Utrecht University

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Project title: **Understanding and suppressing cancer**

Name Principal Investigator: Prof. dr. A. de Bruin
Institute or Department: Cell Biology, Metabolism and Cancer
Research Programme: Regenerative Medicine, Stem Cells and Cancer
Website: http://www.uu.nl/staff/AdeBruin/0

Short description of research area

Our research is centered on exploring the **function of tumor suppressor genes** utilizing single cell genomics and genetic animal models. We have developed unique models to study the function of the Retinoblastoma/E2F signaling pathway, which is altered in almost every type of cancer in animals and humans. A major obstacle in creating novel anticancer drugs is that many tumor suppressor genes are mutated and therefore cannot be reactivated to block cancer. However we discovered novel tumor suppressor genes, the atypical E2Fs, that are very rarely mutated in cancer and therefore represent a novel and promising therapeutic target that can be activated in cancer cells to inhibit cell divisions. To develop novel strategies it is essential to understand how these tumor suppressor genes function and how they are regulated. This knowledge will allow us to develop novel therapy strategies by hyper-activating these tumor suppressor genes in cancer cells.

Furthermore we have developed unique expertise in the pathological analysis of genetic modified animals, and established in 2010 the **Dutch Molecular Pathology Center (DMPC)**, which collaborates worldwide with more than 50 different research groups or industry partners to analyze their transgenic animal models of human diseases. In 2016, we created a novel **Single Cell Analysis Center (SCAC),** which has the unique expertise and innovative equipment to isolate, image, select and analyze single cells for global genomic and transcriptomic alterations. This novel technology platform can be used to identify novel subsets of cell populations in normal or diseased tissues. Moreover it can be used to determine the heterogeneity within tumors or aged tissues.

5 most important publications in the past 3 years

E2f8 mediates tumor suppression in postnatal liver development.
Kent LN, Rakijas JB, Pandit SK, Westendorp B, Chen HZ, Huntington JT, Tang X, Bae S, Srivastava A, Senapati S, Koivisto C, Martin CK, Cuitino MC, Perez M, Clouse JM, Chokshi V, Shinde N, Kladney R, Sun D, Perez-Castro A, Matondo RB, Nantasanti S, Mokry M, Huang K, Machiraju R, Fernandez S, Rosol TJ, Coppola V, Pohar KS, Pipas JM, Schmidt CR, **de Bruin A**, Leone G.
J Clin Invest. 2016 Aug 1;126(8):2955-69.

Synergistic functions of E2F7 and E2F8 are critical to suppress stress-induced skin cancer.
Thurlings I, Martínez-López LM, Westendorp B, Zijp M, Kuiper R, Tooten P, Kent LN, Leone G, Vos HJ, Burgering B, **de Bruin A.**
Oncogene. 2016 Jul 25

Feedback regulation between atypical E2Fs and APC/CCdh1 coordinates cell cycle progression.
Boekhout M, Yuan R, Wondergem AP, Segeren HA, van Liere EA, Awol N, Jansen I, Wolthuis RM, **de Bruin A,** Westendorp B.
EMBO Rep. 2016 Mar;17(3):414-27.

E2F8 is essential for polyploidization in mammalian cells.
Pandit SK, Westendorp B, Nantasanti S, van Liere E, Tooten PC, Cornelissen PW, Toussaint MJ, Lamers WH, **de Bruin A.**
Nat Cell Biol. 2012 Nov;14(11):1181-91

Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice.
Vermeij WP, Dollé ME, Reiling E, Jaarsma D, Payan-Gomez C, Bombardieri CR, Wu H, Roks AJ, Botter SM, van der Eerden BC, Youssef SA, Kuiper RV, Nagarajah B, van Oostrom CT, Brandt RM, Barnhoorn S, Imholz S, Pennings JL, **de Bruin A**, Gyenis Á, Pothof J, Vijg J, van Steeg H, Hoeijmakers JH.
Nature. 2016 Aug 24;537(7620):427-431

Keywords

Cancer, single cell genomics, mouse models, cell cycle, tumor suppressor genes, Rb/E2F/p53

What do we offer?

You will have the opportunity to work on cutting-edge research, network with internationally renowned scientists and enjoy the benefits of joining a supportive researcher community. The aim of the program is to prepare researchers for careers as independent academic scientists. Our lab has already recruited successfully multiple Chinese student with a CSC fellowship.

What are we looking for?

Strong English speaking and writing skills are absolute essentials. Previous laboratory experiences for example in cell culture technique, molecular cloning, PCR, flow cytometry, Western blotting and immunofluorescent staining would be of advantageous, but are not essential for this position.

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Project title: **Understanding and blocking drug resistance in human cancer**

Name Principal Investigator: Dr. Bart Westendorp
Institute or Department: Biomedical Health Sciences
Research Programme: Regenerative Medicine, Stem Cells & Cancer
Email: b.westendorp@uu.nl
Website: https://www.uu.nl/staff/BWestendorp

Short description of research area

In our research we try to understand how human cancer cells can become resistant to targeted inhibitors of cell cycle checkpoints. We have developed single cell RNA-sequencing and live cell imaging techniques to study heterogeneity in drug responses. Our model systems include human-derived cancer cell lines, organoids, and mouse models.
We study various cancers, including muscle-invasive bladder cancer, hepatocellular carcinoma, and large B-cell lymphomas. We have a long-standing interest in understanding the interplay between E2F transcription factors, RAS signaling and P53 signaling in drug-tolerant tumor cells. Ultimately we aim to develop new combination therapies to overcome drug resistance.

Furthermore we have developed unique expertise in the pathological analysis of genetic modified animals, and established in 2010 the **Dutch Molecular Pathology Center (DMPC)**, which collaborates worldwide with more than 50 different research groups or industry partners to analyze their transgenic animal models of human diseases. In 2016, we created a novel **Single Cell Analysis Center (SCAC),** which has the unique expertise and innovative equipment to isolate, image, select and analyze single cells for global genomic and transcriptomic alterations. This novel technology platform can be used to identify novel subsets of cell populations in normal or diseased tissues. Moreover it can be used to determine the heterogeneity within tumors or aged tissues.

5 most important publications in the past 3 years

1. E2F7 is a potent inhibitor of liver tumor growth in adult mice.
Moreno E, Toussaint MJM, van Essen SC, Bongiovanni L, van Liere EA, Koster MH, Yuan R, van Deursen J, **Westendorp B***, de Bruin A.
Hepatology 2020. doi: 10.1002/hep.31259. Online ahead of print.

2. Excessive E2F transcription in single cancer cells precludes transient cell cycle exit after DNA damage.
Segeren HA, van Rijnberk LM, Moreno E, Riemers FM, Yuan R, Wubbolts R, de Bruin A, **Westendorp B***
bioRxiv 2020.03.19.998674; doi: https://doi.org/10.1101/2020.03.19.998674; Cell Reports, revision under review.

3. Cyclin F-dependent degradation of E2F7 is critical for DNA repair and G2-phase progression.
Yuan R, Liu Q, Segeren HA, Yuniati L, Guardavaccaro D, Lebbink RJ, **Westendorp B***, de Bruin A.
EMBO J 2019;38(20):e101430. doi: 10.15252/embj.2018101430.

4. Chk1 and 14-3-3 proteins inhibit atypical E2Fs to prevent a permanent cell cycle arrest.
Yuan R, Vos HR, van Es RM, Chen J, Burgering BM, **Westendorp B***, de Bruin A.
EMBO J 2018;37(5):e97877. doi: 10.15252/embj.201797877.

5. Feedback regulation between atypical E2Fs and APC/C^{Cdh1} coordinates cell cycle progression.
Boekhout M, Yuan R, Wondergem AP, Segeren HA, van Liere EA, Awol N, Jansen I, Wolthuis RM, de Bruin A, **Westendorp B*.** EMBO Rep. 2016;17(3):414-27. doi: 10.15252/embr.201540984.

* corresponding author

Keywords

Cancer, single cell RNA sequencing, mouse models, cell cycle, tumor suppressor genes.

What do we offer?

You will have the opportunity to work on cutting-edge research, network with internationally renowned scientists and enjoy the benefits of joining a supportive researcher community. Our lab is housed in the Hubrecht Institute, with access to excellent facilities. The aim of the PhD program is to prepare researchers for careers as independent academic scientists. You will be enrolled in a course program offered by the Graduate School of Life Sciences. Our lab has already recruited successfully multiple Chinese student with a CSC fellowship.

What are we looking for?

Strong English speaking and writing skills are absolute essentials. The candidate must be a creative and independent thinker with a keen interest in basic cell/molecular biology research. Previous laboratory experience, for example in cell culture techniques, molecular cloning, PCR, flow cytometry, Western blotting, live cell imaging or RNA sequencing would be of advantageous, but are not essential for this position.

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Project title:	**Molecular Host Defence and Anti-infective Drugs**
Name Principal Investigators:	Prof. Henk P. Haagsman / Dr. Edwin J. A. Veldhuizen
Institute or Department:	Biomolecular Health Sciences
Research Programme:	Infection & Immunity
Website:	https://www.uu.nl/staff/EJAVeldhuizen

Short description of research area

Our research group focuses on finding new (alternatives to) antibiotics to combat the current rise in resistance development to conventional antibiotics. A promising approach is to use molecules of the innate immune system, such as antimicrobial (host defense) peptides. These are used as templates to develop new molecules which can either kill or neutralize pathogens directly or are able to stimulate the immune system such that the host can more effectively combat infections. Our current research is dedicated in developing novel anti-infective compounds for either human or veterinary applications. As for human applications, novel antimicrobial peptides ('Pepbiotics') have been developed aimed to treat respiratory infections in patients with cystic fibrosis and other patients. In addition, we study the immunomodulatory potential of antimicrobial peptides aimed to develop novel anti-inflammatory strategies for pulmonary bacterial and viral infections in humans. For veterinary applications, we have successfully demonstrated in chickens that peptides can be used prophylactically to stimulate the immune response towards *E. coli* and *Salmonella* infections. These proofs of concept are now being optimized and new applications for the antimicrobial peptides (also as antiviral agents) are being investigated. Several patents have been filed about antimicrobial host defense peptides.

5 relevant publications from the past 5 years

Mookherjee N, Anderson MA, Haagsman HP, Davidson DJ. Antimicrobial host defence peptides: functions and clinical potential. **Nat Rev Drug Disco. 2020** doi: 10.1038/s41573-019-0058-8.

van Harten RM, van Woudenbergh E, van Dijk A, Haagsman HP. Cathelicidins: Immunomodulatory Antimicrobials. **Vaccines 2018** 6(3). pii: E63.

Peng L, Matthijs MGR, Haagsman HP, Veldhuizen EJA. Avian pathogenic Escherichia coli-induced activation of chicken macrophage HD11 cells. **Dev Comp Immunol 2018**; 87:75-83.

Cuperus T, van Dijk A, Matthijs MG, Veldhuizen EJ, **Haagsman HP** Protective effect of *in ovo* treatment with the chicken cathelicidin analog D-CATH-2 against avian pathogenic E. coli. **Sci Rep 2016**, 6:26622.

Schneider VA, Coorens M, Ordonez SR, Tjeerdsma-van Bokhoven JL, Posthuma G, van Dijk A, Haagsman HP, Veldhuizen EJ. Imaging the antimicrobial mechanism(s) of cathelicidin-2. **Sci Rep 2016**, 6:32948.

Keywords

Innate immunity, host defence peptides, alternatives to antibiotics, antimicrobial resistance, pulmonary infection, inflammation

What do we offer?

We offer an enthusiastic environment in which the student can develop him-/herself into an independent researcher. Our research division has a long history in innate immunity research and is one of the leading groups worldwide in both the antimicrobial peptides and collectin fields. We have experience with foreign PhD students and know what it takes to guide and train students to obtain their PhD within the 4-year period. The student will be part of the Graduate School of Life Sciences at our Faculty of Veterinary Medicine where he/she will be taking topic related courses in for example immunology and microbiology, but also courses to improve English presenting and writing skills. Ongoing collaborations with various renowned research institutes and academical medical centres within The Netherlands and abroad, further contribute to a dynamic and stimulating research environment with many opportunities to address important scientific challenges in the exciting field of innate immunity.

What are we looking for?

We are looking for highly motivated students with an interest and experience in the microbiology / immunology field. The student should have excellent communicational skills in English because communication is essential to perform adequately within a research team. In addition, the student should be eager to learn and be capable to adapt to new environments, both socially and work-related. The ultimate goal is to train the student in such a way that towards the final phase of her/his PhD, he/she will be able to design, perform and present high quality research independently and is capable of writing high-quality scientific manuscripts that will be published in top, peer-reviewed journals.

Current projects

<u>1) Antimicrobial host defense peptides as novel future antibiotics</u>

Effective treatment of severe respiratory infections caused by antibiotic-resistant pathogens becomes increasingly challenging and novel antibacterial therapies are urgently needed. Antimicrobial peptides (AMP) are such a promising alternative therapy to antibiotics that combines broad spectrum antimicrobial- and antibiofilm activity with a strong anti-inflammatory activity. Within the group 'Molecular Host Defence' we have designed novel AMPs, termed 'PepBiotics', that exhibit antimicrobial properties against clinically relevant (multi-resistant) bacterial pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* found in cystic fibrosis related infections. Importantly, 'PepBiotics' do not induce resistance development in these bacteria. This project aims to further develop 'PepBiotics' and extend the activity of these peptides. In a 4-year PhD project, the student will develop and test new and existing peptides for their antibiofilm activity, their antibacterial specificity and elucidate their antimicrobial mechanism of action using microscopic and biochemical techniques. In parallel, the immunomodulatory activities of 'PepBiotics' (for example activation of immune cells and effects on Toll Like Receptor-mediated immune responses) will be determined to discover whether and how they can influence the immune response towards bacterial infections. The

obtained results will contribute to the development of AMP-based compounds into clinically ready antimicrobial candidate drugs (drug development for human medicine).

2) Characterization and modulation of chicken macrophages

Bacterial infections (for example *E. coli* lung infection) of chickens are one of the major problems in the field leading to major economic losses due to mortality or growth reduction of chickens. Immune cells such as macrophages and immune effector molecules such as Host Defence Peptides (HDPs) are the part of the first response in respiratory infections in chickens. Our group has described a wide array of antimicrobial and immune functions for chicken HDPs, however not much is known yet about the phagocytic and immune regulating capacity of chicken macrophages. Even less is known about the ability of chicken macrophages to polarize into a pro-inflammatory M1 state, or anti-inflammatory M2 state, while this information is vital to understand the outcome of a bacterial infections *in vivo*. In a 4-year PhD project, the student will develop a culturing system for M1 and M2-like chicken macrophages and determine polarized macrophage responses towards bacterial infections. In addition, the macrophage responses will be further modulated using chicken HDPs which can both dampen or activate pro-inflammatory responses by the macrophage depending on the specifics of the infection. This knowledge will provide further insight into the chicken innate immune system and can aid to develop immune-based prevention strategies for (respiratory) infections in chicken farming (drug development for veterinary medicine).

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Project title:	**Veterinary and comparative mathematical pharmacology**
Name Principal Investigator:	Ronette Gehring
Institute or Department:	Department of Population Health Sciences
Research Programme:	Veterinary Pharmacotherapeutics and Pharmacy
Website:	https://www.uu.nl/staff/RGehring

Short description of research area

Mathematical pharmacology uses *in silico* mathematical models of biological systems and physiological processes to study the interaction between chemical compounds and the body to discover the relationship between chemical exposure and measured effects and outcomes. This approach has many applications in veterinary, comparative and translational medicine, including the discovery of novel veterinary medicines, the optimization of formulations and dosage regimens, and assessing and managing the risks posed by veterinary medicine use to humans and the environment.

In our research group, we apply different approaches to *in silico* mathematical modelling to answer a broad range of clinically relevant research questions regarding the pharmacological treatment of disease in veterinary patients. We study the pharmacokinetics and pharmacodynamis chemical compounds in animals using a range of approaches from simple non-compartmental analysis of experimental data to compartmental modelling of these data and the development of mechanistic, physiologically-based models. These latter models are used to identify key factors responsible for inter- and intra-species differences in treatment outcomes. Approaches vary depending on the specific research questions and goals.

Whilst we generate some experimental data in our own laboratory, a lot of our work is done in collaboration with other groups at Utrecht University, at other universities, veterinary pharmaceutical companies and regulatory authorities.

5 most important publications in the past 3 years

Dehuisser V, Bosmans T, Devreese M, Gehring R, Croubels S, Duchateau L, Polis I. Alfaxalone total intravenous anaesthesia in dogs: pharmacokinetics, cardiovascular data and recovery characteristics. Vet Anaesth Analg. 2019 Sep;46(5):605-612. doi: 10.1016/j.vaa.2019.04.014. Epub 2019 Jun 8. PubMed PMID: 31395484.

Millecam J, van Bergen T, Schauvliege S, Antonissen G, Martens A, Chiers K, Gehring R, Gasthuys E, Vande Walle J, Croubels S, Devreese M. Developmental Pharmacokinetics and Safety of Ibuprofen and Its Enantiomers in the Conventional Pig as Potential Pediatric Animal Model. Front Pharmacol. 2019 May 9;10:505. doi: 10.3389/fphar.2019.00505. eCollection 2019. PubMed PMID: 31143123; PubMed Central

PMCID: PMC6521589.

Li M, Gehring R, Riviere JE, Lin Z. Probabilistic Physiologically Based Pharmacokinetic Model for Penicillin G in Milk From Dairy Cows Following Intramammary or Intramuscular Administrations. Toxicol Sci. 2018 Jul 1;164(1):85-100. doi: 10.1093/toxsci/kfy067. PubMed PMID: 29945226.
Bon C, Toutain PL, Concordet D, Gehring R, Martin-Jimenez T, Smith J, Pelligand L, Martinez M, Whittem T, Riviere JE, Mochel JP. Mathematical modeling and simulation in animal health. Part III: Using nonlinear mixed-effects to characterize and quantify variability in drug pharmacokinetics. J Vet Pharmacol Ther. 2018 Apr;41(2):171-183. doi: 10.1111/jvp.12473. Epub 2017 Dec 11. Review. PubMed PMID: 29226975.

Lin Z, Gehring R, Mochel JP, Lavé T, Riviere JE. Mathematical modeling and simulation in animal health - Part II: principles, methods, applications, and value of physiologically based pharmacokinetic modeling in veterinary medicine and food safety assessment. J Vet Pharmacol Ther. 2016 Oct;39(5):421-38. doi:

Keywords

veterinary, pharmacology, clinical pharmacology, pharmacotherapeutics, pharmacokinetics, pharmacodynamics, modelling and simulation, mathematical pharmacology

What do we offer?

An inspiring and challenging research environment with a growing research group that is embedded within a collaborative network that has links to the veterinary clinical sciences and basic biomedical sciences, as well as human clinical pharmacology, pharmaceutical sciences and toxicology.

We offer a PhD position that requires publication of findings as papers in peer-reviewed scientific journals, that will form the basis of a PhD thesis.

What are we looking for?

A candidate with a background in biology and physiology with an interest and aptitude for mathematics, statistics and model thinking. The candidate should also have strong computer skills, and be willing to learn new software and programming languages. The ideal candidate will be self-motivated and able to work independently, but should also enjoy working on projects collaboratively in a team.

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

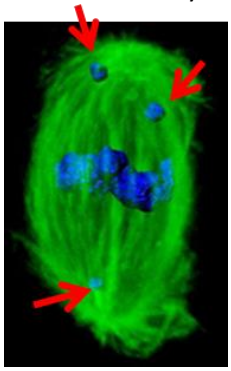
Project title:	**Understanding how advanced maternal age predisposes to chromosome segregation errors in oocytes**
Name Principal Investigator:	M. de Ruijter-Villani
Institute or Department:	Equine Sciences
Research Programme:	Fertility & Reproduction
Website:	https://www.uu.nl/staff/MdeRuijterVillani

Short description of research area

During meiosis, the accurate partitioning of duplicated chromosomes over the daughter cells guarantees genomic integrity. This process is characterized by the formation of a molecular machine consisting of microtubules called 'the spindle', to which the duplicated chromosomes attach via handle-like structures known as the 'kinetochores'. The duplicated chromosomes are held together by cohesion proteins. A 'checkpoint signalling pathway' (the 'spindle assembly checkpoint' or SAC) prevents cohesion breakdown and separation of the duplicated chromosomes until each chromosome is properly attached to the spindle. The spindle then separates the pairs of chromosomes such that each new cell receives one copy of each. This process seems to be particularly prone to errors in oocytes from females of advanced age.

Embryonic aneuploidy of meiotic origin increases markedly in older women and is the leading cause of implantation failure, miscarriage and congenital birth defects. Several hypotheses have been proposed to explain the maternal age-related increase in oocyte aneuploidy, including defective spindle assembly checkpoint and loss of cohesion; however, the mechanisms underlying meiotic segregation errors are still obscure.

While the mouse is a highly tractable model, the infrequency of chromosomal abnormalities in mouse oocytes and embryos renders it suboptimal for studying maternal age-related aneuploidy. Other species, such as the horse, with a time-interval to reproductive senescence more comparable to women, and a comparable increase in the incidence of aneuploidy and early pregnancy loss with maternal age could be more appropriate animal models. Indeed recent results from our group shows that up to 40% of the oocytes of aged mares show chromosome mis-alignment on the metaphase spindle. The aim of the present research project is to understand how advanced maternal age predisposes to chromosome segregation errors in oocytes. In particular we are interested to understand the role of cohesion and different components of the spindle assembly check point in the regulation of the first and second meiotic divisions in oocytes.



Picture: Metaphase II spindle of an oocyte from an aged mare showing mis-aligned chromosomes

5 most important publications in the past 3 years

M. Rizzo, **M. de Ruijter-Villani**, C. Deelen, M. Beitsma, S. Cristarella,M. Quartuccio, T.A.E. Stout Oocytes from aged mares show reduced expression of mRNA for key spindle assembly checkpoint components**. Journal of Equine Veterinary Science** 41:84 June 2016

Gibson C, **de Ruijter-Villani M**, Stout TAE. Negative uterine asynchrony retards early equine conceptus development and upregulation of placental imprinted genes. **Placenta**. 2017 Sep;57:175-182. doi: 10.1016/j.placenta.2017.07.007. Epub 2017 Jul 11.

Cuervo-Arango J, Claes AN, **Ruijter-Villani M,** Stout TA. Likelihood of pregnancy after embryo transfer is reduced in recipient mares with a short preceding oestrus. **Equine Vet J**. 2017 Aug 10.

Swegen A, Grupen CG, Gibb Z, Baker MA, **de Ruijter-Villani M,** Smith ND, Stout TAE, Aitken RJ. From Peptide Masses to Pregnancy Maintenance: A Comprehensive Proteomic Analysis of The Early Equine Embryo Secretome, Blastocoel Fluid, and Capsule. **Proteomics**. 2017 Sep;17(17-18)**.**

de Ruijter-Villani M, Deelen C, Stout TA. Expression of leukaemia inhibitory factor at the conceptus maternal interface during preimplantation development and in the endometrium during the oestrous cycle in the mare. **Reprod Fertil Dev**. 2015 Apr 17.

Keywords

Main research field: Biological sciences / reproductive biology / veterinary medicine
Sub research field: oocyte / meiosis / genetics / aging / medical sciences

What do we offer?

We offer a PhD embedded in the program: “Cancer, Stem Cells & Developmental Biology” (www.csnd.nl). The aim of the programme is to offer research, training and education that builds on novel methodology in genomics, proteomics, metabolomics and bioinformatics technology applied to biomedical processes. The PhD candidate will work in close contact with the equine reproduction clinic, IVF/ICSI lab and will collaborate with other PhD candidates and researchers of the “Fertility and Reproduction” research group (http://www.uu.nl/en/organisation/faculty-of-veterinary-medicine/veterinary-research/research-programmes/fertility-reproduction). Moreover they will have full access to imaging facilities (https://bc-uu.nl/cci/)

What are we looking for?

We are looking for an enthusiastic young scientist or veterinarian with excellent knowledge of the English language and strong writing skills. Previous laboratory experiences for example in cell culture, molecular cloning, PCR, werstern blot and immunofluorescent staining would be advantageous, but are not essential. Prospective PhD candidates should have a MSc degree in life sciences (veterinary medicine, biomedical sciences, biology, chemistry). We expect PhD candidates to be highly motivated, talented and capable of working independently as well as in groups.

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Project title:	**An oviduct-on-a-chip model for equine *in vitro* fertilization and embryo development**
Name Principal Investigator:	Bart M Gadella / Tom AE Stout / Heiko HW Henning
Institute or Department:	Biochemistry & Cell Biology / Equine Sciences / Equine Sciences
Research Programme:	Fertility and Reproduction
Website:	https://www.uu.nl/staff/TAEStout

Short description of research area

Research question and hypothesis

Assisted reproduction techniques play an important economic role in the horse breeding industry worldwide. However, classic *in vitro* fertilization (IVF) is poorly successful in the equine species. Interestingly, when *in vitro* matured oocytes and *in vitro* capacitated sperm are placed in the oviduct of a horse, fertilization does occur. The oviduct is the natural site for fertilization. *In vivo*, oviduct epithelial cells ensure survival of sperm and promote sperm activation i.e. sperm capacitation. Sperm-oviduct interaction induces the development of hyperactive sperm motility, culminating in the release of fertilization competent sperm. Based on earlier observations, we hypothesize that sperm hyperactivation is controlled by endocrine signals at the time of ovulation and that factors secreted from oviduct epithelial cells are required for fertilization, but absent during conventional IVF. This will be tested in a newly developed oviduct cell culture system (oviduct-on-a-chip) because *in situ* observation is no technically feasible.

As an alternative to IVF, intra cytoplasmic sperm injection (ICSI) is used to produce horse embryos. Developmental rates of fertilized oocytes after ICSI are highly variable. As early embryo development also takes place in the oviduct, culture of embryos in an oviduct-on-a-chip may improve developmental rates of fertilized oocytes, and/or embryo quality, after ICSI.

Experimental approach

During primary cell culture, epithelial cells from equine oviducts will be differentiated to an *in vivo*-like appearance. The project will translate cell culture approaches from insert systems to a custom-made perfusable chip system. Chips will be designed and constructed in collaboration with the University of Twente (The Netherlands). By microperfusing the system, hormonal changes during the estrous cycle and around ovulation can be mimicked. The response of the epithelial cells to different culture conditions will be examined by monitoring changes in gene and protein expression, and in cell morphology. Sperm activation and their ability to fertilize *in vitro* will be assessed by live cell imaging of the *in vitro* oviduct system. Effects of co-culture of fertilized oocytes with oviduct cells will be monitored.

Implications of the research

The results may provide molecular insights into factors essential for fertilization and early embryo development in the horse. Homology to other mammalian species including man may exist and provide the basis for improved assisted reproduction procedures.

5 most important publications in the past 3 years

Ferraz MAMM, **Henning HHW, Stout TAE**, Vos PLAM, **Gadella BM.** Designing 3-dimensional in vitro oviduct culture systems to study mammalian fertilization and embryo production. **Annals of Biomedical Engineering (in press)**

Leemans B, **Gadella BM, Stout TA**, De Schauwer C, Nelis H, Hoogewijs M, Van Soom A. 2016. Why doesn't conventional IVF work in the horse? The equine oviduct as a microenvironment for capacitation/fertilization. **Reproduction.** 152:R233-R245.

Leemans B, **Gadella BM, Stout TA**, Nelis H, Hoogewijs M, Van Soom A. 2016. An alkaline follicular fluid fraction induces capacitation and limited release of oviduct epithelium-bound stallion sperm. **Reproduction**. 150:193-208.

Henning HHW, Podico G, **Gadella BM**, **Stout TAE**. 2016. Factors influencing early capacitation responses in equine spermatozoa. **Journal of Equine Veterinary Science** 43, S60.

Leemans B, **Gadella BM**, Sostaric E, Nelis H, **Stout TA,** Hoogewijs M, Van Soom A. 2014. Oviduct binding and elevated environmental pH induce protein tyrosine phosphorylation in stallion spermatozoa. **Biology of Reproduction**. 91:13. doi: 10.1095/biolreprod.113.116418.

Keywords

In vitro fertilization, cell culture, microfluidics, organ-on-a-chip, spermatozoa, oocyte, embryo

What do we offer?

We offer the possibility to enrol for a PhD at the Veterinary Faculty of Utrecht University in a team with members from multiple disciplines (veterinary medicine, biology, chemistry, bioengineering) and nationalities. Different backgrounds of the research team provide the basis for an inspiring work environment for answering clinical questions using basic and applied research. The Veterinary Faculty runs a busy Equine Clinic as well as core facilities for high end microscopy, flow cytometry and lipidomics. The student will receive in depth training in all technical aspects relevant to his/her topic.

What are we looking for?

We are looking for a student with a background in biology, animal reproduction, biomedical science or veterinary medicine. Experience in (primary) cell culture, qRT-PCR and fluorescence microscopy are a plus, but not essential. We expect the student to be able to integrate information from different research fields in his/her experimental approaches. A high level of self-organization and motivation are advantageous. The PhD candidate should be willing to work within a team, but also occasionally independently. Proficiency in written and spoken English is required.

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Project title:	**In vitro ovarian follicle culture**
Name Principal Investigator:	Bernard AJ Roelen
Institute or Department:	Department of Clinical Sciences
Research Programme:	Fertility & Reproduction
Website:	http://www.uu.nl/staff/BAJRoelen/0 http://roelenlab.weebly.com

Short description of research area

- **Problem to be solved**

Girls and young women undergoing cancer treatment generally face fertility problems or premature ovarian failure later in life after survival. Fortunately the success of treatment is increasing, but as a consequence the number of female survivors with fertility problems increases and therefore quality of life decreases. Current options for fertility restoration include oocyte or embryo cryopreservation but these not possible for prepubertal patients. Alternatively, ovarian tissue can be removed and cryopreserved before treatment to be transplanted after treatment but this is still very much an experimental technique and is a rather invasive procedure. In addition there is the risk of reintroducing the cancer with transplantation.

- **Aim of the project idea**

The overall aim is the ex vivo culture of ovarian follicles to be used for oocyte recovery. If we succeed, ovarian samples can be obtained from patients before treatment and cryopreserved. After cure and the women wants to start a family, the ovary can be thawed and in vitro cultured to obtain fertilizable oocytes.
Until so far, very few results have been obtained with in vitro follicle culture. In addition, oocytes from human follicles cannot be fertilized (In the Netherlands not allowed by law). However, the bovine ovary is an excellent model for the human ovary. Bovine ovaries are available in large quantities from slaughterhouses, and bovine oocytes derived from follicles can be fertilized in vitro.

- **What is the novelty?**

Use of bovine ovaries to establish ex vivo follicle development. Since the timing of bovine follicle development is very similar to those of human, the bovine is an excellent model system. As an advantage, bovine ovaries can be routinely obtained as left-over products from slaughterhouses, no experimental animals are needed.

The first preliminary, promising, results have been gathered.

5 most important publications in the past 5 years

Uhde K, van Tol HTA, Stout TAE, **Roelen BAJ**. Metabolomic profiles of bovine cumulus cells and cumulus-oocyte-complex-conditioned medium during maturation in vitro. Sci Rep. 2018 Jun 21;8(1):947

Brinkhof B, van Tol HT, Groot Koerkamp MJ, Wubbolts RW, Haagsman HP, **Roelen BA**. Characterization of bovine embryos cultured under conditions appropriate for sustaining human naïve pluripotency. **PLoS One** 2017;12(2):e0172920.

Aardema H, van Tol HTA, Wubbolts RW, Brouwers JFHM, Gadella BM, **Roelen BAJ**. Stearoyl-CoA desaturase activity in bovine cumulus cells protects the oocyte against saturated fatty acid stress. **Biol Reprod** 2017;96(5):982-992

Brinkhof B, van Tol HT, Groot Koerkamp MJ, Riemers FM, IJzer SG, Mashayekhi K, Haagsman HP, **Roelen BA**. A mRNA landscape of bovine embryos after standard and MAPK-inhibited culture conditions: a comparative analysis. **BMC Genomics** 2015;16:277

Roovers EF, Rosenkranz D, Mahdipour M, Han CT, He N, Chuva de Sousa Lopes SM, van der Westerlaken LA, Zischler H, Butter F, **Roelen BA***, Ketting RF*. Piwi proteins and piRNAs in mammalian oocytes and early embryos. **Cell Rep** 2015;10(12):2069-82

Keywords

Ovary, follicle, oocyte, embryo, in vitro development

What do we offer?

An enthusiastic research group that is currently composed of 2 PhD students and 3 undergraduate students. The lab is well equipped and the location within the university campus provides ample opportunities for collaboration. In addition we have a number of ongoing collaborations with Leiden University Medical Center (Netherlands) and the Institute of Molecular Biology in Mainz (Germany).

What are we looking for?

A PhD candidate that is curious and motivated to do research on ovaries, oocytes and early embryos. Creativity is appreciated.

Utrecht University

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Title	**The impact of the metabolic status of the mother on embryo quality**
Name Principal Investigator:	Dr Hilde Aardema, Dr Peter Vos and Dr Bart Gadella
Institute or Department:	Farm Animal Health
Research Programme:	Fertility & Reproduction
Website:	https://www.uu.nl/staff/HAardema

Short Description of Research Area

Metabolic stress condition, like obesity, diabetes-type II , but also a negative energy balance, are related with reduced fertility in both human and animal. The metabolic condition is reflected at the level of the genital tract and can also affect the quality of the oocyte and embryo. A major characteristic of metabolic stress conditions are elevated levels of free fatty acids (NEFAs) in the circulation, which also results in an increase in the level of NEFAs in the follicular fluid that surrounds the cumulus-oocyte-complex (COC). We demonstrated that in particular saturated NEFAs have a dose-dependent negative impact on the competence of the oocyte to develop into an embryo (Aardema et al., Biol of Reprod, 2011). Interestingly, the relatively high level of mono-unsaturated oleic acid and the surrounding cumulus layer can protect the oocyte against lipotoxicity (Aardema et al., Biol of Reprod, 2011, 2013 and 2017). However, how the metabolic condition of the mother affects the embryo that resides in the oviduct is unknown. Furthermore, studies of the "Dutch Hunger Winter" between 1944-1945 demonstrate that the metabolic condition of the mother during the periconception period had a lifelong impact on the offspring via epigenetic modifications* (Heijmans et al., PNAS, 2008). In the current project we will focus on the interaction between the oviduct environment and embryo during metabolic stress conditions. In our *in vitro* embryo culture laboratory the student will design experiments, based on the metabolic conditions in the oviduct, to unravel the mystery of early life.

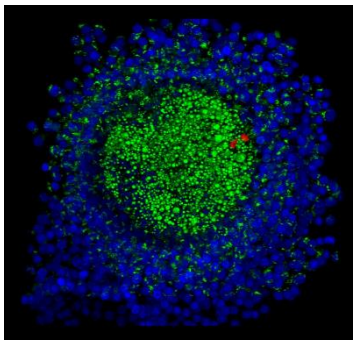
*"Epigenetic changes, such as DNA methylation and histone modification, alter how genes are expressed without altering the underlying DNA sequence"

Keywords

Embryo, oviduct, metabolic stress, NEFA, oocyte

What do we offer

An inspiring research environment at the faculty of Veterinary Medicine in a group of researchers with a focus on oocyte and embryo development. The group is embedded in an international group of collaborators and international contacts. You will be working with state of the art techniques, like bisulfite sequencing for epigenetic analysis, fluorescent confocal microscopy and a 3D-oviduct system (Ferraz et al., Nat Commun, 2018).



Fluorescent detection of the cumulus-oocyte complex. DNA cumulus cells (blue), DNA oocyte (red) and lipid droplets (green).

We offer a full-time position for 4-years at the Reproduction division of the Department of Population Health Sciences, division Farm Animal Health that should result in a PhD thesis. Your thesis will comprise 4-5 published papers, preparing you for a next step in your career!

What are we looking for

An enthusiastic and creative candidate with a MSc degree in biomedical sciences or veterinary sciences, preferably with skills in developmental biology. You are a team player with excellent social and English speaking/writing skills and interested in working in a multidisciplinary research group.

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Utrecht University

Utrecht University Graduate School of Life Sciences
Utrecht – The Netherlands

Title	**Predicting transition diseases in dairy cows using sensor technology**
Name Principal Investigator:	Dr M. Hostens
Institute or Department:	Farm Animal Health
Research Programme:	Applied Veterinary Research
Website:	https://www.uu.nl/staff/MMHostens

Short description of research area

The period in which a dairy cow transfers from a dry cow to a lactating cow, the transition period, is a period with a relatively high incidence of different types of diseases. These diseases can be described as **transition diseases.** This complex of diseases obviously has an impact on animal health, but also leads to suboptimal milk production and impaired welfare (Probo et al., 2018; Hostens et al., 2012). The transition period, defined as the time from 3 weeks before until 3 weeks after calving, is characterized by marked changes in the cow's behavior such as a reduction in feed intake (Huzzey et al., 2012), resulting in a negative energy balance. Commonly acknowledged transition diseases in high yielding dairy cows are hypocalcemia and ketonemia both known to have a strong association with impaired subsequent fertility, udder health, lameness and other diseases.

In the last decade the use of **sensor technology** has been widely introduced in dairy farm management, introducing the possibility to monitor cow behavior such as eating, standing and laying time and intervals. These time periods can be related to important characteristics such as dry matter intake, resting and ruminating time and other important parameters for dairy health. Although there are obviously differences in behavior per parity, farm, milk production level, combining data from sensor technology with other data sources offers the possibility to optimally account for these differences and optimally examine available information.

We hypothesize that cow behavior such as eating time measured in the transition period considering parity and milk production could be used to predict transition disease (hypocalcemia and ketonemia) in dairy cows using novel **data science** techniques such as **machine learning** and **artificial intelligence**.

The aim of the proposed PhD study is to use an existing dataset of the **SenseOfSensor** project (Hut et al., 2019). In that project, 8 Dutch dairy farms with free stall barns in the Netherlands were equipped with the Nedap Smarttag Neck sensor from at least 28d before until at least 28d after calving. Furthermore, in the first two weeks after calving, incidence data of clinical and subclinical diseases were collected, as well as blood samples to measure calcium and β-hydroxy-butyrate levels which are known predictors for hypocalcemia and ketonemia.

The PhD candidate will be challenged to explore the existing big dataset and find the most appropriate algorithms to detect **hypocalcemia** and **ketonemia** and the related diseases using animal behavior recorded through this sensor technology.

The data collected required for this project have already been collected as a part of the SenseOfSensor project of the division of Farm Animal Health of Utrecht University. No additional data collection is required, all data storage and processing infrastructure **are already available now**.

Information

The institute/department at which the project will be carried out is
Department of Population Health Science – division Farm Animal Health

The principal investigator are:
Miel Hostens & Theo Lam

This is a collaborative project with the

- Nedap, Groenlo, the Netherlands
- WUR, Wageningen, the Netherlands
- Vetvice, Bergharen, the Netherlands

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