Zhou *et al. Cell Communication and Signaling* (2025) 23:150
https://doi.org/10.1186/s12964-025-02137-8

Cell Communication and Signaling

RESEARCH Open Access

BDNF secreted by mesenchymal stem cells improves aged oocyte quality and development potential by activating the ERK1/2 pathway

Jing Zhou[1,2,3,4,5,6,7,8†], Cheng Li[1,2,3,4,5,6,7†], Xin Mi[1,2,3,4,5,6,7], Jiali Zhou[1,2,3,4,5,6,7], Caiyi Chen[1,2,3,4,5,6,7], Jiayi Ren[1,2,3,4,5,6,7], Ran Liu[1,2,3,4,5,6,7], Gang Liu[9,10], Zi-Jiang Chen[1,2,3,4,5,6,7,11,12], Yingying Qin[1,2,3,4,5,6,7*], Keliang Wu[1,2,3,4,5,6,7*] and Shidou Zhao[1,2,3,4,5,6,7*]

Abstract

Background Reduced oocyte quality is a key factor in age-related fertility decline, and there are no effective treatments available. The secretome of mesenchymal stem cells (MSC-sec) contains various bioactive factors and has the potential to improve oocyte quality. This study aimed to investigate the effective component and molecular mechanism of MSC-sec involved in improving oocyte quality from aged mice and humans.

Methods Immunofluorescence and chromosome spread were performed to investigate the effects of secretome from human umbilical cord-MSC on spindle assembly and aneuploidy in aged mouse oocytes. Brain-derived neurotrophic factor (BDNF) and its neutralization antibody was supplemented in both in vitro and in vivo experiments to verify the effective component in MSC-sec. RNA-seq analysis was used to reveal the alterations in maternal mRNA degradation in aged mouse oocytes after MSC-sec treatment. In vitro culture of oocytes from aged women was also used to verify the effectiveness of BDNF in improving oocyte quality.

Results MSC-sec treatment significantly increased first polar body emission, improved spindle assembly, promoted maternal RNA degradation, and reduced aneuploidy rate in aged mouse oocytes. While the addition of BDNF neutralization antibody blocked the effects of MSC-sec, BDNF alone also increased the oocyte quality from aged mice. Mechanistically, both MSC-sec and BDNF rescued the quality of aged mouse oocytes by activating the ERK1/2 signaling pathway to increase the expression of DAZL and BTG4. In situ injection of MSC-sec or BDNF into aged

[†]Jing Zhou and Cheng Li contributed equally to this work.

*Correspondence:
Yingying Qin
qinyingying1006@163.com
Keliang Wu
wukeliang_527@163.com
Shidou Zhao
shidouzhao@sdu.edu.cn

Full list of author information is available at the end of the article

mouse ovaries significantly improved oocyte quality and early embryonic development. Finally, we demonstrated that BDNF treatment increased both the fertilization rate and blastocyst formation rate of aged human oocytes.

Conclusion These findings demonstrate that BDNF secreted by mesenchymal stem cells can improve the quality and development potential of oocytes from both aged mice and humans by activating the ERK1/2 signaling pathway, suggesting that it has the potential to mitigate age-related declines in oocyte quality.

Keywords Mesenchymal stem cells, Oocyte quality, BDNF, ERK1/2 pathway, Aneuploidy

Introduction

Female fertility decreases with age, and this can be attributed to both reduced oocyte quantity and decreased oocyte quality [1]. Increasing evidence shows that the decline in oocyte quality is largely due to aneuploidy arising during meiosis I [2, 3]. Recently, it was reported that insufficient maternal mRNA degradation might also reduce the developmental competence in aged human and mouse oocytes [4]. Supplementation with various antioxidants or metabolites which are reduced in aged ovary, have been found to enhance oocyte quality [5–8]. However, there is currently no effective clinical approach to addressing the decline in oocyte quality caused by aging.

Mesenchymal stem cells (MSCs) possess distinct advantages that make them promising contenders for addressing a range of refractory diseases, including premature ovarian insufficiency and ovarian aging [9]. Emerging research has demonstrated that MSCs mainly act via paracrine mechanisms to improve ovarian function. The secretome of MSCs (MSC-sec) contains various bioactive factors that have the potential to inhibit cell apoptosis, stimulate cell proliferation and angiogenesis, and alleviate oxidation and fibrosis [10]. We previously found that human umbilical cord-MSCs (hUC-MSCs) enhanced primordial follicle survival and activation by secreting hepatocyte growth factor (HGF) to activate the PI3K-AKT signaling pathway [11, 12]. It has also been reported that MSC-sec improves human oocyte maturation and development [13]. Nevertheless, the precise mechanisms through which MSC-sec improves oocyte quality remain poorly understood, and further investigation is required to identify the active components.

It is well known that the extracellular signal-related kinase (ERK1/2) signaling pathway plays a pivotal role in governing oocyte meiotic maturation [14], and disruption of the ERK1/2 activity in mouse oocytes results in significant impairment of both spindle assembly and maternal mRNA degradation [15]. Furthermore, oocytes derived from older mice exhibit decreased ERK1/2 activity in comparison to oocytes from younger mice [16]. Consistent with a previous study [17], we also found that MSC-sec is enriched in factors that activate the ERK1/2 pathway [11]. Therefore, MSC-sec might improve aged oocyte quality by activating the ERK1/2 signaling pathway.

In this study, we determined the therapeutic effects of MSC-sec on aged oocytes and identified Brain-derived neurotrophic factor (BDNF) as the effective factor. Mechanistically, MSC-sec and its effective factor BDNF activated the ERK1/2 pathway to promote spindle assembly and maternal mRNA decay, thus providing the basis for the clinical use of BDNF to improve the quality of aged oocytes.

Materials and methods

Experimental animals

The 2- and 9-month-old female ICR mice were obtained from Beijing Vital River Laboratory Animal Technology Co. Ltd. All mice lived in a temperature-controlled facility with a 12-h light/dark cycle and free food and drink. The animal experiments were conducted according to the guidelines of Animal Care and Research Committee of Shandong University.

Cell culture

hUC-MSCs and human fibroblasts were cultured in α-minimum essential medium (Gibco, 523315) or minimum essential medium (Gibco, 11095080) with 5% ultra-advanced GRO (Helios) and 1% penicillin/streptomycin until confluency. All procedures were aseptic. The isolation and characterization of hUC-MSCs were described in our previous studies [11, 12].

Preparation of hUC-MSC-sec and Fib-sec

hUC-MSCs and fibroblasts between passages 6–9 were seeded separately in 75 cm^2 cell culture flasks (Corning, 430641) and cultivated to 90% confluency. After three washes with PBS, M16 medium (Sigma, M7292) was added for 48 h. Then, the supernatant was collected, centrifuged at 1500 × *g* for 5 min at room temperature, and filtered through a 0.22 μm filter. Then, the secretome of hUC-MSCs (hUC-MSC-sec) and fibroblasts (Fib-sec) were stored at − 80 °C or used for subsequent experiments. For ovarian in situ injection, hUC-MSC-sec was collected using DMEM/F12 (Gibco, C11330500BT) media and concentrated 20-fold using ultra-filtration centrifuge tubes (Millipore, UFC900308) with a molecular weight cut-off of 3 KDa at 6000 × *g* for 40–50 min.

In vitro oocyte maturation

Young and aged mice were injected intraperitoneally with 10 IU of pregnant mare serum gonadotropin (PMSG, Ningbo Sansheng Biological Technology Company, 110044564), to stimulate follicle development. After 46–48 h, germinal vesicle (GV)-stage oocytes were collected and cultivated in mineral oil-covered M16 medium (Sigma, M7292), or with addition of hUC-MSC-sec, Fib-sec, hUC-MSC-sec plus BDNF neutralizing antibody ($BDNF^{ab}$, HUABIO, ET1606-42,1ug/ml) or BDNF (peprotech, 450-02-10, 250 pg/ml). To measure first polar body (PB1) extrusion, the culture system was incubated at 37 °C with 5% CO_2, 5% O_2, and 90% N_2 for 16–18 h.

Immunofluorescence and confocal microscopy

Immunofluorescence labeling for α-tubulin and DAPI staining were used to examine the spindle shape and chromosomal distribution in MII-stage mouse oocytes. Oocytes were firstly fixed in 4% paraformaldehyde (PFA) (Sigma, P-6148) in PBS at room temperature for 30 min and then permeabilized with Triton X-100 (0.5%, Sigma, T8787) for 30 min. The non-specific binding was blocked with 1% bovine serum albumin (BSA, Sigma, B2064) at room temperature for 1 h. Next, oocytes were treated with anti-α-tubulin FITC antibody (1:500 dilution, Sigma, F2168) at room temperature for 2 h. After incubation, the oocytes were washed three times with washing buffer (PBS containing 0.01% Triton X-100 and 0.1% Tween-20) and mounted on glass slides with DAPI-containing mounting media (Abcam, ab104139). Using a Dragonfly confocal laser-scanning microscope (Andor Technology, UK) with a 63× oil objective, a z-axis scanning of stained oocytes was performed.

Chromosome spreads

In vitro matured MII oocytes were treated with acidic M2 media at room temperature to remove the zona pellucida for aneuploidy detection. The oocytes were placed in a drop of 1% PFA, 3 mM dithiothreitol, and 0.15% Triton X-100 on a glass slide after washing in pre-warmed M2 media. After 2 h, the oocytes were kept in a half-open moist box until the fixing solution dried. After three washes with PBS, fixed chromosomes were blocked with 25% donkey serum (Sigma, D9663) at room temperature for 1 h. Subsequently, chromosomes were treated overnight at 4℃ with the centromere antigen CREST (1:200 dilution, Antibodies Incorporated, 15-234-001) and topoisomerase II (TOP2, 1:200 dilution, Abcam, ab109524) antibodies. Following three washes with PBS, the slides were treated with Alexa Fluor 647-conjugated Affinipure donkey anti-human antibody (1:200 dilution, Jackson Immuno Research, 709-605-149) and Alexa Fluor 568-conjugated donkey anti-rabbit antibody (1:500 dilution, Proteintech, A10042) for 1 h at room temperature. The chromosomes were mounted in DAPI-containing medium and examined under a confocal laser-scanning microscope (Dragonfly, Andor Technology, UK).

RNA sequencing

To detect maternal mRNA degradation, young, aged, and aged + MSC-sec-treated MII-stage oocytes were subjected to global RNA-seq analyses. To determine the mRNA expression differences between groups, a fold change of ≥ 2 was used.

Western blot

GV-stage oocytes from young and old mice were cultured in M16 media for 8 h. For total protein extraction, 50 oocytes from each group were lysed in SDS sample buffer with protease and phosphatase inhibitors and boiled for 10 min. Protein was transferred to a polyvinylidene fluoride membrane (Millipore, ISEQ00010) after 10–12% SDS-PAGE gel (Beyotime, P0012A) electrophoresis. The membrane was blocked with 5% non-fat dry milk diluted in PBST for 1 h at room temperature, then incubated with the primary antibody overnight at 4 °C. After that, the membrane was incubated for 1 h at room temperature with IRDye 680RD (Li-Cor, 926-32211), 800CW (Li-Cor, 926-68070), or IgG-HRP goat anti-rabbit antibodies (Beyotime, A0208). Chemiluminescence or LI-COR Odyssey CLx9140 (LI-COR Bioscience, USA) was used to visualize immunoreactive bands. The relationship between BDNF and the ERK1/2 signaling pathway was investigated utilizing the MEK1/2 inhibitor U0126 (5 μm). The antibodies used are listed in Supplementary Table 1.

Ovarian in situ injection and in vitro fertilization of mouse oocytes

Twenty 9-month-old female mice subjected to ovarian injection were randomly assigned to four groups: hUC-MSC-Sec (15-fold concentrated), hUC-MSC-sec + $BDNF^{ab}$ (1 μg/ml), BDNF (3.75 ng/ml), and PBS control. Autocrosslinked hyaluronan gel (0.3 mg/ml, Bioregen, 6952181200332) as a medication carrier was used in each group according to our previous study [11]. After anesthesia with tribromoethanol (Sigma, T48402-5G) injections, the mice were operated to expose ovaries gently through dorsal incisions. After injection of 10 μl solution with the chemicals into each ovary using a microsyringe, the surgical wounds were closed. The mice were housed under controlled conditions for 7 days before superovulation.

At 48 h after PMSG injection, 10 IU Chorionic Gonadotrophin for Injection (hCG) (Ningbo Sansheng Biological Technology Company, 110041282) was injected intraperitoneally to induce superovulation. After 16 h, the cumulus-oocyte complexes were collected from

oviductal ampullae, which were denudated for the PB1 extrusion rate or fertilized with sperm from adult ICR male mice in G-IVF medium (Vitrolife, 10136). To observe embryonic development, the zygotes were cultivated in small drops of G1 medium (Vitrolife, 10128) covered in mineral oil under the same circumstances as in vitro oocyte maturation.

Enzyme-linked immunoassay (ELISA)

BDNF protein levels in MSC-sec and Fib-sec were quantified using a commercial ELISA kit (Cusabio, CSB-E04501h) following the manufacturer's instructions. The 96-well plates were analyzed using a microplate reader at 450 nm in accordance with the manufacturer's protocol, and sample concentrations were quantified by comparison with standard concentration curves.

Human oocyte culture with BDNF and embryo development

Immature (GV or germinal vesicle breakdown (GVBD)) human oocytes were donated by patients undergoing intracytoplasmic sperm injection (ICSI) treatments. These discarded oocytes from 42 IVF patients aged 35–44 were randomly divided into two groups. One group received 0.25 ng/ml BDNF treatment, while the other did not, under conditions of 6% CO_2, 5% O_2 at 37 °C for 24 h during in vitro maturation (IVM). Supplementary Table 2 shows basic information of the patients. G-IVF PLUS medium (IVM medium) with 0.29 mmol/L sodium pyruvate (Sigma, S8636) was equilibrated overnight. On oocyte pick-up day, the medium was supplemented with 0.75 IU/mL recombinant follicle-stimulating hormone (Merck Serono, S20160040), 10–11 mol/L melatonin (Sigma, M5250), 10 ng/mL epidermal growth factor (STEMCELL Technologies, 78136.2), and 0.75 IU/mL luteinizing hormone (Merck Serono, S20181004). Next, MII-stage oocytes were counted, using the first polar body emission as a nuclear maturation marker. ICSI was then utilized to inseminate MII oocytes under an inverted microscope (Nikon, Japan). The zygotes were sequentially grown in G1 PLUS (Vitrolife, 10128) and G2 PLUS medium (Vitrolife, 10132) at the above conditions. Photomicrography was used to record the embryo development. Gardner's criteria were used to assess blastocyst stage at 16–18 h and 5–6 days after fertilization. The Institutional Review Board of Reproductive Medicine of Shandong University approved the study, and all participants gave written informed consent.

Statistical analyses

The data were analyzed using SPSS 26.0 software and reported as mean ± standard deviation (SD). Student's t-test, Chi-square test or one-way ANOVA followed by Tukey's test was used to determine statistical significance between two groups when appropriate. The difference was considered to be statistically significant when $P < 0.05$.

Results

MSC-sec improved the quality of oocytes from aged mice

We initially examined in vitro maturation of oocytes from young (2 months) and reproductively aged (9 months) female mice. hUC-MSC-sec or Fib-sec was added into the culture medium of aged oocytes. Fib-sec treatment was used as a control due to the significant differences observed in the contents between hUC-MSC-sec and Fib-sec in our previous study [11]. The rate of emission of PB1 was found to be lower in aged oocytes compared to young oocytes, and it was significantly increased when adding hUC-MSC-sec to IVM medium at a 1:1 ratio. However, there was no significant change when supplementing with Fib-sec (Fig. 1A-B). Therefore, the Fib-sec treatment group was removed in the following experiments. Elongated spindles, pole absence, and chromosomal misalignment were identified as spindle abnormalities in aged oocytes. Treatment with hUC-MSC-sec greatly reduced spindle abnormalities (Fig. 1C-D). Furthermore, aneuploidy was greater in older oocytes than young ones, however hUC-MSC-sec supplementation attenuated this trend (Fig. 1E-F). These findings imply that MSC-sec therapy improves the quality of aged mouse oocytes.

BDNF mediated the function of MSC-sec in improving oocyte quality

Next, we explored the effective factor in hUC-MSC-sec that improved aged oocyte quality. MSC-sec contains a variety of cytokines, chemokines, and growth factors [18], and cytokines enriched in hUC-MSC-sec compared with Fib-sec were analyzed by a cytokine array in our previous study [11]. Specifically, 47 cytokines related to the ERK1/2 pathway exhibited a fold change equal to or exceeding 2 in hUC-MSC-sec (Supplementary Table 3). Four cytokines – HGF, G-CSF, BDNF, ICAM-1 – were chosen based on their high content and significantly differential expression between hUC-MSC-sec and Fib-sec. While HGF, G-CSF, and ICAM-1 promoted oocyte maturation by exerting their effects on cumulus cells [19–21], brain-derived neurotrophic factor (BDNF) was considered to be the most potential effective component. BDNF is a neurotrophic factor secreted by granulosa and cumulus cells in the ovary, and its expression is decreased in follicles derived from elderly females [22, 23]. Meanwhile, the BDNF receptor TrkB is predominantly expressed in oocytes within the ovary [24], and the interaction between BDNF and TrkB triggers the activation of the ERK1/2 pathway [25]. Furthermore, we demonstrated by

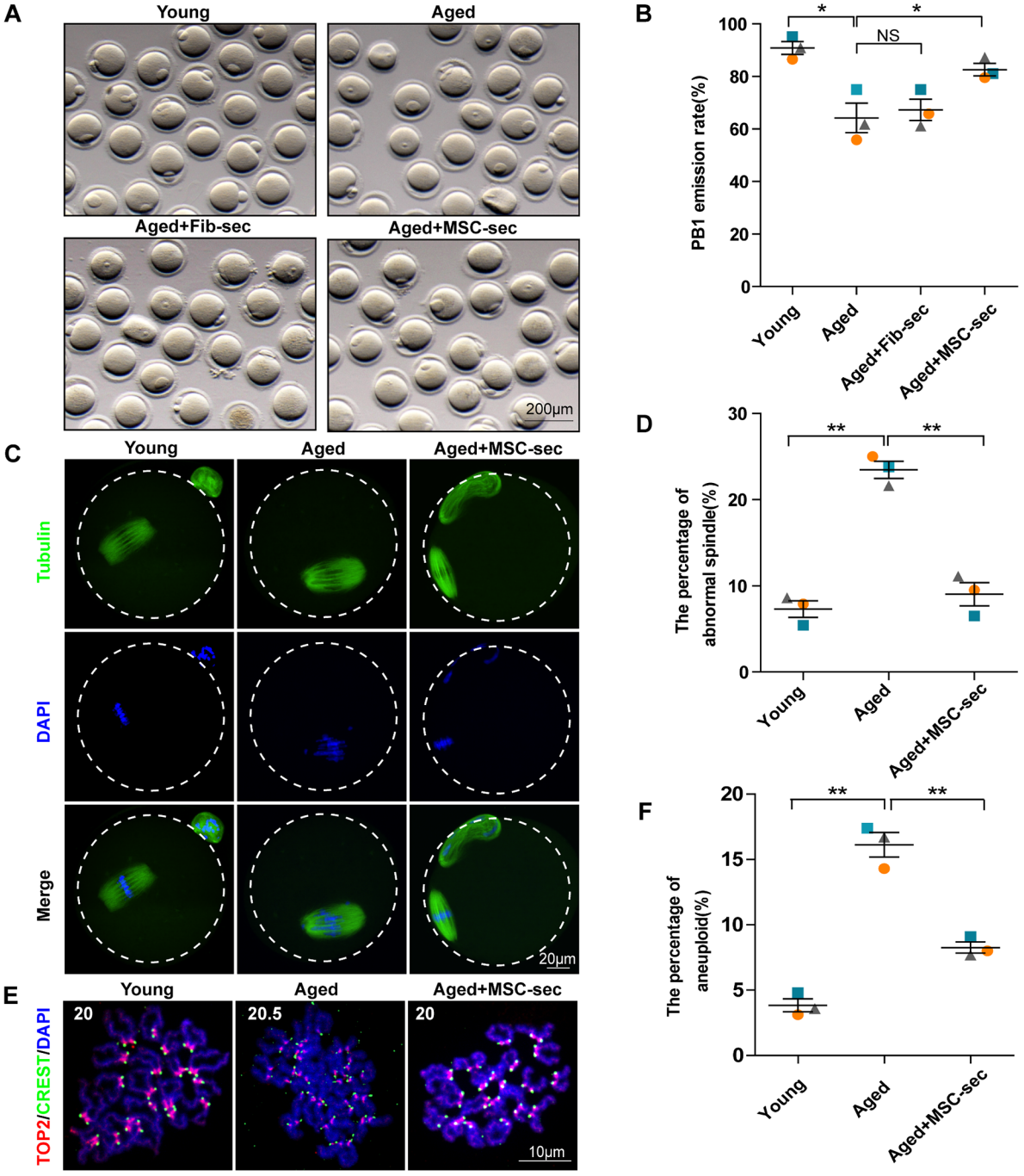


Fig. 1 MSC-sec improved the quality of oocytes from aged mice. **A** Bright-field images of young oocytes, aged oocytes, MSC-sec–treated aged oocytes, and Fib-sec–treated aged oocytes. Scale bars: 200 μm. **B** Maturation rates after in vitro culture. The emission rate of PB1 was found to be significantly higher in MSC-sec–treated aged oocytes in comparison with aged oocytes, but it could not be increased by Fib-sec. **C** Characteristic pictures of MII oocytes with DNA (blue) and labeled spindles (green). Scale bars: 20 μm. **D** Proportions of oocytes exhibiting abnormal spindles. The abnormal spindle incidence was decreased in MSC-sec–treated aged oocytes relative to aged oocytes. **E** Characteristic chromosomal spread pictures of young oocytes, aged oocytes, and aged oocytes treated with MSC-sec were analyzed for the presence of topoisomerase II (TOP2) and the centromere antigen CREST, which indicate the chromosome arms and centromeres, respectively. The upper left corner displays the number of paired sister chromatids. Scale bars: 10 μm. **F** Aneuploidy rates of MII oocytes. The aneuploidy incidence was decreased in MSC-sec–treated aged oocytes relative to aged oocytes. Data information: Statistical analysis was performed using one-way ANOVA followed by Tukey's test, with data presented as the mean ± SD in **B**, **D**, and **F**. Each point in **B**, **D**, and **F**, distinguished by unique colors and shapes, represents results from three independent experiments. Significance levels were indicated as follows: NS (no significance), $^{*}P < 0.05$, $^{**}P < 0.01$

ELISA assay that the BDNF level in MSC-sec was significantly higher than that in Fib-sec (Fig. 2A).

To determine the role of BDNF, BDNF^{ab} was introduced to the hUC-MSC-sec. BDNF^{ab} significantly inhibited PB1 emission when compared to the hUC-MSC-sec group after 16–18 h of culture (Supplementary Fig. 1A-B). The rate of abnormal spindles in the hUC-MSC-sec with BDNF^{ab} group was significantly higher

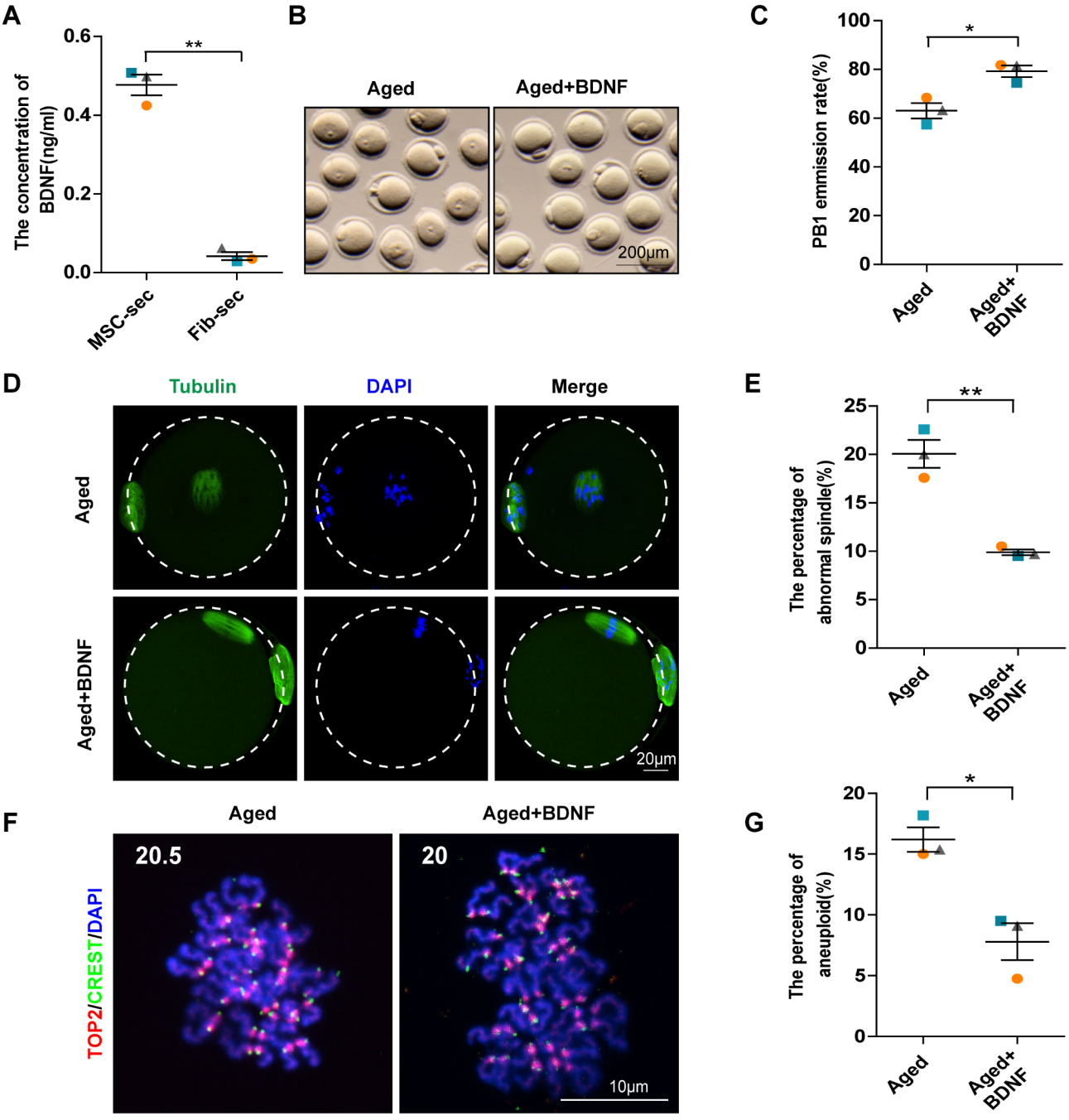


Fig. 2 BDNF mediated the effect of MSC-sec on improving oocyte quality. **A** The concentration of BDNF was significantly higher in MSC-sec than that in Fib-sec by ELISA. **B** Bright-field images of aged oocytes and BDNF-treated aged oocytes. Scale bars: 200 μm. **C** Rates of maturation following in-vitro culture. BDNF alone increased the PB1 emission rate. **D** Characteristic pictures of MII oocytes with DNA (blue) and labeled spindles (green). Scale bars: 20 μm. **E** Proportions of oocytes exhibiting abnormal spindles. The abnormal spindle incidence was decreased in BDNF-treated aged oocytes relative to untreated aged oocytes. **F** Representative chromosome spread images of aged oocytes and BDNF-treated aged oocytes with labeled TOP2 and CREST indicating the chromosome arms and centromeres, respectively. Scale bars: 10 μm. **G** The aneuploidy incidence was decreased in BDNF-treated aged oocytes relative to aged oocytes. Data information: The data are presented as the mean ± SD in **B**, **D**, and **F**, in which each point, distinguished by unique colors and shapes, represents results from three independent experiments. *$P < 0.05$, **$P < 0.01$ by Student's t-test

than that in the hUC-MSC-sec group (Supplementary Fig. 1C-D). In addition, we used exogenous BDNF alone to further determine its function. The BDNF concentration used (250 pg/ml) was similar to its concentration in follicular fluid from in-vitro fertilization (IVF) patients (~240 pg/ml) [26]. BDNF treatment increased the PB1 emission rate and reduced abnormal spindles and aneuploidy in aged oocytes (Fig. 2B-G). In contrast, the addition of K252a, which is an inhibitor of the BDNF receptor TrkB, to hUC-MSC-sec significantly blocked its effects (Supplementary Fig. 2), and this was in line with the results in the hUC-MSC-sec with $BDNF^{ab}$ group (Supplementary Fig. 1). Collectively, these results suggest that BDNF is the main effective factor of hUC-MSC-sec for improving the quality of aged oocytes.

MSC-sec promoted maternal mRNA decay

To detect the degradation of maternal mRNA, we performed RNA-seq and found that there were 303 transcripts with increased expression in aged oocytes (fold change ≥ 2) but with decreased expression after MSC-sec treatment (fold change ≤ 0.5) (Supplementary Table 4, Supplementary Fig. 3A). After overlapping with the results from the abnormal degradation of maternal mRNAs from the GV to MII-stage after knockout of the *Btg4* gene [27], a total of 32 transcripts were found (Supplementary Table 5, Supplementary Fig. 3B). These data suggest that MSC-sec can facilitate maternal mRNA decay.

MSC-sec and BDNF activated the ERK1/2 signaling pathway

To determine the potential of hUC-MSC-sec to activate the ERK1/2 pathway, which regulates both spindle assembly and maternal mRNA degradation [15], the expression of phosphorylated ERK1/2 (p-ERK1/2) was detected in oocytes. After 8 h of in vitro culture, p-ERK1/2 was lower in the aged oocytes when compared to the young oocytes, but it was elevated upon supplementation with hUC-MSC-sec (Fig. 3A-B). We then measured the expression of DAZL and BTG4, which are downstream target genes of the ERK1/2 pathway and regulate the assembly of the meiotic spindle and maternal mRNA degradation, respectively [15]. As expected, we detected a reduction in the expression of these genes in aged oocytes compared to young ones, but an increase after treatment with hUC-MSC-sec (Fig. 3C-F). We next demonstrated that BDNF alone also activated the ERK1/2 signaling pathway (Fig. 3G-H). Furthermore, the MEK1/2 inhibitor U0126 was found to block the increase in DAZL and BTG4 expression induced by BDNF (Fig. 3I-L). These results suggest that BDNF improves oocyte quality by activating the ERK1/2 signaling pathway.

Both MSC-sec and BDNF intraovarian injection enhanced the quality and development potential of aged mouse oocytes

To determine the impact of MSC-sec and BDNF on oocyte quality in vivo, injections of MSC-sec, MSC-sec + $BDNF^{ab}$, or BDNF were administered into aged mouse ovaries. The results showed that PB1 emission rates were higher in the MSC-sec group and BDNF group in comparison to the control group, but the rate of PB1 emission was decreased in the MSC-sec with $BDNF^{ab}$ group (Fig. 4A, B). The results of the immunofluorescent staining showed a decrease in the proportion of abnormal spindles in oocytes following the administration of MSC-sec or BDNF compared to the control group (Fig. 4C, D). The neutralization of BDNF in the MSC-sec using $BDNF^{ab}$ resulted in a significantly higher proportion of abnormal spindles (Fig. 4D), thereby confirming the function of BDNF in hUC-MSC-sec in improving aged oocyte quality.

In vitro fertilization was performed to determine the fertilization rate and subsequent development potential of the aged oocytes. The results showed that there was a substantial enhancement in 2 pronuclei (2PN), two-cell embryos and blastocyst formation rates in the MSC-sec and BDNF experimental groups as compared to the control group (Fig. 4E, F, G and H). In MSC-sec treatment group, the rates of 2PN, two-cell embryos and blastocyst formation were increased (97.57%, 97.57% and 81.97%, respectively) compared to the control group (74.37%, 72.53% and 64.83%). The BDNF group also had high rates of 2PN, two-cell embryos and blastocyst formation at 93.9%, 92.73% and 82.27%, respectively. Furthermore, the MSC-sec with $BDNF^{ab}$ group exhibited lower rates of 2PN, two-cell embryos and blastocyst formation (78.33%, 77.47% and 67.47%, respectively) when compared to the MSC-sec group (97.57%, 97.57% and 81.97%). These findings showed that the BDNF present in MSC-sec has a noteworthy impact on the embryonic development potential of aged mouse oocytes.

BDNF increased both the fertilization and blastocyst formation rates of aged human oocytes

Based on the potential of BDNF to enhance the oocyte quality of aged mouse, we subsequently evaluated the effects of BDNF on the maturation and development competence of aged human oocytes in vitro. Although supplementation of BDNF during IVM of human oocytes did not lead to a significant rise in the maturation rate, the combined analysis of oocytes at the stages of GV and GVBD revealed a noteworthy increase in fertilization and blastocyst formation rates (Fig. 5A-C). In the case of GV-stage oocytes, the BDNF group exhibited higher rates of maturation and embryonic development in comparison to the control group, although the disparity did not

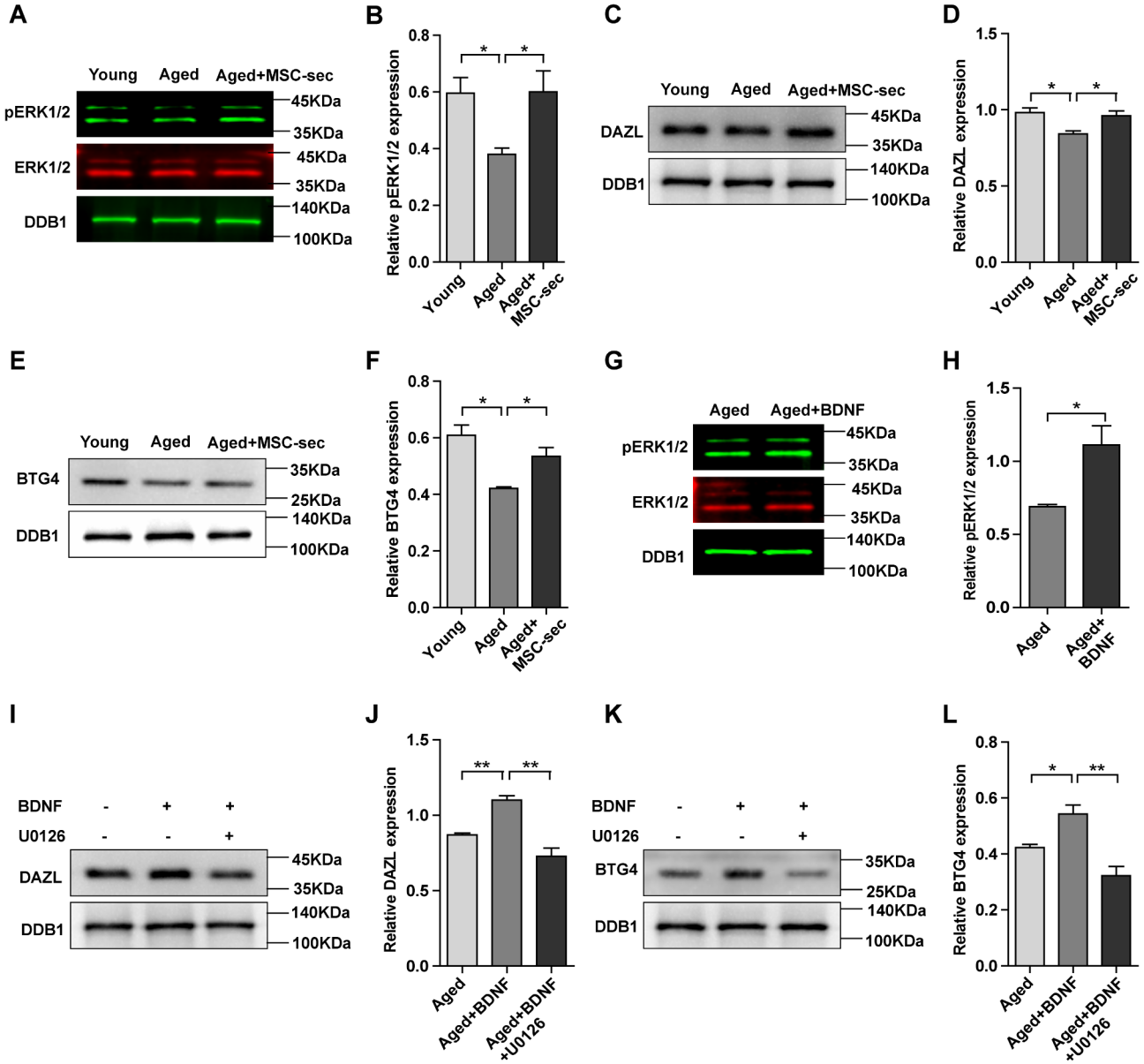


Fig. 3 Both MSC-sec and BDNF activated the ERK1/2 pathway in aged oocytes. **A**, **C**, **E** Western blot demonstrating the expression of p-ERK1/2, DAZL, and BTG4 in aged oocytes cultured in vitro. When comparing the MSC-sec-treated group to the aged group, there was a significant increase in the expression of p-ERK1/2, DAZL, and BTG4. **B**, **D**, **F** Immunoblot densitometry graphs to quantitatively compare p-ERK1/2, DAZL, and BTG4. **G**, **I**, **K** In aged oocytes, BDNF upregulated the expression of p-ERK1/2, DAZL, and BTG4. U0126, an inhibitor of MEK1/2 that inhibits the ERK1/2 signaling pathway, blocked the increase in DAZL and BTG4 expression caused by BDNF treatment. **H**, **J**, **L** Immunoblot densitometry graphs to quantitatively compare p-ERK1/2, DAZL, and BTG4. Data information: The data are presented as the mean ± SD in **B**, **D**, **F**, **H**, **J**, and **L**. Tukey's test was used to assess differences between two groups after one-way ANOVA or Student's t-tests were performed. *$P < 0.05$, **$P < 0.01$. Three replicate experiments' results are shown

achieve statistical significance (Supplementary Table 6). Similarly, GVBD-stage oocytes also showed significantly higher fertilization rates in the BDNF group than in the control group (Supplementary Table 6). The observation of only two high-quality blastocysts in cultured embryos treated with BDNF, attributed to the limited sample size, also indicates the potential of BDNF to improve the quality of aged oocytes and early embryonic development in humans.

Discussion

Although aging affects nearly every aspect of female reproduction, reduced oocyte quality is considered to be a major contributor of reproductive decline [16]. However, there are no effective treatment modalities that specifically target the decline in oocyte quality associated with aging. There is a growing body of evidence suggesting that stem cell-based therapy has the potential to mitigate ovarian damage and ameliorate age-related

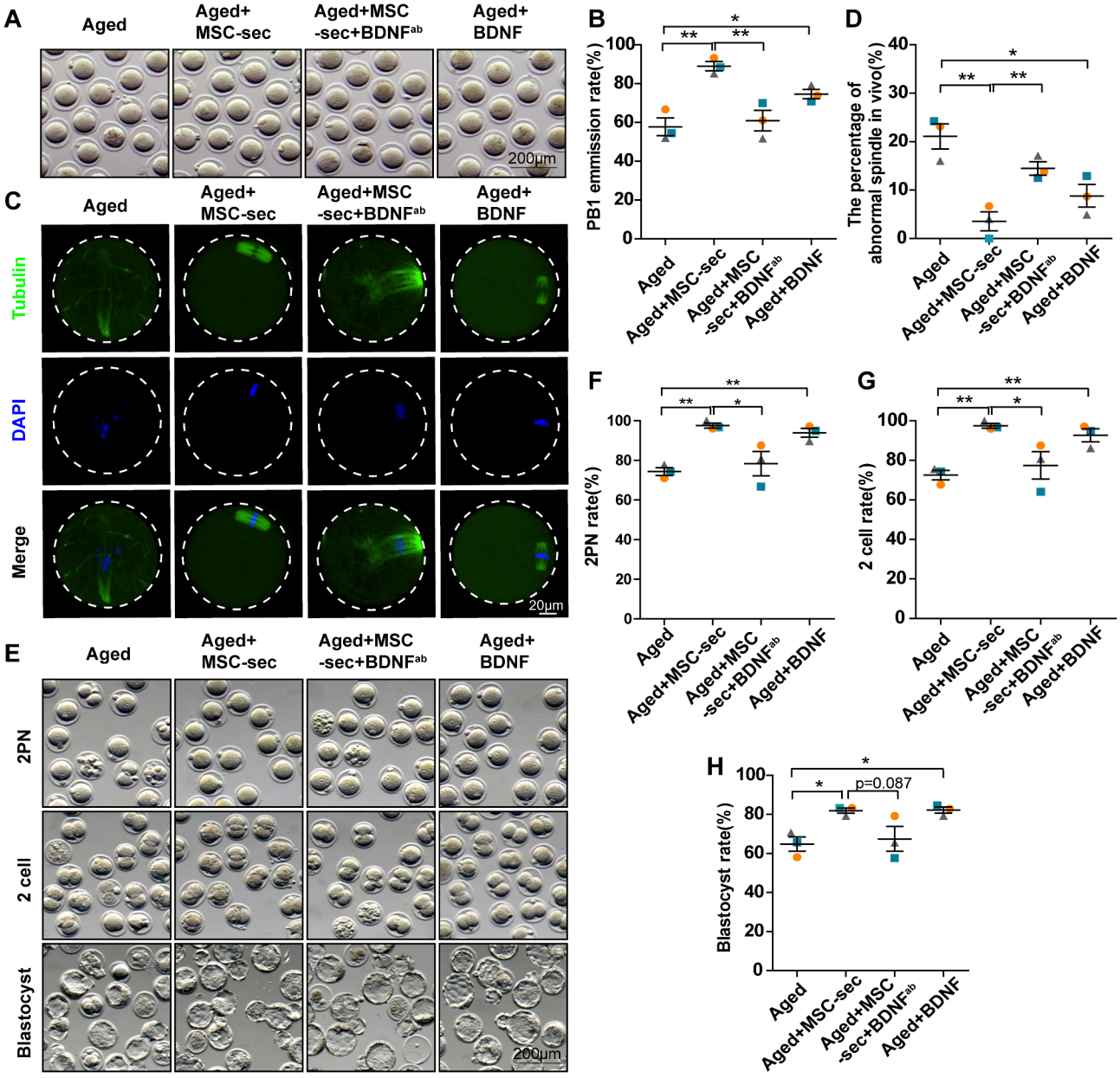
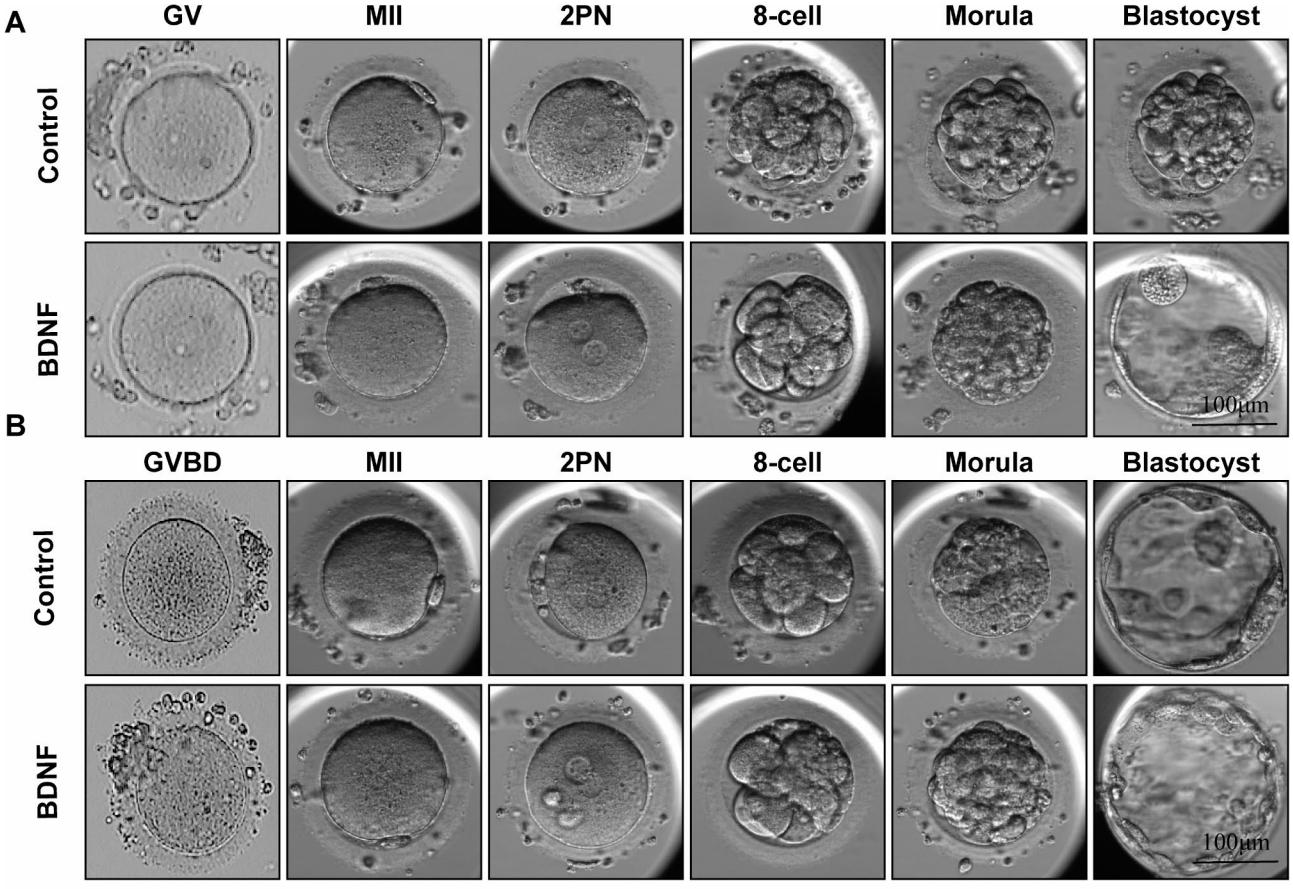


Fig. 4 Both MSC-sec and BDNF intraovarian injection enhanced the quality of aged mouse oocytes. **A** Bright-field images of aged oocytes, MSC-sec–treated aged oocytes, MSC-sec-BDNF^{ab}–treated aged oocytes, and BDNF-treated aged oocytes. Scale bars: 200 μm. **B** Maturation rates after intraovarian injection and in vivo culture. When compared to aged oocytes, the PB1 extrusion rate was considerably higher in aged oocytes treated with MSC-sec and BDNF, but not in aged oocytes treated with MSC-sec-BDNF^{ab}. **C** Characteristic pictures of MII oocytes with DNA (blue) and labeled spindles (green). Scale bars: 20 μm. **D** The percentage of oocytes with abnormal spindles. The abnormal spindle incidence was decreased in MSC-sec-treated and BDNF-treated aged oocytes relative to aged oocytes, but increased in MSC-sec-BDNF^{ab}–treated aged oocytes. **E** Bright-field pictures of early embryonic development in each group. Scale bars: 200 μm. **F**, **G**, **H** The rates of 2PN, two-cell and blastocysts following intraovarian injection and in vivo culture followed by IVF. When compared to aged oocytes, the rates of 2PN, two-cell and blastocysts were considerably higher in aged oocytes treated with MSC-sec or BDNF, but they decreased in aged oocytes treated with MSC-sec-BDNF^{ab}. Data information: The data are presented as the mean ± SD in **B**, **D**, **F**, **G** and **H**, in which each point, distinguished by unique colors and shapes, represents results from three independent experiments. Tukey's test was used to assess differences after one-way ANOVA. *$P < 0.05$, **$P < 0.01$

fertility decline in female mammals [28–30]. To avoid the safety concerns of stem cell therapy, cell-free therapies using the secretome or extracellular vesicles (EVs) from stem cells can also improve ovarian function in various animal models [31, 32]. However, previous studies have mainly only focused on the ability of MSC-sec to enhance somatic cell attributes and improve the microenvironment of the ovary [10]. The present study primarily



C

	No. of GV/GVBD	No. of MII(%)	No. of MII to perform ICSI	No. of 2PN(%)	No. of blastocysts (%)	High-quality blastocysts (%)
Control	44	32 (74.42%)	32	12 (37.50%)	1 (3.13%)	0 (0%)
BDNF	45	34 (77.78%)	33	25 ** (75.76%)	9 * (27.27%)	2 (6.06%)

Fig. 5 Aged human oocytes treated with BDNF had higher rates of fertilization and blastocyst development. **A**, **B** Images of oocytes and embryos at the MII, 2PN, 8-cell, morula, and blastocyst stages that were cultivated in vitro. Scale bar: 100 μm. **C** MII, 2PN, blastocyst, and high-quality blastocyst rates were quantitatively analyzed when BDNF was present. Data information: In (**C**) the 2PN and blastocyst rates were significantly different. *$P < 0.05$, **$P < 0.01$ by chi-squared test

examined the involvement and underlying mechanism of hUC-MSC-sec to improve aged oocyte quality in both nuclear and cytoplasmic maturation. Our results showed that hUC-MSC-sec enhanced the quality of aged oocytes through the increase of PB1 emission, the reduction of abnormal spindle assembly and aneuploidy, as well as through the induction of maternal mRNA degradation, thus providing a potential novel therapeutic approach for the age-related decline in oocyte quality.

Because the components of the MSC-sec are rather complex, it is crucial to determine the crucial effective factor or factors. In this study, we identified BDNF as the effective factor of hUC-MSC-sec that enhances the quality of aged oocytes, thereby improving embryo development potential. The addition of BDNF^{ab} or K252a to the hUC-MSC-sec led to a notable reduction in PB1 emission and a marked increase in abnormal spindle assembly. BDNF, a neurotrophic factor that has been extensively investigated [33, 34], is an ovarian factor produced by granulosa cells within the ovary [22]. Its receptor TrkB is mainly expressed on oocytes within the ovary [24], and the binding of BDNF to TrkB can activate the ERK1/2 pathway [25]. Considerable evidence supports the involvement of BDNF in the development of ovarian follicles [35]. Furthermore, BDNF expression is notably lower in follicles derived from elderly females

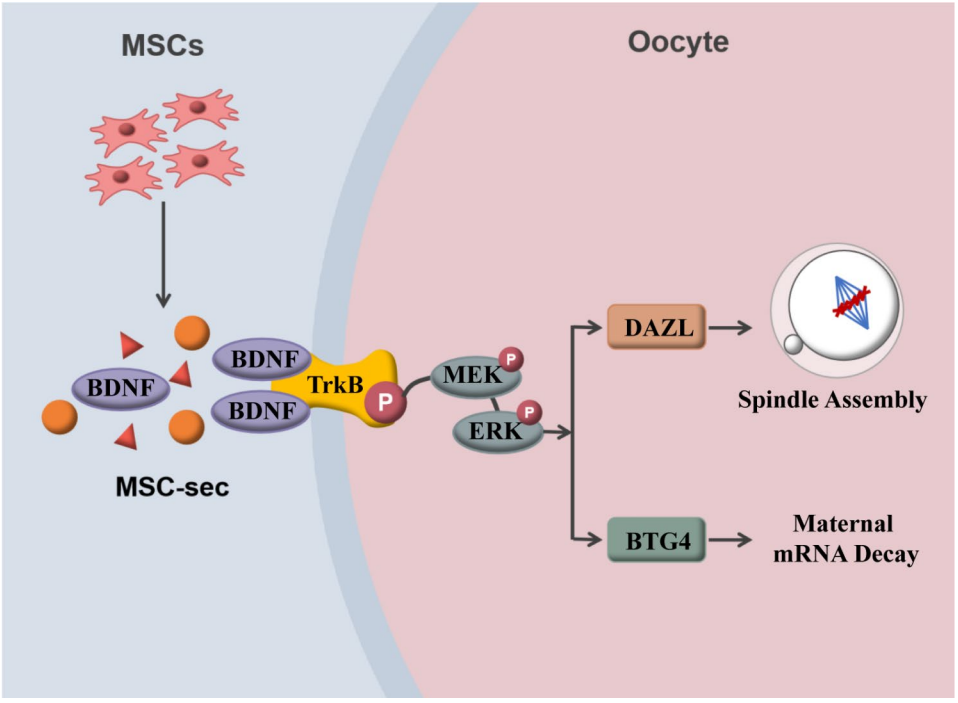


Fig. 6 Molecular mechanisms through which hUC-MSC-sec improves aged oocyte quality. MSC-sec and its functional component BDNF improves the quality of aged oocytes by activating the ERK1/2 pathway to promote spindle assembly and maternal mRNA decay

in comparison to those from younger females, suggesting that it may be associated with poor oocyte quality [23]. While it has been reported that BDNF enhanced PB1 emission or promoted oocyte development into preimplantation embryos in mice, cows, and buffalos [36–38], other studies have yielded negative results [37, 39]. The differences in these results may be due to inappropriate concentrations of BDNF [39, 40]. The concentrations of BDNF used in in vitro cultures have varied in different studies, including 3–10 ng/ml in mouse and rat oocytes [41, 42], 10 ng/ml in human oocytes [39, 43], and higher concentrations in buffalos [38]. In the present study, we found that a relatively low BDNF concentration (250 pg/ml), which is similar to its concentrations in human follicle fluid [26], was sufficient to improve aged oocyte maturation and embryonic development potential. Our results showed that BDNF treatment significantly improved the PB1 emission rate, reduced the aneuploidy rate, and promoted maternal mRNA degradation in aged mouse oocytes in vitro. The impact of BDNF on the maturation of oocytes, assembly of spindles, and competence of embryo development was further confirmed through in vivo experiments. These results underscore the crucial role of BDNF secreted by hUC-MSCs in improving aged oocyte quality.

Although it is well known that stem cells can improve ovarian function, there is limited knowledge about the mechanism through which MSCs enhance oocyte quality [44, 45]. It is widely recognized that the ERK1/2 signaling pathway plays a critical role in oocyte maturation by regulating spindle assembly and maternal mRNA degradation through its downstream genes *Dazl* and *Btg4*, respectively [15]. Our study demonstrated that both hUC-MSC-sec and BDNF activated the phosphorylation of ERK1/2 to improve aged oocyte quality during maturation. Accordingly, as the important downstream targets of the ERK1/2 signaling pathway, both DAZL and BTG4 were increased after hUC-MSC-sec or BDNF treatment. Furthermore, inhibition of the ERK1/2 signaling pathway blocked the impacts of BDNF on increasing DAZL and BTG4 expression in oocytes. These findings suggest that BDNF secreted by MSCs improves oocyte quality by activating the ERK1/2 signaling pathway (Fig. 6).

The oocyte maturation process is the foundation for embryonic development and clinical outcomes [46], and a number of studies have shown that IVM produces oocytes with lower quality and poorer development potential than those matured in vivo [47, 48]. In the current study, we further determined the effects of BDNF on the maturation and embryo development competence of oocytes derived from middle-aged women. Our findings revealed that BDNF treatment significantly improved the fertilization rate and blastocyst formation, underscoring the potential for BDNF as a therapeutic reagent for enhancing IVM of oocytes from aged patients with reproductive dysfunction. Clinical trials with BDNF-related drugs have shown significant advancements in subjects with hearing loss [49]. However, further

mechanistic investigations and safety evaluations of BDNF for improving oocyte quality are still necessary before it can be applied in the clinic.

Conclusion

In summary, our findings show that the administration of hUC-MSC-sec can mitigate the oocyte quality decline associated with aging by improving spindle assembly and promoting maternal mRNA degradation through activation of the ERK1/2 signaling pathway. Furthermore, its functional component BDNF alone also improved oocyte quality in aged mice through activation of the ERK1/2 signaling pathway. Consequently, this process promotes oocyte maturation and early embryonic development in aged mouse oocytes. Furthermore, our investigation on human oocytes suggests that BDNF has the potential to counteract age-related declines in oocyte quality and enhance the efficacy of assisted reproductive technologies in older female patients. However, it is imperative that any clinical intervention utilizing BDNF must first undergo sufficient safety evaluations and be closely monitored for any unforeseen effects on pregnancy.

Abbreviations

2PN	2 pronuclei
MSC-sec	Secretome of mesenchymal stem cells
BDNF	Brain-derived neurotrophic factor
MSCs	Mesenchymal stem cells
hUC-MSCs	Human umbilical cord-MSCs
HGF	Hepatocyte growth factor
ERK1/2	Extracellular signal-related kinase
hUC-MSC-sec	Secretome of hUC-MSCs
Fib-sec	Secretome of fibroblasts
PMSG	Pregnant mare serum gonadotropin
$BDNF^{ab}$	BDNF neutralizing antibody
PB1	First polar body
PFA	Paraformaldehyde
BSA	Bovine serum albumin
hCG	Chorionic Gonadotrophin for Injection
ICSI	Intracytoplasmic sperm injection
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown
IVM	In vitro maturation
EVs	Extracellular vesicles

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-025-02137-8.

Supplementary Material 1

Supplementary Material 2: Supplementary Fig. 1 $BDNF^{ab}$ repressed the function of MSC-sec to improve oocyte quality. A Bright-field images of aged oocytes, MSC-sec–treated aged oocytes, and MSC-sec-$BDNF^{ab}$–treated aged oocytes. Scale bars: 200 μm. B Rates of maturation following in-vitro culture. $BDNF^{ab}$ repressed PB1 extrusion. C Characteristic pictures of MII oocytes with DNA (blue) and labeled spindles (green). Scale bars: 20 μm. D Percentage of oocytes with abnormal spindles. $BDNF^{ab}$ repressed the abnormal spindle incidence relative to MSC-sec–treated aged oocytes. Data information: The data are presented as the mean ± SD in B and D, in which each point, distinguished by unique colors and shapes, represents results from three independent experiments. Tukey's test was used to assess differences after one-way ANOVA. $*P < 0.05$, $**P < 0.01$

Supplementary Material 3: Supplementary Fig. 2 K252a repressed the ability of MSC-sec to improve oocyte quality. A Bright-field images of aged oocytes, MSC-sec–treated aged oocytes, and MSC-sec-K252a–treated aged oocytes. Scale bars: 200 μm. B Rates of maturation following in-vitro culture. K252a repressed the effect of MSC-sec on PB1 extrusion. C Characteristic pictures of MII oocytes with DNA (blue) and labeled spindles (green). Scale bars: 20 μm. D Percentage of oocytes with abnormal spindles. K252a repressed the abnormal spindle incidence relative to MSC-sec–treated aged oocytes. Data information: The data are presented as the mean ± SD in B and D, in which each point, distinguished by unique colors and shapes, represents results from three independent experiments. Tukey's test was used to assess differences after one-way ANOVA. $*P < 0.05$, $**P < 0.01$

Supplementary Material 4: Supplementary Fig. 3 MSC-sec promoted maternal mRNA decay. A There were a total of 303 transcripts with increased expression in aged oocytes that decreased after MSC-sec treatment. B A total of 32 transcripts overlapped with abnormally degraded maternal mRNA after ***Btg4*** knockout in mouse oocytes

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Acknowledgements

The authors thank their colleagues for their technical assistance.

Author contributions

The study was designed by SZ, YQ, and KW, with JZ conducting the majority of experiments. CL completed the human oocyte and embryo culture; XM and JR helped with chromosome spreads; and XM, CC, GL, and RL helped with ovarian in situ injection. The manuscript was drafted by JZ and reviewed by XM, JZ, SZ, YQ, and ZC.

Funding

This study was supported by grants from the National Key Research and Development Program of China (2021YFC2700100 and 2022YFC2703800), the Natural Science Foundation of Shandong Province for Distinguished Young Scholars (ZR2023JQ031) and Grand Basic Projects (ZR2021ZD33), the National Natural Science Foundation for Distinguished Young Scholars (82125014), the Basic Science Center Program of the NSFC (31988101), the National Natural Science Foundation of China (32170867 and 32370906), the CAMS Innovation Fund for Medical Sciences (2021-I2M-5-001), the Shandong Provincial Key Research and Development Program (2020ZLYS02), the specific research fund of The Innovation Platform for Academicians of Hainan Province (YSPTZX202310), and the Taishan Scholars Program for Young Experts of Shandong Province (tsqn202211370).

Data availability

The bulk RNA sequencing data can be accessed in the Gene Expression Omnibus (GEO) database under the accession number GSE263864 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE263864). To review the data, please enter the token mhafsswklxuzpyp in the designated box.

Declarations

Ethics approval and consent to participate

All experimental procedures received approval from the Animal Care and Research Committee of Shandong University, China. Additionally, the Institutional Review Board of Reproductive Medicine at Shandong University approved the study, and all participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]State Key Laboratory of Reproductive Medicine and Offspring Health, Center for Reproductive Medicine, Institute of Women, Children and Reproductive Health, Shandong University, Jinan, Shandong 250012, China
[2]National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Shandong University, Jinan, Shandong 250012, China
[3]Key Laboratory of Reproductive Endocrinology (Shandong University), Ministry of Education, Jinan, Shandong 250012, China
[4]Shandong Technology Innovation Center for Reproductive Health, Jinan, Shandong 250012, China
[5]Shandong Provincial Clinical Research Center for Reproductive Health, Jinan, Shandong 250012, China
[6]Shandong Key Laboratory of Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250012, China
[7]Research Unit of Gametogenesis and Health of ART-Offspring, Chinese Academy of Medical Sciences (No.2021RU001), Jinan, Shandong 250012, China
[8]Department of Obstetrics and Gynecology, Laboratory of Laparoscopic Technology, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, The First Affiliated Hospital of Shandong First Medical University, Jinan, Shandong 250014, China
[9]Department of Nephrology, Multidisciplinary Innovation Center for Nephrology, The Second Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong 250033, China
[10]Nephrology Research Institute of Shandong University, Jinan, Shandong 250033, China
[11]Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai, China
[12]Department of Reproductive Medicine, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Received: 14 November 2024 / Accepted: 4 March 2025
Published online: 23 March 2025

References

1. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocr Rev. 2009;30(5):465–93. https://doi.org/10.1210/er.2009-0006.
2. Hunt PA, Hassold TJ. Human female meiosis: what makes a good egg go bad? Trends Genet. 2008;24(2):86–93. https://doi.org/10.1016/j.tig.2007.11.010.
3. Jessberger R. Age-related aneuploidy through cohesion exhaustion. EMBO Rep. 2012;13(6):539–46. https://doi.org/10.1038/embor.2012.54.
4. Wu YW, Li S, Zheng W, Li YC, Chen L, Zhou Y, et al. Dynamic mRNA degradome analyses indicate a role of histone H3K4 trimethylation in association with meiosis-coupled mRNA decay in oocyte aging. Nat Commun. 2022;13(1):3191. https://doi.org/10.1038/s41467-022-30928-x.
5. Miao Y, Cui Z, Gao Q, Rui R, Xiong B. Nicotinamide mononucleotide supplementation reverses the declining quality of maternally aged oocytes. Cell Rep. 2020;32(5):107987. https://doi.org/10.1016/j.celrep.2020.107987.
6. Cao Y, Zhao H, Wang Z, Zhang C, Bian Y, Liu X, et al. Quercetin promotes in vitro maturation of oocytes from humans and aged mice. Cell Death Dis. 2020;11(11):965. https://doi.org/10.1038/s41419-020-03183-5.
7. Liu C, Zuo W, Yan G, Wang S, Sun S, Li S, et al. Granulosa cell mevalonate pathway abnormalities contribute to oocyte meiotic defects and aneuploidy. Nat Aging. 2023;3(6):670–87. https://doi.org/10.1038/s43587-023-00419-9.
8. Zhang Y, Bai J, Cui Z, Li Y, Gao Q, Miao Y, et al. Polyamine metabolite spermidine rejuvenates oocyte quality by enhancing mitophagy during female reproductive aging. Nat Aging. 2023;3(11):1372–86. https://doi.org/10.1038/s43587-023-00498-8.
9. Salem HK, Thiemermann C. Mesenchymal stromal cells: current Understanding and clinical status. Stem Cells. 2010;28(3):585–96. https://doi.org/10.1002/stem.269.
10. Jiao W, Mi X, Qin Y, Zhao S. Stem cell transplantation improves ovarian function through paracrine mechanisms. Curr Gene Ther. 2020;20(5):347–55. https://doi.org/10.2174/1566523220666200928142333.
11. Jiao W, Mi X, Yang Y, Liu R, Liu Q, Yan T, et al. Mesenchymal stem cells combined with autocrosslinked hyaluronic acid improve mouse ovarian function by activating the PI3K-AKT pathway in a paracrine manner. Stem Cell Res Ther. 2022;13(1):49. https://doi.org/10.1186/s13287-022-02724-3.
12. Mi X, Jiao W, Yang Y, Qin Y, Chen ZJ, Zhao S. HGF secreted by mesenchymal stromal cells promotes primordial follicle activation by increasing the activity of the PI3K-AKT signaling pathway. Stem Cell Rev Rep. 2022;18(5):1834–50. https://doi.org/10.1007/s12015-022-10335-x.
13. Akbari H, Eftekhar Vaghefi SH, Shahedi A, Habibzadeh V, Mirshekari TR, Ganjizadegan A, et al. Mesenchymal stem Cell-Conditioned medium modulates apoptotic and Stress-Related gene expression, ameliorates maturation and allows for the development of immature human oocytes after artificial activation. Genes (Basel). 2017;8(12):371. https://doi.org/10.3390/genes8120371.
14. Das D, Arur S. Regulation of oocyte maturation: role of conserved ERK signaling. Mol Reprod Dev. 2022;89(9):353–74. https://doi.org/10.1002/mrd.23637.
15. Sha QQ, Dai XX, Dang Y, Tang F, Liu J, Zhang YL, et al. A MAPK cascade couples maternal mRNA translation and degradation to meiotic cell cycle progression in mouse oocytes. Development. 2017;144(3):452–63. https://doi.org/10.1242/dev.144410.
16. Tatone C, Carbone MC, Gallo R, Delle Monache S, Di Cola M, Alesse E, et al. Age-associated changes in mouse oocytes during postovulatory in vitro culture: possible role for meiotic kinases and survival factor BCL2. Biol Reprod. 2006;74(2):395–402. https://doi.org/10.1095/biolreprod.105.046169.
17. Xia X, Chan KF, Wong GTY, Wang P, Liu L, Yeung BPM, et al. Mesenchymal stem cells promote healing of nonsteroidal anti-inflammatory drug-related peptic ulcer through paracrine actions in pigs. Sci Transl Med. 2019;11(516):eaat7455. https://doi.org/10.1126/scitranslmed.aat7455.
18. Tran C, Damaser MS. Stem cells as drug delivery methods: application of stem cell secretome for regeneration. Adv Drug Deliv Rev. 2015;82–83:1–11. https://doi.org/10.1016/j.addr.2014.10.007.
19. Wang DH, Ren J, Zhou CJ, Han Z, Wang L, Liang CG. Supplementation with CTGF, SDF1, NGF, and HGF promotes ovine in vitro oocyte maturation and early embryo development. Domest Anim Endocrinol. 2018;65:38–48. https://doi.org/10.1016/j.domaniend.2018.05.003.
20. Cai L, Jeon Y, Yoon JD, Hwang SU, Kim E, Park KM, et al. The effects of human Recombinant granulocyte-colony stimulating factor treatment during in vitro maturation of Porcine oocyte on subsequent embryonic development. Theriogenology. 2015;84(7):1075–87. https://doi.org/10.1016/j.theriogenology.2015.06.008.
21. Aftabsavad S, Noormohammadi Z, Moini A, Karimipoor M. Effect of bisphenol A on alterations of ICAM-1 and HLA-G genes expression and DNA methylation profiles in cumulus cells of infertile women with poor response to ovarian stimulation. Sci Rep. 2021;11(1):9595. https://doi.org/10.1038/s41598-021-87175-1.
22. Chang HM, Wu HC, Sun ZG, Lian F, Leung PCK. Neurotrophins and glial cell line-derived neurotrophic factor in the ovary: physiological and pathophysiological implications. Hum Reprod Update. 2019;25(2):224–42. https://doi.org/10.1093/humupd/dmy047.
23. Qin X, Zhao Y, Zhang T, Yin C, Qiao J, Guo W, et al. TrkB agonist antibody ameliorates fertility deficits in aged and cyclophosphamide-induced premature ovarian failure model mice. Nat Commun. 2022;13(1):914. https://doi.org/10.1038/s41467-022-28611-2.
24. Harel S, Jin S, Fisch B, Feldberg D, Krissi H, Felz C, et al. Tyrosine kinase B receptor and its activated neurotrophins in ovaries from human fetuses and adults. Mol Hum Reprod. 2006;12(6):357–65. https://doi.org/10.1093/molehr/gal033.
25. Li C, Sui C, Wang W, Yan J, Deng N, Du X, et al. Baicalin attenuates Oxygen-Glucose Deprivation/Reoxygenation-Induced injury by modulating the BDNF-TrkB/PI3K/Akt and MAPK/Erk1/2 signaling axes in Neuron-Astrocyte cocultures. Front Pharmacol. 2021;12:599543. https://doi.org/10.3389/fphar.2021.599543.
26. Wang X, Sun Z, Zhen J, Yu Q. Brain-derived neurotrophic factor from follicular fluid is positively associated with rate of mature ooocytes collected and cleavage rate in intracytoplasmic sperm injection patients. J Assist Reprod Genet. 2011;28(11):1053–8. https://doi.org/10.1007/s10815-011-9635-4.
27. Yu C, Ji SY, Sha QQ, Dang Y, Zhou JJ, Zhang YL, et al. BTG4 is a meiotic cell cycle-coupled maternal-zygotic-transition licensing factor in oocytes. Nat Struct Mol Biol. 2016;23(5):387–94. https://doi.org/10.1038/nsmb.3204.

28. Esfandyari S, Chugh RM, Park HS, Hobeika E, Ulin M, Al-Hendy A. Mesenchymal stem cells as a bio organ for treatment of female infertility. Cells. 2020;9(10):2253. https://doi.org/10.3390/cells9102253.
29. Na J, Kim GJ. Recent trends in stem cell therapy for premature ovarian insufficiency and its therapeutic potential: a review. J Ovarian Res. 2020;13(1):74. https://doi.org/10.1186/s13048-020-00671-2.
30. Vanni VS, Viganò P, Papaleo E, Mangili G, Candiani M, Giorgione V. Advances in improving fertility in women through stem cell-based clinical platforms. Expert Opin Biol Ther. 2017;17(5):585–93. https://doi.org/10.1080/14712598.2017.1305352.
31. Zhang S, Zhu D, Li Z, Huang K, Hu S, Lutz H, et al. A stem cell-derived ovarian regenerative patch restores ovarian function and rescues fertility in rats with primary ovarian insufficiency. Theranostics. 2021;11(18):8894–908. https://doi.org/10.7150/thno.61690.
32. Zhou Y, Li Q, You S, Jiang H, Jiang L, He F, et al. Efficacy of mesenchymal stem Cell-Derived extracellular vesicles in the animal model of female reproductive diseases: A Meta-Analysis. Stem Cell Rev Rep. 2023;19(7):2299–310. https://doi.org/10.1007/s12015-023-10576-4.
33. Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci. 2003;4(4):299–309. https://doi.org/10.1038/nrn1078.
34. Lu B, Pang PT, Woo NH. The Yin and Yang of neurotrophin action. Nat Rev Neurosci. 2005;6(8):603–14. https://doi.org/10.1038/nrn1726.
35. Streiter S, Fisch B, Sabbah B, Ao A, Abir R. The importance of neuronal growth factors in the ovary. Mol Hum Reprod. 2016;22(1):3–17. https://doi.org/10.1093/molehr/gav057.
36. Kawamura K, Kawamura N, Mulders SM, Sollewijn Gelpke MD, Hsueh AJ. Ovarian brain-derived neurotrophic factor (BDNF) promotes the development of oocytes into preimplantation embryos. Proc Natl Acad Sci U S A. 2005;102(26):9206–11. https://doi.org/10.1073/pnas.0502442102.
37. Martins da Silva SJ, Gardner JO, Taylor JE, Springbett A, De Sousa PA, Anderson RA. Brain-derived neurotrophic factor promotes bovine oocyte cytoplasmic competence for embryo development. Reproduction. 2005;129(4):423–34. https://doi.org/10.1530/rep.1.00471.
38. Zhao X, Du F, Liu X, Ruan Q, Wu Z, Lei C, et al. Brain-derived neurotrophic factor (BDNF) is expressed in Buffalo (Bubalus bubalis) ovarian follicles and promotes oocyte maturation and early embryonic development. Theriogenology. 2019;130:79–88. https://doi.org/10.1016/j.theriogenology.2019.02.020.
39. Anderson RA, Bayne RA, Gardner J, De Sousa PA. Brain-derived neurotrophic factor is a regulator of human oocyte maturation and early embryo development. Fertil Steril. 2010;93(5):1394–406. https://doi.org/10.1016/j.fertnstert.2009.04.007.
40. Zhao P, Qiao J, Huang S, Zhang Y, Liu S, Yan LY, et al. Gonadotrophin-induced paracrine regulation of human oocyte maturation by BDNF and GDNF secreted by granulosa cells. Hum Reprod. 2011;26(3):695–702. https://doi.org/10.1093/humrep/deq390.
41. Kim JH, Lee HJ, Yu EJ, Jee BC, Suh CS, Kim SH. Dose-dependent embryotrophic effect of Recombinant granulocyte-macrophage colony-stimulating factor and brain-derived neurotrophic factor in culture medium for mouse preimplantation embryo. Obstet Gynecol Sci. 2014;57(5):373–8. https://doi.org/10.5468/ogs.2014.57.5.373.
42. Zhang Q, Liu D, Zhang M, Li N, Lu S, Du Y, et al. Effects of brain-derived neurotrophic factor on oocyte maturation and embryonic development in a rat model of polycystic ovary syndrome. Reprod Fertil Dev. 2016;28(12):1904–15. https://doi.org/10.1071/rd15131.
43. Yu Y, Yan J, Li M, Yan L, Zhao Y, Lian Y, et al. Effects of combined epidermal growth factor, brain-derived neurotrophic factor and insulin-like growth factor-1 on human oocyte maturation and early fertilized and cloned embryo development. Hum Reprod. 2012;27(7):2146–59. https://doi.org/10.1093/humrep/des099.
44. Cacciottola L, Vitale F, Donnez J, Dolmans MM. Use of mesenchymal stem cells to enhance or restore fertility potential: a systematic review of available experimental strategies. Hum Reprod Open. 2023;2023(4):hoad040. https://doi.org/10.1093/hropen/hoad040.
45. Nejabati HR, Nikzad S, Roshangar L. Therapeutic potential of mesenchymal stem cells in PCOS. Curr Stem Cell Res Ther. 2024;19(2):134–44. https://doi.org/10.2174/1574888x18666230517123256.
46. Li Y, Liu H, Wu K, Liu H, Huang T, Chen ZJ, et al. Melatonin promotes human oocyte maturation and early embryo development by enhancing clathrin-mediated endocytosis. J Pineal Res. 2019;67(3):e12601. https://doi.org/10.1111/jpi.12601.
47. Barnes FL, Kausche A, Tiglias J, Wood C, Wilton L, Trounson A. Production of embryos from in vitro-matured primary human oocytes. Fertil Steril. 1996;65(6):1151–6. https://doi.org/10.1016/s0015-0282(16)58330-7.
48. Lonergan P, Fair T. Maturation of oocytes in vitro. Annu Rev Anim Biosci. 2016;4:255–68. https://doi.org/10.1146/annurev-animal-022114-110822.
49. Foster AC, Szobota S, Piu F, Jacques BE, Moore DR, Sanchez VA, et al. A neurotrophic approach to treating hearing loss: translation from animal models to clinical proof-of-concept. J Acoust Soc Am. 2022;151(6):3937. https://doi.org/10.1121/10.0011510.

Publisher's note