Vascular Remodeling and Reactivity in PPHN

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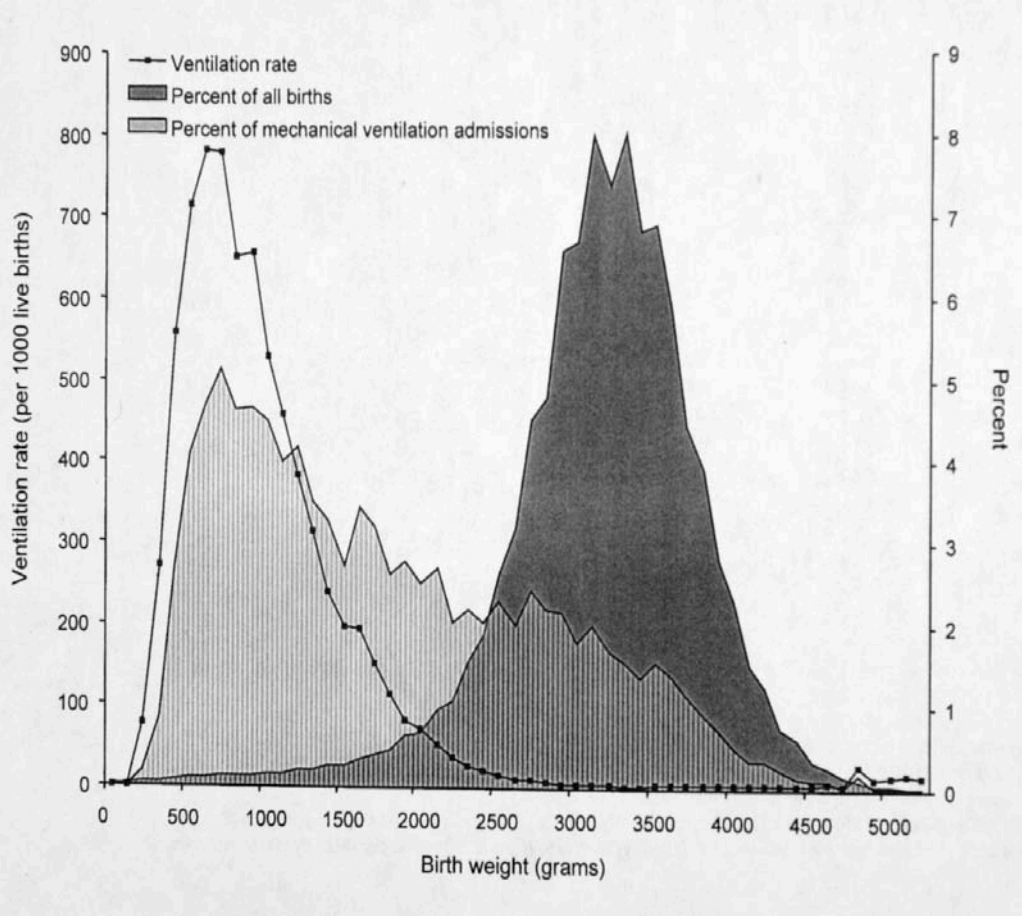
Neonatal Respiratory Failure in the United States

- 18/1000 live births
- 80,000 neonates/year (2% of all live births)
  - ARDS 40-150,000/year in the US
  - High cost: $4.4 billion
- Overall mortality 11.1%
- 8,500 deaths
  - 1/3-1/2 of all neonatal deaths in the US

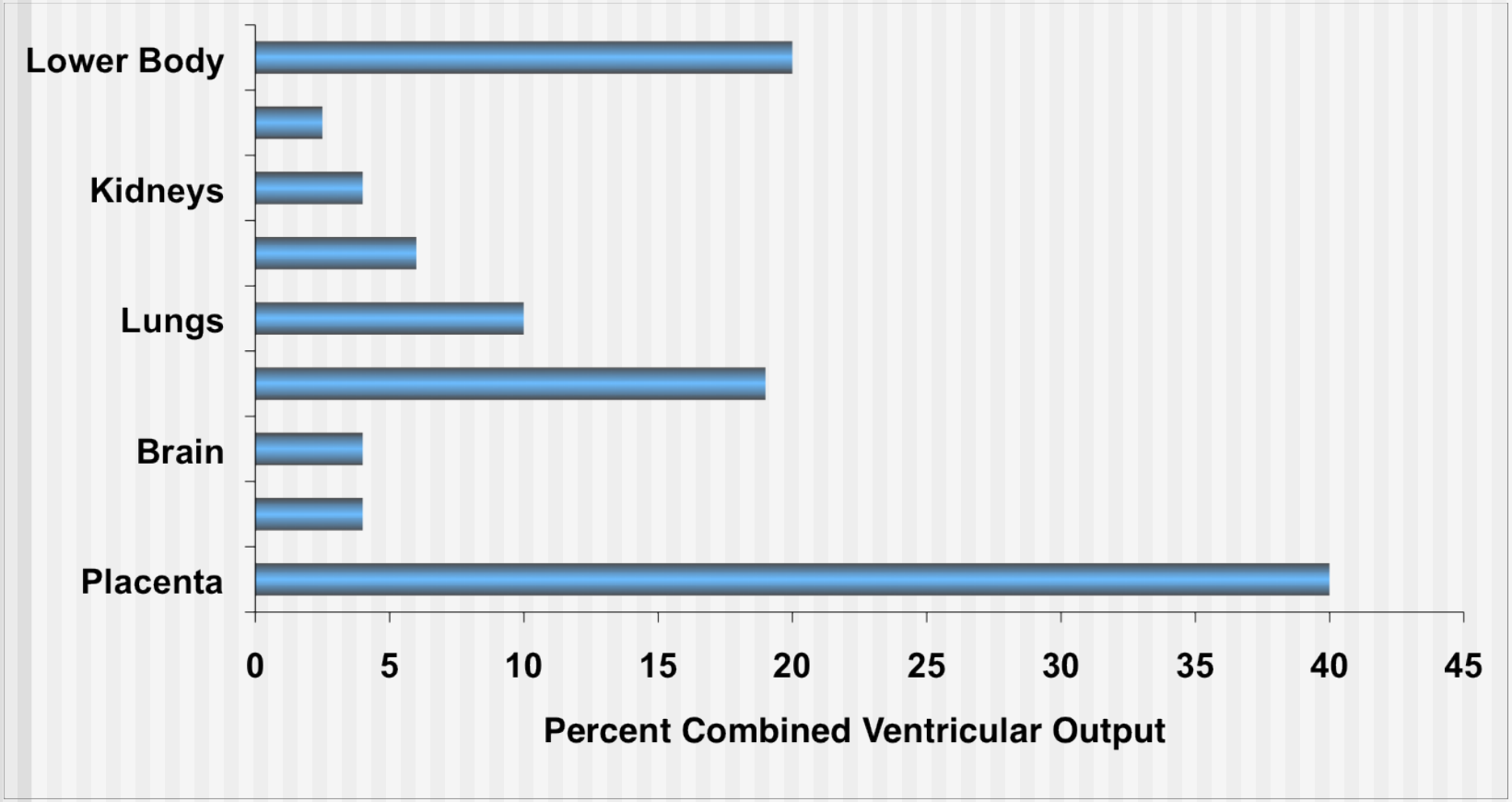
Neonatal Respiratory Failure in the United States



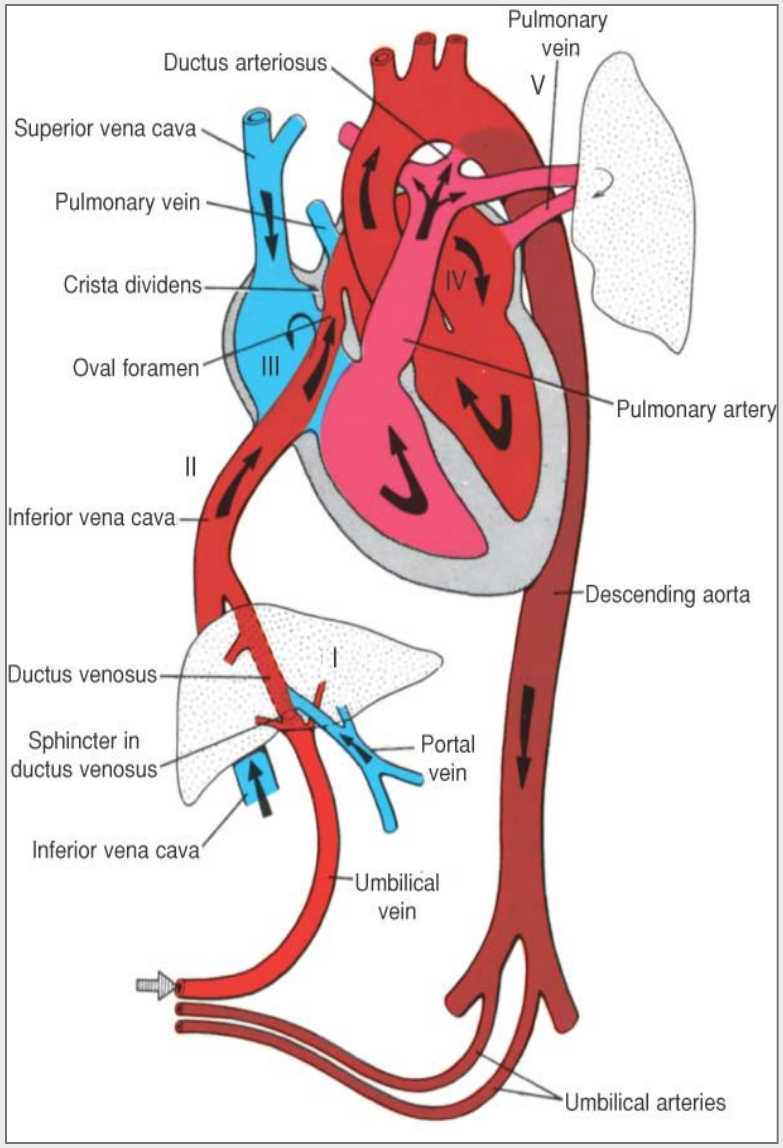
- Approximately 2% of all live births will require ventilator support
- ~ 1/3 of ventilated babies are term or near term

Angus et al; AJRCCM 2001;164: 1154.

Normal Fetal Circulation



Normal Fetal Circulation

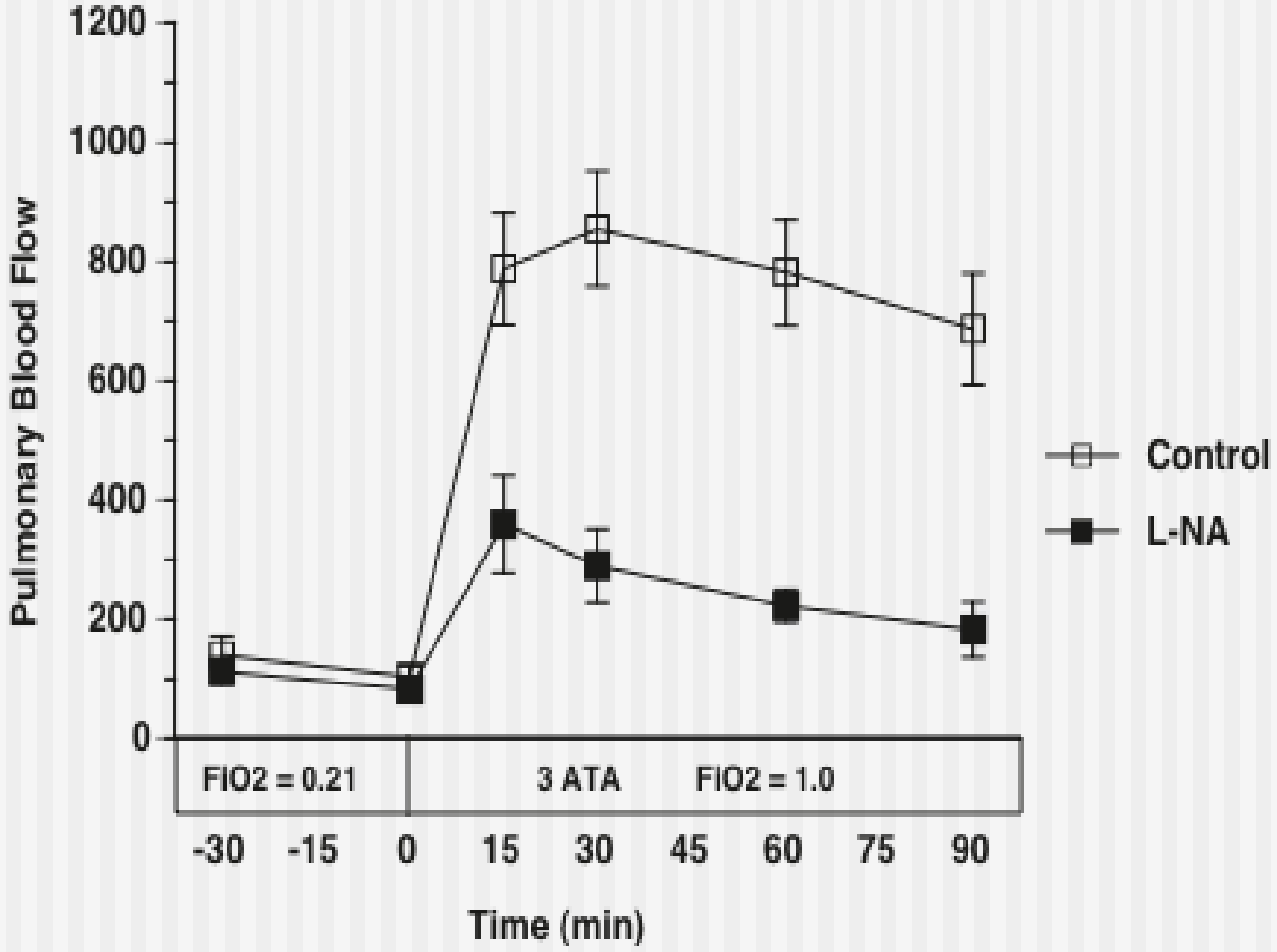


- Gas Exchange by placenta
- 30-50% of venous blood passes through ductus venosus to bypass blood directly to heart
- Right ventricular output maintained by R⇒L shunts across the foramen ovale and ductus arteriosus

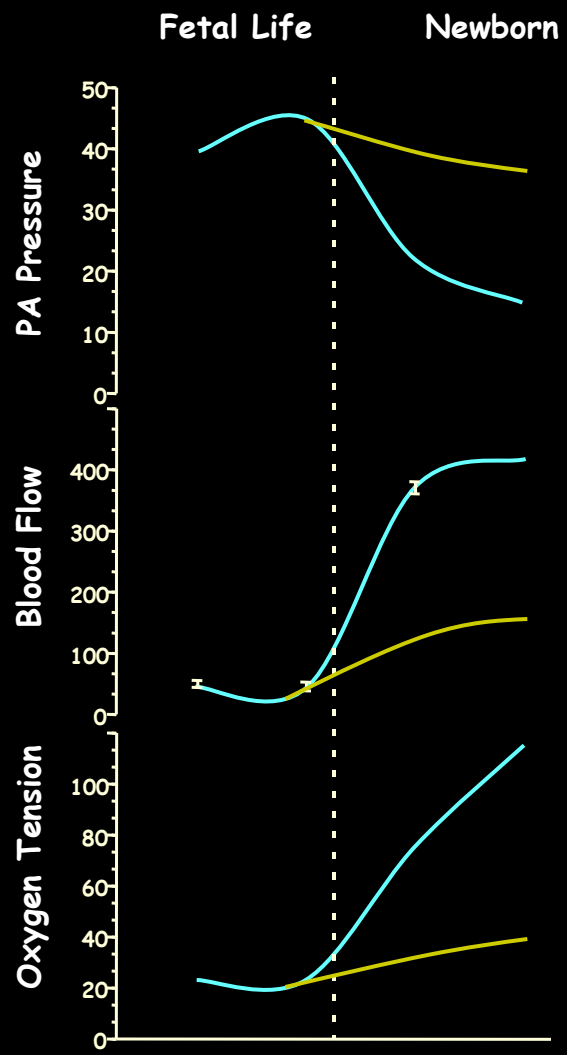
Regulation of Fetal PVR

- Low oxygen tension
- Decreased production of vasodilators
  - Nitric Oxide
  - Prostacyclin
  - Adenosine
- Increased production of vasoconstrictors
  - Endothelin-1
  - Leukotrienes

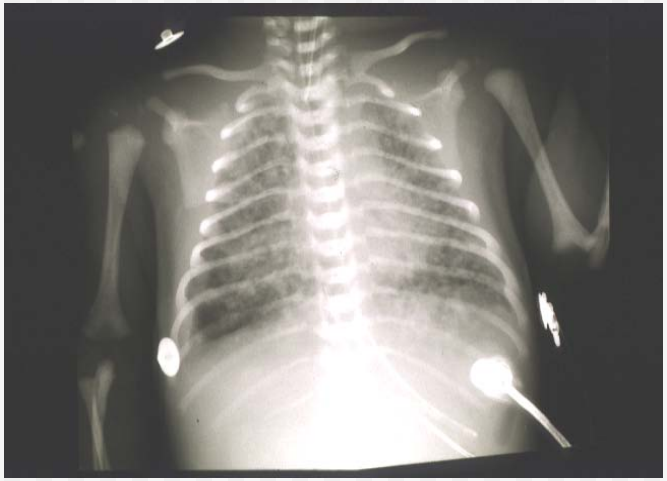
Fetal Pulmonary Vascular Response to Oxygen

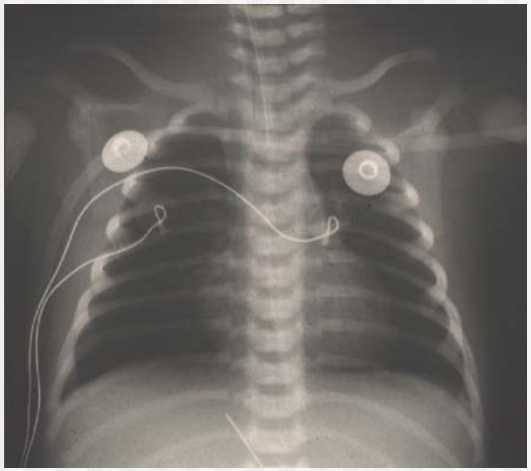


The Transitional Pulmonary Circulation



Persistent Pulmonary Hypertension in the US



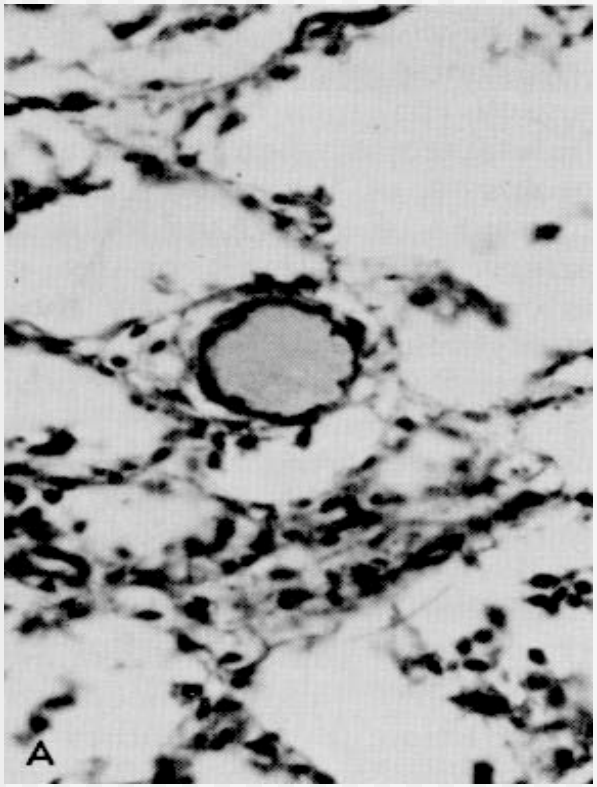


- 1.9/1000 infants (range 0.5-6/1000)
- ~10% of all neonates with respiratory failure
- No genetic factors identified
- High morbidity, mortality
- At risk for long-term sequelae

Pathophysiology of Newborn Pulmonary Hypertension

- Maladaptation: vasoconstriction of a normal vasculature
- The abnormally remodeled vasculature
- The hypoplastic vasculature
- Postnatal remodeling

Antenatal Remodeling Associated With PPHN

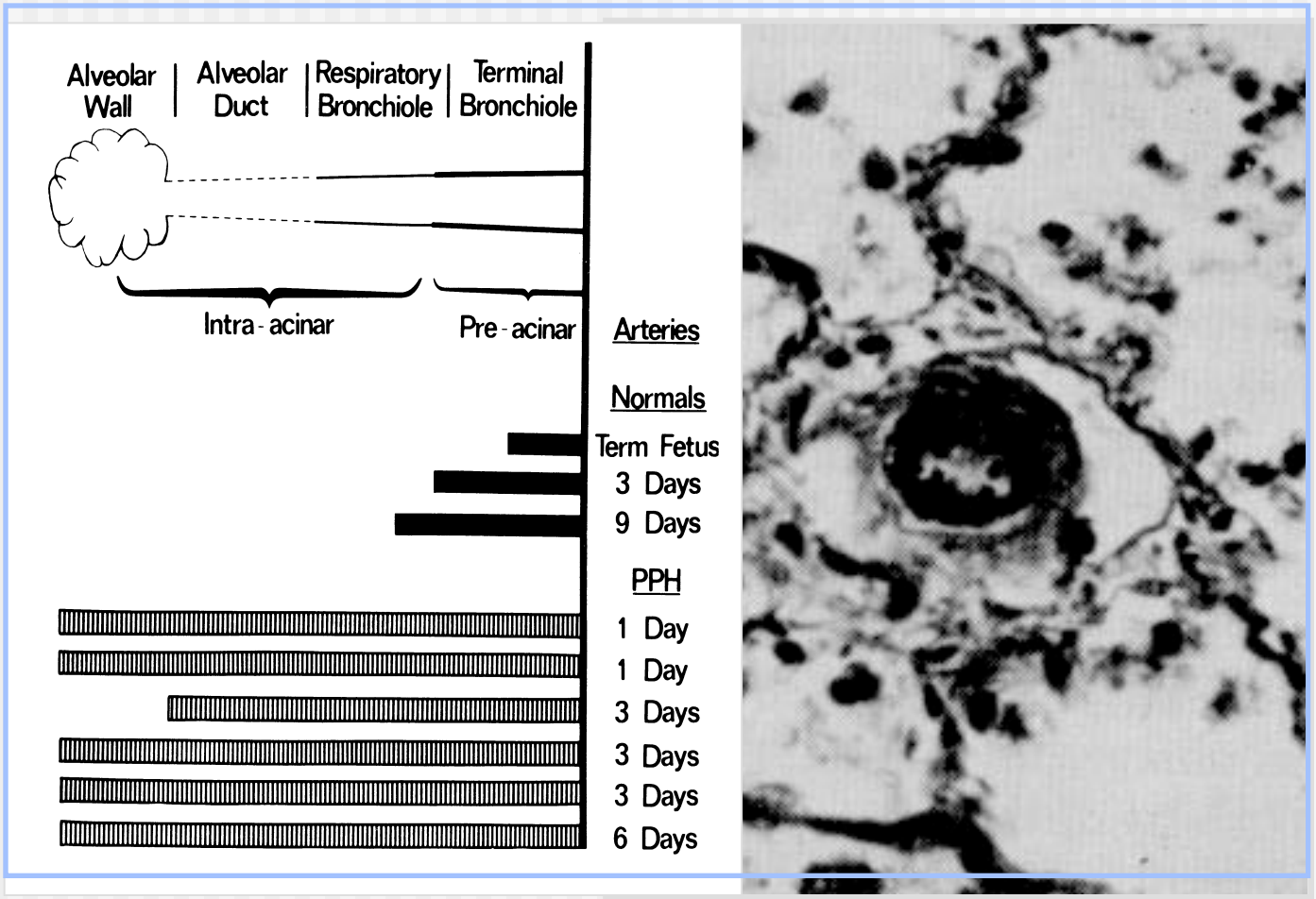


Normal

PPHN

Murphy JD et al. *J Pediatr*. 1981;98:962-967.

Histological Changes Associated With PPHN

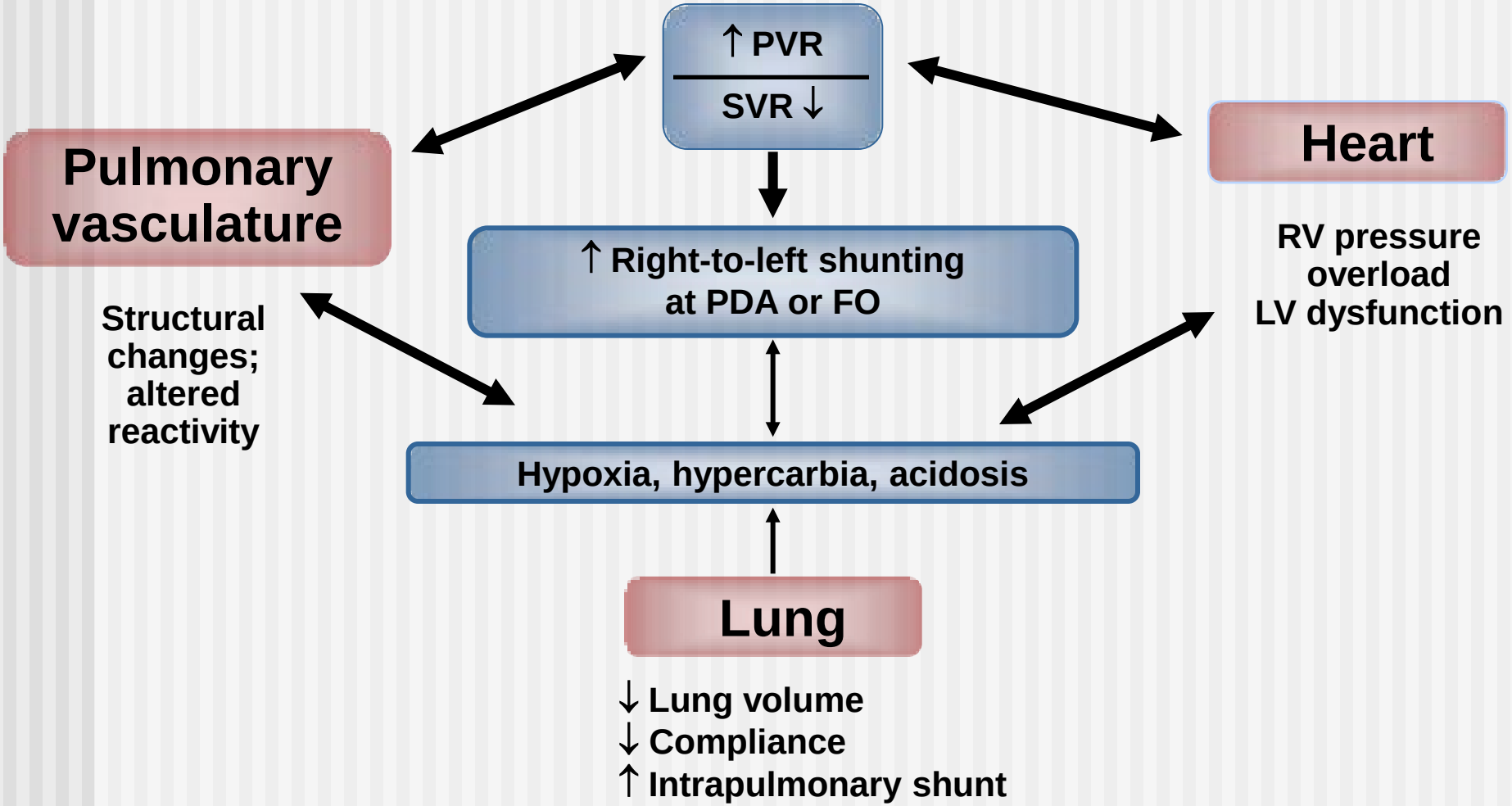


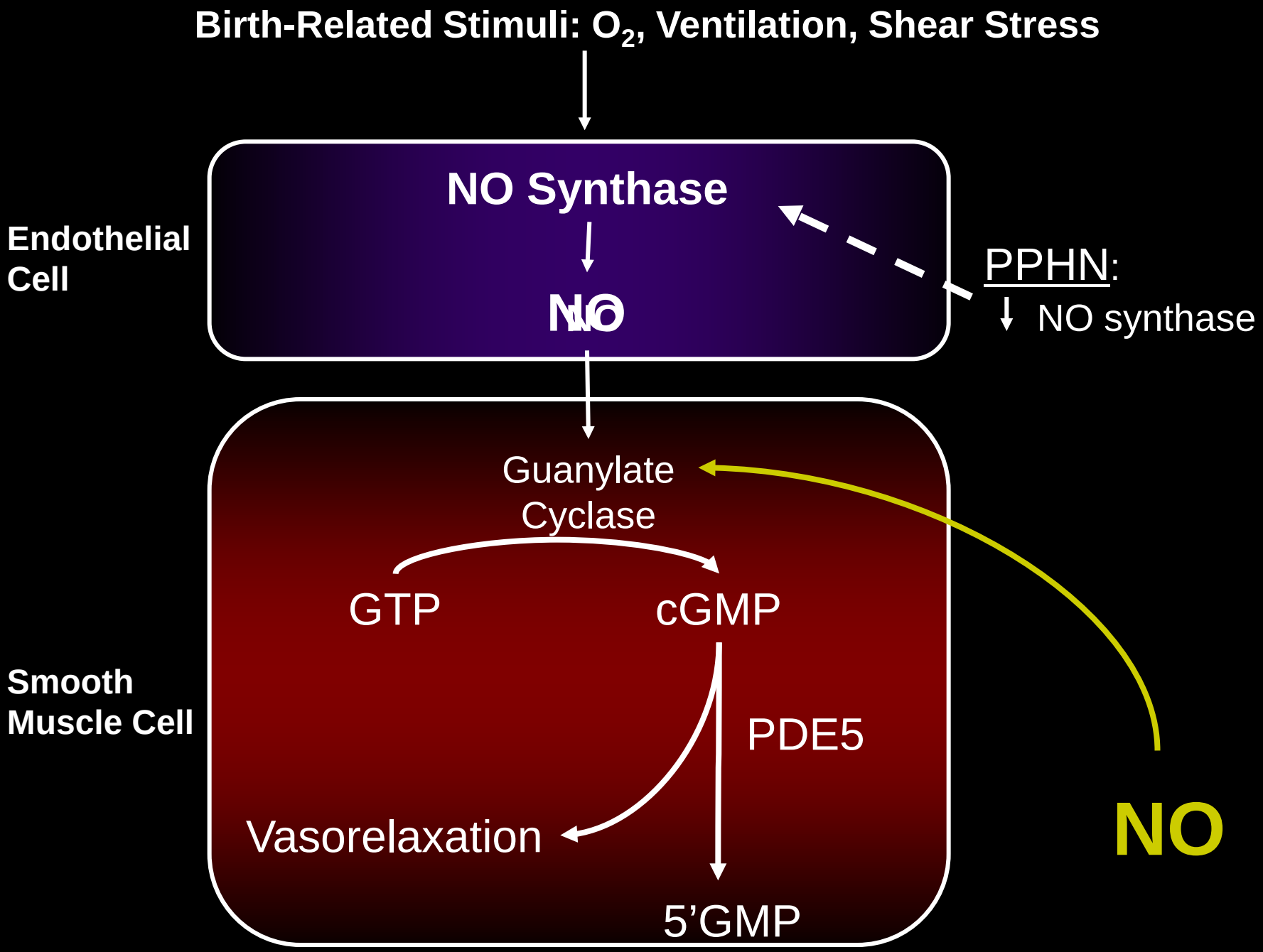
Murphy JD et al. *J Pediatr*. 1981;98:962-967.

PPHN Therapies

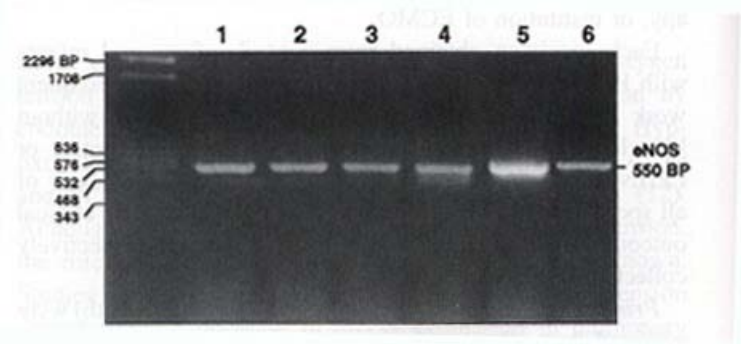
- Optimal lung recruitment
  - High frequency ventilation
  - Surfactant
- Cardiovascular support
- Pulmonary Vasodilation:
  - Oxygen
  - Inhaled Nitric Oxide
- ECMO

Cardiopulmonary Interactions in PPHN

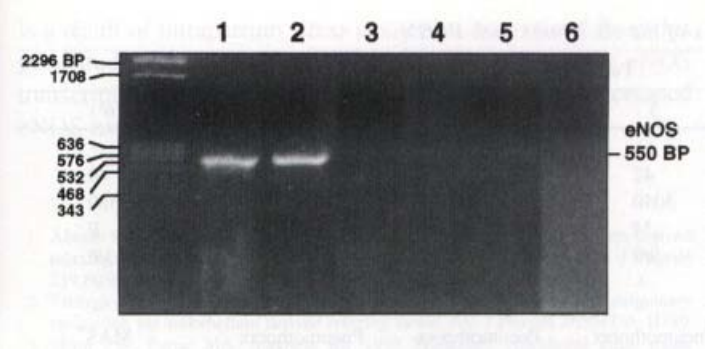



Birth-Related Stimuli: O_2, Ventilation, Shear Stress
Endothelial Cell
NO Synthase
NO
PPHN:
NO synthase
Smooth Muscle Cell
Guanylate Cyclase
GTP
cGMP
PDE5
Vasorelaxation
5'GMP
NO

eNOS Expression is Decreased in Infants that Develop PPHN



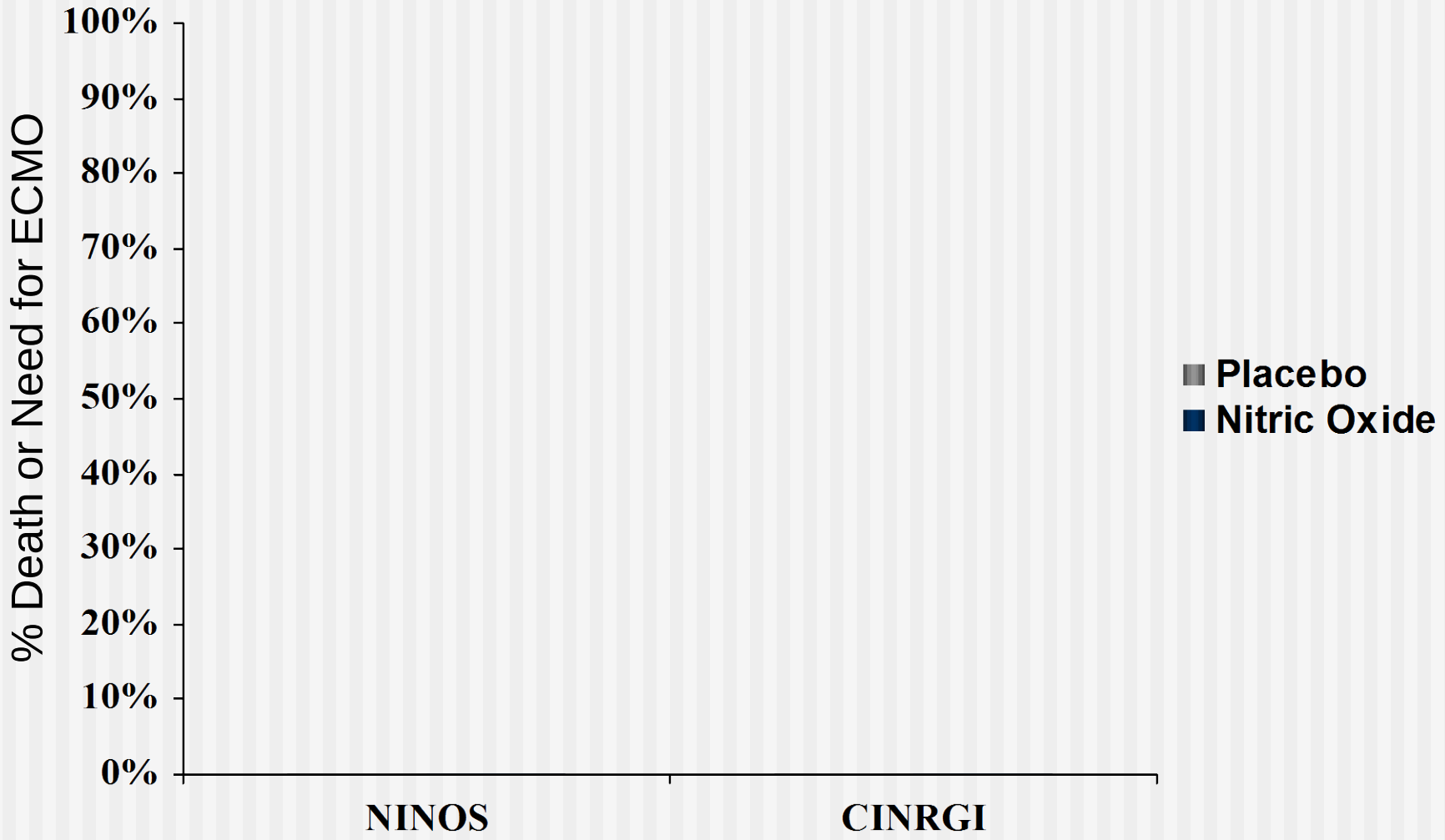
Control



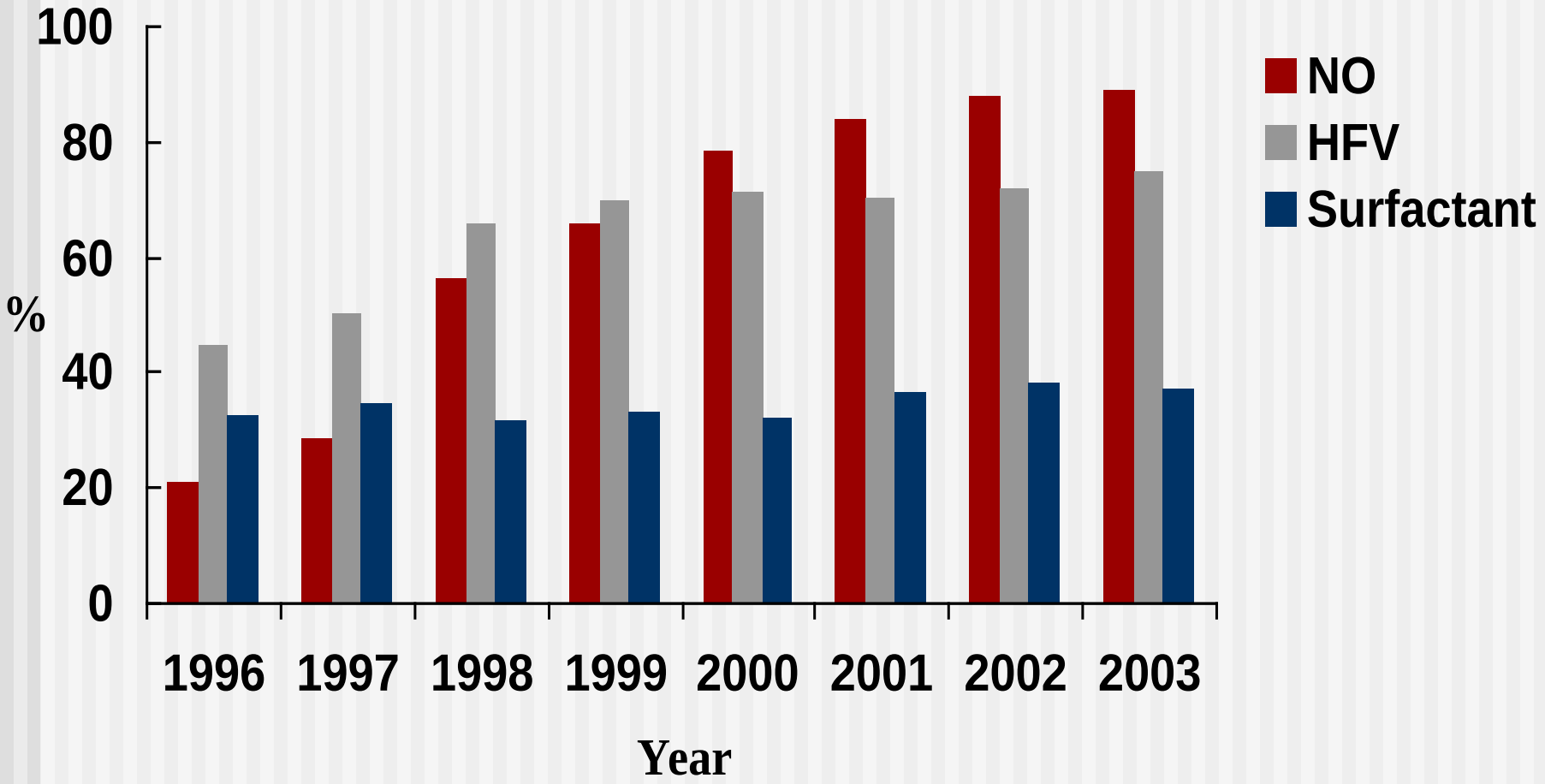
PPHN

Villanueva et al, Pediatr Res 44: 338, 1998

Effect of iNO on Incidence of Death or ECMO



Therapies Prior to ECMO



Fliman et al. *J Pediatr*. 2006

Inhaled NO and PPHN Outcome

			% ECMO		% Mortality	
	n	OI	Control	iNO	Control	iNO
NINOS	235	44	55	39*	17	14
Roberts	58	44.4	71	40*	7	7
Clark	248	39	65	48*	8	7
Davidson	155	24.7	34	22	2	8
Konduri	299	19.2	12	10	9	7

Neurodevelopmental Outcomes: Early NO Trial

	iNO n=121	Control n=113
NDI	27.9%	24.6%
MDI<70	25.2%	22.9%
Hearing Impairment	23%	24%

Konduri et al, J Pediatr 2007

Mechanisms for Poor NO Response

- Poor Lung Inflation
- Anatomic Lung Disease
- Anatomic Heart Disease
- Right Ventricular Failure
- Left Ventricular Failure
- Alterations in Downstream Signaling

Emerging Insights

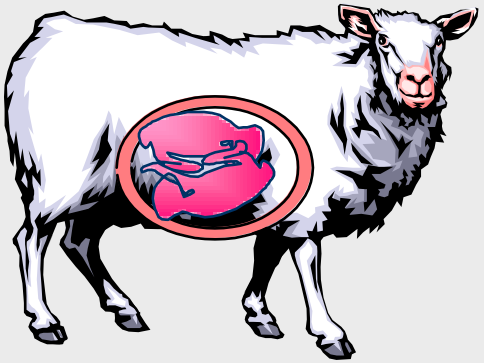
Vascular Abnormalities

- NOS Dysfunction
- sGC Dysfunction
- Abnormal cAMP signaling
- Increased phospho-diesterase activity
- Increased rho-kinase activity
- Oxidant stress

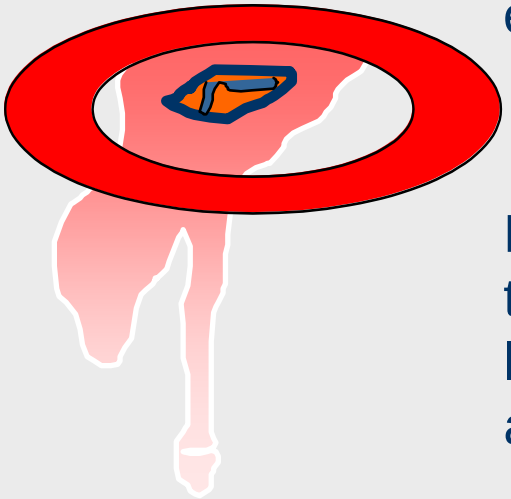
New therapies

- NOS Enhancers
- Direct sGC activators
- Prostacyclin analogues, PDE inhibitors
- Phosphodiesterase Inhibitors
- Rho-kinase inhibitors
- Antioxidants

Ductal Ligation Lamb Model

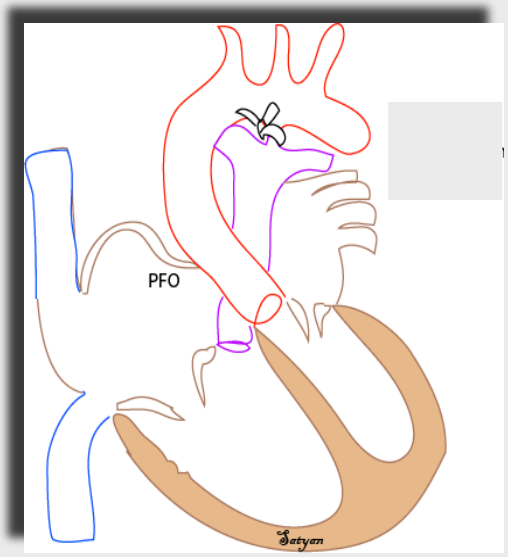


126-128 day gestation pregnant ewe



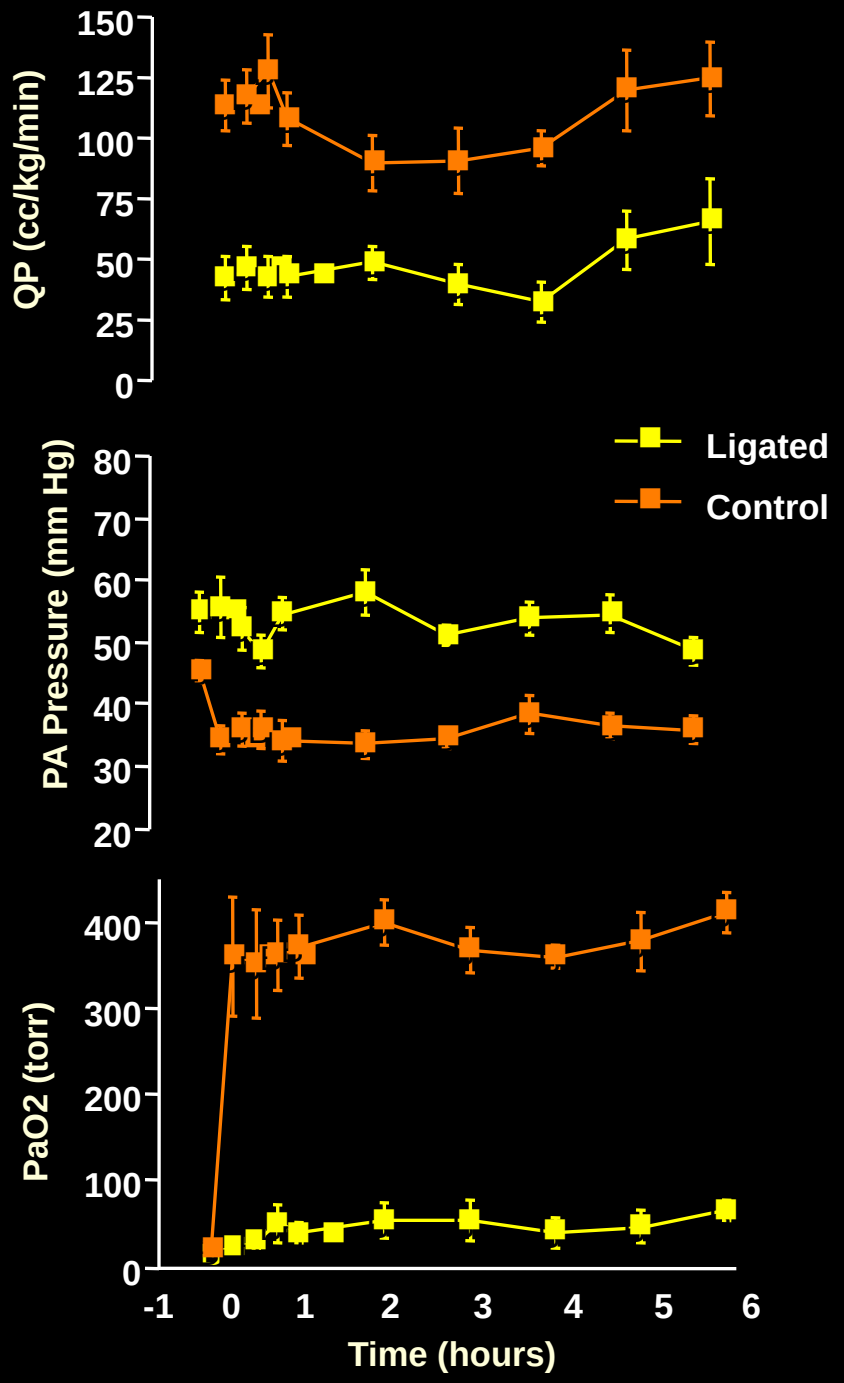
Fetal exteriorization

Lateral thoracotomy and ligation of ductus arteriosus



Induces an acute increase in PBF and development of PPHN

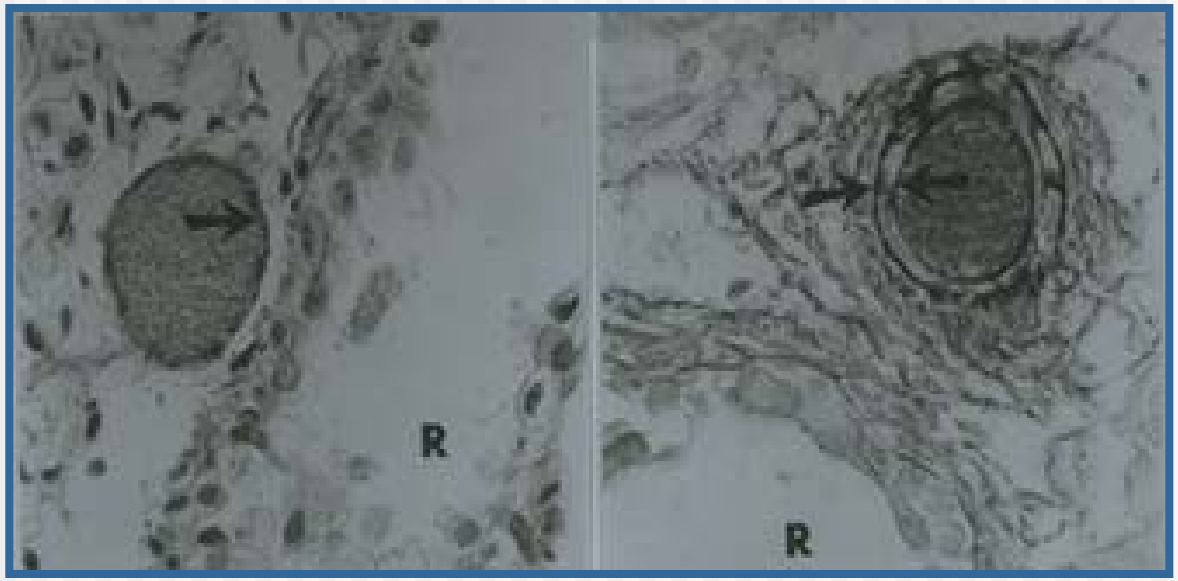
Cesarean delivery 8-13 days after ligation



Ductal Ligation Model of PPHN:

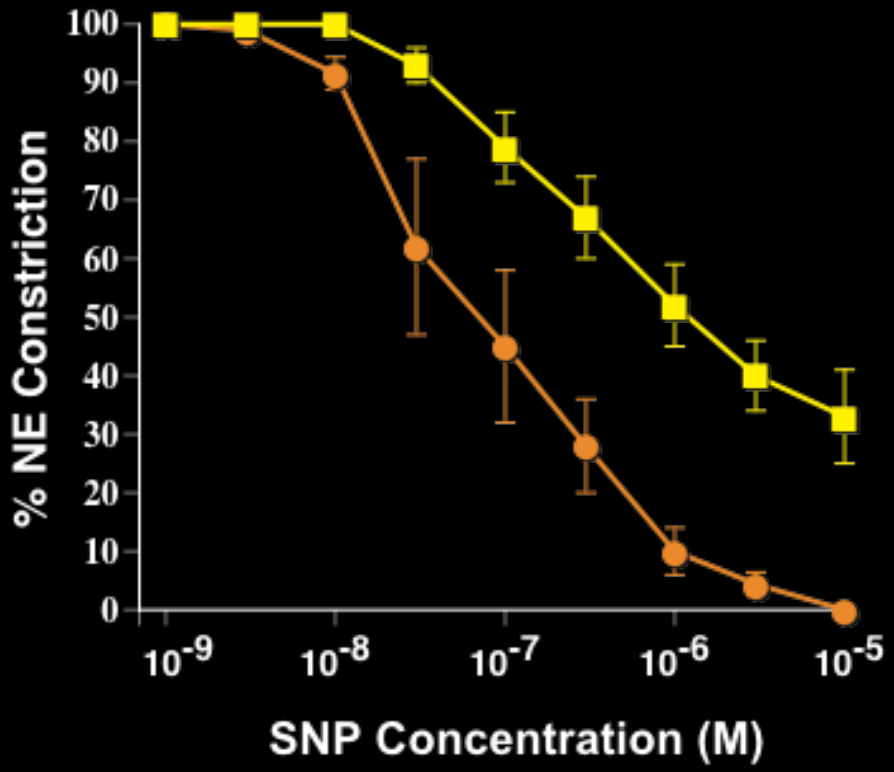
- Severe pulmonary Hypertension
- No parenchymal lung disease
- Hypoxemia unresponsive to O_2 and ventilation
- Mortality >50% in first 24 hours of life without specific therapy

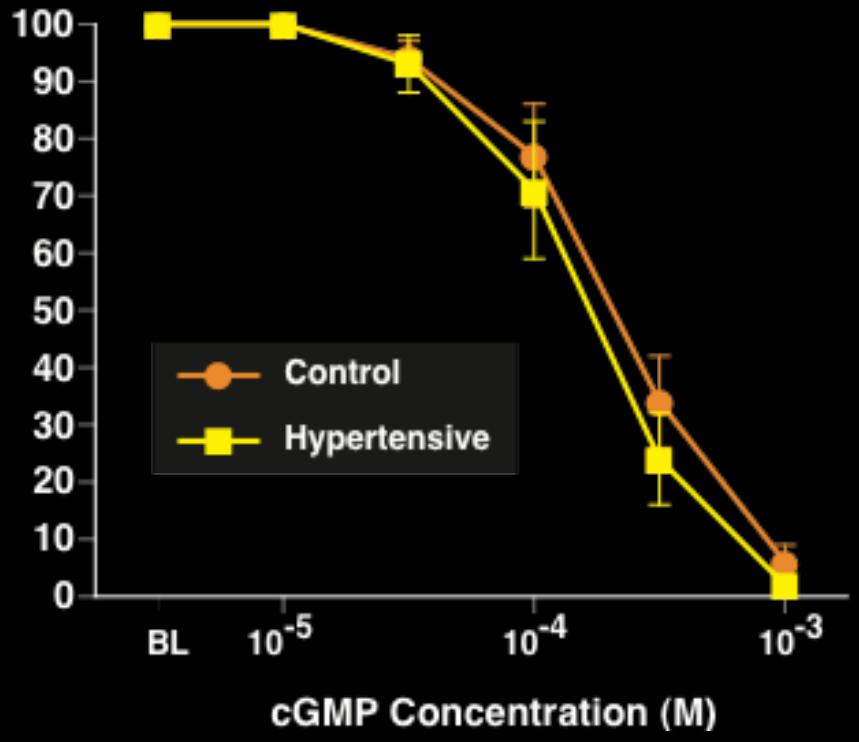
Remodeled Pulmonary Vasculature in PPHN Lambs



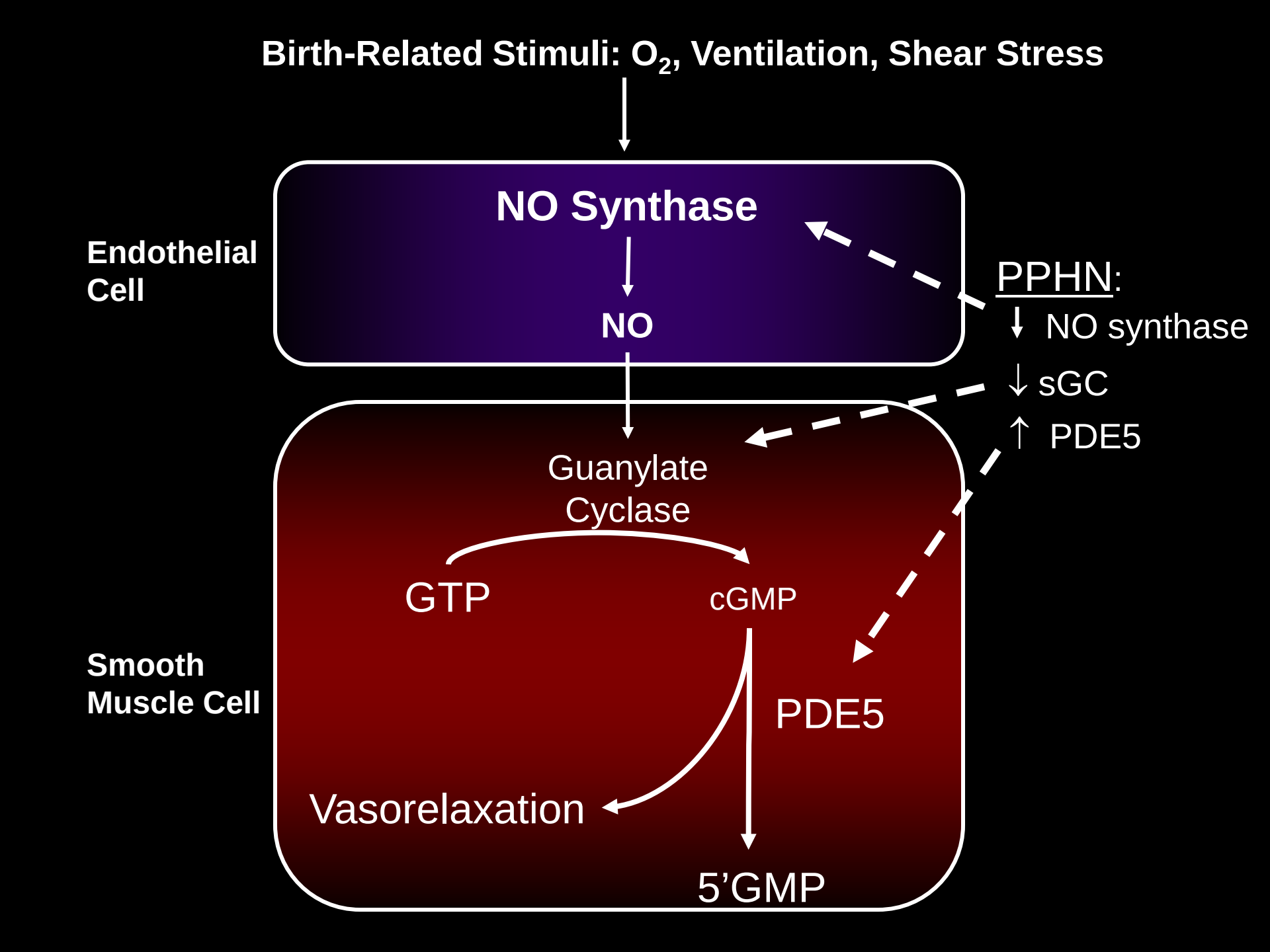
Control **Ductal Ligation**

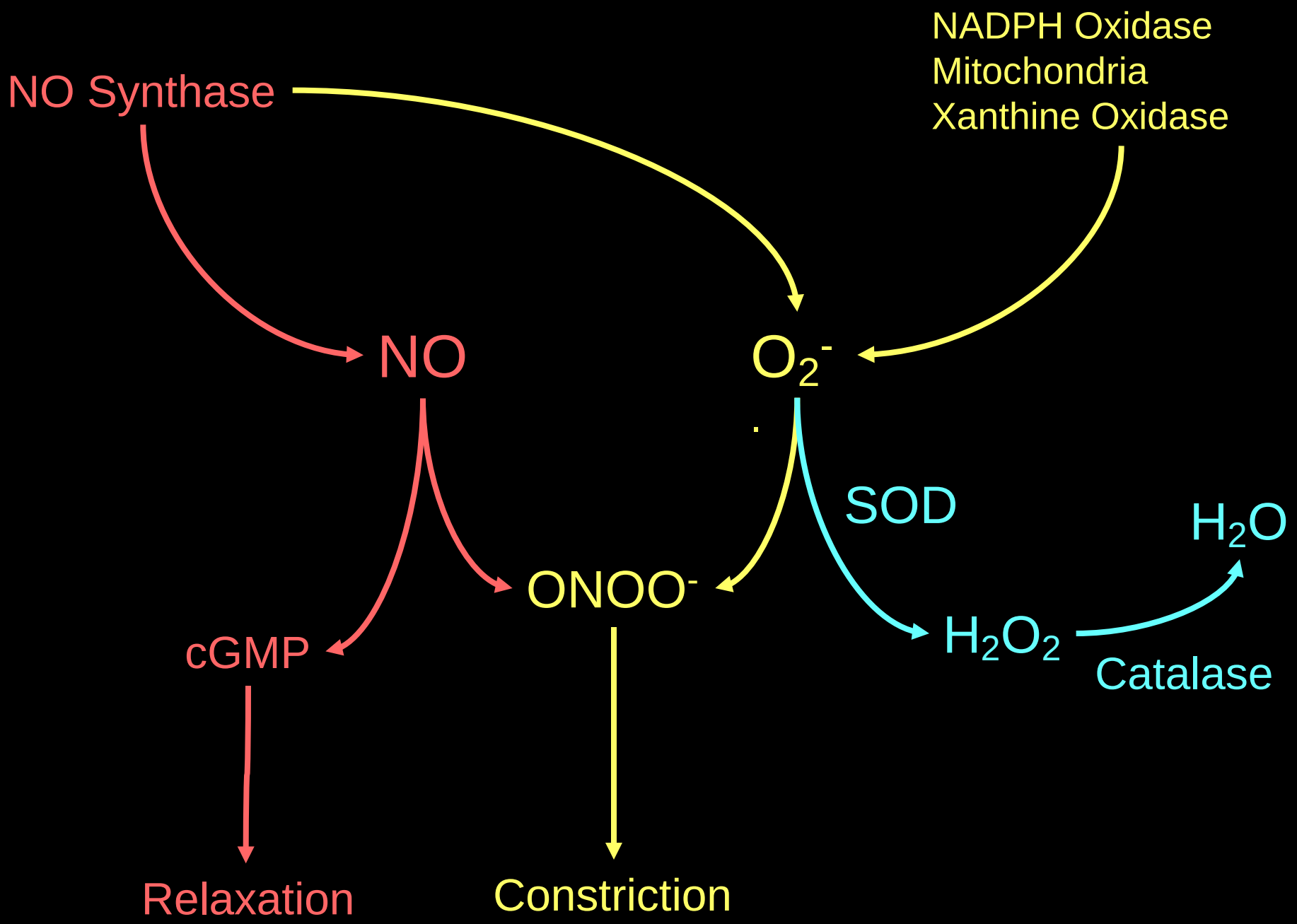
Pulmonary Artery Relaxations in PPHN



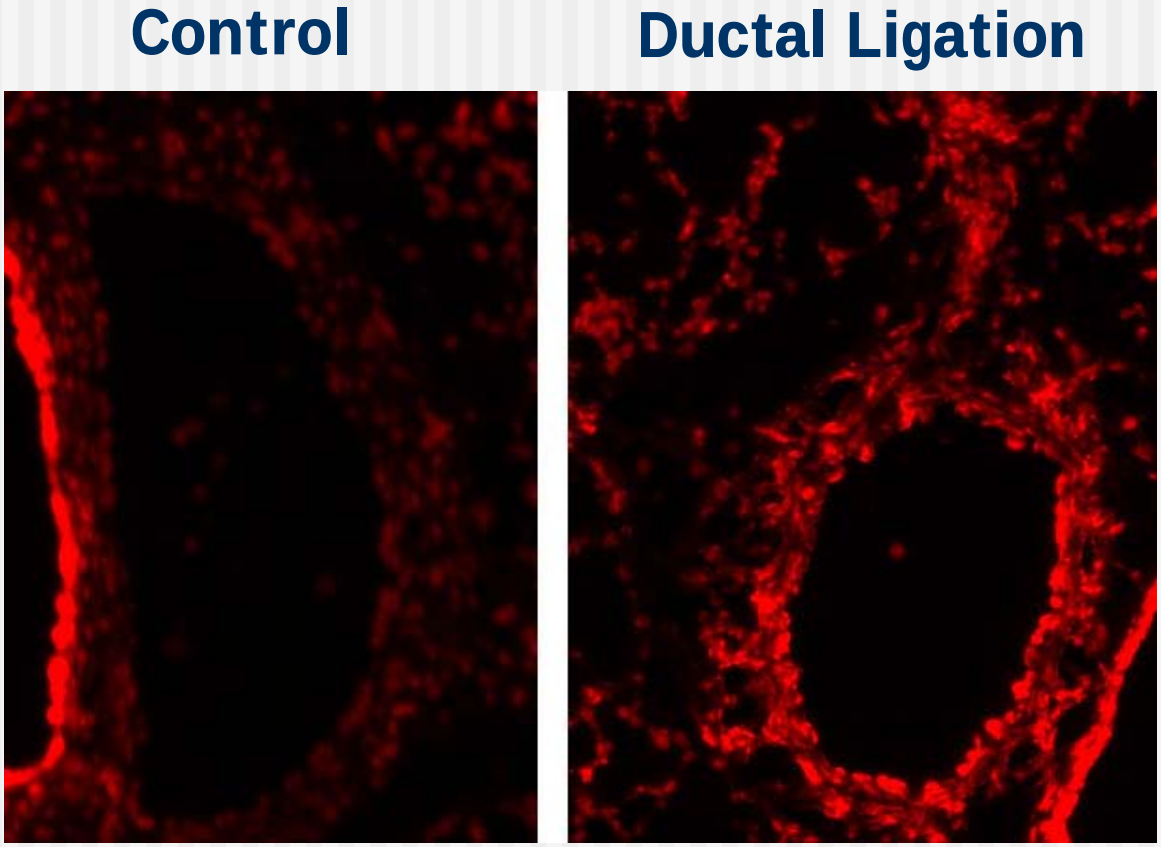


Steinhorn et al; Am J Physiol 1994


Birth-Related Stimuli: O_2, Ventilation, Shear Stress
NO Synthase
NO
Endothelial Cell
PPHN:
↓ NO synthase
↓ sGC
↑ PDE5
Guanylate Cyclase
GTP
cGMP
PDE5
Vasorelaxation
5'GMP
Smooth Muscle Cell

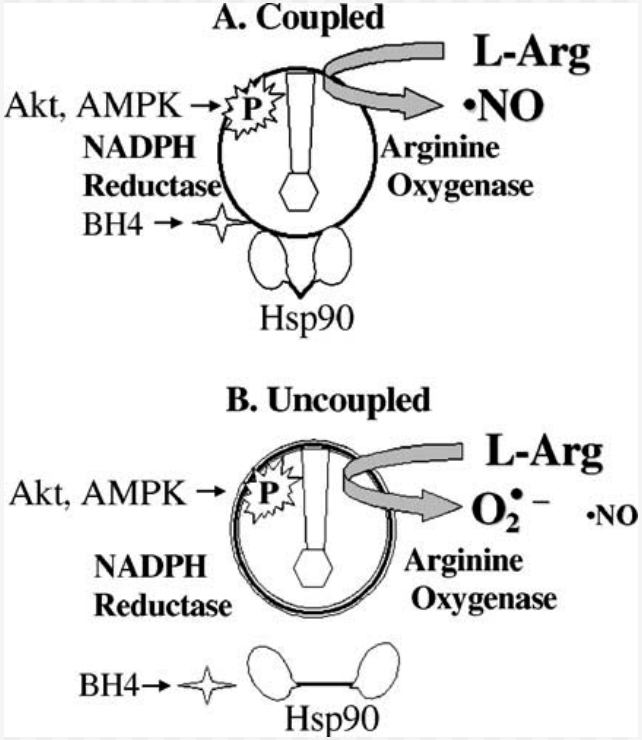

NADPH Oxidase
Mitochondria
Xanthine Oxidase
NO Synthase
NO
O_2^{-}
.
SOD
H_2O
$ONOO^-$
cGMP
H_2O_2
Catalase
Relaxation
Constriction

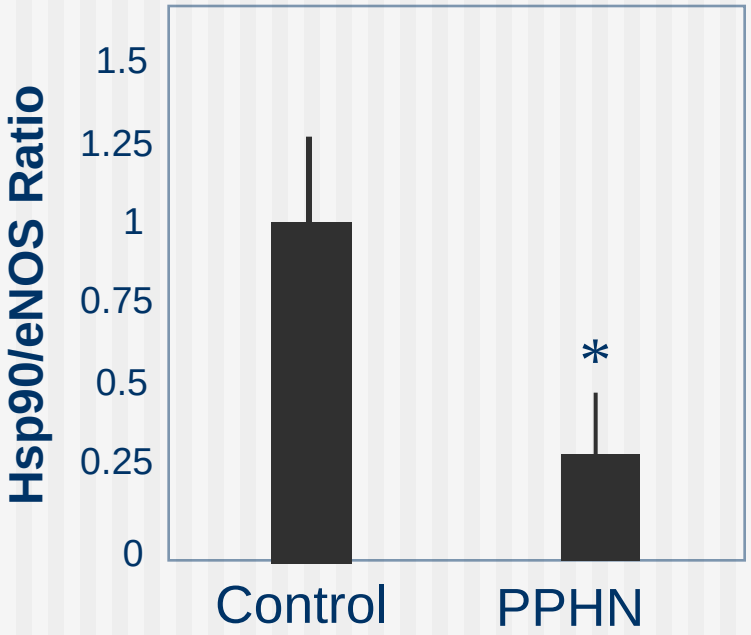
Vascular Superoxide Production is Increased in PPHN Lambs



Brennan et al; Circ Res 2003

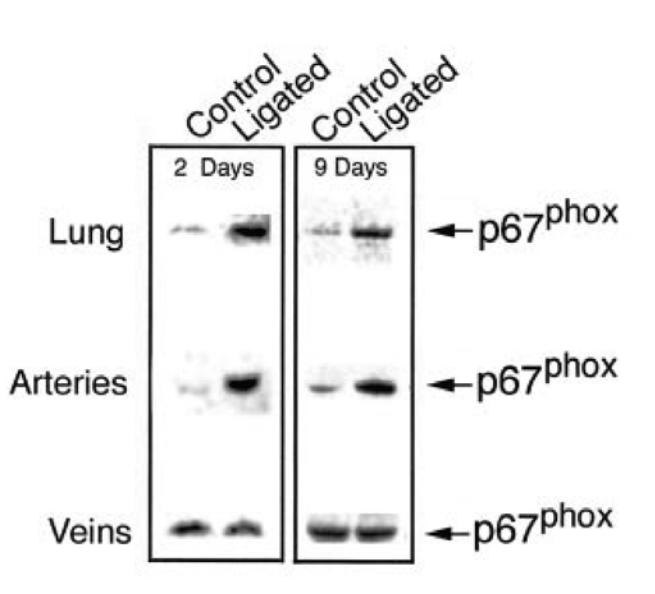
NOS: A Potential Source of Superoxide Production

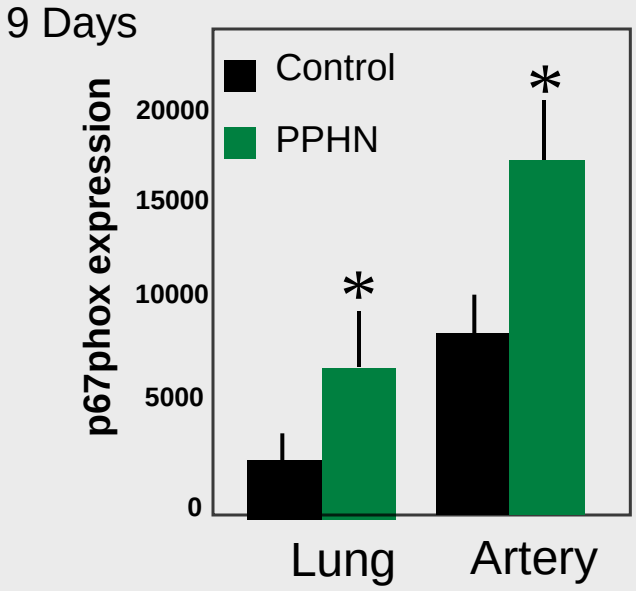


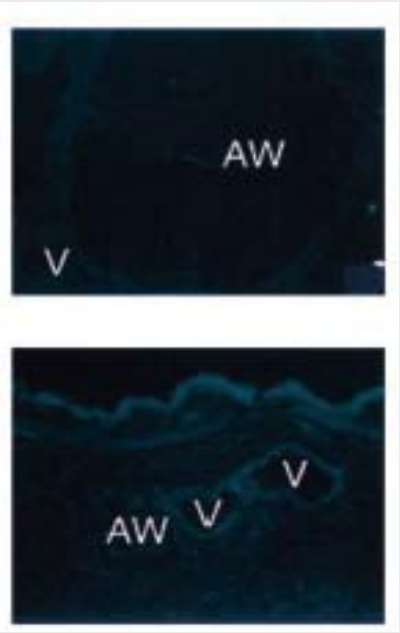


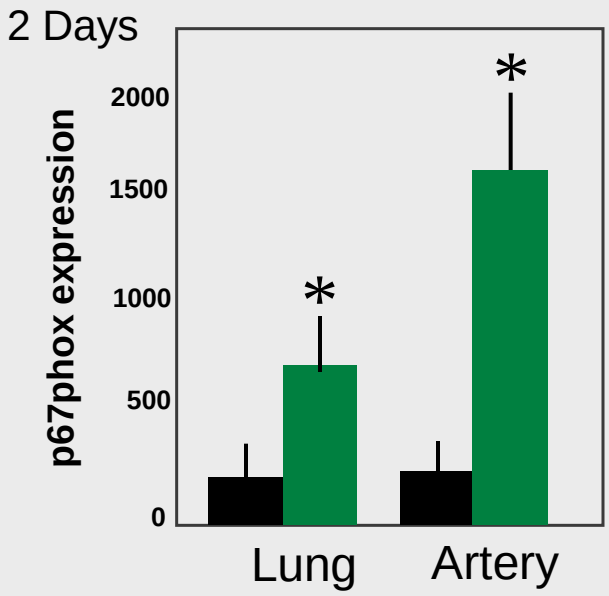
Konduri et al; Am J Physiol 2003

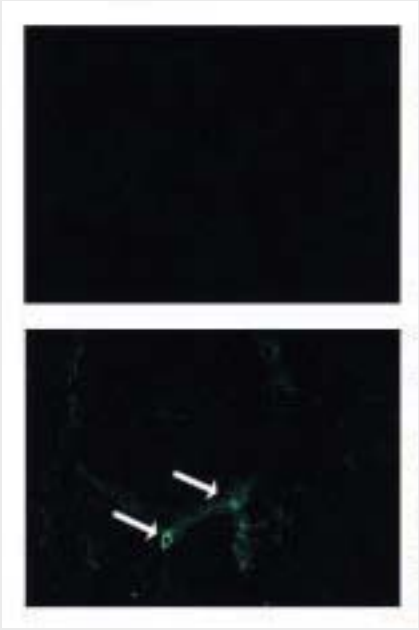
p67phox Expression is Increased in PPHN Lambs





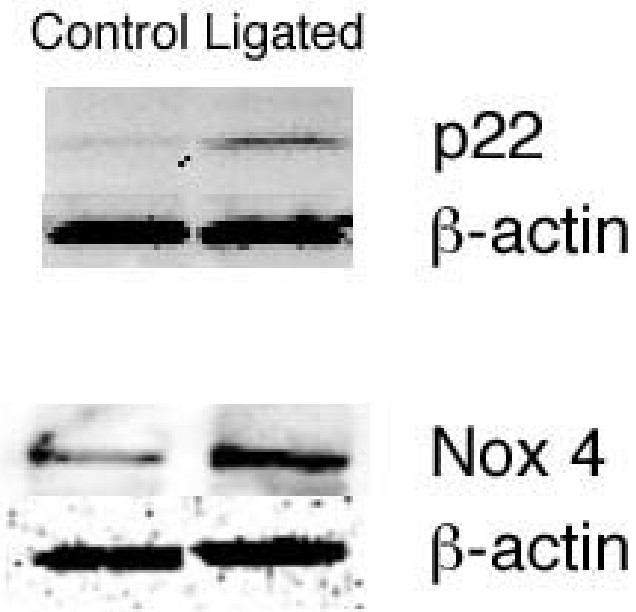


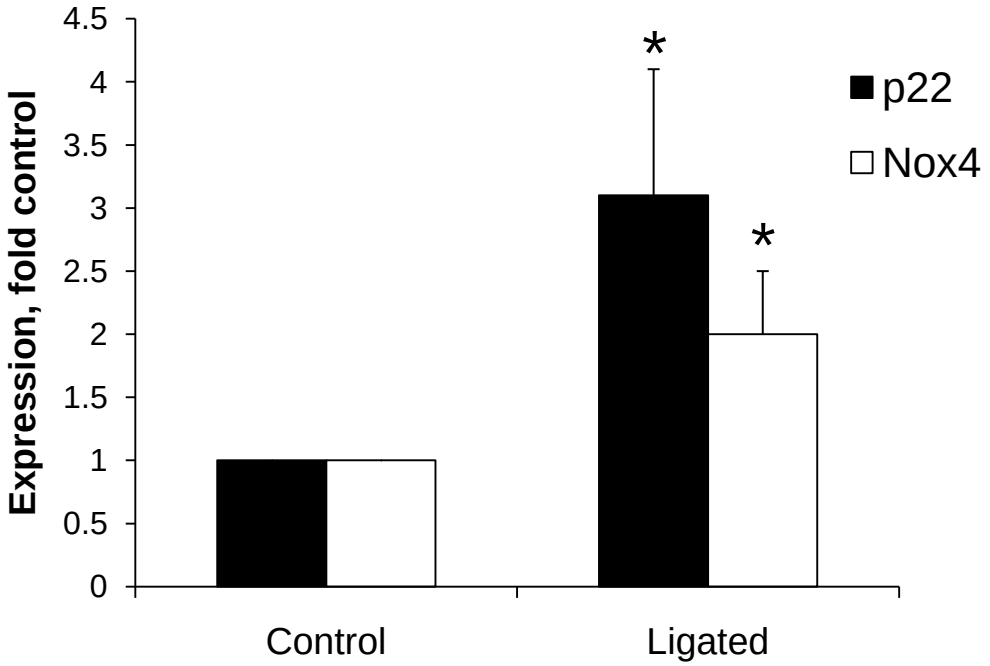




Brennan et al; Circ Res 2003

p22 and Nox4 Levels are Increased in PPHN PASMC

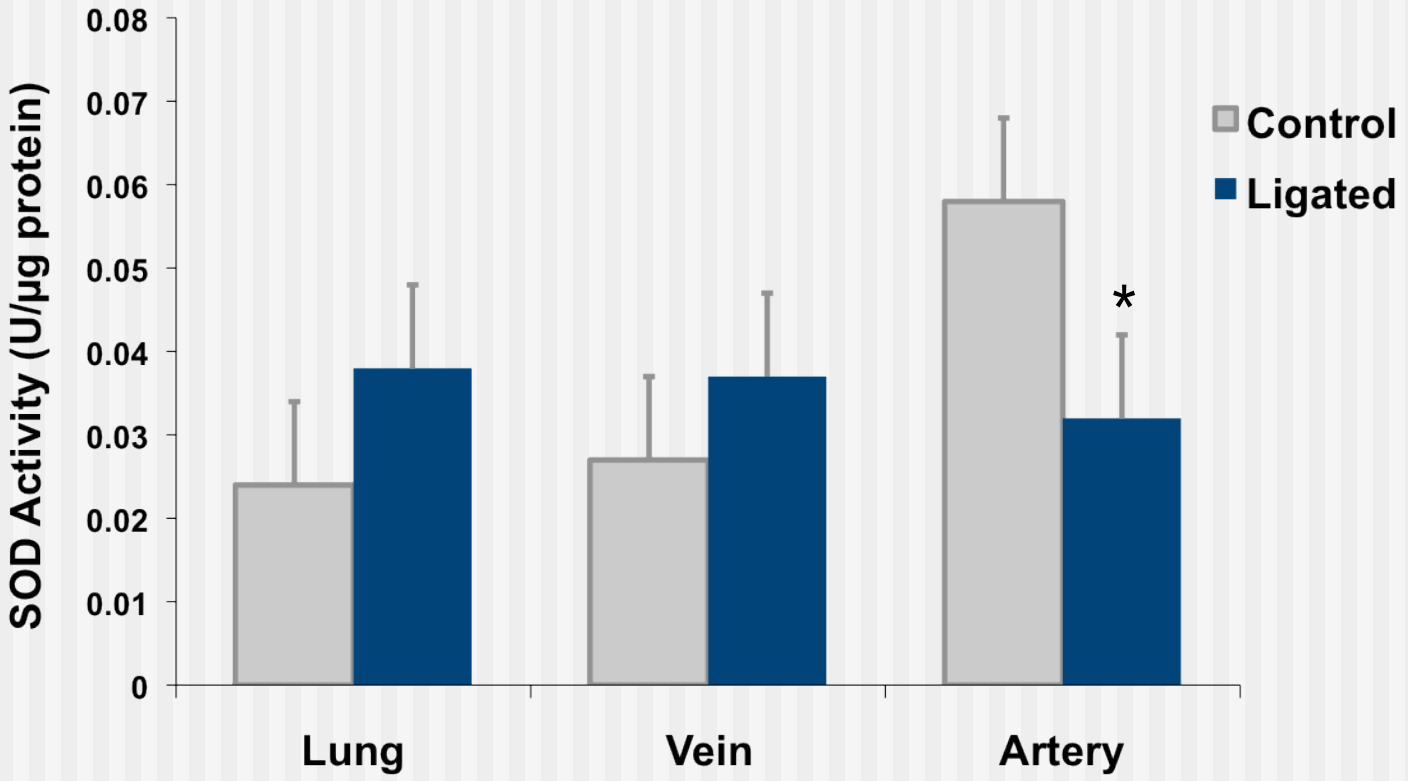




p22 IP Nox 4 WB

Wedgwood et al; PAS 2010

Total SOD Activity in PPHN Lambs



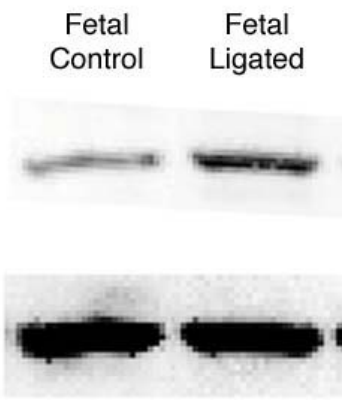
Brennan et al; Circ Res 2003

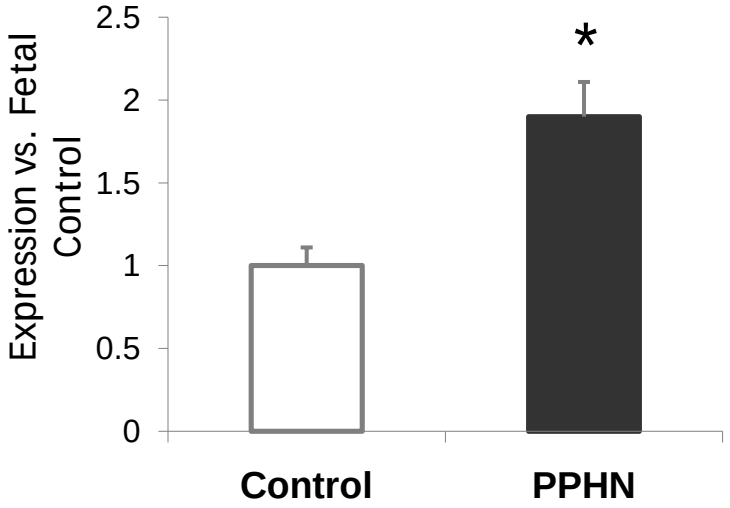
SOD Isoforms

- SOD1 – CuZn SOD, cytosolic
- SOD2 – MnSOD, mitochondrial
- SOD3 – ecSOD, extracellular

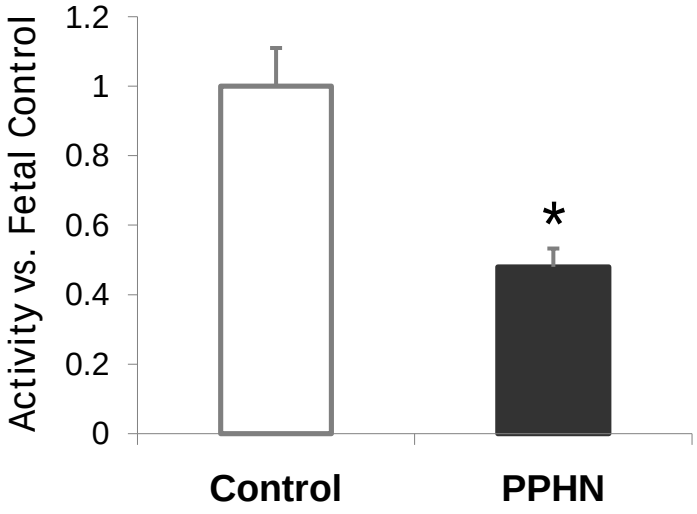
ecSOD Activity in PPHN Lambs

Expression

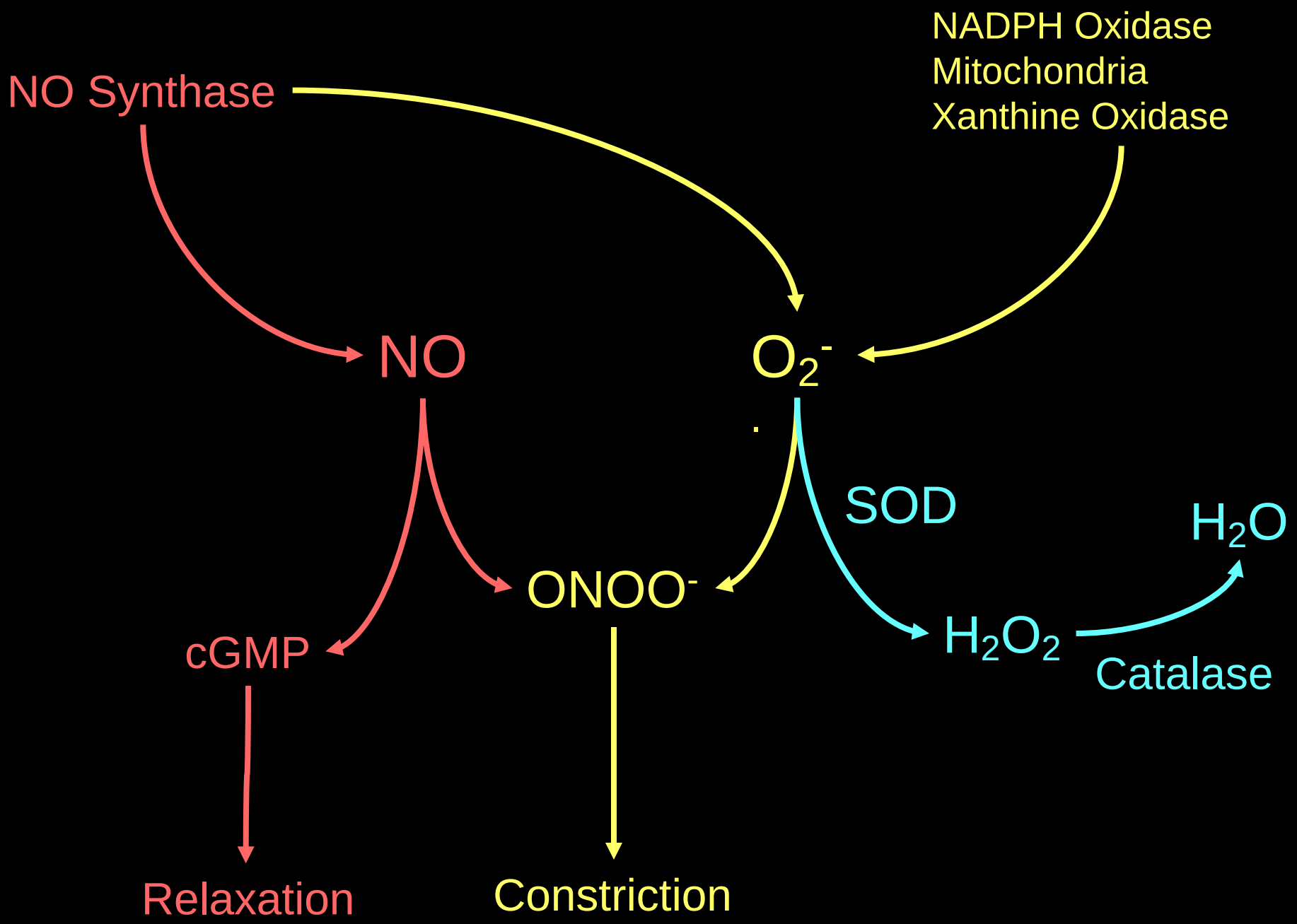




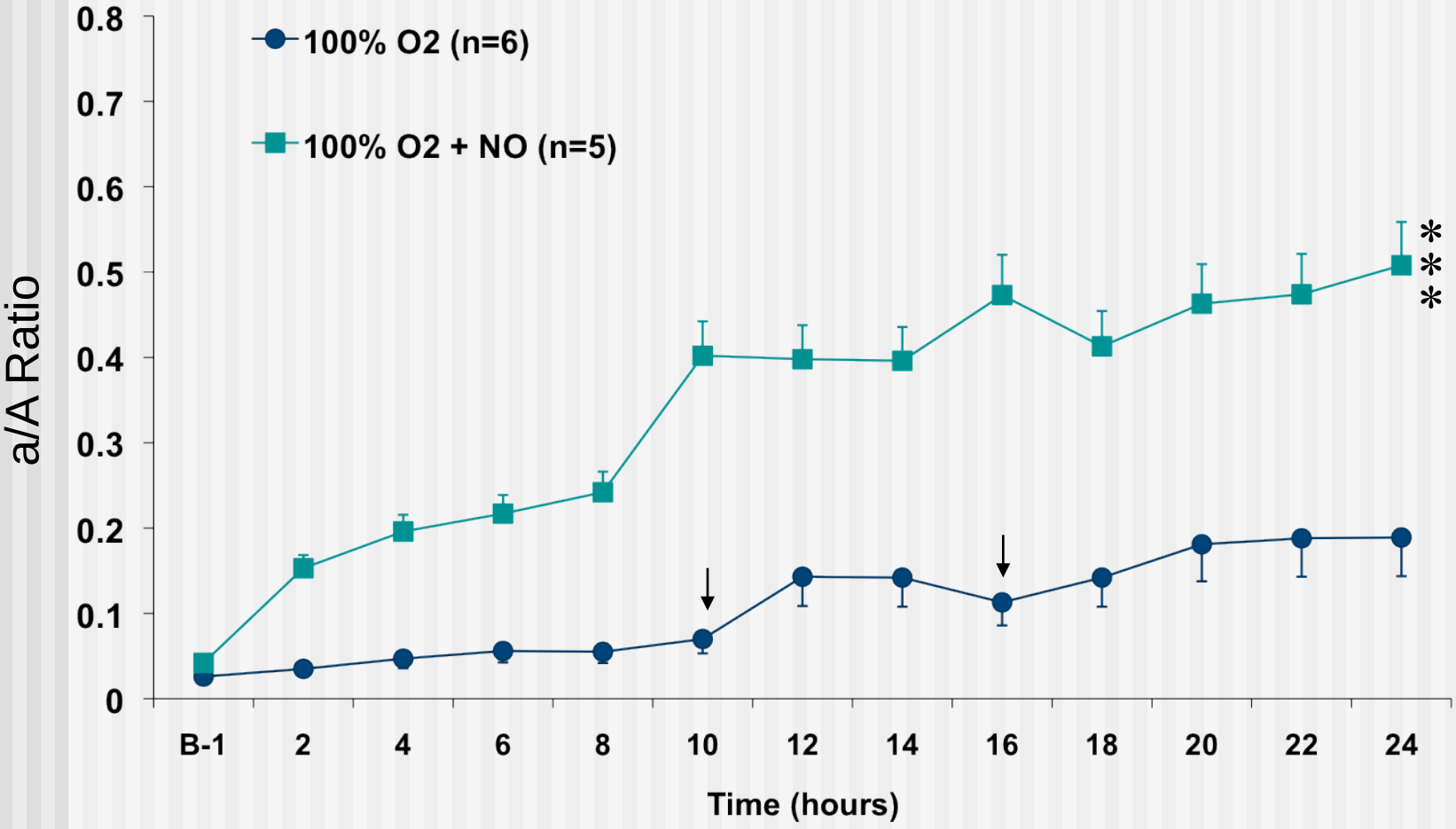
Activity



Wedgwood et al, PAS 2010

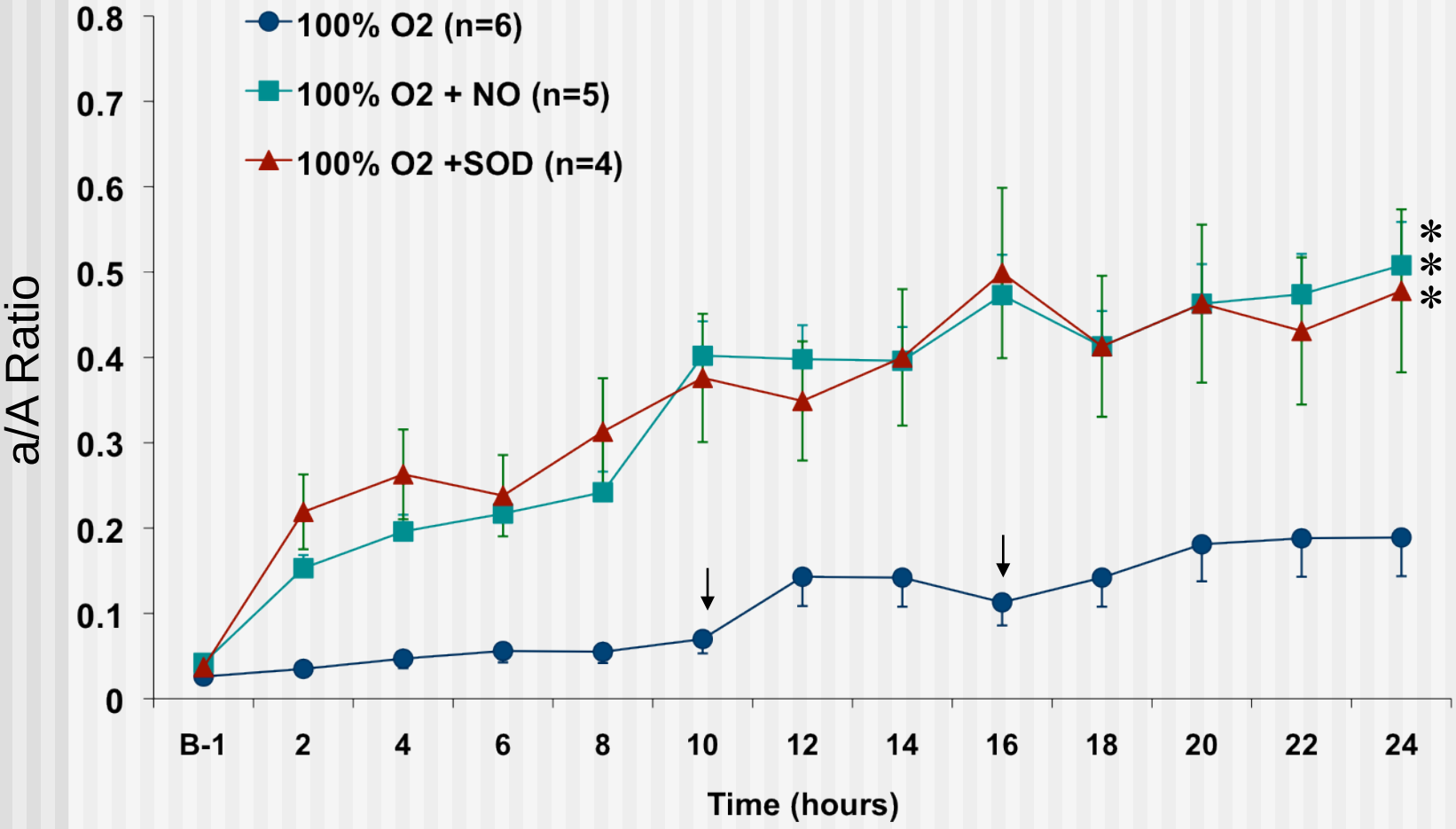

NADPH Oxidase
Mitochondria
Xanthine Oxidase
NO Synthase
NO
O_2^{-}
.
SOD
H_2O
$ONOO^-$
cGMP
H_2O_2
Catalase
Relaxation
Constriction

rhSOD Treatment of PPHN



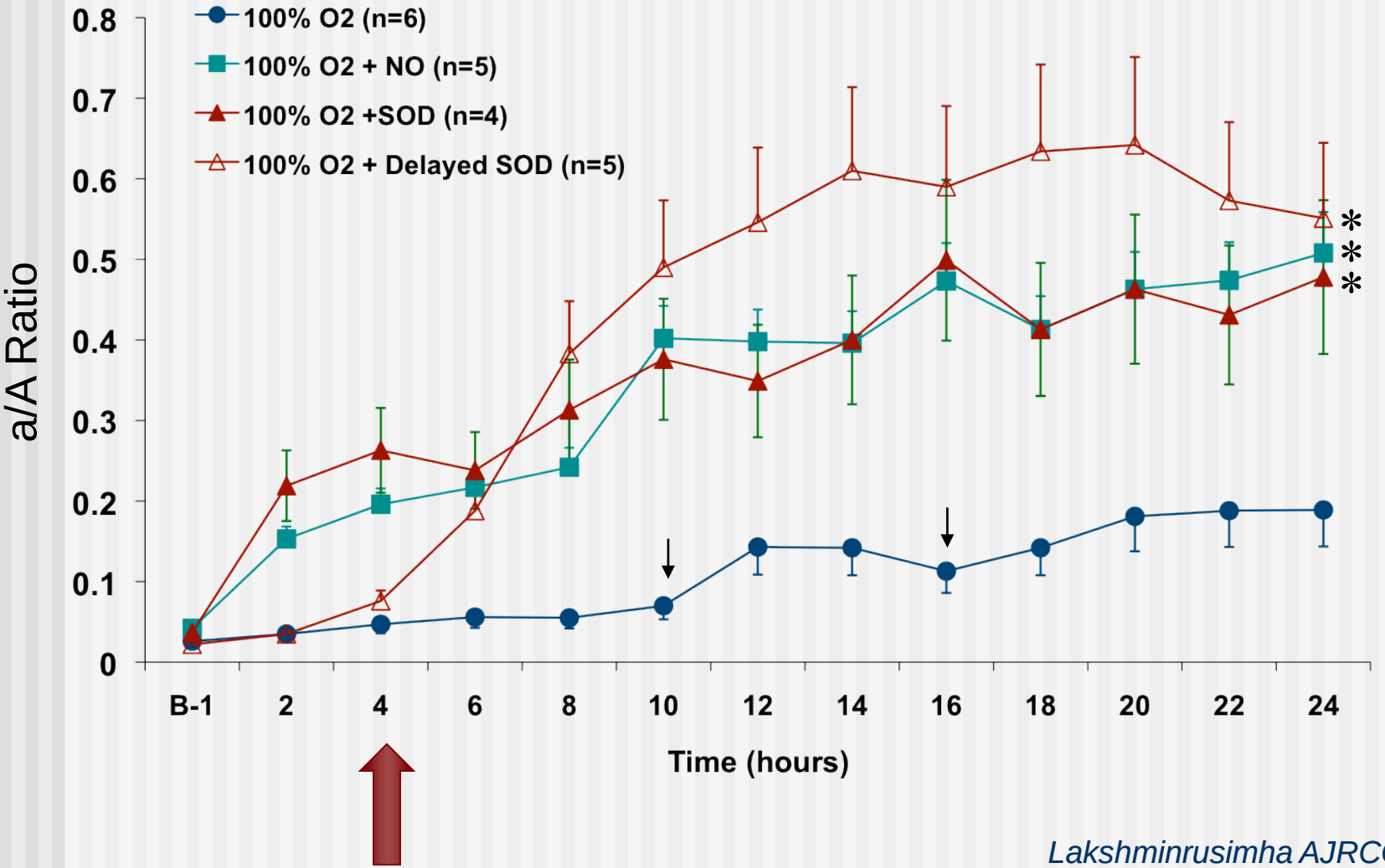
Lakshminrusimha AJRCCM 2006

rhSOD Treatment of PPHN



Lakshminrusimha AJRCCM 2006

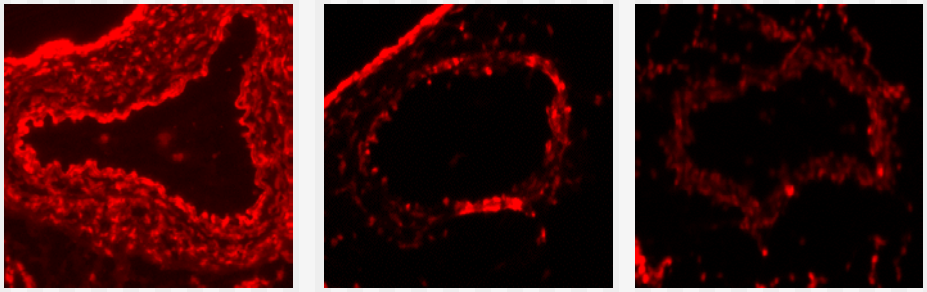
rhSOD Treatment of PPHN



Lakshminrusimha AJRCCM 2006

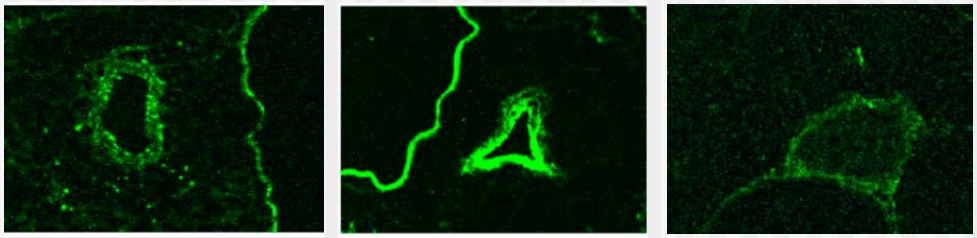
Vascular Effects of rhSOD in PPHN Lambs

DHE



100% O2 NO rhSOD

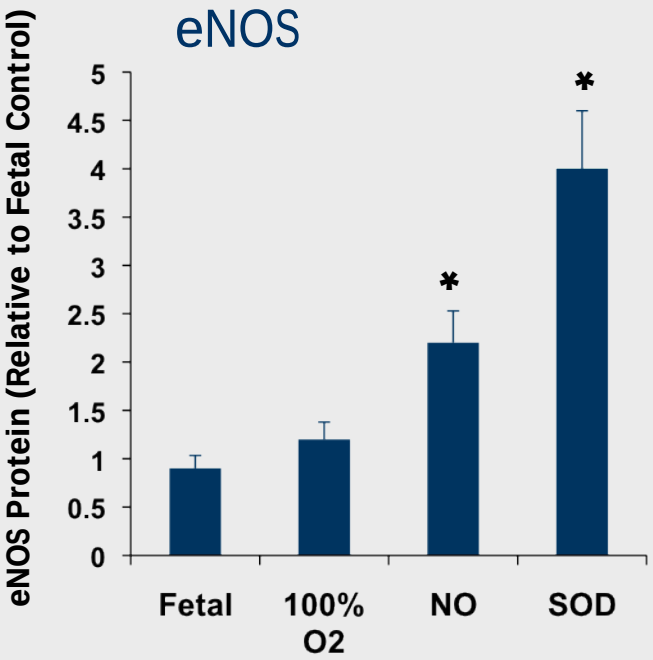
3NT

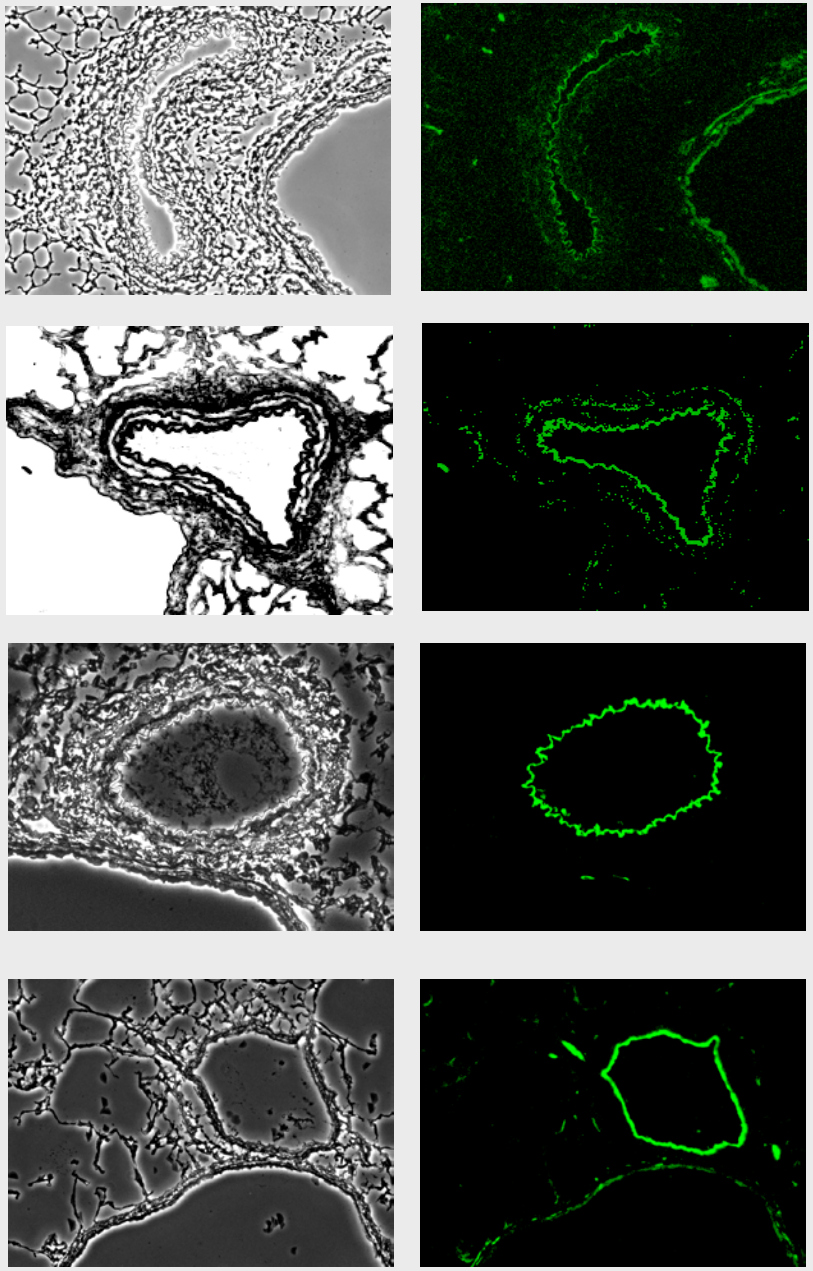


100% O2 NO rhSOD

Lakshminrusimha et al, AJRCCM 2006

rhSOD Restores eNOS Expression



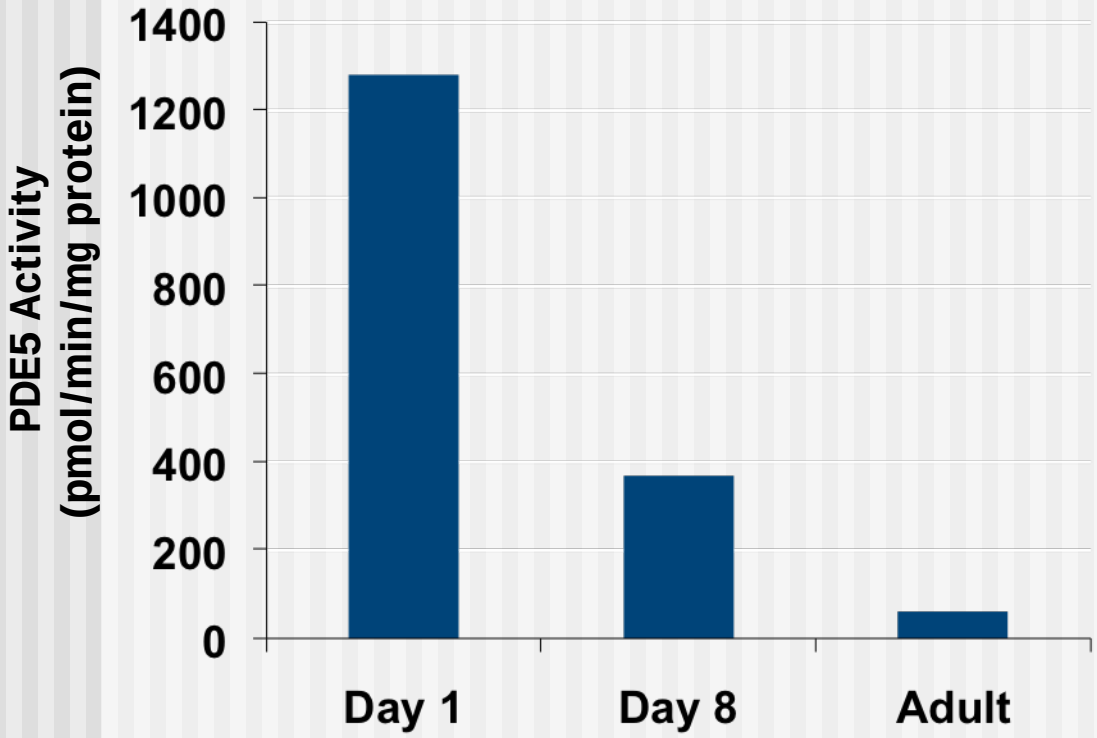


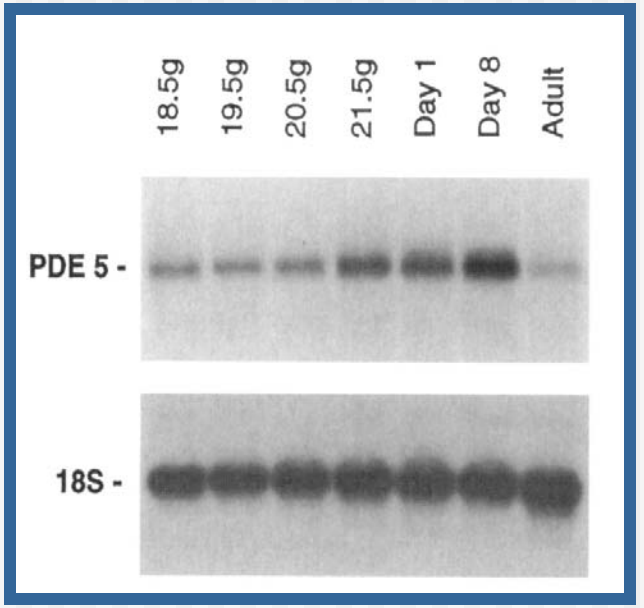
Farrow et al. AJP Lung Cell 2008

PDE5 Inhibition: Rationale

- Enhancement of NO effect
- Protection from NO Toxicity
- Protection from rebound pulmonary hypertension
- Potential for pulmonary vasodilatation

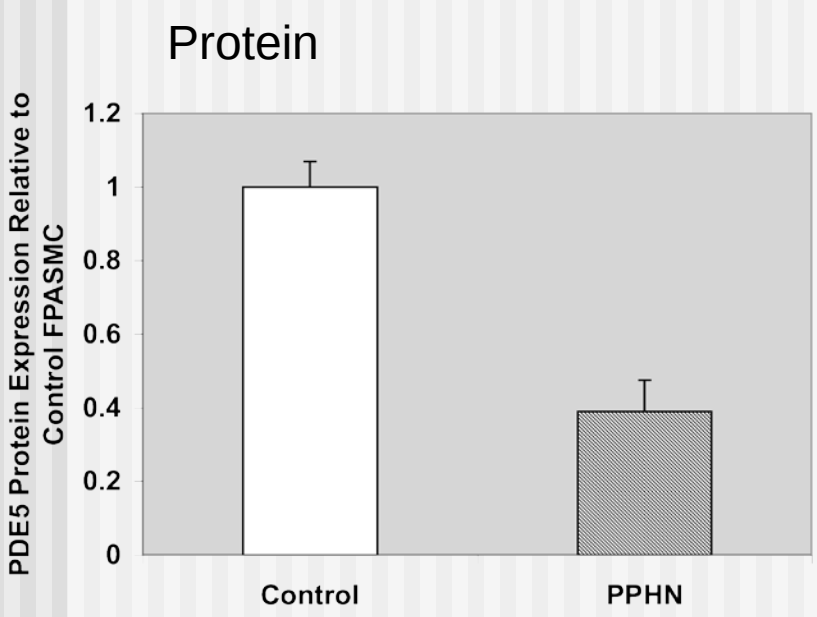
PDE5 Expression During Development

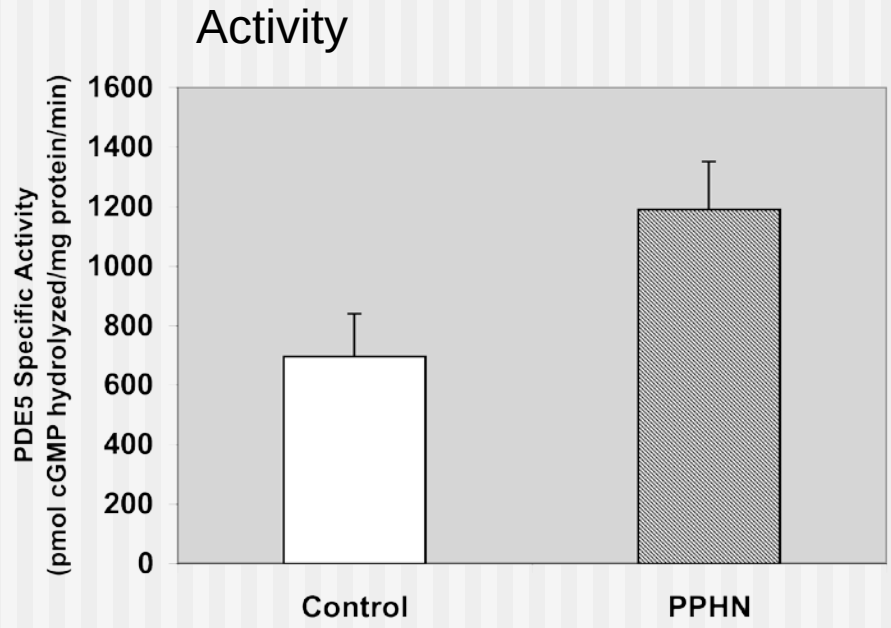


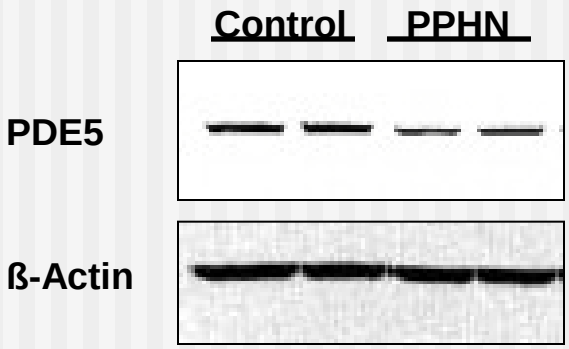


Sanchez et al, Pediatr Res 1998

PDE5 Expression and Activity in PPHN Fetuses

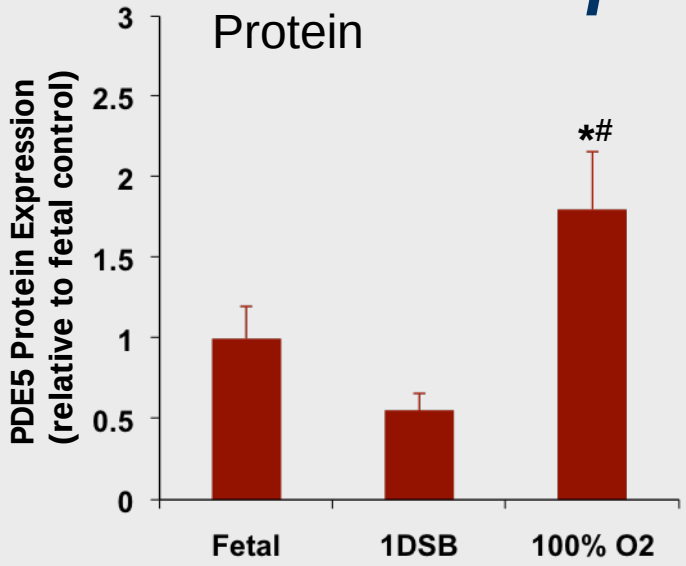


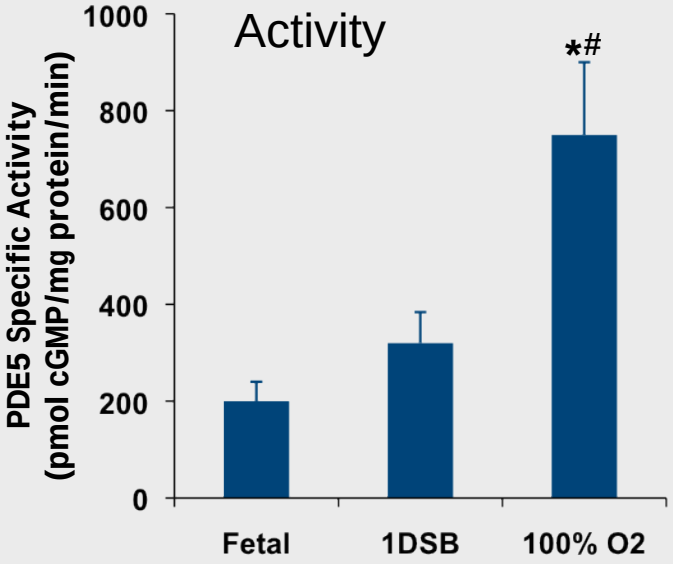


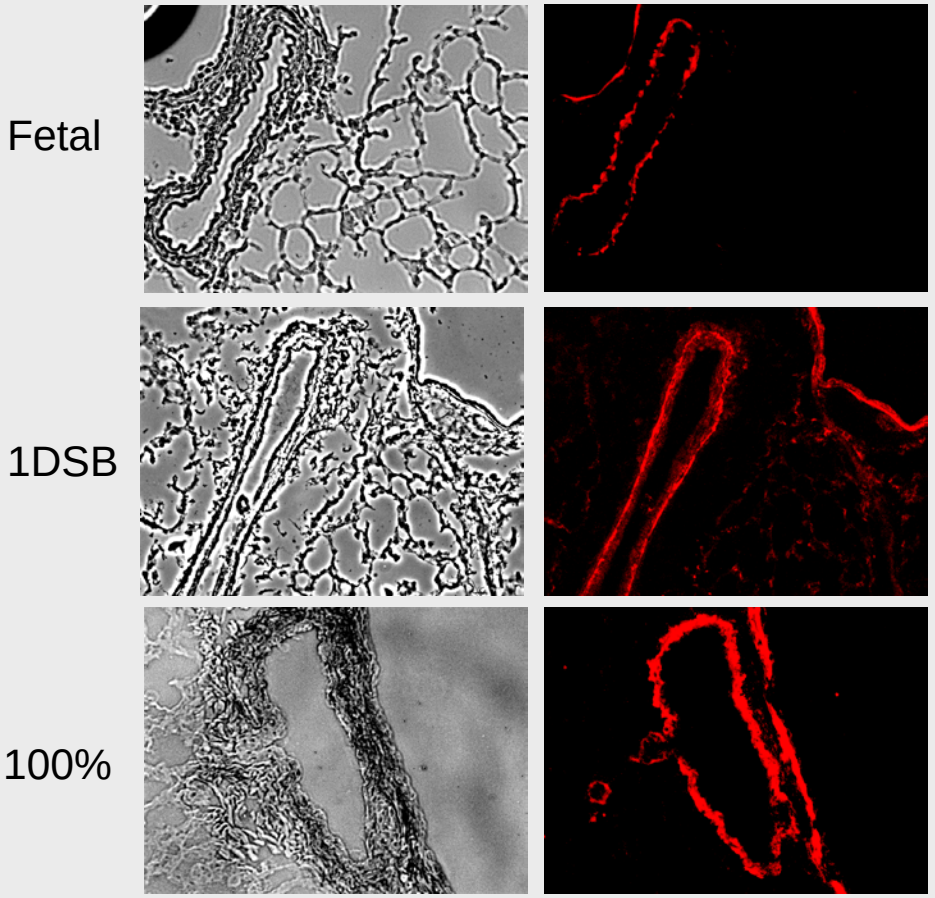


Farrow, et al., Am J Physiol 2010

Hyperoxic Ventilation Increases PDE5 Expression in Normal Lambs

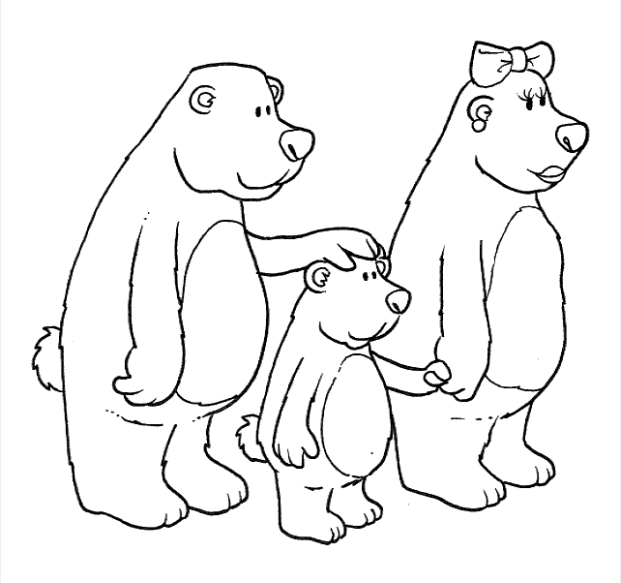


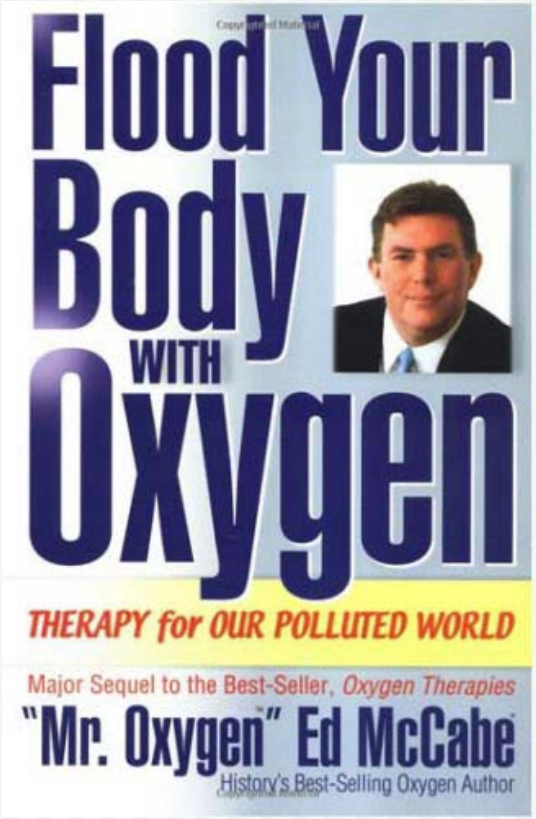




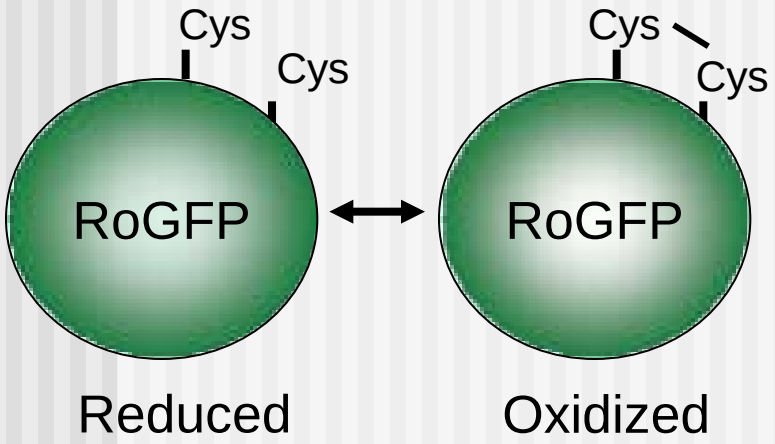
Farrow et al; Circ Res 2008

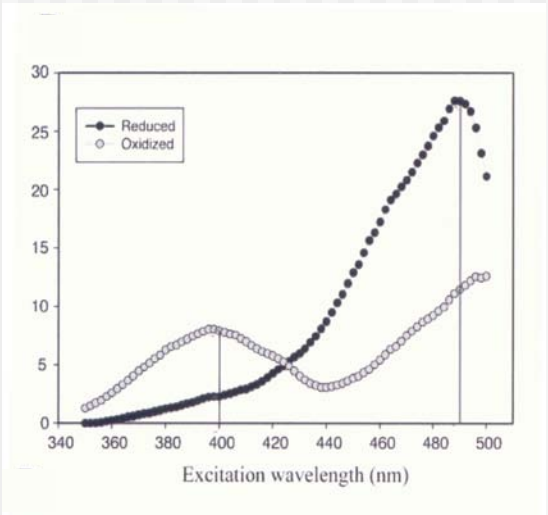
Oxygen: The Treatment or the Problem?

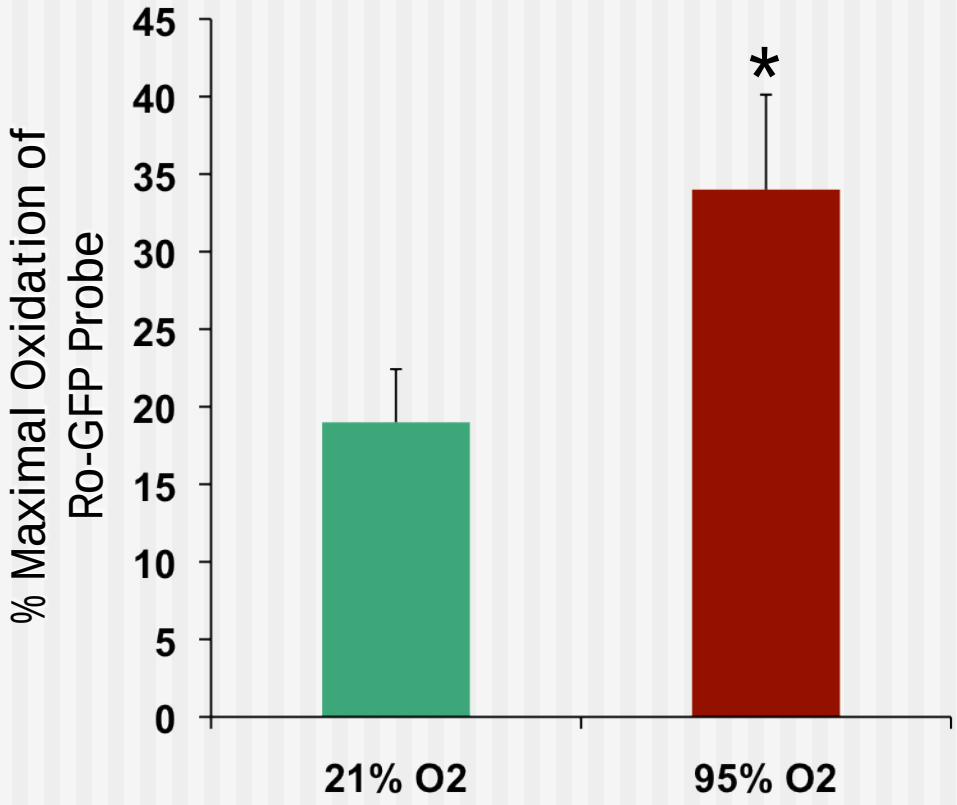




Exposure to 95% O_2 Increases ROS in FPASMC

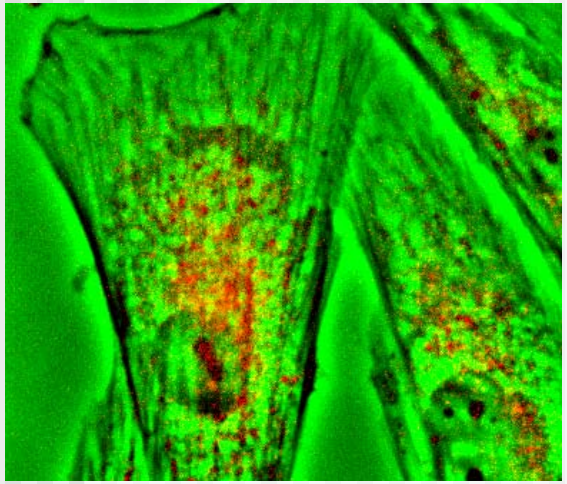




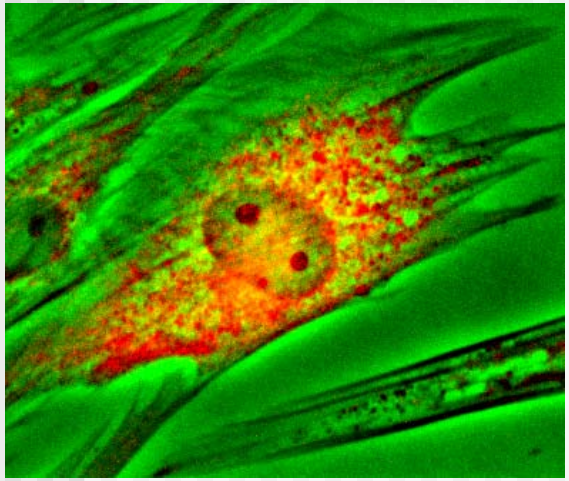


Farrow et al; Circ Res 2008

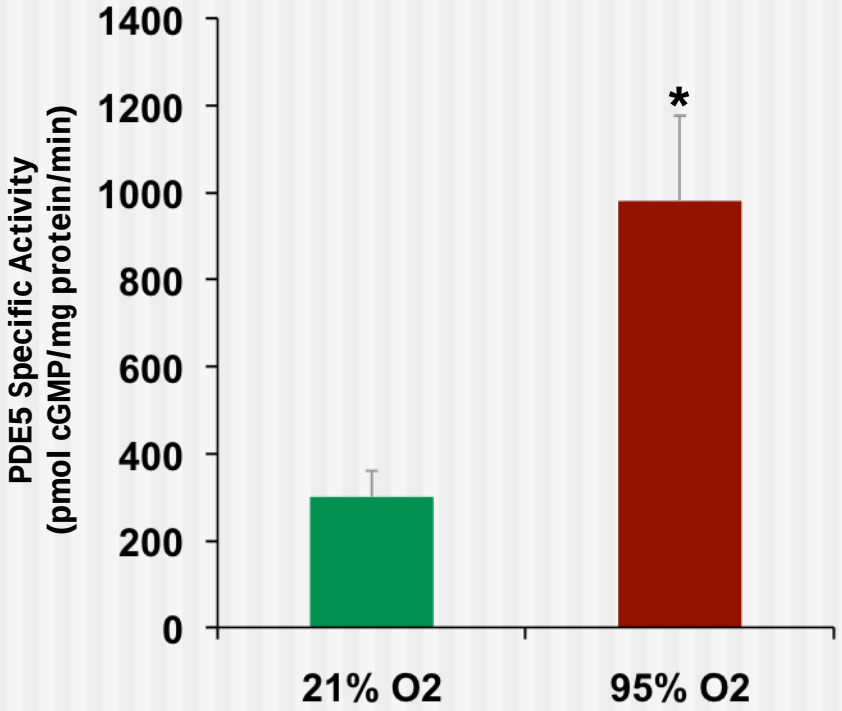
Hyperoxia Increases PDE5 Expression and Activity in FPASMC



21% O_2

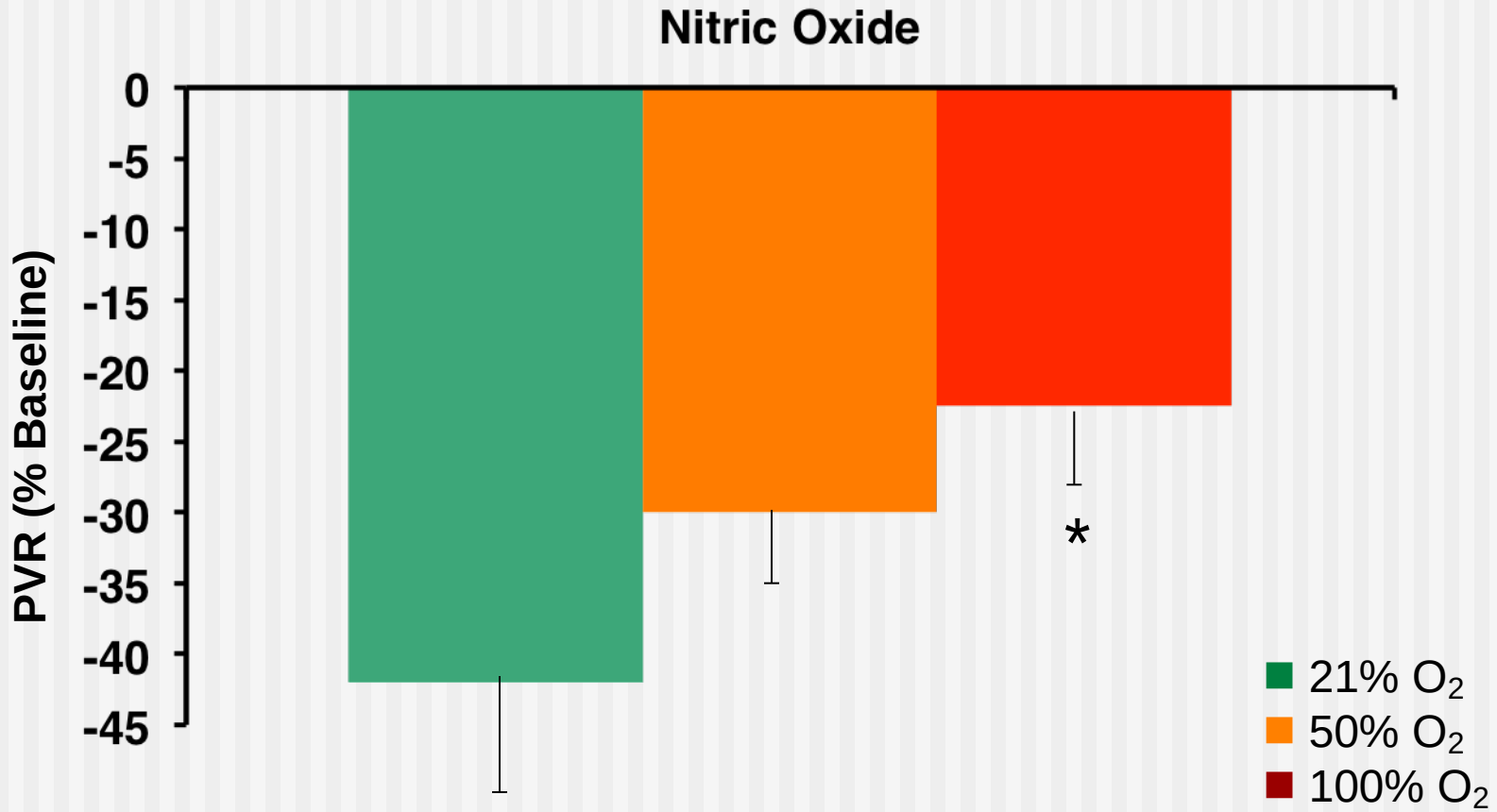


95% O_2



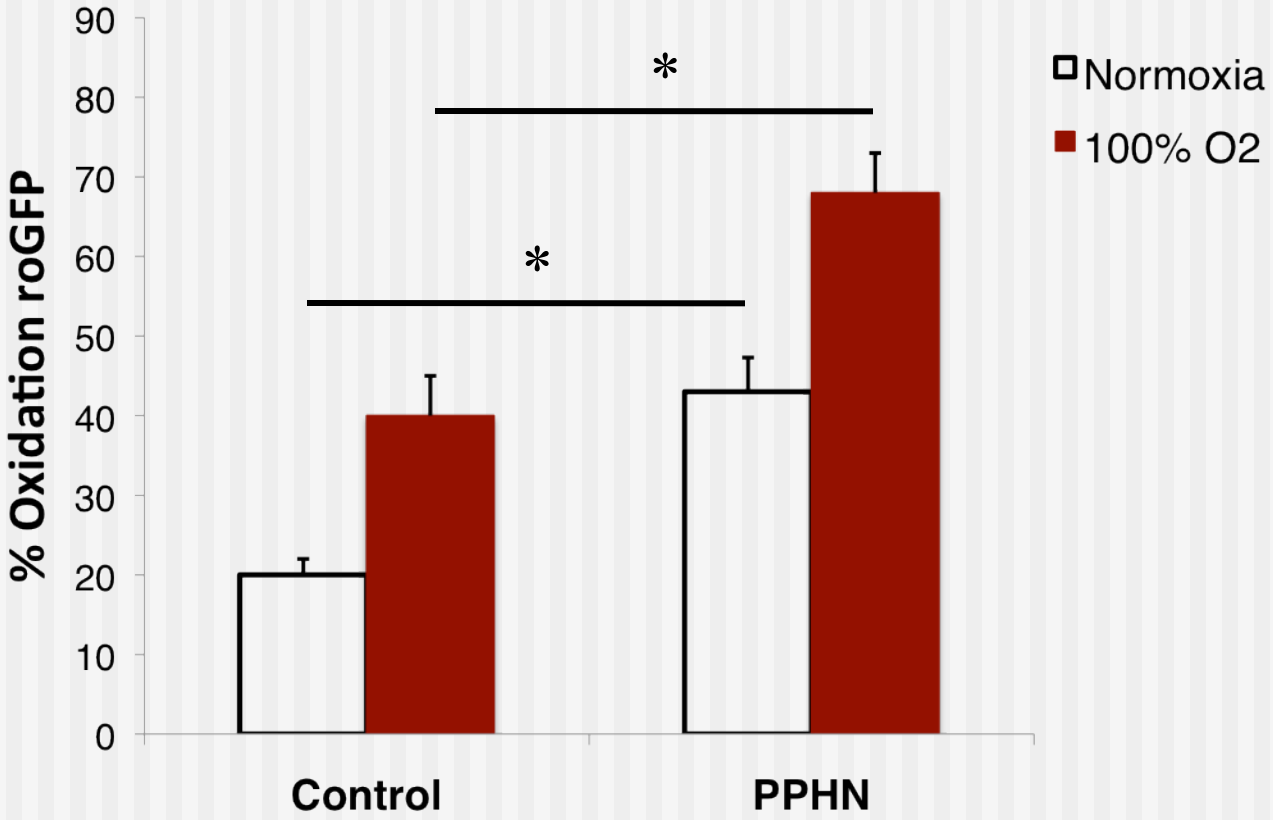
Farrow, et. al. *Circ Res*. 2008

100% Oxygen Blunts Response to NO in PPHN Lambs



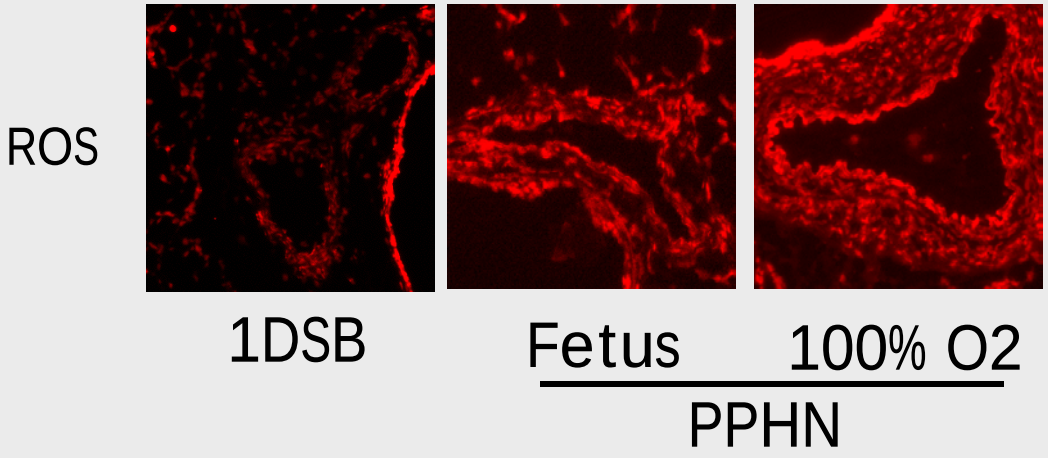
Lakshminrusimha et al; Pediatr Res 2009

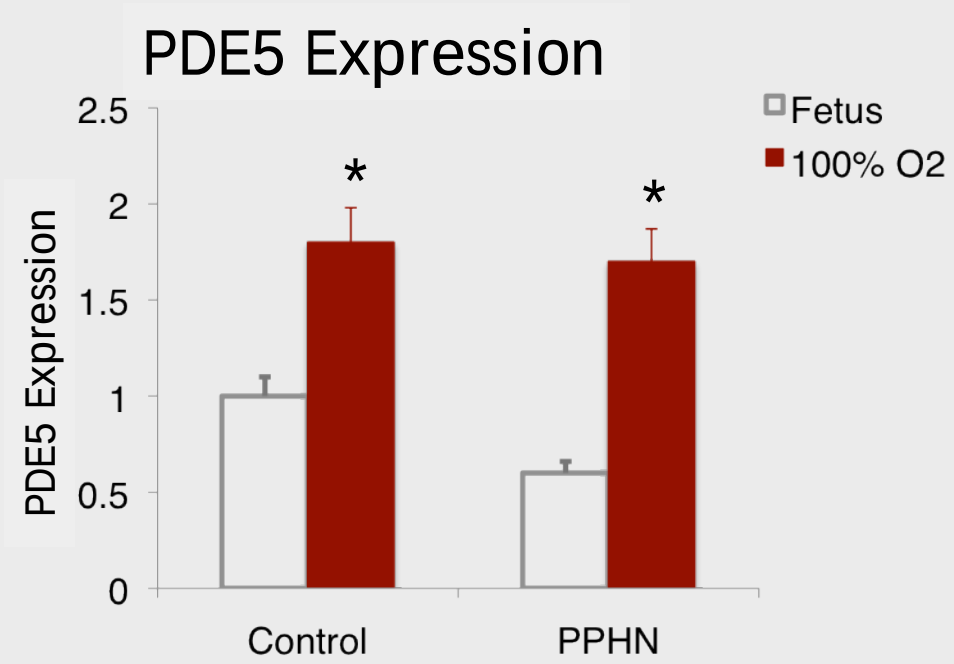
Increased ROS in PPHN PASMC

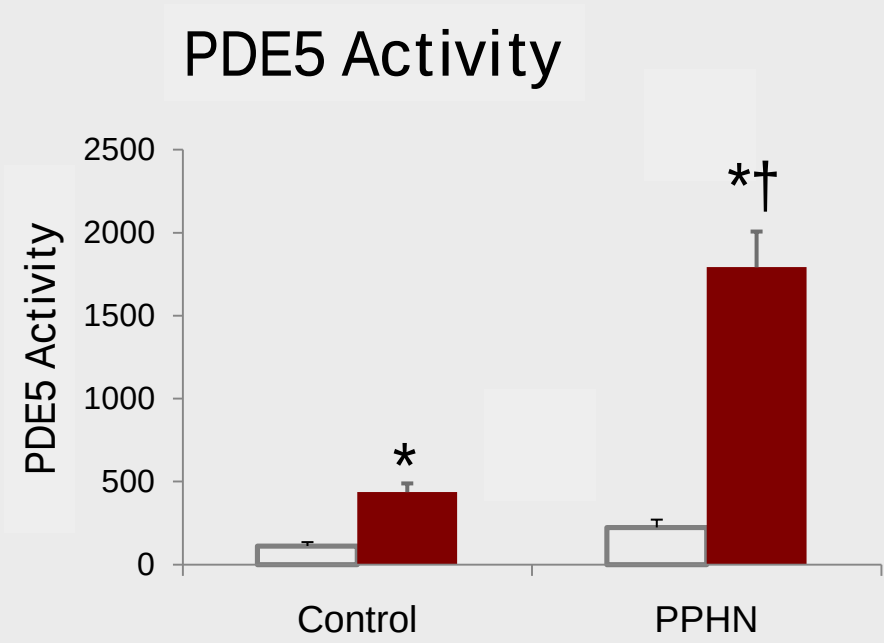


Farrow et al, Resp Physiol Neurobiol 2010 in press

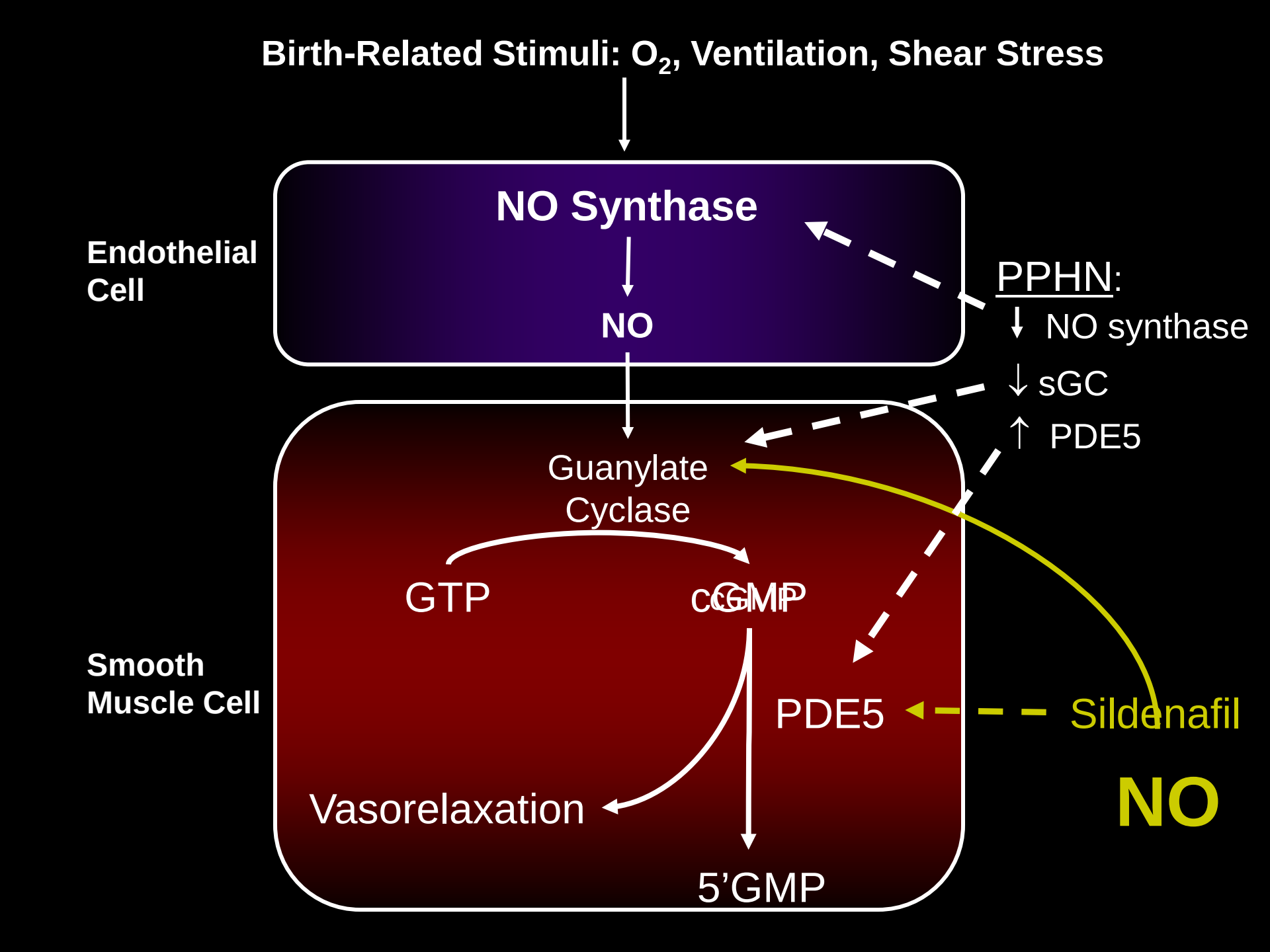
Ventilation with 100% O_2 in PPHN Lambs

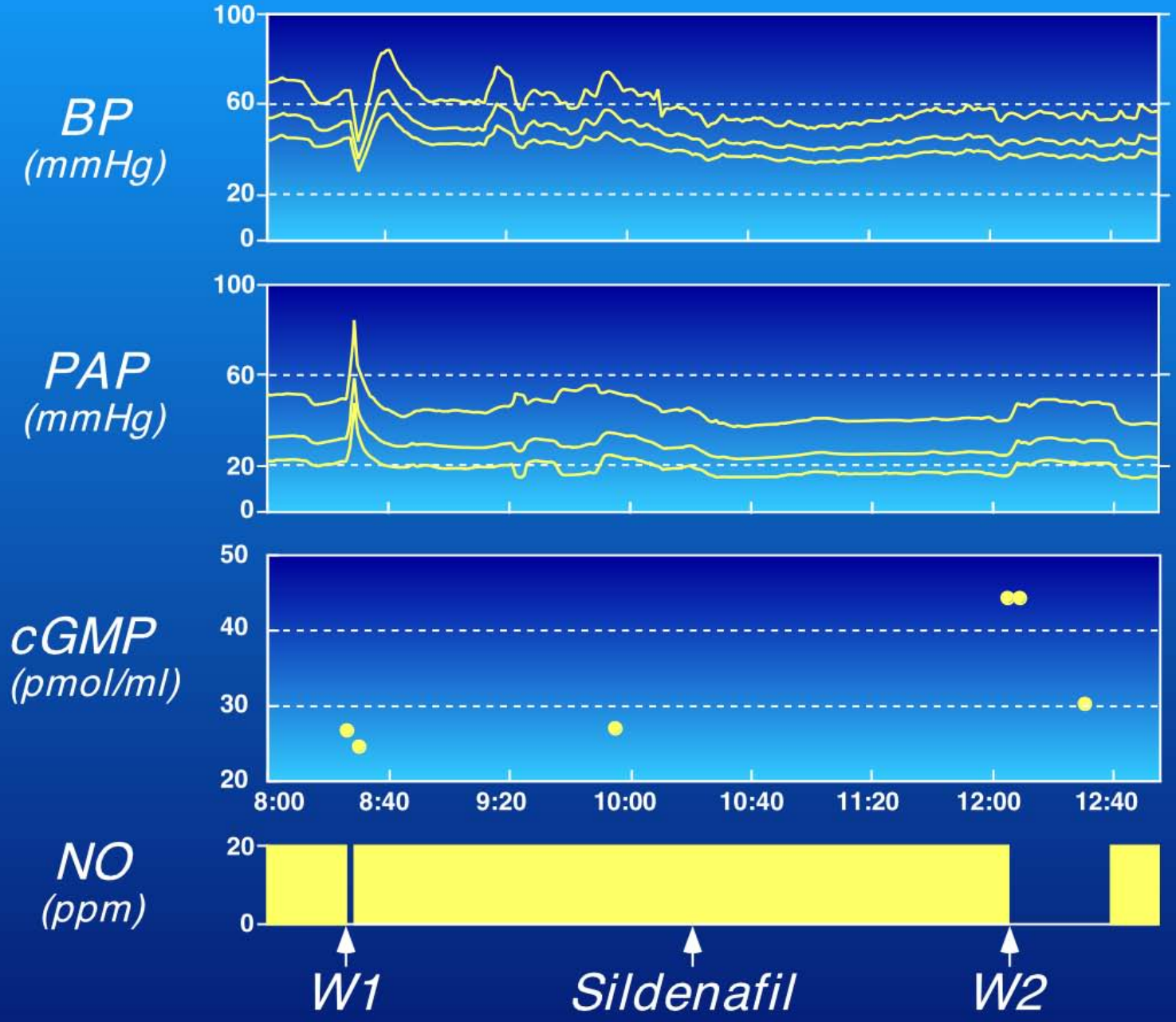




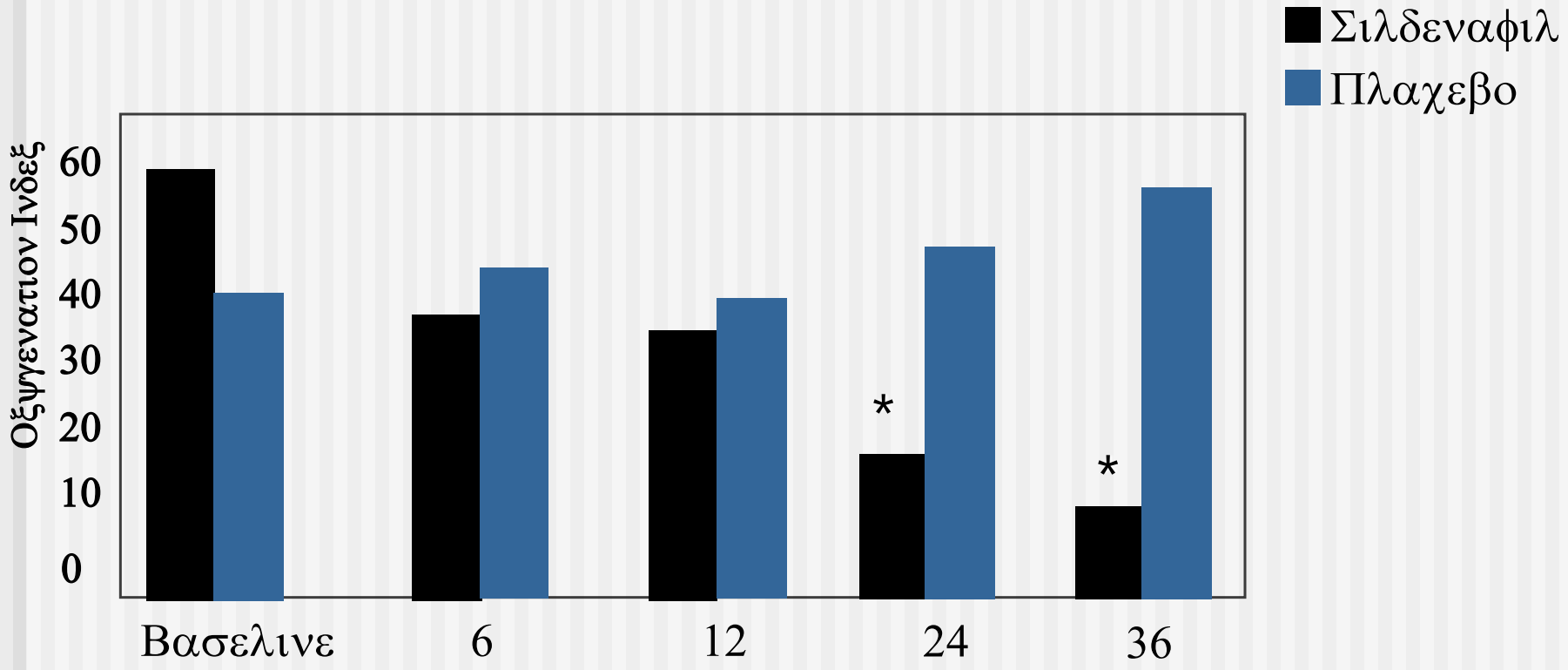


Farrow et al; Am J Physiol 2010


Birth-Related Stimuli: O_2, Ventilation, Shear Stress
Endothelial Cell
NO Synthase
NO
PPHN:
↓ NO synthase
↓ sGC
↑ PDE5
Guanylate Cyclase
GTP
cGMP
Smooth Muscle Cell
PDE5
Sildenafil
NO
Vasorelaxation
5'GMP


BP
(mmHg)
100
60
20
0
PAP
(mmHg)
100
60
20
0
cGMP
(pmol/ml)
50
40
30
20
8:00
8:40
9:20
10:00
10:40
11:20
12:00
12:40
NO
(ppm)
20
0
W1
Sildenafil
W2

Oral Sildenafil for PPHN

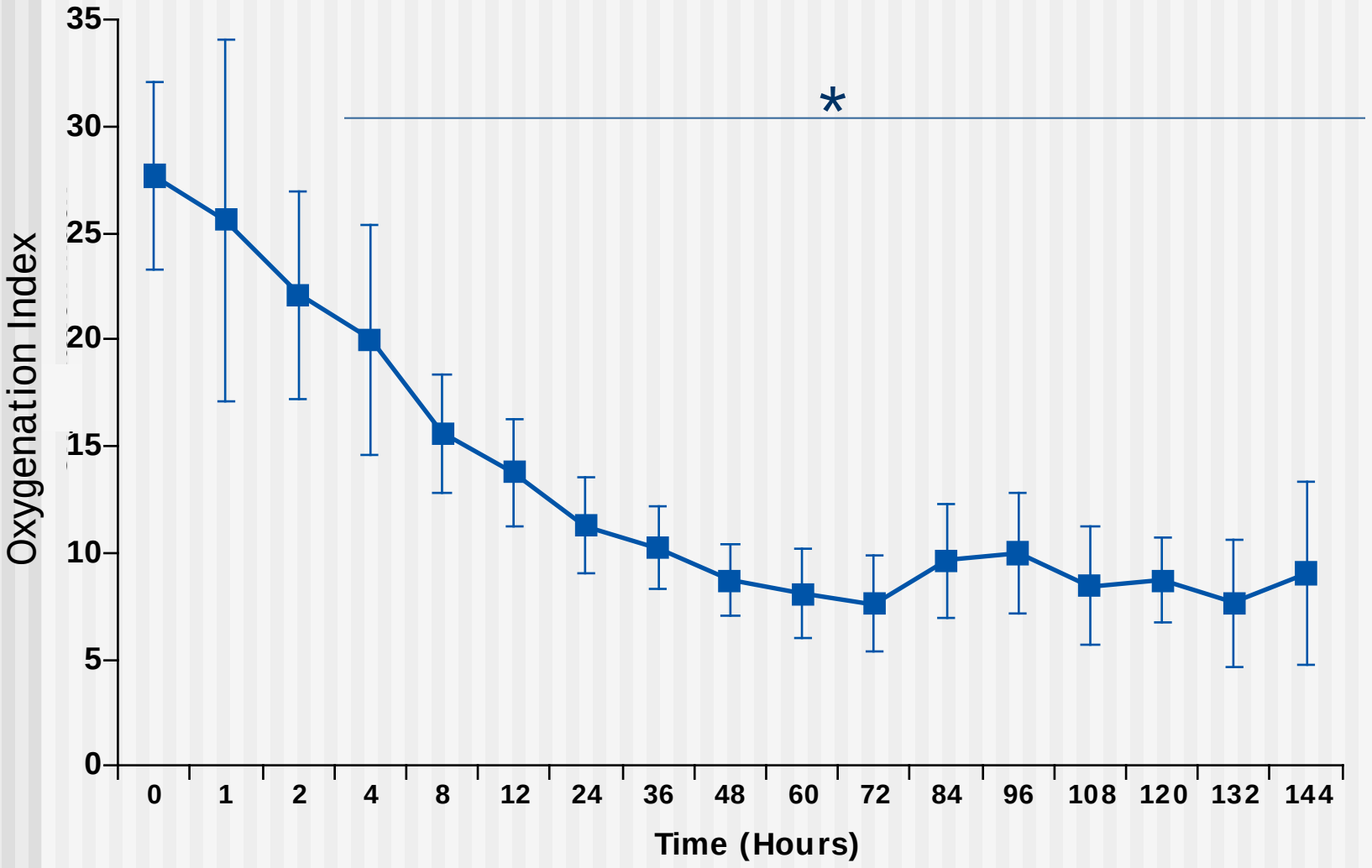


Baquero et al, Pediatrics, 2006

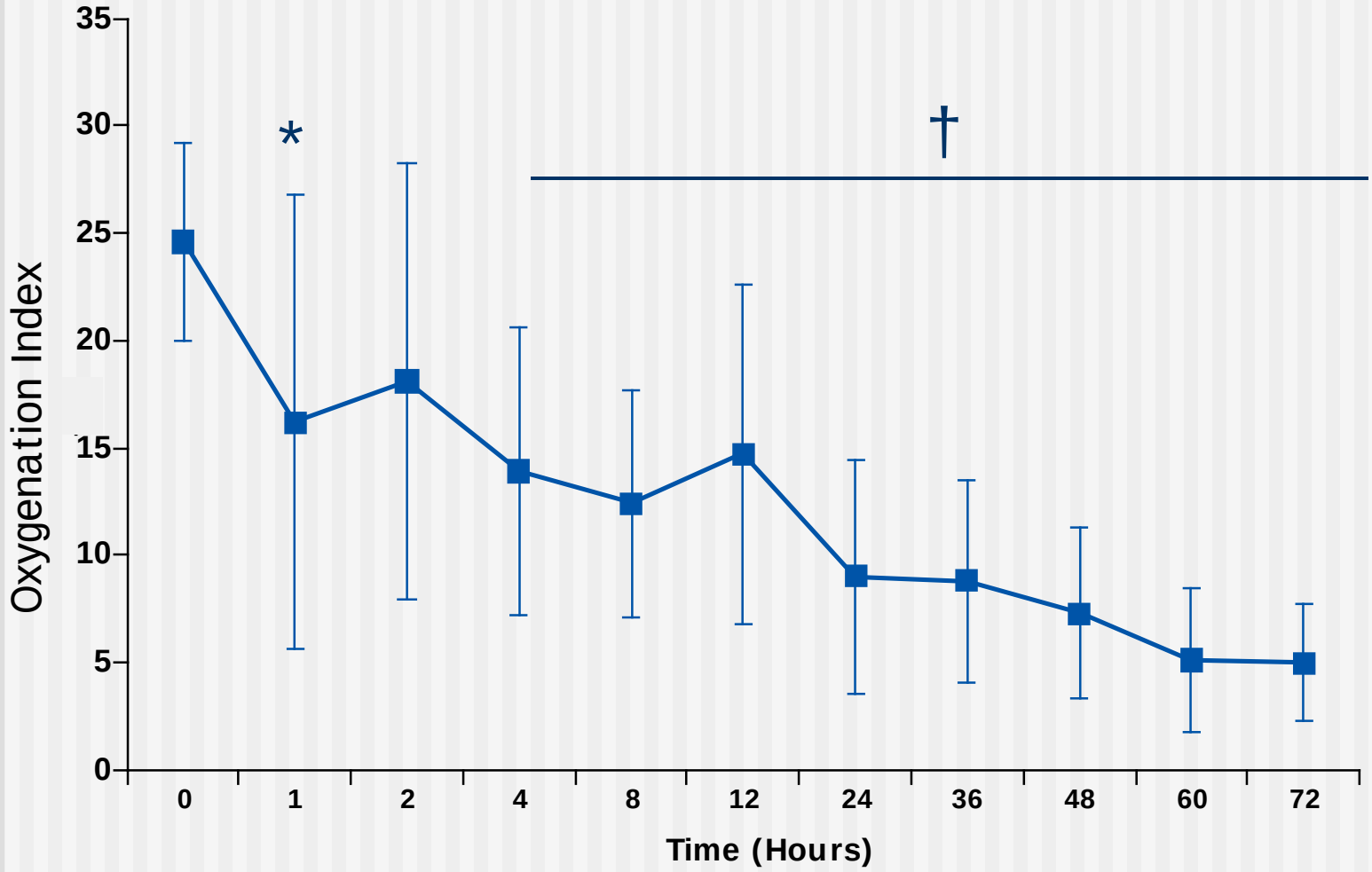
Intravenous Sildenafil for PPHN

- Five centers enrolled 36 neonates with PPHN or hypoxemic respiratory failure in eight ‘step up’ treatment groups.
- Demographic data for infants:
  - 39±2 weeks gestation
  - 3.44 ± 0.51 kg
  - 34±17 hours of age at enrollment
- Of the 36 infants, 29 were enrolled while already receiving inhaled NO.

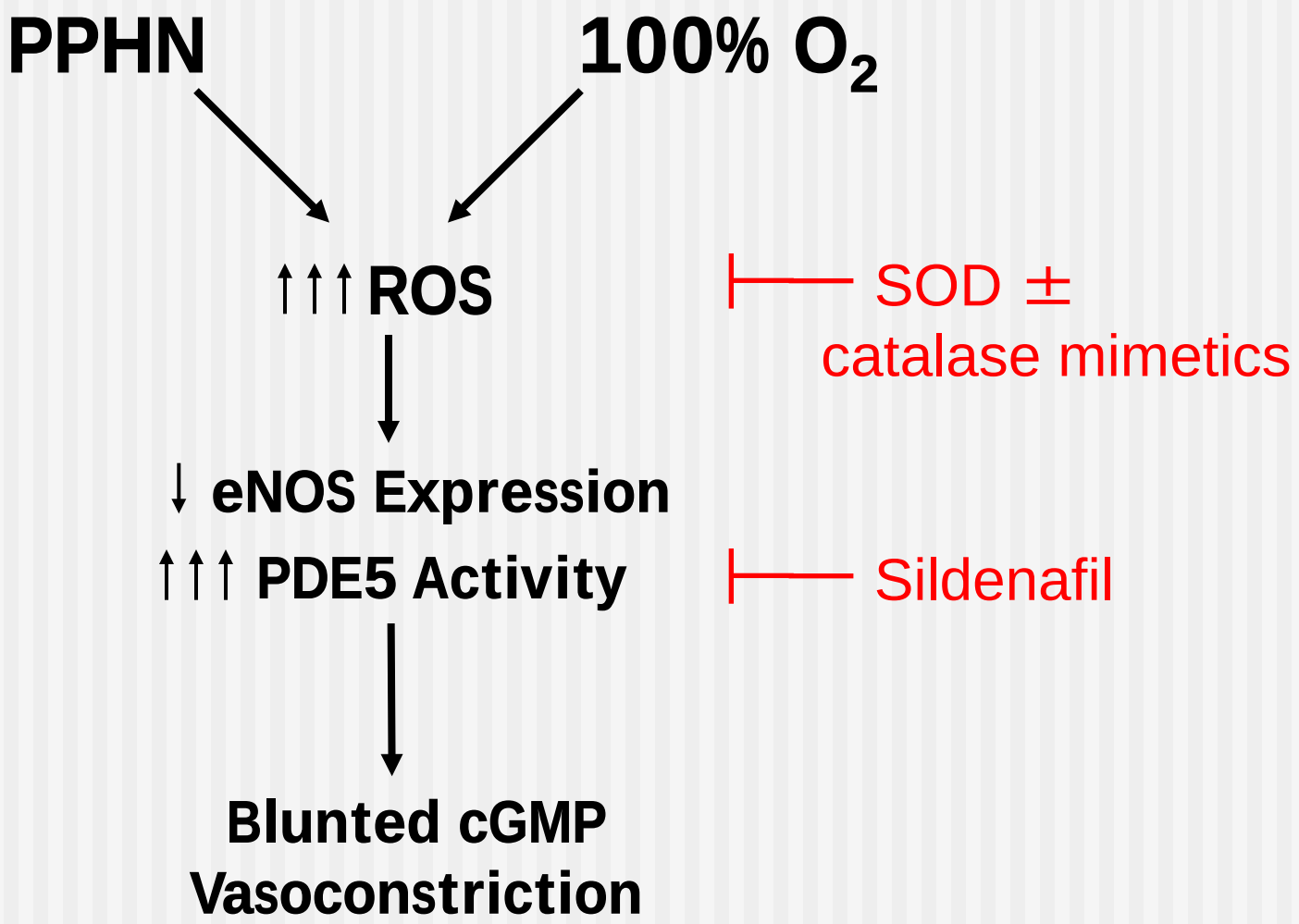
Intravenous Sildenafil Improves Oxygenation in PPHN



Response to Sildenafil Infusion without iNO

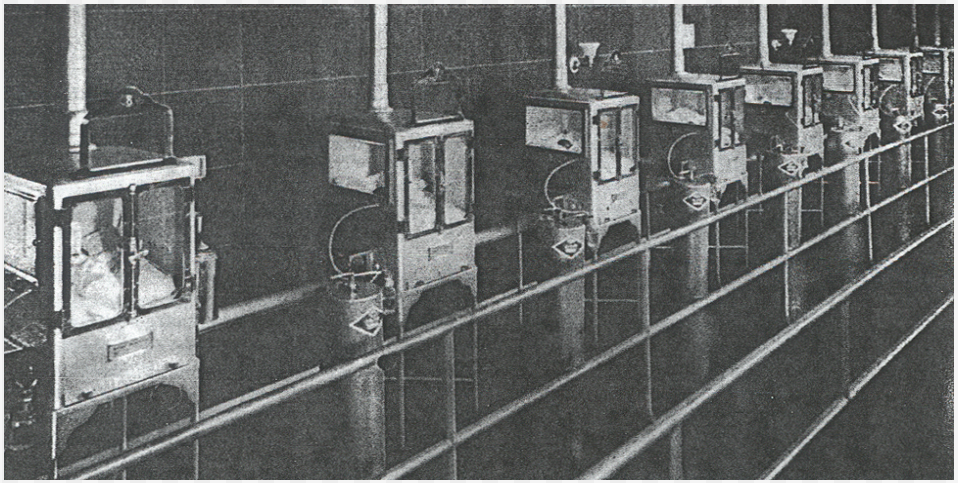


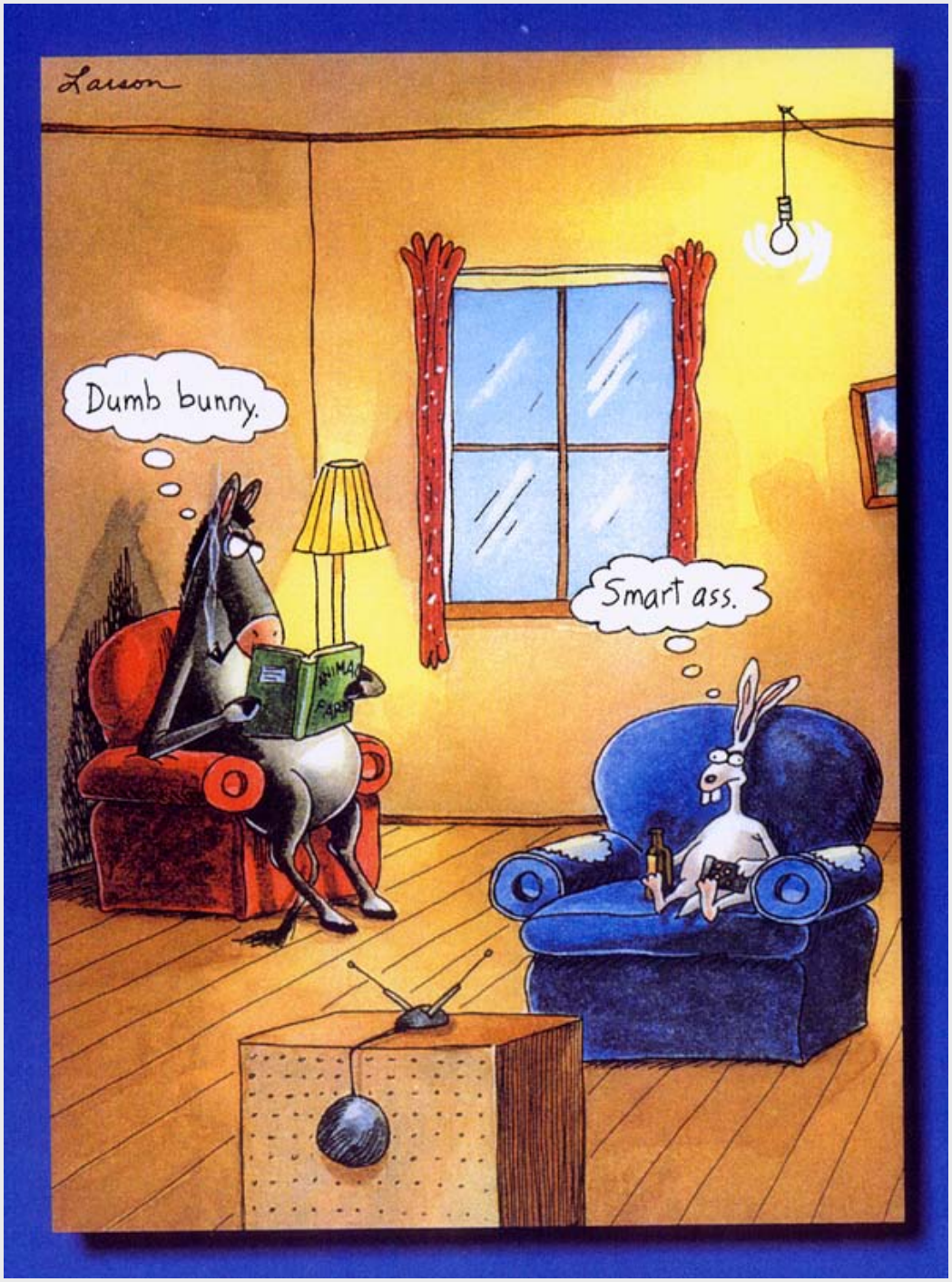
ROS Impair Normal Transition



Joseph Priestly, 1774

"…..the new air might not be proper for use in the healthy state of the body…….the air which nature has provided us may be as good as we deserve."





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Stephen Abman