

Published in final edited form as:

Clin Perinatol. 2014 March ; 41(1): 47–67. doi:10.1016/j.clp.2013.09.007.

Pathogenesis and Prevention of Intraventricular hemorrhage

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SYNOPSIS

Intraventricular hemorrhage (IVH) is a major neurological complication of prematurity. Pathogenesis of intraventricular hemorrhage is attributed to intrinsic fragility of germinal matrix vasculature and to the fluctuation in the cerebral blood flow. Germinal matrix exhibits rapid angiogenesis orchestrating formation of immature vessels. The angiogenic vessels lack pericytes, display immature basal lamina low in fibronectin, and have astrocyte end-feet coverage deficient in glial fibrillary acidic protein. These factors contribute to the fragility of the germinal matrix vasculature. Fluctuation in the cerebral blood flow results from a wide range of respiratory and hemodynamic instability associated with the preterm infants. Prenatal glucocorticoid exposure remains the most effective means of preventing IVH. Therapies targeted to enhance the stability of the germinal matrix vasculature and minimize fluctuation in the cerebral blood flow might lead to more effective strategies in preventing IVH.

Keywords

germinal matrix hemorrhage; intraventricular hemorrhage; astrocytes; pericytes; angiogenesis; glucocorticoids; premature infants; indomethacin

Introduction

In the United States, about 12,000 premature infants develop intraventricular hemorrhage (IVH) every year. The incidence of moderate-to-severe IVH has remained almost stationary during the last two decades.^{1, 2} IVH is a major problem in premature infants, as a large number of them develop neurologic sequelae.³ Approximately 50–75% of preterm survivors with IVH develop cerebral palsy, mental retardation, and/or hydrocephalus.^{3, 4} Approximately, a quarter of non-disabled survivors develop psychiatric disorders and problems with executive function.^{5–7} According to the U.S. Census Bureau and the NICHD Neonatal Research Network, over 3600 new cases of mental retardation each year are children who were born premature and suffered IVH.^{8, 9} Hence, IVH and its resultant neurologic and psychiatric sequelae continue to be a major public health concern worldwide.

IVH typically initiates in the periventricular germinal matrix.¹⁰ This brain region is known to developmental neurobiologists as the ganglionic eminence (Fig. 1A). The germinal matrix

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Conflict of interest: None.

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consists of neuronal and glial precursor cells (Fig. 1B, C) and is most prominent on the head of caudate nucleus. The subependymal germinal matrix is highly vascular and is selectively vulnerable to hemorrhage. After 24 gestational weeks (gw), thickness of the germinal matrix decreases, and it almost disappears by 36–37 gw. When hemorrhage in the germinal matrix is substantial, the underlying ependyma breaks and germinal matrix hemorrhage progresses to IVH, as blood fills the lateral cerebral ventricle.

PATHOGENESIS OF INTRAVENTRICULAR HEMORRHAGE

Pathogenesis of IVH is multifactorial, complex, and heterogeneous. An inherent fragility of the germinal matrix vasculature sets the ground for hemorrhage and fluctuation in the cerebral blood flow induces the rupture of vasculature (Box 1). If there are associated platelet or coagulation disorders, the homeostasis mechanisms are impaired which might accentuate the hemorrhage. Vaginal delivery, low Apgar score, severe respiratory distress syndrome, pneumothorax, hypoxia, hypercapnia, seizures, patent ductus arteriosus, infection, and others seem to increase primarily the fluctuations in the cerebral blood flow and thus, represent important risk factors to the development of IVH.

Box 1

Pathogenesis of germinal matrix vasculature

1. Fragility of germinal matrix vasculature
2. Fluctuation in the cerebral blood flow
3. Platelet and coagulation disorder

What renders the germinal matrix vasculature fragile?

Blood vessels in the brain are unique as they form a blood-brain barrier (BBB). The BBB is a complex dynamic interface between blood and the brain, and consists of endothelial tight junctions, basal lamina, pericytes, and astrocyte end-feet in inside-out fashion (Fig 1D).^{11, 12} Logically, deficiency in any of the components of the BBB can weaken the vasculature and increase the propensity to hemorrhage. We have evaluated each of these components in the human germinal matrix and have unraveled a number of dissimilarities in the cellular and molecular components of this germinal matrix vasculature compared to the embryonic white matter and the cortical plate (Box 2).

Box 2

Fragility of germinal matrix vasculature

1. Paucity of pericytes
2. Reduced fibronectin in the basal lamina
3. Reduced GFAP expression in the astrocyte endfeet

High vascular density and rapid angiogenesis in the germinal matrix—The germinal matrix exhibits rapid angiogenesis in contrast to other brain regions.¹³ This rapid endothelial proliferation contributes to the high vascular density of the germinal matrix. Both the vascular density and the cross-sectional area of the vasculature are higher in the human germinal matrix compared to the cortical plate (cerebral cortex) and embryonic white matter from 17–35 gw.¹⁴ In addition, the abundance of vessels and cross sectional area of

vasculature increases with advancing gestational age in the second and third trimester of pregnancy.¹⁴ Intriguingly, vessels in the germinal matrix are circular in coronal sections whereas blood vessels in cerebral cortex and white matter are flat and elongated. The circular shape of the vessels suggests vasculature immaturity, which is consistent with the rapid ongoing angiogenesis in the germinal matrix. The high vascularity and rapid endothelial turnover is unique to the germinal matrix and can be attributed to the high metabolic demand of this brain region, which has a preponderance of proliferating, maturing, and migrating neuronal and glial precursor cells.

Paucity of pericytes in the germinal matrix vasculature—Pericytes are perivascular cells of the capillaries, venules, and arterioles (Fig. 2).¹⁵ They are enclosed in the basal lamina and wrap around the endothelial cells. They have complex and critical role to play in regulating angiogenesis, providing structural support to the vasculature, maintaining the BBB, and controlling neurovascular unit—endothelium, astrocytes, and neurons.^{16–18} Pericytes are the key players in the various stages of angiogenesis, including initiation, sprout extension, migration, and maturation of blood vessels.¹⁹ In response to angiogenic stimuli, they degrade basal lamina and migrate out of the microvasculature and upon completion of angiogenesis, they resume their position to strengthen vessels by synthesizing extracellular matrix and inducing endothelial maturation. Pericyte recruitment is regulated primarily by four ligand-receptor systems; the ligands include transforming growth factor β (TGF β), platelet derived growth factor-B (PDGF-B), angiopoietin, and sphingosine-1-phosphate.²⁰ Deficiency of any of these ligands in transgenic animals results in failure of pericyte recruitment and consequent dilation of the vessels with increased propensity to hemorrhage.²¹

Quantification of pericyte coverage and density in the brain of human preterm infants and fetuses has been performed in immuno-labeled sections and electron micrographs. These studies have revealed that the density of perivascular cells and their peri-endothelial coverage in the germinal matrix are reduced compared to the white matter and cerebral cortex on both ultrastructural and histochemical evaluation.²² Importantly, TGF β expression is reduced in the germinal matrix microvasculature, which might be contributing to the paucity of pericytes.²² Indeed, low TGF β concentration promotes endothelial proliferation and conversely, high TGF β levels enhance capillary stabilization by facilitating pericyte recruitment. To conclude, rapid angiogenesis in the presence of low TGF β results in abundance of angiogenic vessels deficient in pericytes, which leads to fragility of the germinal matrix microvasculature.

Deficient fibronectin in the basal lamina—Basal lamina is a key component of the BBB that surrounds pericytes and separates pericytes from astrocyte end-feet and endothelium.^{12, 23} Its formation and maintenance is assured by the endothelium, astrocytes, and pericytes. This contributes to the structural integrity of vasculature by virtue of its anchoring function. Basal lamina is composed of laminin, collagen, fibronectin, heparan sulfate proteoglycan, and perlecan.^{12, 24, 25} Knock out experiments have revealed the necessity of these molecules in the formation of blood vessels and maintenance of the vascular stability.^{26–28} Quantification of the constituents of basal lamina in human fetuses and preterm infants (postmortem) has revealed that fibronectin levels are significantly reduced in the germinal matrix vasculature compared to the cortex and white matter (Fig. 3A).²⁹ However, other components of the basement membrane, including laminin ($\alpha 1$, $\alpha 4$, and $\alpha 5$), and $\alpha 1$ (IV) collagen, $\alpha 5$ (IV) collagen, and perlecan, are similarly expressed in the three brain regions. Given that polymerization of fibronectin into extracellular matrix controls stability of the vasculature and that fibronectin null mice exhibit cerebral hemorrhage, deficient fibronectin in the germinal matrix is likely to contribute to the fragility of germinal matrix vasculature.^{30, 31} Since TGF β upregulates fibronectin and other

components of the extracellular matrix, diminished TGF β in the germinal matrix is consistent with low levels of fibronectin in the germinal matrix. Thus, upregulating TGF β might elevate fibronectin levels in the basal lamina and strengthen the germinal matrix vasculature. However, TGF β is a ubiquitously expressed growth factor; and there is no available strategy to increase the expression selectively of this molecule in the germinal matrix vasculature.

Consistent with these studies, another report has shown that there is no significant difference in the expression of Collagen I, II, and IV between the germinal matrix and other brain regions of premature human infants.³² However, in beagle puppies, immunoreactivity of laminin and collagen V in the germinal matrix is greater at postnatal d4 compared to d1, and indomethacin treatment further increases the intensity of immunosignals for laminin and collagen V in the germinal matrix.^{33, 34} This suggests that deficiencies of these two molecules in germinal matrix vasculature of beagle puppies might contribute to the vascular weakness of this brain region. Together, the constituents of the basal lamina add to the stability of the vasculature; and a deficiency of fibronectin level in the basal lamina of the human germinal matrix might contribute to the vascular fragility of this brain region.

Reduced GFAP expression in the astrocyte end-feet—Astrocytes extend processes from the soma in all directions that cover the blood vessels. These astrocytic processes are known as endfeet, which unsheath 99% of the BBB endothelium in adult brains.³⁵ The astrocytes contribute to the development of the BBB and regulate its function. Specifically, they provide structural integrity and control permeability of the BBB.¹² Moreover, they are essential for function of neurons and the neurovascular unit. Astrocyte end-feet contain intermediate filaments, which form the cytoskeleton of the astrocytes. GFAP is a key component of these intermediate filaments. Studies in autopsy materials from fetuses and premature infants have shown that perivascular coverage by GFAP⁺ astrocyte end-feet is less in the germinal matrix compared to the cerebral cortex and the embryonic white matter (Fig. 3B).³⁶ Since GFAP provides structural integrity and mechanical strength to the astrocyte end-feet,^{37–39} fragility of the germinal matrix vasculature can also be ascribed to reduced GFAP expression in the astrocyte end-feet.

Overall mechanism of germinal matrix vascular fragility

Germinal matrix exhibits rapid angiogenesis, in contrast to the other brain regions. The accelerated endothelial proliferation of this brain region is triggered by high levels of vascular endothelial growth factor (VEGF) and angiopoietin-2, and reduced expression of TGF- β (Fig. 4).¹³ Since hypoxia is a key stimulant of these growth factors,⁴⁰ we have evaluated hypoxia inducible factor-1 α and have performed hypoxyprobe test. We have noted that hypoxia inducible factor-1 α levels are elevated in the human germinal matrix compared to the cerebral cortex and white matter (unpublished data). In addition, hypoxyprobe [1-(2-hydroxy-3-piperidiny)propyl 2-nitroimidazole hydrochloride] test shows lower oxygen gradient in the germinal matrix compared to the adjacent white matter.¹⁰ It seems that oxygen demand and utilization of this brain region is high as a result of its high metabolic activity. Indeed, germinal matrix harbors neuronal and glial precursor cells, which are in various phases of proliferation, migration, and maturation. Angiogenic vessels in the germinal matrix are naked endothelial tubes exhibiting paucity of pericytes and immature basal lamina low in fibronectin. In addition, GFAP is weakly expressed in the astrocyte endfeet. Together, a scarcity of pericytes, low fibronectin levels in the basement membrane, and reduced GFAP expression in the astrocyte endfeet contributes to the weakness of the germinal matrix BBB and to the vulnerability to hemorrhage. Importantly, IVH develops in the first three days of postnatal life and premature infants are relatively immune to hemorrhage after this period irrespective of the gestational age. This reduced propensity to

hemorrhage after 3 days in preterm infants might be because of an increase in blood and tissue oxygen concentration after birth suppressing VEGF, angiopoietin-2 levels and the angiogenesis. A shutdown in angiogenesis shortly after birth would mature the angiogenic vessels making them resistant to rupture despite fluctuation in the CBF.

Disturbance in Cerebral Blood Flow

Fluctuation in the CBF—In premature infants with respiratory distress syndrome, two patterns of CBF velocity have been demarcated: one, stable pattern with equal peak and trough of systolic and diastolic flow velocity and other, fluctuating waveform with continuous alteration in systolic and diastolic flow velocity.^{41, 42} Fluctuating cerebral blood flow velocity during the first day of life strongly correlates with the occurrence of IVH and more importantly, elimination of this fluctuation in the CBF by intravenous pancuronium infusion markedly reduces the incidence of IVH.^{41–43} Since these studies were done in mid 1980s, it is possible that fluctuation in the CBF was related to the use of intermittent mandatory ventilation, giving rise to asynchrony between infant and ventilator breath, which can be eradicated by the use of paralytic agents. However, most of the neonatal units currently use synchronized ventilator modes, which minimizes infants “fighting” the ventilator and thus reduces fluctuations in the CBF velocity.⁴⁴ Moreover, routine use of neuromuscular blocking agents in ventilated infants is not recommended because of uncertain long-term neurologic adverse effects of these agents. Other factors that contribute to the fluctuation of CBF velocity include patent ductus arteriosus, hypercarbia, hypotension, severe respiratory distress syndrome, and rapid infusion of sodium bicarbonate (Table 1).^{43, 45–47}

Impaired autoregulation in premature infants—Cerebral autoregulation is the capability of the blood vessels in the brain to retain a constant CBF in spite of fluctuations in the blood pressure. The pressure passivity--impaired autoregulation—of CBF is associated with lower gestational age and birth weight, and is commonly noted in sick, ventilated, and hemodynamically unstable premature infants. Cerebral autoregulation has been evaluated in preterm infants using a number of methods, including Doppler, xenon clearance, near infrared spectroscopy (NIRS), and spatially resolved spectroscopy (SRS).^{48,49,50, 51} Although these techniques involve continuous and repeated monitoring, the results of these studies have not been consistent. It was reported that NIRS identifies infants with impairment in cerebral autoregulation and that this is associated with high likelihood of severe IVH.⁵² Subsequent investigators realized continuous monitoring of CBF over extended period, because cerebral blood pressure passivity is not an “all-or-none phenomena”, but it can occur over varied time intervals.⁴⁹ In VLBW infants, continuous monitoring of cerebral perfusion by NIRS and mean arterial blood pressure has shown that CBF is pressure passive for an average of 20% of the time; and there is strong correlation between pressure passive state and hypotension with lower birth weight and gestational age.⁴⁹ Additionally, there is no association between impaired autoregulation and the occurrence of IVH.⁴⁹ Another subsequent report, in which CBF was measured by SRS, showed strong association between diminished autoregulation and subsequent mortality; however, impaired autoregulation was not associated with the development of IVH.⁵⁰ In a recent study on continuous monitoring of VLBW using NIRS, it has been shown that the correlation of regional cerebral oxygen saturation and mean blood pressure indicates pressure passivity of the CBF and these metrics may predict the occurrence of IVH.⁵³ Hence, the role of impaired autoregulation in the development of IVH needs further evaluation.

Perinatal risk factors for IVH, fluctuation in the CBF, and germinal matrix fragility—A number of risk factors are associated with IVH, which directly or indirectly increase either the fluctuation in the CBF or the fragility of the germinal matrix

microvasculature (Table 1). Hypotension is common in premature infants; however, there is conflicting data on the association between IVH and hypotension.^{49, 54, 55, 56, 57} A correlation between hypotension and CBF seems to be complex and hypotension may not indicate reduced or disturbed CBF.⁵⁸ A rise in central venous pressure (CVP) might contribute to the onset of IVH. Indeed, CVP is elevated in pneumothorax and in infants on mechanical ventilation using high mean airway pressure. Moreover, germinal matrix hemorrhage has been demonstrated to be mainly of venous origin in a study on autopsy materials from premature human infants.⁵⁹ Prolonged positive pressure ventilation is also known to increase BBB permeability.⁶⁰ A rapid infusion of sodium bicarbonate might contribute to the development of IVH. It is possible that a rapid infusion of a large dose of sodium bicarbonate will increase serum osmolality and arterial CO₂ resulting in vasodilation and rupture of the microvasculature in the germinal matrix. However, there is disagreement on a causative role of bicarbonate in the development of IVH.^{61–63} Other risk factors for IVH include early onset sepsis, maternal chorioamnionitis, development of respiratory distress syndrome, recurrent tracheal suctioning, prolonged labor, hypoxia, hypercarbia and others (Table 1). It appears that most of these conditions contribute to the occurrence of IVH by disturbing the CBF. However, sepsis and hypoxia-ischemia might cause molecular and morphological changes in the microvasculature which may weaken the vessels of the germinal matrix. Coagulopathy does not seem to play a key role in pathogenesis of IVH, but can modify the risk and severity of IVH. A Cochrane systematic review showed that vitamin K administration did not affect the incidence of IVH.⁶⁴ Fresh frozen plasma administration has also been tried without success.⁶⁵ However, several studies have reported that thrombocytopenia is a risk factor for IVH.^{66–68}

Genetic factors and IVH

Mutations in the type IV procollagen gene, COL4A1, are associated with IVH in dizygotic preterm twins.⁶⁹ Since inflammatory mediators and coagulation factors might have a role in the development of IVH, polymorphisms of pro-inflammatory cytokines and mutations in the coagulation factors have been evaluated as candidate genes that modify the severity and risk of IVH. Mutations in factor V Leiden, prothrombin G20210A, and IL1 β have been implicated in the development of grade I and II IVH.^{70–72} Polymorphisms of IL-6 and TNF α are proposed as genetic modifiers of IVH risk.^{73, 74} In addition, polymorphism of methylene tetrahydrofolate reductase (MTHFR) gene has been reported in infants with IVH. C677T polymorphism in MTHFR enzyme is associated with high plasma homocysteine levels, which is a known risk factor for vascular disease.⁷⁵ Together, mutations in coagulation, thrombophilia, and inflammation related genes might contribute to the development of IVH.

PREVENTION OF INTRAVENTRICULAR HEMORRHAGE

Rationale of preventive therapies

Since IVH is primarily attributed to increased vascular fragility and disturbance in CBF, our strategies are directed to strengthening the germinal matrix vasculature and to stabilizing the CBF. Germinal matrix has a subset of vessels that are angiogenic, immature, and lack pericytes; and they thrive because of high levels of VEGF and angiopoietin2.^{13, 22} These blood vessels exhibit high fragility and propensity to bleed. It appears that the immature neovasculature are pruned within a few days of premature delivery, thus stabilizing the germinal matrix microvasculature. This is because oxygen concentration increases above intrauterine level shortly after birth, which possibly downregulates the VEGF levels in the germinal matrix. Indeed, preterm infants become relatively immune to the development of IVH after postnatal day 3. Prenatal glucocorticoids (GCs) or antenatal celecoxib also downregulates VEGF levels, which leads to apoptosis of naked endothelial cells, lacking

pericyte coverage.^{13, 76} Hence, prenatal use of angiogenic inhibitors—GCs or celecoxib—trims the nascent vessels, but not the functional vessels protected by pericytes, thereby orchestrating a vascular network consisting of stable blood vessels.^{22, 76, 77, 78} Fluctuations in the CBF is related to routine procedures performed in neonatal units, such as suctioning, handling, placing intravenous lines, and common problem associated with prematurity, including respiratory distress syndrome, patent ductus arteriosus, apneic episodes, seizures, hypoxemia, hypercarbia and others (Table 1). Hence, fluctuations in the CBF can be minimized by reducing the stimulation to the infant and appropriately managing the common complications of prematurity. Overall preventive approach is listed in Table 2.

Prenatal Pharmacological treatments to prevent IVH

Glucocorticoids (GCs)—In the United States, the preterm birth rate is about 12.5% and approximately 75% women in premature labor with gestational age of less than 34 weeks are treated with either betamethasone or dexamethasone.⁷⁹ A number of studies have confirmed that prenatal GC reduces both severity and incidence of IVH.^{80, 81} The beneficial effect of prenatal GC is attributed to stabilization of the microvasculature of the germinal matrix and alleviation of disturbance in the CBF. Prenatal GC, as discussed above, suppresses angiogenesis in the germinal matrix microvasculature and thus trims the nascent and fragile vasculature, which are vulnerable to hemorrhage. Thus, GC exposure stabilizes the BBB of the germinal matrix; and infants treated with prenatal GC exhibit greater pericyte coverage, higher fibronectin levels and more GFAP in the astrocyte end-feet of the blood vessels of the germinal matrix compared to untreated infants.^{22, 29, 76} Moreover, it reduces the incidence and severity of respiratory distress syndrome, which might minimize fluctuation in the CBF. Postnatal betamethasone (0.1 mg/kg) also reduces cerebral flow velocity possibly by exerting a vasoconstrictor effect on cerebral vessels in preterm infants.⁸² Similarly, prenatal betamethasone has been shown to reduce cerebral blood flow by increasing cerebrovascular resistance in fetal sheep model.⁸³

The optimal effects of prenatal GC have been observed after a complete course of 2 doses of betamethasone or 4 doses of dexamethasone when administered within a week of delivery of the premature newborn.⁸⁴ However, benefits have also been noted with incomplete courses of GCs.⁸⁵ Comparison of the two GCs—betamethasone and dexamethasone—has not conclusively shown superiority of one over the other and clinicians should choose whatever is available.⁸⁵ Betamethasone exposed infants exhibit less severe respiratory distress syndrome, but more IVH, compared to prenatal dexamethasone treated infants.^{84, 86} There has been concern that prenatal dexamethasone might increase the incidence of periventricular leukomalacia.⁸⁷ However, a subsequent study on a larger population clearly showed that there is no difference in the incidence of cystic PVL between dexamethasone and betamethasone exposed infants.⁸⁸ This study also noted that both GCs are equally efficacious in preventing severe IVH, however there is a trend toward better efficacy for dexamethasone compared to betamethasone.⁸⁸ Importantly, prenatal betamethasone is associated with a reduced risk for neonatal death compared with dexamethasone.⁸⁸ Together, there is no recommendation for the use of one GC over the other, despite multiple clinical trials undertaken. Another key issue related to the use of prenatal steroid is single versus repeated course. Unfortunately, there is no agreement among the experts on the advantage of single vs. multiple course of GCs.⁸⁵ There are concerns that multiple course of prenatal GC might have adverse effects on brain and other organ systems.

Phenobarbital and Magnesium sulfate—As etiopathogenesis of IVH was more mysterious in 80s than now, a number of agents without a concrete rationale were tried to prevent IVH. Phenobarbital and vitamin K are important to mention here, as these medications attracted the attention of investigators. Initial studies showed some protective

effect of phenobarbital, however, subsequent clinical trials failed to confirm the protective effect of phenobarbital in preventing IVH.^{89–93} Maternal treatment of vitamin K or magnesium sulfate to prevent IVH did not demonstrate any benefit either.^{64, 94–96}

Postnatal pharmacological treatment to prevent IVH

Indomethacin—Indomethacin, commonly used in premature neonates to close patent ductus arteriosus, has been shown to prevent IVH in several clinical trials. Indomethacin, a non-selective cyclo-oxygenase (COX) inhibitor, suppresses both COX1 and COX2 isoforms to reduce prostaglandin synthesis. It attenuates cerebral vascular hyperemic responses induced by hypoxia, hypercapnia, hypertension, and asphyxia.^{97, 98} It reduces alterations in the BBB permeability after cerebral ischemia and promotes maturation of basement membrane by increasing the expression of laminin and collagen V.^{33, 34, 99} The maturation of basal lamina upon indomethacin treatment can be attributed to COX2 inhibition, which suppresses angiogenesis and matures the germinal matrix vasculature.¹³

In a number of clinical trials, indomethacin treatment has shown short-term benefit of reducing the incidence of IVH.^{100,101, 102} Secondary analyses of a multicenter study based on gender have shown that indomethacin treatment reduces the rate of IVH in male infants, but not in female infants.¹⁰³ However, another study on a larger population of preterm infants showed just a weak differential effect of indomethacin by sex.¹⁰⁴ Since indomethacin reduces the occurrence of IVH, it was anticipated that this treatment would improve the neurodevelopment outcome of the infants. However, indomethacin treatment failed to reduce the rate of cerebral palsy, deafness and blindness on long term follow up.^{105, 106} A meta-analysis of 19 clinical trials also did not show any improvement in the long term outcome of indomethacin treated infants.^{107, 108} Together, indomethacin prophylaxis has immediate benefits of reduction in symptomatic patent ductus arteriosus, and severe IVH; however, this does not impact long term neurodevelopmental outcomes. Hence, indomethacin is not recommended for routine prophylaxis against IVH. However, indomethacin is still being used in some neonatal units depending on clinical circumstances and personal preferences.

Ibuprofen is another non-selective COX inhibitor that has shown promise in closing patent ductus arteriosus. This compound does not reduce CBF, in contrast to indomethacin. More importantly, ibuprofen does not prevent IVH in premature infants.^{109, 110}

Other clinical trials of unproven benefit—Postnatal phenobarbital has been used in a number of clinical trials to prevent IVH in 1980s, based on the premise that it might reduce abrupt changes in the CBF during tracheal suctioning, handling, and motor activity.^{111–114, 115} However, phenobarbital did not reduce the incidence of IVH significantly. Another agent that drew the attention of investigators in 1980s was etamsylate. This compound reduces prostaglandin synthesis and promotes platelet adhesiveness. In addition, etamsylate induces polymerization of hyaluronic acid in the vascular basement membrane which might promote homeostasis and minimize bleeding. However, large clinical trials showed that etamsylate neither reduced the incidence of IVH nor enhanced the neurodevelopmental outcome of premature infants.^{116, 117} Pathogenesis of IVH has always puzzled the investigators and thus, a role of free radicals in the etiology of IVH cannot be totally excluded. Hence, vitamin E—a potent antioxidant—has been tried to prevent IVH in preterm infants without appreciable benefits.^{118, 119} To address the need to minimize asynchrony between infant and ventilator breath, pancuronium has been tried in 1980s and was found to reduce fluctuation in CBF and also the incidence of IVH.⁴² A relatively recent meta-analysis identified 6 clinical trials in which the use of neuromuscular blocking agent during mechanical ventilation was compared to no paralysis in newborn infants.¹²⁰ It was

concluded that neuromuscular paralyzes with pancuronium reduced the rate of IVH. However, owing to complications associated with neuromuscular paralysis, routine use of pancuronium in extremely premature infants has not been recommended. The role of activated recombinant factor VII (rVIIa) in promoting coagulation is promising and it has been hypothesized that early (prophylactic) administration of rVIIa to extremely premature infants would reduce the incidence of IVH. However, IVH is not primarily a coagulation disorder and this hypothesis does not seem to be logical.

Optimizing care of fetuses and premature newborns

Prenatal interventions—Inter-hospital transport of extremely premature infants is associated with increased incidence and severity of IVH. This correlation has remained constant in recent years.¹²¹ Hence, pregnant mothers in preterm labor should be transported to a tertiary care center specializing in high-risk delivery. Prolonged labor might increase the risk of IVH and should be managed appropriately. Data on the incidence of IVH in Cesarean section vs. vaginal delivery is inconsistent^{122, 123} and thus, infants are delivered based on the decisions made by obstetricians.

Postnatal interventions—There is no specific recommendation about neonatal resuscitation of premature infants to prevent IVH. However, restoration of normal oxygenation and ventilation immediately at birth is important as hypoxemia and hypercarbia can cause fluctuation in the CBF which might contribute to IVH. While preventing metabolic acidosis and accomplishing normal perfusion is important, a rapid sodium bicarbonate infusion might add to the risk of IVH. After infants have been transferred to the neonatal unit, gentle and synchronized ventilation, prompt closure of ductus arteriosus, and maintenance of normal O₂ and CO₂ levels in the arterial blood are important preventive measures. Preventing pneumothorax, apneic episodes, seizures, as well as minimizing tracheal suctioning and handling will prevent disturbances in the CBF. Reducing stimulation and gentle caretaking decreases the incidence of IVH.¹²⁴ Effect of surfactant treatment on the development of IVH is unclear. Use of high frequency ventilators does not increase the risk of IVH¹²⁵ and therefore, an individualized approach in selection of appropriate ventilator should be pursued.

Together, the incidence of IVH has remained unchanged over the last couple of decades, despite advances in care of newborns. Use of prenatal GCs remains the most effective strategy in the prevention of IVH.

SUMMARY

IVH is a major complication of prematurity. IVH usually initiates in the periventricular germinal matrix and progresses to IVH upon the rupture of the underlying ependyma. The pathogenesis of IVH is ascribed to the intrinsic fragility of germinal matrix vasculature and to fluctuations in the CBF. The germinal matrix exhibits accelerated angiogenesis, which orchestrates formation of nascent vessels that lack pericytes, display immature basal lamina low in fibronectin, and have astrocyte endfeet coverage deficient in GFAP. These morphological and molecular factors contribute to the fragility of the germinal matrix vasculature. Importantly, pressure passive circulation is frequent in the premature infants and CBF fluctuates with the changes in internal milieu (O₂, CO₂, pH, osmolarity), CVP, blood pressure, and cardiac output secondary to inadequate ventilation, pneumothorax, severe lung disease, hemodynamic instability, sepsis, dehydration, patent ductus arteriosus, frequent suctioning, increased handling, and other factors. Recent studies have suggested roles of genes encoding coagulation factors, inflammatory cytokines, and collagen in the pathogenesis of IVH. Prenatal GCs are the most effective in preventing IVH and are the standard care. There is no long term advantage of using postnatal indomethacin. There is a

need of improved therapy to prevent IVH and its neurodevelopmental sequelae. Therapies designed to enhance the stability of the germinal matrix vasculature and reduce fluctuation of CBF might lead to more effective strategies in preventing IVH.

Acknowledgments

Source of funding: NIH/NINDS grant RO1 NS071263 (PB)

Authors thank Drs. Laura Ment and Barabara Stonstreet for the critical review of the manuscript and Joanne Abrahams for the assistance with images.

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KEY POINTS

1. Pathogenesis of intraventricular hemorrhage (IVH) is ascribed to the intrinsic weakness of germinal matrix vasculature and to the fluctuation in the cerebral blood flow.
2. The germinal matrix displays accelerated angiogenesis that orchestrates formation of nascent vessels that lack pericytes, display immature basal lamina low in fibronectin, and have astrocyte end-feet coverage deficient in glial fibrillary acidic protein (GFAP). These morphological and molecular factors contribute to the fragility of the germinal matrix vasculature.
3. The fluctuations in the cerebral blood flow is attributed to the cardiorespiratory and hemodynamic instability frequently associated with extremely premature infants, including hypotension, hypoxia, pneumothorax, patent ductus arteriosus, and others.
4. Prenatal glucocorticoids have emerged as the most effective intervention to prevent IVH. Therapies designed to enhance the stability of the germinal matrix vasculature and reduce fluctuation of CBF could lead to strategies that are more effective in preventing IVH.

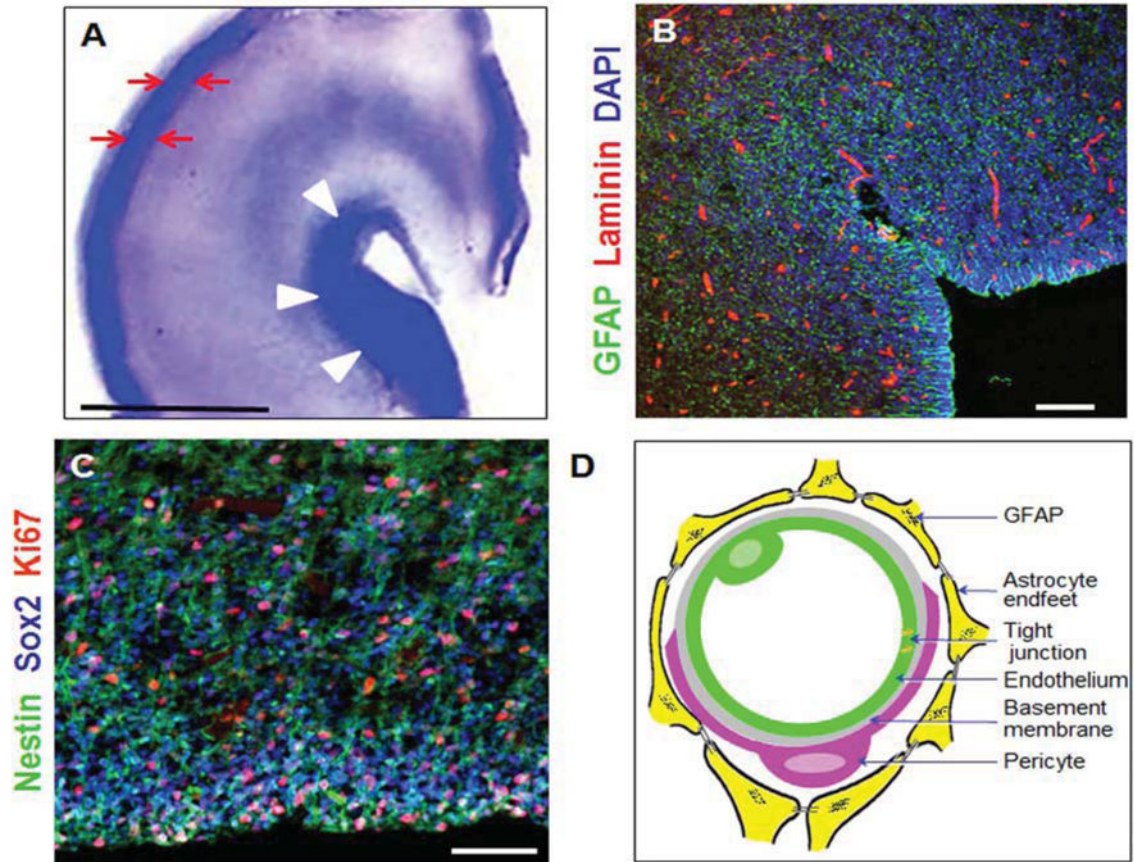


Figure 1. Morphology of germinal matrix

A) Representative cresyl violet staining of coronal section of the right-sided cerebral hemisphere of a 23 week preterm infant. Note cortical plate (arrows) and germinal matrix (arrow heads). Germinal matrix (violet staining) surrounds the whole ventricle, but is most conspicuous at the head of caudate nucleus. V, ventricle. Scale bar, 0.5 cm. B)

Representative immunofluorescence of cryosection from germinal matrix of a 23 week premature infant labeled with DAPI (blue), GFAP (green), and laminin (vascular marker, red). Note germinal matrix is highly vascular (vascular endothelium in red) and enriched with GFAP (+) glial cells (green). C) Coronal brain section was double labeled with nestin (progenitor cells, green), Sox2 (radial glia, blue), and Ki67 (red, proliferation marker). Note nestin and Sox2 positive cells are abundant in the germinal matrix. Scale bar; 100 (B) and 50 μ m (C). D) Schematic drawing of the blood brain barrier in cross section showing endothelium, endothelial tight junction, basal lamina, pericyte, and astrocyte endfeet.

D) Schematic drawing of the blood brain barrier in cross section showing endothelium, endothelial tight junction, basal lamina, pericyte, and astrocyte endfeet.

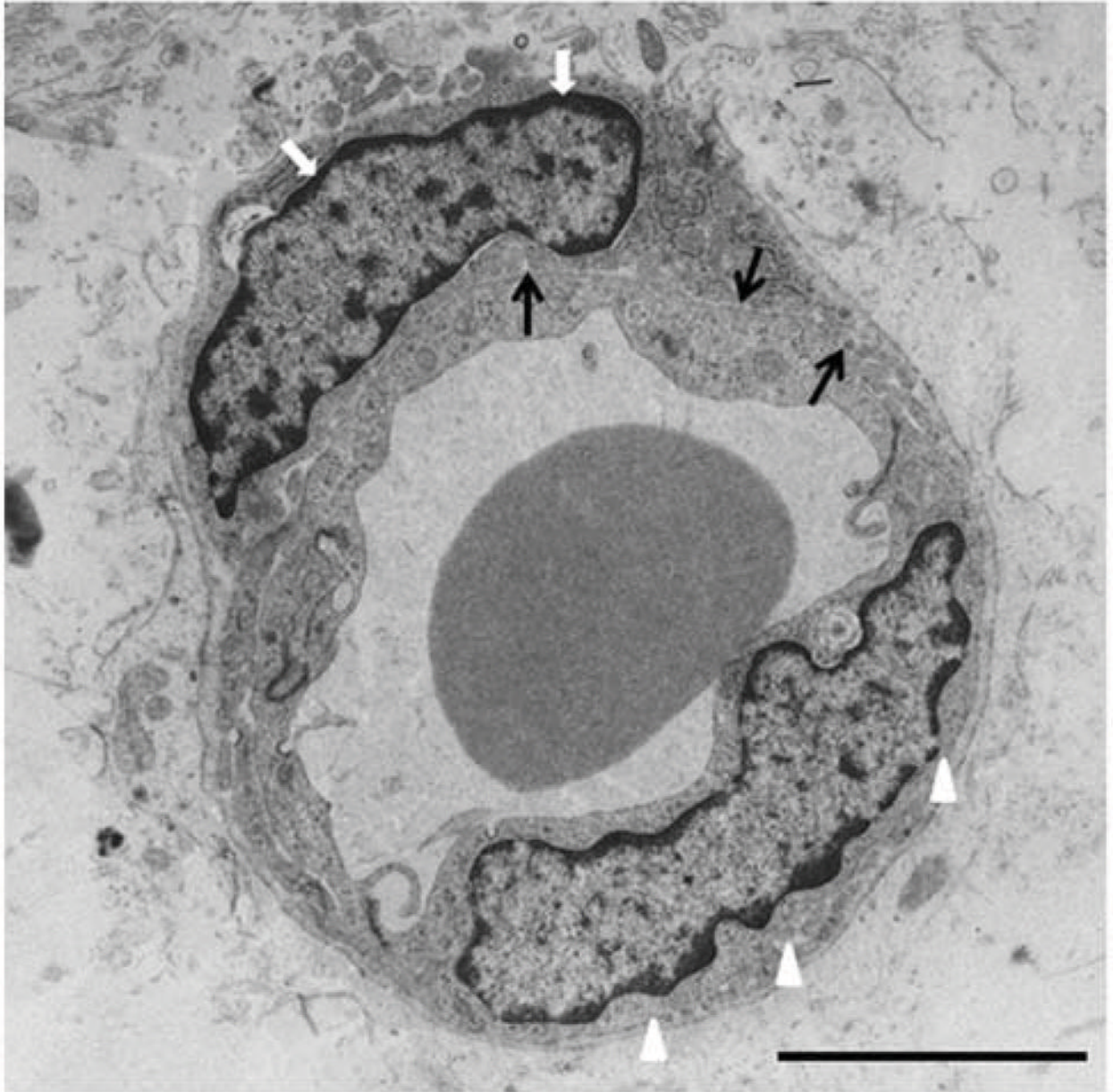


Figure 2. Electron micrograph showing endothelium (arrow heads) and pericyte (white arrows) separated by basal lamina (black arrows) in the white matter of 3 day old preterm rabbit pup (E29). Note pericyte wraps around the endothelium and is outer to the basal lamina. Scale bar, 4 μ m.

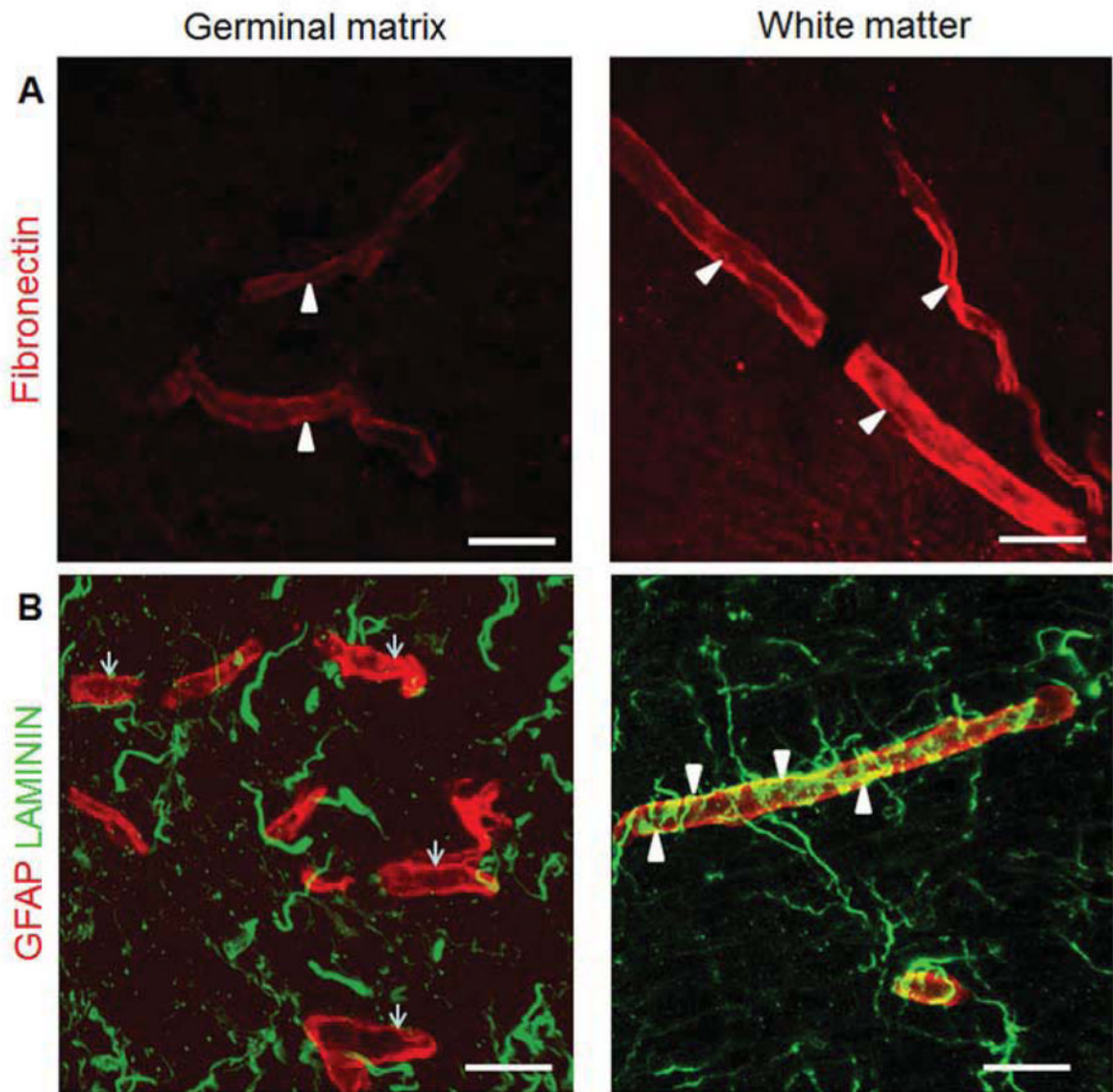


Figure 3. Germinal matrix vasculature is deficient in fibronectin and lack GFAP+ astrocyte endfeet coverage

A) Representative immunofluorescence of cryosection from germinal matrix and white matter of a 24 week premature human infant labeled with fibronectin (red) specific antibody. Fibronectin is strongly expressed in the white matter whereas it is weakly expressed in the germinal matrix (arrowheads). Scale bar; 20 μ m. B) Cryosection from germinal matrix and white matter of a 24 week premature infant was double-labeled with CD34 (endothelium, red) and GFAP (astrocyte, green) specific antibody. GFAP positive astrocyte endfeet are intimately associated with the outer endothelial surface in the white matter (arrowheads). However, GFAP positive astrocytes are not covering endothelium in the germinal matrix (arrows).

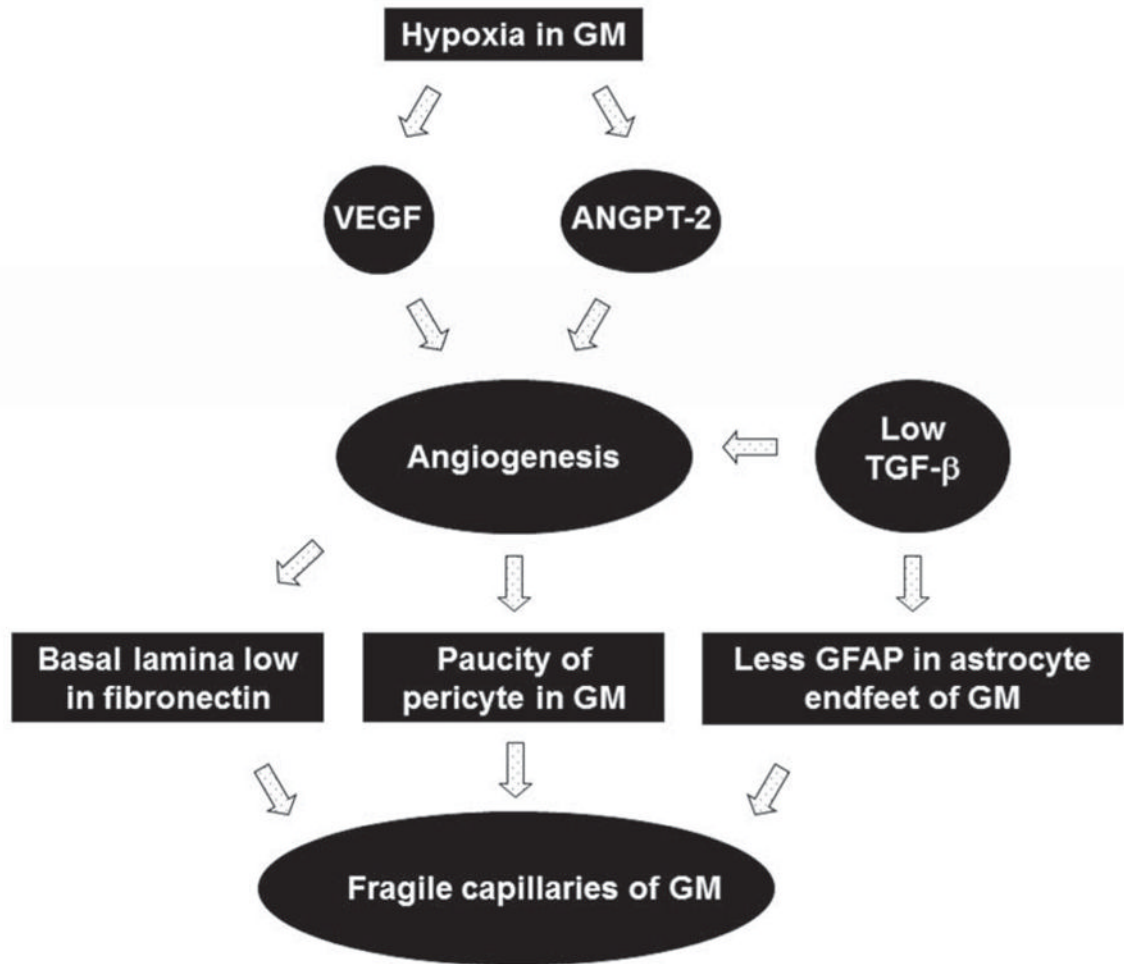


Figure 4. Mechanisms underlying fragility of germinal matrix vasculature

Hypoxic germinal matrix triggers upregulation of VEGF and angiopoietin-2 expression. These growth factors induce angiogenesis. The angiogenic vessels of the germinal matrix exhibit paucity of pericytes and deficiency of fibronectin in immature basal lamina. Additionally, astrocyte endfeet in the germinal matrix vasculature display reduced expression of GFAP. These factors contribute to the fragility of the germinal matrix.

Table 2

Prevention of Intraventricular hemorrhage

A. Prenatal interventions:	<ul style="list-style-type: none"> a. Prevent preterm delivery b. Maternal transport to regional neonatal center c. Prenatal glucocorticoids d. Agents of unproven benefit: Prenatal phenobarbitone, vitamin K, and magnesium sulfate
B. Care during infant delivery:	Optimize obstetric care and prevent prolonged labor
C. Postnatal interventions:	<ul style="list-style-type: none"> a. Optimize neonatal resuscitation b. Reduce fluctuation in cerebral blood flow: Minimize handling and suctioning, synchronized and gentle ventilation, prompt treatment of patent ductus arteriosus, maintaining normal O₂ and CO₂, preventing apneic episodes and seizures. c. Correction of coagulation and bleeding disorders d. Indomethacin: Reduces IVH, but does not enhance long term outcome. e. Agents of unproven benefit: Phenobarbitone, vitamin E, etamsylate