

Investigation of the effect of experimental polycystic ovarian syndrome induced by stradiolvalerate on oocyte quality and in vitro fertilization potential and evaluation of vitamin E supplementation to emryo culture in mouse model

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Abstract

Background & Aims: One of the most common causes of infertility in women is ovarian causes, especially polycystic ovary syndrome (PCOS). However, in patient with PCOS, the number of oocytes taken increased during the IVF, but these oocytes have often low quality, which ultimately leads to reduce fertilization potential. The aim of this research was investigation of PCOS on oocytes quality and potential of IVF and and evaluation of the effect of vitamin E supplementation to embryonic culture medium, as well.

Materials & Methods: In this experimental syudy100 female mice were divided into 2 groups: PCOS and control. To create an experimental PCOS stradiolvalerate injected itraperitonealy (100mg/kg). After 8 weeks IVF process was performed and the quality of oocytes, fertilization rate and embryo development process were evaluated. Finally, the effect of vitamin E with doses of 100, 200, 400 micromol, (p<0.05) were investigated in embryo culture.

Results: Comparison of results showed that experimental PCOS group showed a significant decreasein oocyte quality and fertilization, dualcell, blastocyst and significant increase in lysis and fragmentation in stopped embryos, on the other hand lysis and fragmentation, reduction of percentage and type of stopped embryos in presence of 100 and 200 micromole vitamin E concentrations were significantly in compare with PCOS group (p<0.05).

Discussion & Conclusion: Finally it can be concluded that experimental PCOS decreases the quality of oocytes and IVF and the addition of vitamin E to fetal culture medium as antioxidant can improve fertility.

Keywords: Polycystic ovary syndrome, Stradiolvalerate, Oocyte quality, in Vitro fertilization, Vitamin E

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Introduction

Infertility with an incidence of about 15% is one of the major problems that sometimes threatens the continuity of family life. One of the most common causes of infertility in women is ovarian causes of

polycystic ovary syndrome. Polycystic ovary syndrome (PCOS) is a complex situation due to high levels of androgens, irregularities in the endometrial course and small cysts on one or both ovaries. These irregularities can often be biochemical or morphological form of

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hyperandrogenism. Hyperandrogenism is polycystic ovarian syndrome symptoms which can inhibit follicular growth. Small cysts appear in the ovaries in the form of non-ovulation and menstrual changes (1).

5-10% of women in childbearing age have polycystic ovary syndrome due to hormonal irregularities. This syndrome has a very large negative effects on physiology and metabolism, such as: hyper insulinemia resistance, obesity and high blood pressure and in the long leads to endometrial hyperplasia and cardiovascular disease. The dangers of this disease can be infertility, metabolic syndrome and endometrial tumors (2).

Women with PCOS treated by IVF are somehow related to the disease and its complications. Although the number of oocytes obtained in the process of IVF increases in patients with PCOS, the oocytes are often of poor quality, which ultimately lead to the reduction of fertilization, cleavage and implantation and increase the rate of abortion (3-8).

This is likely to increase the probability of which mal-quality oocytes and embryos increase the aneuploidy rate (9). However, data from recent studies suggest that women with PCOS produce more oocytes and more euploid embryos in the process of IVF but nevertheless still have low pregnancy rates and high abortion which are genetically associated with an increased risk of aneuploidy embryos (6).

So other factors are significantly involved in increased risk of pregnancy in patients with PCOS besides genetic factors (6). Oocyte maturation and poor fetal development ability in women with PCOS may be associated with abnormal paracrine-endocrine factors, metabolic disorders and changes in the inter-follicular microenvironment during follicular and follicular maturation (10)

In addition, the quality of the embryos resulting from in vitro fertilization embryos is less than the normal method (11-12). A variety of factors such as oocyte

quality of protein source, somatic cells, culture media, oxygen levels, the number of embryos per culture (density fetus), energy source and oxidative stress may affect the quality of the embryo before implantation and influence latter evolution (11).

Although many different factors have been reported for low quality laboratory embryos, it seems that the production of reactive oxygen species (ROS) is one of the main causes of stunted growth of embryos cultured in the laboratory. ROS can stop meiosis in oocytes, inhibit embryonic development and cell death. Various antioxidants have been used to cope with the adverse effects of ROS in the embryos. (14-16)

Damaging effects of free radicals can be controlled or inhibited by the system of intracellular antioxidants such as glutathione, ascorbic acid and enzymes such as superoxide dismutase, catalase and glutathione peroxidase (13). However, it seems that the production of free radicals exceeds the antioxidant capacity of the embryo for in vitro culture of embryos of mammals, therefore different antioxidants and exogenous have been investigated to overcome the imbalance of oxidants (17).

The new findings show that non-enzymatic antioxidants such as vitamin E plays an important role in the development of the human reproductive system and fertility. Physiological vitamin E (alpha-tocopherol) is in biological membranes, and plays an important role in reducing oxidative damage in a cell membrane (18) and stops damage caused by free radicals by converting peroxide fatty acid free radical to hydroperoxide (19).

While the products reduce the lipid peroxidation, they significantly increase the content of antioxidant enzymes such as glutathione, superoxide dismutase and catalase activity (20).

The success rates of in vitro fertilization in experimentally induced mice with polycystic ovary syndrome by estradiol valerate compared to control

samples and the effect of adding vitamin E to the embryos in embryonic development have been studied.

Materials and Methods

In this study, 100 young female mice (NMRI strain) were selected and kept for 6-8 weeks under standard conditions at $22 \pm 2^\circ \text{C}$, humidity of 30-60% and a cycle of 14 hours in light and 10 they in the dark and also food and water was freely available to them.

The animals were divided randomly into two groups of control and experimental PCOS. 20 mice were in the control group and 80 were in PCOS group. Single step internal injection of estradiol valerate at a dose of 100 mg per kilogram of body weight was used to create experimental PCOS. Then female mice in each group were prepared for ovulation induction to perform in vitro fertilization. The required dishes and medium were prepared the day before conception and incubated for 12 hours for balance before fertilization. The research was done on oocytes, zygote and embryo obtained by IVF in both control and PCOS experimental groups by adding vitamin E.

The injection of 7.5 IU PMSG (Folligon, Netherlands) intraperitoneally was done around 7 pm To stimulate ovulation after ensuring the light cycle. 48 hours after the first injection, 7.5 units (Iu) hCG (Folligon, Netherlands) was injected intraperitoneally. Ovulation usually occurs 10-13 hours after hCG injection. In this study, the fallopian tubes were remove 10-13 hours after the injection of hCG (the next morning) after the euthanized the animal using ketamine and xylazine anesthesia and put in the hot medium already prepared and tubal ovum were removed using Dissecting technique in the area of injection and then were studied after washing in the warm culture medium (HTF + 3-5 mg / mlBSA) 37°C with 5% CO_2 . Then male rats euthanized using anesthesia and the abdomen sterilized with 70% ethanol and the epididymis, along with some of the channel vas deferens from the testicles

after removing the connective surrounding tissue through incision in the abdomen and were put in the container for sperm containing medium HTF supplemented with 4 mg of BSA (Sigma, America) which was previously incubated and sperms were removed after a cut in the tail epididymis and pressure in the deferens channel and incubated for 1 hour in the carbon dioxide at 37°C to determine the capacity and spermatozoa were removed after 5 hours and distributed in the environment. Then the motile spermatozoa isolated using Swimup method and incubated for 1 hour after determining their viability power and densities to access sperm capacity. Then one million motile able spermatozoa were added per ml culture medium and fertilize was determined 5-6 hours after adding sperm, by observing last two weeks. After fertilization, the fertilized ovule (zygote) is obtained and the fertilized ovule (zygote) were transferred into fresh medium already reached equilibrium after washed after the evaluation of fertilization in different groups. Evaluation of cleavage was done 24 hours of cultivation and embryonic development in over 120 hours were evaluated and the success rate of fertilization and embryonic development of oocytes in PCOS group was evaluated by adding dosages of 100 -200-400 μmol of vitamin E in vitro fertilization and embryo in the next phase.

The growth of the fetal stages over 120 hours were evaluated to evaluate the in vitro fertilization under the microscope contrast phase, and embryos divided in terms of fragmentation, the stages of embryonic development, stopped embryo development and typing of stopped embryos is done based on various factors such as lysis embryo, being necrotic, fragmentation and vesicles cytoplasmic:

Type I: lysis-based embryos, fragmented

Type II: lysis-based fragmented embryos in a number of blastomer,

Type III: embryos with low blastomer, lysis, fragmane and Cytoplasmic vesicle

Eventually, in order to survey the percentage of 2-cell embryos, blastocyst rate and the rate of lysis and fragmentation of embryos, software Minitab (MinitabCo., USA) and statistical methods to compare the ratio with a significant level ($P < 0.05$) have been used.

Results

In the study of the obtained number and quality of oocytes in PCOS group, the number per animal was statistically significant compared to the control group and high percentage of oocytes was lacking good quality compared with the control group in terms of the quality of oocytes for IVF and lots of oocyte had pressed cumulus masses and was seen as lysed. A small percentage of oocyte had good quality for fertilization compared with the control group ($P < 0.05$) (Table 1, Figure 1)

Significant decrease was observed in analyzing the percentage of oocytes for fertilization in the PCOS group and control group. Also, the investigation of two-cell embryos (demonstrating a certain state of embryo splitting) and blastocyst rate, a significant decrease was observed in PCOS group compared with the control group ($P < 0.05$.) Table 1, Figure 1)

Overall percentage of embryos stopped at different stages of development which did not reach the blastocyst stage significantly increased in the PCOS group compared with the control group and the quality of the majority of embryos stopped in PCOS compared to controls had a high percentage of lyse and fragmentation and most of the embryos in this group were type I and II ($P < 0.05$).

The results of adding various concentrations of vitamin E in vitro fertilization, embryonic, fetal development and quality comparison showed that adding vitamin E to medium increases the fertilization

rate, embryo quality and their morphology so as blastocyst created in the presence of vitamin E in the medium morphology were better than PCOS group and have fewer lysis, fragmentation and vacuolation. Evaluation of two cell embryos created in the PCOS group showed that in the presence of various concentrations of vitamin E in the culture, the percentage of two cell embryos was increased compared with PCOS and the increase of the percentage of two cell embryo was 78.26 percent in 100 mmol and the percentage of two cell embryo in concentration of 200 micromoles was 83.78percent and at a concentration of 400 micromoles, the percent of two cell embryos was 76 compared to the PCOS group as 60.29 percent ($P < 0.05$). (Table 3, Figure 1).

Comparing the percentage of blastocysts showed that adding various concentrations of vitamin E in vitro embryos resulted in a significant increase in the percentage of blastocysts which was more in concentrations higher than 100 and 200 micromoles. So that it was 42.03 percent at a concentration of 100 mmol, and 41.71 percent in 200 micromolar and this increasing the percentage of blastocysts in the presence of 100 and 200 micromoles of vitamin E were statistically significant compared with PCOS groups ($P < 0.05$).

Also the comparison of stopped embryos is showing that the addition of various concentrations of vitamin E reduces the percentage of stopped embryos compared to PCOS and the types of stopped embryos in the presence of vitamin E often are Type 3 with low lyse and fragmentation compared with PCOS which represents the antioxidant and the effect of vitamin E on the suffering and the process of fertilization and culture of embryos in the laboratory. And the reduction in lyse and fragmentation and reduction in the percentage of lyse and stopped embryos in the presence of 100 and 200 micromoles of vitamin E was more significant and statistically is highly significant compared with PCOS ($P < 0.05$).

Table 1. Comparison of quality of oocytes, in vitro fertilization and embryonic development both in control groups and PCOS.

Group	Number of rats	The total number of oocytes (oocytes per animal)	The number of suitable oocytes (%)	The number of unsuitable oocytes (%)	Fertilization (%)	2-cell (%)	Blastocyst (%)
Control	20	259 (12.95)	255 (%98.45)	4 (%1.54)	238 (%93.33)	214 (%89.92)	147 (%61.76)
PCOS	20	402 ^a (20.01)	271 ^a (%77.12)	131 ^a (%32.58)	209 ^a (%77.12)	126 ^a (%60.29)	57 (%27.27) ^a

a: Represents render meaningless compared to the control group (P<0.05)

Table 2. Comparison of various groups in the percentage of embryos and stop control and PCOS.

Group	The number of stopped embryos (%)	Type I (%)	Type II (%)	Type III (%)
Control	91 (38.24)	0 (00.00)	3 (1.26)	88 (36.97)
PCOS	152 ^a (72.73)	51 ^a (24.40)	56 ^a (26.79)	45 ^a (21.53)

a: Represents the significance compared to the control group (P<0.05)

Table 3. Compares the effect of different doses of vitamin E to the experimental PCOS mice embryos in vitro fertilization and embryonic development

Group	Total number of oocytes	Fertilization rate	Mono-cell	Blastocyst	stopped	Type I	Type II	Type III
Control	255	238 (%93.33)	214 (%89.92)	147 (%61.76)	91 (38.24)	0 (00.00)	3 (1.26)	88 (36.97)
PCOS	271	209 ^a (%77.12)	126 ^a (%60.29)	57 (%27.27) ^a	152 ^a (72.73)	51 ^a (24.40)	56 ^a (26.79)	45 ^a (21.53)
Vit E 100 Mmol	234	207 ^b (%88.46)	162 ^{ab} (%78.26)	87 ^{ab} (%42.03)	120 ^{ab} (%57.97)	18 ^{ab} (%8.70)	15 ^{ab} (%7.25)	87 ^b (%42.03)
Vit E 200 Mmol	242	222 ^b (%91.73)	186 ^b (%83.78)	95 ^{ab} (%41.79)	127 ^{ab} (%58.21)	21 ^{ab} (%9.46)	17 ^{ab} (%7.66)	89 ^b (%40.09)
Vit E 400 Mmol	255	225 ^b (%88.24)	171 ^{ab} (%76.00)	92 ^{ab} (%40.89)	133 ^{ab} (%59.11)	33 ^{ab} (%14.67)	12 ^{ab} (%5.33)	88 ^b (%39.11)

a: represents the significance compared to the control group (P <0.05)

b: represents the significance compared to PCOS experimental (P <0.05)

c: represents the significance compared to Badz 100 mmol vitamin E group (P <0.05)

d: represents the significance compared to 200 micromolar dose vitamin E group (P <0.05)

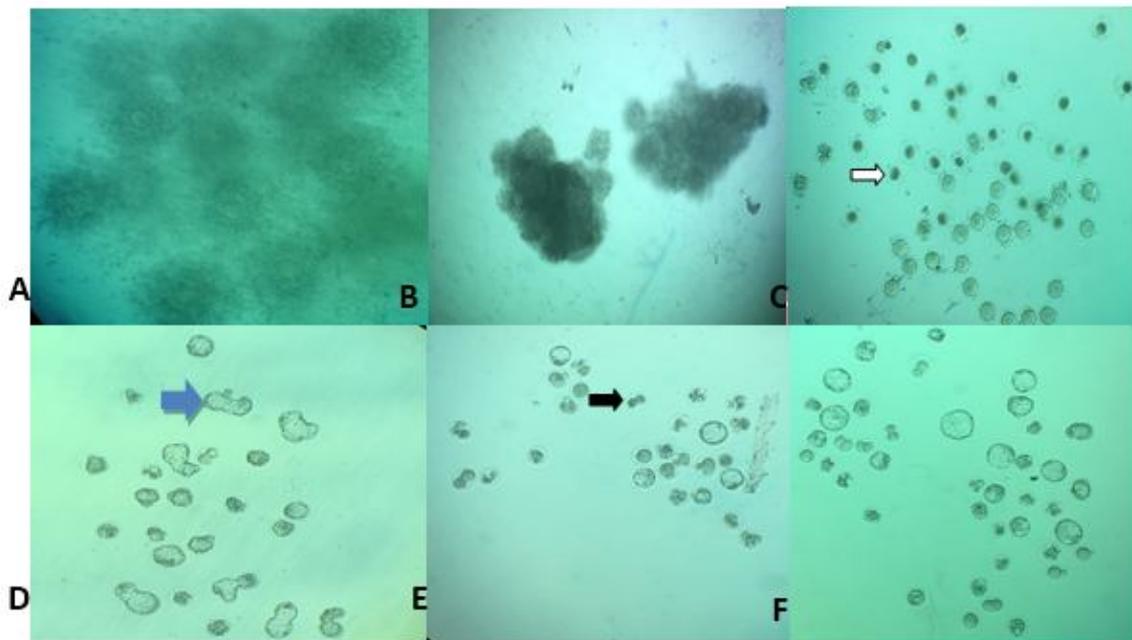


Fig 1. Oocyte embryos in different groups studied over 120 hours of embryos culturing, macro magnification 100 ×.

A: oocytes along with cumulus masses with adequate quality controls, B: oocytes in PCOS group with cumulus masses with poor quality and compressed cumulus masses (Compact); C: oocyte in PCOS group without cumulus masses that a large number of them have lyse and fragmentation (white arrow), D: embryos in the control group that a lot of them have reached to the blastocyst stage (blue arrow). And embryos with good quality in terms of morphology, E: embryos in the PCOS group that only few of them have reached the blastocyst stage and lots of them stopped in various stages of embryonic (black arrow) and the resulting embryos are not of good quality in terms of morphology, F: Embryos in the PCOS group of which vitamin E is added to embryonic culture and the number of blastocysts obtained are increased in comparison with PCOS and the resulting embryos are of good quality in terms of morphology.

Discussion

Polycystic ovary syndrome (PCOS) in terms of clinical and public health is of paramount importance

because it is very common and affects one in five women of childbearing age. This syndrome has a significant impact on fertility which contains forms of reproduction such as infertility, excessive production of androgens, hirsutism and metabolic problems: insulin resistance, impaired glucose tolerance, diabetes mellitus type II, unwanted risks of cardiovascular and psychological problems: anxiety, depression and deteriorating quality of life.

Polycystic ovary syndrome is a heterogeneous condition and clinical and research programs in the field is wide and includes branches. Phenotype of the disease is depending on the stage of one's life, genotype and environmental factors, including lifestyle, race and body weight (21).

The results of this study show that although the number of mature oocytes obtained in PCOS ovulation is acceptable, they are poor quality oocytes which have reduced IVF success rates that which was caused by increased oxidative stress.

Oxidative stress in biology generally used to express conditions that the amount of oxidant is high or low

levels of antioxidants are in the cells. This condition is such that the concentration of oxygen free radicals is the higher the amount of biological (22).

Recently, an increase in the level of malondialdehyde (MDA, as an indicator of lipid peroxidation) and superoxide dismutase activity (SOD, the antioxidant defense) has been reported in women with PCOS, and the content of GSH of red blood cells (GSH) decreased (23-24, 25). Based on these observations, oxidative stress and reduced antioxidant levels have been suggested for polycystic ovary syndrome (26). The results of the studies of Fenkci and collaborate on women with PCOS on a reduction in antioxidant capacity (TAC) and increased levels of protein carbonylation (CO, as free radical attacks proteins) confirm this hypothesis (26).

Increased oxidative stress markers in blood flow in women with PCOS showed up the metabolism of fats, which are responsible for increasing cardiovascular effects (27, 23). This leads to increased oxidative stress in ovarian tissue loss and loss of ovarian function in gonadotropin receptor (28). Luchetti et al also reported that ovarian cyst formation leads to ovarian tissue damage, which is along with an increase in lipid peroxidation and a significant reduction in tissue glutathione (29)

The patients with PCOS produce more oocytes during ovarian stimulation in IVF cycle, however, the women have poor quality oocytes and embryos, in vitro fertilization, less cleavage and implantation and suffering higher abortion (30, 31, 32). Despite the significant increased number of oocytes collected and fertilized in women with PCOS, the overall pregnancy rate or live birth has been reported less than women with normal ovaries (32). Results of other studies in women with PCOS who are undergoing assisted reproductive technology (ART) are in addition to increasing the risk of hyperstimulation syndrome than ovarian (OHSS) (32, 33), despite the increase in the number of follicles and

oocytes (33), the eggs are immature, they have shown poor fertilization, and low pregnancy rates (33).

A series of external and the ovarian factors have been identified that impairs follicle formation processes, follicular growth and maturation of oocyte meiotic, but these disorders have a direct effect on oocyte meiotic maturation interactions of granulosa cells - oocytes, fertilization, embryo development and pregnancy, or the effects applied topically through the mechanism of endocrine and paracrine in circulation / autocrine, which is unclear and warrants further investigation (31).

The results of this study also showed that the number of oocytes taken from PCOS after ovulation was more than the control group but in terms of quality oocytes were of poor quality. So that they show low quality of oocytes for fertilization. According to Hardy and colleagues, a high level of oxidative stress in patients with PCOS may be harmful for oocyte maturation and embryo development. Oxidative changes of cell components caused by oxygen free radicals are among damaging potentially very destructive processes in the cell which may lead to cell death through the necrosis or apoptosis (34). Oxygen concentration during in vitro culture is much higher compared with in vivo. This increases the production of oxygen free radicals in vitro (35).

Badyavi et. al analyzed the relationship between human embryos and the levels of ROS in the medium and showed that high levels of ROS on the first culture day was along with cleavage cut and increased fetal fragmentation and a reduction in the number of blastocyst and consequently decreasing fertility.

The researchers also reported that increased ROS levels in follicular fluid is a prognostic marker of the success rate of in vitro fertilization (36).

Yunda and his colleagues reported the effects of increasing concentrations of oxygen and H₂O₂ on the quality of embryos and showed that reduced amount of

H₂O₂ in the culture conditions can improve the quality of the embryo in the blastocyst stage (38-37).

Wang and colleagues reported that the addition of antioxidants improves the blastocyst formation in mice (39). Kit and his colleagues showed that the use of antioxidants improves in vitro fertility and increases the implantation (40).

Damaging effects of free radicals are controlled or restrained by intracellular antioxidant system. There are two types of antioxidants in body: enzymatic antioxidants as natural antioxidants that can neutralize ROS and protect the cells from damage caused by it and includes superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic antioxidants that are actually synthetic antioxidants or dietary supplements, are including vitamin C, vitamin A, selenium, and zinc (42-41).

But it seems that the production of free radicals exceeds the antioxidant capacity of embryos in vitro culture of embryos of mammals, therefore antioxidants and exogenous Forecast have been presented to overcome the imbalance of oxidants (13. 17).

On the other hand, it seems the antioxidant defense of in vitro embryos to be different in different developmental stages to the blastocyst stage. A group of researchers believe that the original capacity of antioxidant fetus in the early stages of research zygote is related to the storage of the inter-oocyte mother inherited mRNA and is gradually in the phase of the embryonic genome. The genome of the fetus is able to operate against oxidants produced by the cell or medium oxidants. Therefore, it seems that the specific needs of the fetus as foreign antioxidant levels vary before and after the embryonic genome (41 and 36).

The results of this study showed that the addition of Vit E to culture embryonic improves embryonic development and improves the quality of embryos cultured in vitro, so that adding vitamin E to culture embryonic increases the percentage of 2 cells embryos,

and also significantly increases the percentage of blastocysts with indicative morphology that demonstrates the fact that vitamin E slightly increases the quality of fetal growth and the splitting of the embryo and reduces the stop fetus and reduces fragmentation, Liz, and vacuolization of the stopped embryos.

It can be concluded that polycystic ovary decreases the quality of oocytes and increases sterility and adding vitamin E as an antioxidant to in vitro fertilized embryos can improve fertilization and embryonic development.

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