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# Use of biochemical tests of placental function for improving pregnancy outcome (Review)

**Heazell AEP, Whitworth M, Duley L, Thornton JG**

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[Intervention Review]

# Use of biochemical tests of placental function for improving pregnancy outcome

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## ABSTRACT

### Background

The placenta has an essential role in determining the outcome of pregnancy. Consequently, biochemical measurement of placentally-derived factors has been suggested as a means to improve fetal and maternal outcome of pregnancy.

### Objectives

To assess whether clinicians' knowledge of the results of biochemical tests of placental function is associated with improvement in fetal or maternal outcome of pregnancy.

### Search methods

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (31 July 2015) and reference lists of retrieved studies.

### Selection criteria

Randomised, cluster-randomised or quasi-randomised controlled trials assessing the merits of the use of biochemical tests of placental function to improve pregnancy outcome.

Studies were eligible if they compared women who had placental function tests and the results were available to their clinicians with women who either did not have the tests, or the tests were done but the results were not available to the clinicians. The placental function tests were any biochemical test of placental function carried out using the woman's maternal biofluid, either alone or in combination with other placental function test/s.

### Data collection and analysis

Two review authors independently assessed trials for inclusion, extracted data and assessed trial quality. Authors of published trials were contacted for further information.

**Main results**

Three trials were included, two quasi-randomised controlled trials and one randomised controlled trial. One trial was deemed to be at low risk of bias while the other two were at high risk of bias. Different biochemical analytes were measured - oestrogen was measured in one trial and the other two measured human placental lactogen (hPL). One trial did not contribute outcome data, therefore, the results of this review are based on two trials with 740 participants.

There was no evidence of a difference in the incidence of **death of a baby** (risk ratio (RR) 0.88, 95% confidence interval (CI) 0.36 to 2.13, two trials, 740 participants (*very low quality evidence*)) or the **frequency of a small-for-gestational-age** infant (RR 0.44, 95% CI 0.16 to 1.19, one trial, 118 participants (*low quality evidence*)).

In terms of this review's secondary outcomes, there was no evidence of a clear difference between women who had biochemical tests of placental function compared with standard antenatal care for the incidence of **stillbirth** (RR 0.56, 95% CI 0.16 to 1.88, two trials, 740 participants (*very low quality evidence*)) or **neonatal death** (RR 1.62, 95% CI 0.39 to 6.74, two trials, 740 participants, *very low quality evidence*)) although the directions of any potential effect were in opposing directions. There was no evidence of a difference between groups in **elective delivery** (RR 0.98, 95% CI 0.84 to 1.14, two trials, 740 participants (*low quality evidence*)), **caesarean section** (one trial, RR 0.48, 95% CI 0.15 to 1.52, one trial, 118 participants (*low quality evidence*)), change in anxiety score (mean difference -2.40, 95% CI -4.78 to -0.02, one trial, 118 participants), **admissions to neonatal intensive care** (RR 0.32, 95% CI 0.03 to 3.01, one trial, 118 participants), and **preterm birth before 37 weeks' gestation** (RR 2.90, 95% CI 0.12 to 69.81, one trial, 118 participants). One trial (118 participants) reported that there were no cases of **serious neonatal morbidity**. **Maternal death** was not reported.

A number of this review's secondary outcomes relating to the baby were not reported in the included studies, namely: umbilical artery pH < 7.0, neonatal intensive care for more than seven days, very preterm birth (< 32 weeks' gestation), need for ventilation, organ failure, fetal abnormality, neurodevelopment in childhood (cerebral palsy, neurodevelopmental delay). Similarly, a number of this review's maternal secondary outcomes were not reported in the included studies (admission to intensive care, high dependency unit admission, hospital admission for > seven days, pre-eclampsia, eclampsia, and women's perception of care).

**Authors' conclusions**

There is insufficient evidence to support the use of biochemical tests of placental function to reduce perinatal mortality or increase identification of small-for-gestational-age infants. However, we were only able to include data from two studies that measured oestrogens and hPL. The quality of the evidence was low or very low.

Two of the trials were performed in the 1970s on women with a variety of antenatal complications and this evidence cannot be generalised to women at low-risk of complications or groups of women with specific pregnancy complications (e.g. fetal growth restriction). Furthermore, outcomes described in the 1970s may not reflect what would be expected at present. For example, neonatal mortality rates have fallen substantially, such that an infant delivered at 28 weeks would have a greater chance of survival were those studies repeated; this may affect the primary outcome of the meta-analysis.

With data from just two studies (740 women), this review is underpowered to detect a difference in the incidence of death of a baby or the frequency of a small-for-gestational-age infant as these have a background incidence of approximately 0.75% and 10% of pregnancies respectively. Similarly, this review is underpowered to detect differences between serious and/or rare adverse events such as severe neonatal morbidity. Two of the three included studies were quasi-randomised, with significant risk of bias from group allocation. Additionally, there may be performance bias as in one of the two studies contributing data, participants receiving standard care did not have venepuncture, so clinicians treating participants could identify which arm of the study they were in. Future studies should consider more robust randomisation methods and concealment of group allocation and should be adequately powered to detect differences in rare adverse events.

The studies identified in this review examined two different analytes: oestrogens and hPL. There are many other placental products that could be employed as surrogates of placental function, including: placental growth factor (PlGF), human chorionic gonadotrophin (hCG), plasma protein A (PAPP-A), placental protein 13 (PP-13), pregnancy-specific glycoproteins and progesterone metabolites and further studies should be encouraged to investigate these other placental products. Future randomised controlled trials should test analytes identified as having the best predictive reliability for placental dysfunction leading to small-for-gestational-age infants and perinatal mortality.

## PLAIN LANGUAGE SUMMARY

**Using biochemical tests to measure placental function and improve pregnancy outcomes**

**What is the issue and why is it important?**

The placenta (afterbirth) develops in the uterus during pregnancy to provide oxygen and nutrients to the growing baby and to remove waste products from the baby's blood. The placenta attaches to the wall of the uterus and is linked to the baby via the umbilical cord. The placenta plays a critical role in determining the health of the baby and mother. The health of the placenta can be assessed by performing tests on mothers' blood or urine to measure chemicals made by the placenta. Having this information could improve the outcome of pregnancy as professionals could intervene to prevent outcomes such as stillbirth or babies being born too small.

**What evidence did we find?**

We included three randomised controlled studies. Two trials were at a high risk of bias and one was at a low risk of bias. One study did not contribute any data towards this review. Therefore, this review is based on data from two studies involving 740 mothers. The evidence from these studies was graded as either low or very low quality evidence.

We found insufficient evidence to draw any conclusions about the effectiveness of tests that measure placental health in reducing the number of babies that die before birth (*very low quality evidence*) or shortly after birth (*very low quality evidence*), or in reducing the number of babies that are born small for their gestational age (*low quality evidence*).There was no evidence to suggest that measurement of placental health could cause harm by increasing intervention (planned delivery or caesarean section (*low quality evidence*) or increasing mothers' anxiety levels. There was no change in the number of babies admitted to the neonatal intensive care unit or the proportion of babies born before 37 weeks gestation (*low quality evidence*). There were no reports of serious disease for babies (as reported in one study only) or maternal deaths in any of the studies. A number of this review's other outcomes of interest were not reported in the included studies.

More research is needed to determine the most useful test for placental health as a way of predicting poor pregnancy outcome, and then to investigate whether performing this test on mothers improves pregnancy outcomes.

## SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

**Test of placental function compared with standard care for improving pregnancy outcome**

**Patient or population:** women in the third trimester of pregnancy
**Settings:** antenatal clinic or antenatal assessment unit
**Intervention:** test of placental function
**Comparison:** standard care

| Outcomes | Illustrative comparative risks* (95% CI) | | Relative effect (95% CI) | No of participants (studies) | Quality of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| | Assumed risk | Corresponding risk | | | | |
| | Standard care | Test of placental function | | | | |
| **Death of a baby (stillbirth or neonatal death)** report of perinatal death | **Study population** | | **RR 0.88** (0.36 to 2.13) | 740 (2 studies) | ⊕◯◯◯ **very low**[1] | |
| | **27 per 1000** | **24 per 1000** (10 to 58) | | | | |
| | **Low** | | | | | |
| | **15 per 1000** | **13 per 1000** (5 to 32) | | | | |
| | **High** | | | | | |
| | **29 per 1000** | **26 per 1000** (10 to 62) | | | | |
| **Stillbirth** report of stillbirth | **Study population**[2] | | **RR 0.56** (0.16 to 1.88) | 740 (2 studies) | ⊕◯◯◯ **very low**[1,3,4] | |
| | **19 per 1000** | **11 per 1000** (3 to 36) | | | | |
| | **Low**[2] | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | **15 per 1000** | **8 per 1000** (2 to 28) | | | |
| | **High**[2] | | | | |
| | **29 per 1000** | **16 per 1000** (5 to 55) | | | |
| **Neonatal death** report of neonatal death | **Study population** | | **RR 1.62** (0.39 to 6.74) | 740 (2 studies) | ⊕○○○ **very low**[1,4,5] |
| | **8 per 1000** | **13 per 1000** (3 to 55) | | | |
| | **Low** | | | | |
| | **0 per 1000** | **0 per 1000** (0 to 0) | | | |
| | **High** | | | | |
| | **29 per 1000** | **47 per 1000** (11 to 195) | | | |
| **Small-for-gestational age (below 10th centile on customised birthweight chart or as defined by trialists)** birthweight centile chart | **Study population** | | **RR 0.44** (0.16 to 1.19) | 118 (1 study) | ⊕⊕○○ **low**[4,6] |
| | **190 per 1000** | **83 per 1000** (30 to 226) | | | |
| | **Moderate** | | | | |
| | **190 per 1000** | **84 per 1000** (30 to 226) | | | |
| **Preterm birth (before 37 weeks' gestation)** reported gestation at birth | **Study population**[7] | | **RR 2.90** (0.12 to 69.81) | 118 (1 study) | ⊕⊕○○ **low**[4,8] |

| | | | | | |
|---|---|---|---|---|---|
| | **0 per 1000** | **0 per 1000** (0 to 0) | | | |
| | **Low**[7] | | | | |
| | **60 per 1000** | **174 per 1000** (7 to 1000) | | | |
| | **High**[7] | | | | |
| | **80 per 1000** | **232 per 1000** (10 to 1000) | | | |
| **Elective delivery (induction of labour or non-labour caesarean section)** report of mode of delivery | **Study population** | | **RR 0.98** (0.84 to 1.14) | 740 (2 studies) | ⊕⊕◯◯ **low**[1,4] |
| | **485 per 1000** | **475 per 1000** (407 to 553) | | | |
| | **Moderate** | | | | |
| | **533 per 1000** | **522 per 1000** (448 to 608) | | | |
| **Caesarean section** report of mode of delivery | **Study population** | | **RR 0.48** (0.15 to 1.52) | 118 (1 study) | ⊕⊕◯◯ **low**[4,9] |
| | **138 per 1000** | **66 per 1000** (21 to 210) | | | |
| | **Moderate** | | | | |
| | **138 per 1000** | **66 per 1000** (21 to 210) | | | |

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
**CI:** Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
**High quality:** Further research is very unlikely to change our confidence in the estimate of effect.
**Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
**Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
**Very low quality:** We are very uncertain about the estimate.

[1] Duenhoelter 1976 had a high risk of bias in the following domains: random sequence generation and allocation concealment, and unclear for blinding of participants. Heazell 2013 had a high risk of bias in the blinding of participants domain.
[2] Risk of stillbirth in women presenting with reduced fetal movements reported to be three-fold greater than infants with normal movements (~1.5%). Population in Duenhoelter 1976 very heterogenous population, but the overall perinatal mortality rate at the unit was 2.9%.
[3] Few stillbirths in included studies.11 stillbirths in Duenhoelter 1976 and none in Heazell 2013. Total sample size for comparison = 740.
[4] At least one study known to have commenced but discontinued (Grudzinskas 1990).
[5] Few neonatal deaths in included studies; 8 neonatal deaths in Duenhoelter 1976 and none in Heazell 2013. Total sample size for comparison = 740.
[6] Few small-for-gestational-age births in included studies. Heazell 2013 included 16 small-for-gestational-age births in total sample size for comparison = 120.
[7] O'Sullivan 2009 report a preterm delivery rate of 6% in women attending with reduced fetal movements. ~8% of births occur before 37 weeks' gestation.
[8] One preterm birth reported in included study (Heazell 2013) from total sample size for comparison = 120.
[9] Few caesarean deliveries (n = 12) reported in one study (Heazell 2013) with a total of 120 participants.

# BACKGROUND

In a healthy pregnancy, the placenta is a metabolically active endocrine organ secreting many different hormones and metabolites into maternal blood; this profile may alter with pregnancy complications (Conde-Agudelo 2013). The outcome of pregnancy is closely linked to placental function; placental dysfunction has been documented in complications of pregnancy including: fetal growth restriction, small-for-gestational-age infants, pre-eclampsia, preterm birth, reduced fetal movements and stillbirth (Brosens 2011; Ness 2006; Pinar 2014; Warrander 2012).

Biochemical tests of placental function measure released placental factors in maternal biofluid(s), including urine and blood. A previous Cochrane systematic review found no evidence that measuring oestriol improved pregnancy outcome (Neilson 2012). Since Neilson 2012 was published, there has been increased interest in the measurement of biomarkers of placental function. Our review updates the Neilson 2012 review on this topic, and includes more recently developed biomarkers.

## Description of the intervention

Prior to the widespread use of ultrasound to assess fetal biometry or biophysical profile from the mid-1970s onwards, biochemical tests of placental function including: oestriol, human placental lactogen (hPL) and human chorionic gonadotrophin (hCG) were used in antepartum assessment of the fetus in late pregnancy (Greene 1965). These biochemical factors were measured in maternal plasma, serum or urine. Levels of these factors may change through pregnancy; factors which are synthesised by the placenta tend to increase in proportion to placental mass throughout pregnancy. Important exceptions to this are hCG which peaks in the first trimester and free placental growth factor (PlGF), which declines after 36 weeks (Saffer 2013). Therefore, performance of specific biochemical tests may depend on the gestation at sampling.
Recently, biochemical markers related to placental function have been used as part of maternal serum screening for trisomy 21 in the first and second trimester including alpha fetoprotein (AFP), hCG, unconjugated oestriol, pregnancy-associated plasma protein A (PAPP-A), and inhibin. Observational studies have demonstrated that in the absence of chromosomal or structural anomalies, dysregulation of these placental biomarkers is associated with altered risks of fetal death, fetal growth restriction, small-for-gestational-age infants or pre-eclampsia (Dugoff 2004; Smith 2007a; Smith 2007b). These were either case-control or cohort studies which focused on samples obtained in first trimester screening. Serum PAPP-A below 5th centile (0.42 MoM) was associated with an increased risk of spontaneous loss before 24 weeks' gestation (odds ratio (OR) 2.50, 95% confidence interval (CI) 1.76 to 3.56), stillbirth (OR 2.15, 95% CI 1.11 to 4.15), small-for-gestational age below 10th centile (OR 2.47, 95% CI 2.16 to 2.81) and pre-eclampsia (OR 1.54, 95% CI 1.16 to 2.03); hCG below 5th centile was related to small-for-gestational-age infant below 10th centile (OR 1.55, 95% CI 1.33 to 1.80) (Dugoff 2004). Measurements obtained in the second trimester (15 to 21 weeks) found that women with increased AFP greater than 95th centile had an elevated risk of stillbirth (OR 2.79, 95% CI 2.09 to 3.73); this was also true for hCG greater than 95th centile (OR 1.93, 95% CI 1.39 to 2.66) (Smith 2007a).
Recently, placentally-derived factors in maternal blood including hPL (Dutton 2012), placental protein 13 (PP-13) (Schneuer 2012), soluble FMS-like tyrosine kinase (sFLt-1) (Smith 2007b), PlGF (Benton 2012), and various metabolites (Horgan 2011), have been measured by a variety of different experimental approaches including: enzyme-linked immunosorbent assay, mass spectrometry or developed point of care tests. Elevated sFlt-1 in the first trimester is associated with a reduced risk of a small-for-gestational-age infant (OR 0.92, 95% CI 0.88 to 0.96), and stillbirth associated with a placental cause (OR 0.77, 95% CI 0.61 to 0.95). Likewise, high PlGF in the first trimester is associated with a reduction in small-for-gestational-age infant (OR 0.95, 95% CI 0.90 to 0.99) (Smith 2007b). Measurement of PlGF in the third trimester differentiated placental intrauterine growth restriction (IUGR) (n = 9) from constitutionally small fetuses (n = 7) with 100% sensitivity and 86% specificity (Benton 2012).
Currently, ultrasound assessment of fetal well-being provides only modest benefits in selected populations (Alfirevic 2013; Alfirevic 2015). This has increased interest in other methods of predicting or identifying fetal compromise. It is hypothesised that measurement of biochemical factors in maternal blood or urine reflects placental function, which is closely linked to fetal outcome compromise.

## How the intervention might work

Many pregnancy complications are related to abnormal placental function; methods which assess placental function may identify pregnancies where placental dysfunction is sufficiently severe that it leads to fetal demise. It is hypothesised that revealing the results of these biochemical measurements to clinicians may improve detection of complications, which could improve pregnancy outcome by targeting intervention (e.g. delivery). However, it is also possible that the intervention could have negative effects including: increased maternal anxiety due to increased testing or abnormal results, or increased intervention such as induction of labour or caesarean section.

## Why it is important to do this review

Observational studies relating abnormal levels of placentally-derived factors to increased risk of stillbirth, fetal growth restriction and pre-eclampsia have re-ignited interest in biochemical markers of placental dysfunction. Therefore, it is important to determine

the value of biochemical tests of placental function in improving fetal and maternal outcome of pregnancy.

## OBJECTIVES

To assess whether clinicians' knowledge of the results of biochemical tests of placental function is associated with an improvement in fetal or maternal outcome of pregnancy in high-risk, low-risk or unselected pregnancies.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

We included randomised and quasi-randomised trials that assessed the effects of biochemical testing of placental or feto-placental function in pregnancy. Cluster-randomised trials were eligible for inclusion. Cross-over randomised trials were not eligible for inclusion as this is not an appropriate study design for this question. We included studies reported only as abstracts, provided there were sufficient data to evaluate study quality.

#### Types of participants

All pregnant women, regardless of whether deemed to be high risk or low risk for pregnancy complications (e.g. fetal growth restriction, perinatal mortality or pre-eclampsia), or unselected participants by the study investigators. Women who had pregnancies complicated by chromosomal or structural anomaly were excluded.

#### Types of interventions

Studies were eligible if they compared women who had placental function tests and the results were available to their clinicians with women who either did not have the tests, or the tests were done but the results were not available to the clinicians. The placental function tests were any biochemical test of placental function carried out using the woman's maternal biofluid, either alone or in combination with other placental function test/s.

#### Types of outcome measures

##### Primary outcomes

1. Death of a baby (stillbirth or neonatal death)
2. Small-for-gestational age (below 10th centile on customised birthweight chart, or as defined by trialists)

##### Secondary outcomes

**For the baby**

1. Stillbirth
2. Neonatal death
3. Umbilical artery pH < 7.0
4. Neonatal intensive care unit admission
5. Neonatal intensive care for more than seven days
6. Preterm birth (before 37 weeks' gestation)
7. Very preterm birth (before 32 weeks' gestation)
8. Need for ventilation
9. Organ failure
10. Serious neonatal morbidity (e.g. necrotising enterocolitis, chronic lung disease, intraventricular haemorrhage, sepsis, seizures)
11. Fetal abnormality
12. Neurodevelopment in childhood (cerebral palsy, neurodevelopmental delay)

**For the women**

1. Elective delivery (induction of labour or non-labour caesarean section)
2. Caesarean section
3. Intensive care admission
4. High-dependency unit admission
5. Hospital admission for ≥ seven days
6. Pre-eclampsia
7. Eclampsia
8. Maternal death
9. Women's perception of care

### Search methods for identification of studies

The following methods section of this review is based on a standard template used by the Cochrane Pregnancy and Childbirth Group.

#### Electronic searches

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register by contacting the Trials Search Co-ordinator (31 July 2015).

The Cochrane Pregnancy and Childbirth Group's Trials Register is maintained by the Trials Search Co-ordinator and contains trials identified from:

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE (Ovid);

3. weekly searches of Embase (Ovid);
4. monthly searches of CINAHL (EBSCO);
5. handsearches of 30 journals and the proceedings of major conferences;
6. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Details of the search strategies for CENTRAL, MEDLINE, Embase and CINAHL, the list of handsearched journals and conference proceedings, and the list of journals reviewed via the current awareness service can be found in the 'Specialized Register' section within the editorial information about the Cochrane Pregnancy and Childbirth Group.

Trials identified through the searching activities described above are each assigned to a review topic (or topics). The Trials Search Co-ordinator searches the register for each review using the topic list rather than keywords.

### Searching other resources

We searched the reference lists of retrieved studies.

We did not apply any language or date restrictions.

## Data collection and analysis

### Selection of studies

Two review authors (Alexander Heazell (AEPH) and Melissa Whitworth (MKW)) independently assessed studies identified by the search strategy for inclusion. Disagreement was resolved by discussion or, if required, consultation with a third review author (Lelia Duley (LD) or Jim Thornton (JT)). Where there were conflicts of interest due to authorship of an included trial, studies were selected for inclusion by a review author who was not an author of the relevant trial report.

### Data extraction and management

A form was designed to extract data. For eligible studies, AEPH and MKW extracted the data using the agreed form. Discrepancies were resolved through discussion or, if required, consultation with LD or JT. Where there were conflicts of interest due to authorship, data were extracted by a review author who was not an author of the relevant trial report. Data were entered into Review Manager software (RevMan 2014) and checked for accuracy.

When information regarding any of the above was unclear, we attempted to contact authors of the original reports to provide further details.

### Assessment of risk of bias in included studies

AEPH and MKW independently assessed each study for risk of bias using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). Disagreement was resolved by discussion. Where there were conflicts of interest due to authorship, the risk of bias was assessed by a review author who was not an author of the relevant trial report.

#### (1) Random sequence generation (checking for possible selection bias)

For each included study, we described the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.

We assessed the method as:

- low risk of bias (any truly random process, e.g. random number table; computer random number generator);
- high risk of bias (any non-random process, e.g. odd or even date of birth; hospital or clinic record number);
- unclear risk of bias.

#### (2) Allocation concealment (checking for possible selection bias)

For each included study, we described the method used to conceal allocation to interventions prior to assignment and assessed whether intervention allocation could have been foreseen in advance of, or during recruitment, or changed after assignment.

We assessed the methods as:

- low risk of bias (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk of bias (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear risk of bias.

#### (3.1) Blinding of participants and personnel (checking for possible performance bias)

For each included study, we described the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We considered that studies are at low risk of bias if they were blinded, or if we judged that the lack of blinding was unlikely to affect results.

We assessed the methods as:

- low, high or unclear risk of bias for participants;
- low, high or unclear risk of bias for personnel.

#### (3.2) Blinding of outcome assessment (checking for possible detection bias)

For each included study, we described the methods used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Where relevant, we assessed blinding separately for different outcomes or classes of outcomes.

Methods used to blind outcome were assessed as:

- low, high or unclear risk of bias.

#### (4) Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)

We described the completeness of data including attrition and exclusions from the analysis for each included study. We stated whether attrition and exclusions were reported and the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported, or could be supplied by the trial authors, we re-included missing data in the analyses which we undertook.
Methods were assessed as:

- low risk of bias (e.g. no missing outcome data; missing outcome data balanced across groups);
- high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups; 'as treated' analysis done with substantial departure of intervention received from that assigned at randomisation);
- unclear risk of bias.

#### (5) Selective reporting (checking for reporting bias)

We investigated the possibility of selective outcome reporting bias. We assessed the methods as:

- low risk of bias (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- high risk of bias (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest were reported incompletely and so could not be used; study failed to include results of a key outcome that would have been expected to have been reported);
- unclear risk of bias.

#### (6) Other bias (checking for bias due to problems not covered by (1) to (5) above)

For each included study we described any important concerns about other possible sources of bias.
We assessed whether each study was free of other problems that could put it at risk of bias:

- low risk of other bias;
- high risk of other bias;
- unclear whether there is risk of other bias.

#### (7) Overall risk of bias

We made judgements about whether studies were at high risk of bias, according to the criteria given in the *Handbook* (Higgins 2011). With reference to (1) to (6) above, we assessed the likely magnitude and direction of the bias and whether we considered it is likely to impact on the findings. We explored the impact of the level of bias by undertaking sensitivity analyses - *see* Sensitivity analysis.

### Using the GRADE approach to assess the quality of the body of evidence

For this review, we assessed the quality of the evidence using the GRADE approach as outlined in the GRADE handbook in order to assess the quality of the body of evidence relating to the following outcomes for the main comparison (tests of placental function versus standard care).

1. Death of a baby (stillbirth or neonatal death)
2. Small-for-gestational age (below 10th centile on customised birthweight chart or as defined by trialists)
3. Stillbirth
4. Neonatal death
5. Preterm birth (before 37 weeks' gestation)
6. Elective delivery (induction of labour or non-labour caesarean section)
7. Caesarean section

GRADEpro Guideline Development Tool was used to import data from Review Manager (RevMan 2014) in order to create a 'Summary of findings' table. A summary of the intervention effect and a measure of quality for each of the above outcomes was produced using the GRADE approach. The GRADE approach uses five considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the quality of the body of evidence for each outcome. The evidence can be downgraded from 'high quality' by one level for serious (or by two levels for very serious) limitations, depending on assessments for risk of bias, indirectness of evidence, serious inconsistency, imprecision of effect estimates or potential publication bias.

### Measures of treatment effect

#### Dichotomous data

For dichotomous data, results are presented as summary risk ratio with 95% confidence intervals.

#### Continuous data

For continuous data, we planned to use the mean difference if outcomes were measured in the same way between trials and the standardised mean difference to combine trials that measured the same outcome, but used different methods.

### Unit of analysis issues

#### Cluster-randomised trials

We did not identify any cluster-randomised for inclusion in the analysis. In future updates, if trials are identified and found to be eligible, we will include cluster-randomised trials in the analyses along with individually-randomised controlled trials. We will adjust their standard errors using the methods described in the *Handbook* [Section 16.3.6] using an estimate of the intracluster correlation co-efficient (ICC) derived from the trial (if possible), from a similar trial or from a study of a similar population. If we use ICCs from other sources, we will report this and conduct sensitivity analyses to investigate the effect of variation in the ICC. If we identify both cluster-randomised trials and individually-randomised trials, we plan to synthesise the relevant information. We will consider it reasonable to combine the results from both if there is little heterogeneity between the study designs and the interaction between the effect of intervention and the choice of randomisation unit is considered to be unlikely.
We will also acknowledge heterogeneity in the randomisation unit and perform a sensitivity analysis to investigate the effects of the randomisation unit.

#### Cross-over trials

Studies with a cross-over design were not eligible for inclusion, as this design is not appropriate for this question.

#### Studies with multiple treatment groups

We did not identify any studies with multiple treatment groups. In future updates, if such trials are identified and found to be eligible, we will include them if any pair-wise comparisons of the intervention groups are relevant to the review and meet the inclusion criteria. We will report all the intervention groups involved in the index study in the Characteristics of included studies table, but will include only those intervention groups relevant to the analysis. We will address pair-wise comparisons from multi-arm trials in meta-analyses, if they are eligible. We will ensure that data from individual participants are only included once when pooling data. If there are multiple intervention groups in a particular meta-analysis, we will combine all relevant experimental intervention groups of the study into a single intervention group and combine all relevant control intervention groups into a single control group (Higgins 2011).

### Dealing with missing data

For included studies, we noted levels of attrition. We had planned to explore the impact of including studies with high levels of missing data in the overall assessment of treatment effect by using sensitivity analysis if a sufficient number of studies were identified.
For all outcomes, we carried out analyses, as far as possible, on an intention-to-treat basis, i.e. we attempted to include all participants randomised to each group in the analyses, and all participants were analysed in the group to which they were allocated, regardless of whether or not they received the allocated intervention. The denominator for each outcome in each trial was the number randomised minus any participants whose outcomes were known to be missing.

### Assessment of heterogeneity

We assessed statistical heterogeneity in each meta-analysis using the $T^2$, $I^2$ and $Chi^2$ statistics. We regarded heterogeneity as substantial if an $I^2$ was greater than 30% and either a $T^2$ was greater than zero, or there was a low P value (less than 0.10) in the $Chi^2$ test for heterogeneity.

### Assessment of reporting biases

Had there been 10 or more studies in the meta-analysis, we planned to investigate reporting biases (such as publication bias) using funnel plots. No meta-analysis had more than 10 studies. In future updates, if there are 10 or more trials we will assess funnel plot asymmetry visually. If asymmetry is suggested by a visual assessment, we will perform exploratory analyses to investigate it.

### Data synthesis

We carried out statistical analysis using the Review Manager software (RevMan 2014). We used fixed-effect meta-analysis for combining data where it was reasonable to assume that studies were estimating the same underlying treatment effect: i.e. where trials were examining the same intervention, and the trials' populations and methods were judged sufficiently similar. In future updates, if there is clinical heterogeneity sufficient to expect that the underlying treatment effects differed between trials, or if substantial statistical heterogeneity is detected, we will use random-effects meta-analysis to produce an overall summary, if an average treatment effect across trials is considered clinically meaningful. The random-effects summary will be treated as the average range of possible treatment effects and we will discuss the clinical implications of treatment effects differing between trials. If the average treatment effect is not clinically meaningful, we will not combine trials.
In future updates, if we use random-effects analyses, the results will be presented as the average treatment effect with its 95% confidence interval, and the estimates of $T^2$ and $I^2$.

### Subgroup analysis and investigation of heterogeneity

We did not identify substantial heterogeneity in our analyses. However, in future updates, if we identify substantial heterogeneity, we will investigate it using subgroup and sensitivity analyses. We will consider whether an overall summary is meaningful, and if so, will use random-effects analysis to produce it.
We will carry out the following planned subgroup analyses based on:

1. risk at trial entry: women at high risk, women at low risk; women with mixed low and high risk or unselected risk; women with risk status unknown;

2. risk of bias: low risk of bias; high risk of bias; risk of bias unclear;
3. type of placental function tests;
4. timing of placental function tests divided by trimester.

Subgroup analysis will be restricted to the review's primary outcomes.

We will assess subgroup differences by interaction tests available within RevMan (RevMan 2014) and will report the results of subgroup analyses quoting the $\chi^2$ statistic and P value, and the interaction test $I^2$ value.

### Sensitivity analysis

We did not perform sensitivity analysis due to the small number of trials included. In future updates, if more studies are included, we will conduct sensitivity analyses to explore the effect of particular aspects of study quality (e.g. randomised controlled trials versus quasi-randomised controlled trials) or statistical treatment of data looking at primary outcomes only.

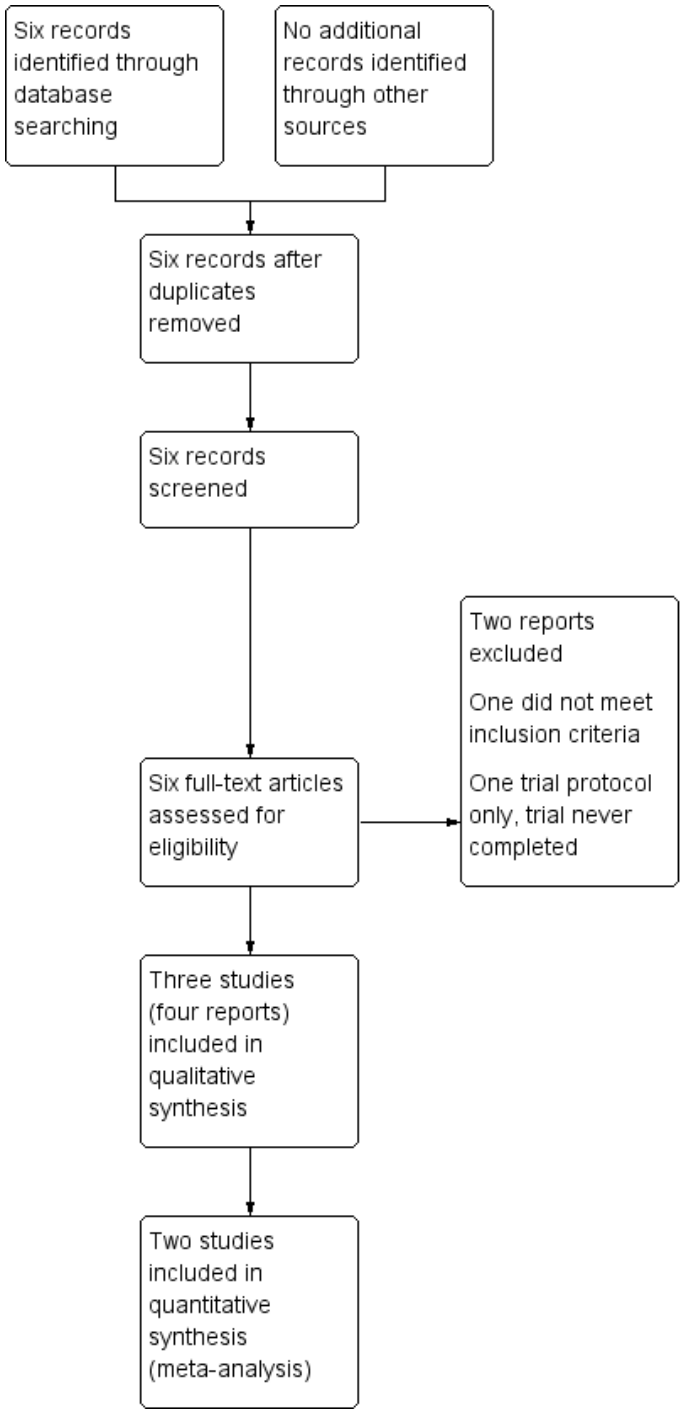
## RESULTS

## Description of studies

### Results of the search

The search of the Cochrane Pregnancy and Childbirth Group's Trials Register in September 2014 retrieved six reports relating to five studies (see:Figure 1). Three studies were included (Duenhoelter 1976; Heazell 2013; Spellacy 1975), and two were excluded (Grudzinskas 1990; Sharf 1984).

**Figure 1. Study flow diagram.**



## Included studies

We included three studies (Duenhoelter 1976; Heazell 2013; Spellacy 1975). Bernatavicius 2013 was a preliminary report of an included study (Heazell 2013). The characteristics of these studies are shown in Characteristics of included studies.

### Design

We included one randomised controlled trial (Heazell 2013) and two quasi-randomised controlled trials (Duenhoelter 1976; Spellacy 1975). All of the trials tested a form of biochemical test in addition to standard antenatal practice compared with standard antenatal practice alone.

### Sample sizes

The studies were of varying size, the smallest had 120 participants (Heazell 2013), the next had 622 participants (Duenhoelter 1976), and the largest study had 2733 participants (Spellacy 1975).

### Setting

Two of the three included studies were conducted in the United States of America (Duenhoelter 1976; Spellacy 1975), and the third one in the UK (Heazell 2013). All of the studies were conducted in a single centre.

### Participants

Two studies included women attending "high-risk" antenatal clinics or inpatient antenatal service with a variety of different complications, including: hypertension, diabetes, fetal growth restriction, postmaturity, Rhesus disease and a history of stillbirth (Duenhoelter 1976; Spellacy 1975). The remaining study focused on women attending the antenatal service of a tertiary maternity service with maternal perception of reduced fetal movements after 36 weeks of pregnancy (Heazell 2013).

### Intervention

One study measured oestrogens (Duenhoelter 1976), and two measured human placental lactogen (hPL) (Heazell 2013; Spellacy 1975). All studies performed biochemical tests in addition to routine antenatal care in that clinical setting at the time of that study.

### Outcomes

One study did not report on any of the primary or secondary outcomes of interest for all participants undergoing biochemical testing (Spellacy 1975). The other two trials reported on the death of a baby (either stillbirth or neonatal death), and the rate of elective delivery (Duenhoelter 1976; Heazell 2013). Only one trial reported information on the frequency of caesarean section, preterm birth before 37 weeks' gestation, admission to the neonatal intensive care unit and levels of maternal anxiety (Heazell 2013). There were no cases of serious neonatal morbidity reported in any study. Maternal death was not reported in any study.

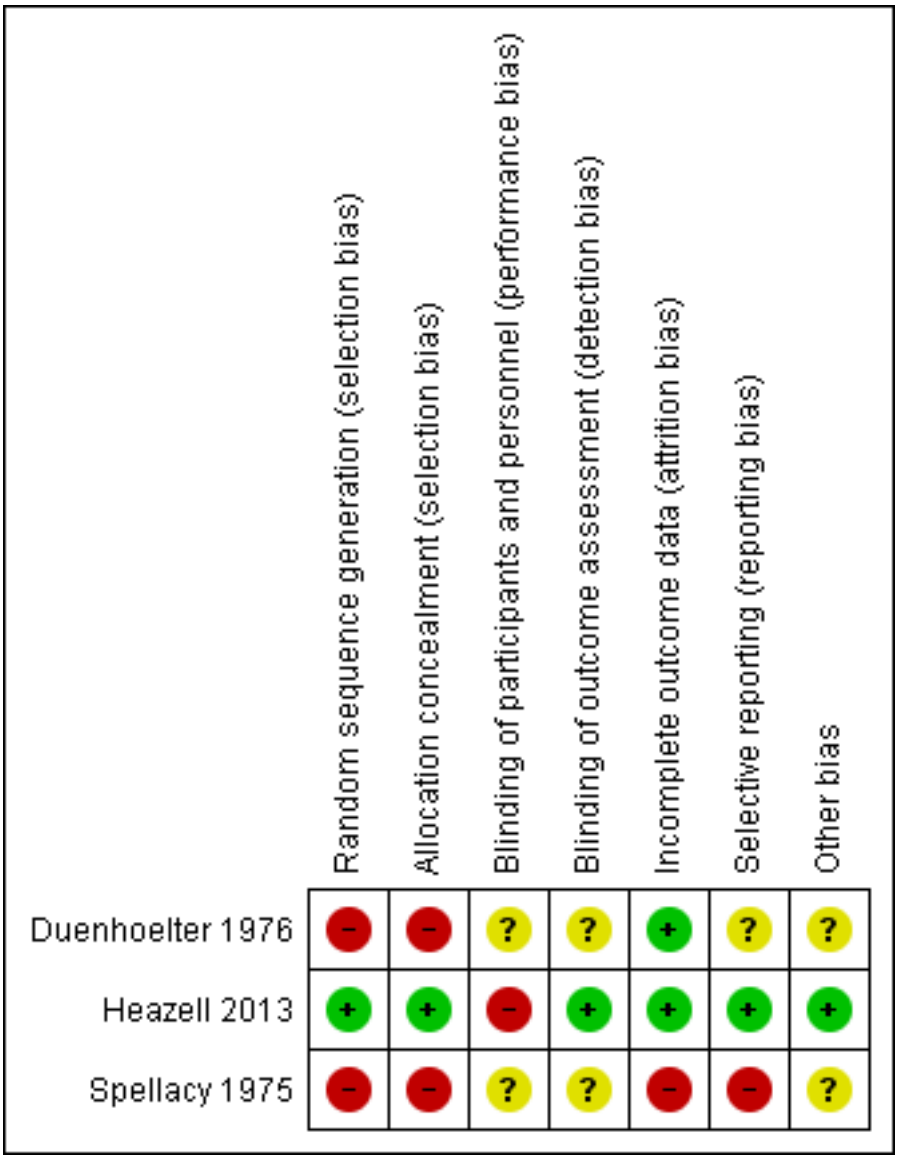
## Excluded studies

Two studies were excluded (Grudzinskas 1990; Sharf 1984). Sharf 1984 was excluded as it did not meet the inclusion criteria as it was not a randomised or quasi-randomised trial. The other study was excluded because the trial was abandoned before completion with no results available for the 160 participants (Grudzinskas 1990).

## Risk of bias in included studies

The 'Risk of bias' assessment for included studies is shown in Figure 2.

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



### Allocation

All included studies were randomised. Two studies were quasi-randomised as participants were assigned to different treatment based upon casenote number given by administrative staff and allocation was not concealed at the point of randomisation (Duenhoelter 1976; Spellacy 1975). The other study used computer-generated individual randomisation in a 1:1 ratio with random variable block size and it was stated that allocations were concealed from those enrolling participants to the trial (Heazell 2013).

### Blinding

There was an attempt to blind women and staff to group allocation in two studies, in which venepuncture was performed in all cases, with the result concealed from the clinicians for participants in the control group, although it was not clear if blinding was successful, and staff would be aware which women were in the intervention group once test results were revealed (Duenhoelter 1976; Spellacy 1975). The group allocation was not directly revealed in the other study, but only participants in the intervention (testing) arm of the trial had venepuncture performed (Heazell 2013). Therefore, clinicians providing care for these participants would be aware of participants' group allocation.

### Incomplete outcome data

Two out of three trials reported complete outcome data for all participants (Duenhoelter 1976; Heazell 2013). The other trial only reported outcome from women who had reduced levels of hPL, interpreted as being in the "danger zone" (Spellacy 1975). Due to the incomplete outcome reporting in this study, the rates of outcomes could not be calculated, so no results could be extracted.

### Selective reporting

Two studies were conducted in the 1970s and we were unable to access the protocols (Duenhoelter 1976; Spellacy 1975). However, Spellacy 1975 did not report all of the data specified in the methods section of the paper, so this was judged to be at high risk of bias. Heazell 2013 reported on primary and secondary outcomes specified in the ISRCTN Registry entry.

### Other potential sources of bias

In general, the included studies had an unclear risk of other potential sources of bias. None of the studies included information about how many participants were screened to be in the study or who were excluded and for what reason. One study (Heazell 2013) described the number of women and their reasons for non-participation in the trial.

## Effects of interventions

See: **Summary of findings for the main comparison** Test of placental function compared with standard care for improving pregnancy outcome

We included three studies but one study (Spellacy 1975) did not report on the outcomes of interest in this review. Consequently, only two studies (740 participants) contributed data towards our analyses. Due to the small number of trials and outcomes of interest reported, differences between studies depending on risk of bias or biochemical analyte could not be assessed.

#### Test of placental function versus standard care (comparison 1)

#### Primary outcomes

The included studies of a biochemical test of placental function do not show evidence of a clear difference in the incidence of the **death of a baby** (risk ratio (RR) 0.88, 95% confidence interval (CI) 0.36 to 2.13, two trials, 740 participants (Analysis 1.1)) or the frequency of a **small-for-gestational-age** infant (RR 0.44, 95% CI 0.16 to 1.19, one trial, 118 participants (Analysis 1.2)).

#### Secondary outcomes

There was no evidence of a clear difference between the incidence of **stillbirth** (RR 0.56, 95% CI 0.16 to 1.88, two trials, 740 participants (Analysis 1.3)), or **neonatal death** (RR 1.62, 95% CI 0.39 to 6.74, two trials, 740 participants (Analysis 1.4)) when women had biochemical tests of placental function compared with standard care, although the directions of any potential effect were in opposing directions. There was no evidence of a difference in any of the secondary outcome measures between women who had biochemical tests of placental function or standard care, including: **neonatal intensive care admission** (RR 0.32, 95% CI 0.03 to 3.01, one trial, 118 participants (Analysis 1.5)), **preterm birth (before 37 weeks' gestation)** (one trial, RR 2.90, 95% CI 0.12 to 69.81, one trial, 118 participants (Analysis 1.6)), **serious neonatal morbidity** (one trial, but RR not estimable as no events (Analysis 1.7)), **elective delivery (induction of labour or non-labour caesarean section)** (RR 0.98, 95% CI 0.84 to 1.14, two trials, 740 participants (Analysis 1.8)), or **caesarean section** (RR 0.48, 95% CI 0.15 to 1.52, one trial, 118 participants (Analysis 1.9)). **Maternal death** was not reported in any study.

### *Outcomes not reported in the included studies*

A number of this review's secondary outcomes relating to the baby were not reported in the included studies: umbilical artery pH < 7.0, neonatal intensive care for more than seven days, very preterm birth (< 32 weeks' gestation), need for ventilation, organ failure, fetal abnormality, neurodevelopment in childhood (cerebral palsy, neurodevelopmental delay). Similarly, a number of this review's maternal secondary outcomes were not reported in the included studies, these are: admission to intensive care, high dependency unit admission, hospital admission for > seven days, pre-eclampsia, eclampsia, and women's perception of care.

### Non-prespecified secondary outcome

There was evidence of a reduction in the mean **anxiety score** of women who had biochemical tests of placental function compared with standard antenatal care (one trial, mean difference -2.48, 95% CI -4.78 to -0.02; Analysis 1.10).

## DISCUSSION

### Summary of main results

The utility of a biochemical test of placental function has two components, i) the predictive reliability of the test and ii) the potentially beneficial or harmful consequences of intervention (delivery). There are an insufficient number of randomised controlled trials describing both the primary and secondary outcomes to evaluate the utility of biochemical tests of placental function. There was no clear evidence of any difference between groups for death of a baby, or in the components of this outcome, stillbirth and neonatal death where the directions of any potential effect were in opposing directions. Critically, this meta-analysis is underpowered to identify a significant difference in all three of these outcomes.

There was insufficient evidence to evaluate whether biochemical tests of placental function altered the frequency of a small-for-gestational-age infant. The use of biochemical tests of placental function did not appear to be associated with potential harms such as an increase in obstetric intervention (elective delivery or caesarean section), preterm birth (< 37 weeks) or admission to the neonatal intensive care unit.

Data from one trial that assessed maternal anxiety (a non-prespecified outcome) suggest that this was lower in women who had tests of placental function. However, a reduction in the state trait anxiety score of 2.4 is unlikely to be clinically significant as the scale ranges from 20 to 80, and no threshold has yet been set for a significant reduction in state anxiety.

### Overall completeness and applicability of evidence

Two of the trials were performed in the 1970s on women with a variety of antenatal complications, some of which are unrelated to placental dysfunction (e.g. rhesus isoimmunisation). The evidence from these studies cannot be generalised to women at low-risk of complications or groups of women with specific pregnancy complications (e.g. fetal growth restriction). Furthermore, outcomes described in the 1970s may not reflect what would be expected at present. For example, neonatal mortality rates have fallen substantially, such that an infant delivered at 28 weeks would have a greater chance of survival were those studies repeated; this may affect the primary outcome of the meta-analysis.

As this review included data from only two studies with 740 participants overall, it is underpowered to detect a difference in the incidence of death of a baby or the frequency of a small-for-gestational-age infant as these have a background incidence of approximately 0.75% and 10% of pregnancies, respectively. Similarly, this review is underpowered to detect differences between serious and/or rare adverse events such as severe neonatal morbidity such as hypoxic-ischaemic encephalopathy. This limitation must be considered when developing adequately powered future clinical trials to evaluate biochemical tests of placental function and performing subsequent meta-analyses.

### Quality of the evidence

Two out of the three studies included in this review were quasi-randomised so had significant risk of bias from group allocation. In addition, there may be performance bias as in one study participants receiving standard care did not have venepuncture, so clinicians treating participants could identify which arm of the study they were in. Future studies should consider more robust randomisation methods and concealment of group allocation e.g. venepuncture could be performed on all participants with restricted measurement of the analyte or disclosure of results in the treatment group.

### Potential biases in the review process

There were no biases identified in the review process.

### Agreements and disagreements with other studies or reviews

Although this analysis identified and included one more trial, the review's findings are in agreement with the previous systematic review and meta-analysis conducted by Neilson (Neilson 2012). We are not aware of other studies that have systematically reviewed this topic.

## AUTHORS' CONCLUSIONS

### Implications for practice

Based on the available data, there are insufficient data to evaluate whether biochemical tests of placental function can reduce perinatal mortality or increase identification of small-for-gestational-age infants. We were only able to identify data from two studies (involving a total of 740 participants) that measured oestrogens and human placental lactogen (hPL). These studies were underpowered to detect differences in pregnancy outcome.

### Implications for research

Biochemical tests of placental function offer an opportunity to evaluate placental health in utero which is inextricably linked with fetal well-being (Heazell 2015).The studies identified in this review described prospective studies of two different analytes: oestrogens and hPL. There are many other placental products that could be employed as surrogates of placental function, including: placental growth factor (PlGF), human chorionic gonadotrophin (hCG), pregnancy-associated plasma protein A (PAPP-A), placental protein 13 (PP-13), pregnancy-specific glycoproteins and progesterone metabolites. None of these have been tested in prospective randomised studies. Such randomised controlled trials should test analytes identified as having the best predictive reliability for placental dysfunction leading to small-for-gestational-age infants and perinatal mortality. If further studies are conducted then meta-analyses could address whether there are differences in perinatal outcome alter depending on the analyte or type of biochemical test.

It is important to appreciate that any test of fetal or placental compromise alone is insufficient to alter pregnancy outcome; a positive test must be combined with an intervention to prevent an adverse outcome. This may take the form of increased antenatal surveillance, e.g. umbilical artery Doppler or delivery. Therefore, further diagnostic test-accuracy studies should be encouraged to determine the optimal measurements or combination of measurements to identify placental dysfunction in utero and then intervention studies conducted to determine whether these measurements combined with appropriate intervention (increased screening or delivery) lead to improved pregnancy outcome for mother and baby.

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As part of the pre-publication editorial process, this review has been commented on by three peers (an editor and two referees who are external to the editorial team) and the Group's Statistical Adviser.

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* *Indicates the major publication for the study*

# CHARACTERISTICS OF STUDIES

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

**Duenhoelter 1976**

| | |
|---|---|
| Methods | Parallel group quasi-randomised trial. |
| Participants | 622 women attending obstetric complications outpatient clinic or inpatients on high-risk obstetric unit. The results of oestrogen levels were reported in 315 women and not reported in 307 women |
| Interventions | Plasma oestrogen measured and results reported to individual physicians. Delivery advised if concentration of oestrogen was consistently low, < 20 ng/mL after 34 weeks or levels suddenly decreased. Comparison group had oestrogen measured but not reported |
| Outcomes | Stillbirths, neonatal deaths, spontaneous labour, primary induction of labour, primary caesarean section |
| Notes | Unable to assess overall caesarean section rate as mode of delivery not reported for women who went into spontaneous labour |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | High risk | Numbers assigned by administrative staff based on casenote number |
| Allocation concealment (selection bias) | High risk | Randomised based on casenote number, so allocation not concealed |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Women in both arms had oestrogen measured but results were not reported in the control group |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether outcome assessors were blinded to group allocation |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Outcome data reported for all participants. |
| Selective reporting (reporting bias) | Unclear risk | Cannot assess as authors did not state what outcomes they would analyse |
| Other bias | Unclear risk | No evidence of how many potential participants were screened. No evidence of differences in baseline characteristics between intervention and control groups. No evidence of different |

**Duenhoelter 1976** (*Continued*)

| | | |
|---|---|---|
| | | diagnostic activity between the 2 groups |

**Heazell 2013**

| | |
|---|---|
| Methods | Parallel-group randomised trial. |
| Participants | 120 women attending a tertiary centre with maternal perception of reduced fetal movements after 36 weeks' gestation; 60 women were randomised to each arm of the study. Women were excluded if there was a known congenital anomaly, multiple pregnancy, fetus required immediate delivery, maternal age < 17 or unable to give informed consent |
| Interventions | Measurement of serum human placental lactogen and ultrasound assessment of fetal biometry, umbilical artery Doppler and liquor volume compared to ultrasound biometry, umbilical artery Doppler and liquor volume alone if met unit protocol |
| Outcomes | Stillbirth, neonatal death, small-for-gestational age (< 10th centile on customised birthweight chart), umbilical artery pH ≤ 7,1, unexpected admission to the neonatal intensive care unit, maternal anxiety (STAI score) |
| Notes | Preliminary data from this study was also reported in Bernatavicius 2013. |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Sequence generation by computer algorithm,using varying block size |
| Allocation concealment (selection bias) | Low risk | Clinicians enrolling to the trial were unable to predict participant allocation, which was achieved using a secure web-based randomisation system using individual randomisation in a 1:1 ratio with random variable block size. Upcoming allocations were concealed from those enrolling participants to the trial |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | Personnel and participants were not blinded to group allocation |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Assessor was blind to group allocation. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Primary and secondary outcomes were reported for all participants |
| Selective reporting (reporting bias) | Low risk | All data specified in the trial registration and protocol were reported |

| | | |
|---|---|---|
| Other bias | Low risk | Number of participants approached to participate and number of participants consented presented. No report of the number of potential participants screened for eligibility. No evidence of differences in baseline characteristics between intervention and control groups. No evidence of different diagnostic activity between the 2 groups |

**Spellacy 1975**

| | |
|---|---|
| Methods | Quasi-randomised trial. |
| Participants | 2733 women attending a high-risk pregnancy clinic with conditions including: hypertension, diabetes mellitus, fetal growth restriction, rhesus isoimmunisation, previous stillbirth, postmaturity and collagen diseases. The result was revealed to clinicians for the 1362 women in the intervention group and not revealed for the 1371 women in the control group |
| Interventions | Measurement of human placental lactogen reported in intervention group, results were concealed in women in control group |
| Outcomes | Stillbirth, neonatal death, Apgar scores at 1 and 5 minutes of age |
| Notes | Although this study met the inclusion criteria, data could not be extracted as they were only reported for women who had a low (fetal danger zone) hPL result |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | High risk | Quasi-randomised trial with sequence based on casenote number (odd or even) |
| Allocation concealment (selection bias) | High risk | Not concealed as case number known. |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Both groups had similar case notes and both had venepuncture performed |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Blinding of outcome assessor not stated. |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Outcomes were only reported for participants who had low hPL levels (referred to as fetal danger zone) |
| Selective reporting (reporting bias) | High risk | No reporting of Apgar results, specified in the methods section |

**Spellacy 1975** (*Continued*)

| | | |
|---|---|---|
| Other bias | Unclear risk | No evidence of how many potential participants were screened. Unable to assess whether there was evidence of differences in baseline characteristics between intervention and control groups. No evidence of different diagnostic activity between the 2 groups |

hPL: human placental lactogen
STAI: state trait anxiety index

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

| Study | Reason for exclusion |
|---|---|
| Grudzinskas 1990 | Trial protocol only. Letter indicating that trial commenced but that it ceased to recruit after 160 women were recruited; the trial was abandoned prior to completion |
| Sharf 1984 | Although stated to be a randomised study in the abstract, the methods section describes a non-randomised study with patients assigned to a control group or intervention arm. Therefore, study excluded as not a randomised or quasi-randomised trial |

## DATA AND ANALYSES

**Comparison 1. Test of placental function versus standard care**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Death of a baby (stillbirth or neonatal death) | 2 | 740 | Risk Ratio (M-H, Fixed, 95% CI) | 0.88 [0.36, 2.13] |
| 2 Small-for-gestational age (below 10th centile on customised birthweight chart or as defined by trialists) | 1 | 118 | Risk Ratio (M-H, Fixed, 95% CI) | 0.44 [0.16, 1.19] |
| 3 Stillbirth | 2 | 740 | Risk Ratio (M-H, Fixed, 95% CI) | 0.56 [0.16, 1.88] |
| 4 Neonatal death | 2 | 740 | Risk Ratio (M-H, Fixed, 95% CI) | 1.62 [0.39, 6.74] |
| 5 Neonatal intensive care unit admission | 1 | 118 | Risk Ratio (M-H, Fixed, 95% CI) | 0.32 [0.03, 3.01] |
| 6 Preterm birth (before 37 weeks' gestation) | 1 | 118 | Risk Ratio (M-H, Fixed, 95% CI) | 2.90 [0.12, 69.81] |
| 7 Serious neonatal morbidity (e.g. necrotising enterocolitis, chronic lung disease, intraventricular haemorrhage, sepsis, seizures) | 1 | 118 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 8 Elective delivery (induction of labour or non-labour caesarean section) | 2 | 740 | Risk Ratio (M-H, Fixed, 95% CI) | 0.98 [0.84, 1.14] |
| 9 Caesarean section | 1 | 118 | Risk Ratio (M-H, Fixed, 95% CI) | 0.48 [0.15, 1.52] |
| 10 Change in state anxiety score | 1 | 118 | Mean Difference (IV, Fixed, 95% CI) | -2.40 [-4.78, -0.02] |

### Analysis 1.1. Comparison 1 Test of placental function versus standard care, Outcome 1 Death of a baby (stillbirth or neonatal death).

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 1 Death of a baby (stillbirth or neonatal death)

| Study or subgroup | Experimental n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Duenhoelter 1976 | 9/315 | 10/307 | | 100.0 % | 0.88 [ 0.36, 2.13 ] |
| Heazell 2013 | 0/60 | 0/58 | | | Not estimable |
| **Total (95% CI)** | **375** | **365** | | **100.0 %** | **0.88 [ 0.36, 2.13 ]** |
| Total events: 9 (Experimental), 10 (Control) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.29 (P = 0.77) | | | | | |
| Test for subgroup differences: Not applicable | | | | | |


### Analysis 1.2. Comparison 1 Test of placental function versus standard care, Outcome 2 Small-for-gestational age (below 10th centile on customised birthweight chart or as defined by trialists).

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 2 Small-for-gestational age (below 10th centile on customised birthweight chart or as defined by trialists)

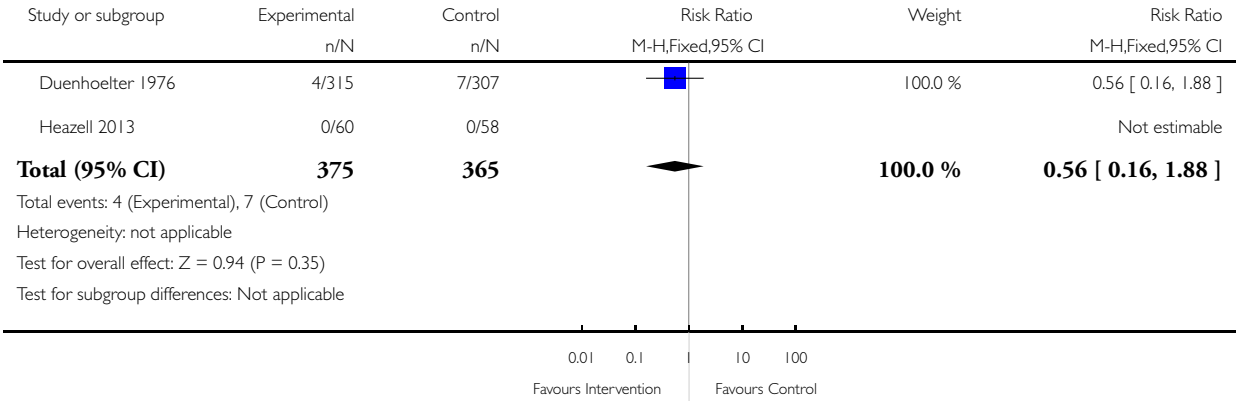
| Study or subgroup | Experimental n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Heazell 2013 | 5/60 | 11/58 | | 100.0 % | 0.44 [ 0.16, 1.19 ] |
| **Total (95% CI)** | **60** | **58** | | **100.0 %** | **0.44 [ 0.16, 1.19 ]** |
| Total events: 5 (Experimental), 11 (Control) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 1.62 (P = 0.10) | | | | | |
| Test for subgroup differences: Not applicable | | | | | |

### Analysis 1.3. Comparison 1 Test of placental function versus standard care, Outcome 3 Stillbirth.

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 3 Stillbirth

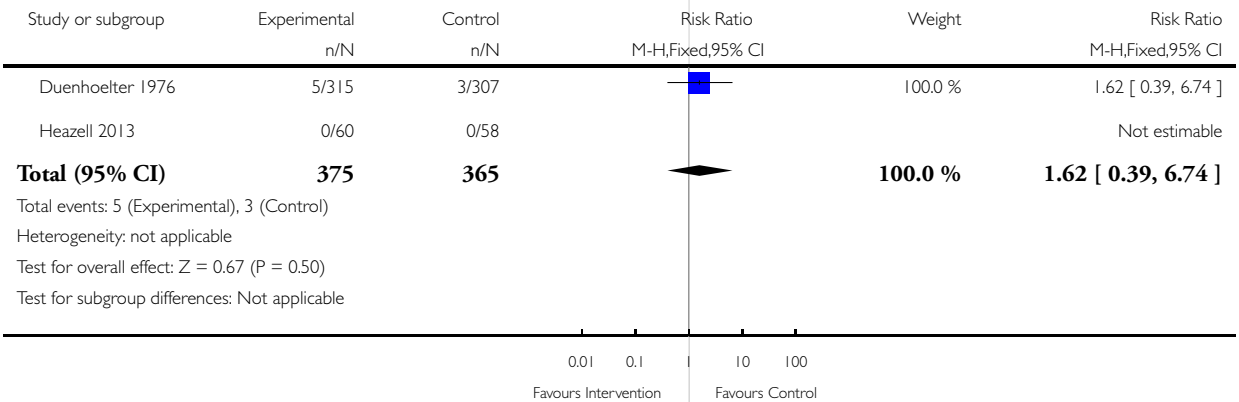



### Analysis 1.4. Comparison 1 Test of placental function versus standard care, Outcome 4 Neonatal death.

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 4 Neonatal death



### Analysis 1.5. Comparison 1 Test of placental function versus standard care, Outcome 5 Neonatal intensive care unit admission.

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 5 Neonatal intensive care unit admission

| Study or subgroup | Experimental n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Heazell 2013 | 1/60 | 3/58 | | 100.0 % | 0.32 [ 0.03, 3.01 ] |
| **Total (95% CI)** | **60** | **58** | | **100.0 %** | **0.32 [ 0.03, 3.01 ]** |

Total events: 1 (Experimental), 3 (Control)
Heterogeneity: not applicable
Test for overall effect: Z = 0.99 (P = 0.32)
Test for subgroup differences: Not applicable


### Analysis 1.6. Comparison 1 Test of placental function versus standard care, Outcome 6 Preterm birth (before 37 weeks' gestation).

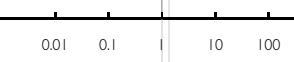
Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 6 Preterm birth (before 37 weeks' gestation)

| Study or subgroup | Experimental n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Heazell 2013 | 1/60 | 0/58 | | 100.0 % | 2.90 [ 0.12, 69.81 ] |
| **Total (95% CI)** | **60** | **58** | | **100.0 %** | **2.90 [ 0.12, 69.81 ]** |

Total events: 1 (Experimental), 0 (Control)
Heterogeneity: not applicable
Test for overall effect: Z = 0.66 (P = 0.51)
Test for subgroup differences: Not applicable



### Analysis 1.7. Comparison 1 Test of placental function versus standard care, Outcome 7 Serious neonatal morbidity (e.g. necrotising enterocolitis, chronic lung disease, intraventricular haemorrhage, sepsis, seizures).

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 7 Serious neonatal morbidity (e.g. necrotising enterocolitis, chronic lung disease, intraventricular haemorrhage, sepsis, seizures)

| Study or subgroup | Experimental n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Heazell 2013 | 0/60 | 0/58 | | | Not estimable |
| **Total (95% CI)** | **60** | **58** | | | **Not estimable** |

Total events: 0 (Experimental), 0 (Control)
Heterogeneity: not applicable
Test for overall effect: not applicable
Test for subgroup differences: Not applicable


### Analysis 1.8. Comparison 1 Test of placental function versus standard care, Outcome 8 Elective delivery (induction of labour or non-labour caesarean section).

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 8 Elective delivery (induction of labour or non-labour caesarean section)

| Study or subgroup | Experimental n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Duenhoelter 1976 | 141/315 | 142/307 | | 80.2 % | 0.97 [ 0.81, 1.15 ] |
| Heazell 2013 | 37/60 | 35/58 | | 19.8 % | 1.02 [ 0.77, 1.36 ] |
| **Total (95% CI)** | **375** | **365** | | **100.0 %** | **0.98 [ 0.84, 1.14 ]** |

Total events: 178 (Experimental), 177 (Control)
Heterogeneity: $Chi^2 = 0.10$, df = 1 (P = 0.75); $I^2 = 0.0\%$
Test for overall effect: Z = 0.29 (P = 0.77)
Test for subgroup differences: Not applicable

### Analysis 1.9. Comparison 1 Test of placental function versus standard care, Outcome 9 Caesarean section.

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 9 Caesarean section

| Study or subgroup | Experimental n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Heazell 2013 | 4/60 | 8/58 | | 100.0 % | 0.48 [ 0.15, 1.52 ] |
| **Total (95% CI)** | **60** | **58** | | **100.0 %** | **0.48 [ 0.15, 1.52 ]** |

Total events: 4 (Experimental), 8 (Control)
Heterogeneity: not applicable
Test for overall effect: Z = 1.24 (P = 0.21)
Test for subgroup differences: Not applicable


### Analysis 1.10. Comparison 1 Test of placental function versus standard care, Outcome 10 Change in state anxiety score.

Review: Use of biochemical tests of placental function for improving pregnancy outcome

Comparison: 1 Test of placental function versus standard care

Outcome: 10 Change in state anxiety score

| Study or subgroup | Experimental N | Mean(SD) | Control N | Mean(SD) | Mean Difference IV,Fixed,95% CI | Weight | Mean Difference IV,Fixed,95% CI |
|---|---|---|---|---|---|---|---|
| Heazell 2013 | 60 | 5.2 (6.7) | 58 | 7.6 (6.5) | | 100.0 % | -2.40 [ -4.78, -0.02 ] |
| **Total (95% CI)** | **60** | | **58** | | | **100.0 %** | **-2.40 [ -4.78, -0.02 ]** |

Heterogeneity: not applicable
Test for overall effect: Z = 1.98 (P = 0.048)
Test for subgroup differences: Not applicable

## CONTRIBUTIONS OF AUTHORS

Dr Alexander Heazell (AEPH) conceived the idea for the review. All authors contributed to the design of the review and writing the protocol. All authors read the studies to determine inclusion in the review. AH, MKW and JT extracted data from the studies. All authors contributed to the final manuscript. AEPH is the guarantor for the review.

## DECLARATIONS OF INTEREST

Jim Thornton and Melissa Whitworth: none known.

Lelia Duley has been awarded an NIHR applied research grant for a programme of work on care at very preterm birth. She is also a collaborator on Alexander Heazell's NIHR Clinician Scientist award which includes funding for a randomised trial (which will be conducted by the Nottingham Clinical Trials Unit) relevant to this review.

Alexander Heazell has received research grants from Alere (UK) and Action Medical Research to investigate placental factors in maternal serum in women with reduced fetal movements. Alexander Heazell holds a Clinician Scientist Award from NIHR and this award includes funding for a randomised trial (which will be conducted by the Nottingham Clinical Trials Unit) relevant to this review. Alexander Heazell was the trialist for one of the included studies (Heazell 2013), he was not directly responsible for decisions involving the inclusion, assessment of quality or data extraction for this study. These tasks were carried out by members of the review team not directly involved with this study.

## SOURCES OF SUPPORT


### Internal sources

- No sources of support supplied

### External sources

- National Institutes of Health Research, UK.

Salary support for Dr Alexander Heazell via Clinician Scientist Award 2013-13-009


## DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Change in state anxiety score has been added as a secondary maternal outcome - this was not prespecified in our published protocol (Heazell 2014). We have also used GRADE to assess the quality of the body of evidence and prepared a 'Summary of findings' table - this was not prespecified in our protocol.

## NOTES

Unpublished data regarding severe neonatal morbidity were obtained from Heazell 2013 for Analysis 1.7. These have been added to the manuscript record held with the Cochrane Pregnancy and Childbirth Group.