Anti-D administration in pregnancy for preventing Rhesus alloimmunisation (Review)

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[Intervention Review]

Anti-D administration in pregnancy for preventing Rhesus alloimmunisation

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Editorial group: Cochrane Pregnancy and Childbirth Group.
Publication status and date: New search for studies and content updated (no change to conclusions), published in Issue 2, 2013.
Review content assessed as up-to-date: 14 November 2012.

Citation: Crowther CA, Middleton P, McBain RD. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. *Cochrane Database of Systematic Reviews* 2013, Issue 2. Art. No.: CD000020. DOI: 10.1002/14651858.CD000020.pub2.

ABSTRACT

Background

During pregnancy, a Rhesus negative (Rh-negative) woman may develop antibodies when her fetus is Rhesus positive (Rh-positive). These antibodies may harm Rh-positive babies.

Objectives

To assess the effects of antenatal anti-D immunoglobulin on the incidence of Rhesus D alloimmunisation when given to Rh-negative women without anti-D antibodies.

Search methods

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (30 September 2012).

Selection criteria

Randomised trials in Rh-negative women without anti-D antibodies given anti-D after 28 weeks of pregnancy, compared with no treatment, placebo or a different regimen of anti-D.

Data collection and analysis

Two review authors independently assessed trial eligibility and risk of bias and extracted the data.

Main results

Two trials with moderate to high risk of bias, involving over 4500 women, compared anti-D prophylaxis with no anti-D during pregnancy. When women received anti-D at 28 and 34 weeks' gestation, risks of immunisation were not significantly different than for women not given antenatal anti-D: risk ratio (RR) of immunisation during pregnancy was 0.42 (95% confidence interval (CI) 0.15 to 1.17); after the birth of a Rh-positive infant the RR was 0.42 (95% CI 0.15 to 1.17); and within 12 months after birth of a Rh-positive infant the RR was 0.39 (95% CI 0.10 to 1.62).

However, women receiving anti-D during pregnancy were significantly less likely to register a positive Kleihauer test (which detects fetal cells in maternal blood) in pregnancy (RR 0.60, 95% CI 0.41 to 0.88) and at the birth of a Rh-positive infant (RR 0.60, 95% CI

0.46 to 0.79). No data were available for the risk of Rhesus D alloimmunisation in a subsequent pregnancy. No significant differences were seen for neonatal jaundice, and no adverse effects were reported in either trial.

Authors' conclusions

The risk of Rhesus D alloimmunisation during or immediately after a first pregnancy is about 1%. Administration of 100 µg (500 IU) anti-D to women in their first pregnancy can reduce this risk to about 0.2% without, to date, any adverse effects. Although unlikely to confer benefit in the current pregnancy, fewer women may have Rhesus D antibodies in any subsequent pregnancy, but the effects of this needs to be tested in studies of robust design.

PLAIN LANGUAGE SUMMARY

Anti-D administration in pregnancy for preventing Rhesus alloimmunisation

Anti-D given during pregnancy at 28 and 34 weeks of pregnancy reduces the incidence of antibody formation and probably also reduces Rhesus alloimmunisation of women.

Women whose blood group is Rh-negative sometimes form Rh-antibodies when carrying a Rh-positive baby, in response to the baby's different red blood cell make-up. This sensitisation is more likely to happen during birth, but occasionally occurs in late pregnancy. These antibodies can cause anaemia, and sometimes death, for a Rh-positive baby in a subsequent pregnancy. Giving the mother anti-D after the first birth is known to reduce this problem. This review assessed two trials and found that giving anti-D during pregnancy is likely to help as well, although more research is required to confirm these possible benefits and identify any possible harms.

BACKGROUND

Description of the condition

Rhesus incompatibility and haemolytic disease of the fetus/newborn

Haemolytic disease of the fetus and newborn can occur when the baby's red blood cells are destroyed. The most common cause is rhesus incompatibility, when antibodies from a Rh-negative mother target and destroy 'foreign' red blood cells from a Rh-positive fetus (Chilcott 2002). Haemolytic disease was a major cause of perinatal mortality, morbidity and long-term disability until the 1970s.

Pathogenesis

Rh-negative mothers carrying a Rh-positive fetus may produce anti-D antibodies (anti-D) following small feto-maternal haemorrhages at birth (Chown 1954; Chilcott 2002). The production of anti-D antibodies occurs in response to the presence of fetal red blood cells in the maternal circulation; this maternal immune response towards the fetal Rh antigen is known as 'sensitisation' or immunisation. It is believed to take between five and 15 weeks for such antibodies to appear in the maternal circulation following a sensitising event such as birth (Gunson 1976). Sensitisation is believed to have no adverse health effects for the mother, and the first baby is usually not harmed, as the pregnancy is generally complete by the time that sensitisation has occurred. These maternal antibodies (directed against antigens inherited from the father) may, however cause haemolytic disease in subsequent pregnancies with Rh-positive fetuses.

In addition to feto-maternal haemorrhage at birth, events during pregnancy may lead to the production of maternal anti-D antibodies, and thus sensitisation may also occur during the antenatal period. As there is a direct, proportional relationship between the volume of fetal Rh-positive red blood cells to which a Rh-negative mother is exposed and the incidence of immunisation (Jones 2004; Zipursky 1967), sensitising events (in addition to birth) may include termination of pregnancy, miscarriage, and some invasive investigative procedures (Bowman 1996). The majority of sensitisations are however, thought to be caused by occult or 'silent', transplacental haemorrhage (Chilcott 2002).

Health consequences for the fetus or newborn

In a Rh-negative mother, maternal anti-D antibodies may cross the placenta and lead to the immune-mediated destruction of fetal red blood cells. This more rapid destruction of the fetal red blood cells than normal, known as haemolytic disease of the fetus or newborn, can lead to anaemia and jaundice and in very severe cases, kernicterus (a form of brain damage caused by very high levels of bilirubin), or even death. A survey of 124 sensitised women showed that about 70%of their pregnancies were affected by some degree of haemolytic disease (Craig 1998). It has been estimated that approximately half of newborn infants with haemolytic disease are mildly affected, requiring no treatment. Of the remainder, half will become hydropic in utero, and half will be born apparently healthy but without treatment may die of kernicterus or be left severely disabled (Bowman 1965).

Description of the intervention

Anti-D administration for preventing Rhesus alloimmunisation

In the 1960s, Stern found that sensitisation to Rhesus positive blood could be prevented by administering anti-D (Stern 1961). Anti-D gammaglobulin is a sterile solution containing anti-D immunoglobulin G (IgG) antibodies manufactured from a pooled source of plasma of males and post-menopausal women. The donors must be Rh-negative and can be immunised to stimulate their immune system to produce anti-D or to increase their anti-D titre.

When anti-D gammaglobulin became available in the early 1970s, deaths from haemolytic disease dramatically reduced, with postpartum administration effectively protecting against Rhesus alloimmunisation when properly used (Gravenhorst 1989; Crowther 1997). Postpartum prophylaxis has been shown to be effective in reducing the incidence of alloimmunisation six months after administration and in a subsequent pregnancy in a Cochrane review of six randomised controlled trials (Crowther 1997). The benefits were seen when anti-D was given within 72 hours of birth, regardless of the ABO blood group status of the mother and baby. Higher doses were more effective than lower doses.

However, as sensitising events may also occur during pregnancy, postpartum anti-D will not prevent Rhesus alloimmunisation which occurs in the antenatal period. Although Zipursky and Israels (Zipursky 1967) first proposed that anti-D could reduce the incidence of Rhesus alloimmunisation during pregnancy in Rh-negative mothers nearly forty years ago, it may still occur, either because insufficient anti-D is given after known sensitising events during pregnancy (or after birth), it is not given soon enough (within 72 hours), or due to silent feto-maternal haemorrhage.

A transplacental haemorrhage from fetus to mother can be detected by the Kleihauer test (which detects the presence and estimates the amount of fetal cells in maternal blood). Injection of anti-D will destroy these fetal cells and thus prevent sensitisation of the mother. The Kleihauer test will indicate how much anti-D is likely to be required.

Antenatal prophylaxis - routine or universal anti-D administration in pregnancy

As occult or 'silent' sensitising events are thought to constitute the majority of sensitisations (Chilcott 2002), routine anti-D prophylaxis during pregnancy for Rh-negative mothers has been proposed and implemented in many countries (Engelfriet 2003). This is intended to supplement the practices of postpartum administration of anti-D, and of offering anti-D prophylaxis to Rh-negative women who experience a known potentially sensitising event (such as miscarriage or threatened miscarriage) during their pregnancy. A recent meta-analysis, including studies with historical controls in addition to those with concurrent controls suggested that there is strong evidence for the effectiveness of routine antenatal anti-D prophylaxis for preventing sensitisation, in support of offering routine prophylaxis to all non-sensitised pregnant Rh-negative women (Turner 2012).

About 10% of all pregnancies involve a Rh-negative mother with a Rh-positive fetus; and in a first pregnancy, about 60% of Rh-negative women will have a Rh-positive baby (Chilcott 2002). Clearly if the father is known to be Rh-negative, the baby will also be Rh-negative and therefore anti-D would not be needed. However, for antenatal prophylaxis, the Rh status of the fetus is usually not yet known, and so all non-sensitised Rh-negative mothers would generally need to be offered routine anti-D. This means that approximately 40% of women carrying Rh-negative babies would have anti-D unnecessarily.

Routine antenatal anti-D prophylaxis is usually not administered until 28 weeks' gestation, since transplacental haemorrhages large enough to cause sensitisation do not usually occur until the third trimester (Contreras 1998) and thus Rhesus antibodies usually develop after the 28th week of gestation (Davey 1979). The half-life of anti-D antibodies is estimated to be, on average, 17-22 days (Bishler 2003). The two main approaches, a single 1500 IU dose at 28 weeks and 500-625 IU doses at 28 and 32 weeks, each theoretically ensure that 12 weeks after administration there is enough anti-D to protect against 1 mL of red blood cells or 2 mL of whole blood (Mackenzie 2006). It is considered extremely unlikely that the volume of an antenatal transplacental haemorrhage would exceed 1 mL of fetal red blood (Mackenzie 2006).

Anti-D administration has been widely regarded as a safe prophylactic intervention. Numerous studies have suggested that while small amounts of passive anti-D may cross the placenta, the antenatal administration of anti-D IgG does not have adverse consequences for the fetus (Liumbruno 2010). However, since anti-D is derived from pooled donor plasma, there is a potential, or at least theoretical, risk of transmission of blood-borne diseases (National Blood 2003).

Some countries experience problems in obtaining sufficient supplies of anti-D, and so antenatal prophylaxis may be restricted to Rh-negative women expecting their first baby (partial rather than universal prophylaxis).

The effects of offering routine or universal antenatal anti-D prophylaxis to non-sensitised Rh-negative women is the focus of this systematic review.

How the intervention might work

The precise mechanism whereby administration of anti-D immunoglobulin prevents alloimmunisation remains unclear (Kumpel 2001). Passive anti-D causes rapid and non-inflammatory clearance of passive anti-D coated red blood cells which stops the inflammatory destruction of fetal red blood cells, evoking a natural immune response (Coopamah 2003). In addition, antibody-mediated immune suppression is believed to lead to the down-regulation of maternal immature dendritic cells or anti-D-specific B cells before the anti-D response develops (Kumpel 2002; Boruchov 2005).

It is considered unlikely that epitope masking (coating the fetal red blood cells with passive anti-D to allow them to evade detection by the maternal immune system), plays a significant role in the prevention of an anti-D response, as a significant number of Rhesus D antigen sites on fetal red blood cells in the maternal circulation are not bound by passive anti-D (Kumpel 2002).

Why it is important to do this review

The benefit of postpartum anti-D prophylaxis in reducing the incidence of alloimmunisation after administration and in a subsequent pregnancy has been established (Crowther 1997). As occult or 'silent' sensitising events are thought to constitute the majority of sensitisations, it is important to assess whether routine or universal antenatal anti-D prophylaxis (to non-sensitised Rh-negative women) is effective in preventing Rhesus alloimmunisation and the potential adverse health consequences for the fetus and infant in the current pregnancy and/or in subsequent pregnancies.

OBJECTIVES

To assess the effects of administering anti-D immunoglobulin at 28 weeks or more of pregnancy on the incidence of Rhesus D alloimmunisation during pregnancy (and/or in subsequent pregnancies) when given to Rhesus-negative women without anti-D antibodies.

METHODS

Criteria for considering studies for this review

Types of studies

All published, unpublished and ongoing randomised and quasi-randomised trials. We will include studies published as abstracts only. We will exclude cluster-randomised trials and cross-over trials.

Types of participants

Rhesus-negative women without anti-D antibodies at 28 weeks' gestation.

Types of interventions

Anti-D immunoglobulin at 28 weeks or more of gestation (regardless of timing, dose and route of administration), compared with no treatment or a placebo; and comparisons of different anti-D regimens.

Types of outcome measures

Primary outcomes

- Incidence of Rhesus D alloimmunisation (during pregnancy, postpartum, and in subsequent pregnancies)

Secondary outcomes

- Incidence of positive Kleihauer test (a test that detects fetal cells in the maternal blood)
- Neonatal morbidity (e.g. neonatal jaundice, anaemia and kernicterus) in current or subsequent pregnancies
- Adverse events attributed to anti-D treatment

Search methods for identification of studies

Electronic searches

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register by contacting the Trials Search Co-ordinator (30 September 2012).

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;
3. weekly searches of EMBASE;

4. handsearches of 30 journals and the proceedings of major conferences;

5. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Details of the search strategies for CENTRAL, MEDLINE and EMBASE, 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.

We did not apply any language restrictions.

Data collection and analysis

Selection of studies

Two review authors independently assessed for inclusion all the potential studies we identified as a result of the search strategy. We resolved any disagreement through discussion or, when required, we consulted a third review author.

Data extraction and management

We designed a form to extract data. For eligible studies, at least two review authors extracted the data using the agreed form. We resolved discrepancies through discussion or, when required, we consulted a third review author. We entered data into Review Manager software (RevMan 2011) 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

Two review authors independently assessed the risk of bias for each study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We resolved any disagreement by discussion or by involving a third assessor.

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

We described for each included study 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)

We described for each included study 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)

We described for each included study the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We considered that studies were at a low risk of bias if they were blinded, or if we judged that the lack of blinding would be unlikely to affect results. We assessed blinding separately for different outcomes or classes of outcomes.

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)

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

We assessed methods used to blind outcome assessment 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 for each included study, and for each outcome or class of outcomes, the completeness of data including attrition and exclusions from the analysis. 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 planned to re-include missing data in the analyses which we undertook.
We assessed methods 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 described for each included study how we investigated the possibility of selective outcome reporting bias and what we found. We assessed the methods as:

- low risk of bias (where it was clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review were reported);
- high risk of bias (where not all the study's pre-specified outcomes were 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)

We described for each included study any important concerns we had 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 explicit judgements about whether studies are at high risk of bias, according to the criteria given in the *Cochrane 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 was likely to impact on the findings. We planned to explore the impact of the level of bias through undertaking sensitivity analyses - *see* Sensitivity analysis.

Measures of treatment effect

Dichotomous data

For dichotomous data, we presented results 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. We planned to use the standardised mean difference to combine trials that measured the same outcome, but used different methods.

Unit of analysis issues

We considered cluster-randomised trials, and cross-over trials inappropriate for this review question.

Dealing with missing data

We noted levels of attrition for the included studies. We 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.
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 planned to assess statistical heterogeneity in each meta-analysis using the T^2, I^2 and Chi^2 statistics. We planned to regard heterogeneity as substantial if I^2 was greater than 30% and either 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

If there were 10 or more studies in the meta-analysis, we intended to investigate reporting biases (such as publication bias) using funnel plots. We planned to assess funnel plot asymmetry visually. If asymmetry was suggested by a visual assessment, we planned to perform exploratory analyses to investigate it.

Data synthesis

We carried out statistical analyses using the Review Manager software (RevMan 2011). 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. If there was clinical heterogeneity sufficient to expect that the underlying treatment effects differed between trials, or if substantial statistical heterogeneity was detected, we used random-effects meta-analysis to produce an overall summary if an average treatment effect across trials was considered clinically meaningful. The random-effects summary was treated as the average range of possible treatment effects and we have discussed the clinical implications of treatment effects differing between trials. If the average treatment effect had not been clinically meaningful, we would not have combined trials.
Where we used random-effects analyses, the results have been presented as the average treatment effect with 95% confidence intervals, with the estimates of T^2 and I^2.

Subgroup analysis and investigation of heterogeneity

If we had identified substantial heterogeneity, we planned to investigate it using subgroup analyses and sensitivity analyses. We planned to consider whether an overall summary was meaningful, and if it was, use random-effects analysis to produce it.
If possible, we planned to carry out the subgroup analyses considering aspects of the regimen for administration.

1. Timing of administration (e.g. single 1500 IU dose at 28 weeks versus 500-625 IU doses at 28 and 32 weeks).
2. Route of administration (intramuscular versus intravenous).
3. Dose administered.

We planned to use only the primary outcomes in subgroup analyses. We were able to perform subgroup analyses by dose administered only. We planned to assess differences between subgroups by interaction tests available within (RevMan 2011). In future updates, if more trials are included, we 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 planned to carry out sensitivity analysis on the primary outcomes to explore the effect of adequacy of allocation concealment (including quasi-randomisation) and other risk of bias components, by excluding those studies rated as 'high risk of bias' for these components. We would have restricted this to the primary outcomes only.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of ongoing studies.
See Characteristics of included studies; Characteristics of excluded studies and Characteristics of ongoing studies.

Results of the search

The updated search of the Cochrane Pregnancy and Childbirth Group's Trials Register found one ongoing trial (Manjunath 2008). We had previously included two trials (Huchet 1987; Lee 1995) and excluded one paper (Ismail 2002).

Included studies

Two trials of anti-D immunoglobulin met our inclusion criteria, one from France (Huchet 1987), and one from the UK (Lee 1995). Both trials compared routine antenatal anti-D prophylaxis with no routine anti-D prophylaxis; and neither study used a placebo.
Huchet 1987 recruited 1969 Rh-negative pregnant women without anti-D antibodies who attended antenatal clinics in the Paris region. Of the 1882 women with results available, 1450 were primigravid and 432 were multigravid. These women gave birth to 599 Rh-positive babies in the anti-D group and 590 Rh-positive babies in the control group.
Lee 1995 recruited 2541 Rh-negative primigravidae; 1273 to the control group and 1268 to the treatment group. No further data were available for 469 women (205 in the control group and 264 in the treatment group). A further 52 women allocated to the treatment group did not receive both doses of anti-D, leaving 2020 women with results available for analysis. Of these women, 1108 gave birth to Rh-positive infants and were tested at the time of birth (595 in the control group and 513 in the treatment group); and 72 women with Rh-positive babies were not tested for anti-D at the time of the birth.
Huchet 1987 administered 100 µg (500 international units (IU)) anti-D at 28 and 34 weeks' gestation (total dose of 200 µg). Lee 1995 administered 50 µg (250 IU) anti-D at 28 and 34 weeks' gestation (total dose of 100 µg).
For further details of the two included studies, see Characteristics of included studies.

Excluded studies

One paper was excluded (this was a plan for a trial, which is not proceeding at this stage, see Characteristics of excluded studies).

Risk of bias in included studies

Summaries for the risk of bias of the included studies are given in Figure 1 and Figure 2.

Figure 1. 'Risk of bias' graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

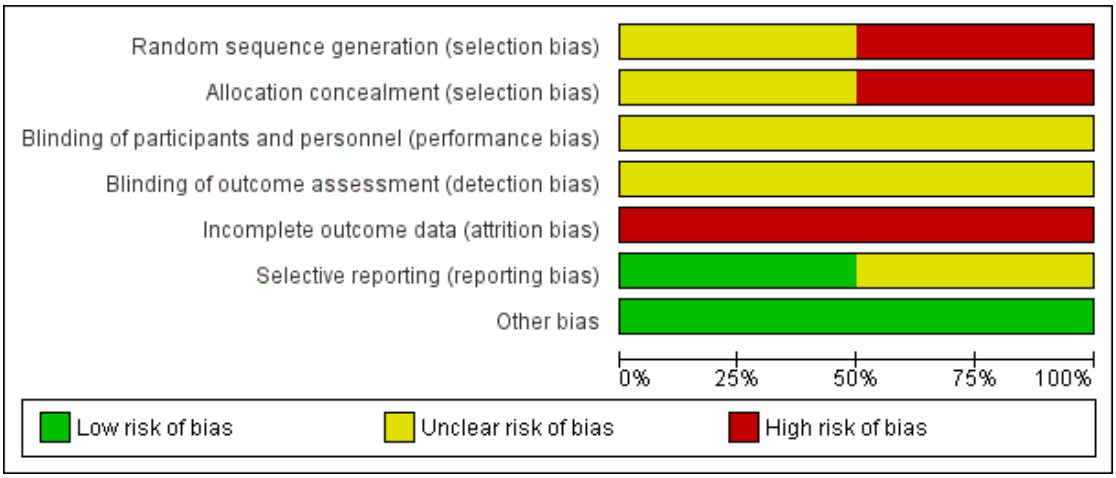
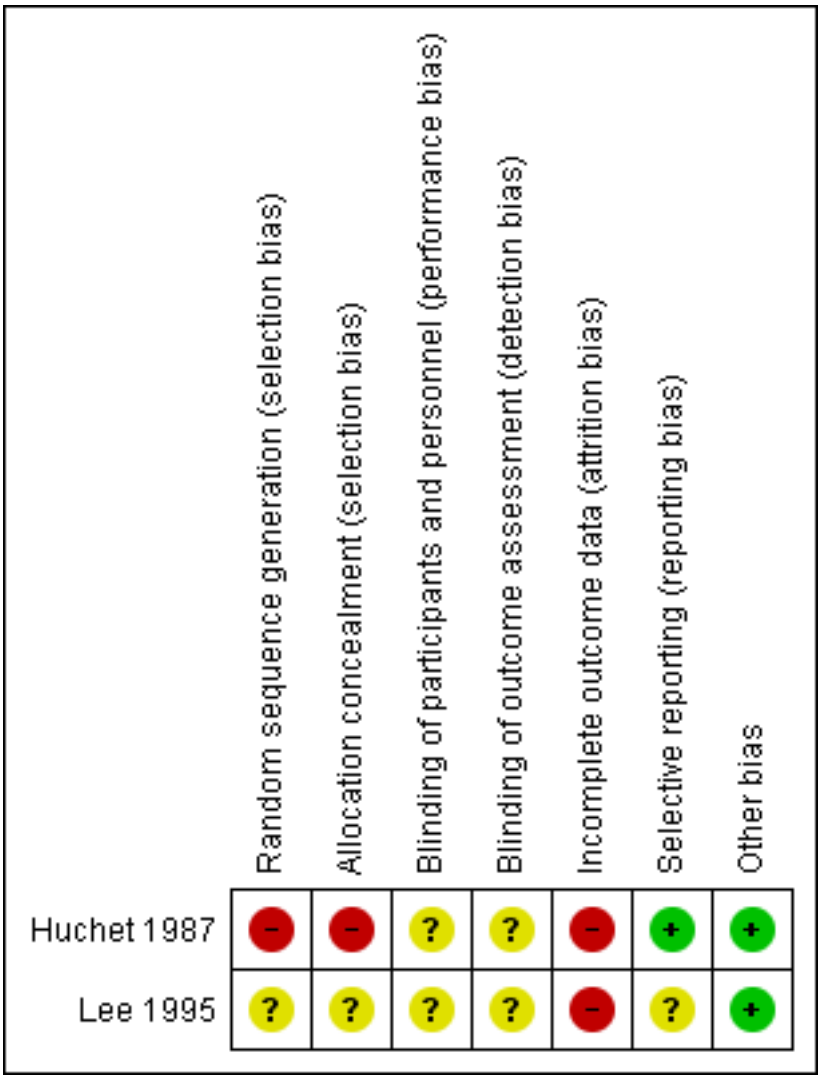


Figure 2. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



Allocation

In the Huchet 1987 trial, women were allocated to the two treatment groups by even or uneven year of birth, and thus the trial was judged to be at a high risk of selection bias due to an inadequate method use to generate a random sequence, making allocation unable to be concealed. In Lee 1995, no detail was provided regarding the random sequence generation, and whilst sealed envelopes were used to conceal allocation, no detail was provided regarding how the envelopes were numbered (i.e. if they were numbered consecutively), and if they were opaque, and thus we judged selection bias to be unclear for this trial.

Blinding

In both trials (Huchet 1987; Lee 1995), no placebo was used in the control group, and no further details were provided regarding blinding of the trial personnel or outcome assessors. It was considered somewhat unclear as to whether the objectively measured outcomes would have been affected by a lack of blinding. Therefore, both trials were judged to be at an unclear risk of bias due to performance and detection bias.

Incomplete outcome data

Outcome data at the time of birth were not available for 4.4% (87/1969) of women who entered the French trial (Huchet 1987) and for more than 23% (593/2541) of women in the UK trial (Lee 1995); with more losses to follow-up in the treatment group (335/1268; 26%) than the control group (258/1273; 20%). In the Huchet 1987 trial, additional women were lost to follow-up at two to 12 months, leaving only 940 of the 1189 women who gave birth to a Rh-positive baby available for analysis for these outcomes.

In Huchet 1987 an intention-to-treat analysis was possible for some outcomes, but this was not the case for Lee 1995. Both trials were judged to be at high risk of attrition bias due to incomplete outcome data.

Selective reporting

All outcome measures reported appear to have been pre-specified in the Huchet 1987 trial, and it was thus judged to be at a low risk of selective reporting. In Lee 1995 a number of outcomes that may have been expected were not reported, including for example, positive Kleihauer during pregnancy/delivery/postpartum and neonatal morbidity, and thus the trial was judged to be at an unclear risk of selective reporting. Neither trial reported adverse effects related to treatment.

Other potential sources of bias

No other obvious source of bias for the included studies was apparent.

Overall risk of bias was judged to be high for Huchet 1987 and unclear for Lee 1995.

Effects of interventions

Primary outcome

Rhesus D (RhD) alloimmunisation during pregnancy; after birth; in a subsequent pregnancy

When women received anti-D at 28 and 34 weeks' gestation, the data pooled over both trials (Huchet 1987; Lee 1995) did not show significant differences between anti-D and no anti-D but suggested a trend to a reduced incidence of RhD alloimmunisation, during pregnancy (risk ratio (RR) 0.42, 95% confidence Interval (CI) 0.15 to 1.17; 3902 women) (Analysis 1.1), after the birth of a Rh-positive infant (RR 0.42, 95% CI 0.15 to 1.17; 2297 women) (Analysis 1.2), and within 12 months after birth of a Rh-positive infant (RR 0.39, 95% CI 0.10 to 1.62; 2048 women) (Analysis 1.3). The moderate heterogeneity (I^2 = 39%) in the latter result may partly reflect the different doses used in the two randomised controlled trials, although the interaction test was not statistically significant.

These results were of borderline significance when calculated as a risk difference (RD) across the two randomised controlled trials (RD -0.01, 95% CI -0.02 to 0.00) for the 100 microgram dose for immunisation after the birth of a Rh-positive baby, or an absolute reduction from 1% to 0.2%.

In Huchet 1987, the use of 100 µg (500 international units (IU)) anti-D immunoglobulin at 28 and 32 weeks in the third trimester in non-sensitised pregnant women reduced the incidence of RhD alloimmunisation during or immediately after pregnancy from 1.02% (6/590) to 0.17% (1/599). One additional woman in the control group developed immunity in the two- to 12-month period after giving birth to a Rh-positive baby.

In the French trial (Huchet 1987), where the larger dose of anti-D (100 µg or 500 IU) was used, there was a non significant reduction in the incidence of RhD alloimmunisation at two to 12 months after birth in women who had received prophylactic anti-D at 28 and 34 weeks (RR 0.14, 95% CI 0.02 to 1.15; 940 women). In this trial, for primigravidae only who had received anti-D antenatally, there was also a non-significant reduction in the incidence of RhD alloimmunisation at two to 12 months following birth (RR 0.11, 95% CI 0.01 to 2.04; 722 women) (Analysis 1.4).

No other trials reported RhD alloimmunisation in a subsequent pregnancy.

Secondary outcomes

Positive Kleihauer test

In Huchet 1987, a positive Kleihauer result was found less commonly during the pregnancy at 32 to 35 weeks (RR 0.60, 95% CI 0.41 to 0.88; 1884 women) (Analysis 1.5); and after the birth of a Rh-positive infant (RR 0.60, 95% CI 0.46 to 0.79) (Analysis 1.6) in 1189 women treated at 28 and 34 weeks' gestation with anti-D. After the birth of a Rh-positive infant, no difference was seen in the number of women with a Kleihauer result greater than 1 in 10,000 in these 1189 women (RR 0.95, 95% CI 0.59 to 1.54) (Analysis 1.7).

Neonatal morbidity in current pregnancy; or in subsequent pregnancies

Huchet 1987 reported neonatal jaundice. There was only one case in the group whose mothers had received anti-D, and three cases in the control group (RR 0.26, 95% CI 0.03 to 2.30) (Analysis 1.8). No further outcomes relating to neonatal morbidity were reported in either trial.

Adverse events in treatment

Neither study reported any data relating to adverse effects of treatment.

Planned subgroup and sensitivity analyses (in addition to considering dose administered) were not able to be conducted due to paucity of data.

DISCUSSION

Summary of main results

While a policy of routine antenatal prophylaxis with anti-D is unlikely to confer benefit or improve outcome in the current pregnancy, fewer women will have Rhesus D antibodies in a subsequent pregnancy. As Chilcott 2002 points out, the clinical benefit sought is the avoidance of haemolytic disease in subsequent babies; if the mother "has a RhD-positive infant *and* she would have been sensitised, *and* she goes on to have a further infant who is also Rh-D positive".

The quantity of available evidence to answer such an important question of policy was disappointingly low and there was a moderate to high risk of bias in the included studies. We included only two studies in this review, with a total of over 4500 women; one trial was quasi-randomised. However, the reduction in the risk of alloimmunisation seen in this review in the Huchet 1987 trial for Rh-negative women with a Rh-positive baby from about 1% to 0.2% with anti-D is consistent with the findings of two non-randomised community studies reported in Chilcott 2002 where the sensitisation rate was reduced from 0.95% to 0.35%. From these figures, Chilcott has calculated that 278 women would need to be treated antenatally with anti-D to avoid one case of sensitisation (based on all Rh-negative women; all will require treatment, since the 60% of women with Rh-positive babies would not yet be identified). Based on the findings of this review, the number needed to treat would be slightly lower, at 213 women.

Use of a smaller dose of anti-D (50 µg or 250 international units) in Lee 1995 at similar gestational ages failed to show any benefit. Women in the intervention and the control groups in both trials also received anti-D after the birth of a Rh-positive baby.

Anti-D does not appear to be harmful to the fetus, although there is a theoretical risk of passive anti-D in the mother causing fetal anaemia (Chilcott 2002). In 1994, batches of anti-D used in Ireland in 1977 and 1978 were found to be contaminated with hepatitis C virus, but additional safety features were introduced and no further instances of transmission of infectious disease have been reported (National Blood 2003). Neonatal jaundice was the only outcome relating to neonatal morbidity reported in the Huchet 1987 trial. No maternal adverse effects related to treatment were reported in either trial.

The costs of prophylaxis need to be considered against the cost of antenatal monitoring and treatment of any affected infant whose mother develops antibodies. The National Institute for Health and Clinical Excellence (NICE) in the UK has calculated that universal routine antenatal anti-D prophylaxis to prevent sensitisation, fetal loss and fetal morbidity is cost-effective (NHS 2011).

Although the evidence for postpartum prophylaxis is stronger (perhaps because more studies of higher quality have been completed), antenatal prophylaxis is also likely to decrease the number of sensitisations, without adverse effects and should be considered to be complementary to postpartum prophylaxis. The decision to implement a policy of antenatal prophylaxis may be influenced by the availability of anti-D.

In some countries, supplies of anti-D gammaglobulin are limited and, on occasions, temporarily exhausted. Before adoption of a programme of anti-D prophylaxis in pregnancy, consideration would need to be given as to how to maintain an adequate supply of anti-D gammaglobulin for women in more urgent need. In many countries, including the UK and Australia, guidelines now advise routine universal antenatal anti-D prophylaxis (RCOG 2011; RANZCOG 2004).

Overall completeness and applicability of evidence

This review is limited with the inclusion of only two trials (Huchet 1987; Lee 1995), that did not report on immunisation in subsequent pregnancies, important secondary review outcomes including neonatal morbidity (Huchet 1987 reported only neonatal jaundice), or maternal adverse effects related to the anti-D treatment. We were unable to perform subgroup analyses based on timing, number of treatments required, and on route of administration, due to the paucity of data.

Quality of the evidence

The two trials included in the review (with over 4500 women) were judged to be at a moderate-to-high risk of bias overall. The Huchet 1987 trial was quasi-randomised, and thus at high risk of selection bias; the Lee 1995 trial did not clearly detail its selection methods. Neither trial used a placebo.

Potential biases in the review process

The evidence for this review has been derived from trials identified through a detailed search process. It is possible (but unlikely) that additional trials assessing routine anti-D prophylaxis in pregnancy have been published but not identified. It is also possible that other studies have been conducted but not published. Should such studies be identified, we will include them in future updates of

this review. Data from Huchet 1987 was obtained from a partial translation of the paper.

AUTHORS' CONCLUSIONS

Implications for practice

In Rh-negative women carrying a Rh-positive baby, the risk of Rhesus D (RhD) alloimmunisation during or immediately after a first pregnancy is about 1%. Administration of 100 µg (500 international units) anti-D at 28 weeks' and 34 weeks' gestation to women in their first pregnancy can reduce this risk to about 0.2% without, to date, any observed adverse effects. Although such a policy generally will not confer benefit or improve outcome in the present pregnancy, fewer women are likely to have RhD antibodies in any subsequent pregnancy.

Adoption of such a policy will need to consider the costs of prophylaxis against the costs of care for women who become sensitised and their affected infants, and local adequacy of supply of anti-D gammaglobulin.

Another Cochrane review has shown that postpartum administration of anti-D gammaglobulin is effective as prophylaxis against RhD alloimmunisation (*see* review on anti-D prophylaxis postpartum, Crowther 1997).

Implications for research

Further trials are warranted to determine the optimal timing, number of treatments, and effective dosage of anti-D administration in pregnancy. The cost-effectiveness of such a policy requires further evaluation. However, one of the most important areas to investigate is the effect of antenatal anti-D on subsequent pregnancies. The move towards more universal antenatal anti-D policies may provide an environment for more robust research to be carried out.

ACKNOWLEDGEMENTS

We thank Emily Bain for her assistance with this most recent update of the review.

For the previous versions of this review, we thank Professor Marc Keirse for his contributions, and Gill Gyte for her very helpful comments and suggestions. We also thank Lynn Hampson and Denise Atherton for their help with the previous updates.

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), one or more members of the Pregnancy and Childbirth Group's international panel of consumers and the Group's Statistical Adviser.

REFERENCES

References to studies included in this review

Huchet 1987 *{published data only}*
Huchet J, Dallemagne S, Huchet C, Brossard Y, Larsen M, Parnet-Mathieu F. The antepartum use of anti-D immunoglobulin in rhesus negative women. Parallel evaluation of fetal blood cells passing through the placenta. The results of a multi-centre study carried out in the region of Paris. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 1987;**16**:101–11.

Lee 1995 *{published data only}*
Lee D, Rawlinson VI. Multicentre trial of antepartum low-dose anti-D immunoglobulin. *Transfusion Medicine* 1995; **5**:15–9.

References to studies excluded from this review

Ismail 2002 *{published and unpublished data}*
Ismail K, Kilby M, Alfirevic Z, Khan K, Whittle M. Prospective randomised trial of alloimmunisation management (PRAM). *Journal of Obstetrics and Gynaecology* 2002;**22**(2 Suppl):S33.

References to ongoing studies

Manjunath 2008 *{published data only}*
Manjunath AP. A clinical trial to study the effect of injection anti D administered during pregnancy for Rh negative mothers. Clinical Trials Registry - India (CTRI) (http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=172) 2008 (accessed 14 March 2012).

Additional references

Bishler 2003
Bishler J, Schondorfer G, Pabst G, Andresen I. Pharmacokinetics of anti-D IgG in pregnant Rh-D negative women. *British Journal of Obstetrics and Gynaecology* 2003; **110**:39–45.

Boruchov 2005
Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, Young JW. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *Journal of Clinical Investigation* 2005;**115**:2914–23.

Bowman 1965
Bowman JM, Pollock JM. Amniotic fluid spectrophotometry and early delivery in the management of erythroblastosis fetalis. *Pediatrics* 1965;**35**:815.

Bowman 1996
Bowman JM. Hemolytic disease of the newborn. *Vox Sanguinis* 1996;**70**:62–7.

Chilcott 2002
Chilcott J, Lloyd Jones M, Wight J, Forman K, Wray J, Beverley C. *A review of the clinical effectiveness and cost effectiveness of routine anti-D prophylaxis for pregnant women who are Rhesus (RhD) negative*. London: National Institute of Clinical Excellence, 2002.

Chown 1954
Chown B. Anaemia from bleeding of the fetus into the mother's circulation. *Lancet* 1954;**263**(6824):1213.

Contreras 1998
Contreras M. The prevention of Rh haemolytic disease of the fetus and newborn - general background. *British Journal of Obstetrics and Gynaecology* 1998;**105**:7–10.

Coopamah 2003
Coopamah MD, Freeman J, Semple JW. Anti-D initially stimulates an Fc-dependant leukocyte oxidative burst and subsequently suppresses erythrophagocytosis via interleukin-1 receptor antagonist. *Blood* 2003;**102**:2862–7.

Craig 1998
Craig JS, McClure BG, Tubman TRJ. Services should be centralised for pregnancies affected by RhD haemolytic disease. *BMJ* 1998;**316**:1611.

Crowther 1997
Crowther C, Middleton P. Anti-D administration after childbirth for preventing Rhesus alloimmunisation. *Cochrane Database of Systematic Reviews* 1997, Issue 2. [DOI: 10.1002/14651858.CD000021]

Davey 1979
Davey MG, Zipursky A. Proceedings of the McMaster Rh Conference. *Vox Sanguinis* 1979;**36**:50–64.

Engelfriet 2003
Engelfriet CP, Reesink HW, Judd WJ, Ulander VM, Kuosmanen M, Koskinen S, et al.Current status of immunoprophylaxis with anti-D immunoglobin. *Vox Sanguinis* 2003;**85**(4):328–37.

Gravenhorst 1989
Gravenhorst JB. Rhesus isoimmunisation. In: Chalmers I, Enkin MW, Keirse MJNC editor(s). *Effective Care in Pregnancy and Childbirth*. Oxford: Oxford University Press, 1989:565–77.

Gunson 1976
Gunson HH, Stratton F, Philips PK. The primary Rho(D) immune response in male volunteers. *British Journal of Haematology* 1976;**32**:317–29.

Higgins 2011
Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Jones 2004
Jones ML, Wray J, Wright J, Chilcott J, Forman K, Tappenden P, et al.A review of the clinical effectiveness of routine antenatal anti-D prophylaxis for rhesus-negative women who are pregnant. *British Journal of Obstetrics and Gynaecology* 2004;**111**:892–902.

Kumpel 2001
Kumpel BM, Elson CJ. Mechanism of anti-D mediated immune suppression - a paradox awaiting resolution?. *Trends in Immunology* 2001;**22**:26–31.

Kumpel 2002
Kumpel BM. On the mechanism of tolerance to the Rh D antigen mediated by passive anti-D (Rh D prophylaxis). *Immunology Letters* 2002;**82**:67–73.

Liumbruno 2010
Liumbruno GM, D'Alessandro A, Rea F, Piccinini V, Catalano L, Calizzani G, et al.The role of antenatal immunoprophylaxis in the prevention of maternal-foetal anti-Rh(D) alloimmunisation. *Blood Transfusion* 2010;**8**(1): 8–16.

Mackenzie 2006
Mackenzie IZ, Roseman F, Findlay J, Thompson K, Jackson E, Scott J, et al.The kinetics of routine antenatal prophylactic intramuscular injections of polyclonal anti-D immunoglobin. *British Journal of Obstetrics and Gynaecology* 2006;**113**:97–101.

National Blood 2003
National Blood Authority. *Guidelines on the prophylactic use of Rh D immunoglobulin (anti-D) in obstetrics*. Canberra, ACT: NHMRC, 2003.

NHS 2011
NHS National Institute for Health and Clinical Excellece. Routine antenatal anti-D prophylaxis for women who are rhesus D negative: Review of NICE technology appraisal guidance 41. http://www.nice.org.uk/ (accessed 14 November 2012).

RANZCOG 2004
Royal Australian and New Zealand College of Obstetricians and Gynaecologists. Guidelines for the use of RhD immunoglobulin (Anti-D) in obstetrics in Australia. www.ranzcog.edu.au/ (accessed 2 November 2012).

RCOG 2011
Royal College of Obstetricians and Gynaecologists. The use of anti-D immunoglobulin for rhesus D prophylaxis. Green-top guideline. http://www.rcog.org.uk/ (accessed 14 November 2012).

RevMan 2011
The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011.

Stern 1961
Stern K, Goodman HS, Berger M. Experimental isoimmunisation to hemoantigens in man. *Journal of Immunology* 1961;**87**:189.

Turner 2012
Turner RM, Lloyd-Jones M, Anumba DO, Smith GC, Spiegelhalter DJ, Squires H, et al.Routine antenatal anti-d prophylaxis in women who are rh(d) negative: meta-

analyses adjusted for differences in study design and quality. *PLoS ONE* 2012;**7**(2):e30711.

Zipursky 1967

Zipursky A, Israels LG. The pathogenesis and prevention of Rh immunization. *Canadian Medical Association Journal* 1967;**97**:1245–57.

References to other published versions of this review

Crowther 1999

Crowther CA, Middleton P. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. *Cochrane Database of Systematic Reviews* 1999, Issue 2. [DOI: 10.1002/14651858.CD000020]

* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

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

Huchet 1987

Methods	Quasi-randomised trial.
Participants	1969 women were randomised from January 1983 to June 1984. **Setting:** women were recruited from 23 maternity units in the Paris region, France **Inclusion criteria:** women who were primipara and were Rh-negative. **Exclusion criteria:** none detailed.
Interventions	**Treatment group (Anti-D) (n = 927)** Women received two anti-D immunoglobulin injections (100 micrograms by intramuscular injection (500 IU)) at 28 and 34 weeks of pregnancy, after blood samples had been taken **Control group (no Anti-D) (n = 955)** No placebo was given. In both groups, women who gave birth to a Rhesus positive baby were administered postpartum (intravenously in almost all cases) anti-D immunoglobulin (100 micrograms), with possible re-treatment following review of fetal red blood cell test results 1450 women were primigravid and 432 were multigravid.
Outcomes	Incidence of immunisation during pregnancy, immunisation at 2-12 months following pregnancy, positive Kleihauer during pregnancy, at delivery, or postpartum. Cost-effectiveness data also provided
Notes	From the translation received for this manuscript, 1969 women began the study, with 1882 monitored until they went into labour The blood groups ABO and Rhesus D were determined using standard techniques

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Not detailed - women were allocated to groups on the basis of their birth year (even/odd)
Allocation concealment (selection bias)	High risk	As above.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Blinding was not detailed, however considered unlikely in view of the intervention. The lack of blinding, however, may be considered unlikely to affect the objectively measured outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	As above.

Huchet 1987 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	High risk	From the translation received - 1969 women began the study, of those 1882 were monitored until they were in labour (the 87 women not followed up to birth were not accounted for) In the control group, 2/957 women were excluded due to fetal-maternal haemorrhage, leaving 955 who were monitored until labour. Of these women, 590/955 gave birth to a Rh-positive baby, however two died at birth; 468 women were followed up postpartum (no reasons given for the 122 women not followed up postpartum). In the treatment group 599/927 gave birth to a Rh-positive baby; 472 women were followed up postpartum (no reasons given for the 127 women not followed up postpartum)
Selective reporting (reporting bias)	Low risk	No clear evidence of selective reporting - outcome measures reported appear to have been pre-specified
Other bias	Low risk	No obvious risk of other bias.

Lee 1995

Methods	Randomised controlled trial.
Participants	2541 women were randomised. **Setting:** obstetric units throughout the UK. **Inclusion criteria:** Rh-negative primigravidae before 28 weeks' gestation. **Exclusion criteria:** any woman with anti-D other than passive found at a 28-week blood sample was excluded from the trial. Women who had already received anti-D to cover a potentially sensitising event were not excluded - where such an event took place after 28 weeks, the patient received anti-D in the usual way
Interventions	**Treatment group (Anti-D) (n = 1268)** Women in the treatment group received 50 micrograms (250 IU) anti-D intramuscularly at 28 and 34 weeks' gestation (n = 952) **Control group (no Anti-D) (n = 1068)** Women received no placebo. Women in both groups "were considered for anti-D Ig in the normal way at delivery"
Outcomes	Presence of anti-D at birth and 6 months postpartum (repeated if equivocal); also reported "potentially sensitizing events."
Notes	Sample size needed to detect five fold reduction in sensitisation: 5200 women

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Generation of random sequence was not detailed.

Lee 1995 (*Continued*)

Allocation concealment (selection bias)	Unclear risk	Quote - "sealed envelopes" were used; no further detail provided regarding how the envelopes were numbered, or whether they were opaque
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Blinding was not detailed, however considered unlikely in view of the intervention. The lack of blinding, however, may be considered unlikely to affect the objectively measured outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	As above.
Incomplete outcome data (attrition bias) All outcomes	High risk	1273 women were controls and 1268 were in the treatment group; no data was provided for 205 controls and 264 women from the treatment group (no details provided). In the control group 649 women gave birth to Rh-positive infants (398 infants were Rh-negative; unknown for 21 infants). One additional woman was excluded from the control group after she was found to have immune anti-D at randomisation with a history of threatened abortion. Therefore, only 648 women were included in the analysis. In the treatment group 532 women and infants were included in the analysis (393 infants were Rh-negative, and 52 women did not receive both doses of anti-D and these women were excluded from further analyses - unknown whether infants were Rh-positive/negative). Not an intention-to-treat analysis
Selective reporting (reporting bias)	Unclear risk	Trial reported only presence of anti-D at birth and 6 months postpartum; a number of outcomes that may have been expected, such as positive Kleihauer during pregnancy/delivery/postpartum, or neonatal morbidity were not reported
Other bias	Low risk	No other obvious sources of bias identified.

IU: international units

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

Study	Reason for exclusion
Ismail 2002	This is only the plan for a trial. Trial not proceeding at this stage (Z Alfirevic, personal communication March 2004)

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

Manjunath 2008

Trial name or title	A clinical trial to study the effect of injection of anti-D administered during pregnancy for Rh-negative mothers
Methods	Randomised controlled trial.
Participants	100 women. **Setting:** Dr TMA Pai Rotary Hospital, Karkala, India. **Inclusion criteria:** all Rh-negative and indirect agglutinin test negative primigravida and un-sensitised multigravida who are willing to participate in the study **Exclusion criteria:** all Rh-negative mothers with Rh-negative husbands. Indirect agglutinination test positive
Interventions	**Treatment group (n = ?)** antenatal administration of 300 micrograms (1500 IU) of Rh-D immunoglobulin **Control group (n = ?)** no intervention.
Outcomes	**Primary outcomes:** incidence of immunisation during pregnancy at term, at delivery and at 6 months **Secondary outcomes:** incidence of neonatal hyperbilirubinaemia, need for exchange transfusion, and need for phototherapy
Starting date	1/12/2008 (on the trial registry however, the trial is listed as "not yet recruiting")
Contact information	**Scientific queries:** Dr A P Manjunath Associate Professor Department of Obstetrics and Gynaecology 576104, India Phone: 09845913140 Fax: 080257061 Email: manjunanth.ap@manipal.edu
Notes	

IU: international units

DATA AND ANALYSES

Comparison 1. Anti-D administration in pregnancy

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Immunisation in pregnancy	2	3902	Risk Ratio (M-H, Fixed, 95% CI)	0.42 [0.15, 1.17]
1.1 100 micrograms at 28 and 34 weeks	1	1882	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.02, 1.42]
1.2 50 micrograms at 28 and 34 weeks	1	2020	Risk Ratio (M-H, Fixed, 95% CI)	0.64 [0.19, 2.18]
2 Immunisation after birth of a Rhesus-positive infant	2	2297	Risk Ratio (M-H, Fixed, 95% CI)	0.42 [0.15, 1.17]
2.1 100 micrograms at 28 and 34 weeks	1	1189	Risk Ratio (M-H, Fixed, 95% CI)	0.16 [0.02, 1.36]
2.2 50 micrograms at 28 and 34 weeks	1	1108	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.20, 2.25]
3 Immunisation at 2-12 months	2	2048	Risk Ratio (M-H, Random, 95% CI)	0.39 [0.10, 1.62]
3.1 100 micrograms at 28 and 34 weeks	1	940	Risk Ratio (M-H, Random, 95% CI)	0.14 [0.02, 1.15]
3.2 50 micrograms at 28 and 34 weeks	1	1108	Risk Ratio (M-H, Random, 95% CI)	0.64 [0.22, 1.91]
4 Immunisation at 2-12 months - primigravidae alone	1	722	Risk Ratio (M-H, Fixed, 95% CI)	0.11 [0.01, 2.04]
5 Positive Kleihauer at 32-35 weeks	1	1884	Risk Ratio (M-H, Fixed, 95% CI)	0.60 [0.41, 0.88]
6 Positive Kleihauer at birth of a Rhesus-positive infant	1	1189	Risk Ratio (M-H, Fixed, 95% CI)	0.60 [0.46, 0.79]
7 Kleihauer > 1/10,000 - Rhesus-positive infant	1	1189	Risk Ratio (M-H, Fixed, 95% CI)	0.95 [0.59, 1.54]
8 Neonatal jaundice	1	1882	Risk Ratio (M-H, Fixed, 95% CI)	0.26 [0.03, 2.30]

WHAT'S NEW

Last assessed as up-to-date: 14 November 2012.

Date	Event	Description
14 November 2012	New citation required but conclusions have not changed	One trial added to ongoing studies (Manjunath 2008) .
30 September 2012	New search has been performed	Format of review updated, including background format, methods, and results and discussion format. Characteristics of studies and 'Risk of bias' tables updated. Search updated and one report identified

HISTORY

Protocol first published: Issue 2, 1996

Review first published: Issue 2, 1996

Date	Event	Description
10 November 2008	Amended	Contact details updated.
6 March 2008	Amended	Converted to new review format.
26 June 2007	New search has been performed	Search updated. No new trials identified.
30 April 2004	New search has been performed	No new studies found in current update. One paper placed in 'excluded studies' (plan for a trial unlikely to proceed). Odds ratio changed to relative risk. Text expanded (e.g. 'Background')
31 August 2000	New search has been performed	New search for trials conducted but none found.
21 January 1999	New search has been performed	Search updated.

CONTRIBUTIONS OF AUTHORS

Caroline A Crowther and Marc JNC Keirse both contributed to the development of the protocol, identification and selection of trials for inclusion and the preparation of the text of the first publication of the review.

Rosie McBain, Caroline A Crowther and Philippa Middleton updated the current version of the review.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- ARCH: Australian Research Centre for Health of Women and Babies, Robinson Institute, Discipline of Obstetrics and Gynaecology, The University of Adelaide, Australia.

External sources

- Australian Department of Health and Ageing, Australia.
- National Health and Medical Research Council, Australia.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We have updated the methods including assessment of risk of bias. We have separated the outcomes into primary and secondary outcomes for this review update, and have added adverse effects attributed to treatment as a secondary outcome. We have clarified that cluster trials, and cross-over trials will be excluded, that comparisons will be no treatment or placebo or a different anti-D regimen.

INDEX TERMS

Medical Subject Headings (MeSH)

Immunologic Factors [*therapeutic use]; Pregnancy Trimester, Third; Randomized Controlled Trials as Topic; Rh Isoimmunization [*prevention & control]; Rho(D) Immune Globulin [*therapeutic use]

MeSH check words

Female; Humans; Pregnancy