OOCYTES FROM INDUCED PLURIPOTENT STEM CELLS

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Bachelor's Degree Final Thesis
Genetics 2021
Bibliographical Review

INTRODUCTION

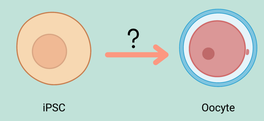
Stem cells are non-(fully) specialised cells that can differentiate into different cell types under certain conditions.

Although embryonic stem cells (ESCs) have been classically proposed as a therapeutic alternative, **induced pluripotent stem cells (iPSCs) have further potential applications.**

→ Auto-immune cells can be generated from patient's own somatic cells, thus avoiding ethical concerns and the possible post-transplantation immune rejection.

Artificial oocytes are mature eggs generated *in vitro* by specification and maturation of **primordial germ cells** (PGCs) or by direct differentiation of **pluripotent cells** to the female germ-cell lineage.

Can oocytes be generated from iPSCs?



→ They should be able to undergo **fertilisation** and **embryogenesis**, in order to transmit their genetic and epigenetic information to the next generation.

OBJECTIVES

The main objective is to **COLLECT THE MAIN STRATEGIES TO DERIVE OOCYTES FROM iPSCs *IN VITRO***, as well as the most important results to date. Moreover, there are secondary objectives:

1. Define the term artificial oocyte and its potential applications.
2. Provide an overview of the main pluripotent stem cells used to derive oocytes, emphasising in why iPSCs are a promising source.
3. Review the limitations of this technology and the barriers that need to be overcome before applying this approach in clinic.

METHODOLOGY

An extensive search was made mainly in **PubMed** and **Google Scholar** databases, using the appropriated combination of keywords and Boolean operators. Results were filtered by date in order to obtain the latest results, and by impact factor of the journal.

RESULTS

STRATEGIES TO DERIVE GERM CELLS FROM PLURIPOTENT STEM CELLS

Suspension culture
Embryoid bodies (EBs) formation
Co-aggregation with somatic ovarian cells
EBs formation with supplementary factors

Spontaneous differentiation

Adherent culture
Feeder-cell free
On feeder cells
With supplementary factors

iPSCs

Epiblast-like cells / incipient Mesoderm-like cells intermediate state

Gene transduction: overexpression of key genes

***In vivo* transplantation of iPSCs to the ovaries**

FUTURE APPLICATIONS OF ARTIFICIAL OOCYTES

→ **Clinical application**: generation of gametes from infertile patients, allowing them to have genetically related offspring.

→ **Scientific application**: creation of an *in vitro* system to reconstitute/study human female germ cell development.

STATE OF THE ART

Table 1. Relevant results of the state of the art about the obtention of oocytes from mouse and human iPSCs (miPSCs / hiPSCs).

iPSCs source	Differentiation method	Derived cells	Offspring	Remarks	Reference
miPSCs	Adherent culture: EBs formation + PFF + RA	OLCs	✗	Expression of ZP3. Ovarian-like structures that seemed to contain follicle-like structures.	Niu *et al.*, 2013
	Spontaneous differentiation	OLCs	✗	Expression of ZP1 and early gametogenesis and ovarian markers. miPSCs from granulosa cells.	Anchan *et al.*, 2015
	EpiLCs and PGCLCs induction, through rOvaries and *in vivo* transplantation	Metaphase II oocytes	✓	Dependent on an *in vivo* system.	Hayashi *et al.*, 2012
	EpiLCs and PGCLCs induction, through rOvaries	Metaphase II oocytes	✓	Complete *in vitro* process.	Hikabe *et al.*, 2016
	EpiLCs and PGCLCs induction, through rOvaries and *in vivo* transplantation	Metaphase II oocytes	✓	Dependent on an *in vivo* system. miPSCs from granulosa cells.	Tian *et al.*, 2019
hiPSCs	Adherent culture: overexpression of germ line genes + induction with BMPs	PGCLCs	✗	Expression of PGCs markers.	Panula *et al.*, 2011 Medrano *et al.*, 2012
	Spontaneous differentiation + culture with germ line inductors	PGCLCs	✗	Expression of PGCs markers, but increased methylation of *H19*.	Eguizabal *et al.*, 2011
	iMeLCs and PGCLCs induction	PGCLCs	✗	Gene expression similar to day 7 PGCs.	Sasaki *et al.*, 2015
	iMeLCs and PGCLCs induction, through xrOvaries	Oogonia-like cells	✗	Starting of the epigenetic reprogramming, elimination of parental imprinting and reactivation of X chromosome.	Yamashiro *et al.*, 2018

Abbreviations. Embryoid bodies (EBs), porcine follicular fluid (PFF), retinoic acid (RA), Epiblast-like cells (EpiLCs), primordial germ cell-like cells (PGCLCs), reconstituted ovaries (rOvaries), bone morphogenetic proteins (BMPs), incipient mesoderm-like cells (iMeLCs), xenogeneic reconstituted ovaries (xrOvaries), oocyte-like cells (OLCs), zona pellucida (ZP), primordial germ cells (PGCs).

CONCLUSIONS

1. **iPSCs are a powerful and promising source for future oocyte obtention**, with clinical and scientific applications.
   However, the efficiencies of obtaining mature cells are low, so this field is still in experimentation and more research is needed.
2. **Oogenesis is a dynamic process** involving the ovarian niche and a continuous interaction between oocyte and somatic cells.
   ↓
   It is important to mimic this niche as much as possible.
3. Before applying these findings to clinic, further research will be needed to **ensure the safety of the process** in terms of oocyte viability and functionality.
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   Ensure a healthy offspring.

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