In vitro male germ cells from embryonic and induced pluripotent stem cells: A promising treatment for male infertility

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1 Introduction

Infertility is a disease affecting the reproductive system that concerns up to 15% of the couples worldwide and one third of the cases are caused by a male factor. Even though assisted reproductive technology (ART) can help more than 80% of patients suffering from infertility to have a child, individuals without functional gametes cannot take benefit from these techniques to have genetically related progeny. **Artificial gametes**, which are germ cells produced in vitro, could be a potential treatment to overcome this issue. These in vitro-generated cells can be obtained from two main sources of stem cells**: embryonic stem cells** (ESCs), which are derived from the inner cell mass of embryos at the blastocyst stage, and **induced pluripotent stem cells** (iPSCs) that are somatic cells genetically reprogrammed with transcription factors that provide pluripotency such us Oct4, Sox2, c-Myc, Klf4, Nanog and Lin28.

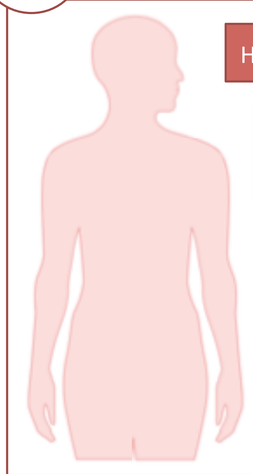
2 Objectives

- Determine what artificial gametes are and delve into the future applications of these in vitro obtained germ cells.
- Acquire knowledge about the existing strategies for the generation of male artificial gametes from ESCs and iPSCs.
- Have an overview about the most important studies performed in the last years regarding the in vitro generation of male germ cells from ESCs and iPSCs, knowing the advances achieved and the limitations that have to be overcome.

3 Methodology

- The first step was looking for papers, specially reviews, through the Pubmed database provided by the National Center for Biotechnology Information (NCBI). Nevertheless, the online search tool called Àrea de Recursos Electrònics (ARE) of Universitat Autònoma de Barcelona (UAB) was also used.
- Reports were found using appropiate combination of key words (e.g.) and the search results were always filter by publication date to see only those published between 2006 and 2016.
- After reading the articles of interest, the relevant information was highlighted and used as a point of reference to elaborate the present written.

4 Future applicability of male artificial gametes



- Help people having genetically related offspring
- Improve our knowledge about the factors modulating human gametogenesis and the mechanisms involved in infertility
- Provide a large amount of gametes for further experiments
- Preserve rare species and transgenic animals with reproductive problems
- Offer training to students with the performance of reproductive techniques

5 Strategies to obtain male germ cells in vitro

Currently, there are two feasible methods to obtain male germ-like cells from ESCs and iPSCs. One of them consists in generating male gametes directly in vitro while the other combine in vitro differentiation with in vivo transplantation to obtain these cells.

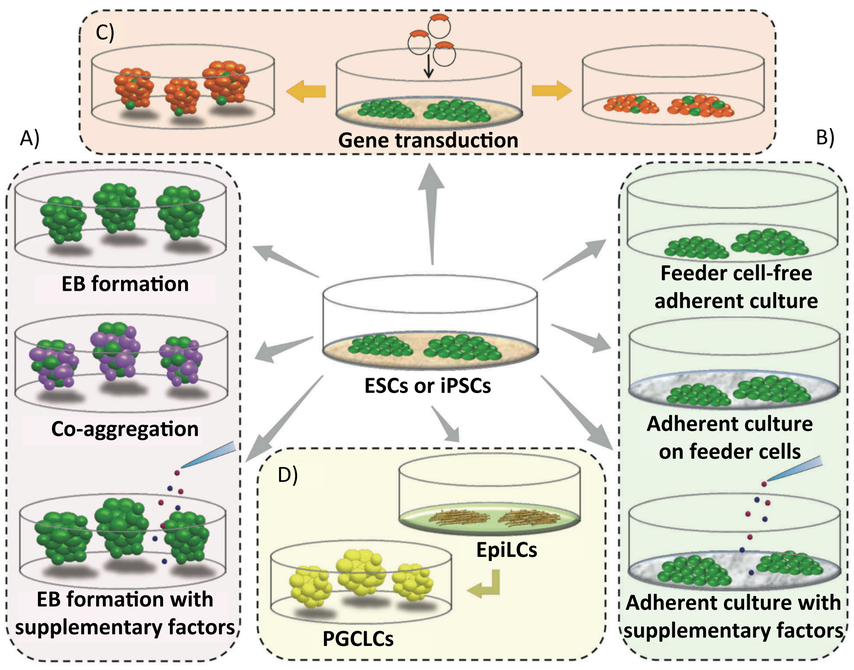


Figure 1. Methods existing for in vitro male germ cells generation from ESCs and iPSCs (adapted from Imamura et al., 2014). A) Suspension cultures can be carried out through EB formation or co-aggregation with other supporting cells (e.g. gonadal cells or fibroblasts) and its efficiency can be increased with additional factors like BMPs and RA. B) Adherent cultures can be performed in combination with feeder cells like fibroblasts or with supplementary factors (e.g. BMPs, RA, T, etc.). C) Gene transduction includes the overexpression of germ cell genes (e.g. STRA8 or DAZL) into ESCs or iPSCs before culturing these pluripotent stem cells in adherent or suspension cultures. D) Pluripotent stem cells can also be differentiated into PGCLCs through an EpiLCs intermediate differentiation stage when exposed to several factors (e.g. bFGF, ActA and BMPs).

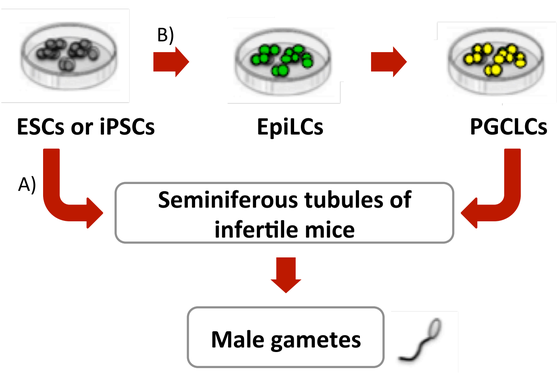


Figure 2. In vitro and in vivo differentiation protocols to obtain male gametes from ESCs and iPSCs (adapted from Mouka et al., 2016). A) Directly transplantation of ESCs or iPSCs into the seminiferous tubules of immunodeficient mice depleted of germ cells gives rise to male germ cells. B) Transplantation into mice testis of PGCLCs, obtained after an EpiLCs intermediate differentiation stage from pluripotent stem cells, contributes to spermatogenesis and generates male gametes in vivo.

Abbreviations: EB, embryoid bodies: three-dimensional structures of pluripotent stem cells; BMPs, bone morphogenetic proteins; RA, retinoic acid; T, testosterone; STRA8, stimulated by retinoic acid gene 8; DAZL, deleted in azoospermia-like; PGCLCs, primordial germ cell-like cells; EpiLCs, epiblast-like cells; bFGF, basic fibroblast growth factor; ActA, activin A.

6 State of the art

Starting cells	Differentiation strategy	Cell type obtained	Remarks	References
Human ESCs and iPSCs	Adherent culture with RA, FRSK, rLIF, bFGF and R115866.	Spermatid-like cells	Cells exhibited deficient imprinting erasure with a strong but incomplete paternal reimprinting tendency.	Eguizabal et al., 2011
Mouse iPSCs	EB formation + RA + Transplantation into mice testis.	Spermatogonial stem cells (in vitro) and spermatid-like cells (in vivo)	-	Zhu et al., 2012
Mouse ESCs	Transfection with Prdm1, Prdm14 and Tfap2c + Induction in adherent culture of EpiLCs (with ActA and bFGF) and PGCLCs (with BMP4, BMP8b, LIF, SCF and EGF).	PGCLCs (in vitro) and spermatozoa (in vivo)	Obtained cells displayed correct imprinting in H19 allele and in vivo generated spermatozoa fertilized oocytes via ICSI and gave rise to healthy offspring.	Nakaki et al., 2013
Human iPSCs	Transfection with VASA + Adherent culture with BMP4, BMP8, RA and hrLIF + Transplantation into mice testis.	PGCLCs (in vitro and in vivo)	PGCLCs showed global DNA demethylation in vivo.	Ramathal et al., 2014
Mouse ESCs	Transfection with Prdm1 and Stella + Induction in adherent culture of EpiLCs (with ActA and bFGF) and PGCLCs (with BMP4, BMP8a, LIF, SCF and EGF) + co-culture with testicular cells, RA, BMPs, ActA, FSH and T.	Spermatid-like cells	Cells showed correct initiation of imprinting erasure in some alleles (e.g. Snrpn and H19). After ICSI into recipient oocytes offspring was obtained and it developed healthy to adulthood.	Zhou et al., 2016

Abbreviations: FRSK, forskolin; (h)rLIF, (human) recombinant leukaemia inhibitory factor; R115866, CYP26 inhibitor; Prdm1, PR domain containing 1; Prdm14, PR domain containing 14; Tfap2c, transcription factor ap2-gamma; SCF, stem cell growth factor; EGF, epidermal growth factor; H19, maternally imprinted gene; ICSI, intra-cytoplasmic sperm injection; VASA/MHV/DDX4, DEAD box polypeptide 4; Stella/Dppa3, developmental pluripotency associated 3; FSH, follicle-stimulating hormone; Snrpn, paternally imprinted gene.

7 Conclusions

- In vitro male germ cells generation from ESCs and iPSCs is still in an experimental stage in humans and mice but these cells could be a future treatment for male infertility.
- Further studies must be done in order to find the safest and most efficient method for generating completely normal male artificial gametes regarding to genetics and epigenetics.
- Before a clinical application in humans, it's crucial to confirm the biological function of in vitro generated human male gametes by creating human embryos and culturing them for a short term.

8 Bibliography

Eguizabal C, et al. Stem Cells. 2011;29(8):1186-1195. **Hou J**, et al. Reproduction. 2014;147(6):179-188. **Imamura M**, et al. Mol Reprod Dev. 2014;81(1):2-19. **Mouka A**, et al. Stem Cells Dev. 2016:25(7):509-521. **Nakaki F**, et al. Nature. 2013;501(7466):222-226. **Ramathal C**, et al. Cell Rep. 2014;7(4):1284-1297. **Zhou Q**, et al. Cell Stem Cell. 2016;18(3): 330-340. **Zhu Y**, et al. Asian J Androl. 2012;14(4):574-579.