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Augusto F. Schmidt, MD PhD, Matthew W. Kemp, PhD, Judith Rittenschober-Böhm, MD, Paranthaman S. Kannan, PhD, Haruo Usuda, MD, Masatoshi Saito, MD, Ms. Lucy Furfaro, BSc, Shimpei Watanabe, MD, Sarah Stock, Boris W. Kramer, MD PhD, John P. Newnham, MD, Suhas G. Kallapur, MD, Alan H. Jobe, MD PhD

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Augusto F SCHMIDT, MD PhD1, Matthew W KEMP, PhD2, Judith RITTENSCHOBER-BÖHM, MD3, Paranthaman S KANNAN, PhD1, Haruo USUDA, MD2, Masatoshi SAITO, MD4, Ms. Lucy FURFARO, BSc2, Shimpei WATANABE, MD4, Sarah STOCK5, Boris W KRAMER, MD PhD6, John P NEWNHAM, MD2, Suhas G KALLAPUR, MD1, Alan H JOBE, MD PhD1

Cincinnati – OH, USA and Perth – Western Australia, Australia

1- Division of Neonatology and Pulmonary Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati - OH, USA
2- School Women’s and Infants’ Health, University of Western Australia, Perth, Australia
3 – Division of Neonatology, Pediatric Intensive Care and Neuropediatrics, Medical University Vienna, Vienna, Austria
4 – Department of Obstetrics and Gynecology, Tohoku University, Sendai, Japan
5 –MRC Centre for Reproductive Health, University of Edinburgh Queen’s Medical Research Institute, Edinburgh, United Kingdom
6 –Maastricht University Medical Center, Maastricht, Netherlands

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Corresponding author:

Alan H Jobe
Division of Neonatology and Pulmonary Biology
Cincinnati Children’s Hospital Medical Center
3333 Burnet ave, ML 7009
P: (513)636-8563 F: (513)636-4830
Cincinnati, OH 45229

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Condensation

A single low-dose injection of betamethasone-acetate promotes fetal lung maturation similarly to the standard treatment with decreased fetal exposure to corticosteroids in preterm fetal sheep.

Short title

Betamethasone-acetate for fetal lung maturation
Abstract

Background: Antenatal steroids (ANS) are standard of care for women at risk of preterm delivery; however ANS dosing and formulation have not been adequately evaluated. The standard clinical 2-dose treatment with betamethasone-acetate+betamethasone-phosphate (Beta-P+Beta-Ac) is more effective than 2 doses of Beta-P for inducing lung maturation in preterm fetal sheep. We hypothesized that the slowly-released Beta-Ac component induces similar lung maturation to Beta-P+Beta-Ac with decreased dose and fetal exposure.

Objective: To investigate pharmacokinetics and fetal lung maturation of antenatal Beta-Ac in preterm fetal sheep.

Study Design: Groups of 10 singleton-pregnant ewes received 1 or 2 intramuscular (IM) doses 24 hours apart of 0.25mg/kg/dose of Beta-P+Beta-Ac (the standard of care dose), or one IM dose of 0.5mg/kg, 0.25mg/kg, or 0.125mg/kg of Beta-Ac. Fetuses were delivered 48 hours after the first injection at 122 days of gestation (80% of term) and ventilated for 30 minutes, with ventilator settings, compliance, vital signs, and blood gas measurements recorded every 10 minutes. After ventilation we measured static lung pressure-volume curves and sampled the lungs for mRNA measurements. Other groups of pregnant ewes and fetuses were catheterized and treated with IM injections of Beta-P 0.125mg/kg, Beta-Ac 0.125mg/kg or Beta-Ac 0.5mg/kg. Maternal and fetal betamethasone concentrations in plasma were measured for 24 hours.

Results: All betamethasone treated groups had increased mRNA expression of surfactant proteins A, B, and C, ABCA3 and aquaporin-5 compared to control. Treatment with 1 dose of IM Beta-Ac 0.125mg/kg improved dynamic and static lung compliance, gas
exchange and ventilation efficiency comparably to the standard treatment of 2 doses of 0.25m/kg of Beta-Ac+Beta-P. Beta-Ac 0.125mg/kg resulted in lower maternal and fetal peak plasma concentrations and decreased fetal exposure to betamethasone compared to Beta-P 0.125mg/kg.

Conclusion: A single dose of Beta-Ac results in similar fetal lung maturation as the 2-dose clinical formulation of Beta-P+Beta-Ac with decreased fetal exposure to betamethasone. A Lower dose of Beta-Ac may be an effective alternative to induce fetal lung maturation with less risk to the fetus.
Introduction

Antenatal corticosteroids (ANS) are a life-saving therapy for premature infants. Despite the well-documented effectiveness and widespread use, questions remain regarding formulation, dosing, route of administration and repeated doses\(^1\), because the ANS used for fetal lung maturation has not been rigorously evaluated\(^2\). In fact, multiple treatment are used around the world based on drug availability and historical use without experimental or clinical evaluation\(^3\). The most commonly recommended and tested regimens are intramuscular (IM) dexamethasone-phosphate 6 mg every 6 hours for 4 doses (total dose 24 mg), or the combination of betamethasone-acetate (Beta-Ac) and betamethasone-phosphate (Beta-P) 12 mg every 24 hours for 2 doses\(^4\)\(^-\)\(^6\). The maturational effects of ANS are likely determined by the length and amplitude of fetal exposure. While several formulations are used interchangeably the pharmacokinetics profile of the formulations are different and they may not all be equally effective.

The 2 dose regimen of Beta-Ac+Beta-P was used by Liggins and Howie in their seminal trial\(^7\) and has been adopted as the standard therapy for most randomized controlled trials\(^8\). The Beta-P component is rapidly dephosphorylated to the active drug, resulting, in the sheep model, in an early maternal peak concentration of about 130 ng/mL at \(<\)1 hour, with a half-life of 4 hours\(^9\)\(^,\)\(^10\). The microparticulate Beta-Ac component is slowly deacetylated resulting in a later peak and more prolonged half-life compared to the phosphate component. The clinical combination of the two drugs results in a complex pharmacokinetics with a half-life of 14 hours\(^10\). Drug levels in humans are limited to paired maternal and cord blood fetal samples collected from deliveries shortly after an ANS treatment. An estimate of the initial half-life in maternal plasma was 9
hours with peak maternal levels of betamethasone at 3 to 4 hours of about 60 ng/mL and fetal plasma beta levels of about 30% of maternal levels\textsuperscript{11}. In contrast, in preterm sheep a single Beta-Ac dose of 0.25mg/kg promotes fetal lung maturation with a fetal drug level of about 2ng/mL at 24h\textsuperscript{9}.

The preterm sheep model has been widely used for studies of fetal lung maturation because the fetus can be catheterized, the gestation length is appropriate for testing exposure to delivery intervals relevant to the human, and for evaluations of physiological responses. Using a preterm fetal lamb model, we showed that maternal IM Beta-Ac+Beta-P was more effective than Beta-P alone for improving lung compliance and increased expression of surfactant protein mRNA\textsuperscript{12}. Low-dose maternal infusions of Beta-P in catheterized pregnant sheep also increased expression of fetal lung maturation markers despite much lower fetal maximal concentrations than the Beta-Ac+Beta-P used clinically\textsuperscript{13}. The pharmacokinetic data suggest that the duration of fetal exposure to a low plasma level of corticosteroids may promote fetal lung maturation better than shorter exposures to the higher plasma concentrations.

Hence, considering the pharmacokinetic profile of Beta-Ac, we hypothesized that a single lower dose of Beta-Ac would promote lung maturation comparable to the standard treatment with lower fetal exposure. Clinical studies of drug and dose are impractical for either pharmacokinetic or pharmacodynamics assessments of new treatment strategies. Therefore, we tested 3 doses of Beta-Ac that were equivalent to the full, one half and one quarter of the total Beta-Ac+Beta-P dose, in a preterm fetal and newborn lamb model as an initial strategy to refine a treatment strategy for clinical evaluation.
Methods

Animal studies

The protocols were approved by the animal ethics committee of The University of Western Australia (RA/3/100/1378). We used chronically catheterized pregnant sheep and their fetuses for drug level measurements, and separate preterm ventilated lambs to assess fetal lung maturation after maternal ANS treatment. In order to reduce the risk of preterm labor from ANS, time-mated ewes with singleton fetuses were treated with one intramuscular (IM) dose of 150 mg medroxyprogesterone acetate (Depo-Provera, Pfizer, New York, NY) at 110 days of gestation for pharmacokinetics studies and at 115 days of gestation for pharmacodynamics studies. No other doses of medroxyprogesterone acetate were given and we did not administer other tocolytics.

For pharmacodynamics studies, animals were randomized to receive either saline (control) or one of the following treatments: 2 doses of Beta-Ac+Beta-P (Celestone Chronodose, gift from Merck & Co., Inc, Kenilworth, NJ) 0.25mg/kg IM 24 hours apart, 1 dose of Beta-Ac+Beta-P of 0.25mg/kg IM, 1 dose of Beta-Ac 0.5mg/kg IM, 1 dose of Beta-Ac 0.25mg/kg IM, or 1 dose of Beta-Ac 0.125mg/kg IM (Figure 1). The Beta-Ac was a gift from Merck & Co. as a preparation of Beta-Ac equivalent to that in Celestone, Merck & Co. did not participate in the design, execution, or analysis of the study. The 0.25mg/kg dose approximates the clinical dose of 12mg of betamethasone for a 50kg woman and was the same dose used for our previous studies.\(^9,^{14}\)

For delivery, pregnant ewes were anesthetized with IV midazolam (0.5 mg/kg) and ketamine (10 mg/kg), followed by spinal anesthesia with 3 mL of 2\% (20 mg/mL)
lidocaine 48 hours after the first IM treatment injection between 121 and 123 days of gestation. The head of the fetus was exposed through abdominal and uterine incisions, the fetal skin was infiltrated with lidocaine and a tracheostomy was performed for insertion of an endotracheal tube. After delivery, fetuses were weighted, dried, and placed on a radiant warming bed (Cozy Cot, Fisher & Paykel Healthcare, New Zealand) and covered with a plastic wrap for temperature control (Neowrap, Fisher & Paykel, NZ) for ventilation.

*Mechanical Ventilation*

Mechanical ventilation (Fabian HFO, Accutronic Medical Systems AG, Switzerland) was immediately started with the intermittent positive pressure ventilation mode with standardized settings: initial peak inspiratory pressure (PIP) of 40 cmH\(_2\)O, positive end expiratory pressure (PEEP) of 5 cmH\(_2\)O, respiratory rate of 50 breaths per minute, inspiratory time of 0.5 seconds, and 100% heated and humidified oxygen. Animals were kept sedated with IM ketamine to avoid spontaneous breathing. We inserted an umbilical artery catheter for blood sampling. Tidal volume (V\(_T\)) was continuously measured and kept between 8.5 and 9.5 mL/kg by adjusting the positive inspiratory pressure only, but with the maximal pressure limited to 40 cmH\(_2\)O. We measured temperature, blood pressure, ventilator data (PIP, V\(_T\), and compliance), and performed blood gas measurements at 10, 20, and 30 minutes of ventilation. Dynamic compliance was recorded from the ventilator. The ventilation efficiency index (VEI), an integrated assessment of ventilation and gas exchange, was calculated using the formula

\[
VEI = \frac{3800}{\text{respiratory rate} \times (\text{PIP} - \text{PEEP}) \times P_{\text{CO}_2} (\text{mm Hg})^{15}}
\]
After 30 minutes of ventilation lambs were disconnected from the ventilator and the endotracheal tube was clamped for 2 minutes to achieve complete atelectasis by oxygen absorption. Lambs were euthanized with pentobarbital, weighed and the chest was opened for visual evaluation of gross lung injury as pulmonary hemorrhage, pulmonary interstitial emphysema, gas pockets within the lung or subpleural dissection of gas. A pressure-volume curve was measured with air inflation of the lungs to a pressure of 40 cmH₂O followed by deflation⁹. Lung samples were snap frozen for molecular analysis.

*mRNA Quantitation by quantitative PCR*

Frozen lung samples were homogenized and total RNA was isolated with TRIzol (Invitrogen, Carlsbad, CA). Reverse transcription was performed using Verso cDNA kit (Thermo Scientific, Waltham, MA) to produce single-strand cDNA. Amplification was performed with sheep-specific primers with Taqman probes (Applied Biosystems, Foster City, CA) for the following genes associated with fetal lung maturation: surfactant protein A (SFTPA), surfactant protein B (SFTPB), surfactant protein C (SFTPC), surfactant protein D (SFTPD), ATP-binding cassette subfamily A member 3 (ABCA3), and aquaporin 5 (AQP5). The mRNA expression for each gene was normalized to the mRNA for the ribosomal protein 18s as internal standard. Final data are expressed as fold increase over the mean control value for animals treated with IM saline.

*Pharmacokinetics*
For the pharmacokinetics studies time-mated ewes had recovery surgery for placement of double lumen maternal jugular and single lumen fetal jugular catheters at 114 to 116 days of gestation as described previously. Animals were allowed to recover for 48 hours and then were assigned to receive IM treatments of Beta-P 0.125mg/kg, Beta-Ac 0.125mg/kg, or Beta-Ac 0.5mg/kg. We chose the dose of Beta-P of 0.125mg/kg as this is the amount of Beta-P in a dose of Beta-P+Beta-Ac 0.25mg/kg. Each animal received a second IM injection 48 hours later to allow for drug clearance after the first treatment and prior to a second injection. Maternal and fetal blood samples of 2 mL were collected 10 minutes before and then 1, 2, 3, 4, 6, 8, 10, 12, 14, and 24 hours after IM injection into chilled K$_3$EDTA vacutainers. The blood was centrifuged at 3000 x g, and the plasma was frozen at -80°C for betamethasone concentration measurements. Maternal and fetal plasma samples and betamethasone standards (500, 250, 100, 50, 25, 12, and 0 ng/mL) in control fetal sheep plasma were extracted as previously described and analyzed by mass spectrometry. The limit of the detection of this assay is 1ng/mL, and a cutoff greater than 30% of the limit was used as a zero point for analysis. Data were fitted to a 1-compartment model with PKSOLVER. All R$^2$ values for calibration curves were >0.98.

**Statistical analysis**

Data are presented as bar graphs of the mean and standard deviations with discrete data points for each animal. Sample sizes were calculated using our previous data in which control animals had a mean V40 of 8mL/kg with standard deviation of 3.5 and treated animals had a mean V40 of 16mL/kg. With alpha set at 0.05 and power of 0.8 the sample size needed to identify differences between groups was 8. Considering loss of
animals due to preterm labor we planned for 10 animals per group. Statistical tests were performed with Prism software (GraphPad Software Inc., San Diego, CA). Initial comparisons were performed with ANOVA followed by multiple groups’ comparison with Holm-Sidak’s post-hoc test to compare control versus each treatment group, and the standard treatment (Beta-Ac+Beta-P 0.25mg/kg x2) versus other treatment groups. Significance was attributed for p-values less than 0.05.

**Results**

Fifty-nine animals completed their protocols. One animal in the Beta-Ac+Beta-P group was excluded due to illness preterm labor prior to receiving any treatment. No animals had signs of preterm labor after ANS treatment. Groups had similar baseline characteristics, including gestational age, sex distribution, birth weight, and cord blood gas values (Table 1). Vital signs did not significantly differ after 30 minutes of ventilation (data not shown).

**Assessment of lung function**

After 30 minutes of ventilation, treatment with any ANS dose improved blood gas measurements and dynamic lung compliance relative to control (Figure 2 and 3). The dynamic compliance increased from a mean of 0.2 ± 0.06 in control animals to 0.5 ± 0.15 for treated animals (p-value <0.01). ANS treatment also improved ventilation efficiency index showing better gas exchange compared to control, except for the group treated with one dose of Beta-Ac+Beta-P in which the difference was not statistically significant.
(unadjusted p-value = 0.03; adjusted for multiple comparisons p-value = 0.052). At 30 minutes the PIP was lower in animals treated with Beta-Ac+Beta-P 0.25mg/kg x 2, Beta-Ac 0.5mg/kg or 0.25mg/kg compared to control, with the PIP required by the Beta-Ac+Beta-P 0.25mg/kg x 2 being significantly lower compared to other treatment groups (p-value<0.01) (Figure 3). It is important to note that all animals in the control group required the maximal positive inspiratory pressure (PIP) of 40 cm H₂O, which was not sufficient to achieve the target Vₜ. All ANS treatment groups had similar Vₜ means at 30 minutes. Treatment with ANS also significantly improved static lung compliance compared to control as demonstrated by the pressure-volume curves and volume at 40 cm H₂O (p-value<0.01), except for animals treated with Beta-Ac+Beta-P 0.25mg/kg x1 (Figure 4). Gross lung injury was present in 3 (30%) animals in the control group, 1 (11%) animal in the Beta-Ac+Beta-P x 2 group, 4 (40%) animal in the Beta-Ac+Beta-P x1 group, 2 (20%) animals in Beta-Ac 0.5mg/kg, 2 (20%) animals in Beta-Ac 0.25mg/kg, and 1 (10%) animals in Beta-Ac 0.125mg/kg. The frequency of gross lung injury was statistically similar in treatment groups compared to control.

All ANS treatments increased mRNA expression of SFTPA, SFTPB, SFTPC, ABCA3, and AQP5, compared to control (Figure 5). Both the one-dose Beta-Ac+Beta-P and the Beta-Ac 0.125mg/kg treatments resulted in lower mRNA quantity of SFTPB, ABCA3, and AQP5, compared to the two doses of Beta-Ac+Beta-P 0.25mg/kg. Compared to the one-dose Beta-Ac+Beta-P, the Beta-Ac 0.125mg/kg treatment had lower mRNA quantity of SFTPA (p-value=0.01), SFTPB (p-value<0.01), ABCA3 (p-
value < 0.01), and AQP5 (p-value = 0.03). There were no differences between the one-dose Beta-Ac+Beta-P and Beta-Ac 0.25mg/kg or 0.5mg/kg. ANS treatment did not increase mRNA expression of SFPTD or SCNN1G.

Pharmacokinetics

Free betamethasone was detected in maternal and fetal plasma after Beta-Ac injections for the 24 hours except for one fetus. After IM Beta-P, betamethasone was not detected (<1 ng/mL) in the maternal plasma at 24 hours in three of five ewes, and in none of the fetuses. In two of the four fetuses betamethasone was not detected at and after 10 hours of the Beta-P injection. For a similar total dose of 0.125mg/kg, Beta-Ac resulted in lower maximal maternal (27.6 vs. 102 ng/mL) and fetal (3.1 vs. 9.3 ng/mL) concentrations and total fetal exposure (AUC 56 vs. 78 ng/mL*h) than Beta-P (p-value=0.04) (figure 6 and table 2). The fetal concentration was about 10% of the maternal concentration for the three treatments. Total fetal exposure to ANS, measured by the area under the curve (AUC), was lower in Beta-Ac compared to a similar dose of Beta-P.

A four-fold increase in the administered dose of IM Beta-Ac, from 0.125mg/kg to 0.5 mg/kg resulted in about a two-fold increase in the mean maximal maternal and fetal concentration (C_{max} 27.6 vs. 57.4 and 3.1 vs. 6 mg/mL, respectively) and in the total fetal and maternal exposure (AUC 443 vs. 941 and 56 vs. 104 ng/mL*h, respectively).

Comment
We demonstrate that a single weight-based maternal dose of IM Beta-Ac effectively induces physiological and biochemical fetal lung maturation at lower doses than the standard of care treatment with Beta-Ac+Beta-P. The Beta-Ac results in decreased fetal exposure to corticosteroids compared to the Beta-P component of the clinically used combination. Corticosteroid toxicity in general results from high dose and long term exposures, primarily when used for control of inflammation. The indication with ANS is for maturational signaling, which results from a prolonged (<24h) low plasma level fetal exposure that can be achieved with Beta-Ac alone, avoiding the two high peak exposures from Beta-P. The other commonly used treatment of 4 doses of 6 mg of dexamethasone-phosphate will expose to fetus to 4 unnecessary high concentration exposures to dexamethasone.17

There have been no short term adverse effects of ANS identified by meta-analysis of the RCTs, except for an increase in hypoglycemia from ANS exposure in infants delivered between 34 and 37 weeks.18 However, most trials were performed in high-income countries with availability of neonatal intensive care. A multicenter randomized trial in low and middle-income countries found that ANS not only did not decrease mortality for infants born with birth weight less than 5th percentile but it also increased neonatal mortality for all infants exposed to ANS prenatally, which could not be attributed to other components of the treatment intervention.19,20

There remain concerns for long term side effects from fetal exposure to glucocorticoids, particularly neurologic, metabolic and cardiovascular changes in later life.21-23 A limitation to the understanding the long-term effects is limited data from randomized trials. The original trials on ANS included preterm infants that were larger
and of a more advanced gestational age than the current population of preterm infants. In a 30-year follow-up of former preterm infants included in a randomized clinical trial of ANS, Dalziel et al. found increased insulin levels in glucose tolerance test among subjects exposed to prenatal betamethasone, suggesting modulation of insulin sensitivity by ANS.

Since very preterm infants have a survival benefit with ANS, it would be unethical to perform new trials to assess for long-term outcomes in this population. Hence, observational studies have attempted to address potential side effects of ANS. Very preterm newborns exposed to betamethasone have lower birth weights, lengths and head circumferences than matched term-born control or infants exposed to preterm labor but not treated with ANS who were born at term. ANS also affects neonatal basal cortisol levels and response to stress. In the neonatal period, prenatal exposure to multiple courses of ANS are associated with lower cortisol levels at baseline and after stress compared to single courses. At school age, history of prenatal exposure to a single course of betamethasone was associated with increased cortisol response to a stress compared to controls. Similar effects of ANS on fetal growth and long-term metabolism have been reported in multiple experimental studies of fetal exposure to glucocorticoids, while others have found no or beneficial effects of ANS. Given these concerns, dosing optimization with minimization of fetal exposure could prevent or decrease long-term side effects. These varied observations are not based on randomized populations.

There has been no optimization of ANS for fetal lung maturation. A Cochrane review compared maternal and neonatal outcomes with different treatments and found no differences in neonatal respiratory outcomes. This meta-analysis included 12 clinical
trials with 7 different treatment strategies, underscoring the wide variation in practice. A randomized controlled trial is a costly and imprecised way to test drug doses. The preterm lamb model can be used as an excellent translational model to test drug dosing as preterm fetal lung maturation and neonatal ventilation due to the similarities in fetal lung development between sheep and humans. Fetal sheep are also extensively used to study maternal-fetal interactions due to the ability to place chronic maternal and fetal catheters. Limitation of the model are the inability to directly assess ANS effects on other neonatal complications such as necrotizing enterocolitis, intraventricular hemorrhage or long term outcome. In this study a single dose of 0.125mg/kg of Beta-Ac resulted in similar improvements in lung compliance, ventilation efficiency and gas exchange as the clinically used Beta-Ac+Beta-P regimen with decreased fetal exposure to corticosteroids. Even though the PIP at 30 minutes was not significantly different for the lowest dose of Beta-Ac compared to control, this may have resulted from limiting the PIP in order to avoid air leaks. Only 4 animals in the Beta-Ac 0.125mg/kg group required the maximal PIP of 40 cmH$_2$O with $V_t$ range of 5.8-7.5mL/kg among those animals, while all animals in the control group required the maximal PIP with a $V_t$ range of 3.1 to 6.4 mL/kg (mean of 4.6 ml/kg). Both dynamic and static lung compliance were improved in the animals treated with Beta-Ac 0.125mg/kg and were similar to the standard dosing. A dose lower than 0.125mg/kg should be tested for efficacy.

The lowest Beta-Ac dosing also increased mRNA expression levels of surfactant proteins A, B, and C, ABCA3, and AQP5 compared to control animals. The mRNA expression of surfactant proteins A and B, ABCA3, and AQP5 was significantly higher in the animals that received two doses Beta-Ac+Beta-P, compared to the lowest dose of
Beta-Ac. We did not perform protein concentration measurements because protein differences will not occur until 4 to 5 days after ANS administration in previous studies\textsuperscript{34}. Given that these 2 groups had similar lung compliance and gas exchange this difference may not be biologically relevant.

The pharmacokinetic findings regarding peak concentrations, half-life, and maternal-fetal transfer are in accordance with previous pharmacological studies\textsuperscript{9,10,13}. Given that the Beta-Ac component is only commercially available in combination with Beta-P, the pharmacological properties of Beta-Ac alone have not been thoroughly explored. Jobe et al. previously reported the pharmacokinetics of the 0.25mg/kg of Beta-Ac alone in 3 pregnant sheep\textsuperscript{9}. In our study a single 0.125mg/kg IM dose of Beta-Ac improved lung mechanics and gas exchange to levels similar to the standard two dose regimen of Beta-Ac+Beta-P, which provides a 0.25mg/kg of Beta-P and 0.25mg/kg of Beta-Ac. Maternal exposure was also significantly reduced with Beta-Ac compared to Beta-P. The Australasian Randomized Trial to Evaluate the Role of maternal Intramuscular Dexamethasone (A*STEROID) trial\textsuperscript{35} is currently comparing 2 doses given 24 hours apart of 12 mg of Beta-Ac+Beta-P to 2 doses of 12 mg of dexamethasone-phosphate, which will yield even higher fetal plasma dexamethasone exposures. The results of this trial will provide more information on the differences between treatments.

Considering the possible long-term effects of ANS to the fetus, optimization of the dose with minimization of exposure is desirable. These concerns are amplified if ANS becomes more widely used for women at risk of preterm delivery in low resource environments, for previable fetuses at risk of preterm delivery, for women who deliver between 34 and 37’ weeks gestational age, and for women who deliver by elective C-
section at term\textsuperscript{18,36,37}. We demonstrate that a single dose of Beta-Ac may be an effective alternative to promote fetal lung maturation in women at risk of preterm delivery while minimizing maternal and fetal exposure to corticosteroids. These experimental evaluations of ANS provide a pathway to the clinical testing of new dosing strategies for ANS.

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Table 1. Summary of animal data for ventilation study.

|                                | Control | Beta-Ac+Beta-P 0.25mg/kg x2 | Beta-Ac+Beta-P 0.25mg/kg x1 | Beta-Ac x1 0.5mg/kg | Beta-Ac x1 0.25mg/kg | Beta-Ac x1 0.125mg/kg |
|--------------------------------|---------|-------------------------------|-------------------------------|---------------------|----------------------|----------------------|
| **Number of animals**          | 10      | 9                             | 10                           | 10                  | 10                   | 10                   |
| **Gestational age (days)**     | 122 ± 0.5 | 122 ± 0.7                    | 122 ± 0.7                    | 122 ± 0.7           | 122 ± 0.5           | 122 ± 0.5           |
| **Weight (kg)**                | 2.7 ± 0.2 | 2.5 ± 0.3                    | 2.6 ± 0.3                    | 2.8 ± 0.4           | 2.6 ± 0.3           | 2.7 ± 0.3           |
| **Sex ratio (M/F)**            | 8/2     | 6/3                           | 6/4                           | 4/6                 | 6/4                 | 7/3                 |
| **Cord blood gas**             |         |                               |                               |                     |                      |                      |
| **pH**                         | 7.34 ± 0.03 | 7.34 ± 0.07                 | 7.33 ± 0.05                  | 7.28 ± 0.14         | 7.34 ± 0.02         | 7.35 ± 0.04         |
| **pCO₂ (mmHg)**                | 51 ± 3  | 51 ± 5                        | 50 ± 5                        | 59 ± 21.2           | 47 ± 3.5            | 51 ± 4.2            |
| **pO₂ (mmHg)**                 | 18 ± 2  | 17 ± 2                        | 18 ± 3                        | 15 ± 2.4           | 16 ± 2.7            | 17 ± 2.7            |
Table 2. Pharmacokinetic measurements of maternal and fetal plasma betamethasone after IM injections of Beta-P or Beta-Ac. \( t\frac{1}{2} \): half-life; \( T_{\text{max}} \): time of maximal concentration; AUC: area under the curve.

|            | Maternal |            | Fetal |            |
|------------|----------|------------|-------|------------|
|            | Beta-P   | Beta-Ac    |       | Beta-P     | Beta-Ac   |
|            | 0.125mg/kg | 0.125mg/kg | 0.5mg/kg | 0.125mg/kg | 0.125mg/kg | 0.5mg/kg |
| \( t\frac{1}{2} \) (hour) | 2.4 | 9.2 | 11.9 | 5.1 | 6.2 | 6 |
| \( T_{\text{max}} \) (hour) | 0.87 | 5.6 | 4 | 2.4 | 8.5 | 7.9 |
| \( C_{\text{max}} \) (ng/mL) | 102 | 27.6 | 57.4 | 9.3 | 3.1 | 6 |
| AUC 0-t (ng/mL*h) | 557 | 443 | 941 | 78 | 56 | 104 |
Figure legends

Figure 1. Experimental design. Negative control animals were treated with IM saline. A group of animals was treated with the clinically used formulation of Betamethasone-acetate + Betamethasone-phosphate (Beta-Ac+Beta-P) either as 2 doses of 0.25mg/kg 24 hours apart (clinical dose) or as a single dose (50% total clinical dose). Another group of animals was treated with Betamethasone-acetate only (Beta-Ac) either with a dose equivalent to the full clinical dose (0.5mg/kg), or 50% of the clinical dose (0.25mg/kg), or 25% of the clinical dose (0.125mg/kg).

Figure 2. Blood gas measurements at 30 minutes of ventilation of preterm lambs. Betamethasone at all doses (A) increased pH, (B) decreased pCO\textsubscript{2}, and (C) increased pO\textsubscript{2} (*p<0.05).

Figure 3. Ventilatory and lung mechanics variables at 30 minutes of ventilation. A: Dynamic compliance; B: Ventilatory efficiency index (VEI); C: Positive inspiratory pressure (PIP); D: Tidal volume (V\textsubscript{t}). Any dose of Beta-Ac, including the lowest dose of 0.125mg/kg improved all measurements compared to control (*p<0.05).

Figure 4. Static lung compliance of exteriorized lungs. (A) Pressure volume curves and (B) volume at 40 cmH\textsubscript{2}O improved with any dose of Beta-Ac (*p<0.05).
Figure 5. mRNA quantitation in fold change relative to control. A: surfactant protein A (SPA); B: surfactant protein B (SPB); C: surfactant protein C (SPC); D: ATP-binding cassette family A member 3 (ABCA3); E: Aquaporin 5 (AQP5); F: Sodium channel epithelium 1 gamma subunit (SCNN1G) (*p<0.05). Single IM dose of Beta-Ac increased the expression of SPA, SPB, SPC, ABCA3, and AQP5. SPD and SCNN1G expression were not changed by any treatment.

Figure 6. Average plasma concentrations of betamethasone after maternal intramuscular administration of antenatal steroids. A: Maternal (●) and fetal (■) plasma concentrations of betamethasone after 0.5 mg/kg of Beta-Ac had a maternal peak concentration of 57 ng/mL at 4 hours and fetal peak concentration of 6 ng/mL at 8 hours; B: Maternal (●) and fetal (■) plasma concentration of betamethasone after 0.125 mg/kg of Beta-Ac had a maternal peak concentration of 27.6 ng/mL at 5.6 hours and fetal peak concentration of 3.1 ng/mL at 8.5 hours after IM injection; C: Maternal (●) and fetal (■) plasma concentrations of betamethasone after 0.125 mg/kg of Beta-P had a maternal peak concentration of 102 ng/mL at <1 hour and fetal peak concentration 11 ng/mL at 2.4 hours after IM injection; D: Comparison of fetal plasma concentrations for IM Beta-P 0.125 mg/kg (◆), Beta-Ac 0.5 mg/kg (○), and Beta-Ac 0.125 mg/kg (□) had a lower peak fetal concentration for a similar total dose of antenatal steroids after IM Beta-Ac compared to Beta-P (3.1 vs. 11, respectively, p<0.05) and lower total fetal exposure measured by the area under the curve.
