Preventing Brain Injury in the Preterm Infant—Current Controversies and Potential Therapies

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Abstract: Preterm birth is associated with a high risk of morbidity and mortality including brain damage and cerebral palsy. The development of brain injury in the preterm infant may be influenced by many factors including perinatal asphyxia, infection/inflammation, chronic hypoxia and exposure to treatments such as mechanical ventilation and corticosteroids. There are currently very limited treatment options available. In clinical trials, magnesium sulfate has been associated with a small, significant reduction in the risk of cerebral palsy and gross motor dysfunction in early childhood but no effect on the combined outcome of death or disability, and longer-term follow up to date has not shown improved neurological outcomes in school-age children. Recombinant erythropoietin has shown neuroprotective potential in preclinical studies but two large randomized trials, in extremely preterm infants, of treatment started within 24 or 48 h of birth showed no effect on the risk of severe neurodevelopmental impairment or death at 2 years of age. Preclinical studies have highlighted a number of promising neuroprotective treatments, such as therapeutic hypothermia, melatonin, human amnion epithelial cells, umbilical cord blood and vitamin D supplementation, which may be useful at reducing brain damage in preterm infants. Moreover, refinements of clinical care of preterm infants have the potential to influence later neurological outcomes, including the administration of antenatal and postnatal corticosteroids and more accurate identification and targeted treatment of seizures.

Keywords: preterm asphyxia; neuroprotection; hypothermia; corticosteroids; erythropoietin; stem cells; anticonvulsants

1. Introduction to Preterm Brain Injury

Premature birth, defined as birth before 37 weeks completed gestation, represents 11.1% of all live births worldwide. The rate of premature birth increased in almost all countries from 1990 to 2010 [1]. Although the mortality after preterm birth has fallen steadily over time, preterm infants continue to have very high rates of neurodevelopmental disability, including severe motor disorders such as cerebral palsy. The etiology of the disability is only partially understood, but is widely considered to be multifactorial, as illustrated in Figure 1. One of the most prevalent risk factors for perinatal brain injury is preterm birth itself, which may result from or interact with perinatal environmental and genetic factors. Disruption of nutrient or oxygen supply can arise from maternal (e.g., nutrient deficiencies, anemia), maternal–fetal (e.g., placental development) or fetal (e.g., abnormal vasculature, metabolic disorders) causes.
1.1. Brain Injury Associated with Hypoxia-Ischemia

Hypoxia-ischemia (HI) before, during or shortly after birth can contribute to brain injury in at least some preterm infants [2–4]. Older studies estimated that the prevalence of hypoxic–ischemic encephalopathy (HIE) in preterm infants was approximately 73/1000 live births, of whom ~50% were moderate to severe compared to approximately 2/1000 cases of moderate to severe encephalopathy in live term births [5]. More recently, small, retrospective studies have suggested lower rates of 1.4/1000 [6], 5/1000 [7] and 9/1000 live births [8]. Nevertheless, a recent large cohort of 115,502 preterm infants delivered in the USA between 2008 and 2011 reported that moderate to severe HIE occurred at a rate of 37.3/1000 babies born before 37 weeks of gestation [9]. Further, this study demonstrated an inverse relationship between lower gestational age at birth and greater mortality and morbidity, such that infants born before 28 weeks gestational age had an overall rate of 120/1000 live births. These studies suggest that acute asphyxia around the time of birth is an important contributor to brain damage in preterm infants.

Some of the controversy surrounding the prevalence of asphyxia in preterm infants may stem from the fact that identifying that events have occurred is more challenging than in infants over 36 weeks gestational age, as reviewed in [2]. For example, healthy preterm infants have lower body tone than term infants, making it more difficult to identify hypotonia. Nevertheless, at least in late preterm infants from 32 weeks gestation onward, it may be feasible to appropriately adapt the clinical neurological criteria used to diagnose HIE in near-term infants. Further, the impacts of HIE on childhood outcomes, particularly in infants born extremely preterm, are difficult to disentangle from the effects of preterm birth itself [2].
In addition to acute HI as discussed above, many preterm infants are exposed to chronic hypoxia before birth, as shown by intrauterine growth restriction (IUGR)/small for gestational age (SGA), defined as birthweight below the 10th percentile [10]. IUGR/SGA is multifactorial. In the developed world, most cases are related to placental insufficiency but malnutrition and chromosomal abnormalities are also potential causes. IUGR/SGA is associated with increased risk of death, developmental impairment, cerebral palsy and cardio-metabolic disease [10–12]. The adverse outcomes associated with IUGR/SGA are at least in part attributed to increased vulnerability to HI at birth [13], likely relating to reduced cardiac glycogen storage and therefore less ability to adapt to repeated severe hypoxia in labor [14].

Preterm infants may also experience intermittent HI after birth, due to conditions such as apnea of prematurity causing repeated periods of mild hypoxia, which are associated with neurodevelopmental and motor impairment [15]. Further, preterm infants may develop bronchopulmonary dysplasia (BPD), a chronic respiratory disease that is associated with frequent periods of hypoxia and neurodevelopmental impairment, particularly if it is moderate or severe [16].

1.2. Brain Injury Associated with Infection and/or Inflammation

Chorioamnionitis (infection of the fetal membranes) is associated with 11–40% of all preterm births [17]. The incidence of chorioamnionitis increases substantially with decreasing gestational age, such that it is associated with only ~4% of term deliveries but 94% of deliveries at 21–24 weeks of gestation [18]. Chorioamnionitis is characterized by invasion of microorganisms including bacteria, viruses and fungal species into the amniotic cavity, by many routes, including ascending from the lower genital tract, from the placenta, accidental introduction via invasive procedures such as amniocentesis, contamination via intrauterine contraceptive devices or retrograde spread via the fallopian tubes [19]. Chorioamnionitis is an independent risk factor for adverse brain development, including intraventricular hemorrhage (IVH), neurological impairment and cerebral palsy [20–23].

During exposure to intrauterine infection/inflammation, the fetal immune system responds by releasing pro-inflammatory cytokines that induce a fetal inflammatory response syndrome (FIRS) [24]. Fetal systemic inflammation is associated with induction of cerebral inflammation and adverse neurodevelopmental outcomes [25].

1.3. Vulnerabilities of the Preterm Brain to Injury

Finally, and not least, the preterm infant is understandably not fully adapted to the ex-utero environment, because of reduced access to suitable nutrition, high oxygen exposure, large blood pressure fluctuations related to fluid shifts, immune system challenges, and exposure to a highly pro-inflammatory environment.

Moreover, there are some unique characteristics that contribute to the vulnerability of the preterm brain to injury. Firstly, cell division and maturation are in progress. There is considerable evidence suggesting that pre-oligodendrocytes are particularly susceptible to injury and death [26]. Oligodendrocytes develop according to a well-characterized lineage, with pre-myelinating oligodendrocyte progenitors developing into pre-oligodendrocytes, which then develop into the myelinating immature and mature oligodendrocytes, as reviewed in [27]. The developmental window associated with the highest risk of periventricular white matter injury (23–32 weeks postconceptional age) coincides with a high proportion of pre-oligodendrocytes and this risk declines with the onset of differentiation of the pre-oligodendrocytes into the myelinating immature oligodendrocytes [28–30]. In 2-day-old rat pups, when pre-oligodendrocytes are the dominant lineage, are more susceptible to hypoxic–ischemic injury of white matter than 7-day-old rat pups, when immature oligodendrocytes are predominant [29]. Further, in 0.65 gestation fetal sheep, there was greater oligodendrocyte cell death in the medial periventricular white matter, in which pre-oligodendrocytes are the predominant lineage, compared to the lateral periventricular white matter, which has a higher proportion of immature oligodendrocytes [31].
The relative vulnerability of the pre-oligodendrocyte to HI compared to the immature oligodendrocyte may, in part, be mediated by greater susceptibility to oxidative stress, as shown in vitro by greater death of pre-oligodendrocytes in response to oxidative stress induced by depletion of intracellular glutathione [32]. Pregnancy itself is associated with increased oxygen demand and rate of production of reactive oxygen species leading to elevated oxidative stress and lipid peroxidation compared with non-pregnant women, as reviewed in [33]. Further, there is an increase in reactive oxygen species generated by the placenta of women experiencing pre-eclampsia. Compared to adults, the newborn infant has low levels of endogenous antioxidant capacity, including lower levels of plasma antioxidants such as vitamin D, beta carotene and sulfhydryl groups and lower levels of plasma metal binding proteins and reduced activity of erythrocyte superoxide dismutase, increasing its vulnerability to elevated oxidative stress, such as that which occurs in response after ischemia-reperfusion [33,34].

Next, the preterm brain has a comparatively underdeveloped vasculature, which matures relatively late in gestation; these increases in vascular density and cross-sectional area continue well into adulthood [35,36]. The most vulnerable brain region is the germinal matrix due to the higher vascular density, greater immaturity, and reduced structural integrity of blood vessels compared to both white and grey matter, at 16 to 32 weeks of gestation. Germinal matrix-IVH (GM-IVH) is a critical complication of prematurity involving hemorrhage that starts at the germinal matrix and progresses to the lateral ventricles [37]. It is inversely associated with both gestational age and birth weight and occurs in approximately 20% of very low birthweight preterm neonates weighing <1000 g at birth. Approximately 30–50% of preterm infants with serious IVH will develop post-hemorrhagic ventricular dilation or hydrocephalus. Although this may spontaneously resolve in some infants, approximately 25% require insertion of a shunt to alleviate progressive hydrocephalus. Need for a shunt after severe IVH has been associated with adverse neurodevelopmental and growth outcomes at 18 to 22 months compared to children who did not require a shunt [38]. Another serious complication of GM-IVH is periventricular hemorrhagic infarction, which is associated with significant cognitive and/or motor abnormalities in two thirds of survivors [39].

2. Neurological Outcomes

Infants born preterm are at high risk for neurodevelopmental disorders, including cerebral palsy [40]. The risk is greatest in extremely preterm infants (<28 weeks gestation), who have highest rates of poor neurological outcomes such as cognitive impairment, hearing loss and retinopathy of prematurity [41].

Preterm infants examined at term equivalent age show widespread changes in white matter diffusivity, indicating compromised white matter integrity, which is often observed in neurodevelopmental disorders such as cerebral palsy [42]. Critically, extremely preterm infants at term-corrected age show reduced cerebral cortical and deep nuclear grey matter volumes, and increased cerebrospinal fluid volumes [43]. Very premature infants (infants born ≤32 weeks’ gestational age) show high rates of white matter (31.6%) and grey matter (21.1%) abnormalities on MRI, which are predictive of poor neurodevelopmental outcomes at 9 years of age [44]. Repeated MRI scans of very preterm infants (23 to 30 weeks GA) show that 49% exhibit changes in brain structure and regional volumes shortly after birth, which increases to 92% by near-term gestational age (GA) [45]. The nature of brain pathology evolves over time, including the appearance of hemorrhagic lesions (germinal matrix and intraventricular), changes in signal intensity (reflecting necrotic or demyelinating processes) and ventricular dilatation [45].

The Quest to Develop Treatment Strategies to Reduce Preterm Brain Injury

A key challenge in developing treatment strategies to reduce brain injury in preterm infants is the large number of different injurious phenomena that these infants can be exposed to before, during or after birth. As highlighted above, these include preterm
birth itself, developmental vulnerability to injury at a particular gestational age, chronic hypoxia in utero, acute asphyxia around the time of birth, infection/inflammation and poor respiratory function after birth. Further, many therapies required for neonatal intensive care such as ventilation, may decrease mortality but increase risk of brain injury [46]. To date therapies for preterm infants have been largely ineffective in improving long-term neurological outcomes and there has been at best modest improvements over time [47–49]. However, exciting potential treatments are being investigated in preclinical or clinical trials that may help to reduce the burden of brain injury in the preterm infant.

3. Treatments Currently in Clinical Use for Preterm Infants

3.1. Antenatal Corticosteroids

Maternal glucocorticoid therapy is recommended for cases of threatened or current preterm labor to promote fetal lung development. The safety and benefits of antenatal glucocorticoids were observed in the EPIPAGE cohort study (Table 1), which showed that very preterm infants (<32 weeks) had a reduction in white matter injury (OR = 0.60, 95% CI = 0.46–0.79) and death (OR = 0.61, 95% CI = 0.41–0.91), but no changes in rates of adverse neurological outcomes at 5 years old [50]. A recent meta-analysis examining 30 trials of antenatal glucocorticoids for women at risk of giving preterm birth found a reduction in infant perinatal death, neonatal death, respiratory distress syndrome, need for mechanical ventilation, and infection therapy [51], with a reduced incidence of IVH (RR = 0.55, 95% CI 0.40 to 0.76, 16 studies, participants = 6093). The only study that reported neurodevelopmental outcomes in childhood did not show a significant effect of antenatal glucocorticoids (RR = 0.64, 9% CI 0.14 to 2.98), but was very underpowered, with only 82 participants [52]. Repeated doses, given more than 7 day apart seem to improve short-term outcomes, without increasing mortality [53]. Importantly, a recent randomized double-blind controlled trial including 1509 fetuses who received antenatal corticosteroids, showed no significant difference in neurodevelopmental disability at 2 years of age between those treated with dexamethasone and those treated with betamethasone [54]. Consistent with this, a recent meta-analysis of 45 trials including 11,227 women and 11,878 infants, found no difference between dexamethasone and betamethasone on neonatal death, neurodevelopmental disability, IVH and birthweight [55]. In contrast to the consistent short-term benefits seen in clinical trials, preclinical studies from a wide range of animal models have raised concerns that antenatal steroids may have adverse effects on neurological outcomes. For example, weekly maternal betamethasone starting as early as 0.63 gestation in sheep reduced myelination in multiple brain regions [56]. In the context of the more rapid developmental of the fetal sheep compared to humans, this represents a relatively more prolonged exposure than it would be for a human. Interestingly, in preterm fetal sheep, exposure to a clinically relevant dose of maternal dexamethasone was associated with transient evolving epileptiform activity, consistent with electrographic and clinical seizures [57]. However, no neural injury or microglial activation was seen at postmortem and maturation of the EEG was enhanced, suggesting there were no adverse outcomes [57]. Reassuringly, clinical MRI studies have demonstrated that antenatal betamethasone or hydrocortisone did not influence regional brain volumes in humans [58,59].

Of greater potential concern, studies in preterm fetal sheep suggest potential deleterious effects after acute HI. For example, maternal dexamethasone given 15 min after fetal asphyxia was associated with more severe neuronal loss in the hippocampus and basal ganglia and greater loss of both total and immature/mature oligodendrocytes in the periventricular white matter [60]. In the same experimental paradigm, maternal dexamethasone treatment was associated with a significantly greater fall in carotid artery blood flow and cerebral oxygenation measured using near infrared spectroscopy, in the first 6 h after asphyxia, despite similar brain activity, suggesting that greater hypoperfusion and impaired cerebral oxygenation during the latent phase may underlie the exacerbation of neural injury [61]. When a single i.m. injection of maternal dexamethasone was given 4 h before asphyxia, induced by 25 min of complete umbilical cord occlusion in preterm fetal sheep, severe,
cystic brain injury was seen, with increased numbers of seizures, worse recovery of brain activity and increased arterial glucose levels compared to diffuse injury after asphyxia alone [62]. Importantly, these findings could be replicated by glucose infusions before asphyxia, suggesting that dexamethasone-induced hyperglycemia may transform diffuse injury into cystic brain injury after asphyxia in the preterm fetal sheep [62]. These data suggest that there is potential for antenatal corticosteroids to have adverse effects on the small subset of preterm infants that may be exposed to asphyxia before or during birth but this has not been specifically investigated in clinical studies. However, we must bear in mind that the occurrence of acute asphyxia in a fetus after administration of antenatal steroids is both unanticipated and unpreventable, while the short-term benefits of antenatal corticosteroids in general are well established.

Table 1. Summary of key clinical studies discussed in this review investigating the neurological effects of current and potential neuroprotective treatments in preterm infants.

| Treatment                      | Study Details                                                                 | Clinical Setting          | Main Findings                                                                                           | Reference |
|--------------------------------|------------------------------------------------------------------------------|----------------------------|----------------------------------------------------------------------------------------------------------|-----------|
| Antenatal corticosteroids      | Observational study 2323 infants (22–32 weeks) (EPIPAGE study)              | Extreme or very preterm birth | Antenatal corticosteroids greatly increased survival with little evidence for effects on neurodevelopmental and behavioural outcomes at 5 years of age | [50]      |
|                                | Meta-analysis of 30 trials 7774 women and 8158 infants                      | Preterm birth              | Antenatal corticosteroids were associated with a reduction in perinatal death, neonatal death, respiratory distress syndrome, intraventricular haemorrhage, need for mechanical ventilation, and infection therapy | [51]      |
|                                | Double-blind randomized controlled trial 1509 fetuses who received dexamethasone or betamethasone | Preterm birth              | No significant difference in the incidence of survival without neurosensory disability at 2 years of age between dexamethasone and betamethasone | [54]      |
|                                | Meta-analysis of forty-five trials (11,227 women, 11,878 infants) dexamethasone vs. betamethasone | Preterm birth              | No difference between dexamethasone and betamethasone on neonatal death, neurodevelopmental disability, IVH and birthweight | [55]      |
Table 1. Cont.

| Treatment                   | Study Details                                                                 | Clinical Setting                                      | Main Findings                                                                                                                                  | Reference |
|-----------------------------|-------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Postnatal corticosteroids   | 18 premature infants (23–31 weeks) 7 treated with postnatal dexamethasone 11 not treated | Preterm infants with chronic lung disease             | Postnatal dexamethasone was associated with impaired brain growth, particularly in cerebral cortical grey matter                              | [63]      |
|                             | 53 extremely low birthweight infants with high-quality MRI 11 infants received postnatal dexamethasone 30 infants received no treatment | Extremely low birthweight infants                    | Postnatal dexamethasone use was associated with smaller total and regional cerebral tissue volumes                                         | [64]      |
|                             | Cochrane Database systematic review of 32 randomised controlled trials including 4395 preterm infants with BPD who received early systemic corticosteroid treatment (< 8 days) | High-risk preterm infants                            | Early postnatal corticosteroids were associated with increased risk of abnormal findings on neurological examination and increased risk of cerebral palsy on long-term follow up | [65]      |
|                             | Cochrane Database systematic review of 21 randomised controlled trials including 1424 preterm infants with BPD who received late systemic postnatal corticosteroids treatment (> 7 days) | Preterm infants with BPD                             | Postnatal steroids were associated with increased retinopathy of prematurity but not blindness, a trend towards a reduction in severe IVH and death but a trend towards increased cerebral palsy or abnormal neurological examination | [66]      |
|                             | Meta-analysis of 16 randomised controlled trials comparing 1136 ventilated preterm infants > 7 days who received dexamethasone or placebo | Ventilated preterm infants                           | Higher cumulative doses of dexamethasone after the first week of life may decrease the risk of BPD without increasing the risk for neurodevelopmental impairment | [67]      |
| Magnesium sulfate           | Cochrane Database systematic review of 5 randomised controlled trials of antenatal magnesium sulfate therapy in women threatening preterm birth at less than 37 weeks gestational age including 6145 babies | Preterm birth                                        | Antenatal magnesium sulfate therapy was associated with a small reduction in the incidence of cerebral palsy, with a number needed to treat of 63 | [68]      |
|                             | Systematic review of 6 trials involving Antenatal magnesium sulphate administration to women threatening preterm delivery before 34 weeks gestation including 5357 infants | Preterm birth                                        | Antenatal magnesium sulphate therapy was associated with a significant reduction in the risk of cerebral palsy and substantial gross motor dysfunction | [69]      |
| Treatment                                                                 | Study Details                                                                 | Clinical Setting          | Main Findings                                                                                                                                                                                                 | Reference |
|-------------------------------------------------------------------------|-------------------------------------------------------------------------------|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Long-term follow up of a randomised controlled trial in women threatening preterm birth before 30 weeks gestation | **Preterm birth**                                                             | Magnesium sulfate was not associated with improvements in neurological, cognitive, behavioral, growth, or functional outcomes in their children at school age (6–11 years) | [48]      |
| 535 received magnesium sulphate                                          |                                                                                |                           |                                                                                                                                                                                                             |           |
| 527 received placebo                                                     |                                                                                |                           |                                                                                                                                                                                                             |           |
| Long-term follow up of a randomised controlled trial of magnesium sulfate in women threatening preterm birth before 33 weeks gestation, including 503 children (7–14 years) | **Preterm birth**                                                             | Magnesium sulfate was not associated with any detrimental effects nor any significant effects on neurological outcome | [47]      |
| Randomized control trial of rEpo versus placebo                          | Extreme or very preterm birth                                                 | rhEpo decreased risk of death and moderate/severe disability at 18 month (13%) compared to control (26.9%) | [70]      |
| 800 infants (≤32 weeks)                                                  | Every other day for 2 weeks starting within 72 h of birth                     |                           |                                                                                                                                                                                                             |           |
| Human recombinant Epo                                                    | Randomized, double-blind trial                                                | rhEpo did not reduce risk of severe developmental impairment at 2 years of age                                                                                                                                  | [71]      |
| 741 infants (24 weeks 0 days to 27 weeks 6 days)                         | Extremely preterm birth                                                       |                           |                                                                                                                                                                                                             |           |
| rEpo started <24 h of birth                                              | 1000 U/Kg every 48 h for 6 doses followed by 400 U/Kg 3 times/week            |                           |                                                                                                                                                                                                             |           |
| Very preterm birth                                                       | 3000 IU/kg iv within 3, at 12–18, and at 36–42 postnatal hours               | rhEpo had no effect on neurodevelopmental outcomes at 2 years or 5 years                                                                                                                                     | [72,73]  |
| 448 infants (26 weeks 0 days’ and 31 weeks 6 days)                       | HIE                                                                            | HT-associated complications in 90% of preterm infants and 81.3% term infants (p = 0.3)                                                                                                                        | [74]      |
| Retrospective study                                                       |                                                                  |                           | Preterms showed increased risk of hyperglycemia, early rewarming, death and white matter injury compared to term infants after cooling                                                                                                                                 |           |
| 31 preterm infants (34–35 weeks)                                        | HIE                                                                            |                           |                                                                                                                                                                                                             |           |
| 32 term infants                                                          |                                                                                |                           |                                                                                                                                                                                                             |           |
| Therapeutic hypothermia                                                  | Retrospective uncontrolled cohort analysis                                    | HIE                       | High incidence of complications, including coagulopathy (50%), thrombocytopenia (20%) and death (18.2%). Death or moderate to severe neurodevelopmental impairment occurred in 50% of infants with known outcomes | [75]      |
| 30 infants (33–35 weeks)                                                 |                                                                                |                           |                                                                                                                                                                                                             |           |
3.2. Postnatal Glucocorticoids

Preterm infants with BPD have a prolonged oxygen requirement after birth, with greatest risk of BPD with decreasing gestational age. Approximately 50% of extremely preterm infants (<28 weeks) and up to 80% of infants born <25 weeks develop BPD [76,77]. Given the strong inflammation associated with BPD, the use of anti-inflammatory agents such as postnatal glucocorticoids was initially widely embraced as a treatment strategy. However, evidence of an association between high-dose postnatal glucocorticoid use and the development of cerebral palsy led to a marked decline in their use in recent decades [78]. Postnatal dexamethasone treatment was associated with negative neurodevelopmental consequences including increased risk of developing CP (OR = 4.45) and developmental delay (OR = 2.87) [45]. Furthermore, high-dose postnatal dexamethasone reduces total intracranial, cerebral tissue, and cortical grey matter volume (Table 1) [63,64].

More recent studies suggest that key factors affecting the relative benefits and risks of postnatal glucocorticoid therapy, include dosing and timing of treatment as well as the underlying relative risk of developing cerebral palsy. High-dose dexamethasone exposure decreased brain weight, delayed neurological development [79], reduced skilled motor coordination and altered posture [80], reduced cerebellar size [81], reduced hippocampal cell proliferation [82], and induced apoptosis in the hippocampus and striatum in rodents [83]. Moreover, high-dose dexamethasone in preterm infants is associated with a trend for increased PVL [84,85]. Although high-dose glucocorticoids are associated with poor neurodevelopment, contemporary low-dose dexamethasone protocols (approximately 10% of the doses used in the 1990s) appear to improve infant ventilation outcomes without significant adverse neurodevelopmental outcomes [86,87]. Recently, a study in moderately preterm lambs (0.86 gestation, full-term brain equivalent) found no obvious risk of neurological harm with high- or low-dose tapered postnatal dexamethasone over 7 days for a cumulative dose 0.27 and 2.67 mg/kg, respectively [88]. There were equivocal outcomes for brain lesions, frontal cortex volumes (white and grey matter), frontal cortex thickness, hippocampus volumes, and gross morphometric measurements [88].

Recent systematic reviews of the relative benefits and risks of both postnatal early (<8 days) or late (>7 days) glucocorticoid therapy concluded that the benefits may not outweigh the risks of treatment [65,66]. Systemic corticosteroids administered < 8 days after birth were associated with beneficial short-term effects for respiratory outcomes but a worrying long-term increase in the risk of cerebral palsy [65]. When systemic corticosteroids were administered > 7 days after birth, similar short-term benefits were seen, without a significant increased risk of cerebral palsy [66]. A meta-analysis by Onland et al. [67] found that with moderately-early (7–14 days after birth) dexamethasone therapy, higher cumulative doses result in reduced rates of BPD without increasing the risk of neurodevelopmental impairment. Further, the risk of mortality and cerebral palsy decreased with each incremental mg/kg cumulative dexamethasone dose. However, this may be explained by the benefit of postnatal dexamethasone interacting with the risk of chronic lung disease and BPD, given that prolonged mechanical ventilation is an independent risk factor for cerebral palsy [89]. The most important finding of this research is that treating preterm infants at a low risk for BPD increases risk of cerebral palsy, whereas treating high-risk infants for BPD lowers risk of cerebral palsy [90,91].

3.3. Magnesium Sulfate (MgSO$_4$)

Magnesium is an endogenous anti-excitotoxic agent that acts by binding to the magnesium site on the NMDA receptor, inhibiting these glutamatergic channels [92]. This has led to interest in the use of magnesium sulfate as neuroprotective treatment for both term and preterm babies. However, the data remain controversial.

Experimentally, some studies in neonatal rodents have suggested neuroprotection with MgSO$_4$ before or after HI. However, it is critical to appreciate that magnesium is a potent vasodilator and so can increase heat loss, leading to iatrogenic hypothermia in small
animals. Systematic review of the literature suggests no benefit in rodents, piglets and fetal sheep in studies with good temperature control [93].

Meta-analysis of large randomized controlled trials of MgSO₄ administered to women in preterm labor found that it was associated with a small but significant reduction in the risk of cerebral palsy and gross motor dysfunction in early childhood (Table 1) [68,69]. However, there was no net effect on overall death and disability [94,95]. Further, two of the five randomized controlled trials included in the original meta-analyses have now followed children up to school age and show no significant improvement in cognitive, behavioral or functional outcomes [47,48]. Given the lack of long-term clinical benefit, there is considerable ongoing controversy around whether MgSO₄ has true neuroprotective effects in the preterm infant.

3.4. Anticonvulsants

The most common age for seizures is <1 year old [96], and is higher still in the neonatal period (<28 days, [97]). Seizures in preterm neonates are particularly common (clinical seizures term vs. preterm incidence 2.0 vs. 11.1 per 1000 live births [98]). Single seizures are unusual in neonates, and recurrent seizures are common if left untreated [99]. Neonatal seizures are associated with poor neurodevelopment and preterm infants with seizures have been reported to have worse outcomes than term infants with seizures [100]. The direction of causality though remains highly unclear.

A recent survey of 193 neonatal physicians indicated that the barbiturates phenobarbital and phenytoin are the most common anticonvulsant medications used to control neonatal seizures [101]. However, the use of these GABAergic drugs is controversial due to potential adverse effects on short- and long-term brain development, and the lack of robust evidence for efficacy [49]. Systematic reviews of barbiturate use show that less than 50% of neonates respond to first line therapy, and none when they are used as a second-line therapy [102]. Indeed, clinical signs of improvements may be due to the sedative effects of these drugs, rather than reducing seizure activity and underlying brain dysfunction [49]. The evidence for a beneficial effect of anticonvulsant drugs in neonates is so limited that a 2007 Cochrane review concluded that “anticonvulsant therapy to term infants in the immediate period following perinatal asphyxia cannot be recommended for routine clinical practice, other than in the treatment of prolonged or frequent clinical seizures” [103].

Preclinical evidence in the developing rat brain suggests that many anticonvulsant drugs are also neurotoxic at clinical and subclinical doses [104]. Phenobarbital treatment was associated with reduced proliferation in the dentate gyrus and reduced expression of neuronal markers and neuronal transcription factors and neurotrophins in P4–P6 neonatal rats [105]. In P7 rats, clinically relevant doses of phenytoin, phenobarbital, diazepam, clonazepam, vigabatrin or valproate were associated with widespread apoptotic degeneration, reduced expression of pro-survival neurotrophins, and a significant reduction in brain weight after eight days [104]. Further, clinical doses of phenobarbital, phenytoin and lamotrigine in P7 neonatal rats were associated with impaired striatal synaptic development between P10 and P18 [106]. Encouragingly, levetiracetam treatment did not impair synaptic development [106], consistent with a previous study showing that it did not induce cell death in the P7 rat brain [107].

The potential harm and lack of efficacy of GABAergic agonists may be due to developmental changes in GABA receptor responses. Early in development, GABA receptors may respond to stimulation with excitation, rather than the inhibition observed in adults [108] due to high intracellular Cl⁻ concentration resulting from high Na-K-2Cl (NKCC1) expression, which mediates Cl⁻ entry and low K-Cl cotransporter KCC2 expression, which mediates Cl⁻ exit from cells [109,110]. With the upregulation of the potassium-chloride co-transporter KCC2 during the early postnatal period, chloride ions can be extruded and GABA and glycine become inhibitory. It has been shown that late gestation HI reduced neuronal KCC2 expression, impairing hippocampal CA3 inhibitory tone in juvenile rats [111]. Impaired inhibitory tone after HI may lower the seizure threshold and contribute to the lack
of efficacy of GABAergic agonists in neonates. This suggests that GABA agonists may be developmentally inappropriate in infants. This led investigators to suggest the use of more developmentally appropriate anticonvulsant treatments, such as activation of NKCC1 with the loop diuretic bumetanide in infants 33–44 weeks postmenstrual age [49,112]. However, an exploratory trial in term infants did not show evidence for seizure control and there was an apparent increased risk of hearing loss [113]. Interestingly, postnatal administration of the potential neuroprotective treatment recombinant erythropoietin (rEpo) has been shown to attenuate the loss of KCC2, associated with late gestation in utero HI brain injury in rats, potentially reversing the deficits in inhibitory circuit formation and greater susceptibility to seizure activity [114].

In summary, there are major challenges in treating preterm infants with seizures. Current first-line anticonvulsants are at best moderately effective, and potentially harmful. Improved understanding of the specific mechanisms underlying neonatal seizures and the development of appropriate anticonvulsant therapies are key research priorities.

4. Potential Neuroprotective Treatments that Have Been Investigated in Clinical and Preclinical Trials for Use in Preterm Infants

4.1. Erythropoietin

Erythropoietin (Epo) has a central role in erythropoiesis and has been widely used to prevent postnatal anemia in premature infants [115]. In addition, rEpo has shown many potentially neuroprotective effects, including promoting expression of anti-apoptotic more than pro-apoptotic genes, inhibiting caspase activation, and attenuating inflammation and oxidative stress, as reviewed in [116]. There is substantial evidence from preclinical models that treatment with rEpo given shortly after HI is neuroprotective in the developing brain. In P7 rats, repeated injections of rEpo (5000 IU/kg on days one, two and three) reduced brain injury and was more effective than a single (5000 IU/kg) dose or three injections with 2500 or 30,000 IU/kg [117]. In preterm-equivalent fetal sheep, treatment with a prolonged infusion of rEpo (5000 IU/kg by slow push, then 832.5 IU/h from 30 min to 72 h) after severe asphyxia induced by complete umbilical cord occlusion, was partially neuroprotective, reduced seizure burden and improved the recovery of EEG power and reduced apoptosis and inflammation, three days after HI [118].

rEpo treatment has shown potential to support the restoration of neural structure and function over the long-term recovery from HI. In P7 rat pups, treatment with repeated injections of rEpo (1000 U/Kg) from 48 h after HI, increased neurogenesis and oligodendrogenesis, improved oligodendrocyte maturation, and restored myelination two weeks after HI [119]. Similarly, postnatal treatment with five injections of rEpo (2000 U/Kg) in rat pups exposed to transient HI at embryonic day 18 restored HI-induced functional deficits in gait and social interaction at P28–30 and attenuated structural abnormalities in the white matter and subcortical grey matter structures seen using diffusion tensor MRI at P35–40 [120].

Importantly, rEpo also appears to be beneficial in models of preterm infection/inflammation. A single injection of rEpo (5000 IU/Kg) after maternal LPS injection in rats at 18–19 days gestation was associated with reduced IL6, IL1 and TNF-α concentrations, apoptosis and demyelination at p7 [121]. In preterm fetal sheep with endotoxin-induced brain damage, intravenous rEpo (5000 IU/Kg) injections administered once daily for 3 days, reduced axonal damage, microglial and astrocytic responses in the white matter and improved myelination [122].

Clinically, phase 1 and 2 trials have provided strong evidence of the safety of rEpo treatment in low birth weight infants, prematurely born infants and full-term neonates with HIE [116,123]. In a small clinical trial in very preterm infants, rEpo (500 U/Kg intravenously every other day for 2 weeks started within 72 h of birth) was associated with improved neurodevelopmental outcomes at 18 months of age (Table 1) [70]. Meta-analysis of 1133 very preterm infants (approximately 32 weeks gestation) from four randomized control trials who received prophylactic rEpo treatment suggested a reduced incidence of severely impaired neurodevelopmental scores at 18–24 months post menstrual age [123]. However, more recently, a randomized, double-blind trial of high-dose rEpo administered
to extremely preterm infants from 24 h after birth until 32 weeks post menstrual age, had no effect on the risk of severe neurodevelopmental impairment or death at 2 years of age [71]. Further, a similar study of 448 infants randomized to receive repeated doses of rEpo started within 3 h of very preterm birth found no effect on neurodevelopmental outcomes at 2 and 5 years of age [72,73]. It is possible that the relatively delayed start (within 24 h) and infrequent dosing regimen in Juul et al. [71] may have contributed to the lack of neuroprotection seen in this study as infants received 1000 IU/Kg rEpo every 48 h for a total of 6 doses followed by 400 IU/Kg 3 times per week, whereas the study in preterm fetal sheep showing partial neuroprotection used early initiation of treatment at 30 min and a prolonged infusion to maintain a stable plasma rEpo concentration [118]. This highlights the importance of optimizing the dosing regimen and therapeutic window of opportunity for treatment in a large animal translational model prior to the start of a clinical trial.

One potential solution to the need for frequent dosing with rEpo could be the use of darbepoetin (Darbe), the long-acting erythropoiesis-stimulating agent, which has been shown to have a half-life that is 3-fold longer than rEpo in term neuroprotection studies [124,125]. In a small clinical study comparing rEpo, Darbe and placebo, administration of either rEpo or Darbe was associated with higher cognitive scores and a reduced incidence of cerebral palsy [126].

4.2. Therapeutic Hypothermia

Mild therapeutic hypothermia induced by cooling the head or body of an infant is now routine clinical care for term infants suffering HIE [127,128]. The success of therapeutic hypothermia as a treatment strategy is likely attributable to its wide array of mechanisms of action, including reducing metabolism, suppressing programmed cell death pathways, and attenuating the inflammatory response and protecting mitochondrial function, as reviewed in [129].

There is compelling clinical and experimental evidence that therapeutic hypothermia can reduce neuronal loss and improve neurological outcome in term and near-term infants with moderate to severe HIE [127,128]. Meta-analysis of 11 randomized controlled trials of either selective head cooling or whole-body cooling initiated within 6 h of birth, involving 1505 term and late preterm (35–36 weeks) infants with moderate/severe HIE found highly consistent beneficial effects after hypothermia [128]. Therapeutic hypothermia was associated with reduced mortality or major neurodevelopmental disability by 18 months of age (relative risk (RR) 0.75 (95% confidence interval (CI) 0.68 to 0.83). Long-term follow up of these studies suggests that improvements in outcome persist into middle childhood after mild induced hypothermia for HIE [130–132]. Although these trials included late preterm infants over the age of 35 weeks gestation, little is known about the safety and efficacy of therapeutic hypothermia for younger preterm infants.

Preclinical evidence suggests that hypothermia is also effective at reducing brain damage in the preterm brain. For example, mild cerebral hypothermia started 90 min after asphyxia induced by complete umbilical cord occlusion and continued for 3 days was associated with a marked reduction in loss of neurons and immature oligodendrocytes, restored carotid artery blood flow and EEG frequency to sham control levels in 0.7 gestation fetal sheep [133]. Similarly, in the same model, hypothermia started 30 min after 25 min of complete umbilical cord occlusion was associated with reduced neuronal loss and microglial induction in the striatum, faster recovery of spectral edge frequency, reduced seizure burden and less suppression of EEG amplitude [134]. However, when treatment was delayed until 5 h after umbilical cord occlusion, no neuroprotection was seen suggesting that the therapeutic window of opportunity may be even narrower than in the term brain, where limited neuroprotection can be achieved with hypothermia started 5.5 h after 30 min of global cerebral ischemia [135].

Although it seems plausible that hypothermia will be effective at reducing brain damage in the preterm brain after an acute hypoxic–ischemic event such as perinatal
asphyxia, concerns remain regarding its safety. Mild hypothermia (33–35 °C) has been associated with slowing of the atrial pacemaker and intracardiac conduction, with sinus bradycardia, decreased left ventricular contractility and cardiac output, as reviewed in [136]. A small retrospective study of 31 preterm neonates born at 34–35 weeks gestation with HIE and treated with hypothermia suggests that use of hypothermia is feasible in this age group but that caution is warranted due to a higher risk of mortality and side-effects, such as hyperglycemia, compared to term neonates treated with hypothermia (Table 1) [74]. In a retrospective uncontrolled cohort analysis of 30 preterm infants 33–35 weeks gestation with HIE who received whole-body hypothermia, there was a concerning incidence of combined outcome of death and neurodevelopmental impairment and complications, including coagulopathy, early clinical seizures, arterial hypotension, persistent metabolic acidosis and thrombocytopenia [75]. However, interpretation of these studies is limited due to their retrospective nature, small cohort size and lack of normothermia controls.

The Preemie Hypothermia for Neonatal Encephalopathy trial (NCT01793129) is currently active, investigating the use of mild therapeutic hypothermia for preterm infants between 33 and 35 weeks gestational age who present at less than 6 h postnatal age with moderate to severe neonatal encephalopathy (Clinical Trial’s Register). Infants are randomized to receive 72 h of whole-body hypothermia or normothermia.

5. Potential Neuroprotective Treatments Showing Promise in Preclinical Studies of Preterm Brain Injury

5.1. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring indolamine secreted by the pineal gland to regulate circadian rhythm that also has antioxidant properties [137]. Exogenous melatonin has potential to be a prophylactic treatment for fetuses at high risk of perinatal hypoxia as it readily crosses the placenta [137]. However, recent large animal studies suggest that a part of the neuroprotective effects observed with melatonin treatment were attributable to the diluent, ethanol.

In preterm fetal sheep at 0.7 gestation, maternal low-dose melatonin infusion from 15 min before asphyxia until 6 h after, was associated with faster recovery of EEG activity, delayed onset of seizures, improved survival of mature oligodendrocytes, and reduced microglial activation in the periventricular white matter [138]. However, the ethanol vehicle was independently associated with reduced duration of fetal seizures and improved neuronal survival in the striatum, albeit with worse neuronal survival in the hippocampus and less white matter proliferation compared to saline treatment. These data suggest complex confounding effects of ethanol. Direct fetal infusion of melatonin (2.6 mg dissolved in ethanol, over 24 h) in preterm (0.7 gestation) fetal sheep, starting 2 h after HI showed region specific improvement in white matter damage 10 days after HI [139].

Further supporting neuroprotective effects of melatonin and ethanol, they have been shown to be similarly neuroprotective in the term brain after HI. In a recent study in neonatal piglets, treatment with high-dose melatonin (18 mg/kg) in conjunction with therapeutic hypothermia, significantly improved recovery of aEEG activity, improved cerebral energy metabolism seen using magnetic resonance spectroscopy and reduced TUNEL-positive cell death after asphyxia [140]. However, this study also showed that the ethanol used to improve melatonin solubility was independently associated with partial protection, including recovery of aEEG and reduced cell death, particularly of oligodendrocytes. Administration of high-dose melatonin (5 mg/kg/h over 6 h) dissolved in ethanol started immediately after HI in postnatal term piglets significantly augmented neuroprotection from therapeutic hypothermia on both magnetic resonance spectroscopy markers of anaerobic stress, and histopathology [141]. When an ethanol-free proprietary melatonin formulation using excipients considered safe for use in neonates was given to neonatal piglets at 2 and 26 h after HI combined with cooling from 2 to 26 h, localized additive protective effects were seen in the sensorimotor cortex, but not other cortical, subcortical or white matter regions examined, with melatonin showing a very narrow window of opportunity for effective treatment [142]. These studies suggest that the combination of melatonin and ethanol has
the potential to effectively reduce brain damage after HI in both the preterm and term brain. The question is whether ethanol will ever be viewed as an acceptable drug to be trialed as a neuroprotective therapy for preterm infants.

Pretreatment with maternal melatonin has been shown to be beneficial in a mouse model of maternal inflammation, with reduced preterm birth and preterm brain injury seen when pregnant mothers of embryonic day 16.5 mice received melatonin before the induction of inflammation using lipopolysaccharide [143]. A limitation of this study is that melatonin was dissolved in dimethyl sulfoxide (DMSO) but no vehicle control group was included to establish whether the vehicle may be contributing to the observed effect given that DMSO has long been known to have neuroprotective effects [144,145].

Melatonin given in combination with erythropoietin prevented post-hemorrhagic hydrocephalus of prematurity in rat pups [120]. In this study, prenatal chorioamnionitis was induced at embryonic day 18 by transient uterine artery occlusion for 60 min followed by intra-amniotic injection of lipopolysaccharide. On postnatal day one, IVH was induced by injection of lysed red blood cells into the lateral ventricles. Pups received 6 doses of rEpo on P2–P5, P7 and P9 and 9 doses of melatonin (20 mg/kg i.p.) on day P2–P10. Combined rEpo and melatonin treatment prevented the development of multiple hallmarks of post-hemorrhagic hydrocephalus of prematurity, including macrocephaly and neurodevelopmental delay and reduced ventriculomegaly. However, rEpo and melatonin were not given as separate treatments in this study, so the relative contribution of melatonin cannot be determined.

Maternal pre-treatment with melatonin has been shown to reduce preterm birth and perinatal brain injury in a mouse model of maternal inflammation induced by lipopolysaccharide [143]. Melatonin pre-treatment was associated with lower pro-inflammatory cytokines in the uterus and placenta as well as a significant reduction in LPS-induced fetal neuro-inflammation.

Small clinical studies suggest that melatonin is not associated with adverse outcomes and may improve survival of neonates with septic shock and reduce lung injury associated with ventilation in preterm infants [146,147]. A small randomized trial in term neonates with HIE reported that five enteral doses of melatonin (10 mg/kg) given in combination with therapeutic hypothermia, reduced seizures and white matter abnormalities on MRI at two weeks of age, and improved survival without neurological abnormalities at six months of age [148]. Long-term follow up has not been reported.

5.2. Vitamin D Supplementation

Maternal vitamin D deficiency appears to be a significant risk factor for preterm birth [149,150], primary cesarean section (a cesarean section performed on a woman for the first time) [151], small for gestational age, and pre-eclampsia [152–154], such that increasingly premature infants are more likely to be vitamin D deficient at birth [155,156]. Meta-analysis has demonstrated an association between preterm birth risk and maternal vitamin D deficiency [153]. Further, a recent study has shown an association between umbilical cord vitamin D levels and adverse neonatal outcomes, including increased risk of preterm birth, neonatal respiratory distress syndrome and increased risk of hospitalization in the first year of life [157]. There is also an association between a vitamin D receptor polymorphism and spontaneous preterm birth [158].

Vitamin D plays several important roles during development. Vitamin D has been shown to influence normal fetal brain development, including cell differentiation, neurotrophic factor expression, cytokine regulation, neurotransmitter synthesis, intracellular calcium signaling, antioxidant activity and expression of genes and proteins involved in neuronal differentiation, structure and metabolism [159]. Maternal deficiency in rodents has been implicated in impaired placental function, increased maternal and fetal glucocorticoid exposure, and changes in behavior [160–162]. Epidemiological data suggests that maternal vitamin D deficiency may be associated with adult schizophrenia [163], poor
child language development [164, 165], and a growing body of evidence for autism-like traits [162, 166, 167]. Nevertheless, the direction of causality is unproven.

Vitamin D supplementation has shown a range of neuroprotective effects in preclinical models of brain injury, including immunomodulatory and anti-inflammatory effects. Indeed, vitamin D has been shown to be a key immunomodulator and regulator of pro-inflammatory Th 17 lymphocytes in adult stroke patients [168, 169]. In adult male mice, administration of the active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25-VitD3) for 5 days prior to stroke induced by middle cerebral artery occlusion for one hour followed by 23 h of reperfusion, was associated with a significant reduction in infarct volume and reduced expression of the pro-inflammatory cytokines interleukin (IL)-6, IL-1β, IL-23a, TGF-β and NADPH oxidase-2 [170]. Interestingly, 1,25-VitD3 was shown to prevent autism-related phenotypes in the adult offspring in a mouse model of maternal immune activation but this was not associated with a reduction in pro-inflammatory cytokines in either the dam or the fetal brain, suggesting an alternative mechanism of action [166].

In a mouse brain endothelial cell culture model designed to mimic the blood-brain barrier in vitro, administration of 1,25-VitD3, prevented the decrease in barrier function as measured by transendothelial electrical resistance, permeability of FITC-dextran, decrease of tight junction proteins, activation of NF-kβ, and increase in matrix metalloproteinase-9 expression after hypoxia/reoxygenation [171]. In a rat model of traumatic brain injury, administration of calcitriol, the active metabolite of vitamin D, at 30 min, 24 h and 48 h after the insult, attenuated neurobehavioral deficits, brain edema and apoptosis as well as down-regulating NADPH oxidase, which may be protective against oxidative stress [172].

Although there is currently no evidence to suggest that vitamin D supplementation is neuroprotective in preterm infants, monitoring vitamin D status should be considered as a focus for future research [173]. Further animal studies investigating possible neuroprotective effects of vitamin D in a variety of preterm brain injury models would help to determine whether vitamin D may be a useful neuroprotective treatment for the preterm infant.

5.3. Cell-Based Therapies

There is increasing preclinical evidence that “stem” cell (i.e., pluripotent cells) therapy may be a viable neuroprotective strategy for the preterm brain, particularly after exposure to HI or infection/inflammation [174–177]. The potential neuroprotective efficacy of stem cell treatments appears to be mediated by multiple protective effects including promotion of proliferation, growth and differentiation through release of trophic factors and chemokines to support host cell survival and development, and their ability to modulate the inflammatory responses post-HI [175–177]. A variety of different stem cells are currently being assessed clinically and preclinically, including human amniotic epithelial cells (hAECs) [174, 177, 178] and umbilical cord blood (UCB).

5.3.1. Human Amnion Epithelial Cells (hAECs)

There are many different stem cells available. hAECs offer a number of practical advantages. They can be readily harvested from the amnion membranes, which surround the fetus in utero and are discarded at birth, therefore they do not require invasive extraction, making them readily available for rapid treatment in the early postnatal phase [155]. hAECs are pluripotent, are capable of differentiating into cell types of all three germ layers, are non-tumorigenic, non-immunogenic and have significant immunomodulatory properties, making them ideal as a generic therapy [175, 179].

In adult mice, hAECs have been shown to reduce infarct development, reduce inflammation and functional deficits after stroke, while in the adult marmoset, hAECs also prevented infarct development [180]. Further, hAECs have been shown to reduce brain swelling and improve motor function after intracerebral hemorrhage and to reduce inflammation in experimental autoimmune encephalitis [181]. In a near-term fetal sheep model of infection/inflammation induced by lipopolysaccharide, hAECs administered at 0, 6 and 12 h after intra-amniotic administration of LPS, have been shown to ameliorate white and
grey matter injury [182], in association with reduced microglial activation, suggesting that hAECs reduced inflammation. Similarly, hAECs reduced ventilation-induced inflammatory white matter injury in newborn lambs [183], as well as lung injury associated with ventilation [184], and hyperoxia [185].

hAECs also appear to be neuroprotective in the preterm brain after asphyxia. Delayed intranasal infusion of hAECs, given at 24 h and 3 and 10 days after 25 min of complete umbilical cord occlusion, was associated with improved brain weight, improved oligodendrocyte maturation and myelination and reduced microglia and astrocyte number after 21 days recovery in the preterm fetal sheep [186]. Further, hAECs have a relatively long therapeutic window of opportunity, showing similar anti-inflammatory effects when administered either 2 or 24 h after 25 min of complete umbilical cord occlusion in the preterm fetal sheep [187]. By contrast, hAECs did not reduce markers of neuroinflammation and injury in preterm lamb brains after mechanical ventilation; however, this was assessed at 48 h after birth and this may be too short a recovery period to see an effect [188].

5.3.2. Umbilical Cord Blood (UCB) Cells

UCB contains a diverse mix of stem cells and progenitor cells, with the potential to generate a wide variety of cell types [189]. Advantages of UCB include that they are an abundant source of non-embryonic stem cells that are easily accessed in a non-invasive and risk-free manner and due to their immature nature, have remarkable proliferative potential [190]. UCB is a rich source of progenitor cells, regulatory T lymphocytes, mesenchymal stem cells, monocytes, endothelial progenitor cells and stromal precursor cells [191].

Early preclinical trials in neonatal rat pups showed that UCB administration at 24 h post-insult, decreased reactive gliosis and normalized connexin 43 expression, increased tissue repair and cognitive improvements and enhanced endogenous neural stem cell proliferation after HI induced by the Rice-Vannucci model of unilateral carotid ligation and inhalational hypoxia [175,192–194]. Further, in preterm fetal sheep, intravenous infusion of umbilical cord blood derived mesenchymal stem/stromal cells at 12 h but not 5 days after asphyxia induced by umbilical cord occlusion, reduced white matter injury and cerebral inflammation [195]. Further, UCB administered at 12 h was associated with a significant systemic increase in the anti-inflammatory cytokine IL-10 ten days after asphyxia as well as a reduction in oxidative stress [195]. When mesenchymal stem cells derived from umbilical cord blood were administered to preterm fetal sheep 12 h after asphyxia induced by 25 min of complete umbilical cord occlusion, preserved myelination, suppressed microglial activation, promotion of macrophage migration and accelerated self-repair within the preterm brain was seen [196].

Interestingly, the gestational age at which UCB cells are harvested may have an impact on the neuroprotective mechanisms. When preterm fetal sheep received UCB cells from either term or preterm sheep, both reduced white matter injury, cell death and microgliosis. However, only preterm cord blood prevented upregulation of plasma tumor necrosis factor alpha, while term cord blood increased the anti-inflammatory cytokine IL-10 and reduced oxidative stress [197].

6. Conclusions

Preterm birth is associated with a high burden of neurological impairment and lifelong disability. Developing therapies for brain injury in the preterm infant is particularly complex given the wide range of potentially injurious phenomena that preterm fetuses and infants may be exposed to before, during and after birth, including chronic antenatal and postnatal hypoxia, perinatal asphyxia, prenatal and postnatal infection/inflammation, poor respiratory function after birth, ventilation-induced brain injury and a variety of treatments with potential side effects, including anticonvulsants and antenatal and postnatal glucocorticoids. It is important to appreciate that each individual infant may be exposed to different combinations of these factors. The heterogeneous nature of preterm brain injury presents many challenges in the quest to develop treatment strategies for these
infants. It is likely that different treatment strategies may be more effective in treating brain damage stemming from particular types of insults. For example, it is plausible that therapeutic hypothermia may alleviate brain damage after acute perinatal asphyxia but not chronic hypoxia.

The multifactorial nature of preterm brain injury also presents a major challenge to the development of suitable preclinical models. Many preclinical studies, particularly those in large animal translational models focus on brain injury induced by acute asphyxia or infection/inflammation but studying the pathology associated with preterm birth itself or combinations of these factors is practically difficult in these paradigms. This has implications for the translation of potential new therapies into clinical trials where infants are likely to have been exposed to a variety of these potentially damaging factors.

Current treatments for preterm infants that may have beneficial effects on neurological outcome include antenatal glucocorticoids, which in addition to improving respiratory function, have been shown to reduce the incidence of IVH in preterm infants. The use of postnatal corticosteroids remains controversial due to concerns around increased risk of cerebral palsy but this appears to be influenced by dose and timing of administration. The use of anticonvulsants to treat seizures in the preterm brain remains controversial, due to poor efficacy and concerns around adverse effects in the developing brain raised by preclinical studies. Better understanding of the cellular mechanisms underlying seizure activity in the developing brain may aid in the development of safer and more effective anticonvulsant treatments for preterm neonates. There is conflicting evidence around the efficacy of MgSO4 for reducing preterm brain injury, with its use having been shown to be associated with a small but significant reduction in the risk of cerebral palsy and gross motor dysfunction in early childhood but no overall improvement in death or disability and studies with long-term follow up to school age to date have shown no long-term benefit.

A number of promising neuroprotective therapies are currently being investigated for use in the preterm brain. Preclinical studies suggest that therapeutic hypothermia is effective at reducing brain damage after asphyxia in the preterm brain but concerns remain around its safety. The results of the randomized controlled trial of therapeutic hypothermia in preterm infants are eagerly awaited to determine whether it is safe and effective for use in preterm infants who may have been exposed to a period of asphyxia around the time of birth.

Further, preclinical studies suggest that recombinant erythropoietin may be a useful treatment option to reduce brain damage in the preterm infant but this has not yet translated successfully in clinical trials, perhaps due to the frequency of treatment. Preclinical studies suggest that melatonin, although the evidence for benefit is confound by the common use of ethanol as a diluent, human amnion epithelial cells and umbilical cord blood cells may also be viable treatment options for preventing brain damage in preterm infants. However, to ensure the greatest chance of these potential neuroprotective treatments translating into clinical practice, well-designed research in a range of translational models are essential to guide the development of evidence-based dosing regimens for use in clinical trials to ensure that they have the highest chance of being successful.

Despite the challenges associated with studying preterm brain injury and the development of treatment strategies, there are now a number of potential neuroprotective treatments showing promise in preclinical studies and/or in clinical trials. With further well-designed, translational research, these treatment strategies may help to reduce the high burden of brain damage and disability associated with preterm birth.

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References

1. Blencowe, H.; Cousins, S.; Oestergaard, M.Z.; Chou, D.; Moller, A.-B.; Narwal, R.; Adler, A.; Garcia, C.V.; Rohde, S.; Say, L.; et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. *Lancet* 2012, 379, 2162–2172. [CrossRef]

2. Laptook, A.R. Birth Asphyxia and Hypoxic-Ischemic Brain Injury in the Preterm Infant. *Clin. Perinatol.* 2016, 43, 529–545. [CrossRef]

3. Gale, C.; Statnikov, Y.; Jawad, S.; Uthaya, S.N.; Modi, N. Neonatal brain injuries in England: Population-based incidence derived from routinely recorded clinical data held in the National Neonatal Research Database. *Arch. Dis. Child. Fetal Neonatal Ed.* 2017, 103, F301–F306. [CrossRef] [PubMed]

4. Huang, J.; Zhang, L.; Kang, B.; Zhu, T.; Li, Y.; Zhao, F.; Qu, Y.; Mu, D. Association between perinatal hypoxic-ischemia and periventricular leukomalacia in preterm infants: A systematic review and meta-analysis. *PLoS ONE* 2017, 12, e0184993. [CrossRef]

5. Low, J.A.; Killen, H.; Derrick, E.J. Antepartum fetal asphyxia in the preterm pregnancy. *Am. J. Obstet. Gynecol.* 2003, 188, 461–465. [CrossRef]

6. Chalak, L.F.; Rollins, N.; Morriss, M.C.; Brion, L.P.; Heyne, R.; S., et al. The effect of antepartum fetal asphyxia in the preterm pregnancy. *Clin. Perinatol.* 2016, 43, 529–545. [CrossRef]

7. Schmidt, B.; Roberts, R.S.; Anderson, P.J.; Asztalos, E.V.; Costantini, L.; Davis, P.G.; Dewey, D.; D’Ilario, J.; Doyle, L.W.; Grunau, R.E.; et al. Academic Performance, Motor Function, and Behavior 11 Years After Neonatal Caffeine Citrate Therapy for Apnea of Prematurity: An 11-Year Follow-up of the CAP Randomized Clinical Trial. *JAMA Pediatr.* 2015, 169, 731–743. [CrossRef] [PubMed]

8. Salhab, W.A.; Perlman, J.M. Severe fetal acidemia and subsequent neonatal encephalopathy in the larger premature infant. *Pediatr. Neurol.* 2003, 29, 1129–1133. [CrossRef] [PubMed]

9. Manuck, T.A.; Rice, M.M.; Bailit, J.L.; Grobman, W.A.; Reddy, U.M.; Wapner, R.J.; Thorp, J.M.; Caritis, S.N.; Prasad, M.; Tita, et al. Neonatal brain injuries in England: Population-based incidence derived from routinely recorded clinical data held in the National Neonatal Research Database. *Arch. Dis. Child. Fetal Neonatal Ed.* 2017, 103, F301–F306. [CrossRef] [PubMed]

10. Ehrenkranz, R.A. Estimated fetal weights versus birth weights: Should the reference intrauterine growth curves based on birth weights be retired? *Arch. Dis. Child. Fetal Neonatal Ed.* 2007, 92, F61–F62. [CrossRef] [PubMed]

11. Streimish, I.G.; Ehrenkranz, R.A.; Allred, E.N.; O’Shea, T.M.; Kuban, K.C.; Paneth, N.; Leviton, A. Birth weight- and fetal weight-growth restriction: Impact on neurodevelopment. *Early Hum. Dev.* 2012, 88, 765–771. [CrossRef] [PubMed]

12. Miller, S.L.; Huppi, P.S.; Mallard, C. The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. *J. Physiol.* 2016, 594, 807–823. [CrossRef]

13. Hayes, B.C.; McGarvey, C.; Mulvany, S.; Kennedy, J.; Geary, M.P.; Matthews, T.G.; King, M.D. A case-control study of hypoxic-ischemic encephalopathy in newborn infants at >36 weeks gestation. *Am. J. Obstet. Gynecol.* 2013, 209, 29.e1–29.e19. [CrossRef] [PubMed]

14. Takahashi, N.; Nishida, H.; Arai, T.; Kaneda, Y. Abnormal cardiac histology in severe intrauterine growth retardation infants. *Pediatr. Int.* 2005, 37, 341–346. [CrossRef]

15. Schmidt, B.; Roberts, R.S.; Anderson, P.J.; Asztalos, E.V.; Costantini, L.; Davis, P.G.; Dewey, D.; D’Ilario, J.; Doyle, L.W.; Grunau, R.E.; et al. Academic Performance, Motor Function, and Behavior 11 Years After Neonatal Caffeine Citrate Therapy for Apnea of Prematurity: An 11-Year Follow-up of the CAP Randomized Clinical Trial. *JAMA Pediatr.* 2015, 169, 731–743. [CrossRef] [PubMed]

16. Goldman, F.; Coppola, M.; De Rose, D.U.; Maggio, L.; Arena, R.; Romano, V.; Cota, F.; Ricci, D.; Romeo, D.M.; Mercuri, E.M.; et al. Neurodevelopmental outcomes in very preterm infants: The role of severity of Bronchopulmonary Dysplasia. *Early Hum. Dev.* 2021, 152, 105275. [CrossRef] [PubMed]

17. Galinsky, R.; Polglase, G.R.; Hooper, S.B.; Black, M.J.; Moss, T.J.M. The Consequences of Chorioamnionitis: Preterm Birth and Effects on Development. *J. Pregnancy* 2013, 2013, 1–11. [CrossRef]

18. Kim, C.J.; Romero, R.; Chaemsaiithong, P.; Chaiyasit, N.; Yoon, B.H.; Kim, Y.M. Acute chorioamnionitis and funisitis: Definition, pathologic features, and clinical significance. *Am. J. Obstet. Gynecol.* 2015, 213, S29–S52. [CrossRef] [PubMed]

19. Romero, R.; Espinoza, J.; Gonçalves, L.F.; Kusanovic, J.P.; Friell, L.A.; Nien, J.K. Inflammation in preterm and term labour and delivery. *Semin. Fetal Neonatal Med.* 2006, 11, 317–326. [CrossRef] [PubMed]

20. Arayici, S.; Simsek, G.K.; Öncel, M.Y.; Eras, Z.; Canpolat, F.E.; Oguz, S.S.; Uras, N.; Zergeroglu, S.; Dilmen, U. The effect of histological chorioamnionitis on the short-term outcome of preterm infants ≤32 weeks: A single-center study. *J. Matern.-Fetal Neonatal Med.* 2014, 27, 1129–1133. [CrossRef] [PubMed]

21. Lu, H.; Zhang, Q.; Wang, Q.-X.; Lu, J.-Y. Contribution of Histologic Chorioamnionitis to Fetal Inflammatory Response Syndrome to Increased Risk of Brain Injury in Infants with Preterm Premature Rupture of Membranes. *Pediatr. Neurol.* 2016, 61, 94–98.e1. [CrossRef]

22. Korzeniewski, S.J.; Romero, R.; Cortez, J.; Pappas, A.; Schwartz, A.G.; Kim, C.J.; Kim, J.-S.; Kim, Y.M.; Yoon, B.H.; Chaiworapongs, T.; et al. A “multi-hit” model of neonatal white matter injury: Cumulative contributions of chronic placental inflammation, acute fetal inflammation and postnatal inflammatory events. *J. Périnat. Med.* 2014, 42, 731–743. [CrossRef] [PubMed]

23. Soraisham, A.S.; Trevenen, C.; Wood, S.; Singhal, N.; Sauve, R. Histological chorioamnionitis and neurodevelopmental outcome in preterm infants. *J. Perinatol.* 2013, 33, 70–75. [CrossRef] [PubMed]

24. Yap, V.; Perlman, J.M. Mechanisms of brain injury in newborn infants associated with the fetal inflammatory response syndrome. *Semin. Fetal Neonatal Med.* 2020, 25, 101110. [CrossRef]
50. Foix-L’Helias, L.; Marret, S.; Ancel, P.; Marchand, L.; Arnaud, C.; Fresson, J.; Picaud, J.-C.; Rozé, J.-C.; Theret, B.; Burguete, A.; et al. Impact of the use of antenatal corticosteroids on mortality, cerebral lesions and 5-year neurodevelopmental outcomes of very preterm infants: The EPIPAGE cohort study. *BJOG* 2008, 115, 275–282. [CrossRef] [PubMed]

51. Roberts, D.; Brown, J.; Medley, N.; Dalziel, S.R. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst. Rev.* 2017, 3, CD004454. [CrossRef]

52. Salokorpi, T.; Sajaniemi, N.; Hallback, H.; Kari, A.; Rita, H.; Wendt, L. Randomized study of the effect of antenatal dexamethasone on growth and development of premature children at the corrected age of 2 years. *Acta Paediatr.* 1997, 86, 294–298. [CrossRef]

53. Crowther, C.A.; McKenzie, J.D.; Middleton, P.; Harding, J.E. Repeat doses of prenatal corticosteroids for women at risk of preterm birth for improving neonatal health outcomes. *Cochrane Database Syst. Rev.* 2015, 2015, CD003935. [CrossRef]

54. Crowther, C.A.; Ashwood, P.; Andersen, C.C.; Middleton, P.F.; Tran, T.; Doyle, L.W.; Robinson, J.S.; Harding, J.E.; Ball, V.; Holst, C.; et al. Maternal intramuscular dexamethasone versus betamethasone before preterm birth (ASTEROID): A multicentre, double-blind, randomised controlled trial. *Lancet Child Adolesc. Health* 2019, 3, 769–780. [CrossRef]

55. Ciapponi, A.; Klein, K.; Colaci, D.; Althabe, F.; Belizán, J.M.; Deegan, A.; Veroniki, A.A.; Florez, I.D. Dexamethasone vs. betamethasone for preterm birth: A systematic review and network meta-analysis. *Am. J. Obstet. Gynecol.* MFM 2021, 1003. [CrossRef]

56. Antonow-Schlorke, I.; Helgert, A.; Gey, C.; Coksaygan, T.; Schubert, H.; Nathanielsz, P.W.; Witte, O.W.; Schwab, M. Adverse Effects of Antenatal Glucocorticoids on Cerebral Myelination in Sheep. *Obstet. Gynecol.* 2009, 113, 142–158. [CrossRef] [PubMed]

57. Davidson, J.O.; Quaedackers, J.S.L.T.; George, S.A.; Gunn, A.J.; Bennet, L. Maternal dexamethasone and EEG hyperactivity in preterm fetal sheep. *J. Physiol.* 2011, 589, 3823–3835. [CrossRef]

58. Parikh, N.A.; Lasky, R.E.; Kennedy, K.A.; McDavid, G.; Tyson, J.E. Perinatal Factors and Regional Brain Volume Abnormalities at Term in a Cohort of Extremely Low Birth Weight Infants. *PLoS ONE* 2013, 8, e62804. [CrossRef]

59. Parikh, N.A.; Kennedy, K.A.; Lasky, R.E.; McDavid, G.E.; Tyson, J.E. Pilot Randomized Trial of Hydrocortisone in Ventilator-Dependent Extremely Preterm Infants: Effects on Regional Brain Volumes. *J. Pediatr.* 2013, 162, 685–690.e1. [CrossRef]

60. Koome, M.E.; Davidson, J.O.; Drury, P.P.; Mathai, S.; Booth, L.C.; Gunn, A.J.; Bennet, L. Antenatal Dexamethasone after Asphyxia Increases Neural Injury in Preterm Fetal Sheep. *PLoS ONE* 2013, 8, e77480. [CrossRef]

61. Lear, C.A.; Koome, M.E.; Davidson, J.O.; Drury, P.P.; Quaedackers, J.S.; Galinsky, R.; Gunn, A.J.; Bennet, L. The effects of dexamethasone on post-asphyxial cerebral oxygenation in the preterm fetal sheep. *J. Physiol.* 2014, 592, 5493–5505. [CrossRef]

62. Lear, C.; Davidson, J.O.; Mackay, G.R.; Drury, P.P.; Galinsky, R.; Quaedackers, J.S.; Gunn, A.J.; Bennet, L. Antenatal dexamethasone before asphyxia promotes cystic neural injury in preterm fetal sheep by inducing hyperglycemia. *J. Cereb. Blood Flow Metab.* 2018, 38, 706–718. [CrossRef] [PubMed]

63. Murphy, B.P.; Inder, T.E.; Hüppi, P.S.; Warfield, S.K.; Zientara, G.P.; Kikinis, R.; Jolesz, F.A.; Volpe, J.J. Impaired cerebral cortical grey matter growth after treatment with dexamethasone for neonatal chronic lung disease. *Pediatrics* 2001, 107, 217–221. [CrossRef] [PubMed]

64. Parikh, N.; Lasky, R.E.; Kennedy, K.A.; Moya, F.R.; Hochhauser, L.; Romo, S.; Tyson, J.E. Postnatal Dexamethasone Therapy and Cerebral Tissue Volumes in Extremely Low Birth Weight Infants. *Pediatrics* 2007, 119, 265–272. [CrossRef] [PubMed]

65. Doyle, L.W.; Cheong, J.L.; Ehrenkranz, R.A.; Halliday, H.L. Early (<8 days) systemic postnatal corticosteroids for prevention of bronchopulmonary dysplasia in preterm infants. *Cochrane Database Syst. Rev.* 2017, 10, CD001146. [PubMed]

66. Doyle, L.W.; Cheong, J.L.; Ehrenkranz, R.A.; Halliday, H.L. Late (>7 days) systemic postnatal corticosteroids for prevention of bronchopulmonary dysplasia in preterm infants. *Cochrane Database Syst. Rev.* 2017, 10, CD001145. [CrossRef]

67. Orland, W.; Offringa, M.; De Jaegere, A.; Van Kaam, A.H. Finding the Optimal Postnatal Dexamethasone Regimen for Preterm Infants at Risk of Bronchopulmonary Dysplasia: A Systematic Review of Placebo-Controlled Trials. *Pediatrics* 2009, 123, 367–377. [CrossRef] [PubMed]

68. Doyle, L.; Crowther, C.; Middleton, P.; Marret, S. Antenatal Magnesium Sulfate and Neurologic Outcome in Preterm Infants: A Systematic Review. *Obstet. Gynecol.* 2012, 113, 1327–1333. [CrossRef]

69. Conde-Agudelo, A.; Romero, R. Antenatal magnesium sulfate for the prevention of cerebral palsy in preterm infants less than 34 weeks’ gestation: A systematic review and metaanalysis. *Am. J. Obstet. Gynecol.* 2009, 200, 595–609. [CrossRef]

70. Song, J.; Sun, H.; Xu, F.; Kang, W.; Gao, L.; Guo, J.; Zhang, Y.; Xia, L.; Wang, X.; Zhu, C. Recombinant human erythropoietin improves neurological outcomes in very preterm infants. *Ann. Neurol.* 2016, 80, 24–34. [CrossRef]

71. Juul, S.E.; Comstock, B.A.; Wadhawan, R.; Mayock, D.E.; Courtney, S.E.; Robinson, T.; Ahmad, K.A.; Bendel-Stenzel, E.; Baserga, M.; LaGamma, E.F.; et al. A Randomized Trial of Erythropoietin for Neuroprotection in Preterm Infants. *N. Engl. J. Med.* 2020, 382, 233–243. [CrossRef] [PubMed]

72. Natalucci, G.; Latal, B.; Koller, B.; Rüegg, C.; Sick, B.; Held, L.; Bucher, H.U.; Fauchère, J.C.; The Swiss EPO Neuroprotection Trial Group. Effect of early prophylactic high-dose recombinant human erythropoietin in very preterm infants on neurodevelopmental outcome at 2 Years: A randomized clinical trial. *JAMA* 2016, 315, 2079–2085. [CrossRef] [PubMed]

73. Natalucci, G.; Latal, B.; Koller, B.; Rüegg, C.; Sick, B.; Held, L.; Fauchère, J.-C. Swiss EPO Neuroprotection Trial Group Neurodevelopmental Outcomes at Age 5 Years After Prophylactic Early High-Dose Recombinant Human Erythropoietin for Neuroprotection in Very Preterm Infants. *JAMA* 2020, 324, 2324–2327. [CrossRef]
74. Rao, R.; Trivedi, S.; Vesoulis, Z.A.; Liao, S.M.; Smyser, C.D.; Mathur, A.M. Safety and Short-Term Outcomes of Therapeutic Hypothermia in Preterm Neonates 34-35 Weeks Gestational Age with Hypoxic-Ischemic Encephalopathy. J. Pediatr. 2017, 183, 37–42. [CrossRef]

75. Herrara, T.I.; Edwards, L.; Malcolm, W.F.; Smith, P.B.; Fisher, K.A.; Pizoli, C.; Gustafson, K.E.; Goldstein, R.F.; Cotten, C.M.; Goldberg, R.N.; et al. Outcomes of preterm infants treated with hypothermia for hypoxic-ischemic encephalopathy. Early Hum. Dev. 2018, 125, 1–7. [CrossRef]

76. Costeloe, K.L.; Hennessy, E.M.; Haider, S.; Stacey, F.; Marlow, N.; Draper, E.S. Short term outcomes after extreme preterm birth in England: Comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ 2012, 345, e7976. [CrossRef]

77. Doyle, L.W.; Ranganathan, S.; Cheong, J.L. Ventilation in Preterm Infants and Lung Function at 8 Years. N. Engl. J. Med. 2017, 377, 1601–1602. [CrossRef] [PubMed]

78. Yoder, B.A.; Harrison, M.; Clark, R.H. Time-Related Changes in Steroid Use and Bronchopulmonary Dysplasia in Preterm Infants. Pediatrics 2009, 124, 673–679. [CrossRef]

79. Flagel, S.B.; Vázquez, D.M.; Watson, S.J.; Neal, C.R. Effects of tapering neonatal dexamethasone on rat growth, neurodevelopment, and stress response. Am. J. Physiol. Integr. Comp. Physiol. 2002, 282, R55–R63. [CrossRef]

80. Gramsbergen, A.; Mulder, E.J.H. The Influence of Betamethasone and Dexamethasone on Motor Development in Young Rats. Pediatr. Res. 1998, 44, 105–110. [CrossRef]

81. Ferguson, S.; Holson, R.R. Neonatal Dexamethasone on Day 7 Causes Mild Hyperactivity and Cerebellar Stunting. Neurotoxicology 1999, 21, 71–76. [CrossRef]

82. Brummelte, S.; Pawluski, J.L.; Galea, L.A. High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behavior of offspring: A model of post-partum stress and possible depression. Horm. Behav. 2006, 50, 370–382. [CrossRef]

83. Haynes, L.; Griffiths, M.; Hyde, R.; Barber, D.; Mitchell, I. Dexamethasone induces limited apoptosis and extensive sublethal damage to specific subregions of the striatum and hippocampus: Implications for mood disorders. Neuroscience 2001, 104, 57–69. [CrossRef]

84. Baud, O.; Foix-L’Helias, L.; Kaminski, M.; Audibert, F.; Jarreau, P.-H.; Papiernik, E.; Huon, C.; Lepercq, J.; Dehan, M.; Lacaz-Masmonteil, T. Antenatal Glucocorticoid Treatment and Cystic Periventricular Leukomalacia in Very Premature Infants. N. Engl. J. Med. 1999, 341, 1190–1196. [CrossRef] [PubMed]

85. Shinwell, E.S.; Karplus, M.; Reich, D.; Weintraub, Z.; Blazer, S.; Bader, D.; Yurman, S.; Dollfin, T.; Kogan, A.; Dollberg, S.; et al. Early postnatal dexamethasone treatment and increased incidence of cerebral palsy. Arch. Dis. Child. Fetal Neonatal Ed. 2000, 83, F177–F181. [CrossRef] [PubMed]

86. Doyle, L.W.; Davis, P.G.; Morley, C.J.; McPhee, A.; Carlin, J.B.; the DART Study Investigators. Outcome at 2 Years of Age from the DART Study: A Multicenter, International, Randomized, Controlled Trial of Low-Dose Dexamethasone. Pediatrics 2007, 119, 716–721. [CrossRef]

87. Doyle, L.W.; Davis, P.G.; Morley, C.J.; McPhee, A.; Carlin, J.B. Low-Dose Dexamethasone Facilitates Extubation among Chronically Ventilator-Dependent Infants: A Multicenter, International, Randomized, Controlled Trial. Pediatrics 2006, 117, 75–83. [CrossRef]

88. Yates, N.J.; Feindel, K.W.; Mehnert, A.; Beare, R.; Quick, S.; Blache, D.; Pillow, J.J.; Hunt, R.W. Ex Vivo MRI Analytical Methods and Brain Pathology in Preterm Lambs Treated with Postnatal Dexamethasone. Brain Sci. 2020, 10, 211. [CrossRef]

89. Walsh, M.C.; Morris, B.H.; Wrage, L.A.; Van Bel, F.; Juul, S.E.; Robertson, N.J.; Mallard, C.; Gunn, A.J. Magnesium Is Not Consistently Neuroprotective for Perinatal Hypoxia-Ischemia in Term-Equivalent Models in Preclinical Studies: A Systematic Review. Dev. Neurosci. 2014, 36, 73–82. [CrossRef]

90. Galinsky, R.; Bennet, L.; Groenendaal, F.; Lear, C.A.; Tan, S.; Van Bel, F.; Juul, S.E.; Robertson, N.J.; Mallard, C.; Gunn, A.J. Magnesium Sulfate for Perinatal Neuroprotection: What Have We Learnt from the Past Decade? Front. Neurol. 2020, 11, 449. [CrossRef]

91. Cahill, A.G.; Caughey, A.B. Magnesium for neuroprophylaxis: Fact or fiction? Am. J. Obstet. Gynecol. 2009, 200, 590–594. [CrossRef]

92. Doyle, L.W. Antenatal magnesium sulfate and neuroprotection. Curr. Opin. Pediatr. 2012, 24, 154–159. [CrossRef]

93. Armstrong, J.F.; Hauser, W.A.; Lee, J.R.-J.; Rocca, W.A. Incidence of Acute Symptomatic Seizures in Rochester, Minnesota, 1935-1984. Epilepsia 1995, 36, 327–333. [CrossRef] [PubMed]

94. Cowan, L.D. The epidemiology of the epilepsies in children. Ment. Retard. Dev. Disabil. Res. Rev. 2002, 8, 171–181. [CrossRef]

95. Ronen, G.M.; Penney, S.; Andrews, W. The epidemiology of clinical neonatal seizures in Newfoundland: A population-based study. J. Pediatr. 1999, 134, 71–75. [CrossRef]
99. Silverstein, F.S.; Jensen, F.E. Neonatal seizures. *Ann. Neurol.* 2007, 62, 112–120. [CrossRef] [PubMed]

100. Ronen, G.M.; Buckley, D.; Penney, S.; Streiner, D.L. Long-term prognosis in children with neonatal seizures: A population-based study. *Neurology* 2007, 69, 1816–1822. [CrossRef] [PubMed]

101. Glass, H.C.; Kan, J.; Bonifacio, S.L.; Ferriero, D.M. Neonatal Seizures: Treatment Practices among Term and Preterm Infants. *Pediatr. Neurol.* 2012, 46, 111–115. [CrossRef]

102. Booth, D.; Evans, D.J. Anticonvulsants for neonates with seizures. *Cochrane Database Syst. Rev.* 2004, CD004218. [CrossRef]

103. Evans, D.J.; Levene, M.I.; Tsaknakis, M. Anticonvulsants for preventing mortality and morbidity in full term newborns with perinatal asphyxia. *Cochrane Database Syst. Rev.* 2007, CD001240. [CrossRef]

104. Bittigau, P.; Sifringer, M.; Genz, K.; Reith, E.; Pospischil, D.; Govindarajalu, S.; Dzietko, M.; Pospischil, E.; Mai, H.; Dikranian, K.; et al. Antiepileptic drugs and apoptotic neurodegeneration in the developing brain. *Proc. Natl. Acad. Sci. USA* 2002, 99, 15089–15094. [CrossRef]

105. Endesfelder, S.; Weichelt, U.; Schiller, C.; Winter, K.; Von Haefen, C.; Bührer, C. Caffeine Protects Against Anticonvulsant-Induced Impaired Neurogenesis in the Developing Rat Brain. *Neurotox. Res.* 2018, 34, 173–187. [CrossRef]

106. Forcelli, P.A.; Janssen, M.J.; Vicini, S.; Gale, K. Neonatal exposure to antiepileptic drugs disrupts striatal synaptic development. *Ann. Neurol.* 2012, 72, 363–372. [CrossRef]

107. Kim, J.; Kondratyev, A.; Gale, K. Antiepileptic Drug-Induced Neuronal Cell Death in the Immature Brain: Effects of Carbamazepine, Topiramate, and Levetiracetam as Monotherapy versus Polytherapy. *J. Pharmacol. Exp. Ther.* 2007, 323, 165–173. [CrossRef]

108. Khazipov, R.; Khalilov, I.; Tyzio, R.; Morozova, E.; Ben-Ari, Y.; Holmes, G.L. Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. *Eur. J. Neurosci.* 2004, 19, 590–600. [CrossRef] [PubMed]

109. Payne, J.A.; Rivera, C.; Voipio, J.; Kaila, K. Cation–chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.* 2003, 26, 199–206. [CrossRef] [PubMed]

110. Pond, B.B.; Berglund, K.; Kuner, T.; Feng, G.; Augustine, G.J.; Schwartz-Bloom, R.D. The Chloride Transporter Na+-K+-Cl– Cotransporter Isoform-1 Contributes to Intracellular Chloride Increases after In Vitro Ischemia. *J. Neurosci.* 2006, 26, 1396–1406. [CrossRef] [PubMed]

111. Jantzie, L.L.; Getsy, P.M.; Denson, J.L.; Firl, D.J.; Maxwell, J.R.; Rogers, D.A.; Wilson, C.G.; Robinson, S. Prenatal Hypoxia–Ischemia Induces Abnormalities in CA3 Microstructure, Potassium Chloride Co-Transporter 2 Expression and Inhibitory Tone. *Front. Cell. Neurosci.* 2015, 9, 347. [CrossRef]

112. Kahle, K.T.; Staley, K.J. The bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 as a potential target of a novel mechanism-based treatment strategy for neonatal seizures. *Neurosur. Focus* 2008, 25, E22. [CrossRef] [PubMed]

113. Pressler, R.M.; Boylan, G.B.; Marlow, N.; Blennow, M.; Chiron, C.; Cross, J.H.; De Vries, L.S.; Hallberg, B.; Hellström-Westas, L.; Jullien, V.; et al. Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): An open-label, dose finding, and feasibility phase 1/2 trial. *Lancet Neurol.* 2015, 14, 469–477. [CrossRef]

114. Jantzie, L.; Getsy, P.M.; Firl, D.J.; Wilson, C.; Miller, R.; Robinson, S. Erythropoietin attenuates loss of potassium chloride co-transporters following prenatal brain injury. *Mol. Cell. Neurosci.* 2013, 54, 152–162. [CrossRef]

115. Ohslos, A.; Aher, S.M. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst. Rev.* 2006, CD004863. [CrossRef]

116. Juul, S.E.; Comstock, B.A.; Heagerty, P.J.; Mayock, D.E.; Goodman, A.M.; Hauge, S.; González, F.; Wu, Y.W. High-Dose Erythropoietin for Asphyxia and Encephalopathy (HEAL): A Randomized Controlled Trial—Background, Aims, and Study Protocol. *Neonatology* 2018, 113, 331–338. [CrossRef]

117. Kellert, B.A.; McPherson, R.J.; Juul, S.E. Comparison of High-Dose Recombinant Erythropoietin Treatment Regimens in Brain-Injured Neonatal Rats. *Pediatr. Res.* 2007, 61, 451–455. [CrossRef] [PubMed]

118. Wassink, G.; Davidson, J.O.; Dhillon, S.K.; Fraser, M.; Galinsky, R.; Bennet, L.; Gunn, A.J. Partial white and grey matter protection with prolonged infusion of recombinant human erythropoietin after asphyxia in preterm fetal sheep. *J. Cereb. Blood Flow Metab.* 2017, 37, 1080–1094. [CrossRef]

119. Iwai, M.; Stetler, R.A.; Xing, J.; Hu, X.; Gao, Y.; Zhang, W.; Chen, J.; Cao, G. Enhanced Oligodendrogenesis and Recovery of Neurological Function by Erythropoietin After Neonatal Hypoxic/Ischemic Brain Injury. *Stroke* 2010, 41, 1032–1037. [CrossRef] [PubMed]

120. Robinson, S.; Corbett, C.J.; Winer, J.L.; Chan, L.A.; Maxwell, J.R.; Anstine, C.V.; Yellowhair, T.R.; Andrews, N.A.; Yang, Y.; Sillerud, L.O.; et al. Neonatal erythropoietin mitigates impaired gait, social interaction and dysfunction within sensory areas in a rat model of prenatal brain injury. *Exp. Neurol.* 2018, 302, 1–13. [CrossRef]

121. Kummer, A.; Baskin, H.; Yesilirmak, D.C.; Ergur, B.U.; Aykan, S.; Genc, S.; Genc, K.; Yilmaz, O.; Tugyan, K.; Giray, O.; et al. Erythropoietin Attenuates Lipopolysaccharide-Induced White Matter Injury in the Neonatal Rat Brain. *Neonatology* 2007, 92, 269–278. [CrossRef] [PubMed]

122. Rees, S.; Hale, N.; De Matteo, R.; Cardamone, L.; Tolcos, M.; Loeliger, M.; Mackintosh, A.; Shields, A.; Probyn, M.; Greenwood, D.; et al. Erythropoietin Is Neuroprotective in a Preterm Ovine Model of Endotoxin-Induced Brain Injury. *J. Neuropathol. Exp. Neurol.* 2010, 69, 306–319. [CrossRef] [PubMed]

123. Fischer, H.S.; Reibel, N.J.; Bührer, C.; Dame, C. Prophylactic Early Erythropoietin for Neuroprotection in Preterm Infants: A Meta-analysis. *Pediatrics* 2017, 139, e20164317. [CrossRef]
148. Aly, H.; Elmahdy, H.; Eldib, M.; Rowisha, M.; Awny, M.; Elghohary, T.; Elbatch, M.; Hamisa, M.; El-Mashad, A.-R. Melatonin use for neuroprotection in perinatal asphyxia: A randomized controlled pilot study. *J. Perinatol.* 2015, 35, 186–191. [CrossRef] [PubMed]

149. Wagner, C.L.; Baggerly, C.; McDonnell, S.; Hamilton, S.; Winkler, J.; Warner, G.; Rodriguez, C.; Shary, J.; Smith, P.; Holllis, B. Post-hoc comparison of vitamin D status at three timepoints during pregnancy demonstrates lower risk of preterm birth with higher vitamin D closer to delivery. *J. Steroid Biochem. Mol. Biol.* 2015, 148, 256–260. [CrossRef]

150. Qin, L.-L.; Lu, F.-G.; Yang, S.-H.; Xu, H.-L.; Luo, B.-A. Does Maternal Vitamin D Deficiency Increase the Risk of Preterm Birth: A Meta-Analysis of Observational Studies. *Nutrients* 2016, 8, 301. [CrossRef]

151. Merewood, A.; Mehta, S.D.; Chen, T.C.; Bauchner, H.; Holick, M.F. Association between Vitamin D Deficiency and Primary Cesarean Section. *J. Clin. Endocrinol. Metab.* 2009, 94, 940–945. [CrossRef]

152. Bodnar, L.M.; Platt, R.W.; Simhan, H.N. Early-Pregnancy Vitamin D Deficiency and Risk of Preterm Birth Subtypes. *Obstet. Gynecol.* 2015, 125, 439–447. [CrossRef] [PubMed]

153. Wei, S.-Q.; Qi, H.-P.; Luo, Z.-C.; Fraser, W.D. Maternal vitamin D status and adverse pregnancy outcomes: A systematic review and meta-analysis. *J. Matern.-Fetal Neonatal Med.* 2013, 26, 889–899. [CrossRef]

154. Wagner, C.L.; Baggerly, C., McDonnell, S.L.; Baggerly, K.; French, C.; Hamilton, S.A.; Hollis, B.W. Post-hoc analysis of vitamin D status and reduced risk of preterm birth in two vitamin D pregnancy cohorts compared with South Carolina March of Dimes 2009–2011 rates. *J. Steroid Biochem. Mol. Biol.* 2016, 155, 245–251. [CrossRef]

155. Burris, H.H.; Van Marter, L.J.; McElrath, T.F.; Tabatabai, P.; Litonjua, A.A.; Weiss, S.T.; Christou, H. Vitamin D status among pregnant women with preterm birth and preterm infants at birth. *Pediatr. Res.* 2014, 75, 75–80. [CrossRef]

156. Monangi, N.; Slaughter, J.L.; Dawodu, A.; Smith, C.; Akinbi, H.T. Vitamin D status of early preterm infants and the effects of vitamin D intake during hospital stay. *Pediatr. Res.* 2016, 79, 166–171. [CrossRef]

157. Treiber, M.; Mujezinovic, F.; Balon, B.P.; Gorenjak, M.; Mave, U.; Dovnik, A. Association between umbilical cord vitamin D levels and adverse neonatal outcomes. *J. Int. Med. Res.* 2020, 48, 300060520955001. [CrossRef]

158. Manzon, L.; Altareescu, G.; Tevet, A.; Schimmel, M.S.; Elstein, D.; Samueloff, A.; Grisaru-Granovsky, S. Vitamin D receptor polymorphism FokI is associated with spontaneous idiopathic preterm birth in an Israeli population. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2014, 177, 84–88. [CrossRef]

159. Eyles, D.W.; Burne, T.; McGrath, J. Vitamin D in fetal brain development. *Semin. Cell Dev. Biol.* 2011, 22, 629–636. [CrossRef]

160. Yates, N.J.; Crew, R.C.; Wyrwoll, C.S. Vitamin D deficiency and impaired placental function: Potential regulation by glucocorticoids? *Reproduction* 2017, 153, R163–R171. [CrossRef]

161. Tesic, D.; Hawes, J.E.; Zosky, G.R.; Wyrwoll, C.S. Vitamin D Deficiency in BALB/c Mouse Pregnancy Increases Placental Transfer of Glucocorticoids. *Endocrinology* 2015, 156, 3673–3679. [CrossRef] [PubMed]

162. Yates, N.J.; Tesic, D.; Feindel, K.W.; Smith, J.T.; Clarke, M.W.; Wale, C.; Crew, R.C.; Wharfe, M.D.; Whitehouse, A.J.O.; Wyrwoll, C.S. Vitamin D is crucial for maternal care and offspring social behaviour in rats. *J. Endocrinol.* 2018, 237, 73–85. [CrossRef]

163. McGrath, J.J.; Burne, T.H.; Féron, F.; Mackay-Sim, A.; Eyles, D.W. Developmental Vitamin D Deficiency and Risk of Schizophrenia: A 10-Year Update. *Schizophr. Bull.* 2010, 36, 1073–1078. [CrossRef] [PubMed]

164. Whitehouse, A.J.; Holt, B.J.; Serralha, M.; Holt, P.G.; Hart, P.H.; Kusel, M.M. Maternal vitamin D levels and the autism phenotype among offspring. *J. Autism. Dev. Disord.* 2013, 43, 1495–1504. [CrossRef]

165. Whitehouse, A.J.O.; Holt, B.J.; Serralha, M.; Holt, P.G.; Kusel, M.M.H.; Hart, P.H. Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurodevelopment. *Pediatrics* 2012, 129, 485–493. [CrossRef] [PubMed]

166. Vuillermot, S.; Luan, W.; Meyer, U.; Eyles, D.W. Vitamin D treatment during pregnancy prevents autism-related phenotypes in a mouse model of maternal immune activation. *J. Autism. Dev. Disord.* 2017, 8, 1–13. [CrossRef]

167. Mazahery, H.; Camargo, C.A.; Conlon, C.; Beck, K.L.; Kruger, M.C.; Von Hurst, P.R. Vitamin D and Autism Spectrum Disorder: A Literature Review. *Nutrients* 2016, 8, 236. [CrossRef] [PubMed]

168. Hu, Y.; Zheng, Y.; Wu, Y.; Ni, B.; Shi, S. Imbalance between IL-17A-Producing Cells and Regulatory T Cells during Ischemic Stroke. *Mediat. Inflamm.* 2014, 1, 1–8. [CrossRef]

169. Li, Q.; Wang, Y.; Yu, F.; Wang, Y.M.; Zhang, C.; Hu, C.; Wu, Z.; Xu, X.; Hu, S. Peripher d Th17/Treg imbalance in patients with atherosclerotic cerebral infarction. *Int. J. Clin. Exp. Pathol.* 2013, 6, 1015–1027.

170. Evans, M.A.; Kim, H.A.; Ling, Y.H.; Uong, S.; Vinh, A.; De Silva, T.M.; Arumugam, T.V.; Clarkson, A.N.; Zosky, G.R.; Drummond, G.R.; et al. Vitamin D3 Supplementation Reduces Subsequent Brain Injury and Inflammation Associated with Ischemic Stroke. *NeuroMolecular Med.* 2018, 20, 147–159. [CrossRef]

171. Won, S.; Sayeed, I.; Peterson, B.L.; Wali, B.; Kahn, J.S.; Stein, D.G. Vitamin D Prevents Hypoxia/Reoxygenation-Induced Blood-Brain Barrier Disruption via Vitamin D Receptor-Mediated NF-kB Signaling Pathways. *PLoS ONE* 2015, 10, e0122821. [CrossRef] [PubMed]

172. Cui, C.; Song, S.; Cui, J.; Feng, Y.; Gao, J.; Jiang, P. Vitamin D Receptor Activation Influences NADPH Oxidase (NOX2) Activity and Protects against Neurological Deficits and Apoptosis in a Rat Model of Traumatic Brain Injury. *Oxidative Med. Cell. Longev.* 2017, 2017, 1–13. [CrossRef] [PubMed]

173. Abrams, S.A.; The Committee on Nutrition; Bhatia, J.J.S.; Corkins, M.R.; De Ferrari, S.D.; Golden, N.H.; Silverstein, J. Calcium and Vitamin D Requirements of Enteraly Fed Preterm Infants. *Pediatrics* 2013, 131, e1676–e1683. [CrossRef]
174. Bennet, L.; Tan, S.; Msc, L.V.D.H.; Derrick, M.; Groenenendaal, F.; Van Bel, F.; Juul, S.; Back, S.A.; Northington, F.; Robertson, N.J.; et al. Cell therapy for neonatal hypoxia-ischemia and cerebral palsy. *Ann. Neurol.* 2011, 71, 589–600. [CrossRef] [PubMed]

175. Castillo-Melendez, M.; Yawno, T.; Jenkin, G.; Miller, S.L. Stem cell therapy to protect and repair the developing brain: A review of mechanisms of action of cord blood and amniotic epithelial derived cells. *Front. Neurosci.* 2015, 7, 194. [CrossRef] [PubMed]

176. Park, H.W.; Kim, Y.; Chang, J.W.; Yang, Y.S.; Oh, W.; Lee, J.M.; Park, H.R.; Kim, D.G.; Paek, S.H. Effect of Single and Double Administration of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Following Focal Cerebral Ischemia in Rats. *Exp. Neurol.* 2017, 289, 55–65. [CrossRef]

177. Zhu, D.; Wallace, E.M.; Lim, R. Cell-based therapies for the preterm infant. *Cytotherapy* 2014, 16, 1614–1628. [CrossRef]

178. Yoshimoto, M.; Koenig, J.M. Stem Cells: Potential Therapy for Neonatal Injury? *Clin. Perinatol.* 2015, 42, 597–612. [CrossRef]

179. Antoniadou, E.; David, A.L. Placental stem cells. *Clin. Perinatol.* 2015, 42, 579–589. [CrossRef]

180. Park, H.W.; Kim, Y.; Chang, J.W.; Yang, Y.S.; Oh, W.; Lee, J.M.; Park, H.R.; Kim, D.G.; Paek, S.H. Effect of Single and Double Administration of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Following Focal Cerebral Ischemia in Rats. *Exp. Neurol.* 2017, 289, 55–65. [CrossRef]

181. McDonald, C.A.; Payne, N.L.; Sun, G.; Moussa, L.; Siatskas, C.; Lim, R.; Wallace, E.M.; Jenkin, G.; Bernard, C.C. Immuno-suppressive potential of human amniotic epithelial cells in the treatment of experimental autoimmune encephalomyelitis. *J. Neuroinflammation* 2015, 12, 1–14. [CrossRef]

182. Yawno, T.; Schuijverwe, J.; Moss, T.J.; Vosdoganes, P.; Westover, A.J.; Afandi, E.; Jenkin, G.; Wallace, E.M.; Miller, S.L. Human Amnion Epithelial Cells Reduce Fetal Brain Injury in Response to Intrauterine Inflammation. *Dev. Neurosci.* 2013, 35, 272–282. [CrossRef]

183. Barton, S.K.; Melville, J.M.; Tolcos, M.; Polglase, G.R.; McDougall, A.R.; Azhan, A.; Crossley, K.; Jenkin, G.; Moss, T.J.M. Human Amnion Epithelial Cells Modulate Ventilation-Induced White Matter Pathology in Preterm Lambs. *Dev. Neurosci.* 2015, 37, 338–348. [CrossRef] [PubMed]

184. Hodges, R.J.; Lim, R.; Jenkin, G.; Wallace, E.M. Amnion Epithelial Cells as a Candidate Therapy for Acute and Chronic Lung Injury. *Stem Cells Int.* 2012, 2012, 1–7. [CrossRef]

185. Yoshimoto, M.; Koenig, J.M. Stem Cells: Potential Therapy for Neonatal Injury? *Clin. Perinatol.* 2015, 42, 597–612. [CrossRef]

186. van den Heuij, L.G.; Fraser, M.; Miller, S.L.; Jenkin, G.; Wallace, E.M.; Davidson, J.O.; Lear, C.A.; Lim, R.; Wassink, G.; Gunn, A.J.; et al. Delayed intranasal infusion of human amnion epithelial cells improves white matter maturation after asphyxia in preterm fetal sheep. *J. Cereb. Blood Flow Metab.* 2017, 39, 223–239. [CrossRef] [PubMed]

187. Davidson, J.O.; Heiju, L.G.V.D.; Fraser, M.; Wassink, G.; Miller, S.L.; Lim, R.; Wallace, E.M.; Jenkin, G.; Gunn, A.J.; Benett, L. Window of opportunity for human amniotic epithelial stem cells to attenuate astrogliosis after umbilical cord occlusion in preterm fetal sheep. *STEM CELLS Transl. Med.* 2020. [CrossRef]

188. Nott, F.; Pillow, J.J.; Dahl, M.; Kelly, S.B.; Melville, J.; McDonald, C.; Nitsos, I.; Lim, R.; Wallace, E.M.; Jenkin, G.; et al. Brain inflammation and injury at 48 h is not altered by human amnion epithelial cells in ventilated preterm lambs. *Pediatr. Res.* 2020, 88, 27–37. [CrossRef]

189. Ali, H.; Bahlbahi, H. Umbilical cord blood stem cells—Potential therapeutic tool for neural injuries and disorders. *Acta Neurobiol. Exp.* 2010, 70, 316–324.

190. Tiwari, A.; Tursky, M.L.; Mushahary, D.; Wasnik, S.; Collier, F.M.; Suma, K.; Kirkland, M.A.; Pande, G. Ex vivo expansion of haematopoietic stem/progenitor cells from umbilical cord blood on acellular scaffolds prepared from MS-5 stromal cell line. *J. Tissue Eng. Regen. Med.* 2013, 7, 871–883. [CrossRef]

191. Pimentel-Coelho, P.M.; Rosado-De-Castro, P.H.; Da Fonseca, L.M.B.; Mendez-Otero, R. Umbilical cord blood mononuclear cell transplantation for neonatal hypoxic–ischemic encephalopathy. *Pediatr. Res.* 2012, 71, 464–473. [CrossRef]

192. Wasielewski, B.; Jensen, A.; Roth-Häber, A.; Dermietzel, R.; Meier, C. Neuroglial activation andCx43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic–ischemic injury. *Brain Res.* 2012, 1487, 39–53. [CrossRef]

193. Krakowiak, P.; Walker, C.K.; Bremer, A.A.; Baker, A.S.; Ozonoff, S.; Hansen, R.L.; Hertz-Picciotto, I. Maternal Metabolic Conditions and Risk for Autism and Other Neurodevelopmental Disorders. *Pediatrics* 2012, 129, e1121–e1128. [CrossRef] [PubMed]

194. Wang, X.L.; Zhao, Y.S.; Hu, M.Y.; Sun, Y.Q.; Chen, Y.X.; Bi, X.H. Umbilical cord blood cells regulate endogenous neural stem cell proliferation via hedgehog signaling in hypoxic ischemic neonatal rats. *Brain Res.* 2013, 1518, 26–35. [CrossRef]

195. Li, J.; Yawno, T.; Sutherland, A.; Loose, J.; Nitsos, I.; Bischof, R.; Castillo-Melendez, M.; McDonald, C.A.; Wong, F.; Jenkin, G.; et al. Preterm white matter brain injury is prevented by early administration of umbilical cord blood cells. *Exp. Neurol.* 2016, 283, 179–187. [CrossRef]

196. Li, J.; Yawno, T.; Sutherland, A.E.; Gurer, S.; Paton, M.; McDonald, C.; Tiwari, A.; Pham, Y.; Castillo-Melendez, M.; Jenkin, G.; et al. Preterm umbilical cord blood derived mesenchymal stem/stromal cells protect preterm white matter brain development against hypoxia-ischemia. *Exp. Neurol.* 2018, 308, 120–131. [CrossRef] [PubMed]

197. Li, J.; Yawno, T.; Sutherland, A.; Loose, J.; Nitsos, I.; Allison, B.J.; Bischof, R.; McDonald, C.A.; Jenkin, G.; Miller, S.L. Term vs. preterm cord blood cells for the prevention of preterm brain injury. *Pediatr. Res.* 2017, 82, 1030–1038. [CrossRef] [PubMed]