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## Appendix



## SUMMARY

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

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 plooï 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 plooï 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

Gomes Fernandes M., Dries R., Roost M., Semrau S., **de Melo Bernardo A.**, Davis R., Ramakrishnan R., Szuhai K., Maas E., Umans L., Albon Escalona V., Salvatori D., Deforce D., Van Criekinge W., Huylebroeck D., Mummery C., Zwijsen A. and Chuva de Sousa Lopes S.M. BMP-SMAD signaling regulates lineage priming, but is dispensable for self-renewal in mouse embryonic stem cells. *Stem Cell Reports*. 2015, *In press*.

**De Melo Bernardo A.**, Thorsteinsdóttir S. and Mummery C.L. Avian as a model for human ovarian cancer: advantages reviewed. *Molecular and Clinical Oncology*. 2015;3: 1191-1198.

**De Melo Bernardo A.**, Heeren A., Anjie S., van Iperen L., Fernandes M., He N., Noce T., Silveira Ramos E. and Chuva de Sousa Lopes S.M. Meiotic wave adds extra asymmetry to the development of female chicken gonads. *Molecular Reproduction and Development*. 2015;9999:1-13.

Heeren A.M., van Iperen L., Klootwijk D., **De Melo Bernardo A.**, Roost M.S., Gomes M., Louwe L., Hilders C., de Graaf R., Helmerhorst F., van der Westerlaken L. and Chuva de Sousa Lopes S.M. High degree of organization marked by the development and dynamics in the 2 follicular basement membrane during human gametogenesis and folliculogenesis. *BMC Developmental Biology*. 2015;15(1):4.

Roost M.S., van Iperen L., **De Melo Bernardo A.**, Mummery C.L., Carlotti F., de Koning E.J.P. and Chuva de Sousa Lopes S.M. Lymphangiogenesis and angiogenesis during human fetal pancreas development. *BMC Vascular cell*. 2014;6:22.

**De Melo Bernardo A.** and Chuva de Sousa Lopes S.M., The involvement of the proamniotic in the development of the anterior amnion fold in the chicken. *PLoS One*. 2014;9(3):e92672.

Caetano L.C., Gennaro F.G.O., Coelho K.C., Araujo F.M., Villa R.A., Oliveira A.A., **De Melo Bernardo A.**, Marcondes C.R., Paz C.C.P., Chuva de Sousa Lopes S.M. and Ramos ES. Differential expression of MHM region and sex-determining-related genes during gonadal development in chicken embryos. *Genet Mol Res*. 2014;13(1):838-49.

**De Melo Bernardo A.**, Sprenkels K., Rodrigues G., Noce T. and Chuva De Sousa Lopes S.M. Chicken primordial germ cells use the anterior vitelline veins to enter the embryonic circulation. *Biology Open*. 2012;1(11):1146-52.





## 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.



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