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Review

In vitro differentiation of primordial germ cells and oocyte-like cells from stem cells

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Summary. Infertility is the result of failure due to an organic disorder of the reproductive organs, especially their gametes. Recently, much progress has been made on generating germ cells, including oocytes, from various types of stem cells. This review focuses on advances in female germ cell differentiation from different kinds of stem cells, with emphasis on embryonic stem cells, adult stem cells, and induced pluripotent stem cells. The advantages and disadvantages of the derivation of female germ cells from several types of stem cells are also highlighted, as well as the ability of stem cells to generate mature and functional female gametes. This review shows that stem cell therapies have opened new frontiers in medicine, especially in the reproductive area, with the possibility of regenerating fertility.

Key words: Adult stem cells, Embryonic stem cells, Induced pluripotent stem cells, Primordial germ cells, Oocyte-like cells

Introduction

It has been estimated that human infertility affects approximately 14% of couples (Boivin et al., 2007) and thus, in recent years, new reproductive medicine technologies have been developed to help to reduce this problem. Women are born with a finite complement of eggs and an ovulated oocyte in a woman has the same age as her. It is known that cellular DNA is not completely invulnerable to the passage of years, the impact of age on oocytes being consistent with its effect on the risk of congenital abnormalities (Balen, 2011). Recently, it was demonstrated that the ovaries contain cells which can be isolated and propagated, and have the characteristics of oogonial stem cells. After reintroduction into the ovary, or in reaggregation models, these primitive cells can form new oocytes and follicles, which can generate healthy offspring (Johnson et al., 2004; White et al., 2012). Stem cell-based strategies for ovarian regeneration and oocyte production have been proposed as future clinical therapies for treating infertility in women (Volarevic et al., 2014). Stem cells are undifferentiated cells that are present in embryonic, fetal, and adult stages of life and give rise to differentiated cells that make up tissues and organs (Volarevic et al., 2014). From the literature, it is known that oocyte-like cells expressing different oocyte-specific genes can be developed *in vitro* from mouse embryonic stem cells (mESCs) (Psathaki et al., 2011) or human embryonic stem cells (hESCs) (Richards et al., 2010; Medrano et al., 2012). In addition, stem cells from human amniotic fluid (Cheng et al., 2012) and from porcine fetal skin (Dyce et al., 2011a) were also found to differentiate into oocyte-like structures. Furthermore, human primordial germ cells have been differentiated from induced pluripotent stem cells (iPS) (Panula et al., 2011; Eguizabal et al., 2011). Hayashi et al. (2012) proposed that hiPS could potentially be utilized to give rise to *de novo* oocytes for use in *in vitro* fertilization (IVF) clinics, thus allowing sterile women to conceive a child.

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In this review, we discuss the advances in our knowledge about the derivation of female differentiated germ cells from several types of stem cells, including ES cells and iPS cells, as well as the mechanisms of reprogramming somatic cells into pluripotent stem cells.

Differentiation of primordial germ cells and oocyte-like cells from embryonic stem cells

ESCs are derived from pluripotent cells of the early mammalian embryo from the inner cell mass (ICM) of blastocysts and can give rise to all three germ layers (ectoderm, endoderm and mesoderm) of the developing embryo (Evans and Kaufman, 1981; Martin, 1981; Singhal et al., 2014). ESCs are capable of self-renewing, but also of exhibiting pluripotency, a feature maintained by the core network of transcription factors comprising *OCT4, SOX2* and *NANOG* (Boiani and Schöler, 2005; Singhal et al. 2014). Dyce et al. (2014) found that expression of the gap junction protein, connexin 43 (Cx43), begins early during embryogenesis and is maintained in many different cell types. Thus, *Cx43* can play an important role in somatic stem cell proliferation and is required in at least some cases for maintaining a pluripotent, undifferentiated stem cell population. Therefore, ESCs have been used in an *in vitro* model of early embryogenesis to investigate the detailed molecular mechanisms for developmental processes (Itoh et al., 2014).

Female reproductive potential is limited in the majority of species due to oocyte depletion. Functional human oocytes are restricted in number and accessibility. A robust system to differentiate oocytes from stem cells would need a thorough investigation of the genetic, epigenetic, and environmental factors affecting human oocyte development. The differentiation of functional oocytes from stem cells may help to restore fertility in women. In mice, Hayashi et al. (2012) demonstrated the generation of primordial germ cell-like cells (PGCLCs) from ESCs with high capacity for differentiation. These authors showed that female ESCs were induced into PGCLCs and underwent proper development in reconstituted ovaries *in vitro* and matured further into fully functional GV oocytes upon transplantation *in vivo* to mouse ovarian bursa. These oocytes then contributed to fertile offspring after *in vitro* maturation and fertilization. Recently, Kurimoto et al. (2015) reported that an intense epigenetic remodeling is observed during differentiation of mouse ESCs to epiblast-like cells and from there into PGCLCs, which provides a foundation for reconstructing regulatory networks of the germline epigenome. This system serves as a robust foundation to investigate and further reconstitute female germline development *in vitro* in mammals (Hayashi et al., 2012), including the human.

Several studies in different species such as mouse (Nagano et al., 2002; Hübner et al., 2003) and human (Clark et al., 2004; Park et al., 2009), indicate that ESCs can differentiate *in vitro* into oocyte- or sperm-like cells. Nicholas et al. (2009) observed endogenous and ESC-derived human oocyte development. Hübner et al. (2003) were the first to report that differentiating mouse ESCs can spontaneously form oocyte-like cells *in vitro*, when cultivated without LIF and feeder cells. Psathaki et al. (2011) indicated that follicle-like structures formed by mouse ESCs *in vitro* consist of a single oocyte-like cell that can grow as large as 70 mm in diameter, surrounded by one or more layers of tightly adherent somatic cells. Besides presenting the expression of genes associated with steroidogenesis, these structures are connected via intercellular bridges with their enclosed germ cells, which may serve to facilitate cell-to-cell interaction required for normal follicle development (Albertini et al., 2001; Hübner et al., 2003; Novak et al., 2006). Lacham-Kaplan et al. (2006) have cultured mouse ESC-derived embryoid bodies (EBs), which formed ovarian-like structures containing oocyte-like cells, surrounded by one or two layers of flattened cells, which expressed specific oocyte marker genes (*FIGα* and *ZP3*). Kerkis et al. (2007) induced oocyte differentiation by stimulating EBs with retinoic acid. Yu et al. (2009a,b) reported that *Deleted in Azoospermia-Like* (*DAZL*) is a master gene controlling germ cell differentiation and that ectopic expression of *DAZL* promotes the dynamic differentiation of mouse ES cells into gametes *in vitro*. Furthermore, transient overexpression of *DAZL* led to suppression of *NANOG*, but induced germ cell nuclear antigen in mESCs, whereas *DAZL* knockdown resulted in reduction in the expression of germ cell markers, including *STELLA, MVH* and *PRDM1* (Yu et al., 2009a). Recently, Hamidabadi et al. (2015) demonstrated that the SSEA1 positive cells, isolated from EBs, differentiate into primordial germ cells and subsequently to oocyte-like cells.

In humans, Clark et al. (2004) demonstrated that differentiation of ESCs into EBs *in vitro* results in formation of cells that express specific markers for gonocytes and thus are capable of forming germ cells *in vitro*. In undifferentiated cells, *NANOG, STELLA, OCT4* and *DAZL* appeared expressed, whereas after differentiation in EBs, mRNAs and proteins for VASA, SCP1, SCP3, BOULE and GDF3 were found, which are germ cell specific markers (Clark et al., 2004). Murakami et al. (2016) recently showed that *NANOG* can induce differentiation of PGCLCs from epiblast-like cells, indicating epigenetic resetting of regulatory elements and genome-wide changes in NANOG-binding patterns between ESCs and epiblast-like cells. Additionally, SOX17 is also important for regulating the establishment of germline gene expression network of human PGCLCs (Irie et al., 2015).

Kee et al. (2006) demonstrated that the addition of recombinant human BMP4 increased the expression of the germ cell-specific markers *VASA* and *SCP3* during differentiation of hESCs to embryoid bodies. In addition, BMP2 and BMP8b associated with BMP-4 demonstrated additive effects on germ cell induction. Thus, several BMPs could induce differentiation of germ cells from human hESCs. Medrano et al. (2012) examined whether

intrinsic germ cell translational, rather than transcriptional factors might drive germline formation and/or differentiation from human pluripotent stem cells *in vitro*. They found that overexpression of *VASA* and/or *DAZL* promoted differentiation of both hESCs into primordial germ cells. Additionally, maturation and progression through meiosis was enhanced. Over the last decade, much progress has been made in the differentiation of human germ cells from both hESCs (Ishii et al., 2013). Nicholas et al. (2009) established fundamental parameters of oocyte development during ESC differentiation. They demonstrated a timeline of definitive germ cell differentiation from ESCs *in vitro* that initially parallels endogenous oocyte development *in vivo* by single-cell expression profiling and analysis of functional milestones, including responsiveness to defined maturation media, shared genetic requirement of *DAZL*, and entry into meiosis (Nicholas et al., 2009). Thus, ESCs can be used in an *in vitro* system to study oocyte development and could be of help in the treatment of female infertility. Fig. 1 shows the differentiation of stem cells in oocyte-like cells, as well as the stimulating substances and markers for each stage of differentiation.

Differentiation of primordial germ cells and oocytes from adult stem cells

After embryonic development, adult stem cells (ASCs) exist in almost all tissues of the body. They are ready for emergencies after tissue injury, as well as for maintenance of tissue homeostasis (Itoh et al., 2014). A vast amount of literature (Johnson et al., 2004, 2005; Lee et al., 2007; Bukovsky et al., 2008; Niikura et al., 2009; Selesniemi et al., 2009; Gong et al., 2010; Bukovsky, 2011; White et al., 2012; Tilly and Sinclair, 2013; Gheorghisan-Galateanu et al., 2014; Hummitzsch et al., 2015) suggests the presence of germ line stem cells in mammalian ovary. However, their ability to replenish the germ cell pool at an adult or postnatal stage during the reproductive phase is not very clear. Sun et al. (2014) reported that ASCs also possess the potential to differentiate into germ cells and oocytes that can be fertilized and give rise to embryos and offspring. In this context, Zou et al. (2009) isolated female germline stem cells in postnatal mammalian ovaries from adult mice. After culture, these stem cells underwent oogenesis, and subsequently the recipient mice produced offspring. White et al. (2012) demonstrated that both adult mice and human ovaries possess mitotically active germ cells that can differentiate into oocytes both *in vitro* and *in vivo*. Germ-line stem cells have been reported in mice and human ovaries by several research groups and have been recently reviewed elaborately (Virant-Klun et al., 2011; Telfer and Albertini, 2012; Woods et al., 2012; 2013; Dunlop et al., 2014; Hanna and Hennebold, 2014; Hummitzsch et al., 2015). However, studies on human ovarian stem cells are relatively few in number because of scarcity of ovarian tissue for research. Bukovsky et al. (2005) demonstrated differentiation of surface epithelium of post-menopausal human ovary and development into oocytes and blastocysts *in vitro*. Thus, those stem cells generated functional oocytes *in vitro* irrespective of age and condition (menopausal and premature ovarian failure) (Bukovsky and Caudle, 2012). Additionally, McLaughlin et al. (2015) developed a model to estimate woman ovarian follicle reserve by non-invasive means, which calculates non-growing

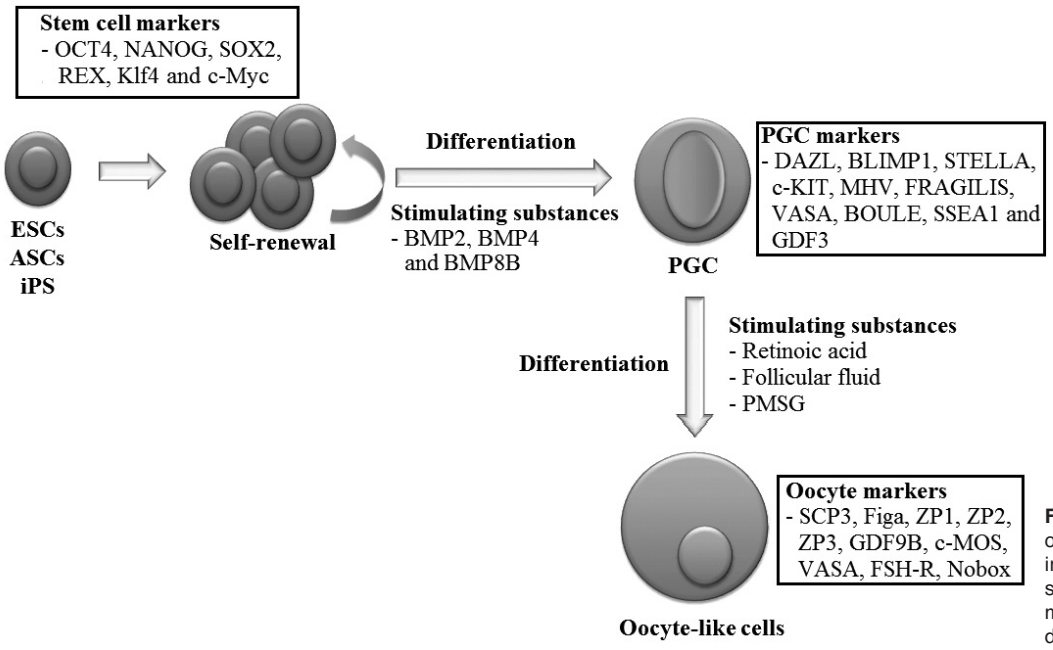

Fig. 1. Schematic presentation of the development of stem cells into oocyte-like cells, and the stimulating substances and markers for each stage of differentiation.

follicle density in the human ovarian cortex, using ovarian biopsies, and could be used in a variety of experimental and pathological situations.

By using various techniques, Virant-Klun et al. (2008, 2009, 2013) and Virant-Klun and Skutella (2010) identified putative stem cells in ovarian sections and in scraped ovarian surface epithelium (OSE) from post-menopausal women and from those with premature ovarian failure (POF), which could differentiate into oocyte-like structures and parthenotes *in vitro*. Parte et al. (2011) reported two distinct stem cell populations, namely, pluripotent very small embryonic-like stem cells expressing nuclear *OCT4* and slightly bigger progenitor stem cells, termed ovarian germ stem cells (OGSCs), which express cytoplasmic *OCT4* in peri-menopausal women and other higher mammalian species. Similar results were observed in adult human (Bhartiya et al., 2010) and mice (Bhartiya et al., 2012) ovaries. The putative stem cells differentiated spontaneously in cultures to give rise to oocyte-like structures and parthenotes (Parte et al., 2011), and expressed both pluripotent (*OCT4, OCT4A, SSEA4, NANOG, SOX2, TERT, STAT3*) and germ cell (*c-KIT, DAZL, GDF9, VASA*) markers.

Bhartiya et al. (2012) reported that mouse ovarian stem cells lodged in the ovarian surface epithelium (OSE) are modulated by pregnant mare serum gonadotropin (PMSG) resulting in neo-oogenesis and postnatal follicular assembly. There is evidence that FSH modulates ovarian stem cells through an alternatively spliced variant of FSH receptor. After *in vitro* culture of OSE, sheep stem cells underwent potential self-renewal and clonal expansion as germ cell cysts (Patel et al., 2013). In mice, PMSG treatment resulted in augmented stem cell activity, neo-oogenesis and primordial follicle assembly (Bhartiya et al., 2012). Stem cells isolated from the skin of porcine fetuses also have the ability to differentiate into oocyte-like cells (OLCs) after stimulation with follicular fluid (Dyce et al., 2006). These cells i) express germ-cell markers, (*OCT4, GDF9B, DAZL* and *VASA*), ii) form follicle-like aggregates that secrete oestradiol and progesterone, iii) respond to gonadotropin stimulation, and iv) express oocyte markers, such as *zona pellucida* (*ZP*), and the meiosis marker, *synaptonemal complex protein 3* (*SCP3*) (Dyce et al., 2006). In mice, Dyce et al. (2011a) reported that newborn mice skin-derived stem cells are also capable of differentiating into early OLCs that express oocyte markers. Dyce et al. (2011b) showed the differentiation of cells derived from porcine fetal skin in OLCs. These authors demonstrated the expression of connexin-37 and connexin-43, which are proteins found in ovarian follicles. Besides that, the expression of meiosis markers *DMC1* and *synaptonemal complex protein*, as well as oocyte specific genes (*OCT4, GDF9B, DAZL, VASA, ZPB* and *ZPC)* were detected in the OLCs formed *in vitro* (Dyce et al. 2011b). Linher et al. (2009) also demonstrated that stem cells isolated from fetal porcine skin have the potential to form primordial germ cells (PGCs) that express specific markers (*OCT4, FRAGILIS, STELLA, DAZL* and *VASA*) and originate putative oocytes after culture. Park et al. (2013) induced overexpression of DAZL using a lentiviral approach in porcine somatic stem cells and observed an increase in the formation of Fragilis-positive cells. In addition, an increase in the levels of mRNA for germ cell markers (*OCT4, STELLA* and *VASA*) was seen. Besides that, high levels of transcripts associated with meiosis (*SCP3, DMC1, REC8* and *STRA8)* were detected. Park et al. (2014) also isolated stem cells from postnatal mouse skin and differentiated into OLCs that were able to restore endogenous serum estradiol levels after transplantation. More recently, Tan et al. (2016) showed that retinoic acid stimulates the differentiation of skin-derived stem cells into PGCLCs.

Stem cells were also isolated from adipose tissue and ovarian stroma from pigs and cultured in the presence of follicular fluid (Song et al., 2011). These cells expressed

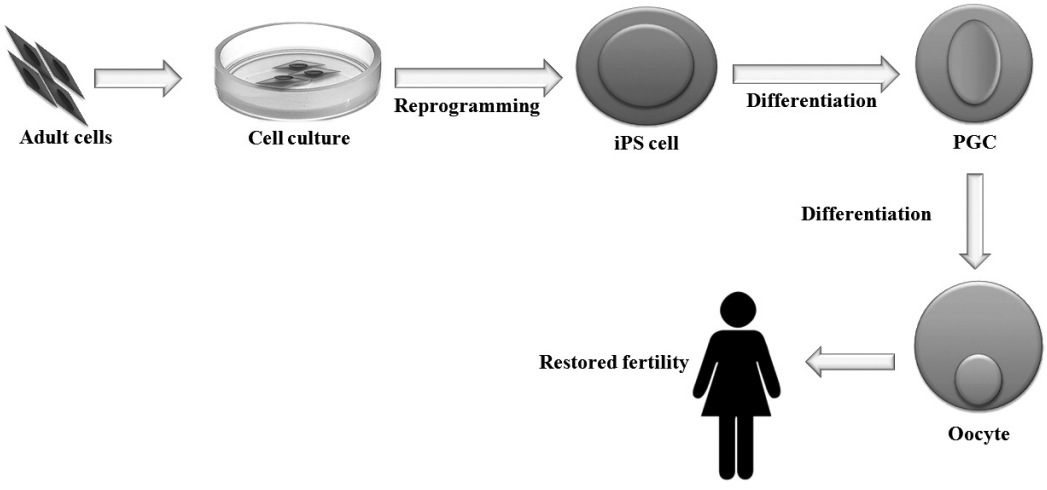

Fig. 2. Procedure for restoring fertility by differentiating iPS into oocytes.

transcription factors, such as *OCT4, NANOG* and *SOX2* and exhibited their potential for *in vitro* oogenesis, since they expressed markers like *OCT4, GDF9B, C-MOS, VASA, DAZL, ZPC* and *FSHR* after culture. The described results indicate that stem cells from ovarian tissue may differentiate into oocytes (Song et al., 2011). Sun et al. (2015) demonstrated that Activin-A increases PGCLCs formation from mouse skin derived stem cells. After 16 days of culture in presence of Activin-A, the cells expressed meiosis markers, such as *STRA8, DMC1, SYCP3* and *SYCP1* (Sun et al., 2015). Ge et al. (2015a) demonstrated that human fetus skin-derived stem cells express germ cell markers, like *DAZL* and *VASA*, and can be differentiated into early germ cell-like cells.

Table 1. Molecular markers for stem cells, PCGs, oocytes and their functions.

Markers	Functions	References
Stem cells		
OCT4	Transcription factor associated with self-renewal of undifferentiated embryonic stem cells, used as a marker for undifferentiated cells.	Lavial et al., 2007; Hu et al., 2015
NANOG	Transcription regulator involved in ICM and ESC proliferation and self-renewal, and prevents the differentiation of ESC in towards extraembryonic endoderm and trophectoderm lineages.	Lavial et al., 2007; Singhal et al., 2015
SOX2	Transcription factor associated with controls the expression of genes involved in embryonic development such as YES1, FGF4, UTF1 and ZFP206. Furthermore, it is essential for early embryogenesis and for embryonic stem cell pluripotency.	Yu et al., 2014
REX1	Marker of pluripotency, is usually found in undifferentiated embryonic stem cells, its regulation is also critical in maintaining a pluripotent state, as the cells begin to differentiate, Rex1 is severely and abruptly downregulated	Lavial et al., 2007
Klf4	Mediator to LIF-Stat3 signal changes, and directly binds to the promoter of Nanog to help Oct4 and Sox2 in regulating the expression of Nanog	Singhal et al., 2015
c-Myc	Regulator the self-renewal and pluripotency of mESC as well as pluripotent cells of the early embryo	Singhal et al., 2015
SSEA4	Cell membrane protein of ESC	Zhao et al., 2012
STAT3	Main extracellular signal that sustains mouse ESCs self-renewal and pluripotency is the activation of leukemia inhibitory factor (LIF) pathway	Niwa et al., 2009
BRG1	Important role in maintaining pluripotency by fine-tuning the expression level of Oct4 and other pluripotency-associated genes.	Singhal et al., 2014
Cx43	Mouse ESC express this connexin and form functional gap junctions during growth	Dyce et al., 2014
PGC		
DAZL	Protein involved in early germ cell differentiation	Panula et al., 2011; Clark et al., 2004; Kerr and Cheng, 2010
BLIMP1 or PRDM1	First indicator of germ cell fate	Yu et al., 2009a,b
STELLA	Gene initial germline	Hu et al., 2015; West et al., 2006
c-KIT	Migration and survival of PGCs	Marqués-Marí et al., 2009; Cai et al., 2013
MVH	Germ pre-meiotic cell-specific marker	Cai et al., 2013; Toyooka et al., 2000
FRAGILIS	Early indicator of germ cell fate	Medrano et al., 2012
VASA	Germ pre-meiotic cell-specific marker	Hu et al., 2015; Medrano et al., 2010; Zhu et al., 2012
BOULE	Genes modulate primordial germ-cell and haploid gamete formation	Kee et al., 2009
GDF3	Protein that modulate TGF-β superfamily members, e.g. potentiates the activity of NODAL	Levine and Brivanlou, 2006
Oocytes		
SCP1, SCP3	Component of the synaptonemal complex, and this complex is involved in synapsis, recombination and segregation of meiotic chromosomes.	Hu et al., 2015; Yuan et al., 2000
Figa	Factor required for progression of germ cells through the pachytene stage of MI	Marqués-Marí et al., 2009; Liang et al., 1997
ZP1, ZP2 e ZP3	Glycoproteins are markers of female germ cells in post-meiotic development which are only secreted by oocytes within primary follicles, to form a glycocalyx known as the zona pellucida	Hu et al., 2015; El-Mestrah et al., 2002
GDF9	Key factor regulating germ cell development, and act as master regulators oocyte-specific transcription factors	Salvador et al., 2008; Sriraman et al., 2015
GDF9B	Expressed in the human fetal ovary at the time of oocyte nest breakdown and primordial follicle formation	Bayne et al., 2015
Nobox	Essential for follicle formation and oocyte survival, and regulates the expression of GDF9 in humans	Singhal et al., 2015
c-MOS	Specifically expressed in male and female germ cells where it appears to play a central role in regulating the meiotic cell cycle	Cooper, 1994
FSH-R	Stimulates proliferation and differentiation of progenitors ovarian germ stem cells to oocytes and primordial follicle assembly	Bhartiya et al., 2012

These cells were differentiated into OLCs after culture in presence of porcine follicle fluid. Regarding proliferation of PGCs, Shen et al. (2012) reported that the heparin-binding growth factor midkine (MK) acts as a mitogenic factor for these cells and it also reduces apoptosis. Besides that, MK induces a reduction in expression of *DAZL* and meiosis-related genes (*SCP3* and *DMC1*), suggesting a role in preventing a shift in the PGC phenotype toward meiosis. Fig. 1 shows the differentiation of adult stem cells into oocytes-like cells, as well as the stimulating substances and markers for each stage of differentiation. The function of markers for stem cells, PGCs and oocytes are shown in Table 1.

Differentiation of primordial germ cells and oocytes from the iPS cells

The differentiation of induced pluripotent stem cells (iPS) into PGCs has been observed in several studies. Park et al. (2009) demonstrated the formation of PGCs from reprogrammed skin fibroblasts of human species. However, PGCs derived from iPS cells did not start imprint erasing efficiently, suggesting that more studies are needed to elucidate the germ cell induction mechanisms from iPS. In another study, Panula et al. (2011) compared the efficiency of human iPS derived from adult and fetal somatic cells to form primordial and meiotic germ cells. They showed that around 5% of human iPS have the potential to differentiate into PGCs after induction with BMPs. In addition, they had the ability to express specific markers of germ cells (*DAZ, DAZL* and *BOULE*) and the iPS cells formed meiotic cells with extensive synaptonemal complexes. In human species, Eguizabal et al. (2011) reported generation of postmeiotic cells from iPS from different origin (keratinocytes and cord blood). These cells demonstrated complete and robust meiotic competence, opening the way for the production of *in vitro* gametes in this species.

Hayashi et al. (2011) demonstrated that mouse iPS cells differentiate into epiblast-like cells, primordial germ cell-like cells (PGCLCs), and fertile oocytes, successively. After reconstitution of ovaries with PGCLCs, these cells differentiated into germinal vesicle-stage oocytes and contributed to fertile offspring after *in vitro* maturation and fertilization (Hayashi et al., 2012). Eguizabal et al. (2011) demonstrated the differentiation of female iPS into haploid cells following the detection of SCP3 and H2AX proteins, which are indicators of meiotic competence. Singhal et al. (2015) demonstrated the generation of germ cell-like cells and oocyte-like cells from goat iPS formed from fibroblast cells. These germ cell-like cells were characterized by expression of germ cell specific markers (*VASA, DAZL, STELLA* and *SCP3*) at transcription and protein level. These iPS differentiated into primordial germ cell-like cells in the presence of retinoic acid and BMP4. Among the differentiated germ cell-like cells, oocyte-like structures were observed. Fig. 2 shows the strategy to restore fertility by differentiating iPS into oocytes.

Mechanisms of cellular reprogramming to produce iPS

Efficient reprogramming methods have been explored since the first report of the generation of human induced pluripotent stem cells (Takahashi et al., 2007; Yu et al., 2007). In differentiated cells (adult cells), absence of expression of the pluripotency genes may be due to changes in chromatin due to DNA methylation or histone acetylation in the promoter region of these genes (Li, 2002; Li et al., 2007; Cedar and Bergman, 2009). Currently, new methods have been applied in the cellular reprogramming process, especially in the production of iPS cells, which include the use of episomal plasmids (Yu et al., 2009b), excisable expression systems (Soldner et al., 2009), recombinant cell-penetrating reprogramming proteins (Kim et al., 2009; Zhou et al., 2009), reprogramming mRNAs (Warren et al., 2010; Yakubov et al., 2010), or microRNAs (Anokye-Danso et al., 2011; Miyoshi et al., 2011). Furthermore, a growing number of compounds have been identified that can functionally replace reprogramming transcription factors, enhance efficiency of iPS generation and accelerate the reprogramming process (Zhang et al., 2012).

Takahashi and Yamanaka (2006) tested 24 different candidate factors that play important roles in the maintenance of pluripotency. They found that four transcription factors (*OCT4, SOX2, C-MYC* and *KLF4*) are involved in the generation of iPS, which are similar to ESCs in morphology, proliferation, and teratoma formation. In regard to aging and rejuvenation, the reprogramming process resets an aged, somatic cell to a more youthful state, elongating telomeres, rearranging the mitochondrial network, reducing oxidative stress, restoring pluripotency, and making numerous other alterations (Rohani et al., 2014). In goats, Singhal et al. (2013) reprogrammed adult female goat fibroblast cells into induced pluripotent stem cells using ectopic expression of *OCT4, NANOG* and *SOX2* genes and the germ-cells-like cells, generated from reprogrammed iPS, could be differentiated into goat oocytes-like structure. However, there have been concerns over the use of integrating retroviruses to deliver the iPS factors, which could potentially compromise the quality of or even cause tumorigenicity in the resultant iPS (Zhang et al., 2012). The efficiency of iPS cell induction is quite low: less than 1% of human fibroblasts that received transcription factors *OCT4*/*SOX2* and the oncogenes *KLF4*/*C-MYC* (together abbreviated to OSKM) become iPS cells. However, Tanabe et al. (2013) showed that, after receiving OSKM (Tanabe et al., 2013; 2014), the reprogramming process initiates in more than 20% of human fibroblasts.

There are several genes related to cell reprogramming, like *OCT4*. This gene is encoded by the gene *POU5F1* (Wu and Schöler, 2014) and is restricted in the blastomeres of the developing mouse embryo, the ICM of blastocysts, the epiblast, germ cells and oocytes. It is also expressed in pluripotent stem cells, including ESCs (Pesce et al., 1998; Yamanaka, 2007). Singhal et al. (2014) demonstrated that *Brahma-Related Gene 1*

(*BRG1*) is essential for early embryonic development, but also enhances the efficiency of reprogramming somatic cells in murine species.

During the reprogramming process, remodeling of the epigenome and modulations of the epigenetic processes may facilitate conversion of cell fate by making cells more permissive to these epigenomic changes. Enzymes that stand out in this process are histone deacetylase (HDAC), histone methyltransferase (HMT), histone demethylase (HDM) and DNA methyltransferase (DNMT) (Zhang et al., 2012).

Among the compounds that can be used in reprogramming, 5-azacytidine, N-Phthalyl-L-Tryptophan (RG108), Valproic acid (VPA), Trichostatin A (TSA), Sodium butyrate (NaB), and other molecules (Federation et al., 2014) can be highlighted. Demethylation of one or more (unknown) loci of DNA is a critical step in the late stages of direct reprogramming. Inhibition of *DNMT1* lowers this kinetic barrier, thereby facilitating the transition to pluripotency (Mikkelsen et al., 2008).

DNA demethylation has been recently reported after use of 5-azacytidine or RG108 during cellular reprogramming (Shi et al., 2008; Okita and Yamanaka, 2011; Pennarossa et al., 2013, 2014; Federation et al., 2014). 5-Azacytidine is a chemical derivative of the DNA nucleoside cytidine, the only difference being the presence of a nitrogen atom at position 5 of the cytosine, the same site at which DNA methylation occurs. Thus, 5-azacytidine causes DNA demethylation or hemi-demethylation. DNA demethylation can regulate gene expression by relaxing chromatin structure. This is detectable as an increase in nuclease sensitivity. This remodeling of chromatin structure allows transcription factors to bind to the promoter regions, assembly of the transcription complex, and gene expression, particularly genes associated with cell pluripotency. As an analog of cytosine, DNA polymerase does not recognize the difference between 5-azacytidine and cytosine and will incorporate 5-azacytidine during DNA replication (Christman, 2002; Federation et al., 2014). Pennarosa et al. (2014) exposed pig dermal fibroblasts to 5-azacytidine for 18 h, followed by a protocol for the induction of endocrine pancreatic differentiation. The results demonstrated changes in cell morphology and expression of pluripotency genes (*OCT4, NANOG, SOX2* and *REX1*) (Pennarosa et al., 2014). The cells expressed insulin and were able to release it in response to a physiological glucose challenge.

Valproic acid (VPA), 2-propyl-pentanoic acid, is a short-chain branched fatty acid (Almutawaa et al., 2014). This VPA, an histone deacetylases (HDAC) inhibitor, increases reprogramming efficiency of human fibroblasts, enabling the efficiency of primary human fibroblasts infected with the transcription factors *OCT4, SOX2* and *KLF4* (Huangfu et al., 2008). The molecular mechanisms of VPA action involves several protein kinase pathways that have been suggested to be the targets for this drug (Monti et al., 2009). VPA has been shown to inhibit the activity of HDACs, resulting in chromatin remodelling and changes in gene expression (Phiel et al., 2001; Gotfryd et al., 2011). Trichostatin A (TSA) is one of the most potent known histone deacetylase inhibitors. This hydroxamic acid is *in vitro* active at nanomolar concentrations and inhibits HDACs with zinc-containing catalytic sites, leading to accumulation of acetylated histones in the nucleus and subsequent activation of target genes (Taddei et al., 2005; Dokmanovic et al., 2007; Kim et al. 2009). Sodium butyrate (NaB) increases histone acetylation and is a potent HDAC inhibitor that causes hyperacetylation of histones H3 and H4 in mammalian cells (Candido et al., 1978; Nör et al., 2013).

Clinical potential

Pluripotent stem cells hold great promise in the field of regenerative medicine, because they can propagate indefinitely. In addition, these cells can lead to every other cell type (sperm, oocytes, neurons, heart, pancreatic, and liver cells), they represent a single source of cells that could be used to replace those lost to damage or disease.

Nearly 72.4 million people have fertility problems, caused by various underlying pathologies, with exposure to toxicants or immune-suppressive treatments, in cases with gonadal insufficiency due to POF or azoospermia (Boivin et al., 2007; Bhartiya et al., 2014; Volarevic et al., 2014). Currently, several advances have been made in assisted reproduction treatment, especially the generation of gametes derived from pluripotent stem cells (Bhartiya et al., 2014). Considering that the use of ESCs has led to deliberate ethical controversy (Klimanskaya et al., 2006), ASCs or iPS can be considered the most suitable type of cells to produce patient-matched oocytes that can be used to recover fertility. In this way, Park et al. (2014) differentiated skin derived stem cells into structures similar to functional ovarian follicles that were capable of producing hormones, such as estradiol. The artificial germ cells proved to be functional because they were capable of differentiating in gametes that give rise to healthy individuals. Ge et al. (2015b) observed similar behavior in mouse PGCs differentiated *in vitro*. These authors suggested that the gonadal ridges produce factors that are essential for PGC survival, proliferation and differentiation, but it is necessary to establish the culture conditions suitable to complete oogenesis *in vitro*. Despite being similar to *in vivo* formed oocytes at molecular levels, using artificial oocytes, differentiated from stem cells, for infertility treatment may need long-term investigation. However, in the short term, these female germ cell like cells, which have the function of hormone secretion, may be utilized to treat hormone disorders, being promising candidates for delaying aging for female.

Offspring of differentiated oocytes from IPs (mice: Hayashi et al., 2012), ESCs (mice: Hayashi et al., 2012)

and ASCs (mice: Zou et al., 2009; Xiong et al., 2015) have been described in the literature. These advances were obtained in mice, by using a combined *in vitro* and *in vivo* system, i.e., the cells were expanded *in vitro* and then transplanted into ovaries of infertile mice. In human species, White et al. (2012) reported that ovaries of reproductive-age women, similar to adult mice, possess mitotically active germ cells that can be propagated *in vitro* and generate oocytes *in vitro* and *in vivo*, but offspring were not reported. It is important to consider that follicular/oocyte development in human species is far longer (~180 days) than in mice (21 days) (Gougeon, 2010). This fact highlights that the *in vivo* step is essential to have complete follicular development. It is well known that a number of different *in vitro* culture systems have been developed to grow follicles from different species, but it is clear that it is vital to optimize these systems, which include the use of supply of hormones, growth factors, as well as co-culture with granulosa and theca cells (Silva et al., 2016).

Final considerations

This review emphasizes the potential of embryonic stem cells, adult stem cells and induced pluripotent stem cells to produce germ cells and oocytes. Recent advances in cellular therapies have led to the understanding how stem cells can give rise to gametes and how somatic stem cells differentiate into germ cells and oocytes. This knowledge gives significant insight into the regulation of developmental gametes and has important implications for female fertility and regenerative medicine. More studies are needed in this area, to clarify what problems affect the achievement of female germ cells. Various studies addressed the potential of stem cells to differentiate into oocytes. However, more information is necessary for obtaining mature gametes of good quality, which subsequently have to go through a process of fertilization to produce viable offspring. It is expected that, in the near future, isolated stem cells could be able to differentiate into viable oocytes in a reproducible manner. These findings will not only contribute to enhancement of female germ cell induction but also to reduction of female infertility rates.

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