Advances in Applied Physiology
2021; 6(2): 47-52
http://www.sciencepublishinggroup.com/j/aap
doi: 10.11648/j.aap.20210602.15
ISSN: 2471-9692 (Print); ISSN: 2471-9714 (Online)

The Effect of Sirtuin 1 Inhibitor Ex-527 and Activator Resveratrol on the Oocytes' Cells Viability in Mice Model of Experimental Systemic Autoimmune Damage

Mariia Stupchuk*, Alina Lytvynenko, Tetyana Voznesenska

Department of Immunophysiology, Bogomoletz Institute of Physiology of National Academy of Sciences of Ukraine, Kyiv, Ukraine

Email address:

mariastupchuk@biph.kiev.ua (M. Stupchuk), alina_lit@ukr.net (A. Lytvynenko), voz@biph.kiev.ua (T. Voznesenska)

*Corresponding author

To cite this article:

Mariia Stupchuk, Alina Lytvynenko, Tetyana Voznesenska. The Effect of Sirtuin 1 Inhibitor Ex-527 and Activator Resveratrol on the Oocytes' Cells Viability in Mice Model of Experimental Systemic Autoimmune Damage. *Advances in Applied Physiology*. Vol. 6, No. 2, 2021, pp. 47-52. doi: 10.11648/j.aap.20210602.15

Received: October 26, 2021; **Accepted**: November 22, 2021; **Published**: November 29, 2021

Abstract: Introduction: Female infertility is a very common pathology for women of reproductive age with autoimmune disorders, which are known to be followed by the abnormal increment of reactive oxygen species levels, moreover, further contributing to follicular atresia and aging of oocytes in the ovaries what directly leads to infertility. Thus, in search for strategies of preventing oxidative threat to ovaries, the current study was aimed to assess the influence of sirtuin 1, a key cellular metabolism regulator and oxidative stress, activator/blocker on the viability of the follicular environment of oocytes (FEO) cells under conditions of experimental systemic autoimmune damage (ESAD). Methods: The study was performed using the model of ESAD on female mice. The FEO cells were cultivated in different conditions: resveratrol 20 µM, Ex-527 20 µM. After 24h, the cells were examined for viability; the ways of cell death via apoptosis, necrosis were estimated using the method of in vivo dual-color with fluorescent nucleic acid dyes Hoechst 33342 and propidium iodide, and the levels of autophagy were estimated using the autophagic vacuoles labeling with monodansylcadaverine assay. Results: The obtained data indicate that a specific inhibitor of sirtuin 1 Ex-527 (20 µM) in vitro inhibits the viability of cells of the FEO cells and increases the percent of cell death via autophagy, apoptosis, and necrosis. On the contrary, the activator of sirtuin 1 - resveratrol, led to an improvement of the viability status of FEO cells, while reducing the negative impact of the inflammatory process. Unidirectional action of Ex-527 and resveratrol compounds at the cellular level has been established. Conclusion: Thus, the results of the present study suggest the involvement of sirtuin 1 in the regulation of the damaging effect of reactive oxygen species on female ovarian cells under the conditions of experimental systemic autoimmune damage.

Keywords: Ovarian Cells, Autophagy, Apoptosis, Sirtuins, Resveratrol, Ex-527, Systemic Autoimmune Damage

1. Introduction

Ovarian failure, which often leads to complete female infertility is a very common pathology of women of reproductive age with autoimmune disorders. Autoimmune disorders are reported to be one of the pivotal factors that directly contribute to the development of female primary ovarian failure. The autoimmune disorders are known to be followed by the abnormal increment of reactive oxygen species levels, moreover, further contributing to follicular atresia and aging of oocytes in the ovaries what directly leads to infertility [1-6].

Therefore, the development of strategies aimed at preventing the oxidative threat to ovaries, caused by autoimmune disorder is a subject of great current relevance. Strong experimental evidence supports the statement that sirtuin1 plays a crucial role in sensing and modulating the redox status of cells providing protective effects in cells and tissues exposed to oxidative stressors *in vitro* and *in vivo* [7-9]. Moreover, a crucial role for sirtuin 1 and sirtuin 3, the main components of the sirtuin family, as sensors and guardians of the redox state in oocytes, granulosa cells, and early embryos has been reported [10]. Thus, in search for strategies of preventing oxidative threat to ovaries, the

current study was aimed to assess the influence of sirtuin 1, a key cellular metabolism regulator and oxidative stress, activator/blocker on the viability of the follicular environment of oocytes (FEO) cells under conditions of experimental systemic autoimmune damage (ESAD).

Sirtuins are the family of high-conserved NAD^+-dependent proteins with deacetylase and Mono-ADP-ribosyl transferase activity that impact multiple somatic cell pathways, which regulate cellular and organismal aging together with metabolism. Mammals express seven sirtuins, sirtuin 1-7 [8, 11].

Ex-527 is a potent and highly selective inhibitor of sirtuin 1 (IC50 38 nm), while not inhibiting the activity of other histone deacetylases class I/II at concentrations below 100 μm. Inhibition of sirtuin 1 is accompanied by the formation of the complex Sirt1/NAD +/Ex-527 [12] Resveratrol (3,5,4-trihydroxystylene) is a specific activator of sirtuin 1 and does not adversely affect the functional state of cells of the reproductive system [8, 13].

In search of ways to prevent oxidative damage to the ovaries, the role of sirtuins, in particular nuclear sirtuin 1, is being actively studied [14-16]. The etiology of this disorder can be investigated on animal models. In recent years, the study of the pathways of programmed cell death, including apoptosis and autophagy, has attracted much attention.

The questions of their occurrence mechanisms in the presence of autoimmune damage in the body and their role in the further functioning of organs and systems of the body still remain open. Therefore, our aim was to investigate the effects of specific activators and inhibitors of sirtuin 1 on the viability of FEO cells and their pathways (autophagy, apoptosis, and necrosis) under the conditions of ESAD followed by oxidative stress.

2. Materials and Methods

2.1. Materials

Dulbecco's modified Eagle medium (DMEM/D2902), 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES) (7365-45-9), propidium iodide (25535-16-4), Hoechst 33342 (875756-97-1), *N*-(5-Amino-pentyl)-5-di-methyl-amino-naphtha-lene-1-sulfon-amide, *N*-(Dimethyl-amino-naphtha-lene-sulfonyl)-1,5-pentane-diamine (Monodansylcadaverine) (10121-91-2) were purchased from Sigma-Aldrich, resveratrol (501-36-0) was purchased from Carl Roth GmbH + Co. Other reagents were obtained from usual commercial sources.

2.2. Animals and Ethics

Experiments were conducted on white laboratory mice at the age of 10 weeks, 18-20 g weight) the maintenance and care of which complies with National Institutes of Health guidelines for the humane use of laboratory animals and have been approved by Bogomoletz Institute of Physiology of NASU Biomedical Ethics Committee.

2.3. Experimental Design

The laboratory mice were divided into following experimental groups: group I – Control, group II – ESAD, group III – Resveratrol 20 μM, group IV - Ex-527 20 μM, group V – ESAD+ Resveratrol 20 μM, group VI - ESAD+ Ex-527 20 μM.

Experimental systemic autoimmune damage

The model of systemic autoimmune damage was reproduced by immunization of Albino I generation mice with a suspension of renal antigen obtained from the mother. Immunization of animals was performed at the rate of 10 μl of suspension per 10 grams of body weight according to the following scheme: 3 times with an interval of 1 day intraperitoneally 1 time per day. Re-immunization was performed after three weeks once intraperitoneally in the same dose. Before and during the experiment, the objective status of the animals and protein levels in the urine were assessed. Control were Albino female mice injected with saline according to the same scheme.

Collection of experimental material (ovaries) was performed under ether anesthesia on the third day after the last injection.

2.4. FEO Cells Cultivation

The FEO cells were isolated mechanically from the ovaries of mice in a non-enzymatic way and distributed in sterile cultivation cameras. All FEO cells were cultured under the same condition: A sterile box, cameras with 0.4 mL culture medium - Dulbecco's modified Eagle's medium (DMEM) and 15 mM HEPES, Ca^{2+} concentration of 1.71 mM, temperature 37°C, duration 24 h. The resveratrol and Ex-527 were stored at -20°C and diluted in cell cultivation media immediately before use to required concentrations (20 μM for resveratrol and 20 μM for Ex-527, doses were chosen according to the results of the preliminary studies).

2.5. Assessment of Autophagy, Apoptotic and Necrotic Cell Death

To examine the levels of apoptosis and necrosis death of FEO cells after being sirtuin's modulators treated, in vivo double staining with fluorescent nucleic acid dyes (propidium iodide and Hoechst 33342) was performed. Chromatin-related dyes make it possible to assess the morphological features of nuclear material. Autophagic cell death was determined by labeling autophagic cell vacuoles with the fluorescent dye monodansylcadaverine. Cells (at least 200) were examined on a fluorescence microscope with a water-immersion lens x85.

2.6. Statistical Analysis

For the statistical analysis of the results, the software package Origin 8Pro (OriginLab Corp., North., MA, USA) and spreadsheets Microsoft®Excel2003 were used. Student's t-test was performed for continuous variables. $P<0.05$ was considered statistically significant. The statistical analysis of

the research results was conducted by using analysis of variance ANOVA followed by a comparison of mean values between groups by Newman-Coles test using the statistic program-5 (program GraphPad Prism 5.0 (GraphPad Software, San Diego, USA).

3. Ex-527 and Resveratrol Effects on FEO Cells Viability

3.1. Apoptotic and Necrotic Death Levels of FEO Cells After Treatment with Sirtuin Modulators

After the exposure to a specific inhibitor of sirtuin 1 Ex-527 *in vitro* at a concentration of 20 μM there was a decrease in the proportion of living FEO cells by 12.85% compared with the control group (P<0.01, N=9). At the same time, the percentage of apoptosis of FEO cells in these conditions increased by 10.68% (P<0.01, N=9), necrosis - by 2.17% compared with the control group, respectively. It was found that the activator of sirtuin 1 resveratrol probably did not lead to a decrease in the proportion of apoptotic and necrotic cells FEO (Figure 1).

3.2. Autophagic Death of FEO Cells After Sirtuin's Modulators Exposure

When assessing the autophagic death of FEO cells after exposure to Ex-527, it was shown that the proportion of autophagic FEO cells after *in vitro* Ex-527 at a concentration of 20 μM increased by 7.33% compared to control (P<0.01, N=9) (Figure 2).

After cultivating in the presence of 20 μM resveratrol activator of sirtuin 1, the number of FEO cells with signs of autophagic death decreased 1.8-fold to 4.66±0.81% compared with 8.33±0.51% in the control group of animals (see Figure 2).

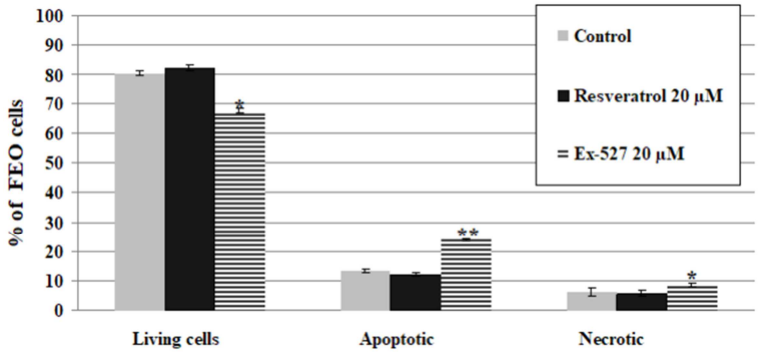

Figure 1. *The viability of the oocyte follicular environment cells and the ways of its cell death after treatment with resveratrol (20 μM) and Ex-527 (20 μM) in vitro.*

Note: ** P<0.01, * P<0.05, the probability of differences in the average data experimental groups (N=9) compared with such values in the control group of animals (N=9).

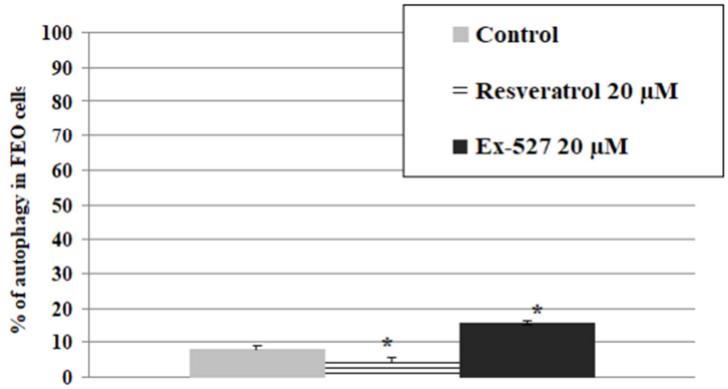

Figure 2. *The levels of autophagy of FEO cells after treatment with resveratrol (20 μM) and Ex-527 (20 μM) in vitro.*

Note: * P<0.05, the probability of differences in the average data experimental groups (N=9) compared with such values in the control group of animals (N=9).

Thus, it has been shown that a specific inhibitor of sirtuin 1 Ex-527 at a concentration of 20 μM led to a decrease in the proportion of living FEO cells and increased levels of autophagy, apoptosis and necrosis of these cells. Specific activator of sirtuin 1 resveratrol at a concentration of 20 μM led to a decrease in autophagy levels in FEO cells. Significant damage to the ovaries of mice was recorded in the simulation of systemic autoimmune damage. Thus, in

animals, a probable (P<0.01; N=9) decrease in the number of mature follicles isolated from the ovary (large follicles with an egg with a pronounced transparent shell and surrounded by many layers of follicular cells) was found.

4. Ex-527 and Resveratrol Effects on FEO Cells Viability Under the Conditions of ESAD

4.1. Apoptotic and Necrotic Death Levels of FEO Cells After Treatment with Sirtuin Modulators Under the Conditions of ESAD

Degradation of follicular cells was also observed in the follicles of mice with ESAD simulation. The next step was to evaluate the effect of the introduction of the inhibitor of sirtuin 1 Ex-527 20.0 μM on the viability of cells of the follicular environment of oocytes and the ways of their death under the conditions of experimental systemic autoimmune damage. It was found that in the conditions of ESAD the share of living FEO cells decreased by 38.61% (P<0.01, N=9) compared with the control group, while the shares of apoptotic and necrotic FEO cells increased by 27.06% (P<0.01, N=9) and 10.55% (P<0.01, N=9), respectively (Figure 3).

After cultivating in the presence of a sirtuin 1 inhibitor Ex-527 20 μM, the number of live FEO cells in animals with ECAU decreased by 10.1% (P<0.01, N=9) compared with those in animals with ECAU without cultivating in nutrient medium, which contained Ex-527 20 μM (see Figure 3). At the same time, the number of apoptotic FEO cells increased by 10.55% (P<0.01, N=9). After cultivating in a medium containing sirtuin activator 1 resveratrol, the number of living FEO cells increased by 10.3% (P<0.01, N=9) compared with those in animals with ECAU without cultivating in a nutrient medium containing resveratrol 20 μM. The proportions of apoptotic and necrotic cells decreased by 8.29% (P<0.01, N=9) and 2.01%, respectively (see Figure 3).

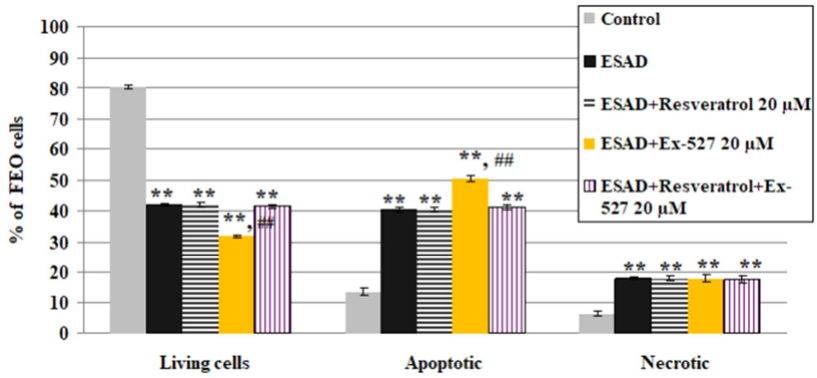

Figure 3. *The viability of the oocyte follicular environment cells and the ways of its cell death after treatment with resveratrol (20 μM) and Ex-527 (20 μM) in vitro in the ESAD conditions.*

Note: ** P<0.01, the probability of differences in the average data experimental groups (N=9) compared with such values in the control group of animals (N=9). ## P<0.01, the probability of differences in the average data experimental groups (N=9) compared with such values in the group of animals with ESAD (N=9).

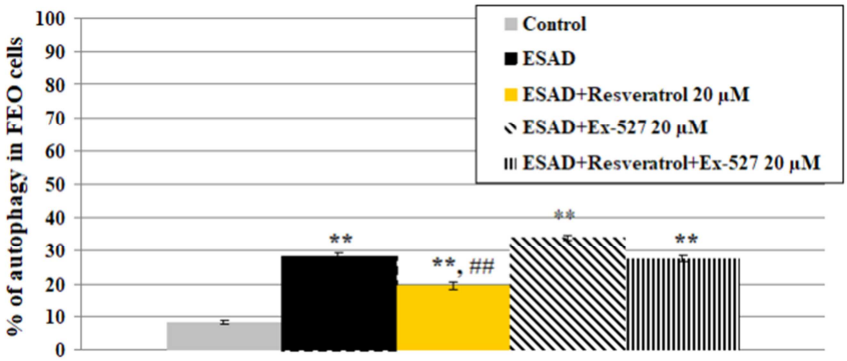

Figure 4. *The levels of autophagy of FEO cells after treatment with resveratrol (20 μM) and Ex-527 (20 μM) in vitro in the ESAD conditions.*

Note: ** P<0.01, the probability of differences in the average data experimental groups (N=9) compared with such values in the control group of animals (N=9). ## P<0.01, the probability of differences in the average data experimental groups (N=9) compared with such values in the group of animals with ESAD (N=9).

That is, it is shown that a specific inhibitor of sirtuin 1 Ex-527 20 μM under the conditions of ESAD has a depressant effect on the viability of FEO cells, and a specific activator of sirtuin 1 - resveratrol 20 μM on the contrary - improves these

indicators.

4.2. Autophagic Death of FEO Cells After Sirtuin's Modulators Exposure Under the Conditions of ESAD

It was found that under the conditions of ESAD the number of autophagic FEO cells increased by 20.0% compared with the control group ($P<0.01$, N=9) (Figure 4).

The activator of sirtuins resveratrol at a concentration of 20 μM led to a decrease in the level of autophagic cell death of FEO animals from ESAD at 9.00% ($P<0.01$, N=9) (see Figure 4). The specific inhibitor of sirtuin 1 Ex-527 (20 μM) *in vitro* reduced the proportion of autophagic FEO cells in animals with ESAD by 5.67%. Evaluating the simultaneous in vitro effect of resveratrol sirtuin activator (20 μM) and Ex-527 inhibitor (20 μM), the following data were obtained. Thus, the simultaneous addition of resveratrol offset the depressant effect of Ex-527 (see Figure 4). Thus, based on the obtained results the same direction of action of resveratrol and Ex-527 and the existence of a common cellular target can be suggested.

5. Discussion

It was shown that mice under the conditions of ESAD, caused by immunization with antigenic suspension of the kidney, develop an inflammatory process, accompanied by the death of FEO cells. This is an important cause of impaired meiotic maturation of oocytes.

Apoptosis, autophagy, and necrosis are types of programmed cell death (PCD) that result from the implementation of its genetic program or the response to external signals that require energy expenditure and *de novo* macromolecule synthesis. Apoptosis is characterized by a decrease in cell size, condensation and fragmentation of chromatin, compaction of the outer and cytoplasmic membranes without release of cell contents into the environment. Apoptosis is carried out caspase-dependent (receptor) or mitochondrial-dependent way [17-19].

Autophagy is characterized by vacuolation of the cell cytoplasm and is accompanied by degradation and intracellular utilization of damaged organelles and proteins without cell damage. In the case of autophagic death, phagophores, autophagosomes, autolysosome, or chaperone-mediated fusion with lysosomes are sequentially formed in the cytoplasm [20]. Autophagy in a normal cell is a way to renew organelles, if autophagy is triggered in the cell after activation of apoptosis, programmed cell death is canceled. Apoptosis eliminates damaged cells by phagocytosis without the development of inflammation, which is detrimental to the macro organism, or accompanies the source of inflammation for its localization and final healing [21, 22]. Another significant difference between autophagy and apoptosis is the absence of a phagocytic link in the mechanism of autophagy. An important characteristic of necrosis is the localization of non-viable tissue by inflammation and the immune response to toxic and harmful effects on the macro organism. Necrosis is accompanied by swelling of the organelles with subsequent rupture of the inner and outer membranes, followed by swelling and further lysis of cells. Phagocytic link in the mechanism of necrosis, as in the case of apoptosis, is very important [17, 18]. Thus, all the above types of PCD play crucial role in maintaining homeostasis at the level both on cellular and macro organismal levels. Therefore, the of our study was to investigate the effects of specific activator and inhibitor of sirtuin 1 on the viability of FEO cells and their pathways (autophagy, apoptosis and necrosis) under the conditions of ESAD followed by oxidative stress.

The intensification of autophagy and apoptosis processes, by which ESAD was shown to be followed, according to our results, is considered to be important mechanisms of regeneration of damaged cell organelles (in case of autophagy) and elimination of damaged cells by phagocytosis without inflammation, or localization of inflammation (in case of apoptosis). Thus, it was found that a specific inhibitor of sirtuin 1 Ex-527 (20 μM) *in vitro* inhibits the viability of cells of the follicular environment of oocytes and increases the proportion of their death by autophagy, apoptosis and necrosis. Such a depressant effect is observed both under normal conditions and under conditions of the presence in the body of experimental systemic autoimmune lesions.

The specific activator of sirtuin 1 - resveratrol, on the contrary, led to an improvement in the viability of FEO cells, while reducing the negative impact of the inflammatory process in ESAD on these cells.

Unidirectional action of Ex-527 and resveratrol compounds at the cellular level has been established. Therefore, sirtuin 1 is involved in the regulation of apoptosis, necrosis and autophagy of FEO cells both in normal conditions and in autoimmune diseases. We hypothesize that the above-mentioned modulators of sirtuin affect the viability of FEO cells due to their effect on the nucleus of these cells (due to the fact that sirtuin 1 is localized in the nucleus).

6. Conclusion

The results of the present study revealed that a specific inhibitor of sirtuin 1 Ex-527 *in vitro* inhibits the viability of cells of the follicular environment of oocytes and increases the proportion of their death by autophagy, apoptosis, and necrosis. Such a depressant effect is observed both under normal conditions and under conditions of the presence in the body of experimental systemic autoimmune lesions. The specific activator of sirtuin 1 - resveratrol, on the contrary, led to an improvement in the viability of FEO cells while reducing the negative impact of the inflammatory process in ESAD on these cells. Thus, we consider the abovementioned sirtuin's modulators to be potent substances that can be used in developing strategies of preventing oxidative threat to ovaries during the inflammation process. Further investigations aimed to study the direct mechanisms of sirtuins modulators prevention the oxidative threat are needed with the subsequent development of the abovementioned threat prevention.

Acknowledgements

Authors would like to thank Bogomoletz Institute of Physiology of National Academy of Sciences of Ukraine for

the financial support of our study.

References

[1] Chen, Z., Shentu, T. P., Wen, L., Johnson, D. A., & Shyy, J. Y. (2013). Regulation of SIRT1 by oxidative stress-responsive miRNAs and a systematic approach to identify its role in the endothelium. *Antioxidants & redox signaling*, *19* (13), 1522–1538. https://doi.org/10.1089/ars.2012.4803.

[2] Chen, Z., Kang, X., Wang, L., Dong, H., Wang, C., Xiong, Z., Zhao, W., Jia, C., Lin, J., Zhang, W., Yuan, W., Zhong, M., Du, H., & Bai, X. (2015). Rictor/mTORC2 pathway in oocytes regulates folliculogenesis, and its inactivation causes premature ovarian failure. *The Journal of biological chemistry*, *290* (10), 6387–6396. https://doi.org/10.1074/jbc.M114.605261.

[3] Gilbert S, Weiner D, Gipson D. National Kidney Foundation's Primer on Kidney Diseases, 6th edition. Elselvier. 2014; 592.

[4] Khizroeva, J., Nalli, C., Bitsadze, V., Lojacono, A., Zatti, S., Andreoli, L., Tincani, A., Shoenfeld, Y., & Makatsariya, A. (2019). Infertility in women with systemic autoimmune diseases. *Best practice & research. Clinical endocrinology & metabolism*, *33* (6), 101369. https://doi.org/10.1016/j.beem.2019.101369.

[5] Ronco, P., & Debiec, H. (2012). Pathogenesis of membranous nephropathy: recent advances and future challenges. *Nature reviews. Nephrology*, *8* (4), 203–213. https://doi.org/10.1038/nrneph.2012.35.

[6] Rives, N., Verhaeghe, F., Di Pizio, P., & Rives, A. (2018). Préservation de la fertilité [Fertility preservation]. *La Revue du praticien*, *68* (2), 213–219.

[7] Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical interventions in aging*, *13*, 757–772. https://doi.org/10.2147/CIA.S158513.

[8] Takeo, S., Sato, D., Kimura, K., Monji, Y., Kuwayama, T., Kawahara-Miki, R., & Iwata, H. (2014). Resveratrol improves the mitochondrial function and fertilization outcome of bovine oocytes. *The Journal of reproduction and development*, *60* (2), 92–99. https://doi.org/10.1262/jrd.2013-102Team, C. F. (2020). Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) - United States, February 12-March 16, 2020. MMWR. Morbidity and mortality weekly report, 69 12, 343-346.

[9] Li, Y., Dai, D., Lu, Q., Fei, M., Li, M., & Wu, X. (2013). Sirt2 suppresses glioma cell growth through targeting NF-κB-miR-21 axis. *Biochemical and biophysical research communications*, *441* (3), 661–667. https://doi.org/10.1016/j.bbrc.2013.10.077.

[10] Tatone C, Di Emidio G, Barbonetti A, Carta G, Luciano AM, Falone S, et al. Sirtuins in gamete biology and reproductive physiology: emerging roles and therapeutic potential in female and male infertility. Hum Reprod Update. 2018 May 1; 24 (3): 267-289. doi: 10.1093/humupd/dmy003.

[11] Fu, H., Wada-Hiraike, O., Hirano, M., Kawamura, Y., Sakurabashi, A., Shirane, A., Morita, Y., Isono, W., Oishi, H., Koga, K., Oda, K., Kawana, K., Yano, T., Kurihara, H., Osuga, Y., & Fujii, T. (2014). SIRT3 positively regulates the expression of folliculogenesis- and luteinization-related genes and progesterone secretion by manipulating oxidative stress in human luteinized granulosa cells. *Endocrinology*, *155* (8), 3079–3087. https://doi.org/10.1210/en.2014-1025.

[12] Gertz, M., Fischer, F., Nguyen, G. T., Lakshminarasimhan, M., Schutkowski, M., Weyand, M., & Steegborn, C. (2013). Ex-527 inhibits Sirtuins by exploiting their unique NAD+-dependent deacetylation mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, *110* (30), E2772–E2781. https://doi.org/10.1073/pnas.1303628110.

[13] Tatone, C., Di Emidio, G., Vitti, M., Di Carlo, M., Santini, S., Jr, D'Alessandro, A. M., Falone, S., & Amicarelli, F. (2015). Sirtuin Functions in Female Fertility: Possible Role in Oxidative Stress and Aging. *Oxidative medicine and cellular longevity*, *2015*, 659687. https://doi.org/10.1155/2015/659687.

[14] Tatone, C., Di Emidio, G., Barbonetti, A., Carta, G., Luciano, A. M., Falone, S., & Amicarelli, F. (2018). Sirtuins in gamete biology and reproductive physiology: emerging roles and therapeutic potential in female and male infertility. *Human reproduction update*, *24* (3), 267–289. https://doi.org/10.1093/humupd/dmy003.

[15] Weng Q, Liu Z, Li B, Liu K, Wu W, Liu H (2016) Oxidative Stress Induces Mouse Follicular Granulosa Cells Apoptosis via JNK/FoxO1 Pathway. PLoS ONE 11 (12): e0167869. https://doi.org/10.1371/journal.pone.0167869.

[16] Zhou, X. L., Xu, J. J., Ni, Y. H., Chen, X. C., Zhang, H. X., Zhang, X. M., Liu, W. J., Luo, L. L., & Fu, Y. C. (2014). SIRT1 activator (SRT1720) improves the follicle reserve and prolongs the ovarian lifespan of diet-induced obesity in female mice via activating SIRT1 and suppressing mTOR signaling. *Journal of ovarian research*, *7*, 97. https://doi.org/10.1186/s13048-014-0097-z.

[17] Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, et al. (2012) Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.*107-20. doi: 10.1038/cdd.2011.96.

[18] Hochreiter-Hufford, A., & Ravichandran, K. S. (2013). Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion. *Cold Spring Harbor perspectives in biology*, *5* (1), a008748. https://doi.org/10.1101/cshperspect.a008748.

[19] Sirotkin, A. V., Kardošová, D., Alwasel, S. H., & Harrath, A. H. (2015). Neuropeptide Y directly affects ovarian cell proliferation and apoptosis. *Reproductive biology*, *15* (4), 257–260. https://doi.org/10.1016/j.repbio.2015.07.004.

[20] Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P et al. (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* 25 (3): 486-541. doi: 10.1038/s41418-017-0012-4.

[21] Janssen, W. J., & Henson, P. M. (2012). Cellular regulation of the inflammatory response. *Toxicologic pathology*, *40* (2), 166–173. https://doi.org/10.1177/0192623311428477.

[22] Kolb, J. P., Oguin, T. H., 3rd, Oberst, A., & Martinez, J. (2017). Programmed Cell Death and Inflammation: Winter Is Coming. *Trends in immunology*, *38* (10), 705–718. https://doi.org/10.1016/j.it.2017.06.009.