

University of Groningen

Fetal programming in pregnancy-associated disorders

Stojanovska, Violeta

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:
2018

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Stojanovska, V. (2018). *Fetal programming in pregnancy-associated disorders: Studies in novel preclinical models*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Summary
Samenvatting in het
Nederlands
Acknowledgments
Research institute SHARE
Publication list
Curriculum vitae

ppendices

Summary

The prenatal period is characterized by rapid growth and development of the fetus. During this period, organogenesis occurs and maturation of all organs and tissues is accompanied by rapid growth of the body. This is also a very critical period of human's life because the environment experienced during pregnancy has the ability to shape the physiology of the fetus in order to prepare it for postnatal optimal functioning. Nevertheless, exposure to unfavorable conditions early in life may have long-lasting consequences on the physical and mental health of the offspring. This observation is known as the 'fetal programming' paradigm or 'the Developmental Origins of Health and Disease' hypothesis.

Throughout pregnancy, the maternal organism goes through local and systemic metabolic, immunological and hemodynamic adaptations that ensure optimal growth and development of the fetus. Failure to achieve optimal nutrient availability or placental functionality will lead to poor growth of the fetus and development of pregnancy-associated disorders. Among the most common pregnancy-associated disorders that contribute to a less optimal *in utero* development are preeclampsia, intrauterine growth restriction, and gestational diabetes. They are characterized by a complex etiology and can increase the risk of developing other pregnancy-associated disorders. Moreover, the exposed fetus is associated with an increased risk of developing cardiovascular and metabolic diseases in adulthood. However, which gestational factors are involved and to what extent this leads to fetal programming is still widely unknown.

Development of comprehensive preclinical models is of major importance for an evaluation of the effects of gestational factors on fetal programming. The research described in this thesis focused on novel models for the most frequent pregnancy-associated disorders and its effects on the fetal health. Ultimately, the results as described in this thesis could help both clinical management and severity evaluation of the fetal programming.

Preeclampsia is associated with increased risk of cardiovascular and metabolic disorders in later life of the offspring. In **chapter 2** we discuss the available human and animal studies on preeclampsia with respect to the cardiovascular and metabolic outcome of the offspring. Human data analyses on the effects of preeclampsia on children's health showed that these children in later age have increased blood pressure and vascular stiffness while the metabolic alterations in terms of increased lipid and glucose concentrations show transient and inconsistent effects. Based on the available animal studies in literature, we could describe the fetal programming effects of three

different pathophysiological mechanisms that are present during preeclampsia with emphasis on angiogenic imbalance, autoimmunity and inflammation. Exposure to angiogenic imbalance during pregnancy can disrupt the blood pressure regulation in the offspring in a sex-specific way with predominant alteration of the males. Angiotensin II type I receptor antibodies were shown to contribute to the pathogenesis of preeclampsia and exposure during pregnancy does not affect the blood pressure of the offspring but can have detrimental effects on the organ formation and insulin resistance. Offspring responses to inflammation, which is also present during preeclampsia, are similar to the responses triggered by angiogenic imbalance with a registered increase in blood pressure without major sex-specific differences and metabolic alterations when high-fat diet is introduced. Furthermore, we discuss novel insights into the mechanisms of fetal programming due to preeclampsia. The placental permeability which can be disrupted due to structural changes and increased apoptosis can serve as an initiator of fetal programming via increased release of signaling molecules such as: antiangiogenic factors, autoantibodies against angiotensin, inflammatory cytokines, leptin or reactive oxygen species. In turn, this will promote unfavorable intrauterine signaling network that can program several aspects of the metabolic and cardiovascular system, mainly via organ remodeling and epigenetic modulation of gene expression.

The angiogenic imbalance has been promoted as a successful model of translational research, elucidating possible mechanism in the genesis of preeclampsia. Therefore, in **chapter 3** we determined the effects of the antiangiogenic factor sFlt-1 (soluble fms like tyrosine kinase 1) on pregnancy and fetal outcomes. We demonstrate that antiangiogenic imbalance solely does not explain the pathophysiology of preeclampsia entirely. However, we show that enforced expression of antiangiogenic factor sFlt-1 results in lower birth weight and length of the fetuses. Moreover, we demonstrate that sFlt-1 can have a direct effect on the liver molecular phenotype, including upregulation of the fatty acid metabolism genes and the *Ppara* targets in the fetal liver.

Preeclampsia is a multifactorial disorder and possibly two different pathophysiological mechanisms might be intertwined in its genesis. Frequently, preeclampsia is associated with inflammation and increased concentrations of antiangiogenic factors. In that order, in **chapter 4** we demonstrate that induction of these two different pathophysiological mechanisms can result in experimental preeclampsia. This novel “double hit” model is characterized by the hallmark clinical symptoms of

preeclampsia, hypertension and proteinuria. As preeclampsia is also associated with metabolic disturbances in the mother, we confirmed that in our model there are prominent differences in the phospholipid fractions in the preeclamptic group with higher concentrations of long-chain fatty acids phosphatidylcholines. Moreover, the fetuses in this double hit preeclampsia model are growth restricted and show sex-specific differences. The male fetuses, on one hand, were asymmetrically growth restricted with sparing of the brain on the expense of other organs. On the other hand, the female fetuses were symmetrically growth restricted without sparing of the brain weight. This was accompanied by different plasma metabolic footprint in the fetuses. The males exposed to preeclampsia had lower concentrations of certain amino acids (proline and threonine), whereas the females showed much more profound differences in the lipid fractions, namely lower levels of several acylcarnitines, sphingomyelins, and glycerophospholipids, which in turn might explain these different growth restriction patterns in our double hit preeclampsia model.

Intrauterine growth restriction is a common complication of preeclampsia but also can be presented as an isolated disorder without manifestation of hypertension and proteinuria in the mother. In **chapter 5** we tested a novel model of growth restriction that has isolated structural changes in the placenta morphology without other maternal symptomatology. This was assessed via conditional deletion in the junctional zone of the transcriptional factor *Tfap2c*, which is important for placenta development. Next, we tested the hypothesis whether growth restriction is associated with obesity and insulin resistance in adulthood. Our growth restricted offspring did not have increased body weight in adulthood and the glucose clearance was not affected in these offspring. However, there were small sex-specific metabolic disturbances at this age. The females had increased plasma free fatty acids and higher mRNA levels of fatty acid synthase gene in the adipose tissue that were associated with increased endoplasmic reticulum stress markers in the adipose tissue. In turn, this shows that while there are molecular changes in adult growth restricted females especially in the white adipose tissue, there no major phenotypic disturbances in the overall glucose and lipid metabolism. Whether this remains to be unchanged when a second environmental stimulus is introduced such as western style diet or other stressors, is yet to be elucidated.

Diabetes during pregnancy is another common pregnancy-associated disorder that is correlated with an increased risk of metabolic and neurological disorders in the offspring. In most cases, diabetes during pregnancy is presented with type 2 diabetes that

is characterized with insulin resistance of the mother. However, to obtain an ideal animal model for type 2 diabetes in pregnancy still remains a challenge. For that cause, in **chapter 6** we explored a novel model of generalized insulin resistance (by conditional global deletion of the insulin receptor (*IR*)) during pregnancy. We demonstrate that this model is characterized by high glucose and high insulin concentrations in the mother with concomitant hypercholesterolemia and hypertriglyceridemia. In turn, the fetuses are growth restricted with lower liver and brain weight with accompanying lower microglial activation in the hippocampus in comparison to the wild-type fetuses. Moreover, this was associated with decreased expression of sterol regulatory binding factor 2 (*Srebf2*) in the fetal liver and brain, which serves as a master regulator of cholesterol metabolism. These gene differences, at least in part, were associated with hypermethylation of the *Srebf2* promoter in the fetal brain and liver. This factor is already known to be affected in diabetic patients, but we for the first time show that it can also serve as a phenotypic mark and potential mediating factor of fetal programming due to the diabetic intrauterine environment.

In this thesis, we show novel preclinical models for pregnancy-associated disorders to study the effects of fetal programming due to a non-optimal intrauterine environment. These models are of extreme importance to unravel the underlying mechanisms which modulate the later health of the offspring. In that way, we can obtain more directions towards the design of proper highly selective and sex-specific diagnostic and therapeutic tools to reverse the fetal programming. The models presented in this thesis recapitulate the clinical course of pregnancy-associated disorders more accurately and aim to bridge the translational research gaps in the fetal programming research.

Samenvatting in het Nederlands

Het prenatale leven wordt gekenmerkt door snelle groei en ontwikkeling van de foetus. Tijdens deze periode worden organen gevormd en de rijping van alle organen en weefsels gaat samen met een snelle groei van het lichaam. Dit is een zeer kritische periode in het mensenleven, omdat de omgeving tijdens de zwangerschap de fysiologie van de foetus kan vormen om het optimaal te laten functioneren in de rest van het leven. Blootstelling aan ongunstige omstandigheden in het vroege leven kan echter een langdurige nadelige invloed hebben op de fysieke en mentale gezondheid. Dit fenomeen wordt ‘foetale programmering’ of ‘Developmental Origins of Health and Disease’ hypothese genoemd.

Tijdens de zwangerschap ondergaat het lichaam van de moeder verschillende lokale en systemische veranderingen in metabolisme, immunologie en hart en vaten. Deze veranderingen zorgen voor optimale groei en ontwikkeling van de foetus. Wanneer de beschikbaarheid van voedingsstoffen of het functioneren van de placenta niet optimaal is, zal dit leiden tot verminderde groei van de foetus en de ontwikkeling van zwangerschapscomplicaties. De meest voorkomende complicaties waarbij de intra-uteriene omstandigheden niet optimaal zijn, zijn pre-eclampsie, intra-uteriene groeivertraging en zwangerschapsdiabetes. Deze complicaties worden gekenmerkt door een complexe etiologie en kunnen het risico op de ontwikkeling van andere complicaties vergroten. Daarnaast wordt de foetus hier ook aan blootgesteld, wat leidt tot een verhoogd risico op cardiovasculaire en metabole aandoeningen later in het leven. Welke factoren tijdens de zwangerschap betrokken zijn bij deze foetale programmering is echter nog grotendeels onbekend.

Het ontwikkelen van preklinische modellen voor zwangerschapscomplicaties is belangrijk om de effecten van verschillende factoren tijdens de zwangerschap op foetale programmering te onderzoeken. Het onderzoek beschreven in dit proefschrift focust op nieuwe modellen voor de meest voorkomende zwangerschapscomplicaties en het effect daarvan op de gezondheid van de foetus. De resultaten in dit proefschrift zouden nuttig kunnen zijn voor het evalueren van ernst van foetale programmering en het bepalen van klinisch beleid op dit gebied.

Pre-eclampsie is geassocieerd met een verhoogd risico op cardiovasculaire en metabole problemen later in het leven bij het nageslacht. In **hoofdstuk 2** bespreken we de beschikbare literatuur op het gebied van cardiovasculaire en metabole uitkomsten voor nageslacht dat aan pre-eclampsie is blootgesteld in de baarmoeder. Humane studies in kinderen die aan pre-eclampsie zijn blootgesteld, hebben aangetoond dat zij later in hun leven een verhoogde bloeddruk en vaatwandstijfheid hebben, maar dat de metabole

gevolgen, verhoogde lipide en glucose concentraties, slechts tijdelijk en inconsistent zijn. Gebaseerd op de beschikbare proefdierstudies konden we de effecten op foetale programmering beschrijven van drie pathofisiologische mechanismen die tijdens pre-eclampsie aanwezig zijn: angiogene onbalans, auto-immuniteit en inflammatie. Blootstelling aan angiogene onbalans tijdens de zwangerschap kan de bloeddrukregulatie in het nageslacht versturen, voornamelijk in het mannelijke geslacht. Angiotensine type 1 receptor autoantilichamen dragen bij aan de ontwikkeling van pre-eclampsie. Blootstelling hieraan heeft geen invloed op de bloeddruk van het nageslacht, maar kan wel schadelijke effecten hebben op de ontwikkeling van de organen en op insulinegevoeligheid. Het gevolg van inflammatie is vergelijkbaar met dat van angiogene onbalans. Blootstelling hieraan leidt tot een verhoogde bloeddruk en, wanneer een hoog vet-dieet wordt geconsumeerd, ook tot metabole veranderingen. In dit hoofdstuk worden daarnaast nog nieuwe inzichten in de mechanismen die een rol spelen bij foetale programmering door pre-eclampsie besproken. Verstoring van de permeabiliteit van de placenta, door structurele veranderingen of apoptose, kan een initiator zijn van foetale programmering door verhoogde afgifte van belangrijke signalerings-moleculen, zoals antiangiogene factoren, angiotensine autoantilichamen, inflammatoire cytokines, leptin en reactieve zuurstofverbindingen. Door deze moleculen worden signalering netwerken bevorderd die een ongunstig effect op foetale programmering kunnen hebben via structurele invloeden of epigenetische beïnvloeding van genexpressie.

Een pre-eclampsie diermodel gebaseerd op angiogene onbalans wordt gezien als een goed model voor translationeel onderzoek, omdat het gebaseerd is op één van de mogelijke oorzaken van preeclampsie. In **hoofdstuk 3** hebben we daarom het effect van de antiangiogene factor sFlt1 (soluble fms like tyrosine kinase 1) op de zwangerschap en de foetus bepaald in de rat. We hebben laten zien dat alleen angiogene onbalans niet de hele pathofisiologie van pre-eclampsie verklaard, maar dat blootstelling aan sFlt1 wel resulteert in lichtere en kortere foetussen. Daarnaast laten we zien dat sFlt1 directe moleculaire effecten heeft in de lever van het nageslacht, waaronder verhoogde expressie van Ppara targets en van genen betrokken bij vetzuur metabolisme.

Omdat pre-eclampsie een multifactoriële aandoening is, is het goed mogelijke dat twee verschillende pathofisiologische mechanismen betrokken zijn bij het ontstaan van pre-eclampsie. Pre-eclampsie is vaak geassocieerd met inflammatie en verhoogde concentraties van antiangiogene factoren. In **hoofdstuk 4** tonen we daarom aan dat de inductie van deze twee pathofisiologische mechanismen tegelijkertijd kan leiden tot

experimentele pre-eclampsie in muizen. Dit nieuwe ‘double hit’ model wordt gekarakteriseerd door de belangrijkste symptomen van pre-eclampsie: hypertensie en proteinurie. Aangezien pre-eclampsie ook geassocieerd is met verstoring van het metabolisme in de moeder, tonen we hier aan dat in het ‘double hit’ model veranderingen zijn opgetreden in de fosfolipiden, met een hogere concentratie lange keten vetzuren fosfatidylcholine in het bloedplasma van pre-eclamptische moeders. Daarnaast zijn de foetussen uit de pre-eclampsie groep groeivertraagd, waarin geslachtsspecifieke verschillen te zien zijn. De mannelijke foetussen zijn asymmetrisch groeivertraagd, wat betekent dat de groei van de hersenen is gespaard ten koste van de andere organen. De vrouwelijke foetussen daarentegen zijn symmetrisch groeivertraagd, bij hen is het hersengewicht relatief net zo veel verlaagd als het gewicht van de andere organen. Dit verschil ging gepaard met een verschil in metabole factoren tussen de geslachten. De aan pre-eclampsie blootgestelde mannelijke foetussen hadden lagere concentraties van bepaalde aminozuren (proline en threonine) dan de controlegroep, terwijl de vrouwelijke foetussen grote verschillen hadden in de lipide fracties, namelijk verlaagde concentraties van verschillende acylcarnitines, sfingomyelines en glycerofosfolipiden. Dit kan het geslachtsspecifieke verschil in groeivertraging in dit preeclampsie model verklaren.

Intrauteriene groeivertraging is een veelvoorkomende complicatie bij preeclampsie, maar dit kan ook voorkomen als een op zichzelf staande complicatie, zonder dat de moeder hypertensie en proteinurie heeft. In **hoofdstuk 5** hebben we een nieuw model van groeivertraging getest, waarin de groeivertraging veroorzaakt wordt door structurele veranderingen in de placenta die geen andere symptomen in de moeder veroorzaken. Dit was bewerkstelligd door een conditionele deletie van de transcriptiefactor Tfabp2c, belangrijk voor ontwikkeling van de placenta, in de junctionele zone van de placenta van de muis. We hebben getest of, in dit model, groeivertraging leidt tot obesitas en insulineresistentie op volwassen leeftijd. Het groeivertraagde nageslacht had op volwassen leeftijd geen verhoogd lichaamsgewicht en de klaring van glucose was ook niet beïnvloed. Er waren wel een aantal kleine geslachtsspecifieke metabole verstoringen te zien op deze leeftijd. De vrouwtjes hadden een verhoogde concentratie vrije vetzuren in het bloedplasma, en in het vetweefsel was de mRNA expressie van het Fasn (fatty acid synthase) gen verhoogd. Daarnaast was de mRNA expressie van verschillende markers voor endoplasmatisch reticulum stress ook verhoogd. Dit toont aan dat, hoewel er moleculaire veranderingen zijn in het vetweefsel, er geen grote fenotypische gevolgen zijn voor glucose en vet metabolisme van groeivertraging op vrouwtjes op volwassen

leeftijd. Of dit nog steeds het geval is als het metabolisme wordt getest met een extra stimulus, zoals een westers dieet, is nog niet onderzocht.

Diabetes tijdens de zwangerschap is een andere veelvoorkomende zwangerschapscomplicatie die geassocieerd is met een verhoogd risico op metabole of neurologische aandoeningen in het nageslacht. In de meeste gevallen wordt zwangerschapsdiabetes gekarakteriseerd door insulineresistentie van de moeder, net als bij diabetes type 2. Desondanks blijft het vinden van het ideale diermodel voor deze complicatie een uitdaging. Daarom hebben we in **hoofdstuk 6** een nieuw model gekarakteriseerd voor insulineresistentie tijdens de zwangerschap, wat ontstaat door conditionele deletie van de insuline receptor in het hele lichaam. Dit model wordt gekenmerkt door hoge glucose- en insuline waardes, hypercholesterolemie en hypertriglyceridemie in de moeder. Voor de foetussen leidt deze zwangerschapsdiabetes tot groeivertraging, een lager levergewicht en een lager hersengewicht met lagere microglia activatie in de hippocampus. Daarnaast is de mRNA expressie van Srebf2 (sterol regulatory binding factor 2), een belangrijke regulator van cholesterol metabolisme, verlaagd in lever en hersenen van foetussen blootgesteld aan zwangerschapsdiabetes. Dit verschil in mRNA expressie was geassocieerd met verhoogde DNA methylatie in de promotor regio van het Srebf2-gen in lever en hersenen. Het is al bekend dat Srebf2 aangedaan is in diabetespatiënten, en hier laten we voor het eerst zien deze factor mogelijk een rol speelt in foetale programmering als gevolg van zwangerschapsdiabetes.

In dit proefschrift laten we nieuwe preklinische modellen van zwangerschapscomplicaties zien die waardevol zijn voor het bestuderen van foetale programmering als gevolg van een niet-optimale intrauterine omgeving. Deze modellen zijn zeer belangrijk om de mechanismen die een rol spelen in het beïnvloeden van levenslange gezondheid te ontrafelen. Het achterhalen van deze mechanismen kan helpen om selectieve en geslachtsspecifieke diagnostische en therapeutische middelen te ontwerpen om nadelige foetale programmering tegen te gaan. De modellen die in dit proefschrift beschreven worden benaderen het klinische verloop van de zwangerschapscomplicaties met als doel de hiaten in translationeel onderzoek naar foetale programmering te overbruggen.

Acknowledgments

First and foremost I would like to thank my supervisors Sicco Scherjon and Torsten Plösch. Dear Sicco, thank you very much for the given opportunity to perform my PhD in the department of Obstetrics and Gynecology. Your enthusiasm always amazes me. Thank you so much for all the fruitful clinical and scientific discussions, kindness and understanding. Dear Torsten, I do not have enough words to thank you for all the support and encouragement you gave me during the past years. Thanks for all the fetal programming discussion, networking and writing tips and most importantly the interest you have shown in Macedonia.

In continuation, I would like to thank the members of the reading committee, Henk-Jan Verkade, Eric Steegers and Elke Winterhager for their willingness and time to review my thesis.

During the past 4 years I was so fortunate to perform my research with many wonderful colleagues. In one or another way you made my PhD journey way more enjoyable. Dear Josée and Rikst Nynke, thanks a lot for all the invaluable technical support in the CDP and in the lab. Dear Claudia, I was so happy and fortunate when you joined our lab. I enjoyed every single moment spent with you (which included lots of conversations about food :) and I truly hope that our paths will cross again. Dear Simone, thanks a lot for being the perfect companion for conferences. Dear Mariette, thanks a lot for the interest you have shown in my research and good luck with combining clinical and experimental work. Dear Dorieke, you joined the lab the last, but soon we became ‘partners in crime’, performing so many experiments together and I wish you lots of luck with the future experiments. Thanks a lot for helping me with the Dutch summary. Also, I would like to thank my students Carolina, Dyonne and Mirjam. I hope I have contributed to some extent in your (research) careers, because for sure you have contributed a lot to my teaching skills.

Throughout the years I was fortunate to collaborate with researchers from other research institutes. Thanks a lot Anne Marijn van der Graaf, Kim Holwerda, Marijke Faas, Mark Boekschoten, Michaela Golic, Ralf Deschend, Hubert Schorle, Neha Sharma, Alexandra Gellhaus and Rebekka Vogtman for your scientific input and fruitful discussions. I have enjoyed a lot while working with all of you. My particular thanks goes to the microsurgery team for all the technical support at the CDP. Also I would like to thank the clinicians from the Department of Obstetrics and Gynecology: Jelmer Prins and Liesbeth Kerkhof for helping me with my clinical study regarding vaginal microbiota and preeclampsia.

I would like to thank, immensely, the members of the Pediatrics and MDL department with whom I have interacted on daily basis either in the lab or at scientific meetings: Niels (no matter my question you always had the answer), Jan Freark, Angelika, Renze, Trijnie, Diane, Tjasso, Manon, Jannette, Janine, Uwe, Hans, Maaike, Han, Tim, Henk, Weilin, Brenda, Irene, Rima, Ana, Lidiya, Onne, Joanne, Mirjam, Fabio, Turu, Archi, Sandra, Sanam, Yu, Marleen, Lori, Maaike, Ivo, Danail, Dicky, Vera, Shiva, Marcela, Fan. Dear Janine, you are the reason I got into doing PhD in the first place. Your master traineeship laid a firm foundation for the rest of my time in the lab. Thanks a lot for everything.

My dear officemates Merel, Aniek, Fenne, Petra, Anne, Federica, Talita, Sterre, Matty, Lotte, Arjen, Jan and Tom thanks a lot for all the scientific and non-scientific talks during nice cup of coffee or tea.

My dearest Irene, Ana and Suru, my gals, thanks a lot for all the amazing dinners, trips, ladies nights and parties we had together. You were my ray of sun when it was raining in Groningen (and that was quite a lot). My dearest Rimini, thanks a lot for all the funny and deep conversations we had. I have learned a lot from you. My dearest Talita, I miss you so much in the office! Your calmness and tranquility was my daily elixir. Dear Maartje, you showed me a new way of cooking (meat, gluten and lactose free). Dear Tereza, thanks for being an amazing roommate. Dear Ena, your perseverance was very motivating to me. Dear Jane, Mina & Max, thanks for all the nice events we spent together, you are a fresh breeze to our small Macedonian community.

Even more people made my life in Groningen hospitable, entertaining and appetizing. Dear Turhan, Tamara, Visnja, Kushi, Neli, Murat, Aysegul, Mina T, Neha, Roberta, Martin, Marcin, Ivan, Faris, Lorena, Lusi, Victor and the ones I forgot, thanks a lot for everything.

Dear Fundita, you are so considerate and fun and thanks for all the endless conversations we had. You were the most amazing host to me in Boston! My dear Constanza, my sister on another continent, your lively and charming nature is unforgettable. Walking through Greenwich Village and Crown Heights with you and Funda, was once in a lifetime experience.

Dear Ilche, thanks a lot for all the tips&tricks In Groningen and of course for all the mild and heated discussions we had throughout the years. Your support and trust was invaluable to me. Dear Bibi (and Viktor), thanks a lot for all the dinners, parties and science

breaks we had. Your positivity and calmness is truly an inspiration. Dear Jovan, thanks a lot for all the technical practicalities you shared with me and for being so cheerful and patient. Dearest Marija, no matter the trouble you always find a solution, and I admire that a lot. Your constant encouragement and objectiveness (in most of the situations), was such a motivation. Thanks for being always there for me, no matter what.

Dear Tanja and Stole, thanks a lot for all the up to date information you shared about Macedonia and keeping me in touch with the clinics. My dearest Igor and Bici, thanks a lot for your contagious enthusiasm and endless help when I needed something from far away. Dear Ivana and Igor, thanks for all the experiences we shared together. Moe другарче, thanks for being so expressive and considerate all throughout the years. Miss you a lot.

Dearest Joco, my tough, little-big brother. Thanks for being such an honest critic to me (*I will always be an outlier*). I admire the way you solve things, show what you think and dispatch from the rest. Dear Ane, you are the most positive, funniest and nicest girl I have ever meet. Joco is truly fortunate to have you in his life. Your sense of humor and the way you look at life should be an inspiration for everyone.

My dearest and beloved parents, your eternal support and positivity was my driving force throughout the years. Најмил тато, фала ти за сите животни лекции, неизмерната верба во мене и за тоа што ми ја всади љубовта кон медицината и истражувањето. Најмила мами, фала ти што си најискрена пријателка во секоја ситуација. Фала ти што неизмерно ме разбираш, што ми покажа како е да бидеш бестрашен во многу ситуации и што ми ја покажа моќта на литературата. Ве сакам неизмерно.

My dearest Nikola, my perfect mix&match, thanks a lot for all the understanding in the world you had for me. Your patience and calmness were the perfect counterbalance to my impulsivity and temper during the PhD. Can't wait to see what other temptations we will have to cross soon.

Violeta

February 2018

Research Institute SHARE

This thesis is published within the Research Institute SHARE (Science in Healthy Ageing and healthcare) of the University Medical Center Groningen/University of Groningen.

Further information regarding the institute and its research can be obtained from our internet site: <http://share.umcg.nl/>

More recent theses can be found in the list below ((co-)supervisors are between brackets).

2017

Zhan Z

Evaluation and analysis of stepped wedge designs; application to colorectal cancer follow-up

(*prof GH de Bock, prof ER van den Heuvel*)

Hoeve Y ten

From student nurse to nurse professional

(*prof PF Roodbol, prof S Castelein, dr GJ Jansen, dr ES Kunnen*)

Ciere Y

Living with chronic headache

(*prof R Sanderman, dr A Visser, dr J Fleer*)

Borkulo CD van

Symptom network models in depression research; from methodological exploration to clinical application

(*prof R Schoevers, prof D Borsboom, dr L Boschloo, dr LJ Waldorp*)

Schans J van der

ADHD and atopic diseases; pharmacoepidemiological studies

(*prof E Hak, prof PJ Hoekstra, dr TW de Vries*)

Berhe DF

Challenges in using cardiovascular medications in Sub-Saharan Africa

(*prof FM Haaijer-Ruskamp, prof K Taxis, dr PGM Mol*)

Heininga VE

The happy, the sad, and the anhedonic; towards understanding altered reward function from a micro-level perspective
(*prof AJ Oldehinkel, dr E Nederhof, dr GH van Roekel*)

Oers AM van

Lifestyle intervention in obese infertile women
(*prof A Hoek, prof BWJ de Mol, dr H Groen*)

Magnée T

Mental health care in general practice in the context of a system reform
(*prof PFM Verhaak, prof FG Schellevis, prof DH de Bakker, dr DP de Beurs*)

Zon SKR van

Socioeconomic inequalities in work and health
(*prof SA Reijneveld, prof U Bültmann*)

Most PJ van der

Development of bioinformatic tools and application of novel statistical methods in genome-wide analysis
(*prof H Snieder, Prof P van der Harst, dr IM Nolte*)

Fleurke-Rozema H

Impact of the 20-week scan
(*prof CM Bilardo, prof E Pajkrt, dr RJM Snijders*)

Schripsema NR

Medical students selection; effects of different admissions processes
(*prof J Cohen-Schotanus, prof JCC Borleffs, dr AM van Trigt*)

Ven HA van de

Shift your work; towards sustainable employability by implementing new shift systems
(*prof JJL van der Klink, prof MP de Looze, prof U Bültmann, prof S Brouwer*)

Hoekstra F

ReSpAct: Rehabilitation, sports and active lifestyle
(*prof LHV van der Woude, prof CP van der Schans, dr R Dekker, dr FJ Hettinga*)

De Carvalho Honorato T

Diminished ovarian reserve and adverse reproductive outcomes
(*prof A Hoek, prof HM Boezen, dr H Groen, dr ML Haadsma*)

Olthof M

Patient characteristics related to health care consumption; towards a differentiated capitation model

(*prof SK Bulstra, prof MY Berger, dr I van den Akker-Scheek, dr M Stevens*)

Bessem B

The young athlete's heart; an electrocardiographic challenge

(*prof J Zwerver, prof MP van den Berg, dr W Nieuwland*)

For more 2017 and earlier theses visit our website

Publication list

1. **Stojanovska V**, Scherjon SA, Plösch T. Preeclampsia As Modulator of Offspring Health. *Biol Reprod* 2016; 94:53–53.
2. Golic M, **Stojanovska V**, Bendix I, Wehner A, Herse F, Haase N, Kräker K, Fischer C, Alenina N, Bader M, Schütte T, Schuchardt M, van der Giet M, Henrich W, Muller DN, Felderhoff-Müser U, Scherjon SA, Plösch T, Dechend R. Diabetes in pregnancy leads to growth restriction and epigenetic modification of the sterol regulatory binding factor 2 gene in rat fetuses. *Hypertension*, In press.
3. Engels G, Hierweger AM, Hoffmann J, Thieme R, Thiele S, Bertram S, Dreier C, Resa-Infante P, Jacobsen H, Thiele K, Alawi M, Indenbirken D, Grundhoff A, Siebels S, Fischer N, **Stojanovska V**, Muzzio D, Jensen F, Karimi K, Mittrucker HW, Arck PC, Gabriel G. Pregnancy-Related Immune Adaptation Promotes the Emergence of Highly Virulent H1N1 Influenza Virus Strains in Allogenically Pregnant Mice. *Cell Host Microbe* 2017; 21:321–333.
4. Kühnel E, Kleff V, **Stojanovska V**, Kaiser S, Waldschütz R, Herse F5, Plösch T, Winterhager E, Gellhaus A. Placental-Specific Overexpression of sFlt-1 Alters Trophoblast Differentiation and Nutrient Transporter Expression in an IUGR Mouse Model. *J Cell Biochem* 2017; 118(6): 1316-1329.

Submitted publications

1. **Stojanovska V**, Dijkstra DJ, Vogtmann R, Gellhaus A, Scherjon SA, Plösch T. A double hit preeclampsia model results in sex-specific growth restriction patterns. Submitted
2. **Stojanovska V**, Sharma N, Dijkstra DJ, Scherjon SA, Jäger A, Schrole H, Plösch T. Placental insufficiency contributes to fatty acid metabolism alterations in aged female mouse offspring. Under revision.
3. **Stojanovska V**, Holwerda KM, van der Graaf AM, Boekschooten MV, Faas MM, Scherjon SA, Plösch T. In utero sFlt-1 exposure differentially affects gene expression patterns in fetal liver. Under revision.

Curriculum vitae

Personal information

Name: Violeta Stojanovska
Date and place of birth: Skopje, Macedonia
Email address: stojanovskavioletaviki@gmail.com

Education

Aug 2013- Apr 2018	PhD Fetal Programming University of Groningen, University Medical Center Groningen
Sep 2011- Aug 2013	Dept of Obstetrics and Gynecology MSc Medical Pharmaceutical Sciences University of Groningen
Oct 2004- Jul 2010	MD General Medicine Ss Cyril and Methodius University, Skopje, Macedonia

Work experience

Aug 2013- Apr 2018	PhD candidate, University Medical Center Groningen Fetal programming in pregnancy-associated disorders
Dec 2012- Jul 2013	Master thesis, University of Groningen Impact of ageing on nuclear integrity in beta pancreatic cells
Jan 2012- Jun 2012	Master thesis, University of Groningen Genomic targets of polycomb genes Cbx7 and Cbx8 in hematopoietic stem cells
Mar 2011- Jul 2011	Medical doctor, Red Cross Macedonia
Aug 2010- Feb 2011	Medical internship, Mother Teresa Clinical Center Skopje, Macedonia
Jul 2010- Aug 2010	Medical internship, St. Anne's University Hospital Brno, Czech Republic

Attended meetings

DOHaD 10th conference (15-18.10.2017, Rotterdam, the Netherlands)

title : A double hit preeclampsia model results in sex-specific growth restriction patterns
type: oral presentation

Experimental biology 2017 (22-26.04.2017, Chicago, USA)

title: High sFlt-1 Concentrations During Pregnancy Modulate the Ppara Promoter Methylation in the Fetal Liver
type: oral and poster presentation

Power of programming (13-15.11.2016, Munich, Germany)

title: Intrauterine growth restriction predisposes to alterations in free fatty acid metabolism and endoplasmic reticulum stress in aged female mice
type: poster presentation

DOHaD 9th conference (08-11.11.2015, Cape Town, South Africa):

title: Aged female mice suffering from IUGR show increased plasma free fatty acids and endoplasmic reticulum stress in adipose tissue
type: poster presentation

Dutch Neuroscience meeting DN2015 (11-12.06.2015, Lunteren, the Netherlands)

title: Adverse in utero conditions and offspring epigenetic status
type: oral presentation

EpiGeneSys, annual meeting (27-29.11.2014, Barcelona, Spain)

title: Maternal diabetes influences the methylation of glucokinase in the offspring
type: oral presentation