

# Prenatal stress and the fetal gut

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# Chapter 8

## Valorization



## Valorization

Preterm birth is the most important cause of neonatal morbidity and mortality in the developed world (1). The WHO estimates that each year, 15 million babies are born premature and approximately one million children die due to complications of prematurity (1, 2). For a large proportion of the surviving preterm neonates, there will be a lifetime of adverse health outcomes including respiratory problems, poor nutritional uptake, intestinal dysmotility, learning disabilities, motor and visual impairments (3-5). This indicates an enormous economic and social burden. Medical care costs have reversed relationship with the neonate's gestational age (6). Recently, a report by the Institute of Medicine in the US estimated that the economic burden related to preterm birth was approximately 26.2 billion USD in 2005 (~ 51,600 USD per neonate born premature) (7). Around 33,000 USD per preterm neonate were utilized for medical care services, with more than 85% of those provided between 0 - 5 years of age (7). In Europe, recent evidence from a study in Finland has shown that the standard costs for surviving preterm (<1000 g) neonates are ~ 100,000 EUR within 2 years whereas for term neonates are ~ 3,000 EUR (7). Besides the costs for hospitalization and associated neonatal intensive care for preterm babies, premature birth and related complications constitute additional costs including expenses for special education or costs due to travelling, accommodation, neonatal care (parental time and related lower income from parents' perspective) (6, 7). In addition to financial considerations, the birth of a premature infant can affect physical, emotional and social status of the parents and relatives (6, 7). In particular, mothers who have given birth to a preterm infant are at high risk of psychological distress, anxiety and depression and thus, they are in need for social support (7, 8).

The adverse health effects of a preterm birth can be further intensified by chorioamnionitis and perinatal asphyxia, which constitute independent risk factors for postnatal pathologies (9, 10). Chorioamnionitis, defined as inflammation of the fetal membranes, is the most frequent cause of preterm labor. It is estimated that approximately 25% of the premature infants are born by a mother with

chorioamnionitis (11, 12). This percentage is even higher in lower gestational ages where approximately 60% of extremely premature (< 25 weeks of gestations) births are associated with chorioamnionitis (13, 14). Similar to chorioamnionitis, perinatal asphyxia causes fetal inflammation and is a frequent fetal/neonatal medical complication accounting for approximately one million deaths each year (15, 16) with an equal amount of survivors who are at high risk to develop lifelong physical and neurological disabilities. Both perinatal pathological conditions affect multiple fetal organs including the gut. The most serious, life threatening postnatal intestinal disease associated with chorioamnionitis is NEC (17, 18). In addition to chorioamnionitis, prematurity is an independent risk factor for NEC as it has been shown that NEC is inversely correlated with gestational age (19, 20). Increased incidence of NEC has also been linked to perinatal asphyxia (21). Given the impact of chorioamnionitis and perinatal asphyxia on intestinal functioning, it is crucial to unravel the underlying mechanisms which may explain the correlation between chorioamnionitis/perinatal asphyxia and intestinal pathologies. Therefore, the studies described in this dissertation aimed at characterizing the intestinal pathological changes induced by chorioamnionitis and fetal HI. In addition, we investigated the potential of antenatal therapeutic interventions to prevent intestinal injury caused by these pathological conditions.

The findings in **chapter 2** extended our previous knowledge in respect to first, the fundamental role of IL-1 $\alpha$  signaling in chorioamnionitis-induced adverse intestinal effects and second, the relative contribution of selective IL-1 $\alpha$  exposure to fetal mucosal surfaces (i.e. lung, gut and chorioamnion/skin) in the induction of intestinal inflammation and injury in the context of experimental chorioamnionitis. We provided mechanistic evidence that chorioamnionitis-driven intestinal pathological changes primarily require direct IL-1 $\alpha$ -mediated intestinal activation. However, extraintestinal IL-1 $\alpha$ -driven immune responses (i.e. lung and chorioamnion/skin) also contributed to intestinal inflammation. This increased insight is essential for the design of future immunomodulatory therapeutic strategies where both intestinal and extraintestinal IL-1 $\alpha$ -driven immune responses will be targeted to protect the fetal gut after antenatal

inflammation.

Using our preclinical ovine model, we explored the therapeutic potential of systemic administration of IL-2 to prevent chorioamnionitis-induced adverse intestinal outcomes. In **chapter 3**, the principal concept was to use the immunomodulatory properties of IL-2 to preferentially expand the Treg cell population which is necessary to control the intestinal immune responses under physiological and pathophysiological conditions. We revealed that IL-2, a drug that is already in clinical use, can be used to prevent chorioamnionitis-driven adverse intestinal outcomes. The application of IL-2 to protect the fetal gut in the context of experimental chorioamnionitis has been filed as a patent in January 2015 (22). This innovative in utero approach to prevent gastrointestinal diseases associated with chorioamnionitis has the potential to be beneficial in numerous neonates who are born with chorioamnionitis each year worldwide. Importantly, in this study prophylactic administration of IL-2 was not correlated with adverse health outcomes of the fetus including immunosuppression and vascular leak syndrome. Considering the current beneficial effects of IL-2 on the fetal gut, additional studies are needed to explore the therapeutic efficiency of IL-2 after the onset of chorioamnionitis.

Before prophylactic IL-2 administration can be translated into the clinic, there are several aspects that should be considered. The results need to be tested and validated under the scope of regulatory affairs. Toxicology testing of IL-2 on fetal tissues should be conducted. All products need to be prepared in good clinical practice (GCP) and standard operating procedures (SOP) need to be developed. The investigational medical product dossier (IMPD) needs to be written and the permission of ethics committee on research involving human subjects needs to be approved. The idea of using IL-2 to prevent gastrointestinal disease caused by chorioamnionitis can be further commercialized by companies which can support and provide services for clinical implementation.

Besides bacteria, fungi including *C.albicans* have been associated with chorioamnionitis. Therefore, in this dissertation (**Chapter 4**) we investigated the adverse

intestinal effects of *C.albicans*-mediated chorioamnionitis in a translational ovine model. In addition we tested the therapeutic effects of the antifungal fluconazole to protect the fetal gut after *C.albicans*-induced chorioamnionitis. Our results demonstrated that intra-amniotic *C.albicans* infection causes intestinal infection, injury and inflammation within a short period of time (~ 3 days). These intestinal pathological features were associated with systemic inflammation and candidemia. These findings imply that in case of *C.albicans*-mediated chorioamnionitis the progression of the infection is rapid indicating that it is of significant importance to early detect and immediately treat the IA fungal infection to reduce morbidity. In this study, the infected animals were treated with fluconazole at a single time point after IA *C.albicans* infection which although resulted in decreased mucosal injury and mortality, failed to ameliorate *C.albicans*-mediated mucosal inflammation. This indicates that the applied *in utero* therapeutic strategy needs to be optimized by considering several aspects including but not limited to the timing, frequency and dosing of the antifungal treatment.

In addition to chorioamnionitis, the present dissertation (**Chapter 5**) addressed the adverse intestinal outcomes as a consequence of fetal HI. We revealed that global HI in fetal sheep induced intestinal inflammation which was associated with structural changes and impaired development of the ENS. In addition, we used an innovative approach to test whether the adverse effects of global HI on the fetal gut would be ameliorated by intravenous administration of MSCs. Our findings showed that the detected pathological intestinal features as a consequence of global HI were not attenuated by the chosen therapeutic MSC-based therapeutic strategy. We chose to administer MSC systemically as this remedy was previously shown to protect the fetal brain from inflammation, structural and functional impairment which was associated with reduced mobilization and invasion of T cells in the preterm brain (23). As this therapeutic strategy provides selective organ protection, additional investigation should focus on a MSC-based therapy which will collectively protect the fetal brain and gut after global HI. These strategies should focus on timing, route and dose of MSC administration.

The acquired knowledge from the translational studies described in this dissertation are of significant interest not only for researchers but also for clinicians including neonatologists, gynecologists, surgeons, perinatologists and pediatricians who are interested in understanding the basic mechanisms underlying gastrointestinal diseases in the newborn. In addition, this dissertation helps to raise parent's awareness of preterm neonates affected by chorioamnionitis and HI and their relationship (infant-parent interaction). Moreover, the effects of the described innovative *in utero* therapeutic interventions can form the basis for scientists to investigate their therapeutic potential in depth and develop additional strategies which will collectively reduce fetal organ morbidity after chorioamnionitis and global HI.

In conclusion, the studies described in this dissertation contribute to our understanding concerning the effects of prenatal inflammatory stimuli (such chorioamnionitis and fetal HI) and intestinal complications. In addition, this dissertation presents potential interventions to protect the fetal gut in the context of chorioamnionitis. Nevertheless, additional research is required for clinical implementation and our translational model is suitable to test the efficacy and feasibility of more comprehensive therapeutic strategies to prevent and/or treat gastrointestinal complications after antenatal inflammation.

## References

1. Blencowe H, Cousens S. Addressing the challenge of neonatal mortality. *Trop Med Int Health*. 2013;18:303-312.
2. Blencowe H, 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.
3. Boyle EM, et al. Effects of gestational age at birth on health outcomes at 3 and 5 years of age: population based cohort study. *BMJ*. 2012;344:e896.
4. Lee AC, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. *Pediatr Res*. 2013;74 Suppl 1:50-72.
5. Moster D, Lie RT, Markestad T. Long-term medical and social consequences of preterm birth. *N Engl J Med*. 2008;359:262-273.
6. Zupancic JAF. Preterm Birth: Causes, Consequences, and Prevention. A Systematic Review of Costs Associated with Preterm Birth. Behrman RE BA, editor. Washington (DC): National Academies Press (US); 2007.
7. Hodek JM, von der Schulenburg JM, Mittendorf T. Measuring economic consequences of preterm birth - Methodological recommendations for the evaluation of personal burden on children and their caregivers. *Health Econ Rev*. 2011;1:6.
8. Eutrope J, et al. Emotional reactions of mothers facing premature births: study of 100 mother-infant dyads 32 gestational weeks. *PLoS One*. 2014;9:e104093.
9. Czikk MJ, McCarthy FP, Murphy KE. Chorioamnionitis: from pathogenesis to treatment. *Clin Microbiol Infect*. 2011;17:1304-1311.
10. Aslam HM, et al. "Risk factors of birth asphyxia". *Ital J Pediatr*. 2014;40:94.
11. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. 2014;345:760-765.
12. Horvath B, Lakatos F, Toth C, Bodecs T, Bodis J. Silent chorioamnionitis and associated pregnancy outcomes: a review of clinical data gathered over a 16-year period. *J Perinat Med*. 2014;42:441-447.
13. Romero R, Espinoza J, Chaiworapongsa T, Kalache K. Infection and prematurity and the role of preventive strategies. *Semin Neonatol*. 2002;7:259-274.
14. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *N Engl J Med*. 2000;342:1500-1507.
15. Black RE, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375:1969-1987.
16. Lawn JE, Kerber K, Enweronu-Laryea C, Cousens S. 3.6 million neonatal deaths—what is progressing and what is not? *Semin Perinatol*. 2010;34:371-386.

17. Been JV, Lievense S, Zimmermann LJ, Kramer BW, Wolfs TG. Chorioamnionitis as a risk factor for necrotizing enterocolitis: a systematic review and meta-analysis. *J Pediatr.* 2013;162:236-242 e232.
18. Coggins SA, Wynn JL, Weitkamp JH. Infectious causes of necrotizing enterocolitis. *Clin Perinatol.* 2015;42:133-154, ix.
19. Gephart SM, McGrath JM, Effken JA, Halpern MD. Necrotizing enterocolitis risk: state of the science. *Adv Neonatal Care.* 2012;12:77-87; quiz 88-79.
20. Derikx JP, Kramer BW, Wolfs TG. Are EGF and TLR-4 crucial to understanding the link between milk and NEC? *Mucosal Immunol.* 2015;8:979-981.
21. Thornton KM, Dai H, Septer S, Petrikin JE. Effects of whole body therapeutic hypothermia on gastrointestinal morbidity and feeding tolerance in infants with hypoxic ischemic encephalopathy. *Int J Pediatr.* 2014;2014:643689.
22. Tim Guillaume Anna Marie WOLFS BWWK, inventor Method for the treatment or prevention of gastrointestinal disease caused by chorioamnionitis. Universiteit Maastricht, Academisch Ziekenhuis Maastricht, Netherlands 2015.
23. Jellema RK, et al. Mesenchymal stem cells induce T-cell tolerance and protect the preterm brain after global hypoxia-ischemia. *PLoS One.* 2013;8:e73031.