NEWBORN TRANSITION

Denise Cole, RNC-NIC, MS

Transition Defined

- The period in which a fetus becomes a neonate, becoming an independent organism
- Adaptation to extrauterine life
- Carried out over hours (or days)
- Most difficult period in the human life cycle


MEDIUM
PRODUCT OF CHINA
55058

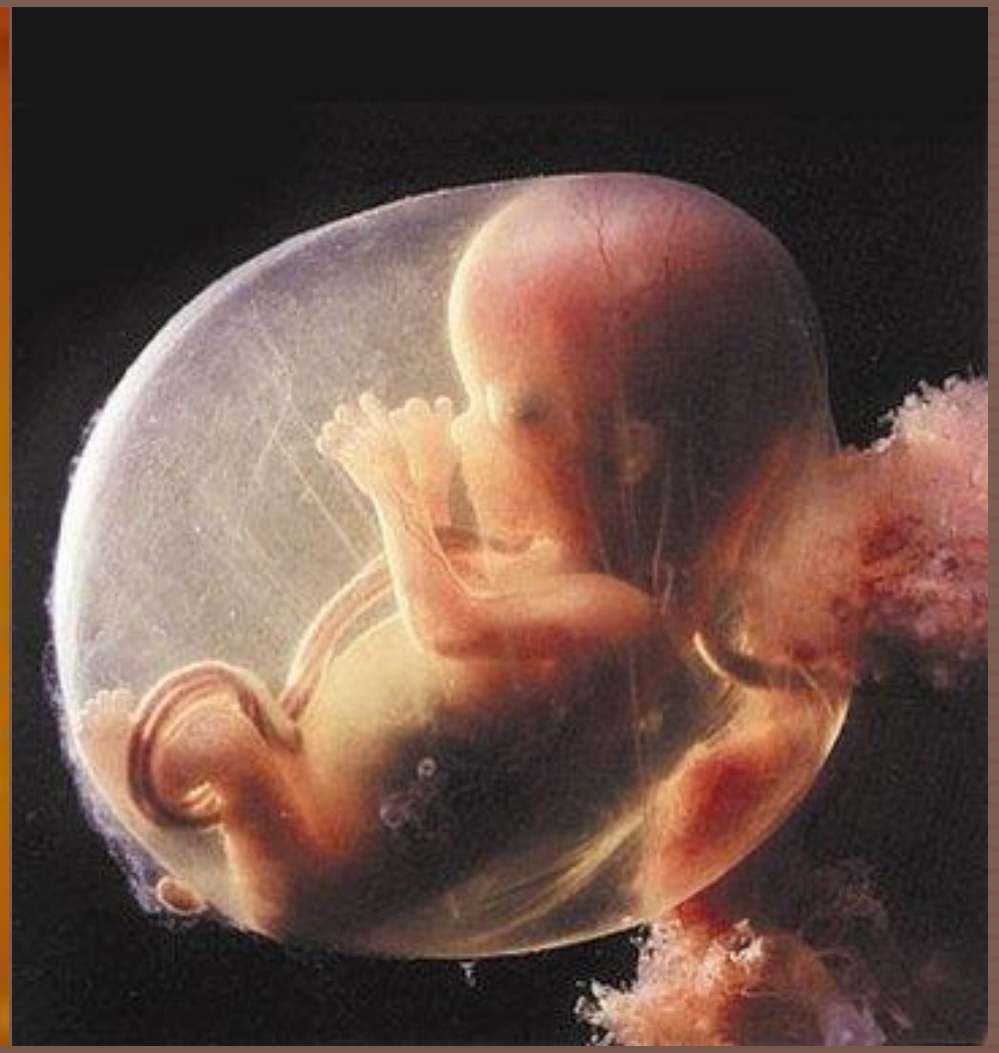
Physiologic Transition

- Most immediate are respiratory and cardiovascular changes
- Each body system goes through a transition cycle
- The successful transition of each system is dependent on the transition of the others

Stages of Transition

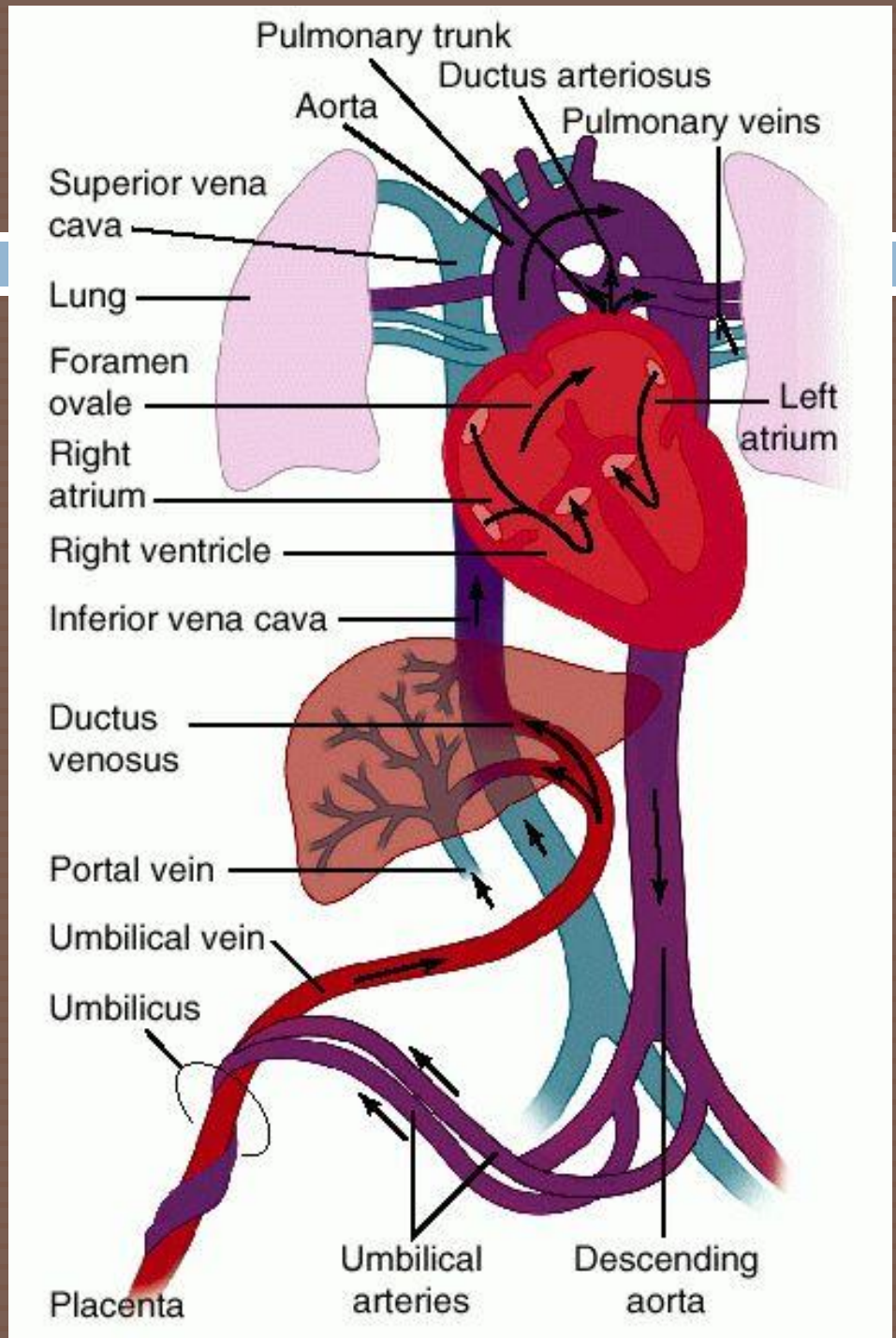
- Triggers of labor
  - Fetal adrenaline
  - Stress of labor (vaginal delivery of the head)
- Pressure changes related to loss of amniotic fluid and uterine contractions
- Entrance into the world, exposed to new stimuli
- First breath
- Clamping the cord

In the beginning . . .

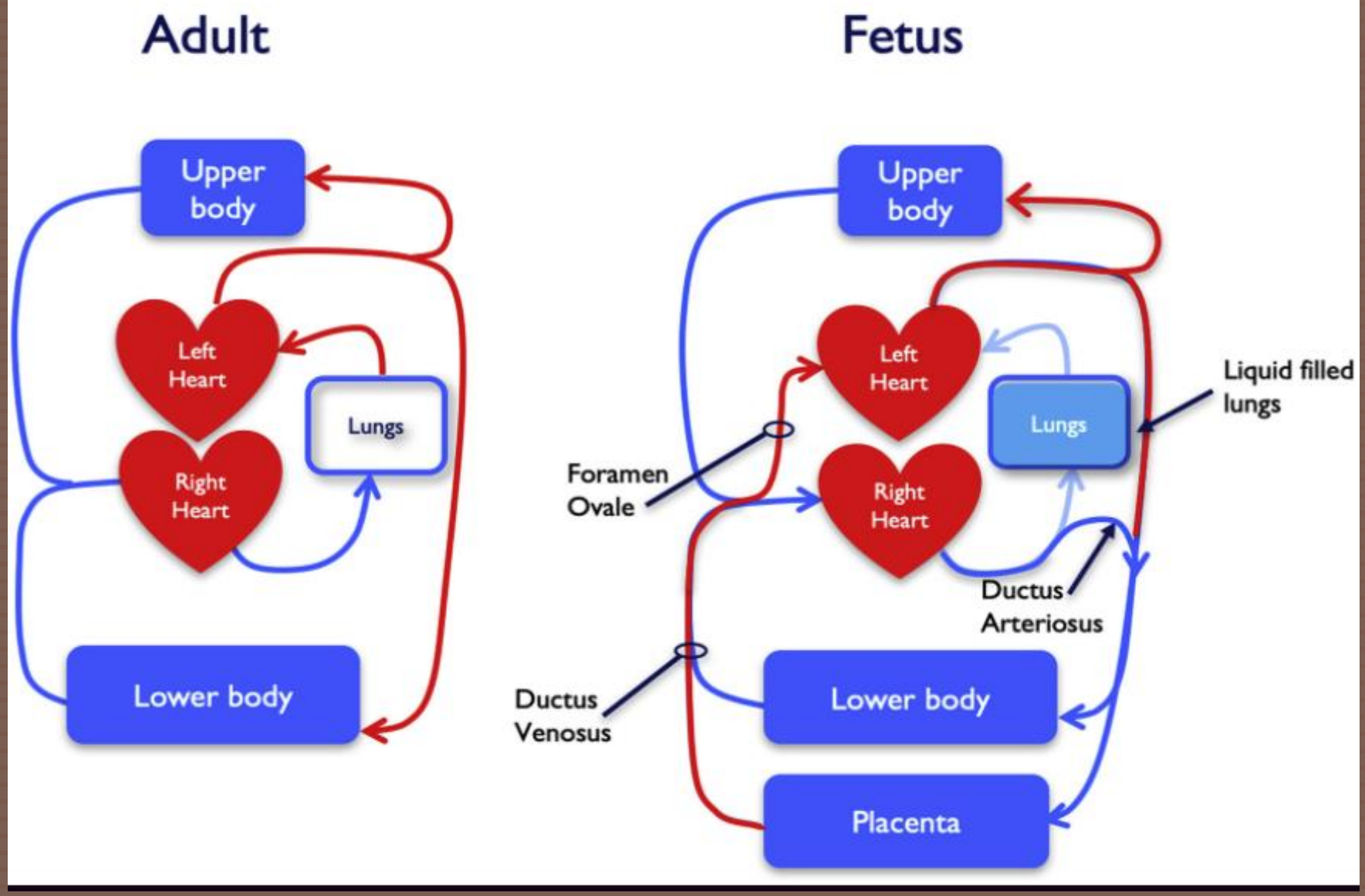


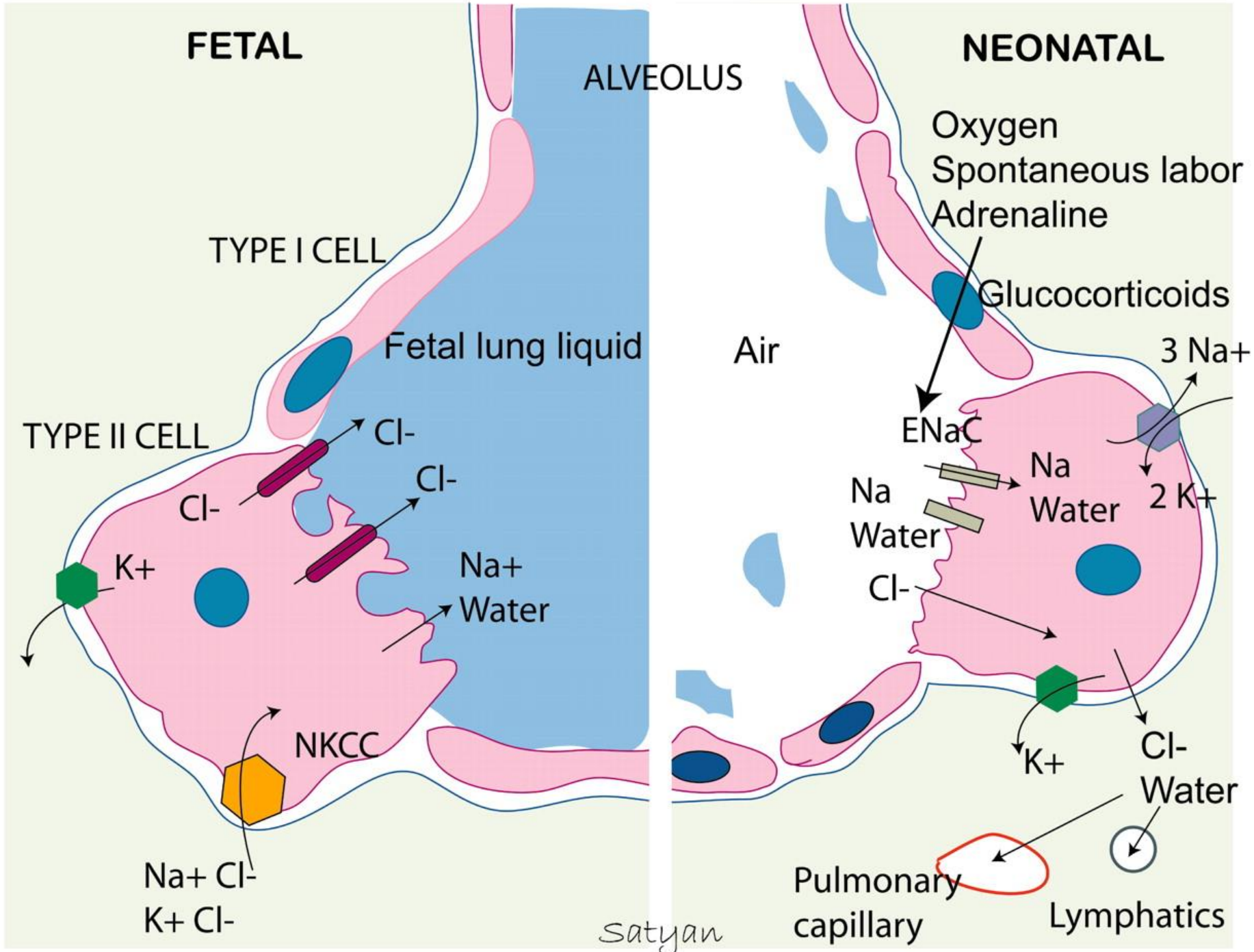
Fetal Circulation

- Lower PaO_2
  - Enters UV at 30-35 mmHg
  - Leaves UAs at 20-25 mmHg
- Fetal Hemoglobin (HbF)
- Presence of 3 ducts:
  - Ductus venosus
  - Foramen ovale
  - Ductus arteriosus



Fetal and Adult circulations




FETAL
ALVEOLUS
NEONATAL
TYPE I CELL
Fetal lung liquid
Air
Oxygen
Spontaneous labor
Adrenaline
Glucocorticoids
TYPE II CELL
Cl-
Cl-
Cl-
K+
Na+
Water
NKCC
Na+ Cl-
K+ Cl-
ENaC
3 Na+
Na
Water
Na
Water
2 K+
Cl-
K+
Cl-
Water
Pulmonary
capillary
Lymphatics
Satyan

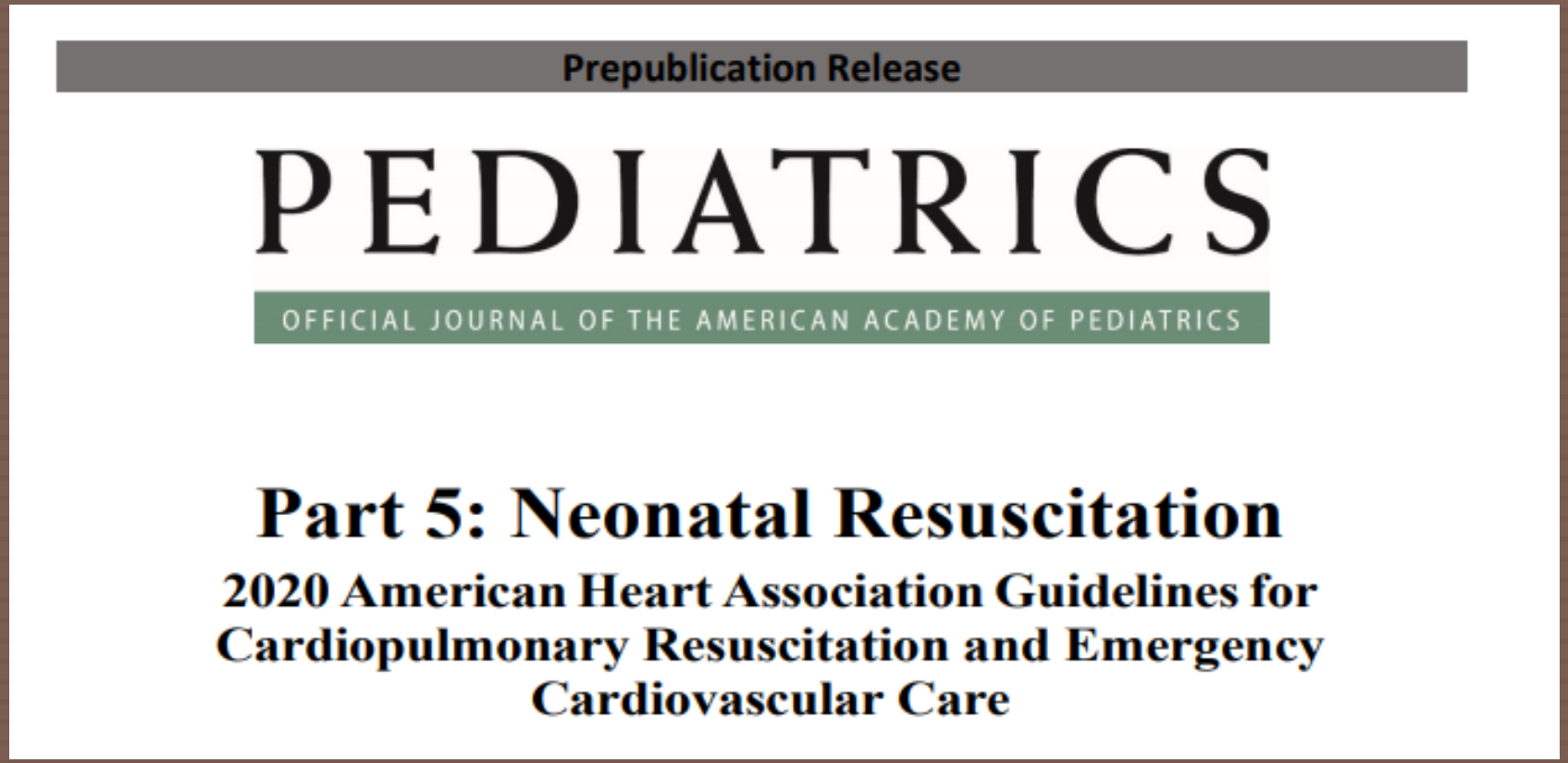
Transition of the Cardiovascular System

- Onset of ventilation
- Loss of placental circulation
  - Results in increase in systemic vascular resistance
- Rise in blood oxygen content
  - Causes drop in pulmonary vascular resistance
  - Flow through DA dramatically decreases

Speaking of the cord . . .

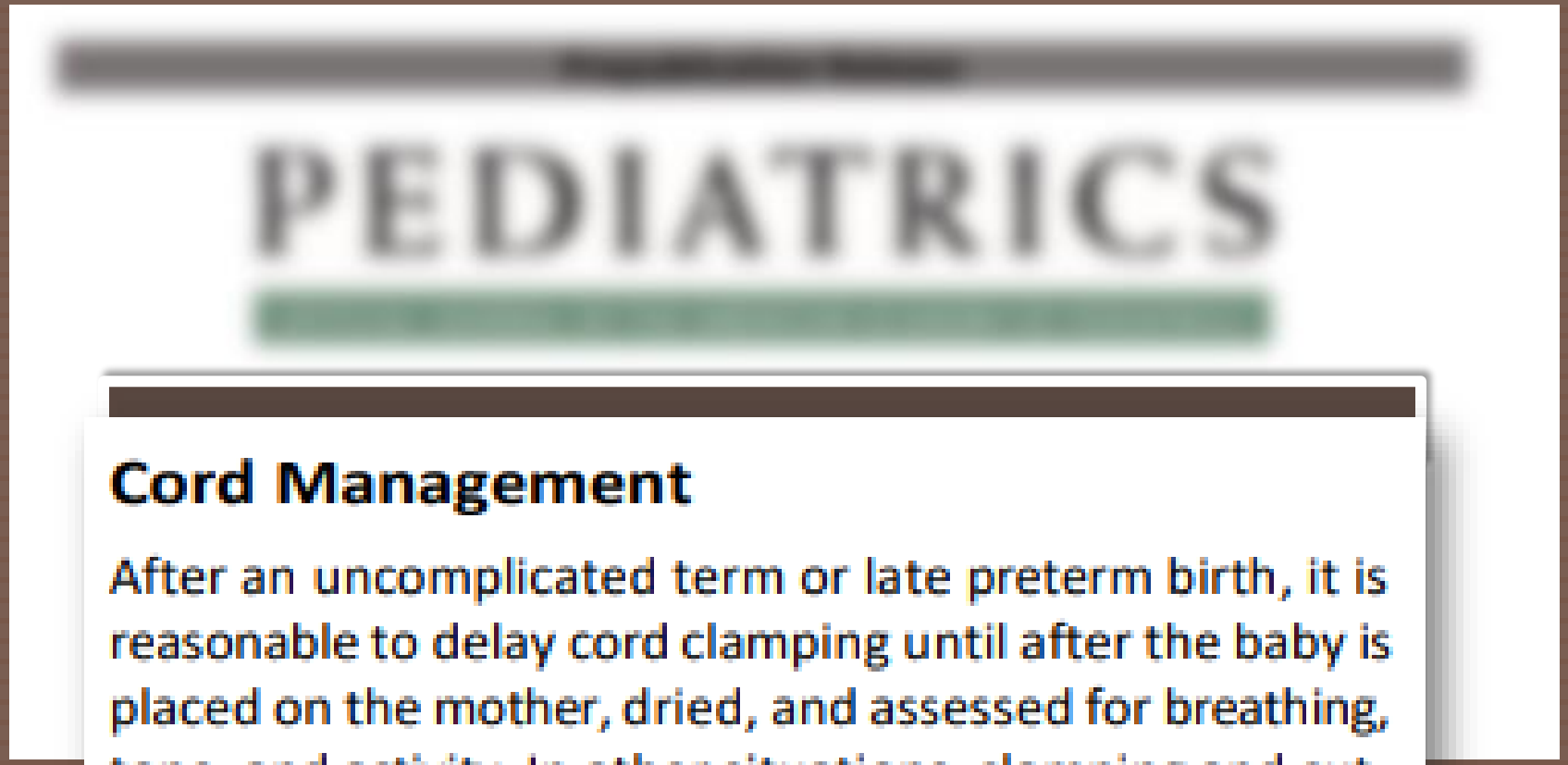
When should it be clamped?

October 2020



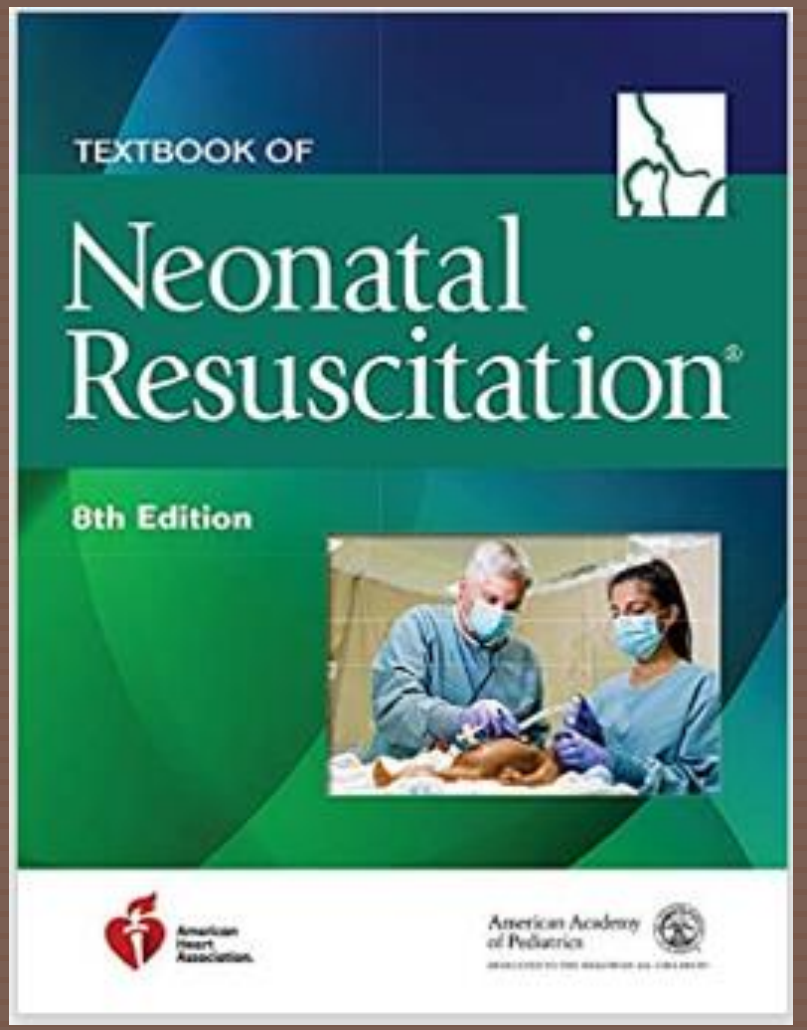
Part 5: Neonatal Resuscitation 2020 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care
Khalid Aziz, et al. *Pediatrics* Jan 2021, 147 (Supplement 1) e2020038505E; **DOI:** 10.1542/peds.2020-038505E

October 2020



“For most vigorous preterm newborns, the current evidence suggests that clamping should be delayed for at least 30 to 60 seconds. Among vigorous term newborns, the evidence suggests that a similar delay may be reasonable. During this time, the baby may be placed skin-to-skin on the mother’s chest or abdomen, or held securely in a warm, dry towel or blanket.”

“If the placental circulation is not intact, such as after a placental abruption, bleeding placental previa, or cord avulsion, the cord should be clamped immediately after birth.”



8th Ed., 2021

The American College of
Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

COMMITTEE OPINION

Number 684 • January 2017

(Replaces Committee Opinion Number 543, December 2012)

Committee on Obstetric Practice

The American Academy of Pediatrics and the American College of Nurse–Midwives endorse this document. This Committee Opinion was developed by the American College of Obstetricians and Gynecologists' Committee on Obstetric Practice in collaboration with committee members Maria A. Mascola, MD; T. Flint Porter, MD; and Tamara Tin-May Chao, MD.

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

Delayed Umbilical Cord Clamping After Birth

ABSTRACT: Delayed umbilical cord clamping appears to be beneficial for term and preterm infants. In term infants, delayed umbilical cord clamping increases hemoglobin levels at birth and improves iron stores in the first several months of life, which may have a favorable effect on developmental outcomes. There is a small increase in jaundice that requires phototherapy in this group of infants. Consequently, health care providers adopting delayed umbilical cord clamping in term infants should ensure that mechanisms are in place to monitor for and treat neonatal jaundice. In preterm infants, delayed umbilical cord clamping is associated with significant neonatal benefits, including improved transitional circulation, better establishment of red blood cell volume, decreased need for blood transfusion, and lower incidence of necrotizing enterocolitis and intraventricular hemorrhage. Delayed umbilical

The American College of

Given the benefits to most newborns and concordant with other professional organizations, the American College of Obstetricians and Gynecologists now recommends a delay in umbilical cord clamping in vigorous term and preterm infants for at least 30–60 seconds after birth.

jaundice that requires phototherapy in this group of infants. Consequently, health care providers adopting delayed umbilical cord clamping in term infants should ensure that mechanisms are in place to monitor for and treat neonatal jaundice. In preterm infants, delayed umbilical cord clamping is associated with significant neonatal benefits, including improved transitional circulation, better establishment of red blood cell volume, decreased need for blood transfusion, and lower incidence of necrotizing enterocolitis and intraventricular hemorrhage. Delayed umbilical

ACOG
The American College of Obstetricians and Gynecologists

INTERIM UPDATE
DECEMBER 2020

ACOG COMMITTEE OPINION

Number 814

(Replaces Committee Opinion 684, January 2017)

Committee on Obstetric Practice

The American College of Nurse-Midwives endorse this document. This Committee Opinion was developed by the American College of Obstetricians and Gynecologists' Committee on Obstetric Practice in collaboration with committee members Maria A. Mascola, MD; T. Flint Porter, MD; and Tamara Tin-May Chao, MD.

INTERIM UPDATE: The content in this Committee Opinion has been updated as highlighted (or removed as necessary) to reflect a limited, focused change in the data and language regarding cord milking.

Delayed Umbilical Cord Clamping After Birth

ABSTRACT: Delayed umbilical cord clamping appears to be beneficial for term and preterm infants. In term infants, delayed umbilical cord clamping increases hemoglobin levels at birth and improves iron stores in the first several months of life, which may have a favorable effect on developmental outcomes. There is a small increase in

Delayed umbilical cord clamping after birth. ACOG Committee Opinion No. 814. American College of Obstetricians and Gynecologists. Obstet Gynecol 2020;136:e100–6.

small study showed no difference between preterm infants exposed to delayed umbilical cord clamping compared with umbilical cord milking (29). A 2019 study of umbilical cord milking was halted early, with 474 infants enrolled, because extremely preterm infants (23–27 weeks of gestation) in the cord milking arm more often developed intraventricular hemorrhage compared with similar infants in the delayed cord clamping group (30). Given this most recent data, cord milking should not be used for extremely preterm infants (less than 28 weeks of gestation), and there is insufficient evidence to either support or refute umbilical cord milking in infants born at 32 weeks of gestation or more, including term infants.

Benefits of Delayed Cord Clamping

- Preterm:
  - improved transitional circulation
  - better establishment of red blood cell volume
  - decreased need for blood transfusion
  - lower incidence of necrotizing enterocolitis and intraventricular hemorrhage

- Term:
  - increases hemoglobin levels at birth and improves iron stores in the first several months of life
  - Favorable effects of developmental outcomes

“Delayed” Cord Clamping

Old View

- Delayed resuscitation

New View

- Support during transition
- Can focus on temperature control and stimulating spontaneous respiration

Table 1. Clinical Situations in Which Immediate Umbilical Cord Clamping Should Be Considered or Care Should Be Individualized

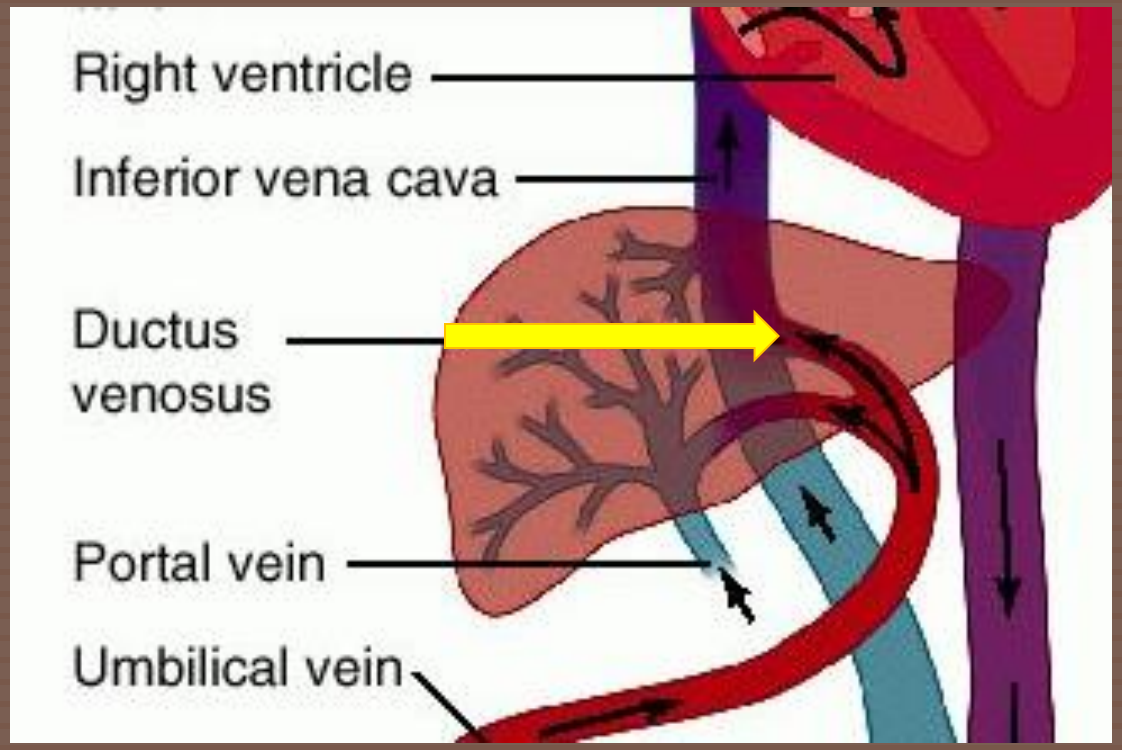
Maternal	Hemorrhage, hemodynamic instability, or both Abnormal placentation (previa, abruption)
Fetal/neonatal	Need for immediate resuscitation Placental circulation not intact (abruption, previa, cord avulsion, IUGR with abnormal cord Doppler evaluation)

Abbreviation: IUGR, intrauterine growth restriction.

What about during Cesarean Delivery?

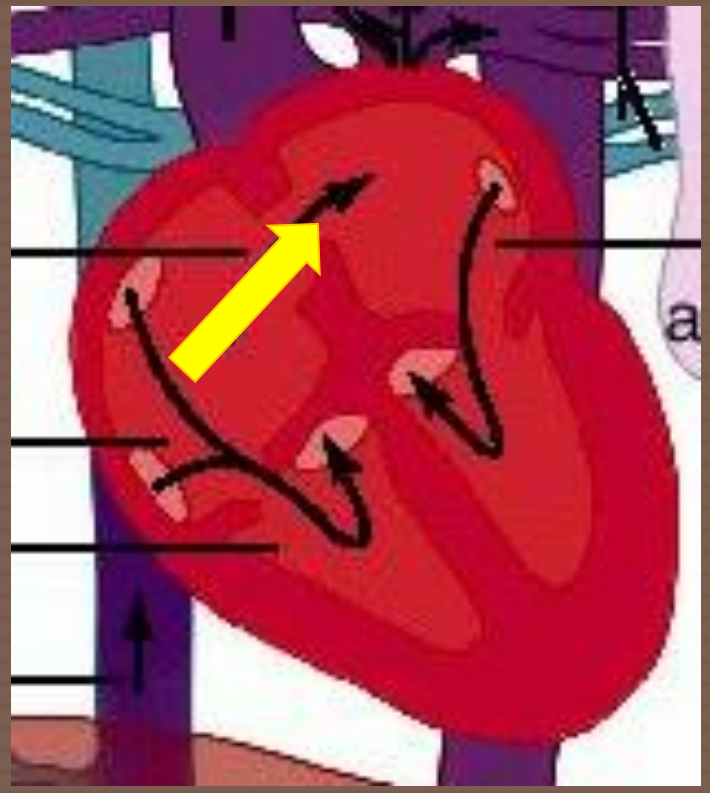
Closure of Ductus Venosus

- In late pregnancy, only ~20% flows through the DV
- Functionally closed within minutes of birth with clamping of the umbilical cord
- Anatomically closed 7-14 days
- Becomes Ligamentum venosus



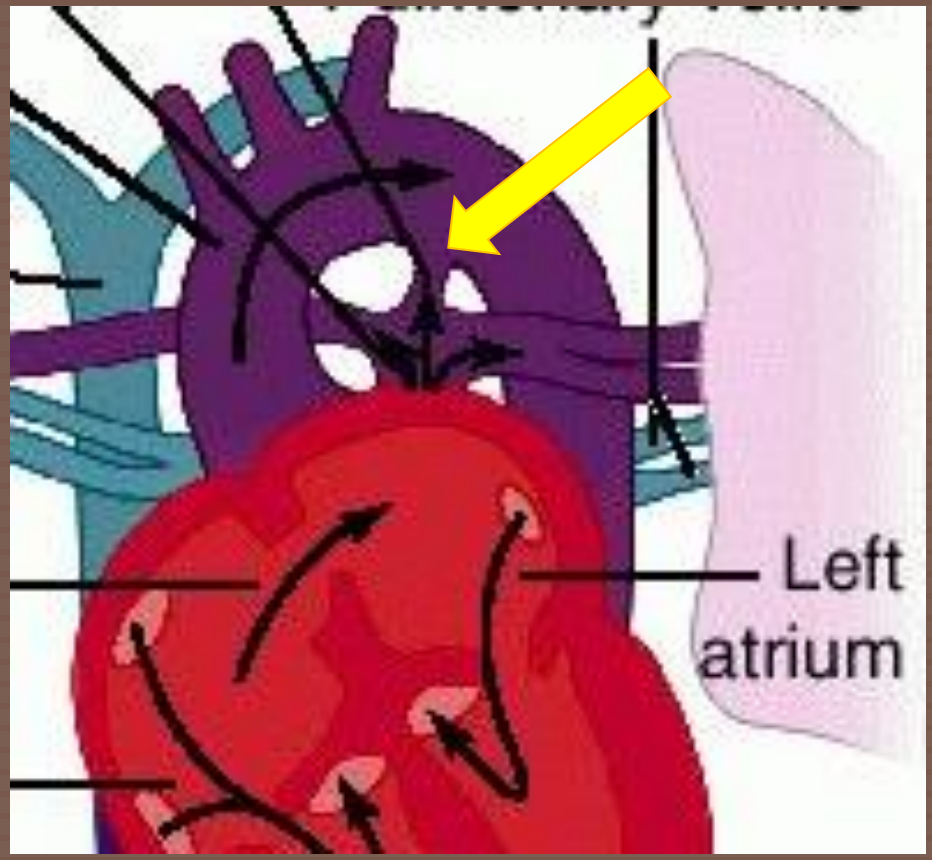
Closure of the Foramen Ovale

- After clamping the cord, left atrial pressure rises above that of right atrial pressure and the flap valve closes
- Anatomically closed by 1 month
- May remain patent in some individuals



Closure of the Ductus Arteriosus

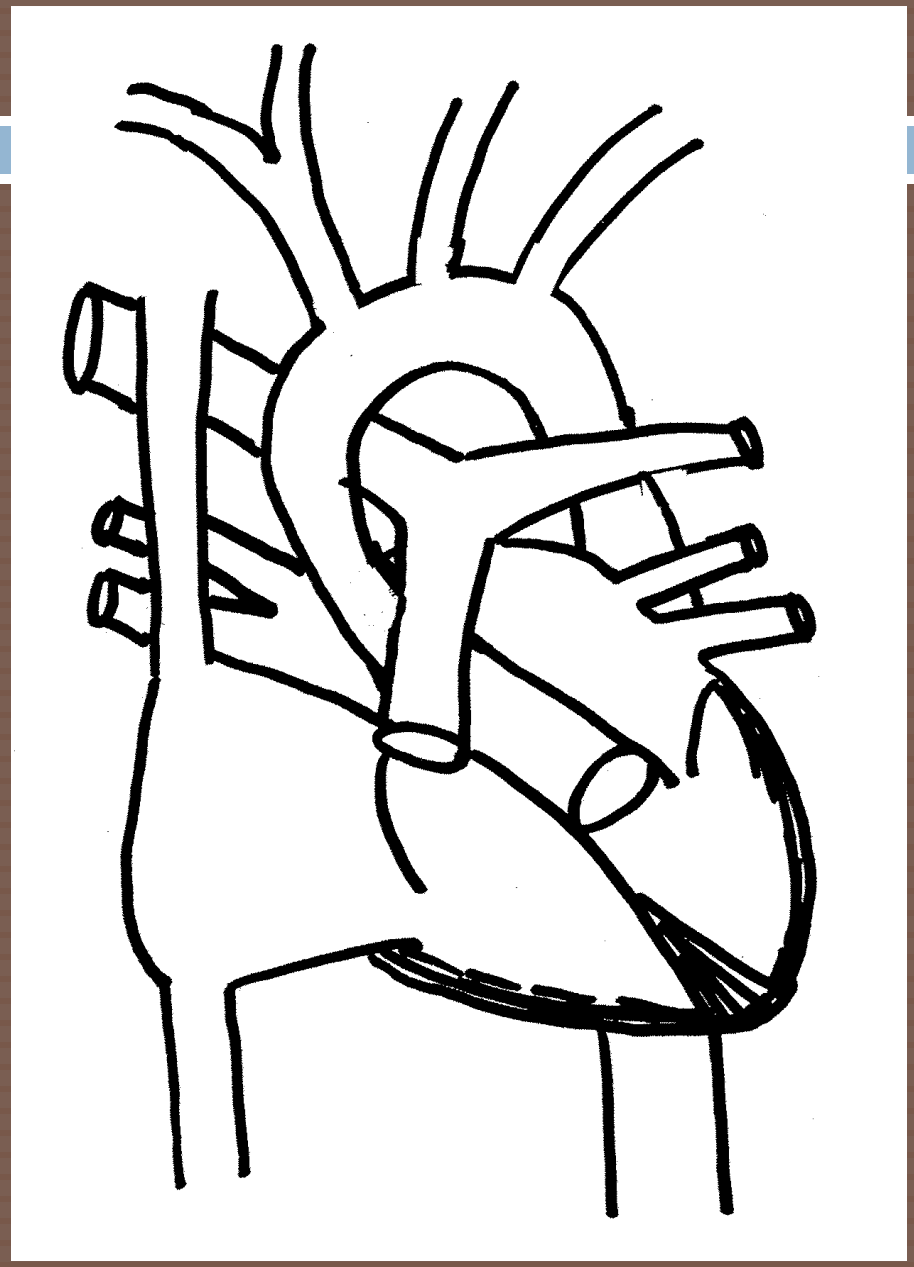
- Flow through the DA reverses due to increasing SVR
- Functionally closed by most at 12-14 hours of extrauterine life
  - Closed in 96 hours in nearly all infants
- Murmur may be auscultated during closure
- Anatomically closed at 2-3 months



Label the Heart

1. Right Common Carotid
2. Left Common Carotid
3. Right Subclavian
4. Left Subclavian

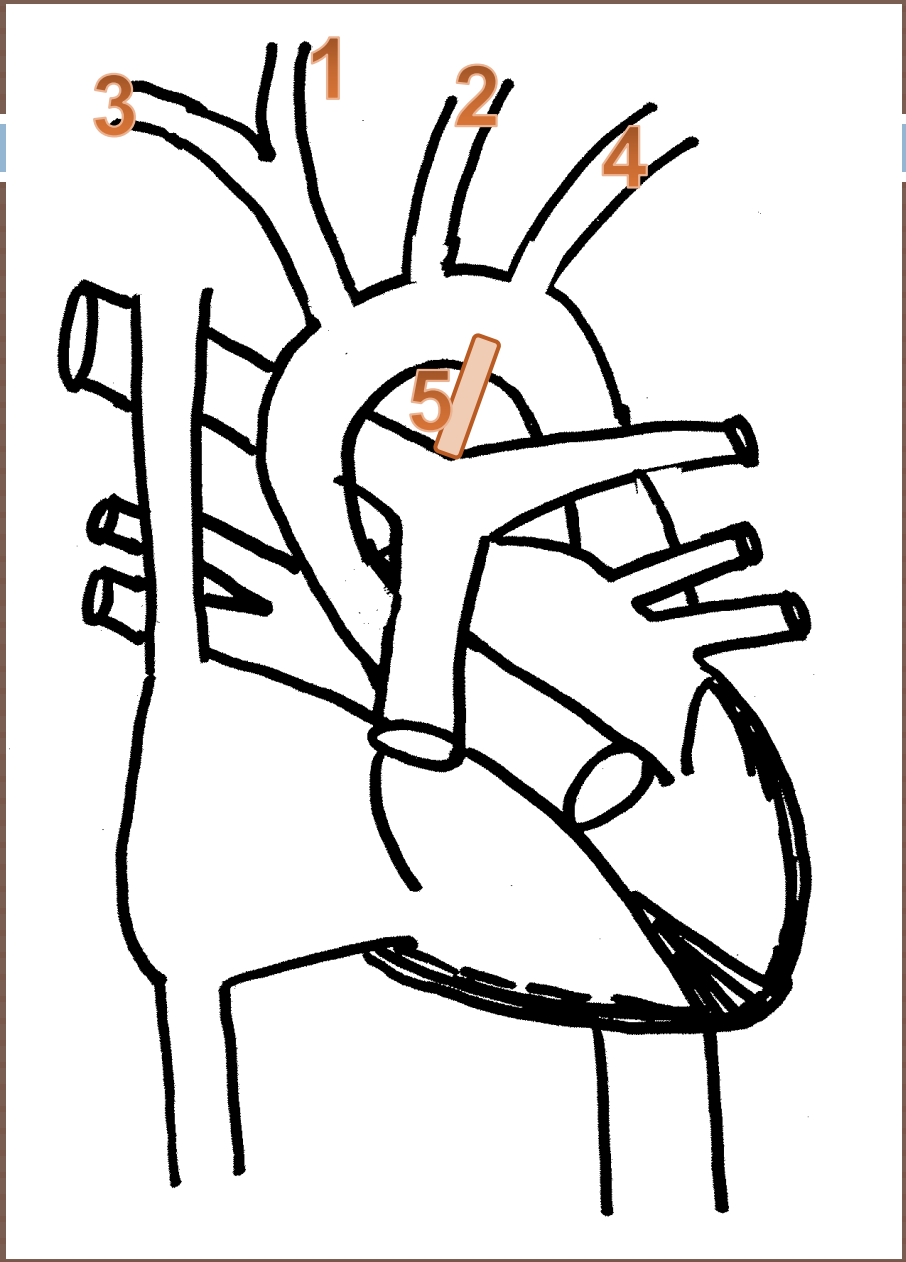
5. Ductus Arteriosus



Label the Heart

1. Right Common Carotid
2. Left Common Carotid
3. Right Subclavian
4. Left Subclavian

5. Ductus Arteriosus

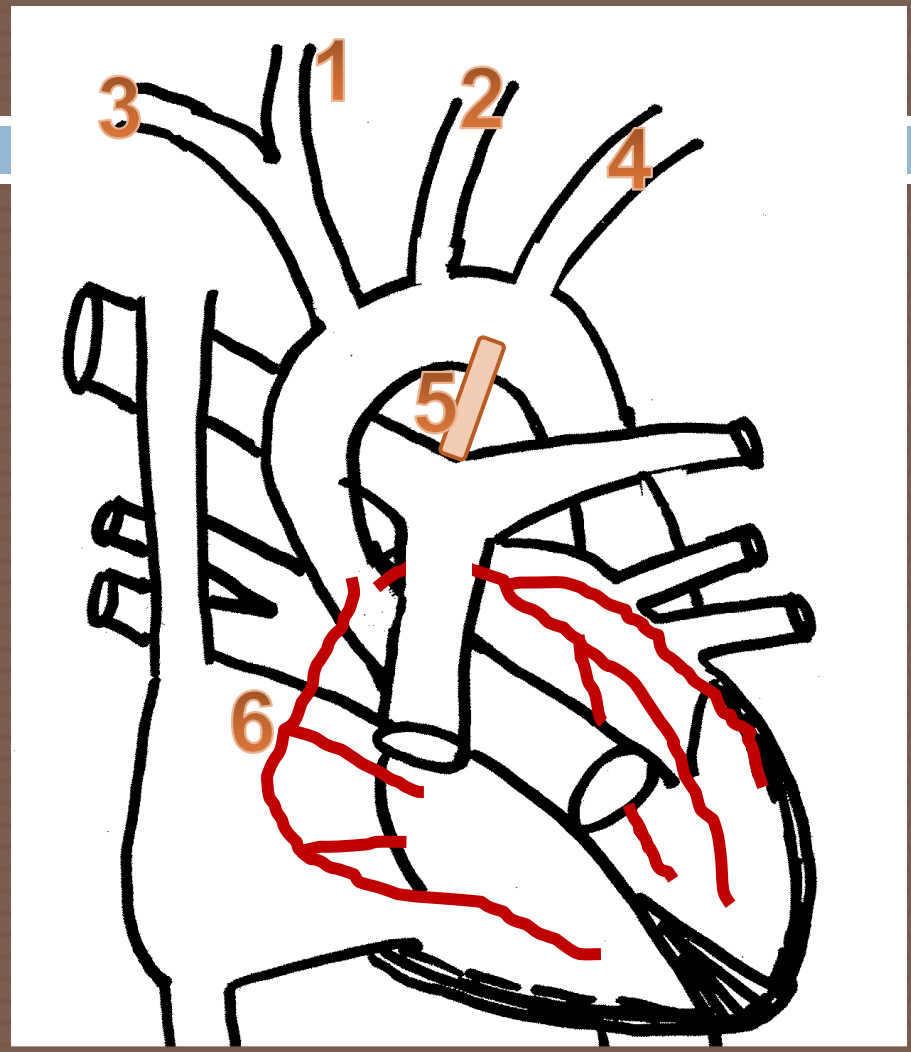


Label the Heart

1. Right Common Carotid
2. Left Common Carotid
3. Right Subclavian
4. Left Subclavian

5. Ductus Arteriosus

6. Coronary Arteries
   - Most oxygenated blood
   - HR good indicator of O_2 status



Transition of the Respiratory System

- First breath is extremely critical in all of transition processes
  - Must occur within seconds of placental separation
  - Interdependent with cardiovascular events
  - Only term, healthy babies can do this well
- Lungs begin absorbing fluid as opposed to secreting fluid
  - Begins in early labor
  - Expedited by increased pulmonary blood flow

8th IFAC Symposium on Biological and Medical Systems
The International Federation of Automatic Control
August 29-31, 2012. Budapest, Hungary

Analysis of the First Cry of the Newborns in Case of Vaginal Delivery and Caesarean Section

Zita Makoi *, Gyorgy Takacs *

**Budapest, Hungary; (e-mail: zita.makoi@gmail.com).*
***Peter Pazmany Catholic University Budapest, Hungary, (e-mail: takacs.gyorgy@itk.ppke.hu)*

Abstract: Nearly all of the newborns are coming into the world with crying. The first cry carries significant information about the baby's wellbeing, about his successful adaptation to the extrauterin life. The quality of first cry is a part of the Apgar score system which is used to assess the condition of newborns after delivery. The powerful first cry can carry the message for parents about the arrival of a healthy baby or the feeble cry immediately arises anxiety. In many countries the number of caesarean section has been increasing. The differences in the first cry can represent a subtle parameter which can reflect the start of life in a different way. The first cry samples of 10 vaginally born babies and 10 babies born by caesarean section were analysed. The analysis took place by subjective test with the participation

8th IFAC Symposium on Biological and Medical Systems
The International Federation of Automatic Control
August 29-31, 2012. Budapest, Hungary

Analysis of the First Cry of the Newborns in Case of Vaginal Delivery and Caesarean Section

Zita Makoi *, Gyorgy Takacs *

**Budapest, Hungary; (e-mail: zita.makoi@gmail.com).*
***Peter Pazmany Catholic University Budapest, Hungary, (e-mail: takacs.gyorgy@itk.ppke.hu)*

Abstract: Nearly all of the newborns are coming into the world with crying. The first cry carries significant information about the baby's wellbeing, about his successful adaptation to the extrauterin life. The quality of first cry is a part of the Apgar score system which is used to assess the condition of newborns after delivery. The powerful first cry can carry the message for parents about the arrival of a healthy baby or the feeble cry immediately arises anxiety. In many countries the number of caesarean section has been increasing. The differences in the first cry can represent a subtle parameter which can reflect the start of life in a different way. The first cry samples of 10 vaginally born babies and 10 babies born by caesarean section were analysed. The analysis took place by subjective test with the participation

8th IFAC Symposium on Biological and Medical Systems
The International Federation of Automatic Control
August 29-31, 2012. Budapest, Hungary

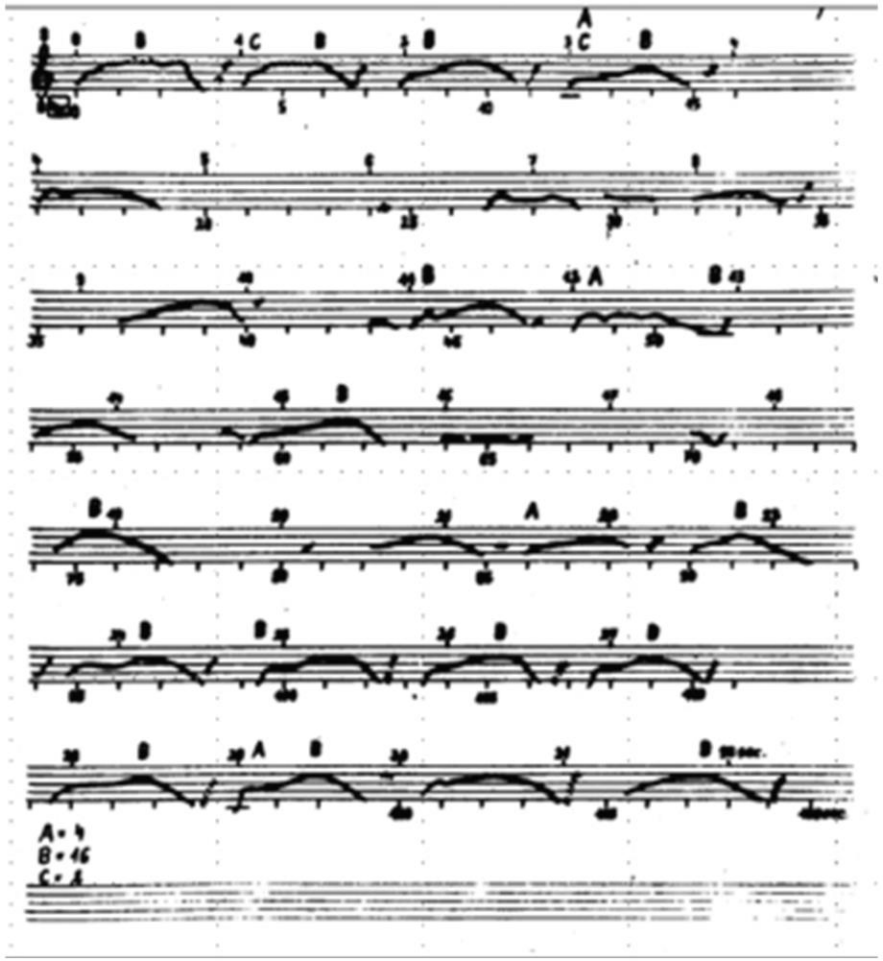
Analysis of the First Cry of the Newborns in Case of Vaginal Delivery and Caesarean Section

Zita Makoi *, Gyorgy Takacs *

**Budapest, Hungary; (e-mail: zita.makoi@gmail.com).*
***Peter Pazmany Catholic University Budapest, Hungary, (e-mail: takacs.gyorgy@itk.ppke.hu)*

Abstract: Nearly all of the newborns are coming into the world with crying. The first cry carries significant information about the baby's wellbeing, about his successful adaptation to the extrauterin life. The quality of first cry is a part of the Apgar score system which is used to assess the condition of newborns after delivery. The powerful first cry can carry the message for parents about the arrival of a healthy baby or the feeble cry immediately arises anxiety. In many countries the number of caesarean section has been increasing. The differences in the first cry can represent a subtle parameter which can reflect the start of life in a different way. The first cry samples of 10 vaginally born babies and 10 babies born by caesarean section were analysed. The analysis took place by subjective test with the participation

4.2 *Analysis of first cry in case of delivery by natural wa*
ınd by caesarean section



Available online at www.sciencedirect.com

ScienceDirect

Speech Communication 77 (2016) 28–52

www.elsevier.com/locate/specom

Cry-based infant pathology classification using GMMs

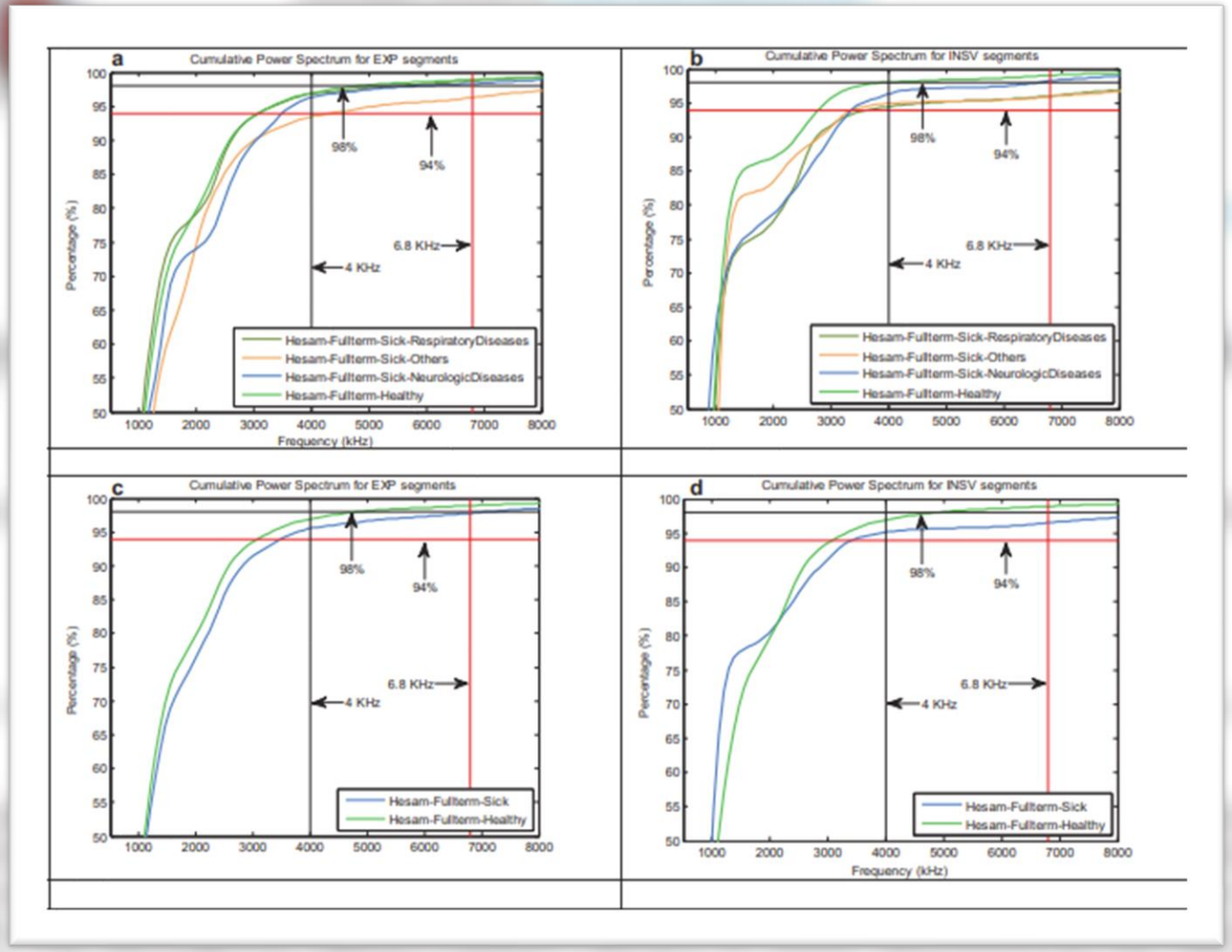
Hesam Farsaie Alaie[1,*], Lina Abou-Abbas, Chakib Tadj

MMS Lab, Department of Electrical Engineering, École de Technologie Supérieure, Université du Québec, 1100 rue Notre-Dame Ouest, Montréal, QC, Canada, H3C 1K3

Received 1 February 2015; received in revised form 13 October 2015; accepted 2 December 2015
Available online 11 December 2015

Abstract

Traditional studies of infant cry signals focus more on non-pathology-based classification of infants. In this paper, we introduce a noninvasive health care system that performs acoustic analysis of unclean noisy infant cry signals to extract and measure certain cry characteristics quantitatively and classify healthy and sick newborn infants according to only their cries. In the conduct of this newborn cry-based diagnostic system, the dynamic MFCC features along with static Mel-Frequency Cepstral Coefficients (MFCCs) are selected and extracted for both expiratory and inspiratory cry vocalizations to produce a discriminative and informative feature vector. Next, we create a unique cry pattern for each cry vocalization type


a
Cumulative Power Spectrum for EXP segments
Percentage (%)
Frequency (kHz)
98%
94%
6.8 KHz
4 KHz
Hesam-Fullterm-Sick-RespiratoryDiseases
Hesam-Fullterm-Sick-Others
Hesam-Fullterm-Sick-NeurologicDiseases
Hesam-Fullterm-Healthy
b
Cumulative Power Spectrum for INSV segments
Percentage (%)
98%
94%
6.8 KHz
4 KHz
Hesam-Fullterm-Sick-RespiratoryDiseases
Hesam-Fullterm-Sick-Others
Hesam-Fullterm-Sick-NeurologicDiseases
Hesam-Fullterm-Healthy
c
Cumulative Power Spectrum for EXP segments
Percentage (%)
98%
94%
6.8 KHz
4 KHz
Hesam-Fullterm-Sick
Hesam-Fullterm-Healthy
d
Cumulative Power Spectrum for INSV segments
Percentage (%)
Frequency (kHz)
98%
94%
6.8 KHz
4 KHz
Hesam-Fullterm-Sick
Hesam-Fullterm-Healthy

the iterative formula can be derived to adapt the model parameters. The adapted parameters, $\hat{\lambda}_K = (\hat{c}_i, \hat{\Phi}_i,\ i = 1, \ldots.., K)$, can be estimated in the $(n+1)$th[th] equation as follows:

$$w^n(X_t) = \frac{f_k\left(X_t \middle| \Phi_k^{(n)}\right)}{c_k^n f_k\left(X_t \middle| \Phi_k^{(n)}\right) + \left(1 - c_k^n\right) F_{k-1}\left(X_t \middle| \lambda_{k-1}\right)} = \frac{f_k\left(X_t \middle| \Phi_k^{(n)}\right)}{F_k\left(X_t \middle| \lambda_k\right)}$$

$$\gamma_t\left(\Phi_k^{(n)}\right) = \frac{w^n\left(X_t\right)}{\sum_{t=1}^{T} w^n\left(X_t\right)}$$

$$\hat{c}_k^{n+1} = \frac{1}{T} \sum_{t=1}^{T} \hat{c}_k^n w^n\left(X_t\right)$$

$$\hat{\mu}_k^{n+1} = \sum_{t=1}^{T} \gamma_t\left(\Phi_k^{(n)}\right).X_t$$

$$\hat{\Sigma}_k^{n+1} = \sum_{t=1}^{T} \gamma_t\left(\Phi_k^{(n)}\right).\left(X_t - \hat{\mu}_k^{n+1}\right)\left(X_t - \hat{\mu}_k^{n+1}\right)^{Tr} \qquad (11)$$

The First Cry

- Bottom line: it matters

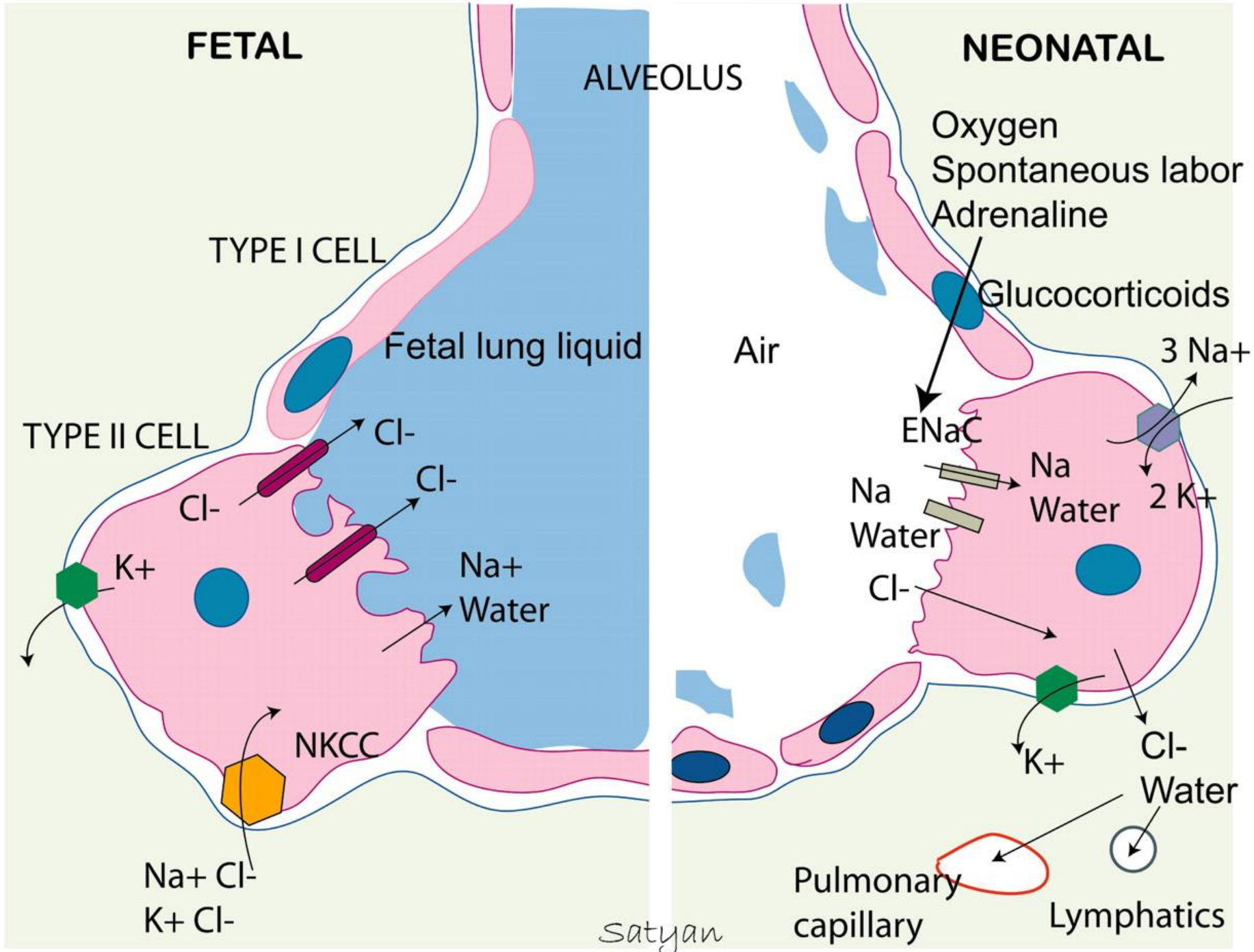
↑ fluid clearance

↑ gas exchange

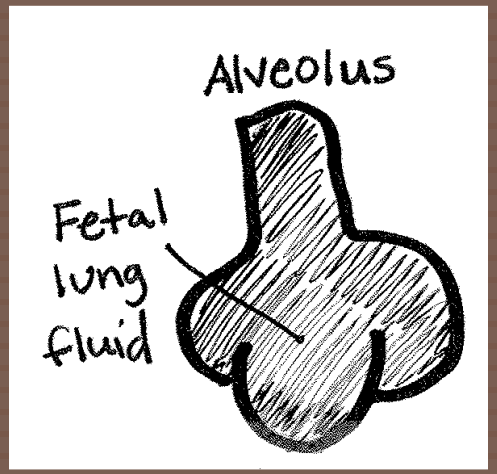
↑ PaO_2

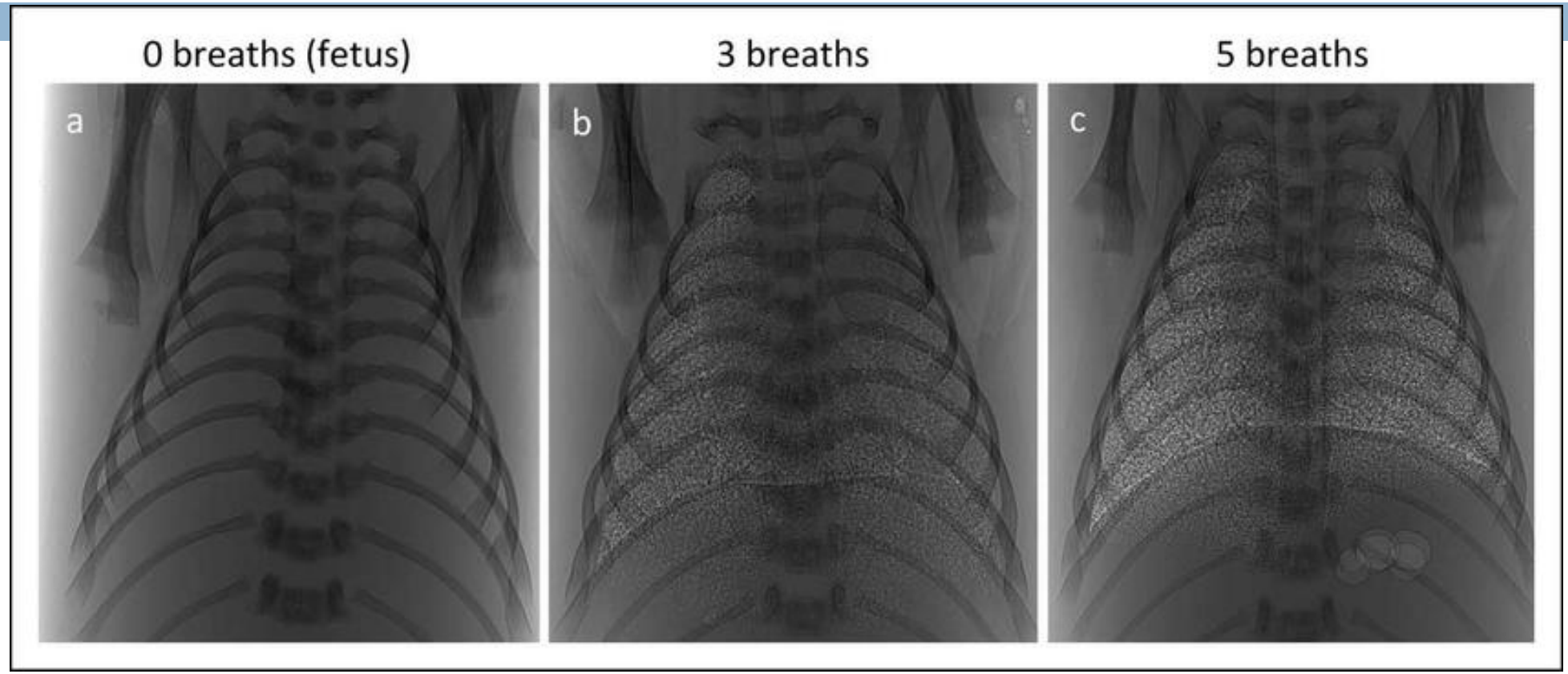
↓ PVR

↑ pulmonary blood flow


FETAL
ALVEOLUS
NEONATAL
TYPE I CELL
Fetal lung liquid
Air
Oxygen
Spontaneous labor
Adrenaline
Glucocorticoids
TYPE II CELL
Cl-
Cl-
Cl-
K+
Na+
Water
NKCC
Na+ Cl-
K+ Cl-
ENaC
3 Na+
Na
Water
Na
Water
2 K+
Cl-
K+
Cl-
Water
Pulmonary
capillary
Lymphatics
Satyan

Fetal Lung Fluid Clearance



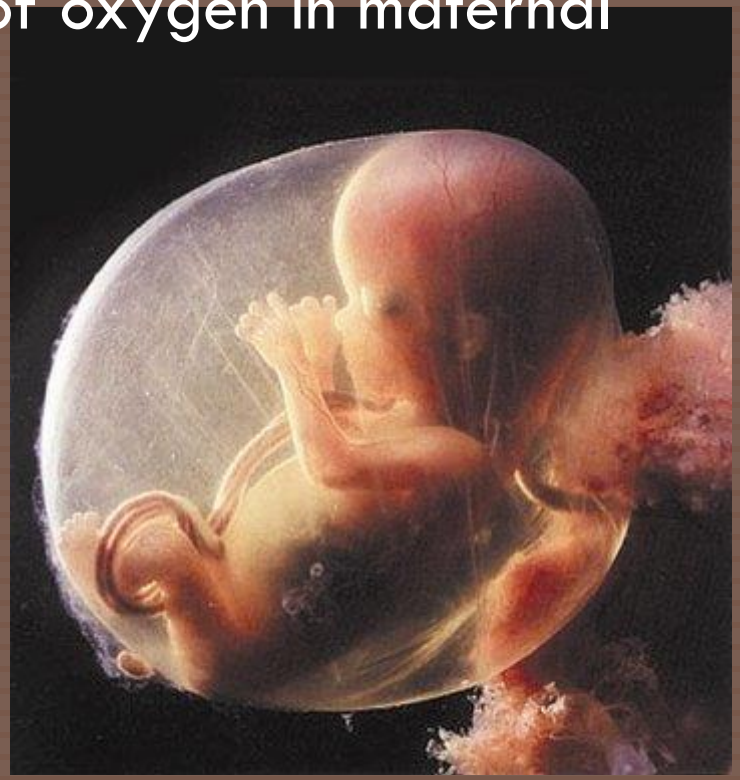


Hooper, Stuart & Pas, Arjan & Lewis, R. & Morley, Colin. (2010). Establishing Functional Residual Capacity at Birth. NeoReviews. 11. e474-e483. 10.1542/neo.11-9-e474.

Let's talk about Oxygen . . .

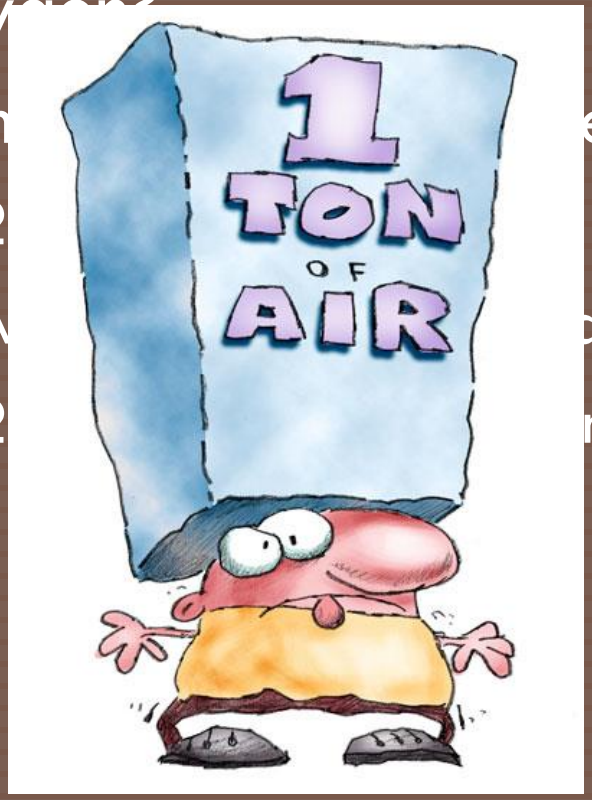
- Where does the fetus get his oxygen from?
- What is the partial pressure of oxygen in maternal mixed venous blood?
  - About 40 mmHg
  - Placenta takes some,

  fetus gets about 30-35 mmHg
- What is the fetal SpO2?
  - About 60%



Let's talk about Oxygen . . .

- After birth, where does the newborn get his oxy
- Wh essure of oxygen in room air?
  - 2
  - A at
  - 2 mm



FETUS

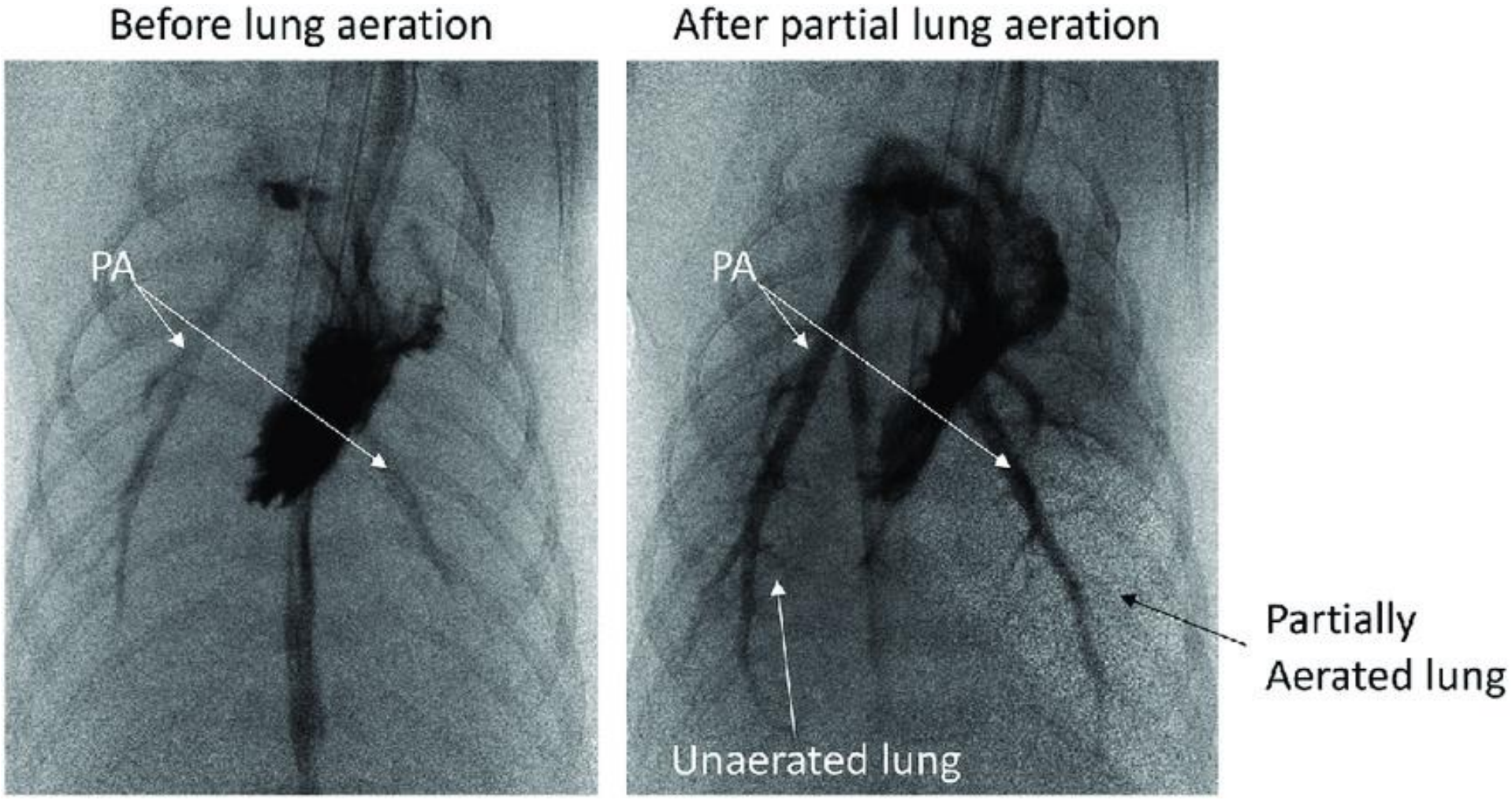
- Source of oxygen:
  - Umbilical Artery
- PaO2 = ?
  - **30-35 mmHg**

NEWBORN

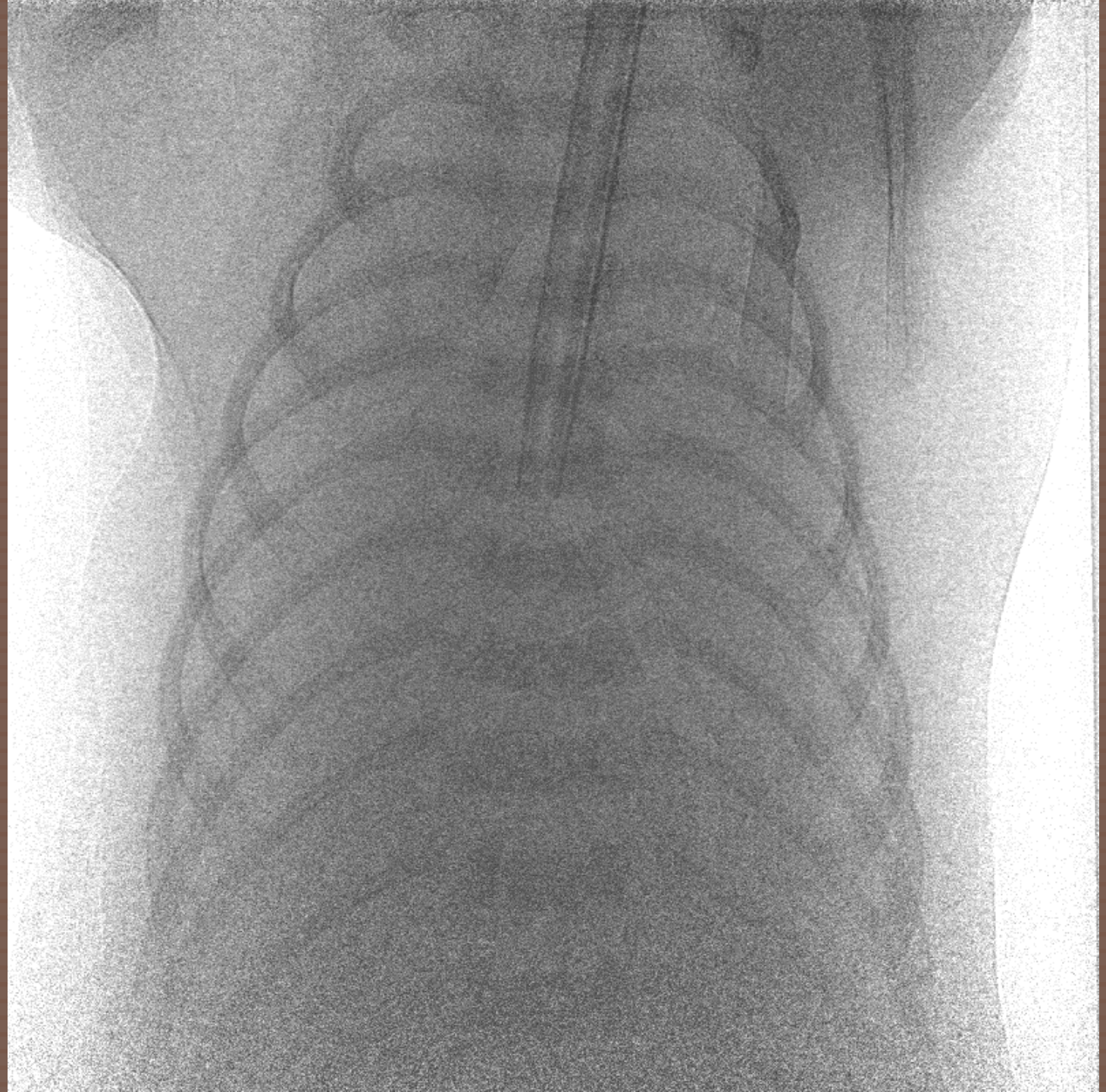
- Source of oxygen:
  - Room Air
- PaO2 = ?
  - **160 mm HG**

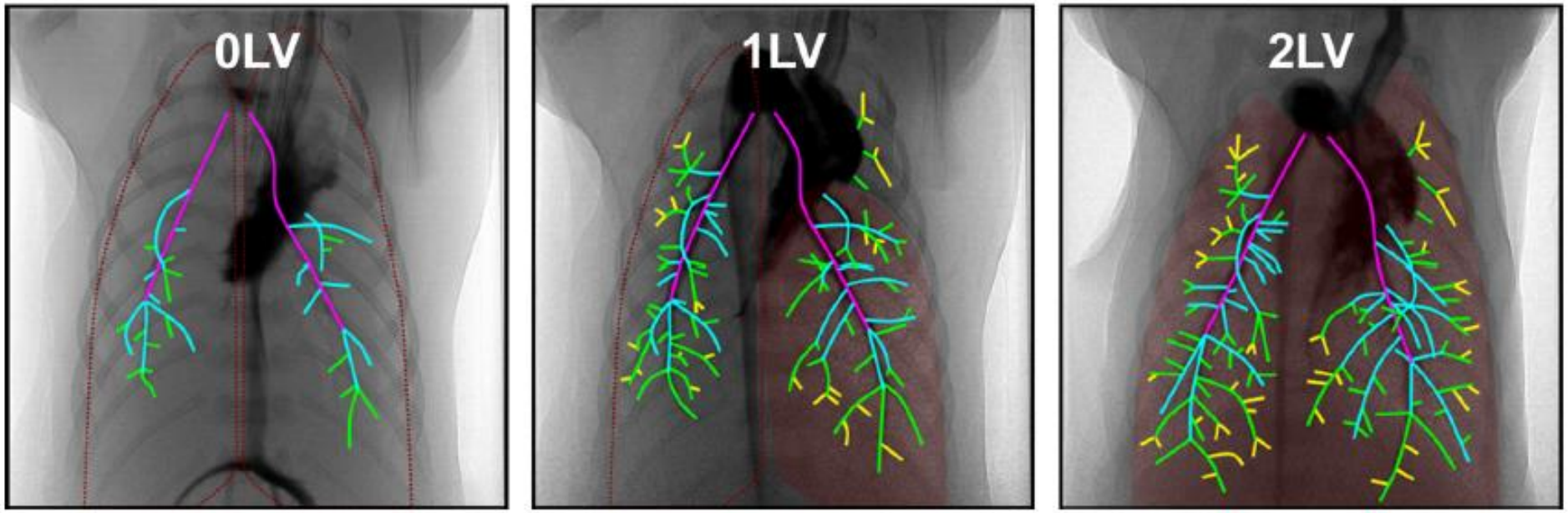
The newborn doesn't necessarily need supplemental oxygen . . .

VENTILATION



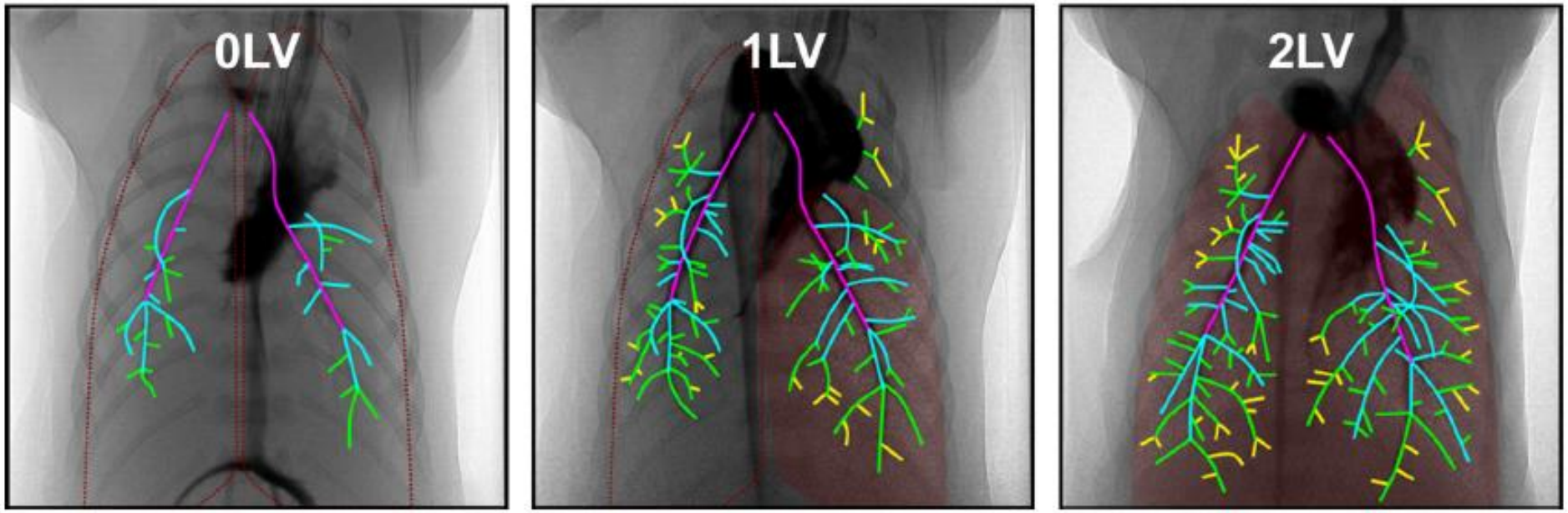
Hooper, Stuart & Roberts, Calum & Dekker, Janneke & Pas, Arjan. (2019). Issues in cardiopulmonary transition at birth. Seminars in Fetal and Neonatal Medicine. 24. 101033. 10.1016/j.siny.2019.101033.



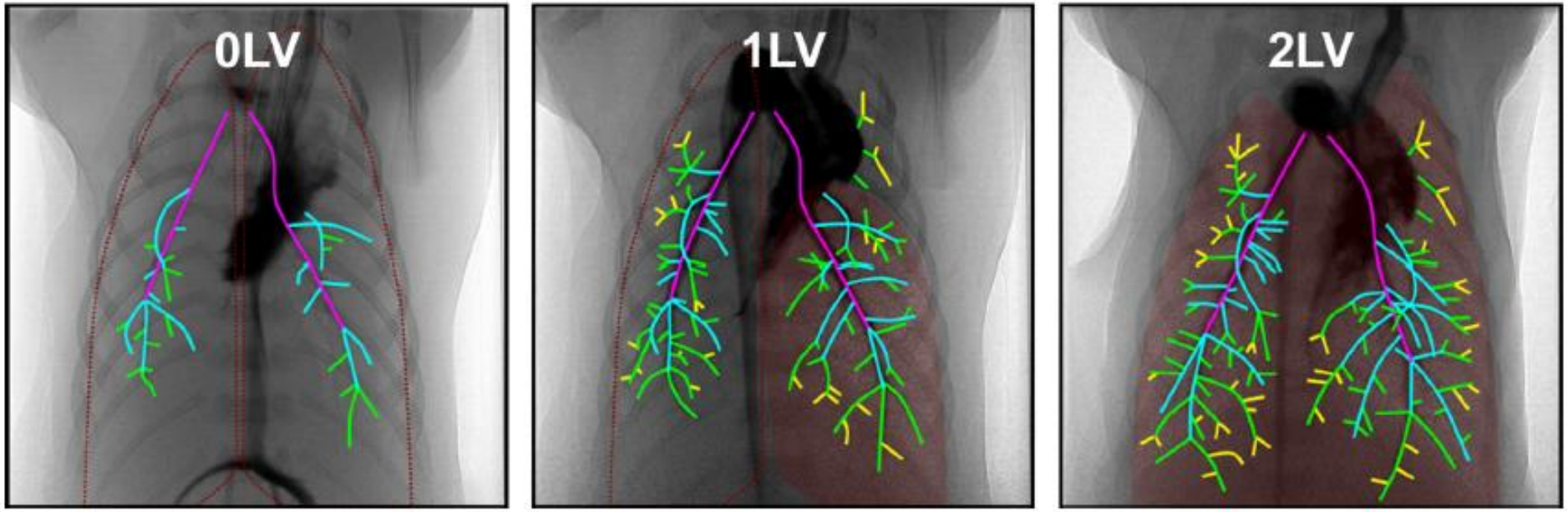


Traced overlay of all visible blood vessels (filled with iodine) superimposed over the X-ray images of the lung acquired before ventilation onset (0LV), during unilateral ventilation of the right lung (1LV), and during ventilation of both lungs (2LV) in the same kitten. Outlined are the approximate boundaries of nonaerated regions of the lungs (red dotted line) and aerated regions of the lungs (solid red background).

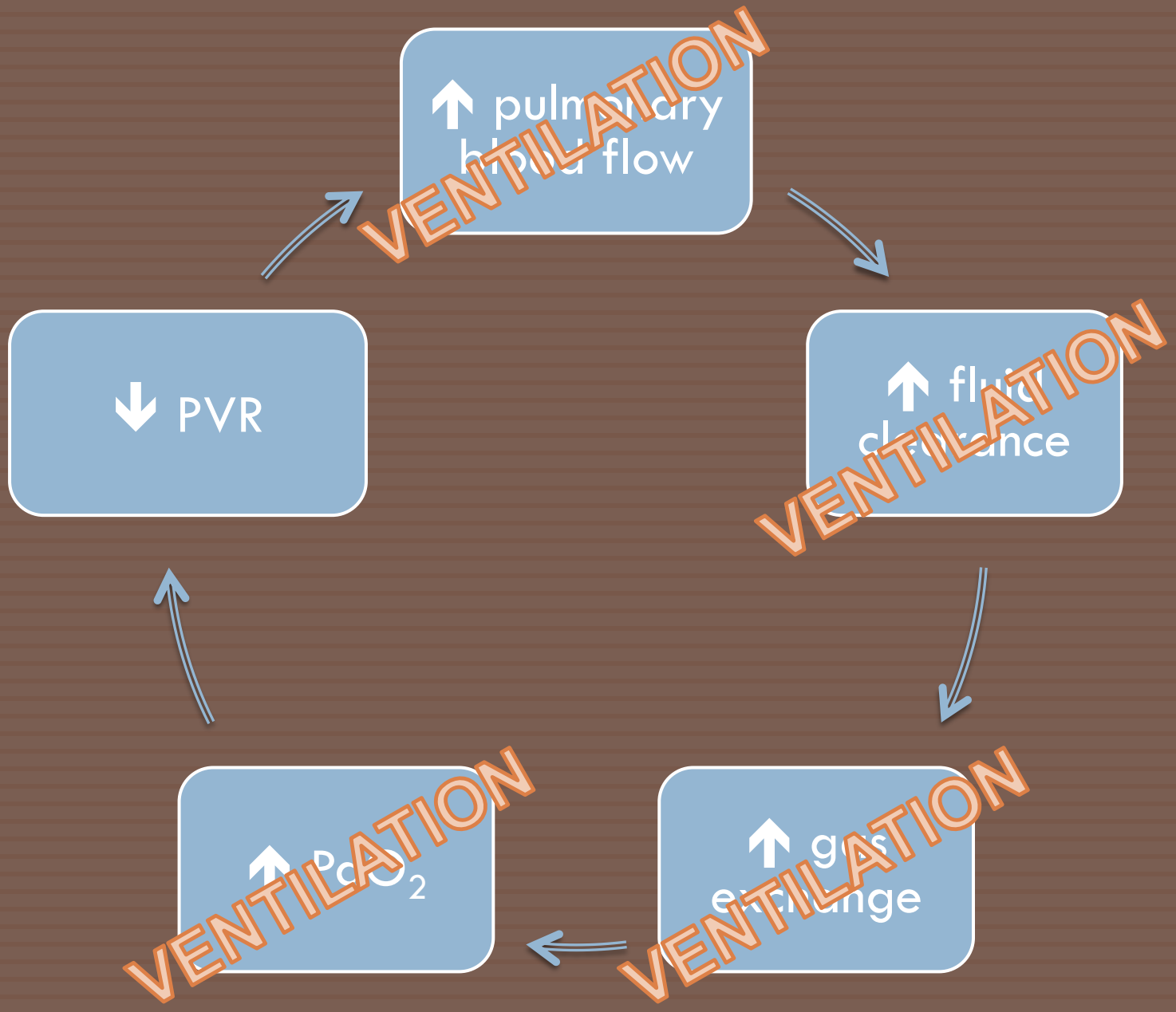
- **L&R pulmonary arteries**
- **1st generation branches**
- **2nd generation branches**
- **3rd generation branches**



Stuart Hooper: “The message is quite simple. That is, **lung aeration** stimulates the increase in pulmonary blood flow at birth and even if only part of the lung is aerated, pulmonary blood flow increases even in both aerated and unaerated lung regions.”



. . . aerating the lung and increasing pulmonary blood flow **before umbilical cord clamping** (known as physiological based cord clamping), can avoid the loss of preload and reduction in cardiac output that normally accompanies immediate cord clamping

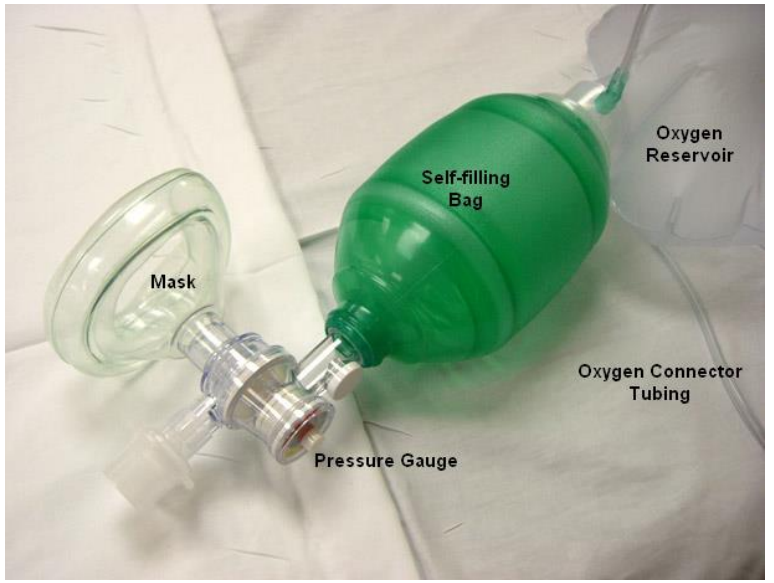

↑ pulmonary blood flow
VENTILATION
↑ fluid clearance
VENTILATION
↑ gas exchange
VENTILATION
↑ PaO_2
VENTILATION
↓ PVR

VENTILATION

$$V = a + b$$

bag valve mask (bvm)

- valve between mask and bag
- can provide positive pressure ventilation (PPV)
- a pressure relief valve prevents overinflation of the lungs

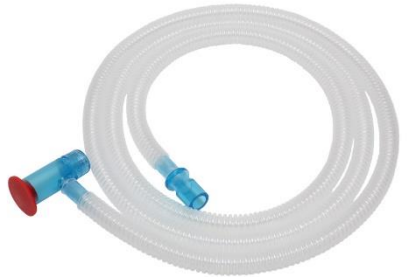


flow-inflating bag

- needs a source of gas to inflate
- prolonged inflation can be provided
- can provide PEEP (but inconsistent)
- danger to give too high a volume

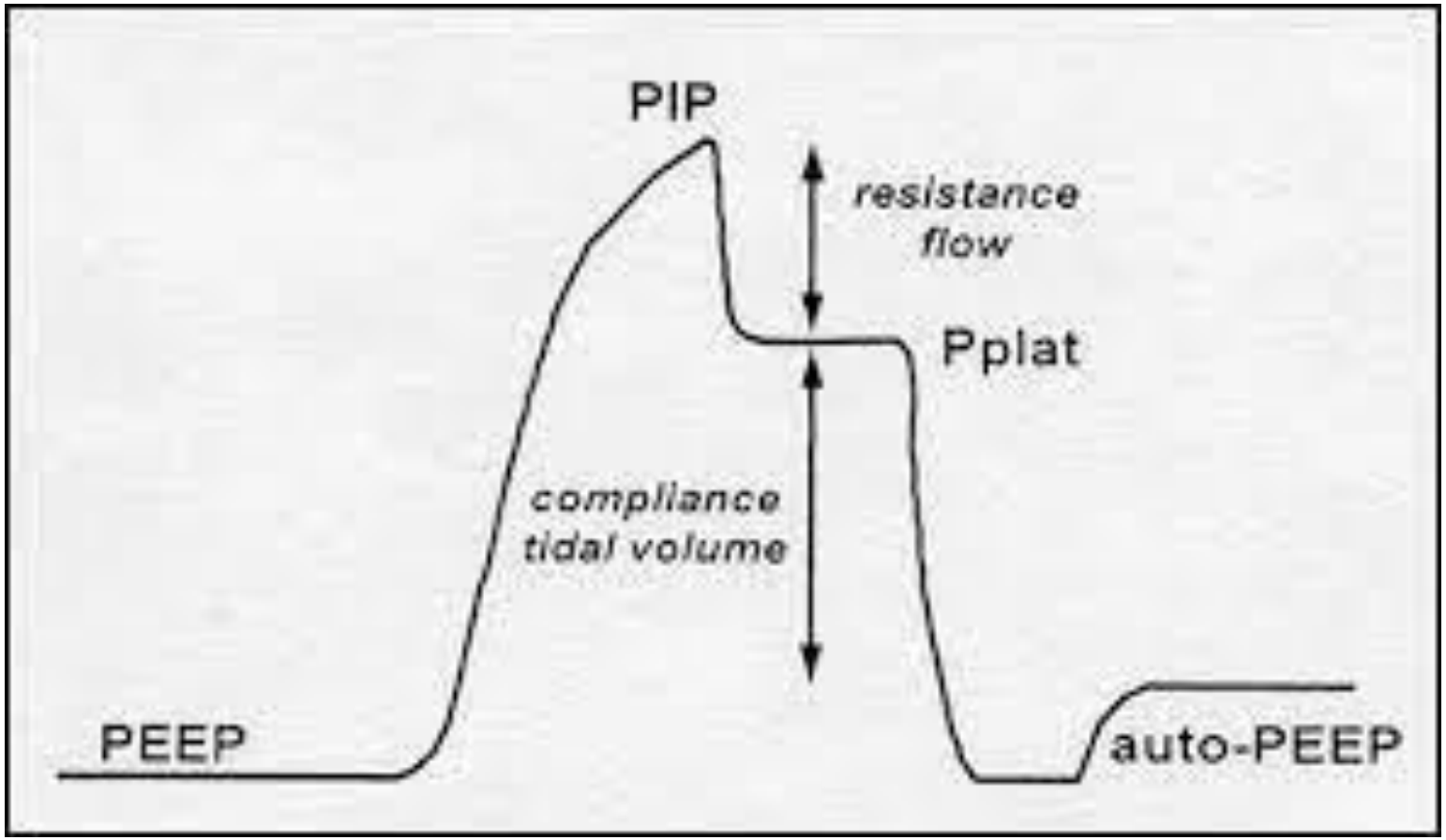
T-piece devices

- gas flows through a T-piece
- can give PPV and be adjusted to a set PIP and PEEP
- good device to give CPAP to a breathing infant
- Neo-Tee is disposable, easily transported
- Neopuff is bigger and more cumbersome




I
O
L/min
% O2
L/min

PEEP vs PIP



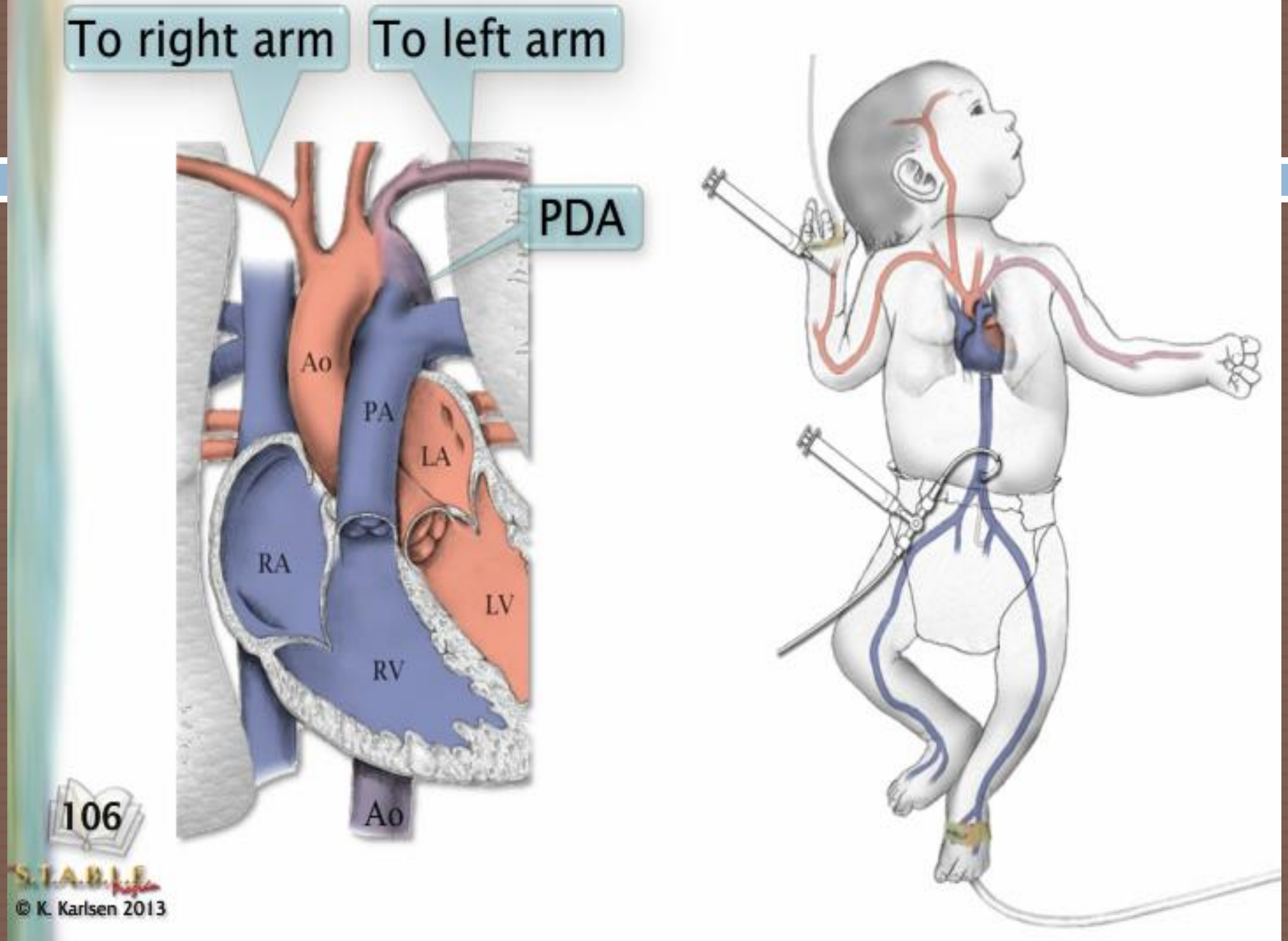
Positive **E**nd **E**xpiratory **P**ressure

Peak **I**nspiratory **P**ressure

CPAP

- **C**ontinuous **P**ositive **A**irway **P**ressure (start at 5 cm h2o)
- Usually delivered at equivalent of PEEP to maintain PEEP
- Prevents collapse of alveoli following expiration
- Not delivered with PIP, patient must be breathing spontaneously with a HR > 100

How do you know if the newborn needs oxygen?


To right arm
To left arm
PDA
Ao
PA
LA
RA
LV
RV
Ao
106
S.T.A.B.L.E.
© K. Karlsen 2013

Normal SpO2 Values

1 minute: 60%

3 minutes: 70%

5 minutes: 80%

10 minutes: 90%

Normal SpO2 Values

1 minute: 60%

3 minutes: 70%

5 minutes: 80%

10 minutes: 90%

Oxygen for ≥ 35 weeks

- Recommendations—Updated 2019
- In term and late-preterm newborns (≥35 weeks of gestation) receiving respiratory support at birth, the initial use of 21% oxygen is reasonable (Class 2a; Level of Evidence B-R).

- 100% oxygen should not be used to initiate resuscitation because it is associated with excess mortality (Class 3: Harm; Level of Evidence B-R).

Escobedo, et al. (2019). American Heart Association Focused Update on Neonatal Resuscitation: An Update to the American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care, *Circulation*, 140(24),

Oxygen for <35 weeks

- Recommendations—Updated 2019
- In preterm newborns (<35 weeks of gestation) receiving respiratory support at birth, it may be reasonable to begin with 21% to 30% oxygen with subsequent oxygen titration based on pulse oximetry (Class 2b; Level of Evidence C-LD).

Escobedo, et al. (2019). American Heart Association Focused Update on Neonatal Resuscitation: An Update to the American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care, *Circulation*, 140(24),

Neonatal Ventilation Videos

- PPV: https://youtube.com/watch?v=aAlMreEBKYU
- PPV: https://bcove.video/35Gb65Z
- T-Piece: https://bcove.video/3spLBj6
- T-piece set-up: https://bcove.video/2LqsTr4
- Heart Rate Assessment during PPV: https://bcove.video/3oLt6TQ
- MR SOPA: https://bcove.video/3soVBJf
- CPAP: https://www.youtube.com/watch?v=Wo-YgHTzees

References

Hooper, Stuart & Roberts, Calum & Dekker, Janneke & Pas, Arjan. (2019). Issues in cardiopulmonary transition at birth. Seminars in Fetal and Neonatal Medicine. 24. 101033. 10.1016/j.siny.2019.101033.

Askin, D.F. (2002). Complications in the transition from fetal to neonatal life. *JOGNN, 31*(3), 318-327.

Jain, L., & Eaton, D.C. (2006). Physiology of fetal lung fluid clearance and the effect of labor. *Seminars in Perinatology*, 30, 34-33.

Part 5: Neonatal Resuscitation 2020 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Khalid Aziz, et al. Pediatrics Jan 2021, 147 (Supplement) e2020038505E; DOI: 10.1542/peds.2020-038505E

References

Blackburn, S.T. (2013). *Maternal, fetal, and neonatal physiology: A clinical perspective* (4th ed.). St. Louis: Mosby.

Escobedo, et al. (2019). American Heart Association Focused Update on Neonatal Resuscitation: An Update to the American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care, *Circulation*, 140(24), e922–e930. DOI: https://doi.org/10.1161/CIR.0000000000000729

Sansoucie, D.A., & Cavaliere, T.A. (1997). Transition from fetal to extrauterine circulation. *Neonatal Network, 16*(2), 5-12.

Delayed umbilical cord clamping after birth. ACOG Committee Opinion No. 814. American College of Obstetricians and Gynecologists. Obstet Gynecol 2020;136:e100–6.