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## Ovarian Stem Cells—the Pros and Cons

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**Abstract:** The potential for postnatal de novo oogenesis in mammals and in humans has become very controversial in the fields of reproductive science and biology.

Historically, it has been thought that females of most mammalian species lose the ability to produce oocytes at birth. A contemporary understanding of stem cell biology together with novel experimental methods has challenged the model of a prenatal fixed ovarian primordial follicle pool that declines with age. Researchers have suggested replenishment of post-natal oocytes by germ-line stem cells (GSCs). According to this theory, GSCs produce oocytes and primordial follicles throughout the lifetime of the adult female.

This review describes recent approaches supporting the revolutionary idea of de novo oogenesis in mammals and humans of reproductive-age and provides counter arguments from opponents of this novel and innovative concept.

**Keywords:** ovarian stem cells, germline stem cells, de novo oogenesis, primordial follicle pool

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## Introduction

In most mammalian females, the ovaries undergo age-related dysfunction and failure, which has historically been thought to occur as a consequence of depleting the nonrenewable pool of female germ cells (primordial follicles) established before birth. This cornerstone of mammalian female reproductive biology was challenged by the work of Johnson et al, who suggested that mouse ovaries retain the capacity to produce germ cells throughout life.<sup>1-4</sup> It remained unclear whether these cells originated from within or from outside of the ovary.<sup>1,2</sup> Johnson et al<sup>2</sup> demonstrated that oocytes could be generated in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. However, other attempts to reproduce the findings reported by Johnson et al were unsuccessful, leading to intense controversy in the field of reproductive sciences.<sup>5-7</sup>

Recently, White et al<sup>8</sup> reported the successful isolation and characterization of germ-line stem cells (GSCs) from the ovaries of reproductive-age women. However, this break-through study lacks proof of concept regarding the occurrence of de novo oogenesis in adult women. Additional studies are needed to determine whether the idea of a fixed, nonrenewable pool of female germ cells will be replaced by the new concept of de novo oogenesis in adult women. If germ cell regeneration is demonstrated, new methods for treating infertile women can be developed.

## Pros

A recently published study reported the isolation of cells from both adult mouse and reproductive-age human ovaries that are capable of forming oocyte-like structures and that become incorporated into follicles under specific in vitro and in vivo conditions.<sup>8</sup> Thus, this study represents a significant step towards the revolutionary idea of neo-oogenesis in reproductive-age women through the isolation and characterization of GSCs. White et al<sup>8</sup> isolated GSCs using fluorescence-activated cell sorting (FACS) with an antibody against the carboxyl (–COOH) terminus of the germ cell-specific marker Ddx4, which is expressed on the cell surface of GSCs. Analysis showed that isolated GSCs only expressed markers specific for germline cells and not markers specific for oocytes. Hence, the isolated cells were not contaminated by oocytes, which express cytoplasmic Ddx4. This FACS-based

isolation protocol represents significant experimental progress compared to GSC isolation using immunomagnetic sorting with the Ddx4 COOH antibody.<sup>9</sup> Thus, use of the Ddx4 COOH antibody with FACS provides a strategy for obtaining GSCs free of oocytes. This study also used immunofluorescence staining of human and mouse ovarian samples with the germ cell-specific marker Ddx4 to reveal the presence of germ cells inside of the ovaries. However, GSCs could not be detected in situ within ovarian samples since oocytes arrested in the diplotene stage were also stained with Ddx4. Thus, the study conducted by White et al<sup>8</sup> was unable to detect GSCs in situ within adult human ovaries.

Questions arise as to whether the GSCs isolated by White et al<sup>8</sup> are only activated in vitro, or whether they indeed contribute to de novo oogenesis in vivo.<sup>4</sup> Further studies are required to resolve these issues.

The origins of these recently isolated GSCs are under investigation. It is currently unknown whether they are generated through differentiation of pluripotent stem cells residing inside the adult ovary, or whether they are primordial germ cells (PGCs) that segregated during gastrulation from the epiblast and underwent mitotic arrest, rather than entering meiosis during the late stages of embryonic development. This has been investigated by several research groups who demonstrated the isolation of stem cells from the ovarian surface epithelium (OSE) of adult and even menopausal mouse and human ovaries; under certain in vitro culture conditions, these cells differentiated into oocyte-like cells.<sup>10-13</sup> Somatic stem cells obtained from different sources, such as the pancreas, fetal skin, and amniotic fluid cells, were shown to differentiate into oocyte-like cells under defined in vitro culture conditions by several groups.<sup>14-18</sup> Hence, stem cells isolated from the OSE may be somatic stem cells and not the GSCs isolated by White et al.<sup>8</sup> These assumptions lead to new questions, such as whether the ovary contains different kinds of stem cells, such as somatic stem cells, that give rise to ovarian somatic cells and whether GSCs are involved in the strongly debated postnatal de novo oogenesis. In other animal species, such as the *Botryllus schlosseri* from the subphylum Urochordata, it has been documented that GSCs segregate early during embryonic development, remain in adult as GSCs, and give rise to new oocytes through de novo oogenesis under specific conditions.<sup>19,20</sup>



Therefore, from an evolutionary point of view, the GSCs isolated by White et al<sup>8</sup> may have been PGCs that underwent mitotic arrest rather than entering meiosis. Wang and Tilly<sup>21</sup> investigated possible mechanisms of how GSCs could become activated in adult ovaries. They demonstrated that activation of the *Strad* promoter in premeiotic germ cells is repressed through an epigenetic mechanism involving histone deacetylation and a not yet identified coactivator. This model may lead to future investigations of how key events such as *Strad* expression, the sex-specific timing of embryonic germ cell meiotic commitment, and putative postnatal gametogenesis are regulated. Another hypothesis is that GSCs are actually oogonia that failed to enter meiosis, but did not undergo apoptosis for an unknown reason and remain in the postnatal ovary. During normal embryonic development, nearly all oogonia have normally entered meiosis and become primordial oocytes. However, those failing to enter meiosis or undergo apoptosis may have the capacity to be “activated” under in vitro conditions. Thus, under normal in vivo conditions, de novo oogenesis would not occur in the adult ovary, despite the presence of these oogonia.

It is clinically important to consider the possible involvement of ovarian somatic stem cells,<sup>10–13</sup> or recently discovered GSCs in the pathogenesis of ovarian cancer.<sup>8</sup> Circumstantial evidence supports the role of stem cells in ovarian cancer. The high rate of chemoresistance recurrence observed in ovarian cancer correlates with the property of stem cells to remain in a quiescent state, rendering stem cells resistant to cytotoxic drugs that target mitotic cells.<sup>22</sup> It is known that stem cells share many characteristics with cancer cells. They both are able to self-renew and proliferate for a long period of time under specific conditions.<sup>8,23,24</sup> Analogous to normal stem cells, cancer cells are thought to possess the capacity for unlimited self-renewal through symmetric cell division, the ability to give rise to progeny cells through asymmetric division, and an innate resistance to cytotoxic therapeutics.<sup>23</sup> While the process of differentiation initiated by a normal stem cell ultimately results in a specialized progeny with no proliferative potential, a cancer cell gives rise to progeny that do not undergo terminal differentiation but instead exhibit uncontrolled proliferation.<sup>25</sup> The normal interplay between somatic cells and stem cells is crucial for

maintaining normal stem cell function.<sup>26</sup> A disturbance in the balance between these two compartments may lead to abnormal stem cell behavior, eventually leading to cancer.<sup>25</sup>

Ovarian stem cells have been shown to exist; however, the presence of GSCs in situ within adult human ovaries has not been demonstrated. Additional studies are necessary to determine whether GSCs are important in the controversial idea of putative de novo oogenesis in adult mammals and humans. Future studies will provide insight regarding possible involvement of ovarian somatic stem cells or GSCs in the pathogenesis of ovarian diseases such as ovarian cancer.

## Cons

Critical commentaries have been published to argue against the possibility of postnatal de novo oogenesis in female mammals.<sup>7,27,28</sup> Mathematical predictions of follicular dynamics in adult mice led to the conclusion that such results reflect the dynamics of a fixed and nonrenewable pool of primordial follicles during adult life.<sup>27</sup> Another study refuting the concept of putative de novo oogenesis in adult female mammals was a primarily reverse transcription-polymerase chain reaction-based analysis of gene expression of various markers of germ cell replication or meiotic entry in adult human ovarian tissue biopsies.<sup>29</sup> In this study, Liu et al<sup>29</sup> concluded that active meiosis, neo-oogenesis, and GSCs are unlikely to exist in normal, adult human ovaries. John et al<sup>30</sup> reported that inactivation of the *Foxo3* gene in mice results in accelerated onset of ovarian failure due to increased rates of primordial follicle growth activation.<sup>30</sup> The authors concluded that their findings are inconsistent with the occurrence of postnatal de novo oogenesis and follicular renewal.

A new study conducted by Zhang et al<sup>28</sup> contradicts the results obtained by White et al.<sup>8</sup> Their study used fluorescent proteins to identify GSCs in the ovaries of mice; however, these cells failed to divide or differentiate into oocytes.

Supporters of postnatal de novo oogenesis disagree with the study conducted by Zhang et al,<sup>28</sup> and state that the study investigated oocytes and not GSCs in their applied experimental setting; thus, the researchers never observed mitosis in Ddx4-positive cells since oocytes expressing cytoplasmic Ddx4



do not divide.<sup>31</sup> According to White et al,<sup>8</sup> Ddx4 is found on the cell surface of GSCs and thus enables FACS-based isolation of living GSCs from adult mouse and human ovaries.<sup>8</sup> This is in contrast to the opinion of Zhang et al,<sup>28</sup> who argue that Ddx4 is expressed only in the cytoplasm and not on the cell surface and hence FACS-based isolation of GSCs is problematic.<sup>28</sup>

Studies conducted in mice helped to increase the understanding of basic mechanisms involved in the process. However, human and mouse biology can differ to some degree. For ovarian physiology and reproduction in general, studies conducted using available human samples are preferable. However, due to the shortage of available human samples and ethical restrictions, investigations conduct studies using the appropriate mouse models.

While the debate continues, only future experiments will help to clarify this issue.

## Conclusion

The potential clinical applications of putative ovarian derived stem cells are apparent. If a viable source of oocyte production remains in infertile women with a reduced ovarian follicle pool, for example due to chemotherapy or advanced age, the potential exists to restore fertility in these women. The identification of GSCs gives hope to these women and suggests the potential for fertility restoration.

However, the isolation and culture of GSCs must be optimized. The time required in vitro to obtain oocytes is very long and, consequently, this procedure would be very expensive in clinical fertility settings. Hence, freezing of the ovarian cortex before chemotherapy or at a young age to delay onset of menopause currently appears to be a more suitable approach.

Understanding the possible involvement of somatic ovarian stem cells or GSCs in the pathogenesis of ovarian cancer may provide new therapeutic strategies for such patients.

A further understanding and potential manipulation of adult female GSCs may provide these answers.

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## Author Contributions

Conceived and designed the experiments: AE and ZB. Analysed the data: AE and ZB. Wrote the first draft of the manuscript: AE and ZB. Contributed to the writing of the manuscript: AE and ZB. Agree with manuscript results and conclusions: AE and ZB. Jointly developed the structure and arguments for the paper: AE and ZB. Made critical revisions and approved final version: AE and ZB. All authors reviewed and approved of the final manuscript.

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