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Brief Report

Effect of Melatonin on the Outcome of Assisted Reproductive Technique Cycles in Women with Diminished Ovarian Reserve: A Double-Blinded Randomized Clinical Trial

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What's Known

- Subfertility with increasing female age is widely accepted and is related to a decrease in the number and quality of oocytes; called diminished ovarian reserve (DOR).
- DOR has adverse effects on the outcomes of assisted reproductive technology (ART) used for the treatment of infertile couples.

What's New

- Women with severe DOR that used melatonin, produced significantly more mature MII oocytes and top quality (grade I) embryos compared to the control group that used placebo. However, other ART outcomes were not different between the two groups.

Abstract

Diminished ovarian reserve (DOR) significantly decreases the success rate of the assisted reproductive technique (ART). In this study, we assessed the effect of melatonin on the ART outcomes in women with DOR. A double-blinded, randomized, clinical trial was performed on 80 women with DOR as a pilot study in Shiraz, between 2014 and 2015. DOR was defined as the presence of 2 of the following 3 criteria: 1) anti-Müllerian hormone ≤1, 2) follicle-stimulating hormone ≥10, and 3) bilateral antral follicle count ≤6. The women received 3 mg/d melatonin or a placebo since the fifth day of one cycle prior to gonadotropin stimulation and continued the treatment up to the time of ovum pickup. The ART outcomes were compared between the groups using SPSS software. Finally, there were 32 women in the case and 34 in the placebo groups. The mean age and basal ovarian reserve test were the same between the groups. The serum estradiol level on the triggering day was significantly higher in the case group (P=0.005). The mean number of MII oocytes was higher in the case group, but the difference did not reach statistical significance. Number of the patients who had mature MII oocytes (P=0.014), top-quality embryos with grade 1 (P=0.049), and embryos with grades 1 and 2 (P=0.014) was higher among the women who received melatonin. However, the other ART outcomes were not different between the groups. The serum estradiol level was higher and more women with DOR had good-quality oocytes and embryos after receiving melatonin; however, no other outcome was different between the case and control groups.

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Keywords ● Melatonin ● Ovarian reserve ● Assisted reproductive techniques ● Embryo ● Oocytes

Introduction

Subfertility with increasing female age is a widely accepted concept and is considered to be related to diminished ovarian reserve (DOR). DOR is defined as a decrease in the number and quality of oocytes in the ovaries. The postponement of childbearing in the modern lives have led to an increasing proportion of couples who need assisted reproductive technique (ART) due to aging and DOR.

DOR has adverse effects on the ART outcomes such as ovarian response to gonadotropins, number and quality of oocytes and embryos, implantation rates, and live birth rates.

Ovarian reserve does not always correlate directly with a woman's chronological age. Screening for ovarian reserve is helpful in predicting the outcome of the ART programs and choosing the best stimulation protocol. Several tests have been suggested to screen ovarian reserve such as evaluation of antral follicle count (AFC), anti-Müllerian hormone (AMH), basal follicle-stimulating hormone (FSH), estradiol, and inhibin B.[1,2] However, for the assessment of ovarian reserve in selected cases by day 3, the American Society for Reproductive Medicine (ASRM) recommends FSH, estradiol, clomiphene citrate challenge test, AFC, or serum AMH.[3] The establishment of normal values for ovarian reserve tests is another challenging factor in the interpretation of the tests. Oocyte donation and adoption are commonly suggested to women with poor DOR test results. Nonetheless, these methods have poor acceptance by many couples who wish to bear their own biologic and genetic offspring.

In order to increase ovarian response in women with DOR in the ART cycles, investigators have tried several different strategies such as various ovarian stimulation protocols and adjuvants like DHEA.[4] Melatonin (N-acetyl-5-methoxytryptamine), a hormone primarily secreted from the pineal gland, is known to regulate physiologic reproductive behaviors in vertebrate animals in different seasons.[5] Melatonin is proved to work as a direct free radical scavenger.[6] The fact that the follicular fluid of preovulatory follicles has large amounts of melatonin compared to that of small immature follicles is suggestive of a possible role for melatonin in follicular maturation.[7,8] Melatonin is suggested to be involved in the pathophysiology of premature ovarian failure endometriosis and polycystic ovary syndrome (PCOS) when deficient.[9]

Melatonin supplementation added to *in-vitro* maturation culture media was reported to improve the clinical outcomes of the ART program for PCOS women in a recent study.[10] The effect of melatonin supplement on the ART cycles has been evaluated by only a few studies.[11,12] In the present study, we sought to perform a double-blinded, randomized, clinical trial to compare the effect of melatonin supplement with that of a placebo on the outcome of the ART cycles in women with DOR.

Patients and Methods

The present study, conducted for a 12-month period commencing in June 2014, recruited infertile women who were referred to the Infertility Center, Mother and Child Hospital, affiliated to Shiraz University of Medical Sciences. The inclusion criteria were comprised of the ART for the first time, normal male factor, normal uterine cavity, and presence of 2 of the following 3 criteria: 1) summation of bilateral AFC≤6, 2) AMH≤1, and 3) basal FSH on the third day of menstrual cycle ≥10. These cases are routinely candidated for egg donation in our center. Couples who insisted on having the ART by using their own oocytes were enrolled in this project. The double-blinded nature of the study was explained to the couples. If the couple declined to participate, the protocol was not exactly followed, or the ovaries showed poor responses to gonadotropins, the cases were excluded from the study. The sample size was calculated to be 38 cases in each group using the following formula with α=0.05 and β=0.2:

$$n' = \frac{\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2}{\left(\frac{d}{2\delta}\right)^2}$$

Eighty couples signed the informed consent forms and were enrolled. Ten women dropped out because of very poor ovarian responses to gonadotropins, and 4 women were excluded because they did not exactly follow the protocol. Finally, 66 women completed the study and their data were analyzed as a pilot study. The CONSORT flow diagram is depicted in figure 1.

Capsules containing 3 mg melatonin (Nature Made Nutritional Products, Mission Hills, USA) and placebos with the same shapes were prepared for the study by the Pharmaceutics Division of Shiraz University of Medical Sciences. To design a double-blinded, simple randomization, the pharmaceutics specified every package that contained the placebo or melatonin with a code number from 101 to 200 based on a randomization table. The prepared packages, each containing 50 capsules, were offered to the participants orderly based on their entrance into the project. Accordingly, the patients or the clinicians did not know that which package contained the placebo or melatonin. The subjects were recommended to take 1 capsule every night orally before going to bed starting from the fifth day of the menstrual cycle prior to the cycle that was planned for gonadotropin stimulation. Transvaginal ultrasound scan was performed for all the participants before starting the supplement, and they were recommended to continue the supplement up to the time of ovum pickup. The downregulation protocol was

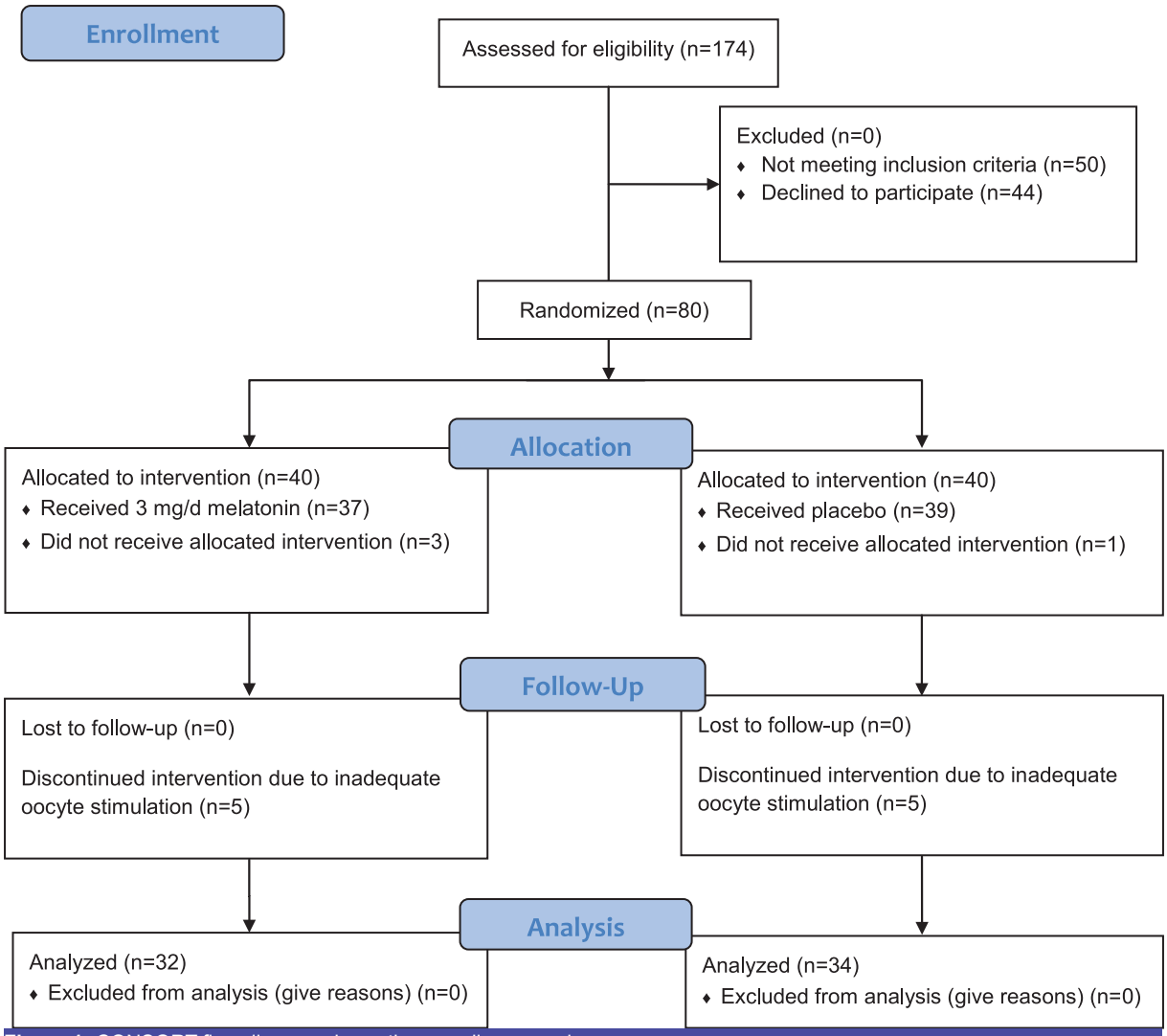

Figure 1: CONSORT flow diagram shows the sampling procedure.

used for all the patients via a subcutaneous injection of GnRH agonists (0.2 mg buserelin, Suprefact, Serono, Rome, Italy), starting from the 21st day of the same cycle when melatonin was started. Gonadotropin stimulation by rFSH (Gonal-F®, Serono, Rome, Italy) was commenced as from the second day of the next menstrual cycle. Ovarian response to gonadotropin stimulation was monitored by transvaginal ultrasound scan starting from the seventh day and the measurement of serum estradiol levels every 3 days. Final oocyte maturation was triggered by 10000 IU hCG (Gonasi® HP, IBSA Italia, Rome, Italy) when at least 2 follicles reached a 17–18 mm diameter. Oocyte retrieval was performed 34–36 hours after triggering. The luteal phase was supported with 100 mg intramuscular progesterone (Iran Hormone, Tehran, Iran) daily, from the day of oocyte retrieval for 3 days, and was continued with vaginal progesterone (400 mg, Cyclogest®, Actavis UK Ltd., Barnstaple, UK) once daily.

The patients were followed up, and their cycle outcomes were documented. The oocytes were classified into 3 groups of germinal vesicle, metaphase I (MI), and metaphase II (MII) according to the standard criteria.[12,13] The embryos were classified to 4 grades (1–4, with grade 1 indicating the best quality) according to the standard criteria proposed by Steer.[14] The embryos with <20% fragmentation (grade 1 or 2) and >6 blastomeres on day 3 were considered as top-quality embryos. Embryo grading was performed just before embryo transfer. An elevation in serum β-hCG levels 16 days after embryo transfer was considered as a biochemical pregnancy. However, a clinical pregnancy was determined by the visualization of an embryo with cardiac activity at 6–7 weeks of pregnancy. After the collection of all the data, the code numbers of the packages were broken by the pharmaceutics and the data were analyzed. The case and control groups were compared in terms of the outcomes such as duration of stimulation, dosage

of gonadotropins, serum estradiol level on the triggering day, number and quality of oocytes and embryos, clinical pregnancies, and miscarriages.

Statistical analysis was performed using SPSS software, version 21 (IBM, Armonk, NY, USA). The independent sample *t* test was used to compare the means; and for the small samples, the Mann-Whitney U-test was performed. The chi-square test was employed to compare proportions, which were presented as numbers (%). A P value <0.05 was considered statistically significant.

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (# ct-p-92-5220).

Results

This double-blinded, placebo-controlled study was finalized on 66 women with DOR in their first ART cycle. Thirty-two women took melatonin and 34 women took the placebo as the control group. There were 32 (91%) women with primary and 3 (9%) with secondary infertility in the case group and 33 (94%) women with primary and 2 (6%) with secondary infertility in the control group. The demographic and hormonal data of the study groups are presented in table 1.

Comparison of the stimulation data and the number and quality of oocytes and embryos and also the ART outcomes between the case and control groups are presented in table 2.

Two women in the case group and 1 in the control group had clinical pregnancies. However, the pregnancy in the placebo group was an ectopic one, which was treated by methotrexate therapy at 7 weeks. Two pregnant women in the case group miscarried in weeks 8–9.

Discussion

This double-blinded, placebo-controlled, clinical trial was performed on women with DOR to evaluate the possible positive effects of melatonin compared to a placebo on the ART outcomes. We found that the mean serum estradiol level on the triggering day was significantly higher in the women who took melatonin (table 2). However, in previous studies, higher values for the mean estradiol level were reported after the consumption of melatonin, although the values were not statistically significant.[11,12] Our study showed that the mean number of MII oocytes was higher in the case group, which is consistent with the previous reports.[11,12] In the present study, the number of the patients who had mature MII oocytes and top-quality embryos with grade 1 was significantly higher among the group who received melatonin (table 2). This finding supports the idea that melatonin has synergistic effects with gonadotropins on the ovaries and promotes oocyte maturation and development.

We found no differences in the clinical pregnancy and miscarriage rates between the groups. This finding is also in agreement with the 2 previously mentioned studies.[11,12] Furthermore, this finding confirms the concept that the normal continuation of a pregnancy and take-home-baby cannot always be predicted only by oocyte and embryo scorings during the ART programs. Although the total days of the administration of melatonin and the resultant cumulative dosages are different in the 3 studies, the ART outcomes are consistent. Nevertheless, in a recent study, melatonin was added to *in vitro* maturation culture media (10 µmol/L) for women with PCOS and higher clinical pregnancy rates were reported, albeit still not significant. In our research and the 2 aforementioned studies, 3 mg/d melatonin orally was used. However, the effective daily value for melatonin is not established yet. The melatonin dose that we used (3 mg/d) may not have been adequate for the follicular fluid to reach a sufficient concentration needed for a maximum result. Although evidence shows that melatonin is possibly safe when taken orally in different doses and time frames, several adverse effects have been reported such as nausea, headache, dizziness, drowsiness, abdominal pain, and short-term feelings of depression.[15] In contrast to *in vitro* or animal investigations, in human studies ethical considerations prevent the researchers from designing projects with multiple increasing doses.

There were only 2 clinical pregnancies in the melatonin group in our study compared to 1 in the placebo group. The small number of

Table 1: Demographic and hormonal data of the study groups

	Melatonin Group (n=32)	Placebo Group (n=34)	P value*
Age (y)	35±5.1 (24–42)	35.1±5.1 (22–42)	0.970
Infertility duration (y)	6±4.6 (1–21)	7.05±5.7 (1–25)	0.409
FSH (IU/L)	14.9±14.2 (4.5–86)	11.07±3.6 (4.7–19)	0.126
AMH (ng/mL)	0.98±0.63 (0.1–2.2)	0.81±0.48 (0.1–2)	0.223
AFC (n)	6.1±3.7 (2–9)	5.2±2.5 (1–9)	0.302

*Analysis was performed with the *t* test. Data are presented as mean ± SD (range). FSH: Follicle-stimulating hormone; AMH: Anti-Müllerian hormone; AFC: Antral follicular count

Table 2: Comparison of the stimulation data and the number and quality of oocytes and embryos and also the ART outcomes between the case and control groups

	Melatonin Group (n=32)	Placebo Group (n=34)	P value
Stimulation duration (d)	11±2.3	10.3±2.4	0.277**
Estradiol on HCG injection day (pg/mL)	2133±1272	1193±1396	0.005*
Human menopausal gonadotropin dose (IU)	3975±1269	3860±1384	0.727**
Oocytes in germinal vesicle stage	3±1	2.6±1.5	1.000*
Oocytes in maturation stage I (MI)	2.5±2.6	2.16±2.4	0.525*
Oocytes in maturation stage II (MII)	5.38±2.37	3.7±2.7	0.053*
No. of embryos transferred	3±1.3	2.3±1.58	0.174*
Embryos in grade 1	2.7±1.3	1.88±1.6	0.027*
Embryos in grade 2	3±2	1	0.200*
Embryos in grade 3	0	3	Not applicable
Embryos in grade 4	0	0	Not applicable
Patients who had mature oocytes (MII)	21 (65%)	12 (35%)	0.014•
Patients who had grade-1 embryos	16 (50%)	9 (26.4%)	0.049•
Patients who had top-quality embryos (grades 1 and 2)	18 (56%)	9 (26.4%)	0.014•
Patients whose embryos were transferred	19 (59%)	11 (32.3%)	0.028•
Biochemical pregnancy	2 (6.2%)	1 (2.9%)	Not applicable
Clinical pregnancy	2 (6.2%)	1 (2.9%)	Not applicable
Miscarriage	2 (6.2%)	1 (2.9%)	Not applicable

*Analysis was performed with the Mann-Whitney U-test. **Analysis was performed with the *t* test. •Analysis was performed with the Chi-square test. Data are presented as mean ± SD or n(%)

clinical pregnancies in our study may be due to the severely decreased ovarian reserves of the subjects from the beginning. We enrolled the women if 2 of the following 3 criteria were present: 1) bilateral AFC≤6, 2) AMH≤1, and 3) basal FSH≥10. By these strict criteria, women with significantly poor ovarian reserves were selected. Oocyte donation is suggested to these women with severe DOR according to the routine protocols in our center. Nonetheless, if the patients insisted on trying their own oocytes for their first ART cycle, we enrolled the women in this project after providing them with complete explanations and obtaining written consent from them. Compared to the overall pregnancy rate of about 30% for the ART in our center, the very poor rate of 2.9–6.2% obtained in this study was not acceptable. Due to this very poor outcome results, we did not continue this project to be completed by including 10 more cases.

The major limitation of the present pilot study is its small sample size, precluding an accurate statistical result. However, we do not recommend that melatonin at this dosage be repeated in larger sample sizes of women with DOR because there were only a few pregnancies, all of which ended in miscarriage. Melatonin might be proven by future studies to be beneficial when used as a supplement in cases with normal ovarian function to improve the ART outcomes. Also, studies might be designed with different dosages and simultaneous measurement of the melatonin concentration in the follicular fluid of oocytes with different qualities to find the optimal effective melatonin dosage or even melatonin could be added to an investigational culture medium and observe whether better ART outcomes would be achieved.

Conclusion

In the current study, the women in the case group had a higher serum estradiol level on the triggering day and also had more good-quality oocytes and embryos after receiving melatonin. However, the other ART outcomes were not different between the case and control groups among the women with DOR.

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Conflict of Interest: None declared.

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