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Review

Ovarian stem cells—Potential roles in infertility treatment and fertility preservation

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ABSTRACT

One of the principal beliefs in reproductive biology is that women have a finite ovarian reserve, which is fixed from the time they are born. This theory has been questioned recently by the discovery of ovarian stem cells which are purported to have the ability to form new oocytes under specific conditions post-natally. Almost a decade after their discovery, ovarian, or oogonial, stem cells (OSCs) have been isolated in mice and humans but remain the subject of much debate. Studies in mice have shown that these cells can be cultured to a mature oocyte stage in vitro, and when injected into germ-cell depleted ovary they can form follicles and have resulted in the birth of healthy offspring. There are few data from human OSCs but this finding would open the door to novel fertility preservation strategies for women with both age-related and premature ovarian insufficiency (POI). As the number of girls and young women surviving cancer increases worldwide, POI secondary to gonadotoxic treatments, such as chemotherapy, is becoming more common. The ideal fertility preservation approach would prevent delays in commencing life-saving treatment and avoid transplanting malignant cells back into a woman after treatment: OSCs may offer one route to achieving this. This review summarises our current understanding of OSCs and discusses their potential clinical application in infertility treatment and fertility preservation.

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1. Introduction

The dogma that female mammals are born with all of the oocytes they will ever possess has its foundations in a paper from Sir Solomon Zuckerman published in 1951 [1]. Simply put, Zuckerman failed to find any experimental evidence available at that time that he felt was inconsistent with an earlier hypothesis [2] that germ cell production in female mammals ceases prior to birth (reviewed by Zuckerman) [3]. This paper and its main conclusion profoundly affected the subsequent interpretation of experimental and clinical observations relating to ovarian development, function and failure for the next 50 years. A paper published by Jonathan Tilly's laboratory in 2004 reignited this debate by reporting the presence of a

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population of mitotically active germline stem cells (GSCs) in the mouse ovary which, the authors postulated, maintain oocyte and follicle production in the ovary after birth [4]. The finding of GSCs, or oogonial stem cells (OSCs) as they are now more commonly known, has generated a lively debate in the field over the last decade as it is in direct opposition to the dogma that female mammals have a non-renewable oocyte reserve from birth. This debate has been perceived as representing two clearly opposing viewpoints with no common ground (reviewed by Powell) [5], but there is the possibility that both views can co-exist, with the formation of a population of oocytes at birth that is the main contributor to ovarian function and fertility and subject to little, if any, renewal and the existence of OSCs in adult ovaries that can only be activated under specific circumstances. It is impossible to prove the absence of any given cell in a tissue but the debate cannot be resolved until the presence and function of OSCs within adult ovaries can be unequivocally demonstrated.

Regardless of the physiological significance of these cells what is undeniable are the possible clinical applications of OSCs in infertility and fertility preservation if their potential can be harnessed; this review will address the background to current understanding of OSCs, and provide a speculative discussion of their potential clinical applications. If human OSCs can be grown into fully functional oocytes, can this be harnessed to address the age-related decline in oocyte quality? Could girls and young women about to undergo gonadotoxic therapy, e.g. for cancer, be able to cryopreserve some OSCs within their ovarian cortex prior to commencing treatment? Instead of concentrating on the finite number of primordial follicles within that ovarian tissue, it is conceivable that OSCs could subsequently be retrieved from this tissue and either cultured to form mature oocytes for use in in vitro fertilisation (IVF), or injected back into the woman's ovarian cortex for in vivo development. The number of new follicles that could be generated from OSCs could be much larger than the number of follicles in the stored ovarian tissue, and certainly much larger than the number of mature oocytes that a woman could store using the conventional approach of ovarian stimulation and aspiration of mature oocytes.

1.1. Identification and isolation of OSCs

Johnson et al. identified cells they considered OSCs whilst investigating follicular atresia in the mouse ovary [4]. They discovered that follicles were dying at a rate such that the ovary would be deplete of oocytes far earlier than is found in vivo. Analysis of the ovary revealed ovoid cells that both immunostained for a germ-cell specific marker (mouse vasa homologue or MVH, a germ-cell specific RNA helicase) and demonstrated incorporation of 5-bromodeoxyuridine (BrdU), indicative of proliferating cells. Furthermore, these cells expressed a meiosis-specific protein (synaptonemal complex protein 3, SCP3) required to initiate meiosis for the production of oocytes. In their final set of experiments, ovarian tissue from wild-type mice was transplanted onto the ovaries of mice which ubiquitously expressed green fluorescent protein (GFP). After 3–4 weeks, the wild-type ovary contained GFP-positive oocytes surrounded by wild-type granulosa cells, persuading the authors that OSCs from the GFP mouse had initiated folliculogenesis in the wild-type mouse and that they had discovered mitotically active OSCs that had the ability to form new oocytes after birth [4].

However, scepticism surrounded the idea of OSCs amongst reproductive biologists [6,7]. A key finding supporting claims that adult mouse ovaries retain the capacity for oogenesis came in a paper that reported that OSCs had been isolated and cultured from neonatal and adult mouse ovaries [8]. These cells, termed female germline stem cells (FGSC), were initially identified using the same criteria used by Johnson et al. [4] i.e., expression of MVH and BrdU

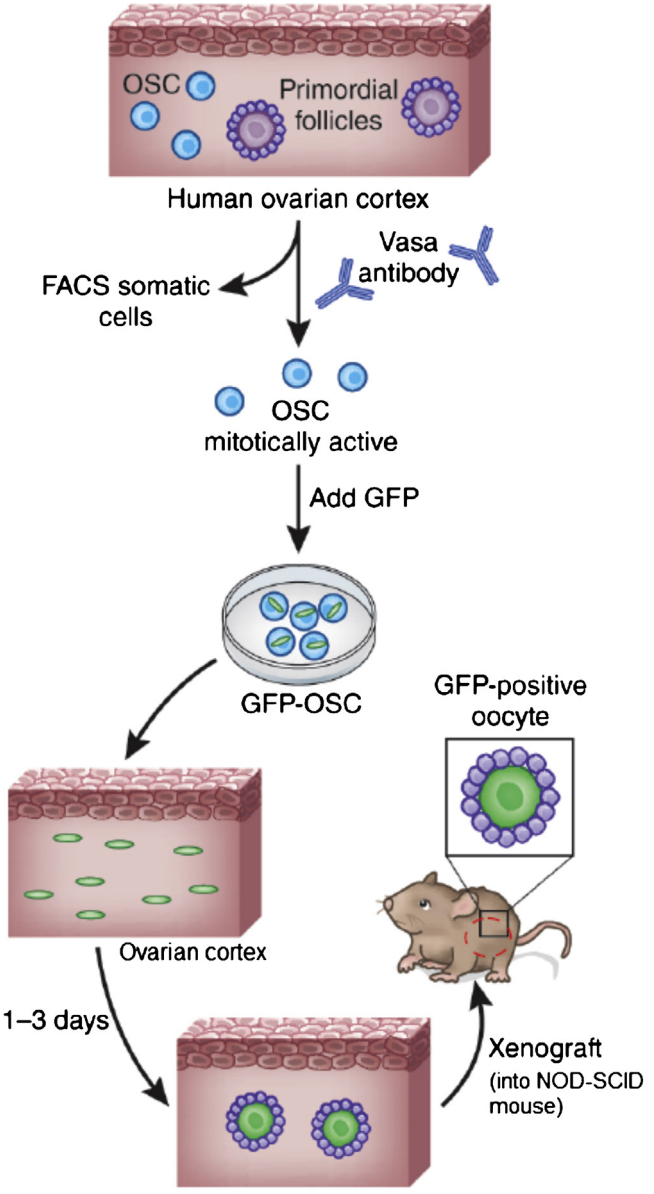

Fig. 1. White et al.'s method of isolating, purifying and culturing human OSCs using a xenograft. FACS: fluorescent-activated cell sorting.
From Telfer and Albertini [43].

incorporation. By employing a cell-sorting approach using an antibody against Ddx4 (DEAD box polypeptide 4, another name for MVH), the authors reported the ability to isolate and purify OSCs in mice. Furthermore, by transplanting GFP-positive OSCs into the ovaries of infertile mice, they were able to produce live GFP-positive offspring.

The main findings of this key study were developed further by White et al. [9] who not only managed to isolate human OSCs using DDX4 (the human orthologue of MVH, or VASA), but they were able to isolate, culture and form early follicle-like structures after injection of both mouse and human OSCs into ovarian tissue which was xenotransplanted into NOD-SCID (non-obese diabetic – severe combined immunodeficiency) mice to provide a suitable environment for early folliculogenesis (Fig. 1) [9].

Interestingly, and from an entirely separate line of evidence, the case for post-natal neo-oogenesis has been bolstered by a recent analysis of the accumulation of microsatellite mutations

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