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Effect of Simvastatin on Improvement of Follicular Apoptosis through Interaction with Tumor Necrosis Factor-α and Changes in Anti-Mullerian Hormone Levels in Female Rats with Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is the most frequent female endocrine disorder affecting about 5%–10% of women and causing infertility due to dysfunctional follicular maturation and ovulation. **Objective:** Simvastatin has an anti-inflammatory impact by trace on leukocytes and cytokines, which inhibit cellular proliferation, reduce androgen production and leads to apoptosis creation in antral follicle.**Methods:** The total of 60 wistar female rats were divided into three experimental groups. The experimental groups were injected each evening with DHEA (6 mg/100 g body weight/0.2 ml sesame oil) for 20 days. Then were divided into two groups (comparison group (PCOS) and treatment group (PCT)). The treatment group received of simvastatin (10 mg/kg body weight 1.5 ml of saline) for 4 weeks. The investigation of ovarian tissue was done with immunohistochemical and histopathologic methods. **Results:** Simvastatin drug led to a significant decrease in the serum level of AMH in the PCT compared to the PCOS ($P<0.001$) and the level of expression of TNF-α in PCT compared to the PCOS ($P<0.05$). Significant decrease in the thickness of theca follicle layer in PCT compared to the PCOS ($P<0.05$). **Conclusion:** The investigation of apoptosis in ovary tissue showed a significant increase in apoptosis index in PCOS compared to PCT with simvastatin. The effects of simvastatin in PCOS may be due to the inhibitory trace on TNF-α and AMH.

Keywords*:* Polycystic ovary syndrome, Simvastatin, Tumor Necrotic Factor-α, Rats.

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Introduction

Polycystic ovary syndrome (PCOS) is the most frequent female endocrine disorder, affecting 5%–10% of women, causing infertility due to dysfunctional follicular maturation and ovulation (1). Regulation of growth of ovarian mesenchymal tissues is essential for normal ovarian development and function (2). One possible explanation of this observation, or at least a significant contributor, may be related to the presence of increased oxidative stress demonstrated in several studies on women with PCOS (3). Excessive growth of tissues may be due to increased cellular proliferation and/or reduced apoptosis. These observations are consistent with the concept that reactive oxygen species (ROS) at moderate levels may function as second messengers, stimulating signal transduction pathways and inducing increased cell growth and reduction of apoptosis (4). Effects of moderate levels of ROS often closely resemble actions of insulin, an important modulator of growth and apoptosis in many cell types, including theca-interstitial cells and anti-inflammatory substance is secreted by white blood cells (5). The increase in the amount of androgen leads to the increase in lipolysis and released fatty acid, and on the other hand it leads to macrophage immigration to adipose tissue and consequently inflammation of astromal arteries and expression of TNF-α and other cytokines such as interleukin-6, 1, 18 in granulosa cells and antral theca follicle (6). The increase of androgen and IGF that promotes the expression of such cytokines as TNF-α, IL_1, and IL_6 (7). Increase in insulin resistance derived from the activity of NF-KB is one of the causes of the creation of ovary cysts, and on the other hand insulin itself causes an unnatural increase in androgens that, in turn, leads to the

production of AMH (8). It is observed that TNF-α by sitting on the receptor of granulosa cells leads to apoptosis creation in antral follicle and on the other hand leads to steroidogenesis and growth of sheath of follicular cells (9). It is the most important cause of the decrease of ovulation or anovulation in infertile women, which was first identified by increased secretion of ovary and adrenal androgen, hyper-insulinism, irregular menstruation, fibrotic and large ovary (10).

AMH is known to be associated with follicle growth, and its levels are two to three times higher in women with PCOS than in those with normal ovaries (11).

In addition, prenatally androgenized sheep, which manifest PCOS-like features, exhibited reduced AMH expression by prenatal and antral follicles (12). In PCOS, the relationship between hyperandrogenism and AMH remains unclear. It is thought, however, that androgen and AMH are closely related, and both affect folliculo-genesis. Statins are competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase, the rate-limiting enzyme of the cellular production of cholesterol and other products of the mevalonate pathway. Statins exert hepatic and extrahepatic effects, modulating the function of various tissues and organs, including ovaries. Previously, we have demonstrated that simvastatin inhibited cellular proliferation and reduced androgen production by ovarian theca-interstitial cells. Among tested statins, simvastatin exerted the greatest inhibitory effects on all tested parameters. However, different statins may have distinctly different profiles of effects on cholesterol and androgens. In another study, the anti-inflammatory impact of simvastatin and its impact on leukocytes and cytokines in the circulation was assessed in a live human with a mild inflammation. In this study, simvastatin with a descending effect on factors such as TNF-α and IL_1 could reduce inflammation (13).

The objective of this research was to evaluate the effect of simvastatin on the expression of TNF-α in theca layers and granulosa as well as apoptosis created by this factor in granulosa layer of follicle and the reduction of the serum level of AMH produced by the granulosa cells of PCOS induced by DHEA in the mature female rat.

Materials and Methods

Animals and The treatments

The study was carried out using 60 wistar rats, with body weights of 170–200 g and regular (4–5 days) ovulatory cycles. The animals were housed in an air-conditioned environment and a 12L:12D cycle and

received standard rat chow and water ad libitum. We divided rats into three groups: the experimental groups and the control group. Immature rats (approximately 21-22 days old) were treated with daily s.c. DHEA injections (rats; 6 mg/100 g body weight) for 20 days. This dose of DHEA is sufficient to induce a hyper-androgenized state similar to that in PCOS women. This model is also reliant on artificial hyper-androgenemia and does not help identify abnormalities upstream of hyper-androgenemia.

After 20days, the experimental groups were divided into two groups (comparison group (PCOS) and treatment group (pct)).

The treatment group received simvastatin (10 mg/kg body weight 1.5 ml of saline) for 4 weeks. Animals used in this study were maintained in accordance with the guidelines of the Animal Resources Centre of Guilan University Medical of Sciences.

Immunohistochemistry

For immunohistochemical analysis, ovaries were dissected, weighed, fixed in 4% paraformaldehyde, embedded in paraffin, and serially sectioned longitudinally throughout the largest diameter of the ovaries (thickness, 6 μm). Then after deparaffinization in dimethylbenzene and rehydration through an ethanol series, the other sections were placed on slides, TNF-α expression was detected immunohistochemically. Immerse dewaxed paraffin section into the 3% H2O2 at room temperature for 10 min wash the section 3X to 5X with distilled water (total 3 to 5 min). Antigen Retrieval (Heat Induced Epitope Retrieval: HIER). Immerse the paraffin sections in citrate buffer, heat the buffer in microwave and turn it off when the buffer has boiled, keep the boiled buffer in microwave for 5 to 10 min, add 5% BSA blocking solution or normal goat serum to the HIER treated samples, and incubate the samples at 37°C for 30 min. primary antibody add to the samples and incubate overnight at 4℃ or at 37℃ for 1 hour. secondary antibody with antibody diluent to the concentration recommended by the antibody manufacturer. Antibody add to the samples and incubate at 37°C for 30 min. Strept-Avidin Biotin Complex (SABC) HRP- or AP-conjugated reagents to the samples, incubate the samples at 37°C for 30 min). The sections were counterstained with Mayer's hematoxylin.

Determination of Apoptosis

Determination of Staining with Acridine Orang

10 ml (0.2M) of NaH2Po4 (sodium phosphate) with PH = 2.5 was prepared, and

then 90 ml (0.1) of citric acid was added. Finally, 19 mg/ml of acridine orange was added to these materials. For staining, after deparaffinization in xylene and rehydration through an ethanol, and then the sample was put in a dark environment in acridine orange for 20 minutes. Therefore, the observed under fluorescence microscope, acridine orange through the normal cell membrane to the nucleus was green or yellow-green uniform fluorescence. In apoptotic cells because of chromatin condensation or fracture fragments ranging from the size, formation of apoptotic bodies. Acridine orange stain to the infected dense green fluorescent yellow or yellowish green debris particles; the yellow fluorescence cell necrosis weakens or even disappears.

Tissue Sample Collection to Measure the Thickness of Theca Follicle Layer

Immediately following the collection of blood samples, both ovaries were quickly removed and cleaned. Thereafter, one ovary was quickly frozen and stored at −80°C; and the other ovary was fixed in 10% formalin solution and stored at 4°C for staining. After fixation in 10% formalin for ~24 h, the ovarian tissue was dehydrated with ethanol and xylene, embedded in paraffin and sliced into 6-μm sections, stained with hematoxylin and eosin (H&E), and analyzed with an optical microscope (Olympus Bx50). To measure the thickness of theca follicle layer, (Micrometer Eye Piece).

Serum Hormone Assays (AMH)

After treatment for 4 weeks, all the rats were anesthetized, and blood samples were obtained from the inferior vena cava. Sera were immediately separated and kept at −20°C for subsequent hormone determination by enzyme-linked immunosorbent assay (ELISA)**:** AMH ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-AMH antibody and an AMH-HRP conjugate.

The assay sample and buffer are incubated together with AMH-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells were then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the AMH concentration since AMH from samples and AMH-HRP conjugate compete for the anti-

AMH antibody binding site. Since the number of sites is limited, as more sites are occupied by AMH from the sample, fewer sites are left to bind AMH-HRP conjugate.

Statistically Analysis

Data were analyzed using Excel Statistics, 2010. The quantitative results (the thickness of theca follicle layer and hormonal parameters) are presented as median [interquartile range (IQR)] and were compared using nonparametric analysis (Mann–Whitney U test). The proportions of the follicles apoptosis and TNF-α expression scores were compared using the χ^2 test.

Results

Simvastatin inhibits activity of TNF-α and reduces cholesterol synthesis in a concentration-dependent fashion. The effect of simvastatin on the reduction of growth of theca-interstitial cells TNF-α is expressed in theca cells. Although oocyte morphology was poorly preserved in these paraformaldehyde -fixed sections, TNF immunostaining was detected in more than half of the oocytes. As shown in Figure1 the control group, a little scattered protein TNF-α were seen. The average expression of TNF-α in the control group, PCOS, and simvastatin-treated (PCT) is (1.58 ± 21.65), (3.03 ± 40.10), and (2.97 ± 28.90), respectively. In the group suffering from PCOS, a significant increase in the amount of expression of this protein was observed compared with the control group ($P<0.05$). This increase in expression in the simvastatin-treated (PCT) showed a significant decrease (Figure1,2).

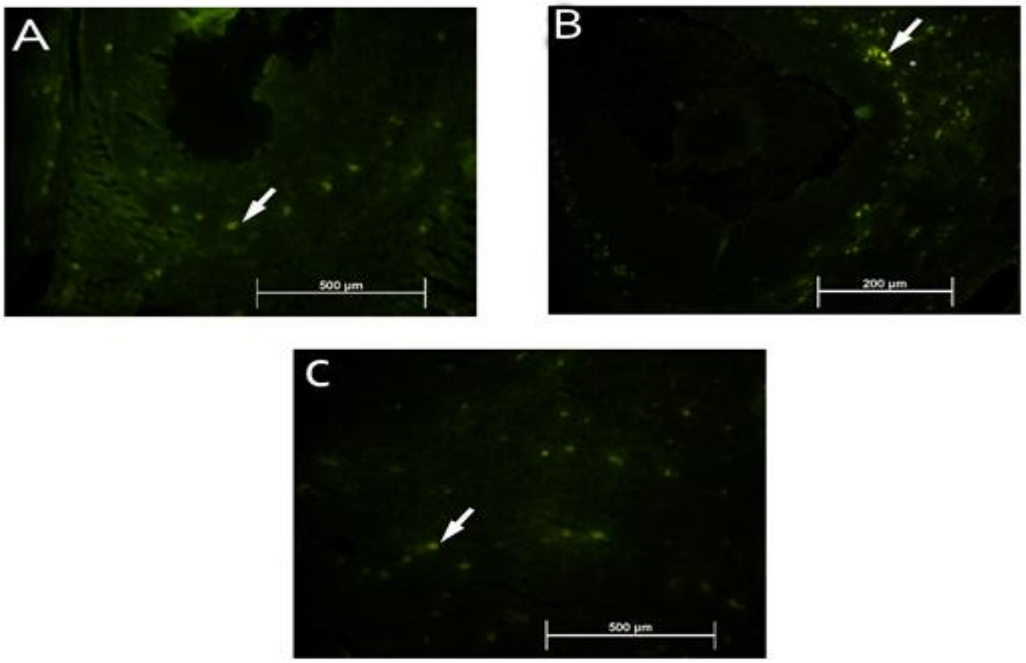

Figure 1: Ovaries and follicles immunostained for TNF-α in 3 groups. Representative sections from ovary of a blank control rat (A), the group induced by DHEA (PCOS) (B), and a simvastatin-treated rat (C).

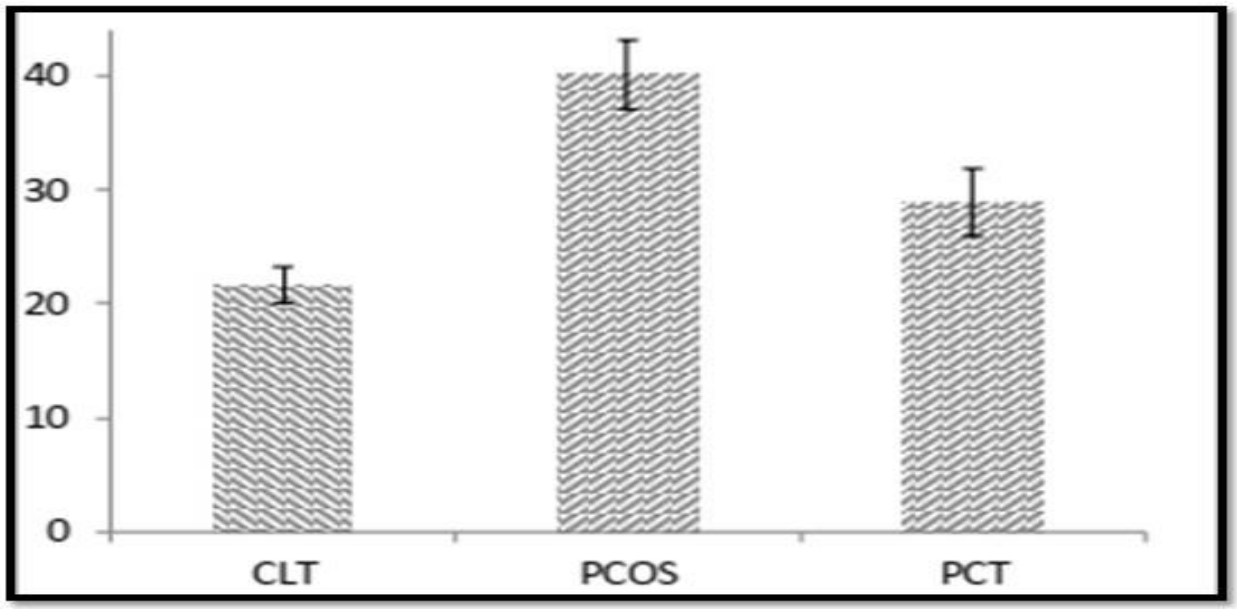

Figure2: Simvastatin treatment following DHEA exposure significantly reduced the level of the TNF-α. In the amount of the expression of TNF-α, a significant increase was observed in the group induced by DHEA (PCOS) compared to the control group (CTL) ($P<0.05$), which decreased significantly in the simvastatin-treated group (PCT)($P<0.001$).

Simvastatin Inhibits TNF-α Effects in Apoptosis

Acridine orange is a fluorescent pigment, the detection wavelength of 488 nm excitation filter. Its blocking filter wavelength is 515 nm. With cell DNA and RNA-binding capacity gaps exist, it may issue a different color fluorescence, and DNA binding of less green fluorescence, and the amount of RNA-binding orange or orange red fluorescence. Therefore, the observed under fluorescence microscope, acridine orange through the normal cell membrane to the nucleus was green or yellow-green uniform fluorescence; In apoptotic cells as a result of chromatin condensation or fracture fragments ranging from the size, formation of apoptotic bodies. Apoptotic nuclei turned to be orange and bright yellow surrounding follicles and some in granulosa cells of the antral follicle. In the control group, a few scattered apoptotic cells are shown in (Figure 3).

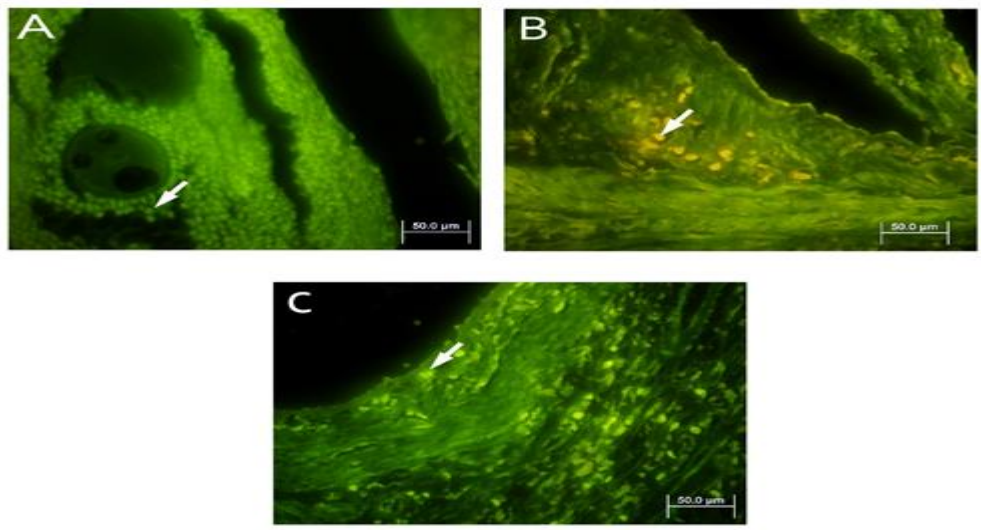

Figure 3: The effect of simvastatin on apoptosis in ovary tissue in areas around the follicle and granulosa cells. PCOS show an up-regulated level of apoptosis in ovarian. The PCOS group, an increase in apoptotic cells compared to the CTL group. The simvastatin-treated group (PCT), has decrease in apoptosis in ovary tissue compared to the group suffering from polycystic ovary syndrome (PCOS) .Acridine orange staining.

Apoptosis index in this group was 1.46 ± 0.78. In the PCOS group, apoptosis index significantly increased ($P<0.05$) compared with the control group. Layer simvastatin-treated and controls showed that ovaries from rats with PCOS possessed a relatively enhanced proportion of stromal tissue, and a thickened theca cell layer. Simvastatin Action on the Morphometric Investigations in order to compare the thickness of the theca (Figure4).

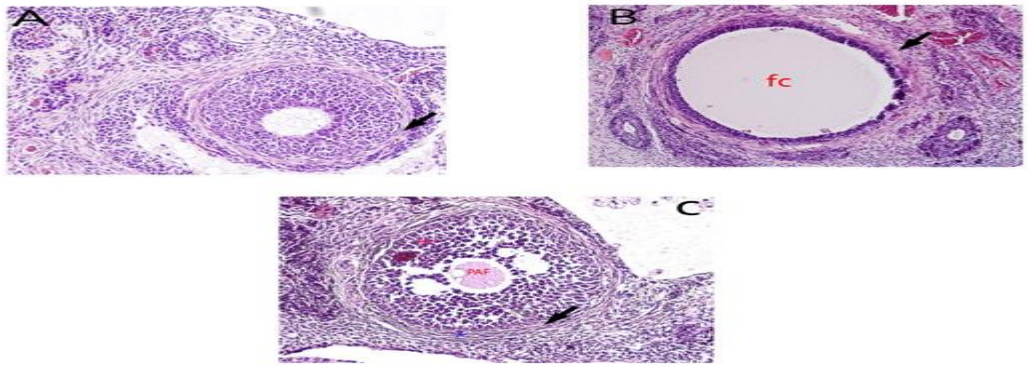

Figure 4: H and E staining of ovarian tissues from DHEA-exposed rats, simvastatin-treated, and controls showed that ovaries from rats with PCOS possessed a relatively enhanced proportion of stromal tissue, and a thickened theca cell layer (A-C). Staining revealed a markedly higher level of collagen in DHEA-exposed ovaries, especially at the region around the follicles (A); Cystic follicles (B); and follicle treated with the simvastatin (C). *a thickened theca layer and thin granulosa layer.

The results of the serum AMH levels were higher in the DHEA group (PCOS) than the control group (CTL) is shown in (Figure 5). The result described indicate that serum AMH level can be used as a marker for the number of growing follicles profiles of serum.

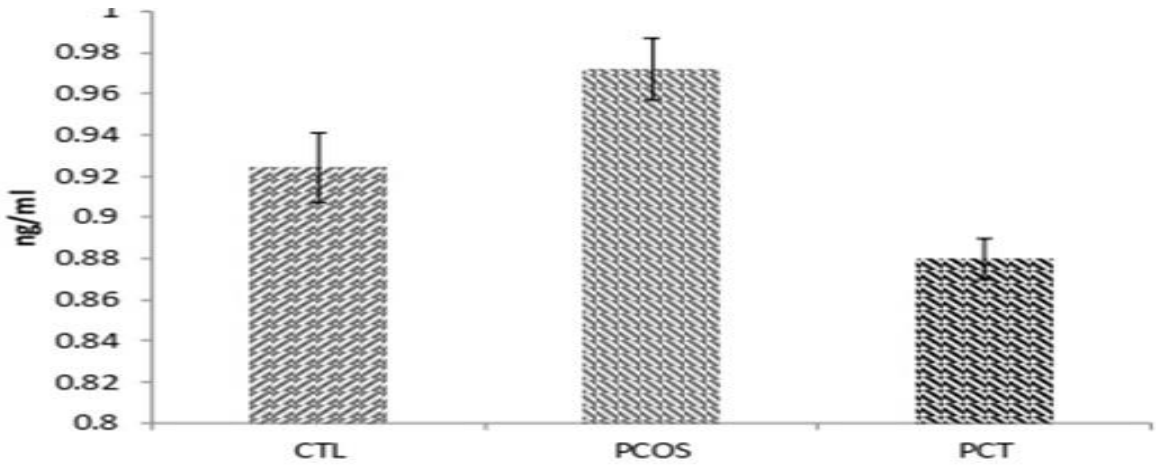

Figure 5: the serum AMH levels were higher in the DHEA group (PCOS) than the control group (CTL)

Hormones from Experimental and Control Rats

AMH's role as a peripheral signal of the size of the growing follicle pool may have important clinical benefits. In women undergoing treatment for infertility, ovarian aging is characterized by decreased ovarian responsiveness to exogenous gonadotropin administration and poor pregnancy outcome. In women with PCOS, serum AMH levels

are two to three times higher than in women with normal ovaries.

Discussion

This study presents the effect of simvastatin on improvement of follicular apoptosis through interaction with TNF-α and changes in AMH levels in female rats with polycystic ovary syndrome. A number of studies have been done to evaluate the impacts of statins on women with PCOS, and they have reported remarkable improvement in many clinical, metabolic, and endocrine aspects of this disorder. PCOS was defined according to the Rotterdam criteria. Specifically, an eligible patient was presented with at least two of the three following criteria: chronic anovulation, hyperandrogenism (hirsutism, acne) and/or hyperandrogenemia and polycystic ovaries (14). Also, two separate studies by Sathyapalan et al. (2009, 2010) have shown that atrovastatin improves biochemical hyperandrogenamia, insulin resistance, and inflammatory markers in patients with PCOS, Also, they have illustrated that atrovastatine decreases the levels of DHEA and androstendione.

Previously, we have demonstrated that one of the statins, simvastatin, inhibits cellular proliferation and reduces androgen production by ovarian theca-interstitial cells (15).

We also found that in these cells simvastatin inhibits activity of TNF-α and reduces cholesterol synthesis in a concentration-dependent fashion. The effect of simvastatin on the reduction of growth of theca-interstitial cells is not reversed in the presence of the cell- and mitochondrion-permeable forms of cholesterol (22-hydroxycholesterol and 25-hydroxycholesterol), indicating that the inhibition of proliferation of theca-interstitial cells is not due to reduced availability of cholesterol (13). However, the effects of simvastatin on cellular proliferation are partly abrogated in the presence of geranylgeranyl pyrophosphate and farnesyl pyrophosphate, indicating that simvastatin acts by inhibiting isoprenylation (16). Mechanisms of action of simvastatin on steroidogenesis are more complex. A simvastatin-induced decrease of androgen production is likely to be due to a combination of two separate effects: reduction of the number of theca-interstitial cells and inhibition of *Cyp17a1* mRNA expression that is independent of the number of cells. Inhibition of androgen production is reversed by 22-hydroxycholesterol as well as by geranylgeranyl pyrophosphate and farnesyl pyrophosphate, indicating that this action of simvastatin is the result of the

reduced availability of cholesterol and substrates of isoprenylation (17). In clinical trials, we have demonstrated that administration of simvastatin to women with PCOS results in significant reduction of ovarian size, decrease of androgen levels, and restoration of ovulatory function (18). Simvastatin also improved the profile of cardiovascular risk factors by reducing total cholesterol, LDL cholesterol, and hs-C-reactive protein level (19). The present observations indicate that theca-interstitial cells are sensitive to the level of ROS and that reduction of oxidants triggers apoptosis. Several studies have shown that AMH is an excellent marker to determine ovarian responsiveness also in an IVF program. Hormone measurements in the early follicular phase (day 3 of spontaneous cycle), retrospectively or in a group of unselected patients, revealed that AMH levels are lower in patients with poor ovarian response than in women with normal response ovarian responsiveness being defined as the number of oocytes retrieved, or as cancellation due to impaired or absent follicular growth. In agreement with the studies described above, AMH serum levels were shown to be highly correlated with the number of antral follicles before treatment and number of oocytes retrieved upon ovarian stimulation (20) (21).

However, a significant relationship between AMH and androgens could not be found. Of note, the available evidence highlights a direct involvement of androgens in follicular development and in the dysregulated folliculogenesis of PCOS (22). AMH is secreted by the granulosa cells of small antral and pre-antral follicles in the ovary. AMH diminishes aromatase induction by FSH in antral follicles and inhibits recruitment of primordial follicles (23). Previous studies have shown that circulating AMH levels are significantly higher in subjects with PCOS and correlate with circulating androgens but not with fasting insulin. In keeping with this observation, it has been demonstrated that granulosa cells from PCOS patients secrete more AMH *in vitro* (24). Furthermore, the increase in circulating AMH is accompanied by, and is related to, an increase in the antral follicle count (AFC). The concentration of AMH is higher in the follicular fluid from unstimulated ovaries of women with PCOS (25). In the present study, we further demonstrated that simvastatin inhibits TNF-α effects in apoptosis. TNF-α is a potent inflammatory cytokine and is produced by many cell types, such as macrophages and $CD4^+$ lymphocytes. Upon interaction with its ligand, TNF-α activates NF-κB (nuclear

factor kappa-light-chain-enhancer of activated B cells) pathway that is involved in apoptosis. The primary role of TNF-α is in the regulation of immune cells and is able to induce apoptosis (26) (27).

Conclusion

Results of this study demonstrated that taking simvastatin may improved the ovary tissue in terms of inflammation, fibrosis, apoptosis caused by the increase of cytokines such as TNF-α, the reduction of the thickness of theca follicle layer, balancing hormone levels such as AMH, which is one of the basic indices in diagnosis of ovary reserves and recovery process from PCOS, and also the reduction of the expression of tumor necrosis factor-α (TNF-α), which is a potential actor in the apoptosis process in tissue of polycystic ovary. Treatment with simvastatin reduces testosterone levels of theca-interstitial cells, which can be considered related to the improvement of the ovary tissue.

Reduced levels of androgens can affect improvement in hormonal levels involved in PCOS.

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References

1. Repaci A, Gambineri A, Pasquali R. The role of low-grade inflammation in the polycystic ovary syndrome. **Molecular and cellular endocrinology**. 2011;335(1):30-41.

2. Benson S, Janssen O, Hahn S, Tan S, Dietz T, Mann K, et al. Obesity, depression, and chronic low-grade inflammation in women with polycystic ovary syndrome. **Brain, behavior, and immunity**. 2008;22(2):177-84.

3. Mannerås L, Cajander S, Holmäng A, Seleskovic Z, Lystig T, Lönn M, et al. A New Rat Model Exhibiting Both Ovarian and Metabolic Characteristics of Polycystic Ovary Syndrome. **Endocrinology.** 2007;148(8):3781–91.

4. Pizarro JG, Verdaguer E, Ancrenaz V, Junyent F, Sureda F, Pallàs M, et al. Resveratrol inhibits proliferation and promotes apoptosis of neuroblastoma cells: role of sirtuin 1. **Neurochemical research**. 2011;36(2):187-94.

5. McGrath K-C, McRobb L, Heather AK. Androgen therapy and atherosclerotic cardiovascular disease. **Vascular health and risk management.** 2008;4(1):11.

6. Shi D, Dyck MK, Uwiera RR, Russell JC, Proctor SD, Vine DF. A unique rodent model of cardiometabolic risk associated with the metabolic syndrome and polycystic ovary syndrome. **Endocrinology.** 2009;150(9):4425-36.

7. Park HJ, Lee HJ, Choi MS, Son DJ, Song HS, Song MJ, et al. JNK pathway is involved in the inhibition of inflammatory target gene expression and NF-kappaB activation by melittin. **Journal of inflammation.** 2008;5(1):1-13.

8. Matsuoka M, Pham Nt, Tsukamoto H. Differential effects of interleukin-1α, tumor necrosis factor α, and transforming growth factor β1 on cell proliferation and collagen formation by cultured fat-storing cells. **Liver.** 1989;9(2):71-8.

9. MO J-S, Matsukawa A, Ohkawara S, Yoshinaga M. Involvement of TNF α, IL-1β and IL-1 receptor antagonist in LPS-induced rabbit uveitis. **Experimental eye research**. 1998;66(5):547-57.

10. Sathyapalan T, Atkin SL. Mediators of inflammation in polycystic ovary syndrome in relation to adiposity. **Mediators of inflammation.** 2010;2010.

11. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. **Endocrinology.** 2002;143(3):1076-84.

12. Masse V, Ferrari P, Boucoiran I, Delotte J, Isnard V, Bongain A. Normal serum concentrations of anti-Müllerian hormone in a population of fertile women in their first trimester of pregnancy. **Human reproduction.** 2011;26(12):3431-6.

13. Sokalska A, Stanley SD, Villanueva JA, Ortega I, Duleba AJ. Comparison of effects of different statins on growth and steroidogenesis of rat ovarian theca-interstitial cells. **Biology of Reproduction.** 2014;90(2):44, 1-6.

14. Stewart D, Dombroski B, Urbanek M, Ankener W, Ewens K, Wood J, et al. Fine mapping of genetic susceptibility to polycystic ovary syndrome on chromosome 19p13. 2 and tests for regulatory activity. **The Journal of Clinical Endocrinology & Metabolism.** 2006;91(10):4112-7.

15. Altieri DC. Statins' benefits begin to sprout. **J Clin Invest**. 2001;108(3):365–6.

16. Tamayo E, Alvarez F, Alonso Q, Bustamante R, Castrodeza J, Soria S, et al. Effects of simvastatin on systemic inflammatory responses after cardiopulmonary bypass. **Journal of Cardiovascular Surgery.** 2009;50(5):687.

17. Ortega I, Cress AB, Wong DH, Villanueva JA, Sokalska A, Moeller BC, et al. Simvastatin reduces steroidogenesis by inhibiting Cyp17a1 gene expression in rat ovarian theca-interstitial cells. Biology of reproduction. 2012;86(1):20, 1-9.

18. Ikeda K, Baba T, Morishita M, Honnma H, Endo T, Kiya T, et al. Long-term treatment with dehydroepiandrosterone may lead to follicular atresia through interaction with anti-Mullerian hormone. Journal of ovarian research. 2014;7(1):46.

19. Sparrow C, Burton C, Hernandez M, Mundt S, Hassing H, Patel S, et al. Simvastatin has anti-inflammatory and anti-atherosclerotic activities independent of plasma cholesterol-lowering. **Atherosclerosis.** 2000;151(1):188.

20. Nelson SM, Yates RW, Fleming R. Serum anti-Müllerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles-implications for individualization of therapy. **Human Reproduction.** 2007;22(9):2414-21.

21. Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Mullerian hormone: a new marker for ovarian function. **Reproduction.** 2006;131(1):1-9.

22. Chao K-C, Ho C-H, Shyong W-Y, Huang C-Y, Tsai S-C, Cheng H-Y, et al. Anti-Mullerian hormone serum level as a predictive marker of ovarian function in Taiwanese women. **Journal of the Chinese Medical Association.** 2012;75(2):70-4.

23. Nardo LG, Yates AP, Roberts SA, Pemberton P, Laing I. The relationships between AMH, androgens, insulin resistance and basal ovarian follicular status in non-obese subfertile women with and without polycystic ovary syndrome. **Human Reproduction**. 2009;24(11):2917.

24. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. The **Journal of Clinical Endocrinology & Metabolism.** 2006;91(11):4237-45.

25. Spanos N, Tziomalos K, Macut D, Koiou E, Kandaraki EA, Delkos D, et al. Adipokines, insulin resistance and hyperandrogenemia in obese patients with polycystic ovary syndrome: cross-sectional correlations and the effects of weight loss. **Obesity facts.** 2012;5(4):495-504.

26. Ergenoglu M, Yildirim N, Yildirim AGS, Yeniel O, Erbas O, Yavasoglu A, et al. Effects of resveratrol on ovarian morphology, plasma anti-mullerian hormone, IGF-1 levels, and oxidative stress parameters in a rat model of polycystic ovary syndrome. **Reproductive Sciences.** 2015;22(8):942-7.

27. Krysko DV, Berghe TV, Parthoens E, D'Herde K, Vandenabeele P. Methods for distinguishing apoptotic from necrotic cells and measuring their clearance. **Methods in enzymology.** 2008; 442:307-41.