Resveratrol improves in vitro maturation of oocytes in aged mice and humans

Mei-Ju Liu, M.D., Ph.D.,[a,b,c,d] Ai-Gang Sun, M.D.,[e] Shi-Gang Zhao, M.D., Ph.D.,[a] Hui Liu, M.S.,[a] Shui-Ying Ma, M.D., Ph.D.,[a] Mei Li, M.D., Ph.D.,[a] Ying-Xue Huai, M.S.,[a] Han Zhao, M.D., Ph.D.,[a] and Hong-Bin Liu, Ph.D.[a]

[a] Center for Reproductive Medicine and [b] Key Laboratory of Reproductive Endocrinology, Shandong University, Jinan; [c] National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Jinan; and [d] Department of Reproductive Medicine and [e] Department of Neurological Surgery, Linyi People's Hospital, Linyi, People's Republic of China

Objective: To evaluate the effects of resveratrol on oocyte maturation in aged mice and humans.
Design: Experimental laboratory study.
Setting: University-based reproductive medicine center.
Patient(s): A total of 64 women 38–45 years of age undergoing intracytoplasmic sperm injection (ICSI) and 48–52-week-old female C57BL/6J mice.
Intervention(s): In vitro culture in the presence of three different concentrations of resveratrol (0.1, 1.0, and 10 μm) or dimethylsulfoxide.
Main Outcome Measure(s): Parameters of oocyte nuclear maturation, fertilization, immunofluorescence intensity of mitochondria, and normal morphology of spindle and chromosome of oocytes undergoing in vitro maturation (IVM) in aged mice and humans; blastocyst formation and levels of *SRIT1*, *CAT*, *SOD1*, and *GPX4* gene expressions in aged mice.
Result(s): Resveratrol at 1.0 μm significantly increased first polar body emission rate in oocytes derived from aged mice and humans, and an increased percentage of fertilization and blastocyst formation was observed in aged mice. In addition, immunofluorescence intensity of mitochondria and normal morphology of spindle and chromosome of oocytes undergoing IVM were notably improved compared with control samples in aged mice and human. Furthermore, the use of resveratrol exhibited enhanced expression patterns of *SRIT1*, *CAT*, *SOD1*, and *GPX4* in aged mice.
Conclusion(s): Resveratrol induced oocyte maturation and blastocyst formation in aged mice, and improved oocyte maturation and quality was examined in aged humans. In conclusion, 1.0 μm resveratrol was the appropriate concentration in IVM medium. (Fertil Steril® 2018;109:900–7. ©2018 by American Society for Reproductive Medicine.)
Key Words: Oocyte, IVM, resveratrol, aged mice, aged humans

Mitochondria play important roles in cellular energy metabolism in oocytes (1). Adenosine triphosphate (ATP) is produced in oocytes by oxidative phosphorylation and is necessary for several functions including maintenance of cellular homeostasis, meiosis regulation of apoptosis, and calcium signaling (1–4).

It is well established that female fecundity declines with increasing maternal age. Such reproductive aging seems to be largely due to age-related changes in ovarian function and decay in oocyte quality. Decreasing numbers of oocytes, coinciding with diminished quality, dictate the gradual changes in reproductive aging (5, 6) and the final occurrence of natural sterility (7, 8). As an indispensable contributor to cellular energy metabolism in oocytes, mitochondria are also necessary for maintenance of calcium homeostasis and regulation of apoptosis. Consequently, mitochondrial dysfunction is responsible for molecular and cellular failures of aged oocytes and infertility (9). In addition, an increased rate of aneuploidy results from anomalies in

Received November 1, 2017; revised January 10, 2018; accepted January 16, 2018.
M.-J.L. has nothing to disclose. A.-G.S. has nothing to disclose. S.-G.Z. has nothing to disclose. H.L. has nothing to disclose. S.-Y.M. has nothing to disclose. M.L. has nothing to disclose. Y.-X.H. has nothing to disclose. H.Z. has nothing to disclose. H.-B.L. has nothing to disclose.
Supported by the National Key Research and Development Program of China (2017YFC1001500), the National Natural Science Foundation of China (31371453, 31571548, 81490743, and 81771538), the Young Scholars Program of Shandong University (2015WLJH54, 2016WLJH50), and the Shandong Medical and Health Technology Development Program (2016WS0226).
Reprint requests: Hong-Bin Liu, Ph.D., 44 Wenhua Xi Road, Jinan, Shandong, 250012, People's Republic of China (E-mail: liuhongbin@sduivf.com).

Fertility and Sterility® Vol. 109, No. 5, May 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.01.020

the meiotic spindle, and chromosome misalignment leads progressively to age-related loss of fertility (10).

With the significantly decreased number of mature oocytes in aged humans, growing follicles in the ovaries constitute a resource for embryos. Decreased oocyte quality (cytoplasmic and nuclear) is one of the main factors causing infertility in aged humans. In clinic, many approaches have been taken for improving/rescuing fertility in aged women, including in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI). It is important to develop oocytes from early antral follicles (EAFs) to fully grown follicles with current improvements in vitro culture techniques. However, the maturation and formation rates of blastocysts from germinal vesicle (GV)–stage oocytes are low. Because improvement of blastocyst maturation and developmental competence of EAFs is important to promote fertility, further exploration of culture techniques is undoubtedly necessary (11).

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally potential antiaging polyphenolic compound from plants and is most notably present in red wine. It has been reported that resveratrol enhances the activity of Sirt1, inhibits phosphodiesterase, and promotes mitochondrial function (12). Over the past couple of decades, in vitro and in vivo studies have highlighted a variety of biologic properties including antioxidant, antiinflammatory, anticarcinogenic, and antiproliferative effects (13, 14). Because the effective role of antioxidants in preventing aging-associated oocyte aneuploidy has been demonstrated, numerous studies have attempted to identify the potential benefits of resveratrol in oocytes. A recent in vivo study showed that resveratrol significantly improved the number and quality of oocytes (15). However, whether or not resveratrol has a role in maturation of GV-stage oocytes and in the developmental ability of embryos and blastocyst formation in aged animals still needs to be studied. In the present study, we assessed the effects of resveratrol on in vitro maturation (IVM) of oocytes and subsequent formation of blastocysts with the use of animal models. The results were further verified in oocytes from aged humans.

MATERIALS AND METHODS

Ethics Approval

This study was approved by the Institutional Review Board (IRB) of Reproductive Medicine, Shandong University. All procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutional Research Committee and with the 1964 Helsinki declaration and its later amendments or similar ethical standards. Every human participant provided written informed consent.

Ovarian Stimulation and Oocytes Collection

Sixty-four patients undergoing ICSI from February 2014 to February 2015 at Shandong Provincial Hospital Affiliated with Shandong University donated their immature oocytes. The ages of the patients varied from 38 to 45 years. The clinical protocol of retrieving GV-stage oocytes from patients was previously described (16). Briefly, the patients were prepared for ICSI with the use of a standard ovarian stimulation protocol including down-regulation of the pituitary gland by means of a GnRH agonist (Decapeptyl; Ferring) followed by ovarian stimulation with the use of exogenous FSH (Gonal-F; Serono Laboratories). Ultrasound-guided follicle drilling and aspiration was performed 36 hours after the administration of 10,000 IU hCG (Profasi; Serono Laboratories), two or three follicles of 18–20 mm in diameter were observed via ultrasound examination. After removal of corona-cumulus cells with hyaluronidase and mechanical pipetting, the meiotic status of the oocytes was assessed. Immature oocytes were defined as either GV stage, representing oocytes arrested at prophase of meiosis I or as metaphase I (MI) stage as evidenced by the absence of a polar body and no discernable GV nucleus; 75 GV-stage oocytes retrieved from 64 patients were collected.

In Vitro Maturation and Fertilization

GV-stage oocytes were retrieved by puncturing the ovary with sterile needles and washed thoroughly in M199 medium. Cumulus cells were aspirated by means of gentle pipetting in hyaluronidase medium. Denuded oocytes were cultured in the presence of three different resveratrol concentrations (0.1, 1.0, and 10 μm) or dimethylsulfoxide (DMSO)–supplemented IVM medium, in a humid environment containing 5% CO_2 at 37°C. MI- and MII-stage oocytes were assessed after 16 hours. A total of 1,138 oocytes from aged mice (ages 48–52 wk) were randomly divided into four groups. Sperm were obtained from the ductus deferens of male mice >8 weeks old, washed with IVF medium, and incubated for 2 hours for capacitation, at 2–5 × 10^6/mL density at 37°C in a humidified atmosphere containing 6% CO_2. The resulting MII-stage oocytes were moved into a sperm droplet. Fertilization was assessed after 5–6 hours; 2-cell stage was checked after 24 hours, and blastocysts after 108 hours.

A total of 75 GV oocytes from 64 patients >38 years of age were randomly divided into two groups. GV-stage oocytes were divided randomly into 1.0 μm resveratrol– and DMSO-supplemented IVM media and incubated at 37°C in a humidified atmosphere with 5% CO_2. MI- and MII-stage oocytes were checked after 24 and 36 hours, respectively.

Media and Chemicals

Unless otherwise stated, all chemicals and reagents in the study were purchased from Sigma-Aldrich Chemical Company. Resveratrol was dissolved in DMSO and stored at −20°C until use. The M199 medium for IVM culture was supplemented with 10% human serum albumin, 50 IU/mL penicillin, 5 μg streptomycin, 0.29 μm sodium pyruvate, 0.15 IU/mL hCG, 0.075 U/mL recombinant FSH, 10 ng/mL recombinant human epidermal growth factor, and 10 μg/mL E_2.

Immunofluorescence and Confocal Analysis

MII-stage oocytes were fixed in 2% paraformaldehyde for 10 minutes at 37°C and then permeabilized with 0.2% Triton X-100 for 45 minutes. Next, oocytes were incubated with