Antenatal corticosteroids (ANS) are the major intervention to decrease respiratory distress syndrome and mortality from premature birth and are standard of care. The use of ANS is expanding to include new indications and gestational ages, although the recommended dosing was never optimized. The most widely used treatment is two intramuscular doses of a 1:1 mixture of betamethasone-phosphate (Beta-P) and betamethasone-acetate (Beta-Ac) – the clinical drug. We tested in a primate model the efficacy of the slow release Beta-Ac alone for enhancing fetal lung maturation and to reduce fetal corticosteroid exposure and potential toxic effects. Pregnant rhesus macaques at 127 days of gestation (80% of term) were treated with either the clinical drug (0.25 mg/kg) or Beta-Ac (0.125 mg/kg). Beta-Ac alone increased lung compliance and surfactant concentration in the fetal lung equivalently to the clinical drug. By transcriptome analyses the early suppression of genes associated with immune responses and developmental pathways were less affected by Beta-Ac than the clinical drug. Promoter and regulatory analysis prediction identified differentially expressed genes targeted by the glucocorticoid receptor in the lung. At 5 days the clinical drug suppressed genes associated with neuronal development and differentiation in the fetal hippocampus compared to control, while low dose Beta-Ac alone did not. A low dose ANS treatment with Beta-Ac should be assessed for efficacy in human trials.
improved water clearance resulting in increased lung compliance. Both the clinical drug and Beta-Ac (0.125 mg/kg) ACS induce fetal lung maturation by a combination of increased surfactant production, structural changes, and to be associated with “lung expression”, “respiratory disease”, and “lung cell line” (Fig. 3A).

Regulated or interact with the NR3C1 receptor. Several common regulated genes are also reported in the literature transcription factor binding site prediction and protein regulation or interaction prediction, several of the common expression of only 393 genes, of which 372 were common to both treatments. The common differentially expressed concentration the clinical drug caused differential expression of 1,779 genes while Beta-Ac caused the differential expression of 432 genes. These commonly regulated genes at 5 days also had a similar magnitude of differential expression and highly correlated log fold-change values (Fig. 3A, C). By tran-

scriptional), with 185 genes differentially expressed in common. These commonly regulated genes at 5 days also had a similar magnitude of differential expression and highly correlated log fold-change values (Fig. 3A, C). By tran-

scriptomic effects of the clinical drug and Beta-Ac on the fetal lung.

Results

Beta-Ac enhances fetal lung maturation at 5 days. To compare Beta-Ac with the clinical drug for fetal lung maturation, pregnant Rhesus macaques were treated with a single IM injection of either Beta-Ac (0.125 mg/kg or 0.06 mg/kg), saline (control) or the clinical drug (0.25 mg/kg) and delivered 5 days after treatment (Table 1). ACS induce fetal lung maturation by a combination of increased surfactant production, structural changes, and improved water clearance resulting in increased lung compliance. Both the clinical drug and Beta-Ac (0.125 mg/kg) improved static lung compliance (Fig. 1A, B). Beta-Ac 0.06 mg/kg inconsistently increased lung compliance with 3 out of 5 treated animals having pressure-volume curves similar to control. The main lipid component of surfactant is saturated phosphatidylcholine (SatPC), which can be used as a marker of lung surfactant content and indicator of biochemical lung maturation24. Both Beta-Ac (0.125 mg/kg) and the clinical drug increased the SatPC in the bronchoalveolar lavage fluid compared to control, while the increase with Beta-Ac (0.06 mg/kg) was not significant (Fig. 1C).

Confocal microscopy of immunofluorescent staining of the fetal lung for the epithelial cell marker TTF-1, smooth muscle actin (SMA), and type II alveolar cell markers pro-SPC, and ABCA3 showed no differences in the relative numbers of cells positive for these markers 5 days after treatment (Supplemental Fig. 1). Further, there were no differences in the proportion of cells expressing the cell cycle marker Ki-67.

Table 1. Weight, gestational age, sex and cortisol of the animals. The groups were comparable. Cord plasma cortisol concentration measured for control animals and 5 days. After ANS treatment were not different among groups.

|                      | Control     | Clinical drug | Beta-Ac     |
|----------------------|-------------|---------------|-------------|
| Birth weight (g)     | 330 ± 35    | 317 ± 22      | 338 ± 39    |
| Gestational age (days)| 132 ± 2     | 131 ± 2       | 133 ± 2     |
| Sex (M/F)            | 6/2         | 3/0           | 3/4         |
| Fetal plasma cortisol (µg/dL) | 2.5 ± 0.6  | —             | 2.6 ± 1.0   |

Any pharmacological intervention for treating a pregnant woman to benefit the fetus should be considered high risk. To translate a new ANS therapy from animal models to humans, a validation in primates is desirable. We evaluated a lower dosing strategy using Beta-Ac for fetal lung maturation and transcriptional effects on the fetal lung and brain in the Rhesus macaque.

Table 2: Birth weight, gestational age, sex and cortisol of the animals. The groups were comparable. Cord plasma cortisol concentration measured for control animals and 5 days. After ANS treatment were not different among groups.
Figure 1. (A) Pressure-volume curves showing comparable improved static compliance after treatment with Beta-Ac (0.125 mg/kg) and the clinical drug. (B) Lung gas volumes were increased significantly for Beta-Ac 0.125 mg/kg and the clinical drug. (C) Saturated phosphatidylcholine (SatPC) concentration in the bronchoalveolar lavage fluid (BALF) increased with Beta-Ac 0.125 mg/kg and the clinical drug compared to control. *p-value < 0.05 vs. control.

Figure 2. Transcriptomic analysis was performed on whole lung RNA 4 hours and 5 days after administration of the clinical drug, and 6 hours and 5 days after Beta-Ac 0.125 mg/kg and from saline-treated animals (n = 3 animals per group). 3-dimensional principal component analysis (PCA) was generated using log-transformed read counts. (A) At the early timepoint PCA separated the animals treated with the clinical drug at 4 h, Beta-Ac at 6 h, and controls. (B) At 5 days there is separation of control animals and overlap of animals treated with the clinical drug and Beta-Ac. (C) Sample correlation heatmap displaying the similarity in gene expression profile. Red color indicates increasing sample correlation and yellow color indicates decreasing sample correlation. Dendrogram clustering indicates the overall similarity of the samples. Both analyses showed separation of the clinical drug at 4 h and overlap of animals in the other treatment groups.
Genes differentially regulated by either of the ANS treatments were associated with several similar biological processes. At the early time point induced genes were associated with “cellular localization”, “developmental process”, “ion and protein transport” while suppressed genes were associated with “cellular morphogenesis”, “chemotaxis”, and “immune responses”.

Despite the similarities, there were large differences between biological processes and pathways that were differentially regulated in the lung by the clinical drug and Beta-Ac relative to control at the time of peak drug concentration in the fetal plasma (Fig. 4). The clinical drug had larger inhibitory effects on immune system processes and development, including suppression of Th1, Th2 and Th17 lymphocyte differentiation, lymphocyte proliferation and activation, and cytokine signaling. The clinical drug also modulated biological processes related to organ development and morphogenesis, which were only weakly associated with Beta-Ac treatment. Specifically, genes inhibited by the clinical drug were strongly enriched for angiogenesis and vascular development, endothelial cell proliferation, epithelial tube morphogenesis, and canonical Wnt signaling. Genes differentially expressed by the clinical drug at the early time point may not be contributing to lung maturational responses as the 2 treatments resulted in similar improvement in lung gas volume and similar RNA profiles at 5 days.

Transcriptomic effects in the fetal hippocampus at 5 days. To evaluate potentially toxic effects of ANS on the fetal brain development we compared the transcriptome of the fetal hippocampus 5 days after ANS treatment. The principal component analysis and the correlation heatmap demonstrate that control animals cluster separately and distant from animals treated with the clinical drug (Fig. 5A,B). In contrast, animals treated with Beta-Ac 0.125 mg/kg are interspersed between the other groups but with wide variation among samples. By differential expression analysis 1612 genes were differentially regulated (788 induced, 824 suppressed) in the hippocampus by the clinical drug at 5 days compared to control (Fig. 5C). There were no statistical differences between gene expression levels in the Beta-Ac treated animals compared to control. Genes induced by the clinical drug were associated with the regulation of synapse maturation and semaphorin interactions, among others. Suppressed genes were associated with regulation of neurogenesis, neuron, projection development, cellular morphogenesis and nervous system development (Figs 6 and 7).
Discussion
Since 1972, use of ANS for women at risk of preterm labor at 24–34 weeks gestation decreased the incidence of respiratory distress syndrome and mortality in preterm newborns, but the formulation and dosing was never optimized by pharmacokinetic analyses or for safety. In fact, the expanding use of ANS beyond the 24 to 34 week gestational has identified previously unrecognized risks such as hypoglycemia in late preterm infants and increased mortality in middle and low-resource countries. We report that fetal lung maturation in the primate can be achieved with a single weight-based dose of Beta-Ac that avoided fetal exposure to higher Beta levels from the Beta-P component of the clinical drug. Beta-Ac minimized early transcriptional changes in the fetal lung associated with immunity and morphogenesis, and at 5 days in the fetal hippocampus associated with nervous system development despite similar pulmonary maturation. As even routine short-term use of corticosteroids is associated with risks, and complications are dose related, the lowest fetal exposure sufficient to get the maturational benefit should be the goal.

Different steroid formulations and dosing intervals are used around the world based on a total dose of 24 mg with minimal experimental or clinical data to support equivalency. We previously showed with fetal sheep that a single dose of Beta-Ac that was 25% of the standard 2-dose treatment with the clinical drug yielded similar improvements in gas exchange and lung compliance 2 days after treatment. This treatment strategy in sheep exposes the fetus to a peak drug concentration that is 10% of the peak drug concentration with the clinical drug, while providing continuous fetal exposure to betamethasone for 24 hours.

In the primate, treatments with Beta-Ac alone and the clinical drug resulted in similar mechanical induction of lung maturation as demonstrated by the pressure-volume curves and similar increase in the surfactant content, measured by the SatPC concentration in the BALF. Increased surfactant production by the premature lung decreases the severity and incidence of RDS in preterm newborns. Moreover, there were no differences between the lung transcriptomes 5 days after treatment. Interestingly, the proportions of cells expressing the epithelial marker TTF-1 or the type 2 alveolar cell markers, ABCA3 and SPC, were similar among treatment groups and control at 5 days, suggesting that ANS induced transient changes in expression of mRNA but not persistent cellular differentiation to increase the number of surfactant producing cells. This finding is consistent with the observation that the effects of ANS are transient and clinical trials have not consistently demonstrated benefit in decreasing RDS beyond 7 days after treatment.

The early transcriptomic changes in the lung 4 to 6 hours after ACS exposure offers new insight into the complex signaling mechanism of lung maturation induced by corticosteroids. While most studies have focused on the effects of ACS on the endpoints of increased surfactant production and improved lung structure here we show that ACS has large effects on developmental, vascularization, cell signaling, and cell cycle pathways. These multiple effects likely all contribute to the improved lung function seen in preterm newborns after ACS treatment. More interestingly, 5 days after ACSs treatment, transcriptional changes were limited and were associated with ion transport and cytoskeletal organization. These changes could be associated with continued structural and tissue water balance maturation. However, most of the effects of ACS on gene expression had disappeared by 5 days.

We provide new information that the high peak drug level from the clinical drug was associated with more suppression of lung immune responses, angiogenesis and developmental pathways based on transcriptome analyses. While the clinical drug regulated a larger number of genes and pathways than Beta-Ac, the genes that were commonly differentially expressed had similar magnitudes of change, indicating that the additional steroid exposure may not be contributing to more maturational signaling. Changes in expression of other genes may
only have harmful harmful side effects. While in high resource environments ANS has not been associated with increased risk of maternal or neonatal infection or sepsis, in the subgroup of patients with preterm rupture of membranes the risk of chorioamnionitis was increased with repeated treatments with ANS. In middle and low-resource countries the increased infant mortality associated with ANS treatment may be caused by increased the risk of infection. Due to cost and availability, the international cluster-randomized trial used Dex-P given as 4 intramuscular doses of 6 mg every 12 hours. This dosing strategy, provides fetal exposure to ANS for greater than 48 hours but with 4 high peak fetal plasma levels that are not necessary in animal models. Avoiding high fetal concentrations of corticosteroids should minimize the treatment effects on the maternal and fetal immune responses and the risk of perinatal infection.

More concerning are the reports of direct effects of glucocorticoids on the fetal brain. Preterm newborns exposed to glucocorticoids had decreased number of neurons in the hippocampus, consistent with a previous report of increased apoptosis of neuron in the hippocampus of macaques after treatment with glucocorticoids. We found that fetal exposure to the clinical drug resulted in suppression of genes associated with neurogenesis and nervous system development 5 days after treatment. There were no differences in the hippocampus transcriptome between animals treated with Beta-Ac compared to control but we did observe a wide variability in the Beta-Ac group with some animals clustering with controls and others with the clinical drug. This variability could be due to individual variations regarding drug metabolism affecting the fetal exposure to the treatment or genetic variants affecting the molecular response to corticosteroids. In our limited sample size the sex of the animal did not seem to affect the response. The most recent meta-analysis of antenatal corticosteroids showed

Figure 5. Transcriptomic analysis was performed for fetal hippocampus from saline-treated animals and 5 days after administration of ANS (n = 5 animals per group). (A) 3-dimensional principal component analysis (PCA) was generated using log-transformed read counts. (B) Sample correlation heatmap displaying the similarity in gene expression profile. Red color indicates increasing sample correlation and yellow color indicates decreasing sample correlation. Dendrogram clustering indicates the overall similarity of the samples. Both analyses showed separation of the control from animals treated with the clinical drug, while animals treated with Beta-Ac are interspersed between the controls and clinical treatment animals. (C) Top differentially induced and suppressed genes by the clinical drug in the hippocampus at 5 days. Genes are ordered by magnitude of gold change; p values are adjusted by the Benjamin-Hochberg method.
Figure 6. Gene set enrichment analysis comparing differentially expressed genes for the clinical drug compared to control in the fetal hippocampus. There was no differential expression between Beta-Ac and control. Selected gene ontology terms are displayed with the bar chart representing log p-values. Positive p-values denote induced genes and negative p-values denote suppressed genes.

Figure 7. Network of genes suppressed by the clinical drug in the fetal hippocampus at 5 days associated with the biological processes of neurogenesis and neuron differentiation.
±

2 days of gestation (n =
other groups of fetuses were treated 5 days before delivery at 132

group). Lung samples were frozen for RNA-sequencing. To assess the maturational effects of the interventions,

After delivery, pressure-volume curves were measured with a syringe and pressure manometer by inflating the

right lower lobe of fetal lung and the hippocampus were snap frozen for RNA-sequencing

Research, Rochester Hills, MI).

described35. Cord blood plasma cortisol levels were measured using an ELISA kit (EA65; Oxford Biomedical

(SatPC) was isolated after exposure to osmium tetroxide and quantified by phosphorus assay as previously

from the left lung and lipids were extracted with chloroform-methanol (2:1). Saturated phosphatidylcholine

Hills Bioreagents, Cincinnati, OH; bSanta Cruz Biotechnologies, Dallas, TX; cLifeSpan Biosciences, Seattle, WA.

Primary antibodies and dilutions for immunofluorescence of paraffin-embedded lung sections. aSeven

Table 2. Primary antibodies and dilutions for immunofluorescence of paraffin-embedded lung sections. aSeven

Hills Bioreagents, Cincinnati, OH; bSanta Cruz Biotechnologies, Dallas, TX; cLifeSpan Biosciences, Seattle, WA.

| Antibody | Cellular marker | Species | Dilution |
|----------|----------------|---------|----------|
| anti-TTF-1 | Epithelial | Rabbit | 1:500 |
| anti-TTF-1 | Epithelial | Guinea pig | 1:200 |
| anti-pro-surfactant protein C (SPC)| Alveolar type II | Rabbit | 1:100 |
| anti-ABCA3 | Alveolar type II | Guinea pig | 1:100 |
| anti-smooth muscle actin (SMA) | Smooth muscle, myofibroblast | Mouse | 1:2000 |
| Anti-Ki67 | Cell cycle | Rat | 1:50 |

a trend towards reduced neurodevelopmental impairment after a single course of ANS in infants less than 34
weeks gestation in high resource countries36. There are no data on neurodevelopmental outcomes for late preterm
infants where the clinical benefits of ANS are small and may not outweigh the risks. This benefit to risk ratio may be
even less for elective C-sections. Even more problematic are reports of increased renal disease, obesity and
metabolic syndrome at advanced ages in sheep and baboons exposed as fetuses to ANS32–34. These effects cannot be
evaluated in human populations being treated with ANS today and may be at long-term risk of fetal effects on
adult outcomes.

Here we demonstrate that a clinically relevant dose of ANS used for fetal lung maturation caused profound and
early changes in transcriptional networks that control lung development and immunity and persistent changes
on brain development pathways. Many of the changes can be avoided by low-dose Beta-Ac while preserving the
physiological maturational effects in a nonhuman primate model. This strategy should be considered for clinical
trials to optimize ANS treatment in preterm infants and decrease potential toxic effects.

Methods

Animals. The Institutional Animal Care and Use Committee at the University of California Davis approved
all animal procedures, which were performed at the California National Primate Research Center according to
the approved protocol. Time-mated pregnant Rhesus macaques were given the clinical drug as intramuscular
Celestone Soluspan® 0.25 mg/kg (6 mg/ml containing 3 mg/mL betamethasone as Beta-P and 3 mg/mL of
Beta-Ac; Merck Sharp & Dohme, Kenilworth, NJ), 0.125 mg/kg Beta-Ac (a gift from Merck Sharp & Dohme,
Kenilworth, NJ), 0.06 mg/kg Beta-Ac or saline prior to preterm delivery at 132 ± 2 days gestational age (term is 165 days). To investigate the early transcriptional effects, fetuses were delivered at the time of peak fetal blood
Beta levels based on measurements in fetal sheep35: 4h after the clinical drug and 6 h after Beta-Ac (n = 3 animals/group).
Lung samples were frozen for RNA-sequencing. To assess the maturational effects of the interventions,
other groups of fetuses were treated 5 days before delivery at 132 ± 2 days of gestation (n = 5–8 animals/group).
After delivery, pressure-volume curves were measured with a syringe and pressure manometer by inflating the
lungs to 40 cm H2O pressure and followed by deflation with measurements of lung volumes. The right upper lobe of
the fetal lung was inflation fixed with formalin at 30 cm H2O pressure for histology; tissue samples from the
right lower lobe of fetal lung and the hippocampus were snap frozen for RNA-sequencing

Saturated phosphatidylcholine and cortisol measurements. Alveolar lavage fluid was recovered from the left lung and lipids were extracted with chloroform-methanol (2:1). Saturated phosphatidylcholine (SatPC) was isolated after exposure to osmium tetroxide and quantified by phosphorus assay as previously described36. Cord blood plasma cortisol levels were measured using an ELISA kit (EA65; Oxford Biomedical Research, Rochester Hills, MI).

Immunofluorescence and confocal microscopy. Sections from paraffin-embedded tissues underwent heat-assisted antigen retrieval with citrate buffer (pH 6.0), followed by blocking with donkey or goat serum and incubation with primary antibodies overnight (Table 2). The following day, sections were incubated with species-specific Alexa Fluor antibody (Life Technologies, Carlsbad, CA), followed by DAPI (Life technologies, Carlsbad, CA, dilution 1:2000). Sections were mounted with ProLong Gold (Life technologies, Carlsbad, CA). Stained slides were imaged by confocal microscopy for co-localization of fluorescent antibodies at 40x magnification, 1024 × 1024 pixels resolution on a Nikon Eclipse A1RSi inverted microscope (Nikon Instruments Inc., Melville, NY). Confocal images were analyzed using Nikon NIS Elements software (Nikon Instruments Inc., Melville, NY), for object count and colocalization.

Statistical analyses. Statistical analyses of morphological and immunofluorescence data were performed with GraphPad Prism software (Carlsbad, CA). Values for continuous variables were compared by t-test or ANOVA followed by Holm-Sidak post-hoc analysis for multiple comparisons. Data are presented as bars with individual data points and standard deviation.

RNA isolation and sequencing. Total RNA was extracted from frozen lung tissues using the RNeasy Universal Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA quality and integrity were verified using the Agilent 2100 Bioanalyzer (Agilent, Agilent Technologies, Santa Clara, CA).
RNA-sequencing was performed by the Cincinnati Children's Hospital Medical Center DNA Sequencing and Genotyping Core with a read depth of 20–30 million reads per sample for 75 bp paired-end reads. The raw sequence reads in FASTQ format were aligned to the Rhesus (Macaca mulatta) genome build MMUL1.0 using Bowtie 2.6.4. Reads were counted using featureCounts27. After checking data quality, raw read counts were filtered to exclude genes with low expression (<7 reads) and normalized using the trimmed mean of M values method38. Differential expression analyses comparing treatment groups to control and between each other were performed using Edger26 followed by false discovery rate adjustment using Storey's method40. Genes were considered differentially expressed based on their fold-change relative to control (= or > 1.5), p-value (<0.05) and q-value (<0.1).

Functional enrichment and pathway analysis. Differentially expressed genes were used for functional enrichment analysis of Gene Ontology and pathway terms using the ToppCluster web server41. Only unique terms associated with either induced or suppressed genes and at least 2 genes are reported. Negative log p-values represent terms associated with suppressed gene expression and positive log p-values are associated with induced gene expression. Promoter GRE cis-element was scanned using the Msig-DB motif gene sets within 4 kb around their transcription starting sites (http://software.broadinstitute.org/gsea/msigdb). The evidence that NR3C1 regulates or interacts with genes in the top hits list was obtained via literature mining using Genomatix co-citation database (Genomatix Inc.) and IPA knowledge base (Ingenuity Pathway Analysis, QIAGEN). Annotation of genes expressed in the lung or associated with respiratory disease were collected from IPA knowledge base.

Data Availability
The gene expression data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series Accession Number GSE118438 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE118438).

References
1. In WHO Recommendations on Interventions to Improve Preterm Birth Outcomes WHO Guidelines Approved by the Guidelines Review Committee (2015).
2. Jobe, A. H. & Goldenberg, R. L. Antenatal corticosteroids: an assessment of anticipated benefits and potential risks. Am J Obstet Gynecol 219, 62–74, https://doi.org/10.1016/j.ajog.2018.04.007 (2018).
3. Gyamfi-Bannerman, C. et al. Antenatal Betamethasone for Women at Risk for Late Preterm Delivery. N Engl J Med 374, 1311–1320, https://doi.org/10.1056/NEJMoa1516775 (2016).
4. Stutchfield, M. N., Whitaker, R., Russell, I. & Antenatal Steroids for Term Elective Caesarean Section Research Team. Antenatal betamethasone and incidence of neonatal respiratory distress after elective caesarean section: pragmatic randomised trial. BMJ 331, 662, https://doi.org/10.1136/bmj.38547.416493.06 (2005).
5. Nada, A. M. et al. Antenatal corticosteroid administration before elective caesarean section at term to prevent neonatal respiratory morbidity: a randomized controlled trial. Eur J Obstet Gynecol Reprod Biol 199, 88–91, https://doi.org/10.1016/j.ear.2016.01.026 (2016).
6. Committee Opinion No. 713 Summary: Antenatal Corticosteroid Therapy for Fetal Maturation. Obstet Gynecol 130, 493–494, https://doi.org/10.1097/AOG.0000000000002331 (2017).
7. Betran, A. P. et al. The Increasing Trend in Caesarean Section Rates: Global, Regional and National Estimates: 1990–2014. PLoS One 11, e0148343, https://doi.org/10.1371/journal.pone.0148343 (2016).
8. Blencowe, H. et al. Born too soon: the global epidemiology of 15 million preterm births. Reprod Health 10(Suppl 1), S2, https://doi.org/10.1186/1742-4755-10-S1-S2 (2013).
9. Althabe, F. et al. A population-based, multifaceted strategy to implement antenatal corticosteroid treatment versus standard care for the reduction of neonatal mortality due to preterm birth in low-income and middle-income countries: the ACT cluster-randomised trial. Lancet 385, 629–639, https://doi.org/10.1016/S0140-6736(14)61651-2 (2015).
10. Althabe, F. et al. The Antenatal Corticosteroids Trial (ACT)'s explanations for neonatal mortality - a secondary analysis. Reprod Health 13, 62, https://doi.org/10.1186/s12978-016-0175-3 (2016).
11. Epstein, M. F. et al. Maternal betamethasone and fetal growth and development in the monkey. Am J Obstet Gynecol 127, 261–263 (1977).
12. Uno, H. et al. Neurotoxicity of glucocorticoids in the primate brain. Horm Behav 28, 336–348, https://doi.org/10.1006/hibeh.1994.1030 (1994).
13. de Vries, A. et al. Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function. J Clin Invest 117, 1058–1067, https://doi.org/10.1172/JCI99082 (2007).
14. Huang, W. L. et al. Effect of corticosteroids on brain growth in fetal sheep. Obstet Gynecol 94, 213–218 (1999).
15. Gramsbergen, A. & Mulder, E. J. The influence of betamethasone and dexamethasone on motor development in young rats. Pediatr Research 44, 105–110, https://doi.org/10.1203/00006450-199807000-00017 (1998).
16. Jobe, A. H., Wada, N., Berry, L. M., Ikegami, M. & Ervin, M. G. Single and repetitive maternal glucocorticoid exposures reduce fetal growth in sheep. Am J Obstet Gynecol 178, 880–885 (1998).
17. Braun, T. et al. Fetal and neonatal outcomes after term and preterm delivery following betamethasone administration. Int J Gynaecol Obstet 130, 64–69, https://doi.org/10.1016/j.ijgo.2015.01.013 (2015).
18. Tijsseling, D. et al. Effects of antenatal glucocorticoid therapy on hippocampal histology of preterm infants. PLoS One 7, e33369, https://doi.org/10.1371/journal.pone.0033369 (2012).
19. Effect of corticosteroids for fetal maturation on perinatal outcomes. NIH Consensus Statement 12, 1–24 (1994).
20. Samtani, M. N., Lohle, M., Grant, A., Nathanielsz, P. W. & Jusko, W. J. Betamethasone pharmacokinetics after two prodrug formulations in sheep: implications for antenatal corticosteroid use. Drug Metab Dispos 33, 1124–1130, https://doi.org/10.1124/dmd.105.004309 (2005).
21. Jobe, A. H. et al. Betamethasone dose and formulation for induced lung maturation in fetal sheep. Am J Obstet Gynecol 201, 611 e611–617, https://doi.org/10.1016/j.ajog.2009.07.014 (2009).
22. Schmidt, A. F. et al. Antenatal dexamethasone vs. betamethasone dosing for lung maturation in fetal sheep. Pediatric research 81, 496–503, https://doi.org/10.1038/pr.2016.249 (2017).
23. Schmidt, A. F. et al. Low-dose betamethasone-acetate for fetal lung maturation in preterm sheep. Am J Obstet Gynecol 218, 132 e131–e139, https://doi.org/10.1016/j.ajog.2017.11.560 (2018).
24. Jobe, A. H. et al. Endotoxin-induced lung maturation in preterm lambs is not mediated by cortisol. Am J Respir Crit Care Med 162, 1656–1661, https://doi.org/10.1164/ajrccm.162.5.2003044 (2000).
Author Contributions

A.F.S. participated in the conception of the experimental design and animal experiments, analyzed the bioinformatics and the confocal data, P.S.K. performed and analyzed the cortisol assay and performed the immunofluorescence experiments, J.P.B. and A.F. performed the surfactant measurement experiments, D.L. participated in the bioinformatics analysis, M.K. participated in the experimental design and data interpretation, S.G.K. participated in the conception of the experimental design and animal experiments, analyzed the
biomarkers, A.F. S. participated in the design of the experimental evaluation and effect interpretation
of the data. A.H.J. conceived the idea, participated in the animal experiments and interpretation of the data. A.F.S. wrote the manuscript with support from J.A.W. and A.H.J. All authors read and approved the final manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-45171-6.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.