Brain Outcomes in Runted Piglets: A Translational Model of Fetal Growth Restriction

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Introduction

Fetal growth restriction (FGR) is one of the most significant causes of perinatal morbidity and mortality after prematurity. It occurs in approximately 5–10% of pregnancies in middle to high income countries with even higher rates (21%) in low-income countries [1]. FGR may affect up to 30 million infants per year worldwide [2]. FGR is commonly caused by chronic placental insuffi-
ciency that results in an inadequate supply of oxygen (hypoaxia) and nutrients delivered to the fetus in utero leading to abnormal fetal development which particularly affects the fetal brain. This chronic hypoxic event results in a fetal adaptive response whereby cardiac output is redistributed towards the brain at the expense of peripheral organs such as muscle and liver, resulting in “brain sparing” or asymmetric FGR where the body is disproportionately smaller than the head [3]. Asymmetric FGR is the most common form of growth restriction and occurs in around 70–80% of all FGR cases [4] where the compromised supply of nutrients and oxygen to the fetus develops in the third trimester. However, brain sparing does not ensure normal brain development in FGR fetuses and newborns [5]. Multiple neurodevelopmental disorders present in FGR infants that can persist into adulthood, such as learning and behavioral disorders, attention deficit hyperactivity disorder (ADHD), autism, psychiatric disorders, and cerebral palsy [6–8].

Brain impairment does not only persist during fetal development but is also evident following birth in the FGR newborn and infant. Clinical imaging studies identify grey and white matter (WM) disruption in the FGR newborn [9–11]. Decreased cortical thickness, delayed cortical development, and altered brain connectivity have been reported in preterm FGR infants. These changes persist and have been shown to be associated with developmental disabilities at 1 year of age [9, 10]. However, the mechanisms underlying these grey and WM changes remain to be determined. There is currently no therapeutic intervention available to protect the FGR newborn brain. However, clinical trials are underway in an effort to protect the FGR baby whilst in utero. With the wide range of long-term neurodevelopmental disorders associated with FGR, determining mechanisms of injury is vital in the development and/or choice of treatments for these babies. As 40% of FGR cases are only detected around the time of birth [12], early intervention following birth is critical to stem the ongoing injury these babies face. The use of translatable animal models to understand mechanisms of injury in the FGR newborn is crucial to the development of therapies for these babies.

**Animal Models of FGR**

Progress into understanding underlying factors influencing brain development in FGR infants has been relatively slow as human studies are either impossible due to ethical considerations, or extremely difficult. Human tissue donations from nonsurviving FGR fetuses/neonates are rare with the only available tissue at various stages of prenatal development and often exposed to pathology. Difficulty in estimating the timing of an FGR insult as well as variables of gestational age on brain development, insults such as pregnancy hypertension and other factors confound interpretation from human FGR autopsy findings. Animal models are a reliable and replicable means to explore specific mechanism of pathology associated with FGR at a physiological, cellular, and molecular level; however, limitations exist.

Results from FGR rodent models commonly used to investigate neurodevelopment are difficult to translate to FGR human infants due to the substantial differences in brain development and morphology. Rodents’ lissencephalic cortical structure is vastly different to the gyrencephalic, highly folded brain of humans. Rodents do not commonly demonstrate asymmetric FGR, yet a recent study demonstrated a head sparing phenotype following 50% calorie restriction [13]. However, various other animal species demonstrate the characteristics of asymmetric FGR such as pigs, rabbits, sheep, and guinea pigs [14–22]. Most of these models of FGR have a specific insult that is chemical, surgical, or physical (interventions include nutrient restriction and uterine artery ligation) at a known time in gestation, whereas in large litter pigs, spontaneous FGR (relevant to the human situation) can be studied. Furthermore, brain growth spurt occurs prenatally in the guinea pig and lamb’s brain and postnatally in rabbits and rats [23], thus leading to difficulties in interpreting brain outcomes in comparison to the human newborn. Aside from the aforementioned, there are many advantages and disadvantages of different animal models of FGR; for further details, see review by Swanson and David [24]. This review will summarize the utility of the pig in studying brain injury in FGR newborns.

**FGR in the Piglet**

The piglet brain resembles the newborn human brain in terms of cortical surface area, histology, myelination, and vascularization [25, 26]. The newborn piglet brain is gyrencephalic with a grey matter and WM ratio similar to that of human infants [27]. Human brain growth is greatest from a few weeks before to a few weeks after birth. Similarly, the piglet has a period of rapid brain growth extending from late prenatal to early postnatal life [23, 25]. Therefore, the piglet is an ideal animal to exam-
ine altered brain development arising from compromising perinatal events. Conrad and Johnson [28] provide a comprehensive review on neurodevelopment of the piglet.

Approximately, 15–20% of piglets in a litter are born growth restricted [29]. Growth restriction in the piglet occurs spontaneously [30] obviating the need for surgical induction of growth retardation. This is important, as brain outcomes can be studied without confounding impacts of experimental interventions. The FGR piglet mimics many of the human pathophysiological outcomes associated with FGR including asymmetrical growth restriction with brain sparing [31]. Inadequate fetal growth in pigs is caused by alterations associated with placental insufficiency similar to the human population [31]. This may be due to certain regions of poor placental development. Bauer et al. [30] suggest the most likely region is located at the crossing of second to third part of the uterine horns, where an overlap (compression) of vascular supply exists.

FGR piglets are classically identified by their lower birth weights [30]. However, just as in human pregnancies, birth weight alone does not indicate exposure to growth restriction during fetal development. In FGR piglets, relatively more nutrients are redirected toward growth of the brain and heart, compared with a normal piglet as part of a fetal adaptive reaction to placental insufficiency [32]. As with the human FGR situation, brain sparing or asymmetric FGR does not ensure neuroprotection [33, 34]. Due to asymmetrical growth, FGR piglets can be recognized by their head shape and lower body weight [35, 36]. Morphologically, FGR piglets can be characterized according to three criteria based on head morphology: “dolphin-like” head shape, bulging eyes, and wrinkles perpendicular to the mouth [36]. Other measurements of growth restriction in the piglet in addition to <10th centile birth weight include relative brain to body weight and brain to liver weight ratio.

**Studying Perinatal Brain Outcomes in the Piglet**

Piglets are ideal animals to explore how perinatal insults affect brain structure and function. Studies have demonstrated substantial postnatal brain growth in domestic pigs [27]. The piglet brain is approximately one-10th the size of a human newborn brain and 1 week of life in the piglet is similar to 1 month of life in humans [28]. Owing to the anatomy, physiology, and size of the piglet, it is a useful model for research into areas of pediatric research. The ability to examine histological (cellular, molecular) and magnetic resonance imaging (MRI) outcomes in the pig makes it an ideal animal to determine the effects of perinatal insults on brain outcomes for not only FGR newborns but also hypoxic-ischemic encephalopathic newborns [14, 37–39].

**Brain Histology in FGR Piglet**

Examining histological changes in the FGR piglet brain to reveal mechanisms of injury will assist in determining treatment options for FGR newborns.

**Neuronal and WM Impairment**

There is a considerable paucity of data from human autopsy tissue of the pathology of the human FGR brain. A classical study of six-term FGR infants demonstrated a reduction in myelin lipids and DNA content (used as an estimate of cell number) in cerebrum-brainstem and cerebellum fractions [40]. Cerebral ischemia was evident in 28/31 FGR fetuses >26 weeks gestation [41]. More recently in nine FGR fetuses, a significant decrease in cell number in developmental zones of the cortex has been reported [42]. Third trimester stillborn FGR fetuses showed neuronal apoptotic changes in the frontal cortex, temporal cortex, pons, and subiculum [43]. In the fetal and neonatal FGR piglet, neuronal and WM impairment has also been observed at the microscopic level [14, 39, 44, 45]. Significant WM and neuronal impairment is evident from 104 days of gestation (term = 114 days) through to postnatal day 7 in both the parietal cortex and hippocampus [39]. Disruption to the WM tracts and neuronal morphology were also evident in newborn FGR piglet brain [14].

Interestingly in the human FGR fetal study, the authors noted if they omitted subjects older than 27 weeks of gestation, no difference between the groups was observed [42]. They interpreted this finding to suggest that the changes resulting from FGR do not occur until after this time. They speculate the impact of FGR on brain development is not apparent until the third trimester. A similar phenomenon is observed in an FGR piglet study where significant disruption to both neurons and WM is observed starting at 104 days gestation (not prior, at 100 days gestation) [39] and continues until postnatal day 7; representing sustained impairment. 104 days of gestation in the piglet is similar to 26–28 weeks of gestation in the human [46]. At 26–28 weeks, in the human, the brain is growing at a rapid rate with concurrent increase in my-
elination [47]. Myelination in the fetal piglet brain has also been shown to increase at a rapid rate between 100 days and 110 days [25]. Whether disruption at this time point in the FGR fetus is due to cellular degeneration or reduction in cellular proliferation requires further exploration. However, a body of evidence states alterations in myelination in the FGR brain arise due to stalling of oligodendroglial cell maturation [48]. Further immunohistochemistry studies on oligodendrocyte lineage markers and immature neuronal markers would be useful to determine the mechanisms of neuronal and WM impairment and the potential for recovery of these cells in the FGR brain.

**Cellular Degeneration and Proliferation**

As previously mentioned, a decrease in cell numbers is apparent in human FGR postmortem brains [40, 42]. Whether this is due to an increase in cellular degeneration or reduction in cellular proliferation has not been examined in the human. In FGR piglet studies, contrasting results are demonstrated [14, 45, 49, 50]. No difference in apoptotic cell counts or antiapoptotic protein levels are demonstrated in FGR piglets compared with controls in the thalamus and the frontal lobe in the term newborn piglet less than 36 h old [49, 50]. Yet, the FGR piglets who underwent hypoxia (as an additional insult) did show a greater amount of cerebral apoptosis [49]. Clinically, FGR infants are at increased risk for acute hypoxic brain injury. The presence of apoptosis was determined morphologically using hematoxylin and eosin and verified using TUNEL and caspase-3. However, at both postnatal days 1 and 4 an increase in caspase-3 positive cells and Fluoro-Jade C staining was observed in the FGR piglet brain compared with controls in the parietal cortex [14, 45]. This discrepancy between FGR piglet studies reporting either no increase or a significant increase in apoptosis could be due to brain region examined, time points examined, or markers used to detect cell death. However, the degree of brain growth asymmetry as determined by brain to liver weight ratios was not marked in Burke et al. [49] and Moxon-Lester et al. [50], which may explain why increased cellular apoptosis was not pronounced. The FGR piglets in the Wixey et al. [14] studies did have asymmetric growth restriction, which may explain the significant degree of apoptosis observed in the FGR piglet brains in these studies [14, 45].

Cellular proliferation demonstrated variable results in the FGR piglet brain. Initial increases are evident on day of birth [14]; however, by postnatal day 4, a significant decrease in Ki67-positive cell numbers is evident in the parietal cortex compared with controls [14, 45]. This initial increase could be due to immature neurons attempting to differentiate as has been demonstrated in the FGR newborn guinea pig [51]. The subsequent reduction in Ki-67 proliferating cells at postnatal day 4 in the FGR piglet brain could be due to an increase in cellular degeneration as has been demonstrated following birth [14]. Cerebral blood perfusion is not uniform in the FGR brain and redirection from regions such as the frontal lobe towards the basal ganglia occurs with worsening placental blood flow indices [33]. This redistribution may lead to a disturbance in regional brain development that may account for certain disabilities in the FGR infant. Cerebral blood perfusion studies in the FGR piglet during development could decipher the potential spatial vulnerability of the FGR newborn brain. Despite clear demonstration of neurologic injury in the FGR piglet, very few studies have examined specific neuronal populations that would impact the release of neurotransmitter subtypes.

**Neurotransmitters**

Disruption to neurotransmitters, in particular dopamine and serotonin, can have an impact on multiple neurologically functions such as cognition (learning, memory), mood (depression, anxiety, schizophrenia), motor (coordination), and behavior (ADHD, autism) [52–54]. Disturbances to these neurotransmitters may be even more critical during the perinatal period where the brain is still developing rapidly. FGR infants show increased risk of ADHD, depression, cerebral palsy, learning, and behavioral issues [55–58]. Although no studies, to date, have examined levels of amine neurotransmitters in the human FGR newborn brain, evidence from limited FGR piglet studies suggest there may be alterations to these systems during fetal and postnatal development [59–61], and thus, behavioral abnormalities may be related to alterations in neurotransmitter activity of the FGR fetus/newborn. FGR piglet studies show both dopamine and the key enzymes of dopamine metabolism (aromatic amino acid decarboxylase activity; l-dihydroxyphenylalanine; 3,4-dihydroxyphenylacetic acid; and homovanillic) are disrupted in the fetal and neonatal FGR piglet brain [59–61].

A study of serotonin in growth restricted rat fetus (uterine artery ligation on day 18 of gestation) found marked decreases in serotonin levels and its metabolite, 5-hydroxyindole acetic acid (5-HIAA) in the forebrain on day 22/23 of gestation [62]. The authors note the level of serotonin disturbance depends on the degree of growth
restriction and perinatal stress. In contrast, a recent study in the pig fetus found no alterations in 5-HT or 5-HIAA in the hypothalamus at either 70 or 90 days of gestation [61], while term FGR pigs demonstrated higher concentrations of 5-HIAA in the hippocampus of male newborns [60]. These contrasting findings may be reflective of the developmental profile of pig and rodent. As the piglet brain development more closely aligns with human newborn brain development [23, 25], whether similar serotonin levels are evident in the human FGR brain remains to be determined. There are clear gaps in our knowledge regarding alterations in neurotransmitters in FGR during fetal and postnatal development. Comprehensive studies need to be conducted using consistent and comparable brain regions and developmental time points to establish the role of this critical mediator of development.

**MRI Brain Outcomes in FGR Piglet Studies**

Although histological assessments are invaluable in determining mechanisms of injury in the FGR brain, the downside to these techniques is that they are performed at postmortem and therefore provide limited information on the surviving FGR brain. Furthermore, many of these histological techniques cannot be used longitudinally in animal studies. In contrast, MRI can be used longitudinally in both human and animal studies. MRI is valuable as it is noninvasive and can be performed in human infants to characterize disease and injury.

The piglets physical size allows for human clinical neuroimaging to examine brain morphology of structural MRI and positron emission tomography [63–65]. Structural MRI protocols have been developed for neonatal and adult pigs [27, 66] and more advanced techniques including functional MRI and diffusion tensor imaging (DTI) have also been used [64, 67]. Similarities between pig and human brain development have been established using MRI methods with longitudinal characterization of volumetric changes from 2 to 24 weeks of age in the pig [27].

Three studies, to date, have used MRI to examine brain alterations in SGA/FGR piglets [44, 50, 68] with varying results. It is important to note the definition of SGA is different to FGR as SGA only considers weight at birth and is defined as a birth weight below the 10th percentile [4]. While birth weight is taken into account for the FGR newborn, it is further defined by physiological determinants and features of malnutrition and in utero growth retardation [4]. Even with these differences, we can extrapolate information from SGA piglets to mimic the FGR condition.

**MRI Volumetrics**

Two studies show similar findings to human SGA/FGR studies with lower total brain volume in SGA piglets [44, 68]. Caputo et al. [68] reported lower total brain volumes and regional brain volumes in SGA piglets at postnatal days 26–29 in all regions assessed except aqueduct, third and fourth ventricle. Regional relative brain volumes (i.e., regional brain volumes expressed as a fraction of total brain volume) however were lower in SGA for only for 7 of the 21 assessed regions, namely the cerebral aqueduct, left cortex, midbrain, pons, putamen, thalamus, and third ventricle [68]. Radlowski et al. [44] also assessed regional brain volumes of 22 brain regions and found that only global grey matter volume was statistically significantly reduced in SGA at postnatal day 28. Volumetric MRI studies of human infants show similar reductions in grey and WM volumes [9, 69].

**Voxel-Based Morphometry**

Voxel-based morphometry enables the spatial localization of group differences in grey or WM concentration. Caputo et al. [68] showed greater grey matter in olfactory bulb and bilateral cortices in SGA piglets, but no differences in WM between SGA and appropriate for gestational age (AGA). Radlowski et al. [44], also identified increased grey matter in the olfactory bulbs and bilateral cortices and additionally reported increased WM in the cerebellum and midbrain and decreased WM in the olfactory bulbs, left and right cortices, and internal capsule.

Padilla et al. [70], used voxel-based morphometry to compare grey and WM of FGR preterm infants and AGA preterm infants at 1-year corrected age. They reported decreased grey matter in the bilateral temporal lobes and insular lobes and left frontal and parietal lobes; as well as increased WM in the bilateral temporal lobes in the FGR group. In a nonoverlapping cohort, Padilla et al. [71], also used voxel-based morphometry to compare FGR and AGA at 12 months. They reported reduced grey matter in the amygdala, basal ganglia, thalami, insula, bilateral angular gyri, left parietal and occipital lobes, and the right periorlal area.

**Diffusion Tensor Imaging**

DTI is commonly used in humans to examine WM microstructure and brain connectivity using tractography. Fractional anisotropy (FA) and mean diffusivity
(MD) derived from DTI are commonly used to investigate microstructural properties. Lower FA and higher MD are generally associated with poorer WM microstructure. Radlowski et al. [44], reported decreased global WM FA in SGA piglets, but observed no differences in corpus callosum or grey matter FA; no differences in MD were observed. Caputo et al. [68], also reported lower global FA, and additionally found lower FA in the right cortex, lower MD (and axial and radial diffusivity) in the corpus callosum, and lower axial diffusivity in the thalamus. Another study employing diffusion weighted imaging (3-directions) found that apparent diffusion coefficient values of the frontoparietal region of the piglet brain showed no difference between control and FGR piglets (less than 36 h in age) [50]. This lack of differentiation could be due to the variability in age of animals examined and small sample size. DTI studies in human SGA infants also found lower FA values for WM compared with AGA controls immediately after birth [72, 73], suggesting reduced WM development and connectivity.

Magnetic Resonance Spectroscopy
Magnetic resonance spectroscopy (MRS) is used as a noninvasive method to quantify metabolite concentrations in the brain and can be a useful tool to test for different pathological conditions. In MRS, metabolites appear in the spectrum with peaks in positions (measured in units of ppm) that are determined by the local chemical and magnetic environment of the molecule. Signal intensity and line width of peaks are related to metabolite concentration. Caputo et al. [68], reported lower concentrations of large macromolecules and lipids at 12 ppm, 13 ppm, and 14 ppm in the hippocampi in SGA piglets. In contrast, Radlowski et al. [44] reported no significant differences in metabolite concentrations measured in the hippocampus and corpus callosum of SGA piglets. A human study concurs whereby SGA preterm infants at 32 and 41 weeks postmenstrual age demonstrated no difference in metabolites to AGA preterm infants [74]. However, a human study on SGA and late onset FGR fetuses demonstrate differences in MRS brain metabolic ratios (n-acetylaspargate/choline; NAA/Cho) in the frontal lobe at 37 weeks gestation [75]. NAA is a neuronal marker, Cho reflects myelination and cell membrane turnover, inositol (Ino) plays an important role in osmoregulation and cellular nutrition and is an astrocytic marker. Esteban et al. [10] demonstrated Ino/Cho ratio significantly higher in human SGA fetuses at 37 weeks gestation in the left frontal lobe. Ino is a marker for astrocyte activation and reactive gliosis that is a feature of FGR brain pathology [14]. A further study on SGA fetuses showed reduced NAA:Cho and NAA:Cr in SGA in comparison with AGA group in a central region of interest in the brain [76]. This may be due to alterations in mitochondrial metabolic status. The differences observed between these human and piglet studies could be due to multiple reasons; timing of analysis, brain region examined, sensitivity of the MRI technique/machine, severity of SGA/FGR. Methodical studies addressing the inconsistencies above, which can be performed in FGR piglets, would reveal the true nature of these results.

Adverse brain outcomes in the FGR infant may be due to a combination of neuronal and WM disturbances as demonstrated using MRI. In the human FGR infant a reduction in both grey matter and WM volume is evident [9, 10, 71]. Structural brain changes underlying altered neurodevelopment in FGR and SGA have been described using different MRI-based methods that include changes in whole structural brain networks. These have been evidenced not only in the prenatal period [71, 75, 77, 78] but at neonatal and early infancy [9, 10, 70, 79–81], persisting into adolescence [82, 83]. More specifically, altered brain connectivity in FGR infants was demonstrated by altered connectivity in motor and cortico-striatal-thalamic networks, with reduced FA. Reduced FA is also demonstrated in the FGR/SGA piglet [44, 68]. Furthermore, structural brain networks of 1-year-old FGR infants have reduced level of organization together with a pattern of regional network features associated with later neurodevelopmental outcomes [84–86]. This suggests the existence of altered maturation and organization of the fiber tracts within these networks in FGR infants.

Limitations to the Piglet MRI/MRS Studies
The relatively small sample size per group for the piglet studies (e.g., n = 5 per group in [44]) may have resulted in a reduced power to detect between-group differences. While group differences can be observed with small sample sizes when the effect size is large, smaller effects require larger sample sizes, which will differ for different brain regions and metrics in question. In addition, lack of adverse findings in Radlowski et al. [44], may also be due to the SGA piglets undergoing catch up growth with a similar weight to the AGA group at postnatal day 15. Catch up growth in the SGA human is associated with decreased mortality compared to SGA infants without catch up growth. Therefore, this cohort of SGA piglets could have a milder brain impairment and therefore it would be difficult to detect subtle differences. However, in the Caputo et al. [68] study brain sparing effect had oc-
Table 1. Brain outcomes in piglet models of FGR

| Author                  | Piglet breed                        | Intervention                      | Age at assessment          | Brain region examined       | Key findings                                                                                                                                 |
|-------------------------|-------------------------------------|-----------------------------------|-----------------------------|------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Caputo et al. [68]      | PIC Camborough (dam) × PIC 359 (sire)| Spontaneous FGR                   | Postnatal day 14 (RNA-seq); 1 month (MRI) | MRI: whole brain; RNA-seq hippocampus | **MRI/MRS**: reduced total brain volumes. Regional relative brain volumes (regional/brain volume) were reduced for the cerebral aqueduct, left cortex, midbrain, pons, putamen, thalamus, and third ventricle. No difference in WM volume using voxel-based morphometry. **RNA-seq**: increased synapse and cell signaling pathways and decreased ribosomal pathways. Asymmetric FGR |
| Wixey et al. [14]       | Large-White                         | Spontaneous FGR                   | Postnatal day 4             | Parietal cortex              | **Neurons and WM**: significant neuronal and WM disruption. Decreased cellular proliferation and increased apoptosis. **Inflammatory markers**: increases in activated microglia, astrocytes, and proinflammatory cytokines in the brain. Ibuprofen reduced inflammation and provided neuroprotection. Asymmetric FGR |
| Wixey et al. [14]       | Large-White                         | Spontaneous FGR                   | Newborn, postnatal day 4    | Parietal cortex              | **Neurons and WM**: significant neuronal and WM disruption on P1 and P4. Decreased cellular proliferation and increased apoptosis on P4. **Inflammatory markers**: increases in activated microglia, astrocytes, and proinflammatory cytokines in the brain on P1 and P4. Asymmetric FGR |
| Kalanjati et al. [39]   | Large-White                         | Spontaneous FGR                   | 100 days gestation, 104 days gestation, newborn, postnatal day 7 | Parietal cortex and hippocampus | **Neurons and WM**: significant neuronal and WM disruption from 104 days to P7. **GABA_A**: alterations in GABA_A alpha 1 and 3 subunits at P7 in hippocampus. Asymmetric FGR |
| Garcia-Contreras et al. [61] | Iberian                           | Nutrient shortage from day 35 of pregnancy | Gestational day 70 and day 90 | Hypothalamus                 | **Neurotransmitters**: catecholamines and indolamines: Day 70: FGR showed higher dopamine precursor and lower dopamine metabolite and higher serotonin precursor and lower serotonin metabolite. Day 90: differences between sexes were more evident. Female FGR showed lower dopamine precursor and metabolites. Higher brain sparing was related to lower serotonin metabolite degree of fetal development and sex affected neurotransmitter profile at both ages |
| Vazquez-Gomez et al. [60] | Iberian × Duroc                  | Spontaneous FGR                   | Newborn                     | Hippocampus and amygdala     | **Neurotransmitters**: catecholamines and indolamines: FGR affected dopamine metabolism, with low concentrations of noradrenaline at the hippocampus and higher dopamine metabolites at both the hippocampus and amygdala. FGR males had higher dopamine metabolites at the amygdala and higher serotonin metabolite at the hippocampus than control males. Sex was a key modulator of catecholamine concentrations |
| Author                  | Piglet breed                          | Intervention       | Age at assessment | Brain region examined       | Key findings                                                                                                                                 |
|------------------------|---------------------------------------|--------------------|-------------------|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Radlowski et al. [44]  | University of Illinois’s swine herd   | Spontaneous FGR    | 1 month           | Whole brain                 | MRI/MRS: reduction in global grey matter, however increases in volumes in olfactory blub and left and right cortices and decreases in WM vol in these regions; grey matter and internal capsule significant difference. Not modelling asymmetric FGR LBW |
| Moxon-Lester et al. [50]| Large White                           | Spontaneous FGR    | Newborn           | MRS: frontoparieto region; IHC: frontal cortex and thalamus | MRS: no difference between mean lactate/NAA ratio in FGR and control piglets Apoptosis: no difference in apoptotic cell counts; no difference in ADC values between FGR and controls. Asymmetric FGR |
| Burke et al. [49]      | Large White                           | Spontaneous FGR    | Newborn           | Frontal lobe and thalamus   | Apoptosis: no difference in apoptotic cell counts or antiapoptotic protein expression between control and FGR. Did not account for asymmetric FGR |
| Bauer et al. [31]      | Not specified                         | Spontaneous FGR    | Newborn, postnatal day 1 | Striatum Frontal cortex Mesencephalon | Neurotransmitters: elevated dopamine production based on increased AADC activity |
| Bauer et al. [59]      | German Landrace                       | Spontaneous FGR    | Postnatal day 2–5  | Striatum Frontal cortex Mesencephalon Cerebellum | Neurotransmitters: increased dopamine production, upregulation of AADC activity in striatum and frontal cortex independent of oxygen delivery to the brain. FGR not asymmetric |
| Bauer et al. [30]      | Deutsches Land-Edelschwein            | Spontaneous FGR    | Newborn, postnatal day 1 | Lower brain stem Cerebellum Forebrain | General characterization: asymmetrical FGR – brain sparring associated with decreased liver, thymus, and pancreas organ weight. Increased brain to liver ratio found to be a strong indicator of growth restriction in animals with body weight <10th centile |

MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; FGR, fetal growth restriction; WM, white matter; LBW, low birth weight; IHC, immunohistochemistry; PIC, pig improvement company; P, postnatal day; GABA, γ-aminobutyric acid type A; ADC, apparent diffusion coefficient; AADC, aromatic L-amino acid decarboxylase.
curred and the piglets did not undergo catch up growth that may indicate more severe brain impairment. In a human study, children with FGR have difficulty with learning and memory, which is more pronounced when catch up growth does not occur [87].

**Conclusion**

FGR can have lasting effects on brain structure and function resulting in long-term adverse neurological outcomes. The use of an animal model appropriate to investigate mechanisms of injury in the FGR newborn is crucial to the development of therapies for these babies. This review demonstrated the similarities in brain outcomes between the human and piglet FGR. The clinically relevant piglet model of FGR provides the opportunity to determine and track mechanisms of brain injury (shown in Table 1). With this information, a targeted approach to postnatal interventions may result in rapid translation to clinic to protect these vulnerable newborns and improve their lifelong outcomes.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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