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SIRT1 participates in the response to methylglyoxal-dependent glycative stress in mouse oocytes and ovary

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ABSTRACT

Methylglyoxal (MG), a highly reactive dicarbonyl derived from metabolic processes, is the most powerful precursor of advanced glycation end products (AGEs). Glycative stress has been recently associated with ovarian dysfunctions in aging and PCOS syndrome. We have investigated the role of the NAD^+-dependent Class III deacetylase SIRT1 in the adaptive response to MG in mouse oocytes and ovary. In mouse oocytes, MG induced up-expression of glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2) genes, components of the main MG detoxification system, whereas inhibition of SIRT1 by Ex527 or sirtinol reduced this response. In addition, the inhibition of SIRT1 worsened the effects of MG on oocyte maturation rates, while SIRT1 activation by resveratrol counteracted MG insult. Ovaries from female mice receiving 100 mg/kg MG by gastric administration for 28 days (MG mice) exhibited increased levels of SIRT1 along with over-expression of catalase, superoxide dismutase 2, SIRT3, PGC1α and mtTFA. Similar levels of MG-derived AGEs were observed in the ovaries from MG and control groups, along with enhanced protein expression of glyoxalase 1 in MG mice. Oocytes ovulated by MG mice exhibited atypical meiotic spindles, a condition predisposing to embryo aneuploidy. Our results from mouse oocytes revealed for the first time that SIRT1 could modulate MG scavenging by promoting expression of glyoxalases. The finding that up-regulation of glyoxalase 1 is associated with that of components of a SIRT1 functional network in the ovaries of MG mice provides strong evidence that SIRT1 participates in the response to methylglyoxal-dependent glycative stress in the female gonad.

1. Introduction

Methylglyoxal (MG) belongs to a heterogeneous group of low-molecular weight dicarbonyls derived from metabolic processes and, in particular, from glycolysis. Dicarbonyl species are highly reactive and promote posttranslational modification of proteins by glycation, a non-enzymatic reaction with free amino groups of proteins, lipids, and nucleic acids [1]. The early stage of this process involves a complex series of reactions, often referred to collectively as the Maillard reaction, leading to formation of intermediates that are initially reversible but ultimately form stable end-stage adducts called advanced glycation end-products (AGEs) [2]. MG is known to react primarily with arginine residues to form hydroimidazolones and argpyrimidine [3,4], here referred to as MG-AGEs. Detoxification of MG mainly occurs *via* glyoxalase 1 (GLO1) and glyoxalase 2 (GLO2), with GLO1 catalysing the glutathione (GSH)-dependent formation of S-D-lactoylglutathione from MG, and GLO2 hydrolysing S-D-lactoylglutathione to D-lactate and GSH [5]. MG accumulation may arise from increased glycolytic metabolism and/or reduced glyoxalase-mediated removal. The latter may occur as a consequence of reduced GSH availability (*e.g.* oxidative stress) and/or decreased glyoxalase expression (*e.g.* aging) [5]. The build-up of MG affects mitochondrial proteins and increases AGEs, which can also activate receptor-mediated pro-oxidant signalling pathways, thus generating a vicious cycle. Therefore one of the main aspects of the

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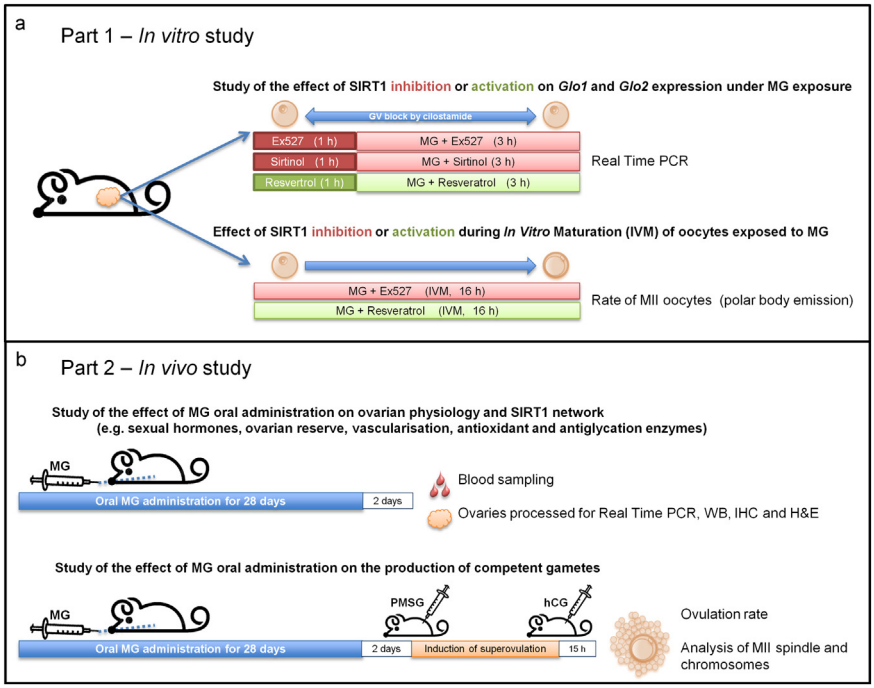

Fig. 1. Experimental design.

glycative burden is oxidative stress [5].

Both MG and protein glycation (dicarbonyl stress) have been attracting considerable attention because of their role in the chronic side effects in diabetic patients with enduring hyperglycaemia and in the pathogenesis of numerous diseases associated with altered redox homeostasis [5]. About ten years ago, Diamanti-Kandarakis' research group proposed the involvement of AGEs in ovarian dysfunctions based on the observation of increased levels of AGEs in the serum and ovary of women affected by polycystic ovary syndrome (PCOS) [6–8]. Specific serum levels of AGEs were established to indicate diminished fertility, and AGEs were shown to correlate positively with altered glucose metabolism, age, and factors related to obesity, dyslipidaemia, hyperglycaemia, and insulin resistance in patients undergoing *in vitro* fertilization (IVF) [9]. In addition, elevated MG and MG-AGEs were reported in ovaries of reproductively aged mice [10–12]. A preliminary analysis of proteome revealed increased glycation of specific polypeptides in concomitance with decreased activity and expression of GLO1 [10]. Further evidence for the role of AGEs in ovarian aging was provided by measurements in follicular fluid and serum of soluble RAGE (s-RAGE), a circulating isoform of RAGE that can neutralize the ligand-mediated damage [13,14]. The crucial role of glyoxalases in the ovary has been confirmed by the finding that dietary glycotoxins and hyperandrogenic status decrease GLO1 activity in rat ovaries, possibly contributing to increased AGE accumulation in granulosa cells [15].

The effects of a possible dicarbonyl overload with PCOS, aging or diabetes have been explored in mouse oocytes by means of MG cytotoxicity assays [16–18]. *In vivo* and *in vitro* experiments have shown that MG induced a significant reduction in the rate of oocyte maturation, fertilization, and embryonic development. MG was found to cause disturbances in redox regulation and distribution of mitochondria, aberrant and delayed spindle formation, epigenetic alterations and DNA damage which probably explain resorption of post-implantation embryos and decreased foetal weight observed after embryo transfer [16–18]. Nevertheless, the expression of glyoxalases in mouse oocytes and cumulus cells suggests that female germ cells are competent to deal with reactive carbonyl compounds [17].

Overall, the effects of dicarbonyl stress in the female reproductive system have been well characterized [19,20]. Nevertheless, the adaptive response orchestrated at molecular level to cope with glycation injury in female reproductive cells and ovaries has been poorly investigated. Glycation-related oxidative challenge may result in altered activity of sirtuins, NAD^+-dependent enzymes with deacetylase and/or mono-ADP-ribosyltransferase activity [21,22]. SIRT1, one of the seven members of the mammalian sirtuin family, plays a key role during different stages of folliculogenesis by regulating energy homoeostasis, mitochondrial biogenesis, chromatin remodelling and protection against oxidative stress [23,24]. The SIRT1 network includes transcription factors, which control genes encoding for antioxidant enzymes such as catalase (CAT) and mitochondrial superoxide dismutase (SOD2), and genes required to meet energetic demands during cellular stresses [25]. Indeed a key SIRT1 substrate is PGC1α (peroxisome proliferator-activated receptor gamma co-activator 1 alpha), which is known to act as a master regulator of mitochondrial biogenesis and functions [26,27]. A downstream effector of SIRT1/PGC1α is the mitochondrial transcription factor A (mtTFA), which plays a central role in transcription, replication and maintenance of mtDNA [28,29]. The SIRT1 functional network also includes the mitochondrial sirtuin, SIRT3 [30]. This sirtuin deacetylates a range of mitochondrial target enzymes, including SOD2 and components of oxidative phosphorylation system thus improving the efficacy of reactive oxygen species (ROS) removal from the mitochondrial compartment and energy production [31]. Evidence of beneficial effects of resveratrol, a SIRT1 activator, have suggested that this sirtuin may be involved in defence against *in vitro* oocyte MG toxicity as well as in attenuating PCOS development and progression [18,32].

In the present study, we investigated the adaptive response to glycation insult in female reproductive cells and ovaries by focusing on SIRT1 network. In the first part we investigated whether modulation of SIRT1 activity influences the effects MG on glyoxalases expression and *in vitro* maturation (IVM) of mouse oocytes (Fig. 1a). In the second part, we aimed to study the *in vivo* effects of supraphysiological MG on the ovary and ovarian SIRT1 network as well as on the production of competent gametes (Fig. 1b). To this end we selected the regimen of MG oral administration in mice previously established by Ghosh et al. [33]. According to this model, blood MG concentrations rapidly increased to reach a five-fold increase to remain stable during the 28 day-MG-

administration so ensuring a MG overload during the overall follicle growth time of about 17–19 days in the ovary of mature mice [34].

Results obtained from the experimental models employed in the present research demonstrated that MG triggers in oocytes and ovary an adaptive response involving SIRT1 functional network and antiglycation enzymes, thus representing a valuable contribution to the knowledge of the effects on fertility of MG and AGE overload during aging, PCOS and diabetes.

2. Materials and methods

2.1. Part 1

2.1.1. Oocyte collection

Outbred CD-1 mice (Charles River Italia s.r.l., Calco, Italy) at the age of 4–8 weeks were used to obtain oocytes at germinal vesicle (GV) stage, employed for expression studies and *in vitro* maturation assays. Animals were maintained in a temperature-controlled environment under a 12 h light/dark cycle (7.00–19.00) and free access to feed and water *ad libitum*. All the experiments were carried out in in conformity with national and international laws and policies (European Economic Community Council Directive 86/609, OJ 358, 1 Dec 12, 1987; Italian Legislative Decree 116/92, Gazzetta Ufficiale della Repubblica Italiana n. 40, Feb 18, 1992; National Institutes of Health Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23, 1985). The project was approved by the Italian Ministry of Health and the internal Committee of the University of L'Aquila. After vaginal smears, mice in diestrous were sacrificed by an inhalant overdose of carbon dioxide (CO_2, 10–30%), followed by cervical dislocation. All efforts were made to minimize suffering. Ovaries were excised and denuded fully grown GV oocytes were obtained by puncturing large antral follicles [17]. Healthy GV oocytes were pooled and randomized before distribution into the experimental groups (Fig. 1a). Each experiment was performed three times.

2.1.2. Oocyte treatments and RNA extraction

In order to study gene expression of SIRT1-related pathway and antiglycation enzymes under carbonyl stress, oocytes were maintained at GV stage by addition of 0.5 μM cilostamide (Sigma-Aldrich, St. Louis, MO, USA) [35] in the culture media, M2 or M16 (Sigma-Aldrich), according to each procedure. At first, to accomplish SIRT1 inhibition or activation, GV oocytes were cultured for 1 h in the presence of 20 μM Ex527 (Sigma-Aldrich) [35] or to 100 μM sirtinol (Sigma-Aldrich) [36], which are two SIRT1 inhibitors, or in the presence of 2 μM resveratrol (Sigma-Aldrich), a SIRT1 activator [37]. Then, GV oocytes were exposed to 75 μM MG (Sigma-Aldrich) for 180 min at 37 °C, 5% CO_2 in M16 medium in the presence 20 μM Ex527, 100 μM sirtinol or 2 μM resveratrol. Controls were cultured in the presence of 0.001% DMSO (Sigma-Aldrich) to evaluate a possible toxic effect of DMSO in which Ex527, sirtinol and resveratrol were dissolved.

Pools of 30 oocytes were washed in RNase free water, then transferred in 10 μl of RNase free water and sunk in liquid nitrogen. Samples were stored at −80 °C. RNA extraction was performed by Picopure kit (Applied Biosystems, Foster City, CA, USA).

2.1.3. cDNA synthesis and Real-time quantitative PCR (qPCR)

Genomic DNA was removed using Riboclear plus (cat. 313-150), following the manufacturer's protocol (GeneAllBiotechnology CO., Ltd). The resulting RNA was used to obtain cDNA *via* reverse transcription (cat. NP100041, OriGeneTechnologies, Inc., Rockville, MD).

The cDNA was used (1:30) for the quantitative PCR reactions using the Applied Biosystems 7300 system (ThermoFisher Scientific, Inc., Rockford, IL) and the TaqMan® Gene Expression Master Mix (Applied Biosystems cat. 4369016) and TaqMan gene expression assays — FAM-MGB, according to the manufacturer's instructions. The Assay IDs used were: 18S-Hs99999901-S1, GLO1-Mm00844954_s1, GLO2-Mm00600347_m1. Amplification steps were set as follows: 2 min at 50 °C and 10 min at 95 °C for the initial holding stage, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C. Gene expression was calculated by using the ΔΔCt method for relative quantitation, using 18S as the reference mRNA and "CTRL" as the calibrator.

2.1.4. Oocyte IVM assay

Since timing of meiosis progression is considered a marker of oocyte quality [38], in order to study the impact of SIRT1 activity under MG exposure during IVM, GV oocytes were placed into M16 medium in presence or absence of 75 μM MG. MG medium was supplemented with the specific SIRT1 inhibitor Ex527 at concentrations of 5 or 10 μM or SIRT1 activator, the resveratrol at a concentration of 2 μM. GV oocytes were cultured for 16 h at 37 °C and 5% CO2. Controls were matured in the presence of 0.001% DMSO (Sigma-Aldrich) to evaluate a possible toxic effect of DMSO in which Ex527 and resveratrol were dissolved. Numbers of oocytes that emitted first polar body (MII, metaphase II), oocytes that had resumed meiosis (GVBD, germinal vesicle breakdown), immature oocytes and degenerated oocytes were recorded.

2.2. Part 2

2.2.1. Animal treatment

2.2.1.1. MG administration. Eighteen 4 week-old, body weight 20–21 g, young CD-1 female mice were randomly divided into two groups (9 for each): mice received water by using gastric gavage for 4 weeks (control mice); mice received MG (100 mg/Kg) by using gastric gavage for 4 weeks [33] (MG mice) (Fig. 1b).

2.2.1.2. Blood collection. Forty-eight hours after the last treatment, six mice per group underwent submandibular facial vein blood collections (survival bleeds) [39]; 100 μl of blood were collected in a glass micropipette capillary tube.

2.2.1.3. Ovary collection. Then mice were sacrificed as described above and ovaries were removed. Part of the ovaries was conserved in 3.7% paraformaldehyde (PFA) for Hematoxylin and Eosin (H&E) staining and immunohistochemistry analysis. The remaining ovaries were sunk in liquid nitrogen and stored at −80 °C until processed for RNA or protein extraction.

2.2.1.4. Superovulation induction and oocyte collection. In order to obtain mature oocytes, at 48 h after the last treatment, the remaining three mice per group were treated for the induction of super ovulation by intraperitoneal injection of 10 IU PMSG (Folligon; Intervet-International, Boxmeer, Holland), followed by 10 IU hCG (Profasi HP 2000; Serono, Roma, Italy) 48 h apart. Fifteen hours after hCG, oviducts were removed and oocytes arrested at metaphase II (MII) stage were isolated after a brief exposure to 0.3 mg/ml hyaluronidase (Sigma-Aldrich).

2.2.2. Hormone assays

Serum progesterone and androstenedione levels were measured by Architect specific Kit (Abbott) following the manufacturer's instructions. Estradiol was measured by VIDAS EII2 Kit (BioMarieux, SA) following the manufacturer's instructions.

2.2.3. H&E staining and ovarian follicle classification

Ovarian sections were stained with Hematoxylin and Eosin (H&E) and analysed under a light microscope for differential follicle counts as previously described [40]. Briefly, blind follicle counts were conducted on every fifth section of entire ovaries by two independent researchers. Follicle stage was classified according to Pedersen and Peters [41]. Primordial follicles are quiescent follicles characterized by a small oocyte, with a diameter of < 20 μm, with up to 20 follicle cells attached to its surface on the largest cross-section. Growing follicles include

primary follicles, characterized by one complete ring of follicle cells (21 to 60 cells on the largest cross-section) that surround a growing oocyte (diameter between 20 and 70 μm); secondary follicles, with two or three layers of follicle cells (61 to 200 cells on the largest cross-section) surrounding a growing oocyte (diameter between 20 and 70 μm); antral follicles, a fully grown oocyte (diameter 70 μm) surrounded by many layers of follicle cells separated by scattered areas or a cavity containing follicle fluid. Only follicles showing the nucleus of the oocyte were counted.

2.2.4. Ovarian immunohistochemical analysis

Fixed ovaries were embedded in low temperature-fusion paraffin, and serial sections of 3 μm were obtained for several histological staining. Ovarian sections were incubated in methanol and 3% hydrogen peroxidase solution for 40 min and then rinsed in PBS. Thereafter, samples were incubated overnight at 4C with polyclonal antibodies to MG-AGE (AGE06B, BioLogo; 1:200), RAGE (PA1-075, ThermoFisher Scientific; 1:200), PCNA (NB500-106, Novus Biologicals, 1:200) and CD34 (PA1334, Boster Biological Technology; 1:200). Samples were then rinsed with PBS for 5 min and incubated with a labelled streptavidin-biotin-peroxidase conjugate kit (Dako LSAB plus, DakoCytomation; Milan, Italy). After rinsing in PBS for 10 min, the sections were incubated with 3,3′-diaminobenzidine-tetrahydrochloride (DAB; DakoCytomation) for 1–3 min. Last, the samples were counterstained with Mayer's Hematoxylin and observed under a photomicroscope Olympus BX51 Light Microscope (Olympus Optical Co. Ltd.). To demonstrate the immunoreaction specificity, negative controls were performed for all immunoreactions. For negative controls, the primary antibody was replaced (same dilution) with normal serum from the same species. Observations were processed with an image analysis system (IAS; Delta; Rome, Italy) and were independently performed by two pathologists (A.V., R.S.) in a blinded fashion.

2.2.5. Ovarian RNA extraction

Total RNA from ovaries was isolated using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. cDNA synthesis and Real-time quantitative PCR (qPCR) were performed as already described under the cDNA synthesis and Real-time quantitative PCR (qPCR) section.

2.2.6. Western blot analysis

Ovarian tissues were homogenized in RIPA buffer by repeated freeze/thaw cycles in liquid nitrogen. After centrifugation (33.000 rpm for 1 h at 4 °C), the supernatants were collected for protein analysis. Protein concentration was determined by BCA protein assay kit (Pierce, Rockford, IL). Protein samples were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Sigma-Aldrich, St. Louis, MO, USA). Non-specific binding sites were blocked for 1 h at room temperature with 5% not fat dry milk (Bio-Rad Laboratories, Italy) in Tris-buffered saline containing 0.05% Tween 20 (TBS-T). Membranes were incubated with polyclonal rabbit anti-SIRT1 antibody (Ab12193, Abcam; 1:700), anti-SIRT3 (Ab86671, Abcam, Cambridge, UK; 1:500), anti-CAT antibody (200-4151, Rockland; 1:10.0000), anti-SOD2 antibody (Ab86087, Abcam; 1:1000), anti-GLO1 antibody (MA1-13029, Thermo Fisher; 1:600), anti-GLO2 antibody (Ab154108, Abcam; 1:500), anti-PGC1α antibody (SC-13067, Santa Cruz Biotechnology Inc., 1:500), anti-AMH (Ab103233, Abcam, 1:100), anti-CD34 (PA1334, Boster Biological Technology, 1:700), anti-RAGE (PA1-075, ThermoFisher Scientific, 1:750), mouse anti-Methylglyoxal (MG)-AGE (argpyrimidine) monoclonal antibody (AGE06B, BioLogo; 1:200), anti-PCNA (NB500-106, Novus Biologicals, 1:1000), anti-mtTFA (SC-166965, Santa Cruz, 1:250), anti-β-actin antibody (Ab8226, Abcam; 1:3000) or anti-GAPDH (TA802519, OriGene Technologies Inc., 1:750) overnight at 4 °C, followed by incubation with horseradish peroxidase (HRP) conjugated anti-rabbit (BA1054, Boster Biological Technology Co., Ltd., 1:3000) or anti-mouse secondary antibody (Ab6728, Abcam, 1:2000) for 1 h at room temperature. After washing, specific immunoreactive complexes were detected by ECL kit (Thermo Scientific, Waltham, MA, USA) and Uvitec Cambridge system (Alliance series, Cambridge, UK). The bands were normalized for β-actin or GAPDH using ImageJ 1.44p software and values were given as relative units (RU). The experiment was performed in triplicate.

2.2.7. Analysis of DNA distribution and spindle configuration of in vivo matured MII oocytes

Immunofluorescence experiments were performed according to Di Nisio et al. [42]. *In vivo* matured MII oocytes were fixed for immunofluorescence and labelled by mouse anti-α-tubulin (T9026, Sigma Aldrich, 1:200) primary antibody overnight at 4 °C and secondary goat anti mouse- antibody conjugated with Alexa 594 (A90-137D4, Bethyl Laboratories Inc., 1:500) for 1 h at room temperature. Chromatin staining was performed by 5 μg/ml Hoechst 33342 (Sigma-Aldrich) for 5 min at room temperature. In negative control oocytes, the primary antibody was omitted. Oocytes were mounted on slides and analysed under epifluorescence microscope at 100× magnification.

Digital images were analysed using ImageJ 1.44p to obtain measurements of spindle length (from pole to pole), spindle width at the equator and spindle pole width. These values were used to calculate the total area of the spindle for each oocyte as follows [43,44].

$$area\ of\ the\ spindle = \frac{\left(\frac{spindle\ lenght}{2}\right)(equator\ width + pole\#1\ width)}{2} + \frac{\left(\frac{spindle\ lenght}{2}\right)(equator\ width + pole\#2\ width)}{2}$$

2.3. Statistical analysis

All data are presented as mean ± SEM. Statistical analysis was assessed by OneWay ANOVA followed by Tukey's multiple comparisons, or *t*-test. Analyses were performed using the SigmaStat software (Jandel Scientific Corporation). P-values < 0.05 were considered statistically significant.

3. Results

3.1. Part 1

3.1.1. Exposure of GV oocytes to MG induces up-regulation of Glo1 and Glo2 gene and SIRT1 inhibition reduces this response

In order to investigate the role of SIRT1 in the antiglycation response, GV-arrested oocytes were exposed to 75 μM MG [17,18] in the presence of two different SIRT1 inhibitors, Ex527 [35] or sirtinol [36] or in the presence of the SIRT1 activator, resveratrol [37] and analysed for the levels of Glo1 and Glo2 transcripts (Fig. 1a). Results from Real Time RT-PCR revealed that transcript levels of Glo1 and Glo2 increased significantly following 3 h exposure to MG. When the medium containing MG was supplemented with SIRT1 inhibitors, Ex527 or sirtinol, the levels of Glo1 and Glo2 transcripts were significantly lower than MG oocytes, although they did not reach levels similar to controls. After 3 h of MG exposure, supplementation with resveratrol did not influence Glo1 and Glo2 gene expression in comparison to culture with MG alone (Fig. 2a,b).

3.1.2. Modulation of SIRT1 activity influences MG effects on oocyte in vitro maturation

Given the effects of SIRT1 inhibition on the glyoxalase system, we investigated whether this condition aggravated the effects of MG on meiosis progression. To this end, GV oocytes were exposed to 75 μM MG in the presence of different Ex527 concentrations during a 16 h incubation. According to previous results [17,35], both these compounds

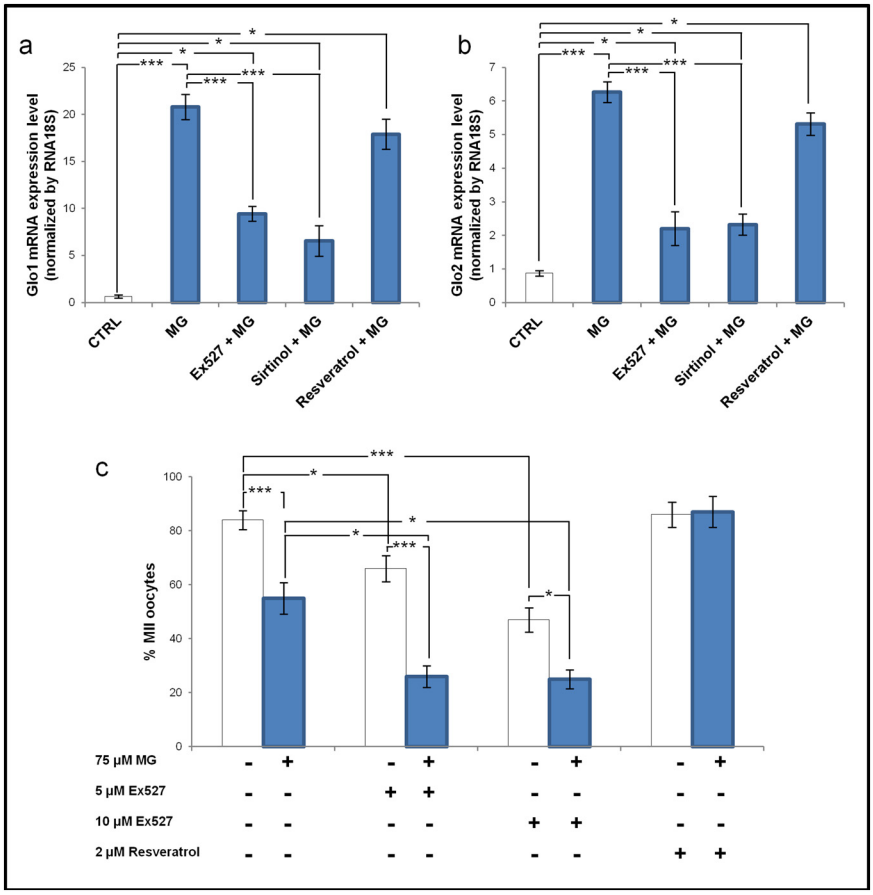

Fig. 2. Modulation of SIRT1 activity affects oocyte Glo1 and Glo2 transcript level and oocyte *in vitro* maturation (IVM) upon MG insult. Glo1 (a) and Glo2 (b) gene expression in oocytes exposed to MG under SIRT1 inhibition by Ex527 and sirtinol, or SIRT1 activation by resveratrol. Data are presented as means ± SEM. (c) GV oocytes exposed to 75 µM MG in the presence of two concentrations of SIRT1 inhibitor Ex527 (5 µM and 10 µM), or in the presence of the SIRT1 activator resveratrol at 2 µM concentration. Data represent the mean percentage of oocytes emitting polar body after IVM (± SEM). Experiments were performed three times. *P < 0.05; ***P < 0.001, One Way ANOVA, Tukey's multiple comparisons.

are known to negatively affect meiotic maturation. Accordingly, our results showed that oocyte exposure to 75 µM MG alone resulted in about 45% polar body extrusion. As expected, a negative effect was also observed when oocytes were exposed to 5 µM and 10 µM Ex527 (65% and 45% polar body extrusion, respectively). When oocytes were challenged with both 75 µM MG and 5 µM or 10 µM Ex527 a synergistic detrimental effect on oocyte maturation was observed. In particular, under both the Ex527 treatments, maturation rate of MG exposed oocytes decreased to 25% (Fig. 2c).

To confirm the contribution of SIRT1 to MG response, oocytes were exposed to 75 µM MG in the presence of the SIRT1 activator resveratrol at 2 µM concentrations [37]. Under this condition the inhibitory effect of 75 µM MG was reverted. Indeed in this group we observed normal meiotic progression, with a maturation rate similar to that of untreated oocytes (Fig. 2c).

3.2. Part 2

3.2.1. MG mice showed normal levels of ovarian hormones and ovarian follicle populations but revealed abnormal ovarian stroma

Given the involvement of SIRT1 in the response of oocytes to *in vitro* MG exposure, we hypothesized a role for this sirtuin in the antiglycation response at ovarian level. Thus we relied on a mouse model based on oral MG administration previously established by Ghosh et al. [33]. Control and MG mice were healthy in behaviour and aspect and body weight of MG mice was similar to control at the end of the treatment. As shown in Fig. 1b, at 48 h from the end of MG administration, serum levels of ovarian hormones of control and MG mice were assessed. According to results in Table 1, steroidogenesis was slightly affected by MG intake with a significant decrease in progesterone levels and a slight increase in androstenedione, whereas 17-β estradiol concentration did not change.

Table 1
Anthropometric and hormonal parameters of control and MG mice (n = 6, per experimental group).

	CTRL	MG	P
Weight day 1 (g)	21.05 ± 0.39	20.95 ± 0.36	n.s.
Weight day 30 (g)	25.80 ± 0.78	22.97 ± 1.00	n.s.
Progesterone (ng/ml)	19.4 ± 1.1	7.3 ± 0.8	***
Androstenedione (ng/ml)	0.32 ± 0.01	0.49 ± 0.05	*
Estradiol (pg/ml)	9.10 ± 0.12	9.28 ± 0.45	n.s.

Values are means ± S.E.M.
n.s., not statistically significant; *t*-test.
* P < 0.05.
*** P < 0.001.

As shown in Fig. 3c, no significant differences were observed in the number of primordial, primary, secondary and antral follicles in the ovaries of MG mice when compared to control. The absence of effects on ovarian reserve (primordial follicles) was confirmed by the assessment of AMH expression by immunoblotting analysis revealing similar levels of this protein in control and MG groups (Fig. 3d,e).

Western blotting and immunohistochemical analysis demonstrated a reductive distribution and expression of PCNA within the ovaries of MG mice in comparison to control (Fig. 4a,b,d,f), whereas ovarian CD34 protein, a vascular endothelial cell marker, did not undergo significant changes (Fig. 4a,c,e,g).

3.2.2. In vivo MG intake increases ovarian expression of SIRT1 network proteins

To investigate the involvement of SIRT1 in the ovarian response to MG, we compared the levels of SIRT1 and proteins of SIRT1 network in control and MG mice (Fig. 1b). Data reported in Fig. 5 revealed a

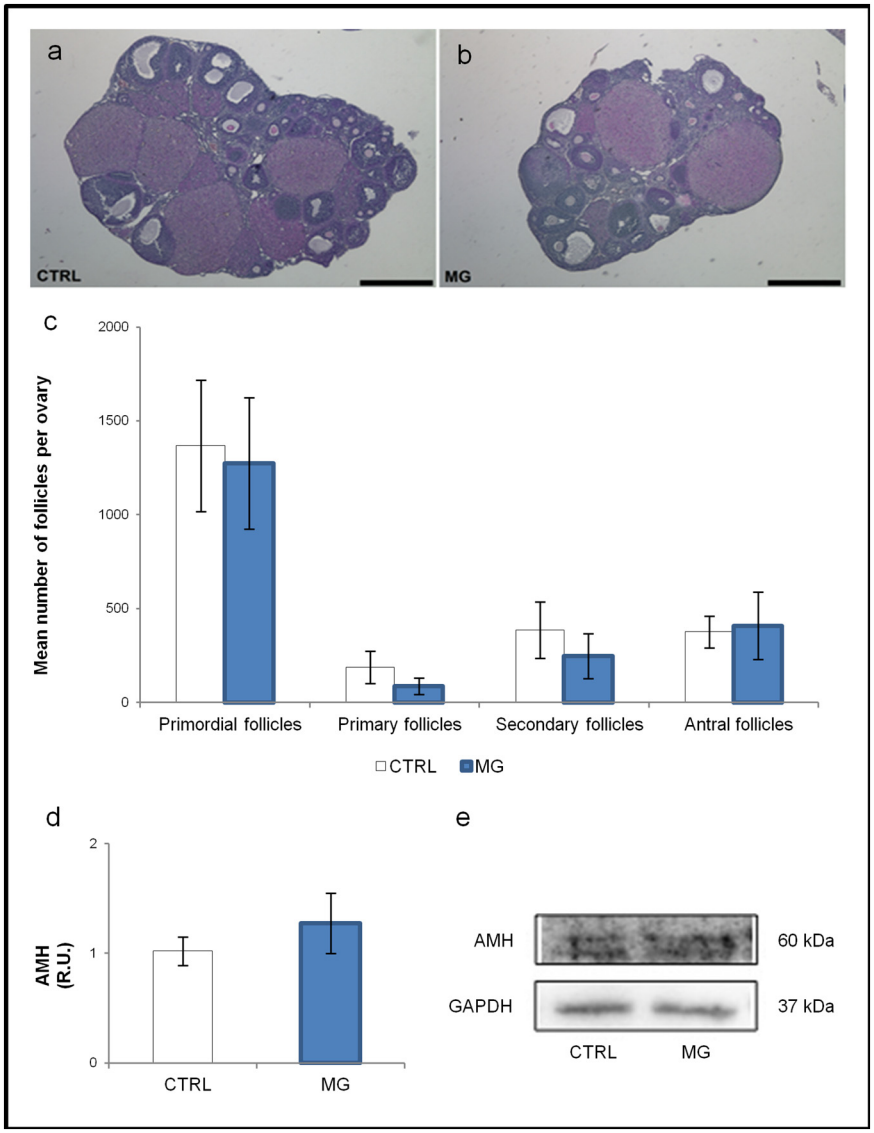

Fig. 3. MG intake did not influence ovarian follicle populations. Representative images of histological sections of control (a) and MG (b) ovaries are shown; scale bars: 400 μm. Data represent means ± SEM. Follicles were classified as primordial, primary, secondary and antral follicles (c). Western blot analysis (d) and representative images (e) of ovarian AMH. Data are presented as means ± SEM of densitometric analysis of immunoreactive bands normalized to internal reference protein (GAPDH). Three mice per experimental group were employed. Experiments were done in triplicate. *t*-test: n.s., not statistically significant.

significant increase of SIRT1 in MG mice. Moreover, we detected an up-regulation of protein levels of SIRT3, the antioxidant enzymes SOD2 and CAT, of PGC1α, the main regulator of mitochondrial biogenesis and function, and mtTFA, which reflects the rate of mtDNA transcription and mtDNA content [45].

3.2.3. In vivo MG intake increases ovarian protein expression of GLO1 and does not result in increased levels of MG-AGEs and RAGE

To investigate the activation of the MG detoxification system, we assessed gene and protein expression of glyoxalase 1 and glyoxalase 2, together with the level and localization of MG-AGEs and RAGE. Our results showed no differences in Glo1 and Glo2 transcript levels (Fig. 6a,b). GLO1 protein expression was significantly higher in ovaries from MG mice when compared with controls, whereas protein levels of GLO2 remained unchanged (Fig. 6c,d,e). Semiquantitative analysis of all the immunoreactive bands revealed similar levels of MG-AGEs in the experimental groups. Moreover, the immunohistochemical analysis did not evidence any differences in intraovarian MG-AGE localization. The absence of MG-AGE accumulation was confirmed by similar protein expression and intraovarian distribution of RAGE in MG and control mice (Fig. 7).

3.2.4. MG intake does not affect ovulation rate but negatively affects oocyte quality

In order to evaluate the impact of changes provoked by MG in ovarian microenvironment on the production of competent gametes, ovulation rate and oocyte quality were assessed (Fig. 1b). Although similar amount of ovulated and mature MII oocytes in both MG and control groups, immunocytochemistry analysis revealed the presence of abnormal spindles in oocytes isolated from MG mice (Table 2). In particular, when we examined spindle dimensions we observed a significant increase in the estimated spindle area due to differences in length, width and pole width in oocytes from MG mice. Indeed the oocytes from MG mice presented barrel shaped spindles that were larger than small focused spindles showed by oocytes from control group (Fig. 8).

4. Discussion

Recently, it has emerged that overload of reactive dicarbonyl

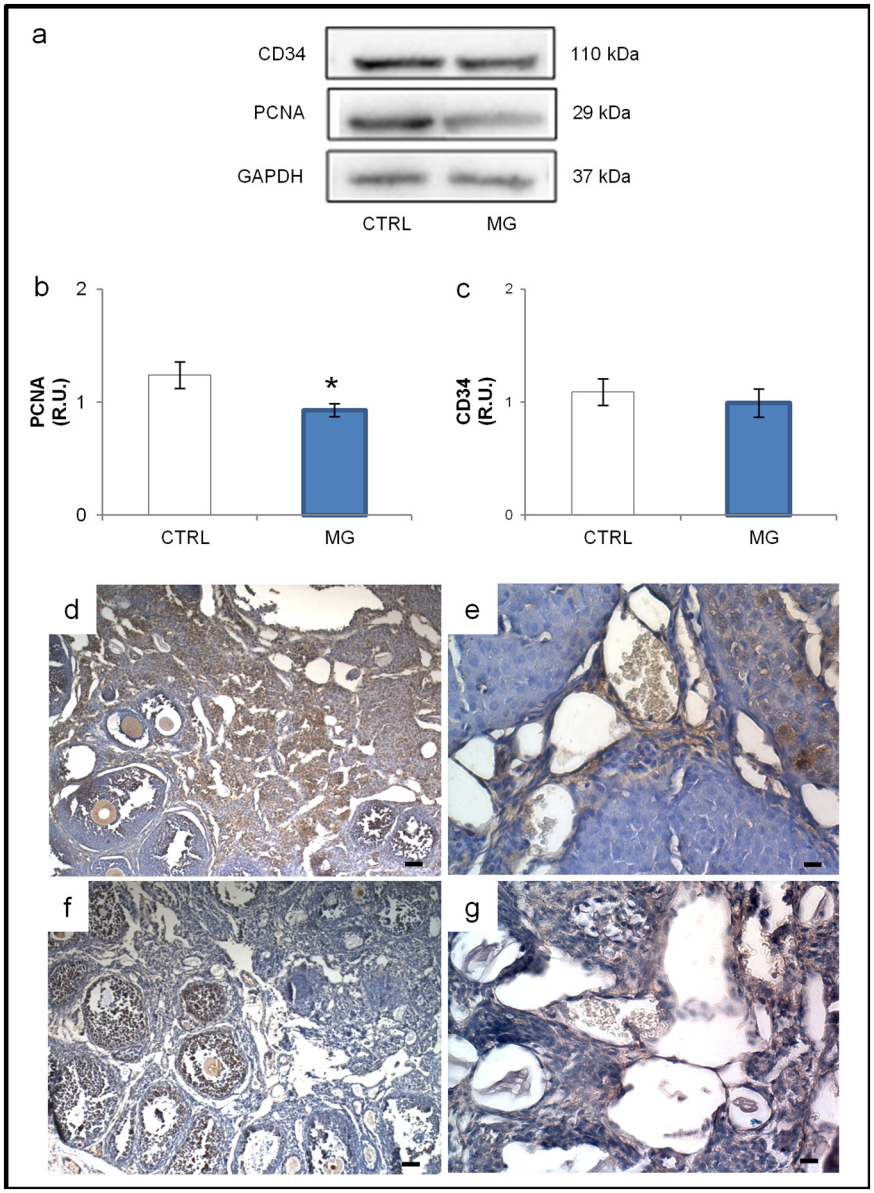

Fig. 4. MG intake affects ovarian stroma and vasculature. Western blot representative images (a) and analysis of PCNA (b) and CD34 (c) are shown. Data are presented as means ± SEM of densitometric analysis of immunoreactive bands normalized to internal reference protein (GAPDH). Representative images of immunolocalization of PCNA and CD34 in control and MG ovaries. PCNA: control (d); MG (f), scale bar, 100 μm. CD34: control (e); MG (g), scale bar, 25 μm. Three mice per experimental group were employed. Experiments were done in triplicate. *P < 0.05, *t*-test.

compounds and AGEs are key factors in ovarian dysfunctions and reduced fertility associated with aging, PCOS and oxidative stress [10,46–49]. Consumption of glycotoxins contained in thermally processed foods and various beverages and foodstuffs typical of a Western diet contribute to increase AGE-related dysfunctions [50–52]. In search for mechanisms underlying an efficient scavenging of MG and AGEs at ovarian level, the present study provides evidence for the involvement of SIRT1 in the antiglycation response orchestrated by female germ cells and ovary.

4.1. SIRT1 participates in the modulation of MG scavenging in mouse oocytes

It is known that glyoxalases are expressed in mouse oocytes at gene and protein level in physiological conditions [17,53]. Here we revealed for the first time that exposure to supraphysiological levels of MG induced up-expression of Glo1 and Glo2 genes in mouse GV oocytes. Moreover we showed that inhibition of SIRT1 by Ex527 or sirtinol reduced this response. Although SIRT1 activation by resveratrol seems not to potentiate glyoxalase expression, data obtained from SIRT1 inhibition represent a strong evidence of the involvement of this sirtuin in the MG detoxification response. Glo1 gene promoter contains numerous regulatory elements including antioxidant responsive elements (AREs) [54,55]. Positive transcriptional regulators of Glo2 gene include p63 and p73, members of the p53 family involved in female germ cell quality control [56,57]. Therefore, Glo2 gene up-regulation in oocytes exposed to MG may be related to the accumulation of DNA double strands breaks observed in previous papers [17,18]. Whether SIRT1 exerts direct or indirect effects on Glo1 and Glo2 promoters remains to be investigated together with the reasons why resveratrol did not enhance Glo1 and Glo2 expression (Fig. 2).

The finding that the negative effects of MG on *in vitro* oocyte maturation were potentiated by the exposure to the SIRT1 inhibitor and reverted by the SIRT1 activator, resveratrol, further supports the notion

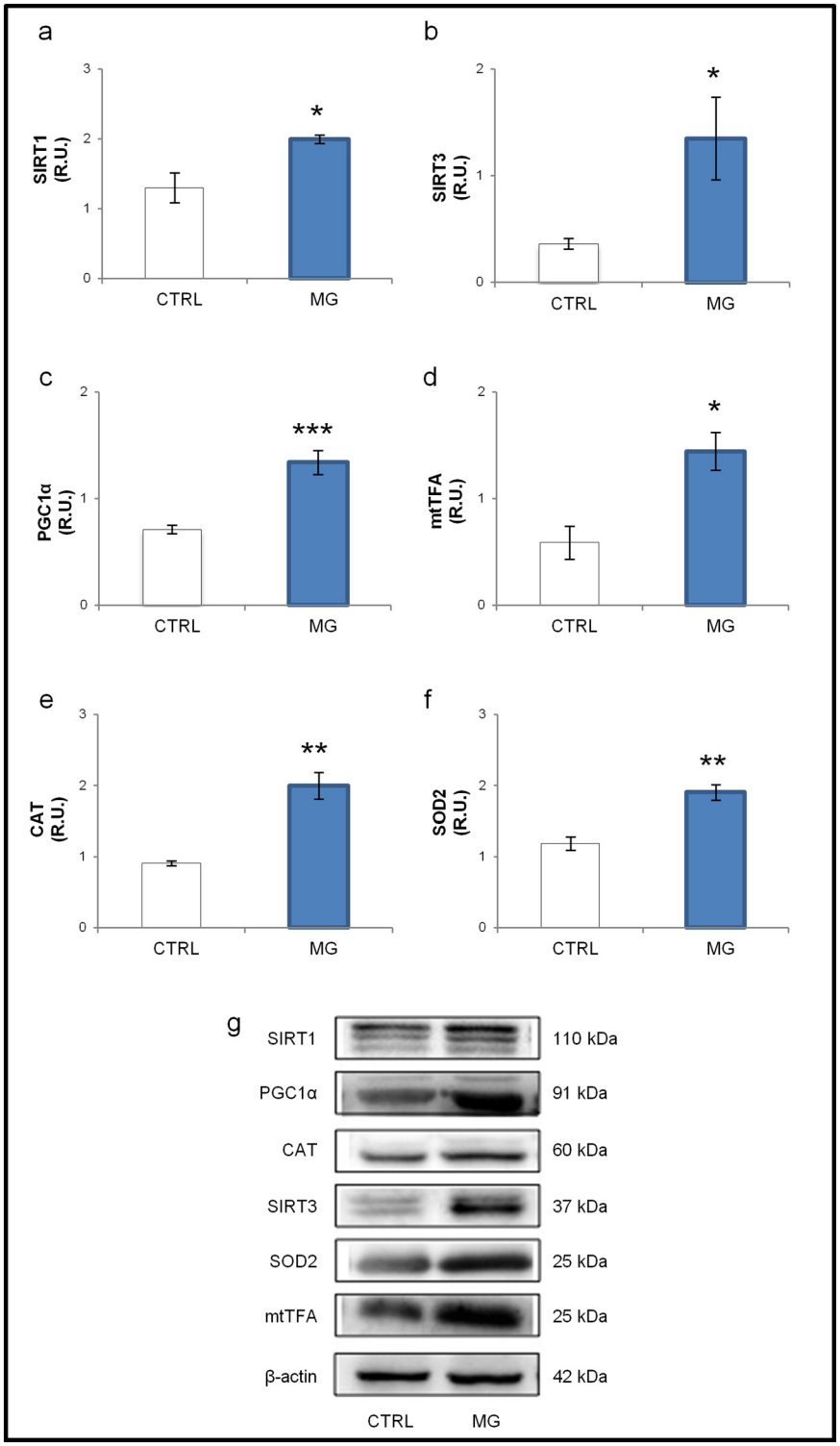

Fig. 5. MG intake increases ovarian expression of SIRT1 and proteins of SIRT1 network. Western blot analysis of SIRT1 (a), SIRT3 (b), PGC1α (c), mtTFA (d), CAT (e) and SOD2 (f) and representative images of immunoreactive bands (g) are shown. Data are presented as means ± SEM of densitometric analysis of immunoreactive bands normalized to internal reference protein (β-actin). Three mice per experimental group were employed. Experiments were done in triplicate. ***P < 0.001, **P < 0.01, *P < 0.05, *t*-test.

that SIRT1 is involved in the adaptive response to MG. According to Liu et al. [18], oocyte MG exposure resulted in increased levels of ROS and oxidative damage, which were prevented by antioxidant effects of resveratrol. Based on our observation that Ex527 aggravates MG cytotoxicity, it is likely that protective actions exerted by resveratrol reported by us and Liu et al. [18] are also linked to its activity as SIRT1 activator.

4.2. *In vivo exposure to MG does not affect ovarian folliculogenesis but influences stromal cell proliferation and ovarian hormones*

Given the involvement of SIRT1 in the response of oocytes to *in vitro*

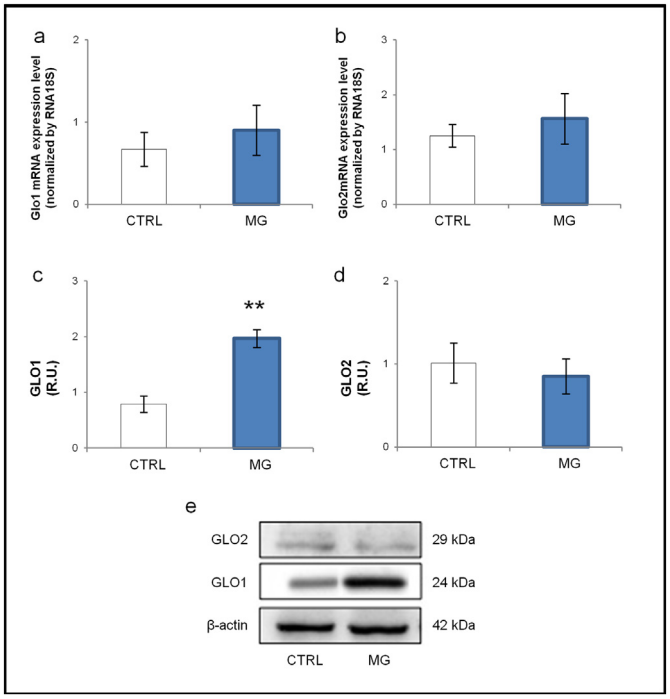

Fig. 6. *In vivo* MG intake increases ovarian protein expression of GLO1 and does not influence the levels of GLO2. Gene expression of Glo1 (a) and Glo2 (b) genes. Data are presented as means ± SEM. Western blot analysis of GLO1 (c) and GLO2 (d) and representative images of immunoreactive bands (e) are shown. Data are presented as means ± SEM of densitometric analysis of immunoreactive bands normalized to internal reference protein (β-actin). Three mice per experimental group were employed. Experiments were done in triplicate. **P < 0.01, *t*-test.

MG exposure, we evaluated a role for this sirtuin in the antiglycation response at ovarian level. The study of the effects of ingested MG has represented the best option to accomplish this objective. *In vivo* MG effects on female fertility have already been described by Chang and Chan [16]. However, the lack of evaluation of the amount of MG ingested and MG plasma concentrations, along with the short duration of treatment, rendered this protocol unsuitable for our study. Indeed we selected the regimen of MG administration by Ghosh et al. [33], which induced an increase in blood MG during the overall follicle growth time. Moreover, under this treatment, animals remained healthy, no changes in body weight were observed and histological examination of several organs did not reveal any adverse effect [33]. Accordingly, in our study MG mice were healthy in behaviour and appearance and showed body weight similar to control at the end of MG treatment (Table 2). Nevertheless, MG mice presented decreased levels of progesterone and increased androstenedione, which may represent an early sign of the hyperandrogenic status associated with PCOS and ovarian AGE deposition [58–60]. In spite of these effects on ovarian steroidogenesis, MG mice did not show alteration folliculogenesis as demonstrated by normal ovarian follicle count, AMH expression and ovulation rate. Nevertheless, reduced ovarian cell proliferation based on PCNA expression may represent evidence for altered homeostasis which is supported by activation of SIRT1 functional network, antiglycation response and oocyte defects discussed below.

4.3. SIRT1 functional network is involved in the antiglycation response to MG at ovarian level

We found that *in vivo* ovarian exposure to MG achieved by means of MG intake increased ovarian expression of SIRT1 functional network. This points to the notion that MG perturbed ovarian homeostasis by generating a condition of redox imbalance. Indeed increased expression of sirtuins is considered an adaptive response to mild oxidative stress, whereas severe oxidant conditions determined posttranslational modifications resulting in sirtuin degradation [21]. Previous studies by our group have reported that SIRT1 mRNA and protein increase in oocytes, granulosa cells and ovary in response to pro-oxidant stimuli. In these systems changes in SIRT1 expression have been linked to the mRNA binding protein HuR and the micro-RNA miR-132 [35,40]. Since oxidative conditions are known to reduce the interaction of SIRT1 with its enzymatic inhibitor DBC1, it is likely that the increased SIRT1 expression observed in this study is associated with increased SIRT1 activity [21].

The recruitment of SIRT1 in the ovary exposed to MG is also supported by the rise in protein level of elements of its functional network reported in this study. SIRT1 is known to promote activation of Cat and Sod2 genes throughout deacetylation of FOXO transcription factors [25,61]. Moreover, SOD2 protein activity is under positive regulation of SIRT3, which also deacetylates a range of mitochondrial target enzymes thus improving energy production in concomitance with the activity of the SIRT1 substrate PGC1α [31]. The finding of increased protein levels of mtTFA, a key regulator of mitochondrial DNA copy number, may represent an evidence of mitochondrial biogenesis activated by SIRT1 as reported in other systems [28,29]. Although we have not investigated the levels of the de-acetylated forms of sirtuin targets, the pattern of expression here described strongly suggests that SIRT1 changes are associated with a complex response that promotes antioxidant defence, mitochondrial biogenesis and functions [23–28].

When we focused on the antiglycation response, no effects were observed on the expression of Glo1 and Glo2 genes. This may be a part of an adaptive response to prolonged exposure to supraphysiological MG levels. Differences between activation of the glyoxalase system in oocytes and ovaries upon MG insults may be ascribed to many factors. Among them, it is likely that in oocytes we observed an early response whereas in the ovary we described the effects of a prolonged exposure. Nevertheless our results indicated that MG detoxification has been activated in the ovary of MG mice. This conclusion is supported by the observation of increased protein expression of GLO1 along with normal level of MG-AGEs and RAGE. The notion that GLO1 plays a key role in antiglycation response at ovarian level is supported by our previous study showing that reduced activity, protein and gene expression are associated with increased MG-AGEs in ovaries from reproductively aged mice [10].

4.4. The ovarian antiglycation response does not prevent negative effects of MG on oocyte competence

Here we reported that MG mice ovulated oocytes with a reduced biological competence. This conclusion arises from the presence of abnormal meiotic spindles. Although chromosomes were correctly aligned on the MII plate, the oocytes ovulated by MG mice presented barrel shaped spindles that were larger than small focused spindles showed by oocytes from control group. The barrel-shaped spindle phenotype has been described in oocytes subjected to *in vitro* maturation under suboptimal conditions and reflected deregulation of molecular components of spindle poles and microtubule organizing centres (MTOCs) probably associated with GSH imbalance [43,44]. Spindle defects here observed in oocytes exposed to MG during *in vivo* folliculogenesis are less pronounced that those found in oocyte matured *in vitro* in the presence of MG in previous papers [17,18]. This difference may be ascribed to the absence of surrounding somatic cells during *in vitro* MG exposure, as supported by our previous finding that cumulus cells mitigated cytotoxic effects of MG on IVM rate, although this effect was lost during reproductive aging [17]. Overall, our observations from *in vitro* and *in vivo* studies on oocytes along with the evidence for reduced ovarian cell proliferation lead to the hypothesis that MG may have multiple targets in the ovarian microenvironment. It is likely that

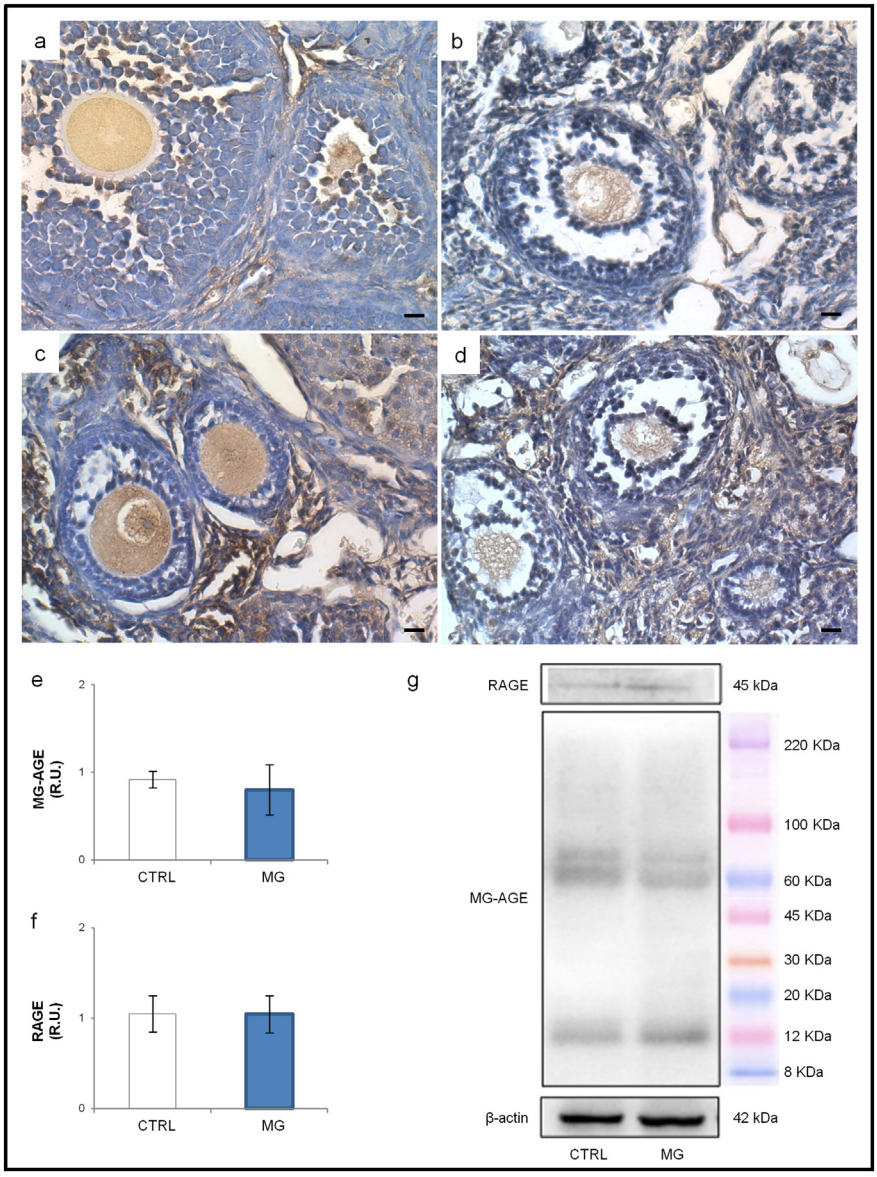

Fig. 7. ***In vivo*** **MG intake influence the expression and intraovarian distribution levels of MG-AGE and RAGE.** Representative images of immunolocalization of MG-AGE and RAGE in control and MG ovaries. MG-AGE: control (a), MG (b), scale bar, 25 μm; RAGE: control (c); MG (d), scale bar, 25 μm. Western blot analysis of MG-AGE (e) and RAGE (f) and representative images (g) are shown. Data are presented as means ± SEM of densitometric analysis of immunoreactive bands normalized to internal reference protein (β-actin). Three mice per experimental group were employed. Experiments were done in triplicate. *t*-test: n.s.

all of them contribute to the reduced ability of the preovulatory follicle to support correct meiotic maturation following gonadotropin stimulation.

5. Conclusions

Although numerous studies support the role of SIRT1 as a protective factor against damages induced by MG or AGE overload in cells and chronically exposed animal models [62–71], our results from mouse

Table 2
Ovulation rate and oocyte quality in control and MG mice.

Experimental group	Ovulation rate	Maturation rate	Meiotic spindle dimensions				
	Oocyte per mouse	% MII	n.	Length (μm)	Width (μm)	Pole width (μm)	Area (μm^2)
CTRL (n = 3)	16.0 ± 0.6	93.5 ± 3.9	32	19.44 ± 0.63	11.23 ± 0.27	3.50 ± 0.16	144.35 ± 7.12
MG (n = 3)	17.3 ± 0.7	94.2 ± 3.2	30	23.75 ± 0.52***	12.78 ± 0.37***	7.07 ± 0.45***	237.26 ± 1.01***

Values are means ± S.E.M.
Pole width indicates the mean width at the two spindle poles. *t*-test.
*** P < 0.001.

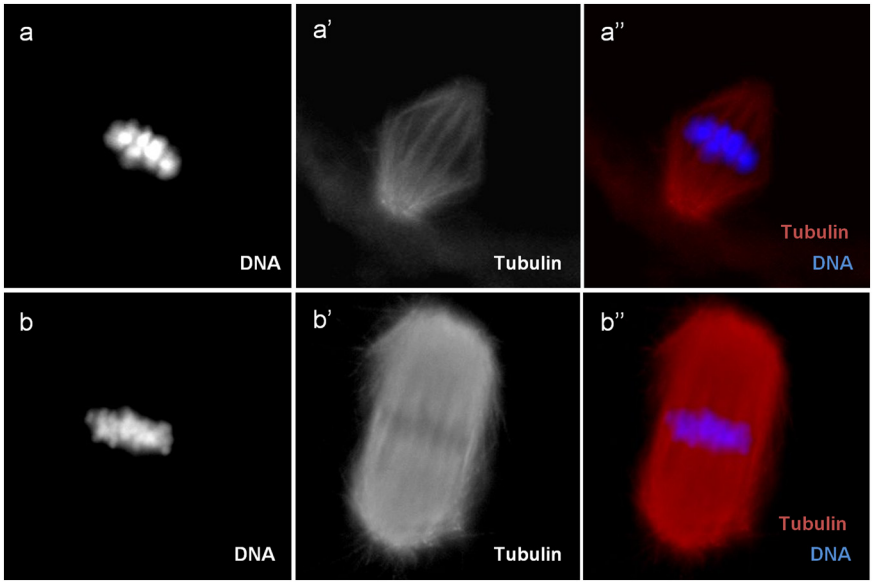

Fig. 8. MG intake influences MII spindle configuration. Representative images of spindles observed in oocytes from control (a–a") and MG (b–b") mice showing differences in dimensions and shape between the two groups. Three mice per experimental group were employed.

oocytes *in vitro* exposed to MG revealed for the first time that SIRT1 can modulate MG scavenging by promoting expression of glyoxalases. Moreover, the finding that up-regulation of glyoxalase is associated with that of components of SIRT1 functional network in the ovaries of MG mice provides strong evidence that SIRT1 participates in the response to methylglyoxal-dependent glycative stress in the female gonad. This response avoids ovarian AGE accumulation and alterations to folliculogenesis, but does not prevent disruption of ovarian and follicle homeostasis required for acquisition of oocyte competence [72,73] and probably responsible for increased embryo aneuploidy and foetal resorption [16].

Further studies are needed to establish the effects of long-term MG *in vivo* administration on the female reproductive system. Although caution should be taken in translating our results in the mouse model to humans, the present study represents a contribution to the knowledge of the effects on fertility of MG and AGE overload during aging, PCOS and diabetes and can help to build up preventive strategies.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Transparency document

The Transparency document associated with this article can be found, in online version.

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References

[1] J. Mano, Reactive carbonyl species: their production from lipid peroxides, action in environmental stress, and the detoxification mechanism, Plant Physiol. Biochem. 59 (2012) 90–97, https://doi.org/10.1016/j.plaphy.2012.03.010.

[2] M. Peppa, J. Uribarri, H. Vlassara, Aging and glycoxidant stress, Hormones (Athens) 7 (2008) 123–132.

[3] I.N. Shipanova, M.A. Glomb, R.H. Nagaraj, Protein modification by methylglyoxal: chemical nature and synthetic mechanism of a major fluorescent adduct, Arch. Biochem. Biophys. 344 (1997) 29–36.

[4] R. Gomes, M. Sousa Silva, A. Quintas, C. Cordeiro, A. Freire, P. Pereira, A. Martins, E. Monteiro, E. Barroso, A. Ponces Freire, Argpyrimidine, a methylglyoxal-derived advanced glycation end-product in familial amyloidotic polyneuropathy, Biochem. J. 385 (2005) 339–345.

[5] N. Rabbani, P.J. Thornalley, Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease, Biochem. Biophys. Res. Commun. 458 (2015) 221–226, https://doi.org/10.1016/j.bbrc.2015.01.140.

[6] E. Diamanti-Kandarakis, C. Piperi, E. Patsouris, P. Korkolopoulou, D. Panidis, L. Pawelczyk, A.G. Papavassiliou, A.J. Duleba, Immunohistochemical localization of advanced glycation end-products (AGEs) and their receptor (RAGE) in polycystic and normal ovaries, Histochem. Cell Biol. 127 (2007) 581–589.

[7] E. Diamanti-Kandarakis, I. Katsikis, C. Piperi, K. Alexandraki, D. Panidis, Effect of long-term orlistat treatment on serum levels of advanced glycation end-products in women with polycystic ovary syndrome, Clin. Endocrinol. 66 (2007) 103–109.

[8] E. Diamanti-Kandarakis, K. Alexandraki, C. Piperi, A. Aessopos, T. Paterakis, I. Katsikis, D. Panidis, Effect of metformin administration on plasma advanced glycation end product levels in women with polycystic ovary syndrome, Metabolism 56 (2007) 129–134.

[9] M. Jinno, M. Takeuchi, A. Watanabe, K. Teruya, J. Hirohama, N. Eguchi, A. Miyazaki, Advanced glycation end-products accumulation compromises embryonic development and achievement of pregnancy by assisted reproductive technology, Hum. Reprod. 26 (2011) 604–610, https://doi.org/10.1093/humrep/deq388.

[10] C. Tatone, M.C. Carbone, G. Campanella, C. Festuccia, P.G. Artini, V. Talesa, R. Focarelli, F. Amicarelli, Female reproductive dysfunction during ageing: role of methylglyoxal in the formation of advanced glycation endproducts in ovaries of reproductively-aged mice, J. Biol. Regul. Homeost. Agents 24 (2010) 63–72.

[11] S. Falone, S.J. Santini, V. Cordone, M. Grannonico, M. Cacchio, G. Di Emidio, C. Tatone, F. Amicarelli, Regular and moderate exercise counteracts the decline of antioxidant protection but not methylglyoxal-dependent glycative burden in the ovary of reproductively aging mice, Oxidative Med. Cell. Longev. 2016 (2016) 3837623, , https://doi.org/10.1155/2016/3837623.

[12] M. Pertynska-Marczewska, E. Diamanti-Kandarakis, Aging ovary and the role for advanced glycation end products, Menopause 24 (2017) 345–351, https://doi.org/10.1097/GME.0000000000000755.

[13] K. Malicková, R. Jarosová, K. Rezábek, T. Fait, J. Masata, I. Janatková, T. Zima, M. Kalousová, Concentrations of sRAGE in serum and follicular fluid in assisted reproductive cycles–a preliminary study, Clin. Lab. 56 (2010) 377–384.

[14] E.Y. Fujii, M. Nakayama, The measurements of RAGE, VEGF, and AGEs in the plasma and follicular fluid of reproductive women: the influence of aging, Fertil. Steril. 94 (2010) 694–700, https://doi.org/10.1016/j.fertnstert.2009.03.029.

[15] E. Kandaraki, A. Chatzigeorgiou, C. Piperi, E. Palioura, S. Palimeri, P. Korkolopoulou, M. Koutsilieris, A.G. Papavassiliou, Reduced ovarian glyoxalase-I activity by dietary glycotoxins and androgen excess: a causative link to polycystic ovarian syndrome, Mol. Med. 18 (2012) 1183–1189, https://doi.org/10.2119/molmed.2012.00293.

[16] Y.J. Chang, W.H. Chan, Methylglyoxal has injurious effects on maturation of mouse oocytes, fertilization, and fetal development, via apoptosis, Toxicol. Lett. 193

(2010) 217–223, https://doi.org/10.1016/j.toxlet.2010.01.007.
[17] C. Tatone, T. Heizenrieder, G. Di Emidio, P. Treffon, F. Amicarelli, T. Seidel, U. Eichenlaub-Ritter, Evidence that carbonyl stress by methylglyoxal exposure induces DNA damage and spindle aberrations, affects mitochondrial integrity in mammalian oocytes and contributes to oocyte ageing, Hum. Reprod. 26 (2011) 1843–1859, https://doi.org/10.1093/humrep/der140.
[18] Y. Liu, X.Q. He, X. Huang, L. Ding, L. Xu, Y.T. Shen, F. Zhang, M.B. Zhu, B.H. Xu, Z.Q. Qi, H.L. Wang, Resveratrol protects mouse oocytes from methylglyoxal-induced oxidative damage, PLoS One 8 (2013) e77960, , https://doi.org/10.1371/journal.pone.0077960.
[19] C. Tatone, F. Amicarelli, The aging ovary–the poor granulosa cells, Fertil. Steril. 99 (2013) 12–17, https://doi.org/10.1016/j.fertnstert.2012.11.029.
[20] C. Tatone, U. Eichenlaub-Ritter, F. Amicarelli, Dicarbonyl stress and glyoxalases in ovarian function, Biochem. Soc. Trans. 42 (2014) 433–438, https://doi.org/10.1042/BST20140023.
[21] M. Buler, U. Andersson, J. Hakkola, Who watches the watchmen? Regulation of the expression and activity of sirtuins, FASEB J. 30 (2016) 3942–3960.
[22] W. Cai, M. Ramdas, L. Zhu, X. Chen, G.E. Striker, H. Vlassara, Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 15888–15893.
[23] C. Tatone, G. Di Emidio, M. Vitti, M. Di Carlo, S. Santini Jr., A.M. D'Alessandro, S. Falone, F. Amicarelli, Sirtuin functions in female fertility: possible role in oxidative stress and aging, Oxidative Med. Cell. Longev. 2015 (2015) 659687, , https://doi.org/10.1155/2015/659687.
[24] C. Tatone, G. Di Emidio, A. Barbonetti, G. Carta, A.M. Luciano, S. Falone, F. Amicarelli, Sirtuins in gamete biology and reproductive physiology: emerging roles and therapeutic potential in female and male infertility, Hum. Reprod. Update 24 (2018) 267–289, https://doi.org/10.1093/humupd/dmy003.
[25] Y. Olmos, F.J. Sánchez-Gómez, B. Wild, N. García-Quintans, S. Cabezudo, S. Lamas, M. Monsalve, SirT1 regulation of antioxidant genes is dependent on the formation of a FoxO3a/PGC-1α complex, Antioxid. Redox Signal. 19 (2013) 1507–1521, https://doi.org/10.1089/ars.2012.4713.
[26] H.C. Chang, L. Guarente, SIRT1 and other sirtuins in metabolism, Trends Endocrinol. Metab. 25 (2014) 138–145, https://doi.org/10.1016/j.tem.2013.12.001.
[27] S. Imai, L. Guarente, NAD+ and sirtuins in aging and disease, Trends Cell Biol. 24 (2014) 464–471, https://doi.org/10.1016/j.tcb.2014.04.002.
[28] K. Aquilano, P. Vigilanza, S. Baldelli, B. Pagliei, G. Rotilio, M.R. Ciriolo, Peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis, J. Biol. Chem. 285 (2010) 21590–21599, https://doi.org/10.1074/jbc.M109.070169.
[29] T. Zhang, Y. Chi, Y. Kang, H. Lu, H. Niu, W. Liu, Y. Li, Resveratrol ameliorates podocyte damage in diabetic mice via SIRT1/PGC-1α mediated attenuation of mitochondrial oxidative stress, J. Cell. Physiol. (2018), https://doi.org/10.1002/jcp.27306.
[30] J. Brenmoehl, A. Hoeflich, Dual control of mitochondrial biogenesis by sirtuin 1 and sirtuin 3, Mitochondrion 13 (2013) 755–761, https://doi.org/10.1016/j.mito.2013.04.002.
[31] A. Chalkiadaki, L. Guarente, Sirtuins mediate mammalian metabolic responses to nutrient availability, Nat. Rev. Endocrinol. 8 (2012) 287–296, https://doi.org/10.1038/nrendo.2011.225.
[32] I. Ortega, A.J. Duleba, Ovarian actions of resveratrol, Ann. N. Y. Acad. Sci. 1348 (2015) 86–96, https://doi.org/10.1111/nyas.12875.
[33] M. Ghosh, D. Talukdar, S. Ghosh, N. Bhattacharyya, M. Ray, S. Ray, In vivo assessment of toxicity and pharmacokinetics of methylglyoxal. Augmentation of the curative effect of methylglyoxal on cancer-bearing mice by ascorbic acid and creatine, Toxicol. Appl. Pharmacol. 212 (2006) 45–58.
[34] T.R. Hoage, I.L. Cameron, Folliculogenesis in the ovary of the mature mouse: a radioautographic study, Anat. Rec. 184 (1976) 699–709.
[35] G. Di Emidio, S. Falone, M. Vitti, A.M. D'Alessandro, M. Vento, C. Di Pietro, F. Amicarelli, C. Tatone, SIRT1 signalling protects mouse oocytes against oxidative stress and is deregulated during aging, Hum. Reprod. 29 (2014) 2006–2017, https://doi.org/10.1093/humrep/deu160.
[36] S.S. Kwak, S.A. Cheong, J.D. Yoon, Y. Jeon, S.H. Hyun, Expression patterns of sirtuin genes in porcine preimplantation embryos and effects of sirtuin inhibitors on in vitro embryonic development after parthenogenetic activation and in vitro fertilization, Theriogenology 78 (2012) 1597–1610, https://doi.org/10.1016/j.theriogenology.2012.07.006.
[37] S.S. Kwak, S.A. Cheong, Y. Jeon, E. Lee, K.C. Choi, E.B. Jeung, S.H. Hyun, The effects of resveratrol on porcine oocyte in vitro maturation and subsequent embryonic development after parthenogenetic activation and in vitro fertilization, Theriogenology 78 (2012) 86–101, https://doi.org/10.1016/j.theriogenology.2012.01.024.
[38] G.G. Leoni, M.G. Palmerini, V. Satta, S. Succu, V. Pasciu, A. Zinellu, C. Carru, G. Macchiarelli, S.A. Nottola, S. Naitana, F. Berlinguer, Differences in the kinetic of the first meiotic division and in active mitochondrial distribution between prepubertal and adult oocytes mirror differences in their developmental competence in a sheep model, PLoS One 10 (2015) e0124911, , https://doi.org/10.1371/journal.pone.0124911.
[39] W.T. Golde, P. Gollobin, L.L. Rodriguez, A rapid, simple, and humane method for submandibular bleeding of mice using a lancet, Lab Anim. (NY) 34 (2005) 39–43.
[40] G. Di Emidio, G. Rossi, I. Bonomo, G.L. Alonso, R. Sferra, A. Vetuschi, P.G. Artini, A. Provenzani, S. Falone, G. Carta, A.M. D'Alessandro, F. Amicarelli, C. Tatone, The natural carotenoid crocetin and the synthetic tellurium compound AS101 protect the ovary against cyclophosphamide by modulating SIRT1 and mitochondrial markers, Oxidative Med. Cell. Longev. 2017 (2017) 8928604, , https://doi.org/10.1155/2017/8928604.
[41] T. Pedersen, H. Peters, Proposal for a classification of oocytes and follicles in the mouse ovary, J. Reprod. Fertil. 17 (1968) 555–557.
[42] V. Di Nisio, G. Rossi, M.G. Palmerini, G. Macchiarelli, G.M. Tiboni, S. Cecconi, Increased rounds of gonadotropin stimulation have side effects on mouse fallopian tubes and oocytes, Reproduction 155 (2018) 245–250, https://doi.org/10.1530/REP-17-0687.
[43] A. Sanfins, G.Y. Lee, C.E. Plancha, E.W. Overstrom, D.F. Albertini, Distinctions in meiotic spindle structure and assembly during in vitro and in vivo maturation of mouse oocytes, Biol. Reprod. 69 (2003) 2059–2067.
[44] E. Ibáñez, A. Sanfins, C.M. Combelles, E.W. Overström, D.F. Albertini, Genetic strain variations in the metaphase-II phenotype of mouse oocytes matured in vivo or in vitro, Reproduction 130 (2005) 845–855.
[45] C.T. Campbell, J.E. Kolesar, B.A. Kaufman, Mitochondrial transcription factor A regulates mitochondrial transcription initiation, DNA packaging, and genome copy number, Biochim. Biophys. Acta 1819 (2012) 921–929, https://doi.org/10.1016/j.bbagrm.2012.03.002.
[46] O. Papalou, E. Diamanti-Kandarakis, The role of stress in PCOS, Expert. Rev. Endocrinol. Metab. 12 (2017) 87–95, https://doi.org/10.1080/17446651.2017.1266250.
[47] C. Tatone, F. Amicarelli, M.C. Carbone, P. Monteleone, D. Caserta, R. Marci, P.G. Artini, P. Piomboni, R. Focarelli, Cellular and molecular aspects of ovarian follicle ageing, Hum. Reprod. Update 14 (2008) 131–142, https://doi.org/10.1093/humupd/dmm048.
[48] G. Di Emidio, A. D'Alfonso, P. Leocata, V. Parisse, A. Di Fonso, P.G. Artini, F. Patacchiola, C. Tatone, G. Carta, Increased levels of oxidative and carbonyl stress markers in normal ovarian cortex surrounding endometriotic cysts, Gynecol. Endocrinol. 30 (2014) 808–812, https://doi.org/10.3109/09513590.2014.938625.
[49] Z. Merhi, Advanced glycation end products and their relevance in female reproduction, Hum. Reprod. 29 (2014) 135–145, https://doi.org/10.1093/humrep/det383.
[50] D. Garg, Z. Merhi, Advanced glycation end products: link between diet and ovulatory dysfunction in PCOS? Nutrients 7 (2015) 10129–10144, https://doi.org/10.3390/nu7125524.
[51] M. Hellwig, S. Gensberger-Reigl, T. Henle, M. Pischetsrieder, Food-derived 1,2-dicarbonyl compounds and their role in diseases, Semin. Cancer Biol. 49 (2018) 1–8, https://doi.org/10.1016/j.semcancer.2017.11.014.
[52] K. Nowotny, D. Schröter, M. Schreiner, T. Grune, Dietary advanced glycation end products and their relevance for human health, Ageing Res. Rev. 47 (2018) 55–66, https://doi.org/10.1016/j.arr.2018.06.005.
[53] S. Wang, Z. Kou, Z. Jing, Y. Zhang, X. Guo, M. Dong, I. Wilmut, S. Gao, Proteome of mouse oocytes at different developmental stages, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 17639–17644, https://doi.org/10.1073/pnas.1013185107.
[54] S. Ranganathan, P.J. Ciaccio, E.S. Walsh, K.D. Tew, Genomic sequence of human glyoxalase-I: analysis of promoter activity and its regulation, Gene 240 (1999) 149–155.
[55] M. Xue, N. Rabbani, H. Momiji, P. Imbasi, M.M. Anwar, N. Kitteringham, B.K. Park, T. Souma, T. Moriguchi, M. Yamamoto, P.J. Thornalley, Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation, Biochem. J. 443 (2012) 213–222, https://doi.org/10.1042/BJ20111648.
[56] Y. Xu, X. Chen, Glyoxalase II, a detoxifying enzyme of glycolysis byproduct methylglyoxal and a target of p63 and p73, is a pro-survival factor of the p53 family, J. Biol. Chem. 281 (2006) 26702–26713.
[57] J. Gebel, M. Tuppi, K. Krauskopf, D. Coutandin, S. Pitzius, S. Kehrloesser, C. Osterburg, V. Dötsch, Control mechanisms in germ cells mediated by p53 family proteins, J. Cell Sci. (2017), https://doi.org/10.1242/jcs.204859 (pii: jcs.204859).
[58] M. Amiri, F. Ramezani Tehrani, F. Nahidi, R. Bidhendi Yarandi, S. Behboudi-Gandevani, F. Azizi, Association between biochemical hyperandrogenism parameters and Ferriman-Gallwey score in patients with polycystic ovary syndrome: a systematic review and meta-regression analysis, Clin. Endocrinol. 87 (2017) 217–230, https://doi.org/10.1111/cen.13389.
[59] M.W. O'Reilly, A.E. Taylor, N.J. Crabtree, B.A. Hughes, F. Capper, R.K. Crowley, P.M. Stewart, J.W. Tomlinson, W. Arlt, Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione, J. Clin. Endocrinol. Metab. 99 (2014) 1027–1036, https://doi.org/10.1210/jc.2013-3399.
[60] D. Garg, Z. Merhi, Relationship between advanced glycation end products and steroidogenesis in PCOS, Reprod. Biol. Endocrinol. 14 (2016) 71.
[61] H. Yao, I.K. Sundar, T. Ahmad, C. Lerner, J. Gerloff, A.E. Friedman, R.P. Phipps, P.J. Sime, M.W. McBurney, L. Guarente, I. Rahman, SIRT1 protects against cigarette smoke-induced lung oxidative stress via a FOXO3-dependent mechanism, Am. J. Phys. Lung Cell. Mol. Phys. 306 (2014) L816–L828, https://doi.org/10.1152/ajplung.00323.2013.
[62] E.M. Choi, K.S. Suh, S.Y. Rhee, Y.S. Kim, Sciadopitysin alleviates methylglyoxal-mediated glycation in osteoblastic MC3T3-E1 cells by enhancing glyoxalase system and mitochondrial biogenesis, Free Radic. Res. 48 (2014) 729–739, https://doi.org/10.3109/10715762.2014.903562.
[63] K.S. Suh, E.M. Choi, W.W. Jung, Y.J. Kim, S.M. Hong, S.Y. Park, S.Y. Rhee, S. Chon, Deoxyactein protects pancreatic β-cells against methylglyoxal-induced oxidative cell damage by the upregulation of mitochondrial biogenesis, Int. J. Mol. Med. 40 (2017) 539–548, https://doi.org/10.3892/ijmm.2017.3018.
[64] K.S. Suh, S. Chon, W.W. Jung, E.M. Choi, Magnolol protects pancreatic β-cells against methylglyoxal-induced cellular dysfunction, Chem. Biol. Interact. 277 (2017) 101–109, https://doi.org/10.1016/j.cbi.2017.09.014.

[65] H. Vlassara, W. Cai, E. Tripp, R. Pyzik, K. Yee, L. Goldberg, L. Tansman, X. Chen, V. Mani, Z.A. Fayad, G.N. Nadkarni, G.E. Striker, J.C. He, J. Uribarri, Oral AGE restriction ameliorates insulin resistance in obese individuals with the metabolic syndrome: a randomised controlled trial, Diabetologia 59 (2016) 2181–2192, https://doi.org/10.1007/s00125-016-4053-x.

[66] Y.F. Zhang, W. Wei, L. Li, G. Tu, Y. Zhang, J. Yang, Y. Xing, Sirt1 and HMGB1 regulate the AGE-induced pro-inflammatory cytokines in human retinal cells, Clin. Lab. 61 (2015) 999–1008.

[67] P. Li, L. Zhang, C. Zhou, N. Lin, A. Liu, Sirt 1 activator inhibits the AGE-induced apoptosis and p53 acetylation in human vascular endothelial cells, J. Toxicol. Sci. 40 (2015) 615–624, https://doi.org/10.2131/jts.40.615.

[68] H.Q. He, Y. Liu, H. Zeng, X.L. Sun, L. Zhang, X.L. Zhang, W.J. Liao, X.Y. Zhou, Y.Z. He, Advanced glycation endproducts regulate smooth muscle cells calcification in cultured HSMCs, Int. J. Clin. Exp. Pathol. 8 (2015) 12260–12267.

[69] K. Huang, C. Chen, J. Hao, J. Huang, S. Wang, P. Liu, H. Huang, Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs-induced upregulation of fibronetin and transforming growth factor-β1 in rat glomerular messangial cells, Mol. Cell. Endocrinol. 399 (2015) 178–189, https://doi.org/10.1016/j.mce.2014.08.014.

[70] K.P. Huang, C. Chen, J. Hao, J.Y. Huang, P.Q. Liu, H.Q. Huang, AGEs-RAGE system down-regulates Sirt1 through the ubiquitin-proteasome pathway to promote FN and TGF-β1 expression in male rat glomerular mesangial cells, Endocrinology 156 (2015) 268–279, https://doi.org/10.1210/en.2014-1381.

[71] W. Cai, J. Uribarri, L. Zhu, X. Chen, S. Swamy, Z. Zhao, F. Grosjean, C. Simonaro, G.A. Kuchel, M. Schnaider-Beeri, M. Woodward, G.E. Striker, H. Vlassara, Oral glycotoxins are a modifiable cause of dementia and the metabolic syndrome in mice and humans, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 4940–4945, https://doi.org/10.1073/pnas.1316013111.

[72] M.L. Hennet, C.M. Combelles, The antral follicle: a microenvironment for oocyte differentiation, Int. J. Dev. Biol. 56 (2012) 819–831, https://doi.org/10.1387/ijdb.120133cc.

[73] A.P. Sobinoff, J.M. Sutherland, E.A. Mclaughlin, Intracellular signalling during female gametogenesis, Mol. Hum. Reprod. 19 (2013) 265–278, https://doi.org/10.1093/molehr/gas065.