The handle http://hdl.handle.net/1887/37294 holds various files of this Leiden University dissertation

Author: Melo Bernardo, Ana de
Title: Primordial germ cells and amnion development in the avian embryo
Issue Date: 2016-01-26
Appendix
Primordial germ cells (PGCs) are the progenitors of the gametes, responsible for transmitting genetic information from generation to generation. Although there is a long history of gamete biology research, there is still a lot to be learned about many of the mechanisms underlying germ cell development. This dissertation describes and discusses the dynamics of PGCs in the chicken, with a focus on their migration to the gonads and meiosis that takes place when PGCs are already settled there. We also discussed the advantages of using the avian model in epithelial ovarian cancer research. Moreover, we revisited an often overlooked model for amniogenesis in which the proamnion plays a crucial role.

Chapter 1 presents the state of the literature on PGC development in different model organisms, with a particular focus on the avian model. It also explains the development of extraembryonic membranes.

Chapter 2 explores differences in the specificity of two different markers of germ cells used in chicken, CVH and SSEA1, through different developmental stages. This chapter presents a functional study that shows the role of the anterior vitelline veins as the main vehicles of the migration of PGCs from the extraembryonic circulation into the embryo, where PGCs become established in the gonads at stage HH15.

Chapter 3 describes in detail the asymmetric dynamic of chicken germ cells in the right and left gonads after sexual differentiation. In this chapter it is shown that there is an asymmetric migration of germ cells, which preferentially migrate to the left gonad. Moreover, it is shown that the meiotic development of chicken germ cells is affected by their position in the right or left gonad, cortex versus medulla, and their distribution in the left cortex. Moreover, we suggest that germ cells located in the right gonad are not apoptotic, contrary to what has been suggested before, but able to start their differentiation into oogonia.

Chapter 4 is a review of the use of avian models in ovarian cancer, one of the most lethal types of cancer among women. After an overview of different models used in epithelial ovarian cancer research, the review argues the value of avian models by showing its relevance to the disease compared to other models. The model captures some of the issues that have so far remained unclear in epithelial ovarian cancer, such as its cellular origin and possibilities for drug development.

Chapter 5 is an anatomical study of the role of the proamnion in the development of the anterior amnion fold in chickens. The importance of the proamnion is further demonstrated by two functional essays ex ovo. This chapter provides evidence
that transient sinkage of the head in the proamnion is of paramount importance to the localization of the anterior amnion fold on top of the head and that it is crucial for correct amniogenesis.

Finally, **Chapter 6** is a general discussion linking the results described in the different chapters of this thesis. It presents and discusses future perspectives on the use of avian models in stem cell research, disease and development of germ cells.
SAMENVATTING

Primordiale geslachtscellen (PGCs) zijn de voorgangers van de gameten, verantwoordelijk voor het doorgeven van genetische informatie van generatie op generatie. Hoewel biologisch onderzoek naar gameten een lange geschiedenis kent, is er nog veel te ontdekken over veel van de mechanismen die de ontwikkeling van geslachtscellen sturen. Deze dissertatie beschrijft en bespreekt de dynamiek van PGCs in de kip, met een focus op hun migratie naar de gonaden en de meiose die plaatsvindt wanneer PGCs daar zijn aangeland. We bespreken ook de voordelen van het gebruik van het vogelmodel in onderzoek naar epitheliale eierstokkanker. Tevens hebben wij een model voor amniogenese, vaak over het hoofd gezien, waarin het proamnion een cruciale rol speelt, opnieuw bestudeerd.

**Hoofdstuk 1** zet de staat van de literatuur over de ontwikkeling van PGCs in verschillende modelorganismen uiteen, met een focus op het model van de vogel. Het legt tevens de ontwikkeling van extraembryonische membranen uit.

**Hoofdstuk 2** verkent verschillen in de specificiteit van twee verschillende markers van kiemcellen die in de kip worden gebruikt, CHV en SSEA1, gedurende verschillende ontwikkelingsstadia. Dit hoofdstuk presenteert de resultaten van een functioneel onderzoek dat de rol van de voorste vitelline aderen als de belangrijkste vehikels voor de migratie van PGCs van de extraembryonische circulatie naar het embryo, waar PGCs zich vestigen in de gonaden tijdens stadium HH15.

**Hoofdstuk 3** geeft een gedetailleerde beschrijving van de asymmetrische dynamiek van geslachtscellen van kippen in de linker- en rechtergonade na seksuele differentiatie. In dit hoofdstuk wordt aangetoond dat er een asymmetrische migratie van geslachtscellen plaatsvindt, waarbij deze bij voorkeur naar de linker gonade migreren. Bovendien wordt aangetoond dat de meiotische ontwikkeling van geslachtscellen van de kip wordt beïnvloed door hun positie in de rechter- of linkergonade, cortex of medulla, en hun verdeling in de linkercortex. Tevens suggereren wij dat geslachtscellen in de rechtergonade niet apoptotisch zijn, in tegenstelling tot wat tot nu toe gesuggereerd is, en in staat zijn hun differentiatie in oogonia te starten.

**Hoofdstuk 4** is een overzicht van het gebruik van vogelmodellen in onderzoek naar eierstokkanker, een van de meest dodelijke vormen van kanker onder vrouwen. Na een overzicht van de verschillende modellen die worden gebruikt in onderzoek naar epitheliale eierstokkanker te hebben gegeven, geeft het een betoog voor het belang van vogelmodellen door hun relatieve belang ten opzichte van andere modellen te tonen, ten aanzien van sommige kwesties die tot zover onduidelijk zijn gebleven, zoals de cellulaire
Hoe de oorsprong van epitheliale eierstokkanker en mogelijkheden voor de ontwikkeling van medicijnen.

**Hoofdstuk 5** is een anatomisch onderzoek naar de rol van het proamnion in de ontwikkeling van de voorste plooi van het amnion in de kip. Het belang van het proamnion wordt verder gedemonstreerd door twee functionele essays *ex ovo*. De resultaten in dit hoofdstuk gepresenteerde bewijzen dat een tijdelijke daling van het hoofd in het proamnion van doorslaggevend belang is voor de lokalisatie van de voorste plooi van het amnion bovenop het hoofd en dat het cruciaal is voor correcte amniogenese.

**Hoofdstuk 6**, ten slotte, is een algemene bespreking die de in de verschillende hoofdstukken beschreven resultaten verbindt. Het zet toekomstige perspectieven op het gebruik van vogelmodellen in onderzoek naar stamcellen, ziektes en de ontwikkeling van geslachtscellen.
LIST OF PEER-REVIEWED PUBLICATIONS


List of peer-reviewed publications | 131
ABOUT THE AUTHOR

Ana de Melo Bernardo was born on 27 March 1988 in Covilhã, Portugal. After receiving her high school diploma in 2006, with honors, she started her Bachelor’s studies in Biology at the Faculty of Sciences, University of Lisbon (FCUL). During her BSc she had the opportunity to do lab rotations in different research groups at Instituto Gulbenkian Ciência (Oeiras, Portugal) and Instituto Medicina Molecular (Lisbon, Portugal). In 2008, her last year, Ana was awarded a research grant by Fundação Amadeu Dias/University of Lisbon, allowing her to develop a project on chicken somitogenesis in Dr. Sólveig Thorsteinsdóttir’s group in FCUL, under the supervision of Dr. Gabriela Rodrigues.

In 2009, Ana started her Master’s degree in Evolutionary and Developmental Biology at the Faculty of Sciences, University of Lisbon. In 2010, she represented FCUL at a workshop in developmental biology at Curie Institute - University Pierre Marie Curie, Paris, France, which broadened her experience with animal models in developmental biology. In the same year, Ana was granted an ERASMUS scholarship for her Master’s thesis on chicken germ cells at the Department of Anatomy and Embryology at Leiden University Medical Center (LUMC), in the group of Dr. Susana Chuva de Sousa Lopes. In 2013, she started her PhD training, continuing her work on chicken germ cells at the same group, with Prof. Dr. Christine L. Mummery as her promotor and Dr. Susana Chuva de Sousa Lopes as co-promotor.

Since January 2015, Ana has been working with Dr. Valeria Orlova at LUMC on human-induced pluripotent stem cells for vascular disease modelling.
ACKNOWLEDGEMENTS

I would like to start by thanking Prof. Dr. Christine L. Mummery, my promotor, for her advice and guidance and Dr. Susana Chuva de Sousa Lopes, my co-promotor, for investing her time and resources in this thesis.

Maria, isto é que foi uma aventura, e tu és concerteza a maior testemunha disso. Together with Matthias and Liesbeth, you were my fellows and direct colleagues in the lab; I am very thankful for your scientific but also your non-scientific support. Nannan, David, Kaylee, Heiko, Sara, Michael, Filipe, Cristiana and Nezha, my colleagues for short but very intense periods in this lab. You helped me to make the best of this time.

Richard, Saskia, Bert and Conny were the people in the Department of Anatomy and Embryology from whom I had the opportunity to learn techniques or how to operate software and hardware. I am very thankful for that. Also Daniël and Paul, thank you very much for the IT support. Another person in the department who was fundamental was Atie: thank you for always keeping the door of your office open and for helping me to handle all the paperwork behind this thesis.

Because science also happens in the Department’s corridors, I would like to thank specially to Teya, Kostas, Giorgos, Tim, Marcelo, Sandra, Oleh, Luca, Elisa, Milena, Catarina, Simone and Margot (to name a few): thank you for the coffee-mug-in-hand talks about science but also about life in general.

I would like to thank Valeria for the opportunity to work with her for the last year. It was a pleasure to be part of your research.

The impact waves of this thesis went beyond the Department of Anatomy and Embryology. From the MCB department I would like to thank Annelies and Joop, for instructing me how to handle all those microscopes, and Karoly for his knowledge on cytogenetics. From Belgium, I want to thank Prof. Dr. An Zwijsen and her group in Leuven. It was great to have the opportunity to work with and learn from such a nice team.

Mariateresa, thank you for being my paranymph, for being my lunch fellow at LUMC and most of all for being a very good friend.

Obrigada Gabriela, por me ter despertado o gosto pela investigação, e por, mesmo estando longe, me continuar a acompanhar nestas aventuras durante todos estes anos.

And because as a scientist, science does not leave you even when you are at home, I would like to thank my roomie, Brechtje. Thank you for listening to the after-work
outpourings solved over a glass of wine or a zen yoga morning. Thank you Vincent, for your awesome chicken pictures: they are a perfect match with my thesis!

I would also like to thank the unconditional support from my friends. The ones from my childhood in Covilhã, from the FCUL times, from the LISFE adventures or from the Netherlands and India’s random corners. So many couches around this world where I can always feel at home. Lia e Ricardo, para vocês um muito especial, não só pela amizade mas também pela vossa disponibilidade em acorrer aos meus pedidos de socorro durante o design desta tese.

Gostaria de agradecer aos meus tios, primos, avó Carmita e Pedro: obrigada pelo espírito de família em cada um de vós. I also want to thank Caroline and Willem, my Dutch family, a very important support on the ground.

Aos meus pais, pelo vosso amor incondicional, mas sobretudo por me mostrarem sempre mais uma perspectiva da vida.

Bastiaan, thank you for putting your rigorous academic eye to good use, but most of all thank you so much for all the love and understanding. They were fundamental to me during this period.

A ti, presente em todos os momentos e em especial naqueles de necessária solidão.