

Investigations of Methods for Non-Viral Generation of Bovine Cells to Pluripotency, With a View on Potential Use in Reproductive Technologies

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Abstract

Spermatogonial stem cells (SSCs) from livestock species have the potential to be used for reproductive technologies and for the production of transgenic animals. The isolation of a relatively pure population of SSCs from livestock species has proven difficult; however, the recent advances in the production of induced pluripotent stem (iPS) cells may provide an alternative source of SSCs through the differentiation of iPS cells toward the germline.

The general aim of this thesis was to produce bovine iPS cells that may be differentiated toward the germ line for potential use in reproductive technologies such as germ cell transplantation. Due to difficulties in isolating a pure population of bovine SSCs, methods to improve the enrichment of these cells are of interest in order to improve the success of bovine germ cell transplantation. A number of different methods to enrich bovine spermatogonia were trialled to determine which of the method for enrichment was most effective. The combination of enrichment by differential plating followed by separation of cells by discontinuous Percoll gradient centrifugation, was found to isolate the most enriched population of un-differentiated spermatogonia.

Additionally, the ability to better identify and characterise bovine testis cells, specifically SSCs, by the identification of new markers that identify SSCs or different sub-sets of spermatogonia in the bovine testis, would also contribute to knowledge of characterisation of the cells and may provide better methods for enrichment. Towards the first goal, the potential markers for bovine SSCs previously identified by a global proteomic screen and comparative analysis of gene expression levels in SSC enriched and depleted cell populations, DDX6, NAP1L4 and TKTL1, were investigated by gene expression analysis and immunohistochemistry. The three markers were shown to identify different stages of spermatogenesis.

Examining methods to produce bovine iPS cells, different combinations of reprogramming factors resulted in differences in reprogramming efficiency as expected. Encouragingly, bovine dermal fibroblasts showed comparable reprogramming potential to bovine embryonic fibroblasts. Non-viral methods of transformation proved to be an effective alternative to viral transduction, with a commercially available non-integrating minicircle system providing particularly promising results. A systematic investigation of the effects of small molecules on reprogramming events identified combinations with significant effects on reprogramming efficiency and pluripotency gene activation. Though the cells described in this thesis are not considered to be fully reprogrammed, they are described as being putative iPS cells, referring to their characteristics being iPS cell 'like'.

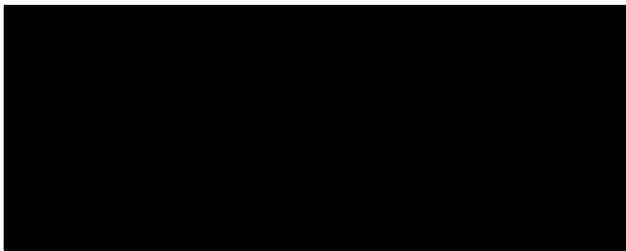
Abstract

In vitro differentiation of the generated putative iPS cells lead to expression of some markers expressed by the germline which is a promising result for future research.

Candidate's Certification

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



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Table of Contents

Acknowledgements.....	II
Abstract.....	III
Candidate’s Certification.....	V
Table of Contents.....	VI
List of Figures.....	XI
List of Tables.....	XX
List of Abbreviations.....	XXI
Gene Synonyms.....	XXIII
Chapter 1 : Literature review.....	1
1.1 Introduction to Stem Cells.....	1
1.2 Pluripotent Stem Cells.....	1
1.2.1 Embryonic stem cells.....	3
1.2.2 Nuclear Reprogramming of Somatic Cells to Pluripotency.....	4
1.2.3 Reprogramming Factors.....	8
1.2.4 Factors affecting iPS cell generation.....	10
1.2.5 Methods for Factor Delivery.....	14
1.2.6 Signalling Pathways in Reprogramming.....	19
1.2.7 Enhancing Pluripotency by Pathway Targeting.....	22
1.2.8 Evaluation of iPS Cells.....	24
1.2.9 Induced Pluripotent Stem Cells from Livestock Species.....	28
1.3 Stem Cells from Adult Tissue.....	30
1.3.1 Spermatogonial Stem Cells.....	31
1.3.2 Characterisation and Molecular Markers of Male Germ Cells.....	33
1.3.3 Spermatogonial Stem Cell Transplant.....	35
1.3.4 Enrichment of Spermatogonial Stem Cells.....	38
1.4 Germ Cells from Pluripotent Stem cells.....	41
1.5 Conclusions.....	43
1.6 Aims.....	44
Chapter 2 : Enrichment of Bovine Spermatogonia and Spermatogonial Stem Cells.....	46
2.1 Introduction.....	46
2.2 Materials and Methods.....	52
2.2.1 General.....	52

List of Figures

2.2.2 Testis Collection and Enzymatic Isolation of Testis Cells	52
2.2.3 Differential Plating Enrichment	53
2.2.4 Discontinuous Percoll Gradient Enrichment.....	53
2.2.5 Immunocytochemistry.....	55
2.2.6 Flow Cytometry Analysis.....	55
2.2.7 MACS Enrichment of Undifferentiated Spermatogonia in the Presence of C-CPE.....	56
2.2.8 Data Analysis.....	57
2.3 Results.....	58
2.3.1 Enrichment of Bovine Spermatogonia by Percoll Discontinuous Density Gradient Centrifugation Further Enhances Enrichment of Differentially Plated Cells	58
2.3.2 Treatment of Bovine Testis Cell Populations with C-CPE _{290.319} Does Not Result in Enhanced Enrichment of Spermatogonia by MACS Isolation.....	61
2.4 Discussion.....	62
2.5 Conclusion.....	67
Chapter 3 : Characterisation of Putative Spermatogonial Markers.....	69
3.1 Introduction	69
3.2 Materials and Methods.....	73
3.2.1 General.....	73
3.2.2 Collection of samples and Enzymatic Isolation of Testis Cells	74
3.2.3 Differential Plating for Enrichment.....	74
3.2.4 RNA Extraction and cDNA Production	75
3.2.5 qRT PCR.....	75
3.2.6 Immunohistochemistry of Testis Sections.....	76
3.2.7 Immunocytochemistry of Cell Smears	77
3.2.8 Data Analysis.....	77
3.3 Results.....	79
3.3.1 Differential plating results in enrichment of bovine spermatogonia.	79
3.3.2 Putative bovine spermatogonia markers <i>DDX6</i> , <i>NAP1L4</i> and <i>TKTL1</i> are expressed in spermatogonia enriched fraction of bovine testis isolations.	80
3.3.3 <i>DDX6</i> , <i>NAP1L4</i> and <i>TKTL1</i> are Expressed by Different Subsets of Spermatogonia in Pre-Pubertal, Pubertal and Post-Pubertal Bovine Testis.....	81
3.3.3 Quantification of Expression of <i>DDX6</i> , <i>NAP1L4</i> and <i>TKTL1</i> in Pre-Pubertal, Pubertal and Post- Pubertal Bovine Testis	92
3.4 Discussion.....	97
3.5 Conclusion.....	103
Chapter 4 : Comparison of reprogramming factor combinations in producing Bovine iPS cells.....	105
4.1 Introduction	105

List of Figures

4.2 Materials and Methods.....	114
4.2.1 General.....	114
4.2.2 Derivation of Bovine Fibroblasts.....	114
4.2.3 Plasmid Preparation.....	115
4.2.4 Lentiviral Production.....	116
4.2.5 Lentiviral Infection of Fibroblasts	116
4.2.6 iPS Cell Culture	117
4.2.7 Analysis of Putative iPS Colonies	119
4.2.8 Data Analysis.....	121
4.3 Results.....	124
4.3.1 Lentiviral Transduction Results in Gene Expression of Bovine Fibroblasts	124
4.3.2 Different Combinations of Transcription Factors have no Effect on Colony Formation Kinetics but do have an Effect on Reprogramming Efficiency.....	125
4.3.3. Reprogramming with a Different Combination of Transcription Factors has an Effect on Colony Morphology	127
4.3.4 Different Combinations of Transcription Factors have Cell-Type Dependent Effects on Pluripotency Gene Induction	129
4.4 Discussion.....	131
4.5 Conclusion.....	141
Chapter 5 : Comparison of Viral and Non-Viral reprogramming of Bovine Fibroblasts to Pluripotency	142
5.1 Introduction	142
5.2 Materials and Methods.....	150
5.2.1 General.....	150
5.2.2 Derivation of Bovine Fibroblasts.....	150
5.2.3 Production of bovine iPS cells.....	151
5.2.4 Embryoid Body (EB) Production.....	153
5.2.5 Analysis of putative bovine iPS colonies.....	154
5.2.6 mRNA Extraction and qRT-PCR analysis.....	155
5.2.7 Statistical Analysis.....	155
5.3 Results.....	158
5.3.1 Electroporation and Lentiviral Transduction results in Gene Expression by Bovine Fibroblasts.....	158
5.3.2 Non-viral Reprogramming Increases Reprogramming Kinetics Compared to Lentiviral Reprogramming	159
5.3.2 Reprogramming Efficiency is not Effected by the use of Non-Viral or Lentiviral Reprogramming, but is Influenced by Cell Type and Choice of Transcription Factor Combination	160

List of Figures

5.3.3 Reprogramming Method Influences iPS Colony Morphology	162
5.3.4 Lentiviral Transduction Results in Transgene Integration into the Host Genome, While Non-Viral Electroporation does not.....	164
5.3.5 Expression of Pluripotency Markers Differs Between Lentiviral and Non-Viral iPS Cultures	165
5.3.6 Some EBs Derived from Putative Non-Viral Bovine iPS Colonies Express Markers from all Three Germ Lineages	168
5.4 Discussion.....	170
5.5 Conclusion.....	176
Chapter 6 : Effect of Small Molecules on Bovine iPS production	178
6.1 Introduction	178
6.2 Materials and Methods.....	183
6.2.1 General.....	183
6.2.2 Derivation of Bovine Fibroblasts.....	184
6.2.3 Non-Viral Reprogramming of Fibroblasts	184
6.2.5 Embryoid Body (EB) Production.....	185
6.2.6 Analysis of Putative Bovine iPS Colonies	185
6.2.6 mRNA Extraction and qRT-PCR analysis.....	186
6.2.7 Statistical Analysis.....	186
6.3 Results.....	189
Experiment 1- The Effect of the addition of VPA to small molecule cocktail NaB-SB-PD.	189
6.3.1 Valproic Acid Accelerates Reprogramming Kinetics and Improves Reprogramming Efficiency.....	189
6.3.2. Colony Morphology is Improved by the Addition of Valproic Acid	193
6.3.3. Putative iPS Colonies Express Alkaline Phosphatase Activity when Supplemented with Valproic Acid	194
6.3.4. VPA Supplementation does not Consistently Increase the Expression of Pluripotency Markers.....	195
6.3.5. Embryoid Bodies Express Makers from all Three Germ Lineages.	199
Experiment 2- Effect of Individual and Combinations of Small Molecules on Bovine iPS Cell Generation	203
6.3.6 Supplementation of Different Combinations of Small Molecule Influences Reprogramming Kinetics and Efficiency	204
6.3.7 Supplementation of Different Combinations of Small Molecules Effects Colony Morphology	206
6.3.8 The Combination of Four Small Molecules VPA-NaB-SB-PD, Consistently Results in Increased Expression of Pluripotency Markers	208
6.3.8 Embryoid Bodies Derived from iPS Colonies Supplemented with Four Small Molecules VPA-NaB-SB-PD, Expressed Markers from all Three Germ Lineages.....	211

List of Figures

6.4 Discussion	214
6.5 Conclusion.....	223
Chapter 7 : Production of Bovine iPS Cells by Non-Viral Minicircle Transfection.....	225
7.1 Introduction	225
7.2 Materials and Methods.....	232
7.2.1 General.....	232
7.2.2 Derivation of Bovine Fibroblasts.....	233
7.2.3 Minicircle and Plasmid Transfection of Bovine Fibroblasts	233
7.2.4 Analysis of Putative Bovine iPS Colonies	235
7.2.5 Embryoid Body (EB) Production and Differentiation of putative iPS Cells Toward the Germline	235
7.2.6 mRNA Extraction and qRT-PCR Analysis	236
7.2.7 Statistical Analysis.....	236
7.3 Results.....	238
7.3.1 Minicircle Vectors are Expressed in Bovine Fibroblasts Following Transfection.....	238
7.3.2 Minicircle Vector Transfection Improves Reprogramming Efficiency	239
7.3.3 Minicircle and Episomal Plasmid Derived Putative Bovine iPS Colonies Express Alkaline Phosphatase.....	240
7.3.4 Minicircle and OSNL Episomal Plasmid Derived Putative iPS Colonies Express Pluripotency Markers.....	242
7.3.5 Minicircle and Episomal Plasmid Derived Embryoid Bodies Express Markers from all Three Germ Lineages	244
7.3.6 Spontaneously Differentiating iPS Colonies Express Markers from all Three Germ Lineages	250
7.4 Discussion.....	251
7.5 Conclusion.....	259
Chapter 8 : General Discussion and Conclusions	260
8.2 Conclusions	269
References	271

List of Figures

Figure 1-1. Three methods used for nuclear reprogramming of somatic cells to a pluripotent state, nuclear transfer (SCNT), fusion of somatic and embryonic stem cells, and reprogramming by defined factors (Yamanaka, Shinya & Blau, 2010).	5
Figure 1-2. Signalling pathways and the influence of small molecules and growth factors on promoting pathways involved in reprogramming (Sumer et al., 2010).	24
Figure 1-3: Basic schematic of A_s model of spermatogenesis.	32
Figure 1-4: Molecular markers expressed by human stem cells and germ cells at different stages of differentiation, established by <i>in vitro</i> and <i>in vivo</i> studies. The gene name is indicated on the left of the arrow, with the temporal expression of the gene indicated by the length of the arrow (Figure adapted from (Roode et al., 2012; Schuh-Huerta & Pera, 2011).	34
Figure 1-5. Schematic representation of the process of germ cell transplantation in cattle (Hill & Dobrinski, 2006).	37
Figure 2-1. Diagram illustrating the preparation of Percoll discontinuous density gradient used in enrichment experiments for bovine testis cells.	54
Figure 2-2. Examples of cell smears from testis cell isolates. a, b) No primary antibody, smears stained with secondary antibodies for mouse and rabbit IgG respectively (controls). c) Smear showing specific staining for DBA-HRT, brown cells circled cells exhibit DBA binding activity (undifferentiated spermatogonia). d) Smear showing specific staining for GATA4, brown cells positively express GATA4 (Sertoli cells). Scale bars represent 50µm.	58
Figure 2-3. a) Percent (%) of DBA positive cells observed in initial isolate, and MACS bound and unbound fractions of testis cells treated with C-CPE (C-CPE ₂₉₀₋₃₁₉) control peptide (C-CPE _{ALA}) or no peptide treatment. b) Percent (%) of propidium iodine (PI) positive cells observed in initial isolate, and MACS bound and unbound fractions of testis cells treated with C-CPE (C-CPE ₂₉₀₋₃₁₉) control peptide (C-CPE _{ALA}) or no peptide treatment. Different characters above treatment means indicate a significant difference in DBA or PI positive cells between treatments (n=4, P<0.05). Error bars represent ± SEM. c) Table showing fold change in undifferentiated spermatogonia as determined by flow cytometry analysis (% DBA positive cells) and cell death (% PI positive cells) compared to initial isolate cells with no peptide treatment or treatment with control or C-CPE ₂₉₀₋₃₁₉ peptides following MACS separation.....	62
Figure 3-1. Expression of known spermatogonia markers a) <i>DDX4</i> (<i>VASA</i>), b) <i>UCHL1</i> (<i>PGP9.5</i>) and c) <i>ZBTB16</i> (<i>Plzf</i>), in adherent and non-adherent populations of differentially plated bovine testis cells relative to housekeeper gene <i>RPS26</i> . * indicates a significant difference in the mean expression of adherent and non-adherent populations of testis cells. (P<0.05 n=7) Error bars represent ± SEM. ...	79
Figure 3-2. Expression of known Sertoli cell markers a) <i>GATA4</i> and b) <i>Vimentin</i> , in adherent and non-adherent populations of differentially plated bovine testis cells, relative to housekeeper gene <i>RPS26</i> . * indicates a significant difference in the mean expression of adherent and non-adherent populations of testis cells (P<0.05 n=7). Error bars represent ± SEM.	80
Figure 3-3. Expression of potential markers for bovine spermatogonia, a) <i>FSCN1</i> , b) <i>IQGAP1</i> , c) <i>TNL1</i> , d) <i>DDX6</i> , e) <i>NAP1L4</i> , and f) <i>TKTL1</i> , in adherent and non-adherent populations of differentially plated bovine testis cells, relative to housekeeper gene <i>RPS26</i> . * indicates a significant difference in the mean expression of adherent and non-adherent populations of testis cells. (P<0.05 n=7). Error bars represent ± SEM.....	81

Figure 3-4. Brightfield images of bovine pre-pubertal testis sections stained for antibodies against putative spermatogonia markers a) No primary antibody (control). b) DDX6 staining of large cells located in the centre of the tubule. c) NAP1L4 staining of large cells located in the centre of the tubule, migrating toward the basement membrane. d) TKTL1 staining of large cells located in the centre of the tubule, migrating toward the basement membrane. Scale bars represent 50 μm 82

Figure 3-5. Immunohistochemistry control sections for background staining of (a-d) pre-pubertal, (e-h) pubertal, and (i-l) post-pubertal bovine testis. No primary antibody applied, secondary mouse and rabbit antibodies applied. Scale bars represent 50 μm 83

Figure 3-6. Expression of DBA-Biotin, Vimentin and putative markers DDX6, NAP1L4 and TKTL1 in bovine pre-pubertal testis sections. (A, E, I) Merge images of tissue stained with Vimentin, candidate marker (NAP1L4, DDX6 or TKTL1) and DBA-Biotin. (C) Tissue stained with DDX6, (G) Tissue stained with NAP1L4, and (K) tissue stained with TKTLI. (B, F, J) Tissue stained with Vimentin. (D, H, L) Tissue stained with DBA-Biotin. (C, D) Co-staining of DDX6 and DBA-Biotin. (G, H) Co-staining of NAP1L4 with DBA-Biotin. (K, L) Co-staining of TKTLI with DBA-biotin. Scale bars represent 50 μm 84

Figure 3-7. Expression of DBA, ZBTB16 and putative markers in bovine pre-pubertal testis sections. (A, E, I) Merge images of tissue stained with ZBTB16, candidate marker (NAP1L4, DDX6 or TKTLI), and DBA-Biotin. (C) Tissue stained with DDX6, (G) NAP1L4, and (K) tissue stained with TKTLI. (B, F, J) Tissue stained with ZBTB16. (D, H, L) Tissue stained with DBA-Biotin. (A, B, C) Co-staining of DDX6 and ZBTB16 shown by circled cells. (E) Co-staining of NAP1L4 with ZBTB16 shown by circled cells. (I) Co-staining of TKTLI with DBA-biotin shown by circled cells. Scale bars represent 200 μm 85

Figure 3-8. Brightfield images of bovine pubertal testis sections stained for antibodies against putative spermatogonia markers. a) No primary antibody (control) .b) DDX6 weak staining of cells on the basement membrane. c) NAP1L4 staining of cells located on the basement membrane and expression pattern toward the centre of the lumen. d) TKTL1 staining of cells one cell layer removed from the basement membrane. Scale bars represent 50 μm 86

Figure 3-9. DBA-Biotin, Vimentin and putative markers DDX6, NAP1L4 and TKTL1 in bovine pubertal testis sections. (A, E, I) Merge images of tissue stained with Vimentin, candidate marker (NAP1L4, DDX6 or TKTLI), DBA-Biotin. (C) Tissue stained with DDX6, (G) Tissue stained with NAP1L4, (K) tissue stained with TKTLI, arrow shows cells located above basement membrane. (B, F, J) Tissue stained with Vimentin. (D, H, L) Tissue stained with DBA-Biotin. (C, D) Co-staining of DDX6 and DBA-Biotin shown by circled cells. (G, H) Co-staining of NAP1L4 with DBA-Biotin shown by circled cells. Scale bars represent 50 μm 87

Figure 3-10. Expression of DBA, ZBTB16 and putative markers in bovine pubertal testis sections. (A, E, I) Merge images of tissue stained with ZBTB16, candidate marker (NAP1L4, DDX6 or TKTLI), and DBA-Biotin. (C) Tissue stained with DDX6, (G) NAP1L4, and (K) tissue stained with TKTLI. (B, F, J) Tissue stained with ZBTB16. (D, H, L) Tissue stained with DBA-Biotin. (A, B, C) Co-staining of DDX6 and ZBTB16 shown by circled cells. (F, G) Co-staining of NAP1L4 with ZBTB16 shown by circled cells. (I, J, L) Co-staining of ZBTB16 and DBA shown by circled cells. (K) TKTLI cells appear to be one cell layer removed from the basement membrane as indicated by the arrow. Scale bars represent 200 μm 88

Figure 3-11. Brightfield images of post-pubertal testis sections stained for antibodies against putative spermatogonia markers. a) No primary antibody (control). b) DDX6 staining of cells located on the basement membrane. c) NAP1L4 staining of cells located on the basement membrane and

List of Figures

expression pattern toward the centre of the lumen. d) TKTL1 staining of cells one cell layer removed from the basement membrane. Scale bars represent 50 μm 89

Figure 3-12. Expression of DBA-Biotin, Vimentin and putative markers DDX6, NAP1L4 and TKTL1 in bovine post-pubertal testis sections. (A, E, I) Merge images of tissue stained with Vimentin, candidate marker (DDX6, NAP1L4 or TKTLI), DBA-Biotin. (C) Tissue stained with DDX6, (G) Tissue stained with NAP1L4, (K) tissue stained with TKTLI, arrow shows cells located above basement membrane. (B, F, J) Tissue stained with Vimentin. (D, H, L) Tissue stained with DBA-Biotin. (C, D) Co-staining of DDX6 and DBA-Biotin, shown by circled cells. (G, H) Co-staining of NAP1L4 with DBA-Biotin, shown by circled cells. Scale bars represent 50 μm 90

Figure 3-13. Expression of DDX6, ZBTB16 and putative spermatogonia markers bovine adult testis tissue. (A, E, I) Merge images of tissue stained with ZBTB16, candidate marker (NAPIL4, DDX6 or TKTLI) and DBA-Biotin. (C) Tissue stained with DDX6, (G) Tissue stained with NAP1L4, (K) tissue stained with TKTLI, arrow shows cells located above basement membrane. (B, F, J) Tissue stained with ZBTB16. (D, H, L) Tissue stained with DBA-Biotin. (A) Co-staining of DDX6 and DBA-Biotin, no co-staining with ZBTB16 was observed (E) Co-staining of NAP1L4 with DBA-Biotin and ZBTB16, shown by circled cells. (I) Co-staining of ZBTB16 and DBA shown by circled cells. (K) TKTL1 positive cells do not co-stain with DBA-biotin or ZBTB16 and appear to be one cell layer removed from the basement membrane as shown by the arrow. Scale bars represent 200 μm 91

Figure 3-14. IgG controls of bovine testis cell smears from a) Pre-Pubertal and b) Pubertal animals, stained with mouse and rabbit IgG. No cells are stained positive, indicating no non-specific staining occurred. Scale bars represent 50 μm 92

Figure 3-15. Samples of smear photographs of Pre-Pubertal, Pubertal and Post-Pubertal bovine testis stained with a) DDX6, b) NAP1L4 and c) TKTL1. Cells that are stained brown indicate cells expressing the marker of interest. Scale bars represent 50 μm 93

Figure 3-16. Samples of smear photographs of Pre-Pubertal, Pubertal and Post-Pubertal bovine testis stained with a) DDX4, b) DBA-HRT and c) GATA4. Cells that are stained brown indicate cells expressing the marker of interest. d, e) Staining for GATA4 positive cells was weak, circled cells indicate cells staining positively for GATA4. Scale bars represent 50 μm 94

Figure 3-17. a) Proportion of positively stained cells for pre-pubertal and pubertal testis cell smears stained with putative markers DDX6, NAP1L4 and TKTL1, known spermatogonia markers DDX4 (VASA), and DBA-HRT, and Sertoli cell marker GATA4. Different characters above treatment means indicates a significant difference in the percentage of cells expressing markers ($P < 0.05$, $n = 4$, Errors bars represent \pm SEM). b) Table shows percent positively stained cells of individual animals in pre-pubertal and pubertal age group testis cell smears stained with putative and known testis cell makers. Group means and SEM are given for each marker for pre-pubertal and pubertal age groups, the mean and SEM for total cells expressing markers in pre-pubertal and pubertal samples is also given. 96

Figure 4-1. Timeline showing the process of lentiviral production and transfection and culture of lentiviral iPS cells in the experiments outlined in Chapter 4. 118

Figure 4-2. Lenti-X™ GoStix™ cassette loaded with lentiviral supernatant from OSNL lentiviral cultures. A faint line at the ‘test position’ indicates the presence of lentivirus. 124

Figure 4-3. Expression of GFP in bovine fibroblast cells lentivirally transduced with eGFP plasmid (Addgene # 21210). a) florescent image for eGFP activity. b) Brightfield image of same field of cells. Scale bars represent 50 μm 125

List of Figures

- Figure 4-4: Time to colony formation (days) of putative bovine iPS like colonies produced from lentiviral infection of Bovine Embryonic Fibroblasts (BEFs) or Bovine Dermal Fibroblasts (BDFs) with reprogramming constructs coding for different combinations of transcription factors, Tet-OSKM, OSNL or OSKMNL. There were no differences in time to colony formation observed between treatments ($P>0.05$, $n=6$). Error bars represent \pm SEM. 126
- Figure 4-5: The average number of putative bovine iPS colonies observed per 10cm dish after 3 weeks of culture, for BDF or BEFs cultures lentivirally transduced with reprogramming constructs coding for different combinations of transcription factors, Tet-OSKM, OSNL or OSKMNL. There were no differences in the number of colonies observed after 21 days of culture between treatments ($P>0.05$, $n=6$). Error bars represent as \pm SEM. 127
- Figure 4-6: Putative bovine iPS colonies representative of different treatments. a) BDF Tet-OSKM, b) BDF OSNL, c) BDF OSKMNL, d) BEF Tet-OSKM, e) BEF OSNL, f) BEF OSKMNL. Scale bars represent 100 μ m. N.B. No colonies were observed in control cultures during the 21 days of culture. 128
- Figure 4-7: PCR for verification of integration of transgene from Tet-OSKM, OSNL or OSKMNL reprogramming constructs into host genome. PCR was conducted on genomic DNA from pooled colonies of BDFs and BEFs transfected with different constructs. +ve indicates positive plasmid control, -ve indicates genomic DNA from uninfected BEFs. 129

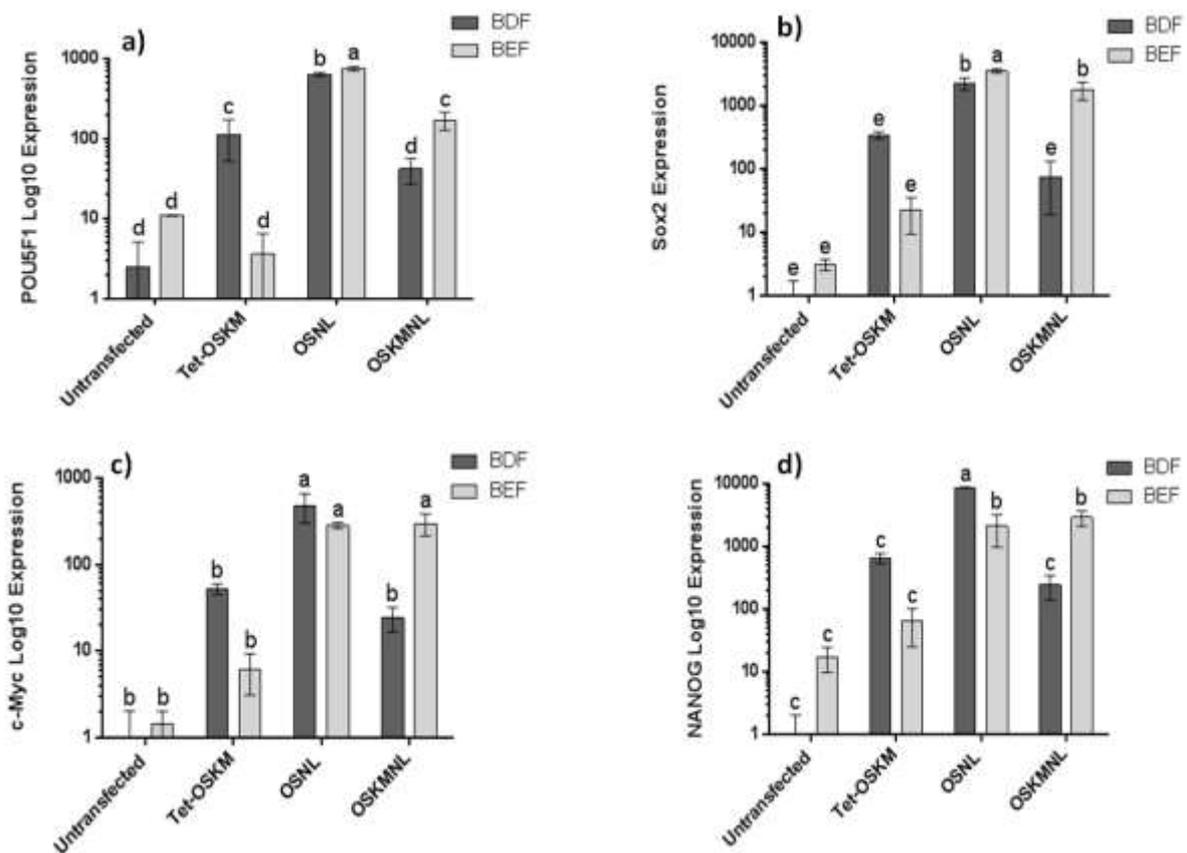


Figure 4-8. Log10 Expression of pluripotency markers relative to housekeeper gene *RPS26*, in putative bovine iPS colonies, produced by lentiviral transduction of reprogramming constructs coding for Tet-OSKM, OSNL or OSKMNL. (a) *POU5F1* (OCT4), (b) *SOX2*, (c) *c-MYC* and (d) *NANOG*. Different characters above treatment means indicate a significant difference between expression of pluripotency markers ($P<0.05$ $n=3$). Error bars represent as \pm SEM. 131

List of Figures

Figure 5-1. Timelines showing the processes of lentiviral and non-viral production of bovine iPS colonies in the experiments outlined in Chapter 5..... 153

Figure 5-2. Expression of GFP protein by bovine fibroblast cells non-virally electroporated with the eGFP plasmid Scale bars represent 50 μm 159

Figure 5-3. Time to formation of putative bovine iPS colonies (in days) of BEFs or BDFs transfected or infected with different combination of transcription factors; Tet-OSKM, OSNL or OSKMNL. a) complete data set for each treatment type showing the effect of cell type, combination of transcription factors and reprogramming method on the time to colony formation ($P < 0.05$, $n = 6$). b) Effect of cell type on time for colony formation of using lentiviral and non-viral reprogramming ($P < 0.05$, $n = 18$). c) Effect of combination of transcription factors on time to colony formation using lentiviral and non-viral reprogramming ($P < 0.05$, $n = 12$). Different characters above treatment means indicate a significant difference in observed colony number between treatments. Error bars are represented as \pm SEM. 160

Figure 5-4. Comparison of number of putative bovine iPS colonies observed per 10cm dish after 21 days of culture for BEFs or BDFs transfected or infected with different combinations of transcription factors, Tet-OSKM, OSNL or OSKMNL. a) raw data for each treatment type showing the effect of cell type, combination of transcription factors and reprogramming method on the number of colonies produced ($P < 0.05$, $n = 6$). b) Effect of cell type using lentiviral or non-viral reprogramming on the average number of colonies produced per 10cm dish ($P < 0.05$, $n = 18$). c) Effect of combination of transcription factors on the average number of colonies produced by lentiviral or non-viral transfection ($P < 0.05$, $n = 6$). Different characters above treatment means indicate a significant difference in observed colony number between treatments, error bars are represented as \pm SEM. 162

Figure 5-5. Colony morphology representative of Lentivirally and non-virally derived putative bovine iPS colonies. a) Example of lentivirally derived colony composed of cells with ES cell like morphology. b) Lentiviral colony that does not display good iPS colony morphology, with un-clear borders. c) Example of putative bovine non-viral colony. Colony is composed of ES like cells around the perimeter of the colony, while the centre has begun to differentiate. d) Non-virally derived colony that has differentiated into a large embryoid body like structure following 21 days of culture. Scale bars represent 100 μm 163

Figure 5-6: Gel showing integration of transgene in host genome of bovine embryonic stem cells for lentivirally transduced cells and non-virally transfected cells. Integration is absent in BEFs and BDFs transfected with Episomal plasmids (BDF-N or BEF-N), but is present in lentivirally transduced cells (BDF-L or BEF-L). +ve indicates the positive control plasmid, -ve indicates the negative control where the OCT4 plasmid was used in PCR rather than DNA from transfected cells. 164

Figure 5-7: Log₁₀ Expression of pluripotency markers, relative to housekeeper gene *RPS26*, of putative lentiviral and non-viral bovine iPS colonies, derived by transfection of BEFs or BDFs with transcription factor combinations Tet-OSKM, OSNL or OSKMNL s. a) *POU5F1* expression by BDF derived bovine iPS colonies, b) *POU5F1* expression by BEF derived bovine iPS colonies, c) *SOX2* expression by BDF derived bovine iPS colonies, d) *SOX2* expression by BEF derived bovine iPS colonies, e) *c-MYC* expression by BDF derived bovine iPS colonies, f) *c-MYC* expression by BEF derived bovine iPS colonies, g) *NANOG* expression by BDF derived bovine iPS colonies, h) *NANOG* expression by BEF derived bovine iPS colonies. Different characters above treatment means indicate a significant difference in the expression of pluripotency markers ($P < 0.05$, $n = 3$), error bars are represented as \pm SEM. 167

Figure 5-8. Log₁₀ Expression, relative to housekeeper gene *RPS26*, of lineage markers *Tubb3* (Ectoderm), *Nestin* (Ectoderm), *Desmin* (Mesoderm), *FoxA2* (Endoderm) expressed by embryoid bodies derived from lentiviral or non-viral transfection of BDF or BEFs with different combinations of transcription factors, Tet-OSKM, OSNL and OSKMNL. a) *Tubb3* expression by BDF derived iPS colonies, b) *Tubb3* expression by BEF derived iPS colonies, c) *Nestin* expression by BDF derived iPS colonies, d) *Nestin* expression by BEF derived iPS colonies, e) *Desmin* expression by BDF derived iPS colonies, f) *Desmin* expression by BEF derived iPS colonies, g) *FoxA2* expression by BDF derived iPS colonies, h) *FoxA2* expression by BEF derived iPS colonies. Different characters above treatment means indicate a significant difference in the expression of lineage makers (P<0.05, n=3). Error bars are represented as ± SEM. 169

Figure 6-1. Time to colony formation of colonies supplemented with small molecule combinations NaB-SB-PD compared to colonies supplemented with VPA-NaB-SB-PD. a) Time to colony formation of BDF or BEFs transfected with different combination of transcription factors Tet-OSKM, OSNL or OSKMNL, supplemented with small molecule combinations NaB-SB-PD or VPA-NaB-SB-PD (P<0.05, n=6). b) The effect of small molecule combinations NaB-SB-PD and VPA-NaB-SB-PD time to colony formation in two cell types BDF and BEFs (P<0.05, n=18). c) Effect of small molecule combinations NaB-SB-PD and VPA-NaB-SB-PD on time to colony formation of cultures transfected with different combination of transcription factors, Tet-OSKM, OSNL and OSKMNL (P<0.05, n=12). *VPA= Valproic acid (1 mM), NaB= Sodium butyrate (0.5 mM), SB= SB431542 (2 µM), PD= PD0325901 (0.5 µM), BDF= bovine dermal fibroblasts, BEF= bovine embryonic fibroblasts. Different characters above treatment mean indicate a significant difference between treatments. Error bars are represented as ± SEM. 190

Figure 6-2. Number of putative non-viral bovine iPS colonies produced by supplementation with small molecule combinations NaB-SB-PD and VPA-NaB-SB-PD. a) Average number of colonies produced by transfection of BDF or BEFs with different combination of transcription factors, Tet-OSKM, OSNL or OSKMNL with iPS cultures supplemented with small molecule combinations NaB-SB-PD or VPA-NaB-SB-PD (P<0.05, n=6). b) The effect of small molecule combinations NaB-SB-PD and VPA-NaB-SB-PD the average number of colonies produced by transfection of two cell types BDF and BEFs (P<0.05, n=18). c) Effect of small molecule combinations NaB-SB-PD and VPA-NaB-SB-PD on the average number of colonies produced from cultures transfected with a different combination of transcription factors, Tet-OSKM, OSNL or OSKMNL (P<0.05, n=12). *VPA= Valproic acid (1 mM), NaB= Sodium butyrate (0.5 mM), SB= SB431542 (2 µM), PD= PD0325901 (0.5 µM), BDF= bovine dermal fibroblasts, BEF= bovine embryonic fibroblasts. A different character above treatment means indicates a significant difference between treatments. Error bars are represented as ± SEM. 192

Figure 6-3. a) Example of typically good colony morphology observed at higher frequencies in cultures supplemented with the VPA-NaB-SB-PD small molecule combination. b) Colony showing typical differentiation in the centre, ES like cells were still observed around the edges of the colony. c) Colony with poorer morphology, cells are less tightly packed and the colony boarder is not clear. d) Colony with poor morphology that has become overgrown and differentiated. Scale bars represent 100 µm. 194

Figure 6-4. Colonies supplemented with the VPA-NaB-SB-PD small molecule combination stain positive for alkaline phosphatase using the alkaline phosphatase live staining kit (Life Technologies). a) Bright field image of alkaline phosphatase positive colony. b) Fluorescent image of colony showing alkaline phosphatase live staining. Scale bars represent 100 µm. 195

List of Figures

Figure 6-5. Expression of pluripotency markers, by BDF (a, c, e) or BEF (b, d, f) putative iPS cultures, transformed by transfection with Tet-OSKM, OSNL or OSKMNL, and supplemented with small molecule cocktails VPA-NaB-SB-PD or NaB-SB-PD. Gene expression relative to housekeeper gene *RPS26*, is shown for a) and b) *POUF1*, c) and d) *SOX2*, e) and f) *c-MYC* and g) and h) *NANOG*. *VPA= Valproic acid (1 mM), NaB= Sodium butyrate (0.5 mM), SB= SB431542 (2 μ M), PD= PD0325901 (0.5 μ M), BDF= bovine dermal fibroblasts, BEF= bovine embryonic fibroblasts. Different characters above treatments means indicates a significant difference in expression between treatments ($P<0.05$, n=3 Error bars are represented as \pm SEM)..... 198

Figure 6-6. Photograph of embryoid bodies' representative of those formed from colonies derived by supplementation with NaB-SB-PD or VPA-NaB-SB-PD. Scale bar represents 100 μ m. 199

Figure 6-7. Expression of lineage markers by EBs derived from putative BDF (a, c, e) or BEF (b, d, f) iPS cultures, transformed by transfection with Tet-OSKM, OSNL or OSKMNL, that were initially supplemented with small molecule cocktails VPA-NaB-SB-PD or NaB-SB-PD. Gene expression relative to housekeeper gene *RPS26*, is shown for a) and b) *Tubb3*, c) and d) *Nestin*, e) and f) *Desmin* and g) and h) *FoxA2*. *VPA= Valproic acid (1 mM), NaB= Sodium butyrate (0.5 mM), SB= SB431542 (2 μ M), PD= PD0325901 (0.5 μ M), BDF= bovine dermal fibroblasts, BEF= bovine embryonic fibroblasts. Different characters above treatments means indicates a significant difference in expression between treatments ($P<0.05$, n=3 Error bars are represented as \pm SEM)..... 202

Figure 6-8. Time to colony formation of putative non-viral bovine iPS cultures supplemented with different combinations of the small molecules Valproic acid (VPA (1 mM)), Sodium Butyrate (NaB 0.5 mM)), SB431542 (SB (2 μ M)) and PD0325901 (PD (0.5 μ M)). Legend shows treatments divided into different numbers of small molecules supplemented in combination with each other, diagonal lines highlight treatments with VPA supplementation. Different letters above treatments indicates they are significantly different from one another ($P<0.05$, n=6). Error bars are represented as \pm SEM. .. 205

Figure 6-9. Average number of putative bovine iPS colonies observed per 10 dish of cultures supplemented with different combinations of the small molecules. Supplementation of small molecules occurred at the following concentrations; Valproic Acid (VPA (1 mM)), Sodium Butyrate (NaB 0.5 mM)), SB431542 (SB (2 μ M)) and PD0325901 (PD (0.5 μ M)). Legend shows treatments divided into different numbers of small molecules supplemented in combination with each other, diagonal lines highlight treatments with VPA supplementation. Different letters above treatments indicates they are significantly different from one another ($P<0.05$, n=6). Error bars are represented as \pm SEM. 206

Figure 6-10. Examples of colony morphology observed in putative bovine iPS cultures supplemented with different combinations of small molecules. Figures 10 a-d show examples of good colony morphology, typically observed in iPS cultures supplemented with VPA-SB, VPA-PD, NaB-SB-PD and VPA-NaB-SB-PD a, b) photographs of colonies with good morphology, colonies have clear borders and are comprised of tightly packed round ES like cells. c) small/young colony comprised of round ES like cells. d) Colony that has begun to differentiate in the centre but still has ES like cells around the colony perimeter. Figures 10 e-h show examples of colonies with poor morphology typically observed in cultures not supplemented with small molecules, or supplemented with only one small molecule or with NaB-VPA in combination. e) Poor colony morphology, colony does not have clear borders. f) Colony that has differentiated. g) Differentiated colony typically observed around plate edges, shows large areas of differentiation. h) Colony displaying embryoid body-like appearance. Scale Bars represent 100 μ m. 208

List of Figures

Figure 6-11. Log ₁₀ expression of Pluripotency Markers a) <i>POU5F1</i> , b) <i>SOX2</i> and c) <i>NANOG</i> by putative bovine iPS colonies after 21 days of culture. Legend shows treatments divided into different numbers of small molecules supplemented in combination with each other, diagonal lines highlight treatments with VPA supplementation. Different characters above treatments indicates a significant difference in the expression of pluripotency marker (P<0.05, n=3). Error bars are represented as ± SEM.	210
Figure 6-12. Photographs showing typical embryoid body morphology after a) 7 days and b) 14 days of culture. Scale bars represent 100 µm.....	211
Figure 6-13. Log ₁₀ expressions of lineage markers a) <i>Tubb3</i> , b) <i>Desmin</i> and c) <i>FoxA2</i> , by embryoid bodies derived from putative bovine iPS cultures. Legend shows treatments divided into different numbers of small molecules supplemented in combination with each other, diagonal lines highlight treatments with VPA supplementation. Different characters above treatments indicates a significant difference in the expression of pluripotency marker (P<0.05, n=3). Error bars are represented as ± SEM.	213
Figure 7-1. Expression of Green Fluorescent Protein (GFP) in bovine embryonic fibroblasts, transfected with minicircle vector, 3 days after initial transfection by electroporation) a) Brightfield image b) Fluorescent image. Scale bar represents 50 µm.....	238
Figure 7-2. a) Time to Colony formation in days for putative bovine iPS colonies derived from BEF and BDF cells transfected with minicircle vector or OSNL plasmid (n=6, P>0.05). b) Average numbers of putative bovine iPS colonies present per 10 cm dish after 21 days of culture for BEF or BDFs transfected with minicircle or OSNL plasmid. Different characters above error bars indicate a significant difference in treatment means (n=6, P<0.05). Error bars represent SEM.....	239
Figure 7-3: Examples of alkaline phosphatase positive putative bovine iPS colonies. a) Small colony present in minicircle cultures after 14 days in culture staining positively for Alkaline Phosphatase, similar colonies also present in OSNL cultures. b, c) Larger colonies present in OSNL (b) and minicircle cultures (c) after 21 days in culture, also stain clearly positively for alkaline phosphatase activity in the centre of the colony and slightly weaker or absent at colony edges. Scale bars represent 100 µm.	241
Figure 7-4: Log ₁₀ Expression of pluripotency markers a) <i>POU5F1</i> (<i>OCT4</i>), b) <i>SOX2</i> and c) <i>NANOG</i> , by putative iPS colonies derived from BEF and BDFs transfected with minicircle vector or OSNL episomal plasmid, relative to housekeeper gene <i>RPS26</i> . Different characters above treatment means indicates a significant difference in expression (n=3, P<0.05). Error bars represent as ± SEM.	243
Figure 7-5. Typical morphology of embryoid bodies derived from putative bovine iPS cultures a) Embryoid bodies present after 7 days in culture. b) Large embryoid body present after 14 days of culture. Scale bars represent 100 µm.....	244
Figure 7-6: Expression of lineage markers by embryoid bodies derived from putative bovine iPS cultures, relative to housekeeper gene <i>RPS26</i> . a) Expression of <i>Tubb3</i> by BEF derived cultures b) Expression of <i>Tubb3</i> by BDF derived cultures. c) <i>Desmin</i> expression by BEF derived cultures. d) <i>Desmin</i> expression by BDF derived cultures. e) <i>FOXA2</i> expression by BEF derived cultures. f) <i>FOXA2</i> expression by BDF derived cultures. Different characters above treatment means indicates a significant difference in expression of lineage markers (n=3, P<0.05). Error bars represent as ± SEM.	246
Figure 7-7. Expression of testis cell markers, relative to housekeeper gene <i>RPS26</i> , by embryoid bodies derived from BDF and BEF iPS colonies transfected with minicircle or OSNL episomal plasmid vector, treated with 0mM, 0.5mM or 1mM retinoic acid (RA) for 14 days a) <i>DDX4</i> (<i>VASA</i>), b) <i>GATA4</i> ,	

List of Figures

c) *UCHL1*, d) *DDX6*, e) *NAP1L4*, f) *TKTL1*. Different characters above treatment means indicate a significant difference in expression of testis markers ($P < 0.05$, $n = 3$). Error bars represent \pm SEM. ... 249

Figure 7-8. Large embryoid body like colonies present following 21 days in culture, these do not stain positively for alkaline phosphatase. Scale bars represent 200 μm 250

Figure 7-9. Expression of pluripotency and lineage markers, by embryoid body- like putative bovine iPS colonies, relative to housekeeper gene *RPS26*. a) Expression of pluripotency markers *POU5F1*, *SOX2* and *NANOG*, by embryoid body like colonies observed in BDF iPS cultures compared to un-transfected BDFs. b) Expression of lineage markers *Tubb3*, *Desmin* and *FoxA2* by embryoid body like colonies observed in BDF iPS cultures compared to un-transfected BDFs. * indicates a significant difference in mean expression by BDFs and EB like iPS colonies ($P < 0.05$, $n = 3$). Error bars represent as \pm SEM. 251

List of Tables

Table 2-1. Previous reports of fold increase and final spermatogonia percentage of bovine spermatogonia in enriched populations using different enrichment methods..... 50

Table 2-2. Preparation of Percoll gradient Fractions with 10x DMEM: F12. 54

Table 2-3: Amino acid sequence and characteristics of C-CPE₂₉₀₋₃₁₉ and control peptides. 56

Table 2-4. Cell counts for DBA positive (+) and GATA4 positive (+) cells in initial isolate, differentially plated non-adherent fraction, Percoll gradients interface between 43-35% and 35-20%. Different characters beside treatments indicate a significant difference in the percentage of cells observed between treatments in the same column (n=4, P<0.05). 60

Table 3-1: Primers, expected amplicon size and efficiencies used in Chapter 3. 'Bv' indicates primer is specific for expression of bovine cDNA. 78

Table 4-1. Reported production of iPS cells from different livestock species. 112

Table 4-2. Plasmids sourced from Addgene plasmid repository, for use in reprogramming experiments carried out in chapter 4. 122

Table 4-3: Primers used in Chapter 4..... 123

Table 5-1: General characteristics of vectors used for reprogramming cells to pluripotency. Table indicates reports of methods used for deriving livestock iPS cells. Efficiencies are general and not specific to livestock generation. 148

Table 5-2: Primers used in Chapter 5..... 156

Table 5-3: Plasmids sourced from Addgene plasmid repository, for use in reprogramming experiments carried out in chapter 5. 157

Table 6-1. Primers used in Chapter 6..... 187

Table 6-2. Plasmids sourced from Addgene plasmid repository, for use in reprogramming experiments carried out in chapter 6. 188

Table 6-3. Small molecule combinations tested in experiment 1..... 189

Table 6-4. Small molecule combinations tested in experiment 2..... 204

Table 7-1.Reports of *in vitro* germ cell derivation from embryonic stem cells and induced pluripotent stem cells. (Table revised from Imamura et al. (2014)). ES= embryonic stem cells, iPS= induced pluripotent stem cells, PGCs= primordial germ cells, SSC= Spermatogonial stem cells, EGF=epidermal growth factor, GDNF= glial cell derived neurotropic factor, SCF= Stem cell factor..... 230

Table 7-2. Primers used in Chapter 7..... 237

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

ES cell: Embryonic stem cell

FACS: Fluorescent activated cell sorting

FBS: Foetal bovine serum

GFP: Green fluorescent protein

HDAC: Histone deacetylase

ICM: Inner cell mass

iPS cell: Induced pluripotent stem cell

LIF: Leukaemia inhibitory factor

MACS: Magnetic activated cell sorting

MDF: Modified Davidsons Fixative

MET: Mesenchymal-to-epithelial transition

MEM: Minimum Essential Medium

Mit-C: Mitomycin C

NaB: Sodium Butyrate

NEAA: Non-essential amino acids

OSKM: POU5F1 (OCT4); SOX2; KLF4; c-MYC

OSKMNL: POU5F1 (OCT4); SOX2; KLF4; c-MYC; NANOG; LIN28

OSNL: POU5F1 (OCT4); SOX2; NANOG; LIN28

PD: PD0325901 (Small Molecule)

PCR: Polymerase chain reaction

PGC: Primordial germ cell

qRT-PCR: Quantitative Reverse transcribed polymerase chain reaction

List of Abbreviations

RA: Retinoic acid

SB: SB431542 (Small Molecule)

SCNT: Somatic cell nuclear transfer

SEM: Standard error margin

SCF: Stem cell factor

SSC: Spermatogonial stem cell

TBS: Tris buffered saline

Tet: Tetracycline

VPA: Valproic acid

Gene Synonyms

Gene Symbol	Synonym	Gene Name
<i>c-MYC</i>		v-Myc avian myelocytomatosis viral oncogene homolog
<i>DDX4</i>	<i>VASA</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4
<i>DDX6</i>	<i>RCK/p54</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
<i>DES</i>		Desmin
<i>FOXA2</i>		Forkhead box A2
<i>FSCN1</i>	<i>FAN1, HSN, SNL</i>	Fascin 1, 55 kDa actin-bundling protein, Singed-like protein, p55
<i>GATA4</i>		GATA binding protein 4
<i>IQGAP1</i>	<i>KIAA0051</i>	Ras GTPase-activating-like protein IQGAP1
<i>KLF4</i>		Kruppel-like factor 4
<i>NANOG</i>		Nanog homeobox
<i>NAP1L4</i>		Nucleosome assembly protein 1-like-4
<i>NES</i>		Nestin
<i>LIN28</i>	<i>CSDD1, LIN28A, ZCCHC1</i>	Zinc finger CCHC domain-containing protein 1
<i>POU5F1</i>	<i>OCT4</i>	POU class 5 homeobox 1
<i>SOX2</i>		SRY (Sex determining region Y) box 2
<i>TKTL1</i>		Transketolase-like 1
<i>TLN1</i>		Talin 1
<i>TUBB3</i>		Tubulin, beta 3 class III
<i>UCHL1</i>	<i>PGP9.5</i>	Ubiquitin carboxyl-terminal esterase L1
<i>VIM</i>		Vimentin
<i>ZBTB16</i>	<i>PLZF</i>	Zinc finger and BTB domain containing 16