Stem Cell Technology, A Novel Approach To Derive Artificial Gametes

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Introduction

Between the 10 and 15% of all couples suffer from infertility. If it is due to a primary failure in germinal cells production, non-genetic parenthood is their only option to have children. But with the creation of **artificial gametes** (AGs) this problem could be solved. AGs are engineered cells with paternal or maternal DNA that exhibit the molecular and physiological characteristics of natural gametes.

In this project, normal gametogenesis will be first reviewed; next, in order to ensure that the created AGs are able to give rise to fertile and viable offspring, current used viability markers will be denoted; and finally, an approach using **stem cell technology** will be proposed to derive gametes.

Objectives

- Give a quick sight of the normal germ cell development highlighting the importance of primordial germ cells.
- Stress the importance of epigenetics in gametes production
- Compile the current knowledge about markers of gametogenesis viability.
- Present and review the state of the art in stem cell technology used to create AGs.

Methodology

The used methodology to develop this project is summarized in Figure 1.

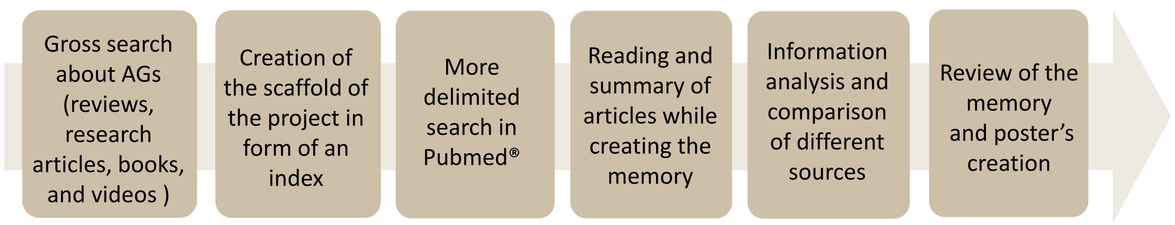

Fig. 1 The methodology followed to create this project.

Gametes creation

Before creating AGs able to produce healthy and fertile offspring, it is important to know the key processes for normal germ cell development to take place.

Normal gametes development and epigenetics

The primary undifferentiated, immortal, totipotent stem cells of spermatozoa and oocytes are called **primordial germ cells** (PGCs). In mice, PGCs originate around embryonic day 6.25 (E6.25) in the proximal epiblast of the post-implantational embryo when a cluster of the future germ cells start expressing the gene PRDM1 in response to **bone morphogenic protein** (BMP) **2**, **BMP4**, and **BMP8b**.

In addition, other transcriptional regulators of PGCs important for their specification, pluripotency maintenance, epigenetic reprograming (shown in green in figure 2), and inhibition of somatic genes; will be induced.

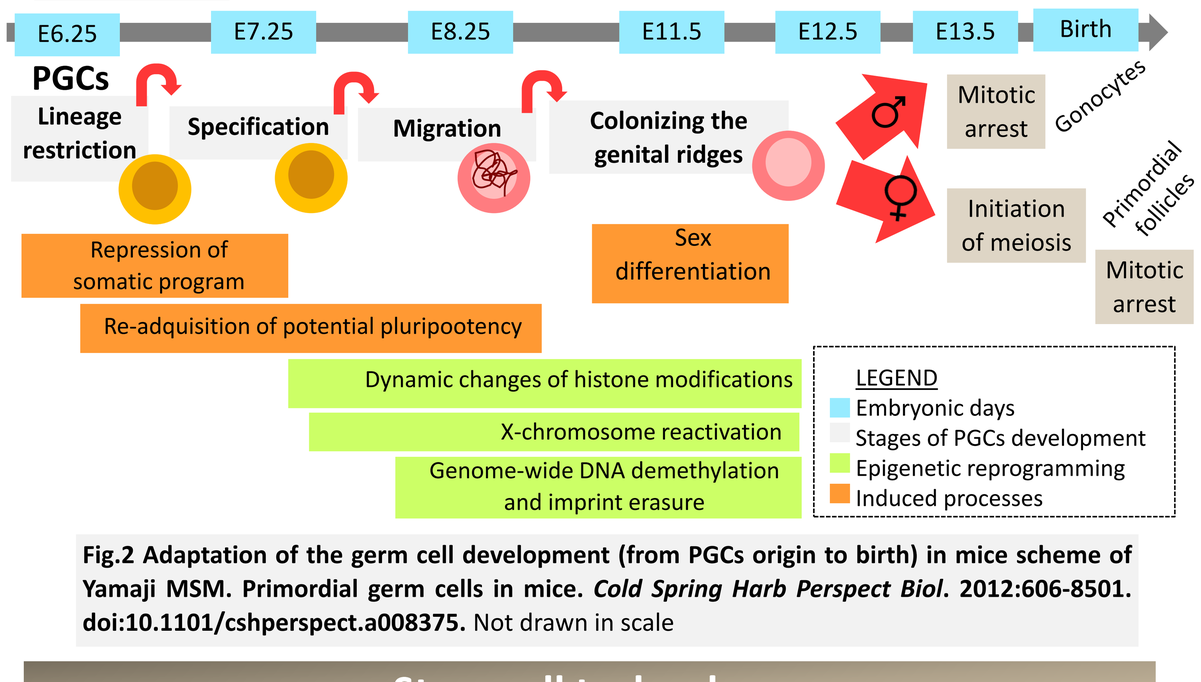

Fig.2 Adaptation of the germ cell development (from PGCs origin to birth) in mice scheme of Yamaji MSM. Primordial germ cells in mice. *Cold Spring Harb Perspect Biol*. 2012:606-8501. doi:10.1101/cshperspect.a008375. Not drawn in scale

Stem cell technology

As well as PGCs are differentiated from epiblast, PGCs could be in vitro derived from **pluripotent stem cells** (SCs) such as embryonic stem cells (ESCs). Several research groups have been looking for the multi-step process to derive PGCs form SCs (Table 1). Different types of cultures have been used to achieve it (Figure 3).

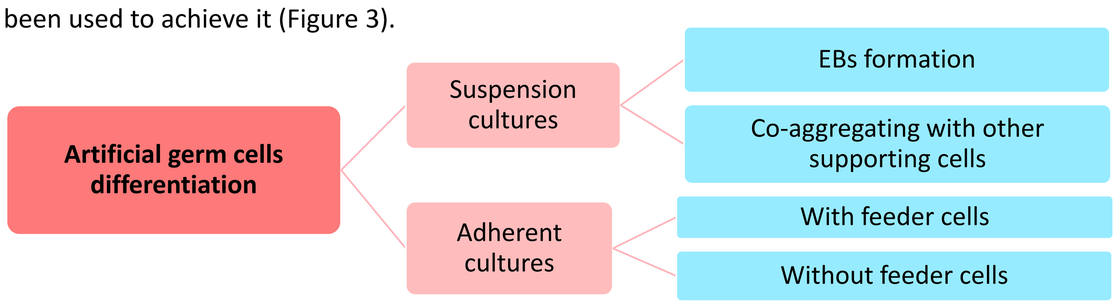

Fig. 3 Types of germ cell cultures Adherent (with or without feeder cells) and in suspension (promoting embryoid bodies (EBs) formation or co-aggregating with other supporting cells).

Table 1. Examples of mice and human *in vitro* germ-cell differentiation studies

AUTHORS	SOURCE CELLS	CULTURE METHOD	*IN VITRO* DERIVED CELLS	EVALUATION
Hübner et al. (2003)	Mouse ESCs (XY, XX)	Adherent culture	Follicle-like structures	Expression of markers Gdf9 and Sycp3; estradiol production
West et al. (2006)	Mouse ESCs (XY)	EBs	PGCs and male haploid cells	Expression of markers Tex14 and Acrosin; genomic imprint (H19,IGF2R); and genome ploidy
Tilgner et al. (2008)	Human ESCs (XX)	Adherent culture with retinoic acid, EBs	PGCs	Expression of markers Stella and Vasa; genomic imprint IFG2
Kee et al. (2009)	Human ESCs (XY, XX)	Adherent culture with BMP4, BMP7, BMP8b, and overexpression of DAZL/DAZ/BOULE	PGCs, spermatids	Expression of markers VASA, and Acrosin; genomic imprint (H19, PEG1); genome ploidy

However, to date, the majority of studies have been carried out using mice ESCs, due to the ethical concerns of using human cells. These ethical drawbacks of using ESCs have been overcome with the discovery by Dr. Yamanaka and colleagues of **induced pluripotent stem cells** (iPSCs) → Adult somatic cells reprogramed into embryonic stem-like cells that express 4 oncogenes OCT4, SOX2, KLF4, and c-Myc.

Several groups claimed to have derived AGs from iPSCs. The most relevant was a protocol made by Katsuhiko Hayashi and Mitinori Saitou, published in *Nature* in 2013 explaining an approach to generate eggs from mouse ESCs and iPSCs.

Once they are differentiated, PGC-like cells have to be isolated and purified. Specific antibodies that detect cell-surface markers can be used to sort them, despite most of them are not exclusive of germ cells.

Limitations of *in vitro* models of ESCs/iPSCs

- ✓ Sometimes cell behaviors and patterns of gene expression are different *in vitro* from *in vivo*
- ✓ Epigenetic alterations at imprinted loci occur when gametes are manipulated with *in vitro* fertilization and intra cytoplasmic sperm injection.

Viability markers

Once AGs are generated, it is utterly important to determine their viability before using them to conceive (Figure 4). But because there is not a consensus yet in which markers use, there is an imminent need to identify gametogenesis and normal physiological function markers.

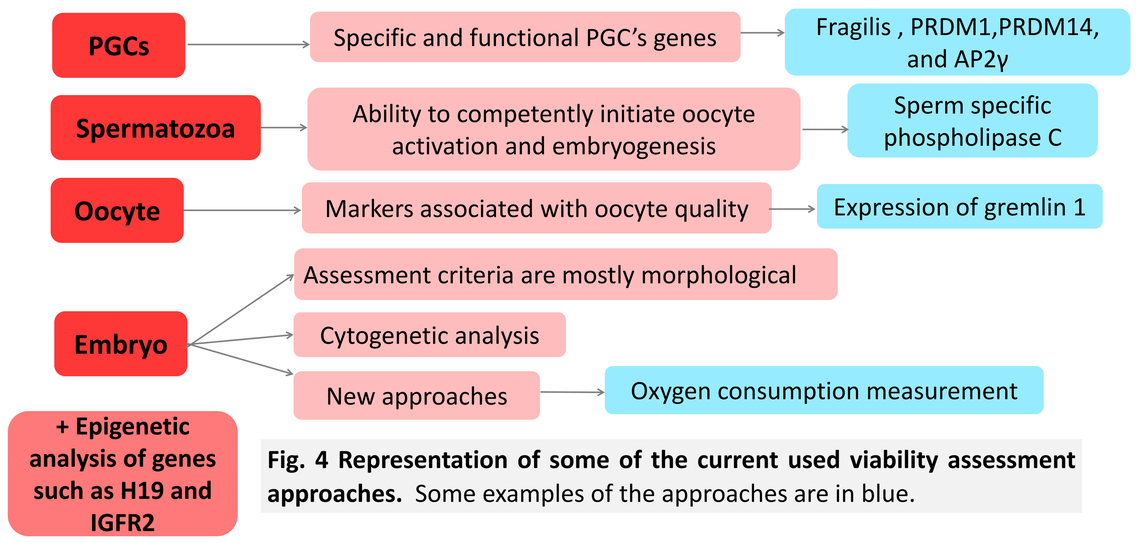

Fig. 4 Representation of some of the current used viability assessment approaches. Some examples of the approaches are in blue.

Future perspectives

› Continue studying normal germ cells development.
› Looking for a more reliable and easier method to assess viability.
› Develop feasible, systematic, and highly efficient germ cells differentiation protocols to produce fertile gametes completely *in vitro*.

Conclusions

- The creation of AGs, seem to be the final solution to genetic parenthood for those individuals who cannot have children.
- PGCs transcriptional program is altered in order to be specified, maintain their pluripotency, reprogram their epigenetic program, and inhibit their somatic genes.
- Using a multi-step process exposing SCs (such as ESCs and iPSCs) to specific stimuli, PGCs, which will give rise to germ cells, can be artificially derived.
- To date, Hayashi and Saitou have been the most successful investigation group creating mice AGs that could give rise to fertile offspring using stem cell technology.
- Before using AGs to conceive, their viability has to be assessed. Depending on the cell stage, several approaches have been proposed to evaluate it.
- Further investigation is needed before being able to use it as regular clinic procedures.