Placental Growth Factor and Prolactin Upregulation in the Placenta are Associated with Improved Lung Development After Antenatal Betamethasone Treatment in Experimental Chorioamnionitis

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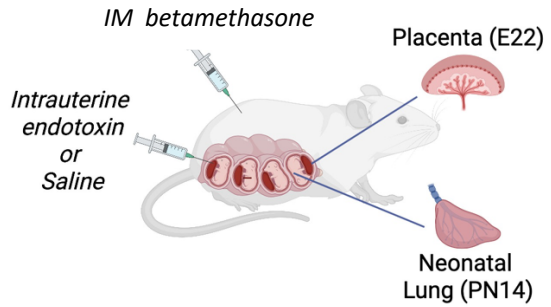
Introduction

- **Bronchopulmonary dysplasia (BPD),** the chronic lung disease of preterm birth, is characterized by impaired lung function, arrested lung development, and increased risk for **pulmonary hypertension (PH).**
- Antenatal stress from **chorioamnionitis (CA**) is strongly associated with risk for developing BPD.
- Administering **endotoxin (ETX)** into the amniotic space to mimic CA impairs lung development and causes (PH).
- **Antenatal steroids (ANS)** improve survival of premature infants, attenuate lung inflammation, and prevent RDS at birth but have not been shown to reduce the incidence of BPD or PH.

Hypothesis

We hypothesize that antenatal betamethasone (BM) treatment of pregnant rats will preserve lung development and prevent PH in experimental CA, and that these effects on the offspring will be associated with alteration of placental signaling factors.

Methods



Methods

Model of CA-induced BPD

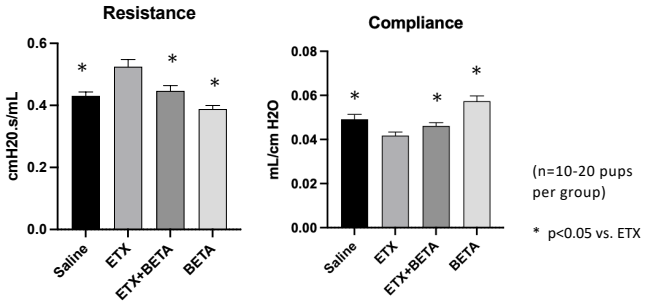
- Intra-amniotic endotoxin (ETX; 10 µg/sac) or saline (CTL; 50ul/sac) was administered to rat pups via laparotomy of pregnant dams at embryonic day 20 (E20; term, 22 days).
- IM BM (0.2mg/kg) was administered to pregnant dams at E20.
- Pups were delivered via C-section at E22.
- **Four subgroups were identified: saline (CTL), ETX, BM, and ETX+BM**.
- Neonatal lung tissue was harvested for functional and morphometric analysis on postnatal day (PN)14

RNASeq Studies

RNA was isolated from fresh frozen rat metrial gland and placental tissues using a Purelink RNA mini kit (Invitrogen). Following isolation, RNA was treated with PureLink DNase (Invitrogen). Preparation of RNA library and transcriptome sequencing was conducted by Novogene (Novogene Co., LTD). Genes with adjusted p-value<0.05 and log2(fold change) >1.5 were considered as differentially expressed between ETX and ETX-Beta groups. Genes enriched for canonical pathways of placental intrauterine signaling were explored using: 1) unsupervised evaluation with Ingenuity Pathway Analysis (IPA) software (Quiagen) and 2) supervised evaluation for established rat placental endocrine, angiogenic, cytokine and chemokine markers.

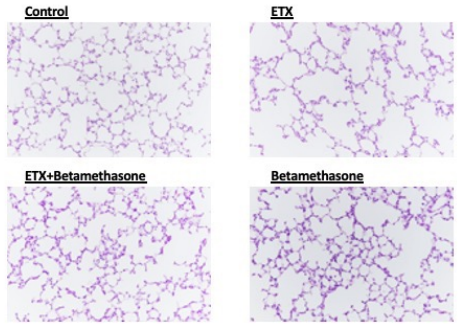
Results

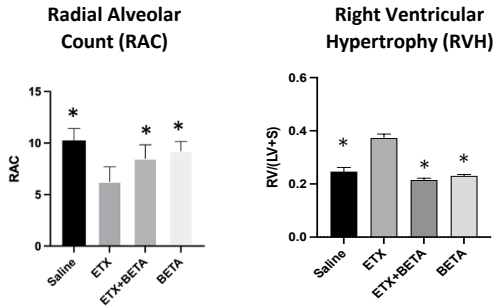
Antenatal Betamethasone Preserves Lung Function in Infant Rats After Intra-amniotic (IA) ETX



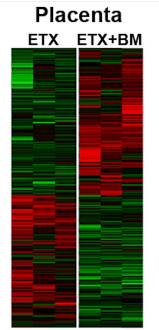
Results

Antenatal Betamethasone Preserves Alveolar Growth in Infant Lungs After IA ETX



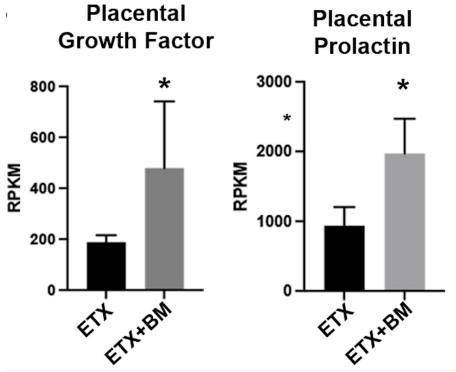


RNAseq analysis of Placental Tissue Identifies Over 330 Genes with Significantly Altered Expression Between ETX vs ETX+BM Pregnancies



Results

Antenatal Betamethasone Upregulates Placental Expression of the Pro-Angiogenic Genes, Placental Growth Factor (PGF) and Prolactin (PRL) After IA ETX



n=3 placentas/group * p<0.05 vs. ETX
RPKM: Reads per kilobase of transcript per million reads mapped

Summary

- Maternal BM treatment of ETX-exposed fetal rats:
  - Reduced total lung resistance ($p<0.05$)
  - Improved compliance ($p<0.05$)
  - Restored alveolarization ($p<0.05$)
  - Prevented RVH ($p<0.05$)

Conclusions and Speculation

We conclude that ANS improves infant lung development and prevents PH in the setting of intrauterine inflammation and speculate that this effect may be mediated by enhanced placental function including the upregulation of pro-angiogenic factors.