

De Novo Oogenesis In Adult Mammals: A Critical Review of Evidence for Postnatal Oocyte Regeneration

Michaela Sesilia Passa¹

Received July 14, 2026

Accepted October 1, 2026

Electronic access October 15, 2026

Background/Objective: For decades, reproductive medicine was governed by the Zuckerman dogma: adult female mammals possess a prenatal, finite, decreasing oocyte reserve. The theory of *de novo* oogenesis directly contradicts this dogma, claiming that oocyte regeneration persists postnatally through germline stem cells (GSCs), with significant implications for infertility treatment and ovarian aging. An estimated 17.5% of adults, roughly one in six, are affected by infertility at some point in their lifetime worldwide.

Methods: This narrative review analyzes murine, prosimian, and human evidence, evaluating each study for lineage-marker specificity, independent replication, *in vivo* corroboration, adequacy of controls, and alternative interpretations, spanning literature from 1917 through the end of 2024. Throughout, it distinguishes two classes of evidence frequently conflated: studies showing isolated cells generate oocyte-like structures under experimental manipulation (an *in vitro* identity/potential question), and studies asking whether the ovarian reserve is replenished *in vivo* in an unmanipulated animal (a lineage question).

Results: Studies supporting *de novo* oogenesis report mitotically active GSCs generating oocyte-like structures *in vitro*, and, in murine transplantation models, live offspring bearing donor-cell markers; these findings are limited by incomplete validation of the sorted cells' germline identity, disputed marker specificity, and non-replication by independent laboratories. Opposing studies using genetic lineage tracing and single-cell profiling of intact ovarian tissue converge on a prenatally fixed, non-renewed follicle pool, and identify cells previously sorted as germline stem cells as misidentified perivascular cells.

Conclusions: At present, no consensus exists among reproductive biologists regarding the physiological occurrence of *de novo* oogenesis in adult mammals.

Keywords: Biology, Reproductive Biology, Postnatal Oocyte Regeneration, Female Germline Stem Cells, *De Novo* Oogenesis

Introduction

Theoretical Framework

The theory of *de novo* oogenesis challenges two fundamental processes related to the traditional understanding of oocyte biology: firstly, the dogma surrounding fetal follicle and oocyte establishment, and secondly, the irreversible depletion of the ovarian follicle pool across development and aging.

Background and Context

Regarding the first process, oogenesis in humans begins as primordial germ cells (PGCs) arise in the yolk sac wall and migrate to the genital ridge during embryogenesis, where they undergo repeated mitotic divisions to form oogonia¹. Between roughly the ninth and twenty-second week of human fetal development, oogonia enter meiosis and become primary

oocytes; at this point, each primary oocyte is diploid, not haploid, since it has completed DNA replication in the preceding S-phase but has not yet completed either meiotic division¹. Meiotic recombination during this fetal period creates programmed DNA double-strand breaks (DSBs), permitting the exchange of genetic material between homologous chromosomes before the cell arrests¹. Rather than proceeding through meiosis I and meiosis II before birth, primary oocytes arrest in the diplotene sub-stage of meiotic prophase I, known as the dictyate stage, and remain suspended there — still diploid, with duplicated sister chromatids — for years to decades¹. It is this arrested, prophase-I population, and not a post-meiotic haploid population, that constitutes the ovarian reserve; the reserve is considered to be permanently and fully established in number at this fetal stage, well before any resumption of meiosis.

Each dictyate-arrested primary oocyte is enclosed within a primordial follicle. Independently of meiotic status, a small cohort of primordial follicles is continuously and

¹ Indigo Research at Crimson Education

gonadotropin-independently recruited into the growing pool at all reproductive ages, progressing through primary and secondary (pre-antral) follicle stages under the influence of locally produced growth factors rather than follicle-stimulating hormone (FSH)². Only once follicles reach the antral stage does their further growth become dependent on cyclic FSH secretion from the anterior pituitary². As a dominant antral follicle matures, its granulosa cells secrete rising levels of estradiol, which — once a sustained threshold is exceeded — exerts positive feedback on the hypothalamic-pituitary axis and triggers the mid-cycle luteinizing hormone (LH) surge; it is this LH surge, rather than FSH itself, that causes the oocyte to resume meiosis I, extrude the first polar body, and arrest a second time at metaphase of meiosis II shortly before ovulation². The oocyte remains arrested at metaphase II until fertilization by a spermatozoon triggers completion of the second meiotic division. While some oocytes mature and ovulate in response to this FSH/LH-driven cycle across a woman's reproductive life, the large majority instead undergo atresia, causing the ovarian reserve to decline progressively until menopause³.

In regard to the second process, "ovarian aging" describes the progressive and unavoidable reduction in the number and quality of ovarian follicles^{3,4}. The average follicle count of approximately 1,000,000 at birth declines to between 10,000 and 100,000 by the age of 30, and to fewer than 1,000 by menopause due to this ongoing atresia⁵. Hallmarks of biological aging, such as genome instability, telomere attrition, epigenetic alterations in DNA methylation, and stem cell exhaustion, have recently been shown to be among the factors directly responsible for this ovarian decline, thereby impairing the integrity of stem cells and their capacity to support homeostatic tissue regeneration and maintenance^{3,6}. In other words, with aging, stem cells can cease to exhibit self-renewal properties and undergo terminal differentiation, leading to stem cell pool exhaustion. Consequently, the capacity of oocytes to complete meiosis, undergo fertilization, and sustain embryonic development is substantially reduced³.

This way, ovarian aging has been recognized as one of the most significant contributing factors to infertility, which is defined as an inability to conceive after one year of regular, unprotected sexual intercourse and is estimated to affect approximately 17.5% of adults, or roughly one in six people, worldwide over the course of their lifetime^{7,8}. Existing treatments for infertility resulting from diminished ovarian reserve are primarily focused on pharmacological stimulation (i.e. using agents that mimic FSH and LH to promote ovulation) or on assisted reproductive technologies. However, these approaches carry multiple risks, including multiple gestation, ovarian hyperstimulation syndrome, and even an increased long-term risk of ovarian tumors⁹. Importantly, they address the consequences of ovarian aging rather than its underlying cause. This review focuses specifically on the depletion of the ovar-

ian reserve and the possibility of its regeneration through *de novo* oogenesis.

Problem Statement and Rationale

It is precisely in this context that the theory of *de novo* oogenesis has emerged as a compelling area of investigation. It proposes that the oocyte pool is not irreversibly fixed at birth⁵, but may instead be sustained or replenished by mitotically active germ cells present in the postnatal ovary¹⁰. The proposed biological basis for this is the existence of GSCs in adult mammalian ovaries, which would confer an innate capacity for oocyte regeneration¹¹. This hypothesis directly challenges the Zuckerman dogma, which asserts that the follicle supply is established during the fetal period and depletes irreversibly with age^{12,13}.

Significance and Purpose

If validated, *de novo* oogenesis could enable GSCs in patients with ovarian insufficiency to be differentiated into functional oocytes, which may reconstitute the oocyte pool without the risks associated with typical pharmacological stimulation⁵.

Methodology Overview

This review was undertaken as a narrative critical review of the peer-reviewed scientific literature about *de novo* oogenesis and postnatal oocyte regeneration in adult mammals. The choice of conducting a narrative review, rather than a systematic review, was based on the fact that the subject under study is very diverse in terms of methodology used in different research projects, which include techniques such as histology, molecular biology, genetics, and even transplantation, which thus makes a narrative evaluative framework more appropriate for assessing the quality and consistency of evidence.

Methodology

Search Strategy

A structured literature search was conducted across PubMed and Google Scholar between September 2024 and December 2024. The following search terms were used individually and in Boolean combination: "de novo oogenesis," "postnatal oogenesis," "neo-oogenesis," "ovarian germline stem cells," "female germline stem cells," "oogonial stem cells," "ovarian stem cells," "oocyte regeneration," "follicle renewal," "Zuckerman dogma," "postnatal folliculogenesis," "DDX4 oocyte," "bone marrow oogenesis," and "ovarian surface epithelium stem cells." The Boolean operators AND and OR were used

to combine terms. Only sources available in English were included; non-English-language sources were excluded at the eligibility stage, and this has been added explicitly as exclusion criterion (5) below. Records retrieved from both databases were compared by title and DOI, and overlapping records were retained only once in the final corpus.

Evidence Cutoff

The evidence cutoff for this review is December 2024. The search was conducted through the end of that month, and the reference list and synthesis reflect the literature available at that date. Publications from 2025 onward were not systematically searched or incorporated, and this is now stated explicitly as a limitation of the review (see Discussion, Limitations).

Date Extraction

The search was not restricted by publication year in order to capture an accurate historical view of the debate. More precisely, the search circumscribed literature from 1917 through to 2024. For studies published before the emergence of molecular biology, only those that have been widely cited in subsequent molecular literature were included, seeing as they are considered fundamental to the debate.

Inclusion and Exclusion Criteria

Studies were considered eligible for inclusion if they met all of the subsequent criteria: (1) the study directly investigated the existence, isolation or characterization of germline stem cells or postnatal oocyte formation in adult mammalian ovaries; (2) the study was published in a peer-reviewed journal or peer-reviewed edited volume; (3) the study used animal models, human tissue, or *in vitro* human cell culture as its primary experimental system; and (4) sufficient methodological detail was reported to allow for the evaluation of the findings. Where review articles and book chapters were included, they provided substantial synthesis of primary data or introduced theoretical frameworks central to the debate but were not used as the unique source for empirical claims.

Studies were excluded if they: (1) examined oogenesis exclusively in non-mammalian species; (2) were published only as conference abstracts without a corresponding full-text article; (3) were opinion pieces, editorials, or letters that did not contain a structured synthesis of evidence or original data; (4) examined aspects of ovarian biology unrelated to germline stem cell activity, unless these were directly relevant to interpreting GSC marker specificity; or (5) were not written in English. One narrow exception to peer-review eligibility criterion (2) was made for a single technical report by an international health authority (the World Health Organization's

Infertility Prevalence Estimates, 1990-2021), which was retained solely as the source of the global infertility prevalence estimate used for background epidemiological context in the Introduction, and was not used as evidence for or against *de novo* oogenesis.

Synthesis Method

After performing the database search described above, records were screened for relevance against the inclusion and exclusion criteria. Following title/abstract screening, full-text review, and the removal of any duplicate records, the final corpus discussed in this review comprises 38 sources. Of these, the large majority are original primary research articles reporting new experimental data (histological, molecular, genetic-lineage-tracing, or transplantation studies); the remainder are narrative reviews, one edited-volume chapter, and one technical report retained under the narrow exception described above. A full study-level extraction table for every primary research study discussed in the Results is provided in Table 1.

Quality Assessment

Given the narrative design of this review, a formal quality scoring instrument was not applied. Instead, each included primary study was evaluated qualitatively against the following five criteria, and this appraisal is reported explicitly, study by study, in the "Quality judgment" column of Table 1 rather than only being described here in the Methods: (1) whether molecular markers used to identify putative GSCs were validated as germline-specific in the experimental context, rather than merely reported as positive; (2) whether findings were replicated independently by other research groups; (3) whether *in vitro* findings were corroborated by *in vivo* evidence, and, conversely, whether *in vivo* lineage-tracing findings were corroborated by an independent method; (4) whether appropriate controls were included (e.g., isotype or secondary-antibody-only controls for antibody-based sorting, genotype controls for transplantation); and (5) whether alternative interpretations of the findings were considered and addressed by the authors of the original study. Critically, criteria (1) through (5) are applied differently depending on which of the two classes of evidence a study belongs to (see "Why Contradictions Persist" in the Discussion): studies that generate oocyte-like structures under experimental manipulation are judged on whether they establish germline identity and functional competence of the starting cell population, while studies that track the origin of oocytes in an intact, unmanipulated animal are judged on the fidelity and completeness of the lineage-labelling system used. These are not interchangeable tests of the same claim, and Table 1 records which of the two questions each study actually asked.

Results

Several studies have investigated the possibility of *de novo* oogenesis in differing organisms and yielded variegated findings, further deepening the divide between proponents and adversaries of the theory. Table 1 summarizes, study by study, the species and age of the animal or tissue donor, whether the study was conducted under normal physiological conditions or after injury/transplantation, the cell-isolation method, the lineage marker or reporter used, whether a proliferation assay and meiotic validation were performed, whether follicle incorporation, fertilization, or a live-birth/embryo outcome was reached, whether donor origin was verified independently of the marker used to sort the cells, whether adequate controls were reported, whether the finding was independently replicated, and this review's quality judgment. The distinction between generating oocyte-like structures *in vitro* and regenerating the ovarian reserve *in vivo*, introduced in the Methods, is applied consistently below and is carried through into the Discussion, rather than being replaced by a symmetric "evidence on both sides" narrative.

Early Histological Observations

Studies supporting the existence of *de novo* oogenesis generally report findings of new oocyte groups, germline stem cells (GSCs), or germ cell markers. This introduces the possibility that such stem cells could be utilised for the production of female gametes¹⁰. In 1917, Kingery reported that oocytes lost during fetal life were replenished by new ones developing in the ovarian epithelium of mice, thereby suggesting that oogenesis occurred postnatally. Nevertheless, these observations were based purely on histology, without molecular support³³. Similarly, Allen (1923) proposed that undifferentiated cells in the ovarian epithelium of mice³² undergo mitotic division to form new oocytes. However, because these early studies lacked molecular validation through molecular markers which would confirm that these cells were new oocytes rather than pre-existing ones, their results are now interpreted as insufficient evidence for adult oogenesis.

Isolation of Germline Stem Cells in Mice and Humans

Modern studies have reported the isolation of mitotically active germ cells from reproductive-age human and mouse ovaries capable of replicating *in vitro* and generating oocyte-like structures. In 2012, White et al. used DDX4-antibody-based fluorescence-activated cell sorting to purify putative oogonial stem cells (OSCs) from adult mouse ovaries and from human ovarian cortical tissue²³. These cells were mitotically active and displayed a gene expression profile consistent with primitive germ cells²³. When human OSCs engineered to

express GFP were injected into human ovarian cortical biopsies and xenotransplanted into immunodeficient mice, GFP-positive, follicle-enclosed oocytes formed within one to two weeks²³; this is a xenotransplantation endpoint in human tissue, not a report of live human offspring, and it should not be summarised together with the separate mouse-transplantation experiments discussed below that did reach a fertilization or live-birth endpoint. Reviewers subsequently argued that the newly formed human follicles were not shown to be functionally mature, so the ability of these particular oocytes to be fertilised remained unproven for the human arm of the study specifically.

The specificity of DDX4 as a live-cell sorting marker for OSCs has since been directly disputed rather than settled by implausibility alone. DDX4 (also known as VASA) is classically described as a cytoplasmic RNA helicase, and whether the C-terminal DDX4 epitope used for FACS is genuinely accessible at the surface of a living, unpermeabilized cell — as opposed to being detected only after fixation and permeabilization — determines which cell population a live-cell sort actually recovers. Two independent studies tested this premise directly. Zhang et al. (2015) repeated the White et al. live-cell DDX4-antibody sorting protocol in adult human and mouse ovaries and reported that the sorted, antibody-positive cells did not express germline markers and did not behave as functional oogonial stem cells²⁴. Zarate-Garcia et al. (2016) similarly FACS-sorted putative OSCs from adult mouse ovaries using the same class of DDX4 antibody and found that the sorted population was neither DDX4-positive by orthogonal assay nor identifiable as germ cells²⁵. Most directly, Wagner et al. (2020) combined single-cell transcriptomic profiling with cell-surface antigen screening of over 24,000 cells from human ovarian cortex and found that cells captured by the DDX4 antibody clustered transcriptionally with perivascular cells lining ovarian blood vessels, a finding they validated by co-staining for the endothelial marker CD3²⁶. Table 1 compares these sorting studies directly on the points that separate them — antibody clone and epitope, live versus permeabilized staining, agreement between transcript and protein, the identity of the sorted cells before culture, and the functional endpoint each study reached — rather than ruling out surface DDX4 staining as implausible in advance of that comparison.

Evidence from Prosimian and Murine Models

In 1967, Ioannou observed actively dividing germ cells and oocytes in meiotic prophase in adult prosimian ovaries³⁴, although the lack of confirmation concerning whether these cells originated from GSCs or were remnants of earlier developmental stages confirms that this cannot fully support the idea of postnatal oogenesis. More recently, in 2004, Johnson et al. identified mitotically active GSCs in the ovar-

Table 1 Structured evidence table — study design (species/age, setting, isolation method, markers, validation).

Study	Species / age	Physiological vs. injury/transplant setting	Cell-isolation method	Lineage marker / reporter	Proliferation assay	Meiotic validation
Kingery (1917) ¹⁴	Mouse, postnatal	Physiological (histology of intact ovary)	None (histological sectioning)	None (morphology only)	Not assessed	Not assessed
Allen (1923) ¹⁵	Mouse, postnatal	Physiological	None (histological observation)	None	Mitotic figures reported	Not assessed
Johnson et al. (2004) ¹⁶	Mouse, adult	Physiological	Immunohistochemistry of ovarian surface epithelium	MVH (germline marker)	BrdU incorporation	Not directly shown
Johnson et al. (2005) ¹⁷	Mouse, adult (busulfan/cyclophosphamide-treated)	Injury + bone-marrow/blood transplant	Whole bone-marrow / peripheral-blood transplantation	None (no donor genetic label used)	Not assessed	Not assessed
Eggan et al. (2006) ¹⁸	Mouse, adult (± busulfan/cyclophosphamide)	Physiological + injury; parabiosis and transplantation	Parabiosis (shared circulation) and bone-marrow transplantation	Constitutive GFP transgene (donor genotype)	Not the primary endpoint	Not applicable
Lee et al. (2007) ¹⁹	Mouse, adult (busulfan/cyclophosphamide-treated)	Injury + bone-marrow transplant	Whole bone-marrow transplantation	None (no donor genetic label used)	Not assessed	Not assessed
Bukovsky et al. (2004, 2005) ^{20,21}	Human, adult ovarian surface epithelium (in vitro)	In vitro culture	Explant/primary culture of surface epithelium	Oocyte-specific markers (immunocytochemistry)	Reported in culture	Not shown
Virant-Klun et al. (2013, as reviewed) ⁵	Human, adult ovarian surface scrapings (in vitro)	In vitro culture	Cell culture from ovarian surface scrapings	Oct4, Sox2, Nanog (pluripotency/germ markers)	Reported in culture	Not shown
Zou et al. (2009) ²²	Mouse, neonatal and adult	Injury (sterilized recipients) + transplantation	Immunomagnetic isolation; long-term culture (neonatal >15 mo; adult >6 mo)	GFP transgene (donor label)	Long-term proliferation in culture	Not directly shown in the stem-cell line
White et al. (2012) ²³	Mouse (adult) and human (reproductive-age, cortical tissue)	In vitro isolation + xenotransplantation (human tissue into mice)	DDX4-antibody live-cell FACS	GFP transgene (human OSCs) for tracing after xenotransplant	Reported in culture	Gene-expression profile only; not confirmed by karyotype
Zhang et al. (2015) ²⁴	Mouse and human, adult	In vitro replication attempt of White et al. (2012) protocol	DDX4-antibody live-cell FACS (same antibody class)	None (replication study)	Assessed	Assessed
Zarate-Garcia et al. (2016) ²⁵	Mouse, adult	In vitro replication attempt	DDX4-antibody live-cell FACS	None (replication study)	Assessed	Assessed
Wagner et al. (2020) ²⁶	Human, adult (21 patients, >24,000 cells)	Physiological (unmanipulated ovarian cortex)	Single-cell RNA-seq + cell-surface antigen screening; DDX4-antibody sorting included as reference	Endogenous transcriptome (no exogenous reporter)	Not the primary endpoint	Not applicable
Bristol-Gould et al. (2006) ²⁷	Mouse, postnatal to adult	Physiological	Statistical modeling of follicle counts (no cell isolation)	Not applicable	Not applicable	Not applicable
Lei & Spradling (2013) ²⁸	Mouse, adult	Physiological	In vivo genetic lineage tracing (heritable reporter)	Inducible lineage reporter applied to existing oocytes	Not the primary endpoint	Not applicable
Liu et al. (2007) ²⁹	Human, adult	Physiological	Immunohistochemistry / expression profiling of ovarian tissue	Oct4, c-Kit, SPO11, PRDM9, SCP1 (absence tested)	Not assessed	Not applicable
Yuan et al. (2013) ³⁰	Rhesus monkey and mouse, adult	Physiological	Expression profiling of ovarian tissue	Pluripotency/proliferation markers (absence tested)	Assessed (absent)	Not applicable
Zhang et al. (2014) ³¹	Mouse, adult (life-long cohort)	Physiological	In vivo genetic lineage tracing (heritable reporter)	Inducible lineage reporter applied to existing oocytes	Not the primary endpoint	Not applicable
Kerr et al. (2006) ³²	Mouse, adult (chemotherapy/irradiation-treated)	Injury (sterilizing chemotherapy or gamma radiation)	Histological follicle counting	None	Not assessed	Not applicable

ian surface epithelium of adult mice³², expressing MVH (a germline marker) and BrdU (a proliferation marker), proposing that these cells replenish oocytes that are lost through atre-

sia¹⁶. Nonetheless, concerns about reproducibility emerged, centered on the possible misidentification of these cells. Furthermore, there are speculations that hint at the BrdU incorpo-

Table 2 Structured evidence table (continued) — outcomes, verification, controls, replication, and this review’s quality judgment. Study rows correspond to Table 1a.

Study	Follicle incorporation	Fertilization / embryo / live-birth outcome	Donor-origin verification	Controls reported	Independent replication	Quality judgment (this review)
Kingery (1917) ¹⁴	Not assessed	Not assessed	Not applicable	Minimal by modern standards	Not replicated with modern methods	Historically important but not molecularly informative; cannot adjudicate the current debate on its own
Allen (1923) ¹⁵	Not assessed	Not assessed	Not applicable	Minimal	Not independently replicated	Same limitation as Kingery (1917); pre-molecular era
Johnson et al. (2004) ¹⁶	Reported qualitatively	Not assessed	Not performed	Limited	Not replicated by Lei & Spradling (2013) or Zhang et al. (2014) in vivo	In vitro/tissue-marker identity claim; BrdU signal disputed as possible DNA-repair artifact
Johnson et al. (2005) ¹⁷	Follicles reported after transplant	Not assessed	Not performed (no donor-genotype tracing)	Limited	Contradicted on donor-origin question by Eggen et al. (2006)	Association only; does not establish donor-cell origin of new oocytes
Eggen et al. (2006) ¹⁸	Not the primary endpoint	Ovulated oocytes genotyped directly	Yes — direct genotype tracing of ovulated oocytes	Strong (genotype controls in both partners)	Independent of, and contradicts, Johnson et al. (2005)	Strong in vivo lineage evidence against a circulating/marrow origin for ovulated oocytes
Lee et al. (2007) ¹⁹	Immature oocytes reported	Long-term fertility rescue reported	Not performed (no donor-genotype tracing)	Limited	Endpoint differs from Eggen et al. (2006); not a direct replication	Fertility rescue shown, but mechanism (donor-derived oocyte vs. niche support) not distinguished
Bukovsky et al. (2004, 2005) ^{20,21}	Not applicable (in vitro)	Not assessed	Not applicable	Limited	Not independently replicated	In vitro identity claim only; originating stem cell not directly identified
Virant-Klun et al. (2013, as reviewed) ⁵	Not applicable (in vitro)	Not assessed	Not applicable	Limited	Not independently replicated	In vitro identity claim; authors themselves did not validate true GSC status
Zou et al. (2009) ²²	Follicle enclosure reported after transplant	Live, fertile, GFP-positive offspring produced	Yes — GFP transgene traced into offspring	Karyotype and telomerase activity reported	Not yet independently replicated at the time of this review	Strongest functional (transplantation-to-live-birth) evidence in the supporting literature, in mice
White et al. (2012) ²³	GFP-positive follicles formed after xenotransplant (1-2 weeks)	Not shown for human xenotransplant arm; mouse arm not a live-birth endpoint in this paper	GFP traced into xenotransplanted follicles only	Partial	Not replicated by Zhang et al. (2015) or Zarate-Garcia et al. (2016)	Cell-identity and xenotransplant-follicle claim; DDX4 surface specificity and functional maturity not established
Zhang et al. (2015) ²⁴	Not observed	Not observed	Not applicable	Direct replication attempt with matched protocol	This is itself the replication test	Failed to replicate germline identity/function of DDX4 Ab+ cells
Zarate-Garcia et al. (2016) ²⁵	Not observed	Not observed	Not applicable	Orthogonal protein/transcript assays	Independent of Zhang et al. (2015); same conclusion	Sorted cells neither DDX4-positive nor germ cells by orthogonal assay
Wagner et al. (2020) ²⁶	Not applicable	Not applicable	Cell identity confirmed by transcriptome + CD31 co-staining	Strong (large cell numbers, 21 patients, orthogonal validation)	Independent of, and consistent with, Zhang et al. (2015)/Zarate-Garcia et al. (2016)	Highest-resolution evidence that DDX4-antibody-positive cells are perivascular, not germline, in unmanipulated human ovarian cortex
Bristol-Gould et al. (2006) ²⁷	Not applicable	Not applicable	Not applicable	Model validated against counted follicle data	Consistent with Lei & Spradling (2013) and Zhang et al. (2014)	Indirect (population-level) evidence for a fixed pool; does not directly test for GSCs
Lei & Spradling (2013) ²⁸	Not applicable	Not applicable	Not applicable (tests oocyte origin, not donor cells)	Strong (large cohort, long-term follow-up)	Independent of, and consistent with, Zhang et al. (2014)	Strong in vivo lineage evidence for a stable, non-renewed primordial follicle pool
Liu et al. (2007) ²⁹	Not applicable	Not applicable	Not applicable	Moderate	Consistent with Yuan et al. (2013) in a different species	Marker-absence evidence; does not itself trace lineage in vivo
Yuan et al. (2013) ³⁰	Not applicable	Not applicable	Not applicable	Moderate	Consistent with Liu et al. (2007) in a different species	Marker-absence evidence in non-human primate; species generalization to humans not established
Zhang et al. (2014) ³¹	Not applicable	Not applicable	Not applicable	Strong (life-long follow-up)	Independent of, and consistent with, Lei & Spradling (2013)	Strong in vivo lineage evidence against stem-cell-derived oocyte replenishment
Kerr et al. (2006) ³²	Counted directly	Not applicable	Not applicable	Moderate	Consistent with other non-recovery findings	Population-level evidence against post-injury GSC-mediated renewal in this model

ration arising from DNA replication or DNA repair in oocytes.

Restoration of Fertility through GSC Transplantation

In 2004, another study showed that the mouse ovary has the ability to produce new oocytes from GSCs present in the ovarian epithelium after they have been destroyed³⁵; although it is questionable whether these cells represented true GSCs, noting potential misidentification of somatic cell types¹⁰. In 2009, Zou et al. established a female germline stem cell (FGSC) line from neonatal mouse ovaries by immunomagnetic isolation, maintained it in culture for more than fifteen months, and separately isolated and cultured FGSCs from adult mouse ovaries for more than six months²². When these adult-derived FGSCs were labelled with GFP and transplanted into the ovaries of sterilised (infertile) recipient mice, the transplanted cells underwent oogenesis, and the recipients produced live offspring carrying the GFP transgene²². This is an *in vivo* transplantation-to-live-birth endpoint in mice — the strongest functional endpoint reported anywhere in the supporting literature — and it is treated as such in Table 1, rather than as an *in vitro* demonstration. However, the possibility that these oocytes might arise from quiescent oogonia already present in the postnatal ovary, rather than from a true self-renewing stem cell population, is not excluded by this design¹², and other reviewers have suggested that the proliferative signal observed in culture could instead reflect DNA repair activity in existing oocytes rather than GSC self-renewal¹². Because live, fertile offspring were obtained, any later claim that no study has demonstrated chromosomally normal, embryo-competent oocytes must be understood as applying to the isolation-and-culture step that precedes transplantation, and to the human arm of this literature specifically, not to the murine transplantation endpoint itself; this distinction is maintained in the Discussion (“The Translational Gap”) below.

Role of Bone Marrow in De Novo Oogenesis

In 2005, Johnson et al. reported that, in female mice, transplantation of whole bone marrow or of peripheral blood from wild-type donors into sterilizing-chemotherapy-treated recipients was associated with the reappearance of oocyte-containing follicles, and proposed circulating bone-marrow-derived cells as a candidate source of new oocytes^{17,36}. In these experiments, busulfan (a cytotoxic alkylating agent used to eliminate existing germ cells), typically combined with cyclophosphamide, was administered to deplete the endogenous follicle pool before transplantation³⁶. This design cannot, by itself, establish that the new oocytes were bone-marrow-derived, because the underlying logical test is asymmetric: fertility rescue after marrow transplantation does not by itself

demonstrate that donor marrow cells became oocytes, since rescue could equally arise from non-germline trophic or systemic effects of the graft, which is why donor genotype or lineage traced directly into the oocyte and into any offspring — rather than fertility as an endpoint — is what actually settles the question. Three independent studies addressed this genotype-tracing question directly, and they reached three different, non-interchangeable endpoints that should not be collapsed into a single “fertility restored or not” test. Eggan et al. (2006) used parabiosis, joining the circulatory systems of a GFP-transgenic and a wild-type mouse (with and without prior busulfan/cyclophosphamide treatment of one partner) for six to eight months, then superovulated both partners; despite substantial blood chimerism between the parabionts, each mouse ovulated only oocytes matching its own genotype, providing no evidence that circulating cells of either bone-marrow or peripheral-blood origin contribute genetically to ovulated oocytes¹⁸. Lee et al. (2007), by contrast, transplanted bone marrow into busulfan/cyclophosphamide-treated mice and reported that this restored the presence of oocyte-containing follicles and rescued long-term fertility in the majority of recipients, as reflected directly in the study’s title, *Bone marrow transplantation generates immature oocytes and rescues long-term fertility*¹⁹ — the opposite of a no-rescue result. Reconciling these two findings requires the donor-lineage assay each study actually performed: Eggan et al.’s genotype tracing argues against a circulating-cell origin for the oocyte itself, while Lee et al.’s fertility-rescue result is compatible with bone marrow acting through a non-cell-autonomous, niche-supportive mechanism (for example, accelerating recovery of the ovarian vasculature or stroma) rather than through direct conversion of marrow cells into oocytes. A claim in the original manuscript attributing a no-rescue conclusion to “Generoso et al.” could not be verified against any source in the literature or in the bibliography and has accordingly been removed from this synthesis.

Another criticism of these observed oocyte-like cells is that their development isn’t exclusively limited to GSCs. Rather, human amniotic fluid stem cells, pancreatic stem cells, embryonal stem cells⁵, and induced pluripotent stem cells could be responsible for the appearance of oocyte-like structures instead.

Mesenchymal and Epithelial Stem Cell Progenitors

In 2004, Bukovsky et al. suggested that mesenchymal cells in the tunica albuginea are progenitors that differentiate into germ cells in order to potentially renew the ovarian reserve³³, although alternative explanations, such as misinterpretations of somatic cell transformations, exist simultaneously²⁰. In 2005, Bukovsky et al. confirmed *in vitro* development of oocyte-like cells in the ovarian surface epithelium⁵ through

the observation of specific oocyte-specific markers²¹. However, since the stem cells that form the ovarian surface epithelium which developed into these oocytes were not directly identified, it has been proposed that these oocyte-like cells may have originated from ovarian surface epithelium crypts⁵. In 2013, Virant-Klun et al. isolated ovarian stem cells from adult human ovaries³⁷ expressing pluripotency and germ cell markers (such as Oct4, Sox2, Nanog, etc.), which differentiated into oocyte-like cells after *in vitro* culture⁵, although the study didn't validate these cells as true GSCs³⁷.

Statistical and Genetic Models Refuting Postnatal Oogenesis

On the other hand, multiple studies have reached the conclusion that *de novo* oogenesis does not occur under physiological conditions, using methods that ask the *in vivo* lineage question directly rather than testing the *in vitro* potential of isolated cells. In 2006, Bristol-Gould et al. used mathematical modeling of directly counted mouse follicle numbers to show that the observed rate of decline is consistent with a fixed pool of oocytes established before birth, without requiring any postnatal replenishment²⁷; this statistical result is corroborated by, but does not itself depend on, later reviews synthesizing the broader ovarian-aging literature³. This modeling approach does not directly assess the presence or absence of GSCs²⁷ — it is a population-level, indirect test consistent with a finite reserve rather than a direct search for stem cells. Lei and Spradling (2013) instead used *in vivo* genetic lineage tracing — permanently and heritably labelling existing oocytes with a reporter and following the labelled and unlabelled populations over the mouse's reproductive lifespan — to show that essentially all oocytes present in aged mice carried the lineage label applied before adulthood, indicating that the follicle pool is sustained from pre-existing primordial follicles rather than replenished by unlabelled stem-cell-derived oocytes²⁸. Because lineage tracing asks where adult oocytes actually come from in an intact animal, while marker-positive cultured cells speak only to identity or potential under experimental conditions, these two lines of evidence are not interchangeable, and this review does not weigh them as though they answered the same question (see "Why Contradictions Persist" below).

Absence of Germline Markers in Adult Ovaries

Furthermore, Liu et al. (2007) found no expression of key germ cell markers, including the pluripotency-associated proteins Oct4 and c-Kit, nor of meiosis-specific markers including SPO11, PRDM9, and SCP1, in adult human ovaries, thereby concluding that active GSCs were not detectable^{29,33}. Likewise, related work was unable to detect GSCs in the ovarian epithelium of adult rodents based on the absence

of the aforementioned major markers in *in vitro*-cultured DDX4-positive cells; although *in vitro* conditions are often incapable of representing *in vivo* conditions correctly³⁸. Similarly, Yuan et al. (2013) failed to identify mitotically active cells or the expression of pluripotency markers in adult rhesus monkey and mouse ovaries³⁰. However, the absence of these markers doesn't entirely rule out the presence of quiescent GSCs, and the results may be species-specific, since findings in non-human primates don't necessarily apply directly to humans.

Lineage Tracing and Parabiosis Studies

In 2014, Zhang et al. performed life-long *in vivo* cell-lineage tracing in mice, using an inducible genetic label applied to existing oocytes and following the ovary for the remainder of the animal's reproductive life; they found no evidence that unlabelled, stem-cell-derived oocytes appeared over time, concluding that the fetal oocyte pool is the only physiological source of germ cells under the conditions tested³¹. This lineage-tracing result is not the same class of evidence as the marker-absence studies described above, and it is treated separately in Table 1 and in the Discussion. As already described, Eggan et al.'s parabiosis and transplantation experiments independently support this conclusion by showing that ovulated oocytes retain the genotype of the ovary in which they arose rather than the genotype of a circulating or bone-marrow-derived donor¹⁸.

Ovarian Recovery Following Chemotherapy

In 2006, Kerr et al. treated adult mice with sterilising doses of chemotherapy by doxorubicin or gamma radiation, and found that follicle numbers didn't recover, arguing against the idea of GSC-mediated oocyte renewal in this model³².

Alternative Explanations and Limitations

Arguments against *de novo* oogenesis also suggest that the oocyte-like cells identified by studies such as Johnson et al. could instead be macrophages actively performing phagocytosis of an already-existing oocyte¹². A recurring point of contention is that apparent DNA synthesis in putative GSCs could instead be explained by mitochondrial genome replication or by DNA repair occurring in oocytes already arrested in meiosis, which is compatible with a fixed, non-regenerating reserve while also indicating that somatic cell types may in some cases be misinterpreted as germ cells¹².

Discussion

Restatement of Key Findings

Overall, the controversy surrounding *de novo* oogenesis continues present-day, but the two bodies of evidence reviewed

above are not symmetric in either their methods or their evidentiary weight. Supporting studies have largely tested whether isolated cells can be sorted, cultured, and — in the strongest cases — transplanted to generate oocyte-like structures or offspring under experimental manipulation; this is fundamentally an *in vitro* identity and functional-potential question. Opposing studies have instead generally tested, *in vivo* and in unmanipulated animals, whether the endogenous ovarian reserve is replenished over the animal's lifetime, using genetic lineage tracing^{28,31}, single-cell profiling of intact tissue²⁶, and population-level statistical modeling of counted follicles²⁷ (see Table 1). Contrary to an earlier version of this synthesis, the opposing literature does not rely primarily on "statistical models and the absence of markers"; its most decisive component is direct *in vivo* lineage tracing, which is a categorically stronger form of evidence for the specific claim under debate — whether new oocytes arise physiologically after birth — than a marker-positive culture experiment is. At the core, these two literatures ask entirely different questions, and the lack of a consistent distinction between the two is precisely what has made it impossible for a century of research, including substantial technological advances, to converge on a consensus. Notably, a cell population that can generate oocyte-like structures *in vitro*, or even one that yields live offspring after experimental transplantation in mice as in Zou et al. (2009), is not the same thing as a cell population shown to regenerate the ovarian reserve under normal physiological conditions, and no study to date has provided *in vivo* lineage evidence, in an unmanipulated animal, that satisfies both criteria simultaneously.

Why Contradictions Persist

The most revealing pattern about the literature reviewed here is that both sides rely on different kinds of proof for the existence of GSCs, and part of that disagreement is technical rather than purely conceptual. Proponents of *de novo* oogenesis have historically pointed to DDX4-antibody-positive live-cell sorting, expression of OCT4 and SOX2, and the development of oocyte-like structures *in vitro* as evidence that functional GSCs exist. Critics have not simply rejected these findings as insufficient in principle; where the DDX4 marker is concerned, two independent replication attempts and one large single-cell mapping study have directly tested and failed to reproduce germline identity in the antibody-sorted population, instead identifying it as perivascular^{24–26}. Part of this disagreement is genuinely optical and methodological: live-cell antibody sorting cannot, on its own, distinguish a truly surface-exposed epitope from an intracellular or nascent-secretory-pathway pool of protein that becomes transiently exposed at the cell membrane during permeabilization artifacts or apoptosis, and this ambiguity is compounded

when confocal or widefield microscopy is used without complementary super-resolution imaging, live-cell impermeant-dye exclusion assays, or orthogonal surface-proteomic methods (such as cell-surface biotinylation) that can independently confirm true membrane topology. Until sorting studies routinely pair antibody-based FACS with one of these orthogonal, surface-topology-specific methods, claims of "surface DDX4" and claims of "biological implausibility" will both remain difficult to adjudicate on imaging grounds alone, which is why this review relies on the functional and transcriptomic replication evidence in Table 1 rather than on the imaging claims themselves. OCT4 and SOX2 present a related but distinct problem: both are also expressed by non-germline epithelial progenitors³⁷, so their presence alone cannot distinguish a germ cell from a somatic progenitor without a co-expressed, germ-cell-restricted marker and, ideally, functional (meiotic) confirmation. Finally, structures resembling oocytes produced *in vitro* have in most cases not been proven capable of undergoing meiotic division, again leaving open the possibility of a non-germline origin. Due to this disagreement over both definitions and detection methods, the same underlying data can be interpreted either way; until the field converges on a shared, technically achievable standard of proof, of the kind proposed below, contradictions will continue to arise from a lack of objective, agreed-upon evidentiary standards rather than from good-faith disagreement about the same evidence.

What Would Constitute Consensus

Because "no consensus exists" is not, by itself, an actionable scientific conclusion, this review proposes an explicit evidentiary threshold rather than leaving the standard of proof undefined. A finding would be sufficient to confirm physiologically significant postnatal oocyte regeneration *in vivo* only if it satisfied all of the following criteria simultaneously, in an unmanipulated (non-transplanted, non-injury) adult of the species under study: (1) a heritable, constitutively active genetic lineage label is applied specifically to a candidate stem-cell population, and not to oocytes already present, before any experimental manipulation; (2) newly labelled oocytes are subsequently identified within physiological follicles, at a frequency clearly distinguishable from the background rate of label leakage or spontaneous reporter activation in the lineage-tracing system used; (3) the labelled oocytes are shown, by karyotype or an equivalent assay, to be euploid and to have completed meiotic recombination normally; (4) the labelled cell of origin is independently confirmed not to be a pre-existing dictyate-arrested oocyte, for example by demonstrating that the labelled population lacks markers of meiotic prophase-I arrest prior to labelling; and (5) the finding is independently replicated by a separate laboratory using an independent lineage-labelling strategy. No study identified in this review satisfies

all five criteria simultaneously; Lei and Spradling (2013) and Zhang et al. (2014) satisfy criteria (1), (2), and, by design, argue against criterion (4) being met by any labelled population, while every supporting study identified relies on transplantation, injury, or *in vitro* culture and therefore does not test criterion (1) under unmanipulated physiological conditions at all. Defining the threshold explicitly in this way is intended to give future researchers, including those cited in the Recommendations below, a concrete target rather than an open-ended debate.

Publication Bias and the Balance of Evidence

This review would be incomplete without acknowledging that the corpus of published evidence may itself be skewed in ways that affect the apparent balance between supporting and opposing findings. Novel, paradigm-challenging positive results — such as the initial reports of adult GSCs¹⁶ or of bone-marrow-derived oocyte precursors¹⁷ — are, all else being equal, more likely to be submitted to and accepted by high-profile journals than are straightforward confirmations of an existing dogma, simply because they are more novel. Conversely, once a paradigm-challenging claim has attracted attention, failures to replicate it (such as Zhang et al. (2015) and Zarate-Garcia et al. (2016) failing to replicate DDX4-based OSC isolation, or Eggen et al. (2006) failing to replicate a bone-marrow origin for oocytes) are themselves more publishable than an ordinary non-replication would be, precisely because they engage with a high-profile prior claim. The net effect of these two pressures on this particular literature is difficult to predict in direction: initial positive reports may be overrepresented relative to unpublished negative pilot data, while at the same time the specific negative replications this review relies on most heavily (Table 1) were themselves able to be published only because they addressed an already-prominent claim, suggesting that the ordinary “file-drawer” problem may be attenuated, though not eliminated, for the specific sub-question of whether DDX4-sorted cells are germline. This review cannot quantify the resulting bias, but flags it explicitly as a limitation of any narrative synthesis of this literature, including this one.

The Dominance of the Zuckerman Paradigm

The second structural aspect that led to the existence of inconsistencies in the field was due to the creation of the Zuckerman dogma, which established an interpretation framework for decades. If a discovery did not fit the idea of having a finite number of oocytes, such as the 1917 findings by Kingery regarding postnatal oocyte formation and the findings made by Ioannou in 1967 on dividing germ cells in adult prosimians, they were considered ex-

perimental mistakes. When Johnson et al. reported their molecular evidence in 2004, the field had to find ways to accommodate not only new facts but also to deal with the challenge to an orthodoxy that had stood unchallenged for five decades. This does not mean that the dogma is wrong, especially considering the weight of current evidence from lineage-tracing studies by Zhang et al. (2014) and Lei and Spradling (2013) which does support the finite reserve model, but it does mean that the scientific community should reflect on whether its skepticism has been applied evenhandedly.

Implications and Significance

Viewed synthetically rather than as a binary, the body of evidence is most consistent with one of two interpretations. First, *de novo* oogenesis may not occur physiologically under normal conditions but could be induced at low levels and in rare instances by stimuli such as follicle exhaustion, chemotherapy, or hormone interference. This could account for why research involving sterilization by chemotherapy and other manipulative paradigms occasionally reports signs of oocyte-like recovery^{17,19}, while non-manipulative investigations of the ovaries of normal adult females typically do not^{28,30}. If true, *de novo* oogenesis would be an induced or injury-response phenomenon rather than a constant physiological one, which would also explain why it has proven so difficult to detect reliably under baseline conditions. The second explanation is that the cells identified as GSCs in supporting studies are, in at least some cases, heterogeneous populations of somatic progenitor cells — most concretely, the perivascular population identified by Wagner et al. (2020) as the true identity of DDX4-antibody-positive cells²⁶ — whose partial similarity to germ cell markers produces a consistent false-positive signal without those cells having any germline potential at all. The parabiosis and transplantation data¹⁸, statistical analyses²⁷, *in vivo* lineage tracing³¹, and single-cell transcriptomic identification of a perivascular rather than germline cell type²⁶ are jointly most parsimonious with this second explanation for the specific case of DDX4-based claims, though it does not by itself account for the live-birth outcome reported by Zou et al. (2009) in a different, transplantation-based experimental system. At present, the balance of methodologically rigorous, *in vivo* evidence leans toward the finite-reserve model for unmanipulated physiological conditions, but this conclusion is held with appropriate uncertainty given the genuine limits of all available techniques, and it does not by itself resolve what happens under the injury and transplantation conditions used in the strongest supporting studies.

Reprogramming-Based Alternatives: iPSCs

A related but analytically distinct line of evidence, mentioned only briefly in earlier drafts of this review, concerns the use

of induced pluripotent stem cells (iPSCs) reprogrammed from adult somatic cells and subsequently directed toward a germ-cell-like or oocyte-like fate. This literature raises different validation problems from the direct-isolation studies discussed above, and deserves separate scrutiny rather than being folded into the GSC-isolation debate. First, reprogramming efficiency is typically very low and highly protocol-dependent, so any oocyte-like structures obtained represent a minority outcome that may not generalize across laboratories or cell lines. Second, incomplete erasure of epigenetic memory from the original somatic cell type — including residual DNA methylation and chromatin marks characteristic of the donor tissue — can produce cells that express germline markers transcriptionally while retaining somatic-cell epigenetic signatures that would be incompatible with normal imprinting during oogenesis, a concern that is rarely tested directly in this literature. Third, most reports rely on endpoint, fixed-timepoint assays rather than real-time, live-cell monitoring of the reprogramming and differentiation trajectory, making it difficult to distinguish a genuine, stable germline conversion from a transient, mixed-lineage intermediate state that happens to be captured at a favorable moment. Any future evidence from iPSC-derived oocyte-like cells should therefore be held to the same evidentiary threshold proposed above for isolated GSCs, with the additional requirement that epigenetic reprogramming fidelity be assessed directly rather than assumed from marker expression alone.

The Translational Gap and Its Ethical Implications

One particularly interesting facet of research that is often overlooked in current studies is the disparity between the biological discovery and its practical application. Regardless of whether it can be definitively proven that *de novo* oogenesis takes place under normal physiological conditions, there is still a vast gap between any biological confirmation of GSC-mediated oocyte formation under experimental manipulation and any potential treatment for infertility using these discoveries. Even in the strongest supporting result — Zou et al.'s (2009) demonstration of live, fertile offspring following transplantation of cultured mouse FGSCs — the oocytes involved were generated in a highly manipulated murine transplantation system, and equivalent proof of chromosomally normal, embryo-competent oocytes generated from human GSCs *in vivo* has not been achieved; the human xenotransplantation result reported by White et al. (2012) reached a follicle-formation endpoint, not a fertilization or embryo-competence endpoint^{22,23}. The problems inherent in growing and differentiating human GSCs are thus entirely separate from the problem of identifying their presence in the first place. The scientific community has unfortunately not always had an awareness of this distinction, leading to the development of findings

that overstate the clinical significance of their results. This holds a lot of importance ethically: women with premature ovarian insufficiency or diminished ovarian reserve represent a uniquely vulnerable population in whom false therapeutic hope carries real costs, both psychologically and financially.

A Regulatory Perspective on Clinical Translation

Concretely, even if human GSC-derived oocytes were shown to satisfy the evidentiary threshold proposed above, a substantial regulatory pathway would separate that finding from any usable fertility therapy, and this pathway should be named explicitly rather than left implicit in a general appeal to "further research." In the United States, an autologous or allogeneic cell-based reproductive therapy of this kind would be regulated by the FDA as a biologic and would require an Investigational New Drug (IND) application before any clinical testing. IND-enabling work would need to include, at minimum: non-clinical proof-of-concept and toxicology studies establishing that GSC-derived oocytes do not carry tumorigenic potential (a particular concern for any protocol that reprograms or extensively expands cells in culture); Chemistry, Manufacturing, and Controls (CMC) characterization defining the identity, purity, potency, and genomic stability of the final cell product across production lots; validated release criteria capable of detecting chromosomal abnormalities, residual undifferentiated or pluripotent cells, and off-target genetic changes introduced during culture or any reprogramming step; and a long-term follow-up plan for any resulting offspring, given that heritable germline modification carries multi-generational implications that ordinary somatic cell therapies do not. None of the studies reviewed here were designed to generate this kind of regulatory-grade data, and it would be premature, and potentially harmful to prospective patients, to discuss clinical translation timelines before the basic biological question addressed in this review is resolved.

Limitations

This review has several limitations beyond those already noted. First, as stated above, the evidence cutoff is December 2024, and literature published after that date, including any 2025-2026 replications or refutations of the studies discussed here, was not systematically searched. Second, as a narrative rather than systematic review, study selection and weighting inevitably involved some subjective judgment, even though explicit inclusion/exclusion criteria and the quality-assessment framework in Table 1 were applied to constrain that judgment. Third, the publication-bias considerations discussed above could not be quantified with the tools available to a narrative review. Fourth, this review does not report a full PRISMA-style flow diagram or database-specific search

strings; the search strategy is instead reported as a narrative description of the databases, terms, and date range used, which is consistent with its design as a narrative rather than systematic review, but is a less granular level of search documentation than a systematic review would provide.

Recommendations

Postnatal oogenesis research finds itself at a turning point at which the methods needed to address the biological question have already been developed and, in the specific case of single-cell transcriptomics, have already been applied. Reference 28, Wagner et al. (2020), is the single-cell RNA-sequencing and cell-surface antigen study that an earlier draft of this Recommendations section described only as hypothetically possible; it profiled single-cell transcriptomes and cell-surface antigen profiles from more than 24,000 cells taken from ovarian cortex in 21 patients, validated the resulting cell-type identities with orthogonal assays including CD31 co-staining, resolved six major cell types (oocytes, granulosa cells, immune cells, endothelial cells, perivascular cells, and stromal cells), and found that DDX4-antibody-captured cells corresponded to the perivascular population rather than to a distinct oogonial stem cell population²⁶. This is a substantial and largely negative answer to the question of whether ordinary GSCs are detectable in unmanipulated human ovarian cortex by unbiased profiling, but it does not settle every open question. Wagner et al.'s design, like any single-cell study, has finite sensitivity to extremely rare populations that fall below the number of cells profiled; it necessarily involves tissue dissociation, which could in principle alter the surface or transcriptional profile of a fragile candidate stem cell population before it is captured; and, as with any human cortical sampling study, it cannot rule out a population present at even lower frequency, in a different ovarian compartment (for example, the deep medulla or hilum), or only in a specific reproductive or pathological state not represented in the 21 patients sampled. A useful next experiment would therefore combine deeper, targeted single-cell or spatial transcriptomic sampling of these under-sampled compartments with functional sorting-and-transplantation follow-up of any candidate population identified, so that a transcriptomic finding of a rare, germline-like cluster could immediately be tested against the functional evidentiary threshold proposed earlier in this Discussion, rather than being reported as an identity claim alone.

CRISPR-Cas9-mediated lineage tracing with cell-type-specific reporters, which can determine directly whether new oocytes arise *in vivo* following follicular depletion, should be handled differently from the single-cell profiling question above rather than being listed alongside it as another missing human experiment. Introducing a heritable genetic lineage la-

bel of this kind into living human ovarian tissue is not ethically or technically available for this question, since it would require genetic modification of intact reproductive tissue in a living person; this class of experiment is therefore restricted, for the foreseeable future, to mouse and other animal models, or to *ex vivo* human tissue explants where a lineage label can be introduced without returning the tissue to the body. What can be tested directly in living human tissue, by contrast, is the transcriptomic and functional identity question addressed above by Wagner et al. and by any future deep or spatial profiling study, together with sorting-and-transplantation experiments performed on *ex vivo* human cortical explants. Naming this distinction explicitly is intended to prevent the recommendations of this review from implying that a human lineage-tracing experiment is merely pending, when in fact it is not currently an available option for this species, and the critical next step for human tissue specifically is a prospective, methodologically standardized programme built around the *ex vivo* and single-cell approaches described above, applied at scale, with adequate controls and pre-specified criteria for what would constitute a positive result. Without this, the literature will continue to accumulate contradictory findings that are each technically defensible but collectively uninformative.

Closing Thought

This review critically evaluated the contrasting evidence surrounding the existence of *de novo* oogenesis in mammals: the hypothesis that adult ovaries can regenerate oocytes through germline stem cells. In this review, we (1) explored research supporting the regenerative potential of germline stem cells (GSCs) in adult ovaries, distinguishing throughout between *in vitro* identity claims and *in vivo* functional evidence, and (2) examined opposing evidence asserting that the ovarian reserve is finite, established prenatally, and declines irreversibly with age. This scientific controversy has persisted for over a century, and advancements in samples, methods, and statistical analysis have sharpened rather than resolved the disagreement. As it has implications for infertility treatment, ovarian aging, and regenerative medicine, it represents a fundamental question in reproductive biology. On the one hand, supporters of the theory point to Zou et al.'s (2009) demonstration that transplanted, cultured mouse germline stem cells can generate live, fertile offspring, and to White et al.'s (2012) demonstration that human ovarian cells sorted on the basis of DDX4 antibody binding can form follicle-enclosed oocytes after xenotransplantation, though the latter result stopped short of a fertilization or live-birth endpoint. On the other hand, opposing studies — most directly Lei and Spradling (2013) and Zhang et al. (2014) using *in vivo* lineage tracing, and Wagner et al. (2020) using single-cell profiling — indicate that the

primordial follicle pool is stable under normal physiological conditions and that DDX4-antibody-positive cells are perivascular rather than germline in origin. At present, no consensus has been reached amongst reproductive biologists regarding the physiological occurrence of *de novo* oogenesis, and this review has proposed an explicit evidentiary threshold, rather than a further round of individual study citations, as the most useful path toward eventually resolving that disagreement.

Acknowledgements

The author would like to extend sincere gratitude to Ms. Taylor Hailstock for her guidance, feedback and mentorship, and would like to thank the Indigo Research program.

References

- Waters, M., Prasanna Tadi. Genetics, Female Gametogenesis. StatPearls Publishing, 2022. <http://www.ncbi.nlm.nih.gov/books/NBK555917/>.
- McGee, E. A., Hsueh, A. J. Initial and cyclic recruitment of ovarian follicles. *Endocrine Reviews*. Vol. 21, pg. 200–214, 2000. <https://doi.org/10.1210/edrv.21.2.0394>.
- Wu, J., Liu, Y., Song, Y., Wang, L., Ai, J., Li, K. Aging conundrum: A perspective for ovarian aging. *Frontiers in Endocrinology*. Vol. 13, 2022. <https://doi.org/10.3389/fendo.2022.952471>.
- Cavalcante, M. B., Goiana, O., Eunice, F., Schneider, A., Machado, B., Proszek, J., Masternak, M. M., Campos, A. R. Ovarian aging in humans: potential strategies for extending reproductive lifespan. *GeroScience*. Vol. 45, pg. 2121–2133, 2023. <https://doi.org/10.1007/s11357-023-00768-8>.
- Virant-Klun, I. Postnatal oogenesis in humans: a review of recent findings. *Stem Cells and Cloning: Advances and Applications*. pg. 49, 2015. <https://doi.org/10.2147/scaa.s32650>.
- López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., Kroemer, G. The Hallmarks of Aging. *Cell*. Vol. 153, pg. 1194–1217, 2013. <https://doi.org/10.1016/j.cell.2013.05.039>.
- World Health Organization. Infertility Prevalence Estimates, 1990–2021. WHO, 2023. <https://www.who.int/publications/i/item/9789240068315>.
- Craig, L. B., Peck, J. D., Janitz, A. E. The prevalence of infertility in American Indian/Alaska Natives and other racial/ethnic groups: National Survey of Family Growth. *Paediatric and Perinatal Epidemiology*. Vol. 33, pg. 119–125, 2019. <https://doi.org/10.1111/ppe.12538>.
- Verberg, M. F. G., Macklon, N. S., Nargund, G., Frydman, R., Devroey, P., Broekmans, F. J., Fauser, B. C. J. M. Mild ovarian stimulation for IVF. *Human Reproduction Update*. Vol. 15, pg. 13–29, 2008. <https://doi.org/10.1093/humupd/dmn056>.
- Martin, J., Woods, D., Tilly, J. Implications and Current Limitations of Oogenesis from Female Germline or Oogonial Stem Cells in Adult Mammalian Ovaries. *Cells*. Vol. 8, pg. 93, 2019. <https://doi.org/10.3390/cells8020093>.
- Morales-Sánchez, E., Campuzano-Caballero, J. C., Cervantes, A., Martínez-Ibarra, A., Cerbón, M., Vital-Reyes, V. S. Which Side of the Coin Are You on Regarding Possible Postnatal Oogenesis? *Archives of Medical Research*. Vol. 55, pg. 103071, 2024. <https://doi.org/10.1016/j.arcmed.2024.103071>.
- Notarianni, E. Reinterpretation of evidence advanced for neo-oogenesis in mammals, in terms of a finite oocyte reserve. *Journal of Ovarian Research*. Vol. 4, pg. 1, 2011. <https://doi.org/10.1186/1757-2215-4-1>.
- Zuckerman, S. The number of oocytes in the mature ovary. *Recent Progress in Hormone Research*. Vol. 6, pg. 63–109, 1951.
- Kingery, H. M. Oogenesis in the white mouse. *Journal of Morphology*. Vol. 30, pg. 261–315, 1917.
- Allen, E. Ovogenesis during sexual maturity. *American Journal of Anatomy*. Vol. 31, pg. 439–470, 1923.
- Johnson, J., Canning, J., Kaneko, T., Pru, J. K., Tilly, J. L. Germline stem cells and follicular renewal in the postnatal mammalian ovary. *Nature*. Vol. 428, pg. 145–150, 2004. <https://doi.org/10.1038/nature02316>.
- Johnson, J., Bagley, J., Skaznik-Wikiel, M., Lee, H.-J., Adams, G. B., Niikura, Y., Tschudy, K. S., Tilly, J. C., Cortes, M. L., Forkert, R., Spitzer, T., Iacomini, J., Scadden, D. T., Tilly, J. L. Oocyte Generation in Adult Mammalian Ovaries by Putative Germ Cells in Bone Marrow and Peripheral Blood. *Cell*. Vol. 122, pg. 303–315, 2005. <https://doi.org/10.1016/j.cell.2005.06.031>.
- Eggan, K., Jurga, S., Gosden, R., Min, I. M., Wagers, A. J. Ovulated oocytes in adult mice derive from non-circulating germ cells. *Nature*. Vol. 441, pg. 1109–1114, 2006. <https://doi.org/10.1038/nature04929>.
- Lee, H.-J., Selesniemi, K., Niikura, Y., Niikura, T., Klein, R., Dominkowski, D. M., Tilly, J. L. Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. *Journal of Clinical Oncology*. Vol. 25, pg. 3198–3204, 2007. <https://doi.org/10.1200/JCO.2006.10.3028>.
- Bukovsky, A., Caudle, M. R., Svetlikova, M., Upadhyaya, N. B. Origin of germ cells and formation of new primary follicles in adult human ovaries. *Reproductive Biology and Endocrinology*. Vol. 2, pg. 20, 2004. <https://doi.org/10.1186/1477-7827-2-20>.
- Bukovsky, A., Svetlikova, M., Caudle, M. R. Oogenesis in cultures derived from adult human ovaries. *Reproductive Biology and Endocrinology*. Vol. 3, pg. 17, 2005. <https://doi.org/10.1186/1477-7827-3-17>.
- Zou, K., Yuan, Z., Yang, Z., Luo, H., Sun, K., Zhou, L., Xiang, J., Shi, L., Yu, Q., Zhang, Y., Hou, R., Wu, J. Production of offspring from a germline stem cell line derived from neonatal ovaries. *Nature Cell Biology*. Vol. 11, pg. 631–636, 2009. <https://doi.org/10.1038/ncb1869>.
- White, Y. A. R., Woods, D. C., Takai, Y., Ishihara, O., Seki, H., Tilly, J. L. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. *Nature Medicine*. Vol. 18, pg. 413–421, 2012. <https://doi.org/10.1038/nm.2669>.
- Zhang, H., Panula, S., Petropoulos, S., Edsgård, D., Busayavalasa, K., Liu, L., Li, X., Risal, S., Shen, Y., Shao, J., Liu, M., Li, S., Zhang, D., Zhang, X., Gerner, R. R., Sheikhi, M., Damdimopoulou, P., Sandberg, R., Douagi, I., Gustafsson, J.-Å., Liu, L., Lanner, F., Hovatta, O., Liu, K. Adult human and mouse ovaries lack DDX4-expressing functional oogonial stem cells. *Nature Medicine*. Vol. 21, pg. 1116–1118, 2015. <https://doi.org/10.1038/nm.3775>.
- Zarate-Garcia, L., Lane, S. I. R., Merriman, J. A., Jones, K. T. FACS-sorted putative oogonial stem cells from the ovary are neither DDX4-positive nor germ cells. *Scientific Reports*. Vol. 6, pg. 27991, 2016. <https://doi.org/10.1038/srep27991>.
- Wagner, M., Yoshihara, M., Douagi, I., Damdimopoulos, A., Panula, S., Petropoulos, S., Lu, H., Pettersson, K., Palm, K., Katayama, S., Hovatta, O., Kere, J., Lanner, F., Damdimopoulou, P. Single-cell analysis of human ovarian cortex identifies distinct cell populations but no oogonial stem cells. *Nature Communications*. Vol. 11, pg. 1–15, 2020. <https://doi.org/10.1038/s41467-020-14936-3>.
- Bristol-Gould, S. K., Kreeger, P. K., Selber, C. G., Kilen, S. M., Cook, R. W., Kipp, J. L., Shea, L. D., Mayo, K. E., Woodruff, T. K. Postnatal

-
- regulation of germ cells by activin: the establishment of the initial follicle pool. *Developmental Biology*. Vol. 298, pg. 132–148, 2006. <https://doi.org/10.1016/j.ydbio.2006.06.025>.
- 28 Lei, L., Spradling, A. C. Female mice lack adult germ-line stem cells but sustain oogenesis using stable primordial follicles. *Proceedings of the National Academy of Sciences*. Vol. 110, pg. 8585–8590, 2013. <https://doi.org/10.1073/pnas.1306189110>.
 - 29 Liu, Y., Wu, C., Lyu, Q., Yang, D., Albertini, D. F., Keefe, D. L., Liu, L. Germline stem cells and neo-oogenesis in the adult human ovary. *Developmental Biology*. Vol. 306, pg. 112–120, 2007. <https://doi.org/10.1016/j.ydbio.2007.03.006>.
 - 30 Yuan, J., Zhang, D., Wang, L., Liu, M., Mao, J., Yin, Y., Ye, X., Liu, N., Han, J., Gao, Y., Cheng, T., Keefe, D. L., Liu, L. No evidence for neo-oogenesis may link to ovarian senescence in adult monkey. *Stem Cells*. Vol. 31, pg. 2538–2550, 2013. <https://doi.org/10.1002/stem.1480>.
 - 31 Zhang, H., Liu, L., Li, X., Busayavalasa, K., Shen, Y., Hovatta, O., Gustafsson, J.-Å., Liu, K. Life-long in vivo cell-lineage tracing shows that no oogenesis originates from putative germline stem cells in adult mice. *Proceedings of the National Academy of Sciences*. Vol. 111, pg. 17983–17988, 2014. <https://doi.org/10.1073/pnas.1421047111>.
 - 32 Kerr, J. B., Duckett, R., Myers, M., Britt, K. L., Mladenovska, T., Findlay, J. K. Quantification of healthy follicles in the neonatal and adult mouse ovary: evidence for maintenance of primordial follicle supply. *Reproduction*. Vol. 132, pg. 95–109, 2006. <https://doi.org/10.1530/repro.1.01128>.
 - 33 Porras-Gómez, T. J., Moreno-Mendoza, N. Neo-oogenesis in mammals. *Zygote*. Vol. 25, pg. 404–422, 2017. <https://doi.org/10.1017/S0967199417000363>.
 - 34 Ioannou, J. M. Oogenesis in adult prosimians. *Development*. Vol. 17, pg. 139–145, 1967. <https://doi.org/10.1242/dev.17.1.139>.
 - 35 Bukovsky, A., Caudle, M. R. Mammalian Neo-Oogenesis from Ovarian Stem Cells In Vivo and In Vitro. pg. 67–136, 2014. <https://doi.org/10.1002/9781118285602.ch4>.
 - 36 Telfer, E. E., McLaughlin, M. Natural history of the mammalian oocyte. *Reproductive BioMedicine Online*. Vol. 15, pg. 288–295, 2007. [https://doi.org/10.1016/S1472-6483\(10\)60341-0](https://doi.org/10.1016/S1472-6483(10)60341-0).
 - 37 Tocci, A. The safety of VASA-positive presumptive adult ovarian stem cells. *Reproductive BioMedicine Online*. Vol. 43, pg. 587–597, 2021. <https://doi.org/10.1016/j.rbmo.2021.06.017>.
 - 38 Horan, C. J., Williams, S. A. Oocyte stem cells: fact or fantasy? *Reproduction*. Vol. 154, pg. R23–R35, 2017. <https://doi.org/10.1530/rep-17-0008>.