Low-dose antenatal betamethasone treatment achieves preterm lung maturation equivalent to that of the WHO dexamethasone regimen but with reduced endocrine disruption in a sheep model of pregnancy

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PII: S0002-9378(22)00534-8
DOI: https://doi.org/10.1016/j.ajog.2022.06.058
Reference: YMOB 14593


Received Date: 17 January 2022
Revised Date: 8 June 2022
Accepted Date: 28 June 2022


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[Title] Low-dose antenatal betamethasone treatment achieves preterm lung maturation equivalent to that of the WHO dexamethasone regimen but with reduced endocrine disruption in a sheep model of pregnancy

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[Disclosure statement] The authors report no conflict of interest.
[Financial support for the research] This work was supported by grants from the Channel 7 Telethon Trust, the Department of Health, Government of Western Australia (MWK), the Stan Perron Charitable Foundation, and the National Health and Medical Research Council (GNT1162572). The funders had no role in study design, in the collection, analysis or interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

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[Word count of abstract] 451

[Word count of manuscript] 5220
A low-dose antenatal steroid regimen conveys equivalent benefit and less homeostatic disruption to mother and fetus than the standard-dose WHO regimen in a sheep model of pregnancy.
A low-dose antenatal betamethasone regimen matures the fetal lung and minimizes HPA axis disruption.
[At a Glance]

A: Why was this study conducted?

Antenatal corticosteroid (ACS) therapy is now widely used to improve preterm outcomes, with the principal desired benefit being precocious maturation of the fetal lung. Increasing evidence shows that current clinical regimens (i.e. intramuscular injections of betamethasone acetate and phosphate (12 mg; two doses at a 24-hour interval) or dexamethasone phosphate (6mg; four doses at 12-hour intervals) achieve materno-fetal steroid exposures well in excess of those required to elicit lung maturation. We and others have previously suggested that these elevated exposures may increase the risk of harm. In support of the forthcoming ACTION III trial (https://www.who.int/publications/m/item/the-who-action-iii-(antenatal-corticosteroids-for-improving-outcomes-in-preterm-newborns)-trial), we used a sheep model of pregnancy to study the lung maturation and pharmacodynamic effects of a low dose treatment regimen employing betamethasone phosphate regimens (2 mg; four times at 12-hour intervals), and compared it with outcomes deriving from the current WHO-recommended regimen employing dexamethasone phosphate (6mg; four times at 12-hour intervals).

B: Key findings

The primary findings of this study were as follows: (1) The low-dose regimen (four 12-hourly doses of betamethasone phosphate at 2mg; total 8mg) achieved functional maturation of the
ovine preterm lung equivalent to that from the current WHO-recommended dosing regimen (four 12-hourly doses of dexamethasone phosphate at 6mg: total 24mg); (2) The low-dose regimen resulted in less severe disruptions of the materno-fetal HPA axis, circulating immunocyte populations, plasma glucose and insulin. Suppression of the key fetal growth factor, IGF-1, was seen only in the standard-dose dexamethasone phosphate treatment group.

C: What does this add to what is known? This study demonstrates that, in a sheep model of pregnancy, low-dose antenatal steroid treatments (66% reduction in steroid administered) achieve equivalent lung maturation to standard-dose regimens currently in use. The low-dose regimen was predicted to achieve lower peak steroid exposures and an improved side-effect profile (i.e. fewer or more modest endocrine and immunocyte perturbations). Given the significant difference in materno-fetal steroid gradient between sheep and humans, these data strongly suggest that a regimen based on doses of betamethasone significantly less than 2mg will achieve similarly improved outcomes in humans.
Key words

Glossary
Glucocorticoids: synthetic steroid hormones that exert pleiotropic signaling activity via activation of the glucocorticoid receptor and via non-genomic signaling. Widely used in obstetrics to precociously mature the preterm lung in anticipation of preterm delivery.
[Abstract]

[Introduction] The intramuscular administration of antenatal steroids (ANS) to women at risk of preterm delivery achieves high maternal and fetal plasma steroid concentrations, which are associated with adverse effects and may reduce treatment efficacy. We have demonstrated that ANS efficacy is independent of peak materno-fetal steroid levels once exposure is maintained above a low threshold.

[Objectives] We aimed to test, using a sheep model of pregnancy, whether the low-dose ANS regimen proposed as part of the ACTION III Trial would achieve preterm lung maturation equivalent to that of the existing WHO dexamethasone treatment regimen, but with reduced risk of adverse outcomes.

[Study Design] Following ethical review and approval, date-mated ewes with single fetuses received intramuscular injections of either: i) four x 6mg maternal intramuscular injections of dexamethasone phosphate q12h (n=22); or ii) four x 2 mg maternal intramuscular injections of betamethasone phosphate q12h (n=21); or iii) four x 2mL maternal intramuscular injections of saline q12h (n=16). Forty-eight hours after first injection, (124±1 d), lambs were delivered, ventilated for 30 minutes, and euthanised for sampling. Arterial blood gas, respiratory, haematological and biochemical data were analysed for between-group differences with ANOVA according to distribution and variance, with p<0.05 taken as significant.
After 30 minutes ventilation, lambs from both steroid-treated groups had significant and equivalent improvements in lung function relative to saline control ($p<0.05$). There was no significant difference in arterial blood pH, $pO_2$, $pCO_2$, lung compliance, ventilator efficiency index or lung volume at necropsy with a static pressure of 40cmH$_2$O. The mRNA expression of surfactant protein ($Sp)a$, $Spb$, $Spc$, $Spd$, aquaporin ($Aqp$)1, $Aqp$5 and sodium channel epithelial 1 subunit beta ($Scnn1b$) was equivalent between both steroid groups. Maternal and fetal plasma neutrophil, glucose and fetal plasma c-peptide levels were significantly elevated in the dexamethasone group, relative to the betamethasone group. Fetal plasma IGF-1 was significantly reduced in the dexamethasone group compared to the betamethasone group ($p<0.05$). Fetal ACTH ($r=0.53$), maternal glucose value ($r=-0.52$) and fetal glucose values ($r=-0.42$) were correlated with maternal weight in Betamethasone Group ($p<0.05$) while fetal $pCO_2$ and $pO_2$ were not correlated. There were no significant differences between male and female lamb outcomes in any groups for any of the items evaluated.

We report that, in preterm lambs, a low-dose treatment regimen of 8mg betamethasone achieves lung maturation equivalent to that of a 24mg dexamethasone-based regimen, but with smaller perturbations to the materno-fetal HPA axis. These data suggest that, given steroid pharmacokinetic differences between sheep and humans, a betamethasone dose of 2mg may remain above the minimum dose necessary for robust maturation of the preterm
lung. Maternal weight-adjusted betamethasone doses might also be a key to reduce perturbations to the materno-fetal HPA axis.
Following observations made in pregnant sheep\(^1\), and a subsequent clinical trial in New Zealand\(^2\), dexamethasone phosphate and betamethasone (as phosphate alone or in combination with the acetate form) have been used for several decades as antenatal corticosteroid (ACS) treatments for women judged at risk of impending preterm delivery. When targeted judiciously, ACS treatment decreases the incidence of respiratory distress syndrome (RDS) and other neonatal morbidities in preterm infants from 24 to 34 weeks’ gestational age (GA).\(^2,3\) Two maternal intramuscular doses of 11.4 mg betamethasone phosphate (Beta-P) and Beta acetate (Beta-Ac), or 12 mg Beta-P alone at a 24-hour interval are used preferentially in high resource environments. In contrast, four maternal intramuscular injections of 6 mg dexamethasone phosphate (Dex-P) at 12-hour intervals is the WHO-recommended regiment used predominantly in low resource environments.\(^4\) Some meta-analyses of clinical trial data comparing Dex to Beta showed no difference in RDS or neonatal mortality.\(^5,6\) Beta-P and Dex-P are rapidly dephosphorylated, resulting in high maternal and fetal concentrations. We have demonstrated that, in non-human primates and sheep, current ACS treatments expose the fetus to unnecessarily excessive amounts of steroids.\(^7,8\) These elevated exposures seem not to benefit fetal lung maturation and, thus, may only contribute to adverse materno-fetal side effects.\(^5,6,9\)
A recent study with Dex-P in low resource environments showed no benefit for small infants but increased mortality for large infants.\textsuperscript{12,13} A more recent study, again in low-resource jurisdictions, demonstrated benefit from the use of Dex-P.\textsuperscript{14} A particular point of contrast between these two studies, and a potential explanation for the discordant findings, was the far higher quality and standardization of antenatal and neonatal care provided to the majority subjects in the latter study. Given this, more subtle adverse effects of ACS treatments, such as higher rate of maternal infection and hyperglycemia, or fetal hypoglycemia and blunted adrenal function may have a greater impact in low and middle-income countries (LMIC) delivery settings with limited capacity to provide antenatal care and/or post-partum support to mother and infant.\textsuperscript{12,13,15,16,17-19} Optimising the dosing for this critically important therapy is thus one of the most pressing challenges presently facing perinatal medicine.
We aimed to use a sheep model of pregnancy to evaluate the use of a low-dose Beta-P regimen (four 2 mg doses given at 12-hour intervals; equivalent to 24 mg prednisone a day) against the current clinical treatment with Dex-P (four 6 mg doses given at 12-hour intervals; equivalent to 72 mg prednisone a day) in anticipation of the ACTION III (Antenatal Corticosteroids for Improving Outcomes in preterm Newborns) randomized control trial. As the interacting influence of ACS and sex on the singleton fetus is still controversial, sex differences in baseline fetal lung function as well as haematological, biochemical and endocrinological responses to antenatal steroid treatment were also investigated.

As responsiveness to steroids may vary with gestational age at time of exposure, and does alter with treatment to delivery interval, all fetuses were delivered at an equivalent treatment duration and gestational age matched time point. Exposure time and dose, delivery procedure, and postnatal management were all stringently controlled to minimize the effect of these variables on outcome measurements. Investigators responsible for the management of lambs undergoing ventilation were blinded to treatment.
[Material and Methods]

Antenatal Corticosteroid Treatments

The animal ethics committee of Murdoch University reviewed and approved these studies prior to work commencing (R3330/21). Time-mated Merino ewes with singleton fetuses received an intramuscular injection of 150 mg medroxyprogesterone acetate (Depo-Provera, Pfizer, New York, NY) on 117±1 d of gestational age (dGA) to decrease the risk of steroid-induced premature labor. Antenatal progesterone has been previously reported not to impact fetal lung maturation in the sheep. Animals were allocated to one of three groups, with first treatment given on 122±1 dGA (term = 150 dGA): i) Saline Control Group, ewes received four maternal intramuscular injections of sterile normal saline at 12 h intervals; or ii) Dexamethasone Group, ewes received four maternal intramuscular injections of 6mg Dex-PO₄ (DBL Dexamethasone sodium phosphate 4mg/ml, Hospira NZ, New Zealand) at 12 h intervals; or iii) Betamethasone Group, ewes received four maternal intramuscular injections of 2mg Beta-PO₄ (Rinderon injection 4mg/ml, Shionogi & CO., Ltd, Japan) at 12 h intervals. All animals were delivered 48 h after the first injection at 124±1 dGA. The experiment was structured so that at least two animals from each of the three groups was delivered on each study day to assist in controlling for confounding.
Ventilatory Assessment

Prior to delivery, ewes received an intravenous bolus of midazolam (0.5 mg/kg) and ketamine (10 mg/kg) for the deep induction of anaesthesia. A 3 mL injection of 2% (20 mg/mL) lidocaine was given at L6/L7 for spinal analgesia. The head of the fetus was delivered through abdominal and uterine incisions. A 4.5 mm endotracheal tube was secured by tracheostomy. The fetus was then delivered and the ewe euthanized under anaesthesia with pentobarbital. The lamb was weighed, dried, and placed in a radiant warmer (Cozy Cot, Fisher & Paykel Healthcare, New Zealand) bed under a plastic insulating wrap (Neowrap, Fisher & Paykel, NZ). Mechanical ventilation (Fabian HFO, Accutronic Medical Systems AG, Switzerland) was immediately started with the following settings: peak inspiratory pressure (PIP) of 35 cmH₂O, positive end expiratory pressure (PEEP) of 5 cmH₂O, respiratory rate (RR) of 50 breaths per minute, inspiratory time (iT) of 0.5 s, using 100% heated and humidified oxygen. The standard use of 100% oxygen allows the comparison of oxygenation through the partial arterial pressure of oxygen among the groups. An umbilical artery was catheterized for blood sampling and administration of supplemental anesthesia with ketamine (5 mg/kg) if necessary. The tidal volume (Vₜ) was continuously measured and the PIP was adjusted to keep the Vₜ between 7.0 and 8.0 mL/kg but with a maximal pressure limited of 35 cmH₂O. At 10, 20, and 30 min of ventilation we measured temperature, blood pressure, ventilator data (PIP, VT, and compliance), and performed blood gas measurements; pH, PCO₂ (mmHg), PO₂ (mmHg), O₂ saturation
(SO₂, %), total hemoglobin (Hb, g/dL) and glucose (Glu, mg/dL) levels (Siemens RapidPoint 500, Munich, Germany). Dynamic compliance (C_{dyn}, mL/cmH₂O/kg) was recorded as measured by the ventilator. The ventilation efficiency index (VEI) was calculated using the formula: $\text{VEI} = \frac{3,800}{(RR \times (\text{PIP} - \text{PEEP}) \times \text{PCO}_2 \text{ (mm Hg)})}$.\(^{22}\)

**Lung Assessment**

After ventilation for 30 min, lambs were euthanized with pentobarbital, disconnected from the ventilator, and the endotracheal tube was clamped for 2 min to achieve atelectasis by oxygen absorption. The lambs were weighed, and the chest was opened for visual evaluation of gross lung injury—pulmonary hemorrhage, pulmonary interstitial emphysema, gas pockets within the lung or subpleural dissection—performed by the same investigator. A deflation pressure-volume curve was measured after air inflation of the lungs to a pressure of 40 cmH₂O. Volume at a pressure of 40 cmH₂O was calculated using fetal weight after ventilation (kg) as $V_{40} \text{ (ml/kg)}$.\(^{280}\)

**Definition of antenatal steroid responder and non-responder subgroups**

Steroid treated animals were analyzed as a group and sub-divided into responder or non-responder subgroups to confirm overall treatment benefit as described previously.\(^{23-25}\) A value of 2 standard deviations (2 SDs) below the average of normally-distributed PaCO₂ value at 30-minutes of ventilation from Saline Control Group animals was used as an arbitrary, *a priori*
cutoff for subgroup distribution. The arterial pCO2 value (±2SD) for Saline Control Group Animals to determine responders/non-responders was 152.3±34.2. Accordingly, the responder subgroups were defined as including those animals with a PaCO2 level more extreme than two SDs below the Saline Control Group mean. Conversely, the non-responder subgroups were defined as including those animals with a PaCO2 level less extreme than 2 SDs below the Saline Control Group mean.

**Haematological and biochemical data acquisition**

Plasma isolated from maternal and fetal blood samples collected at delivery were used for haematological analyses, including white blood cell counts (WBC, /μL), differential leukocyte counts (%), and biochemical parameters; cortisol (nmol/L), adrenocorticotropic hormone (ACTH, pg/mL), Insulin-like growth factor 1 (IGF-1, μg/dL), glucose (Glu, mg/dL), c-peptide (ng/mL). Those analyses were performed by an independent clinical pathology laboratory (Vetpath, Perth, Australia). Cortisol values under limit of detection (<5.52 nmol/L) were allocated a value of 2.76 nmol/L for statistical analyses. ACTH values under limit of detection (<5.0 pg/mL) were allocated a value of 2.5 pg/mL for statistical analyses.
Quantitation of mRNA

RNA was extracted from frozen lung tissue (right lower lobe) using RNeasy Plus Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. The concentration of extracted RNA was determined using a broad-range acid quantitation kit and a Qubit 2.0 Fluorometer (both supplied by Life Technologies, Carlsbad, CA). All RNA extracts were diluted in nuclease-free water (Life Technologies) to yield a final RNA concentration of 25 ng/μL.

Quantitative polymerase chain reaction (qPCR) cycling was performed using ovine-specific TaqMan probe and primer sets (Applied Biosystems, Foster City, CA) on a StepOne Real-Time PCR System in accordance with the manufacturer’s instructions. Messenger RNA (mRNA) transcripts surfactant protein (Sp)a, Spb, Spc, Spd, aquaporin (Aqp)1, Aqp5 and sodium channel epithelial 1 subunit beta (Scnn1b) were measured. In addition, 18s ribosomal protein RNA was used as internal reference to normalize the amplification data for each gene. Delta quantification cycle values were used to determine relative expression of transcripts for statistical analyses. Final data were expressed as fold increase over the control value.
**Statistical analysis**

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, Armonk, NY). A Chi-Square test was used to test the differences of nominal values between groups. All numerical data were tested for normality with Shapiro-Wilk tests. Extreme outliers were tested for exclusion with Smirnov-Grubbs tests. In the comparison of two groups (males and females, respectively, in the Dexamethasone, Betamethasone and Saline Control Groups), between-group differences in parametric data were tested for significance with t-tests, while Mann-Whitney U tests were used for non-parametric data. In the comparison of three groups (Dexamethasone, Betamethasone and Saline Control Group), between-group differences in parametric data were tested for significance with one-way ANOVA, while Kruskal Wallis tests were used for non-parametric data. Multiple post-hoc comparisons were performed with Tukey’s tests. The Spearman correlation coefficient was calculated to assess the relationship with maternal weight in Dexamethasone and Betamethasone Group. All p values <0.05 were accepted as significant and indicated with an asterisk (*).
[Results]

In the following description, significant differences between experimental groups were expressed as (group comparison; $p$ value, mean difference, [95% confidence interval]). Statistical differences between male and female in each experimental group were expressed as (group; $p$ value).

Delivery data and responder rates

The number of animals, sex distribution, responder rates, gestational age at delivery (dGA), maternal weights and fetal birth weights are given in Table 1. There were no significant differences in sex distribution, dGA, maternal weights and fetal birth weights or lung injury (i.e. emphysema) between the three groups. There was no significant difference in treatment response ratio between the Dexamethasone and Betamethasone Groups. The average weight ($\pm 2$SD) of animals in the steroid treated groups was 68.8±12.8 kg.

Delivery values as a function of fetal sex were assessed for each group (Table 2). There were no significant differences in dGA, maternal weights and fetal birth weights between male and female lambs in any of the three groups. There were no significant differences in response ratio between male and female lambs between the steroid treatment groups.
**Arterial blood gas and respiratory physiological data at 30 min of ventilation (Figure 1)**

Arterial blood gas values for both the Dexamethasone and Betamethasone Group animals were significantly different from those of the Saline Control Group Animals. There were no significant differences in these values between the Dexamethasone and Betamethasone Group animals; pH (Dexamethasone Group vs. Saline Control Group; $p^*<0.001$, 0.29, [0.18-0.40], Betamethasone Group vs. Saline Control Group; $p^*<0.001$, 0.23, [0.12-0.34], Dexamethasone Group vs. Betamethasone Group; $p=0.366$), $PO_2$ (Dexamethasone Group vs. Saline Control Group; $p^*<0.001$, 22.4, [9.4-35.1], Betamethasone Group vs. Saline Control Group; $p^*<0.001$, 32.2, [19.7-45.0], Dexamethasone Group vs. Betamethasone Group; $p=0.122$), VEI (Dexamethasone Group vs. Saline Control Group; $p^*=0.001$, 0.018, [0.007-0.030], Betamethasone Group vs. Saline Control Group; $p^*=0.002$, 0.017, [0.006-0.029], Dexamethasone Group vs. Betamethasone Group; $p=0.977$), $Cdyn$ (Dexamethasone Group vs. Saline Control Group; $p^*<0.001$, 0.11, [0.06-0.16], Betamethasone Group vs. Saline Control Group; $p^*<0.001$, 0.10, [0.05-0.16], Dexamethasone Group vs. Betamethasone Group; $p=0.923$) and $V_{40}$ (Dexamethasone Group vs. Saline Control Group; $p^*=0.009$, 8.1, [1.7-14.6], Betamethasone Group vs. Saline Control Group; $p^*=0.018$, 7.6, [1.1-14.0], Dexamethasone Group vs. Betamethasone Group; $p=0.969$).
While PCO₂ values both of Dexamethasone and Betamethasone group animals were significantly lower than that of Saline Control Group animals, there were no significant differences between those in the Dexamethasone and Betamethasone Group animals (Dexamethasone Group vs. Saline Control Group; \( p^* < 0.001, -65.5, [-89.1--41.9] \), Betamethasone Group vs. Saline Control Group; \( p^* < 0.001, -61.2, [-85.0--37.4] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.885 \)).

There were no significant differences in any of the following items between male and female lambs in the two steroid-treated groups: pH (Dexamethasone Group; \( p=0.411 \), Betamethasone Group; \( p=0.431 \), Saline Control Group; \( p=0.391 \)), PO₂ (Dexamethasone Group; \( p=0.732 \), Betamethasone Group; \( p=0.545 \), Saline Control Group; \( p=0.650 \)), PCO₂ (Dexamethasone Group; \( p=0.574 \), Betamethasone Group; \( p=0.651 \), Saline Control Group; \( p=0.753 \)), VEI (Dexamethasone Group; \( p=0.510 \), Betamethasone Group; \( p=0.469 \), Saline Control Group; \( p=0.804 \)), Cdyn (Dexamethasone Group; \( p=0.203 \), Betamethasone Group; \( p=0.385 \), Saline Control Group; \( p=0.336 \)) and \( V_{40} \) (Dexamethasone Group; \( p=0.367 \), Betamethasone Group; \( p=0.541 \), Saline Control Group; \( p=0.693 \)).
**mRNA Quantitation of Lung tissue (Figure 2)**

Fold changes in the expression of lung maturation-associated transcripts in both of Dexamethasone and Betamethasone Group animals were significantly higher than those of the Saline Control Group Animals. There were no significant differences in any of items between the Dexamethasone and Betamethasone Group animals: Sp-a (Dexamethasone Group vs. Saline Control Group; \( p^* < 0.001, 3.37, [2.53-4.21] \), Betamethasone Group vs. Saline Control Group; \( p^* < 0.001, 2.66, [1.81-3.51] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.082 \), Sp-b (Dexamethasone Group vs. Saline Control Group; \( p^* < 0.001, 1.87, [1.34-2.40] \), Betamethasone Group vs. Saline Control Group; \( p^* < 0.001, 1.66, [1.11-2.19] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.562 \), Sp-c (Dexamethasone Group vs. Saline Control Group; \( p^* < 0.001, 2.08, [1.35-2.81] \), Betamethasone vs. Saline Control Group; \( p^* < 0.001, 2.19, [1.46-2.93] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.911 \), Sp-d (Dexamethasone Group vs. Saline Control Group; \( p^* = 0.012, 0.46, [0.09-0.83] \), Betamethasone vs. Saline Control Group; \( p^* = 0.004, 0.52, [0.14-0.90] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.909 \). Aqp-1 (Dexamethasone Group vs. Saline Control Group; \( p^* < 0.001, 1.09, [0.64-1.55] \), Betamethasone Group vs. Saline Control Group; \( p^* = 0.003, 0.66, [0.20-1.12] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.054 \), Aqp-5 (Dexamethasone Group vs. Saline Control Group; \( p^* < 0.001, 1.06, [0.69-1.48] \), Betamethasone vs. Saline Control Group; \( p^* < 0.001, 0.94, [0.54-1.34] \), Dexamethasone Group vs.
Betamethasone Group; \( p=0.603 \) and Scnn1b (Dexamethasone Group vs. Saline Control Group; \( p^*<0.001 \), 1.70, [0.81-2.60], Betamethasone vs. Saline Control Group; \( p^*<0.001 \), 1.86, [0.96-2.76], Dexamethasone Group vs. Betamethasone Group; \( p=0.889 \)).

There were no significant differences in any of the assessed items between male and female lambs in any of the three groups: \( Sp-a \) (Dexamethasone Group; \( p=0.950 \), Betamethasone Group; \( p=0.562 \), Saline Control Group; \( p=0.126 \)), \( Sp-B \) (Dexamethasone Group; \( p=0.680 \), Betamethasone Group; \( p=0.787 \), Saline Control Group; \( p=0.245 \)), \( Sp-c \) (Dexamethasone Group; \( p=0.169 \), Betamethasone Group; \( p=0.426 \), Saline Control Group; \( p=0.983 \)), \( Sp-dP-D \) (Dexamethasone Group; \( p=0.323 \), Betamethasone Group; \( p=0.335 \), Saline Control Group; \( p=0.978 \)), \( Aqp-1 \) (Dexamethasone Group; \( p=0.065 \), Betamethasone Group; \( p=0.562 \), Saline Control Group; \( p=0.462 \)), \( Aqp-5 \) (Dexamethasone Group; \( p=0.658 \), Betamethasone Group; \( p=0.469 \), Saline Control Group; \( p=0.262 \)), and Scnn1b (Dexamethasone Group; \( p=0.333 \), Betamethasone Group; \( p=0.311 \), Saline Control Group; \( p=0.975 \)).

**Haematological and biochemical data**

**ACTH and cortisol concentrations of maternal plasma at delivery (Figure 3 A, B)**

ACTH concentrations in the Dexamethasone Group ewes were significantly lower than those of ewes in both the Betamethasone and Saline Control Groups. ACTH concentrations in
Betamethasone Group ewes was significantly lower than that of Saline Control Group ewes (Dexamethasone Group vs. Saline Control Group; \( p^*<0.001, -497, [-679--314] \), Betamethasone Group vs. Saline Control Group; \( p^*=0.001, -307, [-491--123] \), Dexamethasone Group vs. Betamethasone Group; \( p^*=0.024, -190, [-359--21] \)).

Maternal cortisol concentrations in both the Dexamethasone Group and Betamethasone Group ewes were significantly lower than those of ewes in the Saline Control Group. There was no significant difference between those of the Dexamethasone and Betamethasone Group ewes (Dexamethasone Group vs. Saline Control Group; \( p^*<0.001, -103, [-123--84] \), Betamethasone Group vs. Saline Control Group; \( p^*<0.001, -96, [-116--76] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.614 \)).

**ACTH, cortisol and IGF-1 concentrations in fetal plasma at delivery (Figure 3 C-E)**

Both ACTH and cortisol concentrations in the Dexamethasone and Betamethasone Group fetuses were significantly lower than those of saline group animals. There were no significant differences between Dexamethasone and Betamethasone Group fetuses for either ACTH (Dexamethasone Group vs. Saline Control Group; \( p^*<0.001, -363, [-513--211] \), Betamethasone Group vs. Saline Control Group; \( p^*<0.001, -352, [-505--200] \), Dexamethasone Group vs. Betamethasone Group; \( p=0.983 \)) and cortisol (Dexamethasone Group vs. Saline
Control Group; \( p^* < 0.001 \), -7.5, [-9.9--5.2], Betamethasone Group vs. Saline Control Group; \( p^* < 0.001 \), -9.9, [-7.5--9.9], Dexamethasone Group vs. Betamethasone Group; \( p = 0.807 \).

Fetal plasma IGF-1 concentrations in Dexamethasone Group fetuses were significantly lower than those of both the Betamethasone and Saline Control Group fetuses. There was no significant difference in fetal plasma IGF-1 levels between Betamethasone Group and Saline Control Group fetuses (Dexamethasone Group vs. Saline Control Group; \( p^* = 0.032 \), -25.5, [-49.2--1.8], Betamethasone Group vs. Saline Control Group; \( p = 0.202 \), Dexamethasone Group vs. Betamethasone Group; \( p^* < 0.001 \), -42.7, [-64.6--20.7]).

There were no significant differences in any of items assessed between male and female lambs across all three groups: ACTH concentration (Dexamethasone Group; \( p = 0.471 \), Betamethasone Group; \( p = 0.266 \), Saline Control Group; \( p = 0.562 \)), cortisol concentration (Dexamethasone Group; \( p = 0.998 \), Betamethasone Group; \( p = 0.911 \), Saline Control Group; \( p = 0.446 \)) and IGF-1 concentration (Dexamethasone Group; \( p = 0.171 \), Betamethasone Group; \( p = 0.880 \), Saline Control Group; \( p = 0.412 \)).
Maternal haematological data at delivery (Figure 4 A, B)

There were no significant inter-group differences in total white blood cell (WBC) counts (Dexamethasone Group vs. Saline Control Group; \( p=0.876 \), Betamethasone Group vs. Saline Control Group; \( p=0.998 \), Dex vs Beta; \( p=0.887 \)).

Neutrophils in the Dexamethasone Group ewes were significantly higher than those of both the Betamethasone Group and Saline Control Group ewes. There was no significant difference in neutrophil counts between the Betamethasone Group and Saline Control Group ewes (Dexamethasone Group vs. Saline Control Group; \( p^{*}<0.001 \), 22.0, [10.3-33.7], Betamethasone Group vs. Saline Control Group; \( p=0.118 \), Dexamethasone Group vs Betamethasone Group; \( p^{*}=0.026 \), 12.1, [1.2-22.9]).

Fetal haematological data at delivery (Figure 4 C, D)

There were no significant differences between any group fetuses in total WBC counts (Dexamethasone Group vs. Saline Control Group; \( p=0.415 \), Betamethasone Group vs. Saline Control Group; \( p=0.824 \), Dexamethasone Group vs. Betamethasone Control Group; \( p=0.747 \)).

Neutrophils in the Dexamethasone Group fetuses were significantly higher than in both the Betamethasone and Saline Control Group fetuses. Neutrophils in the Betamethasone Group fetuses were significantly higher than in the Saline Control Group fetuses (Dexamethasone Group vs. Saline Control Group; \( p^{*}<0.001 \), 13.1, [1.1-23.8], Betamethasone Group vs. Saline Control Group; \( p=0.013 \), Dexamethasone Group vs Betamethasone Group; \( p^{*}=0.009 \), 5.7, [1.2-20.7]).
Group vs. Saline Control Group; \( p^*<0.001, 38.3, [28.0-48.7] \), Betamethasone Group vs. Saline Control Group; \( p^*<0.001, 22.4, [11.9-32.9] \), Dexamethasone Group vs. Betamethasone Group; \( p^*=0.001, 15.9, [6.3-25.6] \). There were no significant differences between male and female fetuses in all groups for total WBC counts (Dex; \( p=0.190 \), Beta; \( p=0.701 \), Saline; \( p=0.261 \)) and neutrophil (Dex; \( p=0.529 \), Beta; \( p=0.659 \), Saline; \( p=0.428 \)).

**Glucose levels in Maternal plasma (Figure 5 A)**

Maternal glucose levels in the Dexamethasone Group animals were significantly higher than in both the Betamethasone Group and Saline Control Group animals. There was no significant difference between glucose levels in the Betamethasone Group and Saline Control Group ewes (Dexamethasone Group vs. Saline Control Group; \( p^*<0.001, 28.6, [14.9-42.3] \), Betamethasone Group vs. Saline Control Group; \( p=0.058 \), Dexamethasone Group vs. Betamethasone Group; \( p^*=0.016, 15.1, [2.4-27.8] \)).

**Glucose and e-peptide concentrations in fetal plasma at delivery (Figure 5 B, C)**

Fetal glucose levels in the Dexamethasone Group fetuses were significantly higher than those in both the Betamethasone Group and the Saline Control Group fetuses. Plasma glucose concentrations in the Betamethasone Group fetuses were also significantly higher than those in the Saline Control Group fetuses (Dexamethasone Group vs. Saline Control Group;
Fetal C-peptide concentrations in Dexamethasone Group fetuses were significantly higher than those both of the Betamethasone and Saline Control Group fetuses. There was no significant difference in C-peptide levels between the Betamethasone Group and Saline Control Group fetuses (Dexamethasone Group vs. Saline Control Group; \(p^*<0.001\), 0.021, [0.006-0.037], Betamethasone Group vs. Saline Control Group; \(p=0.606\), Dexamethasone Group vs. Betamethasone Group; \(p^*<0.001\), 0.028, [0.013-0.042]). There were no significant differences between male and female fetal glucose (Dexamethasone Group; \(p=0.274\), Betamethasone Group; \(p=0.923\), Saline Control Group; \(p=0.531\)) or c-peptide concentrations (Dexamethasone Group; \(p=0.889\), Betamethasone Group; \(p=0.306\), Saline Control Group; \(p=0.782\)) between groups.

**Correlations with maternal weight in Dexamethasone Group animals**

Fetal arterial PCO\(_2\), PO\(_2\), maternal ACTH, fetal ACTH, maternal neutrophils, fetal neutrophils, maternal glucose values and fetal glucose values were calculated to assess the relationship with maternal weight in Dexamethasone Group (data not shown). There were no significant correlations with any of PCO\(_2\) \((r=-0.07, \ p=0.75)\), PO\(_2\) \((r=-0.32, \ p=0.150)\), maternal ACTH \((r=0.13, \ p=0.558)\), fetal ACTH \((r=0.15, \ p=0.519)\), maternal neutrophil \((r=-0.07, \ p=0.766)\), fetal
neutrophil ($r=-0.08$, $p=0.716$), maternal glucose value ($r=-0.25$, $p=0.256$) and fetal glucose value ($r=-0.04$, $p=0.868$).

**Correlations with maternal weight in Betamethasone Group animals (Figure 6 A-G)**

Fetal arterial PCO$_2$, PO$_2$, maternal ACTH, fetal ACTH, maternal neutrophils, fetal neutrophils, maternal glucose values and fetal glucose values were calculated to assess the relationship with maternal weight in Betamethasone Group. There were significant correlations with fetal ACTH ($r=0.53$, $p*=0.013$), maternal glucose value ($r=-0.52$, $p*=0.015$) and fetal glucose value ($r=-0.42$, $p*=0.048$) while there were no significant correlations with PCO$_2$ ($r=0.40$, $p=0.071$), PO$_2$ ($r=-0.10$, $p=0.656$), maternal ACTH ($r=0.39$, $p=0.081$), maternal neutrophil ($r=-0.27$, $p=0.238$) and fetal neutrophil ($r=-0.38$, $p=0.093$).
Principal findings

The primary findings of this study are as follows: i) an antenatal steroid regimen based around four, 2mg doses of betamethasone phosphate given at 12 hour intervals (total 8mg), achieved functional maturation of the ovine preterm lung equivalent to that of four, 6mg doses of dexamethasone phosphate, total 24mg (Table 1, Figure 1,2); ii) relative to standard-dose dexamethasone treatment, lower-dose betamethasone treatment reduced the scope and magnitude of potentially important disruption to HPA axis signaling and circulating immunocyte populations (Figure 3-6); and iii) neither of the two dosing regimens employed elicited differential effects due to the sex of the fetus. It is important to note that the regimens administered to the conservative (dexamethasone phosphate) and experimental (betamethasone phosphate) groups in the present study had quite distinct pharmacokinetic profiles from that of the current standard of care (combined betamethasone phosphate and acetate) used in Australia, the US and much of Europe. As such, additional comparative studies against this regimen are warranted in the future.

Clinical Implications - Functional lung maturation

Fetal pulmonary oxygenation (pO₂), diffusion capacity (pCO₂ and VEI), compliance and lung capacity (Cdyn and V₁₅₀) were equivalent between steroid-treatment Groups. There was no
difference in the treatment response rate. (Figure 1). The expressions of mRNA markers of lung maturation were also equivalent between the two steroid-treatment groups (Figure 2). Together, these findings demonstrate that the two treatments tested were equivalent in terms of enhancing preterm lung function in the sheep; for deliveries occurring at 48h after treatment initiation we conclude that a substantially lower dose of betamethasone phosphate may be used in place of a standard-dose of dexamethasone phosphate without any discernable difference in treatment efficacy. For future studies, it would be of interest to explore the impact of a longer treatment-to-delivery interval on lung function outcomes.

Clinical Implications - Suppression of hypothalamic–pituitary–adrenal (HPA) axis

Both the maternal and the fetal HPA axes were suppressed by ACS treatments (Figure 3). This is perhaps not unexpected given that the biological half-life of Dex-P and Beta-P (which relates to its effect on the HPA axis) is 36-54 h and recovery time from suppression of HPA is approximately 60-72 h in the human, and that even the dose of glucocorticoid used in the Betamethasone Group was still sizable, and within a range of 7.5-30 mg prednisone equivalent a day if viewed as a conventional anti-inflammatory dose. Nevertheless, the use of a low-dose betamethasone regimen resulted in less perturbation of maternal ACTH concentrations, compared to the standard-dose dexamethasone phosphate treatment modelled on the current WHO-recommended regimen. Similar improvements were seen in the fetal HPA axis and in
IGF-1 concentrations in the Betamethasone Group lambs. It is reasonable to conclude that an ANS therapy that minimizes as much as possible impact on the HPA axis would be desirable.

**Clinical Implications - Immunosuppression**

Glucocorticoids are known to increase the circulating WBC count acutely following administration. The increase in circulating WBC count predominantly reflects an increase in circulating neutrophils. The biologic effects that contribute to this increase in circulating neutrophils include de-margination of neutrophils from the endovascular lining (about 60%) and delayed migration of neutrophils into tissue, and a slower rate of apoptosis (about 30%). These effects contribute to the action of glucocorticoids in inducing immunosuppressive effects at inflamed sites. In this study, a low-dose betamethasone regimen blunted the elevation of neutrophils, compared to the dexamethasone regimen (Figure 4, B, D). The data suggest that the use of a lower-dose ACS regimen might ameliorate the immunosuppressive effects associated with current regimens, resulting in reduced risk of infection after ACS treatments - a risk of concern in LMICs.

**Clinical Implications - Fetal hypoglycemia**

The association of ACS treatments with neonatal hypoglycemia, especially for late preterm infants (34-36 weeks of gestation inhuman) is an issue of considerable contemporary
Maternal hyperglycemia leads to fetal hyperglycemia, resulting in fetal pancreatic beta cell hyperplasia and/or fetal hyperinsulinemia (elevation of plasma c-peptide), resulting in subsequent neonatal hypoglycemia after delivery. In this study, animals in the low-dose Betamethasone Group had reduced elevations of maternal glucose, fetal glucose and c-peptide compared to those treated with standard-doses of Dexamethasone (Figure 5, A-C). These data suggest that, assuming a similar degree of sensitivity between sheep and humans, a lower-dose ACS regimen might reduce the risk of steroid-induced fetal hypoglycemia after birth.

**Clinical Implications - Fetal growth restriction**

Preclinical and clinical evidence demonstrates that ACS treatments may exacerbate growth restriction, and an inverse relationship has been demonstrated between the number of corticosteroid courses and fetal growth. Moreover, IGF-1 plays central roles in normal fetal growth, stimulating fetal cell proliferation, differentiation, protein and glycogen synthesis, and it has been noted that reduced serum IGF-1 is correlated with reduced fetal growth and brain development. In contrast to the postnatal situation, the initial driver of fetal IGF-1 regulator is not only growth hormone, but also fetal insulin, which in turn is predominantly under regulation by fetal glucose availability. It’s also reported that the IGF-1 levels of fetuses exposed to synthetic corticosteroids are likely to be reduced. Although the underlying
mechanisms of growth restriction due to ACS treatments are still unclear, the IGF-1 system and glucose metabolism are possibly implicated. In this study, although there was no difference in birth weights between the groups, fetal plasma IGF-I levels were reduced by in the standard-dose Dexamethasone Group, but not in the lower-dose Betamethasone Group (Figure 3 E). Any regimen that conveys a reduced risk of even transient reductions in fetal weight / growth would seem to be a significant improvement over the existing treatments.

Clinical Implications - Gender effects

Although influence of ACS and sex on fetus is still controversial,\textsuperscript{17-19} there were no gender differences identified in baseline fetal lung function and maternal and fetal haematological, biochemical and endocrinological responses either treatment regimen. This is an important observation, as it suggests that reducing the dose of steroids used in antenatal regimens does not increase the risk of reduced efficacy as a function of fetal sex.

Research Implications

The present study raises several important questions for further research efforts in this area. Firstly, it is important to note that the ratio of the maternal-fetal steroid gradient in humans is approximately 3:1, whilst in sheep it is approximately 10:1.\textsuperscript{54,55} Assuming a broadly equivalent degree of responsiveness to dexamethasone or betamethasone stimulation between the ovine
and human lung, these findings suggest that a treatment regimen using further reduced doses of betamethasone phosphate would likely be effective when used in humans. Additional studies in non-human primates to confirm these findings would make a valuable addition to our understanding of antenatal steroid dosing. There is a wealth of data to demonstrate that, broadly speaking, adverse effects in relation to steroid therapy are dose-dependent.\textsuperscript{56,57} As such, in the context of treating the developing fetus, it is important to design a treatment regimen employing the lowest dose of agent possible to reliably induce lasting maturation of the preterm lung.

A large number of ‘at risk’ pregnancies remain undelivered more than 7d after ACS treatment, and are viewed at elevated risk of adverse outcomes relative to age-matched, untreated peers. In addition, recent data have highlighted the potential for increased harms in off-target use of antenatal steroids.\textsuperscript{12,13} It would be of particular interest to determine the durability of the two steroid treatments tested in this protocol at extended treatment to delivery intervals (i.e. 7d and 14d post-first dose) – again with the aim of identifying the optimal intersection of lowest efficacious dose and longest possible period of benefit.

Determining if it is possible to personalize antenatal steroid dosing based on maternal weight may also offer the potential to improve treatment efficacy. In the present study, the distribution of ACS-treated maternal weights was 56.0 - 81.6 kg. There was no correlation between HPA axis markers and maternal weight in the Dexamethasone Group. It is tempting to speculate that
this derives from the higher dexamethasone dose remaining above a threshold / plateau value for reduced suppression over the material weight range (i.e. even at the heaviest maternal weight, the drug dose was still large enough to suppress HPA function). This concept is supported by the fact that there was an inverse correlation between maternal weight and HPA axis disruption in the much lower-dose Betamethasone Group – with lighter maternal weights (and thus higher mg steroid / kg maternal weight doses) correlating with greater glucose disruption (Figure 6).

Low-dose ACS regimens may alleviate suppressive effects on the HPA axis. Data from human studies show that current ACS treatments reduce fetal and infant HPA activity.\textsuperscript{58,59} Although reduced basal HPA function seems to recover within the first 2 weeks postpartum, there is preliminary evidence that blunted HPA axis reactivity to pain-related stress persists throughout the first four months of life.\textsuperscript{58,59} Thus, evaluating fetal recovery from HPA suppression, and the potential differences deriving from low-dose vs. standard-dose (i.e. contemporary) ACS treatments will be an important line of future investigation.

**Limitations**

Some limitations should be considered when assessing the translatability of these data. Although the sheep is an excellent translational model to study ACS therapy and its effect on
lung maturation and fetal development, it should be noted that there are a number of differences between sheep and humans, including length of gestation, maternal-fetal steroid pharmacokinetics, and the distribution of maternal weight between human and sheep. Furthermore, the functional lung assessment reported in this study was limited to 30 minutes after delivery. Although this is an adequate period of time to assess functional lung maturation, it might not necessarily be sufficient to assess interactions between ACS use and complications such as chronic lung injury in preterm babies, or adverse effects such as fetal hypoglycemia and blunted adrenal function. Moreover, the treatment to delivery intervals and gestational ages were tightly controlled in this study – a significant difference from the clinical setting of ACS use.

[Final Comment]

We report that, in a sheep model of pregnancy, a low-dose treatment regimen of 4 x 2mg betamethasone given in equal parts over a 36-hour period achieves lung maturation equivalent to that of a 4 x 6mg dexamethasone-based regimen, but with smaller perturbations to the materno-fetal HPA axis. Further optimization of lower-dose treatments using betamethasone might be effective in driving fetal lung maturation with a reduced risk of maternal and fetal adverse effects. At a standardized 48h treatment to delivery interval (and setting aside drug
cost and availability), a low-dose betamethasone phosphate treatment regimen should be favored over the current, standard-dose dexamethasone phosphate regimen.


Table 1. Comparison of delivery data and responder ratio between groups

<table>
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<tr>
<th></th>
<th>Dexamethasone (6 mg/4 times)</th>
<th>Betamethasone (2 mg/4 times)</th>
<th>Saline</th>
<th>test</th>
<th>p value</th>
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<tr>
<td>Total number</td>
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<td>21</td>
<td>16</td>
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<tr>
<td>Sex (Male / Female)</td>
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<td>13/8</td>
<td>11/5</td>
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<td>Responder / non-responder (responder ratio in total number)</td>
<td>16/6 (72.7%)</td>
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<td>Gestational age</td>
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<td>Kruskal-Wallis</td>
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<td>Maternal weight</td>
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<td>69.4±7.2</td>
<td>One way-ANOVA</td>
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<td>Fetal birth weight</td>
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<td>2.9±0.4</td>
<td>3.0±0.3</td>
<td>One way-ANOVA</td>
<td>0.564</td>
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Values are expressed as the group mean ± SD. p<0.05 were considered as significant. The responder subgroup was defined on the basis of a PCO₂ level more extreme than 2 SDs below the control group mean; the non-responder subgroup was defined as a PCO₂ level within 2 SDs of the control group mean.
<table>
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<tr>
<th></th>
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<th>Female</th>
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<tbody>
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<td>13</td>
<td>8</td>
<td>11</td>
<td>5</td>
<td></td>
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<tr>
<td><strong>Responder / non-responder (responder ration in total number)</strong></td>
<td>7/3 (70%)</td>
<td>9/3 (75%)</td>
<td>10/3 (76.9%)</td>
<td>6/2 (75%)</td>
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<td>0.92</td>
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<tr>
<td><strong>Gestational age</strong></td>
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<td>124.1±0.9</td>
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<td>0.562</td>
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<td>123.4±0.5</td>
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<tr>
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<td>67.9±5.8</td>
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<td>0.921</td>
<td>0.498</td>
<td>67.3±5.8</td>
<td>74±6.7</td>
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<tr>
<td><strong>Fetal birth weight</strong></td>
<td>3.0±0.3</td>
<td>3.0±0.3</td>
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<td>0.706</td>
<td>0.351</td>
<td>3.0±0.3</td>
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</tbody>
</table>

Table 2. Comparison of delivery data and responder ratio between male and female in respective group

Values are expressed as the group mean ± SD. p<0.05 was considered as significant. The responder subgroup was defined as a PCO₂ level less than 2 SDs of the control group mean, and the non-responder subgroup was defined as a PCO₂ level within 2 SDs of the control group mean.
Figure 1. Arterial blood gas and respiratory physiological data at 30 min of ventilation

pH (Panel A), PO\(_2\) (Panel B), PCO\(_2\) (Panel C), Ventilation efficacy index (Panel D), Dynamic compliance (Panel E) and Lung volume on 40cmH\(_2\)O (Panel F).

All values are presented as bar charts with the group mean and with whiskers representing SD. Differences of values between the groups were tested for significance using one-way ANOVA or Kruskal Wallis tests according to statistic distribution followed by Tukey test as post-hoc with p value <0.05 accepted as significant. † indicates p<0.01. * indicates p<0.05

Differences of values between male and female in respective group were tested for significance using t-test or Mann-Whitney U tests according to statistic distribution with p value <0.05 accepted as significant. The ventilation efficiency index = 3,800 / (RR × (PIP – PEEP) × PCO\(_2\) (mm Hg)).

Figure 2. mRNA Quantitation of Lung tissue

SP-A (Panel A), SP-B (Panel B), SP-C (Panel C), SP-D (Panel D), AQP-1 (Panel E), AQP-5 (Panel F) and SCNN 1B (Panel G).

All values are presented as bar charts with the group mean and with whiskers representing SD. Differences of values between the groups were tested for significance using one-way ANOVA or Kruskal Wallis tests according to statistic distribution followed by Tukey test as post-hoc with p value <0.05 accepted as significant. † indicates p<0.01. * indicates p<0.05
Differences of values between male and female in respective group were tested for significance using t-test or Mann-Whitney U tests according to statistic distribution with p value <0.05 accepted as significant.

SP-A-D; Surfactant protein A-D, AQP-1,5; Aquaporins 1,5, SCNN 1B; sodium channel epithelial 1 subunit beta

**Figure 3 Maternal and fetal plasma concentrations of ACTH, cortisol and IGF-1**

Maternal ACTH (Panel A), maternal cortisol (Panel B), fetal ACTH (Panel C), fetal cortisol (Panel D) and fetal IGF-1.

All values are presented as bar charts with the group mean and with whiskers representing SD. Differences of values between the groups were tested for significance using one-way ANOVA or Kruskal Wallis tests according to statistic distribution followed by Tukey test as post-hoc with p value <0.05 accepted as significant. †indicates p<0.01. * indicates p<0.05

Differences of values between male and female in respective group were tested for significance using t-test or Mann-Whitney U tests according to statistic distribution with p value <0.05 accepted as significant.

ACTH; adrenocorticotropic hormone, IGF-1; Insulin-like growth factor 1
Figure 4. Maternal and fetal haematological data at delivery

Maternal WBC counts (Panel A), maternal neutrophil percentage (Panel B), fetal WBC counts (Panel C) and fetal neutrophil percentage (Panel D).

All values are presented as bar charts with the group mean and with whiskers representing SD. The Black bars indicate Dex group. The gray bars indicate Beta group. The white bars indicate the Saline control group.

Differences of values between the groups were tested for significance using one-way ANOVA or Kruskal Wallis tests according to statistic distribution followed by Tukey test as post-hoc with p value <0.05 accepted as significant. †indicates p<0.01. * indicates p<0.05

Differences of values between male and female in respective group were tested for significance using t-test or Mann-Whitney U tests according to statistic distribution with p value <0.05 accepted as significant.

WBC; white blood cells

Figure 5 Maternal plasma glucose level and fetal plasma concentrations of glucose and c-peptide

Maternal glucose level (Panel A), fetal glucose level (Panel B) and fetal c-peptide concentration (Panel C).

All values are presented as bar charts with the group mean and with whiskers representing SD.
Differences of values between the groups were tested for significance using one-way ANOVA or Kruskal Wallis tests according to statistic distribution followed by Tukey test as post-hoc with p value <0.05 accepted as significant. †indicates p<0.01. * indicates p<0.05

Differences of values between male and female in respective group were tested for significance using t-test or Mann-Whitney U tests according to statistic distribution with p value <0.05 accepted as significant.

Figure 6. Correlations with maternal weight in Betamethasone Group animals

Fetal PCO₂ (Panel A), maternal ACTH (Panel B), fetal ACTH (Panel C), maternal neutrophil (Panel D), fetal neutrophil (Panel E), maternal glucose values (Panel F) and fetal glucose values (Panel G) were related with maternal weight in Betamethasone Group using Spearman’s correlation coefficient. p value <0.05 was accepted as significant.

ACTH; adrenocorticotropic hormone
A  Feal ACTH / Maternal weight

B  Maternal glucose / Maternal weight

C  Fetal glucose / Maternal weight

\[ r = 0.53, P = 0.013 \]

\[ r = -0.52, P = 0.015 \]

\[ r = -0.42, P = 0.048 \]