



REVIEW ARTICLE

Targeting Female germline stem cells -an attractive modality in ovarian insufficiency / postponed fertility / therapy of infertility following treated malignant tumors -A narrative review

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ABSTRACT

In the arena of reproductive medicine, postponement of ovarian aging and perpetuating fertility in cancer patients have long been believed to be main botherations in addition to continued efforts have been put in. Female germline stem cells (FGSCs) have been illustrated to cause healing of aging or injured ovarian structures as well as result in restoration of i) ovarian reproductive, along with ii) endocrine working. With their i) limitless proliferation in addition to ii) guided differentiation into oocytes, iii) FGSCs yield innovative enthusiasm to patients with i) ovarian insufficiency, ii) malignant tumors, and iii) others who require fertility preservation. Previously we had reviewed exhaustively etiopathogenesis of premature ovarian insufficiency(POI)/diminished ovarian reserve(DOR) and the manner manipulating signaling pathways helped in associated generation of early/primordial follicles enhanced ART outcomes in POI&DOR patients. Further we thoroughly reviewed part of stem cells inclusive of very small embryonic-like stem cells (VSELs) in oncofertility/ part of silent information regulator 1(Sirt1) signalling in improvement of escalating oocyte quality in women presenting with advanced maternal age. In this review, we debut the part of FGSCs in i) ovarian fertility preservation ii) regenerative healing, highlighting the controlling pathways of FGSCs in resulting in restoration of ovarian working. We detail the distinct benefits of FGSCs in infertility therapy, inclusive of i) fertility preservation, ii) animal gene editing, and, iii) regenerative medicine. This objective of our article is yielding innovative research to gather understanding regarding advancement of the clinical translation of FGSCs by evaluating them from plethora of viewpoints, for instance i) origin, ii) controlling, along with iii) implementation.

Keywords: Female germline stem cells (FGSCs); ovarian insufficiency; malignant tumors; postponement of ovarian aging

1. Introduction

Ovarian aging delineates age- associated reduction in ovarian working as well as infertility concern that takes place secondary to ovarian diseases have assumed meaningful botherations in the field of worldwide reproductive health¹. Primary ovarian insufficiency (POI) possesses the properties of a diminished follicle count in addition to a reduction in oocyte quality, resulting in an irreversible diminishing in ovarian working, along with estrogen quantities in women under 40². In recent years, the incidence of POI has been escalating meaningfully, with a worldwide prevalence of 3.7%. In China, the prevalence has escalated from 2.5% twenty years back to 15%, with ovarian- associated factors working in the form of contributors of 15%to 25% of cases^{3,4}. POI apart from causing amenorrhea as well as infertility, however is further intricately associated with systemic health issues for instance i) osteoporosis, ii) cardiovascular diseases, in addition to iii) cognitive impairments^{5,6}.

The mechanistic modes via which ovarian diseases influence fertility are complicated. For instance, ovarian cysts greater than 5 cm as well as cystectomy have been illustrated to meaningfully affect ovarian reserve six months subsequent to surgery^{7,8}. Chemotherapeutic substances, particularly alkylating compounds, possess the capacity of directly causing injury to follicular structures, resulting in irreversible ovarian injury^{9,10}. i) Radiotherapy, ii) bone marrow transplantation, in addition to iii) autoimmune conditions (such as lupus) stimulates minimally one million POI cases worldwide each year¹¹. In recent times, variable arbitration approaches have been posited in reference to tackling such challenges. Successful employment of ovarian tissue cryopreservation in combination with *in vitro* activation (IVA) has been made in patients with POI who possessed just occasional primordial follicles (PF's)^{12,13} along with healthy pregnancies have been documented. GnRH-a might attenuate chemotherapy stimulated-damage to certain magnitude^{14,15}. Additionally, lifestyle interpositions (for instance antioxidant diet, BMI regulation) as well as early fertility preservation (for instance egg freezing) have been

illustrated to postpone reduction in ovarian PF's^{16,17}. Nonetheless, there is existence of requirement of the manner tackling of the dysfunctional ovarian reproductive in addition to endocrine working remodeling has to be attained. Thereby avoidance of along with treatment approaches regarding i) ovarian aging as well as ii) chemotherapy- associated infertility buttressing is required.

2. Corroboration regarding presence of female germline cells in the ovary

2.1 DEBATE IN REFERENCE TO FEMALE GERMLINE CELLS

The query in reference to if there is presence of female germline cells (FGSCs) in mammals as well as their differentiation takes place into working oocytes has been debatable right from the nineteenth century. Substantial studies have illustrated that the ovarian follicle pool is estimated at the time of the perinatal period, in addition to no self-renewing stem cells or newly produced oocytes are existent in the postnatal mouse ovary. The "canonical posit that "the mammalian ovarian follicle pool is fixed at birth" predominated the field for practically four decades¹⁸⁻²¹. Nevertheless, subsequent to advent of FGSCs, accrual of corroboration has questioned the "fixed follicle pool theory" that had been pursued throughout last century in reproductive medicine.

To start with Johnson et al.²², pointed to the existence of proliferative germ cells in the postnatal mammalian ovary In 2004, as well as assisted in follicular in addition to oocyte regeneration²². Subsequent to performance of quantification of atretic along with non-atretic follicles in C57BL/6 mice, they found a swift augmentation of follicular atresia subsequent to postnatal day 30, with just continuation of the one-third of the seminal follicle pool by day 42.

Dependent on the atresia rate, they anticipated full elimination of the follicle pool amongst weeks. Nonetheless, the full quantity of atretic follicles surpassed the exactitude of elimination, pointing to continuation of the oocyte regeneration. Additionally, transplantation of wild-kind ovaries into transgenic

female mice universally, expressing green fluorescent protein (GFP) documented existence of GFP-positive germ cells infiltrating host ovaries as well as new follicles. Such observations defied the canonical belief that the ovary possesses just 1%~33% immature follicles at any stage, resulting in the pathfinder “oocyte regeneration hypothesis.” Their following work pointed that bone marrow (BM) expresses germline markers in addition to causes rectification of oocyte generation in sterilized mice through transplantation. Donor- obtained oocytes, corroborated by morphology, along with molecular markers, point BM in the form of a plausible germ cell facility maintaining oogenesis in adulthood²³. The rhetorical of those not in agreement with Johnson’s hypothesis²⁴, was that their relative assessment methodologies for atretic as well as non-atretic follicles possess restrictions. The guideline in reference to isolating atretic follicles were believed to be subjective in addition to strain-particular, along with existence of plausible artifacts at the time of tissue fixation might have overdetermined atresia rates. Utilization of single-cell RNA sequencing (scRNA-seq as well as fluorescence-activated cell sorting (FACS) in case of recent studies possessed failure in reference to estimating FGSCs in adult ovarian cortex samples²⁵. Nevertheless, Woods and Tilly²⁷, in 2023 were critical of such observations, emphasizing main lacunae in Wagner et al.’s²⁶, workflow. Their observations regarding the removal of cells with gene expression profiles analogous to FGSCs, remarkable cell damage/demise at the time of sample preparation, as well as the requirement for high cell viability in advanced techniques like scRNA-seq.²⁵ Thus, the lack of FGSCs in such studies continues to be nonconclusive, along with escalating confirmation continues to embrace the existence of FGSCs.

The debate encompassing FGSCs basically revolves on the advocating faction’s corroboration from mouse models, where DEAD-box helicase 4 (DDX4), - positive cells generate oocytes, compared to converse debates that human ovaries possess absence of active germline stem cell niches. This opposes the germaneness for generalizing rodent observations

to humans. Nevertheless, with FGSCs escalatingly identified, along with cultured over variable species in addition to side by side persistent continuous advancements would eventually aid in attainment of greater exhaustive resolution of such controversial arena.

2.2 PRESENCE OF FEMALE GERMLINE CELLS

Bukovsky et al.²⁸, in 2008, identified a minimal quantity of adult ovarian surface tissue as well as cultured it *in vitro* for 5~6 days, finding the generation of follicle-like structures which expelled polar bodies in addition to possess the properties of secondary follicles. Nonetheless, they identified, ovarian surface epithelial cells or ovarian stem cells (OSE), not particular FGSCs. The manner detailed in their study, the identified OSE might differentiate into different cell kinds inclusive of i) mesenchymal, ii) epithelial, iii) granulosa, iv) neural kind cells, along with v) oocytes. The initial identification as well as purification of FGSCs, got performed by Zou et al.²⁹, with success in 2009, demonstrating unique germline stem cell characteristics. Following that Lu, White, along with Zhang autonomously identified germline stem cells from ovaries of different species with the utilization of variable approaches in reference to stem cells working³⁰⁻³². Tackling cynicism, recent observations³³, illustrate that idealized single-cell RNA sequencing (scRNA-seq) workflows possess the capacity of isolation of occasional germline cells in adult ovarian cortical tissues which harmonize ovarian stem cells (OSCs) however vary from other cell kinds, inclusive of i) oocytes in addition to ii) perivascular cells (PVCs). Further evaluation displayed pivotal molecular corroboration of such germline cells beginning the initial phase of meiosis amongst mature ovarian tissues. Uptill now, researchers have consistently isolated ovarian germline stem cells (FGSCs) in the ovaries of i) rats, ii) pigs, iii) sheep, in addition to iv) humans, pointing to their broader existence across mammals³⁴⁻³⁷. Present botherations exist in exhaustively evaluating the properties of cell populations amongst adult mammalian ovaries as well as defining situations which might embrace *in vitro* in addition to *in vivo* neoogenesis.

2.3 PROPERTIES OF FEMALE GERMLINE STEM CELLS

FGSCs apart from perpetuating the follicular pool, however further illustrated canonical properties of

adult stem cells. Pivotal properties of FGSCs are illustrated as follows in Figure. 1³⁸.

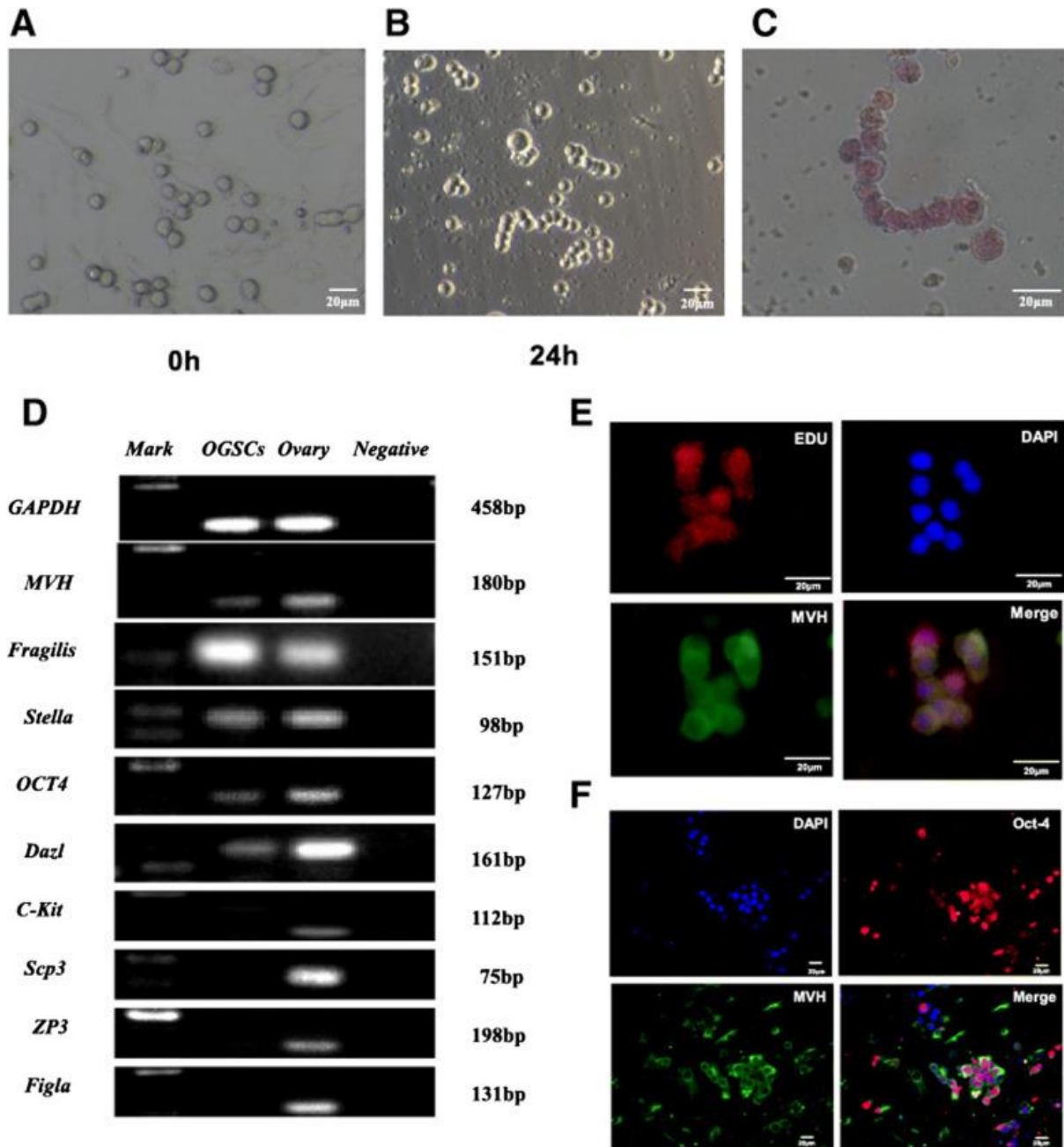


Figure 1. Courtesy ref no-38 - Identification of female germline stem cells. (A-F reproduced with the permission from Fig. 5 A-; Reproductive Biology and Endocrinology; <https://doi.org/10.1186/s12958-021-00699-z>). A, B. The morphology of FGSCs; C Alkaline phosphatase staining; D Reverse transcription polymerase chain reaction (RT-PCR); E, F Immunofluorescence

(1) Morphology as well as Growth designs: FGSCs are analogous spermatogonial stem cells (SSCs), displaying a spherical shape, grape-like clusters, large in addition to bright spherical nuclei, along with distinct boundaries amongst nuclei in addition to cytoplasm³⁹.

(2) Germline-Specific Markers: FGSCs express germ cell-specific markers for instance Mouse Vasa Homologue (MVH), Dazl, octamer-binding transcription-4 (OCT-4), as well as Fragilis, coordinating with the molecular profile of SSCs⁴⁰⁻⁴³.

(3) Proliferative Capacity: Dual immunofluorescence staining for MVH in addition to B5-Bromo-2'-deoxyuridine (BrDU) corroborated that FGSCs possess the retention of mitotic activity whereas conserving identity of germline³⁹.

(4) Cell Cycle controlling: FGSCs express cell cycle associated transcription factors (for instance c-MYC, EGR-1) alongside high telomerase activity, along with TERT in addition to alkaline phosphatase(AP)^{29, 31,39]}.

(5) Probability of Differentiation: subsequent to estrogen supplementation, FGSCs differentiate into oocytes *in vitro*, along with form fertile offspring subsequent to transplantation into mouse ovaries^{29, 41,42,44}.

3. Controlling of female germline stem cells

Sustenance of ovarian reserve in addition to working homeostasis via self-renewal as well as directed differentiation gets attained by FGSCs. FGSCs facilitate follicular regeneration by activating signaling pathways for instance Notch along with Hedgehog (Hh), whereas liberating antioxidant factors (for instance i) superoxide dismutase(SOD), ii) glutathione peroxidase(GPx) to circumvent oxidative stress(OS)-stimulated injury to oocytes.FGSCs control ovarian working via plethora of pathways(see Figure. 2), with crucial mechanistic modes delineated hereafter.

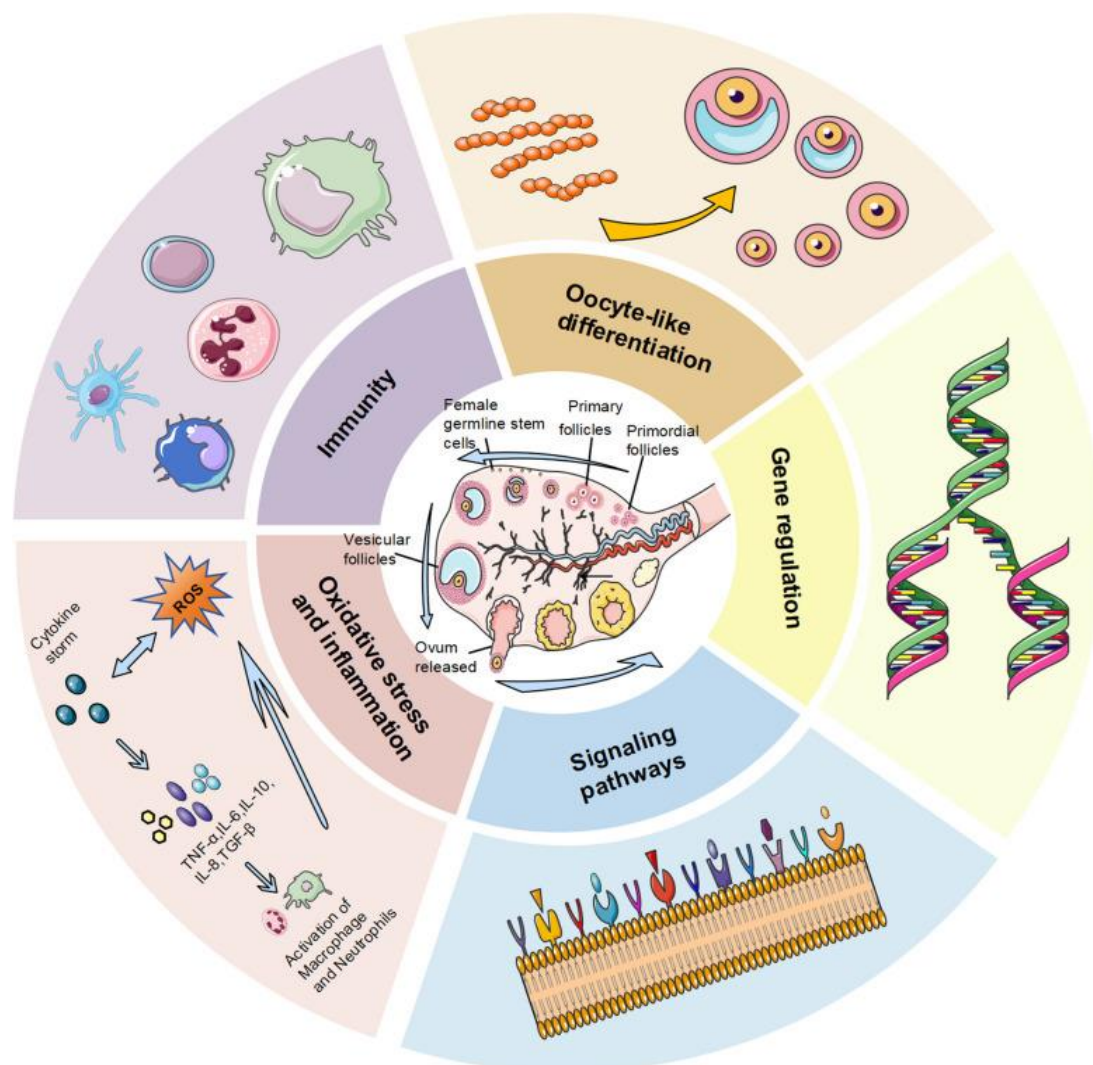


Figure 2. Courtesy ref no-38 - Pathways for FGSCs in reference to rectification of ovarian working. (1) Immune Cell modulated: Immune cells as well as their liberated factors take part in addition to facilitate the proliferation, along with differentiation of ovarian germline stem cells (OGSCs), thereby causes restoration of ovarian endocrine working. (2) Direct Differentiation: FGSCs directly differentiate into primary oocytes, that further generates into mature follicles, resulting in renewal of the follicular pool. (3) Diminishing of Stress & Inflammation: Diminishing oxidative stress (OS)- as well as inflammation

causes restoration of ovarian working in addition to FGSC actions , facilitating FGSC survival. (4) Gene controlling: Subsequent to estrogen (E2) triggering, binding of ER α takes place with the Stra8 gene promoter (that is activated by retinoic acid) to stimulate Stra8 transcription, eventually stimulating oocyte generation. (5) Controlling of signaling pathways: Modulating signaling pathways stimulates an autophagic protection conferring reactions in FGSCs, attenuating cellular senescence

3.1 SIGNALING PATHWAYS

Plethora of signaling pathways control FGSCs, therefore leading to improvement of ovarian working (Fig. 3).

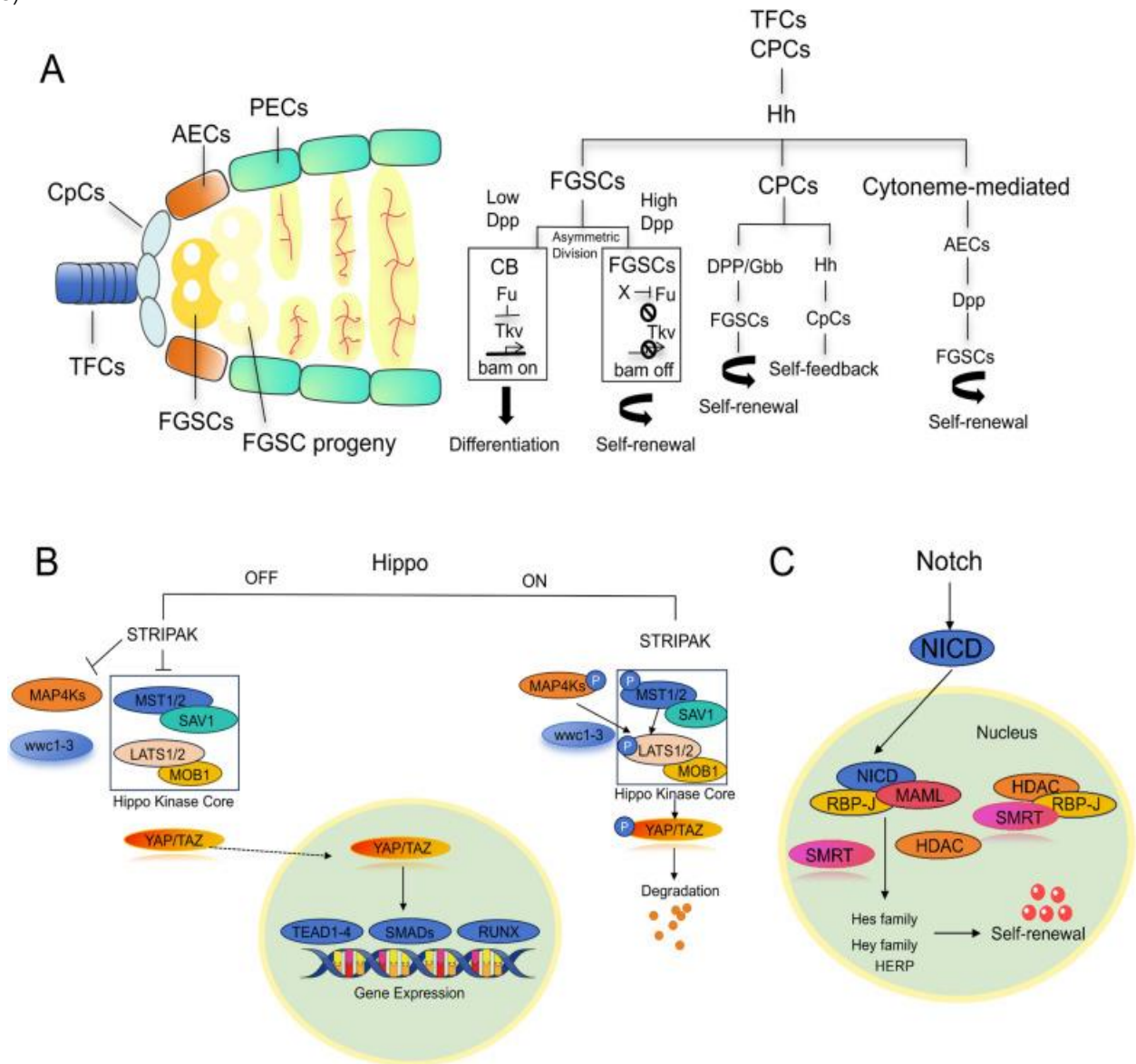


Figure 3. Courtesy ref no-38 - Schematic diagram of the regulatory mechanisms of the Hedgehog/Notch/Hippo signaling pathways. A Hh liberated by TFCs as well as CPCs ,directly possess the capacity of facilitating FGSC self- replenishment, in addition to possess the capacity of further liberating DPP/Gbb to indirectly facilitating FGSC self- replenishment. B The STRIPAK complex serves upstream of kinases MAP4Ks, along with MST1/2, as well as hampers the Hippo pathway. MAP4Ks or MST1/2 along with their scaffold protein SAV1 possess the capacity of phosphorylateLATS1/2 as well as its scaffold MOB1 with the aid of WWC1-3. C In case of existence of Notch ligands, binding takes place with Notch receptors on neighboring cells, creating the Notch intracellular domain (NICD). NICD gains entry into the nucleus, crosstalks with CBF-1/RBP-J κ , displaces SMRT in addition to HDAC enzymes, along with recruits MAML, therefore activating downstream genes as well as facilitating cell proliferation MST1/2; mammalian Sterile 20-like kinase 1/2

3.1A. The Hedgehog (Hh) pathway directly serves +on FGSCs by activating downstream target genes to suppress differentiation as well as maintenance of self-renewal. Hh further indirectly controls FGSCs via micromilieu stepwise patterns. For instance, CpCs (cap cells) activate Hh signaling to stimulate expression of Bone morphogenetic proteins (BMP) family members Dpp in addition to Gbb in anterior epithelial cells (AECs), whereas directly facilitating Dpp transcription in CpCs themselves⁴⁶. Escalated BMP (Dpp) signaling impedes the differentiation factor Bam, further barricading FGSCs differentiation^{47,48}. FGSCs differentiation, is dynamically orchestrated at the time of beginning of differentiation, the Hh pathway's positive governor Fused (Fu) is upregulated. Fu facilitates FGSCs conversion into mature germ cells by modulating ubiquitin- based breakdown of the BMP receptor Tkv, therefore ameliorating BMP-modulated differentiation repression^{46,49,50}.

3.1B. The Notch pathway possesses pivotal parts in i) cell proliferation, ii) differentiation, as well as iii) fate estimation, meaningfully affecting iv) both somatic in addition to v) germline stem cells. Notch controls sustenance of i) FGSCs stemness, along with ii) differentiation through iii) intercellular connection⁵¹.

In *Drosophila*, ovarian stem cell niche development a) depends on Notch signaling. b) Germline stem cells (GSCs) possess placement in somatic niches where Notch is crucial: c) activating Delta in germ cells or Notch in somatic cells escalates niche cell numbers, triggering extra GSC generation. d) On the other hand, GSCs possessing absence of working Notch ligands (Delta/Serrate) illustrated failure to activate transforming growth factor beta (TGF- β) signaling, resulting in differentiation as well as niche exit⁵². e) There is existence of association of diminished Notch signaling i) working with ovarian aging, ii) owing to diminished Notch activity elimination of FGSCs in addition to iii) amplified primordial follicle atresia^{53,54}.

3.1C. The Hippo pathway pivotally controls ovarian working, specifically FGSCs. Via kinase stepwise patterns, Hippo regulates i) cell proliferation, ii)

apoptosis, along with iii) organ size⁵⁵. B) Hippo activation phosphorylates downstream coactivators the Yes-associated protein (YAP) along with transcriptional co-activator with post synaptic density protein, *Drosophila* disc large tumor suppressor [PDZ] and zonula occludens-1-binding motif (TAZ), holding them in the cytoplasm for breakdown, therefore hampering proliferation as well as facilitating apoptosis^{56,57}. C) On the other hand, Hippo hampering aids in translocation of dephosphorylated YAP/TAZ into the nucleus, leading to binding of DNA binding proteins TEA domain (TEAD) family transcription factors to activate genes guiding proliferation as well as stem cell replenishment⁵⁸.

In ovaries, Hippo signaling is imperative for i) FGSCs homeostasis. ii) Decontrolled Hippo signaling is associated with ovarian aging in addition to iii) disease, iv) yielding an understanding into pathology along with v) therapeutic approaches⁵⁹.

In mouse ovarian cortex, co-expression of Hippo constituents Large Tumor Suppressor Kinase 2(LATS2), mammalian Sterile 20-like kinase 1(MST1) as well as germline markers MVH, OCT4 takes place. Their quantities in addition to colocalization reduction takes place with age, whereas YAP1 is prevalent in 2-month-old mice however lacking in 20-month-old mice, pointing to Hippo's part in FGSCs dynamics at the time of physiological , along with pathological ovarian aging⁶⁰.

3.2 STEM CELL NICHE

Schofield's⁶¹, theory in 1978 regarding stem cell niche hypothesized that niches are constituted of i) extracellular matrix(ECM), ii) niche cells, iii) granulocytes, iv) blood vessels, v) immune cells, as well as vi) liberated factors. Escalated confirmation implicates FGSCs niche impairment in the form of a pivotal guide of a) ovarian failure, which probably b) possesses greater influence in contrast to c) FGSCs aging by itself⁴. d) Nevertheless, inflammatory micromilieu disturb niche wholeness⁶². Macrophages, with their multifaceted nature in addition to cytokine-liberating flexibility, might result in stabilization of the niches by removal of senescent red blood cells along with

necrotic tissue through interleukin-10(IL-10), as well as Tumor necrosis factor alpha(TNF α)⁶³.

3.3 OXIDATIVE STRESS (OS) ALONG WITH CHRONIC INFLAMMATION

i) Oxidative stress(OS) as well as ii) chronic inflammation amplify stem cell aging. Follicular hypoxia directs ovarian aging by i) reducing intracellular pH in addition to ii) oocyte metabolism, iii) destabilizing meiotic spindles⁶⁴. Hypoxia activates oxygen sensor hypoxia inducible factor 1 α (HIF 1 α), escalating i) glycolysis, ii) angiogenesis, iii) leukocyte migration, iv) inflammation, along with v) follicular wall rupture⁶⁵.

It further stimulates i) ROS overgeneration, ii) accelerating oxidative ovarian injury⁶⁶. ROS in addition to inflammation generate a vicious cycle: ROS activates i) nucleotide-binding domain, leucine-rich-repeat containing family, pyrin domain-containing (NLRP3) inflammasome as well as ii) nuclear factor κ B(NF- κ B), upregulating iii) IL-1 β , iv) IL-6, in addition to v) TNF- α , that also augment a) OS, along with b) aging⁶⁶⁻⁶⁹. Antioxidant arbitration for instance a) resveratrol (RES) causes restoration of ovarian working as well as viability of FGSCs in POI models by diminishing OS in addition to inflammation⁷⁰⁻⁷⁵. b) Chitosan oligosaccharides result in improvement of ovarian micromilieu along with induce FGSCs proliferation through immune- correlated factors^{76,77}. c) Spermidine (SPD) possesses protection conferring FGSCs actions from H₂O₂- stimulated senescence by stimulating autophagy through phosphatidyl inositol 3 - kinase(PI3K) / protein kinase B (AKT)⁷⁸. Such approaches might postpone reproductive aging as well as conserve fertility.

3.4 OTHERS

Polycystic Ovary Syndrome (PCOS), a common endocrine disorder, is associated with i) hyperandrogenism, ii) glucose intolerance, as well as iii) cystic ovaries. Metformin, a first-line antidiabetic agent, meaningfully escalates i) proliferative FGSCs in addition to upregulates ii) proliferating cell nuclear antigen(PCNA) iii) cyclin D2, iv) phosphorylated mammalian target of rapamycin (p-mTOR), along with v) phosphorylated – 5' AMP-activated protein

kinase(p- AMPK) in PCOS mice, leading to restoration of FGSCs working through AMPK/mTOR signaling⁷⁹. D)Soy isoflavones activate Akt signaling by upregulating C-type lectin domain family 11 member A(Clec11a),promoting FGSCs survival as well as proliferation⁸⁰.

Such observations, along with transplantation in addition to genetic lineage-tracing outcomes, corroborate capability of FGSCs' in creating healthy oocytes, embryos, along with offspring, buttressing their plausibility of tackling female fertility as well as ovarian conditions.

4. Clinical employment along with translational prospects of female germline stem cells

4.1 FERTILITY PRESERVATION

FGSCs offer innovative approaches for fertility preservation along with the postponement of menopause. FGSCs possess the capacity of either getting transplanted into ovaries regarding beginning of the oogenesis in reference to creating fertile offspring or cultured *in vitro* as well as differentiated into oocytes subsequent to injection into human ovarian cortical tissue, with following xenotransplantation into immunodeficient adult female mice⁸¹⁻⁸³. Working oocytes further possess the capacity of getting obtained *in vitro* from SSCs⁸⁴.

White et al.³¹, isolated FGSCs from adult human ovaries in addition to transplanted them into mouse ovarian cortices, finding immature oocytes, along with granulosa cell generation³¹.

Satirapod's along with his coworkers⁸⁵, corroborated that in mouse FGSCs express estrogen receptor- α (ER α), that crosstalks with retinoic acid-induced Stra8 at the time of oogenesis for guiding Stra8 expression⁸⁵. Xiong et al.⁸⁶, illustrated restoration of fertility that resulted subsequent to chemotherapy- in case of infertile mice by transplantation *in vitro*-cultured FGSCs into their ovaries⁸⁶. Wu et al.⁸⁷, illustrated in 2017 that FGSCs start differentiation into early

oocytes subsequent to their arrival to the ovarian cortex, with transplanted FGSCs(F-TFs) leading to restoration of ovarian working in addition to creating offspring.⁸⁷

Taken together, such observations corroborate the presence of proliferative germ cells in postnatal mammalian ovaries, implicated sustaining oocyte as well as follicle development.

Bukovsky and Presl⁸⁸, posited in 1979 that immune system-ovary crosstalk takes place in controlling reproduction in addition to reproductive conditions⁸⁸. Studies that followed embrace this: athymic (nude) female mice illustrated diminished gonadotropin quantities reversible through neonatal thymosin treatment⁸⁹. In case of humans, i) ovarian surface epithelial cells (OSCs) serve in the form of ii) bipotent stem cells iii) producing germ along with iv) granulosa cells. v) Immune controlling takes part in physiological neo-oogenesis as well as vi) follicular replenishment at the time of a) fetal in addition to b) reproductive stages, with i) FGSCS proliferation, along with ii) differentiation manipulated by iii) immune cells as well as their iv) liberated factors^{90,91}.

4.2 ORGANOIDS ALONG WITH IN VITRO MODELS

Li X et al.⁹², in 2021 generated ovarian organoids from mouse FGSCs by utilization of a 3D culture system. Such organoids, analogous to normal ovaries, possessed follicles along with liberated hormones. Single-cell sequencing isolated six cell populations, inclusive of i) germ, ii) granulosa, as well as iii) theca cells, with unique gene expression profiles. Follicles from organoids matured *in vitro* in addition to generated normal offspring through fertilization. One additional group utilized total -organ decellularization to generate 3D bioscaffolds simulating the natural ovarian micromilieu, perpetuating native microarchitecture, along with biochemical tips. Purification of the FGSCs through MACS complied to such scaffolds amongst 24 h, i) that colonized the extracellular matrix (ECM), as well as ii) developed cluster-like structures⁹². iii) In 2023 Luo et al.⁹³, formed semiconductor polymer dot(Pdot)- dependent siRNA

nanocomposites for targeted gene knockdown in FGSCs. Pdots illustrated i) greater fluorescence, ii) making real-time following up of cellular uptake, iii) intracellular trading, in addition to iv) exocytosis. v) Noticeably, Pdots- illustrated least cytotoxicity, along with differentiation disturbance, evading lysosomes for efficacious extracellular liberation, that enables them to be optimal nanocarriers. Further penetration of Pdot- siRNA was brought about in reference to the FGSC- obtained 3D ovarian organoids, efficaciously downregulating target genes.

Therefore, bioengineered ovarian prosthetics (see Figure. 4) utilization of FGSCs delineates an innovative approach regarding rectification of fertility with safety, specifically in cancer survivors.

4.3 STEM CELL ALONG WITH GENE THERAPY

FGSCs are escalatingly getting acknowledged for their plausibility regarding therapy of infertility, perpetuating fertility, aiding in animal gene editing, as well as resulting in advancements in regenerative medicine. Ovarian impairment or insufficiency, resulting from i) genetic abnormalities, ii) autoimmunity, or iii) damage⁹⁴⁻⁹⁶, as well as chemotherapy/radiation-induced ovarian failure⁹⁷⁻⁹⁹, constitute main etiological factors of infertility. Present modalities (oocyte/embryo cryopreservation, ovarian tissue freezing) possess the risk of transportation of the malignant cells back into cancer survivors¹⁰⁰.

Stem cell therapies, are inclusive of i) embryonic stem cells (ESCs), ii) mesenchymal stem cells (MSCs), in addition to iii) induced pluripotent stem cells (iPSCs), are incepting in the form of attractive methodologies for alternatives modes of treatments¹⁰¹. FGSCs, possess the capabilities of for self- replenishment along with differentiation, offer distinct plausibility regarding therapy of the ovarian diseases.

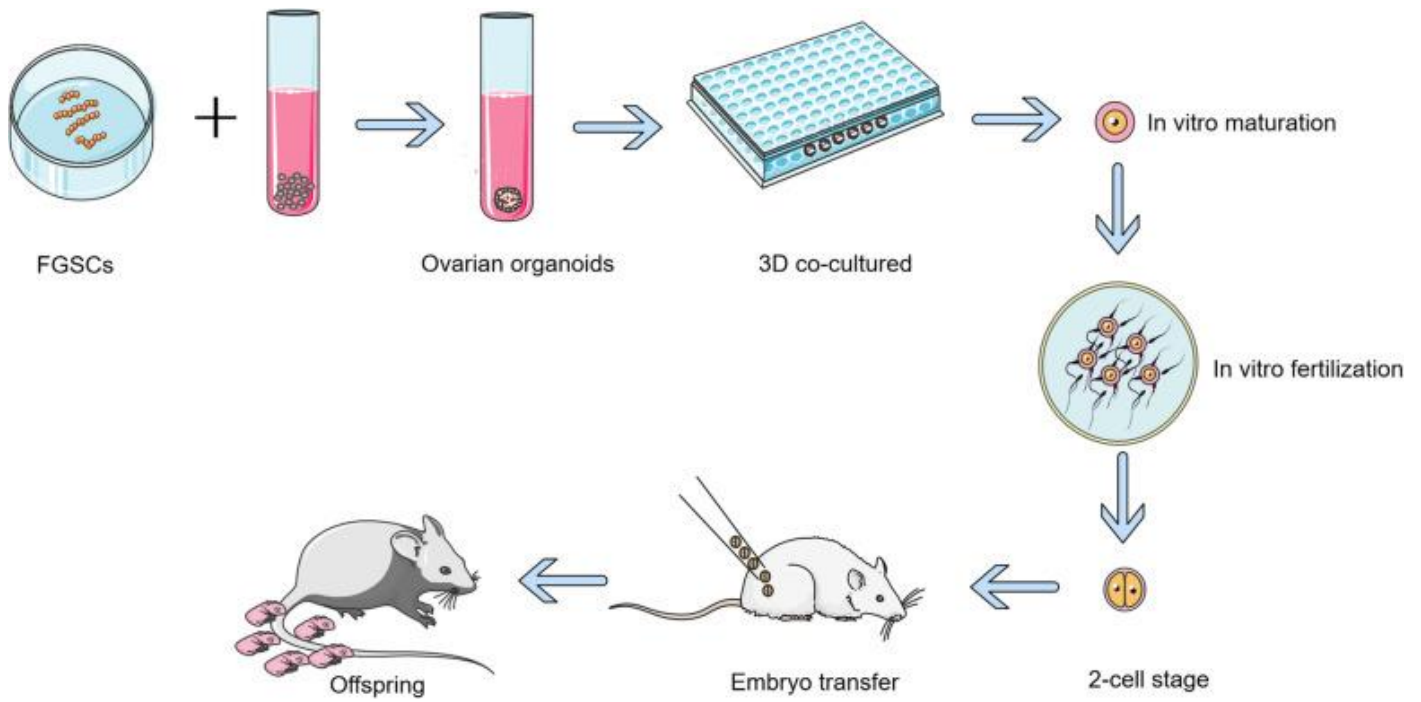


Figure 4. Courtesy ref no-38 - Schematic diagram of ovarian germline stem cell (FGSC)- obtained ovary-like organoid formation. FGSCs were combined with 3Dbioscaffolds as well as co-cultured in 96-well plates. The culture medium was modified in case of differing situations, supplemented with growth factors, until ovary-like organoids possessing oocyte-like follicles were found. Oocytes identified from such organoids were utilized for embryo transfer through assisted reproductive technologies (ART) to form offspring in mice

The intrinsic capability of FGSCs' of differentiating into oocytes enables them optimal for producing transgenic animals, countering greater expenditure along with personnel exhaustive technologies for instance i) pronuclear DNA injection or ii) somatic cell nuclear transfer¹⁰²⁻¹⁰⁵, attained transgenic offspring amongst two months by installation of working genes or knocking down targets in FGSCs identified from neonatal as well as adult mice, with effectiveness (29%~37%) considerably overtaking SSCs (4.5%)¹⁰⁶. Such strategy is widely applicable to mammals, inclusive of livestock, leading to advancements of gene therapy in addition to biotechnology.

A) FGSCs illustrate multifaceted implementations:
i) Ovarian Tissue Engineering: Co-cultured with 3D bioscaffolds, therefore making contrived ovary models regarding restoration of ovarian working.

ii) Oocyte Regeneration: Differentiating into oocytes *in vitro/in vivo*, they renew follicular pools, yielding modalities for POI along with ovarian tissue regeneration.

B) Transgenic Models: Utilized to generate gene-edited animals for evaluating reproduction as well as gene working.

C) Assisted Reproduction: Plausible innovative vistas for women with diminishing fertility. Although, there is existence of botherations, FGSC- dependent therapies illustrate meaningful plausibility in reference to fertility preservation in ovarian injury or cancer patients.

Regenerating human ovary-like micromilieu in addition to clarifying signaling pathways for *in vitro* oogenesis along with folliculogenesis would escalate the effectiveness of FGSCs differentiation, yielding innovative optimism in reference to female fertility preservation.

5. Drawbacks of ovarian germline stem cell research

5.1 TECHNICAL HINDRANCES OF FEMALE GERMLINE STEM CELLS

The isolation as well as identification of ovarian germline stem cells (FGSCs) continues to be bothesome. FGSCs possess absence of considerably particular surface markers, in addition to current identification modalities usually aids in contamination of samples with other ovarian cells, jeopardizing purity. In the clinical scenario identification of FGSCs needs ovarian tissue extraction—an event restrained by restricted accessibility of sample, facilities, along with invasive risks. In humans, FGSCs are yielded from ovarian cortical tissue within follicular aspirates⁸¹. Nevertheless, lesser ovarian tissue obtained in aspirates results in noncommensurate success rates in generating cell lines.

FGSCs illustrated lesser effectiveness in differentiating into mature oocytes *in vitro* as well as usually frequently go through senescence or elimination of plausibility of differentiation at the time of expansion leading to, meaningful omission of stemness subsequent to passaging. Canonical 2-dimensional (2D) culture systems do not possess the capacity of sufficiently mimicking the ovarian microenvironment *in vitro*. Contrasting with mesenchymal stem cells (MSC's), long-term culture protocols for FGSCs continues to be nonidealized. Whereas Matrigel- dependent 3D culture escalates i) cell viability as well as ii) sustenance of stemness, iii) vital frameworks— inclusive of iv) Matrigel content, v) embedding modalities (e.g., coculture with feeder cells), in addition to vi) batch-to-batch consistency—need continued idealization¹⁰⁷.

5.2 CLINICAL SAFETY OF FEMALE GERMLINE STEM CELLS THERAPY

There is absence of the existence of long-term outcomes presently in reference to corroboration of safety of FGSC owing to it might stimulate tumor generation along with possesses the capability of epigenetic memory retention from source cells, which makes it imperative to take into account the

plausible clinical risks¹⁰⁸⁻¹⁰⁹. Whereas transplanted cells need to be incorporated into the i) host ovarian stroma, ii) fibrotic or iii) inflammatory microenvironment iv) might impede FGSC engraftment. Furthermore, allogeneic FGSCs possess the capacity of stimulating host, immune attacks. Germline gene editing in human FGSCs needs considerable precaution owing to inheritance by the offspring. The complicated nature of the ovarian microenvironment, combination with stem cell treatments continues to be in clinical trial/research phases, which leads to meaningful interpersonal discrepancies in reactions to therapy within subjects^{110,111}. The results of post-transplantation are capable of getting impacted by age of the patient as well as the etiological factors that lie beneath.

5.3 MODALITIES OF RESOLUTIONS OF RESEARCH DRAWBACKS OF FEMALE GERMLINE STEM CELLS

Idealization of the culture as well as differentiation systems for FGSCs possess the capacity of getting attained by the utilization of combination of natural stimulators (for instance *Cistanche* polysaccharides) to target the TGF- β pathway, therefore escalating differentiation orchestration¹¹². Efficacious hampering of EED actions leads to improvement of culture effectiveness in addition to cell procured^{41,42,80}. Serum-free along with feeder-free FGSC culture systems possess the capacity of getting generated by the utilization of Matrigel idealization⁸⁰.

Regarding tackling of the botherations in reference to clinical safety, plethora of imaging modalities (for instance MRI-fluorescence dual labeling) aids in realtime monitoring of organization of transplanted cell *in vivo* for early determination of aberrant proliferation. Categorization of patients as per causative factors diminishes multifaceted nature of therapies results. Long-term keeping track of animal transplantation for greater than 6 months requires implementation of mesenchymal stem cells (MSC's) safety protocols evaluation¹¹³, regarding monitoring of teratoma generation along with intergenerational to reproductive actions. Establishing ovarian cell (granulosa/embracing cells)-FGSC co-culture organoids simulates physiological follicular microenvironment, causing

advancing differentiation mechanistic modes research^{41,42}. Furthermore, 3D bioscaffolds possesses the capability of reproducibility of natural ovarian^{niches} possess the capacity of getting engineered. Repopulation of purified FGSCs of decellularized ovarian scaffolds takes place subsequent to triggering that might result in restoration of working⁹². Such modalities delineate attractive technologies regarding assessment of ovarian reproductive in addition to endocrine working glutathione peroxidase(GPx).

Further earlier we had reviewed parthenogenetic embryonic stem cells ,induced pluripotent stem cells , very small embryonic-like stem cells (VSELs)in Oncofertility, targeting SIRT Signaling Pathway in reference to escalating oocyte quality in women presenting with advanced maternal age¹¹⁴⁻¹¹⁹.

6. Role of Proanthocyanidins

Bhartiya D& Sharma D¹²⁰⁻¹²² , isolated two kinds of stem cells in ovary surface epithelial (OSE) of mammals: i) very small embryonic-like stem cells (VSELs) as well as ii) ovarian germline stem cells (OGSCs). Such cells possess the capacity of going through long-term *in vitro* expansion in addition to eventually differentiating iii) into an oocyte-like structure, leading to resulting in the development of fertile pups. OGSCs are robustly associated with the FGSCs that Wu et al. are presently evaluating¹²⁴. Sharma D observed no disappearance of OGSCs subsequent to chemotherapy or aging, as well as outcomes illustrated that some quantity of VSELs survived in ovarian tissue subsequent to chemoablation in addition to possessed the capacity of retention of differentiating into oocyte-like structures¹²³. In future empirical modulations, usable cells from OSE would further get identified for finding in reference to the manner protection gets conferred against age correlated reduction or by external factor in further studies.

Proanthocyanidins (PACs), delineate natural antioxidants broadly acknowledged regarding their i) anti-aging as well as ii) anti-inflammatory characteristics, in addition to are frequently utilized

in variable avenues for instance i) food, ii) medicine iii) environmental protection, iv), cosmetics, along with v) agriculture. PACs portray water-soluble phenolic substances which canonically are present in i) fruits, ii) vegetables, iii) nuts as well as iv) plants. Their antioxidant capability is 40 fold larger than that of vitamin C or E¹²⁴.

Owing to their natural antioxidant characteristics, PACs have been pointed in the form of a plausible protection conferring against oxidative stress (OS) injury to separate organs in addition to tissues, inclusive of the ovaries¹²⁴. Research has illustrated that PACs possess the capacity of efficaciously ameliorating pathological alteration in granulosa cells by inhibiting autophagy, along with apoptosis, that eventually account for postponement of ovarian aging¹²⁴. Nevertheless, studies evaluating the actions of PACs on FGSCs continues to be not revealed. A natural reduction of ovarian follicle pool takes place with ageing of women. Nevertheless, FGSCs possess a distinct capability of differentiating into oocytes as well as indelibly self-replenish, yielding an efficacious vi arena sof postponement of ovarian aging by renewal of the primordial follicle pool. Thereby, activating FGSCs is vital in reshaping in addition to safeguarding ovarian working.

Wu et al.¹²³, conducted the evaluation of the biological actions of proanthocyanidins (PACs), that delineate natural antioxidants that illustrate anti-aging as well as anti-inflammatory characteristics, which is advantageous for both male in addition to female reproduction. Their *in vivo*, along with *in vitro* experiments illustrate that PACs facilitate FGSCs proliferation whereas postponing ovarian aging.

Their observations were that PACs escalated the quantity of i) primordial follicles, ii) primary follicles, iii) corpus luteum whereas diminishing iv) cystic follicles, as well as v) escalated estradiol (E₂) quantities along with anti-mullerian hormone (AMH) quantities in mice. Furthermore, PACs meaningfully buttressed FGSCs proliferation time- in addition to in a dose-based fashion by upregulating mRNA , along with protein expressions for FGSCs- iii) particular markers

for instance i) MVH as well as ii) OCT4 whereas downregulating p53/p21 through activation of iv) silent information regulator 1(Sirt1) signaling pathway. The actions of PACs on FGSCs were observed to be hindered by the Sirt1 hampering agent EX527.

Thereby Conclusion drawn by them were that PACs cause s postponement of premature ovarian insufficiency (POI) via controlling the Sirt1-p53-p21 signaling pathway implicating FGSCs¹²⁴ (see Figure 5).

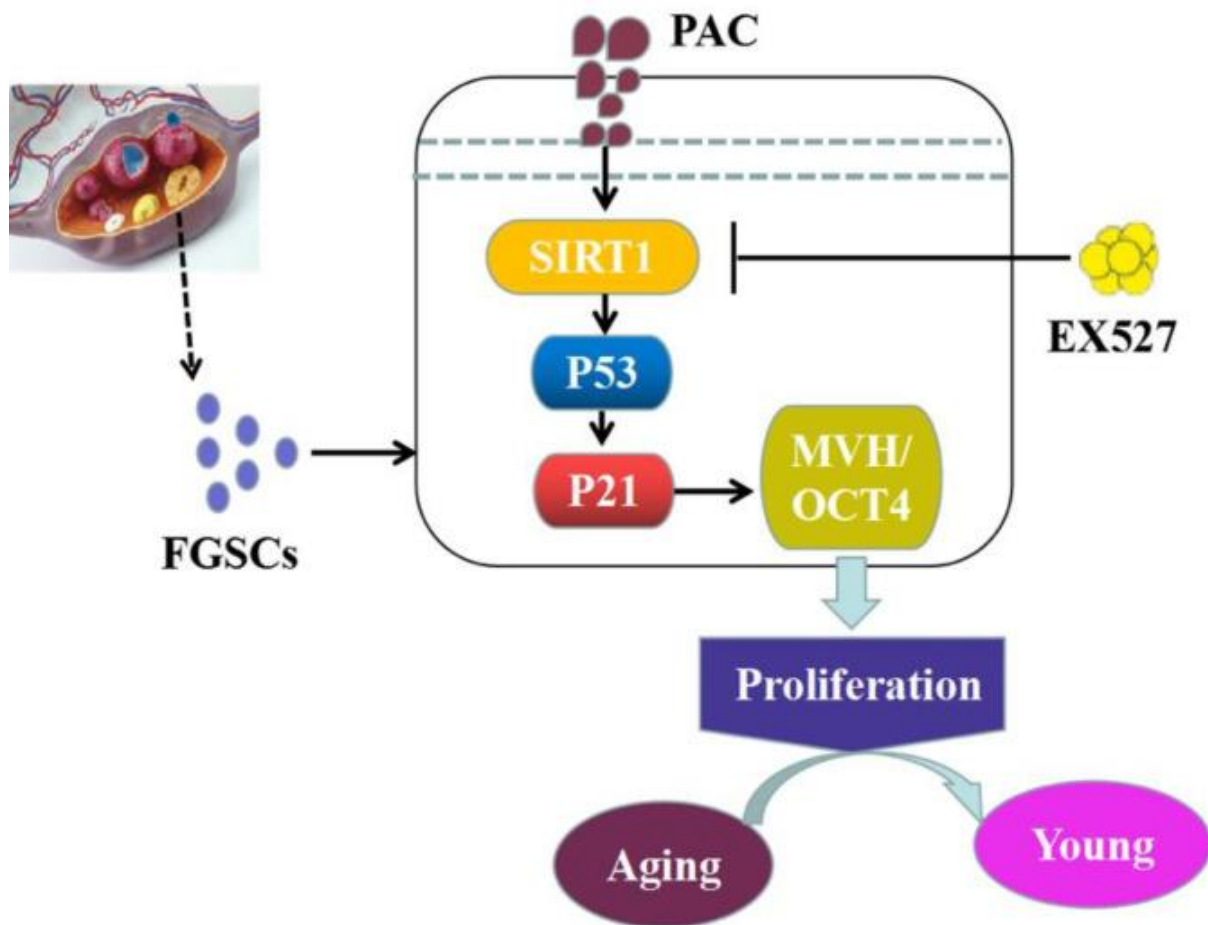


Figure 5. Courtesy ref no-124- The part of PACs in delaying POI through the regulatory Sirt1-p53-p21 signaling Pathway in FGSCs

7. Conclusions

Perpetuating fertility continues to be a crucial concentration in medicine, enabling women to retain reproductive health. Stem cells in adult ovaries hold meaningful repercussions for reproductive medicine. Present fertility preservation modalities are inclusive of i) embryo cryopreservation, ii) oocyte cryopreservation, along with iii) ovarian tissue cryopreservation. Whereas embryo as well as oocyte freezing are well generated, ovarian tissue cryopreservation— is still needing surgical elimination, freezing, in addition to followed by transplantation of thawed tissue— continues to be in its early budding clinical stages^{125,126}.

Advancements in cancer therapy have resulted in improvement in survival rates, yet surgery, chemotherapy, along with radiotherapy usually results in robust injuries in reference to ovarian working. Adolescent or reproductive-age female patients achieving long-term survival subsequent to cancer remission or cure pose reduced or fertility omission. Internationally, ovarian tissue cryopreservation as well as transplantation (OTCT) is acknowledged in the form of the exclusive strategy regarding 'perpetuating ovarian working in addition to fertility in children, along with women who do not possess the capacity of postponement of cancer therapies. OTCT further represents the maximum efficacious as well as

attractive strategy regarding fertility preservation. Decades subsequently, thawed autologous ovarian tissue possess the capacity of getting transplanted back, in reference to restoration of ovarian working in addition to fertility. Nevertheless, present studies have illustrated that double injures from cryopreservation along with ischemia results in robust elimination of ovarian reserve in addition to mitochondrial energy metabolism impairment in oocytes¹²⁷. In reference to tackling the concern, the plausible employment of FGSCs in this field yields extensive attractiveness in reference to reproductive medicine for scientific investigators.

On one side, FGSCs are capable of differentiating directionally into working oocytes; Conversely, they might be co-cultured with other cells or biomaterials, plausibly making the formation of ovarian organoids¹⁰⁷. The ovary possesses the capability of enzymatically getting detached into i) identified cells or ii) primordial follicles. Subsequent to reagglomeration as well as transplantation into recipient animals, such cells regenerate working follicles possessing the capability of ovulation¹²⁸.

At the time of ovarian transplantation as well as tissue healing, i) germ in addition to ii) granulosa cells might redistribute iii) amongst donor, along with iv) host primordial follicles. Noticeably, a) somatic in addition to b) germ cells identified from mouse along with c) rat ovaries possess the capacity of getting recombined *in vitro* in the context of reconstruction of follicles, which get subsequently transplanted into mice which had severe combined immunodeficient (SCID)¹²⁹. Therefore generating innovative contrived ovarian models co-cultured with diverse ovarian cells to study working restoration technologies poses substantial vital botherations. Whereas germline stem cells like FGSCs are believed to be valuable regarding their plausibility in restoration of fertility-, their restricted availability as well as absence of complete insights in oocyte transformation mechanistic modes work in the form of hurdles regarding therapeutic implementation. Furthermore, the tumorigenicity of stem cell treatments continues to be

a botheration. Correspondingly, with spermatogonial stem cells (SSCs) in reference to male infertility therapies, FGSCs possess the retention of risk of malignant conversion¹³⁰. Collectively, FGSCs yield innovative approaches in reference to treatment of ovarian conditions in addition to advancement of scientific investigators work on transgenic animal or breeding of elite livestock. Despite challenges, the scientific community along with public continues to be enthusiastic regarding their plausibility to reform management of ovarian disease as well as infertility treatments.

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