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The clinical use of corticosteroids in pregnancy

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BACKGROUND: The use of antenatal steroid therapy is common in pregnancy. In early pregnancy, steroids may be used in women for the treatment of recurrent miscarriage or fetal abnormalities such as congenital adrenal hyperplasia. In mid-late pregnancy, the antenatal administration of corticosteroids to expectant mothers in anticipation of preterm birth is one of the most important advances in perinatal medicine; antenatal corticosteroids are now standard care for pregnancies at risk of premature delivery in high- and middle-income countries. The widespread uptake of this therapy is due to a compelling body of evidence demonstrating improved neonatal outcomes following antenatal corticosteroid exposure, stemming most notably from corticosteroid-driven maturation of fetal pulmonary function. As we approach the 50th anniversary of landmark work in this area by Liggins and Howie, it is apparent that much remains to be understood with regards to how we might best apply antenatal corticosteroid therapy to improve pregnancy outcomes at both early and mid to late gestation.

METHODS: Drawing on advances in laboratory science, pre-clinical and clinical studies, we performed a narrative review of the scientific literature to provide a timely update on the benefits, risks and uncertainties regarding antenatal corticosteroid use in pregnancy. Three, well-established

therapeutic uses of antenatal steroids, namely recurrent miscarriage, congenital adrenal hyperplasia and preterm birth, were selected to frame the review.

RESULTS: Even the most well-established antenatal steroid therapies lack the comprehensive pharmacokinetic and dose–response data necessary to optimize dosing regimens. New insights into complex, tissue-specific corticosteroid signalling by genomic-dependent and independent mechanisms have not been used to inform corticosteroid treatment strategies. There is growing evidence that some fetal corticosteroid treatments are either ineffective, or may result in adverse outcomes, in addition to lasting epigenetic changes in a variety of homeostatic mechanisms. Nowhere is the need to better understand the intricacies of corticosteroid therapy better conveyed than in the findings of Althabe and colleagues who recently reported an increase in overall neonatal mortality and maternal morbidity in association with antenatal corticosteroid administration in low-resource settings.

CONCLUSIONS: New research to clarify the benefits and potential risks of antenatal corticosteroid therapy is urgently needed, especially with regard to corticosteroid use in low-resource environments. We conclude that there is both significant scope and an urgent need for further research-informed refinement to the use of antenatal corticosteroids in pregnancy.

Key words: corticosteroids / pregnancy / quality control / low-resource settings / recurrent miscarriage / congenital adrenal hyperplasia / preterm birth

Introduction

Improving pregnancy outcomes is essential to the promotion of human health. Approximately 3% of the population are affected by recurrent miscarriage, and of these, 50% of cases are idiopathic (Li *et al.*, 2002). Alleviating of the burden of diseases caused by preterm birth (PTB; live delivery before 37 weeks gestation) remains one of the greatest public health challenges facing perinatal medicine. Some 15 million babies are born preterm each year; of those, approximately 1 million will die and many will have acute and chronic diseases associated with prematurity. Poverty is strongly associated with miscarriage and preterm birth, with Africa and South Asia accounting for nearly two-thirds of the global PTB burden. The risk of death and disease in the preterm population is inversely correlated with gestational age at delivery. Furthermore those born very preterm (28–<32 weeks gestation) or earlier in low-income countries are far more likely to die during the perinatal period than their contemporaries born into developed economies (March of Dimes, 2012).

In their landmark, 1972 report on the effect of antenatal steroids (ANS) on the fetal lung, Liggins and Howie concluded by noting that (p.524) 'it would be surprising if there was no scope for improved results from therapeutic regimens based on a better understanding of the mode of action of corticosteroids, on the better selection of patients and on more effective control of uterine activity' (Liggins and Howie, 1972). In light of recent developments, it is important that we take up this challenge, and attempt to determine how we might better tailor ANS therapy to the characteristics of an individual pregnancy (Romejko-Wolniewicz *et al.*, 2014). Framed around three common therapeutic uses of synthetic corticosteroids in pregnancy (recurrent miscarriage, congenital hyperplasia, and preterm birth) (Fig. 1), this narrative review will draw on laboratory science and animal and clinical studies to advance the argument that developing a more targeted, pregnancy-specific approach to corticosteroid therapy will allow us to improve perinatal outcomes. The review will begin with a discussion of ANS pharmacology and pharmacokinetics.

Methods

Focussing on fetal and neonatal wellbeing, we performed a narrative review of the scientific literature pertaining to the therapeutic use of corticosteroids in pregnancy. Given the importance of therapeutic efficacy, specific attention was paid to corticosteroid pharmacology and signalling. The review was framed around three, well-established therapeutic uses of antenatal steroids in pregnancy, namely recurrent miscarriage, congenital adrenal hyperplasia and preterm birth. Emphasis was placed on: (i) differentiating the evidence available to support the use of corticosteroid therapy in early, mid and term pregnancies; and (ii) differentiating the evidence available to describe the potential risks deriving from the use of corticosteroid therapy in early, mid and term pregnancies.

Corticosteroid pharmacology

Three of the most commonly used corticosteroids in pregnancy are prednisolone, dexamethasone and betamethasone (Fig. 2) (Health, 1994).

Prednisolone is the most commonly used oral corticosteroid in pregnancy, with multiple formulations available. It is predominantly used for immunosuppression and treatment of autoimmune conditions. Prednisolone is a pharmacologically active synthetic steroid with the chemical structure 11β,17α,21-trihydroxy-pregna-1,4-diene-3,20-dione (Czock *et al.*, 2005). Prednisolone, undergoes reversible metabolism to prednisone (17α,21-dihydroxy-pregna- 1,4-diene-3,11,20-trione), thus prednisone is both the inactive metabolite and the prodrug of prednisolone (Bergmann *et al.*, 2012). Prednisolone and prednisone are rapidly absorbed with peak plasma concentrations between 1–3 h after oral administration (Bergmann *et al.*, 2012). Interconversion of prednisolone and prednisone is mediated predominantly by 11β-hydroxysteroid dehydrogenase (11β-HSD), with 11β-HSD1 primarily acting as a reductase converting prednisone to active prednisolone, and 11β-HSD2 acting primarily as an oxidase, converting prednisolone to prednisone (Diederich *et al.*, 2002). In plasma, prednisolone binds to albumin and transcortin, as well as slightly to a1-acid glycoprotein. Protein binding of prednisolone decreases from approximately 95% at plasma concentrations of 200 μg/l to 60–70% at plasma concentrations of 800 μg/l although free prednisolone pharmacokinetics are not thought to be dose dependent (Czock *et al.*, 2005). Prednisolone and prednisone are primarily cleared from the body by hepatic metabolism, but renal tissue may also contribute to metabolism (Bergmann *et al.*, 2012). In healthy pregnant women taking prednisolone, fetal prednisolone

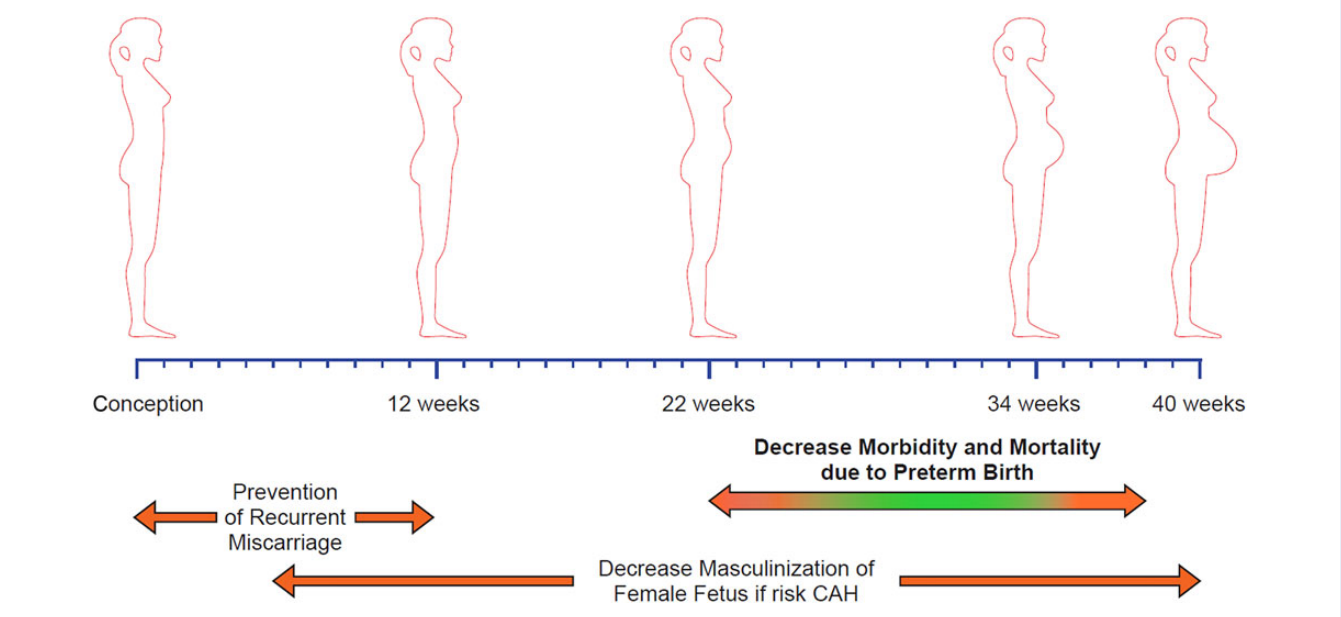

Figure 1 Clinical indications for antenatal corticosteroid use in pregnancy to improve fetal and neonatal outcomes. The strength of evidence of benefit is indicated by the colour of arrow. Green indicates evidence of benefit from randomized control trials and systematic reviews and meta-analyses. Red represents evidence from observational human studies or extrapolated from clinical trials in different populations or animal studies. CAH, congenital adrenal hyperplasia.

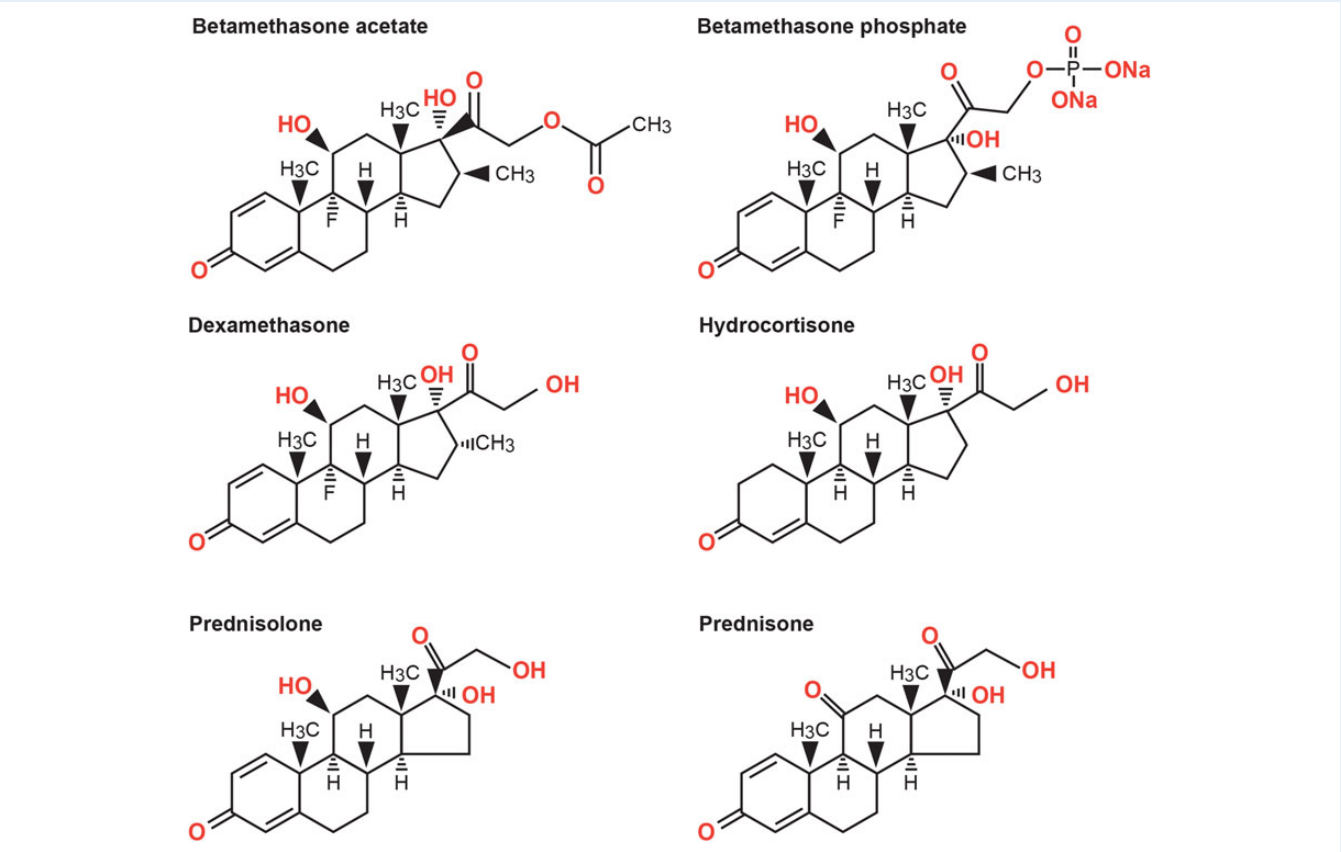

Figure 2 Chemical structures of corticosteroids used in pregnancy.

concentrations are 8- to 10-fold lower than those found in the mother (Murphy *et al.*, 2007). Prednisolone is lipophilic so it can cross the placenta, but its fetal uptake is limited by active retrograde transport by P-glycoprotein, and its conversion to inactive metabolites by placental 11β-HSD2 (Addison *et al.*, 1993; van Runnard Heimel *et al.*, 2005).

Antenatal hydrocortisone administration was investigated in clinical trials (Iams *et al.*, 1985) and experimental studies (Jobe *et al.*, 2003) focused on preterm lung maturation, but due to a lack of effectiveness, experimentally and in clinical data, together with an adverse pharmacokinetic profile (Ballard and Ballard, 1995), hydrocortisone is not recommended for clinical use. Betamethasone and dexamethasone are fluorinated, synthetic corticosteroids with a similar molecular structure and an ability to cross the human placenta from mother to fetus. Betamethasone and dexamethasone are epimers, differing in the orientation (β versus α) of their methyl group at position 16, a difference which allows the two molecules to be resolved with mass spectrometry (De Wasch *et al.*, 2001). The total recommended treatment dose for betamethasone or dexamethasone in anticipated PTB is 24 mg (Health, 1994). Betamethasone is administered as two 12 mg i.m. injections of a 1:1 preparation of betamethasone phosphate and betamethasone acetate, given 24 h apart. In theory, the use of a combined phosphate and acetate preparation allows for rapid exposure to free betamethasone with dephosphorylation of betamethasone phosphate, with extended dosing provided by the slower deacetylation of microparticulate betamethasone acetate (Health, 1994; Ballard and Ballard, 1995). Dexamethasone is commonly prescribed as dexamethasone sodium phosphate, although use of lower doses of the acetate form has also been reported (Senat *et al.*, 1998; Bar-Lev *et al.*, 2004; Jobe and Soll, 2004; Brownfoot *et al.*, 2013). For dexamethasone phosphate, the recommended dosing regimen is four 6 mg i.m. injections at 12 hourly intervals (Jobe and Soll, 2004; Brownfoot *et al.*, 2013).

Free betamethasone and dexamethasone have similar maternal-fetal pharmacokinetic properties. Dexamethasone and betamethasone have higher affinities (7.1 and 5.4 fold, respectively) for the corticosteroid receptor than cortisol. As discussed by Ballard and Ballard, the betamethasone dosing regimen adopted by Liggins and Howie generated a maternal plasma C_{max} of approximately 100 ng/ml 1 hour after treatment, a fetal plasma C_{max} of approximately 20 ng/ml 1 to 2 h after treatment, and a fetal : maternal plasma betamethasone ratio of 0.37 in matched samples. The half-life of betamethasone in fetal plasma was 12 h, approximately twice that in maternal plasma (Ballard and Ballard, 1995).

It is unclear whether betamethasone or dexamethasone is the superior agent for lung maturation. Dexamethasone use has been associated with less alteration in fetal heart rate variability (Senat *et al.*, 1998). In a retrospective analysis of 883 infants born in Paris between 1993 and 1996, Baud and colleagues reported that betamethasone, but not dexamethasone, was associated with a reduced risk of cystic periventricular leukomalacia (Baud *et al.*, 1999). In contrast, a smaller study of 550 infants born in Israel between 1999 and 2001 reported equivalence between the two agents with regard to their ability to reduce the risk of periventricular leukomalacia in low birthweight (≤1.75 kg) infants (Bar-Lev *et al.*, 2004). A retrospective analysis of a US preterm cohort of 334 very-low-birthweight infants (≤1.5 kg) concluded that antenatal betamethasone use was associated with a significant reduction in pulmonary complications when compared with dexamethasone use (Feldman *et al.*, 2007). Jobe and Soll concluded that the weight of clinical evidence supports betamethasone as the antenatal agent of choice although these observation were not based on the randomization of treatment to betamethasone or dexamethasone (Jobe and Soll, 2004). This view has subsequently been reiterated by Lee *et al.* following the analysis of 3600 very-low-birthweight infants (Lee *et al.*, 2006). A recent systematic review and meta-analysis of 12 studies (1557 women and 1661 infants) comparing different antenatal corticosteroid use concluded that it was unclear as to which agent (betamethasone or dexamethasone) was the better choice for antenatal therapy (Brownfoot *et al.*, 2013). What is clear, however, is that there is a need for both animal studies and randomized control trials to determine the most efficacious agent and administration protocol to optimize outcomes for babies born preterm. Importantly, such studies should attempt, as best as possible, to control for differences in gestation, neonatal care and pregnancy-specific factors (e.g. plurality, fetal sex) in the study design.

Signalling and the corticosteroid receptor

In humans, the corticosteroid cortisol is synthesized from cholesterol and released from the *zona fasciculata* of the adrenal cortex under the regulation of the hypothalamic–pituitary–adrenal axis. Corticosteroids are involved in controlling a diverse array of transcriptional targets, theoretically as much as 20% of the human genome (Oakley and Cidlowski, 2013). Classically, corticosteroids are the essential regulators of metabolic processes and stress responses. Much of their contemporary pharmaceutical use derives from their marked anti-inflammatory and immunosuppressive activities. The maturational effects exerted by corticosteroids on multiple organs underpin the rationale for the use of corticosteroids in obstetric medicine (Vandevyver *et al.*, 2014). Endogenous corticosteroids exert their effect predominantly through the glucocorticosteroid receptor (GR), but other effects may result from mineralocorticoid receptor (MR) activation (Funder, 1997). In contrast, synthetic corticosteroids such as dexamethasone and betamethasone exert their effects solely via the glucocorticosteroid receptor. The GR is ubiquitously expressed and is a member of the nuclear receptor superfamily of ligand-dependent transcription factors (Hollenberg *et al.*, 1985; Weinberger *et al.*, 1985), a collection of 18 steroid hormone receptors (Turner *et al.*, 2014).

The human GR gene contains 9 exons (exons 2–9 being responsible for encoding protein) and spans a 150 kb region on chromosome 5q31Y32 (Oakley and Cidlowski, 2013; Turner *et al.*, 2014; Vandevyver *et al.*, 2014). Structurally, the GR is comprised of three primary domains: (i) the N-terminal transactivation domain; (ii) the DNA-binding central domain; and (iii) the C-terminal ligand binding domain (Oakley and Cidlowski, 2013). Nunez and colleagues demonstrated that three different promoter regions, 1A, 1B and 1C contribute to regulating GR gene expression (Nunez and Vedeckis, 2002). The promoters of the GR gene contain binding sites for numerous transcription factors including, among others, AP-1 (Breslin and Vedeckis, 1996), AP-2 (Nobukuni *et al.*, 1995), NF-κB (Webster *et al.*, 2001) and Pu.1 (Geng and Vedeckis, 2005). Interestingly, a number of positive and negative corticosteroid response elements have been identified within the GR gene promotor region, suggesting that the GR has the ability to regulate its own expression (Burnstein *et al.*, 1990).

The GR is primarily an intracellular receptor; both active and inactive forms are thought to constantly shuttle between the cytoplasm and the

nucleus (Vandevyver et al., 2014). However, a membrane-bound GR termed mGR was recently identified (Strehl and Buttgereit, 2014). mGR is found in mouse lymphoma cell membranes (Gametchu, 1987), post-synaptic membranes in rats (Johnson et al., 2005), and in human monocytes and B lymphocytes (Bartholome et al., 2004). As summarized by Strehl and Buttgereit, mGR expression appears to be increased following vaccination, immunological stimulation and in patients with rheumatoid arthritis, although the function and clinical relevance of these observations is not well understood (Strehl and Buttgereit, 2014).

The ability of a single GR to exert precise biological regulation over a discursive array of targets may derive from the tissue-specific expression of a broad collection of GR subtypes originating from differential mRNA splicing and transcriptional isoforms. The two primary GR variants are GRα and GRβ, which are generated by the alternative splicing of GR pro-mRNA exon 9 (Oakley and Cidlowski, 2013; Turner et al., 2014). To date, three minor variants have been identified: (i) GRγ, deriving from the use of an alternative splice donor site located in the intronic region between exons 3 and 4 (Rivers et al., 1999; Beger et al., 2003); (ii) GR-A, deriving from the excision of exons 5, 6 and 7 due to alternate splicing; and (iii) GR-P, which lacks exons 8 and 9 due to the loss of the splice acceptor site in exon 8 (Moalli et al., 1993). The biological role of these three minor variants is not well understood, although an increased GRγ:GR ratio is associated with corticosteroid resistance in leukaemic blast cells from childhood acute lymphocytic leukaemia (Beger et al., 2003). More recently, Saif and colleagues suggested that corticosteroid resistance observed in the male placenta may be due to the increased nuclear localization of the GRβ, GR-A and GR-P variants (Saif et al., 2014).

GRα is the dominant active GR form, and is constitutively found in the cytoplasm where it forms a transcriptionally inactive complex with a number of chaperone proteins including Heat Shock Protein (HSP)90, HSP70, and p23 (Vandevyver et al., 2014). Corticosteroids freely diffuse through cell membranes to access cytoplasmic GRα. Unlike cortisol, dexamethasone and betamethasone are neither bound to cortisol binding globulin, nor inactivated by 11β-hydroxysteroid dehydrogenase 2 (Asztalos, 2012). Following corticosteroid binding, GRα undergoes a conformational change, disassociates from its chaperone proteins, and translocates to the nucleus. Here, in a process known as classical signalling, GRα dimers change in transcriptional activity by interacting directly with conserved sequences containing corticosteroid response elements.

GRα also exerts transcriptional changes by interacting with other DNA-bound transcription factors or by changing the activity of a number of kinases (Vandevyver et al., 2014). GRα has the additional ability to mediate physiological effects via a range of non-genomic mechanisms, collectively termed nonclassical GR signalling. Severe developmental abnormalities and perinatal lethality in GR knock-out mice demonstrate that GR receptor activity is essential for life. However, subsequent work by Reichardt and colleagues suggests that the GR's DNA-binding-independent activities are sufficient for survival in mice. Homozygous mutant mice expressing dimerization impaired (and thus DNA binding defective) GR mutants induced by an A458T mutation were viable, fertile and did not have the fatal lung atelectasis identified in homozygous GR-null mice (Reichardt et al., 1998).

GRβ, presumably by virtue of its distinct C-terminal sequence, does not bind GR agonists and does not activate corticosteroid response genes. GRβ is constitutively resident in the nucleus where it acts as a negative inhibitor of GRα by directly competing for corticosteroid response elements and transcription factors and by forming inactive heterodimers with GRα (Kino et al., 2009b). GRβ, for example, antagonizes corticosteroid-driven silencing of interleukin (IL)-6 and tumour necrosis factor (TNF)-α gene expression (Li et al., 2006). Cells that overexpress GRβ possess a unique transcriptional profile, suggesting that GRβ possesses a native transcriptional activity, independent of the effects that it exerts on GRα (Kino et al., 2009a).

Eight GR isoforms (GR-A, GR-B, GR-C1, GR-C2, GR-C3, GR-D1, GR-D2, and GR-D3) are derived from the use of alternative AUG start codons in the GR mRNA transcript (Lu and Cidlowski, 2005). Sequence analysis suggests that these isoforms are generated by all five GR splice variants (GRα, GRβ, GRγ GR-A and GR-P), and the absolute expression of each of these isoforms has a tissue-specific pattern (Vandevyver et al., 2014). Intriguingly, the ratios of isoform expression appear to influence a cell's responsiveness to corticosteroid stimulation, potentially through common and isoform-specific patterns of gene induction and repression (Lu and Cidlowski, 2005). For example, cells expressing the GR-C3 isoform are more sensitive to corticosteroid killing, whereas GR-D expressing cells have reduced sensitivity (Bender et al., 2013).

The complexity of GR signalling in different organs has not yet been evaluated within the context of the effects of corticosteroids on the developing fetus.

Recurrent miscarriage

In the United States, recurrent miscarriage (RM) is defined as two or more consecutive miscarriages (Anonymous, 2013). RM affects between 0.5 and 3.0% of women, although the precise incidence is difficult to determine due to inter-institutional differences in how RM is classified (Li et al., 2002). Approximately 50% of RM cases are due to uterine disorders, chromosomal abnormalities, endocrine dysfunction or autoimmune conditions, such as anti-phospholipid antibody syndrome (Li et al., 2002; Tang et al., 2009). The remaining 50% of cases have no identifiable aetiology and are termed idiopathic RM (Li et al., 2002; Tang et al., 2009).

The disruption of normal CD4 T-helper cell and circulating/uterine natural killer (NK) cell populations has received increased scrutiny as a potential cause of idiopathic RM and implantation failure (Guerin et al., 2009; Bansal, 2010). As noted by Saito and colleagues, both overt Th1- and overt Th2-dominant immunity is associated with RM (Saito et al., 2010). Furthermore, a ratio slightly favouring the Th2 phenotype may be conducive to pregnancy maintenance, whereas a shift towards an overtly Th1/Th17-dominant or Th2-dominant phenotype may be harmful to the trophoblast and the developing embryo (Bansal, 2010; Bansal et al., 2012).

CD4 T-helper cells include Th1, Th2, T regulatory (Treg), and Th17 cells (Zhu and Paul, 2008). Th1 cells are important in host defence against intracellular pathogens. Their differentiation is characterized by the expression of transcription factors T-bet and STAT-4, and they are functionally characterized by their production of interferon (IFN)-γ, TNF-β and IL-2 (Zhu and Paul, 2008; Saito et al., 2010). Th2 cell differentiation is characterized by the expression of transcription factors GATA3 and STAT-6. Th2 cells play a key role in defence against extracellular infection and are functionally characterized by their production of ILs-4, -5, -13, and transforming growth factor (TGF)-β (Zhu and Paul, 2008). Th17 cells originate from TGF-β and IL-6 co-stimulated naive CD4T cells. They are important for defence against extracellular

infection, and express a range of immunomodulatory mediators including ILs-17a and 17f, both of which recruit and activate neutrophils. In contrast to the co-inhibitory interaction between Th1 and Th2 cells, there appears to be a synergistic relationship between Th1 and Th17 cells (Guerin *et al.*, 2009; Bansal, 2010). Of particular interest is data suggesting reciprocal development pathways between Th17/Th1 and Th17/Treg subsets (for a more comprehensive treatment of this subject, see Saito *et al.*, 2010) and the potential for plasticity in cytokine production and the inter-differentiation of Th cell lineages (O'Shea and Paul, 2010).

Immune responses driven by Th1 and Th2 cells are controlled, in part, by Treg cells which express the transcription factor forkhead box P3 (FOXP3) in addition to CD4 and CD25 (for a detailed review of type-1 Treg cells, see Guerin *et al.*, 2009). Treg cells are believed to be important for the maintenance of host self-tolerance at the feto-maternal interface, effects which are exerted by cell–cell interactions in addition to the expression of IL-10, TGF-β and IL-35. More recently, Treg cells have been shown inhibit cytokine production and proliferation in $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cell cytotoxicity, B-cell immunoglobulin production and antigen presenting cell differentiation and function (Zhu and Paul, 2008; Guerin *et al.*, 2009; Bansal, 2010). A number of studies have identified alterations in T-helper and NK cell populations/activity with RM. Treg cells have been shown to concentrate in the decidua and the lymph nodes draining the uterus after coitus (Saito *et al.*, 2010; Bansal *et al.*, 2011). Decreases in decidual and circulating Treg cells have been identified in women with RM. Similarly, the percentage of activated NK cells, and absolute number of NK cells has been shown to be increased in women with RM. Women with RM have also been found to exhibit alterations in Th1:Th2 ratios (Saito *et al.*, 2010; Bansal *et al.*, 2011, 2012).

Due to the increasing body of evidence suggesting a T-helper cell imbalance in association with RM, a number of studies have attempted to use immunologic approaches to prevent RM in early pregnancy. A Cochrane Review of 20 randomized control trials of immunotherapies (1137 women; including paternal leukocyte transfusions and intravenous immunoglobulin) for RM prevention concluded that there was no benefit over placebo to the live birth rate or a reduction in the future risk of RM. Corticosteroids are also used as a treatment for RM, and are known to be able to exert immunosuppressive effects on T cells and NK cells (Henderson *et al.*, 2003; Novac *et al.*, 2006). However, there is a shortage of data from large, randomized control trials to assist in the determination of therapy safety and efficacy. And as with many corticosteroid applications in pregnancy, there is not a body of rigorous dose–response data to inform the optimal choice of agent, length of treatment, or dose. Confounding this situation is the observation that many studies employ corticosteroids, notably prednisolone, in combination with other agents including folate, aspirin and progesterone, making it difficult to determine the origin of any beneficial or adverse effects that might be observed.

Tempfer *et al.* undertook a case–control study to compare pregnancy outcomes between women with idiopathic RM receiving either no treatment or combination therapy with prednisone (20 mg/day) and progesterone (20 mg/day) for the initial 12 weeks of gestation, followed by a course of aspirin (100 mg/day) and folate (5 mg every 2 days) for the remainder of gestation (Tempfer *et al.*, 2006). The authors reported a significant increase in live birth rates in the intervention group compared with no-treatment control (77 versus 35%; $P = 0.04$). More recently, Gomaa and colleagues reported findings from a study of 160 women with idiopathic RM randomized to low-dose heparin (subcutaneous administration at 10 000 IU/day) and aspirin (81 mg/day), with or without 5 mg/day prednisolone (Gomaa *et al.*, 2014). Combination therapy including prednisolone significantly increased pregnancy success (70.3 versus 9.2%; RR 7.63, 95% CI (3.71–15.7)), defined as pregnancy ongoing beyond 20 weeks' gestation, although they did not follow the pregnancies to term.

Several clinical reports describe beneficial effects of prednisolone therapy to pregnancy outcomes. Ogasarawa *et al.* reported that uterine administration of prednisolone prior to ovulation was associated with a successful pregnancy following 10 previous miscarriages (Ogasawara and Aoki, 2000). Hasegawa and colleagues reported that combined use of prednisolone and low-dose asprin in women with anti-phospholipid antibodies significantly improved pregnancy success, relative to controls. Interestingly, combined treatment was also reported to ameliorate growth restriction identified in previous pregnancies (Hasegawa *et al.*, 1992). These data are in contrast to that reported by Laskin *et al.* from a study of 202 women with an autoantibody and RM, randomized to receive prednisolone 0.5–0.8 mg/kg/day and aspirin 100 mg/day. The primary outcome measure (successful pregnancy) was not statistically different from controls. Moreover, there was a statistically significant increase in hypertension, diabetes mellitus and an increased risk of preterm birth in the treatment group (Laskin *et al.*, 1997). Quenby and co-workers reported that daily administration of 20 mg oral prednisolone resulted in a statistically significant reduction in $CD56^+$ cells in the endometrium (median value of 14% before treatment versus 9% after treatment). The normal range of uterine NK cells (derived from a control group of women without RM undergoing sterilization) was $<5\%$. As such, it is not clear if the NK cell reduction achieved by prednisolone therapy would improve pregnancy outcomes (Quenby *et al.*, 2005).

The safety of corticosteroid exposure in early pregnancy is yet to be conclusively determined. To date, prednisolone has been the corticosteroid of choice for RM treatment. Under normal conditions, metabolism of prednisolone to prednisone, 20α-dihydroprednisone, 20β-dihydroprednisone and 20β-dihydroprednisolone by the human placenta likely reduces fetal exposure (Addison *et al.*, 1993). A prospective controlled cohort study of 311 pregnancies exposed to corticosteroids during at least the first trimester of pregnancy failed to identify an increased risk of birth defects, but did identify a 2-fold increase in preterm birth and reduction in birthweight (Gur *et al.*, 2004). Interpreting these data is difficult due to substantial variation in the type of corticosteroids used (e.g. prednisolone, hydrocortisone, betamethasone, dexamethasone) and the length of corticosteroid exposure. Of interest is recent studies suggesting that, as the pharmacokinetics/pharmacodynamics of prednisolone are impacted by circadian rhythm, the time at which treatment is administered may need to be considered/standardized to optimize outcomes and allow efficacy assessment in research studies (Xu *et al.*, 2008).

In an attempt to answer the question of prednisolone efficacy, Tang and colleagues are presently undertaking a randomized, double-blind placebo controlled trial of prednisolone therapy for idiopathic RM (Current Controlled Trials ISRCTN28090716) (Tang *et al.*, 2009). The lead aim of this study is to determine if first trimester prednisolone therapy increases the live birth rate in women with idiopathic RM and a uterine NK count $>5\%$. Importantly, given concerns over the safety of early pregnancy ANS administration, secondary outcome measures will include ultrasound assessment of fetal growth at 28 and 34 weeks' gestation in addition to pregnancy complications, congenital abnormalities, and a 6-week follow-up.

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia is a group of autosomal recessive disorders characterized by failure of cortisol synthesis by the adrenal cortex. The cause of congenital adrenal hyperplasia is deficiency in 21-hydroxylase in more than 95% of cases (Merke and Bornstein, 2005). It is traditionally classified as classical (severe), which presents in the neonatal period, or non-classical (mild), which has a later presentation. The classical form has an incidence of 1 in 15 000 newborns (Therrell, 2001). The pathophysiology relates to the enzymatic deficiency and disruption in cortisol biosynthesis. A lack of usual negative feedback leads to a compensatory increase in pituitary production of corticotropin and hypothalamic production of corticotropin-releasing hormone (CRH) and excess of cortisol precursors and adrenal androgens (Merke and Bornstein, 2005). Intrauterine androgen excess and adrenal androgens cause virilization of the female fetus and ambiguous genitalia. Characteristically there is enlargement of the clitoris, partially fused labia with rugae, and a common urogenital sinus rather than separate vagina and urethra, but normal internal female organs (Merke and Bornstein, 2005). Complex surgery is required for feminization of the genitalia, and this is frequently carried out in the neonatal period (Clayton *et al.*, 2002). Supraphysiological doses of exogenous steroids are required to supress androgen excess, and in the 1980s, it was shown that prenatal dexamethasone treatment of fetuses at risk of congenital adrenal hyperplasia can prevent masculinization of a female fetus (New *et al.*, 2001). This treatment has since been offered to avoid the need for surgery and the risk of complications, and remains controversial. As masculinization starts as early as 6 weeks post-conception, to prevent development of ambiguous genitalia prophylactic, dexamethasone must be given to all at-risk fetuses as soon as pregnancy is recognized. Chorionic villus sampling should be performed as soon as possible to confirm gender and whether the fetus carries a 21-hydroxylase gene mutation, and treatment should be stopped in unaffected and male fetuses. As the chance of an affected female fetus is only 1 in 8 pregnancies, 7 out of 8 fetuses receive unnecessary dexamethasone. Furthermore, dexamethasone does not prevent virilization in all cases, with success rates of 85% found in follow-up studies (Clayton *et al.*, 2002). As discussed below, the long-term effects of steroids in early pregnancy remain unclear, with potential detrimental effects on fetal programming, brain function and congenital anomalies. Therapy should therefore be only offered at specialist centres, with full counselling about the risks and benefits of antenatal treatment.

Extreme prematurity

Our inability to effectively diagnose and prevent preterm birth places increased importance on treatments to maximize survival and optimize short- and long-term outcomes for preterm infants. In developed countries, antenatal corticosteroids are recommended for women at risk of preterm labour when the fetus is considered viable, i.e. from as early as 22 weeks in certain settings. Fetal maturational responses to corticosteroids occur in animal models of extremely preterm gestations (Bunton and Plopper, 1984). Observational studies have also suggested a reduction in adverse outcomes in babies born at extreme preterm gestations (Costeloe *et al.*, 2000; Carlo *et al.*, 2011; Mori *et al.*, 2011). However, there is little evidence of benefit from randomized control trials. Roberts and Daziel (Roberts and Dalziel, 2006) performed a *post hoc* analysis of clinical trials of corticosteroids on the effect of the gestational age at trial entry. They found no differences for women who received ANS and delivered before 28 weeks gestation compared with those receiving placebo or no treatment with regards to neonatal death (RR 1.9, 95% CI 0.6–5.7, one study, 27 infants), respiratory distress syndrome (RR 2.9, 95% CI 0.4–21.9, one study, 24 infants) or chorioamnionitis (RR 2.2, 95% CI 0.6–7.8, one study, 46 women). However only one trial was included in the analysis, and the lack of significant findings may reflect inadequate power to detect benefit. In addition, ANS at extreme preterm gestations may decrease, but not prevent, complications of prematurity. In a trial of steroids at 24–28 weeks, although there was no difference in the incidence of respiratory distress syndrome (RDS), it was less severe (Garite *et al.*, 1992). The challenge for an evidence-based approach to gestations <28 weeks is that randomized control trials are now considered unethical because of the widely accepted evidence of benefit of ANS treatment at a more advanced gestational age.

Teratogenic effects of synthetic corticosteroids in early pregnancy

There is conflicting evidence regarding a putative link between synthetic corticosteroids use in early pregnancy and teratogenicity. Interpreting these data is difficult due to substantial variation in the type of corticosteroids used (e.g. prednisolone, betamethasone, dexamethasone) and the length of corticosteroid exposure. Of interest are recent studies suggesting that, as the pharmacokinetics/pharmacodynamics of prednisolone are impacted by circadian rhythm, the time at which treatment is administered may need to be considered/standardized to optimize outcomes and allow efficacy assessment in research studies (Xu *et al.*, 2008). Only three studies have included more than ten women exposed to any formulation of corticosteroids, and even in these studies, there are few women exposed to systemic corticosteroids. Pradat *et al.* used data from nine malformation registries collected over 13 years to explore the relationship between oral clefts and first trimester corticosteroid exposure (Pradat *et al.*, 2003). They included 11 150 women with babies with congenital abnormalities, of whom 982 had babies with cleft palate or cleft lip. Single formulation systemic corticosteroid exposure was not associated with increased odds of cleft palate or lip compared with other congenital anomaly (odds ratio 1.25 [95% CI 0.72–2.15]; 15/982 [1.53%] women with cleft lip or palate exposed to systemic corticosteroids versus 155/10168 [1.53%]) although combination systemic corticosteroids were (odds ratio 2.10 (95% CI 1.03–4.26; 9/982 [0.92%] women with cleft lip or palate versus 61/ 10168 [0.6%] women with other congenital abnormalities).

Källen used the Swedish Birth Registry to study the association of antenatal drug use and orofacial clefts over a 6-year period (Källín, 2003). In total there were 576 873 births in the period, and 1044 infants with clefts. The authors compared observed and expected frequencies of clefts in women exposed to glucocorticoids in early pregnancy. No significant increase of clefting was found in association with systemic corticosteroid use (relative risk 1.94 [95% CI 0.78–3.99] observed frequency of clefts in women exposed to corticosteroids 7/2050 [0.34%] versus expected frequency 3.6/2050 [0.17%]). The National Birth Defects Prevention Study is a population based case–control study, which included

1141 mothers of children with cleft lip with or without cleft palate, 628 mothers of children with isolate cleft palate, and 4143 controls. Carmichael *et al.* interviewed these mothers about corticosteroid exposure in early pregnancy (Carmichael *et al.*, 2007). The odds ratio for cleft lift and palate with any steroid exposure was 1.7 (95% CI 1.1–2.6) when compared with no steroid exposure (33/1141 [2.9%] versus 72/4143 [1.7%]). Corticosteroid exposure only during Weeks 1–4 and 5–8 after conception were associated with the highest increase in risk of cleft lip and palate with an OR of 7.3 (95% CI 1.8–29.4). In summary, the evidence suggests there may be a small, but significant association between systemic corticosteroid use and cleft lip and palate, but the absolute risk is small.

Corticosteroids and preterm birth

Antenatal corticosteroids (ANS) are perhaps the most effective therapy for improving short- and long-term outcomes in preterm infants (Jobe and Soll, 2004). Drawing on experimental and clinical data, the 1994 National Institutes of Health consensus statement concluded that ANS therapy reduces mortality, respiratory distress syndrome (RDS) and cerebral haemorrhage in preterm infants born between 24 and 34 weeks' gestation (Health, 1994). Subsequent analyses of clinical trials, conducted predominantly in high- and middle-income countries, confirmed that the use of maternally administered ANS in cases of threatened preterm delivery improves neonatal outcomes with reductions in neonatal death, respiratory distress syndrome, cerebral haemorrhage and necrotising enterocolitis, without risk to maternal wellbeing (Roberts and Dalziel, 2006).

A key feature of PTB is its heterogeneity, both in terms of underlying cause, and effects on the mother and baby (Romero *et al.*, 2014). PTB spans a wide spectrum of fetal development, varying by as much as 15 weeks depending on how the lower limit of prematurity is defined by individual jurisdictions (Kramer *et al.*, 2012). PTB is associated with lifestyle, social, environmental, and genetic factors, many of which stratify against gestational age at delivery in both high- and low-income countries (Blencowe *et al.*, 2012; Shapiro-Mendoza and Lackritz, 2012; Ananth *et al.*, 2013). Yet in stark contrast to the complex clinical picture of PTB, only two standardized treatment regimens for ANS delivery are recommended to improve outcomes (Jobe and Soll, 2004). Although these regimens were initially used as single-course treatments, they have been used for multiple-course treatments when anticipated PTB does not occur within 7 days of the initial treatment (Murphy *et al.*, 2008).

Despite the extensive research on ANS use, a number of controversies remain regarding their use. In particular, there is a contention with regards to the administration of multiple courses of ANS to women at risk of preterm birth, the long-term effects of synthetic corticosteroid exposure on the developing fetus, and their use in resource-poor settings (Crowther and Harding, 2007; Murphy *et al.*, 2008; Asztalos *et al.*, 2013; Stock and Kemp, 2014). Of late, potential limitations of ANS for anticipated PTB in a low-resource setting has been highlighted by Althabe and colleagues' report of an excess of 3.5 neonatal deaths per 1000 women exposed to antenatal corticosteroids in a cluster randomized trial (Althabe *et al.*, 2015).

The administration of synthetic corticosteroids mimics the surge in endogenous corticosteroids seen in late gestation that is essential for normal development of many organs systems and prepares the fetus for ex-utero life. Corticosteroids have well described effects on fetal lung maturation. Alveolar structure, vascularization, surfactant production and airspace fluid clearance have all been implicated in improvements to preterm lung function observed following antenatal corticosteroid therapy (Whitsett and Matsuzaki, 2006). Corticosteroids also have important effects on other organ systems, including heart, brain, circulation hypothalamus, kidneys and thyroid, that support post-natal adaptation.

In key studies, homozygous GR-null mice had normal lung morphogenesis until embryonic day 15.5 (term being approximately 21 days), but died rapidly after birth due to respiratory failure (Cole *et al.*, 1995). The activity of selected populations of GRs might be essential and sufficient for late-gestation lung development. In elegant work using germ layer targeted GR knockout mice, Bird and colleagues reported that isolated mesenchymal GR activity was critical for fetal lung maturation and post-natal survival (Bird *et al.*, 2014). Jobe and Ikegami suggest that the early lung maturation driven by antenatal corticosteroid exposure results initially from thinning of the alveolar walls, which in turn increases the lung volume available for gas exchange prior to an increase in surfactant production (Jobe and Ikegami, 2000). Subsequent work in preterm lambs demonstrated that relative to a saline control, alveolar thinning, higher alveolar volumes and an increased proportion of alveolar ducts followed repeated maternal and intraamniotic antenatal betamethasone administration in sheep (Polglase *et al.*, 2007).

Improved neonatal respiratory function from induced surfactant production by the preterm lung is an important physiological response to the administration of ANS therapy. In the alveolar airspaces, surfactant is produced by type II pneumocytes, specialized epithelial cells that comprise approximately 5% of the alveolar surface. Pulmonary surfactant is a composite of 80% glycerophospholipids (principally dipalmitoyl phosphatidylcholine), 10% cholesterol, and 10% protein (Andreeva *et al.*, 2007). Phospholipids are the primary surface tension-lowering component of pulmonary surfactant (Gunasekara *et al.*, 2005). The surfactant specific protein components include four proteins, the hydrophilic surfactant proteins (SP)-A and SP-D and the hydrophobic SP-B and SP-C (Goss *et al.*, 2013). SP-B and SP-C facilitate lipid recruitment, organization and stability at the air-fluid interface (Gunasekara *et al.*, 2005). SP-A and SP-D are multifunctional collections, innate host defence proteins that participate in surfactant homeostasis and the regulation of pulmonary inflammation. Both SP-A and SP-D recognize a broad range of conserved pathogen-associated molecular patterns, including lipopolysaccharide and lipoteichoic acid (Kingma and Whitsett, 2006; Nayak *et al.*, 2012). Surfactant protein A, for example, binds a number of PTB-associated pathogens including members of the *Pseudomonas, Mycoplasma, Escherichia and Candida* species (Piboonpocanun *et al.*, 2005).

Corticosteroids appear to increase surfactant production by both transcription and post-transcriptional mechanisms, enhancing the rate of phosphatidylcholine and fatty acid biosynthesis in the fetal lung (Bolt *et al.*, 2001; Garbrecht *et al.*, 2006). Interestingly, corticosteroids have been suggested to have differential effects on surfactant protein expression; SP-B, SP-C and SP-D expression is increased at the expense of SP-A, with effects mediated by changes in the rate of absolute transcription and the stability of the mRNA transcripts (Rooney *et al.*, 1994; Bolt *et al.*, 2001; Garbrecht *et al.*, 2006).

Corticosteroid-induced increases in SP-A, SP-B, and SP-C mRNA transcription are acute and transitory in nature, returning to pretreatment

levels within several days. The dynamic nature of SP-expression may contribute to the loss of antenatal corticosteroid efficacy 7 days after treatment (Jobe and Ikegami, 2000). Studies investigating a potential link between genetic variation in surfactant protein genes and neonatal lung disease have demonstrated an association between SP-A, SP-B and respiratory distress syndrome (Hallman et al., 2002). Allelic variants of SP-B have also been linked to an increased risk of bronchopulmonary dysplasia (Pavlovic et al., 2006). Surfactant increases compliance and protects the lung from atelectasis. Surfactant forms a film at the alveolar air/water interface, lowering alveolar surface tension and thus assisting respiration. The surface tension at a clean air/water interface is about 70 mN/m. With surfactant, alveolar air/water surface tension is approximately 23 mN/m at equilibrium, and decreases to close to 0 mN/m with the compression of the alveolar surface area that accompanies expiration (Gunasekara et al., 2005).

Not all the effects of antenatal corticosteroids on preventing respiratory distress are mediated through surfactant production. Rapid removal of fluid from the lung to allow efficient gas exchange at the alveolar surface is key to the fetus' transition to ex-utero life. Corticosteroid signalling influences the function of a number of proteins involved in mediating alveolar fluid clearance (Whitsett and Matsuzaki, 2006); targets include α-epithelial sodium channel (αENaC) subunit and the α1 and β1 subunits of the adenosinetriphosphate (ATP)-dependant basolateral Na^+/K^+ pump, both of which are expressed in the respiratory epithelium and are critical to sodium transport in the rat and human lungs (Matthay et al., 2002). In αENaC-knockout mice, αENaC−/− pups die within 40 h of birth (Hummler et al., 1996). In primary rat lung cells, a 10 hour treatment with 0.1 μM dexamethasone increased hyperpolarisation along with the expression of Na^+ channel mRNA (Champigny et al., 1994). Corticosteroids can also regulate Na^+-K^+-ATPase in rat type-II alveolar cells within 6 h of exposure (Barquin et al., 1997).

Clinical evidence and controversies

There is high-level evidence from systematic reviews and meta-analysis of clinical trials that babies who are born preterm benefit from a single course of maternal ANS. In the most recent Cochrane review, Roberts and Dalzeil concluded that ANS administration to women at risk of preterm birth was associated with a reduction in neonatal death (RR 0.69, 95% CI 0.58–0.81, 18 studies, 3956 infants), RDS (RR 0.66, 95% CI 0.59–0.73, 21 studies, 4038 infants), cerebroventricular haemorrhage (RR 0.54, 95% CI 0.43–0.69, 13 studies, 2872 infants) and necrotising enterocolitis RR 0.46, 95% CI 0.29–0.74, eight studies, 1675 infants) (Roberts and Dalziel, 2006). ANS are one of 13 commodities identified by the UN that could save the lives of more than six million women and children worldwide if more widely accessed and properly used. Appropriate administration of ANS is frequently used as a marker of the quality of antenatal care (Henderson et al., 2014).

The majority of ANS trials included women with singleton pregnancy and moderate prematurity (28–34 weeks), and therefore, the evidence of benefits relate to this group of women. Nevertheless, as discussed below, the benefits of ANS administration to other groups of pregnant women have been extrapolated to a wide range of cases without clear evidence of benefit. Although it seems reasonable to give ANS in most of these cases, the potential for long-term harms must be remembered.

Relatively few follow-up studies of infants who have received ANS have been carried out. A salient reminder of the potential harms of ANS is provided by the use of post-natal dexamethasone to prevent and treat bronchopulmonary dysplasia, a chronic lung disease of preterm neonates. Widespread use of dexamethasone was implemented after reports of short-term benefits to reduce the incidence of bronchopulmonary dysplasia, facilitate extubation and reduce the incidence of other complications such as retinopathy of prematurity in neonates requiring ventilation (Doyle et al., 2014a). However, follow-up studies identified increases in cerebral palsy with early administration (<7 days) of post-natal dexamethasone (Doyle et al., 2014a) and a trend towards adverse neurological outcomes with later (>7 days) administration of dexamethasone (Doyle et al., 2014b). If the risk of bronchopulmonary dysplasia exceeds 50%, the short-term benefits of low-dose corticosteroids may mitigate the long-term risks (Doyle et al., 2014c). The routine administration of post-natal corticosteroids to ventilated neonates is, however, not recommended due to the excess long-term neurological morbidity that may result (Watterberg, American Academy of Pediatrics. Committee on, and Newborn, 2010).

Placental insufficiency and fetal growth restriction

Placental insufficiency is the term used to describe the condition where the placenta fails to meet the oxygen or nutrient demands of the developing fetus. It occurs in approximately 3% of pregnancies, and is thought to result from defective trophoblast invasion in the first trimester leading to increased placental vascular resistance that impairs oxygen and nutrient supply to the fetus (Miller et al., 2008). It can be recognized by progressive alterations in the pattern of fetal growth (fetal growth restriction), followed by changes in cardiovascular, metabolic and behavioural indices, representing increasing hypoxaemia and acidosis. As there are currently no effective interventions to prevent or treat placental insufficiency, the mainstay of management is based on monitoring progression of resulting fetal restriction, and delivering the baby at a time that is thought to minimize risk to the infant (Stock et al., 2012). This means that affected pregnancies are frequently delivered preterm. However, there is conflicting evidence regarding the benefits of antenatal steroid administration in women with suspected fetal growth restriction.

It has been hypothesized that placental insufficiency and fetal growth restriction accelerate fetal lung maturation, based on observations that small for gestational age infants have lower rates of respiratory complications at delivery when compared with appropriately grown infants born at the same gestation (Procianoy et al., 1980; Sharma et al., 2004; Bartels et al., 2005). Furthermore, the lecithin/sphingomyelin (L/S) ratio, a biochemical measure of fetal lung maturity, is higher in growth-restricted fetuses than in gestational age matched appropriately grown fetuses (Torrance et al., 2008). Increased exposure to endogenous steroids may mediate lung maturation, as higher cortisol and lower ACTH is seen in the blood of growth-restricted fetuses, when compared with appropriately grown fetuses at similar gestations (Economides et al., 1988). Fetal growth restriction is associated with a reduction in placental 11-beta-hydroxysteroid dehydrogenase type 2, thus more maternal cortisol may be able to cross to the fetus (McTernan et al., 2001). The potential benefits of exogenous steroids in already stressed, growth restricted fetuses have been questioned, particularly in view of potential

effects on fetal programming and brain development associated with elevated steroid levels.

There are no randomized control trials of the use of antenatal corticosteroids in women with suspected growth restriction that have examined neonatal or longer-term outcomes. Torrance *et al.* performed a review of three observational studies of steroid use in pregnancies with suspected fetal growth restriction and four with small for gestational age (less than 10th centile). In total, 586 fetuses were exposed to corticosteroids and 481 were not exposed to corticosteroids (Torrance *et al.*, 2009). There were no significant differences in incidence of respiratory distress syndrome, brain injury (intraventricular haemorrhage, periventricular leukomalacia, intracranial haemorrhage) or necrotizing enterocolitis between the steroid exposed and non-exposed infants (odds ratios 0.76 [95% CI 0.52–1.11], 1.10 [0.58–2.07] and 0.82 [0.44–1.67] respectively). The authors concluded that, on the basis of this evidence, there is no benefit of antenatal corticosteroid administration in women with suspected fetal growth restriction.

Most of the studies in the Torrance review were retrospective, but one was a carefully designed case–control study of 62 growth-restricted infants who received antenatal corticosteroids between 24 h and 7 days prior to delivery, matched by birthweight, sex and year of birth to 62 infants who did not receive antenatal steroids (Schaap *et al.*, 2001). Infants were prospectively followed up to determine disability. The odds of survival without disability or handicap at 2 years corrected age were higher in the corticosteroid group compared with the control group (OR 3.2, [95% CI 1.1–11.2]). At school age, steroids were associated with a negative effect on physical growth (OR 5.1, [95% CI 1.4–23.8), but no differences in behaviour were identified. The authors concluded that the potential benefits of antenatal corticosteroid use outweigh the potential risks. Current national guidelines on steroid administration, which generally support the use of antenatal corticosteroids in women with suspected fetal growth restriction at risk of preterm delivery, agree with this conclusion.

Animal studies have suggested both potential benefits and harms of clinically relevant doses of corticosteroids in models of growth restriction. For example, in sheep where fetal growth restriction was induced by ligation of a single umbilical artery, betamethasone increased surfactant protein expression and morphological changes in lung tissue, despite higher cortisol levels in the growth-restricted fetuses (Miller *et al.*, 2012). This suggests that exogenous steroids have potential to enhance lung maturity, even when endogenous steroids are high secondary to placental insufficiency and growth restriction. However, betamethasone administration to sheep with growth restriction induced by single umbilical artery ligation has been seen to increase oxidative brain damage when compared with twin controls without growth restriction (Miller *et al.*, 2007), and to significantly reduce weight in both growth restricted and control ovine fetuses (Miller *et al.*, 2012).

Effects of antenatal corticosteroids on feto-maternal blood flow are evident in human growth-restricted fetuses. In Doppler studies, antenatal steroid administration decreases umbilical artery resistance to blood flow in approximately 60–70% of growth-restricted preterm fetuses, as evidenced by restoration of absent end-diastolic flow (Wallace and Baker, 1999; Guerin *et al.*, 2009). Fetuses that do not respond to steroids in this way have worsened neonatal respiratory outcomes (requirement for assisted ventilation, longer duration of assisted ventilation and oxygen requirement) (Guerin *et al.*, 2009). However, it remains to be determined if alterations in umbilical artery blood flow, which may be at the cost of cerebral blood flow, may have other detrimental effects to growth-restricted fetuses.

In summary, there is no conclusive evidence that antenatal corticosteroids benefit growth restricted fetuses, and there is potential for increased effects on fetal programming, compounded by exogenous steroid use on a background of high endogenous steroids. Nevertheless, current recommendations, based mainly on observational studies, endorse the clinical use of antenatal corticosteroids in growth-restricted fetuses delivered preterm, and they are frequently given in clinical practice.

Late preterm and early term gestations and Caesarean delivery

The risk of respiratory morbidity declines as gestation increases, but there is an excess of neonatal unit admissions of newborn infants with respiratory distress or transient tachypnoea at late preterm (34–36 weeks) and early term (37–38 weeks) gestations, particular with surgical deliveries. Although the individual risks are small, with around 8% of babies delivered at 34–37 weeks requiring intensive care, at a population level this is a significant disease burden. The rationale for ANS administration when delivery is anticipated or planned prior to 38 weeks gestation is to decrease respiratory morbidity, although there is a lack of good quality trial data to support this practice.

In the Roberts and Daziel (Roberts and Dalziel, 2006) meta-analysis of clinical trials of ANS on the effects by gestational age at trial entry, RDS was decreased when steroids were administered at 33 to 34 + 6 weeks (RR 0.53, 95% CI 0.31–0.91, two studies, 434 infants), but not at 35 to 36 + 6 weeks (RR 0.61, 95% CI 0.11–3.26, one study, 189 infants) or at greater than 36 weeks. Similarly, there was no benefit for neonatal survival or chorioamnionitis at 33 weeks' gestation onward. Porto *et al.* subsequently performed a small randomized control trial of betamethasone versus placebo in women with imminent late preterm delivery in Brazil (Porto *et al.*, 2011). They found no differences in the primary outcomes of RDS or transient tachypnoea of the newborn between the two groups (Respiratory distress 2/163 [1.4%] in the corticosteroid group versus 1/157 [0.8%] in the placebo group; $P = 0.54$; transient tachypnoea 34/163 [24%] in corticosteroid group versus 29/157 [22%] in placebo group; $P = 0.77$). The NICHD Maternal Fetal Medicine Units Network in the USA is completing a prospective randomized trial of antenatal steroids or placebo in 2800 pregnancies likely to deliver between 34–36 weeks gestation (Clinical Trial ID NCT01222247). This trial will be powered to evaluate ANS effects on common complications including pre-eclampsia and diabetes.

ANS have been administered at even later gestational ages, prior to Caesarean delivery, to avoid a two-fold increased risk of neonatal unit admission for respiratory problems after planned Caesarean delivery when compared with vaginal delivery (Kolas *et al.*, 2006). Stutchfield *et al.* evaluated the benefit of antenatal betamethasone versus no treatment in patients delivered by elective Caesarean section at term in a pragmatic randomized trial (Stutchfield *et al.*, 2005). Betamethasone decreased admissions to the special care nursery for respiratory distress (RR 0.46, CI 0.23–0.93) compared with no treatment. There were no significant reductions in individual respiratory morbidities. The findings are suggestive of benefit, but the study was open label and not placebo controlled, and therefore vulnerable to bias. The absolute risk of respiratory

morbidity at term is low in term neonates, even after elective Caesarean delivery, therefore the number needed to treat to prevent one case of RDS would be high. The number needed to treat has been estimated at 145 for gestations after 34 weeks, versus around 5 for infants at 30 weeks (Kamath-Rayne *et al.*, 2012).

Low-resource settings

Prematurity is a global problem, with middle and low development countries reporting some of the highest rates of preterm birth that result in very high infant mortality (March of Dimes, 2012). Whether antenatal corticosteroid efficacy and safety data from high-resource settings can be applied to the developing world is a question of great importance, especially in light of recent findings associating antenatal corticosteroid administration with an increased risk of death in large fetuses exposed to ANS (Althabe *et al.*, 2015). Of the 21 studies considered in the meta-analysis of Roberts and Daziel, none were from countries represented in the lowest grade (low human development) of the United Nations Development Program 2014 Human Development Index (HDI). Of the 21 studies, 17 were from countries in the highest (very high human development) category of human development and only one study was from a country (South Africa) ranked outside the top 100 developed countries (Program, 2014). Although both the United States and Brazil (from which 11 studies included in the review were drawn) report high rates of preterm birth, there is a lack of efficacy data from low development countries, notably in Southern Asia and Sub-Saharan Africa, which feature strongly in preterm birth and mortality statistics (March of Dimes, 2012).

McLure and colleagues have called for studies to evaluate ANS use in low-resource settings, noting that (p.215) 'For births occurring in hospitals in low-income countries without high-level neonatal care or for births outside hospitals, no studies have been conducted to evaluate prenatal corticosteroid use' (McClure *et al.*, 2011). The South East Asia - Optimising Reproductive and Child Health in Developing Countries (SEA-ORCHID) project performed a retrospective audit of ANS use in nine hospitals from Indonesia, Thailand, Malaysia and the Philippines. The inter-country variation in ANS use was high; 73% of Thai, but less than 10% of Indonesian women who gave birth before 34 weeks' gestation were reported to have received ANS. Infants exposed to ANS were also less likely to be still born or die in hospital prior to discharge compared with those who were not exposed. However, they also found that if an infant was born alive, it was more likely to survive to discharge if it had not been exposed to antenatal corticosteroids, and the infant's mother was less likely to develop either post-partum haemorrhage or pyrexia if not treated with ANS therapy (Pattanittum *et al.*, 2008).

These findings are echoed by the more recent data presented by Althabe and colleagues from their cluster randomized trial to evaluate ANS administration in rural and semi-urban settings in Argentina (49th in 2014 HDI Ranking), Guatemala (125th in 2014 HDI Ranking), India (135th in 2014 HDI Ranking), Pakistan (146th in 2014 HDI Ranking) and Zambia (141st in 2014 HDI Ranking). The authors reported that (p.636) 'among the entire population, the intervention resulted in a significant increase in neonatal deaths of 3.5 per 1000 live births and an increase in perinatal deaths of 5.1 per 1000 live births', with no benefits in infants greater 5th percentile for weight and increased mortality in infants at and above the 25th birthweight percentile. Interestingly, and in keeping with the SEA-ORCHID study data, the intervention was also associated with a statistically significant 0.8% increase in suspected infection among all women (Althabe *et al.*, 2015). The reasons for these concerning outcomes are unclear; a lack of efficacy in the absence of tertiary neonatal intensive care, difficulties in accurately predicting women at risk of preterm birth and errors in determining gestational age may each have contributed to these findings. Socio-economic factors including sub-optimal maternal nutrition, variable access to clean water and quality housing, and variable hospital infection control practices may also contribute to both the lack of efficacy and increase in suspected maternal infection. Similarly, the use of these agents in high-resource settings with improved antibiotic coverage and aseptic practice may camouflage an increased susceptibility to infection. What is clear, however, is that new research into the use of ANS that considers pregnancy-specific factors and medical resources are needed.

In addition to efficacy and safety per se, healthcare providers need to have adequate information to appropriately administer ANS therapy, an additional challenge in low- and middle-resource settings. Potential concerns regarding appropriate use of ANS therapy are highlighted by a study of midwives and physicians in Latin America, which reported substantial differences in ANS administration practice, including in the use of repeated courses of steroids (Aleman *et al.*, 2013). Similarly, the authors of a recent Chinese study concluded that ANS were often underprescribed to women at risk of preterm birth and inappropriately prescribed to women after 35–36 weeks of pregnancy (Wang *et al.*, 2014).

Timing of steroids and multiple courses

ANS are of benefit if delivery occurs between 24 h and 7 days after treatment administration (Roberts and Dalziel, 2006). Delivery outside of this timeframe is associated with increased risk of adverse outcomes (McLaughlin *et al.*, 2003a). However, the optimal timing for administration of antenatal corticosteroids is hampered by the imprecise identification of women at risk of preterm delivery and the likely remaining latency. The majority of preterm births are spontaneous (i.e. preceded by spontaneous contractions or rupture of membranes) (Norman *et al.*, 2009), but the signs and symptoms of preterm labour are non-specific and false positive diagnoses are common. In a randomized control trial of antibiotics for prevention of preterm labour (ORACLE), nearly 80% of women 'diagnosed' with preterm labour delivered after 37 weeks (Kenyon *et al.*, 2001). Conditions such as pre-eclampsia and intrauterine growth restriction that may necessitate medically indicated preterm delivery are variable in presentation and progression. Evidence supports prolonging a preterm gestation until the benefits of delivery clearly outweigh the risks, thus delivery may be delayed for some time after initial diagnosis (Stock *et al.*, 2012). The recommendations are to give ANS at the first signs of impending delivery or potential indication for medically indicated PTB. Given the clear advantages of ANS to babies born preterm, it is understandable that a treat-all approach is often taken, but this is at the expense of optimally-timed ANS administration. The magnitude of the problem of timing ANS administration is demonstrated in a recent population study from Canada, which reported that as uptake of appropriate ANS increased over time, so did inappropriate administration (Razaz *et al.*, 2015). Despite well-developed maternity care systems and guidelines for administration for steroids, more than half of women who received steroids in 2012 delivered at 35 weeks of gestation

or greater (Razaz *et al.*, 2015). Difficulty in predicting preterm delivery results in unnecessary steroid exposure to babies who eventually deliver at term. It also leads to uncertainty regarding the best course of management if delivery does not occurs within 7 days, but high risk of preterm delivery persists.

The administration of multiple courses of antenatal corticosteroids remains a matter of contention. On the one hand, a number of clinical and experimental studies have reported fetal growth restriction and alterations in organ (notably brain) development and childhood behaviour in association with repeated ANS administration (French *et al.*, 1999; Huang *et al.*, 1999; Braun *et al.*, 2013; Moisiadis and Matthews, 2014). On the other hand, the argument for repeated ANS administration is made by a meta-analysis undertaken by McLaughlin and colleagues, which reported that some 40% of women who receive a single course of ANS do not deliver within 7 days of treatment. Two observations support the argument for administering repeated doses of ANS: (i) the beneficial effects of ANS therapy on preterm respiratory function appear to be lost approximately 7 days after completion of the initial treatment; and (ii) delivery more than 7 days after a single course of treatment is associated with an increased risk of perinatal death and maternal infection (McLaughlin *et al.*, 2003b).

A number of large, well-controlled studies have now been repeated to determine the efficacy and safety of repeated ANS administration. Several excellent structured reviews, including that by Crowther and colleagues for the Cochrane Collaboration, provide an in-depth composite picture of the outcomes. The repeated administration of corticosteroids is associated with a small but significant decrease in RDS and a reduction in serious adverse infant outcomes (Crowther *et al.*, 2006; Peltoniemi *et al.*, 2011). One of the central issues relating to the repeated administration of ANS is that the dose for repeated courses of antenatal steroids has not been empirically optimized. Some trials have simply repeated the initial treatment used by Liggins and Howie in 1972 (Liggins and Howie, 1972) whereas others have used modified treatment schedules.

The Australasian Collaborative Trial of Repeat Doses of Steroids (ACTORDS) Study enrolled 962 women who remained at risk of preterm delivery 7 or more days following ANS. This hospital-based study investigated the use of a single, weekly dose of 11.4 mg betamethasone compared with saline placebo for up to 32 weeks gestation. The study reported reduced respiratory distress, and reduced severe lung injury with repeated doses of corticosteroids. Interestingly, the significant reductions in weight, length and head circumference at birth in babies exposed to repeated corticosteroid treatment were not maintained at discharge (Crowther *et al.*, 2006). Of note is the authors' observation that there was no difference in perinatal mortality between the repeated steroid and placebo groups, and that the causes of death were (p.1916) 'much the same between the two groups' (Crowther *et al.*, 2006). This finding is a significant departure from the original trial conducted by Liggins and Howie, which demonstrated a significant reduction in perinatal mortality between a single ANS course infants and those receiving placebo control (3.2 versus 15%, respectively; $P = 0.01$) (Liggins and Howie, 1972). Assuming that repeated ANS administration does indeed convey a benefit to the preterm infant, the reasons for the similarity in risk of death between groups are unclear. Advances in neonatal care over the past four decades (especially with regards ventilation and surfactant therapy) contribute to increased survival and blunt any benefit from repeated corticosteroid therapy. Whether or not this finding of equivalence would be replicated in a patient population drawn from mid and low-resource settings is of interest, given the high preterm birth and mortality burden in the developing world.

In a 2-year follow-up to the ACTORDS study, the authors concluded that aside from an increase in treatment for attention problems, there were no statistically significant differences in body size, blood pressure or health service utilization between children exposed to repeated ANS and those exposed to placebo (Crowther *et al.*, 2007). The long-term outcomes of the small acceleration in post-natal growth identified in a subset of ACTORDS infants exposed to multiple courses of ANS remain unknown, but may be of concern (Battin *et al.*, 2012). There is a strong body of epidemiological data demonstrating an association between low birthweight, accelerated neonatal growth and chronic diseases including type-2 diabetes, obesity, cardiovascular disease, hypertension and depression (van Deutekom *et al.*, 2013). Whilst noting the need for further, long-term follow-up, the authors concluded that the equivalence of 2-year survival free of major neurosensory disability supports consideration of administering a weekly treatment with a single dose of betamethasone in women who remain at risk of very preterm delivery 7 or more days after their initial corticosteroid treatment. Additional reassurance is provided by data from a 6–8 year follow-up, suggesting no association between repeat antenatal corticosteroid exposure and cardio-metabolic disease risk in early school age (McKinlay *et al.*, 2015). However, an alternative view is that these rigorous clinical studies in fact support the adoption of a more conservative position. In light of ongoing concerns regarding administration of multiple courses of ANS discussed below, a demonstrated equivalence in perinatal morbidity and overall childhood wellbeing argues in favour of not administering repeated doses of ANS to women at continued risk of preterm delivery.

The multiple courses of antenatal corticosteroids for preterm birth study (MACS) evaluated the effect of repeated courses of ANS given at two weekly intervals in high-risk women who had failed to deliver 14–21 days after initial treatment (Murphy *et al.*, 2008). The study involved 1858 women drawn, importantly, from a number of countries with differing levels of economic development and large differences in perinatal mortality rates. Repeat dosing was not continued beyond 33 weeks gestation and the majority of women (87%) in the active treatment group received betamethasone. There was no difference in a composite primary outcome (perinatal mortality and morbidities associated with prematurity including severe respiratory distress syndrome, neurological injury, haemorrhage and necrotising enterocolitis) between the corticosteroid and placebo groups. However, there were small but significant reductions in weight, length and head circumference at birth in infants exposed to repeated courses of antenatal corticosteroids. Nearly 75% of women in the repeated corticosteroid group received two or fewer repeated courses; a secondary outcome analysis identified that for each additional course of ANS, birthweight, length and head circumference decreased further (Murphy *et al.*, 2012). A pre-discharge study assessing the effect of repeated ANS exposure on auditory brainstem response in preterm infants found no difference in brain maturity or auditory function but did identify significant reductions in birthweight, length, and head circumference in association with repeated antenatal corticosteroid exposure (Church *et al.*, 2010).

The subsequent 5-year follow-up to the MACS study concluded that there were no significant differences in the risk of neurodevelopmental disability or death (Asztalos *et al.*, 2013). Interestingly, when gestational age at birth was taken into account, exposure to repeated courses of

ANS was associated with a statistically significant increased risk of neurodevelopmental/neurosensory impairment by 5 years of age for infants exposed to ANS and delivered close to term (Asztalos *et al.*, 2014). These conflicting data suggest that, if there are indeed changes in fetal neurodevelopment as a result of repeated antenatal corticosteroid exposure, then any adverse effects may be partially dependent on the gestational age at which the infant was born and also the post-natal age that neurosensory function is assessed. Additionally, sex-dependent effects of antenatal corticosteroid exposure were observed in juvenile baboons (Rodriguez *et al.*, 2011), and dose-dependent delays in auditory nerve and brainstem transmission times were demonstrated in juvenile Wistar rats exposed to repeated doses of betamethasone in late pregnancy (Church *et al.*, 2012).

Neurological effects of synthetic corticosteroids in pregnancy

Long-term follow-up studies of children at risk of congenital adrenal hyperplasia and treated with dexamethasone in early pregnancy have suggested variable effects on brain function. Meyer-Bahlburg performed a questionnaire survey of 174 children exposed prenatally to dexamethasone (including 48 with congenital adrenal hyperplasia) and 313 unexposed children (including 195 with congenital adrenal hyperplasia) between 1 month to 12 years of age (Meyer-Bahlburg *et al.*, 2004). They found no significant effect of dexamethasone treatment on nine different social or developmental scales. These results conflict with those of a prospective follow-up study, of 40 children treated prenatally with dexamethasone and aged 7–17 years, which included neuropsychological testing as well as validated child and parental questionnaires (Hirvikoski *et al.*, 2007). In this study, no differences were seen in intelligence or long-term memory, but children without congenital adrenal hyperplasia who had received prenatal dexamethasone had poorer verbal working memory, poorer self-perception of scholastic competence (both $P = 0.003$), and increased self-rated social anxiety ($P = 0.026$). Related pilot data from this study report that the parents of prenatally treated children described them as being more sociable than controls ($P = 0.042$) and reported effects on gender role behaviour in boys (Hirvikoski *et al.*, 2008, 2011). These studies suggest possible negative cognitive and behavioural effects resulting from steroids in early pregnancy. Although further long-term studies are needed, the potential long-term effects on brain function should be considered when weighing up the risks and benefits of early pregnancy steroid use.

Effects of corticosteroids on fetal programming

The intrauterine environment plays a key role in regulating fetal growth and development; data, predominantly from rodent studies, also suggest that it can also program health throughout life. Corticosteroids are key mediators in this process, and excess exposure to antenatal corticosteroids is associated with adverse pregnancy outcomes, including reduced birthweight, and a host of persistent changes in hypothalamic–pituitary–adrenal (HPA) axis programming that manifest as elevated stress responses, hypertension and changes in glucose metabolism, behaviour and motivation (Reynolds, 2013; Moisiadis and Matthews, 2014).

Precisely how excess exposure to antenatal corticosteroids might affect pathological changes on the fetus remains unclear; however, several excellent reviews have recently highlighted the potential roles of the fetal HPA axis and the placenta (Braun *et al.*, 2013; Moisiadis and Matthews, 2014). Impaired HPA axis function is associated with changes in behaviour and neurodevelopment along with an increased risk of chronic cardiovascular and metabolic diseases. Antenatal steroid exposure appears to differentially affect basal cortisol activity in neonates based on fetal sex, post-natal age at which studies are undertaken, and whether the infant was delivered preterm or at term (Moisiadis and Matthews, 2014). Interestingly, a 30-year follow-up of adults exposed to a single course of antenatal corticosteroids concluded that treatment did not adversely impact cardiovascular function but might cause insulin resistance (Dalziel *et al.*, 2005). There are limited human data available on the long-term effects of repeated antenatal corticosteroid exposure on HPA axis function, although studies in sheep suggest that changes in HPA function associated with repeated antenatal corticosteroid exposure persist into adulthood (Moisiadis and Matthews, 2014).

Antenatal corticosteroids may also unbalance maturational effects in certain systems by selective stimulation of GR. At physiological concentrations, endogenous corticosteroids bind to the MR with 10 times greater affinity for MR than GR (Funder, 1997). In contrast, synthetic corticosteroids selectively activate GR. In general, mineralocorticoid target tissues co-express 11 Beta-HSD-2, which converts cortisol to inactive cortisone to prevent unwanted stimulation of MR by cortisol (Chapman *et al.*, 2013). However, some of the functions of endogenous corticosteroids that mediate maturation of the developing fetus are signalled through MR not GR. For example, fetal heart maturation is mediated by both GR and MR effects (Rog-Zielinska *et al.*, 2013) and hippocampal MR is important in the negative feedback of cortisol. There is thus the potential that dexamethasone or betamethasone may perturb maturation in extra pulmonary systems by only stimulating GR (Rog-Zielinska *et al.*, 2014).

Relative to late pregnancy, surprisingly little is known about the effects of GC exposure on the developing fetus in early pregnancy. Using a sheep model of pregnancy, Dodic and colleagues reported that transient (2 day) exposure to dexamethasone at Day 27 of pregnancy resulted in hypertension, left ventricular hypertrophy, and reduced cardiac function in adult offspring (Dodic *et al.*, 2001). The same group demonstrated changes in nephrogenesis in adult sheep exposed to corticosteroids in early pregnancy (Wintour *et al.*, 2003). Antenatal dexamethasone exposure in early pregnancy has been demonstrated to alter HPA axis function and maturation in fetal lambs (Braun *et al.*, 2009; Li *et al.*, 2012) More recently, Braun and colleagues have demonstrated sex-specific effects on placental binucleate cell numbers and transient changes in pro-apoptotic factors and fetal growth in association with antenatal dexamethasone exposure at 40–41 days' gestation (Braun *et al.*, 2015).

Pregnancy-specific factors and antenatal corticosteroid effects

If we are to understand how we might refine the use of antenatal corticosteroids then we must attempt to clarify how the maternal and fetal responses to such treatment might be modified by preterm birth-associated factors. Factors including gestational age, maternal diet, the presence of intrauterine infection, and fetal sex have each been

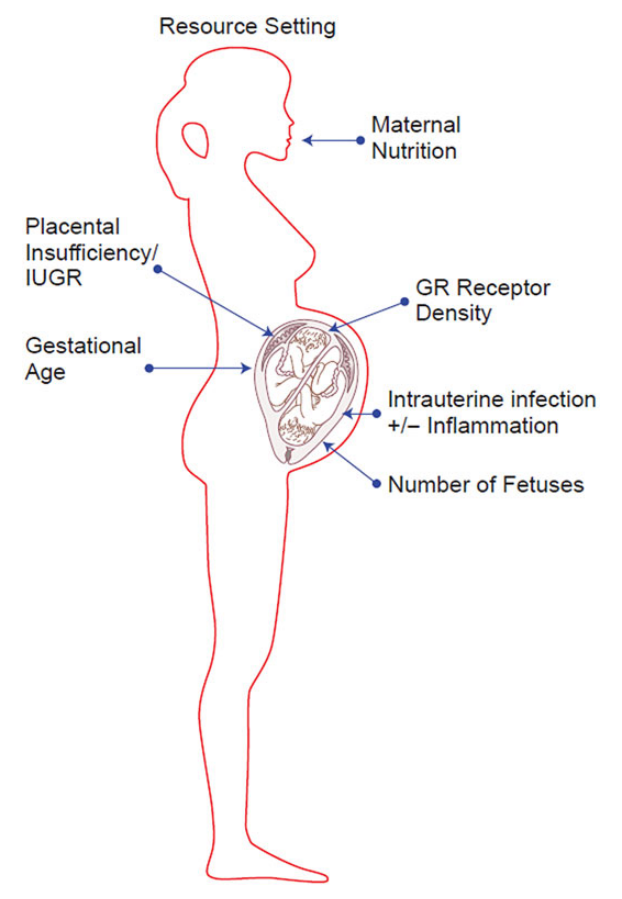

Figure 3 Factors that may influence the effectiveness of antenatal corticosteroids in reducing morbidity and mortality associated with prematurity.

implicated as potential modulators of corticosteroid effects (Fig. 3). The scope for refining the use of ANS is highlighted by the observation that, under current obstetric protocols, a 60 kg women carrying a 33 week fetus would be given the exact same the same dose of ANS as a 120 kg woman carrying a 24 week fetus in an attempt to improve neonatal outcomes.

GR receptor density

There are good data to suggest that GR density in target organs is critical in determining the level of response, or resistance, to corticosteroid stimulation. Accordingly, the ontology of GR receptor expression in the developing fetal lung should also be taken into account in future studies investigating gestation-specific antenatal steroid dosing. mRNA studies in sheep have suggested that GR expression in the fetal lung is higher at 140 days gestation than at 80 days gestation, although it was unclear from this study if differences in mRNA expression materially altered GR receptor density (Gnanalingham *et al.*, 2005). In *in vitro* experiments using lung slices from human fetuses aged between 16 and 20 week' gestation, Ballard and Ballard demonstrated preferential, high-affinity binding of labelled dexamethasone to a cytoplasmic macromolecule. Nuclear localization of labelled dexamethasone was also dependent on the cytoplasmic receptor. The lung is the human fetal tissue with the highest GR concentration and the GR is expressed from as early as 12 weeks gestation through to term. GR expression was also detected in the human fetal liver, kidney, heart, intestine, muscle and skin (Ballard and Ballard, 1974, 1995). GR mRNA expression was detected in the fetal rat brain from embryonic day 12.5 and its expression increased throughout the brain towards the end of the third trimester, underscoring the ubiquitous nature of its expression (Diaz *et al.*, 1998). Interestingly, Ballard and Ballard concluded that with regards the developing lung (p.482), 'there was no apparent effect of fetal age on the concentration of receptor sites' (Ballard and Ballard, 1974). This observation is supported by the findings of Labbe *et al.* who reported that human fetal GR concentration remains high (182 ± 88 fmol/mg protein) irrespective of gestational age (Labbe *et al.*, 1990). More recently, Rajatapati and colleagues surveyed GR expression in human fetal lung samples collected at 16 (pseudoglandular phase), 21.8 (cannicular phase), 30.4 (saccular phase) and 39 (alveolar phase) weeks of gestation. Using immunohistochemistry, they identified nuclear GR reactivity in all samples analysed and, interestingly, that the GR mRNA concentration did not significantly differ with gestational age (Rajatapiti *et al.*, 2005).

Maternal nutrition

Maternal nutrition is one factor that may warrant consideration in any future optimization of antenatal corticosteroid dosing regimens, both in low-resource settings with maternal nutrient restriction and in the increasingly obese developed world. In a study of 1661 Australian women who gave birth between 2002 and 2005, 43% were classified as either overweight or obese (Athukorala *et al.*, 2010). Overweight or obese women are at an increased risk of gestational diabetes, potentially resulting a hyperglycaemic, hyperinsulaemic fetus. Maternal diabetes is associated with suppressed surfactant production, and Dekowski and colleagues have suggested that insulin has an inhibitory effect on SP-A and SP-B mRNA accumulation (Dekowski and Snyder, 1995). Infusion the ovine fetus with glucose over a period of 10 days results in reduced pulmonary 11β-hydroxysteroid dehydrogenase I, GR and surfactant protein mRNA, all changes which may correlate with reduced corticosteroid responses (McGillick *et al.*, 2014).

In sheep, maternal nutrient restriction did not impact lung growth but did result in small increases (relative to animals maintained on normal diets) in lung GR mRNA expression at 80 and 140 days gestation in singleton lambs (Gnanalingham *et al.*, 2005). Interestingly, studies in sheep have suggested that maternal undernutrition is associated with substantial changes in GR methylation and increased hypothalamic GR mRNA and protein expression in both male and female offspring (Begum *et al.*, 2013). In rodent studies, a 50% reduction (9 versus 18% total diet) in maternal dietary protein throughout pregnancy is associated with increased GR mRNA and protein expression in the fetal lung, kidney, liver and brain (Bertram *et al.*, 2001). In baboons, maternal nutrient restriction resulted in sex-dependent changes in GR mRNA, but not protein, expression. There were also increases in 11β-hydroxysteroid dehydrogenase 1 and hexose-6-phosphate dehydrogenase mRNA and protein in female fetal perirenal adipose tissue and male fetal liver tissue (Guo *et al.*, 2013). How sub-optimal maternal nutrition might impact the fetal maturational effects mediated by corticosteroids remains poorly understood. However, the accumulating body of evidence suggests that maternal diet can influence the corticosteroid signalling machinery.

Infection and inflammation

Whether or not infection might alter fetal and maternal responses to antenatal corticosteroid therapy is another important consideration. Intrauterine infection and inflammation are strongly associated with preterm birth, especially those deliveries occurring at or below 32 weeks of gestation (Goldenberg *et al.*, 2008b; Kemp, 2014; Romero *et al.*, 2014). Histologic chorioamnionitis is the hallmark of intrauterine infection most commonly identified in early gestation deliveries (Lahra and Jeffery, 2004). Intrauterine infection is frequently polymicrobial and access of organisms to the fetal environment has been suggested to occur following a discrete breach in the chorioamniotic membranes (Jones *et al.*, 2009; Kim *et al.*, 2009). *Ureaplasma* species are commonly isolated from the gestational tissues in preterm birth (Goldenberg *et al.*, 2006, 2008a) and, in experimental studies, *Ureaplasma* have been shown to have the ability to modulate fetal inflammatory signalling (Kallapur *et al.*, 2010). In the sheep model of pregnancy, chronic *Ureaplasma* infection increased surfactant production and had a surfactant-independent additive effect on lung compliance following a single course of antenatal betamethasone (Moss *et al.*, 2009). Similar additive improvements in fetal ovine lung compliance in the absence of increased surfactant production were identified following the co-administration of maternal betamethasone with intraamniotic lipopolysaccharides from *Escherichia coli* (Newnham *et al.*, 2001).

Differential effects of infection and ANS on pulmonary growth factors may play a role in these observed differences. For example, transforming growth factor (TGF)-βs are a family of three (TGF-β1, TGF-β2, and TGF-β3) soluble signalling peptides, expressed in the developing lung, that influence endothelial-mesenchymal interactions during lung development (Zeng *et al.*, 2001). Ventilated lungs of preterm baboons infected antenatally with *Ureaplasma urelyticum* had increased concentrations of TGF-β1 and more extensive fibrosis than uninfected animals (Viscardi *et al.*, 2006). Overexpression of TGF-β1 inhibits lung morphogenesis and differentiation in addition to inhibiting the expression of SP-B and SP-C (Zeng *et al.*, 2001). Increased expression of TGF-β in the distal lung promotes oedema by reducing fluid clearance and epithelial sodium uptake (Hallman *et al.*, 2002). Experiments in sheep have shown an increase in transforming growth factor (TGF)-β activity following exposure to LPS-induced chorioamnionitis (Kunzmann *et al.*, 2007).

Using cultures of human and rat type-II alveolar epithelial cells, Roux and colleagues demonstrated that IL-1β, a key mediator of pulmonary inflammation, inhibited αENaC expression via p38 mitogen activated protein kinase (MAPK) (Roux *et al.*, 2005). Corticosteroids modulate the production of TGF-β; hydrocortisone and dexamethasone down-regulated TGF-β2- and TGF-β3-driven induction of TGF-β1 (Wen *et al.*, 2003) and dexamethasone was shown to be more effective than hydrocortisone in inhibiting TGF-β2-driven TGF-β1 induction. In fetal sheep, antenatal corticosteroids did not alter TGF-β itself, but did modulate TGF-β signalling; a 7 day exposure to intraamniotic LPS increased levels of the signalling molecule phosphorylated SMAD2 in fetal alveolar tissue and this increase was attenuated by treatment with betamethasone (Kunzmann *et al.*, 2014).

Fetal sex

Fetal sex is known to influence the risk of adverse outcomes in response antenatal corticosteroid therapy. The underlying mechanisms remain poorly understood, although sex-specific differences in the placenta have been hypothesized as being important in determining fetal responses to antenatal insults and prematurity (Clifton, 2010). Female premature infants respond more effectively to antenatal betamethasone exposure than their male counterparts, with lower rates of respiratory distress syndrome (Papageorgiou *et al.*, 1981; Anadkat *et al.*, 2012). This observation from clinical studies has been replicated in a sheep model of prematurity; a retrospective analysis of fetuses that received 0.5 mg/kg betamethasone demonstrated improved lung function irrespective of sex, but with significantly greater increases in compliance, lung volume, arterial oxygen partial pressure and conductance for female compared with male fetuses (Willet *et al.*, 1997). The observed difference in corticosteroid responsiveness between male and female fetuses does not appear to derive from differences in lung structural development or surfactant production between the sexes (Ishak *et al.*, 2014). Studies of the fetal programming of hypertension in sheep have shown that betamethasone exposure has a sex-dependent effect on endothelin-1 responsiveness (Lee *et al.*, 2013). Zuloaga *et al.* have hypothesized that, in the post-natal period, the female brain may be (p.542) 'somewhat more vulnerable to GC (corticosteroid) effects' (Zuloaga *et al.*, 2011). In baboons, repeated antenatal betamethasone exposure reduces motivation in both male and female offspring; interestingly, reductions in reversal task performance were more pronounced in female offspring compared with males (Rodriguez *et al.*, 2011).

Summary and conclusions

The use of antenatal steroids has prevented the loss of thousands of pregnancies, and prevented significant morbidity in many more. However, it is also clear that ANS are not innocuous, and may have significant and long lasting effects on health. Maternal and fetal factors, as well as the healthcare setting, can influence the risk benefit ratio for ANS. There is surprisingly little data available to inform drug dosing and timing for early pregnancy complications such as recurrent miscarriage and congenital adrenal hyperplasia. Even in obstetric medicine, which possesses the strongest empirical evidence base for ANS administration, there are still only two ANS dosing regimens, neither of which have been significantly refined to determine the optimal dose or treatment interval since their clinical introduction. In the era of personalized medicine, we must move away from a one-size fits all approach. There is a need to trial new formulations to maximize benefits and minimize unwanted side effects, provide better delivery systems and dosage, and tailor treatment to individual circumstances, ensuring delivery of the right ANS, to the right pregnancy and at the right time. In this way, we can realize the full potential of this life saving, and morbidity sparing treatment.

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Authors' roles

M.W.K., A.H.J. and S.J.S. wrote the manuscript. M.W.K., A.H.J., J.P.N., J.G.C. and S.J.S. reviewed, edited and approved the manuscript.

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Conflict of interest

The authors declare no conflict of interest. The authors have no financial interest in the contents of this manuscript. Organizations funding the authors not have input to the drafting of this manuscript.

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