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# Supplementation of spermidine enhances the quality of postovulatory aged porcine oocytes

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## Abstract

**Background** Spermidine (SPD) is an intermediate compound in the polyamine metabolism which takes critical part in a variety of cellular processes. In particular, it has been reported to exert anti-aging effects, suppress the age-related diseases, and extend lifespan across species. However, whether it has the favorable influence on the quality of postovulatory aged oocytes remains elusive.

**Methods** Immunostaining and fluorescence intensity measurement were used to evaluate the effects of postovulatory aging and SPD supplementation on the oocyte fragmentation, spindle/chromosome structure, actin polymerization, dynamics of cortical granules (CGs) and ovastacin, mitochondrial distribution and function, as well as autophagy levels. In addition, in vitro sperm binding assay and in vitro fertilization (IVF) experiment were applied to assess the impacts of postovulatory aging and SPD supplementation on the sperm binding ability and fertilization capacity of oocytes.

**Results** Here, we showed that supplementation of SPD during postovulatory aging could relieve the deterioration of porcine oocytes. Specifically, we found that postovulatory aging impaired the oocyte quality by damaging the morphological integrity of oocytes, maintenance of spindle/chromosome structure, and dynamics of actin cytoskeleton. Postovulatory aging also weakened the sperm binding ability and fertilization capacity of oocytes by compromising the distribution pattern of CGs and their content ovastacin. Notably, supplementation of SPD attenuated these defects in postovulatory aged porcine oocytes *via* strengthening mitochondrial function, eliminating excessive reactive oxygen species (ROS), inhibiting apoptosis, and enhancing autophagy levels.

**Conclusion** Altogether, our findings demonstrate that SPD supplementation is a feasible approach to ameliorate the quality of postovulatory aged oocytes, which can be potentially applied to the human assisted reproductive technology (ART) and in vitro production of animal embryos.

**Keywords** Spermidine, Postovulatory aging, Oocyte quality, ROS, Apoptosis, Autophagy

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## Introduction

Ovulated oocytes require prompt fertilization by sperm to form the embryos with high developmental potentials [1]. Failure to achieve fertilization within the optimal timeframe would lead to a time-dependent decline in oocyte quality, termed postovulatory aging [2], which is a pivotal factor contributing to the challenges occurring in the human assisted reproductive technology (ART) and in vitro production of animal embryos [3]. Postovulatory oocyte aging is invariably accompanied by a multitude of adverse biochemical and cellular changes, including chromosomal abnormalities, partial exocytosis of cortical granules (CGs), structural hardening of zona pellucida (ZP), elevated levels of reactive oxygen species (ROS), altered epigenetic modifications, and apoptosis [4–9]. These alterations further result in weakened fertilization ability [10], occurrence of polyspermy [11] and parthenogenesis [12, 13], as well as abnormal embryo/fetus development [14, 15]. Therefore, identification of effective strategies to improve the quality of postovulatory aged oocytes is important for the application of reproductive technologies.

Spermidine (SPD), a polyamine compound widely found in animals and plants [16], takes a critical part in various cellular processes such as DNA transcription, RNA translation, protein synthesis, and apoptosis, owing to its electrostatic interactions with negatively charged structures [17]. In addition, SPD possesses notable antioxidant properties, capable of neutralizing free radicals [18]. Recent studies have revealed its favorable impact on aging and age-related diseases in both animal models and humans by enhancing autophagy [19–22]. It has been also reported that SPD can inhibit cellular senescence in female germline stem cells (FGSCs) by reducing oxidative stress damage and bolstering cytoprotective autophagy [23]. Notably, the studies by us and others have shown that supplementation of SPD or its precursor putrescine enhances the quality of oocytes from reproductively aged mice [24–26]. However, the impacts of SPD on the quality of postovulatory aged oocytes have not been determined.

In the current study, we applied porcine oocytes as a research model to address this question. We found that supplementation of SPD inhibited the occurrence of cytoplasmic fragmentation in postovulatory aged oocytes, accompanied by the recovery of the dynamics of spindle/chromosome structure, actin cytoskeleton, cortical granules and mitochondria. Supplementation of SPD also elevated the sperm binding and fertilization capacity of postovulatory aged oocytes. We further revealed that SPD enhanced the quality of postovulatory aged oocytes by mitigating superfluous ROS to suppress the apoptosis and enhancing autophagy levels.

## Materials and methods

### Antibodies

α-tubulin-FITC antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA; Cat# F2168); Rabbit polyclonal anti-microtubule-associated protein 1 LC3B antibody were purchased from Cell Signaling Technology (Danvers, MA, USA; Cat# 2775s); Rabbit polyclonal anti-human ovastacin antibody was obtained from Dr. Jurrien Dean (National Institutes of Health, Bethesda, MA, USA).

### Dyes

Lens culinaris agglutinin (LCA)-FITC was purchased from Sigma-Aldrich (Cat# L-9296); MitoProbe JC-1 and MitoTracker Red CMXRos were obtained from ThermoFisher Scientific (Waltham, MA, USA; Cat# T3168; Cat# M7512); Actin-Tracker Red, DCFH-DA, Annexin-V-FITC, and Lyso-Tracker Green were purchased from Beyotime (Shanghai, China; Cat# C2205S; Cat# S0035S; Cat# C1062S; Cat# C1047S).

### Collection and in vitro maturation (IVM) of porcine oocytes

The procedures for collection and IVM of porcine oocytes were carried out as described in our previous study [27], and were approved by the Animal Research Institute Committee of Nanjing Agricultural University, China. For postovulatory aging, in vitro matured oocytes were further incubated in the medium for 48 h.

### SPD treatment

SPD (Sigma-Aldrich) was dissolved in the cell culture grade water for a stock solution to 50 mM, and then diluted with the culture medium into final concentrations of 25, 50, 100 or 200 μM, respectively.

### Fluorescence staining and intensity quantification

Antibody staining, actin staining, LCA staining, JC-1 staining, mitochondrion staining, Annexin-V staining, DCFH-DA staining, and fluorescence intensity measurement were carried out as we described previously [27]. Lysosome staining was performed according to our previous study [24].

### Sperm binding assay and IVF

The procedures for sperm binding and IVF were carried out as described in our previous studies [27, 28]. Briefly, the cryopreserved sperm were resuspended in the fertilization medium at 38.5 °C for 1 h to acquire capacitation. Then, $0.25\times10^6$ cells/ml of sperm was incubated with matured oocytes in the fertilization droplets for 1 h to test the sperm binding and for 6 h to perform IVF.

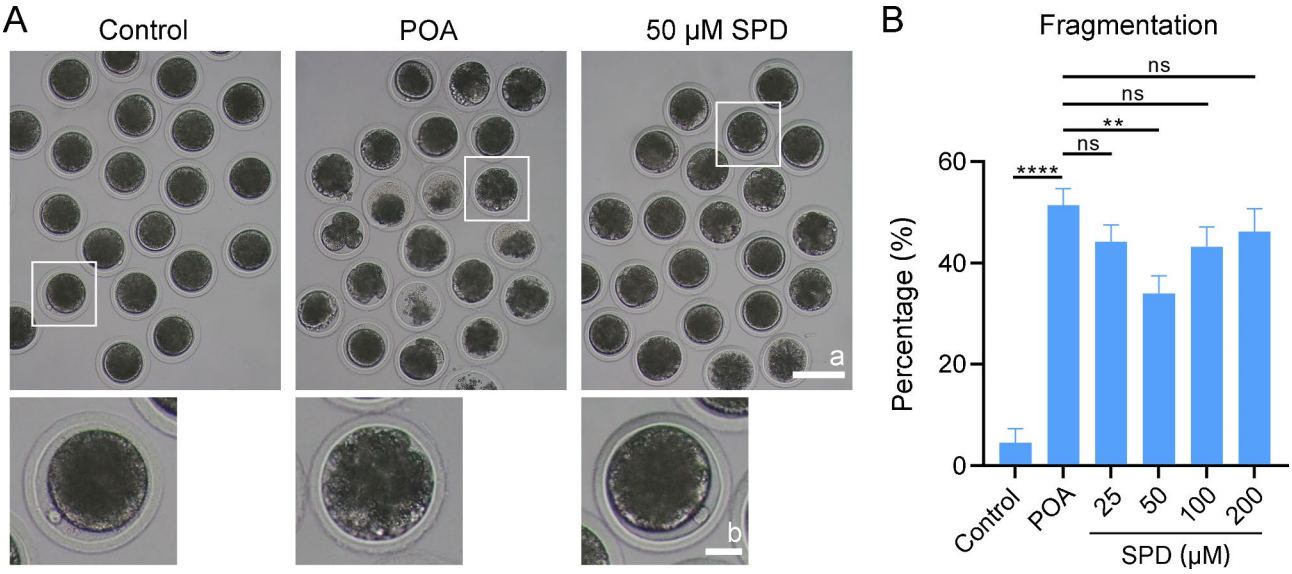


**Fig. 1** SPD supplementation inhibits the fragmentation of porcine oocytes after postovulatory aging. **(A)** Images of matured oocytes in control, postovulatory aged (POA), and SPD-supplemented groups. Scale bars, a: 150 μm, b: 30 μm. **(B)** The proportion of fragmented oocytes was calculated in control ($n=172$), POA ($n=168$), and SPD-supplemented (25 μM: $n=164$; 50 μM: $n=177$; 100 μM: $n=177$; 200 μM: $n=206$) oocytes. Data were represented as mean ± SEM of at least three independent experiments. $^{**}P<0.01$; $^{****}P<0.0001$; ns, no significance

### Statistical analysis

All values or percentages from at least three independent experiments were represented as mean ± SD or mean ± SEM, and the total number of oocytes used in the experiments was indicated in parentheses as (n). Data were analyzed by two-tailed unpaired t-test, which as provided by GraphPad Prism 9. $P<0.05$ was regarded as the statistically significant difference.

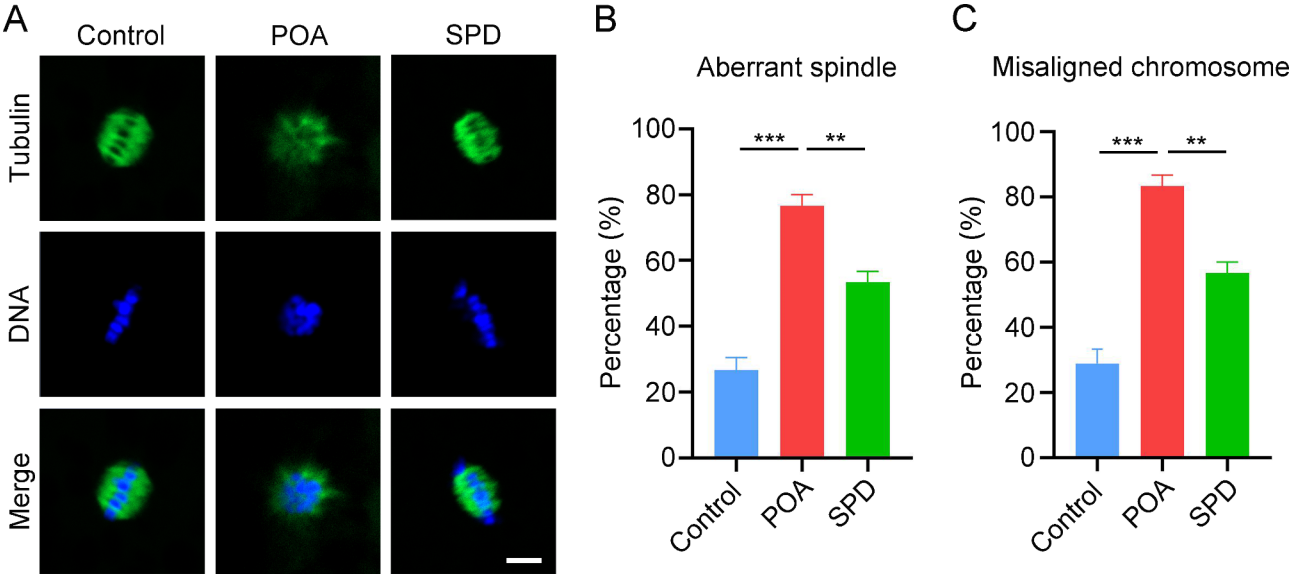


**Fig. 2** SPD supplementation maintains the spindle/chromosome apparatus in postovulatory aged porcine oocytes. **(A)** Fluorescence images of spindle morphology and chromosome alignment in control, POA, and SPD-supplemented oocytes. Scale bar, 5 μm. **(B)** The proportion of disorganized spindles was quantified in control ($n=45$), POA ($n=45$), and SPD-supplemented ($n=45$) oocytes. **(C)** The proportion of misaligned chromosomes was quantified in control ($n=45$), POA ($n=45$), and SPD-supplemented ($n=45$) oocytes. Data were represented as mean ± SEM of at least three independent experiments. $^{**}P<0.01$, $^{***}P<0.001$

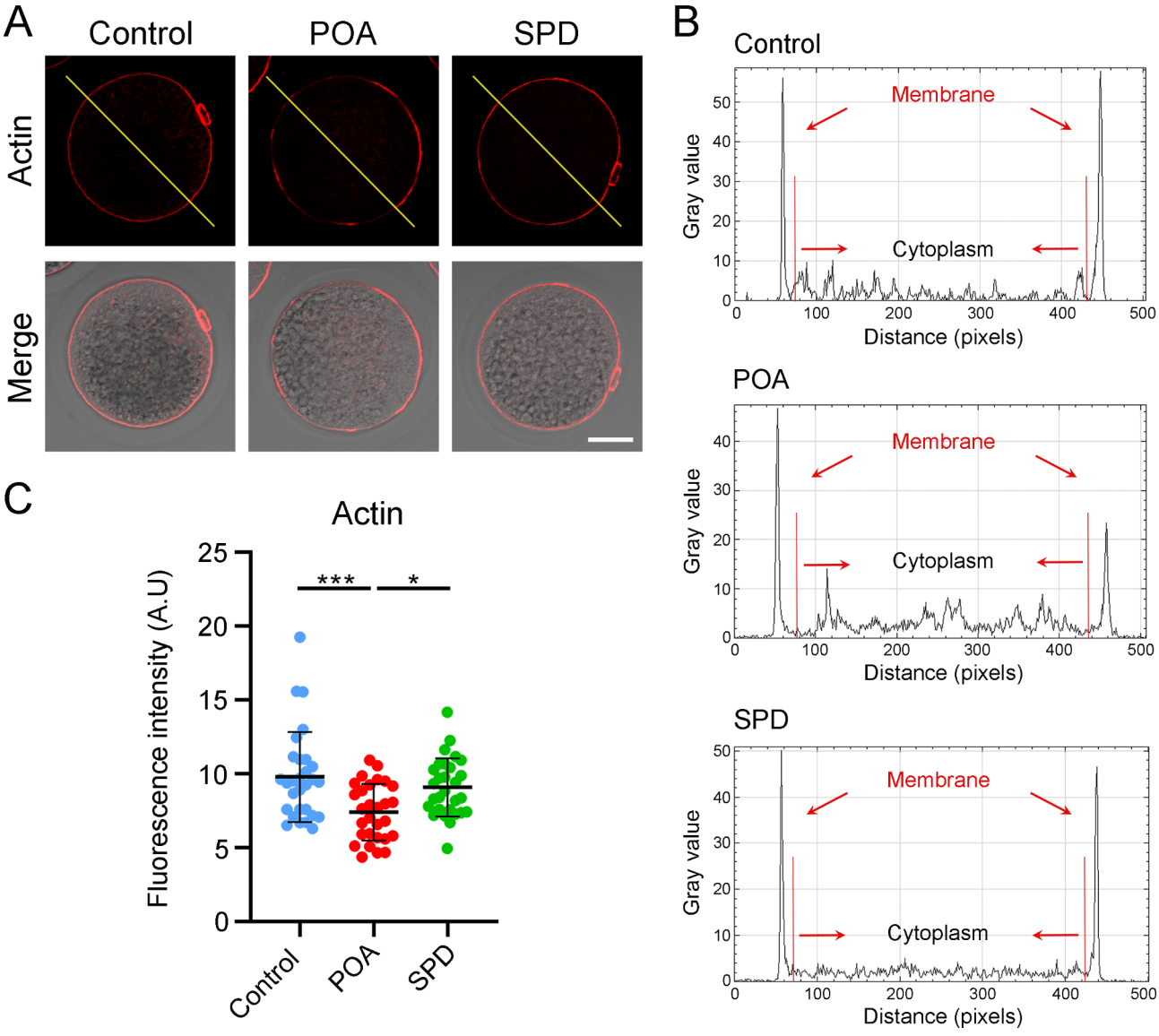


**Fig. 3** SPD supplementation restores the actin dynamics in postovulatory aged porcine oocytes. **(A)** Fluorescence images of actin signals in control, POA, and SPD-supplemented oocytes. Scale bar, 30 μm. **(B)** The fluorescence intensity profiling of actin in control, POA, and SPD-supplemented oocytes. **(C)** The fluorescence intensity of actin on the plasma membrane was measured in control ($n=29$), POA ($n=29$), and SPD-supplemented ($n=29$) oocytes. Data were represented as mean ± SD of at least three independent experiments. $^{*}P<0.05$, $^{***}P<0.001$

## Results

### SPD supplementation inhibits the fragmentation of porcine oocytes during postovulatory aging

To test the impact of SPD on the quality of postovulatory aged oocytes, different concentrations of SPD (25, 50, 100, or 200 μM) were supplemented in the IVM medium during aging. Porcine oocytes at germinal vesicle (GV) stage were cultured in IVM medium for 44 h to achieve maturation, and then transferred to a fresh IVM medium with or without SPD for another 48 h of incubation for postovulatory aging. As shown in Fig. 1A, we observed a large number of morphologically normal oocytes with first polar body in control group (Fig. 1A). By contrast, a substantial proportion of oocytes were fragmented after postovulatory aging, which could be relieved by supplementation of SPD (Fig. 1A). Quantitative data further showed that ~ 50% of fragmented oocytes were present in postovulatory aged group compared with ~ 5% in control group (Fig. 1B). In addition, supplementation of 50 μM SPD significantly decreased the oocyte fragmentation rate after aging (Fig. 1B). We then applied this concentration of SPD for further studies because of its best restorative effect. Taken together, these observations suggest that supplementation of SPD has a favorable effect on the postovulatory aged porcine oocytes.

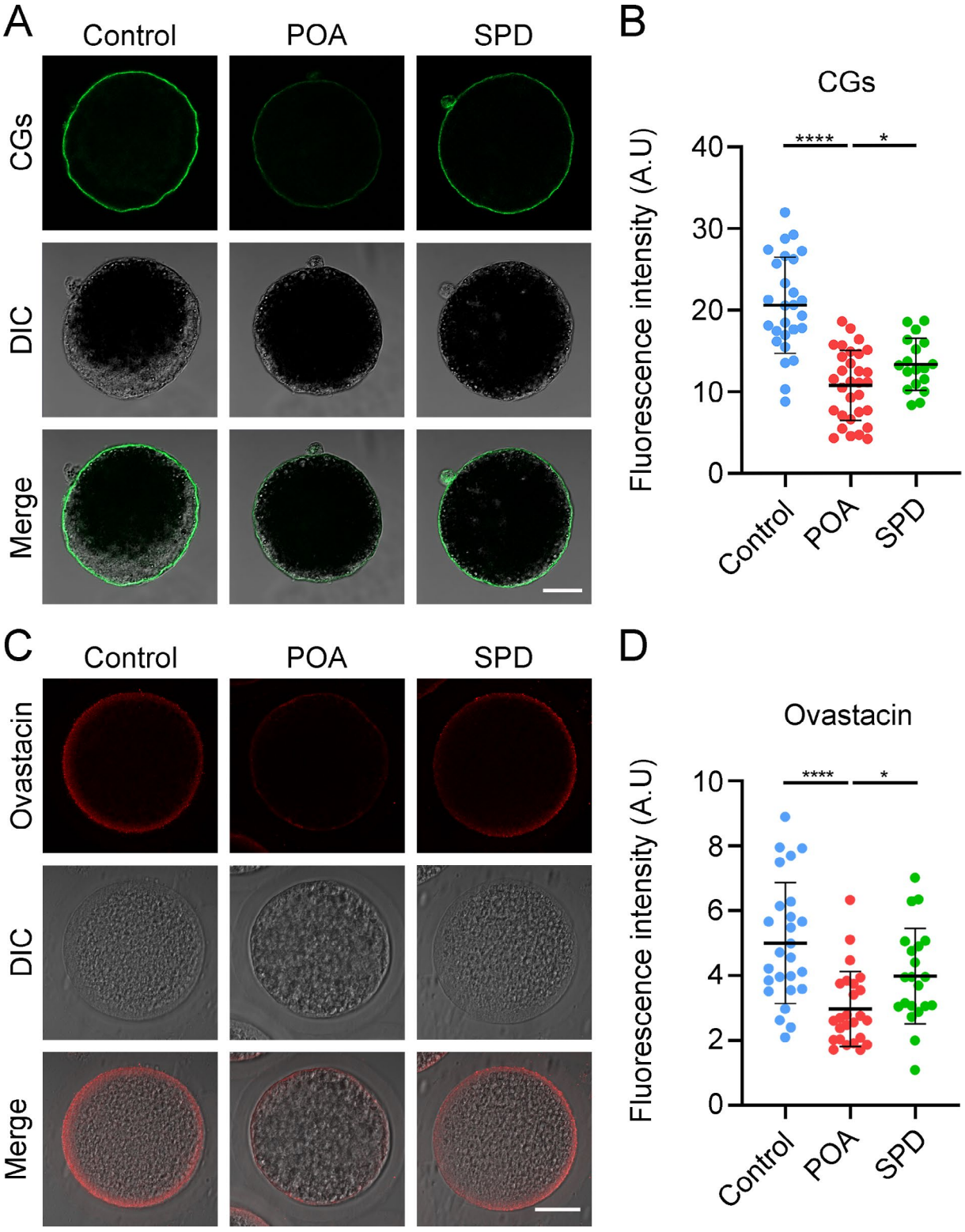


**Fig. 4** SPD supplementation recovers the distribution of CGs and ovastacin in postovulatory aged porcine oocytes. **(A)** Fluorescence images of CGs in control, POA, and SPD-supplemented oocytes. DIC, differential interference contrast. Scale bar, 30 μm. **(B)** The fluorescence intensity of CGs was measured in control ($n=27$), POA ($n=31$), and SPD-supplemented ($n=18$) oocytes. **(C)** Fluorescence images of ovastacin in control, POA, and SPD-supplemented oocytes. Scale bar, 30 μm. **(D)** The fluorescence intensity of ovastacin was measured in control ($n=26$), POA ($n=25$), and SPD-supplemented ($n=21$) oocytes. Data in **(B)** and **(D)** were represented as mean ± SD of at least three independent experiments. *$P<0.05$, ****$P<0.0001$

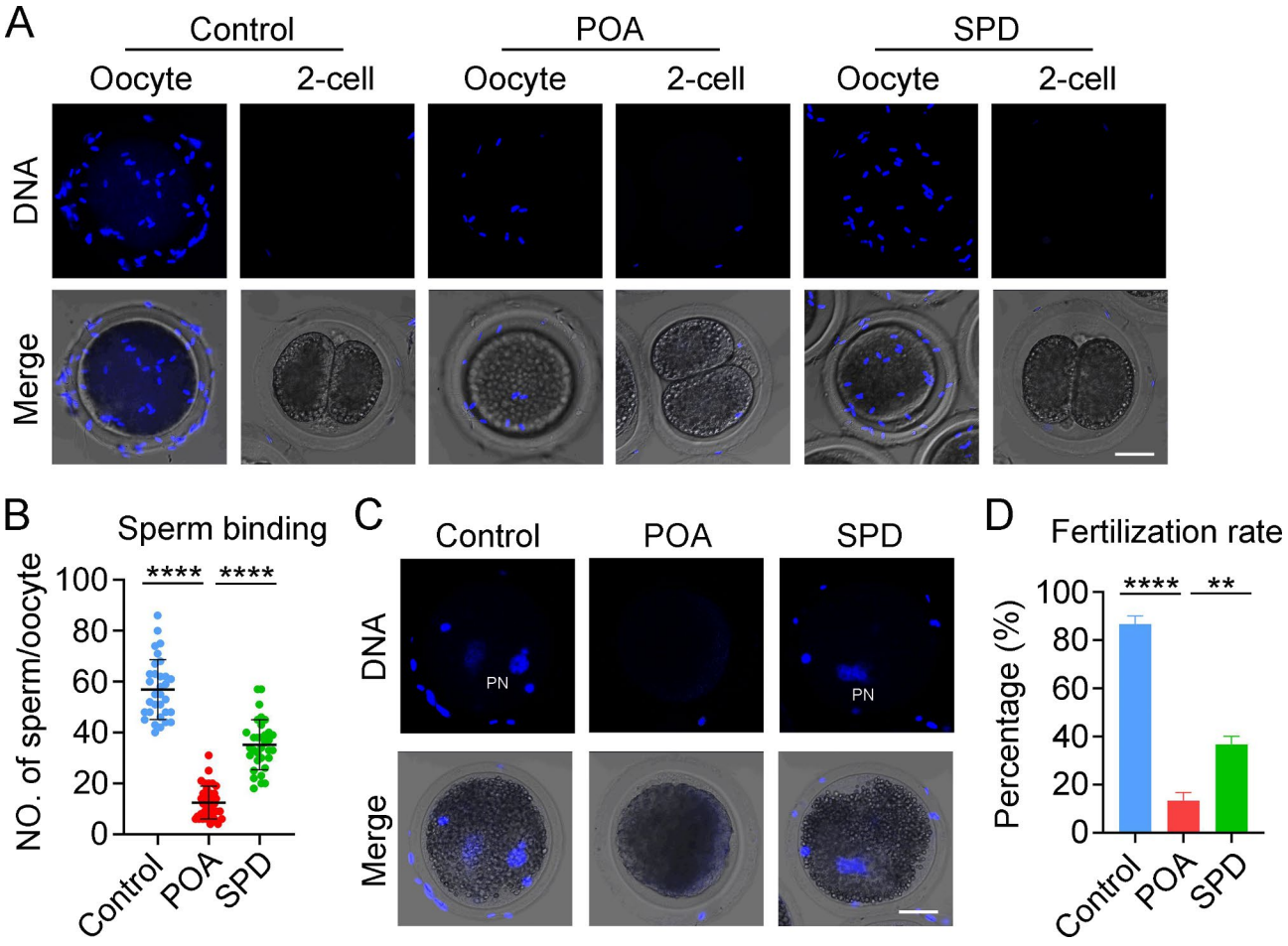


**Fig. 5** SPD supplementation enhances the sperm binding and fertilization ability of postovulatory aged porcine oocytes. **(A)** Images of sperm binding to the ZP surrounding control, POA, and SPD-supplemented oocytes. Scale bar, 30 μm. **(B)** The number of sperm binding to the oocytes was counted in control ($n=32$), POA ($n=35$), and SPD-supplemented ($n=33$) oocytes. **(C)** Images of oocytes after IVF in control, POA, and SPD-supplemented oocytes. PN, pronuclei. Scale bar, 30 μm. **(D)** The fertilization rate was recorded in control ($n=30$), POA ($n=30$), and SPD-supplemented ($n=30$) oocytes. Data in **(B)** and **(D)** were represented as mean ± SD or mean ± SEM of at least three independent experiments. $^{**}P<0.01$, $^{****}P<0.0001$

### SPD supplementation maintains the spindle organization and chromosome alignment in porcine oocytes during postovulatory aging

It has been previously shown that normal spindle organization and chromosome alignment would be impaired by postovulatory aging to weaken the oocyte quality, we thus tested whether supplementation of SPD could alleviate these defects. α-tubulin-FITC antibody was used to stain the spindle apparatus, and Hoechst was applied to counterstain the chromosomes. As shown in Fig. 2, a set of regularly organized spindle was observed with well-aligned chromosomes in most of control oocytes (Fig. 2A). By contrast, a variety of abnormal spindle/chromosome apparatuses were present in postovulatory aged oocytes, and these defects were attenuated by SPD supplementation (Fig. 2A). Quantitatively, a higher frequency of incorrectly assembled spindles and misaligned chromosomes was observed in postovulatory aged oocytes than that in controls, but reduced in SPD-supplemented oocytes (Fig. 2B, C), suggesting that SPD supplementation restores the quality of postovulatory aged oocytes through preserving the spindle/chromosome apparatus.

### SPD supplementation rescues the actin polymerization in porcine oocytes during postovulatory aging

Actin cytoskeleton plays a key role in nuclear positioning, spindle migration and anchoring, and polar body extrusion to promote meiotic progression in mammalian oocytes. We therefore used phalloidin-TRITC to visualize the changes of actin polymerization in postovulatory aged oocytes exposed to SPD. Immunofluorescence imaging and quantitative data revealed that actin integrity in porcine oocytes was impaired by postovulatory aging. In control oocytes, actin signals were strongly distributed on the plasma membrane as shown by the plot of fluorescence intensity along a line drawn across the oocytes (Fig. 3A, B). Whereas, actin filaments on the

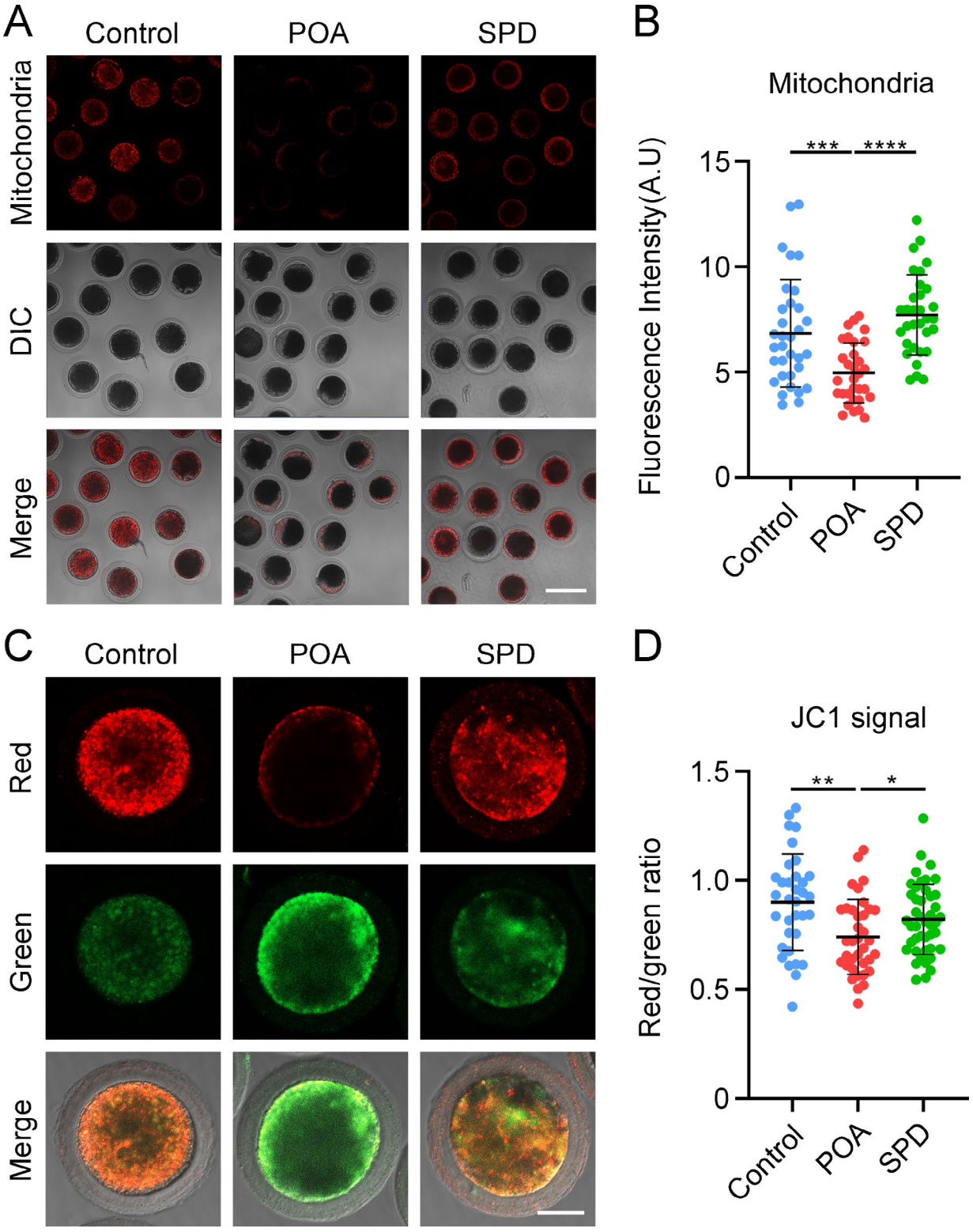


**Fig. 6** SPD supplementation sustains the mitochondrial localization and function in postovulatory aged porcine oocytes. **(A)** Fluorescence images of mitochondria in control, POA, and SPD-supplemented oocytes. Scale bar, 150 μm. **(B)** The fluorescence intensity of mitochondrial signals was measured in control ($n = 33$), POA ($n = 31$), and SPD-supplemented ($n = 32$) oocytes. **(C)** Fluorescence images of J-aggregates and J-mononer in control, POA, and SPD-supplemented oocytes (red, high MMP; green, low MMP). Scale bar, 30 μm. **(D)** The fluorescence intensity of red to green signals was recorded in control ($n = 34$), POA ($n = 36$), and SPD-supplemented ($n = 44$) oocytes. Data in **(B)** and **(D)** were represented as mean ± SD of at least three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

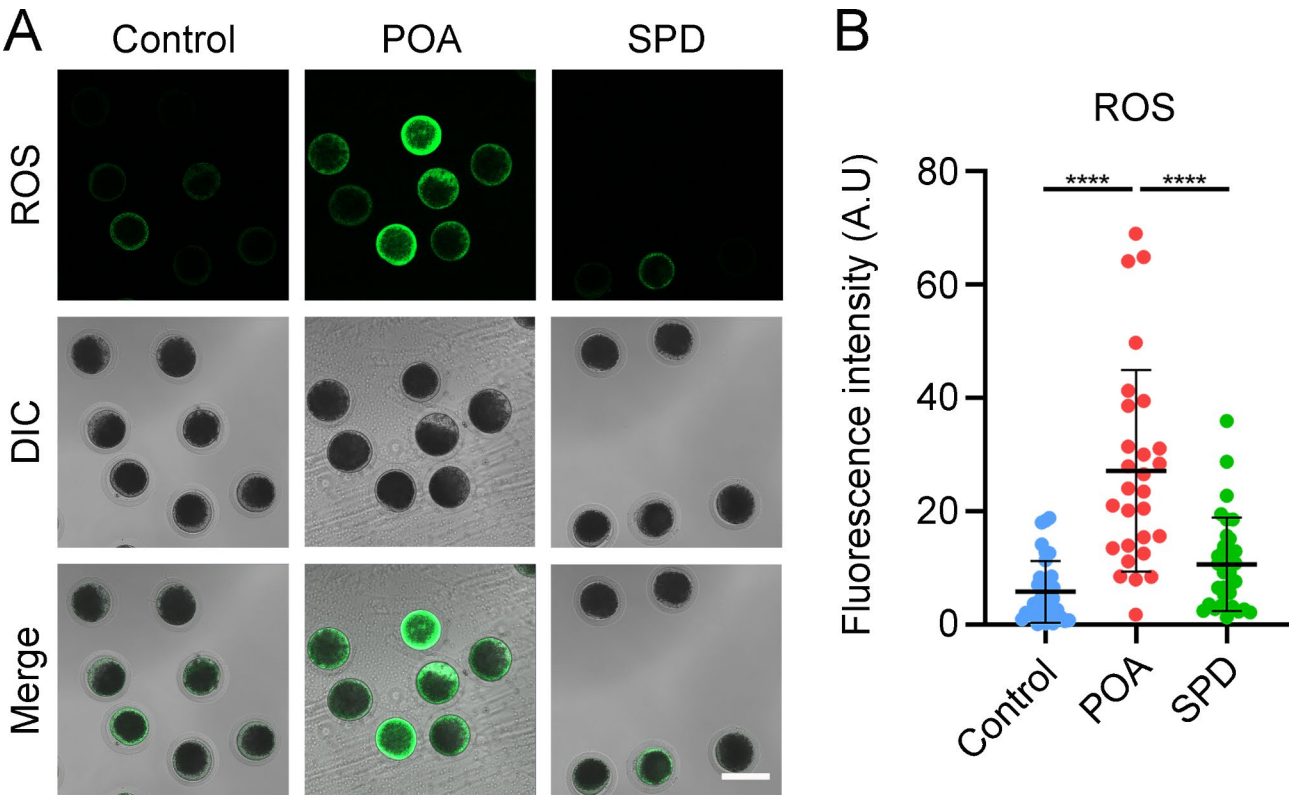


**Fig. 7** SPD supplementation reduces the ROS levels in postovulatory aged porcine oocytes. **(A)** Fluorescence images of ROS signals in control, POA, and SPD-supplemented oocytes. Scale bar, 150 μm. **(B)** The fluorescence intensity of ROS was quantified in control ($n=36$), POA ($n=28$), and SPD-supplemented ($n=32$) oocytes. Data were represented as mean ± SD of at least three independent experiments. ****$P<0.0001$

membrane displayed a discontinuous distribution pattern with significantly weakened signals in postovulatory aged oocytes, instead recovered in SPD-supplemented oocytes. (Fig. 3A, B). Consistently, quantification of actin signals on the entire plasma membrane also verified that SPD supplementation mitigated the defects in actin dynamics in postovulatory aged oocytes (Fig. 3C). Therefore, SPD maintains the actin cytoskeleton during postovulatory aging to protect the oocyte integrity.

### SPD supplementation restores the dynamics of CGs and ovastacin in porcine oocytes during postovulatory aging

Mammalian CGs are a type of oocyte-specific vesicles required for the post-fertilization block to polyspermy, and their distribution pattern has been considered as one of the important indicators for oocyte cytoplasmic maturation. We then stained the CGs using LCA-FITC to observe their dynamics. The fluorescence imaging and intensity quantification data revealed that CG signals in the subcortical region of postovulatory aged oocytes were substantially decreased compared to those in control oocytes, and this reduction was rescued by supplementation of SPD (Fig. 4A, B). In the meantime, we examined the behavior of ovastacin, a component of CGs that takes a key part in the prevention of polyspermy. Similarly, we found that postovulatory aging prominently compromised the distribution pattern of ovastacin, which was manifested by the faded fluorescent signals in the oocyte subcortex, but restored in the SPD-supplemented oocytes (Fig. 4C, D). Collectively, our findings indicate that postovulatory aging might induce the premature exocytosis of CGs and their component ovastacin to impair the fertilization ability of postovulatory aged oocytes, which could be inhibited by SPD supplementation.

### SPD supplementation strengthens the sperm binding and fertilization capacity of porcine oocytes during postovulatory aging

As release of ovastacin in the CGs out of oocytes would remove the sperm binding domain in the ZP, we next assessed the potential role of SPD on the sperm binding and fertilization ability of postovulatory aged oocytes. The head of sperm stained with Hoechst was shown to count the number of sperm binding to the ZP of oocytes (Fig. 5A). As the negative control, two-cell embryos did not support the binding of sperm due to the loss of sperm binding site in the ZP after fertilization (Fig. 5A). Compared to control oocytes, the amount of sperm binding to the postovulatory aged oocytes was substantially

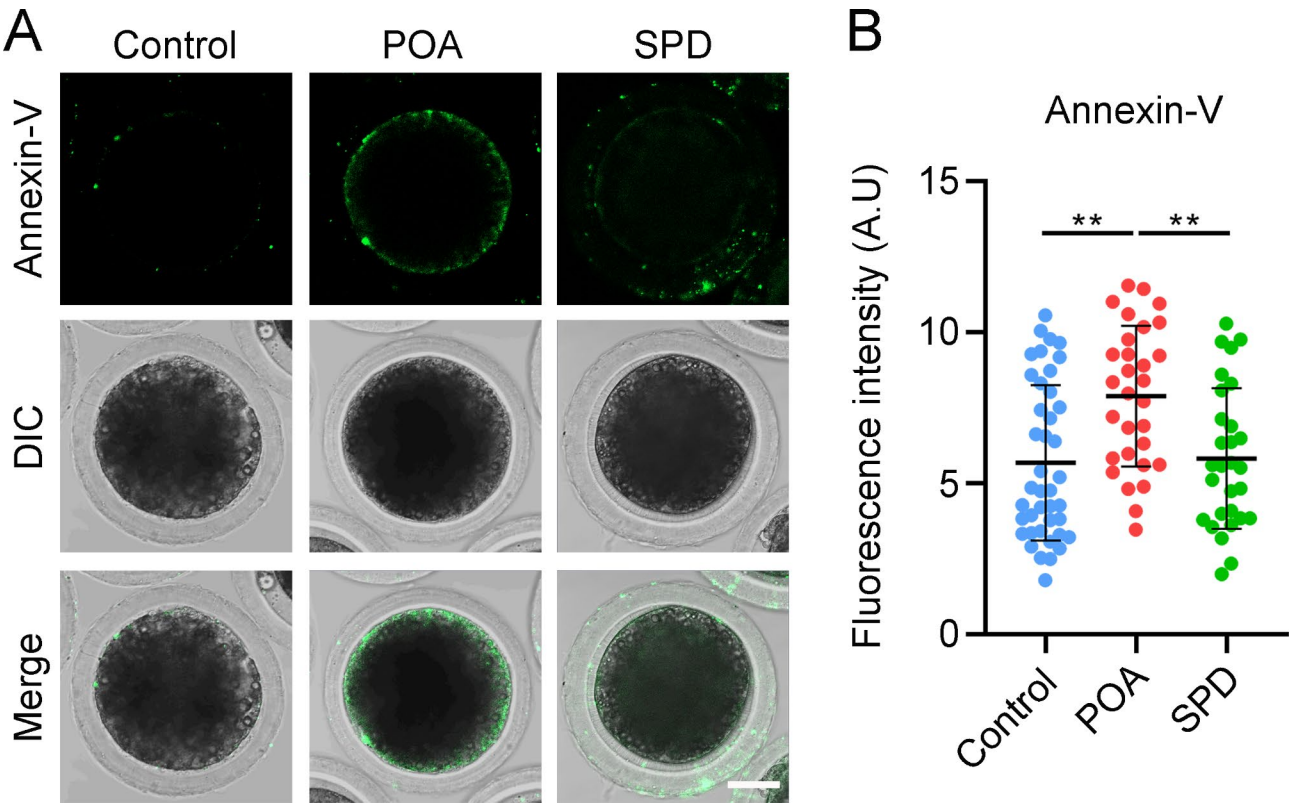


**Fig. 8** SPD supplementation suppresses the occurrence of apoptosis in postovulatory aged porcine oocytes. **(A)** Images of apoptotic oocytes in control, POA, and SPD-supplemented groups. Scale bar, 30 μm. **(B)** The fluorescence intensity of Annexin-V signals was calculated in control ($n=41$), POA ($n=30$), and SPD-supplemented ($n=29$) oocytes. Data were represented as mean ± SD of at least three independent experiments. $^{**}P<0.01$

decreased, whereas increased after SPD supplementation (Fig. 5B). Moreover, it was found that the fertilization rate as evaluated by the presence of pronuclei after IVF was also compromised by postovulatory aging and recovered by SPD supplementation (Fig. 5C, D). Therefore, these observations demonstrate that SPD could enhance the fertilization capacity deteriorated by postovulatory aging.

### SPD supplementation improves the mitochondrial function in porcine oocytes during postovulatory aging

A large number of mitochondria exist in oocytes to provide the energy for various cellular processes, and thus mitochondrial distribution has been considered as another key index for oocyte cytoplasmic maturation. Therefore, MitoTracker Red was employed for locating live mitochondria to determine whether they were affected by postovulatory aging and SPD supplementation. As shown in Fig. 6, the mitochondria in control group were uniformly localized around the subcortical region of oocytes with robust fluorescence signals as assessed by fluorescence staining and quantification (Fig. 6A, B). While the mitochondria in postovulatory aged oocytes lost their normal positioning with considerably decreased MitoTracker signals, and this phenomenon was significantly improved following supplementation of SPD (Fig. 6A, B). Furthermore, we performed an evaluation of mitochondrial membrane potential (MMP) by JC-1 staining. The high MMP was represented by a red fluorescence to show the J-aggregates, and the low MMP was represented by a green fluorescence to show the J-monomer. Compared with the control oocytes, the proportion of red to green signals in postovulatory aged oocytes was dramatically lowered, but significantly increased after SPD supplementation (Fig. 6C, D). These results collectively imply that supplementation of SPD attenuates the mitochondrial dysfunction in porcine oocytes caused by postovulatory aging.

### SPD supplementation reduces the ROS levels in porcine oocytes during postovulatory aging

As mitochondrial dysfunction usually produces excessive ROS in cells, we then used dichlorofluorescein diacetate (DCFH-DA) staining to detect the ROS levels in each group of oocytes. The imaging results showed that quite a low level of ROS signals were observed in control oocytes (Fig. 7A). On the contrary, postovulatory aging remarkably elevated the ROS signals in porcine oocytes, which could be eliminated by SPD supplementation (Fig. 7A). Quantitatively, SPD supplementation significantly decreased the intensity of ROS signals present in

the postovulatory aged oocytes (Fig. 7B). Hence, these results reveal that supplementation of SPD can effectively remove the excessive ROS in oocytes produced during postovulatory aging.

### SPD supplementation inhibits apoptosis in porcine oocytes during postovulatory aging

Mitochondrial damage-induced accumulation of ROS would always lead to the occurrence of cellular apoptosis. To test whether this is the case in postovulatory aged oocytes and whether it could be inhibited by SPD supplementation, we applied Annexin-V to test the presence of phosphatidylserine on the surface of oocytes as it can be translocated from the inner layer to the outer layer of cell membrane when early apoptosis occurs. As displayed in Fig. 8A, the fluorescence signals of Annexin-V were scarcely observed on the outer membrane of control and SPD-supplemented oocytes (Fig. 8A). Nevertheless, they were visibly present on the membrane of postovulatory aged oocytes (Fig. 8A). Compared to the control and SPD-supplemented oocytes, the intensity of Annexin-V signals was markedly higher in postovulatory aged oocytes (Fig. 8B), indicating that SPD supplementation inhibits the occurrence of oocyte apoptosis induced by postovulatory aging.

### SPD supplementation recovers the autophagy in porcine oocytes during postovulatory aging

Our previous study has shown that the autophagy level was reduced in oocytes from reproductively aged mice, and could be restored by spermidine administration [24]. Therefore, we assessed the autophagy in porcine oocytes during postovulatory aging. Microtubule-associated protein 1 A or 1B light chain 3 (LC3) staining and quantification data manifested that the number of LC3 foci was considerably less in postovulatory aged oocytes than that in controls, but elevated in SPD-supplemented oocytes (Fig. 9A, B). Meanwhile, the lysosome signals as assessed by Lyso-Tracker Green staining were also decreased in oocytes by postovulatory aging, while increased following SPD supplementation (Fig. 9C, D). Altogether, these observations imply that the enhanced autophagy levels by SPD might be one of the causes to improve the quality of postovulatory aged oocytes.

## Discussion

It has been known that mammalian oocytes would experience a time-dependent deterioration process after ovulation when they cannot be immediately fertilized by fresh sperm in the ampulla or in a test tube [3], which might result in morphological, molecular, cellular, and epigenetic abnormalities in oocytes [29]. In particular, this phenomenon is often observed during human ART procedures when retrieved oocytes are subjected to extended culture prior to fertilization [3]. Thus, it is necessary to develop effective methods or strategies to enhance the quality of postovulatory aged oocytes and thus improve the reproductive outcome of infertile patients.

In line with our recent studies [27, 29], we first of all demonstrated that postovulatory aging impaired the morphology of matured porcine oocytes through generation of cytoplasmic fragmentation, accompanied by the perturbed dynamics of spindle and actin cytoskeleton, partial exocytosis of CGs and their component ovastacin, as well as mis-distributed and dysfunctional mitochondria. In addition, we observed that these defects further led to the weakened sperm binding and fertilization ability of oocytes, which were highly correlated with the elevated levels of ROS and decreased levels of autophagy in oocytes during postovulatory aging, and thereby inducing the cellular apoptosis. Previous reports have shown that polyamine compound SPD declines with age in a diversity of organisms, including Saccharomyces cerevisiae, Drosophila, mouse, rat, and human [30–34], and supplementation of SPD has an anti-aging property though autophagy-dependent or independent manner and has been ranked among the most promising interventions that may slow aging [35, 36]. Of note, SPD has been shown to suppresses oxidative stress and ferroptosis to alleviate ovarian damage in mice [37], and our recent study documents that SPD rejuvenates the oocyte quality by enhancing mitophagy in mice of advanced maternal age [24]. Accordingly, we proposed that SPD might have beneficial effect on the quality of postovulatory aged oocytes. As anticipated, we evidenced that supplementation of SPD mitigated the oocyte abnormalities caused by postovulatory aging *via* eliminating excessive ROS, inhibiting apoptosis, and increasing autophagy levels. Interestingly, we also found that the restorative effect of SPD on the quality of postovulatory aged oocytes is not concentration-dependent. Higher concentrations of SPD instead could not mitigate the oocyte fragmentation during postovulatory aging. Consistently, SPD at supraphysiological doses induces oxidative stress and granulosa cell apoptosis in mouse ovaries [38].

## Conclusion

To sum up, our study documents that SPD supplementation is a feasible approach to improve the quality of postovulatory aged oocytes by boosting the mitochondrial function and suppressing apoptosis (Fig. 10), which provides scientific evidence for its application in both human ART and in vitro production of agricultural animal embryos.

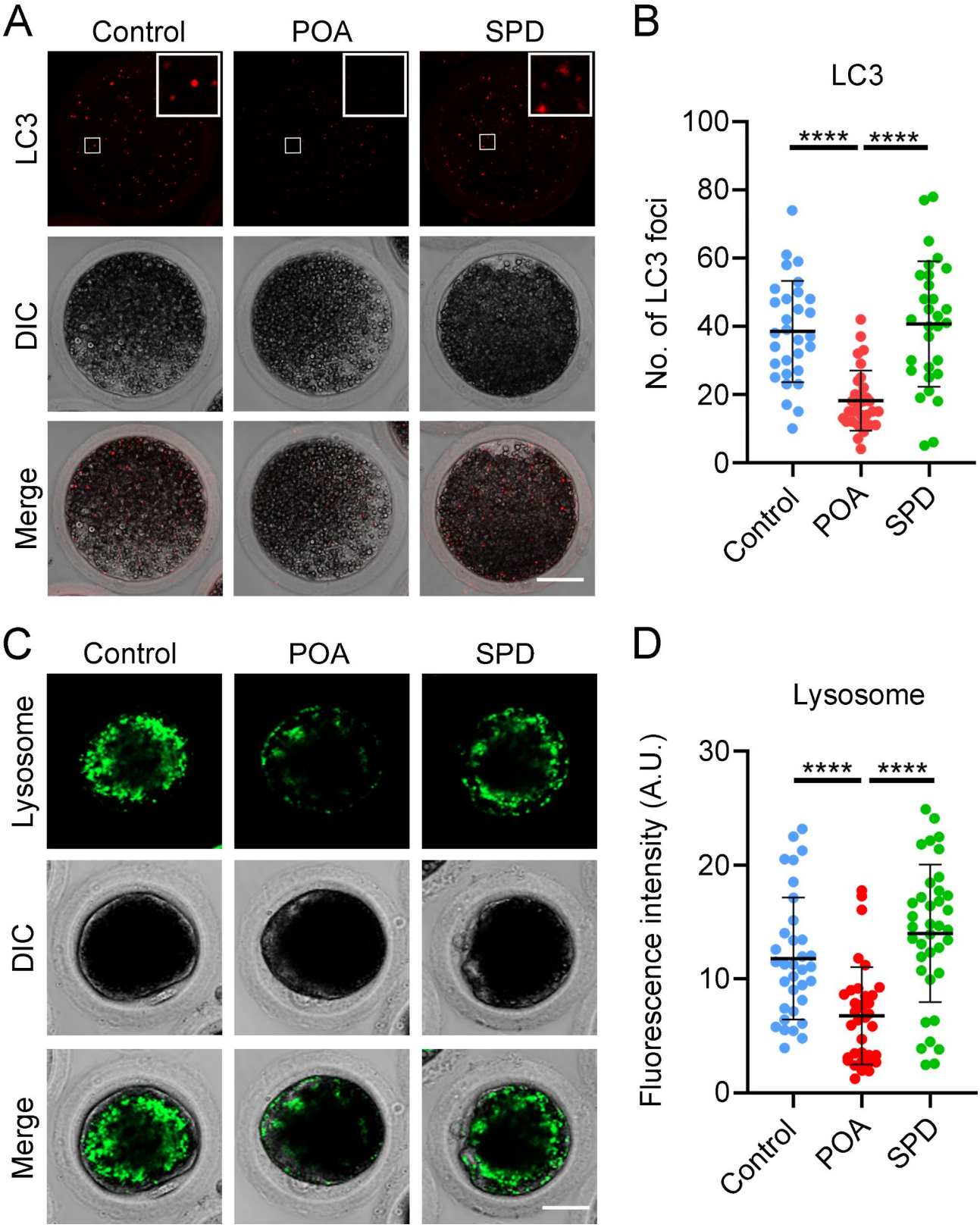


**Fig. 9** SPD supplementation enhances the autophagy levels in postovulatory aged porcine oocytes. **(A)** Images of LC3 foci in control, POA, and SPD-supplemented groups. Scale bar, 30 μm. **(B)** The number of LC3 foci was quantified in control ($n=30$), POA ($n=29$), and SPD-supplemented ($n=30$) oocytes. **(C)** Images of lysosomes in control, POA, and SPD-supplemented groups. Scale bar, 30 μm. **(D)** The fluorescence intensity of lysosome signals was quantified in control ($n=34$), POA ($n=35$), and SPD-supplemented ($n=37$) oocytes. Data in **(B)** and **(D)** were represented as mean ± SD of at least three independent experiments. ****$P<0.0001$

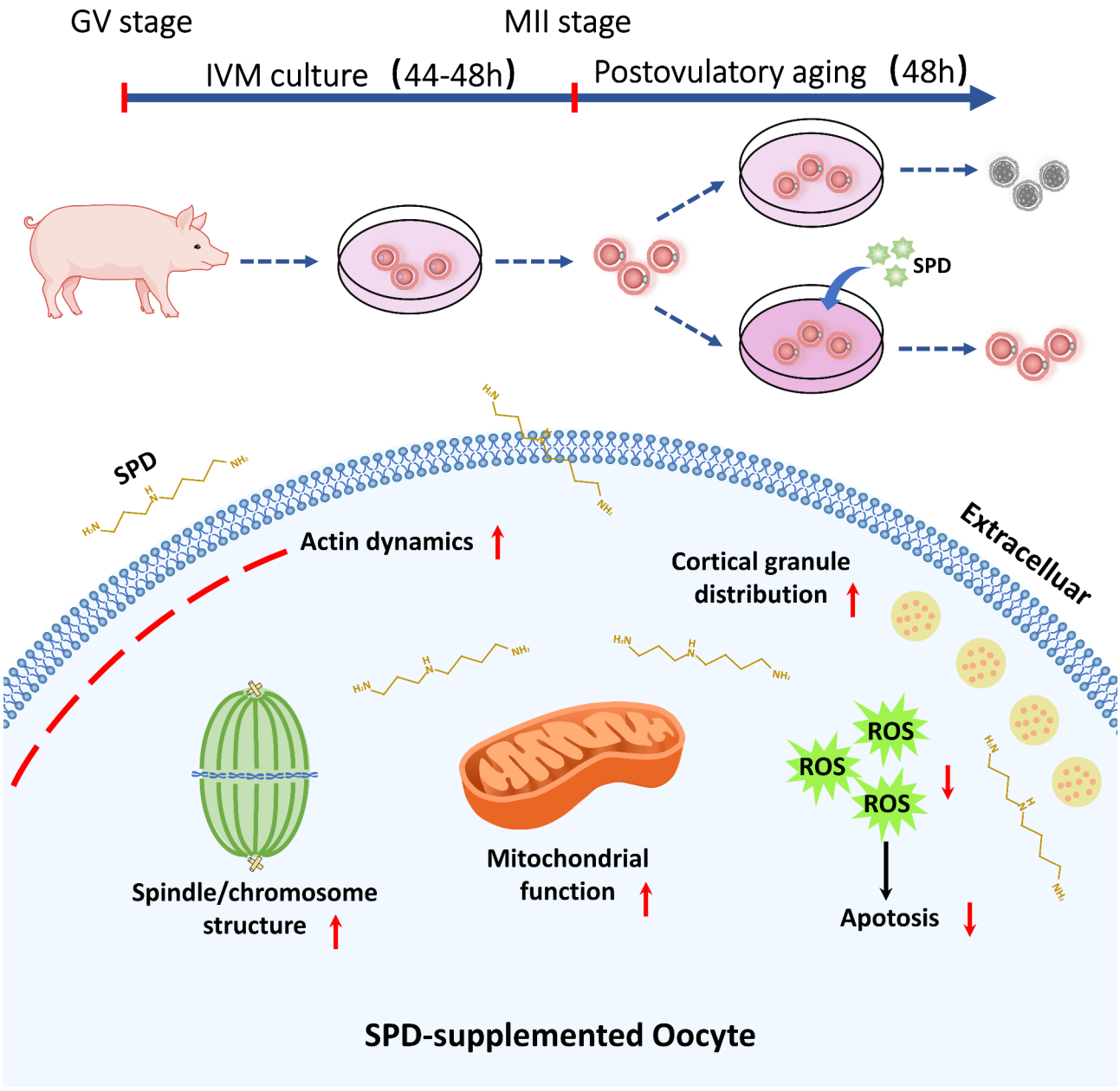


**Fig. 10** Working model for the impact of SPD on the quality of postovulatory aged porcine oocytes

**Abbreviations**

| | |
|---|---|
| SPD | Spermidine |
| CGs | Cortical granules |
| ROS | Reactive oxygen species |
| ART | Assisted reproductive technology |
| ZP | Zona pellucida |
| IVM | In vitro maturation |
| GV | Germinal vesicle |
| MMP | Mitochondrial membrane potential |
| DCFH-DA | Dichlorofluorescein diacetate |
| LCA | Lens culinaris agglutinin |

**Author contributions**
BX designed the research; JB, YZ, NL, ZC, HZ, YL and YM performed the experiments; JB, SS and BX analyzed the data; JB and BX wrote the manuscript.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
All experimental procedures were approved by the Animal Research Institute Committee of Nanjing Agricultural University, China.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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