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7,8-Dihydroxyflavone preserves ovarian follicles during cisplatin injury and aging

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Article

Keywords: Diminished ovarian reserve, premature ovarian insufficiency, 7,8-dihydroxyflavone, TrkB, granulosa cells, SOCS3, FOS

Posted Date: November 18th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11471274/v1

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Additional Declarations: There is no conflict of interest

Abstract

Loss of ovarian reserve reflects both follicle depletion and deterioration of the cellular environment that supports follicular development. Whether reinforcing trophic signaling can protect this environment, and which transcriptional responses mediate such protection, remain incompletely defined. Here, we integrated human oocyte transcriptomes and clinical specimens with mouse models of cisplatin-induced ovarian injury and natural aging to investigate 7,8-dihydroxyflavone (7,8-DHF). Aged human oocytes showed reduced expression of BDNF-TrkB pathway components, while follicular-fluid BDNF was lower in women with diminished ovarian reserve and positively correlated with serum anti-Müllerian hormone. In cisplatin-treated mice, 7,8-DHF preserved primordial and developing follicles, increased serum anti-Müllerian hormone and improved litter size. Treatment also improved follicular profiles in aged mice. In KGN granulosa-like cells, 7,8-DHF reduced cisplatin-induced apoptosis, reactive oxygen species accumulation and mitochondrial depolarization. Integrative transcriptomic analysis identified FOS and SOCS3 as candidate mediators. The TrkB antagonist ANA-12 blocked SOCS3 induction, whereas FOS associated with the SOCS3 promoter and increased its reporter activity. FOS and SOCS3 silencing further implicated this transcriptional response in the regulation of granulosa-cell functional markers. These results link the ovarian-protective effects of 7,8-DHF to trophic signaling and FOS-SOCS3 regulation, identifying support of granulosa-cell function as a strategy for preserving the follicular reserve.

Introduction

Ovarian reserve underpins female fertility and endocrine function. Its decline accompanies aging and can be accelerated by gonadotoxic injury, compromising both reproductive potential and long-term health[1–3]. Diminished ovarian reserve (DOR) and premature ovarian insufficiency (POI) are clinically distinct conditions, rather than obligatory consecutive stages. Hormone therapy remains essential for managing estrogen deficiency in POI, but does not replenish the follicular pool[4]. Preserving existing follicles therefore addresses a different biological need from hormone replacement. AMH provides an indirect readout of the growing follicle compartment[5,6], making structural and functional endpoints important when evaluating interventions intended to preserve ovarian reserve[7].

Follicular maintenance depends on support from surrounding granulosa cells, whose redox and metabolic programs become disrupted with age[8]. Brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB), provide one route for sustaining this support[9,10]. BDNF supplementation promotes follicular development in mice[11], and a TrkB agonist antibody improves reproductive outcomes in aged and cyclophosphamide-treated mice[12]. These studies establish the relevance of ovarian trophic signaling but leave its downstream transcriptional contribution to granulosa-cell protection incompletely resolved.

The flavonoid 7,8-dihydroxyflavone (7,8-DHF), originally identified as a small-molecule TrkB agonist[13], limits oxidative and mitochondrial injury in other cellular systems[14,15]. Its effects on ovarian reserve and the transcriptional mechanisms linking treatment to granulosa-cell function remain unclear. Distinguishing cell protection from stimulation of hormone production is particularly important for interventions intended to sustain residual follicles rather than merely modify endocrine readouts. FOS, an AP-1 component, regulates metabolic and steroidogenic programs in human periovulatory granulosa cells[16]. Suppressor of cytokine signaling 3 (SOCS3), by contrast, controls cytokine responses through receptor-dependent inhibition of JAK activity[17]. Whether FOS-dependent SOCS3 regulation participates in the ovarian response to 7,8-DHF has not been established.

Here, we combined human clinical specimens and transcriptomic analyses with cisplatin-injured and naturally aged mice to examine 7,8-DHF. We show that treatment preserves ovarian follicles and supports granulosa-cell function. Gene-silencing and promoter analyses further identify FOS-dependent SOCS3 regulation as a component of the cellular response, linking trophic intervention to transcriptional programs relevant to follicular support and ovarian reserve preservation.

Materials and Methods

1. Clinical Specimen Collection and Primary Granulosa Cell Isolation

The study was approved by the Ethics Committee of Shanghai First Maternity and Infant Hospital (KS25468), and written informed consent was obtained from all participants. Follicular fluid (FF) and primary human luteinized granulosa cells (hGCs) were collected from patients with diminished ovarian reserve (DOR, n = 16) and age-matched normal controls (n = 16) during oocyte retrieval. FF supernatants were stored at -80°C. Primary hGCs were isolated using 50% Percoll gradient centrifugation, cleared of red blood cells, and cultured in DMEM/F12 containing 10% FBS and 1% penicillin-streptomycin at 37°C with 5%CO2.. Fasting serum AMH, FSH, LH, and E2 were measured by chemiluminescence immunoassay.

2. Animal Models and Treatments

Female C57BL/6J mice (6–8 weeks old for disease models; 10 months old for natural aging models; Shanghai Model Organisms Center) were housed under SPF conditions with IACUC approval.

Chemotherapy-induced POI model: Mice were randomized into Control, POI (cisplatin, 5–10 mg/kg, i.p., every other day for 2 weeks), POI + 5 mg/kg 7,8-DHF, and POI + 10 mg/kg 7,8-DHF groups (n = 4 per group).Natural aging model: Ten-month-old mice received vehicle, 5 mg/kg, or 10 mg/kg 7,8-DHF (i.p., every other day for 2 weeks), with 8-week-old mice as young controls.

Fertility and estrous cycle assessment: Estrous cyclicity was monitored daily by crystal violet-stained vaginal smears. For fertility tests, treated females were co-caged with proven-fertile males (2:1 ratio; n =

6), and mating plugs, gestational length, and litter size were recorded.

3. Histological Evaluation and Follicle Counting

Paraffin-embedded ovaries and major vital organs (heart, liver, spleen, lung, kidney, brain) were serially sectioned (5um). Organ morphology and ovarian follicles were evaluated via H&E staining, and ovarian fibrosis was assessed using Masson's trichrome staining. Follicles with visible oocyte nuclei were staged as primordial, primary/pre-antral, antral, or atretic across every fifth ovarian section.

4. Cell Culture, Transfection, and In Vitro Interventions

Human granulosa cells (KGN) were maintained in DMEM/F12 supplemented with 10% FBS. Cells were pretreated with gradient concentrations of 7,8-DHF (0.5–25 uM) for 1 h, followed by cisplatin (200 uM) challenge for 48 h. The TrkB antagonist ANA-12 (10 uM) was added 2 h prior to 7,8-DHF. For gene silencing, specific siRNAs targeting FOS (si-FOS) or SOCS3 (si-SOCS3) and negative control siRNA (si-NC) were transfected into KGN cells using Lipofectamine 3000 for 48 h before 7,8-DHF stimulation.

5. Cell Viability, Apoptosis, ROS, and Mitochondrial Membrane Potential Assays

Cell proliferation was measured using CCK-8 reagent (450 nm absorbance) at 24, 48, and 72 h. Apoptosis was determined using the Annexin V-APC/7-AAD staining kit on a CytoFLEX flow cytometer. Intracellular ROS was detected using the DCFH-DA probe (10 uM) by flow cytometry (FITC channel). Mitochondrial membrane potential (Δψm) was assessed using JC-1 staining, with CCCP (10 uM) as a positive control, and calculated as the ratio of JC-1 monomers (FITC) to aggregates (PE).

6. ELISA

Concentrations of AMH, FSH, and E2 in mouse serum and cell supernatants, as well as SOCS3 and BDNF in human FF and culture supernatants, were determined using specific commercial ELISA kits strictly according to the manufacturers' protocols.

7. Transcriptomic Profiling and Bioinformatic Mining

Public scRNA-seq datasets of human oocytes (GSE164371) and cisplatin ovarian damage data (GSE248745) were retrieved from GEO. In-house RNA-seq was performed on flash-frozen mouse ovaries (Control, Control + 7,8-DHF, POI, and POI + 7,8-DHF; n = 3) using an Illumina NovaSeq 6000 platform. DEGs were identified using DESeq2 $|\log_2(\text{fold change})| \geq 1.0$ and false discovery rate (FDR)-adjusted $p < 0.05$, followed by GO and KEGG functional enrichment analyses via the clusterProfiler package in R.

8. RT-qPCR and Western Blotting

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Total RNA was extracted, reverse-transcribed, and analyzed by RT-qPCR using TB Green Premix Ex Taq II on a CFX384 system ($2^{-\Delta\Delta Ct}$ method; primers in Supplementary Table S1). Total proteins were extracted in RIPA buffer, resolved by SDS-PAGE, and transferred onto PVDF membranes. After blocking with 5% milk, membranes were incubated overnight at 4°C with primary antibodies.

9. Immunofluorescence, Luciferase Reporter, and ChIP-qPCR Assays

Dual Immunofluorescence: Paraffin sections were subjected to EDTA antigen retrieval (pH 8.0), blocked with 3% BSA, and incubated with primary antibodies against p-c-Fos/SOCS3 or BDNF/SOCS3, followed by Alexa Fluor 488- and Cy3-conjugated secondary antibodies and DAPI. Line-scan fluorescence profiles and Pearson correlation coefficients (r) were evaluated in ImageJ.Dual-Luciferase Reporter Assay: Wild-type (pGL3-SOCS3-WT) or mutated (pGL3-SOCS3-MUT) promoter fragments were co-transfected with pcDNA3.1-FOS and pRL-TK into HEK-293T cells. Firefly and Renilla luciferase activities were quantified at 48 h.ChIP-qPCR: Chromatin from cross-linked KGN cells was enzymatically digested into mono/oligonucleosomes (150–900 bp) and immunoprecipitated using anti-c-Fos or normal rabbit IgG. Purified DNA was analyzed by qPCR using SOCS3 promoter-specific primers, and enrichment was normalized via the percent-input method.

10. Statistical Analysis

Data are presented as mean ± SEM from at least three independent biological replicates. Differences between two groups were analyzed using a two-tailed unpaired Student's t-test; multiple-group comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test. Clinical correlations were determined by Pearson correlation analysis. Statistical significance was defined as *P < 0.05, **P < 0.01, and ***P < 0.001 using GraphPad Prism 9.0 and R (v4.2.2).

Results

1. Human ovarian decline is associated with reduced BDNF-TrkB pathway expression

To identify changes in trophic signaling associated with reproductive aging, we first analyzed public transcriptomic profiles of human germinal-vesicle (GV) and metaphase II (MII) oocytes from young and advanced-maternal-age groups (GSE164371)[18]. Expression of *NTRK2*, which encodes TrkB, was lower in aged oocytes and reached statistical significance at the MII stage (P < 0.05). *PIK3CA* expression was reduced at both the GV (P < 0.01) and MII (P < 0.05) stages (Fig. 1A). *BDNF* and *CREB1* showed downward trends that did not reach statistical significance (Supplementary Figure S1A). Profiling of

BDNF, *BDNF-AS*, *CREB1*, *NTRK2* and *PIK3CA* further illustrated age-associated differences in this network (Fig. 1B).

We next examined whether altered BDNF abundance was also evident in the follicular environment of women with DOR. The clinical cohort included 16 women with DOR and 16 controls (Table 1). Female and male age, body mass index, education, infertility type and infertility duration did not differ significantly between groups. Compared with controls, women with DOR had lower serum anti-Müllerian hormone (AMH; 0.8 ± 0.8 versus 3.7 ± 2.0 ng/mL, $P < 0.001$), higher basal follicle-stimulating hormone (FSH; 12.7 ± 6.3 versus 6.4 ± 2.4 IU/L, $P = 0.003$) and fewer retrieved oocytes (3.7 ± 2.6 versus 10.3 ± 5.1, $P = 0.002$). Basal luteinizing hormone and estradiol (E2) did not differ significantly.

Follicular-fluid BDNF concentrations were lower in the DOR group ($P < 0.0001$; Fig. 1C) and positively correlated with serum AMH ($r = 0.5337$, $P < 0.005$; Fig. 1D). No significant correlation was detected with serum FSH ($r = -0.05639$, $P = 0.7672$) or antral follicle count ($r = 0.04405$, $P = 0.8172$; Supplementary Figure S1B-C). These observations associate ovarian decline with reduced BDNF availability and lower expression of selected TrkB pathway components, providing a rationale for testing trophic intervention in experimental ovarian injury.

2. 7,8-DHF preserves follicles and improves reproductive output after cisplatin exposure

To test whether 7,8-DHF could protect ovarian function, we used a cisplatin-induced ovarian injury model (Fig. 2A). Vaginal cytology showed that cisplatin exposure increased the proportion of time spent in diestrus, whereas 7,8-DHF co-treatment shifted the distribution of estrous stages toward that of controls (Fig. 2B-C). Endocrine profiling showed that cisplatin exposure significantly reduced serum AMH while elevating FSH and E2. Co-treatment with 7,8-DHF restored AMH and E2 levels in POI mice, but failed to significantly reverse the elevated FSH. (Fig. 2D).

We then assessed whether this response was accompanied by follicular preservation. Ovarian sections and follicle counts showed depletion of the follicular pool after cisplatin exposure, with greater retention of primordial, primary, secondary and antral follicles in the co-treated group (Fig. 2E-F). Notably, in healthy mice receiving 7,8-DHF alone, we observed an increased proportion of atretic follicles together with a reduction in antral follicle numbers. Corpora lutea and atretic follicles were included in the histological assessment. The representative sections showed better preservation of follicular architecture with 7,8-DHF, whereas collagen staining revealed no conspicuous difference in stromal deposition among the illustrated groups (Fig. 2G).

To determine whether follicular preservation translated into a reproductive benefit, treated females were mated with fertile males. Fertility assessment revealed that cisplatin treatment significantly reduced litter size compared with the Control group. The number of offspring was markedly increased in the POI + 7,8-DHF group relative to the POI group, restoring litter size to a level comparable to the Control group

(Fig. 2H-I). Histological examination of the heart, liver, spleen, lung, kidney and brain revealed no overt additional lesions attributable to 7,8-DHF over the observation period; the pulmonary septal thickening and disrupted splenic architecture described after cisplatin exposure were less apparent with co-treatment (Fig. 2J). Thus, 7,8-DHF preserved ovarian follicles and improved reproductive output in the cisplatin model, prompting us to investigate the cellular basis of this protection.

3. 7,8-DHF limits oxidative stress, mitochondrial depolarization and apoptosis in granulosa-like cells

Because granulosa-cell injury can compromise follicular support, we examined the direct effects of 7,8-DHF in cisplatin-treated KGN cells. Cisplatin reduced the CCK-8 viability readout, whereas 7,8-DHF at 0.5–10 μM increased it, with the largest response at 5 μM after 72 h (Fig. 3A). Culture-supernatant measurements showed higher E2 and AMH concentrations and lower measured FSH after 5 μM 7,8-DHF treatment (Supplementary Figure S1G-H). Immunoblotting showed that 7,8-DHF opposed the cisplatin-associated reduction in estrogen receptor and AMH abundance and the increase in FSH receptor (FSHR). The TrkB antagonist ANA-12 blocked these functional-marker responses (Fig. 3B).

We next examined cellular injury rather than viability alone. Annexin V/7-AAD analysis showed that 7,8-DHF reduced cisplatin-induced apoptosis (Fig. 3C-D). DCFH-DA fluorescence was also lower after co-treatment, indicating reduced intracellular reactive oxygen species (ROS; Fig. 3E-F). Consistently, JC-1 analysis showed a smaller monomer-positive fraction, indicating attenuation of mitochondrial depolarization (Fig. 3G-H). These results establish a direct cytoprotective effect of 7,8-DHF in KGN cells and implicate TrkB in the associated functional-marker response.

4. Integrative transcriptomics identifies FOS and SOCS3 as candidate mediators of ovarian protection

To identify transcriptional responses associated with the ovarian effects of 7,8-DHF, we profiled ovaries from control and cisplatin-treated mice, with or without 7,8-DHF. Differential-expression analysis identified genes altered by cisplatin whose expression shifted in the opposite direction after co-treatment (Fig. 4A-B). Pathway annotation included cytokine-related pathways, including JAK–STAT signaling, alongside Gene Ontology categories related to immune responses and molecular transducer activity (Fig. 4C-D).

Intersecting genes downregulated by cisplatin with those upregulated by 7,8-DHF yielded 76 reversal-associated candidates. Comparison with the independent cisplatin dataset GSE248745 narrowed this set to 10 candidates, including *Socs3*, *Fos* and *Jun* (Fig. 4E-F). RT-qPCR showed increased ovarian *AMH*, *SOCS3* and *Fos* transcript abundance after 7,8-DHF treatment (Fig. 4G-H). The ovarian assay labeled “FSH” also showed a lower signal after treatment (Fig. 4G).

We returned to the clinical samples to assess the relevance of *SOCS3* to human ovarian reserve. Follicular-fluid *SOCS3* concentrations were higher in women with DOR than in controls and negatively correlated with serum AMH ($r = -0.7244$, $P < 0.0001$; Fig. 4I-J). Immunoblotting of DOR-derived clinical samples showed a lower *SOCS3* signal (Fig. 4K). The transcriptomic and clinical findings nominated *SOCS3* for functional analysis, while its association with FOS in the candidate set suggested a possible transcriptional link.

5. FOS regulates SOCS3 transcription in the granulosa-cell response to 7,8-DHF

We first tested whether the *SOCS3* response was sensitive to TrkB inhibition. Cisplatin reduced SOCS3 protein abundance in KGN cells, whereas 7,8-DHF increased it; ANA-12 blocked this increase (Fig. 5A). The corresponding immunoblots also showed an ANA-12-sensitive increase in S6 phosphorylation after 7,8-DHF treatment (Fig. 5B). These results placed *SOCS3* within the TrkB-associated response to 7,8-DHF and motivated examination of its transcriptional regulation.

FOS silencing reduced *SOCS3* expression in 7,8-DHF-treated cells and impaired the associated ERβ and CYP19A1 responses (Fig. 5C). To determine whether FOS could regulate SOCS3 directly, we identified a candidate AP-1 recognition motif in the SOCS3 promoter (Fig. 5D). FOS overexpression increased SOCS3 promoter-driven luciferase activity in HEK-293T cells (Fig. 5E). ChIP-qPCR detected FOS occupancy at the corresponding promoter region, with increased enrichment in FOS-overexpressing cells (Fig. 5F). Together, the expression, reporter and chromatin-binding results support FOS-dependent regulation of SOCS3 transcription.

We next depleted SOCS3 to examine its contribution to the cellular response. SOCS3 silencing altered ERβ and CYP19A1 responses to 7,8-DHF (Fig. 5G) and was accompanied by changes in S6 phosphorylation (Fig. 5H). Annexin V/7-AAD analysis further showed an increased apoptotic fraction with selected SOCS3-targeting siRNAs, although the response was not significant for every siRNA (Fig. 6I). These perturbations implicate SOCS3 in granulosa-cell function and survival.

Finally, we assessed whether the FOS–SOCS3 response was also evident in ovarian tissue. Phosphorylated FOS (p-c-Fos) and SOCS3 immunofluorescence signals were reduced in cisplatin-injured ovaries and increased after 7,8-DHF treatment (Fig. 5J). Line-scan profiles and pixel-intensity correlations showed greater spatial correspondence between the two signals in treated follicular compartments (Fig. 5K-L). This tissue-level response, together with promoter regulation and gene perturbation in cells, supports a FOS-SOCS3 component of the ovarian response to 7,8-DHF.

6. 7,8-DHF improves follicular profiles in aged ovaries and attenuates senescence-associated responses in granulosa-like cells

Having established protection during chemotherapy-associated injury, we asked whether the ovarian response extended to natural aging. Ten-month-old mice received vehicle or 7,8-DHF at 5 or 10 mg/kg for two weeks (Figure 6A). Follicle counts and ovarian sections showed greater retention of primordial and developing follicles in treated aged mice, together with differences in atretic follicle counts and improved follicular morphology (Figure 6B-C). The serum AMH response differed between doses, whereas E2 and FSH did not differ significantly among the aged groups (Figure 6D).

To examine a cellular response relevant to aging, we exposed KGN cells to H_2O_2. Treatment with 7,8-DHF reduced the H_2O_2-associated increases in p53, p21 and p16, and ANA-12 opposed these effects (Figure 6E). In aged ovaries, BDNF and SOCS3 immunofluorescence showed treatment-associated changes within follicular structures (Figure 6F). Line-scan profiles documented their spatial distributions, and pixel-intensity analysis showed differences in signal correspondence between doses (Figure 6G-H). These findings extend the follicular effects of 7,8-DHF to natural aging and link treatment to TrkB-sensitive changes in senescence-associated protein expression.

Discussion

Ovarian protection requires maintenance of the cellular functions that support follicles as well as preservation of follicle number. Here, 7,8-DHF limited granulosa-cell injury and improved ovarian phenotypes in cisplatin-treated and naturally aged mice. FOS-dependent SOCS3 regulation provides a transcriptional component of this response, linking treatment to granulosa-cell functional programs. Alongside the human BDNF observations, these findings support an approach to ovarian reserve preservation that reinforces the follicular support environment. The combination of tissue-level effects and cell-based regulatory evidence helps explain how trophic intervention can sustain the remaining follicular pool.

The results fit an emerging view of ovarian aging as a multicellular process. Single-cell analyses of primate ovaries have linked aging to disrupted antioxidant programs in oocytes and granulosa cells[19], providing context for the ROS and mitochondrial phenotypes observed here. BDNF supplementation supports mouse follicular development[11,20,21], and Qin et al. demonstrated that a TrkB agonist antibody improves fertility in aged and cyclophosphamide-treated mice[12]. Our study extends this work to 7,8-DHF and focuses on the downstream granulosa-cell response. Identification of FOS-SOCS3 regulation connects the established protective effects of trophic intervention to a defined transcriptional relationship.

FOS offers a potential route through which trophic inputs can engage functional adaptation. Its established roles in human periovulatory granulosa-cell metabolism and cholesterol synthesis[16] place the ERβ and CYP19A1 responses within a broader program of cell function. Maintaining both viability and functional identity may be particularly relevant for granulosa cells, whose contribution to the follicle depends on coordinated steroidogenic and trophic responses rather than survival alone. FOS occupancy and reporter activation support SOCS3 as a transcriptional target in the experimental systems studied,

and gene perturbation links this relationship to treatment-associated cellular responses. SOCS3 controls selected cytokine-receptor pathways by inhibiting receptor-associated JAK activity[17,22].Recruitment of such a feedback regulator could help coordinate trophic support with stress adaptation, an interpretation consistent with its context-dependent signaling role. The accompanying S6 response identifies a further point of convergence, although the biochemical connection between SOCS3 and S6 phosphorylation remains unresolved. The evidence therefore supports a regulatory contribution without assigning a complete linear downstream cascade[23].

The clinical SOCS3 findings bring the local follicular environment into this framework. Follicular-fluid SOCS3 was elevated in DOR and inversely associated with AMH, whereas cisplatin reduced intracellular SOCS3 in KGN cells. These measurements concern distinct compartments and biological contexts: extracellular protein abundance in a clinical condition and intracellular regulation during experimental injury. Their different directions emphasize that a fluid measurement need not mirror cellular activity. The clinical correlation and functional perturbations are thus complementary, linking SOCS3 to both the ovarian milieu and the cellular response to treatment. The origin and turnover of follicular-fluid SOCS3 remain questions for paired cellular and extracellular analyses.

Notably, our endocrine and histological data reveal context-dependent effects of 7,8-DHF in healthy versus injured ovaries. In healthy mice receiving 7,8-DHF alone, serum AMH remained unchanged, while FSH and E2 were significantly elevated, accompanied by reduced antral follicle counts and increased follicular atresia. This observation underscores that 7,8-DHF exerts protective effects in the setting of cisplatin-induced ovarian damage, yet it modulates HPO-axis hormone levels and antral follicle dynamics under physiological conditions, highlighting divergent biological responses depending on the ovarian status.

The intervention has a follicle-preserving rather than a follicle-regenerating scope. Reproductive benefit was demonstrated during cisplatin co-treatment, while the natural-aging experiments extend the follicular findings to a second biological setting. Differences between doses in aged mice also make dose selection an important part of further development. Histological preservation of major organs provides an initial tolerability observation within this preclinical framework. Follicle preservation and correction of estrogen deficiency nevertheless address different clinical needs. Hormone therapy remains an important component of POI care[24–28], and future evaluation of reserve-preserving approaches should consider their place alongside established management rather than assume that they can substitute for hormone replacement.

The principal limitations concern model specificity, target engagement and duration of follow-up. KGN cells do not reproduce all features of primary granulosa cells, and ANA-12 sensitivity supports TrkB involvement rather than direct or exclusive receptor engagement[29]. Quantitative pharmacological studies have questioned the reproducibility of reported small-molecule TrkB agonism[30], making receptor-level measurements and genetic validation particularly important. Granulosa-cell-specific perturbation would help define the in vivo contributions of TrkB, FOS and SOCS3. Longer-term fertility, endocrine and

systemic safety, together with preservation of antitumor efficacy, require evaluation before translation. Within this scope, FOS–SOCS3 regulation provides a testable mechanism for linking trophic intervention to follicular support.

Conclusion

7,8-DHF preserves ovarian follicles and improves reproductive output in a cisplatin injury model, with additional follicular effects in naturally aged mice. Cell-based experiments connect these responses to reduced oxidative and mitochondrial injury, TrkB-associated signaling and FOS-dependent SOCS3 regulation. The findings support granulosa-cell trophic responses as an intervention point for ovarian reserve preservation and establish a basis for further mechanistic and preclinical evaluation.

Declarations

Declaration of competing interest

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 32270952).We thank the core facility team for technical assistance. We appreciate the participants who provided clinical samples for this research. We also thank our laboratory members for helpful discussion.

CRediT authorship contribution statement

JG and JQ conceived the study, conducted the experiments, analyzed the data, and drafted the manuscript. MQ and JC contributed to clinical sample collection and histological evaluation. YL supervised the research, secured funding, guided data interpretation, and critically revised the manuscript. All authors have read and approved the final published version.

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Tables

Table 1 not available with this version.