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Full Length Research Paper

Resveratrol protects against hazardous effects of 2-bromopropane on maturation of mouse oocytes, fertilization and fetal development

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2-Bromopropane (2-BP) is regularly used as an alternative to ozone-depleting cleaning solvents. Previously, we reported the cytotoxic effects of 2-BP on oocyte maturation and subsequent pre- and post-implantation development, both *in vitro* and *in vivo*. In the current study, we further demonstrate that these hazardous effects are suppressed by resveratrol, a grape-derived phytoalexin with known antioxidant and anti-inflammatory properties. Specifically, 2-BP treatment induced a significant reduction in the rates of oocyte maturation, fertilization, and *in vitro* embryonic development. Treatment of oocytes with 2-BP during *in vitro* maturation (IVM) resulted in increased resorption of post-implantation embryos and decreased fetal weights. Experiments with a mouse model disclosed that consumption of drinking water containing 20 µM 2-BP led to decreased oocyte maturation *in vivo* and fertilization *in vitro*, as well as impairment of early embryonic development. Notably, pretreatment with resveratrol prevented 2-BP-induced disruption of oocyte maturation and sequent embryonic development, both *in vitro* and *in vivo*. Our results collectively indicate that resveratrol has the potential to prevent the hazardous effects of 2-BP on embryos derived from pretreated oocytes.

Key words: 2-Bromopropane, resveratrol, apoptosis, oocyte maturation, embryonic development.

INTRODUCTION

2-Bromopropane (2-BP), a cleaning agent, is used as an alternative to ozone-depleting solvents. In 1995, 2-BP was reported to cause a series of reproductive and hematopoietic disorders in both female and male workers following exposure. Moreover, earlier reports revealed a high incidence of oligozoospermia in male workers after long-term exposure to 2-BP (Kim et al., 1996; Li et al., 2001; Park et al., 1997). Several animal studies further confirmed the potential of 2-BP in injuring the reproductive, hematopoietic, central nervous and immune systems (Ichihara et al., 1997; Kim et al., 2004; Omura et al., 1999; Son et al., 1999; Wu et al., 2002; Yu et al., 1999a; Yu et al., 1999b; Zhao et al., 2002). In cytotoxicity experiments, mouse embryos treated with 2-BP displayed micronuclei formation and decreased embryo cell number (Ishikawa et al., 2001). Moreover, 2-BP was recently identified as a potent DNA-damaging agent (Wu et al., 2002; Zhao et al., 2002). These results collectively suggest that 2-BP induces various toxicities via DNA damage.

A reproductive toxicity investigation further demonstrated that exposure to 2-BP induces testicular or ovarian dysfunction, causing injury to early types of spermatogenic cells or primordial follicles and oocytes of rats (Omura et al., 1999; Yu et al., 1999b). However, to date, no clinical or epidemiological studies or case reports have demonstrated a direct relationship between

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Abbreviations: 2-BP, 2-Bromopropane; **COCs,** cumulus-oocyte complexes; **IVM,** *in vitro* maturation; **IVF,** *in vitro* fertilization; **ICM,** inner cell mass; **TE,** trophectoderm; **RSVL,** resveratrol.

2-BP exposure of pregnant workers and reproductive problems. The major exposure route is via inhalation in the workplace or factory (Kim et al., 1996; Park et al., 1997). A recent study by our group showed that 2-BP induces cellular apoptosis in both the ICM and TE of mouse blastocysts, leading to decreased implantation, reduced embryonic development, and loss of embryo viability. We further demonstrated that 2-BP induces developmental injury via induction of apoptosis processes in oocyte maturation and early-stage embryos (Chan, 2010b). These findings clearly suggest that short-term exposure to 2-BP is a risk factor for normal mouse embryonic development, and may lead to inhibition of oocyte maturation in infertile subjects.

Resveratrol, a member of the phytoalexin family found in grapes and other dietary plants, inhibits tumor initiation and progression (Huang et al., 1999; Jang et al., 1997; Mgbonyebi et al., 1998). The compound exerts a wide range of pharmacological effects, including prevention of heart disorders, blocking of lipoprotein oxidation and inhibition of platelet aggregation (Frankel et al., 1993; Gusman et al., 2001; Pace-Asciak et al., 1996). The anti-tumor properties of resveratrol are attributed to its antioxidant activity and ability to inhibit activation of cyclooxygenases (Jang et al., 1997; Lin et al., 2008; Subbaramaiah et al., 1998), and may also be associated with a capacity to cause cell cycle arrest and apoptosis (Ahmad et al., 2001; Clement et al., 1998; Hsieh and Wu, 1999; Mader et al., 2010; Surh et al., 1999). In an earlier experiment, pretreatment with resveratrol prevented ethanol-, methylglyoxal- and 2-BP-induced disruption of embryonic development, both *in vitro* and *in vivo* (Chan, 2011; Chan and Chang, 2006; Huang et al., 2012).

In the current study, we explored whether resveratrol prevents a long-term injurious impact on the pre- and post-implantation stages of embryo development induced by short-term exposure to 2-BP at the oocyte stage. Our results clearly demonstrate that resveratrol effectively prevents the injurious effects of 2-BP exposure during the oocyte stage on oocyte maturation, *in vitro* fertilization and sequent embryonic development.

MATERIALS AND METHODS

Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), 2-bromopropane, resveratrol and pregnant mare serum gonadotropin (PMSG) were obtained from Sigma (St. Louis, MO). Human chorionic gonadotropin (hCG) was purchased from Serono (NV Organon Oss, The Netherlands). TUNEL *in situ* cell death detection kits were acquired from Roche (Mannheim, Germany), and CMRL-1066 medium from Gibco Life Technologies (Grand Island, NY).

COC collection and *in vitro* maturation (IVM)

ICR mice were acquired from the National Laboratory Animal Center (Taiwan, ROC). This research was approved by the Animal Research Ethics Board of Chung Yuan Christian University (Taiwan, ROC). Mice were maintained on breeder chow (Harlan Teklad chow) with food and water available *ad libitum*. Housing was provided in standard 28 cm × 16 cm × 11 cm (height) polypropylene cages with wire-grid tops, and maintained under a 12 h day/12 h night regimen. Cumulus-oocyte complexes (COCs) were obtained according to a previous protocol (Banwell et al., 2007). Briefly, COCs were isolated from female hybrid ICR mice (21 days old) injected with 5 IU human chorionic gonadotropin (hCG) 44 h prior to oocyte collection. COCs were collected in HEPES-buffered α minimum essential medium (MEM) (containing 50 μg/ml streptomycin sulfate, 75 μg/ml penicillin G, and 5% fetal bovine serum) by gently puncturing visible antral follicles present on the ovary surface. Germinal vesicle stage oocytes containing an intact vestment of cumulus cells were collected and pooled in at least 8 to 10 animals. For oocyte maturation, one drop (~100 μL) of buffer (αMEM supplemented with 50 μg/ml Streptomycin, 75 μg/ml penicillin G, 5% FBS and 50 U/ml recombinant human FSH) containing 10 COCs was added under oil in 35 mm culture dishes. COC maturation was analyzed following treatment with or without various concentrations of resveratrol and 2-BP for 24 h under an atmosphere of 5% O_2, 6% CO_2 and balance of N_2 at 37°C.

Maturation status assessment

After *in vitro* maturation (IVM), COCs of each group were treated with 50 U/ml ovine hyaluronidase and gently pipetted for the removal of all cumulus cells. Denuded oocytes were collected, and washed with fresh medium, followed by phosphate-buffered saline (PBS). Oocytes were fixed in ethanol: glacial acetic acid (1:3) for 48 h, and stained with 1% aceto-orcein solution. Nuclear structures were visualized using phase-contrast microscopy.

In vivo maturation

For obtaining *in vivo* matured oocytes, 21 day-old mice were injected with 5 IU equine chorionic gonadotropin (eCG) and 5 IU hCG, 61 and 13 h prior to fertilization, respectively. Mature ova were collected from the oviduct into HEPES-buffered α-MEM medium.

Effects of resveratrol and 2-BP intake on oocyte maturation in an animal model

The effects of 2-BP on oocytes were analyzed in 21 day-old ICR virgin albino mice. Female mice were randomly divided into four groups of 20 animals each, and administered a standard diet with or without 20 to 40 μM resveratrol and 20 μM 2-BP in drinking water for four days. COCs were collected by pre-treatment with 5 IU human chorionic gonadotropin (hCG) for 44 h prior to oocyte collection, and analyzed for oocyte maturation *in vitro* fertilization, and embryonic development.

In vitro fertilization

For *in vitro* fertilization, ova were washed twice in bicarbonate-buffered α-MEM medium (containing 50 mg/ml streptomycin, 75 mg/ml penicillin G and 3 mg/ml fatty acid free bovine serum albumin), and fertilized in the same medium with fresh sperm (obtained from a CBAB6F1 male donor). After incubation with sperm for 4.5 h, eggs were washed three times in potassium simplex optimized medium (KSOM) without amino acids in the presence of L-alanyl-L-glutamine (1.0 mM). Next, eggs were placed in 20 ml drops of KSOM under oil and cultured overnight. During cleavage to the 2-cell stage, embryos were transferred to a fresh drop of KSOM under oil, and cultured for another 72 h. In all

fertilization steps, embryo culture were additionally carried out under 5% O_2, 6% CO_2 and balance of N_2 at 37°C.

Fertilization assessment

For the examination of fertilization, ova were incubated with sperm for 4.5 h, followed by 3 h of culture in fresh medium. Zygotes were assessed for the presence of the male pronucleus with orcein staining as described previously (Banwell et al., 2007).

Cell proliferation

Cell proliferation was analyzed by dual differential staining, which facilitated the counting of cell numbers in inner cell mass (ICM) and trophectoderm (TE) (Chan, 2007; Huang et al., 2007; Pampfer et al., 1990). Blastocysts were incubated with 0.4% pronase in M2–BSA medium (M2 medium containing 0.1% bovine serum albumin) for the removal of zona pellucida. Denuded blastocysts were exposed to 1 mM trinitrobenzene sulfonic acid (TNBS) in BSA-free M2 medium containing 0.1% polyvinylpyrrolidone (PVP) at 4°C for 30 min, and washed with M2 (Hardy et al., 1989). Blastocysts were further treated with 30 µg/ml anti-dinitrophenol-BSA complex antibody in M2-BSA at 37°C for 30 min, followed by M2 supplemented with 10% whole guinea pig serum as a source of complement, along with 20 µg/ml bisbenzimide and 10 µg/ml propidium iodide (PI) at 37°C for 30 min. The immunolysed blastocysts were gently transferred to slides, and protected from light before observation. Under UV light, ICM cells (which took up bisbenzimide but excluded PI) appeared blue, whereas TE cells (which took up both fluorochromes) appeared orange-red. Since multinucleated cells are not common in pre-implantation embryos (Gardner and Davies, 1993), the number of nuclei represent an accurate measurement of cell number.

TUNEL assay of blastocysts

For terminal transferase dUTP nick end labeling (TUNEL) staining, embryos were washed in 2-BP-free medium, fixed, permeabilized and subjected to labeling using an *in situ* cell death detection kit (Roche Molecular Biochemicals, Mannheim, Germany), according to the manufacturer's protocol. Photographic images were obtained with a fluorescence microscope under bright-field illumination.

Blastocyst development following embryo transfer

To determine the ability of expanded blastocysts to implant and develop *in vivo*, embryos generated were transferred to recipient mice. ICR females (6-8 week-old, white skin) were mated with vasectomized males (C57BL/6J; black skin; National Laboratory Animal Center, Taiwan, ROC) to produce pseudopregnant dams as recipients for embryo transfer. To ensure that all fetuses in pseudopregnant mice were derived from embryo transfer (white color) and not fertilization by C57BL/6J (black color), we examined skin color at day 18 post-coitus. To assess the impact of resveratrol and 2-BP on post-implantation growth *in vivo*, COCs were exposed to various concentrations of resveratrol and 2-BP as indicated for 24 h, followed by fertilization and *in vitro* maturation to the blastocyst stage. Subsequently, eight untreated control embryos were transferred to the left uterine horn, and eight 2-BP-treated embryos to the right uterine horn in day 4 pseudopregnant mice. 40 surrogate mice were analyzed and killed on day 18 post-coitus, and the frequency of implantation was calculated as the number of implantation sites per number of embryos transferred. The incidence rates of resorbed and surviving fetuses were calculated as number of fetuses per number of implantations, respectively. The weights of the surviving fetuses and placenta were measured immediately after dissection.

Statistical analysis

Data were analyzed using one-way ANOVA and t-tests, and presented as means ± SEM. Data were considered statistically significant at $P < 0.05$.

RESULTS

In the current study, we observed a lower oocyte maturation rate in the 5 µM 2-BP-treated oocyte group. Moreover, pretreatment with 10 and 20 µM resveratrol effectively prevented 2-BP-induced embryonic development injury (92.6, 61.5 and 74.5% metaphase II maturation in control group, 2-BP-treated and 10 µM resveratrol+2-BP-treated group, respectively) (Figure 1). Male pronucleus formation was assessed for detection of fertilization. The ability of oocytes to be fertilized by fresh sperm was significantly decreased upon pretreatment with 2-BP prior to IVM, and rescued by pretreatment with resveratrol (90.8, 49.5 and 70.8% fertilization rate in control group, 2-BP-treated and 10 µM resveratrol+2-BP-treated group, respectively) (Figure 1). Furthermore, 2-BP pretreatment led to a significant decrease in oocyte cleavage to the two-cell stage and the number of embryos cleaving to form blastocysts, compared to the untreated control group (Figure 1). The cytotoxicity of short-term treatment of oocytes with 2-BP was significantly prevented upon co-incubation with resveratrol (Figure 1).

The effects of resveratrol on cell proliferation in 2-BP-treated oocytes were further investigated. Differential staining, followed by cell counting was employed to assess cell proliferation. Significantly lower blastocyst cell numbers were derived from 2-BP-pretreated oocytes, compared to control oocytes. The observed inhibition of cell proliferation by 2-BP was prevented in the 10 or 20 µM resveratrol-treated groups (Figure 2A). Blastocysts derived from 2-BP-pretreated oocytes were additionally evaluated for apoptosis. TUNEL staining and quantitative analysis disclosed increased apoptosis of blastocysts derived from the 2-BP-pretreated oocyte group, which was significantly prevented by resveratrol (Figure 2B and C).

Embryos were transferred to 50 recipients per group (8 per horn). In total, 40 recipients were pregnant in at least one horn at day 18. The implantation ratio decrease of blastocysts derived from the oocyte group treated with 2-BP was significantly prevented upon co-incubation with resveratrol (Figure 3A). Embryos that implanted but failed to develop were subsequently resorbed in the uterus. The proportion of implanted embryos that failed to develop normally was significantly higher in the 5 µM 2-BP-treated group than the control group. Moreover, treatment with

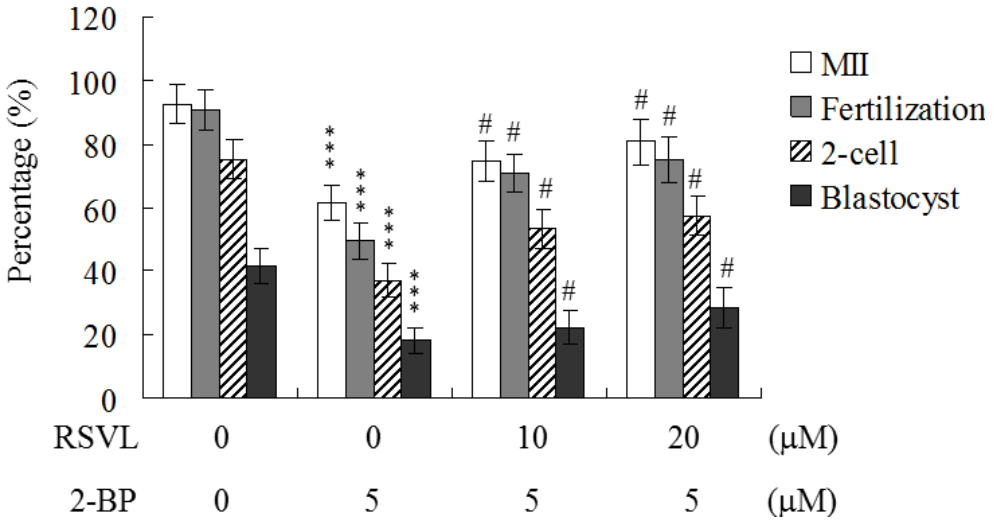

Figure 1. Effect of resveratrol on 2-Bromopropane (2-BP)-induced hazardous effects in mouse oocyte maturation and embryo development *in vitro*. Oocytes were collected from 21 day-old mice, cultured for 24 h in IVM medium containing resveratrol (RSVL; 10-20 μM) and/or 2-BP (5 μM) , fertilized *in vitro*, and transferred to *in vitro* culture (IVC) medium. Oocyte maturation, *in vitro* fertilization, cleavage and blastocyst development were analyzed. Values are presented as means ± SEM of eight determinations. Data are based on 280-300 samples per group. ***P < 0.001 versus the untreated control group. # *P* < 0.001 *vs.* the 5 μM 2-BP-treated only group.

resveratrol rescued this development injury (Figure 3A). Embryo survival rate (surviving fetuses) in the 2-BP-treated group surviving to post-coitus day 18 was markedly lower than that in the untreated control group, and pre-incubation with resveratrol prevented 2-BP-triggered development of hazardous effects (Figure 3A). Interestingly, placental weights of blastocysts derived from 2-BP-treated or resveratrol and 2-BP-treated oocytes in IVM were not significantly different from those of the control group (Figure 3B), while fetal weights were lower in groups treated with 2-BP, relative to the untreated control. Co-incubation with resveratrol effectively prevented fetal weight loss caused by 2-BP (Figure 3C). Our findings clearly indicate that exposure of oocytes to 2-BP during IVM reduces post-implantation development potential, which can be prevented by resveratrol.

In view of the protective effects of resveratrol on 2-BP-induced oocyte maturation and embryo development *in vitro*, we assessed its activity *in vivo* via intake in an animal model. Female mice were fed a standard diet and drinking water supplemented with resveratrol (10-20 μM) and/or 2-BP (20 μM) for 10 days or left untreated, prior to COC collection. Oocyte maturation status, fertilization rate and *in vitro* embryo development were further evaluated. Dietary 2-BP induced a significant decrease in oocyte maturation and fertilization, resulting in inhibition of embryonic development from the zygote to blastocyst stage, which was effectively prevented by intake of resveratrol (Figure 3D).

To further determine the effects of resveratrol on 2-BP-induced embryo implantation and post-implantation development injury, we analyzed resveratrol activity *in vivo* via intake and transfer of blastocyst stage embryos to the uterus horn using the embryo transfer assay in an animal model. Notably, the implantation ratio of blastocysts in the resveratrol/ 2-BP intake group was significantly higher than that of blastocysts in the 2-BP intake group (Figure 4A). The 2-BP intake group displayed a higher overall resorption rate than the resveratrol/2-BP intake group (Figure 4A). Moreover, the embryo survival rate of the resveratrol/2-BP intake group was markedly higher than that of 2-BP intake group (Figure 4A). Interestingly, the placental weights of blastocysts derived from all four groups were not significantly different (data not shown), while fetal weights of the resveratrol/2-BP intake groups were higher than those of the 2-BP intake (20 μM) group (Figure 4B).

DISCUSSION

During the complex and precisely orchestrated process of embryonic development, chemical or physical injury can affect normal progression, leading to malformation or miscarriage of the embryo. Exposure to 2-BP induces degeneration of germ cells via activation of apoptosis, with spermatogonia as the major target cells (Li et al., 2001; Yu et al., 2001). In a previous animal study investigating the cytotoxic effects of 2-bromopropane (2-BP) on rat development, rats were exposed to 0 to 1000 ppm 2-BP via inhalation for 6 h per day, seven days a

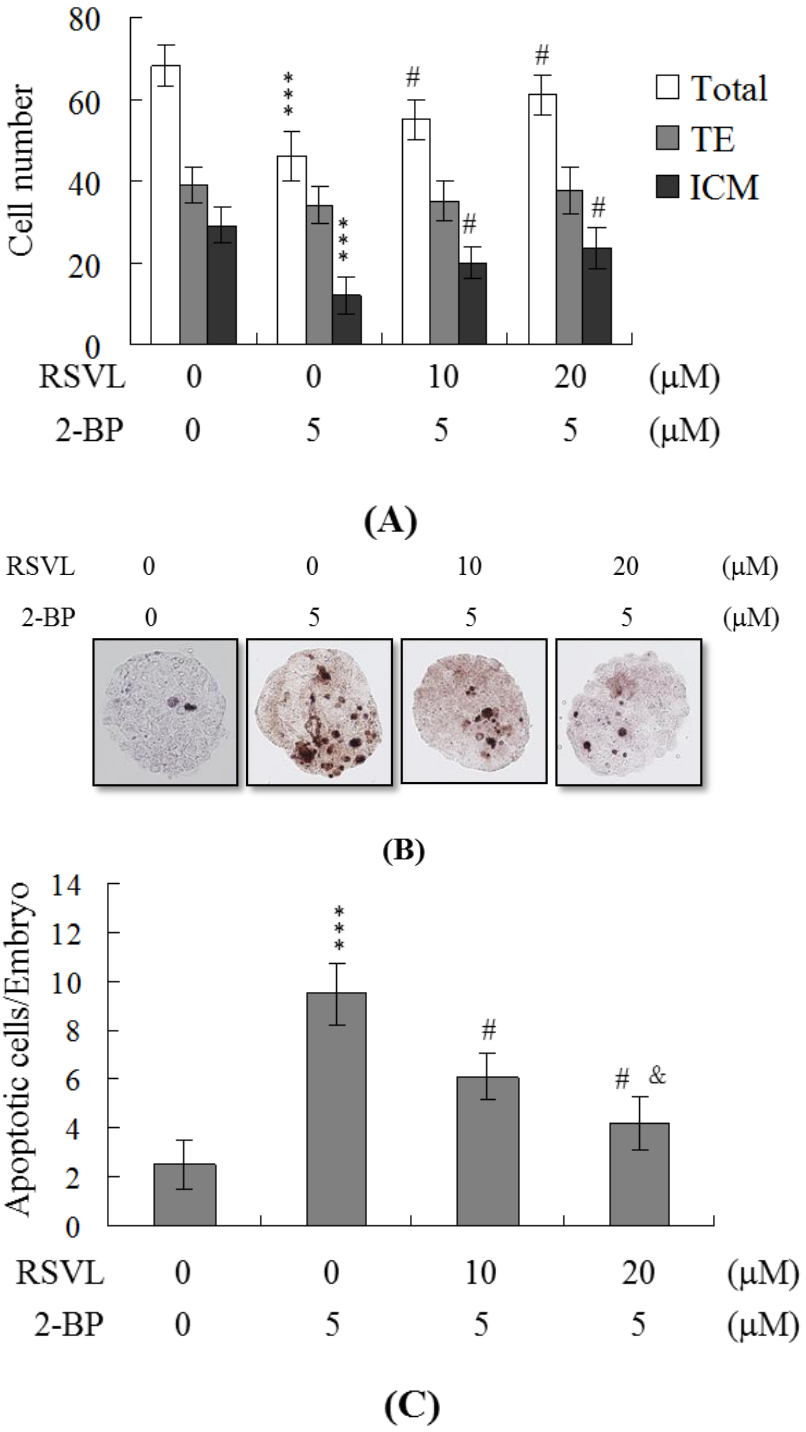

Figure 2. Effects of resveratrol on cell number and apoptosis in embryos during IVM of 2-BP-treated oocytes. Oocytes were cultured for 24 h in IVM medium containing resveratrol (RSVL; 10 to 20 μM) and/or 2-BP (5 μM), fertilized *in vitro* and transferred to *in vitro* culture (IVC) medium for *in vitro* development. (A) Cell numbers of total, trophectoderm (TE) lineages and inner cell mass (ICM) were counted in blastocysts. (B) Apoptotic cells were examined at the blastocyst stage using TUNEL staining, followed by light microscopy. Positive cells are depicted in black. (C) The mean number of apoptotic (TUNEL-positive) cells per blastocyst was calculated. Values are presented as means ± SEM of ten determinations. Data are based on at least 250 samples in each group. ***P < 0.001 versus the untreated control group. $^{\#}P < 0.001$ *vs.* the 5 μM 2-BP-treated only group. $^{\&}P < 0.001$ *vs.* the 10 μM RSVL+5 μM 2-BP-treated group.

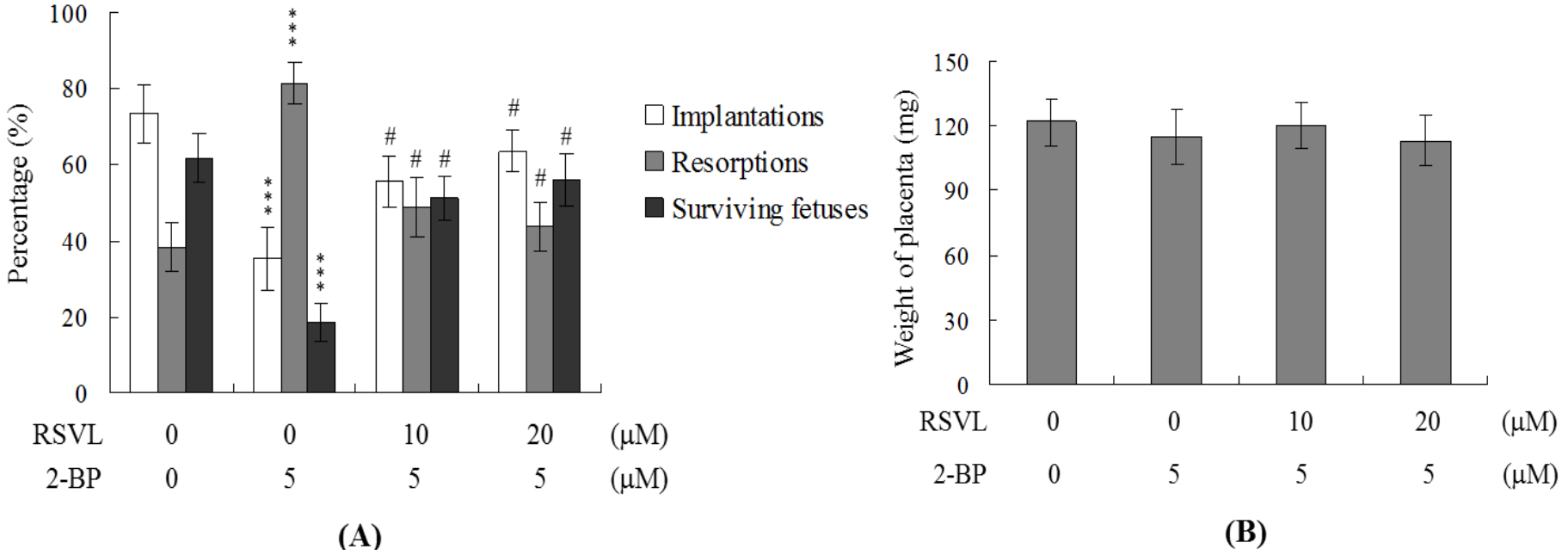

Figure 3. Effects of resveratrol treatment or dietary resveratrol on embryo development during 2-BP-treated oocyte IVM. Oocytes were cultured for 24 h in IVM medium containing resveratrol (RSVL; 10-20 μM) and/or 2-BP (5 μM), fertilized *in vitro*, and transferred to *in vitro* culture medium for development. (A) Implantation, resorption and surviving fetuses were analyzed as described in Materials and Methods. The implantation percentage represents the number of implantations per number of transferred embryos × 100. The percentage of resorption or surviving fetuses represents the number of resorptions or surviving fetuses per number of implantations × 100. (B) Placental weights of 40 recipient mice were measured. (C) Weight distribution of surviving fetuses at day 18 post-coitus. Surviving fetuses were obtained by embryo transfer of control, 2-BP-, resveratrol/2BP-pretreated groups, as described in materials and methods (320 total blastocysts across 40 recipients). (D) Random female mice were fed a standard diet and drinking water supplemented with resveratrol (20-40 μM) or/and 2-BP (20 μM) for 10 days or left untreated. Oocytes were collected for *in vitro* maturation, *in vitro* fertilization, cleavage, and blastocyst development analyses. Data are based on at least 280 samples in each group. ***P < 0.001 versus the untreated control group. $^{\#}$ *P* < 0.001 *vs.* the 2-BP-treated only group. $^{\&}$*P* < 0.001 *vs.* the 10 μM RSVL+5 μM 2-BP-treated group.

week for two weeks, prior to mating, during the mating period until copulation, and during days 0 to 19 of gestation. 2-BP inhaled at a concentration of 1000 ppm significantly suppressed the number of live fetuses, signifying fetal lethality during the post-implantation period (Takeuchi et al., 2004). *In vitro* treatment with 5 or 10 μM 2-BP was associated with increased resorption of post-implantation embryos as well as decreased placental and fetal weights (Chan, 2010a). Moreover, 2-BP induced a significant reduction in the rates of oocyte maturation, fertilization, and *in vitro* embryonic development (Chan, 2010b). Notably, pretreatment of 2-BP-treated oocytes with resveratrol *in vitro* or consumption of drinking water containing resveratrol *in vivo* resulted in effective suppression of 2-BP-induced hazardous effects on oocyte maturation, *in vitro* fertilization and sequent embryonic development (Figures 1 to 3). Previous studies reported that 2-BP induces major ovarian dysfunction by causing primordial follicle damage and suppressing oocyte maturation in rats via cell apoptotic processes (Yu et al., 1999b). A recent study by our group further confirmed that the pretreatment with a caspase-3-specific inhibitor effectively prevented 2-BP-triggered hazardous effects, suggesting that embryonic impairment by 2-BP occurs via a caspase-dependent apoptotic process.

TE arises from the trophoblast at the blastocyst

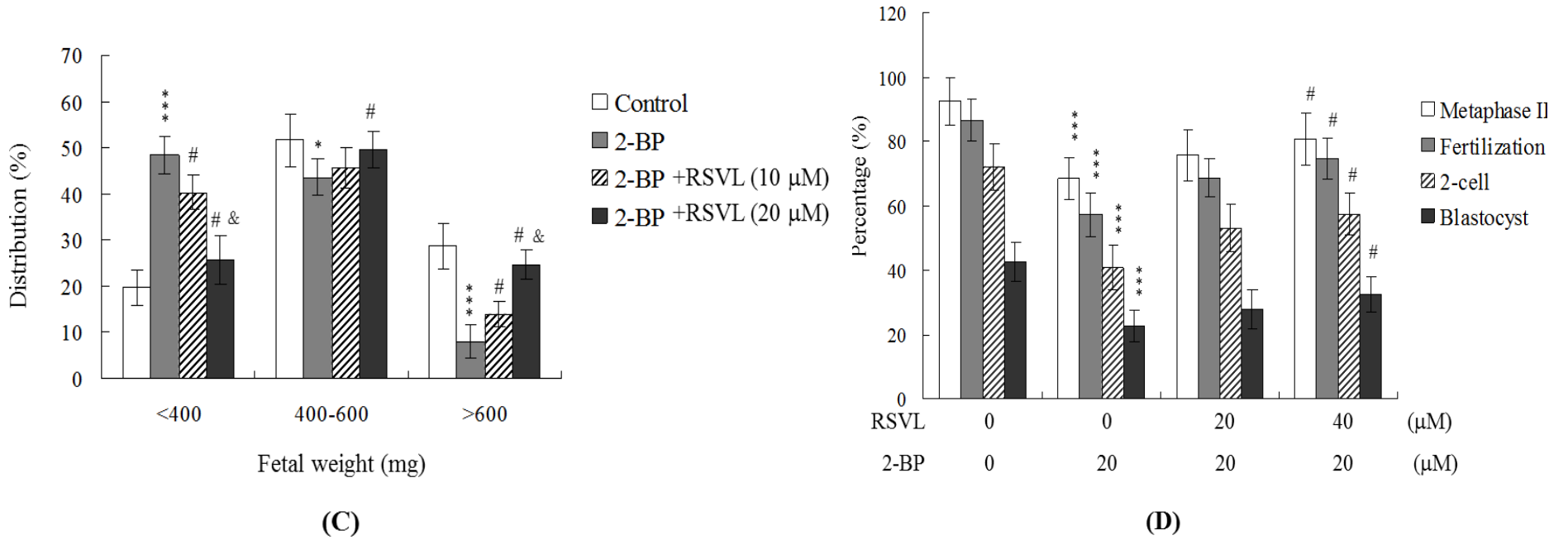

Figure 3. Continued.

stage and develops into a sphere of epithelial cells surrounding the ICM and blastocoel. These cells contribute to the placenta and are required for mammalian conceptus development (Cross et al., 1994). Reduction in cells of TE and/or ICM lineage leads to suppressed implantation and lower embryonic viability (Kelly et al., 1992; Pampfer et al., 1990). ICM and total blastocyst cell numbers were positively correlated with embryonic development during the embryo transfer assay (Lane and Gardner, 1997). In our study, application of 2-BP during oocyte maturation had no effect on the TE cell numbers of blastocysts, but led to a dramatic decrease in ICM and total (TE plus ICM) cell numbers (Figure 2). Thus, it appears that 2-BP treatment during IVM triggers mortality and/or developmental delay in post-implantation mouse embryos via ICM cell death or suppression of proliferation (Figures 2 and 3). Interestingly, blastocysts derived from 2-BP-treated oocytes displayed decreased implantation, increased embryo resorption and a lower fetal survival rate (Figure 3A). Furthermore, pre-treatment with resveratrol prevented 2-BP-triggered decrease of ICM cell numbers (Figure 2A) and embryo implantation (Figure 3A). Our results demonstrate that the decrease in ICM cell number induced by 2-BP during oocyte maturation is the major injurious factor leading to inhibition of embryonic development, and resveratrol is effectively able to rescue 2-BP-induced injury.

A recent study reported that 2-BP induces DNA damage, impairs functional antioxidant cellular defenses, and enhances the lipid peroxidation process in primary cultures of rat Leydig cells (Wu et al., 2002). Melatonin protected against 2-BP-induced sperm injuries via reactive oxygen species (ROS) scavenging and anti-apoptotic effects, as observed from morphological, biochemical and histopathological features and apoptosis assays (Huang et al., 2009). Our group previously showed that blockage of 2-BP-induced hazardous effects on embryonic development by resveratrol in mouse blastocysts (Chan, 2011). We additionally reported that 2-BP can induce ROS generation directly and through mitochondrion-dependent apoptotic pathways to trigger cell death in mouse blastocysts (Chan, 2010a). These results indicate that 2-BP triggers embryonic development injury through ROS generation and apoptosis, and its hazardous

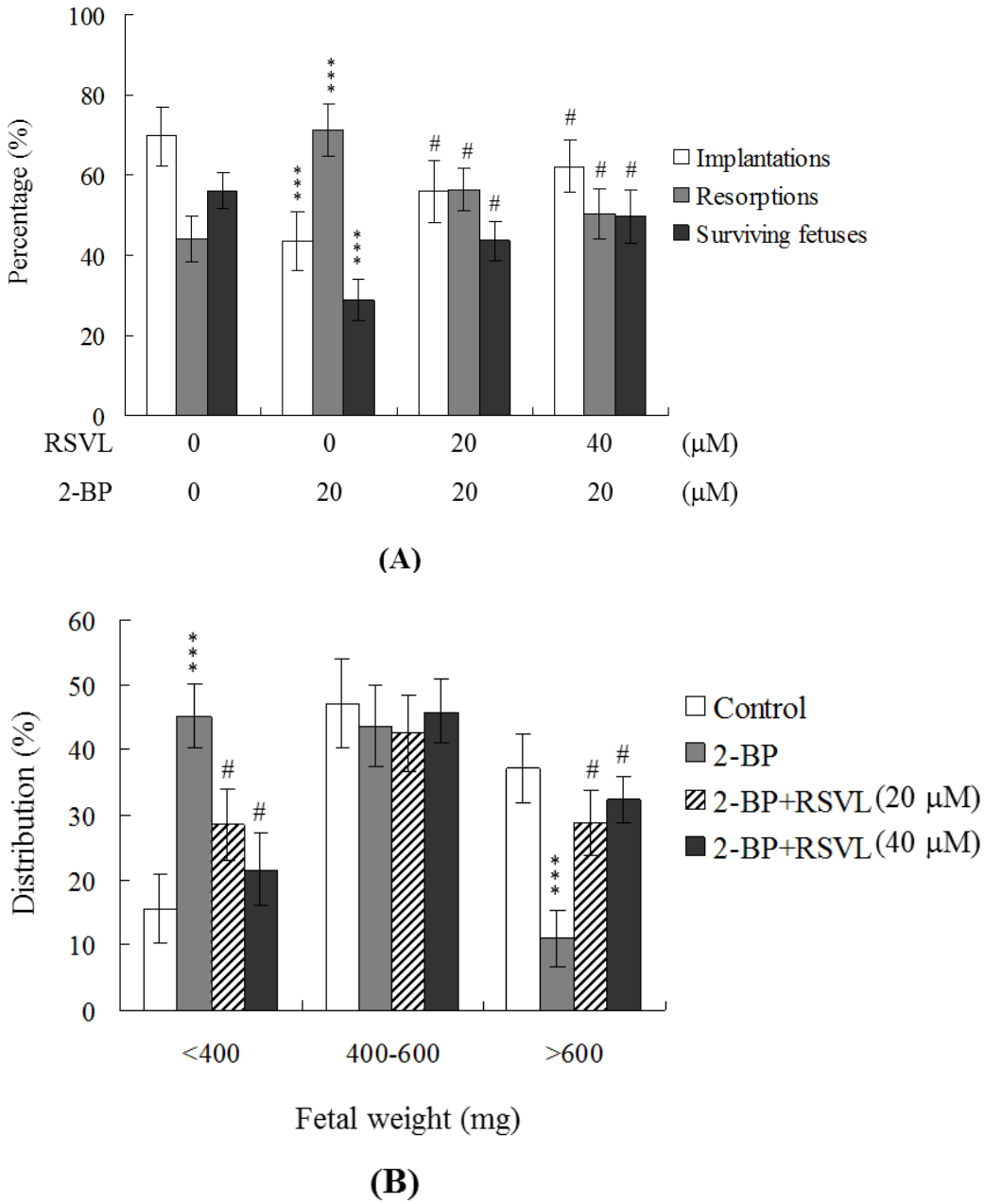

Figure 4. Effects of dietary resveratrol and 2-BP on embryo development in mouse blastocysts. Random female mice were fed a standard diet and drinking water continuously supplemented with resveratrol (RSVL; 20-40 μM) and/or 2-BP (20 μM) or left untreated for 10 days before embryo transfer to the uterus during the experimental period. (A) Implantation, resorption and surviving fetuses were analyzed, as described in Materials and Methods. The implantation percentage represents the number of implantations per number of transferred embryos × 100. The percentages of resorption or surviving fetuses represent the number of resorptions or surviving fetuses per number of implantations × 100. (B) Weight distribution of surviving fetuses at day 18 post-coitus. Surviving fetuses were obtained via embryo transfer of control, resveratrol/2-BP, and 2-BP intake groups, as described in Materials and Methods (320 total blastocysts across 40 recipients). ***P < 0.001 versus the control group. $^{\#}P < 0.001$ *vs.* the 2-BP-intake only group.

effects can be prevented by antioxidants at the blastocyst stage.

Inhibition of the apoptotic biochemical changes triggered by various stimuli by resveratrol is attributed to its antioxidant properties (Chan and Chang, 2006; Chen and Chan, 2009; Huang et al., 2007; Jang and Surh,

2001). Resveratrol exerts a powerful antioxidant effect against multiple forms of ROS (O_2^- and H_2O_2) produced in macrophages stimulated with lipopolysaccharides or phorbol esters, which induce O_2- synthesis via the NADPH oxidase pathway (Martinez and Moreno, 2000). Our recent experiments demonstrated that resveratrol attenuates ethanol- or 2-BP-induced ROS generation and apoptosis in mouse embryos, supporting the hypothesis that the compound acts via inhibition of ROS production (Chan, 2011; Huang et al., 2007). Here, our results implied that resveratrol prevents 2-BP-induced oocyte maturation injury via antioxidant activity, and support its potential for development as a health food to protect against the embryonic hazardous effects of 2-BP.

However, further studies are required to determine the precise regulatory mechanisms of resveratrol.

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