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The Complex Challenge of Antenatal Steroid Therapy Non-Responsiveness

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N/A
The efficacy of antenatal steroid therapy is highly variable such that an important percentage of babies treated do not benefit.
Antenatal steroid (ANS) therapy is standard care for women at imminent risk of preterm delivery. When deliveries occur within seven days of treatment, ANS therapy reduces the risk of neonatal death and improves preterm outcomes by exerting diverse developmental effects on the fetal organs, in particular the preterm lung and cardiovascular system. There is, however, sizable variability in ANS treatment efficacy, and an important percentage of fetuses exposed to ANS therapy do not respond sufficiently to derive benefit. Respiratory distress syndrome (RDS), for example, is a central metric of clinical trials to assess ANS outcomes. In the present analysis, we addressed the concept of ANS non-responsiveness, and defined a failed or sub-optimal response to ANS as being death or a diagnosis of RDS following treatment. For deliveries 24-35 weeks’ gestation, the number needed to treat (NNT) to prevent one case of RDS is 19 (95% CI, 14-28). Reflecting gestation-dependent risk, for deliveries >34 weeks’ gestation the NNT is 55 (95% CI, 30-304), whereas for elective surgical deliveries at term the NNT is 106 (95% CI, 61-421).

We reviewed data from clinical and animal studies investigating ANS therapy to highlight the significant incidence of ANS therapy non-responsiveness (i.e. residual mortality or RDS after treatment), and the potential mechanisms underpinning this outcome variability. Origins of this variability may relate both to the manner in which the therapy is applied (i.e. the treatment regimen itself) and factors specific to the individual (i.e. genetic variation, stress, infection). The primary aims of this review were: i) to emphasise to the obstetric and neonatal communities the extent of ANS response variability and potential impact; ii) to propose approaches by which ANS therapy may be better applied to improve overall benefit; and iii) to stimulate further research towards the empirical optimisation of this important antenatal therapy.
[KEYWORDS]
Preterm birth, fetus, glucocorticoid, betamethasone, dexamethasone, lung maturation, non-responsiveness, variability.
ANS Non-responsiveness: A Pragmatic Definition and Clinical Evidence

The issue for this review is that of preterm babies who do not gain benefit (e.g. still die or have respiratory distress syndrome (RDS)) despite receiving antenatal steroid (ANS) therapy. We have broadly defined non-response as preterm infants exposed to a standard treatment with ANS, but still having mortality and/or RDS. Establishing a definition of ANS non-responsiveness is challenging given the multitude of organ systems modulated by glucocorticoids and difficulty in dissecting a cardiovascular benefit from, for example, a lung maturation benefit in the clinical setting. The task is made further challenging because the benefits of ANS therapy are strongly gestational age-dependent; at 24-35 weeks’ gestation, RDS is a useful marker to treatment efficacy; however, at 35-37 weeks’ gestation, RDS rates in the untreated population are low, and generally benign indications such as transient tachypnea of the newborn are more relevant. Furthermore, it could be reasonably argued that a preterm infant presenting with any form of RDS may have had much more severe RDS (and perhaps other complications, including brain injury), and may subsequently develop bronchopulmonary dysplasia, in the absence of ANS therapy. Similarly, a fetus treated with ANS may die from other preterm birth-related complications, including intrapartum injury, or sepsis. As such, any definition of ANS non-responsiveness will be imperfect.

Our choice of mortality and/or RDS for determining ANS responsiveness is partly pragmatic, as these are among the most readily assessed, clinically-relevant treatment outcomes modulated by ANS therapy. However, this selection is also well aligned with the trial data used to inform ANS recommendations. Mortality and RDS are two key metrics (either individually or as part of a composite outcome) used in the majority of randomised control trials undertaken to date to test ANS treatment efficacy. These are the same data that are then used by various obstetric societies, and policy agencies (including the National Institutes of Health and the World Health Organisation) to make and refine...
clinical recommendations regarding the use of exogenous glucocorticoid treatments in pregnancy. On this basis, we suggest that, whilst acknowledging the inherent limitations of this approach, it is also reasonable to define non-responsiveness in a similar fashion.

The cost-benefit assessment for ANS therapy should include consideration that exogenous glucocorticoids, an agent class with well-established adverse effects on growth and differentiation, are administered to the rapidly developing fetus at a poorly optimised, supra-physiological dose\(^1,2\). US and Canadian Practice Guidelines do not mention ANS non-responders\(^3,4\), suggesting that the neonatal and perinatal community may not appreciate the frequency of non-responders after ANS treatment. Aside from reducing the impetus to further refine this important treatment, the status quo may also result in patients being frequently consented for ANS treatment without being made aware that it may not convey benefit and, in some cases, may increase the risk of harm. ANS non-responders are a substantial problem given the increasing number and gestational range of pregnancies exposed to ANS\(^5,6,7\).

There are two pharmacologic issues that may be used to frame a discussion of ANS non-responsiveness: the pharmacokinetics (PK) of the drugs used for ANS, and the pharmacodynamics (PD) to achieve the desired maturational benefits. We now have a reasonable understanding of the PK of the drugs used for ANS therapy (i.e. dexamethasone phosphate, betamethasone phosphate, and combined betamethasone phosphate and acetate)\(^8,9,10\). We know much less about the optimal drug exposures needed to reliably cause the desired PD effects: lung maturation and other, difficult to isolate effects of steroids that improve neonatal adaptation, decrease intraventricular hemorrhage (IVH), and decrease death.

The magnitude of the clinical problem of ANS non-responsiveness is detailed in Table 1, which lists individual trials and meta-analyses grouped by a 10-year era during which the RCTs were done. In the
most recent meta-analysis (2020)\textsuperscript{11}, for single course treatment, ANS decreased mortality from 5.3% to 4.5% and decreased RDS from 13.2% to 9.9% - of note, this meta-analysis included trials for late preterm infants. Although clearly important, the overall magnitude of the effect results in a large amount of residual mortality and RDS. This meta-analysis also included trials of late preterm pregnancies wherein death, which is not a discriminating outcome with regards ANS treatment efficacy, is rare. The overall number needed to treat for a death benefit was 38 in the McGoldrick et al. 2020 meta-analysis of 22 trials\textsuperscript{11}, which means that many fetuses did not benefit from treatment.

A further challenge is that the PD assessment for death is not stable with time, because death is primarily driven by gestational age (GA) at birth. This outcome is greatly impacted by improving modern obstetric and neonatal practices that decrease death, such as surfactant treatments, new approaches to respiratory support such as continuous positive airway pressure (CPAP), non-invasive ventilation, better in utero surveillance of the fetus, and better delivery timing. It is also important to note that we seldom know the cause of death with any precision to be able to assess if it possibly could have been mitigated by a better ANS response. Causes of death have not been reported in recent studies.

The application of repeat ANS courses also serve to identify the significant potential that exists to improve overall outcomes if the issue of non-responsiveness can be better addressed. Repeat courses should give maximum benefit and minimize non-responders. In the individual patient meta-analysis of a repeat course of ANS, the benefit for avoiding a serious outcome (death, severe respiratory disease) was only a 1.4% absolute difference\textsuperscript{12}. The composite adverse outcome still occurred in 11.4% of the population. For the outcome, ANS decreased respiratory support by 4.8%, again an important difference, but in the single-dose group, 31% still required respiratory support. Clearly, repeated courses of ANS do not obviate the need for respiratory support. We can also conclude that additional doses of corticosteroids do not solve the non-response problem.
The recent WHO trial of 4 doses of 6 mg dexamethasone given every 12 hours for 4 doses versus saline placebo performed effectively in well-resourced hospitals in low and middle-income countries, with death decreased from 331/1406 (23.5%) to 278/1417 (19.6%), a significant reduction p<0.03\(^{13}\). In another trial (the A*Steroid Study) testing the two most commonly used ANS drugs – dexamethasone phosphate (employed in the WHO-recommended standard care protocol in low- and middle-income countries) and combined betamethasone phosphate and acetate (as originally trialled by Liggins and Howie\(^{14}\)), there were no differences in death or neurodevelopment at 2 years\(^{15}\). However, there were also no differences in death or respiratory support requirements, with 24% having RDS after ANS, and equivalent for either drug therapy. Therefore, about one-in-four ANS-treated infants still have RDS, and increasing materno-fetal plasma steroid concentrations (in this instance by the sole use of dexamethasone phosphate) do not improve outcomes. From these data it can be concluded that administering higher doses of steroids is not a means by which treatment responsiveness may be improved.

An additional challenge is that, even when a response is achieved, the durability of benefit (the persistence of the described clinical benefit such as a reduction in death, RDS or IVH) appears to be transient. This is a particular challenge given that the clinical ANS treatment strategy is often to quickly assess a pregnancy in possible preterm labor, administer ANS and then attempt to determine if the presentation was correctly identified as being preterm labor. About 50% of these women will be briefly observed in hospital, discharged, and deliver at term\(^{16}\). For RDS outcome, there is a likely period of benefit lasting from 24 hours to 7 days post-treatment, as originally suggested by Liggins\(^{14}\) and in the 2006 Meta-analysis of Roberts and Dalziel\(^{17}\). The rationale for repeat treatment results from an assumption that one can prevent RDS or death 7 days after initial treatment. For intraventricular hemorrhage (IVH) meta-analyses, IVH was decreased only for a treatment to delivery interval of 24 hours to 48 hours\(^{17}\). These are rough estimates, further confounded by our limited understanding as to how ANS decreases IVH and mortality.
Potential Causes of ANS Non-Responsiveness

Potential causes of the variability observed in responses to ANS treatment may be categorized into two groups: i) those related to the treatment given; and ii) those related to a patient-specific factor or factors (Figure 1). These could involve abnormalities in glucocorticoid pharmacokinetics between mother and fetus, GR signaling, variable responses of the developing lung cells, or the regulation of GR-modulated targets such as surfactant that have been shown to play an important role in functional lung maturation.

As noted above, the treatment to delivery interval is important for ANS treatment efficacy. Although some studies have failed to report a difference in outcome against treatment interval, the general consensus is that the optimal delivery window is 1-7d after initial ANS administration. The gestational age at which ANS are administered also has a pronounced impact on the magnitude and nature of treatment benefit. Considering their assessment of glucocorticoid efficacy against pretreatment amniotic fluid lecithin / sphingomyelin ratios in 1976, Block and colleagues concluded that if the fetal lungs are already mature enough to produce surfactant, then there is nothing to be gained by treating with ANS. Broadly speaking, deliveries at earlier gestations (<34 weeks’ gestation) treated with ANS show significant reductions in RDS and neonatal death, whereas the benefit derived from ANS use at later gestations are largely restricted to reduced oxygen use and improvements in transient tachypnea. Whether protection from these outcomes, which are generally amenable to post-natal management in well-resourced delivery settings, warrant exposure to ANS treatments given even a small risk of deleterious steroid effects, is a topic that deserves further discussion.
Insight into the patient-specific role of ANS non-responsiveness may be gained from the concept of generalized glucocorticoid resistance, defined as a resistance to adrenal suppression following dexamethasone administration\textsuperscript{20}, and observed clinically as Cushing’s syndrome. Resistance to treatment with glucocorticoids has also been identified in a range of diseases including asthma, nephrotic syndrome, and adrenal cortex hyperplasia\textsuperscript{20, 21}. Given the involvement of glucocorticoid resistance in other diseases, it is reasonable to argue that resistance to glucocorticoid signaling may similarly play a role in the efficacy of ANS therapy. Indeed, in light of data linking GR polymorphisms with differential preterm outcomes\textsuperscript{20-24}, and the persistence of RDS in ANS-treated infants, it is not inconceivable that some forms of RDS may eventually be categorized as glucocorticoid-sensitive and glucocorticoid-resistant.

The molecular mechanisms involved in glucocorticoid signaling and regulation are both complex and incompletely understood\textsuperscript{25, 26}. ANS function primarily via their interaction with and activation of the glucocorticoid receptor (GR; \textit{NR3C1}). Free (dephosphorylated) betamethasone and dexamethasone bind with high affinity to the ligand-binding domain of the GR in the cytoplasm. GR/glucocorticoid complexes translocate to the nucleus where they exert transcriptional activation and suppression\textsuperscript{26}.

There are several different GR isoforms, of which GR\textalpha{} and GR\textbeta{} are the primary examples. GR\textalpha{} is the isoform predominantly involved in glucocorticoid signaling. The role of GR\textbeta{} is still not fully understood; it lacks a ligand binding domain, making it unable to bind glucocorticoids or transduce GR-activated signaling. It can, however, dimerize with GR\textalpha{}, and is believed to predominantly exert a negative regulatory effect on GR\textalpha{} activity via the formation of GR\textalpha{}/GR\textbeta{} dimers\textsuperscript{26, 27}.

Potential pregnancy-associated candidates for dysregulation of GR signaling, and thus ANS resistance include in the setting of ANS therapy include: stress\textsuperscript{28}, glucocorticoid receptor mutations resulting in...
loss or reduction in function\textsuperscript{20, 21, 24, 29}, aberrant GR\textalpha or GR\textbeta expression\textsuperscript{30}, interference with GR signaling by immune dysfunction (e.g. inflammation, oxidative stress\textsuperscript{31, 32}), viral infection\textsuperscript{33, 34}, the presence of bacterial proteins including endotoxins\textsuperscript{35}, and variations in mesenchymal cell populations that signal to immature type II alveolar epithelial cells\textsuperscript{5} over gestation.

Maternal stress has been implicated in a range of undesirable pregnancy outcomes, with Cohen and colleagues suggesting that the responses of individual tissues to GR signaling under chronic stress conditions is more important than the circulating level of cortisol itself\textsuperscript{28}. Excess maternal cortisol may cross the placenta and act to modulate fetal development\textsuperscript{36}. Prodanovic and colleagues used a human bronchial epithelial cell line and demonstrated that cortisol acts as a partial agonist at the GR, limiting glucocorticoid-induced GR-dependent transcription\textsuperscript{37}. Interestingly, maternal anxiety has also been associated with reduced placental expression of \textit{ABCB1}, (which encodes multidrug resistance p-glycoprotein (P-gp))\textsuperscript{38} in placental tissue from non-overweight pregnancies and also from placentas of female but not male fetuses\textsuperscript{39}. P-gp is involved in reducing the materno-fetal transfer of exogenous glucocorticoids (including 11\textbeta HSD2-resistant betamethasone and dexamethasone phosphate). Reduced placental P-gp expression occurs in humans with advancing gestational age\textsuperscript{40}, and may correlate with increased materno-fetal transfer of dexamethasone and betamethasone phosphate.

A potential role for glucocorticoid receptor mutations has been assessed in a range of conditions including hypertension\textsuperscript{20}, adrenal hyperplasia\textsuperscript{21}, increased susceptibility to high-altitude pulmonary oedema\textsuperscript{29} and asthma\textsuperscript{41}. Vitellius and Lombes have catalogued thirty one loss-of-function mutations in the GR that are associated with generalized glucocorticoid resistance syndrome (defined as a circulating cortisol concentration of > 50 nmol/L at 8am, the morning after a 1 mg dexamethasone suppression test), including twenty three missense, four frameshift and four nonsense mutations\textsuperscript{20, 21}. Several studies have sought to use \textit{in vitro} approaches (predominantly transfection studies using glucocorticoid response element regulated reporter constructs) to assess the potential functional
impact of these GR mutations. GR functional changes including reductions in transactivation, nuclear importation and DNA binding have all been identified in association with the GR polymorphisms identified to date. For example, nine GR mutations (e.g. R469X, R491X, D641V, Y660X, L672P, I757V) are shown to prevent dexamethasone binding and thus interfere with nuclear translocation.

A study of 117 infants reported a significant association between GR SNP rs41423247 (OR, 2.56; 95% CI, 1.11–5.95; P = .02) and the development of BPD. Schreiner and colleagues also reported an association between the GR polymorphism rs41423247 (BclI) and an increased risk of BPD in very low birthweight infants, whereas Bertalan reported no association between the BclI polymorphism and BPD, but did find a significant association with increased birth weight.

Bacterial or viral intrauterine infection are strongly associated with preterm birth, notably in higher-risk deliveries occurring before 32 weeks’ gestation. Webster and colleagues demonstrated that, in vitro, TNF-α increased the steady state protein levels of GR-β, resulting in glucocorticoid resistance. Given that these same fetuses are likely candidates for ANS treatment, the potential impact of infection and inflammation on ANS efficacy warrants assessment. Endotoxins have been shown to possess the ability to alter GR expression and signaling, although their likely impact on ANS therapy is unclear and appears to be tissue-specific. Toll-like Receptor 3 (TLR-3) upregulation in response to RNA viral infection (human rhinovirus, respiratory syncytial virus) or poly(I:C) (synthetic TLR-3 agonist) exposure are associated with glucocorticoid hypo-responsiveness. TLR-3/Erk1/2 pathway activation inhibits GR phosphorylation, translocation and GRE binding. These data suggest that the presence of a sterile or microbially-driven inflammatory response in utero (both factors both associated with preterm birth) may modulate GR responses to ANS therapy and thus treatment efficacy.
Experimental (animal models) evidence for antenatal steroid non-response

Animal experiments support observations from clinical studies that a sizable number of fetuses exposed to ANS therapy fail to develop a substantial improvement in functional lung maturation\(^6\). These data, generated under highly standardised, well-controlled conditions are of particular importance to our assessment of treatment efficacy in a clinical setting; if there is a degree of glucocorticoid response variability under tightly-controlled experimental conditions, then it is reasonable to expect that this variability would be exacerbated under highly variable clinical conditions, involving comparably heterogenous patient populations.

In his assessment of the impact of glucocorticoid infusions on parturition in the sheep, Liggins reported that (p.515) “partial aeration of the lungs was observed in lambs born vaginally at 117-123 days of gestation after receiving dexamethasone”, with said partial aeration of the lung identified in six out of ten lambs in the relevant dexamethasone group\(^7\). Based on these data, it may be inferred that 40% of animals in this dexamethasone-exposed group did not exhibit an alteration in lung aeration consistent with precocious lung maturation.

Kotas and Avery assessed the accelerated appearance of pulmonary surfactant in the fetal rabbit lung following intraamniotic and (fetal) intramuscular administration of 0.05mg 9-fluoroprednisolone acetate. The mean lung gas volume in steroid-treated fetal rabbits at 27 days’ gestation was 3.5 (range 2.0-3.8) ml/g lung weight at a maximal pressure of 35-40 cmH\(_2\)O. In the age matched saline control group, mean lung volume at a lower maximal pressure of 35 cmH\(_2\)O was 1.4 (range 1.0-1.9) ml/g\(^8\). The range of lung volume ranges observed in the steroid and group under controlled conditions demonstrates significant variability in the magnitude of response, and it can be inferred that at least one animal from the steroid group had lung volumes consistent with animals treated solely with saline.
Using preterm rabbits differentially treated with antenatal betamethasone and/or post-natal surfactant, Ikegami and colleagues measured protein leakage as a marker of lung injury clinically associated with the ventilation of RDS patients\textsuperscript{49}. Mean $\pm$ standard error values for pCO$_2$ after 30 minutes of ventilation, peak inspiratory pressure, and intratracheal recovery of $^{125}$I-labelled albumin post-ventilation overlapped in the saline control and 48-hour steroid treatment groups, suggesting the presence of animals that responded poorly, or not differently than control, to ANS exposure.

Studies in the preterm lamb have similarly highlighted a significant non-response rate to ANS therapy under tightly controlled experimental conditions. Using chronically catheterised animals, Takahashi and colleagues reported that, based on an analysis of cord blood pCO$_2$ values taken after 30 minutes of ventilation, some 40% of preterm lambs exposed to ANS therapy had functional lung maturation that was not significantly different to saline-treated control animals, with a similar pattern observed in lung pressure-volume assessments taken at necropsy (Figure 2A and B). An analysis of serial fetal and maternal plasma samples by LCMS showed that there were no significant differences in the total dose, maximum concentration or duration of betamethasone exposure (unbound and total) between the 60% of ANS-exposed animals that did, and the 40% of ANS-exposed animals that did not functionally respond to treatment (Figure 2C).

Schmidt and colleagues used Rhesus macaques to study the comparable efficacy of oral dexamethasone phosphate administration against two maternal intramuscular injections of 0.25mg/kg betamethasone phosphate and acetate representing current clinical standard of care\textsuperscript{50}. Pressure-volume analyses measured at necropsy demonstrated that one out of six animals in the repeat betamethasone phosphate and acetate group, and two out of eight animals in the three-dose 0.15mg/kg oral dexamethasone phosphate treatment group had static lung volume values at forty centimetres of water (V40; ml/kg) the same or lower than the mean value for the saline negative control group. These data suggest that variability in fetal responsiveness to ANS therapy occurs across
multiple species, and is exhibited an organ function level (i.e. pCO$_2$ levels in blood during preterm lamb ventilation, V40 compliance values in preterm Rhesus macaques at necropsy), and is specific to the individual materno-fetal pair.

### Strategies to Improve ANS Efficacy and Safety

There are several strategies which may be promptly implemented in an effort to improve the overall benefit gained from ANS therapy.

The quality of perinatal care is of paramount importance to improve ANS responsiveness. For example, the ACT cluster randomised trial in 2014 reported that ANS therapy did not derive any benefits for low birthweight infants and increased neonatal mortality (in large, probably term infants) and increased incidence of maternal infection in low-income and middle-income countries. However, later WHO ACTION-1 Trials showed ANS therapy reduced neonatal death without increasing negative effects, like neonatal hypoglycemia or maternal infection in low-resource countries. One key difference between these studies is that former predominantly recruited from un-standardised primary health care and community-level clinics, whereas later was a hospital-based RCT with an emphasis on standardisation of peripartum care protocols, staff training, and equipment access. Another report from a high-resource setting also shows that ANS effects to prevent RDS and IVH for infants in 23-34 weeks were related to quality of perinatal and neonatal intensive care. The quality of perinatal care thus greatly influences the efficacy of ANS therapy.

Secondly, improving the targeting of ANS to women with a high likelihood of preterm delivery within seven days is an obvious, albeit challenging means of improving treatment efficacy. In this setting, the routine application of a minimally invasive test with a high negative predictive value, such as fetal fibronectin (fFN) screening (>99% for delivery within 7d; >98% for deliveries <30 weeks’ gestation in the setting of cerclage), may be of value in guiding acute ANS decision making. Rapid fFN tests are
minimally invasive and may be reported in a little over thirty minutes, limiting the risk of a lost
treatment opportunity to treat. Development of a more discriminating fFN cut-off scale, and the inclusion of
other rapidly-deployed analyses such as cervical length screening may further assist in the optimal
targeting of ANS treatment and, importantly, can be adapted to local populations.

A third immediate approach that can be taken to improve ANS outcomes is for individual units to
regularly audit their ANS use (i.e., treatment to delivery outcomes, repeat course use) to gain a better
understanding of their historical performance in terms of patient selection. There is good evidence
that the optimal benefit of ANS treatment occurs between one- and seven-days post-administration;
as such, minimising missed opportunities to treat, and the administration of ANS to those who present
as at risk but fail to deliver will improve overall benefit. Units may consider an annual audit of their
‘on target’ performance of ANS use, widely communicating the findings to those involved in the care
and assessment of women at risk of preterm delivery, and set a minimum target for ANS-treated
women to deliver within 1-7d post-treatment. Doing so will establish a baseline to inform how
accurately risk of imminent preterm delivery is being assessed, allowing for data-driven refinements
to treatment decision-making protocols over time.

Fourthly, prioritising ANS therapy to the highest risk gestations is an important step in optimising
immediate treatment benefit. The American College of Obstetrics and Gynecologists (ACOG)
recommends ANS therapy is used for at risk pregnancies from 240/7 to 336/7 weeks’ gestation. This
target window has expanded to capture pregnancies at both gestational extremes. ANS therapy is not
recommended (based on high-quality level 1A evidence) as an obstetric intervention for threatened
and imminent preterm birth between 200/7 and 226/7 weeks’ gestation. However, since neonatal care,
and the expectation of survival, has considerably improved recent years, most infants born from 22 to
23 weeks’ are delivered after maternal treatment with ANS. There are scant RCT data specifically for
ANS use in extremely preterm infants, so the size of possible benefits and risks are largely unknown.
Based on cohort study data, there does appear to be benefits of ANS use in peri-viable pregnancies\textsuperscript{61}. However, we should also note that non-ANS patients may have had clinical reasons for being not able to receive steroid treatment, and as such that these ANS or non-treated groups may be biased\textsuperscript{1}.

There is an important discussion to be had about using ANS in late preterm pregnancy\textsuperscript{62}. ACOG, for example, recommends a single course of ANS in untreated women 34$^{0/7}$ to 36$^{6/7}$ weeks' gestation if at risk of delivering within seven days. In contrast, the 2019 update to the European Consensus Guidelines on the Management of Respiratory Distress Syndrome note that ANS are not currently recommended for women in spontaneous preterm labour after 34 weeks' gestation, and that repeat courses after 32 weeks of gestation are unlikely to convey benefit\textsuperscript{63}. Later preterm ANS use was shown to reduce the use of continuous positive airway pressure or high-flow nasal cannula for at least two consecutive hours from 13.1\% to 10.2\% ($p=0.01$). However, the risk of RDS was low even in placebo group at 6.4\% which was similar to treated group at 5.5\% ($p=0.36$). There was no difference in usage of mechanical ventilation between treated or placebo group, and treated group showed significantly higher risk of neonatal hypoglycaemia than placebo group. The balance of the very modest benefits and potential risks of ANS should be considered and communicated to families in the selection of ANS treatment for late preterm infants. Given data showing no difference in death, RDS, or length of hospital stay between steroid treated and steroid naïve late-preterm infants, institutions should at a minimum should clearly articulate and communicate the purpose of ANS treatment at a specific gestation, and on this basis consider the balance of benefits and risks\textsuperscript{62}.

In the longer-term, animal research to inform clinical trials of the minimum efficacious dose of steroids, the optimal agent, and the dosing regimen that conveys the greatest durability of benefit (all presently unknown) is key to optimising ANS treatment benefit. It is now clear that elevated fetal steroid concentrations generated by current ANS dosing regimens are unnecessary for preterm lung maturation\textsuperscript{64}. Maternal intramuscular injections of both combined betamethasone phosphate and
acetate (as per the original Liggins and Howie Trial) or dexamethasone phosphate induces an elevated peak betamethasone or dexamethasone concentration in maternal plasma of around 100 ng/ml and 20 ng/ml in cord blood, soon after injection in humans. In a well-controlled and standardized pregnant sheep model, maternal intramuscular injection of 0.25 mg/kg composite of betamethasone phosphate and acetate induced a peak betamethasone concentration in maternal plasma at around 100 ng/ml and in fetal plasma at around 10 ng/ml, respectively. In keeping with the predictions by Samtani and colleagues, Kemp et al. recently demonstrated experimentally that, once a low baseline is reached, elevated materno-fetal plasma steroid concentrations do not additionally improve preterm lung maturation in the sheep. The duration of exposure necessary to elicit and maintain functional lung maturation is also poorly defined. However, given data showing that benefit is lost more than 7d after treatment, it is reasonable to assume that the minimum duration of exposure should be longer for extended treatment delivery intervals. In the sheep, injections of 0.25 mg/kg Beta-P+Ac could improve fetal lung maturation, relative to saline control, at 48 hours, 5 days and 7 days after treatment, but that much of the benefit was lost 10 days after initial drug administration. Constant maternal betamethasone infusions were also used to explore this phenomenon. Pregnant ewes received continuous Betamethasone phosphate infusions for 36 hours, to targeted 1-4 ng/ml of betamethasone in fetal plasma. The treatment could improve lung maturation in animals delivered two days after treatment initiation (i.e. 12 hours after the infusion ceased) but at 4 or 7 days after the initiation of infusion benefit was lost. That the loss of benefit durability was uniform in these groups suggest that, although there is variability between animals in response initiation, once a maturational occurs it may degrade in a more standardised manner. These findings are in keeping with earlier work showing that the glucocorticoid-driven increases in surfactant protein A mRNA concentration in vitro is predicated on a constant agonist exposure; when stimulation is withdrawn mRNA levels rapidly return to baseline.
With regards to better outcomes from ANS therapy, these data highlight the importance of obtaining:

i) a better understanding of the relationship between the magnitude and duration of glucocorticoid exposure and how this changes temporally, and ii) a better degree of accuracy of identifying women at risk of imminent preterm delivery and targeting ANS administration accordingly. The mechanisms by which steroid-induced lung maturation reverts to a less-mature phenotype after an extended post-treatment period (i.e. >7d) remains unstudied.

Conclusions

ANS therapy is rightly a mainstay of the obstetric management of anticipated preterm delivery. When administered judiciously to the right women at the right time there is overall – but not universal – improvements in preterm outcomes that are impacted by the gestational age at which treatment occurs. There is a significant and perhaps underappreciated percentage of the preterm population that does not receive benefit from the administration of ANS. This of concern not only because of the lost potential benefit to be gained from improved ANS treatments, but because of evidence suggesting elevated risk of harms in association with off-target ANS treatments. Both treatment and patient-specific factors likely interact to drive the variability in ANS treatment efficacy observed in both clinical and experimental studies. As such, a dual approach based around immediate steps to improve the targeting of ANS therapy in the clinic (i.e. better understanding of individual unit success rates, more accurate prediction of preterm labour risk, judicious use of ANS in late preterm pregnancies), combined with longer-term research to optimise dosing regimens may provide a means by which the true therapeutic potential of this important treatment may be achieved. In addition to improving outcomes in high-resource environments, the optimisation of ANS therapy has, given its low cost, ease of application and simple supply-chain requirements, the very real potential to greatly improve perinatal mortality and disease rates in low- and middle-income countries with the highest rates of preterm birth and associated mortality.
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Table 1. Summary of placebo-controlled trials of ANS expressed as number needed to treat for benefit or harm for a neonatal death outcome.

<table>
<thead>
<tr>
<th>Trials and Analyses</th>
<th>NNT for benefit</th>
<th>NNT for harm</th>
<th>RR (Confidence Interval)</th>
<th># of trials</th>
<th># of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT 73</td>
<td>-</td>
<td>293</td>
<td>1.12 (1.02-1.22)</td>
<td>1</td>
<td>100,705</td>
</tr>
<tr>
<td>Action I Trial (WHO) 13</td>
<td>26</td>
<td>-</td>
<td>0.84 (0.72-0.97)</td>
<td>1</td>
<td>2,852</td>
</tr>
<tr>
<td>McGoldrick et al. 11, 17</td>
<td>38</td>
<td>-</td>
<td>0.78 (0.70-0.87)</td>
<td>22</td>
<td>10,609</td>
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<tr>
<td>Meta-analysis of trials from 1970s – 2010s (2020)</td>
<td>56</td>
<td>-</td>
<td>0.83 (0.67-1.04)</td>
<td>7</td>
<td>2,743</td>
</tr>
<tr>
<td>McGoldrick et al. 11, 17</td>
<td>45</td>
<td>-</td>
<td>0.90 (0.55-1.49)</td>
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<td>326</td>
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<tr>
<td>Trials in 1980s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGoldrick et al. 11, 17</td>
<td>19</td>
<td>-</td>
<td>0.60 (0.4-0.9)</td>
<td>5</td>
<td>788</td>
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<tr>
<td>Trials in 1990s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGoldrick et al. 11</td>
<td>4</td>
<td>-</td>
<td>0.46 (0.31-0.66)</td>
<td>2</td>
<td>270</td>
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<tr>
<td>Trials in 2000s</td>
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</tr>
<tr>
<td>McGoldrick et al. 11</td>
<td>55</td>
<td>-</td>
<td>0.83 (0.72-0.96)</td>
<td>4</td>
<td>6,482</td>
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<tr>
<td>Trials in 2010s</td>
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<td></td>
<td></td>
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<tr>
<td>Crowther 12</td>
<td>71*</td>
<td>-</td>
<td>0.92 (0.82-1.04)</td>
<td>11</td>
<td>5,893</td>
</tr>
<tr>
<td>Repeat dose meta-analysis (2019)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Primary Serious Composite Outcome

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Figure Legends

Figure 1. Treatment and Patient-Specific factors potentially impacting the efficacy of antenatal steroid therapy.

Figure 2. Evidence of fetal ANS non-responsiveness from sheep studies. A, Cord arterial PaCO₂ values from preterm lambs after 30 minutes of ventilation and B, Lung pressure-volume values at necropsy after antenatal treatment with either saline (Negative Control, n=9) or a clinical course of betamethasone acetate and phosphate being 2 maternal 0.25mg/kg intramuscular injections spaced by 24h (Positive Control, n=19). Positive Control Group animals were sub-grouped as either ANS responders (n=12) or non-responders (n=7) using a 30-minute arterial cord blood PaCO₂ value 2SD (capturing 95% of normally distributed data) below the mean Control Group value. Thus, animals with a 30-minute arterial cord blood PaCO₂ value lower than the cut-off value were classified as ANS Responders and those greater than the cut-off value were classified as ANS Non-Responders. Despite receiving an identical fetal betamethasone exposure prior to ventilation (C), approximately 40% of animals matched for plurality, gestational age and breeding conditions were classified as ANS Non-Responders. Adapted from Takahashi et al.⁴⁶.
Iatrogenic Factors

Accuracy of risk diagnosis
Steroid chosen
Dose given
Dosing regimen
Tx to delivery interval
Gestational age at Tx
Single vs. repeat courses
Perinatal care available

Patient-Specific Factors

Maternal stress
Intrauterine infection
Inflammation
Oxidative stress
GR polymorphisms
Variability in GR responses
Receptivity of lung mesenchymal cells to ANS signaling