What can stem cell technology offer to IVF patients?

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What can stem cell technology offer to IVF patients?

Running head: Stem cell technology and infertility treatment

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Couples and individuals with fertility issues often embrace the latest advances in medical research and are open to pursuing novel interventions, even those which have little or no evidence concerning efficacy or safety. Stem cell research and regenerative medicine are among the most controversial areas of medical science that have emerged within the last decade, and hold up the hope of providing a panacea for almost any health issue, including fertility. Here, we provide a brief overview of what the stem cell field can offer to patients facing fertility issues, with the aim of helping patients and physicians to make informed decisions and to have a better insight into possible outcomes.

Among multiple types of stem cells, only patient-specific induced pluripotent stem cells (iPSC) and germline stem cells, either male spermatogonial stem cells (SSCs) or female ovarian/oogonial stem cells (OSC) have the potential to be used as therapeutic tools in infertility treatment (Figure 1).

iPSC and gametogenesis

Most work in this area has been carried out using a mouse model. However, translation between mouse and human reproductive biology is not straightforward. Conditions and protocols that work for mice cannot be simply extrapolated to humans. From veterinary medicine, we know that there are species-specific differences in outcomes of much simpler procedures than differentiation of gametes from iPSC lines. For example, in vitro maturation (IVM) of oocytes and blastocyst development following intracytoplasmic sperm injection (ICSI) are relatively successful in cattle, but less so in horses.

Experiments in mice suggest that differentiating oocytes from human iPSCs might be easier than achieving spermatogenesis. However, we are still struggling even with terminal stages of oocyte growth and maturation. IVM is offered to the patients only in some cases and in a limited number of clinics. In vitro growth (IVG), a stage of oogenesis that occurs before IVM,

is a subject of intense research and it is not offered clinically. Only when we resolve the problems of accomplishing successful IVM and IVG in humans, we be able to differentiate oocytes from iPSCs or primordial germ cell-like cells (PGC-LC).

iPSC and chromosomal trisomy

Reprogramming of fibroblasts from sterile trisomic (41, XXY and 41, XYY) mice led to generation of euploid iPSCs.[1] Such euploid iPSC were further differentiated into the male germ cell lineage and functional sperm, which has been used in intracytoplasmic sperm injection (ICSI) to produce chromosomally normal, fertile offspring. The phenomenon of trisomy-biased chromosome loss (TCL) during reprogramming was not restricted only to sex chromosomes, but has also been observed in iPSC from a Down syndrome mouse model. TCL also occurs in humans as confirmed with cells from Down (47, XY,+21 or 47, XX,+21) and Klinefelter (47, XXY) syndromes. Isogenic euploid 46,XY and 46,XX iPSC lines derived from a male with Klinefelter syndrome (47, XXY) would provide a tool for genetic dissection of sexual polymorphism. The strategy will be only useful for 47,XXY males with complete azoospermia; Down syndrome males and females are usually fertile and produce euploid offspring. Regardless, in order to translate TCL into clinic, a complete gametogenesis has to be developed in vitro first. Although both oogenesis and spermatogenesis from iPSC lines have been successfully demonstrated in mice,[2-5] such attempts in humans are still rare and mostly futile.

SSCs and male infertility

About 50% of infertility is attributable to the male and in a majority of cases is caused by impaired spermatogenesis. Spermatogenesis is a process in which SSCs undergo complex

cellular processes of meiosis, DNA compaction and flagellum formation. Reproducing these events in vitro has not been successful yet and there is no cellular replacement therapy available for men who suffer from azoospermia.

Finding that the transplantation of mouse spermatogonial stem cells from an infertile donor to a permissive testicular environment could restore fertility and result in progeny with the genetic makeup of the infertile male donor, raised the hopes of many infertile men.[6] However, this is rather an exception than a rule. In this particular case, the defect causing infertility was azoospermia due to lack of a membrane-bound kit ligand (KITLG, known also as Steel factor or stem cell factor) on Sertoli cells. Spermatogonia from infertile testis with defective Sertoli cells transplanted into mice with a healthy testicular microenvironment was able to undergo spermatogenesis and form mature spermatozoa. Whether and how this strategy could be implemented and executed in infertile men carrying mutation in KITLG gene remains open for discussion. Novel genome editing technology based on clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated enzymes might be an option to repair the mutation.[7]

The most common genetic cause of male infertility, de novo deletion of one or more *azoospermia factor* (*AZF*) regions of human Y chromosome cannot be rescued with a similar strategy. This is due to the defect being in germ cells and not supportive cells. Indeed, human iPSC lines with deleted *AZF* region were compromised in germ cell development in vitro as well as in vivo, following xenotransplantation to murine seminiferous tubules.[8]

OSC and female fertility

In addition to work on human embryonic stem cells (hESCs) and iPSCs to produce gametes in vitro, there have been several studies on isolating and developing putative OSCs from adult women.[9-11] For almost a century, there has been a debate surrounding the ability of

adult female mammals to undergo post-natal germ cell renewal. The current consensus is that the population of female germ cells (oocytes) is fixed before or around the time of birth, depending on species. In 2004 a study proposing germ cell renewal in adult female mice was published[12] and since then the isolation of mitotically active cells expressing germline markers has been reported from ovaries of adult rodents, cows, sheep, primates and humans. Development of these putative OSCs in-vitro and the generation of live young from fully differentiated rodent OSCs has also been described.

OSCs are characterised by expression of primitive germ cell-specific markers and high levels of telomerase. Cells with these characteristics have been isolated from human ovaries and cultured *in vitro* and they form what appear to be oocytes,[9-11] but, so far, the formation of meiotically functional oocytes from human OSCs has yet to be demonstrated. Transplantation of in vitro expanded mouse OSCs back into germ cell depleted ovaries leads to the generation of fertilisation-competent eggs and the production of viable embryos and offspring. The physiological relevance of these cells to adult ovarian function and fertility remains to be determined and it may be that they are only activated under perturbed conditions that cause damage to the germ cells in vivo.

Whilst there remains controversy over the biological significance of these cells, it must be acknowledged that the identification and isolation of populations of cells with germ line potential within the adult human ovary represents a significant advance with the potential to improve infertility treatments. The most significant application of putative OSCs for human fertility would be to produce and develop oocytes in vitro.[10] Methodologies to grow human immature oocytes from primordial stages to mature oocytes entirely in vitro have been developed[13] and the technique of in vitro growth (IVG) could be combined with OSCS to support formation and growth entirely *in vitro*. In order to move this work forward, it is essential to demonstrate which cell populations can form oocytes when given the appropriate support and to demonstrate the developmental potential of the putative oocytes

derived from human OSCs. Many practical and conceptual obstacles remain before clinical application of OSC-based technologies can ever be fully realised, but it is important to move forward from the initial scepticism towards solid testing of the cells found within the adult human ovary.

Same sex partners and genetic parenthood

The newest study using a combination of gametes and hypomethylated mouse embryonic stem cells (mESCs) with deletion of imprinted regions suggests that it might be possible to allow both members of a same-sex couple to become genetic parents.[14] To create bimaternal mice, the scientists deleted three imprinting regions of the genome from haploid female mESCs before injecting the cells into eggs from another female mouse. From 210 embryos, they produced 29 live mice (13.8%), which were physically normal, lived to adulthood, and had offspring of their own. Their cognitive capacity has not been assessed. Creation of bipaternal mice was more complicated and less successful. They deleted seven imprinting regions from haploid male mESCs. The edited haploid X-bearing mESCs and Y-bearing sperm from another male mouse were then injected together into eggs that had their nuclei, and therefore their female genetic material, removed. Although the resulting zygotes had mitochondria originating from the denucleated egg, the embryos created contained nuclear DNA only from the two male parents. None of 1184 bipaternal embryos developed beyond embryonic day (E)8.5 after transplantation into pseudopregnant female mice, and placental development did not pass E10.5. In an attempt to rescue the defects in the extraembryonic tissues, the authors derived androgenic diploid ESC lines from bipaternal embryos and combined them with tetraploid embryos. From 1023 such embryos, only 12 (1.2%) reached full-term, and these died shortly after birth. To consider exploring similar technology for human application in the near future is implausible. Imprinted genes differ between species and in humans the pattern is more complex than in mice. The risk of

severe abnormalities is too high, and it is likely to take years of research in various animal models to fully understand how this could be done safely.[15]

Summary

Stem cell technology is not yet sufficiently advanced to provide treatments for infertile patients. Neither the use of germline stem cells to support in vivo gametogenesis, nor the production of in vitro gametes from germline stem cells or iPSC lines, is close to clinical implementation. Whilst there are still many barriers to recapitulating the processes and overcoming the limiting steps of gametogenesis pathways, animal studies have shown promising avenues for research, which may in time lead to clinical benefits.

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Conflict of interest

The authors declare no conflict of interest. Completed disclosure of interest forms are available to view online as supporting information.

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Figure legend

Figure 1. Patient-specific induced pluripotent stem cells (iPSC) and germline stem cells, either male spermatogonial stem cells (SSCs) or female ovarian/oogonial stem cells (OSC) have the potential to be used as therapeutic tools in infertility treatment. PGCs, primordial germ-cells.