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# The blood-brain barrier; protecting the developing fetal brain

Kate Goasdoué, Stephanie M. Miller, Paul B. Colditz, S. Tracey Björkman*

*The University of Queensland, Perinatal Research Centre, UQ Centre for Clinical Research, Herston QLD 4029, Australia*

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ABSTRACT

While placental function is fundamental to normal fetal development, the blood-brain barrier provides a second checkpoint critical to protecting the fetal brain and ensuring healthy brain development. The placenta is considered the key barrier between the mother and fetus, regulating delivery of essential nutrients, removing waste as well as protecting the fetus from potentially noxious substances. However, disturbances to the maternal environment and subsequent adaptations to placental function may render the placenta ineffective for providing a suitable environment for the developing fetus and to providing sufficient protection from harmful substances. The developing brain is particularly vulnerable to changes in the maternal/fetal environment. Development of the blood-brain barrier and maturation of barrier transporter systems work to protect the fetal brain from exposure to drugs, excluding them from the fetal CNS. This review will focus on the role of the 'other' key barrier during gestation – the blood-brain barrier – which has been shown to be functional as early as 8 weeks' gestation.



**Contents**



## 1. Introduction

There are a number of physiological barriers present in the body throughout life. Arguably the most important during pregnancy and the development of the fetus is the blood-placental barrier. The placenta forms the primary barrier between the maternal environment and the fetus regulating a wide variety of functions required for healthy development including gas exchange, hormone production and secretion and transfer of nutrients and waste [1,2]. The placenta is essential for survival; it is responsible for stimulating the maternal endocrine system to release hormones necessary for the continuation of pregnancy but also functions to provide protection of the fetus from potentially harmful agents. The placenta plays a key role in adaption during pregnancy, responding and adjusting throughout to signals from both the mother and the fetus to ensure optimal growth and is essential to the development of the fetal brain [3]. Both intrinsic and extrinsic factors from the maternal environment result in modulation of intra-uterine development. In addition to the placenta however, other barrier

* Corresponding author.
*E-mail address:* t.bjorkman@uq.edu.au (S.T. Björkman).

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systems exist in the fetus. The blood-brain barrier (BBB) is vital for protection of the brain and fundamental for the effective function of the central nervous system (CNS).

The BBB is the interface between the systemic circulation and brain parenchyma. It is responsible for the regulation of movement between the two compartments and is essential for maintaining homeostasis in the CNS. The brain requires a carefully maintained microenvironment and protection from toxic endogenous and exogenous substances for normal function. While the placenta provides the first regulatory barrier between the mother and fetus, the BBB serves as a highly specific safeguard of the developing fetal brain. The placenta and BBB work to protect the fetus from potentially toxic substances which can have long term pathological consequences. To date, our understanding of the development and functionality of this barrier has been poor, to the point that many believed that the fetal BBB is immature if not altogether absent. Across time this belief has been perpetuated, and it was believed protection of the growing fetus was provided solely by the placenta [4]. However, experiments performed nearly a century ago as well as several recent studies shows not only the presence of the BBB in the developing fetus but that it is functionally capable, possessing many of the barrier properties observed in the fully developed BBB of the adult brain [5]. In this review we will explore the development of the BBB, its function in the growing fetus and, how such changes in the maternal environment may impact the developing brain.

## 2. Development of the blood-brain barrier

The BBB is primarily a diffusional barrier between the systemic vascular system and the brain. Like the placenta, the BBB is responsible for maintaining an optimal environment for development. It does this through the complex cellular structure that makes up the BBB as well via a number of transport mechanisms responsible for molecule transfer and protection of the brain from toxic substances. The BBB is made up of endothelial cells of the vasculature forming cell-to-cell tight and adherens junctions to limit transcellular/paracellular movement between the two compartments. These endothelial cells lack fenestrations, have low turnover and proliferation rates, and have high electrical resistance [6]. Under normal conditions these properties limit the free movement of ions, large proteins, and water allowing tight control of concentration gradients between the blood and brain. This results in the protection of the brain from vasogenic edema, other toxic effects, and regulation of neuronal excitability. However, a fully functional BBB requires the support of a range of cell types including neurons, pericytes, astrocytes, and microglia, all of which contribute to barrier integrity; together these are known as the neurovascular unit (NVU).

Historically it was proposed that the BBB was not mature during early stages of development and that the vulnerable developing brain was fully protected by the barrier properties of the placenta. Early studies using vascularly injected dyes such as trypan blue in animal embryos and fetuses showed permeation into all tissues including the brain, perpetuating the idea that in the immature animal the BBB was undeveloped, leaky, or lacking altogether (for a comprehensive review see Saunders et al., 2014 [5]). However, several studies such as those by Weed (embryonic pig), Cohen and Davies (embryonic guinea pig) and Grazer (rat E10-birth) did not show any evidence of staining in the brain [7–9]. In human, post-mortem tissue from fetuses and neonates showed that from as early as 12 weeks gestation trypan blue did not cross the BBB [10]. Overloading the binding capacity of plasma albumin, to which many dyes such as trypan blue bind, results in excess dye that can easily penetrate into the brain; in many of the early studies such toxic levels of dye were used that many animals died [5,11].

## 3. Structural development of the blood-brain barrier

The precise structure of the BBB is key to its functional ability to protect and maintain the brain microenvironment. Recent studies have demonstrated that humans, rats, and sheep have a number of functional barrier mechanisms in place from early gestational timepoints [12,13]. These include tight junction proteins and several transporters at the cerebral vasculature.

The development of the BBB is a multistep process. Initial vascularisation is followed by tight junction protein and nutrient transporter expression. The BBB then matures further with contact of pericytes and astrocytes of the NVU [14]. Development continues with the increased expression of efflux transporters, decreased levels of transcytosis, and sealing of the inter-endothelial cleft. Across BBB development there are changes to the NVU, electrical resistance of the endothelial cells themselves, tight junction proteins, and influx and efflux transport which may alter the permeability of the BBB to different substances such as water, proteins, or ions. Alterations to barrier structure and function address specific needs of the brain at various developmental stages.

Vascularisation of the human telencephalon begins at approximately week 8 of gestation, by the 12th week tight junction proteins occludin and claudin-5 are expressed in the primary vessels [15]. The appearance of tight junction proteins at this time appears sufficient to prevent endogenous albumin from entering the brain, providing evidence of early functionality of the barrier [15]. By the 18th week of gestation, these tight junction proteins demonstrate similar staining patterns to the tight junctions of the adult BBB [15]. Freeze fracture and thin section electron microscopy in neonatal human tissue demonstrates that tight junctions are organised in complex, linear, near-continuous tracts between endothelial cells of the microvasculature [16]. Similar detailed findings have been reported in rat BBB tight junction development, where tight junctions are abundantly present in late gestation and undergo increases in complexity in terms of integrity and length throughout gestation and after birth [17,18].

There are several studies demonstrating *in vitro* that astrocytes are essential for tight junction formation [19–21]. However, it has been demonstrated in rodents that tight junctions are present and functional at birth [18], whereas the primary period of astrocytic differentiation and vessel encirclement does not occur until the third postnatal week [22]. It is unclear how astrocytes contribute to the development of the barrier phenotype, however there is evidence to support a role in the progressive tightening of tight junctions after birth [23]. Another cell type critical for development and maintenance of the BBB is the pericyte [24]. While pericytes are expressed throughout the systemic vasculature, the highest density of pericytes is found in the brain [24]. Pericytes have also been shown to be essential for the formation of tight junctions [24,25]. Generation of pericytes and their effects on tight junction formation occur as early as P13 in the rodent preceding those of the astrocyte [24]. The process of pericyte encirclement of cerebral vessels is associated with a decrease in BBB solute permeability – demonstrating their functional importance in CNS protection [26].

How the development of the BBB across time affects barrier functionality is not well understood. It is clear that each of these changes is necessary for the tight control at the BBB demonstrable in adults. However, differences in permeability to drugs of differing size or pharmacological properties across development are unknown and likely to be species-dependant. This presents challenges when targeting treatments towards the mother or fetus in the prediction of efficacy and toxic effects. These factors need to be kept in mind when studying the effects of the maternal environment on

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