



Supplementation with *Lycium barbarum* glycopeptide (LbGP) rescues the quality of aged oocytes

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ABSTRACT

Ethnopharmacological relevance: *Lycium barbarum* L. (goji berry), a traditional medicinal and edible herb, has long been employed for its anti-aging, vision-enhancing, and anti-inflammatory properties. *Lycium barbarum* glycopeptide (LbGP), a major bioactive glycoconjugate isolated from this plant, possesses documented antioxidant and immunomodulatory activities. However, its specific therapeutic efficacy in counteracting reproductive aging and the precise mechanisms underlying its protective effects on oocyte quality remain to be fully elucidated.

Aim of the study: To investigate the restorative effects of LbGP on ovarian function and oocyte quality in aged mice and to decipher the underlying mechanisms involving both oocyte-intrinsic metabolic regulation and extrinsic ovarian microenvironment remodeling.

Materials and methods: A reproductive aging model was established using naturally aged female mice supplemented with LbGP. Follicular development and oocyte quality were assessed via histological analysis, in vitro fertilization (IVF), and early embryonic culture. Mitochondrial function and oxidative stress levels were monitored using live-cell imaging. Mechanistic insights were generated through integrated proteomic analysis of oocytes and single-cell RNA sequencing (scRNA-seq) of ovarian tissues to identify key metabolic pathways and cellular composition changes.

Results: LbGP supplementation significantly promoted follicular development, enhanced oocyte maturation competence, and improved subsequent early embryonic potential compared to untreated aged mice. Mechanistically, proteomic analysis revealed that LbGP restored mitochondrial function in aged oocytes by activating PPAR signaling pathways, leading to reduced intracellular reactive oxygen species (ROS) accumulation and DNA damage. Furthermore, ovarian single-cell transcriptomics demonstrated that LbGP systemically remodeled the aged ovarian microenvironment by increasing functional granulosa cell populations, reducing pro-inflammatory immune cells, and repairing intercellular communication networks.

Conclusions: LbGP delays reproductive aging by concurrently restoring mitochondrial function in aged oocytes and remodeling the ovarian microenvironment. These findings provide modern pharmacological evidence supporting LbGP as a promising natural therapeutic candidate capable of improving oocyte quality and enhancing fertility outcomes in women of advanced maternal age.

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1. Introduction

With global socioeconomic development, there is a growing trend for women to delay childbearing, making age-related fertility decline a significant public health and societal challenge. Female fertility begins to decline sharply after the age of 35 (McFalls, 1990). This phenomenon is primarily driven by ovarian aging, which is characterized by both a quantitative decrease in ovarian reserve and a qualitative deterioration of oocyte quality (Faddy et al., 1992; te Velde and Pearson, 2002). A woman's ovarian reserve is established at birth as a finite pool of primordial follicles that undergoes progressive, irreversible attrition throughout reproductive life (Mark-Kappeler et al., 2011; Richardson et al., 2014). This loss accelerates markedly after the mid-thirties, resulting in a diminished cohort of available oocytes and ultimately culminating in menopause (Broekmans et al., 2009).

Concurrently, the decline in oocyte quality constitutes the primary bottleneck for successful pregnancy in women of advanced maternal age. This deterioration leads to reduced fertilization rates, embryonic developmental arrest, increased aneuploidy, and a significantly higher risk of miscarriage and congenital birth defects (Franasiak et al., 2014; Leese et al., 2007; Levi et al., 2001). The process of oocyte aging is driven by a convergence of cellular and molecular changes, with mitochondrial dysfunction widely recognized as a key driver (Bentov and Casper, 2013). As the cell's "powerhouses," mitochondria provide essential ATP for critical events including meiotic division, fertilization, and early embryonic development. (May-Panloup et al., 2005). Aging compromises mitochondrial function, resulting in insufficient energy supply and excessive production of reactive oxygen species (ROS), which cause severe oxidative stress, cumulative DNA damage, and apoptosis (Ge et al., 2012).

Furthermore, oocyte competence is intricately dependent on the surrounding ovarian microenvironment. The proliferation and function of granulosa cells (GCs) constitute fundamental biological processes required to sustain normal follicular development (Fan et al., 2019). However, the aging process disrupts this delicate microenvironment, manifesting as somatic cell dysfunction, chronic low-grade inflammation (termed "inflammaging"), and disordered intercellular communication networks. These extrinsic factors collectively accelerate the decline in oocyte quality (Briley et al., 2016; Lopez-Otin et al., 2013). Therefore, therapeutic strategies that simultaneously target oocyte-intrinsic defects and optimize the extrinsic microenvironment are urgently needed. While exogenous molecules such as nicotinamide mononucleotide (NMN) have shown promise in improving aged oocyte quality (Bertoldo et al., 2020), natural compounds derived from traditional medicine offer an attractive alternative due to their long history of safe human consumption.

Lycium barbarum L. (Goji berry), a traditional medicinal and edible plant, has been widely used in Traditional Chinese Medicine (TCM) for its remarkable anti-aging and antioxidant properties (Amagase et al., 2009; Yao, 2025). While previous studies have demonstrated that *Lycium barbarum* polysaccharides (LBP) protect against ovarian damage induced by external stressors, such as repeated superovulation (Liu et al., 2020), or D-galactose-induced premature ovarian insufficiency (Zheng et al., 2023a), the specific effects of its other primary bioactive components remain less understood. Importantly, in ethnopharmacological practice, *Lycium barbarum* is traditionally administered mainly as whole fruits or decoctions (often within multi-herbal formulations), where multiple constituents may act additively or synergistically. To enhance material traceability and experimental reproducibility, we therefore focused on a defined and standardizable bioactive fraction derived from *Lycium barbarum*, which facilitates mechanistic dissection of key active contributors while acknowledging that it cannot fully recapitulate the holistic effects of traditional preparations (Bao et al., 2026).

Lycium barbarum glycopeptide (LbGP), a stable glycoconjugate distinct from polysaccharides, has been confirmed to possess significant

biological activities, including antioxidant, anti-inflammatory, neuro-protective, and anti-aging effects (Gao et al., 2017; Lam et al., 2015; Niu et al., 2023; Yu et al., 2023; Zheng et al., 2023b). As a purified and batch-traceable constituent from a traditionally used edible/medicinal plant, LbGP provides a practical entry point for mechanistic evaluation with improved standardization and reproducibility. However, unlike acute stress models, the potential of LbGP to counteract the complex, multifaceted processes of natural reproductive aging remains systematically unexplored. Specifically, the high-resolution molecular mechanisms by which LbGP might rescue ovarian function have not been elucidated.

This study aims to systematically evaluate the effects of LbGP supplementation on reproductive aging using a naturally aged mouse model. By integrating multi-omics approaches, including proteomics and single-cell transcriptomics, we sought to elucidate potential mechanisms; similar high-resolution omics approaches have recently been effectively applied to dissect complex tissue microenvironments under stress (Wang et al., 2023). Our findings comprehensively reveal that LbGP significantly improves oocyte quality and fertility potential in aged mice through a dual mechanism: the correction of oocyte-intrinsic metabolic defects and the systemic remodeling of the ovarian microenvironment. This research provides foundational evidence supporting the potential of LbGP as a natural agent for improving reproductive health in women of advanced maternal age.

2. Materials and methods

2.1. Mice and LbGP supplementation

All animal experiments were performed in compliance with animal welfare guidelines and approved by the Institutional Animal Care and Use Committee of Nanjing Drum Tower Hospital (2025AE01013). Young (8-week-old) and aged (10-month-old) female ICR mice were housed in polycarbonate cages under a 12-h light/dark cycle (20–24 °C) with ad libitum access to standard chow and water. For supplementation, *Lycium barbarum* glycopeptide (LbGP; Tianren Bio-engineering, Zhongning, China, Batch No.20240901) was dissolved in phosphate-buffered saline (PBS). Aged mice were randomly assigned to two groups. Unless otherwise specified, all animal-based experiments included at least three mice per group ($n \geq 3$). Mice received daily intraperitoneal injections for 10 consecutive days of either LbGP (125 mg kg⁻¹ body weight) or an equivalent volume of PBS as a vehicle control. On the morning of Day 13, mice were euthanized by cervical dislocation for subsequent tissue collection. The dose of 125 mg/kg was selected based on previously reported safe and pharmacologically active dose ranges of *Lycium barbarum*-derived glycopeptides in murine models (Dai et al., 2023; Wu et al., 2025). As LbGP is a purified bioactive glycopeptide rather than a crude extract, its activity is commonly evaluated at fixed doses in mechanistic studies. Thus, a single-dose regimen was used here to investigate its effects on reproductive aging.

Chemical fingerprint: A chemical fingerprint of LbGP (HPLC chromatogram) provided by the manufacturer is included in [Supplementary Fig. S1](#).

2.2. Oocyte collection and in vitro maturation

For the collection of ovulated metaphase II (MII) oocytes, mice were superovulated by intraperitoneal injection of 10 IU of pregnant mare serum gonadotropin (PMSG), followed 48 h later by 10 IU of human chorionic gonadotropin (hCG). Cumulus-oocyte complexes (COCs) were retrieved from the oviductal ampullae 13.5h post-hCG injection. Cumulus cells were removed by a brief treatment with 1 mg ml⁻¹ hyaluronidase. To collect germinal vesicle (GV)-stage oocytes, ovaries were harvested 48 h after PMSG injection and placed in M2 medium (M1250, Nanjing Aibei Biotechnology) supplemented with 5 μM milrinone. COCs were released by puncturing antral follicles, and cumulus

cells were mechanically denuded by gentle pipetting. For in vitro maturation (IVM), collected GV oocytes were cultured in milrinone-free M2 medium and incubated at 37 °C in a 5% CO₂ atmosphere for 8 h to reach the MI stage or 14 h for the MII stage.

2.3. Immunofluorescence

Oocytes were fixed in 4% paraformaldehyde (PFA) for 30 min at room temperature, permeabilized with 0.5% Triton X-100 for 20 min, and blocked with 5% FBS in PBS for 1 h. The oocytes were then incubated with primary antibodies overnight at 4 °C. Antibodies are listed in Table 1. After washing three times with PBST (PBS + 0.1% Tween-20), they were incubated with diluted secondary antibodies (1:1000) for 1 h at room temperature. Finally, the oocytes were washed again in PBST and mounted on glass slides using an antifade mounting medium containing DAPI. Fluorescence images were captured using a Carl Zeiss LSM 780 laser scanning confocal microscope.

2.4. Live-cell staining

Mitochondrial activity was assessed by incubating oocytes for 30 min with MitoTracker® Mitochondrion-Selective Probes (1:1000; A66444, Thermo Fisher Scientific). For mitochondrial membrane potential ($\Delta\Psi_m$) measurement, oocytes were incubated with JC-1 dye (1:500; 40705ES03, Yeasen Biotechnology) for 30 min. Intracellular reactive oxygen species (ROS) levels were detected using DCFH-DA (1:200; S0033S, Shanghai Biyuntian Biotechnology) for 30 min. After staining, oocytes were washed thoroughly in M2 medium. Fluorescence signals were captured using an inverted fluorescence microscope, and mean fluorescence intensity in regions of interest (ROIs) was quantified using ImageJ software.

2.5. In vitro fertilization and embryo culture

Female ICR mice were superovulated by an intraperitoneal injection of 10 IU of PMSG, followed 48 h later by an injection of 10 IU of hCG. MII oocytes were collected from the oviductal ampullae 13.5 h post-hCG and transferred into pre-equilibrated G-IVF™ PLUS medium supplemented with HSA (Vitrolife, Sweden; REF 10136). Sperm were collected from the cauda epididymis of 12-week-old male ICR mice and capacitated for 1 h in the same medium before being co-incubated with the MII oocytes. After 4–6 h of co-incubation, the embryos were washed and transferred into G-1™ PLUS medium supplemented with HSA (Vitrolife, Sweden; REF 10128), which had been pre-equilibrated overnight at 37 °C in 5% CO₂. The formation of two pronuclei was considered the marker of successful fertilization. Bright-field images of the embryos were captured at 24, 48, 60, 72, and 96 h post-fertilization to monitor their development through the zygote, early 2-cell, late 2-cell, 4-cell, 8-cell, morula, and blastocyst stages; for quantitative comparisons, embryos were scored at 24, 48, 72, and 96 h post-fertilization, consistent with Fig. 2B.

2.6. Ovarian histology and follicle counting

Freshly collected ovaries were cleaned of surrounding fat and tissue, then fixed overnight in 4% paraformaldehyde (PFA, pH 7.5) at 4 °C. The fixed tissues were subsequently dehydrated through a graded ethanol series, cleared with xylene, and embedded in paraffin. The paraffin-embedded ovaries were serially sectioned at a thickness of 3 µm and stained using a standard hematoxylin and eosin (H&E) protocol. For analysis, bilateral ovaries from three mice per group were used. Tissue morphology was observed under a microscope, and follicles at all developmental stages—including primordial, primary, secondary, antral/mature, and atretic—were counted. Follicle quantification was performed using a systematic sampling approach: one of every five consecutive sections was selected for counting, and follicle numbers per

ovary were estimated by multiplying the counted values by five. Follicles were classified as primordial, primary, secondary, antral/mature, or atretic based on standard histological morphology under × 200 magnification. To avoid double counting, only follicles containing a clearly visible oocyte nucleus were included. Follicle counts were performed independently by two investigators (blinded to group allocation).

2.7. Single-cell RNA sequencing

For sample collection, mice were confirmed to be in the diestrus stage of the estrous cycle. Ovaries were rapidly excised following euthanasia, cleaned of surrounding tissues, washed in pre-chilled saline, and immediately transferred into preservation solution for transport. Ovarian tissues were enzymatically and mechanically dissociated into single-cell suspensions. Cell viability and concentration were assessed using a Nexcelom Cellometer Auto2000 with AOPI staining. Approximately 10,000–20,000 viable cells were loaded onto a DNBelab C Series Single-Cell Microbead system (BGI Genomics) to generate single-cell Gel Bead-in-Emulsions (GEMs). Single-cell cDNA and libraries were prepared using the DNBelab C Series Single-Cell Library Prep Set (BGI Genomics) following the manufacturer's instructions. Library quality was evaluated using an Agilent Bioanalyzer High Sensitivity DNA Kit and a Qubit HS DNA assay. Qualified libraries were sequenced on a DNBSEQ-T7 platform (BGI Genomics). Unless otherwise specified, downstream data analyses were performed in R (version 4.2.2) using Seurat v4 and other standard single-cell RNA-seq analysis packages.

2.8. Proteomics analysis

For proteomics analysis, oocytes were collected in phosphate-buffered saline containing 0.1% polyvinyl alcohol. The collected oocytes were washed three times with pre-chilled PBS-PVA, ensuring the final residual volume was less than 5 µL. The samples were then snap-frozen in liquid nitrogen and stored at −80 °C before being shipped on dry ice within 12 h. Proteomic sample preparation was performed by the sequencing facility. Briefly, proteins were extracted from oocytes, reduced with dithiothreitol (DTT), alkylated with iodoacetamide (IAA), and digested with sequencing-grade trypsin. The resulting peptides were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Raw MS data were searched against the UniProt *Mus musculus* protein database for peptide and protein identification. Downstream statistical and functional analyses were conducted in R (version 4.2.2) using multiple packages, including limma for differential expression analysis, clusterProfiler for enrichment analysis, org. Mm.eg.db for mouse gene annotation, and ComplexHeatmap for visualization of expression patterns.

2.9. Statistical analysis

All experiments were performed with at least three biological replicates. Statistical analyses were conducted using GraphPad Prism (v7.0 for Windows). Comparisons between two groups were performed using a two-tailed Student's t-test. Comparisons among three or more groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Data are presented as the mean ± standard deviation (SD). A p-value <0.05 was considered statistically significant.

3. Results

3.1. LbGP improves ovarian morphology and enhances the in vivo maturation competence of oocytes from aged mice

To evaluate the effects of LbGP on female reproductive aging, we administered LbGP to aged mice continuously, followed by

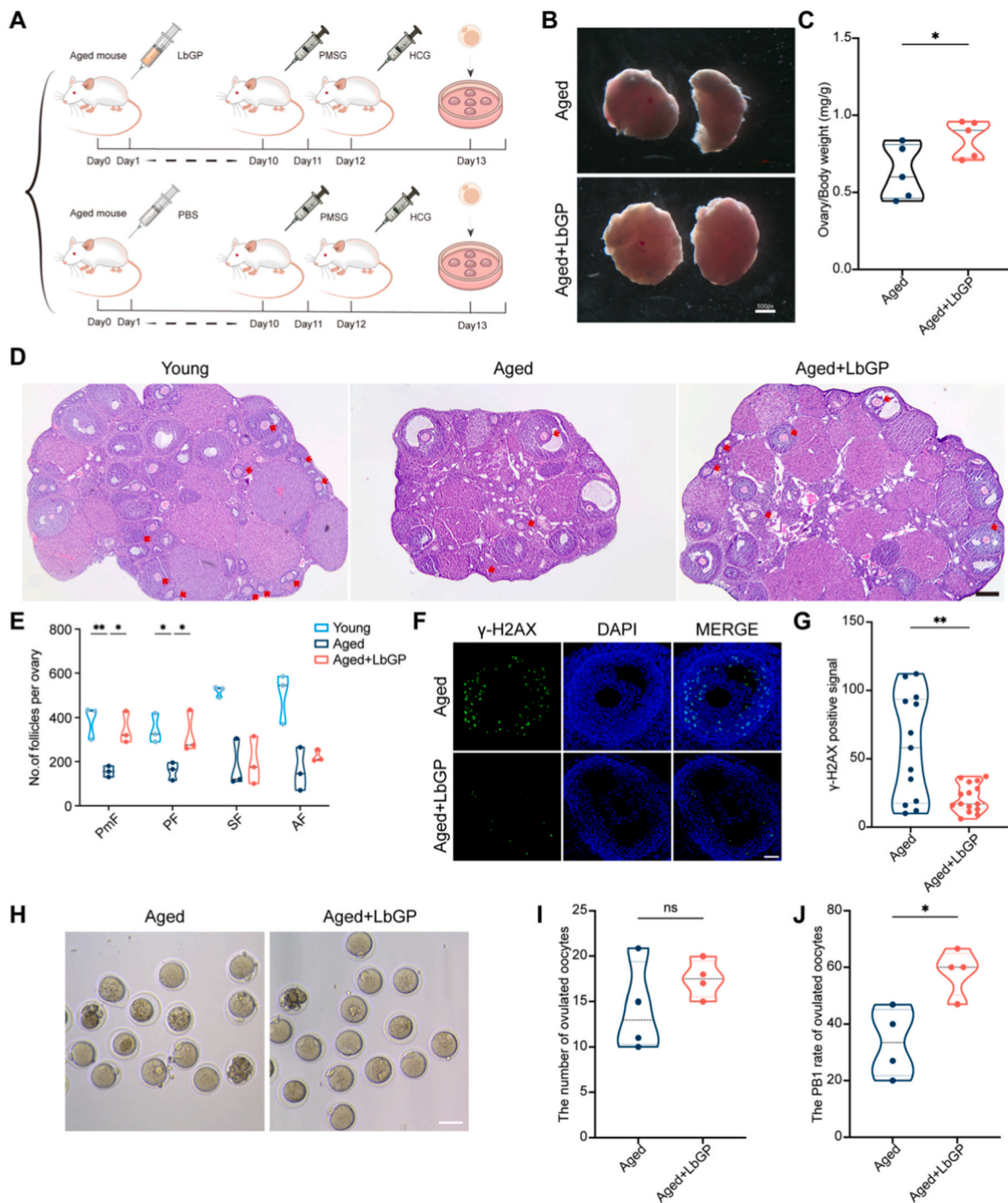


Fig. 1. LbGP improves ovarian morphology and enhances in vivo maturation of oocytes in aged mice. (A) Schematic timeline of LbGP administration, superovulation, and oocyte collection. (B) Representative gross images of ovaries from each group. (C) Ovary-to-body weight ratios in aged control (Aged) and LbGP-treated (Aged + LbGP) mice. * $p < 0.05$. (D) Representative H&E-stained ovarian sections from Aged and Aged + LbGP groups showing follicle structures at different developmental stages (follicles are indicated by arrowheads). Scale bar = 100 μ m. (E) Quantification of follicle numbers per ovary in Aged and Aged + LbGP groups. Follicles were classified as primordial (PmF), primary (Pk), secondary (Sf), and antral (Af). Data are shown as mean \pm SEM. ** $p < 0.01$, * $p < 0.05$. (F) Representative immunofluorescence images of γ -H2AX staining detecting DNA damage in follicles from Aged and Aged + LbGP groups. Scale bar = 50 μ m. (G) Number of γ -H2AX positive signals in follicles. ** $p < 0.01$. (H) Representative morphology of MII oocytes retrieved after superovulation in Aged and Aged + LbGP groups. Scale bar = 100 μ m. (I) Total number of ovulated oocytes in Aged and Aged + LbGP groups. ns, no significant difference. (J) Percentage of oocytes extruding the first polar body (PB1). * $p < 0.05$.

superovulation and oocyte collection according to the experimental timeline (Fig. 1A). Compared with the control group, LbGP treatment increased the ovary-to-body weight ratio (Fig. 1B and C). Hematoxylin and eosin (H&E) staining revealed numerous atretic follicles in the ovaries of aged mice, whereas the follicular structure was markedly

improved after LbGP treatment (Fig. 1D). Follicle counting demonstrated that LbGP treatment significantly increased the number of primordial and primary follicles in the ovaries of aged mice (Fig. 1E). Furthermore, immunofluorescence staining for γ -H2AX showed that LbGP significantly reduced the rate of DNA damage in follicles (Fig. 1F

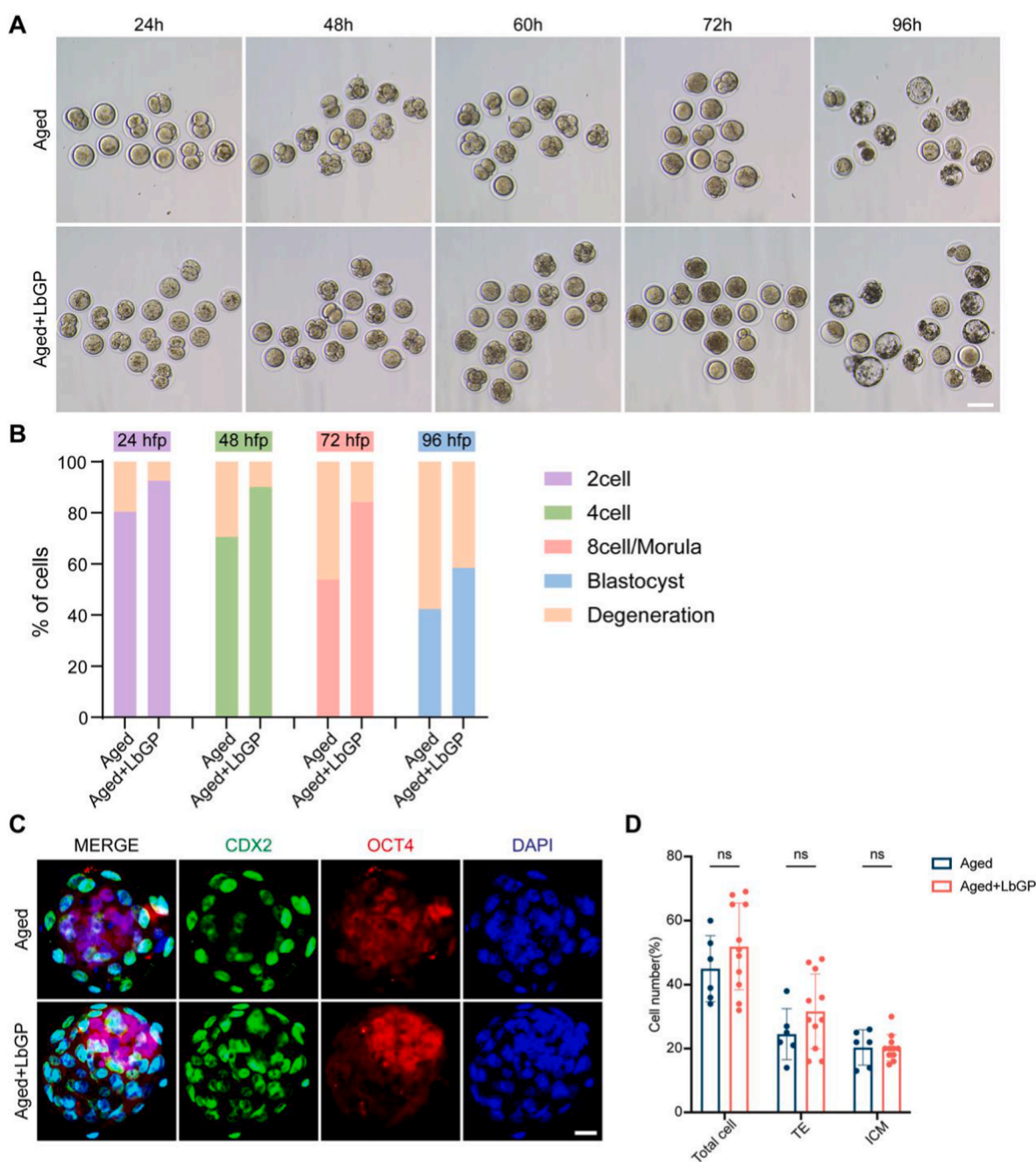


Fig. 2. LbGP promotes early embryonic development of aged oocytes. (A) Representative images of embryos at 24–96 h after IVF in Aged and Aged + LbGP groups. Scale bar = 100 μ m. (B) Quantification of embryonic developmental potential. The stacked bar graph shows the distribution of embryos at 24, 48, 72, and 96 h post-fertilization (hpf). Aged group (n = 78) and Aged + LbGP group (n = 108). (C) Representative immunofluorescence staining of blastocysts showing trophoblast (TE) and inner cell mass (ICM) with DAPI nuclear staining (blue). Scale bar = 10 μ m. (D) Quantification of total cell number, TE cell number, and ICM cell number per blastocyst. ns, no significant difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and G). In the superovulation experiments, morphological observation revealed that oocytes from the LbGP-treated group were more intact and uniform, with a significantly lower fragmentation rate compared to the control group (Fig. 1H). Moreover, while LbGP supplementation did not significantly affect the total number of oocytes obtained via superovulation (Fig. 1I), it markedly increased the extrusion rate of the first polar body (Fig. 1J), indicating that LbGP enhanced the in vivo maturation competence of the oocytes. These results indicate that LbGP can improve the ovarian developmental status, alleviate follicular DNA damage, and enhance the maturation quality of oocytes in aged mice.

3.2. LbGP promotes the early embryonic development of oocytes from aged mice

To investigate the effect of LbGP on the early embryonic developmental potential of oocytes from aged mice, we performed time-series observations of embryos obtained through in vitro fertilization (IVF) (Fig. 2A). The results showed that embryos from the LbGP-treated group exhibited superior morphological uniformity and synchrony of cell division during development compared to the control group. Quantitative analysis revealed that LbGP significantly increased the proportion of embryos at each stage (Fig. 2B). Specifically, the LbGP-treated group

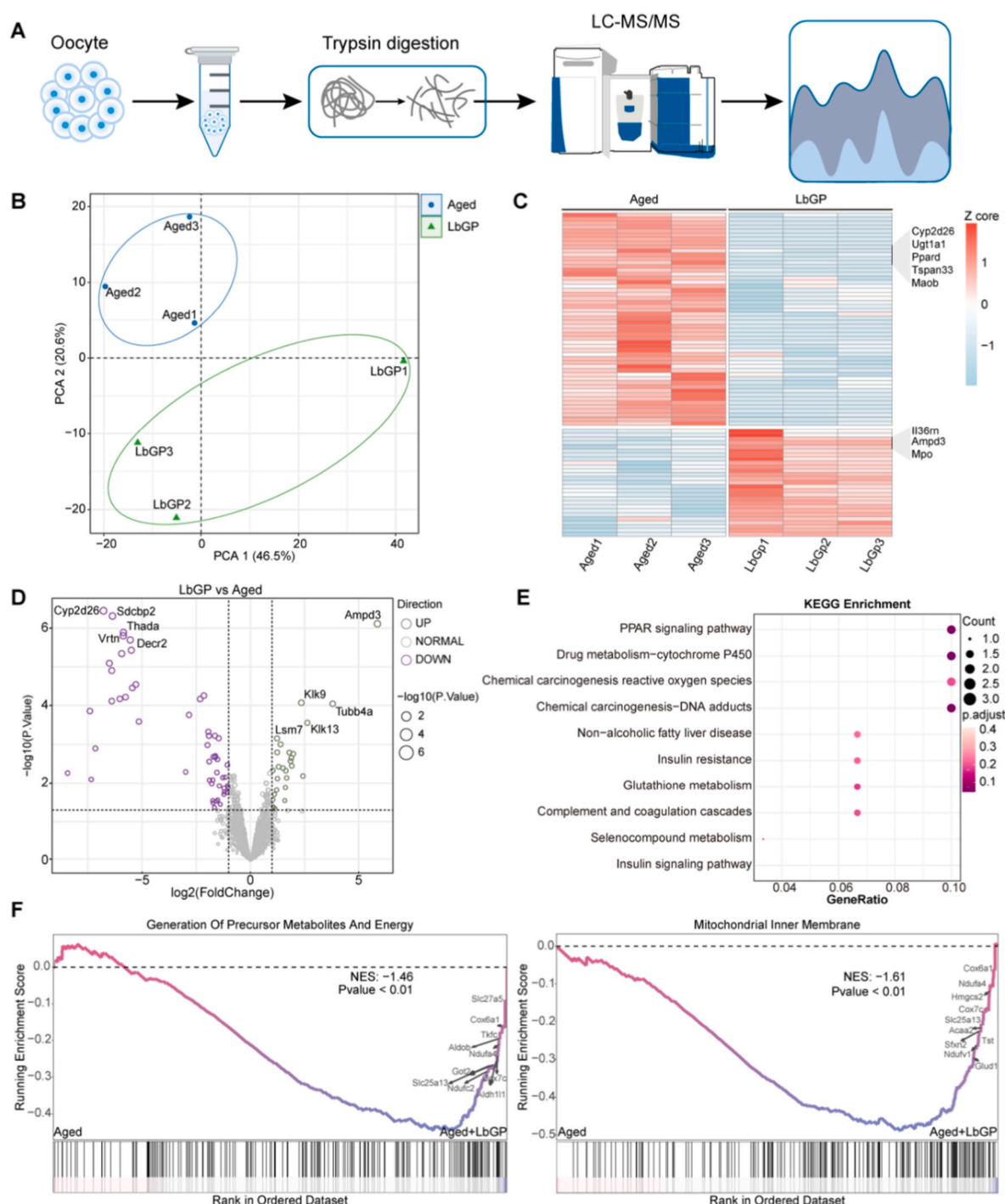


Fig. 3. Proteomic analysis reveals that LbGP regulates key metabolic pathways in aged oocytes. (A) Schematic of experimental design. (B) Principal component analysis (PCA) showing separation of proteomic profiles between Aged and Aged + LbGP oocytes. (C) Heatmap of differentially expressed proteins (DEPs). (D) Volcano plot showing upregulated and downregulated proteins. (E) KEGG enrichment analysis of DEPs. (F) GSEA showing significant downregulation of "Generation of Precursor Metabolites and Energy" and "Mitochondrial Inner Membrane" pathways.

showed a noticeable improvement in embryo development during the cleavage stage. For the Aged group ($n = 78$ oocytes) and Aged + LbGP group ($n = 108$ oocytes), the improved developmental potential in the LbGP-treated group was evident across all stages, leading to a higher number of embryos reaching later stages. To determine whether improved cleavage translated into enhanced blastocyst quality, we examined OCT4- and CDX2-labeled blastocysts to quantify inner cell mass (ICM) and trophectoderm (TE) cells (Fig. 2C). Although LbGP treatment did not induce significant differences in total cell number, TE

cell number, or ICM cell number, LbGP-derived blastocysts showed a consistent upward trend in total and TE cell counts (Fig. 2D). Together, these findings indicate that LbGP enhances the early embryonic developmental potential of aged oocytes by promoting cleavage progression, whereas its influence on blastocyst cellular composition remains limited.

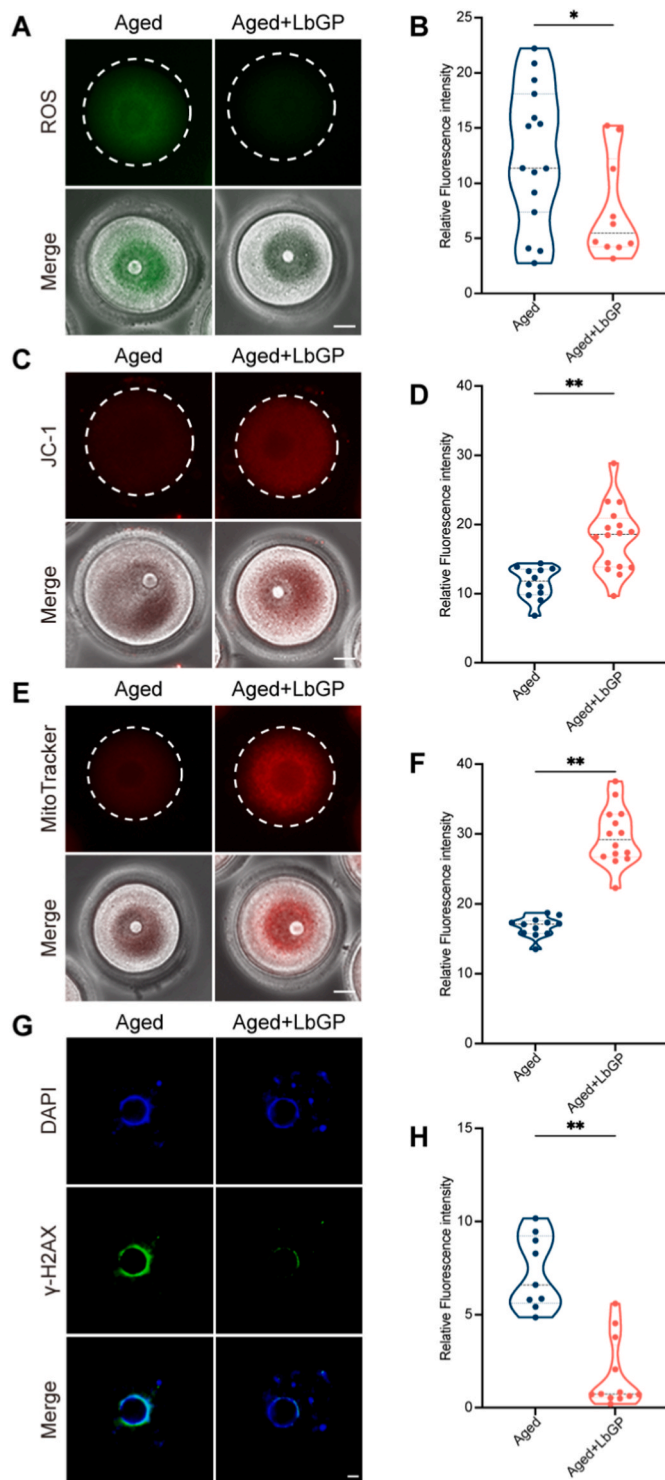


Fig. 4. LbGP improves mitochondrial function, alleviates ROS accumulation, and reduces DNA damage. (A) Representative DCFH-DA staining images of ROS levels in Aged and Aged + LbGP oocytes. Scale bar = 20 μ m. (B) Quantification of ROS fluorescence intensity. * p < 0.05. (C) Representative JC-1 staining images showing mitochondrial membrane potential ($\Delta\Psi$ m). Scale bar = 20 μ m. (D) Quantification of JC-1 fluorescence ratio. ** p < 0.01. (E) Representative MitoTracker staining images showing mitochondrial signals. Scale bar = 20 μ m. (F) Quantification of MitoTracker fluorescence intensity. ** p < 0.01. (G) Representative γ -H2AX staining images showing DNA double-strand breaks. Scale bar = 10 μ m. (H) Quantification of γ -H2AX fluorescence intensity. ** p < 0.01.

3.3. Proteomic analysis reveals the regulatory pathways of LbGP's effect on oocytes from aged mice

To further investigate the potential mechanisms by which LbGP may improve the quality of oocytes from aged mice, we performed proteomic analysis using LC-MS/MS on oocytes from aged mice with and without LbGP supplementation (Fig. 3A). Principal component analysis (PCA) demonstrated a distinct separation between the two sample groups based on their protein expression profiles (Fig. 3B). Heatmap analysis revealed significant differences in the proteomic profiles, highlighting several differentially expressed proteins that may influence oocyte quality (Fig. 3C–Table 2). The volcano plot further showed that, compared to oocytes from aged mice, oocytes from LbGP-supplemented aged mice had 27 upregulated and 53 downregulated proteins (Fig. 3D). KEGG enrichment analysis indicated that DEPs were enriched in metabolic pathways, including the PPAR signaling pathway, oxidative stress response, and insulin resistance pathways, which is consistent with a potential role of LbGP in metabolic remodeling (Fig. 3E). These pathways are crucial for maintaining normal oocyte metabolism and function (Froment et al., 2006; Vitti et al., 2016; Wang et al., 2021), suggesting that LbGP may exert multiple effects in combating the aging process. Furthermore, Gene Set Enrichment Analysis (GSEA) showed that the ‘Generation of Precursor Metabolites and Energy’ and ‘Mitochondrial Inner Membrane’ signaling pathways were significantly downregulated in the LbGP-supplemented group compared to the aged group (Fig. 3F). This downregulation may indicate that LbGP acts to fine-tune mitochondrial function and energy metabolism, possibly by reducing overactive metabolic processes and oxidative stress associated with aging. The downregulation of these pathways could reflect a more balanced metabolic state that promotes mitochondrial health and overall oocyte quality. This study suggests that LbGP may significantly improve the protein metabolic profile and function of aged mouse oocytes. These findings suggest that LbGP could improve oocyte quality by regulating energy metabolism and mitochondrial function. Collectively, these proteomic shifts support an oocyte-intrinsic metabolic remodeling associated with improved mitochondrial function and energy homeostasis.

3.4. LbGP alleviates oxidative stress and DNA damage in aged oocytes by improving mitochondrial function

Mitochondrial dysfunction and the resulting oxidative stress are hallmarks of oocyte aging. To determine whether *Lycium barbarum* glycopeptide (LbGP) could mitigate these age-associated defects, we first assessed reactive oxygen species (ROS) levels in aged oocytes. Fluorescence imaging and quantitative analysis revealed a marked accumulation of ROS in aged oocytes, which was significantly reduced following LbGP treatment (Fig. 4A and B). To explore whether this reduction in ROS was linked to improved mitochondrial health, we next evaluated mitochondrial activity and abundance. Using JC-1 staining to detect mitochondrial membrane potential ($\Delta\Psi$ m), we found that aged oocytes exhibited impaired $\Delta\Psi$ m, a critical indicator of mitochondrial dysfunction. In contrast, LbGP treatment effectively restored $\Delta\Psi$ m, indicating improved mitochondrial function (Fig. 4C and D). Consistently, MitoTracker staining showed a significant increase in mitochondrial fluorescence signals in aged oocytes supplemented with LbGP, suggesting recovery of mitochondrial quantity or activity (Fig. 4E and F). As excessive ROS production and mitochondrial dysfunction can trigger severe cellular damage, we further examined genome integrity by staining for γ -H2AX, a marker of DNA double-strand breaks. LbGP supplementation markedly reduced the accumulation of γ -H2AX foci in aged oocytes (Fig. 4G and H). Together, these findings demonstrate that LbGP enhances mitochondrial function to suppress oxidative stress and maintain genomic stability, thereby improving the quality of aged oocytes.

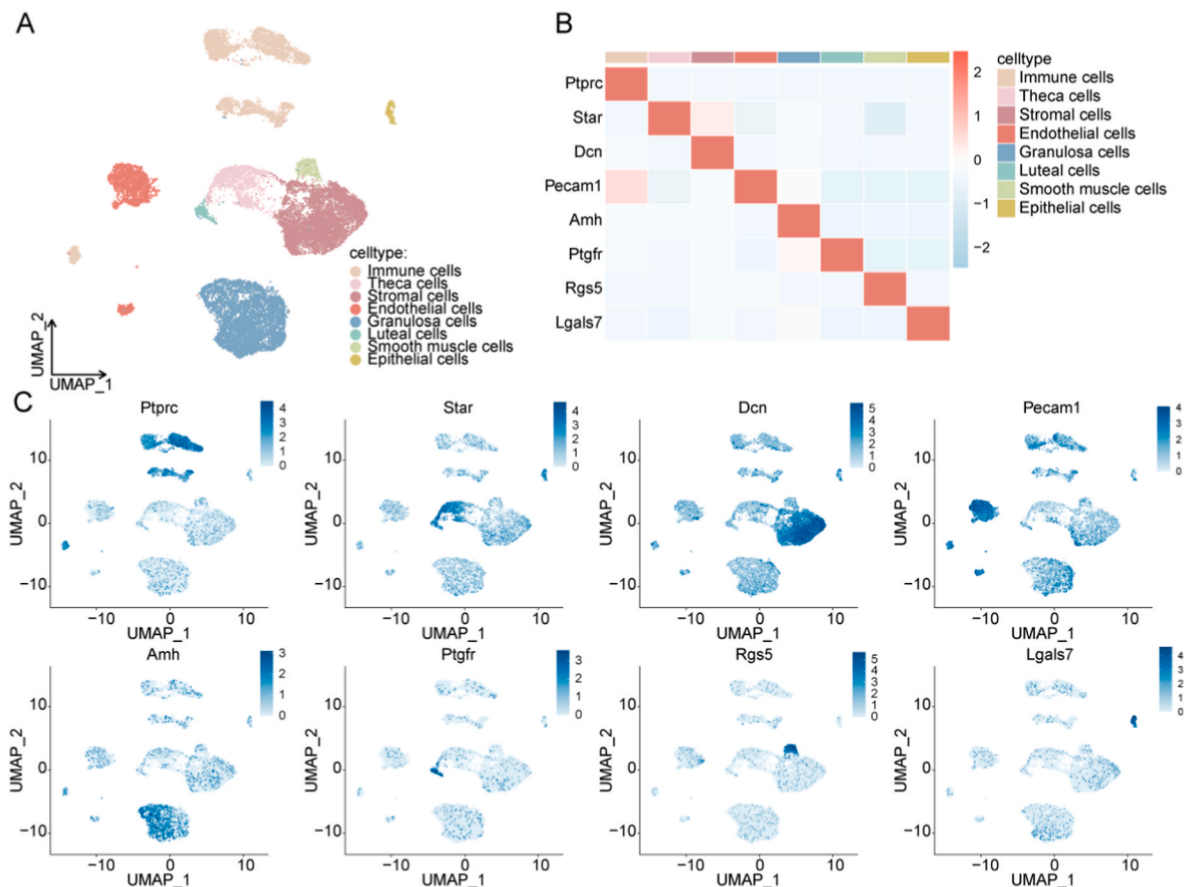


Fig. 5. Single-cell transcriptomic analysis identifies major ovarian cell populations. (A) UMAP plot showing the distribution of eight major ovarian cell types. (B) Heatmap of characteristic gene expression across cell types. (C) UMAP feature plots showing expression of marker genes (*Ptprc*, *Star*, *Dcn*, *Pecam1*, *Amh*, *Ptgr*, *Rgs5*, *Lgals7*).

3.5. Single-cell transcriptomic analysis identifies major ovarian cell populations

To comprehensively resolve cellular heterogeneity and age-associated alterations within ovarian tissue, we performed single-cell RNA sequencing (scRNA-seq). Clustering analysis of all cells followed by dimensionality reduction with Uniform Manifold Approximation and Projection (UMAP) enabled the identification of eight major cell populations (Fig. 5A). Based on their gene expression signatures, these clusters were annotated as immune cells, theca cells, stromal cells, endothelial cells, granulosa cells, luteal cells, smooth muscle cells, and epithelial cells (Fig. 5A). To validate the identities of these populations, we examined the expression of a panel of canonical marker genes. Heatmap visualization (Fig. 5B) together with UMAP feature plots (Fig. 5C) confirmed the specific enrichment of these markers within the corresponding cell clusters. Collectively, our single-cell transcriptomic analysis delineates the principal cellular landscape of the ovary and provides a robust framework for further mechanistic studies into the roles of specific cell types in ovarian physiology and aging.

3.6. LbGP improves the aged ovarian microenvironment by remodeling cellular composition and molecular networks

To further elucidate the mechanisms by which *Lycium barbarum* glycopeptide (LbGP) acts on aged ovaries, we performed comparative single-cell RNA sequencing (scRNA-seq) of ovaries from young, aged, and LbGP-treated aged mice. Aging markedly altered the cellular composition of the ovary (Fig. 6A). Quantitative analysis revealed that, compared with young ovaries, the proportion of granulosa cells in aged

ovaries declined sharply from 36.5% to 19.4%, while immune cells increased from 15.7% to 26.8% (Fig. 6B). LbGP intervention significantly alleviated this imbalance, restoring granulosa cells to 23.2% and reducing immune cells to 22.7% (Fig. 6B), suggesting that LbGP helps re-establish a more youthful ovarian cellular microenvironment.

To uncover the underlying molecular basis, we performed functional enrichment analysis of differentially expressed genes. Gene Ontology (GO) and KEGG pathway analyses showed that in aged ovaries, pathways related to oxidative stress, inflammatory responses (including TNF and IL-17 signaling), and apoptosis were significantly enriched (Fig. 6C–E). By contrast, LbGP treatment enriched pathways associated with steroid hormone responses, apoptotic signaling regulation, autophagy, and AMPK signaling—processes critical for maintaining cellular homeostasis and metabolic health (Fig. 6D–F). Given the importance of intercellular interactions in sustaining tissue function, we further examined cell–cell communication networks. Aging caused a dramatic disruption in the strength and patterns of intercellular interactions (Fig. 6G). We focused specifically on the Thrombospondin (THBS) signaling pathway. As matricellular proteins, THBS family members are critical mediators of cell–matrix interactions and angiogenesis, orchestrating the tissue remodeling required to maintain follicular structural integrity (Bender et al., 2019). Network analysis revealed that aging caused a dramatic collapse in the connectivity and intensity of THBS-mediated signaling (Fig. 6H). Remarkably, LbGP treatment reinvigorated this network, effectively restoring the strength of THBS signaling to support intercellular communication (Fig. 6H). To demonstrate THBS pathway changes at the tissue level, we performed immunofluorescence staining on ovarian sections for representative THBS-related ligands and receptors, including THBS1, THBS4, CD47,

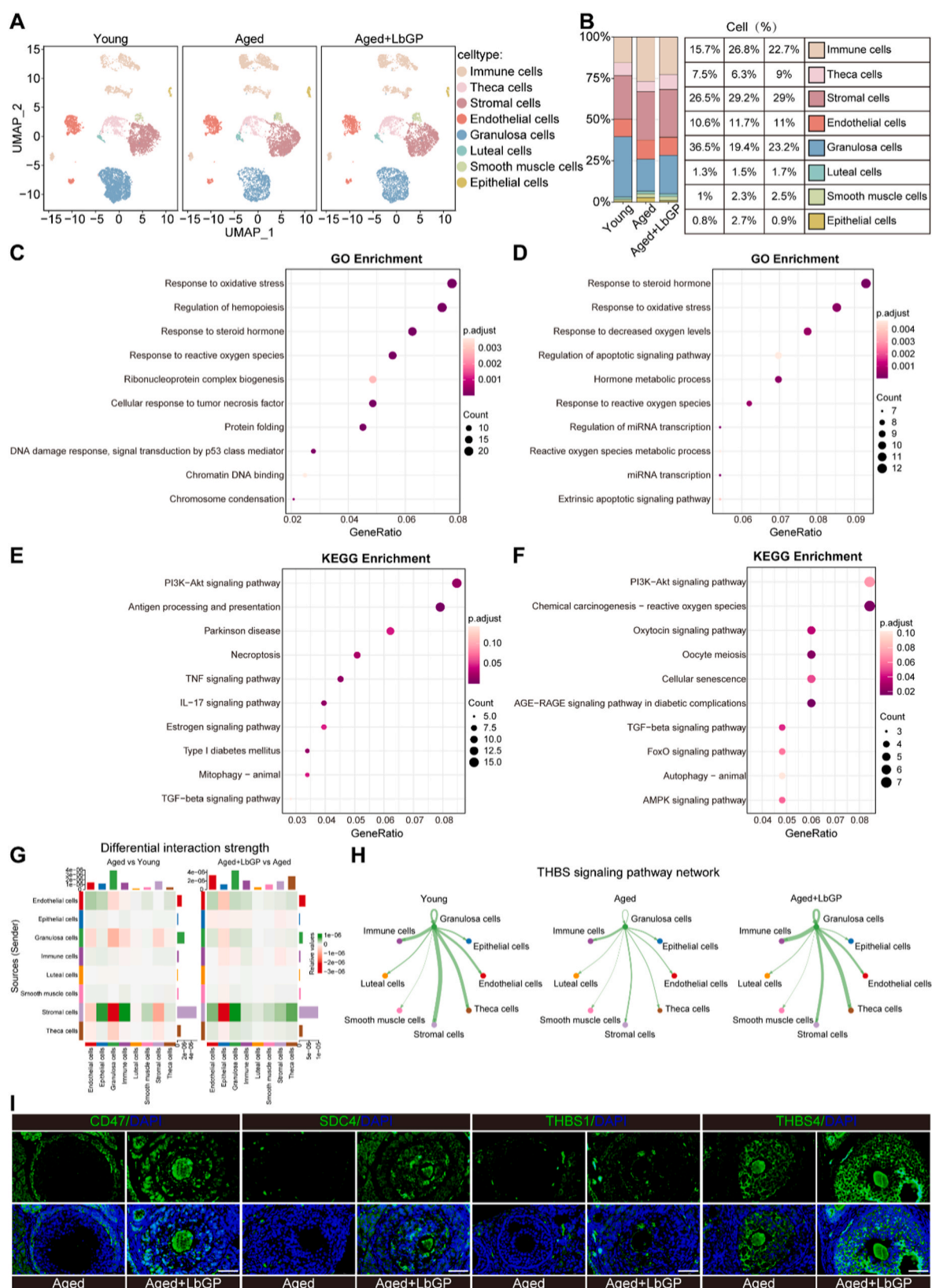


Fig. 6. LbGP remodels cellular composition and molecular networks in aged ovaries. (A) UMAP plots comparing cell type distributions among Young, Aged, and Aged + LbGP groups. (B) Quantification of cell numbers and percentages in each group. (C) GO enrichment analysis of DEGs between Aged and Young groups. (D) GO enrichment analysis of DEGs between Aged + LbGP and Aged groups. (E) KEGG enrichment analysis of DEGs between Aged and Young groups. (F) KEGG enrichment analysis of DEGs between Aged + LbGP and Aged groups. (G) Network analysis of changes in intercellular communication strength among Young, Aged, and Aged + LbGP groups. (H) THBS signaling networks in Young, Aged, and Aged + LbGP groups. (I) Representative immunofluorescence images of ovarian sections showing the expression of CD47, SDC4, THBS1, and THBS4 (green) in the Aged and Aged + LbGP groups. Nuclei were counterstained with DAPI (blue). Scale bar, 50 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and SDC4. Consistent with the cell–cell communication analysis, the spatial distribution and staining patterns of these THBS-related molecules differed between aged and LbGP-treated ovaries, supporting an LbGP-associated remodeling of THBS-related signaling within the ovarian microenvironment (Fig. 6I).

Collectively, these findings demonstrate that LbGP ameliorates the aged ovarian microenvironment through a multidimensional strategy: by rebalancing cellular composition, suppressing aging-associated detrimental pathways, activating protective signaling cascades, and restoring intercellular communication networks, thereby providing a healthier niche for oocyte development.

4. Discussion

A central challenge of female reproductive aging lies in the depletion of oocyte quantity and the deterioration of oocyte quality, the latter directly contributing to infertility, increased miscarriage, and higher risk of congenital defects in advanced maternal age (Broekmans et al., 2009; Navot et al., 1991). Thus, the development of safe and effective strategies to delay or reverse oocyte aging holds substantial clinical importance. In this study, we systematically demonstrate that the naturally derived *Lycium barbarum* glycopeptide (LbGP) markedly improves ovarian function in aged mice, primarily through a dual mode of action: directly repairing mitochondrial function and metabolic networks within oocytes, correcting oocyte-intrinsic defects in mitochondrial function and metabolism, and systemically remodeling and optimizing the ovarian microenvironment that sustains them. Importantly, these two layers are not mutually exclusive; rather, our data support a coordinated “inside–out” mode of action in which intrinsic oocyte repair and niche remodeling may reinforce each other.

At the oocyte level, we first confirmed that LbGP effectively ameliorates multiple hallmarks of aging. The size of the follicular pool is a critical determinant of ovarian lifespan and female reproductive capacity (Delcour et al., 2019). We found that LbGP not only increased follicle numbers across developmental stages and reduced apoptosis to protect ovarian reserve, but also significantly enhanced the maturation competence and genomic stability of aged oocytes. Given that oocyte quality is the cornerstone of subsequent embryonic development, the ability of LbGP to reduce DNA damage and promote meiotic progression provides a mechanistic explanation for the higher fertilization rates and improved blastocyst quality observed, particularly in the balance of inner cell mass (ICM) and trophectoderm (TE) cells. Notably, the apparent preservation of follicle numbers is more plausibly explained by reduced atresia/attrition rather than de novo follicle renewal, consistent with the concept that interventions can slow the depletion of the existing follicular pool.

Mechanistically, mitochondrial remodeling emerged as the central process underlying these effects. Our proteomic data provide a direct molecular blueprint for these oocyte-intrinsic improvements. As the “powerhouse” of the cell, mitochondria are indispensable for oocyte quality, and their decline is a central driver of oocyte aging, leading to energy insufficiency and oxidative stress (Guo et al., 2023; Miwa et al., 2022). Our results demonstrate that LbGP restores mitochondrial membrane potential, enhances mitochondrial activity, and scavenges excessive ROS, thereby directly improving the intracellular state of oocytes. Proteomic analysis further supported this conclusion by revealing LbGP-mediated activation of critical metabolic pathways, including PPAR and insulin signaling, and the enrichment of proteins linked to the “mitochondrial inner membrane” and “energy generation.” The PPAR pathway is a central regulator of lipid metabolism and mitochondrial biogenesis (Chen et al., 2023), while insulin signaling is essential for glucose metabolism (Saltiel and Kahn, 2001). We therefore propose that LbGP rejuvenates aged oocytes by activating these pathways to remodel their metabolic network, partially restoring energy supply and homeostasis. Given that mitochondrial dysfunction and ROS accumulation can amplify DNA damage and meiotic errors, the restoration of PPAR-linked

metabolic homeostasis provides a mechanistic bridge between improved mitochondrial “fitness” and the observed gains in oocyte genome integrity and developmental competence.

Beyond oocyte-intrinsic repair, our single-cell transcriptomic analysis uncovered a second major layer of LbGP action: “ecological restoration” of the ovarian microenvironment. This analysis provides compelling evidence for the extrinsic arm of LbGP's dual mechanism. Aging drastically disrupted ovarian cellular composition, with a sharp loss of granulosa cells that support oocyte development and a marked increase in pro-inflammatory immune cells, consistent with the phenotype of ovarian “inflammaging” (Ben Yaakov et al., 2023; Huang et al., 2019). LbGP effectively reversed this imbalance, recalibrating cell proportions to a more youthful profile that favors follicle survival. On the molecular level, LbGP suppressed aging-associated pathways enriched in oxidative stress, inflammatory signaling such as TNF and IL-17, and apoptosis, while activating protective pathways including AMPK signaling and autophagy (Cairns et al., 2000; Zhang and Shen, 2025). Notably, we also found that LbGP repaired disrupted intercellular communication networks in aged ovaries. For example, the THBS-mediated signaling network was substantially weakened by aging but was restored in strength and connectivity by LbGP, rebuilding granulosa cell-centered interactions that are essential for coordinated ovarian function. We speculate that such ECM/niche communication (including THBS-associated signaling) may contribute to oocyte metabolic recovery by stabilizing the follicular microenvironment, mitigating inflammatory/oxidative stress, and supporting granulosa-cell functions that provide metabolic substrates and redox buffering to the enclosed oocyte. In this view, niche remodeling could create a permissive extrinsic context that may facilitate the engagement or effectiveness of oocyte-intrinsic metabolic programs (e.g., PPAR-linked lipid utilization and mitochondrial biogenesis). Conversely, improved oocyte mitochondrial function may reduce local stress signals and the demand for compensatory inflammatory responses, thereby promoting a feed-forward normalization of the niche. Although our current datasets are correlative and do not prove causality, this bidirectional crosstalk model provides an integrated framework to interpret the proteomic and single-cell findings. Future studies combining pathway perturbation (e.g., pharmacological inhibition of PPAR signaling or blockade of key THBS receptor axes) will be needed to test the directionality and causality of this interaction.

In summary, our findings provide compelling evidence that LbGP, a traditional and safe natural bioactive compound (Dai et al., 2023; Wu et al., 2025), can effectively counteract reproductive aging. Its unique strength lies in its multidimensional and systemic mode of action: acting internally within oocytes by repairing the mitochondrial engine via PPAR and metabolic pathways, and externally within ovarian tissue by rebalancing cellular composition, suppressing inflammation, and reconstructing intercellular communication. Together, these data support a unified model in which LbGP promotes oocyte rejuvenation through coordinated intrinsic metabolic restoration and extrinsic niche remodeling, with potential mechanistic coupling between THBS/ECM signaling and PPAR-linked mitochondrial recovery. This dual “inside–out” mechanism underscores the strong potential of LbGP in reversing the decline of oocyte quality in aged females. Although this work was conducted in mice, future studies in models closer to humans are warranted.

Nevertheless, our results provide new insights into ovarian aging and identify LbGP as a promising natural agent for supporting fertility in advanced maternal age. While our histological and in vivo analyses yielded statistically significant outcomes, we acknowledge that the sample size was limited. Although this sample size aligns with standard practices in oocyte proteomics and single-cell ovarian profiling, future studies incorporating larger cohorts will be essential to further validate and strengthen these findings.

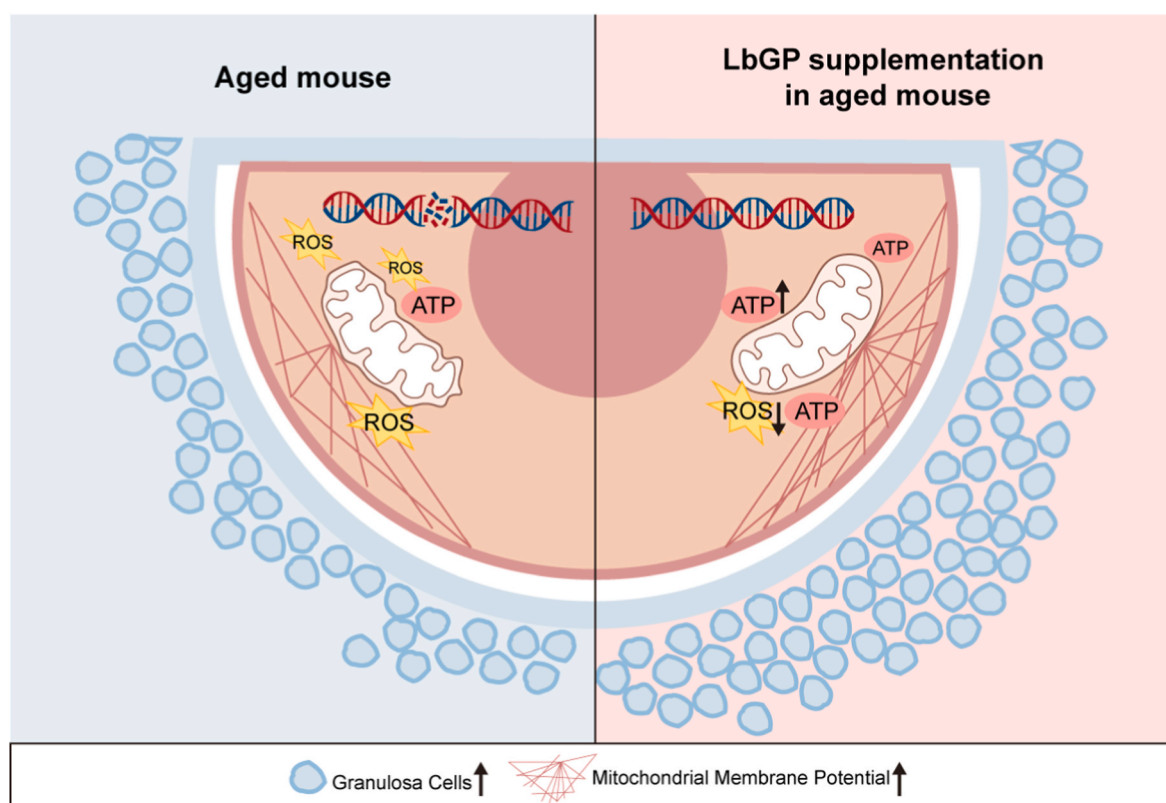


Fig. 7. Schematic model of the mechanism by which LbGP improves the quality of aged oocytes. The left panel illustrates the state of ovarian aging, characterized by oocyte mitochondrial dysfunction (leading to elevated reactive oxygen species (ROS) and reduced ATP production) and an adverse ovarian microenvironment (with decreased granulosa cells). The right panel shows the restorative effects following LbGP supplementation: LbGP not only enhances mitochondrial activity and function but also remodels the ovarian microenvironment into a healthier state. This dual restorative action on both the oocyte and its microenvironment collectively promotes an improvement in the quality of aged oocytes.

5. Conclusion

In conclusion, our study demonstrates that *Lycium barbarum* glycopeptide (LbGP) effectively counteracts the detrimental effects of reproductive aging on ovarian function and oocyte quality in mice. We reveal a novel dual mechanism of action whereby LbGP not only directly rejuvenates the oocyte by restoring mitochondrial function and correcting metabolic defects through pathways such as PPAR signaling, but also systemically remodels the aged ovarian microenvironment. This remodeling includes rebalancing somatic cell populations, suppressing chronic inflammation, and repairing essential intercellular communication networks. These findings establish LbGP as a potent, multi-target agent that addresses both oocyte-intrinsic defects and extrinsic microenvironmental decline (Fig. 7). These findings provide a solid scientific foundation for the potential application of LbGP as a safe, natural approach to supporting reproductive health in women of advanced maternal age.

CRediT authorship contribution statement

Aolei Guo: Writing – original draft, Resources, Methodology, Investigation, Data curation. **Ruixin Shi:** Writing – original draft, Resources, Methodology, Investigation, Data curation. **Sipei Liu:** Methodology, Investigation, Data curation. **Yang Zhang:** Data curation. **Yan Mao:** Funding acquisition. **Guijun Yan:** Writing – review & editing, Validation. **Guangyi Cao:** Writing – review & editing, Funding acquisition.

Ethics approval and consent to participate

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Nanjing Drum Tower Hospital (2025AE01013).

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2026.121318>.

Abbreviations

LbGP	<i>Lycium barbarum</i> glycopeptide
PBS	Phosphate-buffered saline
IVF	In vitro fertilization
ROS	Reactive oxygen species
PPAR	Peroxisome Proliferator-Activated Receptor
ATP	Adenosine triphosphate
NMN	Nicotinamide mononucleotide
MII	Metaphase II
PMSG	Pregnant mare serum gonadotropin
hCG	Human chorionic gonadotropin
GV	Germinal vesicle
GCs	granulosa cells
IVM	In vitro maturation
COCs	Cumulus-Oocyte Complexes
PFA	paraformaldehyde
FBS	Fetal Bovine Serum
PBST	Phosphate-Buffered Saline with Tween 20
DTT	Dithiothreitol
IAA	Iodoacetamide
H&E	Hematoxylin and eosin
TE	trophectoderm
ICM	inner cell mass
CDX2	Caudal-type homeobox 2
OCT4	Octamer-binding transcription factor 4
PCA	Principal component analysis
GSEA	Gene Set Enrichment Analysis
UMAP	Uniform Manifold Approximation and Projection
GO	Gene Ontology
ANOVA	Analysis of variance
AMPK	AMP-activated Protein Kinase
THBS	Thrombospondin
DAPI	4',6-diamidino-2-phenylindole

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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