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Resveratrol attenuates doxorubicin-induced toxicity during *in vitro* culture of mouse-isolated preantral follicles

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Research Article

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Summary

The aims of this study were to evaluate the doxorubicin concentration that induces toxic effects on *in vitro* culture of isolated mouse secondary follicles and to investigate whether resveratrol can inhibit or reduce this toxicity. Secondary follicles were isolated and cultured for 12 days in control medium (α-MEM$^+$) or in α-MEM$^+$ supplemented with doxorubicin (0.1 μg/ml) or different concentrations of resveratrol (0.5, 2, or 5 μM) associated with doxorubicin (0.1 μg/ml) (experiment 1). For experiment 2, follicles were cultured in α-MEM$^+$ alone or supplemented with doxorubicin (0.3 μg/ml) or different concentrations of resveratrol (5 or 10 μM) associated or not with doxorubicin (0.3 μg/ml) (experiment 2). The endpoints analyzed were morphology (survival), antrum formation, follicular diameter, mitochondrial activity, glutathione (GSH) levels and DNA fragmentation. In the first experiment, doxorubicin (0.1 μg/ml) maintained survival and antrum formation similar to the control, while 5 μM resveratrol showed increased parameters, maintained mitochondrial activity and increased GSH levels compared to the control. In the second experiment, doxorubicin (0.3 μg/ml) reduced survival, antrum formation and follicular diameter compared to the control. Resveratrol at a concentration of 10 μM attenuated the damage caused by doxorubicin by improving follicular survival and did not present DNA fragmentation. In conclusion, supplementation of the *in vitro* culture medium with 0.3 μg/ml doxorubicin reduced the survival and impaired the development of mouse-isolated preantral follicles. Resveratrol at 10 μM reduced doxorubicin-induced follicular atresia, without DNA fragmentation in the follicles.

Introduction

Ovarian toxicity is one of the adverse effects triggered by chemotherapy in women receiving antineoplastic agents. Many chemotherapeutic drugs, including doxorubicin, have been implicated in excessive activation of primordial follicles, which constitute the total ovarian reserve, and/or in the apoptosis of follicular cells. This leads to the loss of ovarian function, premature ovarian failure and infertility (Melekoglu *et al.*, 2022; Spears *et al.*, 2019).

Doxorubicin is an anthracycline primarily used in breast cancer treatment (Spears *et al.*, 2019). It targets multiple cellular pathways, including the inhibition of the enzyme topoisomerase II, which is responsible for breaking and joining DNA during replication, leading to DNA fragmentation. Additionally, doxorubicin can generate oxygen free radicals mainly within mitochondria, causing oxidative stress that can damage proteins, lipids and DNA, resulting in cellular and tissue damage (Sritharan and Sivalingam, 2021; Spears *et al.*, 2019). In mice, doxorubicin increased reactive oxygen species (ROS) production, reduced mitochondrial membrane potential and induced apoptosis of granulosa cells cultured *in vitro* (Zhang *et al.*, 2017), as well as inhibited oocyte maturation through DNA damage (Ding *et al.*, 2019). Considering that oxidative stress and subsequent apoptosis are critical determinants of follicle loss during chemotherapy exposure (Assis *et al.*, 2022; Mohan *et al.*, 2021), antioxidant agents may be used as potential protective drugs against the toxic effects of doxor jmubicin in the ovary.

Resveratrol (3,5,4'-trihydroxystilbene) is a secondary metabolite (Berman *et al.*, 2017) found in various natural products, such as grapes, nuts and the medicinal plant *Morus nigra* (Tian *et al.*, 2020; Wang *et al.*, 2021). Even though resveratrol has been shown to sensitize cancer cells to conventional chemotherapeutic drugs, enhancing the antitumour activity of doxorubicin in vivo and in vitro (Kweon *et al.*, 2010; Osman *et al.*, 2013; Rai *et al.*, 2016; Xu *et al.*, 2017), it is also capable of acting as chemoprotective agent. *In vivo* studies with murine models have shown

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that resveratrol attenuated doxorubicin or cyclophosphamide-induced ovarian injury, preserving primordial follicles and reducing apoptosis (Herrero *et al.*, 2023; Nie *et al.*, 2021a). Additionally, resveratrol reduced oxidative stress and apoptosis caused by doxorubicin or cisplatin in ovarian follicles (Herrero *et al.*, 2023; Ibrahim *et al.*, 2021). During *in vitro* culture of granulosa cells, resveratrol reduces oxidative stress and apoptosis induced by hydrogen peroxide (Nie *et al.*, 2021b) and cyclophosphamide (Nie *et al.*, 2020).

Although the importance of the *in vitro* follicle culture model for conducting reproductive toxicology assays is well recongnized (Simon *et al.*, 2020), there are no studies demonstrating the effect of resveratrol against doxorubicin-induced toxicity during *in vitro* culture of mouse secondary follicles. The aims of this study were to evaluate the doxorubicin concentration that induces toxic effects on the *in vitro* culture of isolated mouse secondary follicles and to investigate whether resveratrol can inhibit or reduce this toxicity. This was performed by investigating the following endpoints: follicular morphology and development, active mitochondria, intracellular levels of glutathione (GSH) and ROS and DNA fragmentation.

Material and methods

Chemicals and reagents

The alpha-minimum essential medium (α-MEM), MEM, HEPES, antibiotics, mineral oil, phosphate buffered saline (PBS), fluorescent marker H2DCFDA, resveratrol, supplements and DNAse were obtained from Sigma Aldrich Chemical Co. (St. Louis, United States). Doxorubicin was obtained from Libbs Farmacêutica (São Paulo, Brazil). The recombinant human follicle-stimulating hormone (r-hFSH) was obtained from Merk KGaA (Darmstadt, Germany). The recombinant human chorionic gonadotropin (r-hCG) was obtained from Meizler UCB Biopharma (São Paulo, Brazil). The fluorescent markers CellTracker® Blue and Mitotracker® Red were obtained from Invitrogen Corporation (Carlsbad, United States) and Molecular Probes (Melbourne, Australia), respectively. In Situ Cell Death Detection Kit, Fluorescein was obtained from Roche (Basel, Switzerland).

Animals and ethics

This study was approved by the Ethics Committee on Animal Use of the Federal University of Vale do São Francisco (protocol number: 0007/290519). Twenty-six Swiss mice (*Mus musculus*), 22 days old, with an average weight of 10 g, were housed in a climate-controlled environment at a temperature of 25°C, with alternating cycles of 12 hours of light and 12 hours of darkness. They had free access to food and water. The animals were euthanized by cervical dislocation, and their ovaries were collected and taken to the laboratory in MEM supplemented with HEPES and antibiotics (100 μg/ml penicillin and 100 μg/ml streptomycin).

Experimental design

The procedures used for preantral follicle culture followed previous studies (Lenie *et al.*, 2008; Sun *et al.*, 2008) with some modifications. Secondary follicles, ranging from 100 to 130 μm in diameter, were mechanically isolated through microdissection using 26-gauge (26G) needles coupled to 1 ml syringes. Morphologically normal follicles were selected and cultured individually in 25 μl drops of medium under mineral oil, using 60 mm petri dishes for 12 days, at 37°C with 5% CO_2. Isolated follicles were pooled and then randomly distributed to the treatment groups, with approximately 70 follicles per group (in both experiments).

This work was divided into two experiments conducted at different times. Experiment 1 was followed by experiment 2, and different pairs of ovaries were used for each experiment: 12 pairs for experiment 1, and 14 pairs for experiment 2. In both experiments, the base culture medium (control or α-MEM$^+$) consisted of α-MEM supplemented with 5% heat-inactivated foetal bovine serum, 5 ng/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, 10 mIU/ml r-hFSH and 1 mIU/ml r-hCG. In experiment 1, a low dose of doxorubicin was evaluated, and follicles were cultured in five treatments: control medium (α-MEM$^+$) alone, α-MEM$^+$ associated with 0.1 μg/ml doxorubicin (Morgan *et al.*, 2013), or α-MEM$^+$ supplemented with different concentrations of resveratrol (0.5, 2, or 5 μM; [Han *et al.*, 2020]) associated with 0.1 μg/ml doxorubicin. Based on these results, in experiment 2, a greater dose of doxorubicin was used to impair both follicle survival and growth. In addition, greater concentrations of resveratrol were evaluated alone or in association with doxorubicin. Follicles were cultured in six treatments: control medium (α-MEM$^+$) alone, α-MEM$^+$ associated with 0.3 μg/ml doxorubicin (Assis *et al.*, 2022), or α-MEM$^+$ supplemented with 5 or 10 μM of resveratrol (Han *et al.*, 2020; Nie *et al.*, 2020) alone or associated with 0.3 μg/ml doxorubicin. Exposure of preantral follicles to doxorubicin started on day 1 and lasted for 24 h, after which it was completely removed on day 2 of *in vitro* culture. Subsequently, every 2 days, half of the culture medium was replaced in all experimental groups.

Morphological analysis

During culture (days 0, 4, 8 and 12), the following endpoints were evaluated: morphology (survival), antrum formation and follicular diameter (growth). Follicles were considered morphologically normal when they were translucent, surrounded by two or more compact layers of granulosa cells, and showed no apparent damage to the basement membrane at the beginning of culture. Follicles that showed morphological signs of atresia, such darkening of the oocytes and/or surrounding granulosa cells or misshapen oocytes, were considered atretic. Antral cavity formation was defined as the appearance of a translucent cavity visible within the layers of granulosa cells. Follicular diameter (growth) was measured from the basement membrane, including two perpendicular measures of each follicle using an ocular micrometre attached to an inverted microscope. The growth rate was calculated as the diameter variation during the culture period.

Measurement of intracellular levels of active mitochondria, glutathione and reactive oxygen species

The intracellular levels of active mitochondria, GSH and ROS were measured using methods previously described (Silva *et al.*, 2023). Briefly, Mitotracker Red (Mitotracker® Red), 4-chloromethyl6.8-difluoro-7-hydroxycoumarin (CellTracker® Blue) and 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) were used to detect the levels of active mitochondria, GSH and ROS as red, blue and green fluorescence, respectively. After the culture, a pool of approximately 40 follicles per treatment was incubated in the dark for 30 min in drops of PBS, and with 100 nM Mitotracker Red, 10 μM CellTracker Blue and 10 μM H2DCFDA. Subsequently, the follicles were washed in PBS, and fluorescence was observed under

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an epifluorescence microscope (Nikon E200, Tokyo, Japan) with UV filters (579 nm for active mitochondria, 370 nm for GSH and 460 nm for ROS). The fluorescence intensity was analyzed using Image J software (Version 1.41; National Institutes of Health, Bethesda, Maryland, USA), and normalized to follicles of the control treatment group.

Assessment of DNA fragmentation by TUNEL assay

At the end of the culture in experiment 2, follicles from the control group and those treated with 10 µM of resveratrol combined with 0.3 µg/ml of doxorubicin were subjected to the terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay as previously described (Gouveia *et al.*, 2019). Briefly, after *in vitro* culture, follicles were fixed in 4% paraformaldehyde solution for 1 hour at room temperature and incubated in droplets of 100 µL of permeabilizing solution [0.1% (v/v) Triton X-100 in 10 mM PBS] for 3 hours at room temperature. Positive and negative controls were incubated in drops of 100 µL containing DNase-free Rnase at 37°C for 1 hour and washed three times in 50-µL drops of PBS-polyvinylpyrrolidone (PVP). The TUNEL assay was prepared approximately 15 minutes prior to use and stored at 4°C, as recommended by the manufacturer (In Situ Cell Detection Kit, Fluorescein). To prepare the assay, 12.5 µL of terminal deoxynucleotidyl transferase enzyme and 112.5 µL of the marker solution of 2-deoxyuridine triphosphate (dUTP) Fluorescein Isothiocyanate (5-FITC) were mixed to obtain 125 µL of the TUNEL reaction mixture. The experimental groups and the positive control were incubated with 15 µL of this solution for 1 hour at 37°C in a moist chamber in the dark. The negative control was incubated with 15 µL of the marker solution. Follicles were washed three times in 50-µL drops of PBS-PVP and incubated in droplets of 50 µL containing 10 mM Hoechst 33342 for 15 minutes at room temperature in the dark. Subsequently, follicles were washed in PBS-PVP, and slides were prepared for evaluation using an epifluorescence microscope (Nikon) at a magnification of ×400. DNA fragmentation was observed as green fluorescence chromatin.

Statistical analysis

Data regarding morphologically normal follicles (survival) and antral cavity formation were compared using Chi-square test, and the results were expressed as percentages. Data for follicular diameter, mitochondrial activity, GSH and ROS concentrations were evaluated using D'Agostino–Pearson or Shapiro–Wilk normality test, followed by Kruskal Wallis and Student-Newman Keuls tests. The differences were considered significant when $P<0.05$.

Results

Experiment 1

Follicular morphology (survival) and development

After 12 days of culture, exposure to doxorubicin did not affect ($p>0.05$) the percentage of normal follicles (63.04%) (Figure 1A) and antrum formation (35.87%) (Figure 1B) compared to the control medium (77.42% and 39.78% for survival and antrum formation, respectively). Interestingly, culture with 5 µM resveratrol + doxorubicin showed a greater ($p < 0.05$) percentage of follicular survival (92%) and antral cavity formation (78.87%) compared to the other experimental groups (71.43% and 67.08% of normal follicles and 25% and 62.02% of antrum formation for the 0.5 and 2 µM resveratrol + doxorubicin groups, respectively) (Figure 1A and 1B).

Follicles cultured in all media containing doxorubicin showed a decrease ($p < 0.05$) in diameter as early as day 4 of the culture. By day 12 of culture, doxorubicin decreased ($p < 0.05$) follicular diameter compared to the control medium (119.16 and 86.77 µm for control and doxorubicin groups, respectively) (Figure 1C). However, culture with 2 µM (92.96 µm) and 5 µM (98.12 µm) resveratrol associated with doxorubicin attenuated this damage, resulting in an increase ($p < 0.05$) in follicular diameter compared to the doxorubicin alone and 0.5 µM resveratrol + doxorubicin groups (84.63 µM). Additionally, 5 µM resveratrol + doxorubicin increased ($p < 0.05$) the growth rate compared to the groups cultured with doxorubicin alone or combined with 0.5 and 2 µM resveratrol (data not shown).

Mitochondrial activity and GSH concentration

Follicles cultured with doxorubicin showed lower ($p < 0.05$) mitochondrial activity and GSH concentrations compared to follicles in the control group. Nevertheless, culture with 2 or 5 µM resveratrol + doxorubicin increased ($p < 0.05$) mitochondrial activity and GSH concentrations compared to groups cultured with doxorubicin alone or 0.5 µM resveratrol + doxorubicin (Figure 2).

Experiment 2

Follicular morphology (survival) and development

On day 12, the increased dose of doxorubicin (0.3 µg/ml) dramatically reduced ($p < 0.05$) the percentage of normal follicles (18.05%) compared to the control medium (94.73%) or to the medium supplemented with 5 (93.82%) or 10 µM (72.5%) of resveratrol. The combination of resveratrol + doxorubicin attenuated follicular loss, presenting significantly greater percentages of normal follicles (29.68% and 33.33% for 5 and 10 µM of resveratrol + doxorubicin, respectively) than the group cultured with doxorubicin alone. In addition, 10 µM of resveratrol + doxorubicin increased ($p < 0.05$) the percentage of survival compared to follicles cultured with 5 µM of resveratrol + doxorubicin (Figure 3A).

Follicles cultured with doxorubicin alone did not reach antrum development on day 4 (Figure 3B) and showed a decrease ($p < 0.05$) in diameter as early as day 4 of the culture (Figure 3C). In addition, after 12 days, culture with doxorubicin alone or associated with resveratrol showed lower percentages of antrum formation and smaller follicular diameter compared to doxorubicin-free medium ($p < 0.05$).

Mitochondrial activity and GSH and ROS concentrations

Follicles cultured with doxorubicin alone or in combination with resveratrol showed similar mitochondrial activity ($p>0.05$) as follicles from the control group. However, culture with 5 µM of resveratrol increased ($p < 0.05$) mitochondrial activity compared to the other experimental groups. Intracellular GSH concentrations decreased ($p < 0.05$) after exposure to doxorubicin (alone or in combination with resveratrol) compared to doxorubicin-free medium (Figure 4). There was no difference ($p>0.05$) between the groups in relation to ROS concentrations.

DNA fragmentation after exposure to resveratrol and doxorubicin

All oocyte nuclei were stained by Hoechst 33342 (Figure 5). DNA fragmentation was not observed in the control (MEM+) and in the

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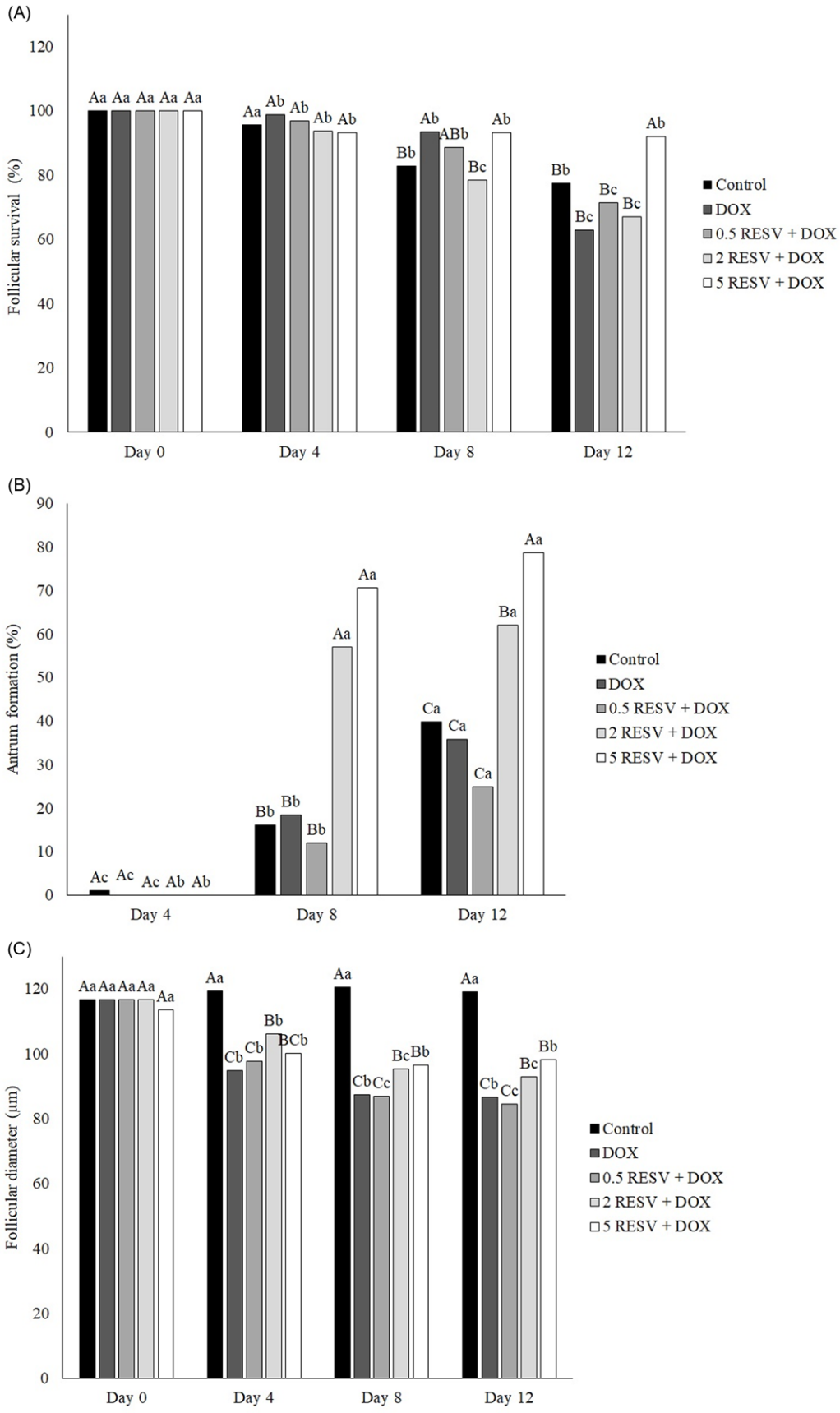

Figure 1. Follicular survival (A), antral cavity formation (B) and follicular diameter (C) after *in vitro* culture in control medium (α-MEM^{+}), medium supplemented with doxorubicin (0.1 µg/ml) or different concentrations of resveratrol (0.5, 2 and 5 µM) associated with doxorubicin. $^{(A, B)}$ indicates significant difference between treatments on the same culture day. $^{(a, b, c)}$ indicates significant difference between culture days within the same treatment ($p<0.05$).

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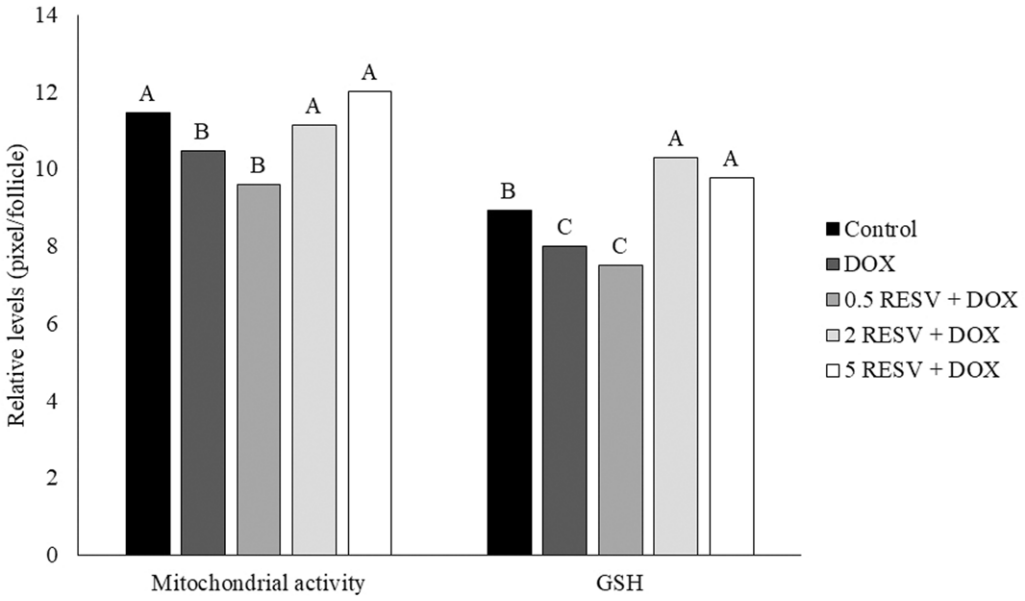

Figure 2. Intracellular levels (pixel/follicle) of mitochondrial activity and glutathione after *in vitro* culture in control medium (α-MEM^{+}) or medium supplemented with doxorubicin only (0.1 µg/ml) or different concentrations of resveratrol (0.5, 2 and 5 µM) associated with doxorubicin. $^{(A, B, C)}$ indicates significant difference between treatments within the same parameter ($p<0.05$).

groups treated with 10 µM resveratrol associated with 0.3 µg/ml doxorubicin.

Discussion

The toxic effects of doxorubicin on ovarian follicles were primarily demonstrated by assessing damage to ovarian tissue and reduced primordial follicles (Assis *et al.*, 2022; Silva *et al.*, 2023; Wang *et al.*, 2019). To our knowledge, this is one of the first studies to show differential effects of doxorubicin alone or in association with resveratrol on *in vitro* mouse follicular survival and growth.

In experiment 1, although it reduced follicular diameter, supplementation of the culture medium with 0.1 µg/ml doxorubicin for 24 h had no effect on the survival and antrum formation of secondary follicles. Contrary to our results, Morgan *et al.* (2013) reported increased percentages of degenerated follicles, especially affecting granulosa cells, when mouse ovarian tissue was cultured in medium with 0.1 µg/ml doxorubicin for up to 24 h. Therefore, it is suggested that the reduction in follicular viability caused by doxorubicin depends on the concentration and *in vitro* culture system used. Furthermore, the decrease in follicle diameter may have been caused by the ability of doxorubicin to inhibit the cell division of follicular cells, which can impair both oocyte growth and granulosa cell proliferation. Depending on the degree of damage caused by this chemotherapeutic agent to the follicular cells, the reduction in follicle diameter may be reversed by the addition of growth factors or hormones to the culture medium, which can stimulate follicular proliferation and growth (Cappeta *et al.*, 2018; Spears *et al.*, 2019; Sritharan and Sivalingam, 2021; Yu *et al.*, 2020).

The greatest concentration of resveratrol (5 µM) associated with doxorubicin increased follicular survival, antrum formation and GSH levels compared to the control while maintaining mitochondrial activity similar to the control (experiment 1). This could be attributed to resveratrol antioxidant properties, which help mitigate the harmful effects of increased oxidative stress, such as elevating levels of the endogenous antioxidant GSH, and preserving the mitochondrial function of follicular cells (Han *et al.*, 2020; Lastra; Villegas, 2007; Nie *et al.*, 2020). These findings suggest that a concentration of resveratrol greater than 5 µM could provide even more significant benefits for *in vitro* follicular survival and development. Furthermore, these results showed the importance of investigating the isolated effect of resveratrol on *in vitro* culture of mouse secondary follicles.

Hence, in the second experiment, a greater dose of doxorubicin was analyzed to impair both follicle survival and growth, in addition to different concentrations of resveratrol (5 or 10 µM), either alone or associated with doxorubicin. In this experiment, doxorubicin at 0.3 µg/ml significantly reduced the percentage of normal follicles and impaired antrum formation and growth (follicular diameter) compared to doxorubicin-free groups. Due to its non-specific action on cells, doxorubicin can harm non-cancerous cells, including ovarian cells, leading to follicle atresia in different stages of development through increased oxidative stress (Assis *et al.*, 2022; Mohan *et al.*, 2021; Silva *et al.*, 2023). Furthermore, doxorubicin induces cellular oxidative stress by increasing ROS production and/or reducing endogenous antioxidants, such as GSH (Spears *et al.*, 2019; Songbo *et al.*, 2019). In the present study, the concentration of GSH decreased in follicles cultured with doxorubicin, which may have contributed to the decline in follicular survival and development.

In experiment 2, supplementation of the culture medium with 5 µM resveratrol maintained follicular survival at levels similar to those in the control medium and significantly enhanced antrum development and mitochondrial activity compared to the other experimental groups. Mitochondria are responsible for regulating essential cellular processes, including antioxidant defence, metabolism, proliferation and apoptosis. These organelles play a fundamental role in modulating the development of oocyte capacity, fertilisation potential and embryonic development (Babayev and Seli, 2015; Grasso *et al.*, 2020). However, under cellular stress, mitochondrial gene suppression occurs, which can

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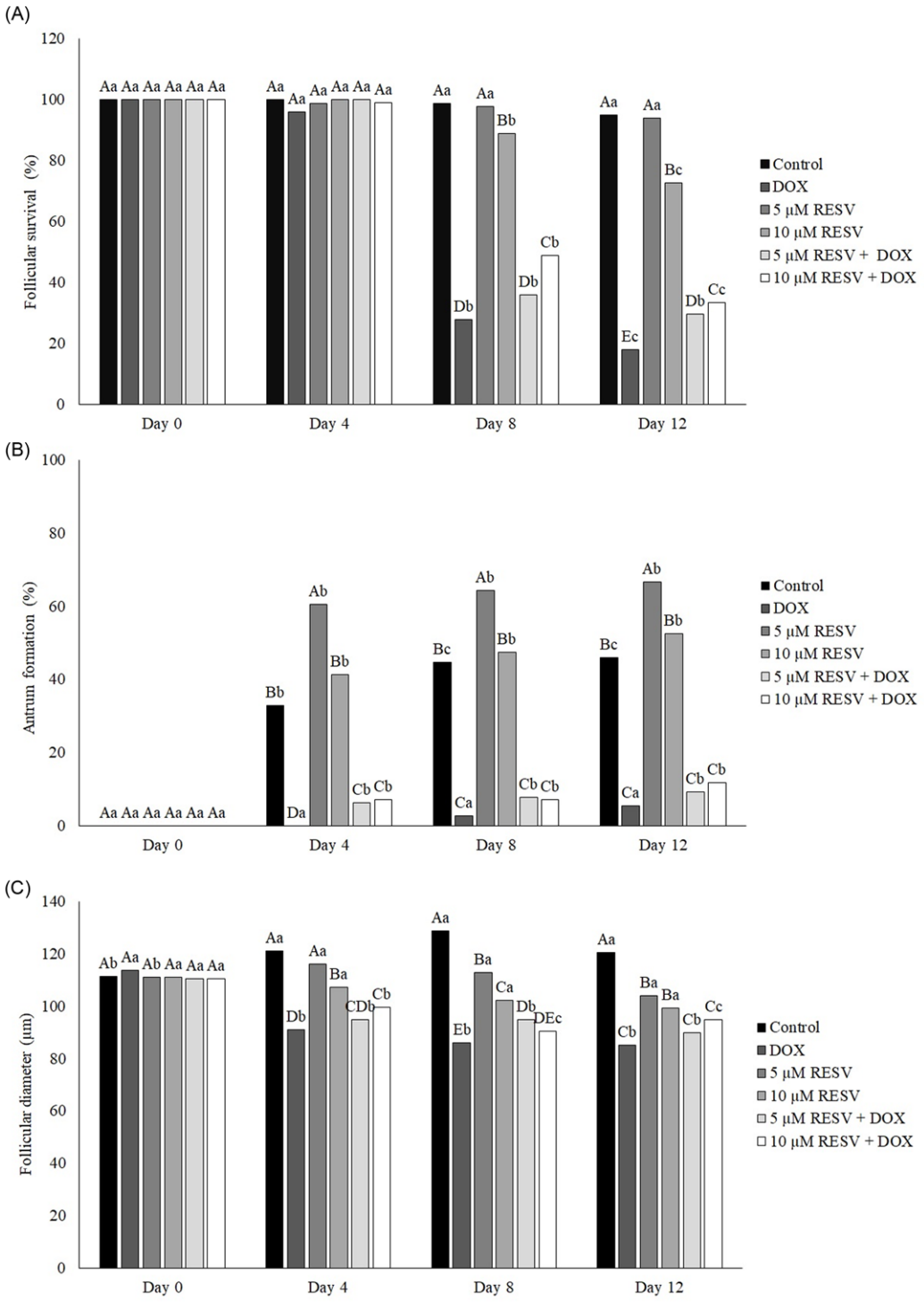

Figure 3. Follicular survival (A), antral cavity formation (B) and follicular diameter (C) after *in vitro* culture in control medium (α-MEM^{+}) or medium supplemented with different concentrations of resveratrol (5 and 10 µM) associated or not with doxorubicin (0.3 µg/ml).$^{(A, B, C, D, E)}$ indicates significant difference between treatments on the same culture day.$^{(a, b, c)}$ indicates significant difference between culture days within the same treatment ($p<0.05$).

result in a decrease in the number of these organelles in the body. In this sense, resveratrol is a notable compound with significant benefits for mitochondrial health. It can activate the Silent Information Regulator 2 homolog 1 (SIRT1) protein, a nicotinamide adenine dinucleotide (NAD+) dependent deacetylase. While SIRT1 primarily acts in the cell nucleus, it promotes mitochondrial biogenesis by deacetylating target proteins, thus reducing cellular stress (Nishigaki *et al.*, 2022). Takeo *et al.* (2014) demonstrated that resveratrol supplementation during *in vitro* maturation of bovine oocytes increased SIRT1 expression, leading to adenosine triphosphate synthesis and enhanced fertilization capacity. Supplementation of the culture medium of sheep ovarian tissue with resveratrol induced the activation of primordial follicles, increased granulosa cell proliferation and reduced DNA

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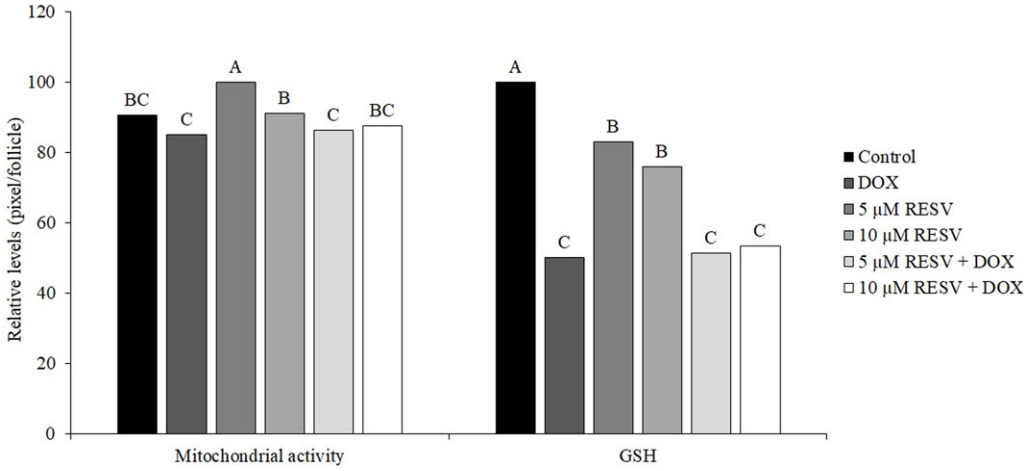

Figure 4. Intracellular levels (pixel/follicle) of mitochondrial activity and glutathione after *in vitro* culture in control medium (α-MEM^{+}) or medium supplemented with different concentrations of resveratrol (5 and 10 µM) associated or not with doxorubicin (0.3 µg/ml). $^{(A, B, C)}$ indicates significant difference between treatments within the same parameter ($p < 0.05$).

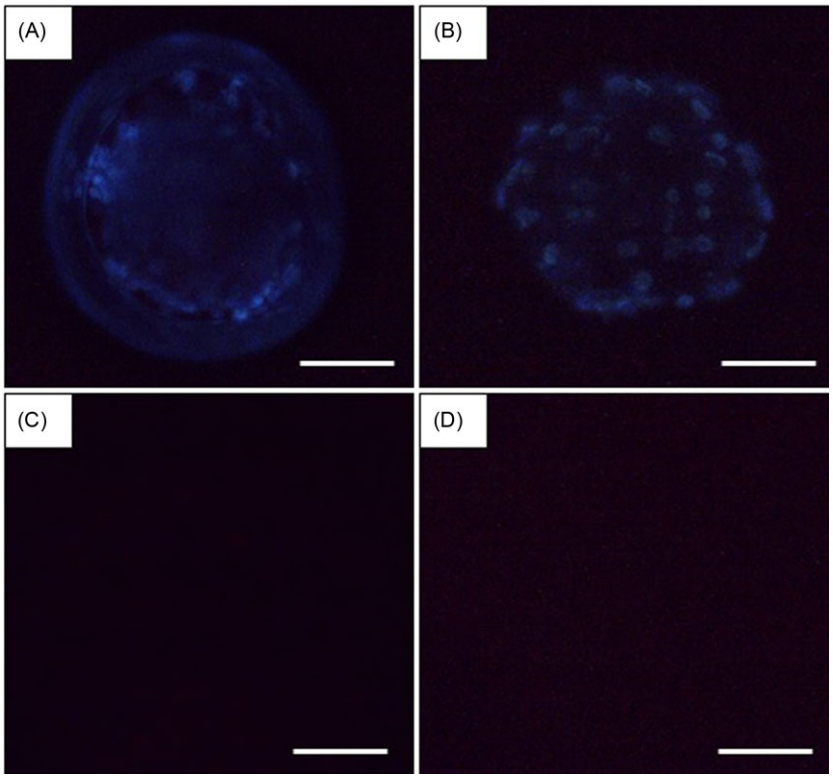

Figure 5. DNA fragmentation of murine follicles after 12 days of culture. Normal follicles in the control group (a, c) and 10 µM resveratrol associated with 0.3 µg/ml doxorubicin group (b-d). Follicles stained with Hoechst 33342 (a-b) and TUNEL (c-d). Scale bars: 50 µm.

damage (Bezerra *et al.*, 2018). Together, these results demonstrate the importance of this antioxidant in maintaining survival and promoting follicular development. However, in the current study, the highest concentration of resveratrol (10 µM) reduced follicular survival compared to the control. At this greater concentration and in the absence of the chemotherapeutic agent, the phenolic compound may be exerting a pro-oxidant effect. Similarly, Macedo *et al.* (2017) showed that the highest concentration of resveratrol (30 µM) during the *in vitro* culture of sheep secondary follicles resulted in DNA fragmentation and oxidative stress due to decreased mitochondrial activity.

Although both concentrations of resveratrol (5 and 10 µM) reduced the damage caused by doxorubicin to ovarian follicles by preserving follicular survival, the concentration of 10 µM resveratrol presented a greater percentage of normal follicles compared to 5 µM resveratrol. Furthermore, follicles cultured with 10 µM resveratrol associated with 0.3 µg/ml doxorubicin did not exhibit DNA fragmentation. These findings are in line with

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previous *in vitro* (50 μM) and *in vivo* (7 and 15 mg/kg) studies demonstrating that resveratrol reduces apoptosis of ovarian cells compared to treatments with only doxorubicin or cyclophosphamide (Herrero *et al*., 2023; Nie *et al*., 2020). In addition, increasing concentrations of resveratrol (0.5, 1 and 5 μM) significantly reduced cell apoptosis and improved oocyte survival compared to cells treated with doxorubicin (Han *et al*., 2020). However, in our study, culture with both concentrations of resveratrol (5 or 10 μM) combined with doxorubicin yielded similar levels of active mitochondria and GSH compared to the chemotherapeutic agent alone. We suggest that resveratrol might have preserved mitochondrial activity and GSH concentration sufficiently to sustain follicular survival, without significantly elevating these endpoints compared to the concentrations induced by doxorubicin. Furthermore, resveratrol may improve follicular survival through various mechanisms, including the activation of SIRT1, which regulates important cellular signalling pathways for follicular survival and apoptosis reduction (Ortega and Duleba, 2015). Thus, these results are promising, as they demonstrate that resveratrol can attenuate the toxic effects caused by doxorubicin by enhancing survival and protecting against DNA fragmentation.

In conclusion, supplementation of the culture medium with 0.3 μg/ml doxorubicin reduced the survival and impaired the development of isolated preantral follicles in mice. Nevertheless, resveratrol at 10 μM reduced doxorubicin-induced follicular atresia, without causing DNA fragmentation in the follicles. These findings suggest that resveratrol could serve as a potential treatment to protect against or attenuate doxorubicin-induced toxicity in the ovaries. Further investigations are necessary to assess whether pre-treatment with resveratrol affects the efficacy of cancer treatment.

Competing interests. None of the authors have any conflict of interest to declare.

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Ethical standards. The authors declare that all procedures were performed according to national and institutional guides and use of animals.

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