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*Research Article*

# Protective activity of *Chlorella vulgaris* microalgae extract on the *in vitro* cultured oocytes

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**Abstract**

Free radical scavengers, which act as antioxidants, have the potential to prevent oocyte aging and preserve fertilization capacity. The microalgae are an important source of antioxidants in the environment. This study examined the protective effects of methanol extracts derived from *Chlorella* sp. microalgae on the viability and maturation of oocytes obtained from mice under *in vitro* conditions. The microalgae were collected from the Persian Gulf region and cultured in Bold's Basal medium. The antioxidant capacity of the extracts was assessed using the ferric reducing ability of plasma (FRAP) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) methods. Oocytes from female Naval Medical Research Institute (NMRI) mice were treated with the microalgae extracts for 16 h. The viability and maturity of the oocytes were evaluated using inverted microscopy. The expression levels of SIRT-3, BMP-15, and GDF-9 genes in the treated oocytes were analyzed using real-time PCR. Both extracts showed antioxidant properties, according to the findings of FRAP and DPPH analyses. Untreated oocytes had a survival percentage of 58% without maturation. However, oocytes treated with either extract at a dosage of 150 µg/ml had a survival rate of over 70%. The gene expression levels of SIRT-3 and BMP-15 were significantly higher in the treatment groups compared to the control group. In conclusion, *Chlorella* sp. extracts demonstrated protective effects on oocyte survival, but they did not significantly impact oocyte maturation. This study emphasizes the potential therapeutic uses of microalgae extracts from the Persian Gulf for disorders associated with infertility.

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## Introduction

Oocytes undergo postovulatory aging when they fail to be fertilized after ovulation, resulting in a range of morphological, genetic, and epigenetic consequences (Di Emidio *et al.*, 2021). The detrimental effects of postovulatory senescence include mitochondrial dysfunction, telomere shortening, and meiotic spindle abnormalities. These well-recognized implications contribute to the reduced number of oocytes and quality of embryos (Cimadomo *et al.*, 2018). The formation of reactive oxygen species (ROS) is a key factor inducing the process of aging in oocytes (Premkumar and Chaube, 2016). *In-vitro* fertilization procedures, particularly gamete collection, oocyte manipulation, and cell culture, may produce ROS, reducing oocyte quality, fertilization rate, and embryonic development (Niu *et al.*, 2023). The balance between ROS production and detoxification plays an important role in fertilization as well as preimplantation embryonic development (Deluao *et al.*, 2022). Antioxidants are substances that neutralize free radicals and remove reactive oxygen species (ROS), thus helping to maintain a balance between antioxidants and oxidative stress in oocytes (Khazaei and Aghaz, 2017). To counteract the harmful effects of oxidative damage, mitochondria employ a range of antioxidants along with many enzymatic defense mechanisms (Jiang *et al.*, 2020). Sirtuin (SIRT) protein family members, which are deacetylases dependent on Nicotinamide adenine dinucleotide (NAD), play a crucial role in regulating the antioxidant activity of mitochondria by inhibiting ROS production via direct and indirect mechanisms (Di Emidio *et al.*, 2021).Mammals possess a total of seven SIRT paralogs, namely SIRT 1-7, which exhibit distinct primary-terminal structures and are distributed throughout several subcellular compartments (Vo *et al.*, 2023). SIRT-3 modulates the intramitochondrial antioxidant environment through deacetylation-mediated activation of superoxide dismutase 2 (SOD2) (Zhang *et al.*, 2020). In addition to limiting reactive oxygen species (ROS) levels within mitochondria, SIRT-3 facilitates the repair of oxidative DNA damage, notably in mitochondrial DNA (mtDNA), by stabilizing 8-oxoguanine-DNA glycosylase 1 (OGG1) (Cheng *et al.*, 2013). Studies have shown that the oocytes of SIRT-3$^{-/-}$ female mice exhibit diminished fertilization capacity and impaired blastocyst formation (Kawamura *et al.*, 2010). Moreover, various experiments employing knock-in or knock-out techniques targeting SIRT-3 in oocytes have underscored its pivotal role in mitigating oxidative stress induced by reactive oxygen species (ROS) in murine models afflicted with obesity or diabetes (Zhang *et al.*, 2015).

The accumulation of reactive oxygen species (ROS) within oocytes results in impairment of their capacity for successful fertilization (Wang *et al.*, 2021). The growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15) proteins are members of the transforming growth factor-beta (TGF-β) superfamily. These proteins, secreted from oocytes, significantly impact oocyte maturation and fertilization processes. When cumulus-oocyte complexes (COCs)

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are subjected to treatment with GDF-9 or recombinant BMP-15 during *in vitro* maturation (IVM), there is a discernible increase in blastocyst formation rates and embryo production in mice (Sudiman *et al.*, 2014). In a study employing a mouse model, it was demonstrated that BMP-15 $^{-/-}$ females exhibited restricted ovulation and fertilization, while GDF-9 $^{-/-}$ mice displayed arrested follicular development at the early follicular stage (Juengel *et al.*, 2004).

Microalgae are photosynthetic microorganisms with antioxidant, anti-inflammatory, anti-cancer, and antimicrobial activities (Khavari *et al.*, 2021). Microalgae-derived antioxidants protect cells from ROS produced in response to biotic or abiotic stressors (Coulombier *et al.*, 2021). *Chlorella vulgaris* is a single-cell green microalga containing various chemical components with antioxidant activity, including phenolic compounds, carotene, ascorbic acid, and tocopherols (Mtaki *et al*., 2020). Apart from their cost-effectiveness and simplicity of culture, *Chlorella vulgaris* microalgae possess antioxidant properties that surpass those observed in industrial antioxidants (Rodriguez-Garcia and Guil-Guerrero, 2008). Furthermore, they are recognized as a useful resource for pharmacological and medical applications, allowing for the acquisition of bioactive metabolites (Kaur *et al.*, 2023). While the beneficial effects of nutrition and supplementation with *Chlorella vulgaris* on improving infertility-related disorders have been proven in some studies (Farag *et al.*, 2023), its effects on the growth and maturation of female germ cells, as well as factors associated with oocyte fertilization, remain to be precisely determined. The identification of these mechanisms may yield innovative therapeutic resources for fertility-related issues (Vaskova *et al.*, 2023). Therefore, the purpose of this investigation was to determine whether methanolic extracts of *Chlorella* sp. N4 and *Chlorella* sp. D1 microalgae affected the maturation and survival of oocytes obtained from Syrian mice. Additionally, in order to assess the precise molecular mechanism of action, the expression levels of the genes GDF-9, SIRT-3, and BMP-15 were measured in oocytes treated with the D1 and N4 extracts.

## Materials and methods

*Culture of Chlorella vulgaris microalgae*

The D1 and N4 microalgae *Chlorella vulgaris* (referred to as *Chlorella* sp. D1 and *Chlorella* sp. N4) were obtained from the Persian Gulf located in the southern region of Iran (26°32N, 53°56E), and their molecular identification was determined and reported (Vahdati *et al.*, 2022). Bold's Basal Medium (BBM) was used for the culture of microalgae; initially, a 10% volume of the purified microalgae strain was added to 250 ml Erlenmeyer flasks containing 150 ml of culture media. The flasks were subjected to rotation at a speed of 110 rpm and maintained at a temperature of 25°C. The exposure conditions involved the use of cold white fluorescent lights with an intensity of 49.9 µmol $^{m-2\ s-1}$. The microalgae were grown under alternating light and dark conditions with a cycle duration of 16:8 hours. The spectrophotometer was utilized to measure the cell density daily, specifically at a wavelength of 620 nm. Following a period

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of 15 days, the concentration of the inoculum was approximately $5.5\times10^7$ cells/mL. In order to process the biomass after the end of the logarithmic phase, the first step involved subjecting the biomass to several washes using distilled water for desalination. Each washing cycle lasted 20 minutes and was followed by centrifugation at 1500 g. Afterward, the biomass was subjected to a drying process using a freeze dryer. The dried biomass was thereafter stored at a temperature of 4°C.

*Extraction of bioactive compounds from microalgae*

To initiate the extraction process, 200 mg of frozen dry microalgae was subjected to grinding for 24 hours. Subsequently, the ground microalgae were extracted with 2 mL of methanol at ambient temperature for 24 hours. The tube was centrifuged at 1500 g for 10 minutes, and the resulting supernatant was collected, filtered, and used. Three rounds of extraction were performed on the residues, and the resulting supernatants were combined.

*Measurement of the antioxidant activity of microalgae extract*

*Ferric reduction activity potential (FRAP) analysis*

To conduct FRAP analysis, solutions containing 125 and 250 µg/mL of the extracts were prepared. The solution had a final volume of 2 mL and comprised the FRAP solution containing 10 mM TPTZ (dissolved in 40 mM HCl) and 20 mM Fe. Furthermore, a 300 mM acetate buffer was introduced into the solution, resulting in a pH of 6.3. The aforementioned sample was subjected to a temperature of 37°C for 10 minutes, after which the intensity of its color was measured at a wavelength of 593 nm in comparison to a blank sample. The standard curve was generated using ferrous sulfate (FeSO4, $7H_2O$) at a concentration of 1000 µM, as well as the concentrations of 125 µM, 250 µM, and 500 µM. The quantification of antioxidant activity in the extracts was carried out by measuring the amount of micromoles of ferrous ion ($Fe^{+2}$) involved.

*2, 2-diphenyl-1-picrylhydrazyl (DPPH) measurement*

Following the preparation of a 100 µM DPPH solution, the absorbance at a wavelength of 517 nm was measured. Appropriate amounts of ethanol and the microalgae extract, with concentrations of 125 and 250 µg/ml, respectively, were introduced into the solution and kept at ambient temperature. The absorbance of the samples was measured at a wavelength of 517 nm after an incubation period of 30 minutes. The calculation of the percentage of inhibition or regeneration of DPPH by an antioxidant molecule was performed using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs Control} - \text{Abs Sample}}{Abs\ control} \times 100$$

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*Ovulation induction and oocyte collection*

To induce ovulation, female NMRI mice, aged 5-6 weeks, were administered an intraperitoneal injection of 10 IU of pregnant horse serum gonadotropin (PMSG) hormone (Hipra Co., Spain). The animals were housed under standard laboratory conditions of temperature and a 12-hour light-dark cycle, with unrestricted access to water and food, and all ethical guidelines in dealing with animals were followed in accordance with the Ethics Code: IR.KHU.REC.1400.044. The animals were sacrificed spinally 44-48 hours following the injection, and their ovaries were extracted and dissected in DMEM culture media containing 10% fetal bovine serum and 1% antibiotics. The oocytes at the germinal vesicle (GV) stage were carefully transferred to a fresh medium using an inverted light microscope and oral pipetting. Subsequently, the samples were put in an incubator for 16 hours.

*Treatment of oocytes with extract*

The collected oocytes were allocated into three distinct groups: a control group with no treatment, a group treated with *Chlorella* sp. D1 extract, and a group treated with *Chlorella* sp. N4 extract. Each group had a minimum of 30 oocytes. The concentrations of the extracts that were tested were 50, 150, and 300 µg/mL.

*Survival rate and maturity of oocytes in the culture medium*

Following a 16-hour incubation period, the control and treated oocytes were observed under an inverted microscope at magnifications of 10× and 40×. The quantification of viable and non-viable oocytes were conducted by assessing their morphological characteristics, and subsequently, the survival rate was calculated. The existence of the polar body was examined to assess the level of maturity. Then, the oocyte maturity percentage was quantified.

*Real-time PCR*

The oocytes from all experimental groups were collected via centrifugation, and their total RNA was subsequently extracted utilizing the Trizol reagent. The cDNA synthesis was performed using the Thermo Scientific Revert Aid RT kit (USA) according to the manufacturer's instructions. Oligo 7 software was used to design the specific primers, which were synthesized by Sina Clone Company (Iran). Table 1 displays the primer sequences used in this research. Real-time PCR was carried out using Amplicon Master Mix (Denmark) and Corbett Rotor Gene-RG 6000 (USA). The temperature cycle was 95°C for 5 minutes, 95°C for 30s, 62°C for 60s, 72°C for 60s, and 40 cycles. In all groups, real-time PCR reactions for the target and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were conducted in duplicate, and the relative expression of genes was evaluated using CTs and REST2009 software.

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**Table 1: Sequence of primers.**

| Name | Seq | Product size | Accession nu | Ta |
|---|---|---|---|---|
| SIRT-3 | F: 5'- ATGTGGTCCAGCTAGGGGAT -3'<br>R: 5'- ACGTTCCCGCTGCATAAGAT -3' | 98 | NM_022433.2 | 59 |
| BMP-15 | F: 5'- TCCTTGCTGACGACCCTACAT-3'<br>R: 5'- TACCTCAGGGGATAGCCTTGG-3 | 100 | NM-001289726/1 | 60 |
| GDF-9 | F: 5'- CTCCCACTCTTCCACCTTCG-3'<br>R: 5'- GCCTCTCTTGCTCAGTGTCC-3' | 136 | NM-008110/2 | 59 |
| GAPDH | F: 5'- CTCCCACTCTTCCACCTTCG-3'<br>R: 5'- GCCTCTCTTGCTCAGTGTCC-3' | 189 | NM_001289726.1 | 60 |

*Statistical analysis*

The experiments were conducted with a minimum of three repetitions, and the data were subjected to statistical analysis using one-way analysis of variance followed by Tukey's post hoc test. A significance level of $p<0.05$ was considered as statistically significant differences. The diagrams were created with the GraphPad Prism software.

## Results

*Antioxidant effect of Chlorella sp. D1 and Chlorella sp. N4 extracts*

The analysis of the antioxidant properties of two extracts derived from *Chlorella* sp. D1 and *Chlorella* sp. N4 microalgae revealed their ability to suppress the activity of free radicals effectively. The results of the FRAP assay revealed that the FRAP value of *Chlorella* sp. D1 extract at a concentration of 125 µg/mL exhibited 23 µM $Fe^{+2}$, whereas the FRAP value of *Chlorella* sp. D1 extract at 250 µg/mL concentration displayed 51.4 µM $Fe^{+2}$. In contrast, it was observed that the FRAP value of *Chlorella* sp. N4 extract at a concentration of 125 µg/mL had a content of 21.4 µM $Fe^{+2}$, whereas the FRAP value of *Chlorella* sp. N4 extract at a dosage of 250 µg/ml had a content of 50.5 µM $Fe^{+2}$. Upon analysis, it was determined that there was no statistically significant difference between the two extracts (Fig. 1a).

The results of the DPPH test showed that the *Chlorella* sp. D1 extract had a free radical scavenging capacity of 10.5% at a concentration of 125 µg/mL and 19.5% at a concentration of 250 µg/mL. Similarly, the *Chlorella* sp. N4 extract displayed a free radical scavenging capacity of 15% at 125 µg/mL and 16.5% at 250 µg/mL. Nevertheless, it is important to highlight that there was no statistically significant difference in the free radical scavenging ability between the two extracts at the corresponding concentrations (Fig. 1b).

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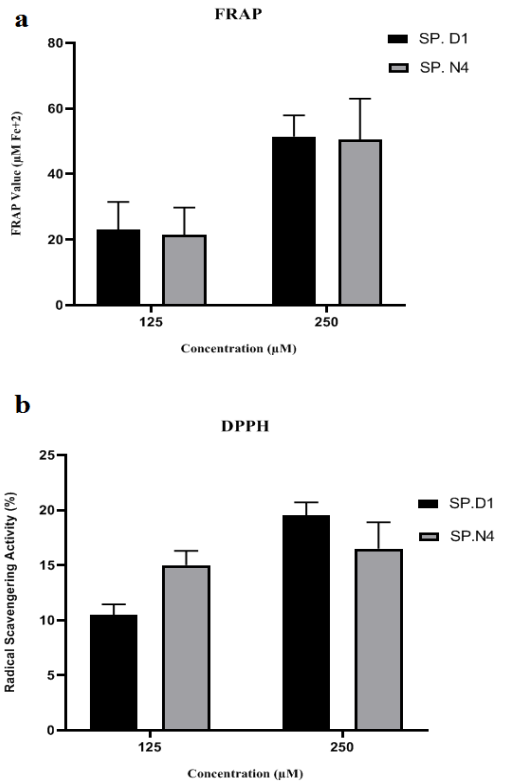



**Figure 1: Comparison the antioxidant activity of *Chlorella* sp. D1 (sp. D1) and *Chlorella* sp. N4 (sp. N4) extracts using FRAP and DPPH assays. There was no significant difference between extracts at 125 or 250 (µg/mL) concentrations.**

*Effects of Chlorella sp. D1 and Chlorella sp. N4 extracts on the survival and maturity of oocytes in the culture medium*

Based on the morphological observations, the *Chlorella* sp. D1 and *Chlorella* sp. N4 extracts exhibited significant levels of toxicity when administered at a concentration of 300 µg/mL and resulted in the degeneration of all oocytes. In contrast, 50 µg/mL concentration yielded outcomes comparable to those of the control group, with no statistically significant distinction (Fig. 2a-c). However, when exposed to a dose of 150 µg/mL, the observed outcomes aligned with the findings presented in Table 2. In addition, after 16 hours of treatment with two microalgae, *Chlorella* sp. N4 and *Chlorella* sp. D1, at a 150 µg/mL concentration, oocytes with polar bodies were counted, and the results are shown in Table 3.

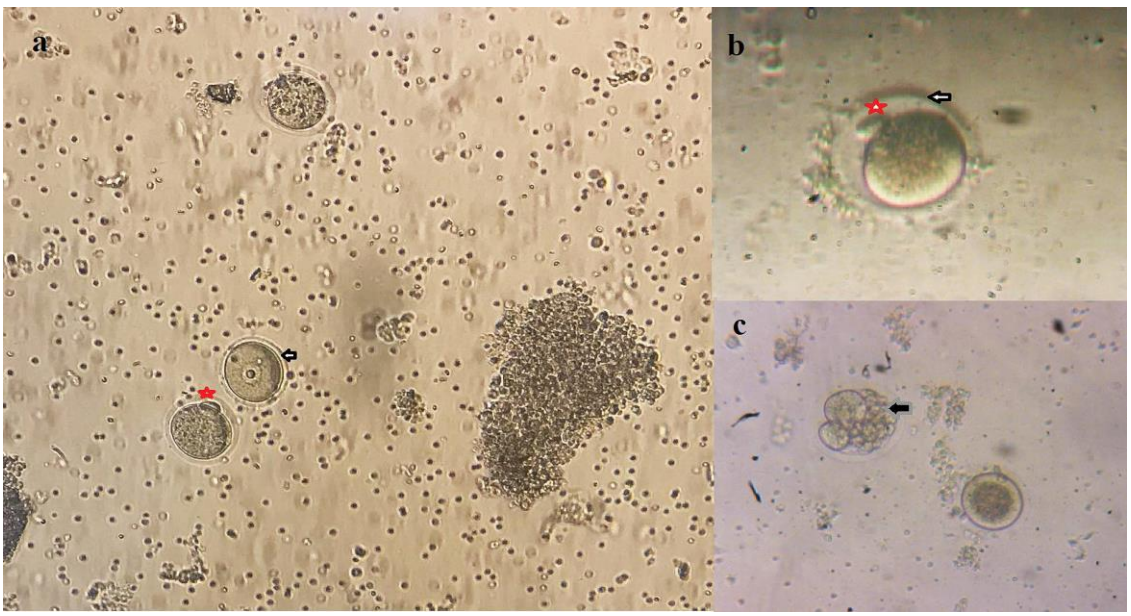



**Figure 2: Morphological characteristic of oocytes isolated from NMRI mice. a and b show intact oocytes; white arrow: zona pellucida, red star: first polar body. c shows an apoptotic oocyte (black arrow). a: observation under 10× objective; b and c: observation under 40× objective.**

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**Table 2: Effect of *Chlorella* sp. D1 and *Chlorella* sp. $N_4$ extracts on the viability of oocytes.**

| | Control | Chlorella SP. D1 ($150\ ^{\mu g}/_{mL}$) | Chlorella SP. $N_4$ ($150\ ^{\mu g}/_{mL}$) |
|---|---|---|---|
| Total Counted Oocytes | 30 | 30 | 30 |
| Viable Oocytes | 17 | 25 | 21 |
| Apoptotic Oocytes | 13 | 6 | 9 |
| Percentage of Viable Oocytes | % 56 | % 83 | % 70 |

**Table 3: Effect of *Chlorella* sp. D1 and *Chlorella* sp. $N_4$ extracts on the maturation of oocytes.**

| | Control | Chlorella SP. D1 ($150\ ^{\mu g}/_{mL}$) | Chlorella SP. $N_4$ ($150\ ^{\mu g}/_{mL}$) |
|---|---|---|---|
| Total Counted Oocytes | 30 | 30 | 30 |
| Oocytes with first polar body | 3 | 2 | 4 |
| Percentage of Maturation | % 10 | % 6 | % 13 |

*Effect of Chlorella sp. D1 and Chlorella sp. N4 extracts on the expression levels of SIRT-3, BMP-15 and GDF-9 genes*

Real-time PCR analysis revealed that the expression of the SIRT-3 gene exhibited a 2.346-fold increase in the group treated with N4 extract compared to the control group ($p$<0.05). The expression of the SIRT-3 gene displayed a 7.086-fold increase in the group treated with the D1 extract compared to the control group ($p$<0.05). The expression of the BMP-15 gene exhibited an 11.35-fold increase in the group treated with the N4 extract compared to the control group ($p$<0.05). Furthermore, upregulation of the BMP-15 gene was observed in the D1 extract-treated group with an average fold change of 3.11 compared to the control group ($p$<0.05). No statistically significant differences were observed with the expression of the GDF-9 gene among N4 treatment groups compared to the control group ($p$>0.05), but the difference was statistically significant between the D1 extract-treated group and the control with an average fold change of 2.73 ($p$<0.05). The comparison between the N4 and D1 groups revealed a significant elevation in the expression of the SIRT-3 gene in oocytes subjected to treatment with N4, as opposed to D1, with an average coefficient of 3.56 ($p$<0.05). The expression of the BMP-15 gene in oocytes treated with N4 was significantly higher compared to oocytes treated with D1, with an average coefficient of 4.64 ($p$<0.05). Also, a significant difference was observed in the expression of the GDF-9 gene between oocytes treated with N4 and those treated with D1 ($p$<0.05) (Fig. 3).

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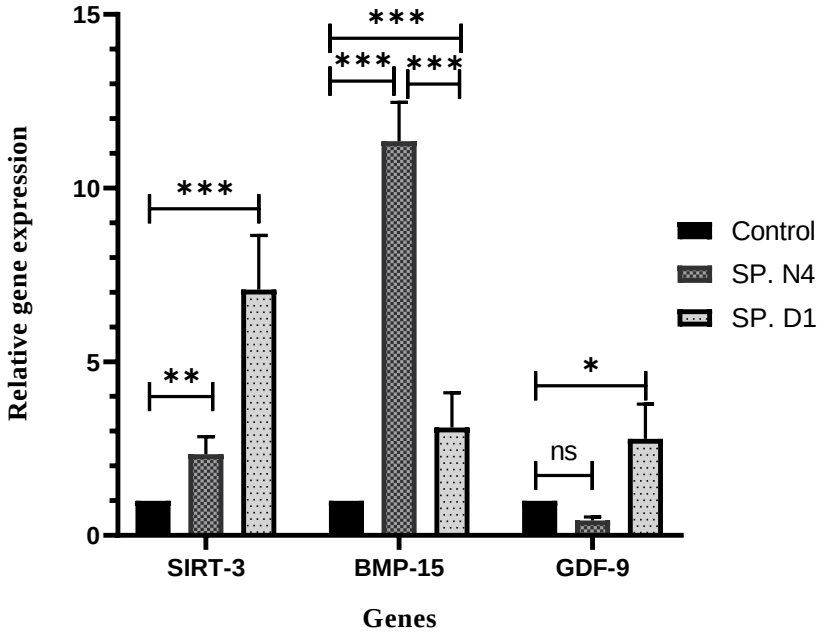



**Figure 3: Effect of *Chlorella* sp. $D_1$ (sp. D1) and *Chlorella* sp. $N_4$ (sp. N4) extracts on the expression of SIRT-3, BMP-15, and GDF-9 genes in the in vitro cultured oocytes. ns: non-significant, **: $p<0.01$, ***: $p<0.001$.**

## Discussion

The present research aimed to explore the impact of methanolic extracts derived from two *Chlorella vulgaris* strains on the viability, maturation, and gene expression related to oocyte function in mice under laboratory conditions. Our research demonstrated that the methanolic extracts obtained from *Chlorella* sp. D1 and *Chlorella* sp. N4 exhibited antioxidant properties and treatment of collected oocytes with *Chlorella* sp. D1 and *Chlorella* sp. N4 extracts resulted in a significant enhancement in oocyte survival under *in vitro* culture. Several studies have indicated the antioxidant properties of *Chlorella vulgaris*. For instance, the impact of *Chlorella vulgaris* supplementation on oxidative stress and the expression of antioxidant genes was assayed in the livers and ovaries of New Zealand white rabbits. The results showed that *Chlorella vulgaris* supplementation increased the overall antioxidant levels in rabbits' blood, uterus, and liver (Sikiru *et al.*, 2019). Also, in a study conducted by Zainul Azlan et al., the administration of *Chlorella vulgaris* resulted in enhanced mitigation of oxidative stress and improved muscle regeneration in both young and aged Sprague-Dawley rats (Zainul Azlan *et al.*, 2020). Vahdati and colleagues isolated the methanolic extract of *Chlorella* sp. D1 and reported its antioxidant activity and protective properties for the first time (Vahdati *et al.*, 2022). Their findings indicated that phytol, di-tert-butyl peroxide (DTBP), and BHT were the primary phytochemicals responsible for the observed antioxidant activities of *Chlorella* sp. D1. Additionally, they demonstrated that the extract derived from *Chlorella* sp. D1 had significant FRAP and DPPH antiradical activity while exhibiting a

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protective effect on nerve cells under oxidative stress. In addition, Mousavian and colleagues previously highlighted the significance of sulfated polysaccharides found in the extracts of *Chlorella* sp. D1 and *Chlorella* sp. N4 concerning their antioxidant properties (Mousavian *et al.*, 2022). Based on these findings, it can be inferred that the Chlorella extracts employed in the current study demonstrated antioxidant activity, likely owing to the presence of compounds such as DTBP or sulfated polysaccharides. These compounds may have played a protective role against toxic factors encountered during in vitro oocyte culture.

We also compared the expression levels of SIRT-3, BMP-15, and GDF-9 genes between oocytes treated with Chlorella extracts and those without any treatment. The observed increase in SIRT-3 gene levels compared to untreated oocytes indicates a positive effect of both extracts on the intracellular antioxidant pathways. The relevance of SIRT-3 to the competence of female germ cells for fertilization and early embryonic development has been validated through the examination of human oocytes extracted from IVF procedures. These observations have revealed a positive association among the abundance of SIRT-3 mRNA, mitochondrial biogenesis, and the effectiveness of oocyte development from the oocyte stage to the blastocyst stage (Zhao *et al.*, 2016). Under non-physiological conditions, the regulation of SIRT-3 expression may vary; factors such as diabetes, aging, and a high-fat diet have been shown to decrease SIRT-3 levels in the ovary (Di Emidio *et al.*, 2021). According to a study performed by Kamalipour and colleagues, the reduction in SIRT-3 gene expression in oocytes caused by polycystic ovary syndrome (PCOS) may account for oocyte fertility issues (Kamalipour *et al.*, 2020). While the absence of SIRT-3 did not impact the population of ovarian follicles and SIRT-3-deficient oocytes exhibited similar mitochondrial mass compared to their wild-type counterparts, they did demonstrate an increased production of ROS, suggesting impaired mitochondrial activity following SIRT-3 abrogation (Iljas and Homer, 2020). The administration of some treatments that positively affect ovarian physiology is correlated with restoring SIRT-3 levels in the ovaries. In this context, the administration of curcumin and melatonin has been shown to boost the expression of SIRT-3 and improve the aging phenotype (Mayo *et al.*, 2017, Saeideh Hasani Azami, 2019). Slipa *et a*l. demonstrated the involvement of SIRT-3 in reproductive features and its potential utility in the selection of viable goat breeds by analyzing single nucleotide polymorphisms (SNPs) within the SIRT-3 gene across several goat breeds (Silpa *et al.*, 2018).

In a similar manner to SIRT-3, the gene expression levels of BMP-15, and GDF-9 genes were notably elevated in oocytes subjected to Chlorella extracts compared to untreated oocytes. Several studies have revealed the positive effects of BMP-15 and GDF-9 on oocyte quality and their ability to enhance blastocyst rate and embryonic development in fertilized oocytes (Dalbies-Tran *et al.*, 2020). The addition of BMP-15 to the culture medium accelerated growth

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and antrum development while maintaining the structural integrity of isolated goat pre-antral follicles (Celestino *et al.*, 2011). A study reported that BMP-15 could facilitate the resumption of oocyte meiosis in mice through the upregulation of natriuretic peptide receptor (Npr2) and Inosine monophosphate dehydrogenase (Impdh) (Qin *et al.*, 2019). Furthermore, Jitjumnong and colleagues demonstrated that BMP-15 supplementation during *in vitro* cultivation of mouse ovarian follicles can promote follicular maturation and growth (Jitjumnong and Tang, 2023). GDF9 has been suggested to reflect oocyte quality (Park *et al.*, 2020), and the use of antioxidant compounds, such as α-tocopherol, sodium selenite, melatonin, and ascorbic acid during *in vitro* maturation of the oocyte has been shown to help maintain GDF-9 expression and improve fertilization outcome (Tripathi *et al.*, 2023). In alignment with the reports on the benefits of antioxidant compounds on oocytes, our findings also demonstrated that the Chlorella extracts utilized in the current research maintained the expression levels of BMP-15 and GDF-9 genes in the *in vitro* cultured oocytes.

## Conclusions

The current research findings indicated that the methanolic extracts derived from *Chlorella vulgaris* microalgae exhibited antioxidant properties and enhanced the viability of oocytes obtained from murine ovaries in the culture medium. The sustained expression of genes such as SIRT-3, BMP-15, and GDF-9 indicates that the analyzed extracts may bolster the mitochondrial antioxidant defense system in oocytes, potentially preserving their quality for successful fertilization.

## Conflicts of interest

The authors declare that they have no conflict of interest.

## References

**Celestino, J.J., Lima-Verde, I.B., Bruno, J.B., Matos, M.H., Chaves, R.N., Saraiva, M.V., Silva, C.M., Faustino, L.R., Rossetto, R., Lopes, C.A., Donato, M.A., Peixoto, C.A., Campello, C.C., Silva, J.R. and Figueiredo, J.R., 2011.** Steady-state level of bone morphogenetic protein-15 in goat ovaries and its influence on in vitro development and survival of preantral follicles. *Molecular and Cellular Endocrinology*, 338**,** 1-9. DOI:10.1016/j.mce.2011.02.007

**Cheng, Y., Ren, X., Gowda, A.S., Shan, Y., Zhang, L., Yuan, Y.S., Patel, R., Wu, H., Huber-Keener, K., Yang, J.W., Liu, D., Spratt, T.E. and Yang, J.M., 2013.** Interaction of Sirt3 with OGG1 contributes to repair of mitochondrial DNA and protects from apoptotic cell death under oxidative stress. *Cell Death and Disease*, 4, e731. DOI:10.1038/cddis.2013.254

**Cimadomo, D., Fabozzi, G., Vaiarelli, A., Ubaldi, N., Ubaldi, F.M. and Rienzi, L., 2018.** Impact of Maternal Age on Oocyte and Embryo Competence. *Frontiers in Endocrinology*, 9, 327-335. DOI:10.3389/fendo.2018.00327

**Coulombier, N., Jauffrais, T. and Lebouvier, N., 2021.** Antioxidant Compounds from Microalgae: A Review. *Marine Drugs*, 19, 549-579. DOI:10.3390/md19100549

**Dalbies-Tran, R., Cadoret, V., Desmarchais, A., Elis, S., Maillard, V.,**

[ Downloaded from jifro.ir on 2026-11-25 ]

**Monget, P., Monniaux, D., Reynaud, K., Saint-Dizier, M. and Uzbekova, S., 2020.** A Comparative Analysis of Oocyte Development in Mammals. *Cells,* 9, 1002-1029. DOI:10.3390/cells9041002

**Deluao, J.C., Winstanley, Y., Robker, R.L., Pacella-Ince, L., Gonzalez, M.B. and Mcpherson, N.O., 2022.** OXIDATIVE STRESS AND REPRODUCTIVE FUNCTION: Reactive oxygen species in the mammalian pre-implantation embryo. *Reproduction,* 164, F95-F108. DOI:10.1530/REP-22-0121

**Di Emidio, G., Falone, S., Artini, P.G., Amicarelli, F., D'alessandro, A.M. and Tatone, C., 2021.** Mitochondrial Sirtuins in Reproduction. *Antioxidants (Basel),* 10, 1047-1073. DOI:10.3390/antiox10071047

**Farag, M.R., El-Naseery, N.I., El Behery, E.I., Nouh, D.S., El-Mleeh, A., Mostafa, I.M.A., Alagawany, M., Di Cerbo, A., Azzam, M.M. and Mawed, S.A., 2023.** The rRole of *Chlorella vulgaris* in attenuating infertility induced by cadmium chloride via suppressing oxidative stress and modulating spermatogenesis and steroidogenesis in male rats. *Biological Trace Element Research*. Vol. 20. DOI:10.1007/s12011-023-03971-y

**Iljas, J.D. and Homer, H.A., 2020.** Sirt3 is dispensable for oocyte quality and female fertility in lean and obese mice. *FASEB Journal,* 34, 6641-6653. DOI:10.1096/fj.202000153R

**Jiang, Q., Yin, J., Chen, J., Ma, X., Wu, M., Liu, G., Yao, K., Tan, B. and Yin, Y., 2020.** Mitochondria-Targeted Antioxidants: A Step towards Disease Treatment. *Oxidative Medicine and Cellular Longevity,* 8837893. DOI:10.1155/2020/8837893

**Jitjumnong, J. and Tang, P.C., 2023.** Bone Morphogenetic Protein 15 (BMP-15) Improves In Vitro Mouse Folliculogenesis. *Animals (Basel),* 13. DOI:10.3390/ani13060980

**Juengel, J.L., Bodensteiner, K.J., Heath, D.A., Hudson, N.L., Moeller, C.L., Smith, P., Galloway, S.M., Davis, G.H., Sawyer, H.R. and Mcnatty, K.P., 2004.** Physiology of GDF9 and BMP15 signalling molecules. *Animal Reproduction Science,* 82, 447-60. DOI:10.1016/j.anireprosci.2004.04.021

**Kamalipour, F., Jalali, H. and Azarnia, M., 2020.** Comparison the Effect of Metformin and Clomiphene Citrate on Sirtuin3 gene Expression in the Oocytes of Mice with Polycystic Ovary Syndrome. *Iranian Journal of Pharmaceutical Research,* 19, 160-168. DOI:10.22037/ijpr.2020.113321.14236

**Kaur, M., Bhatia, S., Gupta, U., Decker, E., Tak, Y., Bali, M., Gupta, V.K., Dar, R.A. and Bala, S., 2023.** Microalgal bioactive metabolites as promising implements in nutraceuticals and pharmaceuticals: inspiring therapy for health benefits. *Phytochemistry Reviews*, 1-31. DOI:10.1007/s11101-022-09848-7

**Kawamura, Y., Uchijima, Y., Horike, N., Tonami, K., Nishiyama, K., Amano, T., Asano, T., Kurihara, Y. and Kurihara, H., 2010.** Sirt3 protects in vitro-fertilized mouse preimplantation embryos against oxidative stress-induced p53-mediated developmental arrest. *Journal of Clinical Investigation,* 120, 2817-28. DOI:10.1172/JCI42020

**Khavari, F., Saidijam, M., Taheri, M. and Nouri, F., 2021.** Microalgae: therapeutic potentials and applications.

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*Molecular Biology Reports*, 48, 4757-4765. DOI:10.1007/s11033-021-06422-w

**Khazaei, M. and Aghaz, F., 2017.** Reactive Oxygen Species Generation and Use of Antioxidants during In Vitro Maturation of Oocytes. *International Journal of Fertility and Sterility*, 11, 63-70. DOI:10.22074/ijfs.2017.4995

**Mayo, J.C., Sainz, R.M., Gonzalez Menendez, P., Cepas, V., Tan, D.X. and Reiter, R.J., 2017.** Melatonin and sirtuins: A "not-so unexpected" relationship. *Journal of Pineal Research*, 62, 1-17. DOI:10.1111/jpi.12391

**Mousavian, Z., Safavi, M., Azizmohseni, F., Hadizadeh, M. and Mirdamadi, S., 2022.** Characterization, antioxidant and anticoagulant properties of exopolysaccharide from marine microalgae. *AMB Express*, 12, 27-43. DOI:10.1186/s13568-022-01365-2

**Mtaki, K., Kyewalyanga, M.S. and Mtolera, M.S.P., 2020.** Assessment of antioxidant contents and free radical-scavenging capacity of chlorella vulgaris cultivated in low cost media. *Applied Sciences*, 10, 1-11. DOI:10.3390/app10238611

**Niu, J., Chang, Q., Xu, J., Li, J., Liu, W., Chen, Z., Jiao, X., Guo, Y. and Teng, X., 2023.** Relationship of the levels of reactive oxygen species in the fertilization medium with the outcome of in vitro fertilization following brief incubation. *Frontiers in Endocrinology*, 14, 1133566-1133572. DOI:10.3389/fendo.2023.1133566

**Park, M.J., Ahn, J.W., Kim, K.H., Bang, J., Kim, S.C., Jeong, J.Y., Choi, Y.E., Kim, C.W. and Joo, B.S., 2020.** Prediction of ovarian aging using ovarian expression of BMP15, GDF9, and C-KIT. *Experimental Biology and Medicine*, 245, 711-719. DOI:10.1177/1535370220915826

**Premkumar, K.V. and Chaube, S.K., 2016.** Increased level of reactive oxygen species persuades postovulatory aging-mediated spontaneous egg activation in rat eggs cultured in vitro. *In Vitro Cellular & Developmental Biology - Animal*, 52, 576-88. DOI:10.1007/s11626-016-0007-3

**Qin, Y., Tang, T., Li, W., Liu, Z., Yang, X., Shi, X., Sun, G., Liu, X., Wang, M., Liang, X., Cong, P., Mo, D., Liu, X., Chen, Y. and He, Z., 2019.** Bone Morphogenetic Protein 15 Knockdown Inhibits Porcine Ovarian Follicular Development and Ovulation. *Frontiers in Cell and Developmental Biology*, 7, 1-7. DOI:10.3389/fcell.2019.00286

**Rodriguez-Garcia, I. and Guil-Guerrero, J.L., 2008.** Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. *Food Chemistry*, 108, 1023-6. DOI:10.1016/j.foodchem.2007.11.059

**Saeideh Hasani Azami, H.N., Mohammad Amin Abdollahifar, Fatemeh Eini, Mehdi Allahbakhshian Farsani and Marefat Ghaffari Novin, 2019.** The antioxidant curcumin postpones ovarian aging in young and middle-aged mice. *Reproduction, Fertility and Development*, 32, 292-303. DOI:10.1071/RD18472

**Sikiru, A.B., Arangasamy, A., Alemede, I.C., Guvvala, P.R., Egena, S.S.A., Ippala, J.R. and Bhatta, R., 2019.** Chlorella vulgaris supplementation effects on performances, oxidative stress and antioxidant genes expression in liver and ovaries of New Zealand White rabbits. *Heliyon*, 5, e02470. DOI:10.1016/j.heliyon.2019.e02470

[ Downloaded from jifro.ir on 2026-11-25 ]

**Silpa, M.V., Naicy, T., Aravindakshan, T.V., Radhika, G., Boswell, A. and Mini, M., 2018.** Sirtuin3 (SIRT3) gene molecular characterization and SNP detection in prolific and low prolific goat breeds. *Theriogenology*, 122, 47-52. DOI:10.1016/j.theriogenology.2018.09.008

**Sudiman, J., Ritter, L.J., Feil, D.K., Wang, X., Chan, K., Mottershead, D.G., Robertson, D.M., Thompson, J.G. and Gilchrist, R.B., 2014.** Effects of differing oocyte-secreted factors during mouse in vitro maturation on subsequent embryo and fetal development. *Journal of Assisted Reproduction and Genetics*, 31, 295-306. DOI:10.1007/s10815-013-0152-5

**Tripathi, S.K., Nandi, S., Gupta, P.S.P. and Mondal, S., 2023.** Antioxidants supplementation improves the quality of in vitro produced ovine embryos with amendments in key development gene expressions. *Theriogenology*, 201, 41-52. DOI:10.1016/j.theriogenology.2022.11.048

**Vahdati, S.N., Lashkari, A., Navasatli, S.A., Ardestani, S.K. and Safavi, M., 2022.** Butylated hydroxyl-toluene, 2,4-Di-tert-butylphenol, and phytol of Chlorella sp. protect the PC12 cell line against H(2)O(2)-induced neurotoxicity. *Biomedicine and Pharmacotherapy*, 145, 112415. DOI:10.1016/j.biopha.2021.112415

**Vaskova, J., Klepcova, Z., Spakova, I., Urdzik, P., Stofilova, J., Bertkova, I., Kloc, M. and Rabajdova, M., 2023.** The Importance of Natural Antioxidants in Female Reproduction. *Antioxidants (Basel)*, 12, 907-916. DOI:10.3390/antiox12040907

**Vo, K.C.T., Sato, Y. and Kawamura, K., 2023.** Improvement of oocyte quality through the SIRT signaling pathway. *Reproductive Medicine and Biology*, 22, e12510. DOI:10.1002/rmb2.12510

**Wang, L., Tang, J., Wang, L., Tan, F., Song, H., Zhou, J. and Li, F., 2021.** Oxidative stress in oocyte aging and female reproduction. *Journal of Cellular Physiology*, 236, 7966-7983. DOI:10.1002/jcp.30468

**Zainul Azlan, N., Mohd Yusof, Y.A. and Makpol, S., 2020.** Chlorella vulgaris Ameliorates Oxidative Stress and Improves the Muscle Regenerative Capacity of Young and Old Sprague-Dawley Rats. *Nutrients*, 12, 3752-3774. DOI:10.3390/nu12123752

**Zhang, J., Xiang, H., Liu, J., Chen, Y., He, R.R. and Liu, B., 2020.** Mitochondrial Sirtuin 3: New emerging biological function and therapeutic target. *Theranostics*, 10, 8315-8342. DOI:10.7150/thno.45922

**Zhang, L., Han, L., Ma, R., Hou, X., Yu, Y., Sun, S., Xu, Y., Schedl, T., Moley, K.H. and Wang, Q., 2015.** Sirt3 prevents maternal obesity-associated oxidative stress and meiotic defects in mouse oocytes. *Cell Cycle*, 14, 2959-68. DOI:10.1080/15384101.2015.1026517

**Zhao, H.C., Ding, T., Ren, Y., Li, T.J., Li, R., Fan, Y., Yan, J., Zhao, Y., Li, M., Yu, Y. and Qiao, J., 2016.** Role of Sirt3 in mitochondrial biogenesis and developmental competence of human in vitro matured oocytes. *Human Reproduction*, 31, 607-22. DOI:10.1093/humrep/dev345

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