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A review of the potential off-target effects of antenatal steroid exposures on fetal development

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Review

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Abstract

Antenatal steroids (ANS) are one of the most widely prescribed medications in pregnancy, being administered to women at risk of preterm delivery. In the setting of preterm delivery at or below 35 weeks' gestation, systematic review data show ANS reduce perinatal morbidity and mortality, primarily by promoting fetal lung maturation. However, with the expanding use of this intervention has come a growing appreciation for the potential off-target, adverse effects of ANS therapy on wider fetal development. We undertook a narrative literature review of the animal and clinical literature to assess current evidence for adverse effects of ANS exposure and fetal development. This review presents a summary of the evidence relating to the potential for wide-ranging, off-target, adverse effects of ANS therapy on fetal development and programming. We highlight an urgent need for further animal and clinical studies investigating the effects of ANS on the fetal immune, cardiovascular, renal and hepatic systems given a current sparsity of evidence. We also strongly suggest an emphasis on open disclosure, discussion and education of clinicians and patients with regard to the potential benefits and risks of ANS therapy, particularly in late preterm and term gestations where infants derive relatively few benefits from these drugs. We also propose further studies on the optimisation of ANS therapy through improved patient selection and improved dosing regimens based on a pharmacokinetic-pharmacodynamic informed understanding of ANS action on the fetal lung.

Introduction

Antenatal steroids (ANS) are one of the most widely prescribed off-label medications in obstetric practice today. Since the first randomised control trial (RCT) of ANS reported in 1972 by Liggins and Howie, these drugs have become routinely administered to women at risk of preterm birth (birth <37 completed weeks' gestation) due to their recognised benefits of reducing perinatal morbidity and mortality, primarily by promoting fetal lung maturation.[1–3] Additional anecdotal evidence suggests an improvement in cardiovascular stability, although these effects are difficult to isolate from improved lung maturation in the clinical setting. Of interest, however, is that the original Auckland Steroid Trial (AST) by Liggins and Howie only reported on a small portion of the entire cohort of patients ($n = 287$ of 1115 patients) when they published their findings of a significant reduction in respiratory distress syndrome (RDS) in those born under 32 weeks' gestation and a survival benefit only under 30 weeks' gestation.[4] Remarkably, it took over 50 years for the findings from the entire AST cohort ($n = 1115$) to be published, which revealed a reduction in RDS, but no survival benefit of ANS at any gestation nor a reduction in the rates of intraventricular haemorrhage and no benefit of doubling the ANS dose.[5] At present, ANS dosing remains largely unchanged around the world since the first publication of the AST.

There are two fluorinated steroids, each with distinct pharmacokinetic profiles in common use for ANS therapy: betamethasone (either solely as the rapidly released phosphate ester or as the phosphate ester in combination with the slow release acetate ester) or rapidly released dexamethasone phosphate.[6,7] Today, all ANS are administered via maternal intramuscular injection. Hydrocortisone is not used for this indication due to its very short half-life and placental inactivation. Despite the findings of the 1994 NIH Consensus Development Panel on ANS administration,[8] sizable variation exists worldwide regarding ANS dosing regimens.

Improved preterm lung function is likely achieved by steroids crossing the placenta and activating the glucocorticoid receptor (GR) in fetal lung tissue resulting in both genomic and non-genomic downstream signalling.[9] The exact molecular mechanisms by which ANS achieve accelerated preterm lung maturation are not yet fully understood. However, there is evidence that ANS therapy results in reduced mesenchymal and epithelial cell proliferation, increased fluid clearance, extracellular remodelling with increased elastin expression and thinning of the

lung septae, as well as enhanced maturation of alveolar type I and type II epithelial cells with the initiation of surfactant production.[10] These changes all contribute to functional maturation via improved lung expansion and gas exchange. However, concerns have also been raised about the potential pluripotent, off-target, adverse effects of ANS exposure on wider fetal development beyond lung maturation, given that corticosteroids may impact the regulation of as much as 20% of the human transcriptome (Fig. 1).[9]

The seventh article of the Declaration of Helsinki notes that *"Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality"*.[11] ANS therapy is an obvious candidate for careful evaluation according to these principles given its extensive use, poorly understood mode of action and lack of dose optimisation. This is especially true given that, since the introduction of ANS into clinical practice in 1972 and widespread global use from the 1990s, there have been significant other advances in the obstetric management of high-risk patients and in the neonatal care of preterm infants including the use of endogenous surfactant and improved neonatal ventilation techniques.[12,13] Indeed, the vast majority of improvements in perinatal outcomes (perhaps with the exception of peri-viable deliveries) seen today were achieved prior to the widespread introduction of ANS therapy (i.e. post-1994).[12] These advances in neonatal care have contributed in their own right to a reduction in preterm morbidity and mortality irrespective of whether an infant has been exposed to ANS. As such, it is important that, rather than solely focusing on short-term outcomes (i.e. RDS risk), there is consideration given to long-term outcomes such as neurodevelopment and adult chronic disease risk.

It is also important to assess benefits and risks as a function of gestational age. This is important due to the increased use of ANS at peri-viable gestations (with clear survival benefits) and in older or late preterm gestations (>35 weeks' gestation) where the respiratory and survival benefits of ANS are, at best, far more modest. For example, based on systematic review data, the number needed to treat to prevent one case of RDS at <34 weeks' gestation is 19, compared to 55 at >35 weeks' gestation and 106 at term prior to Elective Lower Uterine Segment Caesarean Section.[14] Supporting these observations are the findings of the ALPS trial, which demonstrated no significant reduction in RDS risk with ANS compared to placebo after 34 weeks' gestation.[15] Of further concern is that a large proportion (up to 44%) of infants who are exposed to ANS due to an incorrect assessment of preterm birth risk go on to deliver at term.[16] Thereby both mother and fetus are exposed to a therapy conveying them no benefit but potential harms.

In light of the above, this review examined the short- and long-term, off-target, developmental implications of ANS exposure by examining both animal and clinical studies.

Methods

We performed a narrative review of the animal and clinical literature focusing on the short- and long-term effects of ANS on fetal development and the origins of disease. Search terms included "antenatal steroids", "glucocorticoid", "synthetic glucocorticoids", "fetal", "neonatal", "infant", "childhood", "adult", "development", "respiratory", "lung maturation", "cardiovascular", "brain", "neurodevelopment", "programming", "hippocampus", "renal", "kidney", "hepatic", "liver", "metabolic", "immune", "HPA axis", "animal", "human" in the following databases: SCOPUS, PUBMED, MEDLINE, EMBASE and Google Scholar. Searches were conducted between July and October 2024. Data from approximately 200 papers was reviewed with randomized control trials, large animal studies (particularly non-human primates) and systematic reviews being prioritised. A focus was placed on literature from 2010 to 2024, with the inclusion of articles outside this range for historically important studies or those addressing specific knowledge gaps. Articles included in this review were full-text, English language papers.

Results and discussion

Neurodevelopmental and behavioural outcomes

Multiple factors predispose the developing fetal brain to short and long-term development risk from ANS exposure. Firstly, endogenous glucocorticoids such as cortisol are integral to driving normal neurodevelopment by inducing remodelling, programmed apoptosis and proliferation of axons and dendrites in a time-critical manner.[17] Animal models (rodents) have demonstrated that the placental expression of 11β-HSD-2 (which rapidly converts active cortisol to its inactive form) is highly expressed in midgestation, potentially protecting the brain from premature remodelling effects of endogenous glucocorticoids (cortisol).[17] However, at the end of midgestation, placental 11β-HSD-2 is dramatically reduced in both mice and rats leading to cortisol-induced terminal neuronal differentiation as described above.[17] Importantly, unlike cortisol, the exogenous glucocorticoids, dexamethasone and betamethasone are resistant to 11β-HSD-2 and thus readily cross the placenta in their active form to activate the GR in fetal tissues, including the developing brain.[17] Secondly, evidence suggests that the fetal hippocampus and hypothalamus both have a high density of GR expression making these structures potentially susceptible to off-target effects of ANS.[17–20] Furthermore, both clinical and animal models of chronic stress (a condition marked by chronic glucocorticoid elevation) and clinical depression have demonstrated downregulation of GR expression in the hippocampus on pathological examination. These findings indicate an important link between glucocorticoid exposure and long-term behavioural risks.[21] Finally, the third trimester (wherein the majority of ANS exposure occurs) corresponds to a critical stage of fetal neurodevelopment, where there is a dramatic increase in myelination of axons and occipital white matter as well as neural synapse formation.[22,23] Therefore, a comprehensive understanding of the effects of ANS exposures on the developing fetal brain is of high importance given the potential long-term implications on mental, behavioural and learning issues for exposed individuals.

Animal studies

Taking into account variability in the ontogeny of animal model neurodevelopment (especially with regard to rodent models vs. the human), evidence from a range of animal studies has demonstrated alterations in the fetal nervous system following ANS exposures.[24] An early study by Uno *et al.* demonstrated that both term and preterm fetal rhesus monkeys exposed to antenatal dexamethasone had a reduction in neuron density as well as evidence of neurodegeneration characterised by shrinkage of pyramidal neurons and marked atrophy of the zona lucidum within the hippocampus and dentate gyrus.[18] These observed neurodegenerative effects were found to be dose-dependent.[18] Further, work by Dunlop *et al.* in a sheep model of pregnancy also demonstrated that repeat administration of ANS

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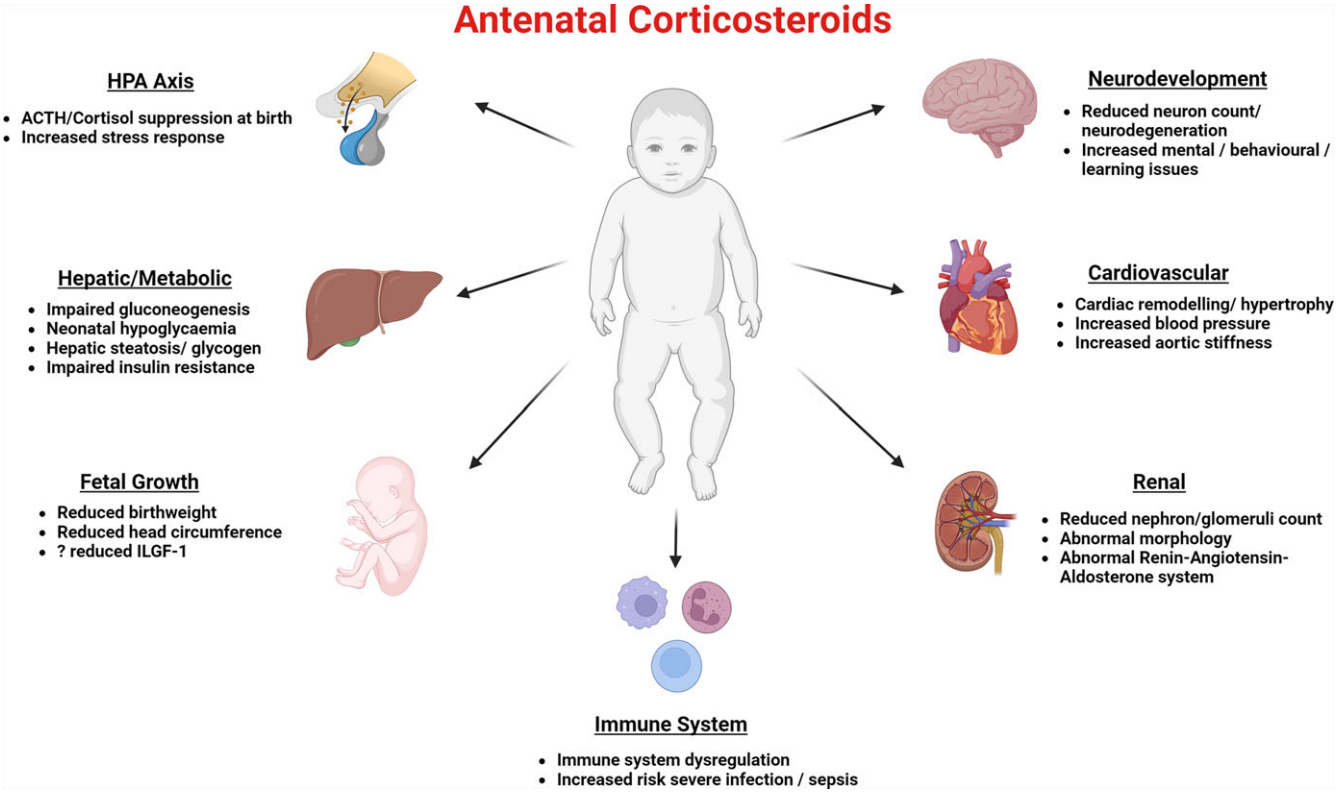

Figure 1. Graphical abstract of the short and long term off target effects of antenatal corticosteroid therapy on fetal development. Created in BioRender.com.

delayed myelination of the fetal optic nerve.[25] The same group then later showed that single and repeat doses of ANS resulted in reduced whole brain weight in the same sheep model of pregnancy.[26] Similar findings have been observed in rats and mice exposed to single and repeat antenatal dexamethasone demonstrating a dose-dependent reduction in brain weight as well as evidence of reduced neurogenesis within the forebrain, hippocampus and cortex.[27–29] Studies of ANS therapy (betamethasone) in juvenile baboons have also shown evidence of impaired cognition including decreased motivation in both sexes and sex-specific differences with females demonstrating impaired learning outcomes.[30] A similar study in baboons investigated structural brain effects of ANS and showed that repeat ANS exposure resulted in reduced myelination within the corpus callosum, the subcortical and cortical deep white matter.[31] Furthermore, evidence of increased astrogliosis indicating possible neuronal injury and damage was also observed in these animals.[31] Despite the limitation that many of these studies were conducted on term-born animals, the findings from these non-human primate studies add considerable translational weight to human outcomes, given the similarities in neurodevelopment and cognitive ability. Our group recently demonstrated that preterm lambs delivered at 122 ± 2 d gestation (term = 150 d gestation) exposed to either dexamethasone or betamethasone had differential gene expression in the fetal hippocampus related to neuropsychiatric and neurostructural disorders.[32] Additionally, lambs exposed to dexamethasone at both a clinical dose (utilised as a first-line treatment in Singapore) or significant dose reduction (75%) had upregulation of transcriptomic pathways within the hippocampus related to neurodegenerative disorders such as Parkinson's, Alzheimer's and Prion disease, a profile which was not seen in betamethasone exposed animals.[32] Schmidt *et al.* reported similar findings of abnormal neurodevelopment in rhesus macaques with ANS (dexamethasone or betamethasone) exposure resulting in alteration of the fetal hippocampal transcriptome, notably in genes related to synaptic transmission, neurogenesis and neuronal maturation.[33,34] Other studies of ANS exposure on pregnant guinea pigs have shown altered DNA methylation patterns, altered GR binding and transcriptomic profiles in the fetal hippocampus.[35–37] These findings suggest the potential for long-term adverse neurodevelopmental changes with ANS exposure. Concerningly many of these changes were seen across multiple generations suggesting that ANS also exert epigenetic neurodevelopmental influences on future offspring.[38,39].

Clinical studies

Clinical and large population cohort studies examining the impact of ANS on neurodevelopment have also raised concerns. Tijsseling *et al.* demonstrated a reduction in neuron density within the hippocampi of ANS-exposed neonates ($n = 10$) compared to non-ANS-exposed neonates ($n = 11$) that delivered preterm.[40] Davis *et al.* reported structural brain changes on MRI examination of healthy, term-born pre-adolescents exposed to betamethasone ANS compared to controls.[41] The MRI findings included bilateral cortical thinning, seen maximally (30% reduction) in the rostral anterior cingulate cortex, which corresponded to an increased risk of affective disorder problems in that cohort.[41]

Of additional interest are the findings from two large population cohort studies demonstrating associations between ANS exposure and mental and behavioural issues later in life. The first, from a population cohort study of >674,000 children born in Finland between 2006 and 2017, showed that ANS exposure was

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associated with a higher risk of mental and behavioural disorders in the entire cohort (12% vs. 6.45%, HR 1.33), in term-born children (8.89% vs. 6.31%, HR 1.47) and in preterm-born children (14.59% vs. 10.71%, HR 1.00).[42] A second population cohort study by Lin *et al.* of all infants born in Taiwan between 2004 and 2010 (>1,160,000 infants) demonstrated a significant association between ANS exposure and childhood mental disorders in the entire cohort (HR 1.13), those born at term (HR 1.11), in late preterm (34–37 weeks gestation) born children (HR 1.15) and in children born <28 weeks' gestation (HR 1.22).[43] They noted a particular increased risk for disorders such as ADHD and developmental delay.[43] Whilst a smaller Chinese cohort study by Tao *et al.* reported the findings of 1759 infants (710 of which were exposed to ANS) and demonstrated increased risk of cognitive delay measured using the Bayley Scales and Toddler Development in 1-year-olds exposed to dexamethasone ANS after cofounders were taken into account ($n = 710$).[44] Reassurance regarding the generalisability of these observed associations can be found given that these studies draw from ethnically and geographically distinct populations (i.e. Northern Europe vs. Asia).

The 5 year follow-up data of 80% of the children from the MACS trial (comparing single to repeat course of ANS) demonstrated that term-born children exposed to repeat courses of ANS had a 3.7-fold increased odds of neurosensory disability, which was not observed in the preterm cohort.[45] However, follow-up of children who were enrolled in a similar trial of repeat compared to single-dose ANS by the Maternal Fetal Medicine Unit network showed no difference in neurocognition (assessed by the Bayley Testing) between groups at age 2–3 years of age.[46] Similarly, participants from the ALPS trial of ANS versus placebo between 34 and <37 weeks gestation showed no adverse neurocognitive or behavioural outcomes at 6 years of age. However, only 42% of participants were included in the analysis.[47] Interestingly, the main findings of the original ALPS trial demonstrated a reduction in transient tachypnoea of the newborn but no significant effect on RDS rates or total duration of hospital stay for infants, suggesting that any benefit from ANS therapy was transient.[15] Concerningly though, this same trial reported a significant increased risk of neonatal hypoglycaemia with ANS exposure, which has been linked to poor neurodevelopmental outcomes later in life including impaired executive and motor functioning.[15,48]

Follow-up data from 51% of the children enrolled in the ASTECS trial (administration of ANS *vs.* placebo prior to term elective caesarean section) demonstrated that those children exposed to ANS were more likely (17.7% vs. 8.5% – number needed to harm = 11) to be in the lowest quartile of academic ability at school at age 8–15 years old.[49] Similarly, follow-up of children at 6 years of age from the original AST ($n = 250$ of 318 enrolled at the start of the trial) demonstrated that ANS-exposed children scored lower in tests of cognitive development (Ravens Progressive Matrices Test) and visual memory (particularly in males) indicating evidence of potential cognitive delay.[50] Supporting these findings is evidence from a cohort of 19-year-olds that demonstrated ANS exposure was associated with poorer IQ scores and more behavioural problems, particularly in individuals with GR subtypes that convey an increased sensitivity to ANS.[51]

Perhaps the longest follow-up of ANS-exposed infants has been reported by Savoy *et al.* This longitudinal study followed up 142 infants who were born at extremely low birthweights and either received ANS ($n = 63$) or were untreated ($n = 79$). Comparisons were made between groups and also matched to normal birthweight, term-born subjects. Confounders such as socio-economic status, neurosensory impairment and postnatal steroid exposure were considered. Concerningly, at 22–26 years of age, those exposed to ANS had a 3–5-fold increase in the odds of having a diagnosed mood disturbance (anxiety or depression), which persisted in later follow-up at 29–36 years of age.[52] In contrast, a long-term follow-up of data from the original AST by Liggins and Howie of ANS versus placebo was published in 2005. They reported no differences between groups in neurocognition or mental and behavioural disorders; however, it is important to note that only 27% of the original trial participants were followed up and reported on.[53] Interestingly, a similar population cohort study from New Zealand of all the babies born at a very low birthweight (<1500 g) in 1986 revealed that on follow-up ($n = 250$ of the original 413 trial participants included), ANS exposure was associated with double the risk of major depression at 26–30 years of age.[54]

Two clear themes emerge from our analysis of the literature in relation to the risk of adverse brain effects in association with ANS exposures. Firstly, from animal models (wherein tissue for histological, proteomic and molecular studies are readily available), it is clear that doses of steroids used clinically elicit both acute and lasting changes in transcriptome, proteome and central nervous system architecture and growth. The molecular and structural changes reported are also consistent with observations from human cohort studies. Although it is indeed reasonable to assess rodent and small animal data with caution due to developmental differences, it is more difficult to dismiss extensive data from longer gestation non-human primates or from sheep studies.

Secondly, greater caution than presently exercised is warranted regarding the use of ANS and neurodevelopmental outcomes. The presently available evidence is of sufficient strength that it should be considered (and disclosed) when consenting patients at risk of preterm delivery for ANS therapy, especially over 35 weeks' gestation.

Hypothalamic pituitary adrenal axis effects

The hypothalamic pituitary adrenal (HPA) axis functions to regulate corticosteroid metabolism and secretion, which is critical for normal homeostasis and the body's ability to respond to stress.[55] The hypothalamus produces corticotropin-releasing hormone (CRH) and vasopressin which in turn stimulates adrenocorticotropic hormone (ACTH) release from the anterior pituitary. ACTH then stimulates the release of cortisol from the adrenal cortex. The HPA axis is then regulated via the negative feedback of cortisol on glucocorticoid and mineralocorticoid receptors (MR) within the hippocampus, hypothalamus and anterior pituitary. Endogenous glucocorticoids have wide-ranging, time-critical effects on fetal development, which include fetal growth, cardiovascular, immune, metabolic and neurobehavioral programming.[55] Unlike the endogenous glucocorticoid cortisol, exogenous glucocorticoids (ANS) have a much higher affinity for the GR and exert a larger and more sustained negative feedback effect on the HPA axis.[56] Therefore, disruption of the HPA axis via ANS has the potential to exert wide-ranging acute and chronic fetal programming effects, thereby providing a possible link to the origins of childhood and adult disease related to ANS exposure.[57–60] Data describing the effects of ANS on the HPA axis from animal and clinical studies are outlined below.

Animal studies

Studies using pregnant sheep have shown both immediate and prolonged maternal and fetal HPA axis suppression with ANS exposure. Suppression of both ACTH and cortisol has been observed in pregnant ewes and fetal lambs exposed to ANS therapies at current

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clinical doses and in regimens with significant dose reduction (~75%).[32,61–64] HPA axis suppression persisted in these animals even when steroid concentration was undetectable in both maternal and fetal plasma circulation.[32] Further studies in guinea pigs have also shown dose-dependent suppression of the fetal HPA axis, including downregulation of CRH mRNA in the fetal hypothalamus, providing further evidence that ANS pass the blood-brain barrier and act directly on the developing fetal brain through the GR.[65] A study by de Vries *et al.* in non-human primates also showed dose-dependent maternal HPA axis suppression in pregnancy and elevated plasma cortisol concentrations to minor stress insults (blood sampling) in the 8-month-old offspring.[60] Similarly, Uno *et al.* demonstrated dose-dependent maternal and fetal HPA axis suppression in rhesus macaques exposed to single and repeat courses of antenatal dexamethasone.[18]

Clinical studies

Clinical studies have also demonstrated evidence of acute HPA axis dysregulation in ANS-exposed neonates at birth. Findings included reduced basal and stress-induced cortisol concentrations.[57,66–69] In contrast, one study of term neonates exposed to ANS demonstrated elevated cortisol concentrations in response to stress.[70] Multiple other studies have suggested that ANS-induced HPA axis suppression at birth is acute and does not persist past the neonatal period.[71,72] However, one high-quality study clearly demonstrated ongoing blunted basal and stress-induced cortisol concentrations at 12 months of age in those children exposed to ANS.[69] Additionally, follow-up studies of children aged 6–11 years[73] and adolescents[74] exposed to ANS have both demonstrated ongoing HPA axis dysregulation and reprogramming effects including significantly higher cortisol response to stress testing.[73,74]

The impact of ANS on the maternal HPA axis is also another question. A study in non-pregnant women administered a 6 mg IM dose of either dexamethasone phosphate, betamethasone phosphate or combined betamethasone phosphate and acetate demonstrated cortisol suppression up to 60 hours, 72 hours and >4 days, respectively.[7] Notably, the prolonged HPA axis suppression identified in the betamethasone acetate and phosphate group occurred at maternal dosing substantially lower than that used clinically and persisted at plasma concentrations now understood to be sub-therapeutic for fetal lung maturation. McKenna *et al.* reported the findings of a prospective case-control trial in pregnant patients between 24 and 34 weeks' gestation, investigating the effects of repeat ANS (at least 2 × weekly course of betamethasone phosphate and acetate, $n = 18$) on maternal HPA axis suppression, demonstrating secondary adrenal insufficiency after corticotropin stimulation test over 48 hours post-ANS exposure.[75] Given that the maternal HPA axis is closely related to fetal HPA axis regulation through the transplacental passage of cortisol and CRH, these findings are of concern. Furthermore, secondary adrenal insufficiency in pregnancy may put patients at risk of falls related to postural hypotension, fatigue and weakness as well as infection related to immune system dysregulation and intrapartum morbidity due to inadequate stress response similar to Addison's disease (adrenal insufficiency).

Immune system modulation

Previous studies have established that corticosteroids exert dose-dependent, immune-modulating and anti-inflammatory effects[76] including increased leukocyte release from bone marrow, impaired leukocyte migration into affected tissue from circulating plasma[77,78] and inhibition of neutrophil apoptosis.[79]

Animal studies

Our group has demonstrated in pregnant sheep that ANS exposure at both current clinical doses and with 75% dose reduction, results in rapid maternal and fetal plasma neutrophilia and lymphocytopenia.[32,61] Similar findings have also been seen in rat models, demonstrating reduced maternal and fetal lymphocyte proliferation and lymphocyte interleukin-2 production after antenatal betamethasone exposure.[80] Mice exposed to antenatal betamethasone also exhibit significant reductions in thymus size and a large reduction in CD^{4+}/CD^{8+} T-cell counts at birth.[81] As the thymus is integral to T-cell production and acts as a primary immune organ, these findings have implications for adaptive immune system functioning.

Clinical studies

Animal data showing ANS-associated immunological disruption are supported by data from clinical studies suggesting potentially serious ANS related immune modulation. For example, Smolders *et al.* reported ANS-exposed infants had an increased rate of hospital admission due to infection during the first year of life.[82] Whilst a secondary analysis of the ACT trial showed a significant association between severe bacterial infection and neonatal mortality in infants exposed to ANS in African nations.[83] Furthermore, a Finnish population cohort study demonstrated that ANS exposure was associated with a higher need for treatment of infectious diseases (respiratory, gastrointestinal and urinary tract) up to 4 years of life, in the entire cohort, term and late preterm-born infants compared to non-exposed infants.[84] Finally, a recent large cohort study in Taiwan demonstrated ANS exposure was associated with a small but significant increased risk of serious childhood infection.[85] At present, there is a lack of evidence on the long-term immune modulation effects of ANS besides the few studies outlined above. However the evidence of increased risk of infection is concerning especially given that many preterm infants are exposed to both intrauterine infection (chorioamnionitis is a leading cause of preterm birth) and ANS.[86]

Growth restriction

Animal studies

Evidence from studies of multiple different species demonstrates that ANS exposure is associated with reduced intrauterine fetal growth. Extensive work has been performed in sheep models of pregnancy on the effects of ANS on fetal growth. Huang *et al.* utilised single or repeat weekly courses (up to 4 courses) of 0.5 mg/kg betamethasone (equivalent to double the current standard clinical dose delivered in a single injection) in preterm and term animals with delivery occurring 24 hours after the last dose of ANS. Analyses showed reduced birthweight in all groups compared to saline control.[26] The largest reduction in growth was seen with repeat courses and in the term gestation groups exposed to ANS.[26] The same group then showed that 1, 2 or 3–4 weekly courses of ANS administration resulted in a 15%, 19% and 27% reduction in birthweight, respectively.[87] Similar findings were reported in a subsequent sheep study showing both single and repeat ANS dosing resulted in reduced lung, heart, liver, thymus and kidney weight in addition to a reduced overall birthweight.[88] Interestingly the study team also administered direct fetal ANS (single and repeat courses) at the same dose which did not affect birthweight, such as that seen in maternal ANS dosing. Although speculative, this may be due to placental export of the drug back across into maternal circulation, fetal hepatic drug metabolism and a lower or shortened overall steroid exposure.[88] Following on from this earlier work, Takahashi *et al.* using a reduced dose of antenatal

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betamethasone (2 × 0.25 mg/kg given 24 hours apart) in a sheep model, demonstrated reduced birthweights of ~ 15% in preterm lambs exposed to ANS compared to saline control.[63] A potential mechanism for ANS-induced growth restriction has been postulated in the same sheep-based model by Usuda *et al.*, who observed that fetal plasma concentrations of insulin-like growth factor 1 (IGF-1) were reduced at birth in lambs exposed to dexamethasone (4 × 6 mg maternal intramuscular injections of dexamethasone phosphate 12 hourly) compared to both saline control and a reduced dose betamethasone regimen (4 × 2 mg maternal intramuscular injections of betamethasone phosphate 12 hourly).[61] This is a significant finding as IGF-1 is associated with fetal growth.[89,90]

Rodent models have also been used to explore a potential link between ANS exposure and intrauterine growth restriction. Noting material differences in gestational length between rodents and humans (and thus a proportionally longer exogenous steroid exposure in rodents), Ozdemir *et al.* showed that mouse pups exposed antenatally to single and repeat courses of betamethasone or dexamethasone had smaller lung, liver and birthweights compared to control.[91] Interestingly, dexamethasone pups exhibited a larger reduction in growth than the betamethasone groups.[91] A study of single-dose antenatal betamethasone or dexamethasone in rats also showed similar findings including reduced birthweights but only in the dexamethasone group. Whilst at 3 weeks of age (time of weening), both ANS-exposed groups had reduced body weights.[92] Another study of rats exposed to a single course of antenatal betamethasone demonstrated reduced birthweights and lower serum glucose, insulin, IGF-1 and leptin levels compared to saline control.[93] Furthermore, males exposed to antenatal betamethasone who then mated with control (unexposed) females gave birth to offspring with lower birthweights, suggesting a possible male-linked, transgenerational epigenetic effect from ANS exposure on fetal growth.[93]

Finally, three different studies in rabbits have investigated the effect of ANS on fetal growth. Sun *et al.* utilised a reduced dose of betamethasone (0.1 mg/kg) compared to clinically utilised doses and demonstrated that two doses (given 24 hours apart) resulted in a 20% reduction in birthweight, whilst a single dose (48 hours prior to delivery) reduced birthweight by 9.4% but significantly did not functionally mature the fetal lung.[94] A study in pregnant rabbits by Pratt *et al.* utilising 1, 2 or 3 courses of betamethasone (2 × 0.1 mg/kg 24 hours apart) demonstrated a progressive reduction in birthweight with increasing ANS courses, representing a clear dose-dependent effect of ANS on fetal growth.[95] Interestingly, the same group also gave a later term course (single and double) of betamethasone and showed a greater reduction in birthweight in these animals compared to preterm gestations.[95] A third study in rabbits utilising the same dose of betamethasone demonstrated that a single course resulted in reduced birthweight that persisted up to 7 weeks of age. Concerningly, these animals also demonstrated evidence of impaired neurocognition on functional testing.[96]

The findings outlined above of reduced fetal growth with ANS exposure are particularly troubling, given they are observed across multiple different species and persist despite significant ANS dose reduction.

Clinical studies

The evidence from clinical studies of ANS-induced growth restriction is unclear, with the most recent Cochrane review of ANS by McGoldrick *et al.* stating that ANS result in little or no difference in mean birthweight and with an uncertain effect on the incidence of small for gestational age (<10th centile) at birth.[1] However, two high-quality clinical trials have demonstrated clear evidence of fetal growth restriction with repeat ANS exposure. Firstly, the ACTORDS trial comparing repeat dosing to single course of ANS demonstrated small reductions in z-scores of birthweight and head circumference in infants exposed to repeat courses of ANS.[97] Secondly, the MACS trial comparing single to repeat courses of ANS showed no improvement of perinatal morbidity or mortality with repeat ANS dosing but demonstrated a reduction in birthweight, length and head circumference with repeat ANS dosing. These findings remained true in a secondary analysis of the MACS trial data when gestational age was accounted for.[98,99]

Cohort studies have also demonstrated an association between ANS exposure and evidence of reduced fetal growth. Term-born infants exposed to a single course of ANS had reduced birthweights, lengths and head circumferences compared to matched unexposed controls.[100] A cohort study of 477 infants exposed to repeat ANS and born preterm (<33 weeks' gestation) also had reductions in birthweight (up to 9%) and reduced head circumferences.[101] Further, Bloom *et al.* reported a retrospective cohort study of preterm infants exposed to a dexamethasone (four × 6 mg maternal intramuscular injections of dexamethasone phosphate 12 hourly) single or repeat regimen ($n = 961$). Dexamethasone-exposed infants demonstrated reduced birthweight compared to a reference population of matched controls from the same institution that did not receive ANS ($n = 2808$). The study also demonstrated significantly reduced birthweight with ANS exposure of up to −161 g (at 30–32 weeks' gestation) when compared to a historically matched cohort ($n = 444$) from the same institution who on retrospective analysis had the same indications for ANS but were born a year before the introduction of ANS.[102] Braun *et al.* published the findings of a retrospective cohort study from all births at an institution in Germany from 1996 to 2008 and showed that preterm antenatal betamethasone exposure ($n = 1799$) was associated with a dose-dependent reduction in birthweight compared to matched controls ($n = 42{,}240$) after confounders were accounted for including gestational age.[103] Those infants exposed to a dose of 24 mg of betamethasone (equivalent to a single course) had a reduction in birthweight of 523g, whilst any dose of betamethasone >24 mg (repeat courses) was associated with an 811g reduction in birthweight.[103] Findings of reduced fetal growth were also detected on routine antenatal ultrasound scan prior to delivery with a dose-dependent reduction in estimated fetal weight (EFW).[103] However, no difference was detected in neonatal morbidity or mortality.[103] Similar findings have been reported from a study performed in Rome, which showed evidence of reduced growth velocity (head circumference, abdominal circumference, EFW) on antenatal ultrasound with a corresponding reduction in birthweight at delivery for a cohort of infants ($n = 262$) exposed to a single course of betamethasone (2 × 12 mg IM 24 hours apart) compared to controls ($n = 270$).[104] However, there was a significant difference in gestational age of delivery between the ANS cohort (mean 37.6 weeks) and control (mean 39.7 weeks) which may have contributed to the observed findings.[104] The longer-term impact of ANS on childhood growth is relatively unexplored. Osteen *et al.* reported the results of follow-up from 3,556 term-born children at 5 years of age, of which 629 children were exposed to ANS (betamethasone) and 2927 children were not (controls).[105] All pregnancies, including the controls were at some point assessed for threatened preterm labour. Those exposed to ANS had higher rates (21.8% vs. 16.4%) of being a small for gestational age infant (birthweight <10th centile) compared to controls which persisted at 5 years of age (13.7% vs. 7.1%, OR 2.00 95% CI 1.22–3.25).[105] However, ANS-exposed mothers were more

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likely to have diabetes or hypertensive disorders which could have affected fetal growth. Further, it is noted that there was a difference in gestational age at delivery (38.5 vs. 39.0 weeks); however, this is unlikely to be clinically significant.[105]

Finally, a large population cohort study of all babies born in Finland between 2006 and 2010 ($n = 278,508$ singleton births, 4,887 of which were exposed to ANS) showed an association between ANS exposure and reduced birthweight, head circumference and length in babies born at preterm gestations up to term.[16]

The longer-term implications of ANS-induced growth restriction observed in these studies are not yet fully understood but warrant further investigation. Especially so, as many ANS trial follow-up studies (including ASTECS and ALPS trials) have not reported on childhood or adolescent growth. Intrauterine growth restriction (IUGR) is however known to be a long-term risk factor for metabolic syndrome and associated cardiovascular disease. Therefore, it is not unreasonable to extrapolate the findings of IUGR to the observed effects of ANS on fetal growth.[106]

Cardiovascular effects

Fetal cardiovascular system maturation is likely a result of both mechanical and hormonal influences.[107] However, the exact mechanisms behind fetal cardiovascular maturation and the programming effects of glucocorticoids on fetal heart development are not yet fully understood. Studies have however shown that the GR and MR are highly expressed in the myocardium, endothelium and vascular smooth muscle.[108]

Animal studies

Key insights into the effects of glucocorticoids on the development of the cardiovascular system have been made in animal models and are explored below. Firstly, a study utilising a mouse model overexpressing cardiomyocyte GR showed no major structural abnormalities within the fetal heart but demonstrated an increased incidence of bradycardia and conduction defects.[109] Other rodent studies have demonstrated that GR knockout results in a small but structurally normal heart, which exhibits diastolic dysfunction and early heart failure in midgestation.[110] On a cellular level, GR knockout mice display features of cardiac immaturity including poorly aligned cardiomyocytes, short, disorganised myofibrils as well as impaired cellular calcium homeostasis, which is important for normal cardiac contraction.[110] The importance of MR activation by glucocorticoids was demonstrated in a study that generated mice that overexpressed cardiomyocyte MR activity. Data showed that these mice had structurally normal hearts but demonstrated early and sudden death due to major arrhythmias.[111] When the cardiomyocyte MR was knocked out, there was no major functional or morphological impact on the heart, demonstrating the variable effect of the MR on heart development.[112]

Rodent models of the cardiovascular effects of ANS show mixed results. Sakurai *et al.* demonstrated evidence of fetal cardiac maturation in rats exposed to antenatal dexamethasone. Histologically, dexamethasone-exposed hearts displayed organisation of ventricular myofibrils, cardiomyocyte hyperplasia and proliferation as well as an increase in cross-sectional area of the myocardium.[113] In contrast, a study in rats exposed to antenatal dexamethasone demonstrated impaired cardiac maturation with an increased heart/body ratio and lack of transition from hyperplastic growth to hypertrophic growth within the ventricles (higher cellular proliferation and lower extracellular matrix composition).[114] De Vries *et al.* reported that adult rats exposed to antenatal dexamethasone had decreased heart weights but with increased cardiomyocyte size suggesting hypertrophy and increased collagen content. The authors suggested these changes were consistent with signs of early onset degeneration, which may cause permanent abnormalities to the cardiac structure.[115]

Sheep studies from the late 1990s have demonstrated that a direct *in utero* fetal infusion for 48 hours of betamethasone or dexamethasone at concentrations similar to clinical exposures results in an increase in fetal blood pressure and femoral vascular resistance.[116] Similar findings were demonstrated by Koenen *et al.* in fetal baboons exposed to maternal antenatal betamethasone. They showed an increase in fetal blood pressure without a change in fetal heart rate, maternal heart rate or maternal blood pressure.[117] Other non-human primate studies have demonstrated persistent cardiovascular effects after ANS exposure including increased blood pressure in juveniles.[60,118] Our group has demonstrated that fetal sheep exposed to an equivalent clinical dose of antenatal betamethasone have evidence of abnormal cardiac compliance on cardiac ultrasound examination (reduced mitral and tricuspid valve E/A ratios) which was associated with differential expression of genes related to myocardial hypertrophy within the fetal heart.[119]

A study using preterm pigs exposed to antenatal betamethasone compared to preterm controls demonstrated findings of cardiac maturation including increased atrial weight as well as evidence of cardiomyocyte terminal differentiation in the left and right ventricles as demonstrated by a shift from mononucleated cells to multinucleated. These findings indicate possible preterm cardiac hypertrophy resulting in acutely improved preterm cardiac function; however, the long-term implications of ANS-induced cardiac hypertrophy are unknown.[120]

Clinical studies

Evidence from cohort studies on the effects of ANS with regard to the cardiovascular system is mixed. Dalzeil *et al.* have reported on the cardiovascular outcomes of individuals from the original AST (ANS vs. placebo) and showed no differences in blood pressure at 6 years of age and no difference in blood pressure, plasma lipids, cardiovascular disease or diabetes at 30 years of age.[121] They did, however, report an increased insulin response to oral glucose tolerance test at 30 years of age, which may indicate a higher risk of developing diabetes (a main cardiovascular disease risk factor) due to insulin resistance later in life.[121] A major limitation of the cohort data from the AST is that it includes only 19% of the study population at 6 years of age and 46% at 30 years of age. The 50-year cardiovascular follow-up of this same cohort was published recently by Walters *et al.* and showed no difference in cardiovascular risk factors (hypertension, hyperlipidaemia, diabetes, pre-diabetes) or major cardiovascular events between the exposed and unexposed cohorts.[122] However, again follow-up rates were low (46% of the original cohort), and outcomes were measured by a patient questionnaire alone which has the potential for limitations and bias.

In comparison, a cohort study of 210 children born prematurely and with a birthweight <1500 g was reported at 14 years of age (>84% follow-up).[123] Those exposed to ANS ($n = 89$) had an elevated systolic and diastolic blood pressure (albeit not in the hypertensive range) when compared to a matched unexposed group ($n = 88$).[123] Furthermore, a prospective cohort study of 23–28-year-olds showed that individuals exposed to ANS had

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evidence of increased aortic arch stiffness on MRI assessment that was equivalent to adults decades older.[124] This is a concerning finding as increased aortic arch stiffness is associated with an increased risk of developing hypertension, stroke and coronary artery disease later in life.[124]

Hepatic and metabolic effects

The programming effects of glucocorticoids on fetal and neonatal liver function is an area that is poorly understood at present. However, early studies have shown the importance of cortisol on fetal hepatic glycogen storage. In 1977, Barnes *et al.* showed that *in utero* control lambs had a rapid rise in hepatic glycogen concentration at 130d gestation (term 150d), whilst hepatic glycogen storage was markedly reduced in lambs that had undergone surgical removal of the adrenal and pituitary glands (removing endogenous fetal glucocorticoid exposure).[125] A direct fetal infusion of cortisol via a fetal catheter was then seen to restore normal hepatic glycogen concentrations.[125] Similar studies using glucocorticoid knock-out mice have shown impaired hepatic gluconeogenesis and glycogen storage after birth.[126] This is significant as after birth and prior to the onset of established breastfeeding the infant must rely on endogenous glucose for metabolism. Therefore, any reduction in an infant's ability to maintain glucose homeostasis immediately after birth (i.e. reduced gluconeogenesis and glycogen storage) may predispose it to hypoglycaemia, a finding that was seen in ANS-exposed infants in the ALPS trial.[15]

Animal studies

Two studies (one in rhesus macaques, one in mice) have reported that antenatal betamethasone exposure resulted in an increase in fetal hepatic glycogen content and hepatic weight.[91,127] Supporting this is evidence from rat models of antenatal exposure to dexamethasone showing an increase in hepatic steatosis without obesity, possibly due to the suppression of key genes (*AMPK, PGC1α*) involved in hepatic fat metabolism.[128,129] An important study in sheep by Franko *et al.* demonstrated a mechanistic link between ANS exposure and increased hepatic glycogen storage. This study showed that a clinically relevant dose of antenatal dexamethasone increased the expression of the fetal hepatic gluconeogenic enzyme glucose-6-phosphatase (G6Pase), which was associated with increased fetal hepatic glycogen storage (2–3-fold), fetal plasma glucose and insulin levels.[130] Another study by de Vries *et al.* in non-human primates (African vervet) exposed to antenatal dexamethasone showed impaired glucose tolerance at 8 months of age based on higher fasting plasma insulin levels and abnormal (slower) glucose clearance on oral glucose tolerance test.[60] At 12–14 months of age, the dexamethasone-exposed non-human primates from this study had a reduction in the number and size of pancreatic β cells (which are responsible for insulin production), as well as a reduction in hepatic expression and mRNA for a key gluconeogenic enzyme phosphoenol-pyruvate carboxykinase.[60] These findings add further weight to the potential issues of glucose homeostasis and adaptation that infants exposed to ANS may face in the postnatal and infant periods. These changes may also predispose to long-term glucose insensitivity and increased risk of type II diabetes mellitus. Furthermore, Kuo *et al.* demonstrated that 10-year-old baboons (equivalent to a 40-year-old human) that were exposed to ANS had increased pericardial fat and hepatic lipid accumulation at a normal weight, indicating evidence of long-term metabolic reprogramming.[131] Conversely, a study utilising a rat model of pregnancy by Zhang *et al.* reported evidence of liver dysplasia, inhibition of hepatocyte proliferation and dose-dependent reduction in liver weight after antenatal dexamethasone exposure.[132] In sheep, our group has shown that preterm lambs exposed to ANS (both dexamethasone and betamethasone) demonstrate elevated neonatal plasma GGT levels,[32] which may be an indication of potential hepatobiliary dysfunction.[133]

In conclusion, normal hepatic homeostasis appears to be significantly affected in these animal models with ANS exposure, resulting in an increased risk of developing hepatic steatosis at a normal body weight (without obesity) in both early and later life.

Clinical studies

The exact mechanism of how ANS-induced fetal hepatic steatosis occurs remains relatively unknown. This is possibly because GR/MR expression and function within the developing fetal liver is poorly understood.[134] However, mechanisms may be postulated from findings in adults, in which exogenous glucocorticoids increase hepatic lipid synthesis through increased expression of fatty acid synthase as well as an increase in hydrolysis of circulating triglycerides leading to a corresponding rise in free fatty acids.[135] Unfortunately, there are very little clinical data at present on the short and long-term effects of ANS on human fetal and adult hepatic functioning. This is of concern given the rising incidence and earlier age of onset of non-alcoholic fatty liver disease around the world in conjunction with evidence in this review of a potential fetal origin for hepatic steatosis.[134] Furthermore, the link between ANS exposure and disordered glucose metabolism outlined in this article suggests a possible link to childhood and adult-onset type II diabetes mellitus. This is important given that the Auckland Steroids Trial Cohort also demonstrated evidence of insulin resistance in later life.[121]

Renal system effects

Endogenous glucocorticoids (cortisol) demonstrate both glucocorticoid and modest MR activity. Presently, the impact of cortisol on fetal renal system development is not fully understood. Rat models have however revealed that pharmacological blockade of 11β-HSD-2 (the placental enzyme that protects the fetus from high levels of maternal glucocorticoids) results in adult-onset hypertension.[136] Further, clinical studies of individuals with a severe genetic deficiency of 11β-HSD-2 display a phenotype of juvenile-onset hypertension.[137] Therefore, it is evident that fetal programming of the renal system must occur in relation to specific endogenous glucocorticoid exposures.

The impact of exogenous glucocorticoids (betamethasone and dexamethasone) on fetal renal programming is similarly not well characterised but requires further investigation especially given that these drugs demonstrate no direct MR action but cause suppression of cortisol and therefore impede cortisol's action on the MR.

Animal studies

Animal models have in part helped to shed some light on the fetal programming effects of ANS exposure. Ortiz *et al.* utilising a rat model of pregnancy demonstrated a 20%–30% reduction in glomerular number as well as hypertension in juveniles who were exposed to two doses of antenatal dexamethasone compared to control.[138] Similar findings have been demonstrated in sheep studies

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of ANS exposure showing a reduction in nephron and glomeruli counts at two months, six months and seven years of age which was accompanied by the development of hypertension.[139–142] Fetal kidney development in sheep closely resembles that of humans, making this animal model highly translatable. Persistent abnormal renal morphology has also been demonstrated in ANS-exposed adult sheep at 7 years of age in the study by Wintour *et al.*[139] Findings from this study included grossly enlarged and dilated proximal renal tubules as well as abnormal collagen accumulation in the renal tubular interstitium and renal cortical vessels. The authors postulated that these findings, particularly increased collagen within cortical vessels, may account for the development of hypertension seen in those animals.[139] Furthermore, other studies in sheep exposed to antenatal betamethasone exhibited hypertension and abnormal angiotensin receptor expression at 1–1.5 years of age.[143]

These findings provide a link between ANS exposure, abnormal renal development and risk of hypertension in adult life . This is a potentially significant finding given the increasing incidence of renal disease and hypertension in human populations today. The exact mechanism by which ANS might cause hypertension remains unknown. However, human adults with essential hypertension have been shown to have a significant reduction in nephron counts similar to that observed in ANS animal studies.[144] Therefore, a reduction in nephron count may impair renal sodium excretion leading to increased blood volume and subsequent blood pressure.

Clinical studies

Evidence from two cohort studies investigating the effect of ANS on renal function and development is unclear. A cohort ($n = 173$) of 14-year-olds who were born at a mean gestation of ~ 28 weeks' and with a very low birth weight <1500 g showed that those exposed to ANS compared to unexposed exhibited alterations in the renin-angiotensin-aldosterone system (RAAS) as measured by urinary and plasma biomarkers.[145] Dysregulation of the RAAS was more marked for those adolescents of black ethnicity.[145] The authors concluded that these changes may increase the risk of developing hypertension and renal inflammation/fibrosis later in life.[145] By comparison, a very similar cohort ($n = 162$) of 14-year-olds who were also born premature with a very low birthweight (<1500 g) showed that ANS exposure was not associated with abnormal kidney function as measured by eGFR.[146] However, biomarkers of the RAAS were not measured in this cohort.

The impact of ANS on mineralocorticoid expression is not well characterised. Kessel *et al.* showed that in a clinical study of women exposed to antenatal betamethasone, plasma aldosterone levels were lower in both mother and infant at delivery compared to controls, which took up to 3–7 days to recover.[147] Aldosterone plays a critical role in the homeostasis of electrolyte (potassium/sodium) excretion and reabsorption in the kidney, which therefore affects blood volume and pressure.[147] The long-term programming effects of aldosterone suppression on the fetus remain unknown and require further investigation in clinical studies. However, this is another potential mechanism by which ANS may contribute to the observed development of hypertension in some individuals.

There is a current paucity of evidence from recent RCT follow-up studies (including ALPS/ASTECs or AST) on the long-term effects of ANS on renal functioning, therefore highlighting an area of need for further investigation.

Conclusion

ANS therapy undoubtedly improves perinatal outcomes when administered to the right patient at the right time. However, in order to maximise the potential benefits and minimise the potential harms of ANS therapy, a comprehensive understanding of the on and off-target effects of ANS on fetal development is required. Improving the current understanding of the potential off-target effects of ANS therapy on fetal development was the aim of this review. In addition, we highlight the urgent need for increased animal and clinical studies investigating fetal developmental programming after ANS exposure, particularly with regard to the immune, cardiovascular, renal and hepatic systems given the current sparsity of high-quality, long-term evidence. We also advocate for increased education, open disclosure and discussion between clinicians and patients on the current evidence of the off-target effects of ANS. We place particular emphasis on the late preterm and term gestations wherein the observed benefits of ANS therapy are very modest. Finally, we urge further studies on the optimisation of ANS therapies through better patient selection and reduced dose regimens based on the current pharmacokinetic understanding.

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