1. **GENERAL INTRODUCTION**
	1. **A brief history of stem cells**

Stem cells are differentiated cells that are defined by their ability to both self-renew indefinitely and differentiate to produce progeny cells. They are classified by their developmental potential as totipotent (give rise to all cells that contribute to the formation of an organism), pluripotent (give rise to all cells of an organism but cannot contribute to the formation of one), multipotent (give rise to a small number of cells that are usually lineage restricted to a germ layer origin), oligopotent (give rise to a limited number of cell types) and unipotent (give rise to only one type of cell). Due to the unique ability of pluripotent stem cells to differentiate into any cell in the body, there has been a large research focus on isolating these cells and examining their therapeutic potential.

During the early developmental stages of an embryo, the cells possess the ability to differentiate into any tissue within the body. These cells are therefore regarded as pluripotent stem cells. In humans and mice, pluripotent stem cells can be isolated from the blastocyst stage of an early embryo in a cluster of cells contained with the inner cell mass (ICM) (Evans and Kaufman, 1981, Thomson et al., 1998). These cells termed embryonic stem cells (ESCs) have the capacity to rapidly self-renew indefinitely and differentiate into all adult cell types (Suda et al., 1987). Pluripotent stem cells can also be captured at later developmental time points during germline development from primordial germ cells (PGCs) (Matsui et al., 1992). Isolated PGCs can be cultured long term on feeder layers and under appropriate conditions to give rise to colonies of cells resembling undifferentiated, pluripotent embryonic stem cells. The use of pluripotent stem cells has great potential for regenerative medicine due to their ability to become any cell type required. However, one of the ethical dilemmas that researchers face is removal of these cells from the ICM of blastocysts renders the embryos nonviable. Therefore, safer isolation techniques or other sources of pluripotent stem cells are required and would aid in avoiding this ethical dilemma.

Induced pluripotent stem cells (iPSCs) were first created in 2006 by reprogramming skin fibroblasts from the adult into cells resembling those of mESCs, which makes them another valuable source for stem cell research (Takahashi and Yamanaka, 2006). This was made possible by introducing four factors, Oct3/4, Sox2, c-Myc and Klf4, genes associated with the signaling network necessary for ES cell pluripotency. As iPSCs can be derived directly from adult tissues, they can be matched in a patient-matched manner resulting in individual pluripotent stem cell lines. However, there are challenges associated with reprogramming cells to pluripotency. One challenge is the low efficiency of conversion into iPSCs which range between 0.01-0.1% (Takahashi and Yamanaka, 2006). Additionally, the viral delivery method used to reprogram adult cells to obtain iPSCs poses an increase in tumorgenicity as the expression of oncogenes may potentially be triggered. A later study by Yamanka reported that iPSCs can be generated without the oncogene c-Myc, however, the process took weeks longer and yielded 100 times fewer cells (Nakagawa et al., 2008). Therefore, although these methods bypass the need to isolate pluripotent stem cells from the developing embryo, further testing is still required to ensure their feasibility in therapeutic transplants.

Due to the difficulties and ethical concerns posed by ESCs and iPSCs; an alternative source of stem cells that may bypass these dilemmas reside within our own bodies. These stem cells named ‘adult’ stem cells are found within specific niches in tissue (Watt and Hogan, 2000). These cells are classified as multipotent, oligopotent and unipotent and most are lineage restricted. They work by maintaining the integrity of tissues by replenishing dying cells and regenerate damaged tissue. A well-studied adult stem cell is the haematopoietic stem cells (HSCs) found in the bone marrow of adults (Birbrair and Frenette, 2016). They give rise to the entire blood cell lineage, classifying them as multipotent stem cells. HSCs are used in bone marrow transplantation to reconstitute the hematopoietic system to replace damaged or destroyed bone marrow with healthy bone marrow stem cells. It is most often performed on patients with certain cancers e.g. leukemia to re-establish hematopoietic function in patients whose bone marrow or immune system is damaged or defective. Other adult stem cells with multipotent potential have been found in culture methods from skin, muscle, heart and brain (Toma et al., 2001, Cao et al., 2003, Jiang et al., 2002, Beltrami et al., 2003). In contrast, cultured unipotent stem cells isolated from mouse testis named spermatogonial stem cells (SSCs), were found to revert to a pluripotent state (Kanatsu-Shinohara et al., 2004, Guan et al., 2006). Therefore, isolation of these adult stem cell populations would be of great benefit for stem cell therapies as individual cell lines could be produced depending on the requirement from the patient. Draw backs on utilising adult stem cells involve the harvesting and culturing these cells up to sufficient numbers, which can prove challenging as they usually have low proliferative ability. Additionally, some cell types have not been successfully cultured yet and some established lines have a shorter life span compared to ESCs. It would be a great benefit if a pluripotent stem cell population could be identified in the adult.

* 1. **Nanog: A pluripotency homeobox transcription factor**

Nanog is a homeobox transcription factor that is critically involved with self-renewal of undifferentiated embryonic stem cells and was named after the mythical Celtic land of the ever-young Tir nan Og (Chambers et al., 2003). Characterization of the Nanog cDNA revealed the presence of the homeobox domain and indicated that Nanog is likely to act as a transcriptional regulator (Gehring et al., 1994). Sequence conversation was most pronounced over this homeodomain region between human, rat and mouse, indicating it shares a similar function across these species (Hart et al., 2004, Chambers et al., 2003). While Nanog or Nanog-like sequences have been found in vertebrate genomes, they have not been found in invertebrates (Theunissen et al., 2011, Hart et al., 2004). In mESCs, it was found Nanog plays a key role in maintaining their pluripotent state as cells deficient in Nanog are prone to differentiate. In mice, leukemia inhibitory factor (LIF) is a cytokine that is utilized to maintain the symmetrical self-renewal of mouse ES cells (Williams et al., 1988). Overexpression of Nanog was found to maintain the pluripotent state of ESCs in the absence of LIF, suggesting it plays a major downstream effect of other genes (Chambers et al., 2003).

***(1.2.1) Expression during embryo development***

Nanog expression was first detected in the interior cells of the compact morulae during mouse embryo development (Chambers et al., 2003). It then becomes confined to the ICM and disappears in the trophectoderm in the blastocyst stage. In later blastocysts, Nanog mRNA becomes restricted to the epiblast and by implantation stage is down regulated (Chambers et al., 2003, Hart et al., 2004). In postimplantation murine embryos, Nanog mRNA continues to be expressed in E6.5 and E7.5, however, it was found Nanog protein did not co-localise with PGC marker PGC7 (Hatano et al., 2005, Hart et al., 2004). By E7.75, it was found that Nanog protein co-localized with a subpopulation of PGC7-positive cells (Yamaguchi et al., 2005). The expression of Nanog continued in PGCs as they migrated to the developing genital ridges. In E11.5 embryos, there was a high number of Nanog expressing PGCs, which continued to be found in both male and females PGCs at E12.5. After this developmental timepoint, Nanog expression was not found in the germ cells of E15.5 female gonads whereas in the male, Nanog expression was not found by E16.5. The differences may lie in the fact Nanog expression is down-regulated in germ cells undergoing meiosis in the female gonads and in the germ cells of male gonads, which have transitioned into gonocytes and undergone mitotic arrest. This study had also investigated the adult gonads, however, they found no expression of Nanog. In contrast, other studies have found Nanog expression in the gonocytes from other species which include human, pig and marmoset (Hart et al., 2005, Hatano et al., 2005, Goel et al., 2008, Mitchell et al., 2008). Additionally, other studies investigated Nanog in the adult gonad in various species and found its expression to the be localized in spermatogonial stem cells (SSCs), spermatocytes and neonatal sertoli cells (Kuijk et al., 2010, Ventela et al., 2012, Goel et al., 2008, Schreiber et al., 2013). The SSCs serve as the foundation of spermatogenesis and undergo a number of steps that include proliferation and differentiation to become spermatozoa that is ready to be released from the seminiferous epithelium (Phillips et al., 2010). There continues to be a debate about the true expression of Nanog in the germ cells of the testis, however, studies have shown pluripotent stem cells can be isolated from neonatal and adult testis (Guan et al., 2006, Kanatsu-Shinohara et al., 2004, Seandel et al., 2007). Therefore, it is possible Nanog may play a functional role in the pluripotent state of germ cells.

***(1.2.2) Regulation of Nanog in maintaining the pluripotent state and its target genes***

Nanog is considered a key to pluripotency as it is highly transcribed in mouse ESCs and genetic deletion of *Nanog* leads to their differentiation into extraembryonic endoderm lineages (Mitsui et al., 2003, Chambers et al., 2003). This led to several investigations into determining how Nanog regulates the pluripotent state of ESCS.

Leukemia inhibitory factor (LIF) has been shown to be required for the undifferentiated self-renewal of mouse ES cells through activation of the JAK/STAT3 pathway *in vitro* (Smith et al., 1988, Williams et al., 1988, Niwa et al., 1998). In contrast, however, it has been demonstrated LIF to not be essential *in vivo* as mutant embryos deficient in the LIF/gp130/Stat3 pathway are still able to form a normal ICM and develop normally (Stewart et al., 1992). Additionally, LIF is not required to maintain the self-renewal of human ESCs (Reubinoff et al., 2000, Daheron et al., 2004). This suggested that additional pathways can compensate in the absence of LIF.

A screening for critical factors that can maintain ES cell pluripotency independently of the LIF/STAT3 pathway led to the discovery of Nanog by two independent research groups that analyzed its function (Chambers et al., 2003, Mitsui et al., 2003). It was found that downregulation of Nanog resulted in differentiation of mESCs, while mouse embryos lacking *Nanog* fail to develop beyond the blastocyst stage due to the absence of epiblasts (Mitsui et al., 2003). It was also found that overexpression of Nanog has the capacity to maintain the pluripotent state of mESCs in the absence of LIF, bypassing the Stat3 pathway (Chambers et al., 2003). Therefore, it was investigated on how other core pluripotency factors regulate its gene expression.

Before the discovery of Nanog, core transcription factors Oct4 and Sox2 were other critical players in the pluripotent state of mESCs. This led to the discovery that the Nanog promoter contains binding sites for the transcription factors Oc4 and Sox2, both of which are essential for maintaining the pluripotent embryonic stem cell phenotype (Rodda et al., 2005). Oct4 is required for regulation of cell fate in the early embryo, is expressed in the ICM and downregulated upon differentiation (Nichols et al., 1998). Sox2 is also expressed in the ICM and epiblast of the blastocyst and required for its development (Avilion et al., 2003). Furthermore, studies have found that Nanog, Oct4 and Sox2 co-occupy the promoters of many genes and collaborate to form a regulatory network in hESCs (Boyer et al., 2005). Their results show that Oct4, Sox2 and Nanog are also bound to their own promoters, thus they form an interconnected autoregulation loop to maintain the ES cell identity. This mechanism was further confirmed by another study in which Oct4 maintains Nanog expression by directly binding to a Nanog promoter when present at a sub-steady level (Pan et al., 2006). In contrast, when the expression of Oct4 rises above the steady level, it represses its own promoter as well as Nanog whereas overexpression of Nanog does not increase Oct4 expression above steady state. Another study done on mESCs identified 1083 and 3006 binding sites for Oct4 and Nanog respectively (Loh et al., 2006). Through depletion of Oct4 and Nanog by integrating RNA interference and integrating with microarray expression profiling, they demonstrated that downstream gene targets are related to pluripotency, self-renewal and cell fate determination. These findings suggest that the key pluripotent factors work together in concert rather than on an individual basis. Therefore, they control a whole set of target genes, as well as each other, to keep the pluripotent properties of ES cells.

**FIGURE\_Nanog stem cells transcriptional network**

* + 1. ***Expression and function during germline development***
		2. ***Expression in adult tissue and cancer***

*Nanog*-null iPSCs contribute to the germline and produce functional germ cells (Carter et al., 2014).

***(1.2.4.1) Human***

***(1.2.4.2) Mouse***

***(1.2.4.3) Other***

* 1. **The germline lineage and testicular development**

**(1.4.1) Development of the germline**

**(1.4.2) Primordial germ cells**

**(1.4.3) Gonocytes**

 **FIGURE\_ development of the germline**

* 1. **Spermatogenesis**

***(1.5.1) Spermatogonial stem cells***

***(1.5.1.2) Pluripotent spermatogonial stem cells***

***The cellular organisation of the seminiferous tubules***

***IFIGURE – The seminiferous tubule***

 ***\_ Asingle – A paired hierarchy/whole mount IHC of tubule***

***(1.5.2) Spermatocytes***

* 1. **The testicular stem cell niche**

The testicular stem cell niche is formed by the sertoli cell-germ cell interaction, which plays an essential role in germ cell development both *in vivo* and *in vitro*. The interaction of these cells allows a certain number of germ cells to reside or repopulate the seminiferous tubules by limiting the expansion of the SSC population. The Sertoli cells provide essential factors for growth, proliferation and differentiation of germ cells into spermatozoa.

***(1.6.1)***

***(1.6.2) The Sertoli cell***

***(1.6.2.1)***

***(1.6.2.2) Sertoli-germ cell junction***

***(1.6.2.3) Functional analysis of the niche ( Knock-out studies and regeneration)***

* 1. **Conclusion**
	2. **Aims and scope of this study**
	3. **References**

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