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Placental Transfusion: May the “Force” be with the Baby

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Key Words: placental transfusion, umbilical cord clamping, vascular perfusion, preterm infants

Abbreviations: DCC, delayed cord clamping; ICC, immediate cord clamping; PAP, pulmonary artery pressure.

Key Points:

1. Placental transfusion provides enhanced vascular perfusion and reduces the risk of death for preterm infants
2. Enhanced vascular perfusion stimulates endothelial cells to release vital angiocrine messengers to guide normal function and development of neonatal organs
3. High progesterone levels and pulmonary artery pressure in the first 12 hours may assist the newborn to adapt to the placental transfusion throughout the body
4. Blood volume conservation is important at birth and during the NICU stay for preterm infants

Running Title: Placental Transfusion and Force

2 **ABSTRACT**

Placental transfusion results in a significant decrease in the risk of death for extremely preterm infants.

4 With immediate cord clamping (ICC), these infants can leave up to one-half of their normal circulating *in utero* blood volume in the placenta. Extremely preterm infants are at highest risk of harm from immediate cord

6 clamping (ICC) yet are currently the most likely to receive ICC. Receiving a placenta transfusion provides infants with life-saving components and enhanced perfusion. We present some lesser known but important effects of

8 placental transfusion. New research reveals that enhanced vascular perfusion causes an organ's endothelial cells to release angiocrine responses to guide essential functions. High progesterone levels and pulmonary artery

10 pressure in the first few hours of life assist with neonatal adaptation. We propose that lack of essential blood volume may be a major factor contributing to inflammation, morbidities, and mortality that preterm infants

12 frequently encounter.

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“it is not widely appreciated that the dysfunction of the inner lining of blood vessels is the single most common cause of human mortality. Endothelial cells control their microenvironments as gate keepers of organ development, homeostasis, and tissue regeneration.”(1)

INTRODUCTION

Facilitation of placental transfusion has become the standard of care for newborns.(2) Emerging data from three systematic reviews reveal that the most striking benefit is a 27 to 30% lower death rate for preterm infants with ~60 seconds delay in clamping compared to those with immediate cord clamping (ICC).(3–5). A recent large (n = 4680) retrospective cohort study of extremely low gestational age neonates (ELGANS, ≤ 26 weeks’ gestational age at birth), reported lower rates of severe neurologic injury as well as death in the infants who had delayed cord clamping (DCC) compared to those with immediate cord clamping (ICC).(6) Placental transfusion has also resulted in better developmental outcomes at 18 and 24 months for preterm infants after a delay in cord clamping and/or milking of the cord.(7–9) No recent studies on delayed cord clamping have reported adverse outcomes although cord milking of infants under 28 weeks’ gestation is under question. Yet, the best time to clamp and cut the umbilical cord at birth remains unknown, and most tiny infants and those infants needing resuscitation still receive immediate cord clamping (ICC).

Placental transfusion at birth provides cardiovascular stability (10,11), a huge allotment of vital stem cells (12–14), a 50% increase in iron-rich red cell volume (15), a large quantity of neuroprotective progesterone(16–19), and many important messengers including cytokines, chemokines, proteins, growth factors, and other hemostasis facilitators essential for a normal transition.(12) Several excellent reviews about the benefits of placental transfusion are available.(20–22)

Although placental transfusion results in a 27-30% decrease in the chance of death for preterm infants, there is no agreement as to how and why receiving some of the residual placental blood volume reduces the

incidence of death. In this review we will not focus on the unanswered questions related to the clinical practice concerning placental transfusion. Instead, we will describe and discuss some important but lesser known effects of placental transfusion in the transition to extra-uterine life of preterm and term infants that may impact the infant's transition and suggest some ideas for addressing some of the most troublesome problems for infants.

BLOOD AS AN ESSENTIAL FACTOR FOR GROWTH AND REGENERATION

Science has not yet revealed exactly how and why placental transfusion reduces the incidence of death for premature infants. We suggest that recent work in biology, physiology, biometrics, and biophysics offers important new clues about how an enhanced blood volume may benefit newborns. The most plausible reason for the decreased incidence of death may be increased blood volume replete with all of its essential components that infants can receive with placental transfusion.(12,23)

The vascular system, a vast network of capillaries lined by endothelial cells, interconnects arteries and veins creating vascular beds in organs throughout the body – a prerequisite of organ development.(24,25) It is known that capillary endothelial cells are not merely passive conduits for the delivery of oxygen or nutrients, but also support organ development and organ regeneration through elaboration of tissue-specific paracrine growth factors. These factors, known as angiocrine factors, are secreted by the endothelial cells and are essential players in all physiologic processes.(26,27) Only recently has research shown that these angiocrine factors can be stimulated by mechanical forces alone.(28) Lorenz et al. have demonstrated that shear forces generated by dynamic blood flow appear to induce mechanical signals in endothelial cells causing release of important angiocrine factors essential for homeostasis, and for normal growth and development (Figure 1).(28)

In liver cells, Lorenz et al. demonstrated that enhanced vascular perfusion induces the proliferation of hepatocytes driving liver development. Using murine embryos, they noted that liver expansion was preceded by initial new blood perfusion in a highly controlled manner starting in the periphery and proceeding towards the center of the liver. They found higher levels of activated forms of two important angiocrine growth factors, β 1-integrin and VEGF-R3 (vascular endothelial growth factor receptor 3), in the periphery of the liver where there

2 was extensive blood perfusion and hyperproliferation of hepatocytes. In subsequent perfusion experiments,
they showed that activation of the angiocrine growth factors was reduced when blood perfusion was slowed by
4 decreasing the embryo's heartbeat and accelerated when the heart rate was increased (pharmacologically).
Next, they provided evidence (*ex vivo* and *in vitro*) that in human liver cells, mechanical stretching alone
6 triggered activation of the same growth factors. Remarkably, culturing human adult liver cells in medium
collected from stretched human liver cells resulted in increased proliferation and decreased apoptotic cell
8 death. The process responsible for these changes, known as *mechano-transduction*, is a mechanism by which
cells convert mechanical stimuli into electrochemical activity. This occurs in most if not all organs throughout the
10 body(24,25) Lorenz clearly demonstrated that enhanced vascular perfusion provides mechanical stimuli
(stretching, shear force) through vasodilation that stimulated organ-specific endothelial cells (ECs) to release
12 angiocrine factors that guide physiologic function and development of the liver (Figure 1).

INSERT FIGURE 1 ABOUT HERE

14 Although Lorenz's work supporting the importance of an ample blood volume was confined to
hepatocytes, advances in our understanding of one organ system can inform us about other systems.(24,29,30)
16 Many of the intercellular signaling networks that control the morphogenesis of the respiratory system are
similarly present in the development of other organs such as the kidney and liver.(29) While angiocrine factors
18 appear to be organ-specific, most seem to depend on mechano-transduction force from vasodilation. For
example, prolonged or severe hypoperfusion in preterm infants is one of the most common causes of acute
20 intrinsic kidney injury (AKI) often leading to tubular dysfunction and acute tubular necrosis.(31) While damage to
tubular epithelial cells is a hallmark of ischemic AKI, "damage to the inner most lining of the renal vascular
22 system, the endothelial cells, has a critical role in the initiation, extension, maintenance, and recovery phases of
AKI...in ischemic AKI, with restoration of renal blood flow, glomerular filtration rate can return promptly to
24 normal."(31) Most organs experience vasodilation—and thus endothelial cell (EC) stretching—when their
physiological function is extensively needed as they start to grow (Figure 1).(24). When damaged, ECs produce a

systemic inflammatory response which directly affects the brain, lung, heart, liver, bone marrow and GI tract.(32). In the presence of hypoperfusion, ECs may be unable to support expected functions including organ growth and development.

The benefits from placental transfusion most likely are related to the autotransfusion of the large volume of residual blood available to the newborn. It is probable that the enhanced blood flow generates mechanical forces within the microcirculation that initiate proper organ health and development.(1,28,30) Immediate (ICC) or early cord clamping (ECC) reduces potential blood volume for the neonate that may contribute to loss of organ-specific vascular competence in the lung, gastrointestinal tract, brain, kidney, and other organs.

EVIDENCE OF ENHANCED PERFUSION FROM PLACENTAL TRANSFUSION

Classic physiologic studies completed over the past 60 years document that placental transfusion results in improved perfusion in the neonate's hematologic, urinary, gastrointestinal, neurological, and respiratory systems.(33–38) The improved perfusion was demonstrated in a 1968 study in which heel capillaries were biopsied at 2 to 5 hours after birth from 12 neonates who had either ICC or DCC (when pulsations ceased).(39) As seen in Figure 2 (electron microscopy), the capillary on the left (2.A.) has a small, irregular lumen with thick endothelium while in the capillary on the right (2.B.) the lumen is large and full and the endothelium is thin with small fenestrations at the top allowing for maximal exchange of gases, nutrients and waste products. Pietra et al found significantly more capillaries resembling the undistended capillary in the neonates who received ICC and more resembling the distended capillary in the neonates who experienced DCC, suggesting better blood volume and perfusion with DCC.(39)

INSERT FIGURE 2 HERE

As heels are usually the last area of the body to be well perfused, it is likely that other more vital areas such as brain and heart are also better perfused. This graphic description of the effect of a full blood volume on vascular

perfusion in the heels of newborns could never be repeated today. Yet, it is important because it so clearly demonstrates the increased vascular perfusion that is a consequence of placental transfusion.

THE IMPACT OF ENHANCED PERFUSION ON FETAL-NEONATAL TRANSITION

We propose that Lorenz's work applies to the transition from fetus to neonate with a major role in inducing the beginning of normal organ development from the mechanical force of a large new blood volume auto-transfused over the first few minutes after birth. Our hypothesis is that the residual blood volume obtained by placental transfusion creates increased systemic and regional organ blood flow and vasodilation and is augmented by high initial levels of progesterone and high pulmonary artery pressure over the first several hours.(16,35) Tenets of this hypothesis include initial distension of the lung capillaries, removal of lung liquid, high pulmonary artery pressure, and the overall effect of high progesterone levels on transition and are discussed in the following paragraphs.

Distension of Alveolar Capillaries

In fetal life, lung growth depends on the lungs (air sacs) being highly distended with a large volume (20-30mL/kg) of fetal lung liquid.(40,41) Without this liquid, the fetus will develop hypoplastic lungs as the special liquid creates lung expansion along with mechanical stress. Blood supply to the fetal lung is ~8% of the cardiac output. Placental transfusion can supply ~30 mL/kg of blood volume to the neonate some of which goes to fill the capillaries surrounding each alveolus for the first time.

We propose that, with a full placental transfusion, stretch is applied within the capillary side of the alveolar-capillary membrane instead of by fetal lung liquid within the alveolus.(42) At birth, the alveolar capillaries adapt to the sudden influx of blood by increasing the diameter of the lumen, but do not change the diameter of the capillary itself thus not effecting the overall lung volume (Figure 3).(43) This allows transfer and

INSERT FIGURE 3 ABOUT HERE

inflow of the large volume of blood formerly used by the placenta for respiration in fetal life. Instead of pressure in the alveolus from fluid, pressure is created by new blood flow in the alveolar capillaries. While in small

preterm in the canaliculi or saccular stage alveoli are not fully developed, there must be approximation with blood source for oxygenation to occur. This is essential as air exchange following birth must occur in the alveolus itself and rapid growth must continue. It is likely that the enhanced vascular perfusion provides mechanical stimuli (stretching) through vasodilation to continue the distension (stretch; shear force), so important in fetal life, in order to now support new neonatal lung growth.(28,30) Effects of the essential stretch over time on the vessels surrounding the alveoli are readily seen in the porcine lung over the first minutes (Figure 3) and hours (Figure 4) following normal birth.(43,44)

INSERT FIGURE 4 ABOUT HERE

The large volume of blood flow in the alveolar capillaries triggers a mechanical force that causes the capillaries to become erect playing a vital role in recruitment of lung tissue and preventing derecruitment.(42,45) It has been said that “capillary erection” as described by Jaykka has been disproven. In fact, the only study that tried used inappropriate subjects (had previously breathed) to try to replicate Jaykka’s work.(46) However, both Jaykka and Avery reported that lungs were easier to inflate if there was fluid in the lung vessels. Thus, waiting to clamp the cord until after pulsations cease allows time for the lungs to fill fully with blood and to augment fetal lung fluid removal.

Removing Acidic Fetal Lung Fluid

Fetal lung liquid has a pH of 6.27 and must be removed rapidly from the lung in order for the infant to breathe air.(40) The anatomy of the alveolus and its blood supply informs the process of removal. While approximately 80% of the alveoli develop after birth, ~50 million alveoli are covered by 56 billion capillary segments in the term neonate.(47) This results in a ratio of 1 alveolus to ~900 million capillary segments accounting for the denseness of the capillaries. The pulmonary capillary networks are so dense that an almost contiguous sheet of blood encircles each alveolus.(48) The interstitial space between an alveolus and the surrounding capillary network is extremely thin, with only a 0.5 µm barrier known as the alveolar–capillary membrane separating air (or fluid) in the alveoli from blood in the pulmonary capillaries.(49) The thinness of this

2 barrier facilitates the exchange of oxygen and carbon dioxide. Filled with rapidly flowing blood, these capillaries
present a powerful force for ridding the lung of the acidic fetal lung liquid as quickly as possible. The blood from
4 the placental “lungs” rapidly enters the newborn lung at birth causing alveolar capillary erection and supports
rapid removal of fetal lung liquid out of the alveoli through various active and passive transport mechanisms
6 (including sodium channel activation) across the very thin layer of tissues (interstitium) into the capillaries
surrounding each alveolus and thus into the general circulation. (42,45)

8 It is probable that the removal of the fetal lung liquid is responsible for the "hidden acidosis" that
Wiberg and others have reported in infants with DCC.(50–52) Wiberg sampled blood in the intact cords and
10 found an increase in acidic blood coming from the intact umbilical arteries at 45 seconds of life but not until 90
seconds following birth in the vein. This indicates that the newborn was the source of the acidosis and not the
12 placenta. These findings occurred in infants born either vaginally or by cesarean section. Giovannini reported
increased acidosis from birth to 3 minutes after examining paired arterial–venous blood gas parameters drawn
14 from the unclamped cord and again after a 3-minute delay in cord clamping.(50) This occurred in spite of good
oxygenation, thereby suggesting that the acidic fetal lung liquid enters the infant’s blood supply rapidly. These
16 reported changes in the acidity of the blood are statistically, but not clinically, significant. However, in an infant
with ICC, the acidic lung liquid will be diluted with ~30% less blood volume which may make removal of the
18 acidic lung liquid more difficult.

Many sources state that the fetal lung liquid moves to the “interstitium” when air is forced into the
20 lungs. The idea of forcing air into this very delicate tissue has been shown to be harmful and resulted in
increased death rates in premature infants in the first 48 hours.(53) Nair found that sustained inflation (SI), even
22 with DCC, prevented blood flow into the lung via the pulmonary artery and inferior vena cava.(54) (Poster
Presentation, PAS 4/28/2019). Also, there would not be enough area in the lung “interstitium” for this large
24 volume of lung liquid, even briefly, without severe compromise. The thick interstitium seen in lung samples of

infants dying from respiratory complications is a pathologic outcome of their condition and not typical in a healthy lung.(55)

Progesterone: The key to incorporation of placental transfusion

The newborn's body is likely primed for capillary erection and enhanced vascular perfusion by high levels of progesterone, a potent vasodilator.(56) Immediately after a term birth, the amount of progesterone in the neonate is almost twice as high as it is in the mother at term (~270 ng/mL versus ~170 ng/mL).(16,17) It is likely that this high level of progesterone assists in the vascular modeling that takes place over the first hours of newborn transition because of its effect of relaxation on the vasculature.(Fig 3 and 4).(43,44,56) Progesterone also plays a role in dampening the response to inflammatory cytokines and is highly neuroprotective for the fetus and the newborn.(18) Progesterone levels quickly fall in the mother and the newborn over the first day after birth and thereafter continue to decline over the first week of life.(16) Thus, the essential initial vascular modeling in the lung is likely to be uniquely facilitated by the high levels of circulating progesterone in the infant in the first 12 hours of life suggesting this may be a critical period in organ development.

Enhanced Perfusion from Placental Transfusion: The Role of Initial High Pulmonary Artery Pressure

Placental transfusion results in enhanced perfusion, thus offering a significant mechanical force to drive transformation in the lung tissue and other organs that are now required to begin to function independently from the placenta.(35) Arcilla compared (using cardiac catheterization) pulmonary artery pressure (PAP) in term infants who received a full placental transfusion caused by a 5-minute delay in umbilical cord clamping (positioned lower than placenta) with infants who received ICC. The PAP in the DCC group remained high for 10+ hours in contrast to those infants with ICC whose PAP fell to 70% of systemic pressure by 2 hours of age and to 50% by 4 hours. (35) The initial high PAP, along with the high progesterone levels and a full placental transfusion, may be necessary to facilitate the essential stretch and mechanotransduction needed for post-birth lung transition.(16,43,44) The combined effect of high progesterone and PAP supports the assimilation of the large placental transfusion at birth and full perfusion to all of the infant's organs in adapting to extra-uterine life.

Together, these factors appear to make the first 10-12 hours of an infant's life a unique critical period for organ development that can be enhanced by a full placental transfusion.

Is Over-Transfusion from Placental Transfusion Possible? Concerns have been raised that a full placental transfusion could cause "over-transfusion" in the neonate. Yet, none of the studies on placental transfusion have found evidence of issues of over-transfusion. All fetal respiration has taken place in the placenta requiring a large part of the fetal cardiac output. Immediately at birth, respiration must switch to the newborn lung. To do so, a similar volume of blood – 40-50% of the cardiac output is required by the newborn lung for adequate oxygenation. The blood an infant receives from placental transfusion is not "extra" – it is the same volume of blood that the fetus's heart was pumping in utero out to the placenta and back again to perfuse his body and the placenta (i.e., the fetal lung). The newborn's body is programmed to receive the transfer or auto-transfusion of the residual placental blood as explained above. At no other time, short of a serious hemorrhage, could a human body incorporate such a large volume of blood. Thus, it is highly unlikely that an infant can be over-transfused from his own placental transfusion. Under-perfusion resulting in hypovolemia may be a much larger problem as discussed in the following section. A one-minute delay may not yield an adequate increase in blood volume to impact rates of other morbidities in the smallest infants. Research is ongoing that allows a longer time for the infant to transition (NCT02671305, NCT03019367, NCT02742454).

WHAT IS LOST DUE TO IMMEDIATE CORD CLAMPING?

Residual placental blood volume is the blood remaining in the placenta after the cord is clamped and cut. This is the blood that is used when stem cells are harvested. Seeking to identify covariates of bronchopulmonary dysplasia (BPD), Chaudhury et al completed a prospective study of 200 preterm infants <32 weeks by collecting residual placental blood volume for analyses.⁽¹²⁾ For infants 29-32 weeks, the amount of whole residual blood collected was 35 mL/kg (IQR 28-41). However, for the 35 infants between 23 and 28 weeks, the amount collected was 46 mL/kg (IQR 37-57) and for the 17 very low birth weight (VLBW) infants <1000 grams, it was 57 mL/kg (IQR 47-62). Given a fetal-placental blood volume of 110 to 115 mL/kg⁽⁵⁷⁾, the tiniest

infants with ICC lost to cord blood collection half or more of the blood volume potentially available to them with DCC.

Higher levels of stem cells (e.g., CD34+, CD90+, CD105+) were found in the residual placental blood of infants who developed BPD, with the highest stem cell levels for those who developed the worst BPD. In the 74 infants whose cord blood was analyzed to measure 12 cytokines and growth factors, higher levels of angiopoietin, IL-8, and PGF, among others were found in the blood of those developing BPD.

Chaudhury et al state that their findings may have important implications for those studying stem and progenitor therapies to use for the prevention of BPD. However, when harvested residual umbilical cord blood is processed for storage, the iron-rich red blood cells (RBCs) and the other protective substances in the blood along with the plasma are discarded. When an infant receives the residual placental blood volume at the time of birth, the stem cells are in the perfect medium along with many cytokines, proangiogenic and antiapoptotic messengers and growth stimulating factors.(12,23) Finding the appropriate medium is a major obstacle confronting stem cell therapies especially when attempting proliferation of stem cells and translation of successful animal studies to humans.(58)

Baker states that in spite of years of successful animal studies, translation of stem cell therapy to human infants has not been productive in preventing, or in treating, the most damaging diseases such as BPD that preterm infants acquire.(58,59) Other issues in postnatal transfusion of stem cells include finding the correct dosage, timing of doses, single or multiple doses, cell suspension density, route of administration, infusion protocols, and regulatory issues and good manufacturing procedures.(58) While these factors are important to resolve for use of stem cells in infants already born, the initial autologous auto-transfusion that occurs with placental transfusion at birth preserves all components of the residual placental and cord blood for the infant. This potentially leads to reduced inflammation and conserves the infant's progenitor cells.(23,58)

The loss of fresh whole fetal blood, and its many components, when ICC or early cord clamping (<30 seconds) occurs is underappreciated. Not only does the concentration of stem cells increase with decreasing

2 gestational age at birth but preterm stem cells also have higher clonogenic activity.(60,61) With ICC, larger
amounts of these essential substances are unavailable to the tiniest infants. Did the infants in Chaudhury's study
4 develop BPD because of an intrinsic problem or because they lacked the cells and substances they needed for a
successful transition? The large volume of blood lost by the smallest infants with ICC would be considered
6 equivalent to severe hemorrhage and has been shown to result in an inflammatory response in adult humans
and rodents.(62,63) Severe blood volume loss alone has been shown to lead to inflammation without any
8 accompanying infectious process or reperfusion.(62,63) Makley reports that after hemorrhage, replacing the lost
blood volume with fresh whole blood reduces inflammation more quickly and efficiently than replacement with
10 donor blood or other transfusion products.(64) Placental transfusion can supply that fresh whole blood to
protect the neonate and is readily available at birth. Given that almost all newborn problems have a large
12 inflammatory component, this information begs further study in human infants.

PREMATURE INFANTS ARE AT THE HIGHEST RISK FROM HARM DUE TO ICC OR ECC

14 Preterm infants have about 50% of their fetal-placental blood volume in the placenta at any point in
time due to the 1:1 placenta to body ratio in contrast to term infants whose placentas are approximately 1/3 the
16 size of the fetus at the time of birth. With ICC, Chaudhury et al have demonstrated that the smallest infants
receive a lower percentage of essential residual cord blood with reduced stem cell levels and other important
18 factors essential for building their hematologic and immune systems after birth.(12) In addition to ICC, the
tiniest infants have a large blood sample for admission laboratory studies and frequent multiple phlebotomies
20 for testing throughout the NICU stay.(65) It is likely that these actions contribute to susceptibility to
hypoperfusion and the devastating and too common illnesses of prematurity.(66) Hellstrom et al reported that
22 preterm infants lose 58% of their own newborn blood volume in the first two weeks due to phlebotomy. While
this is most often replaced with adult blood, many of the components unique to fetal/newborn blood are lost.
24 We suggest that new approaches are needed to protect the smallest infants: a safer, more physiological way to
get more of the available residual placental blood volume into the preterm infant at the time of birth; admission

blood sampling from the umbilical cord or placental veins; and micro-sampling techniques for NICU blood testing.

Placental Transfusion at Birth

If one thinks of death, disease, and health on a continuum, it is not unreasonable to suggest that if a DCC of ~60 seconds reduces the rate of death, obtaining an even greater placental transfusion may further reduce death and illnesses. We have provided evidence suggesting that birth is the time when the infant's body is best prepared to receive the residual placental blood. There is likely greater receptivity and plasticity of the vascular system due to the high levels of progesterone which will decrease within ~12 hours. Longer delayed cord clamping times than the current recommended 30-60 seconds may provide additional benefits and be needed to accomplish a greater transfusion for preterm infants. Katheria has provided a review of current and ongoing studies on longer delays.(67)

Blood Conservation During Transition and NICU Stay

Two other methods to protect the premature infant's blood volume included collection of blood for initial admission studies from the umbilical cord after delivery and use of microsystems for ongoing testing in the NICU. Using cord samples for admission blood work has been shown to result in higher 24-hour hematocrit and hemoglobin and can lower risk of hypotension.(68–70) Phlebotomy losses following birth lead to nearly universal anemia in very low birth weight (VLBW) infants.(65,66,71) Rabe reported that some tiny infants had over 30 mL/kg withdrawn during the NICU stay.(72)(Rabe 2000) More microsystems that can reduce the volume of blood removed from the infant for testing are needed.(65,66,73)

CONCLUSION

The importance of the microvascular system among very preterm infants in disease progression is only beginning to be appreciated for its significant clinical implications. Enhanced vascular perfusion provides mechanical stimuli causing electrochemical activity that seems increasingly likely to be essential for normal function, development and maintenance of newborn organs, especially the lungs. It is probable that immediate

or early cord clamping denies the infant the enhanced vascular perfusion needed to provide the mechanical force to stimulate organ-specific endothelial cells to direct their growth, maintenance, and repair. Methods to enhance and protect neonatal blood volume via placental transfusion at birth and conserve blood in the NICU are under study and may help prevent or lessen the burden some of the morbidities observed in very preterm infants.

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FIGURE LEGENDS

Figure 1. Model of mechanotransduced angiocrine signals in the liver.

6 Simplified drawing of liver sinusoids with liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs),
extracellular matrix (ECM), Kupffer cells (KCs) and hepatocytes: a, Liver sinusoid under normal blood flow; b,
8 magnification of space of Disse; c, Liver sinusoid with enhance blood perfusion; d, magnification of the space of
Disse. When the vascular lumen widens owing to enhanced blood perfusion, circumferential stretching of liver
10 sinusoidal ECs activates endothelial $\beta 1$ integrin and interaction with VEGFR3. The hepatic ECs (in concert with
other cells) subsequently release angiocrine signals (such as HGF, IL-6 and TNF) and activate MMP9 and thus
12 enhance proliferation and survival of the adjacent hepatocytes. Adapted from Lorenz L, Axnick J, Buschmann T,
Henning C, Urner S, Fang S, et al. Mechanosensing by $\beta 1$ integrin induces angiocrine signals for liver growth and
14 survival. Nature. 2018;562(7725):128–32 (with permission).

16 **Figure 2. Electron microscopy photographs of capillaries from heel biopsies of infants** with immediate cord
clamping (ICC) or cord clamping when pulsations stopped (+/- 5 min, DCC). There were more capillaries found
18 resembling the specimen on the left (2A) in the infants with ICC and more of the distended capillaries in infants
with DCC on the right (2B). There are small fenestrations at the top of the red arrow on the DCC capillaries that
20 would aid in transport of gases and nutrients in 2B only. Pietra G, D'Amodio M, Leventhal M, Oh W, Braudo J.
Electron microscopy of cutaneous capillaries of newborn infants: effects of placental transfusion. Pediatr
22 1968;42(4):678-683 (with permission).

24 **Figure 3. Electron micrographs of transverse sections through small muscular lung arterioles in naturally born
piglets.** a stillborn; b aged 5 min taken at the same magnification. At birth the endothelial cells of the intra-

2 acinar arteries showed more rapid and greater changes in shape and thickness than did the cells of more
proximal vessels. IEL = internal elastic lamina; L = lumen. Scale – bar line on lower right = 2 μ m. Adapted from
4 Haworth SG, Hall SM, Chew M, Allen K. *Thinning of fetal pulmonary arterial wall and postnatal remodeling:
ultrastructural studies on the respiratory unit arteries of the pig. Virchows Arch A Pathol Anat Histopathol.*
6 *1987;411(2):161–71 (with permission).*

8 **Figure 4. Diagram illustrating en face shape changes in the endothelial cells of intra-acinar arteries during the
first three weeks of life** (porcine). Two phases of change are shown, a-c stretching during inflation; d-e
10 spreading during dilatation. The endothelial cells of the intra-acinar arteries showed marked changes in cell
shape and relationships after birth while those of large preacinar arteries did not. The first structural changes
12 detected during the first 30 min of life occurred in the endothelial cells lining the intra-acinar arteries. Adapted
from: Hall SM, Haworth SG. *Normal adaptation of pulmonary arterial intima to extrauterine life in the pig:
14 ultrastructural studies. J Pathol. 1986 May;149(1):55–66 (with permission).*