlying causes (female factor, male factor, unexplained; [CDC 2007](#)). Assisted reproduction techniques (ART) refer to procedures involving the handling of human reproductive cells outside the uterus, in concordance with the well-synchronised exogenous ovarian stimulation and endometrial preparation required with the aim of establishing a live birth ([Zegers-Hochschild 2009](#)). Unfortunately, the effectiveness of ART is limited, as only 10% to 30% of all embryos replaced in the uterus will implant and finally result in a live birth ([de Mouzon 2010](#); [Lieberman 2001](#)). Only 30% of embryos created by ART are transferred into the uterus, while the majority of them do not produce a live birth ([Kovalevsky 2005](#)). Success rates range from 4.5% to 40.1%, depending on the woman's age ([SART 2009](#)). Three components are considered crucial to the success of ART [including both intra-cytoplasmic sperm injection (ICSI) and in vitro fertilisation (IVF)]: gamete quality, embryo quality, and endometrial receptivity. In clinical practice, gamete quality is assessed through morphological scoring; in the case of oocytes, their low abundance after retrieval requires a more advanced evaluation system than pure morphology ([Nicoli 2013](#)). In clinical practice, oocytes with poor morphological grade may result in progressing embryos without achieving live birth. Good-quality oocytes may not even become fertilised through intracytoplasmic sperm injection, or may generate poor embryos, or embryos without the potential for endometrial implantation ([Sfontouris 2015](#)). In terms of endometrial receptivity, assessment of the endometrium has been commonly done by measuring its thickness through ultrasonography. Current data indicate that this practice has a limited capacity in identifying women with a low chance to conceive after ART ([Kasius 2014](#)). The results of this meta-analysis also indicate that the use of endometrial thickness as an evaluation tool for endometrial receptivity is not justified, and further research is needed.

One of the methods used to improve current pregnancy rates is the transfer of multiple embryos, resulting in an increased incidence of multiple pregnancies and their associated risks during pregnancy for both the mother and foetuses. It also has an economic impact on national health systems because of the risk of life-threatening complications for newborns, and long-term health effects ([Bromer 2008a](#); [Kallen 2010](#); [Kjelberg 2006](#); [Ledger 2006](#)). In addition, researchers have identified a wide cluster of morphologic parameters, such as pronuclear morphology, polar body structure and placement, appearance of cytoplasm, early cleavage, number of blastomeres in certain days of culture, size, symmetry and fragmentation of blastomeres, compaction and expansion of blastomeres,

and multinucleation, associated with increased viability of embryos at various stages ([Baczowski 2004](#)). The need for noninvasive methods to assess embryo quality emerged at least 10 years ago ([Sakkas 2005](#)). Numerous embryo grading systems have been developed on this basis, as well as specially designed incubators that monitor embryonic development and record morphokinetic parameters during cellular division, by assessing the time required to complete a cell division and reach a successive cell cycle, in the framework of cleavage synchronisation ([Chamayou 2013](#)).

Description of the intervention

In order to overcome the low effectiveness of ART and the high incidence of multiple foetuses, metabolomics has emerged to provide sufficient information on the adequacy of the physiology and function of oocytes, embryos, and the endometrium, to facilitate a targeted subfertility treatment. The dynamics of these three components for a successful outcome remain poorly understood ([Varghese 2007](#)). Metabolomics belong to a wider group of 'omics' technologies: these disciplines include the study of the events and interactions of cellular structures and processes from deoxyribonucleic acid (DNA) to biological function, that is from DNA and genes to metabolites, in a complex and global way ([Egea 2014](#)). The metabolomic profile, also known as the metabolome, represents the tissue, cell, or embryo physiology and functional phenotype, where any variations in the cellular functional capacity are reflected in this terminal cellular product, to assist in distinguishing between normal and pathological states ([Vergouw 2010](#)). Various metabolites are examined during this procedure, through signifying cellular activities and their respective concentrations in the biological material or medium under examination. The final goal is to gain a better understanding of the cellular mechanisms involved, and to obtain the opportunity to 'foresee' the fate of an artificially produced embryo ([Uyar 2012](#)).

The metabolome refers to the complete inventory of small-molecule, non-proteinaceous compounds, including metabolic intermediates, adenosine triphosphate, hormones, and metabolites, which are present in a biological sample ([Gardner 2013](#)). Metabolomics is the systematic study of the dynamic inventory of these compounds, which attempts to determine and quantify metabolites associated with physiologic and pathologic states, using various forms of analytical approaches ([Botros 2008](#); [Egea 2014](#)). As 'cellular information providers', they are more informative than genomics, transcriptomics, or proteomics, because they represent the final products of the cell regulatory process, reflect, and are closer to the functional phenotype ([Allen 2003](#); [Egea 2014](#); [Oliver 1998](#)).

In ART, the term 'metabolomics' refers to the particular metabolic products found in specific biological materials or media. In the endometrium, a timely biopsy or endometrial fluid collection and analysis of the metabolites can reveal inadequacy of its receptivity and other pathological states, such as endometriosis, which may

affect the reproductive process. The assessment of oocyte competence and fertilising capacity is mediated mainly through the metabolomic analysis of follicular fluid, although analysis of cumulus and granulosa cells could also contribute crucial information on gamete functionality. In the embryo, the non-interventional approach is to perform an analysis on spent media culture in order to acquire information on the potency of the embryo to implant. This is conducted through the viability scores that are derived from the previous investigation of the metabolites of viable and non-viable embryos, and processed through formulas. Metabolomic analysis, as a technology, is mainly based on techniques, such as gas chromatography, Raman and near infrared (NIR) spectroscopy, that aim to quantify and determine the nature of metabolites present (Brison 2004; Scott 2008; Seli 2007; Seli 2008; Vergouw 2008). Compared with morphology alone, metabolomic profiling constitutes a more reliable parameter, independent of morphology (Vergouw 2008).

The goal of this intervention is to help in determining a healthy and receptive endometrium, and the selection of viable embryos or competent oocytes to improve ART outcome; enhance the ability to perform selective embryo transfers; and avoid implantation failure, biochemical pregnancies, miscarriages, and the adverse health, emotional, and socioeconomic consequences that accompany them.

How the intervention might work

The intervention may work on three different levels:

1. Oocyte quality

The metabolomic assessment of the oocyte can offer reliable biomarkers, and replace other methods of oocyte selection, such as morphology, microscopy, and polar body biopsy, which have been proven to be inefficient to predict the generation of a competent embryo to produce a live birth (Hillier 2008). Although analysis of cumulus and granulosa cells could also serve this purpose, the review focused mainly on follicular fluid, since this is a normally abundant and easily accessible material in *in vitro* fertilisation (IVF) cycles. Egea and colleagues successfully grouped the metabolites of interest into hormones, transforming growth factor beta, other growth factors and interleukins, reactive oxygen species, anti-apoptotic factors, proteins, peptides and amino-acids, sugars, and prostanoids (Egea 2014). In addition, similar studies showed that the metabolite type and concentration in follicular fluid reflects gamete physiology and fertilising capacity (Piñero-Sagredo 2010), developmental competence (Wallace 2012), reduced ovarian reserve due to advanced maternal age (Pacella 2012), and alterations attributed to body mass index (BMI; Valckx 2012).

2. Embryo viability

The metabolomic assessment of the embryo is performed in a non-invasive manner by analysing spent media culture, so the most competent embryo can be transferred, increasing the potential for implantation (Koot 2013; Montag 2013). Accordingly, nutrients and metabolites within the culture media have been studied as potential predictors of embryo quality by using various techniques with different degrees of effectiveness (Bromer 2008b; Egea 2014; Hillier 2008). It has been shown that amino acid turnover can predict the ability of early cleavage stage embryos to form a blastocyst, and that amino acid turnover is significantly associated with the ability of an embryo to implant and give rise to a clinical pregnancy and live birth (Brison 2004; Houghton 2002). Recently, Seli and colleagues published a viability score, generated by the metabolomic profiling of human embryo culture media using NIR spectroscopy, showing that it was more accurate in predicting pregnancy outcome compared to morphologic grading in women undergoing single embryo transfer on day five (Seli 2010).

3. Endometrium receptivity

Until as recently as 2012, the metabolomic analysis of the endometrium was only reported in mice models, which investigated the lipidomic analysis of endometrial receptivity. Studies demonstrated that several lipids, including triglycerides, prostaglandins, thromboxanes, endocannabinoids, and sphingolipids, played an important role in reproductive biology during early pregnancy, including pre-implantation embryo formation and development, implantation, and post-implantation growth (Egea 2014).

Two studies have now been published on metabolomics in the human endometrium (Altmäe 2014). In Vouk 2012, investigators identified biomarkers in plasma for the diagnosis of endometriosis. They showed that elevated levels of sphingomyelins and ether-phospholipids were associated with endometriosis, and presented eight lipids as novel endometriosis-associated biomarkers. Vilella 2013 studied lipidomics of the human endometrium, and demonstrated, for the first time, a significant increase of lipid levels in endometrial fluid during the window of implantation, which could provide a new tool for endometrial-receptivity prediction.

Why it is important to do this review

Metabolomics could offer a powerful means to assist gamete selection and embryo transfer, and determine endometrial status, in order to improve the procedures linked with assisted reproduction, and increase the success rates of ART, thus reducing the emotional stress of the couples, and the social and economic impact that accompanies this procedure. So far, the few data arising from the literature demonstrate considerable variation, and the resulting scientific views are conflicting. Evidence from preliminary prognostic studies has shown promising results for the use of metabolomics to assess embryo viability (Ahlstrom 2011; Nagy

2008; Scott 2008; Seli 2007; Seli 2010; Seli 2011; Vergouw 2008; Vergouw 2011). There appears to be a lack of evidence about the use of metabolomics to assess oocyte quality and endometrium receptivity.

There is an emerging need to determine whether metabolomics could effectively facilitate an improved and individualised treatment of subfertile couples through ART, in order to assess its utility in clinical practice. We also evaluated the methodological quality of existing and ongoing trials, and informed the conduct of more studies on the topic.

OBJECTIVES

To evaluate the effectiveness and safety of metabolomic assessment of oocyte quality, embryo viability, and endometrial receptivity for improving live birth or ongoing pregnancy rates in women undergoing ART, compared to conventional methods of assessment.

METHODS

Criteria for considering studies for this review

Types of studies

Published and unpublished randomised controlled trials (RCTs) assessing the role of metabolomic investigation of the endometrium, oocytes, and embryos in improving clinical outcomes in women undergoing ART [including both intra-cytoplasmic sperm injection (ICSI) or in vitro fertilisation (IVF)]. We applied no limitations on country of origin or language.

Types of participants

Women and couples referred to assisted reproduction units and undergoing IVF or ICSI cycles (both fresh and frozen). Oocyte donation cycles were excluded.

Types of interventions

Clinical decisions based on endometrial, oocyte, and embryo metabolomic profile assessment, during an artificial reproductive cycle with IVF or ICSI, versus any other assessment (traditional techniques, such as morphology grading). Time-lapse systems (where images of embryos at frequent time intervals are being taken, this allowing assessment of embryos without removing them from the incubator) for assessing the embryo quality were excluded, as it is the topic of another review (Armstrong 2015).

Types of outcome measures

Primary outcomes

1. Effectiveness

Live birth or ongoing pregnancy rates (defined as the delivery of a live foetus after 20 completed weeks of gestation, and the presence of a foetal heart on ultrasound scan after 12 weeks of gestation, respectively) per woman or couple randomised. We counted multiple live births (twins, triplets) as a single live birth event.

2. Adverse events

Miscarriage rates (the loss of pregnancy before 20 completed weeks of gestation) per woman or couple randomised.

Secondary outcomes

3. Effectiveness

Clinical pregnancy rates (the presence of a foetal heart on ultrasound scan at seven weeks of gestation) per woman or couple randomised.

4. Adverse events

Cancellation (defined as the cycle in which ovarian stimulation or monitoring has been carried out with the intention to treat, but which did not proceed to follicular aspiration or, in the case of a thawed embryo, to embryo transfer (Zegers-Hochschild 2009)), multiple and ectopic pregnancy rates, and foetal abnormality rate per woman or couple randomised.

Search methods for identification of studies

In consultation with the Cochrane Gynaecology and Fertility Group Information Specialist, we searched for published and unpublished RCTs that assessed the impact of metabolomic investigation of the oocyte quality, embryo viability, and endometrial receptivity in women undergoing IVF or ICSI. All searches were performed from inception of the relevant databases until 26 February 2018.

Electronic searches

We have presented the search strategies used for the Cochrane Gynaecology and Fertility Group Specialised Register, from inception to 26 February 2018 (PROCITE platform) (Appendix 1), the Cochrane Central Register of Studies, searched 26 February 2018 (CENTRAL CRSO web platform) (Appendix 2), MEDLINE, from 1946 to 26 February 2018 (OVID platform) (Appendix

3), Embase, from 1980 to 26 February 2018 (Ovid platform) (Appendix 4), and CINAHL, from 1961 to 26 February 2018 (Ebsco platform) (Appendix 5). All searches were carried out without any language or date restriction.

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

We searched the World Health Organization International Trials Registry (apps.who.int/trialsearch/Default.aspx) and the ClinicalTrials.gov (ClinicalTrials.gov) registry for ongoing and registered trials (Appendix 6). We searched OpenGrey (www.opengrey.eu/) for grey literature (Appendix 7). These databases were searched on 27 February 2018. We also consulted experienced clinicians to see if they were aware of any ongoing or existing studies.

Searching other resources

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

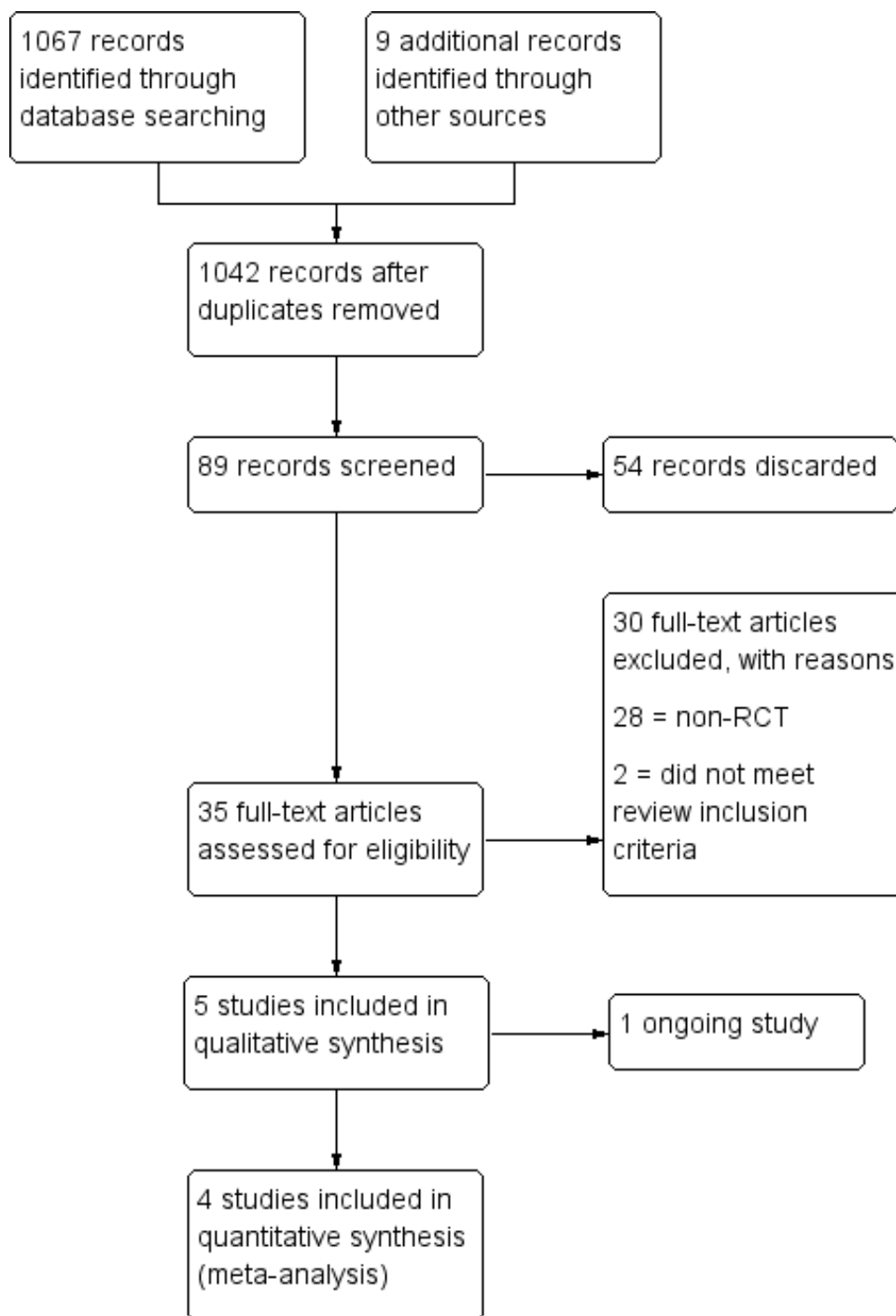
Data collection and analysis

We entered data into Review Manager 5 (RevMan 5; RevMan 2014), and conducted statistical analysis in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Selection of studies

Two review authors independently screened titles and abstracts for each publication and excluded studies irrelevant to the objective of this review. We retrieved the remaining publications in full text, and the same review authors independently appraised them in order to identify RCTs suitable for inclusion. Any potential disagreements were resolved by discussion with the first review author. We outlined the selection process in a PRISMA flow chart (Figure 1).

Figure 1. Study flow diagram.



Data extraction and management

Three review authors independently extracted the data from the included studies using a pre-designed data extraction form. Our intention was to use the main RCT report as the reference in studies with multiple publications, and to supplement it with additional data from the secondary publications.

We corresponded successfully with all authors of the studies that were eligible for inclusion in an attempt to retrieve additional data or methodological details. We resolved potential disagreements through consensus, involving the first review author. One review author imported data into RevMan 5, and a second review author validated the imported values against the data extraction form.

Assessment of risk of bias in included studies

Two review authors independently assessed the included studies for risk of bias, using the Cochrane 'Risk of bias' assessment tool, for selection, performance, detection, attrition, reporting, and other biases (Higgins 2011). We explicitly reported our judgement concerning the risk of bias in the 'Risk of bias' table in the 'Characteristics of included studies' section.

A priori, we seriously considered that a degree of bias might inevitably exist in the studies, due to the difficulty in blinding clinical staff and embryologists, given the nature of clinical processes required for the best possible outcome of the treatment. Considering this, we proceeded with a meticulous evaluation of the methods of each study, and requested further specifications in order to clarify our evaluations.

Measures of treatment effect

All defined outcomes were binary (dichotomous), and we used the numbers of events in the control and intervention groups of each study to calculate Mantel-Haenszel odds ratios (OR). We presented 95% confidence intervals (CI) for the ORs.

Unit of analysis issues

We expected all studies to have the woman (or couple) as the unit of randomisation. When data were not reported per woman (or couple), for example data reported 'per cycle', we retrieved the data from the text, through communication with the authors, or both.

Dealing with missing data

We evaluated the included studies to determine whether missing data were randomly distributed. Our plan was that if data loss exceeded 20%, we would test this with sensitivity analyses; in the current review, data loss did not exceed this cut-off point. Where

data were missing, we added supplementary data, provided by the authors of the original studies through personal communication.

Assessment of heterogeneity

First, we considered whether the clinical and methodological characteristics of the included studies were sufficiently similar to provide a clinically meaningful summary by pooling the data. We assessed statistical heterogeneity by the I^2 statistic. An I^2 of 30% to 60% indicated moderate heterogeneity, and we considered levels 60% to 90% to indicate substantial heterogeneity across studies (Higgins 2011). In the case of substantial heterogeneity for a specific outcome, we would not perform meta-analysis.

In cases that true effect sizes differed among studies, we had planned to explore clinical heterogeneity among studies using a random-effects model; this was not the case in our review.

Assessment of reporting biases

We conducted a comprehensive search in order to minimise the potential impact of publication and other reporting biases. We had planned to use a funnel plot to explore publication bias when the number of included RCTs exceeded 10, but we only included 10 studies in the current review.

We examined the possibility of within-study selective reporting for each included study by comparing, if available, either the protocol outcomes with the published study outcomes, or the outcomes listed in the methods section with the reported results.

Data synthesis

All analyses were carried out as per ITT analyses. All outcomes were dichotomous (binary), and we combined the data from similar outcomes using a fixed-effect Mantel-Haenszel model. We reported the pooled ORs with a 95% CI. In the case of considerable clinical, methodological, or statistical heterogeneity, our intention was to use a random-effects model. Where events were rare, and if all relevant criteria were fulfilled, we would have considered the Peto method for pooling the data. An increase in the odds of the outcome is displayed graphically in the forest plot to the right of the centre-line, and a decrease in the odds of an outcome to the left of the centre-line.

We carried out pooled analyses for the following comparisons:

- Women or couples randomised to undergo metabolomic investigation versus those randomised to routine ART procedures without any metabolomic investigation.

We had planned to stratify this comparison for the three different categories of metabolomic investigation: oocytes, embryos, and

endometrium. However, there were not enough data to use this stratification.

Subgroup analysis and investigation of heterogeneity

In the presence of sufficient data, we had planned to perform subgroup analysis for the primary outcomes set for this review, according to:

1. Subfertility factor (male, tubal, anovulation, endometriosis, unexplained), BMI (between 18.5 and 24.9 kg/m², equal to or greater than 25 kg/m², equal or greater than 30 kg/m², and equal to or greater than 35 kg/m²), and age of the woman (younger than 37, 37 to 39, 40 or older; [ESHRE Task Force on Ethics and Law 2010](#); [NICE 2013](#)).
2. IVF or ICSI cycles, IVF or ICSI protocol, adjuncts used, or both.
3. Technique used for the metabolomic profiling of oocytes, embryos, or endometrium.
4. Age (day) and number of embryos transferred.

Sensitivity analysis

We conducted sensitivity analyses for the primary, and most important, outcome (e.g. clinical pregnancy), in order to determine whether the conclusions were robust, and able to stand up to our arbitrary decisions on eligibility and analysis.

These analyses considered whether the review conclusions would have differed if:

- eligibility were restricted to studies without high risk of bias;
- a random-effects model had been adopted;
- publication type had been considered (abstract versus full text).
- the primary outcome was restricted to live birth.

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

We prepared a 'Summary of findings' table using the browser-based version of GRADEpro ([GRADEpro GDT 2014](#)). This table presents the overall quality of the body of evidence for the main review outcomes (live birth, ongoing pregnancy, miscarriage, clinical pregnancy, cancellation, multiple and ectopic pregnancy, and foetal abnormalities), using five GRADE criteria: study limitations, consistency of effect, imprecision, indirectness, and publication bias ([Higgins 2011](#)). Two authors independently assessed the quality of the evidence for each outcome. We justified, documented, and incorporated judgements about the quality of the evidence when reporting the results for each outcome.

RESULTS

Description of studies

Results of the search

Through the initial database search and other sources, we identified 1076 articles. After removing 15 duplicate records, and screening 89 abstracts, we identified 35 studies as possibly eligible, and retrieved the full-text report. Out of them, we excluded 30 studies with reasons, identified one ongoing study, and selected four studies for analysis. We had no studies awaiting further assessment. We have displayed the summary details in the relevant tables: [Characteristics of included studies](#); [Characteristics of excluded studies](#); [Characteristics of ongoing studies](#); [Figure 1](#).

Included studies

Four studies, including a total of 924 women, met the inclusion criteria ([Economou 2011](#); [Hardarson 2012](#); [Sfontouris 2013](#); [Vergouw 2012](#)).

Study design and setting

Two of the studies were large; [Vergouw 2012](#) randomised 417 women and [Hardarson 2012](#) randomised 327. The remaining two studies were smaller: [Economou 2011](#) randomised 56 women and [Sfontouris 2013](#) randomised 125. The studies were conducted in ART clinics in Greece, the Netherlands, and Sweden. Two trials were terminated prematurely. One study was funded by an unconditional grant from Molecular Biometrics (MB) Inc., USA ([Hardarson 2012](#)), and one by a grant from a National Health Organisation, with MB providing the equipment needed ([Vergouw 2012](#)).

Participants

The mean age of the women in the four studies was 33 years, and did not differ significantly across studies or intervention groups. The studies included subfertile women with a good prognosis for IVF; they excluded women with discouraging factors, such as undergoing frozen-thawed cycles, cycles with oocyte donation, or testicular biopsy. Although cause or duration of subfertility of the women was described in all studies, it was not clarified whether they had previously undergone IVF or ICSI treatments.

Data availability

For [Economou 2011](#) and [Sfontouris 2013](#), for all randomised women data were available.

In [Vergouw 2012](#) 417 women were randomised, of whom 108 were excluded prior to treatment because good quality embryos were not available. We believe that randomisation should have taken place after assessing the eligibility of embryos and not afterwards. However, as we couldn't determine this for the particular

study, we used these data and included them in an intention to treat analysis.

[Hardarson 2012](#) offered data on 312 of the 327 randomised women; for 12 women in the metabolomics group the data losses were due to failed near infrared (NIR) spectroscopy, and for three women in the control group the reasons were referred to as 'protocol violation and admin reasons'.

Interventions

In the intervention group, all good quality embryos were analysed using a near-infrared (NIR) spectroscopy system scheduled for IVF use (Molecular Biometrics Inc., Norwood, MA, USA). According to the technical procedure, a small aliquot was aspirated from the culture medium and injected into a sample cell. Then, using a non-invasive screening technology based on NIR spectroscopy to analyse the chemical content of spent culture medium, a viability algorithm was developed. Finally, when the NIR analysis had been performed on the good quality embryos group, the embryo with the highest numerical score was chosen for embryo transfer. In all four studies, the same equipment and algorithm were used.

Outcomes

The primary outcomes measured were live birth or ongoing pregnancy (as a combined outcome), and the adverse event of miscarriage, per woman randomised. Rates of live birth or ongoing pregnancy were reported in all four studies (924 women analysed). Three studies reported on live birth rates only, while one reported on live birth or ongoing clinical pregnancy rates ([Hardarson 2012](#)). Miscarriage rate was reported in three studies (869 women analysed; [Sfontouris 2013](#); [Vergouw 2012](#); [Hardarson 2012](#)). For the primary outcome we had data availability separately for live births on three of the studies, with only [Hardarson 2012](#) reporting only the composite outcome of 'live or ongoing' pregnancy. Hence we have additionally analysed live births only, based on data from three studies ([Vergouw 2012](#), [Economou 2011](#), and [Sfontouris 2013](#)), as a sensitivity analysis for the composite outcome.

The secondary outcomes measured were clinical pregnancy, multiple pregnancy, ectopic pregnancy, cancellation, and foetal abnormalities per woman/couple randomised. Clinical pregnancy was reported in all four studies. Cancellation was reported in two studies (744 women; [Hardarson 2012](#); [Vergouw 2012](#)). Multiple pregnancy was reported in two studies (180 women; [Economou 2011](#); [Sfontouris 2013](#)). The ectopic pregnancy rate was reported in one study (417 women; [Vergouw 2012](#)). Foetal abnormality was reported in one study (125 women; [Sfontouris 2013](#)).

Excluded studies

We excluded 30 studies from the review, mostly because they were not RCTs, or did not explore the outcomes of interest prespecified in this review. We have described details in the [Characteristics of excluded studies](#) table.

Studies awaiting assessment

No studies are awaiting further classification.

Ongoing studies

We found one ongoing study, which was registered as a clinical trial in February 2016 ([NCT02698488](#)); there are no preliminary results so far.

Risk of bias in included studies

As summarised below, and detailed in the 'Risk of bias' tables, [Figure 2](#) and [Figure 3](#), the risk of most types of bias was unclear for all studies. Three of the studies had a registered protocol in international databases ([Hardarson 2012](#); [Sfontouris 2013](#); [Vergouw 2012](#)). Only one trial was assessed at high risk of attrition and reporting bias ([Sfontouris 2013](#)). However, all four studies were assessed at high or unclear risk of other sources of bias. The considerable impact of all types of bias on the reliability of the results contributed to our decision to reduce the quality of the evidence to low to very low for most outcomes.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

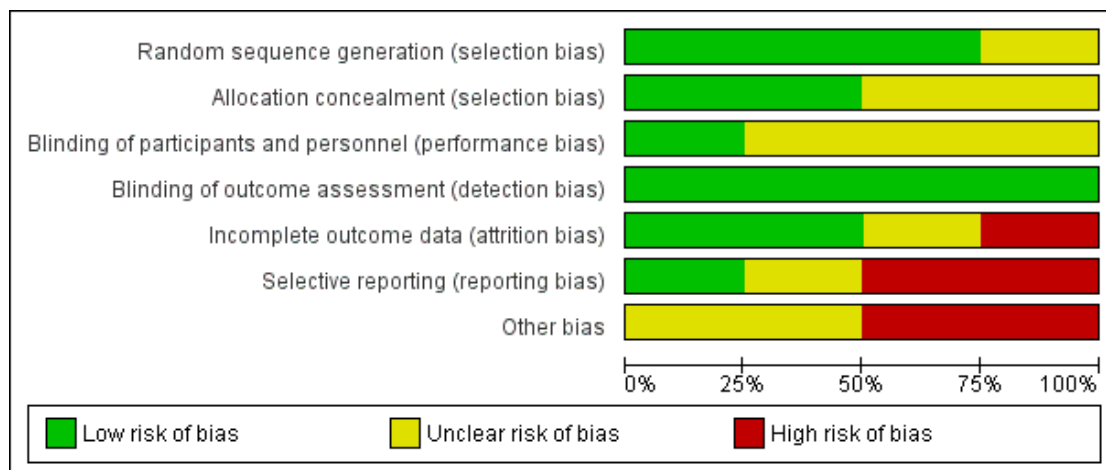


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Economou 2011	?	?	?	+	?	?	?
Hardarson 2012	+	+	?	+	+	-	-
Sfontouris 2013	+	?	?	+	-	-	?
Vergouw 2012	+	+	+	+	+	+	-

Allocation

All four studies were introduced as randomised controlled trials. We assessed three of them at low risk of selection bias for random sequence generation, as the investigators used computerised randomisation sequences for the selection of the women, while one was considered at unclear risk due to lack of information on the methods used (Economou 2011). Two studies used either a secure database or sealed, consecutively numbered opaque envelopes, so we considered them at low risk of bias for allocation concealment (Hardarson 2012; Vergouw 2012). We assessed the other two studies as unclear risk of bias, since they mentioned the use of closed or sealed envelopes, but gave no further details of methods used.

Blinding

One of the studies blinded patients, physicians and laboratory personnel, and we considered it at low risk of performance bias (Vergouw 2012). Three studies did not report blinding of participants and personnel, and were assessed as at unclear risk of performance bias. We rated all studies at low risk of detection bias, as we judged that the outcome measures were not likely to be influenced by lack of blinding.

Incomplete outcome data

We assessed two of the studies at low risk of attrition bias, since both of them performed both intention-to-treat and per-protocol analyses, and provided plausible justification for exclusions; missing outcome data were balanced across the groups, and were unlikely to influence the intervention effect estimate (Hardarson 2012; Vergouw 2012). We considered one study at unclear risk of attrition bias; although outcome data were not missing, the number of women eligible for randomisation and number of exclusions were not stated (Economou 2011). We determined that one trial was at high risk. The investigators stated that the sample size could not achieve adequate power due to premature termination of the study, but there was an excessive number of exclusions and an unequal randomisation of women to the study and control groups, without plausible explanation. Missing outcome data are likely to be related to true outcome, with a significant clinical influence on the intervention effect estimate (Sfontouris 2013).

Selective reporting

We assessed one study at low risk of reporting bias, as all outcomes, pre-specified in the registered protocol, were adequately reported (Vergouw 2012). We assessed one study at unclear risk of reporting bias. Although all primary outcome rates were provided

by communicating with the first author, the primary form of the study was an abstract, and the trial was not registered (Economou 2011). We assessed two studies at high risk since one did not report miscarriage rates, which was expected from such a study, and the other, which ended prematurely, failed to achieve adequate power, and grouped and analysed the data in subsets that had not been pre-specified, despite the fact that all outcomes had been measured.

Other potential sources of bias

We assessed two studies at unclear risk because of a lack of sufficient information to determine otherwise, and two trials at high risk of bias because they were industry-sponsored (financially and in-kind); safety data showed very low conditional superiority for the primary outcome in one, and the lead investigator had financial investment in the other.

Effects of interventions

See: [Summary of findings for the main comparison Metabolomics compared to non metabolomics for embryo quality assessment](#)

We sought RCTs assessing the role of metabolomic investigation of the endometrium, oocytes, and embryos. We did not find any RCTs addressing metabolomic assessment of the endometrium or oocytes. We found four studies (N = 802 women randomised) that examined the assessment of the embryo: Economou 2011; Hardarson 2012; Sfontouris 2013; Vergouw 2012.

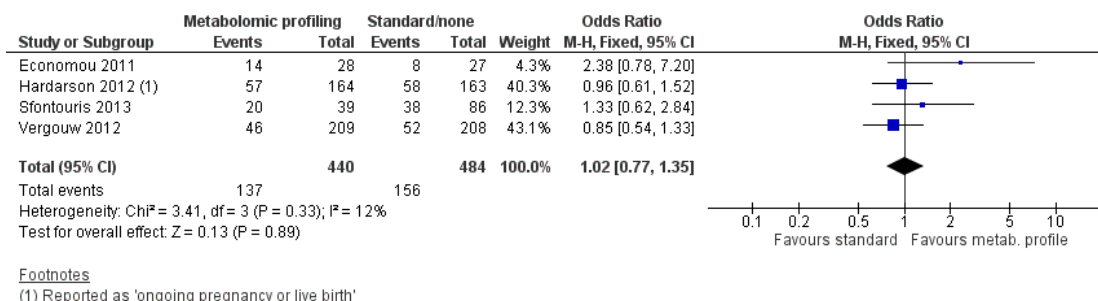
Metabolomic assessment of embryos versus no metabolomic assessment

Primary outcomes

(1) Live birth or ongoing pregnancy rate per woman randomised

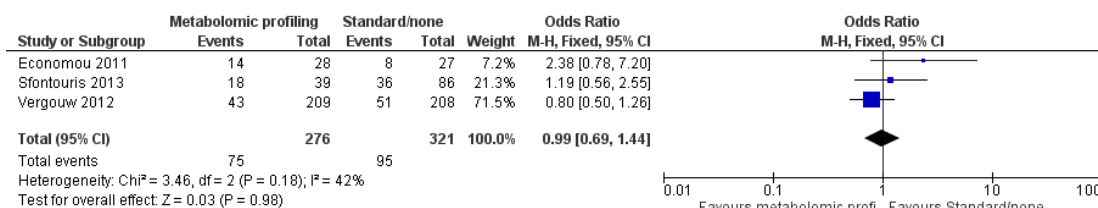
This outcome was reported in all four studies. There was evidence of little or no difference in effect between metabolomic assessment and non-metabolomic assessment of embryos (OR 1.02, 95% CI 0.77 to 1.35, four RCTs, N = 924, $I^2 = 0.0\%$, low-quality evidence; Analysis 1.2; Figure 4). Our findings suggest that if the rate of live birth or ongoing pregnancy was 35% in the non-metabolomic group, it would be between 29% and 42% with the use of metabolomics.

Figure 4. Forest plot of comparison: Metabolomic profile vs standard, outcome: 1.2 Live birth or ongoing pregnancy.



A sensitivity analysis excluding [Hardarson 2012](#), as this study did not report live birth and ongoing pregnancy separately, did not substantially change the effect estimate (OR 0.99, 95% CI 0.69 to 1.44, three RCTs, $N = 597$, $I^2 = 42\%$, low-quality evidence; [Analysis 1.1](#); [Figure 5](#)).

Figure 5. Forest plot of comparison: Metabolomic profile vs standard, outcome: 2.1 Live birth.

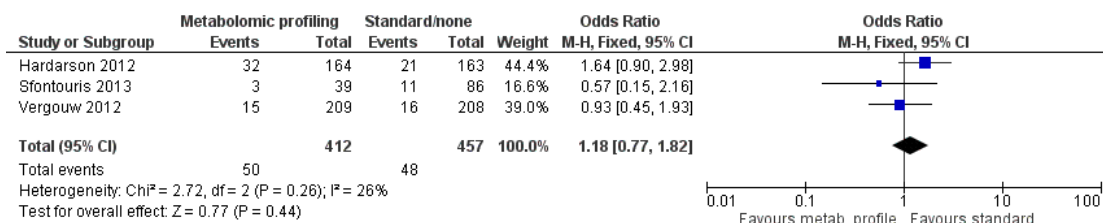


A sensitivity analysis excluding [Economou 2011](#) and [Sfontouris 2013](#) for high risk of bias did not change the results (OR 0.90, 95% CI 0.66 to 1.25, $I^2 = 0\%$; two RCTs; $N = 744$; [Hardarson 2012](#); [Vergouw 2012](#)).

(2) Miscarriage rate per woman randomised

This outcome was reported in three studies ([Sfontouris 2013](#); [Vergouw 2012](#); [Hardarson 2012](#)). There was evidence of little or no difference in effect when comparing metabolomic assessment with non-metabolomic assessment of embryos (OR 1.18, 95% CI 0.77 to 1.82; $I^2 = 0\%$; three RCTs; $N = 869$, low-quality evidence; [Analysis 1.3](#); [Figure 6](#)).

Figure 6. Forest plot of comparison: Metabolomic profile vs standard, outcome: 1.3 Miscarriage.

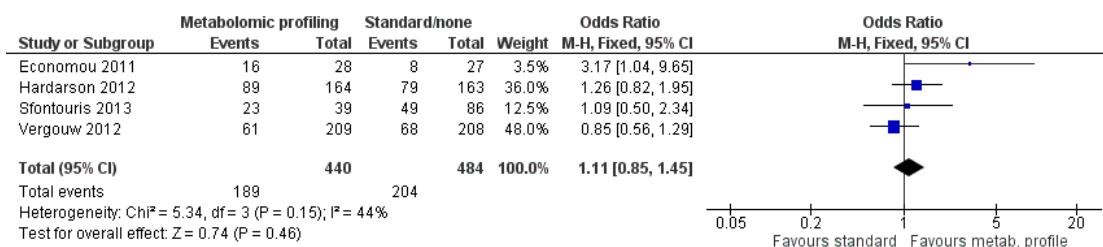


Secondary outcomes

(3) Clinical pregnancy rates (the presence of a foetal heart on ultrasound scan at seven weeks of gestation) per woman or couple randomised

This outcome was reported in all four studies. There was no evidence of effect when comparing metabolomic assessment with non-metabolomic assessment of embryos (OR 1.11, 95% CI 0.85 to 1.45; $I^2 = 44\%$; four trials, $N = 924$, low-quality evidence; [Analysis 1.4](#); [Figure 7](#)).

Figure 7. Forest plot of comparison: Metabolomic profile vs standard, outcome: 1.4 Clinical pregnancy.



A sensitivity analysis excluding [Economou 2011](#) and [Sfontouris 2013](#) for high risk of bias, did not change the interpretation of the results for clinical pregnancy (OR 1.03, 95% CI 0.76 to 1.38; $I^2 = 40\%$; two RCTs; $N = 744$, low-quality evidence).

(4) Cancellation

This outcome was reported in two studies ([Hardarson 2012](#); [Vergouw 2012](#)). There was evidence of a higher cancellation rate in the metabolomic group (OR 1.78, 95% CI 1.18 to 2.69; $I^2 = 51\%$; two RCTs; $N = 744$; low-quality evidence; [Analysis 1.5](#)).

(5) Multiple pregnancy

This outcome was reported in two studies ([Economou 2011](#), [Sfontouris 2013](#)). There was evidence of little or no difference between the groups when comparing metabolomic assessment with non-metabolomic assessment of embryos (OR 1.50, 95% CI 0.70 to 3.19; $I^2 = 0\%$; two RCTs, $N = 180$, low-quality evidence; [Analysis 1.6](#)).

(4) Ectopic pregnancy

This outcome was reported in one study ([Vergouw 2012](#)). There was evidence of little or no difference between the groups when comparing metabolomic assessment with non-metabolomic assessment of embryos (OR 3.00, 95% CI 0.12 to 74.07, one RCT, $N = 417$; very low-quality evidence; [Analysis 1.7](#)).

(5) Foetal abnormalities

This outcome was reported in one study involving 125 women (Sfontouris 2013). No events occurred in either group (very low-quality evidence).

Other analyses

There were insufficient data to conduct subgroup analyses. Sensitivity analyses using a random effects model or restricting the analysis to fully published studies did not substantially affect our main findings.

DISCUSSION

Summary of main results

This Cochrane review evaluated the effectiveness of metabolomic assessment in improving pregnancy outcomes in women undergoing assisted reproduction techniques (ART). The initial objective was to assess the metabolomic profiles of the oocyte quality, embryo viability, and endometrial receptivity during an artificial reproductive cycle with in vitro fertilisation (IVF) or Intracytoplasmic sperm injection (ICSI). However, we did not find any studies evaluating metabolomic profiles of the oocyte quality or endometrium receptivity. We identified and included four randomised controlled trials (RCT), with a total of 802 women, which investigated the metabolomic profile of embryo viability, using near-infrared (NIR) spectroscopy and a pre-determined algorithm, and compared the embryo viability score with the conventional morphology assessment.

We found low-quality evidence from four RCTs of no clear difference between metabolomic and morphology assessment in the rates of live birth or ongoing pregnancy (Economou 2011; Hardarson 2012; Sfontouris 2013; Vergouw 2012), and low-quality evidence from two RCTs of no clear difference in miscarriage between the two assessment types (Sfontouris 2013; Vergouw 2012). We found low-quality evidence from four RCTs of no clear difference between the groups in clinical pregnancy rates. We did not find different results when we removed data from the RCTs with high risk of bias in our sensitivity analyses. There were sparse data for multiple and ectopic pregnancies, and foetal abnormalities, and we found no clear differences between the types of assessment. However, there was low-quality evidence from two trials that cancellation rates were higher in the metabolomic than in the morphology assessment group (Hardarson 2012; Vergouw 2012). We retrieved no evidence on the safety of the metabolomic technique. However, we assessed the overall quality of evidence as low or very low for all comparisons.

Overall completeness and applicability of evidence

We included four studies in this Cochrane review with data relevant to the review question, but they only addressed the metabolomic assessment of embryos; we found no studies that examined the relative assessment of the endometrium and oocytes. Women eligible for randomisation were an average age of 33 years; they were defined as subfertile women with an expected good prognosis (i.e. undergoing ICSI, younger than 38 years, having more than two to five embryos of good quality each). All studies reported rates on the primary outcome of live birth or ongoing pregnancy, and two reported on the adverse event of miscarriage. Given the study populations, the results of this review are widely applicable for women identified as normal responders for ART. However, there is a gap in the literature for other subgroups of subfertile women, including high or poor responders, and women with recurrent implantation failures. More evidence is also needed about spectroscopic platforms besides NIR spectroscopy, and new tools. Of note, this review focused on conventional morphologic assessment, not on more advanced techniques such as time-lapse monitoring, which is the subject of another Cochrane review (Armstrong 2015).

Quality of the evidence

We found 35 potentially eligible studies. Out of them, only four studies were eligible for inclusion and further analysis. The overall quality of the evidence ranged from very low to low. Limitations included serious risk of bias (associated with poor reporting of methods, attrition bias, selective reporting, and other bias), imprecision, and inconsistency across trials.

In addition to the published data collected, we retrieved extra details by communicating with most authors of the original studies; specified information remained missing in many cases. It is important to mention that one study was published only as a conference abstract, but the author provided reliable and detailed data when requested. One study was terminated prematurely due to market withdrawal of the instrument. Another trial was terminated early, as preliminary results for improved birth outcomes were not encouraging, which was the reason that Biotechnology Company funded the study.

We found low-quality evidence for the primary outcome of live birth or ongoing pregnancy because we assessed serious risk of bias in all studies, including: poor reporting of methods, attrition, selective reporting, and other biases; and found serious imprecision in one study (wide confidence intervals, compatible with benefit in either arm, or no difference between the groups). We found low-quality evidence from two trials for the primary outcome of miscarriage, due to incomplete data, premature termination, inappropriate use of subsets for analysing the data, high risk of attrition bias, reporting bias, and other sources of bias, as one au-

thor held shares in the company that supplied the equipment for spectroscopy.

We found low-quality evidence from four trials for the secondary outcome of clinical pregnancy for the same reasons as for live birth or ongoing pregnancy. We found low-quality evidence from two trials for cancellation, not only due to serious risk of bias, but also due to imprecision and moderate heterogeneity across the studies. For multiple pregnancy, we found low-quality evidence from two trials, due to imprecision, and the potential impact of multiple types of bias in both studies reporting this outcome. For ectopic pregnancy, evidence was very low quality with very serious imprecision, as only one study contributed. For foetal abnormalities, we found very low-quality evidence from one trial, at high risk of attrition and reporting biases, which reported no events.

Potential biases in the review process

We made every effort to identify all eligible studies. All trial authors responded to our requests for additional information, with the exception of the authors of the ongoing trial, where reasonably, the data could not be retrieved.

Agreements and disagreements with other studies or reviews

The results of this review are in agreement with the conclusions of an individual patient data meta-analysis performed by all authors of the included studies (Vergouw 2014). Focusing on embryo selection, they found no evidence of a difference in the rates of live births between the use of NIR spectroscopy in combination with morphology assessment and morphology evaluation alone. Authors concluded that there was no evidence of effectiveness of metabolomic assessment in embryo selection in clinical practice. Most narrative reviews published to date are in accordance with our findings (Egea 2014; Gardner 2015; McRae 2013; Nagy 2008; Nel-Themaat 2011; Revelli 2009; Rødgaard 2015; Singh 2007; Uyar 2014). In these papers, it was emphasised that the primary drawback in most studies was a large intra-group variation, which often lead to false positive and false negative results, as well as the continuously different culture media available in the market. Although several possible biomarkers have been identified, no single or composite early biomarker, sensitive for clinical use, has been established. Authors concluded that there were still factors underlying their inconsistent findings; for example, signal threshold, distinguishing between a viable and nonviable embryo being susceptible to signal noise; the algorithms created had not been validated in prospective trials - even a method established and cross-validated on a larger scale can remain problematic if variations are linked to the technical platform itself. An important purpose of these trials was the transfer of a single embryo, capable of carrying the same or higher possibilities of live birth rates than embryos

of a multiple transfer. Finally, we have to reference the systematic review of Bracewell-Milnes 2017, where 21 studies irrespective of their methodology were included, as metabolomics is a relatively recent methodology; authors reported “considerable variation regarding the research question, methodology, study design, sample size and outcome measures” and noted a “paucity of data and inconsistencies in the literature regarding metabolomic studies in the field of fertility”

It is interesting to note that the classic problems of high chemical complexity, widely dynamic production patterns, different chemical properties of the molecules, and the low sensitivity of the methods during the metabolomic assessment of any media, have not yet been overcome Revelli 2009.

AUTHORS' CONCLUSIONS

Implications for practice

According to current trials in women undergoing ART, there is no evidence to show that metabolomic assessment of embryos before implantation has any meaningful effect on rates of live birth, ongoing pregnancy, miscarriage, multiple pregnancy, ectopic pregnancy or foetal abnormalities. The existing evidence varies from very low to low-quality. Data on other adverse events were sparse, so we could not reach conclusions on these. At the moment, there is no evidence to support or refute the use of this technique for subfertile women undergoing ART. Robust evidence is needed from further RCTs, which study the effects on live birth and miscarriage rates for the metabolomic assessment of embryo viability. Well designed and executed trials are also needed to study the effects on oocyte quality and endometrial receptivity, since none are currently available.

Implications for research

Larger and well-designed randomised controlled trials (RCTs) on the metabolomic assessment of oocytes, embryos, and endometrium are needed in order to determine its effectiveness as an adjunct to, or its superiority over, conventional assessment in IVF/ICSI processes. In particular, investigators need to recruit an adequate number of participants to conduct meaningful analyses, and perform subgroup analyses as applicable. Participants so far have been subfertile women, identified as normal responders to ovarian stimulation. Other subgroups, such as high or poor responders (although in the latter there might be a restriction because of the low number of embryos anticipated), or even women with recurrent implantation failure, could be included in the trials.

Accurate documentation of the randomisation, allocation concealment, and blinding methods is highly desirable, so that risks of bias be eliminated. For the reduction of performance bias due to

the lack of blinding of the laboratory personnel, additional measures should be taken, such as the training of all members to the technique, their blinding to the participants and the final results, and the development of a methodological design with a placebo laboratory technique. In addition to the primary outcomes of live birth and miscarriage, study protocols should include the reporting of other adverse effects, and of crucial secondary outcomes.

Other spectroscopic platforms could be used, such as Raman and nuclear magnetic resonance (NMR) spectroscopy. Near infrared (NIR) spectroscopy, used so far, offers special advantages, such as cost and accuracy, over other techniques. New tools include electrospray ionization mass spectrometry (ESI-MS; Cortezzi 2013), and direct injection (DI)-MS (Sheedy 2014). We believe that further advances in both technology and the use of databases could make the currently available spectroscopic methods faster and more effective in predicting success in ART at all three levels of

the oocyte, embryo, and endometrium.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Economou 2011

Methods	<p>Published data:</p> <p>Study design: Prospective, randomised</p> <p>No. of centres involved: Single centre</p> <p>Additional data after communication (email)</p> <p>Method of randomisation: 'closed envelope' method on the day of the treatment</p> <p>Method of allocation concealment: NS</p> <p>Blinding: NS</p> <p>Sample size: NS</p> <p>Intention-to-treat analysis: N/A</p>
Participants	<p>Published data</p> <p>Inclusion criteria: Subfertile patients undergoing ICSI, < 38 years, > 5 embryos per patient</p> <p>Exclusion criteria: > 38 years old and < 5 embryos</p> <p>No. eligible for randomisation: NS</p> <p>No. enrolled in the trial: 56</p> <p>No. randomised to intervention group at the start of the trial: 28</p> <p>No. randomised to control group at the start of the trial: 28</p> <p>No. in the treatment group at the end of the trial: 28</p> <p>No. in the control group at the end of the trial: 28</p> <p>Additional data after communication</p> <p>No. (%) in the treatment group who were lost to follow-up/withdrew: 0</p> <p>No. (%) in the control group who were lost to follow up/withdrew: 0</p> <p>Age of intervention group at the start of the trial: 33 years</p> <p>Age of control group at the start of the trial: 34 years</p> <p>No. (%) in intervention group who had previous IVF treatment: 4/28</p> <p>No. (%) in control group who had previous IVF treatment: 9/28</p> <p>Cause/duration of subfertility of intervention group:</p> <p>Duration: 1 to 10 years; average 2.72 years</p> <p>Causes: 9/28 male infertility, 5/28 tubal infertility, anovulation 4/28, endometrial infertility 0/28, unexplained 7/28 (primary infertility 27/28)</p> <p>Cause/duration of subfertility of control group:</p> <p>Duration: 1 to 8 years (average 2.78 years)</p> <p>Causes: 2/28 male infertility, 4/28 tubal infertility, anovulation 2/28, endometrial infertility 5/28, unexplained 11/28 (primary infertility 6/28)</p> <p>Other relevant demographic information: NS</p>
Interventions	<p>Published data</p> <p>Type of metabolomic analysis in intervention group: Spent culture medium from embryos - Embryo Level</p> <p>Control treatment: No metabolomic analysis</p> <p>Concomitant factors in intervention group: Viability score and morphology assessment</p> <p>Concomitant factors in control group: Morphology assessment alone</p> <p>Method of metabolomic assessment: NIR Spectroscopy</p>

	<p>Embryos transferred in intervention group: 2.9 ± 0.3</p> <p>Embryos transferred in control group: 2.8 ± 0.5</p> <p>Time of commencement of intervention: Prior to embryo transfer, day 3 or day 5 following ICSI</p> <p>Length of study follow-up: NS</p>
Outcomes	<p>Primary outcome rates measured in intervention and control group</p> <p>Data after communication:</p> <p>Live birth (per woman/couple)</p> <p>Ongoing pregnancy</p> <p>Miscarriage; NS</p> <p>Secondary outcome rates measured in intervention and control group</p> <p>Published data:</p> <p>Clinical pregnancy (per woman/couple)</p> <p>Data after communication:</p> <p>Cancellation(per woman/couple); NS</p> <p>Multiple pregnancy on ultrasound (per woman/couple)</p> <p>Ectopic pregnancy (per woman/couple); NS</p> <p>Foetal abnormality (per woman/couple); NS</p>
Notes	<p>Setting of trial: Private IVF Clinic, Athens, Greece</p> <p>Source of funding: Not stated</p> <p>Review authors communicated with the study investigators through email. They requested and received the complete data set; they asked for a description of the methods used for randomisation, for which the answer was: "closed envelope" method on the day of the treatment'; despite a request, they received no information provided on the method of allocation concealment, blinding, or sample size calculation</p>

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	From extra data sent after communication. Use of sealed envelopes reported
Allocation concealment (selection bias)	Unclear risk	From extra data sent after communication, it was stated that a sealed envelope method was used, and participants were randomly allocated to either group
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Not described in the abstract published nor in the extra information sent after communication
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not described in the abstract published nor in the extra information sent after communication, but the review authors judged that the outcome measurement was not likely to be influenced by lack of blinding

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	There was no information on the number of women eligible for randomisation, while no drop-outs were reported
Selective reporting (reporting bias)	Unclear risk	Pre-specified primary outcomes were fully reported, according to extra data sent after communication, although full reporting was not feasible in its primary form as it was published as an abstract. No trial registration
Other bias	Unclear risk	Although specific data on procedures and outcomes were sent, there was insufficient information to assess whether an important risk of bias existed

Hardarson 2012

Methods	<p>Study design: Double-blind RCT</p> <p>No. of centres involved: Single centre</p> <p>Method of randomisation: Performed using a computerised randomisation programme balancing for prognostic variables by way of minimisation (1:1) by laboratory staff</p> <p>Method of allocation concealment: NS</p> <p>Blinding: Blinded from both the patient and physician</p> <p>Sample size: Calculation provided</p> <p>Intention-to-treat analysis: Provided</p> <p>Overall risk of bias rating: Moderate</p>
Participants	<p>Inclusion criteria: Subfertile couples who underwent IVF or ICSI</p> <p>Exclusion criteria: Couples planned for two embryos to be transferred, had less than two good quality embryos on the day of embryo transfer, had a testicular biopsy performed (testicular sperm aspiration/testicular sperm extraction), had the embryo transferred on day 3 or 4, or had previously been randomised to the study.</p> <p>No. eligible for randomisation: 972</p> <p>No. enrolled in the trial: 327</p> <p>No. randomised to intervention group at the start of the trial: 164</p> <p>No. randomised to control group at the start of the trial: 163</p> <p>No. in the treatment group at the end of the trial: 152</p> <p>No. in the control group at the end of the trial: 160</p> <p>No. (%) in the treatment group who were lost to follow-up/withdrew: 12</p> <p>No. (%) in the control group who were lost to follow up/withdrew: 3</p> <p>Age of intervention group at the start of the trial: 35.5 (\pm 4.2)</p> <p>Age of control group at the start of the trial: 35.6 (\pm 4.2)</p> <p>No. (%) in intervention group who had previous IVF treatment: NS</p> <p>No. (%) in control group who had previous IVF treatment: NS</p> <p>Cause/duration of subfertility of intervention group: Duration: NS Causes: Male factor 30.5% (50), Other 22.6% (37), Tubal factor 11.0% (18), Unexplained 36.0% (59)</p> <p>Cause/duration of subfertility of control group: Duration: NS Causes: Male factor 32.</p>

	5% (53), Other 24.5% (40), Tubal factor 9.2% (15), Unexplained 33.7% (55) Other relevant demographic information: BMI, number of previous IVF treatment cycles, number of fertilised oocytes, method used to fertilise oocytes, number of aspirated oocytes, number of follicles aspirated, number of GQE on day of transfer, type of pituitary regulation, total dosage of gonadotrophins administered
Interventions	Type of metabolomic analysis in intervention group: Spent culture medium from embryos - Embryo Level Control treatment: No metabolomic analysis Concomitant factors in intervention group: Viability score Concomitant factors in control group: Morphology assessment alone Method of metabolomic assessment: NIR Spectroscopy Embryos transferred in intervention group: Single ET - day 2 53.0% (87), day 5 47.0% (77) Embryos transferred in control group: Single ET - day 2 50.9% (83), day 5 49.1% (80) Time of commencement of intervention: Prior to embryo transfer, day 2 or day 5 following IVF/ICSI Length of study follow up: NS
Outcomes	Primary outcome rates measured in intervention and control group Live birth (per woman/couple) Ongoing pregnancy Miscarriage: NS Secondary outcome rates measured in intervention and control group Clinical pregnancy (per woman/couple) Cancellation (per woman/couple) Multiple pregnancy on ultrasound (per woman/couple): NS Ectopic pregnancy (per woman/couple): NS Foetal abnormality (per woman/couple): NS
Notes	Setting of trial: Fertility Center Scandinavia, Carlanderska Hospital, Gothenburg Source of funding: This study was supported by an unconditional grant from Molecular Biometrics Inc., USA The study was terminated early as the analysis of the Data Safety Monitoring Board showed a very low conditional power of superiority for the primary outcome

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Through a computerised randomisation programme balancing for prognostic variables performed by laboratory staff
Allocation concealment (selection bias)	Low risk	Remote allocation. Data locked in database
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Blinded from the patient and physician, not stated for the laboratory personnel

Hardarson 2012 (Continued)

Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blinding of outcome assessment not reported but the review authors judged that the outcome measurement is not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	ITT and PP analysis performed by the researchers; the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; Justification on exclusions from both groups provided
Selective reporting (reporting bias)	High risk	The primary outcome of miscarriage was not reported, as expected for such a study, which was terminated prematurely due to unfavourable preliminary results
Other bias	High risk	The study was terminated prematurely as the analysis of the Data Safety Monitoring Board showed a very low conditional power of superiority for the primary outcome. The study was supported by an unconditional grant from Molecular Biometrics Inc., USA

Sfontouris 2013

Methods	Study design: RCT No. of centres involved: Single centre Method of randomisation: Performed by computer-generated random allocation sequence. Unequal randomisation was used (ratio metabolomics + morphology: Routine morphology = 1:2) Method of allocation concealment: Performed by a study nurse using sealed envelopes. Blinding: NS. Doctors and patients not blinded for the treatment. Sample size: Power calculation provided - study was prematurely terminated Intention-to-treat analysis: Not performed Overall risk of bias rating: High risk
Participants	Inclusion criteria: IVF patients ≥ 18 and ≤ 43 years, with at least four fertilised oocytes (2PN ≥ 4) Exclusion criteria: > 43 years old and < 5 embryos, frozen-thawed cycles and cycles with oocyte donation or testicular biopsy were excluded from the study No. eligible for randomisation: 276 No. enrolled in the trial: 125 No. randomised to intervention group at the start of the trial: 39 No. randomised to control group at the start of the trial: 86 No. in the treatment group at the end of the trial: 39

	No. in the control group at the end of the trial: 86 No. (%) in the treatment group who were lost to follow-up/withdrew: 0 No. (%) in the control group who were lost to follow up/withdrew: 0 Age of intervention group at the start of the trial: 34.5 ± 4.7 Age of control group at the start of the trial: 35.7 ± 4.4 No. (%) in intervention group who had previous IVF treatment: 1.2 ±1.6 No. (%) in control group who had previous IVF treatment: 1.5 ± 1.9 Cause/duration of subfertility of intervention group: Duration: 3.5 ± 2.1 Causes: NS Cause/duration of subfertility of control group: Duration: 4.0 ± 3.6 Causes: NS Other relevant demographic information: BMI, basal FSH, oocytes retrieved	
Interventions	Type of metabolomic analysis in intervention group: Spent culture medium from embryos - Embryo Level. Control treatment: No metabolomic analysis. Concomitant factors in intervention group: Morphology assessment and Viability Score Concomitant factors in control group: Morphology assessment alone Method of metabolomic assessment: NIR Spectroscopy Embryos transferred in intervention group: 2.6 ± 0.7 Embryos transferred in control group: 2.9 ± 0.6 Time of commencement of intervention: Prior to embryo transfer, day 2 or day 3 or day 5 following ICSI Length of study follow up: NS	
Outcomes	Primary outcome rates measured in intervention and control group Live birth (per woman/couple) Ongoing pregnancy Miscarriage Secondary outcome rates measured in intervention and control group Clinical pregnancy (per woman/couple) Cancellation (per woman/couple) Multiple pregnancy on ultrasound (per woman/couple) Ectopic pregnancy (per woman/couple): NS Foetal abnormality (per woman/couple): NS	
Notes	Setting of trial: Private IVF Clinic, Athens, Greece Source of funding: Self-funded and part of the cost of the sample cells would have to be covered by the patients The study was terminated prematurely due to the market withdrawal of the instrument	
Risk of bias		
Bias	Authors’ judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Through a computer-generated random allocation sequence with unequal randomisation (1:2)
Allocation concealment (selection bias)	Unclear risk	Sealed envelopes were used

Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Neither doctors nor patients were blinded for the treatment
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blinding of outcome assessment not clarified, but the outcome measurement was not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	High risk	Potentially inappropriate application of simple imputation; sample size for achieving adequate power was not reached, as the study was terminated prematurely due to market withdrawal of the instrument. Probably missing outcome data, excessive number of exclusions and unequal randomisation of participants in study and control group were likely to be related to true outcome; with relevant influence on the intervention effect estimate
Selective reporting (reporting bias)	High risk	All pre-specified primary and secondary outcomes were reported, but with the use of not pre-specified subsets (data subgrouped and analysed per day of embryo transfer, overall analysis not reported). Moreover, the study was terminated prematurely due to the market withdrawal of the instrument, and the sample size failed to achieve adequate power
Other bias	Unclear risk	Insufficient rationale or evidence that an identified problem (premature termination of the study) would introduce bias. Although specific data on procedures and outcomes were sent, information was judged to be insufficient to assess whether an important risk of bias existed

Methods	<p>Study design: RCT</p> <p>No. of centres involved: Single centre</p> <p>Method of randomisation: At oocyte retrieval, using a computerised randomisation programme</p> <p>Method of allocation concealment: The allocations were placed in consecutively numbered, opaque envelopes</p> <p>Blinding: Both patient and physician were blinded</p> <p>Sample size: Power calculation provided</p> <p>Intention-to-treat analysis: Performed</p>
Participants	<p>Inclusion criteria: Subfertile patients undergoing ART with two or more similar best-quality embryos, with ejaculated sperm were included</p> <p>Exclusion criteria: Less than two similar best-quality embryos and the transfer of more than one embryo. Patients were only allowed to participate once.</p> <p>No. eligible for randomisation: 555</p> <p>No. enrolled in the trial: 417</p> <p>No. randomised to intervention group at the start of the trial: 209</p> <p>No. randomised to control group at the start of the trial: 208</p> <p>No. in the treatment group at the end of the trial: 146</p> <p>No. in the control group at the end of the trial: 163</p> <p>No. (%) in the treatment group who were lost to follow-up/withdrew: 63 (30.1%)</p> <p>No. (%) in the control group who were lost to follow up/withdrew: 45 (21.6%)</p> <p>Age of intervention group at the start of the trial: PP: 34.6 (4.1); ITT: 34.5 (4.1)</p> <p>Age of control group at the start of the trial: PP: 34.0 (4.5); ITT: 34.0 (4.4)</p> <p>No. (%) in intervention group who had previous IVF treatment: NS</p> <p>No. (%) in control group who had previous IVF treatment: NS</p> <p>Cause/duration of subfertility of intervention group: Duration: PP: Control Group 3.33 (1.98), Intervention Group: 3.17 (2.39); ITT: Control Group 3.29 (2.07), Intervention Group: 3.14 (2.02) Causes: Analytical provided in the respective tables.</p> <p>Other relevant demographic information: BMI, primary infertility, number of previous IVF attempts, type of pituitary regulation, total dosage of gonadotrophins administered, fertilization method</p>
Interventions	<p>Type of metabolomic analysis in intervention group: Spent culture medium from embryos - Embryo Level.</p> <p>Control treatment: No metabolomic analysis</p> <p>Concomitant factors in intervention group: morphology assessment and Viability Score</p> <p>Concomitant factors in control group: Morphology assessment alone</p> <p>Method of metabolomic assessment: NIR Spectroscopy</p> <p>Embryos transferred in intervention group: PP: 146; ITT: 199</p> <p>Embryos transferred in control group: PP: 163; ITT: 201</p> <p>Time of commencement of intervention: day 3 following fertilization</p> <p>Length of study follow up: NS</p>
Outcomes	<p>Primary outcome rates measured in intervention and control group</p> <p>Live birth rate (per woman/couple; PP & ITT analysis performed)</p> <p>Ongoing pregnancy rates in intervention group: (PP & ITT analysis performed)</p> <p>Miscarriage</p> <p>Secondary outcome rates measured in intervention and control group</p> <p>Clinical pregnancy (per woman/couple)</p>

	Cancellation (per woman/couple) Multiple pregnancy (per woman/couple) : NS Ectopic pregnancy (per woman/couple) : NS Foetal abnormality (per woman/couple) : NS	
Notes	Setting of trial: VU University Medical Center, Amsterdam, The Netherlands Source of funding: This study was supported by grant no. 171001003 from ZonMW, Organization for Health Research and Development, The Hague, The Netherlands. Molecular Biometrics Inc., USA supplied the NIR spectroscopy technology	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed centrally just before ovum pick-up, using a computerised randomisation program
Allocation concealment (selection bias)	Low risk	The allocations were placed in consecutively numbered, opaque envelopes
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Patients, physicians and laboratory personnel were blinded
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Described as double-blinded, and the outcome measurement was not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	ITT (n=417) and PP (n=309) analysis performed by the researchers; missing outcome data were balanced in numbers across intervention and control groups, with similar reasons adequately explained for all cases; exclusions from both groups adequately justified
Selective reporting (reporting bias)	Low risk	The study protocol was available and all of the study's pre-specified (primary and secondary) outcomes that were of interest in the review were reported in the pre-specified way
Other bias	High risk	Equipment for spectroscopy technology was supplied by Molecular Biometrics Inc. , one author held shares in Molecular Biometrics

BMI: Body mass index
ET: Embryo transfer
ICSI: Intracytoplasmic sperm injection
ITT: Intension-to-treat
IVF: In vitro fertilization
N/A: Non applicable
NIR: Near infra-red
NS: Not specified
PP: Per protocol
RCT: Randomised clinical trial

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Ahlstrom 2011	Not RCT
Bellver 2015	No outcome of interest - No control group with no metabolomics- Not RCT
Ciepiela 2015	Not RCT
Cordeiro 2015	Not RCT
de Los Santos 2015	Ovum donation - Not RCT
Drabkova 2016	Not RCT
Fu 2013	Not RCT
Garip 2016	Not true RCT
Gonsalvez-Alvarez 2015	Not RCT - Ovum donation
Hardarson 2008	Not RCT
Kato 2007	Not RCT
Kirkegaard 2013	Comparison of variation in metabolome according to clinical outcome - Not RCT
Kirkegaard 2014	Not RCT
Li 2015	Not RCT
Lian 2010	No review outcome of interest was measured; metabolomic assessment of follicular fluid in both groups was compared
McRae 2012	No review outcome of interest was measured; metabolomic assessment of follicular fluid before oocyte retrieval was compared in both groups

(Continued)

Montani 2016	Not RCT
Montsko 2015	No review outcome of interest was measured - Not RCT
Nagy 2015	Not RCT
NCT01427413	Not RCT
Rødgaard 2015	Not RCT
Scott 2008	Matched controls - Not RCT
Seli 2007	Not RCT
Seli 2010	Not RCT
Simerman 2015	Not RCT
Valckx 2014	No outcome of interest - Not RCT
Vergouw 2008	Not RCT
Xia 2014	No outcome of interest- Not RCT
Yildizfer 2015	Not RCT
Zhao 2013	Not RCT

Characteristics of ongoing studies *[ordered by study ID]*

[NCT02698488](#)

Trial name or title	Embryo selection by metabolomic profiling of embryo culture medium with mass spectroscopy as an adjunct to morphology
Methods	Allocation: Randomised Intervention model: Parallel assignment Masking: Double-blind (subject, investigator) Primary purpose: Basic science
Participants	Estimated Enrollment: 300
Interventions	Embryo selection by morphology and mass spectroscopy
Outcomes	Clinical Pregnancy (Time frame: 3 months) Clinical pregnancy will be defined as the presence of a foetal heartbeat using vaginal ultrasound at 6 weeks of amenorrhoea

NCT02698488 (Continued)

Starting date	2016
Contact information	Tel +90 532 413 41 95, e-mail: ercan.bastu@istanbul.edu.tr
Notes	Study in protocol stage, ClinicalTrials.gov identifier: NCT02698488

DATA AND ANALYSES

Comparison 1. Metabolomic profile vs standard

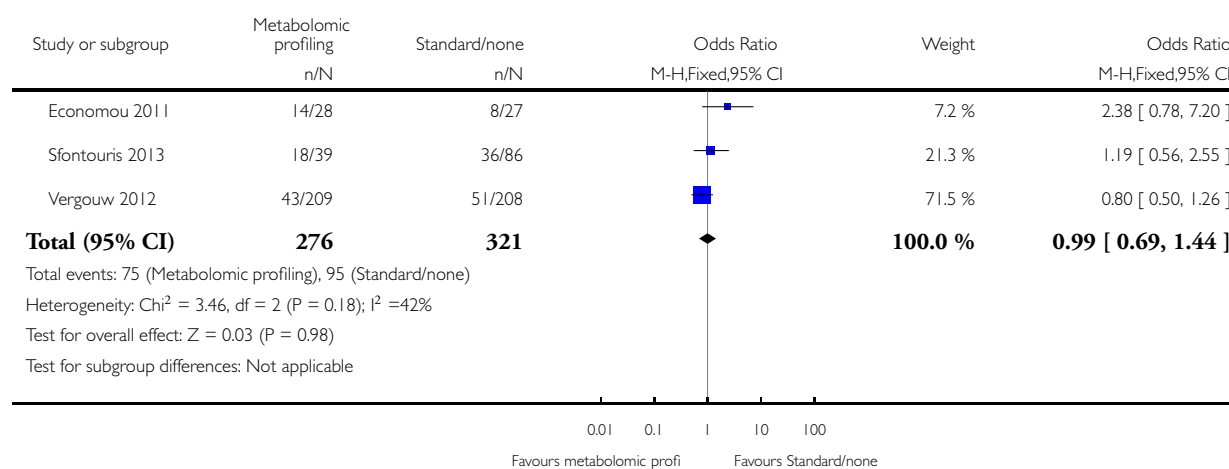
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth	3	597	Odds Ratio (M-H, Fixed, 95% CI)	0.99 [0.69, 1.44]
2 Live birth or ongoing pregnancy	4	924	Odds Ratio (M-H, Fixed, 95% CI)	1.02 [0.77, 1.35]
3 Miscarriage	3	869	Odds Ratio (M-H, Fixed, 95% CI)	1.18 [0.77, 1.82]
4 Clinical pregnancy	4	924	Odds Ratio (M-H, Fixed, 95% CI)	1.11 [0.85, 1.45]
5 Cancellation	2	744	Odds Ratio (M-H, Fixed, 95% CI)	1.78 [1.18, 2.69]
6 Multiple pregnancy	2	180	Odds Ratio (M-H, Fixed, 95% CI)	1.50 [0.70, 3.19]
7 Ectopic pregnancy	1	417	Odds Ratio (M-H, Fixed, 95% CI)	3.0 [0.12, 74.07]

Analysis 1.1. Comparison 1 Metabolomic profile vs standard, Outcome 1 Live birth.

Review: Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

Comparison: 1 Metabolomic profile vs standard

Outcome: 1 Live birth

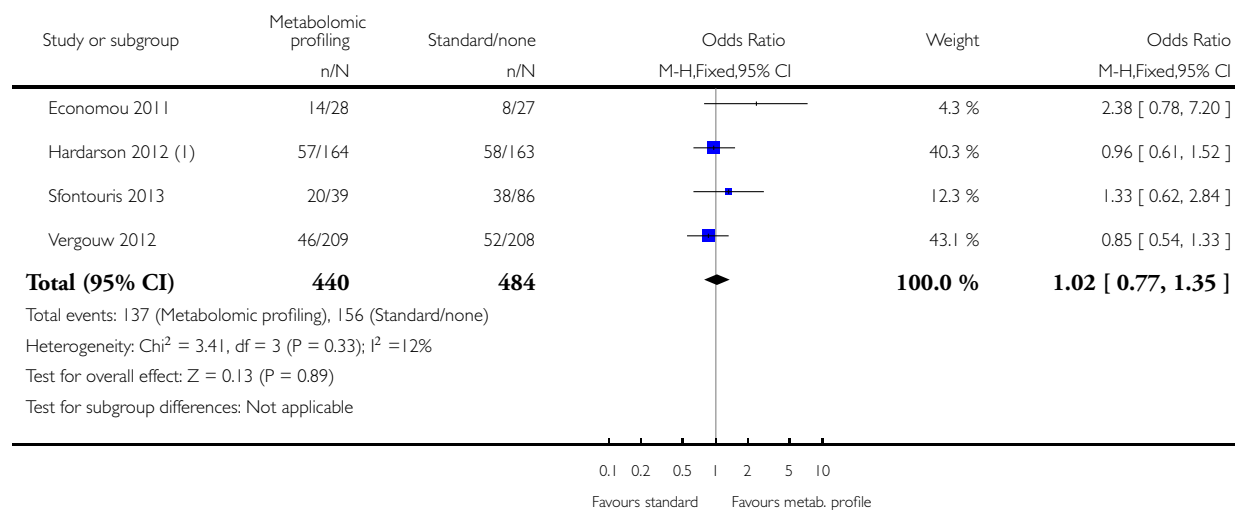


Analysis 1.2. Comparison 1 Metabolomic profile vs standard, Outcome 2 Live birth or ongoing pregnancy.

Review: Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

Comparison: 1 Metabolomic profile vs standard

Outcome: 2 Live birth or ongoing pregnancy



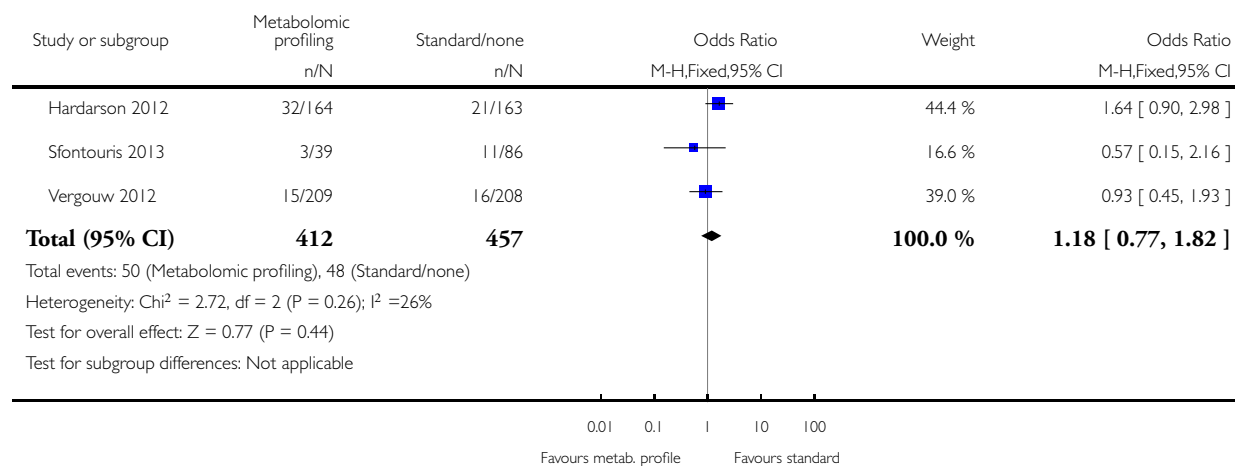
(1) Reported as 'ongoing pregnancy or live birth'

Analysis 1.3. Comparison 1 Metabolomic profile vs standard, Outcome 3 Miscarriage.

Review: Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

Comparison: 1 Metabolomic profile vs standard

Outcome: 3 Miscarriage

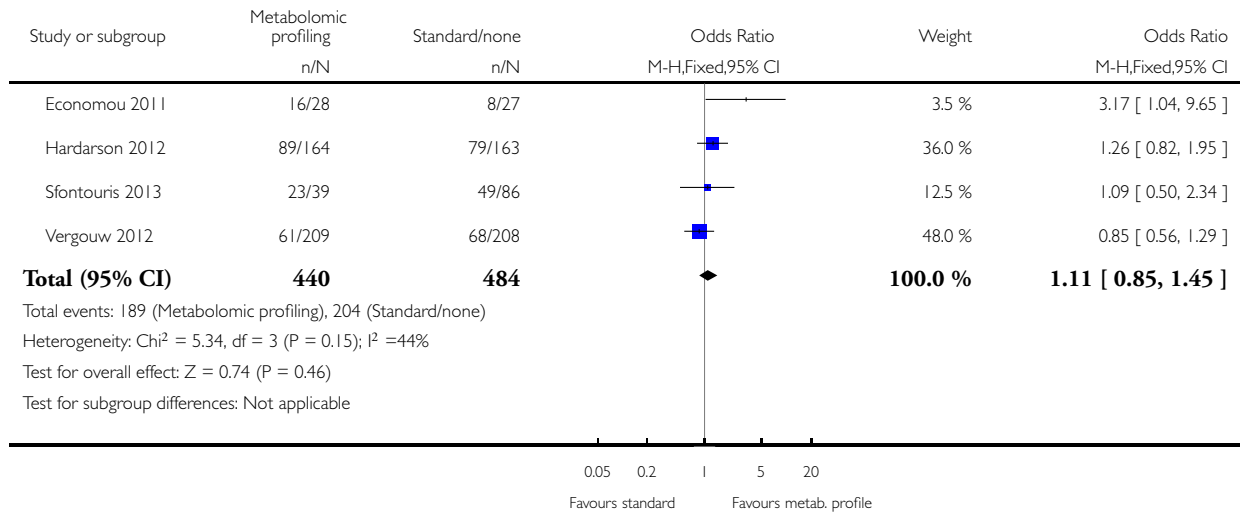


Analysis 1.4. Comparison 1 Metabolomic profile vs standard, Outcome 4 Clinical pregnancy.

Review: Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

Comparison: 1 Metabolomic profile vs standard

Outcome: 4 Clinical pregnancy

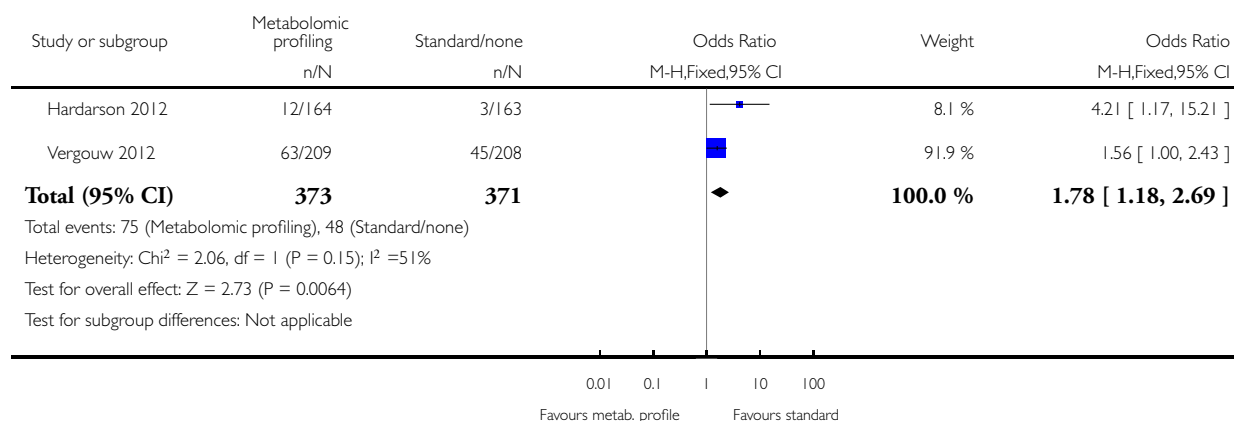


Analysis 1.5. Comparison 1 Metabolomic profile vs standard, Outcome 5 Cancellation.

Review: Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

Comparison: 1 Metabolomic profile vs standard

Outcome: 5 Cancellation

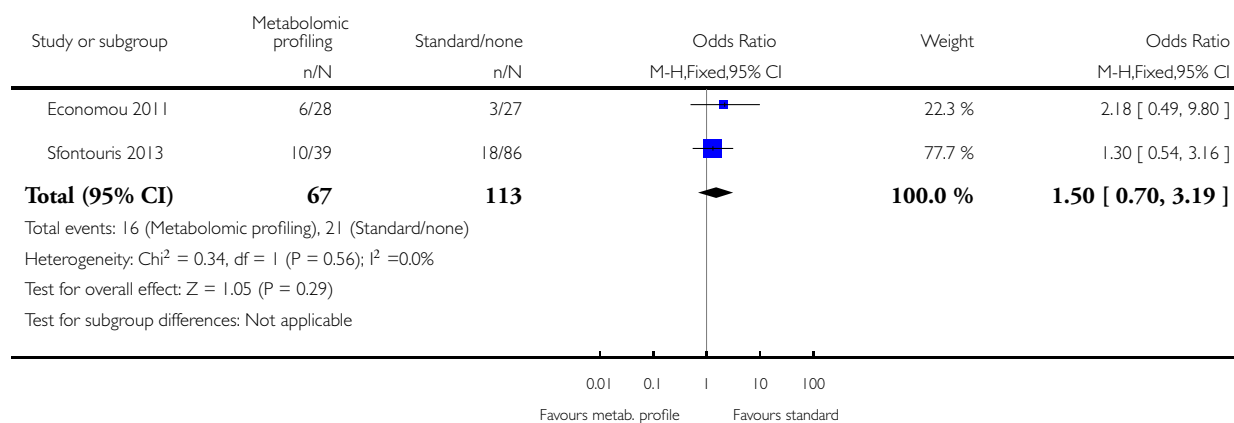


Analysis 1.6. Comparison 1 Metabolomic profile vs standard, Outcome 6 Multiple pregnancy.

Review: Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

Comparison: 1 Metabolomic profile vs standard

Outcome: 6 Multiple pregnancy

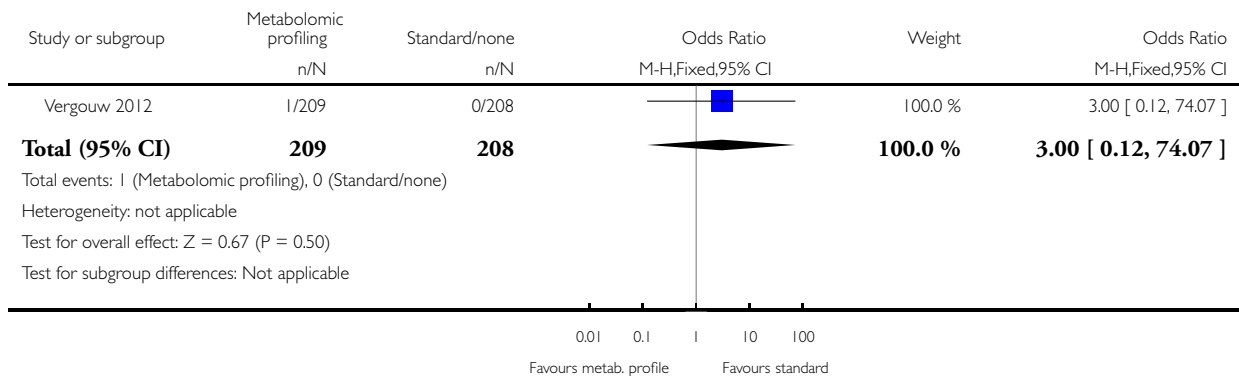


Analysis 1.7. Comparison 1 Metabolomic profile vs standard, Outcome 7 Ectopic pregnancy.

Review: Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies

Comparison: 1 Metabolomic profile vs standard

Outcome: 7 Ectopic pregnancy



APPENDICES

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

From inception to 26 February 2018

PROCITE platform

Keywords CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "ET" or "Embryo" or "in-vitro fertilization" or "Embryo Transfer" or "Embryo Transfer-uterine" or "blastocyst transfer" or "oocyte" or "Follicular Fluid" or "Culture-Media" or "culture" or Title CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "Embryo" or "in-vitro fertilization" or "ET" or "Embryo" or "in-vitro fertilization" or "Embryo Transfer" or "Embryo Transfer-uterine" or "blastocyst transfer" or "oocyte" or "Follicular Fluid" or "Culture-Media" or "culture"

AND

Keywords CONTAINS "metabolomic" or "near infrared spectroscopy" or "NIR" or "infra red" or "biomarkers" or "endometrial receptivity markers" or "carbohydrate metabolism" or "glucose metabolism" or "follicular fluid meiosis activating sterol" or "follicular fluid profile" or "oocyte chemical activation" or "oocyte receptors" or "pyruvate uptake" or "metabolic parameters" or "metabolic profile" or "biochemical indicators" or "biochemical markers" or "biochemical parameters" or Title CONTAINS "metabolomic" or

“near infrared spectroscopy” or “NIR” or “infra red” or “biomarkers” or “endometrial receptivity markers” or “carbohydrate metabolism” or “glucose metabolism” or “follicular fluid meiosis activating sterol” or “follicular fluid profile” or “oocyte chemical activation” or “oocyte receptors” or “pyruvate uptake” or “metabolic parameters” or “metabolic profile” or “biochemical indicators” or “biochemical markers” or “biochemical parameters” (69 hits)

Appendix 2. CENTRAL search strategy

Searched 26 February 2018

Central Register of Studies Online - web platform

- #1 MESH DESCRIPTOR Blastocyst EXPLODE ALL TREES 157
- #2 MESH DESCRIPTOR Embryo Culture Techniques EXPLODE ALL TREES 84
- #3 MESH DESCRIPTOR Embryo Implantation EXPLODE ALL TREES 456
- #4 MESH DESCRIPTOR Embryo Research EXPLODE ALL TREES 1
- #5 MESH DESCRIPTOR Embryo Transfer EXPLODE ALL TREES 984
- #6 MESH DESCRIPTOR Embryology EXPLODE ALL TREES 1
- #7 MESH DESCRIPTOR Oocytes EXPLODE ALL TREES 446
- #8 MESH DESCRIPTOR Reproductive Techniques, Assisted EXPLODE ALL TREES 2892
- #9 MESH DESCRIPTOR Fertilization in Vitro EXPLODE ALL TREES 1893
- #10 MESH DESCRIPTOR Sperm Injections, Intracytoplasmic EXPLODE ALL TREES 491
- #11 (embryo* or blastocyst*):TI,AB,KY 5014
- #12 (in vitro fertilization):TI,AB,KY 2209
- #13 (ivf or icsi):TI,AB,KY 4150
- #14 (intracytoplasmic sperm injection*):TI,AB,KY 1358
- #15 oocyte*:TI,AB,KY 2893
- #16 MESH DESCRIPTOR Follicular Fluid EXPLODE ALL TREES WITH QUALIFIERS CH,CY,EN,ME,MI,PH 102
- #17 (Follicular Fluid):TI,AB,KY 278
- #18 (infertil* or subfertil*):TI,AB,KY 5007
- #19 (assisted reproducti* techn*):TI,AB,KY 495
- #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 10600
- #21 metabolom*:TI,AB,KY 613
- #22 MESH DESCRIPTOR Metabolomics EXPLODE ALL TREES 134
- #23 MESH DESCRIPTOR Spectrophotometry, Infrared EXPLODE ALL TREES 84
- #24 MESH DESCRIPTOR Magnetic Resonance Spectroscopy EXPLODE ALL TREES 604
- #25 MESH DESCRIPTOR Fourier Analysis EXPLODE ALL TREES 201
- #26 MESH DESCRIPTOR Spectroscopy, Fourier Transform Infrared EXPLODE ALL TREES 41
- #27 MESH DESCRIPTOR Spectroscopy, Near-Infrared EXPLODE ALL TREES 260
- #28 MESH DESCRIPTOR Gas Chromatography-Mass Spectrometry EXPLODE ALL TREES 329
- #29 MESH DESCRIPTOR Spectrum Analysis EXPLODE ALL TREES 1802
- #30 ViaMetric*:TI,AB,KY 1
- #31 spectroscop*:TI,AB,KY 3036
- #32 spectromet*:TI,AB,KY 5080
- #33 NIR:TI,AB,KY 118
- #34 chromatograph*:TI,AB,KY 10448
- #35 (nuclear magnetic resonance):TI,AB,KY 8862
- #36 (NMR or HPLC):TI,AB,KY 3533
- #37 transform-infrared:TI,AB,KY 86
- #38 Raman:TI,AB,KY 77
- #39 chemometric*:TI,AB,KY 16
- #40 (Gas Chromatography-Mass Spectrometry):TI,AB,KY 626
- #41 (Gas Chromatography-MS):TI,AB,KY 2
- #42 MESH DESCRIPTOR Biomarkers EXPLODE ALL TREES WITH QUALIFIERS AN,ME 4487

#43 MESH DESCRIPTOR Pyruvic Acid EXPLODE ALL TREES WITH QUALIFIERS ME 21
 #44 MESH DESCRIPTOR Spectrum Analysis EXPLODE ALL TREES WITH QUALIFIERS MT 379
 #45 MESH DESCRIPTOR Principal Component Analysis EXPLODE ALL TREES 198
 #46 (Biological Marker*):TI,AB,KY 6671
 #47 biomarker*:TI,AB,KY 24166
 #48 (biochemical marker*):TI,AB,KY 1618
 #49 (metabolic profile*):TI,AB,KY 844
 #50 (amino acid turnover):TI,AB,KY 3
 #51 (endometri* receptivity):TI,AB,KY 163
 #52 (glucose adj2 metabolism):TI,AB,KY 10728
 #53 (amino acid metabolism):TI,AB,KY 193
 #54 MESH DESCRIPTOR Carbohydrate Metabolism EXPLODE ALL TREES 873
 #55 (Carbohydrate* adj2 metabolism):TI,AB,KY 1616
 #56 Spectrophotometry:TI,AB,KY 1007
 #57 (Fourier Analysis):TI,AB,KY 253
 #58 (spectrum* analysis):TI,AB,KY 232
 #59 #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 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50
 OR #51 OR #52 OR #53 OR #54 OR #55 OR #56 OR #57 OR #58 62583
 #60 #20 AND #59 433

Appendix 3. MEDLINE search strategy

From 1946 to 26 February 2018

Ovid platform; MEDLINE(R) Epub Ahead of Print, In Process & Other Non-Indexed Citations, Ovid MEDLINE (R) Daily, and Ovid MEDLINE

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (37568)
 2 in vitro fertilization.tw. (20518)
 3 ivf.tw. (20716)
 4 icsi.tw. (7210)
 5 intracytoplasmic sperm injection\$.tw. (6261)
 6 blastocyst\$.tw. (19825)
 7 embryo\$.tw. (320987)
 8 oocyte\$.tw. (64481)
 9 Follicular Fluid/an, ch, cy, en, me, mi, ph [Analysis, Chemistry, Cytology, Enzymology, Metabolism, Microbiology, Physiology] (2276)
 10 Follicular Fluid.tw. (4661)
 11 (infertil\$ or subfertil\$).tw. (55949)
 12 assisted reproducti\$ techn\$.tw. (8408)
 13 or/1-12 (431597)
 14 metabolom\$.tw. (17403)
 15 exp Metabolomics/ (9831)
 16 exp Spectrophotometry, Infrared/ (59781)
 17 exp Magnetic Resonance Spectroscopy/ (198292)
 18 exp Fourier Analysis/ or exp Spectroscopy, Fourier Transform Infrared/ (52656)
 19 exp Spectrum Analysis, Raman/ (19057)
 20 near-infrared.tw. (29344)
 21 ViaMetric\$.tw. (2)
 22 spectroscop\$.tw. (323218)
 23 spectromet\$.tw. (247858)
 24 NIR.tw. (12856)
 25 chromatograph\$.tw. (393741)

26 nuclear magnetic resonance.tw. (38044)
 27 (NMR or HPLC).tw. (261332)
 28 transform-infrared.tw. (27546)
 29 Raman.tw. (44077)
 30 chemometric\$.tw. (5246)
 31 exp Gas Chromatography-Mass Spectrometry/ (44127)
 32 Gas Chromatography-Mass Spectrometry.tw. (24594)
 33 Gas Chromatography-MS.tw. (111)
 34 Biological Markers/me [Metabolism] (41855)
 35 lipodomic\$.tw. (14)
 36 (lipid\$ adj3 endometri\$ receptivity).tw. (2)
 37 endometri* receptivity array.tw. (20)
 38 endometri* receptivity analysis.tw. (1)
 39 Microfluorometric enzymatic assay\$.tw. (3)
 40 (Preimplantation adj3 Embryo Metabolism).tw. (11)
 41 Pyruvic Acid/me [Metabolism] (2637)
 42 Spectrum Analysis/mt [Methods] (8806)
 43 Principal Component Analysis/ (20994)
 44 Biological Marker\$.tw. (7683)
 45 biomarker\$.tw. (181286)
 46 biochemical marker\$.tw. (13136)
 47 metabolic profile\$.tw. (8646)
 48 amino acid turnover.tw. (105)
 49 follicular fluid profile\$.tw. (2)
 50 endometri\$ receptivity marker\$.tw. (24)
 51 (glucose adj2 metabolism).tw. (31265)
 52 amino acid metabolism.tw. (5045)
 53 Carbohydrate Metabolism/ (29453)
 54 (Carbohydrate\$ adj2 metabolism).tw. (13142)
 55 metabolic footprint.tw. (38)
 56 Microfluidic Analytical Techniques/ (9578)
 57 or/14-56 (1429849)
 58 13 and 57 (15873)
 59 randomized controlled trial.pt. (454273)
 60 controlled clinical trial.pt. (92178)
 61 randomized.ab. (403817)
 62 randomised.ab. (80559)
 63 placebo.tw. (191743)
 64 clinical trials as topic.sh. (182669)
 65 randomly.ab. (285621)
 66 trial.ti. (178408)
 67 (crossover or cross-over or cross over).tw. (75394)
 68 or/59-67 (1190818)
 69 exp animals/ not humans.sh. (4428797)
 70 68 not 69 (1096812)
 71 58 and 70 (159)

Appendix 4. Embase search strategy

From 1980 to 26 February 2018

Ovid platform

- 1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (58894)
- 2 in vitro fertilization.tw. (26494)
- 3 icsi.tw. (13894)
- 4 intracytoplasmic sperm injection\$.tw. (8322)
- 5 (blastocyst adj2 transfer\$).tw. (1921)
- 6 ivf.tw. (34835)
- 7 exp infertility therapy/ or exp artificial insemination/ or exp intrauterine insemination/ or exp ovulation induction/ (86448)
- 8 assisted reproduct\$.tw. (19034)
- 9 infertil\$.tw. (73038)
- 10 subfertil\$.tw. (6124)
- 11 embryo\$.tw. (360089)
- 12 oocyte\$.tw. (78137)
- 13 Follicular Fluid.tw. (5404)
- 14 or/1-13 (511355)
- 15 exp metabolomics/ (20452)
- 16 metabolom\$.tw. (22637)
- 17 infrared spectrophotometry/ or spectrophotometry/ (83711)
- 18 exp nuclear magnetic resonance spectroscopy/ (105368)
- 19 exp Fourier analysis/ (9410)
- 20 exp infrared spectroscopy/ (87402)
- 21 exp Raman spectrometry/ (29671)
- 22 near-infrared.tw. (29761)
- 23 ViaMetric\$.tw. (7)
- 24 spectroscop\$.tw. (313652)
- 25 spectromet\$.tw. (283161)
- 26 NIR.tw. (12528)
- 27 chromatograph\$.tw. (442948)
- 28 nuclear magnetic resonance.tw. (38294)
- 29 (NMR or HPLC).tw. (320267)
- 30 transform-infrared.tw. (29851)
- 31 Raman.tw. (30426)
- 32 chemometric\$.tw. (6381)
- 33 mass fragmentography/ (34544)
- 34 Gas Chromatography-Mass Spectrometry.tw. (27776)
- 35 biological marker/ec [Endogenous Compound] (85953)
- 36 (lipid\$ adj3 endometri\$ receptivity).tw. (6)
- 37 Microfluorometric enzymatic assay\$.tw. (2)
- 38 Preimplantation Embryo Metabolism.tw. (13)
- 39 pyruvic acid/ec [Endogenous Compound] (4464)
- 40 spectroscopy/ (75096)
- 41 principal component analysis/ or chemometrics/ (36780)
- 42 Biological Marker\$.tw. (10634)
- 43 biomarker\$.tw. (282274)
- 44 biochemical marker\$.tw. (17995)
- 45 metabolic profile\$.tw. (12561)
- 46 amino acid turnover.tw. (119)
- 47 follicular fluid profile\$.tw. (3)
- 48 endometri\$ receptivity marker\$.tw. (58)
- 49 endometri* receptivity analysis.tw. (8)

50 endometri* receptivity array.tw. (55)
 51 glucose metabolism.tw. (38231)
 52 amino acid metabolism.tw. (5268)
 53 carbohydrate metabolism/ (26906)
 54 Carbohydrate metabolism.tw. (11786)
 55 metabolic footprint.tw. (46)
 56 exp microfluidic analysis/ (7239)
 57 or/15-56 (1691092)
 58 14 and 57 (18049)
 59 Clinical Trial/ (962783)
 60 Randomized Controlled Trial/ (485322)
 61 exp randomization/ (77219)
 62 Single Blind Procedure/ (30431)
 63 Double Blind Procedure/ (143745)
 64 Crossover Procedure/ (54244)
 65 Placebo/ (305944)
 66 Randomized controlled trial\$.tw. (174771)
 67 Rct.tw. (27287)
 68 random allocation.tw. (1746)
 69 randomly allocated.tw. (28903)
 70 allocated randomly.tw. (2288)
 71 (allocated adj2 random).tw. (791)
 72 Single blind\$.tw. (20305)
 73 Double blind\$.tw. (178954)
 74 ((treble or triple) adj blind\$.tw. (749)
 75 placebo\$.tw. (262502)
 76 prospective study/ (424687)
 77 or/59-76 (1858047)
 78 case study/ (52084)
 79 case report.tw. (345711)
 80 abstract report/ or letter/ (1019676)
 81 or/78-80 (1409154)
 82 77 not 81 (1810881)
 83 58 and 82 (717)

Appendix 5. CINAHL search strategy

From 1982 to 26 February 2018
 EBSCO platform

#	Query	Results
S53	S40 AND S52	149
S52	S41 OR S42 OR S43 OR S44 OR S45 OR S46 OR S47 OR S48 OR S49 OR S50 OR S51	1,209,073
S51	TX allocat* random*	8,289
S50	(MH "Quantitative Studies")	18,692

(Continued)

S49	(MH "Placebos")	10,680
S48	TX placebo*	49,911
S47	TX random* allocat*	8,289
S46	(MH "Random Assignment")	45,803
S45	TX randomi* control* trial*	143,871
S44	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*))	938,843
S43	TX clinic* n1 trial*	220,952
S42	PT Clinical trial	85,807
S41	(MH "Clinical Trials+")	235,376
S40	S10 AND S39	1,177
S39	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 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38	125,035
S38	TX Microfluidic Analytical Techniques	99
S37	(MM "Microfluidic Analytical Techniques")	54
S36	TX Carbohydrate metabolism	2,545
S35	(MM "Carbohydrate Metabolism/PH")	21
S34	TX amino acid metabolism	1,718
S33	TX glucose metabolism	15,602
S32	TX endometri* receptivity marker*	9
S31	TX amino acid turnover	26
S30	TX metabolic profile	1,474
S29	TX biochemical marker*	2,013

(Continued)

S28	TX biomarker*	32,468
S27	TX Biological Marker*	52,491
S26	TX Pyruvic Acid	26
S25	(MH “Biological Markers”)	39,907
S24	TX Chromatography-MS	17
S23	TX Chromatography-Mass Spectrometry	1,958
S22	(MH “Gas Chromatography-Mass Spectrometry”)	1,058
S21	TX Raman	1,864
S20	TX nuclear magnetic resonance	1,004
S19	TX chromatograph*	19,681
S18	TX NIR	1,584
S17	TX spectrometer	511
S16	TX spectroscop*	14,815
S15	TX near-infrared	2,575
S14	(MM “Spectrum Analysis, Raman”)	84
S13	(MM “Magnetic Resonance Spectroscopy”)	1,526
S12	(MM “Spectrophotometry, Infrared”) OR (MM “Colorimetry”)	210
S11	TX metabolomic*	1,546
S10	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9	28,398
S9	TX (infertil* or subfertil*)	10,688
S8	TX blastocyst*	979
S7	TX follicular fluid	87
S6	TX oocyte*	1,662

(Continued)

S5	TX embryo*	15,634
S4	TX IVF or TX ICSI	2,241
S3	(MM “Fertilization in Vitro”)	1,904
S2	TX vitro fertilization	4,004
S1	TX vitro fertilisation	4,004

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

From inception to 27 February 2018

Web platform

“IVF” and “metabolomic”

Appendix 7. OpenGrey literature

From inception to 27 February 2018

Web platform

“IVF” and “metabolomic”

WHAT’S NEW

Last assessed as up-to-date: 26 February 2018.

Date	Event	Description
27 February 2018	New citation required but conclusions have not changed	The change in analyses has not changed the conclusions of the review
11 February 2018	New search has been performed	An intention to treat analysis was used, 2 new authors (from the protocol stage) were added

CONTRIBUTIONS OF AUTHORS

Protocol development

CS: Conceived the idea, designed and drafted the protocol, and is the responsible author of the review.
CV, DV, and MT contributed to the design and drafting of the protocol.

Contribution to the full review

CS: Review design, co-ordination of data collection and analysis, drafting of manuscript, critical evaluation of final content. lead author in writing the protocol and full review.

DV and ES: Data collection and extraction, Table and figure development.

All authors (CS, ES, DV, CV, MT) critically reviewed the manuscript for content.

DECLARATIONS OF INTEREST

None of the authors have any conflicts of interest to disclose.

SOURCES OF SUPPORT

Internal sources

- None, Greece.

External sources

- None, Other.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We reworded our objectives to reflect our primary outcomes. In the protocol ([Siristatidis 2015](#)), our objective was to evaluate the effectiveness of metabolomics in improving pregnancy outcomes in women undergoing ART. Also, we made changes to the types of interventions (where we have erased the potential comparison of 'no assessment'), and the unit of analysis for miscarriage (per woman not per pregnancy). We added a sensitivity analysis for clinical pregnancy, and more detail about the development of the SoF table, in our effort to improve the quality of the review.

Our primary outcome was revised to live birth OR ongoing pregnancy (as a composite outcome), to improve the statistical power of our primary effectiveness outcome. We conducted an additional sensitivity analysis, to check the effect of restricting the primary outcome to live birth only.

The protocol defined clinical pregnancy as the presence of a foetal heart on ultrasound scan at seven weeks of gestation and 12 weeks of gestation: we corrected this to include only seven weeks. Minor changes were also applied to the definition of adverse events.

We previously published this review ([Siristatidis 2017](#)); at this update we added two authors and used ITT analysis, according to the Group's decision: of note, the final conclusions were similar.

INDEX TERMS

Medical Subject Headings (MeSH)

*Pregnancy Outcome; *Pregnancy Rate; *Reproductive Techniques, Assisted; Abortion, Spontaneous [*epidemiology]; Endometrium [physiology]; Live Birth [*epidemiology]; Metabolomics [*methods]; Oocytes; Pregnancy, Multiple [*statistics & numerical data]; Randomized Controlled Trials as Topic; Sensitivity and Specificity

MeSH check words

Adult; Female; Humans; Pregnancy