doi:10.4149/endo_2011_04_175

Survival role of superoxide dismutase 1 on human granulosa luteinized cells in vitro

Dineva J, Vangelov I, [1]Abrashev R, Todorova K, [2]Gulenova D, [2]Nikolov G, [3]Rangelov I, [3]Stamenov G, [1]Angelova M, Ivanova M

The "K. Bratanov" Institute of Biology and Immunology of Reproduction and [1]The Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences (BAS), Sophia, Bulgaria; [2]Medical center "ReproBioMed", Ltd., Sophia, Bulgaria; [3]Centre of Reproductive health "Nadezhda", Clinic "Dr Malinov", Sophia Bulgaria

Objective. Deleterious effects of free radicals do not only result from the amount of free radicals produced but also are related to the efficiency and to the activities of enzymatic antioxidant systems. We investigated the effect of exogenous superoxide dismutase (SOD1) or Cu-chelating agent diethyldithiocarbamate (DDC) on the apoptosis (caspase-3 activity) of human granulosa luteinized cells (hGLC) *in vitro.*

Methods. The effects of SOD1 and DDC were studied using *in vitro* culture system, caspase-3 and the total SOD activity in hGLCs were measured using AcDEVD-PNA substrate and Beaushap and Fridovich methods, respectively, after 48 h of the culture period.

Results. The activity of SOD1 was the lowest in GLCs treated with 100 μM DDC as compared to control cells and to the cells supplemented with Cu, Zn-SOD or DDC (10 μM). The effect of DDC was associated with elevated caspase-3 activity as compared to control cells.

Conclusions. It was demonstrated for the first time that the supplementation of cultured hGLCs with Cu^2+, Zn^2+-SOD (200 U/ml) maintains the viability of hGLCs via caspase-3 suppression.

Key words: apoptosis, caspase-3, granulosa luteinized cells, superoxide dismutase, diethyldithiocarbamate

There is a continuous contact between cells and high reactive oxygen-derived free superoxide, hydrogen peroxide and hydroxyl radicals. The protection against these free radicals is achieved by natural antioxidant molecules, mainly by antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase which act in a cooperative or synergistic way to ensure the cell protection.

The SOD converts highly reactive and damaging superoxide free radicals to peroxides. Three different SODs are known in humans including CuZn-SOD (SOD1) found in the cytoplasm and nucleus (Crapo et al. 1992), mitochondrial Mn-SOD (SOD2) (Weisiger and Fridovich 1973), and an extracellular SOD (EC-SOD, SOD3) (Marklund 1982). However, SOD1 is also localized in inter membranous space of mitochondria (IMS-SOD) in various types of cells and the active enzyme has been reported to be implicated in the protection of vital mitochondrial functions (Inarrea et al. 2007). SODs were also found in normal cycling human ovaries (Shiotani et al. 1991; Tamate et al. 1995; Suzuki et al. 1999) and SOD activity has been measured in human follicular fluids (Sabatini et al. 1999; Carbone et al. 2003; Kably Ambe et al. 2004, 2005; Pasqualotto et al. 2009). SODs expression has been demonstrated in human follicular granulosa cells (Tatone et al. 2006) and adjacent cumulus oophorus cells (Matos et al. 2009). In GLCs the antioxidant enzymes play a crucial role in the scavenging of superoxide anions and hydrogen peroxide generated during the synthesis of steroid hormones (Tatone et al. 2008).

The oxidative stress starts the action when the generation of reactive oxygen species (ROS) and other reactive radicals go beyond scavenge capacity of antioxidants

and thus provoke many intracellular events including apoptosis.

In humans, oxidative stress-related damages of oocyte and GLCs structures have been described by de Bruin et al. (2004). Apoptosis has since been recognized and accepted as a distinctive and important mode of "programmed" cell death, which involves genetically determined elimination of cells, generally characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms (Elmore 2007). Inhibitors of oxidative stress and antioxidants have been reported to mimic the ability of gonadotrophins to suppress apoptosis in rat ovarian follicles (Tilly and Tilly 1995) and in the rabbit corpus luteum *in vitro* (Dharmarajan et al. 1999).

Diethyldithiocarbamate (DDC) is Cu-chelating agent generally used as an inhibitor of cytoplasmic SOD1 activity. The inhibition of SOD1 with DDC has been found associated with the induction of cell dead in different cell types (Siwik et al. 1999; Moriyama-Gonda et al. 2002; Kimoto-Kinoshita et al. 2004), with enhancement of methylglyoxal-induced apoptosis (Du et al. 2001) or with increased SOD1 mRNA level and decreased catalase and glutathione peroxidase mRNA level (Maitre et al. 1993).

In this study we investigated the effect of exogenous superoxide dismutase (SOD1) supplementation or the effect of Cu-chelating agent diethyldithiocarbamate on the apoptosis (caspase-3 activity) of human granulosa luteinized cells (hGLCs) *in vitro*.

Materials and Methods

Isolation of human GLCs. For each experiment GLCs were isolated from follicular aspirates, macroscopically without blood contamination, obtained after ovum pick-up from at least three women undergoing IVF-ET program, after informed consent was given. Ethical approval of GLCs investigations was obtained by the local clinical ethical committee (ReproBioMed. Center, Sofia). After gradient centrifugation on Histopaque-1077 (Sigma®, Saint Louis, Missouri, USA), GLCs were aspirated from interphase and washed with culture medium (HAM's F-12, 10 % fetal calf cerum (FCS) (Sigma). The cell viability was determined by Trypan blue exclusion method.

Cell culture system and incubation. Isolated GLCs from at least three women were diluted in HAM's F-12 (10 % FCS) supplemented with penicillin (100 IU/ml) and streptomycin (100 mg/ml) at $70x10^4$ cells/ml and cultured in 96-well plates (Linbro® Flow Labs, Virginia, USA) using 50 µl/well for 24 h at 37 °C and 5 % CO_2. After 24 h culture period the medium was removed and cells were supplemented with 10, 100, 200 U/ml SOD1 (Cu^{2+}, Zn^{2+}-SOD; Institute of Microbiology, Bulgarian Academy of Sciences). Purified Cu^{2+}, Zn^{2+}-SOD enzyme is a water-soluble homodimeric glycoprotein with a molecular mass of approximately 31.7 kDa and was isolated from *Humicola lutea 103* (Krumova et al. 2007) in HAM's F-12 (1 % FSC) or with 10, 100 µM DDC (Sigma) in HAM's F-12 (1 % FSC). Control cells were supplemented with HAM's F-12 (1 % FCS) only and all cells were cultured for next 24 h.

SOD activity determination. Cultured human GLCs were suspended in 150 µl ice-cold phosphate buffer (5 mM potassium phosphate, pH 7.0), 5 mM EDTA; 10 % glycerol, 25 mM phenylmethylsulfonyl fluoride and were sonicated on ice. Sonication condition was 90 s bursts, typically 3 times. The sonicated GLCs were centrifuged at 12,000 x g, for 10 min at 4 °C. The activity of total SOD in the supernatants was then determined.

Total SOD activity was measured by nitro-blue tetrazolium (NBT) reduction method according to Beauchamp and Fridovich (1971). The reaction mixture contained 56 µM (NBT), 0.01 M methionine, 1.17 µM riboflavin, 20 µM NaCN and 0.05 M phosphate buffer pH 7.8. Superoxide was measured by increasing absorbance at 560 nm at 30 °C after 6 min incubation from the beginning of illumination. One unit of SOD activity was defined as the amount of enzyme required for inhibition of the reduction of NBT by 50 % (A_{560}) and was expressed as units per mg protein. Protein concentration was estimated according to Lowry (1951) using bovine serum albumin as standard.

Caspase-3 activity. Cultured cells were lysed with a lysis buffer: 50 mM Hepes, 100 mM NaCl, 0.1 M CHAPS, 1 M EDTA, 10 M DTT, 10 % glycerol (Sigma®). Soluble fraction of the cell lysate was assayed for caspase-3 activity using AcDEVD-PNA substrate (Sigma®, Saint Louis, Missouri, USA). After incubation for two hours at 37 °C and 5 % CO_2, the intensity of color reaction being measured by Multiplate reader (LKB, Roma, Italy) at 405 nm.

Statistical evaluation was performed with software package STATISTICA® 6.0 using one way ANOVA followed by Newman-Keuls post hoc test and the results were expressed as mean±SD and significance was assumed at $p< 0.05$.

Results

The supplementation of GLCs with each dose of 10 U/ml, 100 U/ml or 200 U/ml Cu^{2+}, Zn^{2+}-SOD was associated with significant dose dependent increase of GLCs SOD activity compared to the control (Table 1 and Fig. 1, P<0.05).

The measurement of caspase-3 activity showed that the cells treated with 10, 100, 200 U/ml Cu^{2+}, Zn^{2+}-SOD have lower activity of executor enzyme compared to the control cells, but the suppressive effect on caspase-3 activity reached the significance at 200 U/ml Cu^{2+}, Zn^{2+}-SOD treatment (Table 2 and Fig. 2, P<0.05). The GLC's caspase-3 activity after 10 U/ml and 100 U/ml Cu^{2+}, Zn^{2+}-SOD treatment was comparable (Table 2 and Fig. 2, p>0.05) in spite of significantly higher SOD activity as found after 100 U/ml Cu^{2+}, Zn^{2+}-SOD treatment compared to 10 U/ml - treatment (Table 1 and Fig. 1, p<0.05).

Depending of the dose used, dual effect of DDC on GLCs total SOD activity was documented. Thus, the treatment of GLCs with 10 μM DDC induced a significant enhancement in the total SOD activity (Table 1 and Fig. 1, p<0.05), while the treatment with 100 μM DDC significantly inhibited SOD activity compared to the control cells (Table 1 and Fig. 1, p<0.05).

Despite of documented dual effect of DDC on GLCs SOD activity, neither low dose (10 μM) nor high dose of DDC (100 μM) treatment was associated with enhanced GLCs viability; the incubation of GLCs with both dosages of DDC stimulated significantly the activity of caspase-3 as compared to the control cells (Table 2 and Fig. 2, P<0.05).

The highest total SOD activity in cultured GLCs, induced after the supplementation with 200 U/ml Cu^{2+}, Zn^{2+}-SOD, was associated with significantly (p<0.05) lowest caspase-3 activity compared to the cells supplemented with the 10 and 100 U/ml Cu^{2+}, Zn^{2+}-SOD

Table 1

Comparisons of means GLCs SOD activity, Newman-Keuls test. Marked differences (*) are significant at P < .05.

	{1}	{2}	{3}	{4}	{5}	{6}
GROUP	**M=10.475**	**M=11.903**	**M=8.700**	**M=10.925**	**M=20.175**	**M=22.625**
Control {1}		*P<0.05	*P<0.05	*P<0.05	*P<0.05	*P<0.05
10 μM DDC {2}			*P<0.05	*P<0.05	*P<0.05	*P<0.05
100 μM DDC {3}				*P<0.05	*P<0.05	*P<0.05
10 U/ml SOD {4}					*P<0.05	*P<0.05
100U/ml SOD {5}						*P<0.05
200 U/ml SOD {6}						

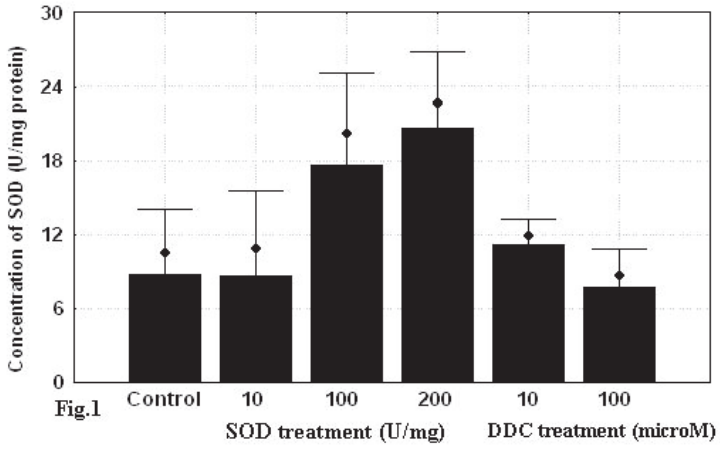

Fig. 1. Comparison of GLCs SOD activity measured in cultured GLCs, after treatment with exogenous SOD and Cu-chelating agent DDC.

and to the cells treated with 10, 100 μM DDC (Table 2 and Fig. 2).

Discussion

ROS are continuously neutralized by antioxidants present in body tissues. Whenever the production of ROS exceeds the scavenging capacity of antioxidants, it leads to oxidative stress (Desai et al 2008). Many studies have shown that the redox state of the cell, resulting from the accumulation of ROS and a decrease of antioxidant levels, is involved in inducing of apoptotic cell death (Hockenbery et al. 1993; Buttke and Sandstrom 1994). We used a 48 h *in vitro* model of human GLCs, to investigate how the supplementation with various concentrations of Cu^{2+}, Zn^{2+}- SOD or with various concentrations of Cu-chelating agent DDC influences GLCs total SOD activity and the GLCs viability (caspase-3 activity) and how these parameters are interrelated. In different cell types dual effects of DDC on intracellular redox state, either prooxidant (Maitre et al. 1993; Siwik et al. 1999; Moriyama-Gonda et al. 2002; Kimoto-Kinoshita et al. 2004) or antioxidant (Ito et al. 2000, 2001) have been observed. DDC inactivates Cu, Zn - SOD and extracellular SOD by chelating the copper ion at the active sites. Based on the measured GLCs total SOD activity, antioxidative effect of low dose DDC (10 μM) and prooxidative effect of high dose DDC (100 μM) were documented: significantly higher GLCs total SOD activity (10 μM DDC) and significantly lower GLCs total SOD activity (100 μM DDC) was measured, respectively, compared to the control cells.

ROS also induce the dissociation of cytochrome C from cardiolipin on the inner mitochondrial membrane (Zhao et al. 2004), oxidize the critical thiol groups in adenine nucleotide translocase (ANT) (Li et al. 2006), cause changes in the inner mitochondrial membrane that resulte in an opening of the mitochondrial permeability transition pore,

Table 2

Comparisons of means GLCs Caspase-3 activity, Newman-Keuls test. Marked differences (*) are significant at P < .05.

	{1}	{2}	{3}	{4}	{5}	{6}
GROUP	**M=.226**	**M=.245**	**M=.287**	**M=.234**	**M=.220**	**M=.204**
Control {1}		*P<0.05	*P<0.05	P=0.26	P=0.44	*P<0.05
10 μM DDC {2}			*P<0.05	P=0.13	*P<0.05	*P<0.05
100 μM DDC {3}				*P<0.05	*P<0.05	*P<0.05
10 U/ml SOD {4}					P=0.14	*P<0.05
100 U/ml SOD {5}						*P<0.05
200 U/ml SOD {6}						

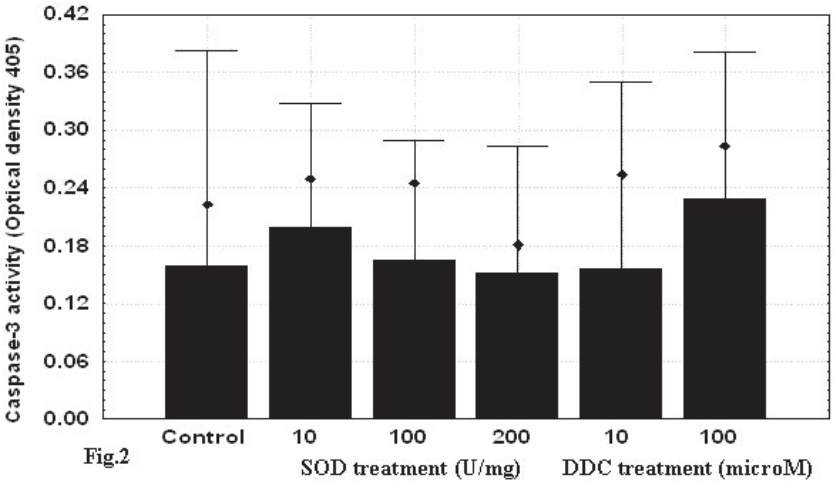

Fig. 2. Comparisons of Caspase-3 activity of cultured GLCs in vitro, after treatment with exogenous SOD and Cu-chelating agent DDC.

loss of the mitochondrial transmembrane potential and release of normally sequestered pro-apoptotic proteins as cytochrome C, Smac/DIABLO, HtrA2/Omi, AIF, endonuclease G and CAD, from the intermembrane space into the cytoplasm (Saelens et al. 2004). Cytochrome C binds and activates Apaf-1 as well as procaspase-9, forming an "apoptosome", which subsequently activates the main executor enzyme caspase-3. Inhibition of GLCs apoptosis within rat follicles incubated in medium at 1000 U/ml exogeneous SOD (Tilly and Tilly 1995) or dose-dependent (1.5-150 U/ml exogeneous SOD) inhibition of rabbit luteal cell apoptosis (Dharmarajan et al. 1998) has been described. In our study for the first time we documented the positive effect of supplementation of culture medium with 200 U/ml Cu, Zn-SOD on the hGLCs viability *in vitro*. The obtained results showed that the elimination of superoxides, generated during the physiological process of oxygen consumption from GLCs, after supplementation with Cu, Zn-SOD, was important condition for the maintaining of human GLCs viability *in vitro* by the suppression of caspase-3 activity.

The apoptosis is a coordinated and often energy-dependent process that involves the activation of caspases and a cascade of events that link the initiating stimuli to the final demise of the cell (Elmore 2007). When the overall balance between physiological ROS production and the total antioxidant defences becomes unbalanced, this disequilibrium may eventually drive the cell to apoptosis (Orrenius et al. 2007). In our study, the highest activity of caspase-3 measured after the treatment with 100 μM DDC was associated with the lowest total GLCs SOD activity, showing the direct role of Cu, Zn- SOD (SOD1) in the prevention of human GLCs from apoptosis as was demonstrated by Inarrea et al. (2007). We demonstrated that after treatment with high dosage DDC, in human GLCs the deleterious effect was relative to the activity of enzymatic antioxidant Cu, Zn-SOD (SOD1) which counteracts the proapoptotic effect of the superoxide anion on the cells *in vitro*. In spite of documented effects of DDC on the total SOD activity in GLCs, the elevated GLCs apoptosis, demonstrated by increased caspase-3 activity, was documented after treatment with either of 10 μM DDC or 100 μM DDC.

In conclusion, obtained results indicated that the Cu, Zn-SOD in GLCs was closely involved in anti-apoptotic mechanisms in human GLCs *in vitro.* In the present study, for the first time, we demonstrated that the supplementation with 200 U/ml Cu, Zn-SOD 200 U/ml maintains the human GLCs viability *in vitro,* by the suppression of caspase-3 activity.

Acknoledgments

We thank to Verka Pesheva for the excellent technical support. The *in vitro* culture of cells was realized in The Lab for cell culture, equipped by the Project DOO – 50/2008, Nat. Sci. Fund, Ministry of Education, Youth and Science.

References

Beauchamp C, Fridovich I: Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 44, 276-287, 1971. http://dx.doi.org/10.1016/0003-2697(71)90370-8

Buttke TM, Sandstrom PA: Oxidative stress as a mediator of apoptosis. Immunol Today 15, 7–10, 1994. http://dx.doi.org/10.1016/0167-5699(94)90018-3

Carbone MC, Tatone C, Delle Monache S, Marci R, Caserta D, Colonna R, Amicarelli F: Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent changes. Mol Hum Reprod 9, 639–643, 2003. http://dx.doi.org/10.1093/molehr/gag090

Crapo JD,Oury T,Rabouille C,Slot JW,Chang LY: Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci 89, 10405-10409, 1992. http://dx.doi.org/10.1073/pnas.89.21.10405

de Bruin JP, Dorland M, Spek ER, Posthuma G, van Haaften M, Looman CW, te Velde ER: Age-related changes in the ultrastructure of the resting follicle pool in human ovaries. Biol Reprod 70, 419–424, 2004. http://dx.doi.org/10.1095/biolreprod.103.015784

Desai N, Sharma R, Makker K, Sabanegh E, Agarwal A: Physiologic and pathologic levels of reactive oxygen species in neat semen of infertile men. Fertil Steril 92, 1626-1631, 2008. http://dx.doi.org/10.1016/j.fertnstert.2008.08.109

Dharmarajan AM, Hisheh S, Singh B, Parkinson S, Tilly KI, Tilly JL: Antioxidants mimic the ability of chorionic gonadotropin to suppress apoptosis in the rabbit corpus luteum in vitro: a novel role for superoxide dismutase in regulating bax expression. Endocrinology 140, 2555-2561, 1999. http://dx.doi.org/10.1210/en.140.6.2555

Du J, Suzuki H, Nagase F, Akhand AA, Ma XY,Yokoyama T, Miyata T, Nakashima I: Superoxide-mediated early oxidation and activation of ASK1 are important for initiating methylglyoxal-induced apoptosis process. Free Radic Biol Med 31, 469-478, 2001. http://dx.doi.org/10.1016/S0891-5849(01)00611-6

Elmore S: Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol 35, 495-516, 2007. http://dx.doi.org/10.1080/01926230701320337

Hockenbery DM, Oltvai ZN, Yin X-M, Milliman CL, Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell 75, 241–251, 1993. http://dx.doi.org/10.1016/0092-8674(93)80066-N

Iñarrea P, Moini H, Han D, Rettori D, AguilΓi I, Alava MA, Iturralde M,Cadenas E: Mitochondrial respiratory chain and thioredoxin reductase regulate intermembrane Cu,Zn-superoxide dismutase activity: implications for mitochondrial energy metabolism and apoptosis. Biochem J 405, 173-179, 2007.

Ito Y, Cai H, Nabekura T, Terao M, Tomohiro M: Preventive effect of Diethyldithiocarbamate on opacification of cultured rat crystalline lenses. Journal of the Eye 17, 113-116, 2000

Ito Y, Cai H, Terao M, Tomohiro M: Preventive effect of diethyldithiocarbamate on selenite-induced opacity in cultured rat lenses. Ophthalmic Res 33, 52-59, 2001. http://dx.doi.org/10.1159/000055642

Kably Ambe A, Ruiz Anguas J, Carballo Mondragon E, Corona de Lau C, Karchmer Krivitsky S: Correlation between follicle levels of superoxide dismutase and oocyte quality, fertilization rates and embryo development. Ginecol Obstet Mex 72, 335-344, 2004.

Kably Ambe A, Ruiz Anguas J, Carballo Mondragon E, Karchmer Krivitsky S: Intrafollicular levels of sexual steroids and their relation with the antioxidant enzymes on the oocyte quality in an in vitro fertilization program. Ginecol Obstet Mex 73, 19-27, 2005.

Kimoto-Kinoshita S, Nishida S, Tomura TT: Diethyldithiocarbamate can induce two different type of death: apoptosis and necrosis mediating the differential MAP kinase activation and redox regulation in HL60 cells. Mol Cell Biochem 265, 123-132, 2004. http://dx.doi.org/10.1023/B:MCBI.0000044366.32073.2d

Li Q, Sato EF, Kira Y, Nishikawa M, Utsumi K, Inoue M: A possible cooperation of SOD1 and cytochrome C in mitochondria-dependent apoptosis. Free Radic Biol Med 40, 173-181, 2006. http://dx.doi.org/10.1016/j.freeradbiomed.2005.09.037

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265–275, 1951.

Maitre B, Jornot L, Junod AF: Effects of inhibition of catalase and superoxide dismutase activity on antioxidant enzyme mRNA levels. Am J Physiol 265, L636-L643, 1993.

Marklund SL, Holme E, Hellner L: Superoxide dismutase in extracellular fluids. Clin Chim Acta 126, 41-51, 1982. http://dx.doi.org/10.1016/0009-8981(82)90360-6

Matos L, Stevenson D, Gomes F, Silva-Carvalho JL, Almeida H: Superoxide dismutase expression in human cumulus oophorus cells. Mol Hum Reprod 15, 411-419, 2009. http://dx.doi.org/10.1093/molehr/gap034

Moriyama-Gonda N, Igawa M, Shiina H, Urakami S, Shigeno K, Terashima M: Modulation of heat-induced cell death in PC-3 prostate cancer cells by the antioxidant inhibitor diethyldithiocarbamate. BJU Int 90, 317-25, 2002. http://dx.doi.org/10.1046/j.1464-410X.2002.02810.x

Orrenius S, Gogvadze V, Zhivotovsky B: Mitochondrial Oxidative Stress: Implications for Cell Death. Pharmacol Toxicol. 47, 143-183, 2007. http://dx.doi.org/10.1146/annurev.pharmtox.47.120505.105122

Pasqualotto EB, Lara LV, Salvador M, Sobreiro BP, Borges E, Pasqualotto FF: The role of enzymatic antioxidants detected in the follicular fluid and semen of infertile couples undergoing assisted reproduction. Hum Fertil (Camb) 12, 166-171, 2009. http://dx.doi.org/10.1080/14647270903207941

Sabatini L, Wilson C, Lower A, Al-Shawaf T, Grudzinskas JG: Superoxide dismutase activity in human follicular fluid after controlled ovarian hyperstimulation in women undergoing in vitro fertilization. Fertil Steril 72, 1027-1034, 1999. http://dx.doi.org/10.1016/S0015-0282(99)00411-2

Saelens X, Festjens N, Vande Walle L, Van Gurp M,Van Loo G, Vandenabeele P: Toxic proteins released from mitochondria in cell death. Oncogene 23, 2861-2874, 2004. http://dx.doi.org/10.1038/sj.onc.1207523

Shiotani M, Noda Y, Narimoto K, Imai K, Mori T, Fujimoto K, Ogawa K: Immunohistochemical localization of superoxide dismutase in the human ovary. Hum Reprod 6, 1349-1353, 1991.

Siwik DA, Tzortzis JD, Pimental DR, Chang DL, Pagano PJ, Singh K, Sawyer DB, Colucci WS: Inhibition of copper-zinc superoxide dismutase induces cell growth, hypertrophic phenotype, and apoptosis in neonatal rat cardiac myocytes in vitro. Circ Res 85, 147-153, 1999.

Suzuki T, Sugino N, Fukaya T, Sugiyama S, Uda T, Takaya R, Yajima A, Sasano H: Superoxide dismutase in normal cycling human ovaries: immunohistochemical localization and characterization. Fertil Steril 72, 720-726, 1999. http://dx.doi.org/10.1016/S0015-0282(99)00332-5

Tamate K, Sengoku K, Ishikawa M: The role of superoxide dismutase in the human ovary and fallopian tube. J Obstet Gynaecol 21, 401-409, 1995.

Tatone C, Carbone MC, Falone S, Aimola P, Giardinelli A, Caserta D, Marci R, Pandolfi A, Ragnelli AM, Amicarelli F: Age-dependent changes in the expression of superoxide dismutases and catalase are associated with ultrastructural modifications in human granulosa cells. Mol Hum Reprod 12, 655-660, 2006. http://dx.doi.org/10.1093/molehr/gal080

Tatone C, Amicarelli F, Carbone MC, Monteleone P, Caserta D, Marci R, Artini PG, Piomboni P, Focarelli R: Cellular and molecular aspects of ovarian follicle ageing. Hum Reprod Update 14, 131-142, 2008. http://dx.doi.org/10.1093/humupd/dmm048

Tilly JL, Tilly KI: Inhibitors of oxidative stress mimic the ability of follicle-stimulating hormone to suppress apoptosis in cultured rat ovarian follicles. Endocrinology 136, 242-252, 1995. http://dx.doi.org/10.1210/en.136.1.242

Weisiger RA, Fridovich I: Mitochondrial superoxide simutase. Site of synthesis and intramitochondrial localization. J Biol Chem 248, 4793-4966, 1973.

Zhao K, Zhao GM, Wu D, Soong Y, Birk AV, Schiller PW, Szeto HH: Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. J Biol Chem 279, 34682-34690, 2004. http://dx.doi.org/10.1074/jbc.M402999200