Research Paper
Study the Effects of N-acetyl Cysteine and Melatonin on the Quality of Oocyte and Embryo in Women With Polycystic Ovary Syndrome

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is related to the presence of low-quality oocytes. This study aims to examine the impact of N-acetyl cysteine (NAC), melatonin, and their combination on the parameters of follicular fluid (FF) and the quality of oocytes and embryos in women with PCOS.

Methods: This is a prospective randomized placebo-controlled clinical trial that was conducted on 60 Iranian women diagnosed with PCOS who were candidates for intracytoplasmic sperm injection (ICSI). They were randomly allocated into four groups of 15: Melatonin (received 3 mg of melatonin daily at 22:00), NAC (received 1800 mg of NAC per day), NAC+ melatonin, and placebo. The treatment started on the fifth day of the menstrual cycle and continued until the day of oocyte retrieval, lasting 40 days. Hormonal status, sperm parameters, oocyte morphology, fertilization rate and embryo quality were assessed. The data were analyzed using one-way ANOVA, considering a significance level of P≤0.05.

Results: The number of immature oocytes (1.27±0.88, P=0.006) and abnormal oocytes (2.1±1.1, P=0.001) were significantly lower in the NAC compared to the placebo group (3.33±1.35 and 3.7±1.7, respectively). Only the NAC group showed a significantly higher number of high-quality (grade I) embryos compared to the placebo group (2.87±1.45 vs 1.53±0.83; P=0.02). Insulin and luteinizing hormone (LH) levels were significantly lower in the melatonin (7±2.4 and 1.5±0.5, respectively) and NAC (7.1±2.5 and 1.5±0.5, respectively) groups (P=0.01) compared to the placebo group (9.5±2.9 and 1.8±0.6, respectively).

Conclusion: Both melatonin and NAC can improve the quality of embryo and oocyte in women with PCOS, but their combination has no significant positive effect.

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Introduction

With a 15% incidence in couples, infertility is a complex disorder caused by several factors [1]. One of the main causes of infertility in women is polycystic ovary syndrome (PCOS), affecting about 10-15% of women before menopause [2]. PCOS is also linked to irregular ovulation, insulin resistance, and elevated androgen levels, which can result in metabolic complications, including diabetes and cardiovascular diseases [3]. Oocyte quality is a critical factor in the success of assisted reproductive technologies. During ovulation, women with PCOS, typically exhibit a higher quantity of oocytes. However, these oocytes frequently possess inferior quality, resulting in diminished rates of fertilization, cleavage, and implantation [4]. Chronic inflammation, increased oxidative stress, and the subsequent increased cell apoptosis lead to follicular growth disorders and an increased number of atretic follicles in PCOS patients [5, 6]. An imbalance between prooxidants and antioxidants ultimately increases the apoptosis of granulosa cells in PCOS patients and causes symptoms of the disease [7]. Oxidative stress and apoptosis can be reduced by using antioxidants.

Melatonin (N-acetyl-5-methoxytryptamine) is primarily produced by the pineal gland at night. However, it can also be found in other organs, including retina, extraorbital lacrimal gland, Harderian gland, gastrointestinal tract's enterochromaffin cells, and gut and bone marrow cells. This hormone plays a crucial role in regulating various central and peripheral functions associated with circadian rhythms and reproductive processes [8, 9]. As an indirect antioxidant, Melatonin influences biological processes indirectly by promoting antioxidative enzymes and suppressing prooxidative enzymes. In addition, it serves as a potent direct scavenger of free radicals [10].

N-acetylcysteine (NAC), as a mucolytic agent derived from the amino acid L-cysteine, is a strong antioxidant, destroying free radicals by being converted into metabolites which stimulate glutathione production. Insulin receptors and insulin secretion are affected by NAC. It also increases stored glucose [11]. NAC is an antioxidant, anti-inflammatory, and anti-apoptotic agent, contributing to maintaining vascular integrity and exhibiting immunological effects [12]. It may represent a promising strategy for enhancing or stimulating ovulation in women experiencing chronic anovulation, such as those with PCOS.

In this study, we aim to examine the impacts of NAC, melatonin, and their combination on the quality of embryos and oocytes, in addition to the biochemical parameters present in the follicular fluid (FF), in women with PCOS candidate for intracytoplasmic sperm injection (ICSI). We hypothesized that the concurrent use of melatonin and NAC can enhance insulin sensitivity and promote ovulation in these women.

Materials and Methods

Study design and participants

This is a randomized placebo-controlled clinical trial conducted on 60 Iranian women aged 25-35 referred to the infertility clinic of Shahid Akbar Abadi Hospital in Tehran, Iran, from May 2015 to August 2017, who were candidates for ICSI due to PCOS-related infertility. The sample size was determined based on previous studies [13, 14]. Patients were required to meet the diagnostic criteria for PCOS defined by the 2003 Rotterdam Consensus Workshop [15], which is based on the presence of at least two of the following criteria: Chronic oligo- or an-ovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries as observed through ultrasound examination. Exclusion criteria were hypersensitivity to melatonin or NAC, infertility unrelated to anovulation, male infertility, pelvic organic pathologies, congenital adrenal hyperplasia, thyroid dysfunction, Cushing's syndrome, hyperprolactinaemia, androgen-secreting tumors, diabetes mellitus, the use of medications that affect carbohydrate metabolism and hormonal analogues except for progesterone in two months prior to inclusion, or severe liver or kidney dysfunction. Semen specimens from male partners were evaluated in accordance with World Health Organization (WHO) guidelines [16], and women with partners having irregular semen characteristics were not included in the study. Lack of cooperation, unwillingness to continue the study, and failure of ovulation induction were the reasons for excluding eight women from the study. Finally, 52 patients underwent analysis (Figure 1). All participants were asked not to make any modifications in their regular bodily activity and food plan, and not to use any new medications during the study.

Treatment protocol

The patients were divided into four groups of 15, (i) placebo group, receiving an oral rehydration solution (Razi, Tehran, Iran) three times daily; (ii) melatonin group, receiving melatonin (KAL, Park City, UT, USA) 3 mg per day at 10:00 PM; (ii) NAC group, receiving 600 mg

NAC (Batch no. 6N5483; Holzkrichen, Bavaria, Germany) three times daily; and (iv) melatonin+NAC group, receiving the same doses of melatonin and NAC simultaneously. A midwife administered the medication to the patients, ensuring that both the patients and the physicians remained unaware of the treatment protocols. The dosage and duration of NAC treatment were determined based on prior research [14, 17]. All treatments were administered for 40 days, commencing from the fifth day of previous menstrual cycle until the day designated for oocyte aspiration. In addition, all participants were prescribed oral contraceptive pills (OCP) for 21 days, which initiated concurrently with the administration of the placebo, melatonin, NAC, or the melatonin+NAC on the fifth day following menstruation, prior to the start of the treatment cycle. During the study, patients did not show any side effects except one in the melatonin group who had a headache.

Sample collection

The Long protocol [15] was used to stimulate ovulation. Among the analogues of gonadotropin-releasing hormone (GnRH), triptorelin (Decapeptyl; Ferring GmbH, Wittland 11, D-24109 Kiel, Germany) or an antagonist of the GnRH, cetrorelix (Cetrotide; Kantstrasse 2, D-33790 Halle, Germany) was used according to the physician's opinion. Triptorelin 0.14 mg for 14 days and cetrorelix 0.25 mg/day were injected to observe at least one follicle or more with a size of 14 mm by transvaginal ultrasound. From the third day of the menstrual cycle, recombinant follicle-stimulating hormone (FSH, Gonal-F 75; Merck Serono S.p.A, 70026-Modugno, Italy) was used to oversaturate ovaries. The daily dose was determined by a gynecologist based on the patient's specifications and history. The growth of follicles was controlled by the ultrasound method. When the size of at least three follicles attained 18 mm or more, human chorionic gonadotropin (hCG) was administered at 1000 IU; 36 hours later, the ovaries were punctured by a physician and through vaginal ultrasonography. Each follicle was meticulously aspirated into a single tube. The aspiration of each follicle was conducted individually, and the FF comprising the oocyte was gathered.

Body mass index (BMI) was recorded, and fasting blood samples were obtained from each participant two times: Once prior to treatment on the fifth day following the menstrual cycle and again on the day of ovum pickup (OPU) during the ICSI cycle. After collecting peripheral blood samples, they were centrifuged at 1500g for 10 minutes at room temperature using a centrifuge (EBA20, Hettich, Tuttlingen, Germany). The resulting serum samples were then preserved at -70 °C until further analysis. Similarly, the FF samples were immediately subjected to centrifugation at 1500 g for 10 minutes at room temperature, with the supernatants collected and stored at -70 °C until analysis.

Commercial enzyme-linked immunosorbent assay (ELISA) kits (Demeditec Diagnostics Inc., Kiel, Germany) were used to measure the concentrations of FF and serum levels of luteinizing hormone (LH; in mIU/mL; catalogue no. DE1289), total testosterone (TT; in ng/mL; catalogue no. DE1559), fasting insulin in mIU/L (catalogue no. DE2935), and estradiol (E2; in pg/mL; catalogue no. DE2693). The concentration of anti-Mullerian hormone (AMH; in ng/mL) in the FF was measured using a second-generation enzyme immunoassay (AMH-EIA kit; catalogue no. A92269C; Immunotech Beckman Coulter, Brea, CA, USA), according to the instructions of the manufacturer.

The level of malondialdehyde (MDA; in mM), a naturally occurring byproduct of lipid peroxidation, was assessed in the FF using the thiobarbituric acid (TBA) colorimetric method and a TBA-reactive substances (TBARS) assay kit (catalogue no. KA1381; Abnova Corp., Taiwan). Prior to the measurement of AMH (1:20) in FF, dilutions were carried out using the phosphate-buffered serum (PBS) buffer based on the calibration range.

ICSI and culture of oocytes

Oocyte retrieval was conducted through ultrasound-guided transvaginal aspiration utilizing a single-lumen needle (Reproline Medical GmbH, Rheinbach, Germany). The cumulus cells surrounding all oocytes were removed by a 30-second exposure to a solution of 20 IU/mL hyaluronidase (ART-4007A; SAGE BioPharma, Pasadena, CA, USA) in a HEPES-buffered medium. This procedure was subsequently followed by thorough washing with HEPES-buffered human tubal fluid (HTF) that contained 5 mg/mL of human serum albumin (ART-3001; SAGE BioPharma, England) and mechanical pipetting. Mature oocytes at the metaphase II stage (MII) were identified by observing the first polar body using a stereomicroscope (Olympus, Tokyo, Japan). The most effective oocytes that successfully extruded the first polar body were selected for ICSI. Prior to the injection, an organized sperm suspension was added to a 50-mL droplet of polyvinylpolypyrrolidone (PVP; ART-4006-A; SAGE BioPharma, England). Four hours after oocyte retrieval, a spermatozoa with good parameters exhibiting normal morphology was immobilized and utilized

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for insemination of the oocyte. The inseminated oocytes were subsequently placed in fertilization medium (ART-1520; SAGE BioPharma, England) and were covered by mineral oil (Reproline Medical GmbH, Rheinbach, Germany). The next day, fertilization was assessed, and fertilized eggs were placed in an equilibrated cleavage medium (ART-1526; SAGE BioPharma, England) if two pronuclei (2PN) were seen. Using an embryo transfer catheter (Labotect, Göttingen, Germany), embryos were transferred 48-72 hours following oocyte retrieval. For each patient, a maximum of four embryos were transferred. Starting from the embryo transfer day and continuing until the pregnancy test, 100 mg progesterone injections were given intramuscularly every day to provide luteal phase support (Gestone, London, UK). Serum β-hCG measurements on days 16–17 post-transfer and the finding of a gestational sac and heartbeat during ultrasound examinations seven weeks following the embryo transfer were used to confirm pregnancy.

Evaluation of oocyte morphology, fertilization, and embryo quality

Using an Olympus inverted microscope (IX71) with a Hoffmann modulation contrast system at a magnification of 400x, the first polar body detected just before the ICSI operation was used to measure the nuclear maturation of oocytes. The MII oocyte morphological scoring system (MOMS) and the grading criteria developed by Rienzi et al. who considered granularity (including the size and distribution of granules, whether they were clustered or homogeneously distributed, and their location within the oocyte), the size of the perivitelline space, and the arrangement of organelles (including vacuoles and endoplasmic reticulum) were used to examine the oocytes' morphology [18]. Oocytes were divided into three groups according to these morphological evaluations: (a) normal oocytes, (b) oocytes with extracytoplasmic abnormalities (like a dark zona pellucida, a large perivitelline space, and a fragmented polar body), and (c) oocytes with intracytoplasmic abnormalities (like vacuolation, structural deformities, cytoplasmic fragments, and dark or granular cytoplasm). The meiotic spindle was visualized using polarized light technology (CRi PolScope Technology, London, UK).

By looking for two different pronuclei, fertilization results were assessed 12-16 hours after ICSI. During 24-36 hours following fertilization, cleavage was observed. On the third day after insemination, the embryos' quality was evaluated and categorized as follows: (a) Uneven blastomeres with less than 30% fragmentation found in grade II, (b) symmetric blastomeres with no fragmenta-

tion found in grade I, and (c) unequal blastomeres with more than 30% fragmentation found in grade III [19].

Statistical analysis

The Kolmogorov–Smirnov test confirmed that continuous variables had a normal distribution. The Mean±SD was used to describe the data. Chi-square test and one-way ANOVA were used for data analysis, followed by post hoc comparisons using Tukey's test and Dunnett's T3 test. P≤0.05 was regarded as statistically significant. Pearson's correlation test was used to evaluate the relationship between the variables. Statistical analyses were conducted in SPSS software, version 13.

Results

Clinical and demographic characteristics

The results showed that BMI, oligomenorrhea, amenorrhea, length of marriage, duration of infertility, age, hirsutism, and levels of insulin, LH, FSH, TT, and E2 were not significantly different among the four groups prior to therapy. Also, there was no significant difference between the groups in the partner's sperm parameters (Table 1).

Comparison based on hormonal parameters of the FF

As seen in Table 2, insulin and LH levels in FF were significantly lower in the melatonin (7±2.4 and 1.5±0.5, respectively) and NAC (7.1±2.5 and 1.5±0.5, respectively) groups than in the placebo group (9.5±2.9 and 1.8±0.6, respectively) (P=0.01). However, TT, E2, and AMH concentrations in FF were not significantly different between treatment groups and the placebo group (P>0.05). The MDA level was significantly lower in the NAC (4.28±0.69) and melatonin + NAC (4.53±0.6) groups than in the melatonin (5.04±0.67) and placebo (6.11±0.82) groups (Table 2). The difference between treatment groups and the placebo group in MDA level was statistically significant (P=0.01).

Comparison based on oocyte and embryo morphology

As seen in Table 3, the number of oocytes retrieved from three treatment groups (NAC: 13±6.6, melatonin: 16.6±7.1, melatonin + NAC: 15.6±8) was not significantly different compared to the placebo group (10.9±4.5) (P=0.06). The NAC group had the lowest number of immature oocytes (in two metaphase I and germinal vesicle stages) and abnormal mature MII oocytes with values of 1.27±0.88 and 2.1±1.1, respectively, among the treat-

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Table 1. Semen characteristics of participants' fertile partners

Parameters	Mean±SD				P
	NAC	Melatonin	NAC + Melatonin	Placebo	
Semen volume (mL)	3±0.8	2.8±0.5	3.2±0.7	3±8	0.59
Total count (×10⁶ per ejaculate)	40.1±9.1	40.6±8.6	39.1±5.4	37.4±6.3	0.62
Sperm concentration (×10⁶/mL)	14.6±5.2	15.8±5.9	13.1±3.9	14.2±4.9	0.52
Total motility (%)	42.9±6.7	44.9±6.4	45.9±6.6	44.3±5.7	0.61
Normal forms (%)	5.8±2.9	6.2±2.9	5.7±2.6	6.6±3.2	0.77

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ment groups compared to the placebo (3.33±1.35 and 3.7±1.7, respectively). In these factors, the difference between treatment groups and the placebo group was significant (P=0.006 and P=0.001, respectively). The number of normal MII oocytes was significantly greater in the melatonin (8.7±5.4) and NAC (8.5±3.7) treatment groups compared to the placebo group (3±1) (P<0.001). Furthermore, all treatment groups showed a significantly higher number of MII oocytes with normal meiotic spindles around the second polar body (NAC: 9.9±5.8, melatonin: 10.5±4.5, melatonin+NAC: 10.6±5.3) compared to the placebo group (5.7±2.3) (P=0.01). The number of fertilized MII oocytes (P=0.09) and cleaved embryos (P=0.06) was not different significantly among the groups. Only the NAC group showed a significantly higher number of high-quality (grade I) embryos on day three compared to the placebo group (2.87±1.45 vs. 1.53±0.83; P=0.02). The melatonin (2.5±0.3) and melatonin+NAC (2.3±0.9) groups did not exhibit such a significant increase compared to the placebo group (P>0.05). There was no significant difference among

the four groups in the number of grade II (P=0.29) and III (P=0.06) embryos and the number of transferred embryos (grade I+II) (P=0.82). Additionally, there was no significant difference in the incidence of endometrial thickness (P=0.30) and ovarian hyperstimulation syndrome (OHSS) (P=0.65) among the four groups (Table 3). There was a strong negative correlation between the fertilization rate and MDA level in FF (r=-0.28; P=0.02) and between the number of mature oocytes with meiotic spindle and MDA level in FF (r=-0.294; P=0.02).

The study of oocyte intracytoplasmic and extracytoplasmic abnormalities revealed that the NAC group had a significantly lower polar body fragmentation rate (13.3%, P=0.02) and lower large perivitelline space (40%) compared to the placebo group (66.7% and 86.7%, respectively) (P=0.01). The difference in polar body fragmentation rate and large perivitelline space of the melatonin and melatonin+NAC groups was not statistically significant compared to the placebo group (P>0.05). Compared to the placebo group (73.3%), the prevalence of vacuolated oocytes was significantly lower in

Table 2. Mean values of biochemical and hormonal parameters in the FF of women with PCOS after treatment

Parameters	Mean±SD				P
	NAC	Melatonin	NAC + melatonin	Placebo	
Insulin (mU/L)	7.1±2.5*	7±2.4*	7.4±2.8	9.5±2.9	0.01
AMH (ng/mL)	505.4±161.5	427.7±118.9	493.1±173.9	549.8±165.6	0.21
MDA (mM)	4.28±0.69*	5.04±0.67	4.53±0.6*	6.11±0.82	0.01
LH (mIU/mL)	1.5±0.5*	1.5±0.5*	1.53±0.6	1.8±0.6	0.01
TT (ng/mL)	8.3±3.6	8.4±4.1	9.1±3.9	9.18±3.3	0.86
E2 (pg/mL)	496.8±145.3	526.8±196.3	500.4±207.7	466±175.57	0.81

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Abbreviations: NAC: N-acetyl cysteine; AMH: Anti-mullerian hormone; MDA: Malondialdehyde; LH: Luteinizing hormone; FSH: Follicular stimulating hormone; TT: Total testosterone; E2: Estradiol.

*P<0.05 compared to the placebo group.

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Table 3. The mean or frequency values for retrieved oocytes, the quality of oocytes and embryos, and pregnancy outcome in women with PCOS after treatment

Parameters	Mean±SD/No. (%)				P
	NAC	Melatonin	Melatonin	NAC + Placebo	
Oocytes retrieved	13±6.6	16.6±7.1	15.6±8	10.9±4.5	0.063
Immature oocytes (GV + MI)	1.27±0.88*†	3±2	2.27±1.94	3.33±1.35	0.006
Mature oocytes (MII)	10.8±5.4*	12.2±5.3*	12.07±6.05*	6.3±2.5	0.006
Normal MII oocytes	8.7±5.4*	8.5±3.7*	7.6±3.6*	3±1	0.0001
Abnormal MII oocytes	2.1±1.1*†	4.4±1.7	4.7±2.8	3.7±1.7	0.001
MII oocytes with normal spindle	9.9±5.8*	10.5±4.5*	10.6±5.3*	5.7±2.3	0.01
Fertilized oocytes (2PN)	7.7±3.9	9.2±4.5	8.3±4.5	5.6±2.2	0.09
Cleaved embryos	7.3±3.4	8.5±4.1	7.53±3.52	5.13±2.13	0.06
Grade i embryos	2.87±1.45*	2.5±0.3	2.3±0.9	1.53±0.83	0.02
Grade II embryos	2.7±1.7	2.7±1.6	2.4±1.2	1.9±0.9	0.29
Grade III embryos	1.7±1.3	3.4±2.3	2.8±2.1	1.7±0.8	0.06
Embryos transferred (grades I+II)	2.07±0.46	2.13±0.35	2.2±0.6	2.2±0.4	0.82
OHSS	2(13.3)	4(26.7)	4(26.7)	5(33.3)	0.65
Endometrial thickness (mm)	9.4±0.9	9.9±1.9	9.6±1.2	8.9±1.6	0.30
Clinical pregnancy	3(20)	4(26.7)	2(13.3)	2(13.3)	0.76

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abbreviations: NAC: N-acetyl cysteine; GV: Germinal vesicle; MI: Metaphase I; MII: Metaphase II; 2PN: 2 Pronuclei; OHSS: Ovarian hyperstimulation syndrome.
*P<0.05 compared to the placebo group, †P<0.05 compared to the melatonin group, *†P<0.05 compared to the melatonin + NAC group.

the NAC (6.7%), melatonin (20%), and melatonin + NAC (26.7%) groups (P=0.001). Compared to the placebo group, there was a tendency for the treatment groups to have decreased rates of dark zona, subzonal fragmentation, and morphological abnormalities; however, these changes were not statistically significant (Table 4).

Discussion

The results of this study showed that the NAC group of women with PCOS had a significantly lower number of immature and abnormal oocytes but a higher number of grade I embryos compared to the placebo group. Furthermore, the NAC and melatonin+NAC groups had significantly lower MDA level, and the melatonin and NAC groups had significantly lower insulin and LH levels compared to the placebo group.

Several studies have been conducted in recent years to reduce the problems associated with PCOS. A primary goal of the proposed treatments is to alleviate oxidative stress through the administration of various antioxidants. Women with PCOS often exhibit persistently elevated levels of LH, which is believed to adversely affect oocyte maturation and embryo quality, potentially hindering implantation and leading to a higher incidence of spontaneous abortions [4]. Our results indicated that administration of NAC and melatonin improves the oocyte quality and implantation rates [20], which are associated with elevated estrogen levels. Additionally, significant declines in oocyte quality, reduced maturation, lower fertilization rate, compromised embryo quality, decreased pregnancy rate, and increased miscarriage rate are linked to high LH concentrations, elevated reactive oxygen species in FF, and diminished total antioxidant capacity [21].
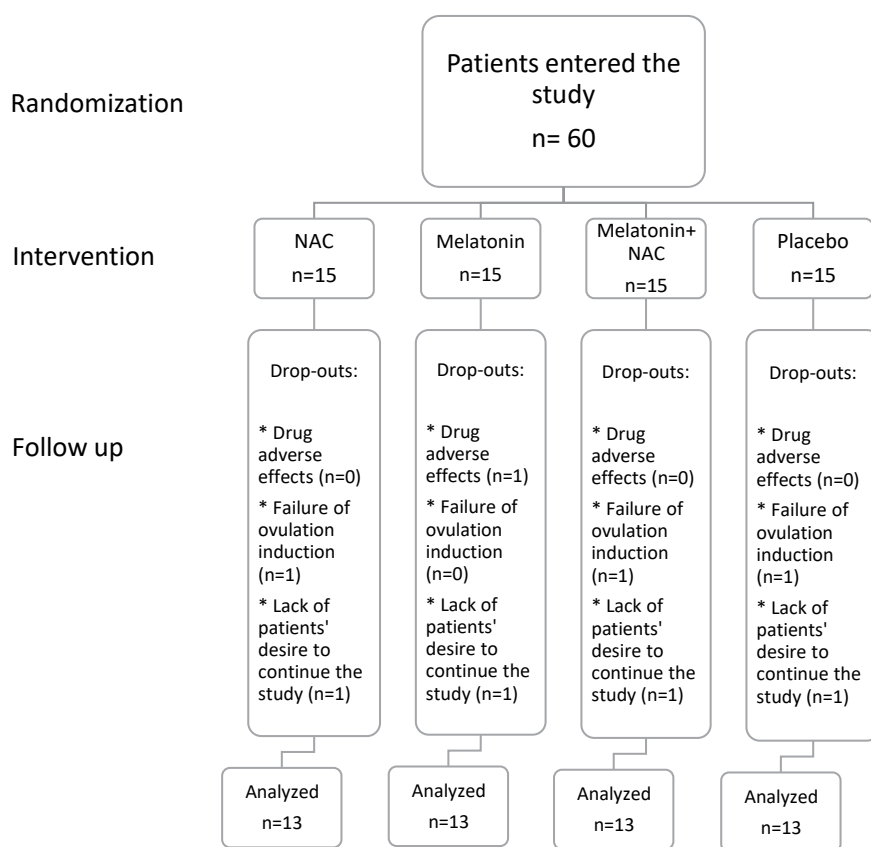
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Randomization

Patients entered the study
n= 60

Intervention

| NAC n=15 | Melatonin n=15 | Melatonin+ NAC n=15 | Placebo n=15 |

Follow up

Drop-outs:

* Drug adverse effects (n=0)
* Failure of ovulation induction (n=1)
* Lack of patients' desire to continue the study (n=1)

Drop-outs:

* Drug adverse effects (n=1)
* Failure of ovulation induction (n=0)
* Lack of patients' desire to continue the study (n=1)

Drop-outs:

* Drug adverse effects (n=0)
* Failure of ovulation induction (n=1)
* Lack of patients' desire to continue the study (n=1)

Drop-outs:

* Drug adverse effects (n=0)
* Failure of ovulation induction (n=1)
* Lack of patients' desire to continue the study (n=1)

| Analyzed n=13 | Analyzed n=13 | Analyzed n=13 | Analyzed n=13 |

Figure 1. Flowchart of the sampling and allocation processes

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Melatonin is found in many organs, but when it is administered regularly, it accumulates in the ovary, eye, and pineal gland [22]. Mature follicles have larger intrafollicular melatonin concentrations than tiny atretic follicles [23]. As a powerful scavenger of free radicals and a complete antioxidant, melatonin is essential for controlling the transcription of genes encoding antioxidant enzymes [24]. In the study by Öztürk et al. [25], rats' livers exhibited elevated superoxide dismutase (SOD) activity after receiving 10 mg/kg of melatonin for seven days.

Table 4. Oocyte abnormalities in women with PCOS after treatment

| Parameters | No. (%) | | | | P |
| | Extracytoplasmic Abnormalities | | | | |
	NAC	Melatonin	NAC + Melatonin	Placebo	
Dark zona	2(13.3)	5(33.3)	6(40)	6(40)	0.34
Large PVS	6(40)*	13(80)	13(80)	13(86.7)	0.01
Fragmented polar body	2(13.3)*	7(46.7)	8(53.3)	10(66.7)	0.02
No. of intracytoplasmic abnormalities (%)	3(20)*	6(40)	6(40)	10(66.7)	0.07
Granulated	1(6.7)*	3(20)*	4(26.7)*	11(73.3)	0.001
Vacuolated	2(13.3)	5(33.3)	4(26.7)	5(33.3)	0.58
Morphological abnormalities Subzonal fragmentation	3(30)	9(60)	7(46.7)	8(53.3)	0.13

PVS: Perivitelline space.

*P<0.05 compared to the placebo group.

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Similarly, Liu and Ng [26] reported that, after injection of melatonin at a single dose of 5 mg/kg, there was an increase in SOD activity in the rats' kidneys, liver, and brain. Moreover, it has been shown that melatonin affects the expression of antioxidant enzymes. Antolín et al. [27] were the first to observe that exogenous administration of melatonin (500 µg/kg) led to elevated mRNA levels in copper/zinc SOD and manganese SOD. Some studies showed that melatonin affected the production of sex steroids at various stages of ovarian follicular maturation [9, 28]. A 100 mM dose of melatonin was shown to enhance the production of progesterone and androgens in mouse preantral follicles after a 12-day incubation period [9]. Elevated level of melatonin in FF are critical for follicle development, ovulation, and the quality of oocytes, while low concentration of melatonin may contribute to anovulation, suboptimal oocyte quality in PCOS, and failures in follicular maturation [28].

Animal studies have shown that NAC is not teratogenic and mutated, with no serious side effects [11]. In 2007, Badawy et al. [29] investigated the effect of clomiphene citrate (CC), NAC, and their combination in PCOS patients, and observed that ovulation was significantly improved in the group received NAC plus CC. They suggested NAC can be used as a supplement to the CC, because NAC is well tolerated by the body and is also cost-effective. Because of its anti-apoptotic and antioxidant qualities, Cheraghi et al. demonstrated that NAC reduces insulin, LH, and leptin levels in FF, improving oocyte maturity and quality and fetal development while lowering the quantity of immature oocytes [30]. According to Javanmanesh et al.'s study in 2015 [31], patients who take NAC in addition to CC have higher levels of progesterone, ovulation, and fertility. Using the extended agonist protocol, Elgindy et al. found that 1200 mg of NAC during ICSI cycles did not significantly increase the number of grade I embryos or the rate of fertilization and pregnancy [13]. Recent findings indicate that mice subjected to a two-month treatment with NAC demonstrated better embryo development and an increase in the number and quality of oocytes. The elevation in telomerase activity and telomere length attributed to NAC can be due to a reduction in follicle atresia, thereby ensuring the quality of oocytes [32].

The findings of this study demonstrated that NAC's antioxidant and anti-apoptotic qualities significantly reduced the levels of insulin, LH, and MDA in the FF of women with PCOS. In addition to improving embryo development, it lowers the frequency of immature oocytes and increases the maturation and quality of oocytes. These results support the findings of other studies [29,

32, 33]. Furthermore, our results suggested that there may be no synergistic effect when NAC and melatonin are administered concurrently, or the doses of NAC or melatonin might be inadequate.

Our findings are consistent with those of Rajani et al. [34], who found that a higher number of oocytes with a normal meiotic spindle and high MDA level resulted in lower fertilization rate and lower-quality embryos. Our research showed a strong negative association between fertilization rate and MDA level in FF and between the number of mature oocytes with a meiotic spindle and MDA level in FF. Limitations of this study include quantity of patients needed, the time needed for patients to get on the cycle, lack of cooperation of patients to enter the study, and lack of funding.

Conclusion

When administered alone to women with PCOS, melatonin and NAC have positive effects on oocyte and embryo quality; however, their combination has no positive synergistic effects. Using NAC along with anti-insulin resistance drugs, such as metformin, may have positive effects on oocyte and embryo quality.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (Code: IR.IUMS.REC.1394.27418). All participants declared informed consent.

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Authors' contributions

Supervision, project administration, and funding acquisition: Reza Shiraz and Sajed Khaledii; Data collection, investigation, resources, writing: Sajed Khaledi ; Review & editing: Mehrdad Ghorbanlou; Software, validation, formal analysis: Maryam Najafpour Pitka and Zahra Sadat Hoseini.

Conflict of interest

The authors declared no conflict of interest.

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Acknowledgments

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