

**Towards *in vitro* produced germline stem cells in
the bovine**

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BSc (Hons) University of New England, 2009

**A thesis submitted for the degree of Doctor of Philosophy of the University of
New England**

Submitted July 2012

Acknowledgements

Firstly I would like to acknowledge the financial support I have received through an F. D. McMaster PhD Scholarship. I would also like to acknowledge the project funding received through the CSIRO Food Futures National Research Flagship. In addition, I would also like to acknowledge financial support provided by the University of New England and Meat & Livestock Australia, which allowed me to attend an international conference, which was an invaluable experience.

I would like to thank my principal supervisor Dr Sabine Schmoelzl, and my associate supervisors Dr Nick Andronicos and Professor Geoff Hinch, for their constant support, advice and assistance. Without your valuable input completing this thesis would have been an impossible task. Thank you must also go to all my friends, co-workers and fellow students at the CSIRO who have provided friendship, laughter and advice on a daily basis. Although I am happy to be completing this thesis, I will miss seeing you all every day.

I would also like to thank all of my family and friends, for their constant support throughout the last three years. Your encouragement has been particularly appreciated throughout the last few months as I have finished writing this thesis. I know I have been living in my own little world for the last few years, and I am excited about joining you all in the real world again!

Finally, I owe a heartfelt thankyou to Aaron. You have been my rock throughout this whole process. Words aren't enough to express how much your encouragement, support and endless patience mean to me. I couldn't have done it without you.

Abstract

Bovine spermatogonial stem cells (SSCs) have potential to be used in advanced reproductive technologies such as testis cell transplantation, where identification and purification of large numbers of SSCs is required. There are at least two possible sources of SSCs: isolation from the testis, or *in vitro* differentiation from pluripotent stem cells. The long-term goal of this thesis was to work towards the generation of SSCs from bovine somatic cells using induced pluripotent stem (iPS) cell technology. In order to do so, it was first important to characterise molecular markers expressed by bovine SSCs to allow for their identification in culture, and secondly to explore the feasibility of producing bovine iPS cells.

In order to achieve the first goal, a screening platform was developed based on comparative analysis of gene expression levels in SSC enriched and depleted cell populations. Expression of established testis cell markers was used to confirm the validity of the screening platform. This method was then used to examine expression of candidate spermatogonial markers in the bovine testis. STRA8, KIT, GFRA1, CLDN8, DDX6 and NAP1L4 were shown to be putative markers for bovine spermatogonia. Further analysis of CLDN8 showed expression by both a subset of spermatogonia and a subset of Sertoli cells, leading to the hypothesis that CLDN8 plays a role in the maintenance of SSCs in the SSC niche.

Reprogramming of bovine somatic cells was undertaken by introducing canonical reprogramming factors through a lentiviral vector. Initial experiments found that the reprogramming protocol was sufficient to produce cells exhibiting stem cell-like characteristics. Analysis of these cells indicated partial reprogramming had been achieved. A number of small molecules were then tested for their ability to enhance the success of cell reprogramming. A combination of three small molecules was found to accelerate the kinetics

of the reprogramming process and also promoted further reprogramming where cells could differentiate to the three different germ layers. Further research is now required to define the optimal culture conditions for the maintenance and expansion of bovine pluripotent cells in long term culture, and to test whether they can be differentiated towards the germ line.

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List of Abbreviations

- BDF: Bovine dermal fibroblast
- BEF: Bovine embryonic fibroblast
- bFGF: basic fibroblast growth factor
- BMP: Bone morphogenetic protein
- BTB: Blood-testis barrier
- DBA: *Dolichos biflorus* agglutinin
- DMEM: Dulbecco's Modified Eagle Medium
- Dox: Doxycycline
- DPBS: Dulbecco's phosphate buffered saline
- EB: Embryoid body
- ESC: Embryonic stem cell
- FACS: Fluorescent activated cell sorting
- FBS: Foetal bovine serum
- GFP: Green fluorescent protein
- HDAC: Histone deacetylase
- iPSC: Induced pluripotent stem cell
- LIF: Leukemia inhibitory factor
- MACS: Magnetic activated cell sorting
- MDF: Modified Davidsons Fixative
- MEF: Mouse embryonic fibroblast
- MET: Mesenchymal-to-epithelial transition
- NEAA: Non-essential amino acids
- OSKM: POU5F1 (OCT4); SOX2; KLF4; c-MYC
- PCR: Polymerase chain reaction
- PGC: Primordial germ cell
- RA: Retinoic acid
- RT-PCR: Reverse transcribed polymerase chain reaction

SEM: Standard error margin

SCF: Stem cell factor

SSC: Spermatogonial stem cell

TBS: Tris buffered saline

Tet: Tetracycline

Gene Synonyms

<u>Gene Symbol</u>	<u>Synonym</u>	<u>Gene Name</u>
<i>AFP</i>		<i>Alpha-fetoprotein</i>
<i>ALPL</i>		<i>Alkaline phosphatase</i>
<i>ASB9</i>		<i>Ankyrin repeat and SOCS box containing 9</i>
<i>ATIC</i>		<i>5-aminoimidazole-4-carboxamide ribonucleotide methyltransferase/ IMP cyclohydrolase</i>
<i>BCL6B</i>		<i>B-cell CLL/lymphoma 6 member B</i>
<i>BMP4</i>		<i>Bone morphogenetic protein 4</i>
<i>CLDN8</i>		<i>Claudin-8</i>
<i>c-MYC</i>		<i>v-myc avian myelocytomatosis viral oncogene homolog</i>
<i>CSF1R</i>		<i>Colony stimulating factor 1 receptor</i>
<i>DDX4</i>	VASA	<i>DEAD (Asp-Glu-Ala-Asp) box polypeptide 4</i>
<i>DDX6</i>		<i>DEAD (Asp-Glu-Ala-Asp) box polypeptide 6</i>
<i>DES</i>		<i>Desmin</i>
<i>FOXA2</i>		<i>Forkhead box A2</i>
<i>GATA4</i>		<i>GATA binding protein 4</i>
<i>GFRA1</i>		<i>GDNF family receptor alpha 1</i>
<i>KIT</i>	<i>c-KIT</i>	<i>v-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog</i>
<i>KLF4</i>		<i>Kruppel-like factor 4</i>
<i>MTHFD1</i>		<i>Methylenetetrahydrofolate dehydrogenase 1</i>
<i>NANOG</i>		<i>Nanog homeobox</i>
<i>NAP1L4</i>		<i>Nucleosome assembly protein 1-like-4</i>
<i>NES</i>		<i>Nestin</i>
<i>PARK7</i>	<i>DJI</i>	<i>Parkinson protein 7</i>
<i>PFN1</i>		<i>Profilin 1</i>
<i>PHGDH</i>		<i>Phosphoglycerate dehydrogenase</i>
<i>POU5F1</i>	<i>OCT4</i>	<i>POU class 5 homeobox 1</i>
<i>PRDX1</i>	<i>PRX1</i>	<i>Peroxiredoxin 1</i>
<i>REX1</i>		<i>RNA exonuclease 1 homolog</i>
<i>SEPT7</i>	<i>CDC10</i>	<i>Septin 7</i>
<i>SOX2</i>		<i>SRY (Sex determining region Y) box 2</i>
<i>STRA8</i>		<i>Stimulated by retinoic acid gene 8</i>
<i>THY1</i>		<i>Thy-1 cell surface antigen</i>
<i>TKTL1</i>		<i>Transketolase-like 1</i>
<i>TUBB3</i>		<i>Tubulin, beta 3 class III</i>
<i>UCHL1</i>	<i>PGP9.5</i>	<i>Ubiquitin carboxyl-terminal esterase L1</i>
<i>ZBTB16</i>	<i>PLZF</i>	<i>Zinc finger and BTB domain containing 16</i>