Female fertility preservation and oogonial stem cells (OSCs): current status and future perspectives

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Introduction

Recent studies have proposed that the adult mammalian ovary's oocyte-containing follicles is not definite, but rather they posess oogonial stem cells (OSCs) that provide for its renewal. Although their existence is not widely accepted by the scientific community, as it challenges the principle that the number of oocytes in a mammal's ovaries is fixed at birth, the isolation and development promotion of such cells is specially interesting in infertility treatment procedures in women.

Materials and methods

Databases of citations and abstracts were consulted, such as Pubmed and Scopus, in search of scientific articles relevant to the subject with a high impact factor and recent publication dates in order to avoid out-dated information.

What are OSCs?

These cells were discovered due to the initial suspicion that primordial follicle numbers were discordant with the rate of follicle atresia in mice ovaries [1]. Immunohistochemical analysis of **mouse Vasa homologue** (MVH, also known as Ddx4), expressed exclusively in germ cells, confirms the presence of cells in the ovarian surface epithelium. They are also **mitotically active**, as they appear positive for 5-bromodeoxyuridine (BrdU) injection.

OSCs can be isolated from ovary tissue using a **flourescence-activated cell sorting** (FACS)-based protocol using immunomagnetic beads targeting surface-expressed domain of Ddx4. It appears OSCs exhibit cell-surface expression of this protein, unlike oocytes [2].

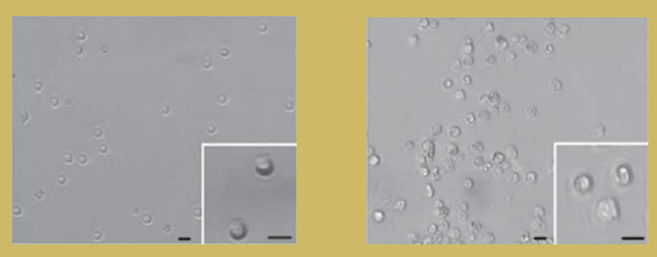


Fig 1. Viable cells isolated by FACS based on cell-surface expression of DDX4 (human, left) or Ddx4 (mouse, right) [2]

Freshly isolated OSCs posess a gene expression pattern consistent with that of **primitive germ cells**

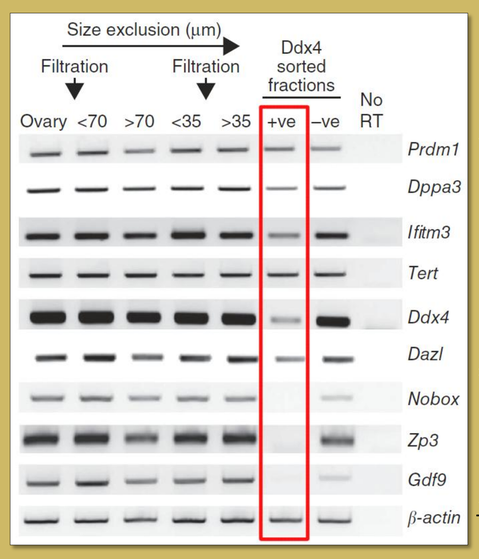


Fig 2. Gene expression of mouse cell fractions obtained by FACS. [2]

Isolated OSCs can be cultured in vitro for months and **spontaneously** generate large (35-50 µm) spherical cells **resembling oocytes** by morphology and gene expression analysis

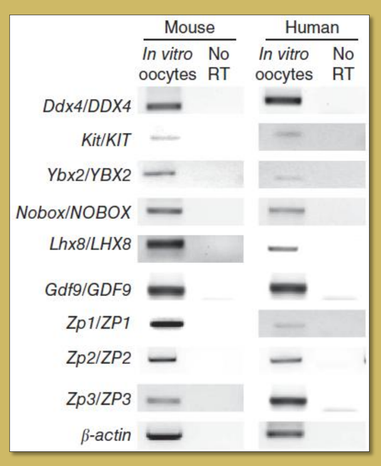
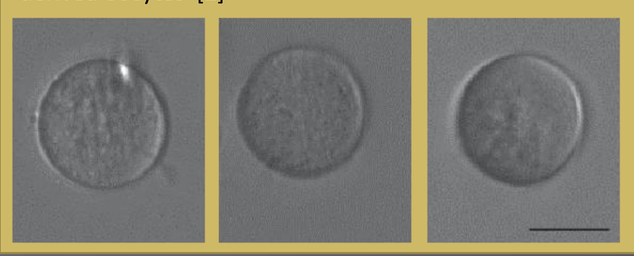


Fig 3. Expression analysis of oocyte markers in oocyte-like cells spontaneously generated from mouse and human OSCs [2]

Fig 4. Morphology of OSC-derived oocytes [2]



Injection of OSCs engineered to express GFP into mouse ovaries results in ovulation of GFP-positive oocytes

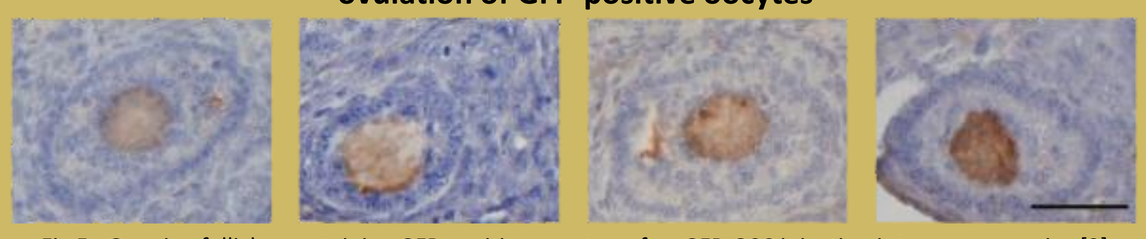


Fig 5. Growing follicles containing GFP-positive oocytes after GFP-OSC injection into mouse ovaries [2]

Ovulated GFP-positive oocytes can be fertilized in vitro, reaching the hatching blastocyst stage.

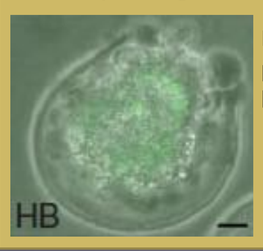


Fig 6. In vitro fertilized GFP-positive oocyte at the hatching blastocyst stage [2]

Offspring generated from sterile mice injected with GFP-OSCs and naturally mated.

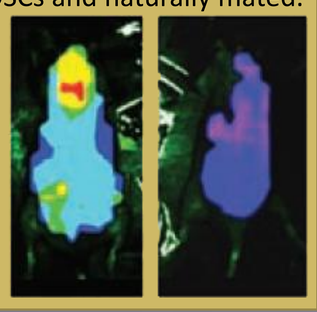


Fig 7. A GFP-positive F1 mouse (left) and a GFP-negative F1 mouse (right) [3]

Human OSCs generate oocytes in xenografted human tissue

When GFP-hOSCs are re-aggregated with dispersed adult human ovarian cortical tissue, large GFP-positive cells become enclosed by smaller GFP-negative cells in structures resembling follicles. When xenografted into NOD-SCID mice, after 1-2 weeks follicles containing both GFP-negative oocytes and GFP-positive oocytes can be observed.

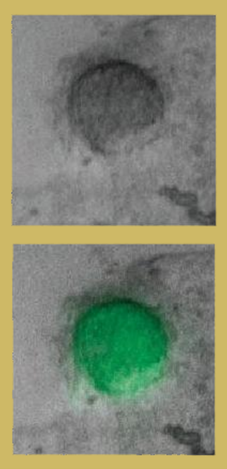


Fig 8. Large GFP-positive cells surrounded by smaller GFP-negative cells in structures resembling oocytes. [2]

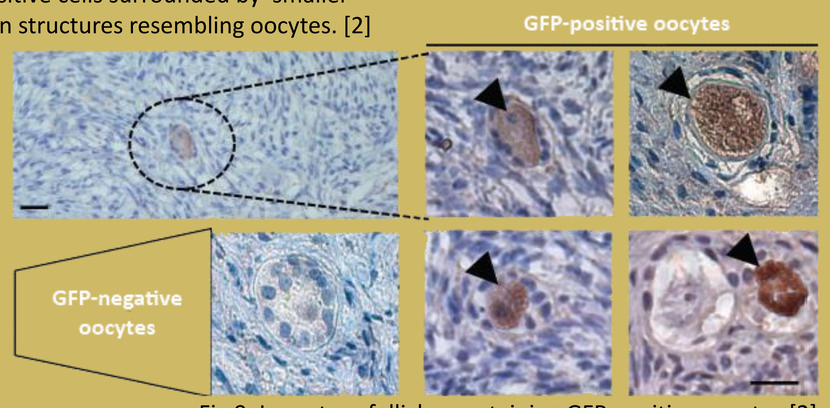


Fig 9. Immature follicles containing GFP-positive oocytes [2]

Potential clinical applications

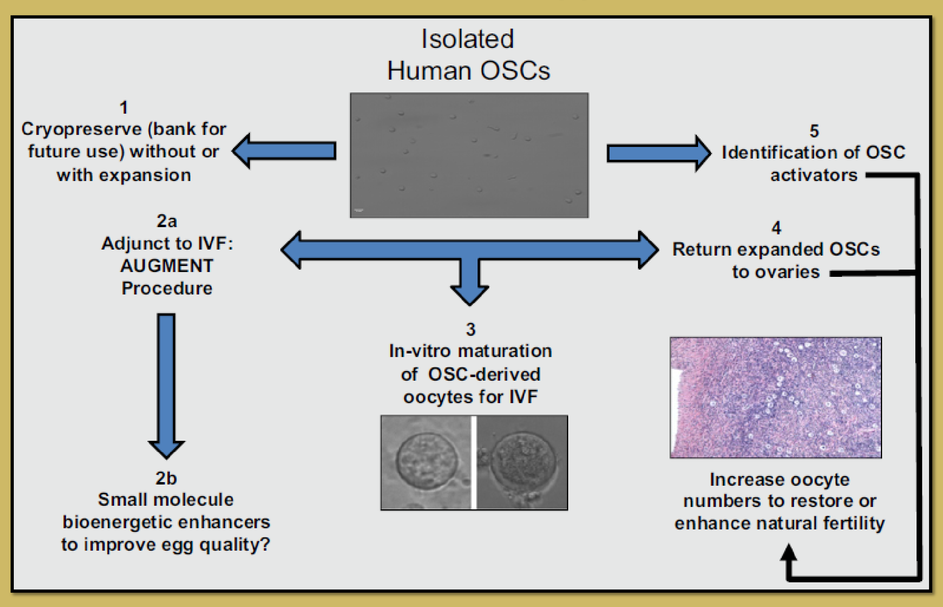


Fig 10. OSCs could be used in many ways to restore fertility in women. [4]

	Age-related infertility	Iatrogenic premature ovarian insufficiency	Non-iatrogenic premature ovarian insufficiency
Current strategy	Oocyte donation	Cryopreservation of oocytes, embryos or ovarian tissue → involves hormonal medication, delays in treatment, risk of reintroducing malignant cells.	Spontaneously concieve (only 5% of cases) or oocyte donation and IVF.
OSC strategy	OSC isolation and proliferation → age-related aneuploidy risk unexistant?	OSC isolation → avoids delay in commencing life-saving treatments. No teratoma formation.	OSC isolation, culture and use in IVF → can use one's own oocytes.

Table 1. Current strategies for infertility due to different causes and advantages OSCs could offer [5]

Conclusions

Much evidence has been provided that mammals do indeed possess OSCs, there is still a long way until they are accepted, and more studies are needed to determine their exact function in the ovary. Several questions need to be answered:

- Under what mechanisms do these cells spontaneously generate oocytes *in vitro*?

- Why do OSCs, unlike oocytes, exhibit cell surface expression of Ddx4?

- Do these cells contribute actively to *de novo* neo-oogenesis *in vitro* to mantain follicle numbers, or are they activated only under certain circumstances?

Regardless, what is truly of interest is whether or not these cells are relevant clinically. Additional work is needed to map the exact relationship between OSC and oocyte numbers in vivo, as well as improve the efficiency of isolation.

Bibliography

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