Summary

Mycotoxins are toxic secondary metabolites produced by various fungi that contaminate food and feed, posing significant health risks to humans and animals. In Burkina Faso, the primary contributors to mycotoxin prevalence include suboptimal pre- and post-harvest practices, climate change, lack of enforcement of mycotoxin regulations, low awareness, and a poor dietary diversity. Given their potentially carcinogenic, teratogenic, hepatotoxic, mutagenic, and immunosuppressive health impacts, dietary mycotoxin exposure requires special attention, especially for vulnerable groups such as pregnant and lactating women and newborns. This exposure has been associated with numerous negative birth and infant growth outcomes, including stillbirths, low birth weight (LBW), fetal growth restriction, and stunted growth. However, previous research in low- and middle-income countries (LMICs) has yielded inconsistent and low-quality evidence regarding the causal relationships between mycotoxin exposure and the increased rates of these adverse birth and infant growth outcomes. The primary aim of this PhD dissertation was to design and implement the innovative Biospecimen study (BioSpé) in rural Burkina Faso. As a sub-study of the MIcronutriments pour la SAnté de la Mère et de l'Enfant III trial, it focused on collecting various biological specimens from mother-infant pairs at multiple prenatal and postnatal time points. The first purpose was to perform biomonitoring of environmental contaminants in these samples to assess their effects on maternal health (including gestational weight gain and anemia) and on infant characteristics linked to adverse birth outcomes, such as LBW, neonatal mortality, small-for-gestational-age (SGA), preterm birth (PTB), stunting, underweight, and wasting. The study also explored associations with continuous birth measurements (such as birth length, weight, and chest, head, and mid-upper arm circumferences) and growth metrics (such as infant length, weight, and chest, head, and mid-upper arm circumferences) during postnatal follow-up from birth to 6 months of age. The second objective of this PhD research was to analyze whole blood microsamples to investigate the relationship between maternal mycotoxin exposure during pregnancy and newborn exposure at birth, and their effects on the aforementioned maternal health, adverse birth outcomes, and infant growth outcomes in rural Burkina Faso.

Additionally, providing essential nutritional interventions, such as multiple micronutrient (MMN) supplementation, through high-quality antenatal care can help improve birth outcomes and support infant growth. Key nutrients like protein, essential fatty acids, B vitamins, vitamin A, vitamin D, iron, and zinc play a vital role in regulating key processes of fetal

growth, with iodine, folic acid, and zinc being especially important for brain development. Considering the potential benefits of nutrition interventions improving pregnancy outcomes—including reductions in maternal and neonatal deaths and stillbirths—there is an increasing need to evaluate the effects of prenatal nutritional interventions on newborn biomarkers, rather than focusing solely on anthropometric outcomes. It is possible that improvements are not visible through birth anthropometry and biological biomarkers are needed to assess an intermediate effect of the fortified BEP supplement. Therefore, the third objective of this PhD dissertation was to assess the impact of a daily maternal multiple MMN-fortified balanced-energy protein (BEP) and iron-folic acid (IFA) supplementation on newborn telomere length (TL) and mitochondrial DNA content (mtDNAc).

Chapter 1 defines the concept of the exposome and describes the epidemiological study to assess intrauterine growth restriction and birth outcomes. This chapter also gives a general overview of the different types of mycotoxins and their producing fungi, the distinctive features of the different mycotoxins, and their overall impact on human and animal health. Moreover, a description of the current status of the magnitude of the mycotoxins problem in sub-Saharan Africa and Burkina Faso is included in this chapter. Further, the impacts of mycotoxins on adverse birth outcomes and infant growth are highlighted, as well as the different mycotoxin analytical techniques. In the end, the different biological aging markers are outlined, as well as the sample sources and analytical techniques used for their measurement.

Chapter 2 emphasizes the research gaps concerning mycotoxins and biological aging markers in pregnant and lactating women and newborns in Burkina Faso, which prompted the formulation of the research questions in this PhD dissertation. The chapter also contains the objectives of the PhD research.

Chapter 3 outlines the BioSpé protocol, detailing the collection of whole blood microsamples, plasma, umbilical cord blood, urine, breast milk, and feces from pregnant and lactating women and their newborns during the prenatal and postnatal periods in rural Burkina Faso. This chapter also highlights the multi-omics and human biomonitoring analyses conducted to uncover biological pathways and discover novel biomarkers for assessing maternal health, as well as maternal and infant features associated with adverse birth outcomes and continuous metrics of birth anthropometry and growth from birth to six months of age. The expected outcome of this work is to generate evidence-based strategies for health prevention and intervention, improving maternal health, birth outcomes, and infant growth while addressing related

health issues like accelerated biological aging. This research further aims to elucidate mechanistic pathways, providing valuable insights to inform public health policy decisions and translate research findings into policies and protocols.

Chapter 4 presents the findings for the analysis of multiple biomarkers and metabolites of exposure to mycotoxins during pregnancy and their associations with birth outcomes and infant growth in 305 pregnant participants, between 30 and 34 completed weeks of gestation. In this study, whole blood microsamples collected using volumetric absorptive microsampling (VAMS) devices were analyzed using ultraperformance liquid chromatography coupled to tandem mass spectrometry. Ochratoxin A (OTA) exposure was detected in 50.8 % of the study participants, with aflatoxin G1 (AFG1), aflatoxin M1 (AFM1), cyclopiazonic acid, deoxynivalenol (DON) and T-2-toxin being detected in the range of 0.33 % and 2.31 % of the population. Though OTA was found in a high prevalence, no statistically significant associations were observed between maternal OTA exposure during pregnancy and adverse birth outcomes or infant growth. Likewise, OTA exposure was not significantly associated with infant growth and nutritional status at 6 months of age, and no significant correlation between OTA exposure and growth trajectories from birth to 6 months.

In **chapter 5**, findings from the analysis of 274 newborn whole blood microsamples extracted from VAMS at birth are discussed. OTA exposure was detected in 38.3% of newborns, with other mycotoxins such as aflatoxin B1, AFG1, AFM1, DON, citrinin, zearalanone and zearalenone being detected in the range of 0.36% and 4.01% of newborns. OTA exposure was significantly associated with adverse birth outcomes, such as lower birthweight (p= 0.042) and ponderal index (p = 0.034), and marginally significant lower height growth trajectories during the first 6 months (p = 0.057). The results highlight the crucial need to implement food safety measures to reduce OTA exposure thus resulting in improved maternal and fetal health in Burkina Faso.

Chapter 6 examines the impact of a daily MMN-fortified BEP supplement and an IFA tablet on newborns' relative TL and mtDNAc. At birth, whole arterial blood samples were collected from the umbilical cord of 104 newborns in the control group (whose mothers only received the IFA tablet) and 90 newborns in the intervention group (whose mothers received the MMN-fortified BEP supplement and IFA tablet). Average relative TL and mtDNAc were measured using quantitative polymerase chain reaction. The findings indicate no significant differences in TL or mtDNAc between newborns in the intervention or control groups. However, the results suggest that offspring with adverse birth phenotypes (e.g., SGA , LBW and PTB) tended to have higher mtDNAc, likely indicating mitochondrial dysfunction. In the future, further research is

required to assess the effects of preconception and prenatal nutritional supplementation on absolute TLs and more granular measures of mitochondrial bio-energetic functioning (e.g., oxygen consumption rate, mtDNA mutations and deletions), while larger observational studies might further explore the TLs and mtDNAc of infants born with adverse phenotypes.

Chapter 7 summarizes the key findings from this PhD study and presents the general conclusions made.

Chapter 8 addresses the issue of mycotoxins and the research on biological aging markers from a global perspective, highlighting the contributions of this PhD dissertation in filling the identified research gaps. It presents recommendations for future research on multiple mycotoxins in Burkina Faso and emphasizes the importance of pre- and post-harvest practices in reducing and controlling mycotoxins. The chapter also discusses nutrition-sensitive strategies to mitigate mycotoxins in the food supply and the need for improvements in public health policies, such as food safety regulations and their enforcement, as well as enhancing academic collaborations and laboratory equipment in LMICs settings. Additionally, it suggests measures to improve lifestyle factors in LMICs to improve biological aging and calls for further research on the exposome effect on biological aging markers in Burkina Faso.