# Week 3: Neonatal Cardiopulmonary

## Neonatal Pulmonology: What's a Little Hyperoxia Among Friends?

**Tuesday, June 23  2:30-4:00 pm EDT**

**Moderators**
- Shu Wu
- Trent Tipple

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Note: Schedule subject to change based on presenter availability.
CONTROL ID: 3377610
TITLE: Cannabidiol reduces lung damage following meconium aspiration in newborn piglets
PRESENTER: Luis Arruza

AUTHORS (LAST NAME, FIRST NAME): Arruza, Luis1; Barata, Lorena2; Rodriguez, Maria Jose1; Vierge, Eva1; Vargas, Carlos1; Gutierrez-Rodriguez, Ana1; del Pozo, Aaron1; Villa, Maria1; Martinez-Orgado, Jose1


CURRENT CATEGORY: Neonatology
CURRENT SUBCATEGORY: Neonatal Pulmonology
KEYWORDS: cannabidiol, meconium aspiration syndrome, lung injury.
SESSION TYPE: Platform|Webinar

ABSTRACT BODY:
Background: Neonatal Meconium Aspiration Syndrome (MAS) is a potentially devastating complication with significant mortality and morbidity in term and near-term newborns. Currently there is no specific treatment other than supportive management. Cannabidiol (CBD) is a non-psychoactive cannabinoid with potent anti-inflammatory and antioxidant properties. CBD has demonstrated its beneficial effects on animal models of lipopolysaccharide (LPS) or cerebral hypoxia-ischemia mediated acute lung injury.

Objective: To assess the effects of CBD on lung damage in a piglet model of MAS.

Design/Methods: Animals were randomly assigned to each experimental group. Thirty min after intratraqueal instillation of 3 mL/kg of meconium (20% dilution in saline) to 2-3 day-old mechanically ventilated and sedated piglets, animals received i.v. vehicle (VEH, n=9) or CBD 5 mg/kg (CBD, n=6). Thereafter, hemodynamic (mean arterial blood pressure - MABP) as well as respiratory parameters (including arterial pH, pCO2, inspired air oxygen fraction -FiO2, and tidal volume -Vt) were continuously monitored for six hours. Similarly managed piglets without MAS served as controls (SHM, n=6). After 6 hours of observation animals were killed and lung samples were obtained. Histological damage was assessed by a blinded investigator in hematoxylin-eosin stained lung specimens using a semi-quantitative lung injury severity score. Concentration of TNFa was measured by western blot in lung tissue to assess inflammation. One- or two-way ANOVA was used to compare groups and different timepoints, with Bonferroni’s post-hoc test for multiple comparisons. A p<0.05 was accepted for statistical significance.

Results: Post-insult administration of CBD reduced MAS-induced deterioration of pH and pCO2, and improved haemodynamic stability (Table 1). These beneficial effects on gas exchange were obtained despite lower tidal volume requirements for CBD-treated animals. (Table 1).

Histological and biochemical studies evidenced reduced lung damage and inflammation in piglets treated with CBD (Figures 1 and 2, respectively).

Conclusion(s): CBD reduces histologic lung damage and inflammation in a piglet model of MAS. This is translated clinically into improved gas exchange and hemodynamic stability.
Figure 1. Histopathological score (Merz U, et al. Intensive Care Med 2000;26:109–16). MAS-VEH: Meconium Aspiration and i.v. vehicle; MAS-CBD: Meconium Aspiration and i.v. cannabidiol; SHM: Sham animals. (*) p<0.05 vs. SHAM by one way ANOVA.

Figure 2. Concentration of TNFa in lung tissue. MAS-VEH: Meconium Aspiration and i.v. vehicle; MAS-CBD: Meconium Aspiration and i.v. cannabidiol; SHM: Sham animals. (*) p<0.05 vs. SHAM by one way ANOVA.
**TITLE:** Angiopoietin 1 Protects Against LPS-induced Acute Lung Injury and Remodeling in Neonatal Mice

**PRESENTER:** Heather Menden

**AUTHORS (LAST NAME, FIRST NAME):** Menden, Heather¹; Salimi, Umar¹; Mabry, Sherry M.²; Xia, Sheng²; Sampath, Venkatesh⁴

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**CURRENT CATEGORY:** Neonatology

**CURRENT SUBCATEGORY:** Neonatal Pulmonology

**KEYWORDS:**


**SESSION TYPE:** Platform|Webinar

**ABSTRACT BODY:**

**Background:** Sepsis-induced acute lung injury (ALI) in premature newborns is a risk factor for bronchopulmonary dysplasia (BPD). Angiopoietins are vascular growth factors that regulate endothelial homeostasis and inflammation. During sepsis-induced ALI, angiopoietin 2 (ANGPT2) is upregulated and antagonizes normal angiopoietin 1 (ANGPT1)-mediated Tie2 receptor phosphorylation resulting in endothelial (EC) immune activation, and pro-inflammatory signaling. In this study, we investigated the hypothesis that recombinant ANGPT1 will attenuate sepsis-induced ALI and subsequent alveolar remodeling by restoring EC quiescence.

**Objective:**

1) Determine whether ANGPT1 antagonizes ANGPT2-mediated Tie2 dephosphorylation to suppress LPS-induced ALI.

2) Assess the effect of ANGPT1 therapy on LPS-induced lung remodeling and alveolar simplification.

**Design/Methods:** 6-day-old C57BL/6 mice were given i.p. LPS (2mg/kg) ± 2hr pretreatment with i.p. recombinant mouse ANGPT1 (rmAngpt1, 1µg). Lungs were harvested after 24hrs for mRNA, protein, and immunohistochemistry. A similar approach was used for bronchoalveolar lavage (BAL) in 10-day-old mice. To assess alveolar remodeling, 7-day-old mice were given i.p. LPS ± 2hr pretreatment with i.p. rmANGPT1 and inflation-fixed lungs were collected on day of life 14 for morphometry. Separately, HPMEC (ScienCell) cultures were stimulated with recombinant human ANGPT2 (rhANGPT2, 25ng/mL) or LPS ± recombinant human ANGPT1 (rhANGPT1, 200ug/mL) for 1 or 24hrs.

**Results:** rmAngpt1 protected against LPS-induced Tie2 dephosphorylation and ANGPT2 upregulation in mouse lungs and HPMEC. In HPMEC, exposure to rhANGPT2 induced inflammatory mediators and dephosphorylated Tie2, while co-stimulation with rhANGPT1 attenuated both effects. In mice, rmAngpt1 pretreatment acutely suppressed lung NF-kB activation, cytokines, adhesion molecules, and apoptosis. rmAngpt1 also suppressed LPS-induced lung permeability as indicated by less alveolar neutrophils, WBC counts, and protein levels in BAL. Early LPS-induced lung tissue destruction was attenuated by rmAngpt1, evidenced by reduced MMP9:TIMP1 levels and elastin fiber degradation. rmAngpt1 also protected against LPS-induced alveolar simplification.

**Conclusion(s):** Exogenous ANGPT1 attenuates LPS-induced ALI and alveolar remodeling in newborn mice while antagonizing ANGPT2-induced pro-inflammatory Tie2 dephosphorylation. Restoring balance in Angiopoietin-Tie axis signaling during sepsis with therapeutic ANGPT1 has the potential to counter neonatal sepsis-induced ALI and prevent BPD.

![Image](image-url)
Figure 2: rmAngpt1 pretreatment in 10-day-old mice suppresses LPS-induced alveolar protein content and cell counts. (A) Albumin was quantified in Bronchoalveolar (BAL) fluid after LPS and rmAngpt1 treatments. B) Cell counts were quantified in BAL fluid, and cell differential evaluated by Diff-Quik staining.

Figure 3: Effect of rmAngpt1 on LPS-induced elastin architecture at 24 h and LPS-induced alveolar remodeling on P14. (A) Elastic fiber staining done in experimental groups 24 h after LPS treatment on P7. (B) H&E stained images done on inflamed lungs on P14 after LPS and rmAngpt1 treatments on P7 with RAC (C) and MLT (D) quantified. (E) Graph depicting the radial alveolar counts at P14. (D) Graph depicting mean linear intercepts at P14. Red arrows show elastin fiber staining that is normal (C, LPS+rmAngpt1) or interrupted (LPS).

Figure 4: rhAngpt1 suppresses LPS-induced inflammation, NFkB activation, and Tie2 dephosphorylation at 24 h in human pulmonary microvascular endothelial cells (HPMEC) in vitro. HPMEC in culture were treated with LPS and rhAngpt1 for experiments. (A) PCR on HPMEC lysates was done for cytokines gene expression after treatments. (B) Western blotting was done in HPMEC lysates for Tie2, phospho-Tie2, p65NFkB and NFkB.

IMAGE CAPTION:
CONTROL ID: 3376861
TITLE: Longer term airway hyperreactivity following neonatal hyperoxia can be attenuated with both levalbuterol and S-nitrosoglutathione reductase inhibition
PRESENTER: Thomas M Raffay

AUTHORS (LAST NAME, FIRST NAME): Sopi, Ramadan B.¹; Gaston, Benjamin²; Desai, Amar³; MacFarlane, Peter M.¹; Martin, Richard J.¹; Raffay, Thomas M.¹

AUTHORS/INSTITUTIONS: R.B. Sopi, P.M. MacFarlane, R.J. Martin, T.M. Raffay, Pediatrics, Case Western Reserve University, Rainbow Babies & Children's Hospital, Cleveland, Ohio, UNITED STATES; B. Gaston, Indiana University, Riley Children's Hospital, Indianapolis, Indiana, UNITED STATES; A. Desai, Case Western Reserve University, Case Comprehensive Cancer Center, Cleveland, Ohio, UNITED STATES;

CURRENT CATEGORY: Neonatology
CURRENT SUBCATEGORY: Neonatal Pulmonology
KEYWORDS: Airway Hyperreactivity, S-Nitrosoglutathione, Bronchopulmonary Dysplasia.
SESSION TYPE: Platform|Webinar

ABSTRACT BODY:

Background: Bronchopulmonary dysplasia survivors display longer term obstructive lung disease with airway hyperreactivity (AHR). While often treated with β2 adrenergic receptor agonist asthma medications, patient responses are variable and may induce receptor tachyphylaxis with repeated use. We have previously shown that the endogenous smooth muscle relaxant molecule, S-nitrosoglutathione (GSNO), is degraded in neonatal hyperoxia by upregulated GSNO reductase (GSNOR) in the murine lung (Raffay, et al. Molecular Pharmacology 2016; 90(4):418-26).

Objective: We hypothesize that inhibition of GSNOR is a novel approach to attenuate neonatal hyperoxia-induced AHR in room air recovered mice.

Design/Methods: Newborn C57BL/6 mice were randomized on the first day of life and assigned to room air (21% O₂) or hyperoxic (60% O₂) groups for three weeks to induce bronchopulmonary dysplasia AHR. Animals were then recovered in room air until six weeks of age. AHR was assessed in vitro using precision-cut living lung slice preparations in response to increasing doses of bath-applied methacholine (MCh, 0.25-64 μM). Lung slices were pre-incubated with or without a GSNOR inhibitor (100 μM N6022) or a β2 adrenergic receptor agonist (10 or 100 µM levalbuterol). AHR is reported as percent change in airway lumen area from baseline (+ SEM). 2-3 airways were imaged and averaged per animal per condition.

Results: Neonatal hyperoxia significantly increased airway contractile responses to MCh in six week mice with a mean maximal effect (E_max) of 71.9 ± 2.7% of baseline compared to the room air control E_max of 39.8 ± 5.2 (p<0.01). GSNOR inhibition significantly attenuated airway contractile responses in hyperoxic slices to 32.2 ± 2.0 (p<0.001). Pre-incubation of hyperoxic slices with either a GSNOR inhibitor or a β2 adrenergic receptor agonist (100 μM) or a β2 adrenergic receptor agonist (10 or 100 µM levalbuterol). AHR is reported as percent change in airway lumen area from baseline (+ SEM). 2-3 airways were imaged and averaged per animal per condition.

Conclusion(s): These studies show that neonatal hyperoxia-exposed mice display longer term in vitro AHR to MCh and that inhibition of GSNOR attenuates hyperoxia-induced AHR comparable to a β2 adrenergic receptor agonist. We speculate that GSNO-based therapies may serve as novel treatments for AHR in bronchopulmonary dysplasia survivors that do not respond to β2 adrenergic receptor agonists.

(No Image Selected)
**Results:** GF mice in hyperoxia show protected lung structure and mechanics, and decreased neutrophilic inflammation. In normoxia BPD-GF mice demonstrated similar lung structure and function as both GF and Non-BPD-GF mice. However in hyperoxia, BPD-GF mice demonstrated severe BPD phenotype marked by alveolar hypoplasia (RAC), worse pulmonary function (low compliance, high resistance), increased neutrophilic inflammation, as compared to both GF mice and Non-BPD-GF mice. *Hmox1* and *Nqo1* expression were lower in NGF mice compared to GF mice in hyperoxia. All significance = p<0.05.

**Conclusion(s):** Human BPD microbiome transplantation predisposes GF mice to hyperoxic lung injury. As lung dysbiosis plays a critical role in BPD, manipulation of lung microbiome may be a potential therapeutic intervention. GF mice are protected and exhibit an upregulation of antioxidant responses in hyperoxia. Our novel data indicates an interaction between airway microbiome and Nrf2-dependent antioxidant responses. Future studies will examine the mechanisms by which antioxidant responses contribute to microbiome-based BPD therapeutics.
Objective: To evaluate the role of RAGE signaling using in vitro and in vivo hyperoxia models of BPD.

Design/Methods: Mouse lung epithelial cells (MLE-15) were exposed to 21% oxygen (normoxia) or 85% oxygen (hyperoxia) during culture conditions. In some studies, cells were also treated with the RAGE ligand, S100A12, with or without sRAGE after which cells were harvested for qRT-PCR and western blot analysis. In additional experiments, RAGE knockout (-/-) and wild type mice were exposed to normoxia (21% oxygen) or hyperoxia (85% oxygen) from birth till day 14 of life. At day 14, lungs were harvested and morphometric measurements including mean linear intercept, radial alveolar count and alveolar surface area were quantified.

Results: In vitro experiments: Hyperoxia exposure for 24 hours resulted in increased RAGE mRNA and protein expression in MLE-15 cells (P < 0.05 compared to cells cultured in 21% oxygen). Treating hyperoxia exposed cells with the RAGE ligand S100A12 increased mRNA expression of inflammatory cytokines by these cells. This effect was blocked by pre-treatment of cells with the decoy receptor sRAGE. In vivo experiments: Hyperoxia exposure for 14 days resulted in dilated distal airspaces, fewer alveoli and a BPD lung phenotype. Genetic deletion of RAGE (RAGE KO) did not prevent hyperoxia-mediated BPD changes in the neonatal mouse lung.

Conclusion(s): Hyperoxia exposure increases RAGE mediated inflammatory signaling in mouse lung epithelial cells. Contrary to our hypothesis, genetic deletion of RAGE (RAGE KO) did not prevent hyperoxia-mediated BPD changes in the neonatal mouse lung. These data suggest that along with promoting inflammation, the RAGE axis may have additional roles in maintaining homeostasis in the developing lung. Further studies to better understand the role of RAGE/sRAGE axis during lung development are needed.
M. Sharma, S. Kulandavelu, Pediatrics, University Of Miami Miller School of Medicine, Miami, Florida, UNITED STATES;

CURRENT CATEGORY: Neonatology
CURRENT SUBCATEGORY: Neonatal Pulmonology
KEYWORDS: Mesenchymal Stem Cell Exosomes, Pulmonary Hypertension, Bronchopulmonary Dysplasia.
SESSION TYPE: Platform/Webinar

ABSTRACT BODY:

Background: Previously, we demonstrated that early administration of Wharton’s Jelly Mesenchymal Stem Cell-derived (WJ-MSC) exosomes attenuates pulmonary hypertension (PH) and improves lung structure in experimental bronchopulmonary dysplasia (BPD). The optimal dose is however not known. The purpose of this study was to determine the optimal WJ-MSC exosome dose for lung regeneration in severe BPD.

Objective: To elucidate the most effective MSC exosome dose to prevent neonatal hyperoxia-induced lung injury and PH.

Design/Methods: Newborn Sprague-Dawley rats (N=75) exposed to normoxia (RA) or hyperoxia (85% O2; HYP) from postnatal day (P) 1- P14 were assigned to receive varying doses of intra-tracheal (IT) WJ-MSC derived exosomes (low dose: 2 x 10^8; medium dose: 12 x 10^8; high dose: 60 x 10^8 particles per gram body weight in 50 ul) or exosome free media (PL) on P3. The degree of pulmonary hypertension (PH), vascular remodeling, alveolarization, as well as lung angiogenesis was assessed at P14. PH was determined by measuring right ventricular systolic pressure (RVSP) and pulmonary vascular remodeling was evaluated by quantifying the percentage of muscularized peripheral pulmonary vessels. Right ventricular hypertrophy (RVH) was determined by right ventricle to left ventricle plus septum weight ratio (RV/LV+S). Alveolarization was evaluated by measuring mean linear intercept (MLI) and radial alveolar count (RAC). Angiogenesis was determined by measuring vascular density. Data are expressed as mean ± SD, and analyzed by ANOVA.

Results: Exposure of neonatal rats to hyperoxia impaired lung alveolarization as evidenced by increased MLI and reduced RAC (Fig. 1 and Table 1). This was accompanied by decreased vascular density and increased percent muscularized vessels (Fig. 1 and Table 1). In contrast, IT administration of low, medium or high dose WJ-MSC exosomes significantly improved alveolar structure, vascular density and pulmonary vascular remodeling and there was no difference in beneficial effects among the three doses (Fig.1 and Table 1). Moreover, whereas hyperoxia-PL exposed animals had increased RVSP and RV/LV+S and decreased survival, IT administration of all three WJ-MSC exosome doses significantly attenuated PH and RVH (Fig.2). However, only high dose WJ-MSC exosome improved survival (Fig 3).

Conclusion(s): In an experimental model of severe BPD, IT WJ-MSC exosomes preserves lung structure and attenuates PH in a non-dose dependent manner. However, only high-dose WJ-MSC exosome improved survival.
CONTROL ID: 3382464
TITLE: Mitochondrial DNA Variation Modulates Hyperoxia Induced Pulmonary Mitophagy and ATP generation in Newborn Mice.
PRESENTER: jegen kandasamy
AUTHORS (LAST NAME, FIRST NAME): kandasamy, jegen¹; Li, Rui¹; Rezonzew, Gabriel²; Olave, Nelida²; Jilling, Tamas³; Ambalavanan, Namasivayam²
AUTHORS/INSTITUTIONS: J. kandasamy, R. Li, University of Alabama at Birmingham, Birmingham, Alabama, UNITED STATES; G. Rezonzew, N. Olave, N. Ambalavanan, Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama,
ABSTRACT BODY:

Background: Using C57BL6 (C57\textsuperscript{WT}), C3H/HeN (C3H\textsuperscript{WT}) mice wild type (WT) mice and Mitochondrial-Nuclear eXchange (MNX) strains – C57MNX (C57\textsuperscript{MNX}) and C3HMNX (C3H\textsuperscript{MNX}) - we have previously found that mice with C3H mtDNA are protected against hyperoxia induced neonatal lung injury when compared to mice that carry C57 mtDNA, irrespective of nuclear background. Hyperoxia-exposed newborn mice with C3H mtDNA also have higher pulmonary fibroblast oxygen consumption and lower superoxide generation vs. mice with C57 mtDNA.

Objective: In this study, we aimed to identify possible mechanisms through which mtDNA variations in inbred neonatal mice modify hyperoxic neonatal lung injury susceptibility by assessing pulmonary mtDNA damage, mitophagy, and ATP generation in this model.

Design/Methods: Newborn mice belonging to all 4 strains (C57\textsuperscript{WT}, C3H\textsuperscript{WT}, C57\textsuperscript{MNX} and C3\textsuperscript{HMNX}) were exposed to hyperoxia (85\% \textsubscript{O}_2) or room air from P1-P14. Lungs were isolated, homogenized and used to assess mtDNA damage using qPCR, PINK1 expression using immunoblot (mitophagy marker) and total lung ATP content using colorimetry.

Results: Hyperoxia-exposed C57\textsuperscript{MNX} mice (which are less susceptible) had decreased pulmonary mtDNA damage when compared to C57\textsuperscript{WT} mice which are more susceptible to hyperoxic neonatal lung injury (Figure 1). Lungs from hyperoxic C57\textsuperscript{MNX} mice also showed increased PINK1 expression as quantified through Western Blot, indicating higher levels of pulmonary mitophagy vs. C57\textsuperscript{WT} mice (Figure 2) as well as higher pulmonary tissue ATP content vs. C57\textsuperscript{WT} mice (Figure 3). Similarly, hyperoxic C3\textsuperscript{HMNX} mice which are more susceptible to hyperoxic neonatal lung injury. A minimum of 14 animals were used per group per exposure for all analyses, with p-values < 0.05 indicated by an asterisk.

Conclusion(s): Lungs from hyperoxic newborn mice with C3HmtDNA have increased mitophagy but decreased mtDNA lesions, indicative of improved clearance of mtDNA damaged by hyperoxia induced oxidant stress vs. mice with C57 mtDNA. Such differences could lead to improved mitochondrial efficiency that underlies the increased lung ATP content that was also noted in mice with C3H mtDNA vs. mice with C57 mtDNA. Possible mechanisms for these findings including changes in mitochondrial transition pore permeability, membrane potential and protein import from the cytosol as well as alterations in nuclear gene expression are ongoing.
Representative PINK1 immunoblot (2 samples per exposure per group)

Lung ATP content

IMAGE CAPTION:
mtDNA damage

Representative PINK1 immunoblot (2 samples per exposure per group)

Lung ATP content

CONTROL ID: 3380237
TITLE: Sex-specific Differences in Bronchopulmonary Dysplasia: Is it the Sex Chromosome or Gonadal Hormones?
PRESENTER: Krithika Lingappan
AUTHORS (LAST NAME, FIRST NAME): Lingappan, Krithika¹; Grimm, Sandra L.²; Coarfa, Cristian²
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CURRENT CATEGORY: Neonatology
CURRENT SUBCATEGORY: Neonatal Pulmonology
KEYWORDS: Bronchopulmonary Dysplasia, Sex-specific, RNA-Seq.
SESSION TYPE: Platform/Webinar
ABSTRACT BODY:
**Background:** Male sex is considered an independent predictor for the development of bronchopulmonary dysplasia (BPD), but the main mechanisms underlying sexually dimorphic outcomes are unknown. To distinguish sex differences caused by gonadal hormones versus sex chromosome complement (XX versus XY), we used the four Core Genotype mice (FCG). In the FCG model (Fig 1), *Sry* is a transgene and is not present on the Y chromosome, so that XX and XY mice can each have either testes (with *Sry*, XXY or XYM) or ovaries (without *Sry*, XXF or XYF).

**Objective:** Our objective was to test the hypothesis that hormonal and sex chromosome mechanisms interact in the sex-specific modulation of neonatal hyperoxic lung injury.

**Design/Methods:** Neonatal FCG mice were exposed to hyperoxia (95% FiO₂, P1-4: saccular stage) or room air and euthanized on P5 for RNA-Seq analysis and analysis of lung morphometry and lung vascular development on P21 (Mean Linear Intercept and immunohistochemistry for vWF). Pulmonary gene expression was studied using RNA-seq on Illumina HiSeq 2500 platform. Data were mapped onto the UCSC mm10 using Hisat2 and quantified using feature counts and the GENCODE mouse reference. We compared transcriptomic changes for each genotype between room air and hyperoxia exposure (FDR<0.05, fold change exceeding 1.5). We evaluated enriched pathways using hypergeometric distribution (FDR<0.05).

**Results:** Significant differences in the pulmonary transcriptome were observed in numerous genes for each genotype at P5 (Fig 1 shows significant differences between XX and XXY mice). The overlap of gene expression between XXF and XXY was only ~18%, while it was ~40% between XXY and XYF mice. While differentially expressed genes in XXF mice enriched for cell cycle and proliferation pathways (*Mki67, Stmn1, Incenp, Plk1*), XXY mice showed enrichment for morphogenesis and development pathways (*Lif, Col11a1, Zfpm2, Myl2*). Differentially expressed genes in XXY mice mapped to muscle development pathways (*Myom1, Acta1, Tmod2, Des*), whereas XYF mice were enriched for immune response pathways (*Ccl2, Aoc3, Serpina3n, Adora1*). Significantly, lung alveolarization and vascular development were more severely impacted in XXY and XYF compared to XXF and XXY indicating that the Y chromosome may be mediating the deleterious effects of the male sex as a biological variable in BPD.

**Conclusion(s):** The Y chromosome may predispose to neonatal lung injury secondary to hyperoxia exposure in neonatal mice.

In the FCG model, *Sry* is a transgene on chromosome 3 and is not present on the Y chromosome, so that XX and XY mice can each have either testes (with *Sry*, XXY or XYM) or ovaries (without *Sry*, XXF or XYF; Figure 1

**IMAGE CAPTION:**
In the FCG model, *Sry* is a transgene on chromosome 3 and is not present on the Y chromosome, so that XX and XY mice can each have either testes (with *Sry*, XXY or XYM) or ovaries (without *Sry*, XXF or XYF; Figure 1