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The Effect of Serum and Follicle Fluid Melatonin Levels on In-Vitro Fertilization Success Between Polycystic Ovary Syndrome and Unexplained Infertility Patient Groups in In-Vitro Fertilization

Tüp Bebek Tedavisinde Polikistik Over Sendromu ve Açıklanamayan İnfertilite Hasta Gruplarında Serum ve Folikül Sıvısı Melatonin Düzeylerinin Tüp Bebek Başarısı Üzerine Etkisi

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The Effect of Serum and Follicle Fluid Melatonin Levels on In-Vitro Fertilization Success Between Polycystic Ovary Syndrome and Unexplained Infertility Patient Groups in In-Vitro Fertilization

ABSTRACT

Objective: To investigate the difference in the serum melatonin and follicle fluid melatonin levels at unexplained infertility and polycystic ovary syndrome (PCOS) patients who underwent in-vitro fertilization (IVF) treatment, and to investigate whether the melatonin level affects oocyte quality, embryo number, and clinical pregnancy.

Material and Method: Women with unexplained infertility (n=26) and women with the polycystic ovarian syndrome (n=26) who started IVF treatment were included in this prospective trial. The levels of melatonin in the groups' were tested using the enzyme-linked immunosorbent (ELIZA) method. In addition, the effect of the difference in melatonin levels between the groups on IVF success was investigated.

Results: Patients with the polycystic ovarian syndrome had significantly low serum melatonin levels (*p=0.018*). Melatonin levels in follicular fluid were similar in both groups (*p=0.701*). Total oocyte, M2 oocyte, PN2 oocyte, grade A embryo, second-day embryo count, and the number of transferred embryos did not differ across the groups (*p>0.05)*. There was no significant relationship between serum and follicular fluid melatonin levels (*p>0.05*). There was no significant relationship between clinical pregnancy and melatonin level (*p>0.05*). There was no statistical difference between the groups regarding age, weight, height, and body mass index (*p>0.05*).

Conclusion: In our study, serum melatonin levels were lower in infertile women with polycystic ovary syndrome. This may be due to high melatonin consumption. However, melatonin levels in the serum and follicular fluid had no direct effect on IVF outcomes.

Keywords: Follicular fluid, melatonin, PCOS, unexplained infertility.

ÖZET

Amaç: Açıklanamayan infertilite ve tüp bebek tedavisi gören PKOS (Polikistik Over Sendromu) hastalarında serum ve folikül sıvısı melatonin düzeyleri arasındaki farkı araştırmak ve melatonin düzeyinin oosit kalitesi, embriyo sayısı ve klinik gebelik üzerine etkilerini araştırmak.

Gereç ve Yöntem: Bu prospektif çalışmaya, açıklanamayan infertilitesi olan kadınlar (n=26) ve polikistik over sendromlu (n=26) tüp bebek tedavisine başlayan kadınlar dahil edildi. Grupların serum ve foliküler sıvısındaki melatonin düzeyleri ELIZA yöntemi kullanılarak test edildi. Ayrıca gruplar arasındaki melatonin düzeyi farklılığının IVF başarısına etkisi araştırıldı.

Bulgular: Polikistik over sendromlu hastaların serum melatonin düzeyleri anlamlı düzeyde az idi (*p=0.018*). Foliküler sıvıdaki melatonin düzeyleri her iki grupta da benzerdi (*p=0.701*). Toplam oosit, M2 oosit, PN2 oosit, A sınıfı embriyo, ikinci gün embriyo sayısı ve transfer edilen embriyo sayısı açısından gruplar arasında anlamlı fark yoktu (*p>0.05*). Serum ve foliküler sıvı melatonin düzeyleri arasında anlamlı bir ilişki saptanmadı (*p>0.05*). Klinik gebelik ile melatonin düzeyi arasında anlamlı bir ilişki bulunmadı (p>0.05). Gruplar arasında yaş, kilo, boy ve vücut kitle indeksi açısından istatistiksel fark yoktu (*p>0.05*).

Sonuç: Çalışmamızda polikistik over sendromlu infertil kadınlarda serum melatonin düzeyleri daha düşüktü. Bunun nedeni yüksek melatonin tüketimi olabilir. Ancak serum ve foliküler sıvıdaki melatonin düzeylerinin IVF sonucuna doğrudan bir etkisinin olmadığı görüldü.

Anahtar Sözcükler: Açıklanamayan infertilite, foliküler sıvı, melatonin, PKOS.

Introduction

Traditional definitions of infertility include the failure to conceive with unprotected intercourse for longer than 12 months. This 12-month criteria is accepted as six months for women over 35 years old (1). Approximately 10-30% of infertile couples have no identifiable cause and are termed unexplained infertility (2). Unexplained infertility patients can have a wide range of treatment options, from expectant management to IVF treatment.

Polycystic ovarian syndrome (PCOS) is a complicated endocrinopathy that affects around 10% of women at reproductive age and involves various metabolic processes (3). Free oxygen radicals and anovulation, which develop as a result of oxidative stress in PCOS, are thought to have a negative impact on reproductive function.

The pineal gland produces melatonin (N-acetyl-5-methoxytryptamine), released into the circulatory system. Melatonin has central and peripheral activities and affects the gastrointestinal, cardiovascular, endocrine, and reproductive systems. It has an antigonadotropic effect on the reproductive system. Furthermore, it affects the oocyte's granulosa and luteal cells, suppressing their steroidogenesis (4). Follicular atresia can be observed in PCOS patients due to follicular damage as a result of increased oxidative stress and decreased intrafollicular melatonin levels. Oxidative stress is one of the primary reasons for infertility in women with PCOS, and melatonin is known to have antioxidant properties and inhibitory effects on prooxidant enzymes and proinflammatory cytokines. Melatonin and its metabolites are known to reduce oxidative damage in addition to their direct action. Its anti-inflammatory activity reduces the generation of free radicals and helps to reduce free radical damage. This study aimed to investigate if melatonin affects fertility in subgroups like PCOS and unexplained infertility.

Material and Methods

The study includes 52 infertile women who were admitted to the university hospital's IVF center between September 2021 and March 2022. The Hitit University Faculty of Medicine Clinical Research Ethics Committee authorized this study according to the Declaration of Helsinki (approved number: 488). The study included patients who completed written informed consent forms after receiving extensive verbal and written information. The research was carried out in line with the Helsinki Declaration.

A total of 52 patients were divided into two groups which are PCOS (n=26) as the study group and unexplained infertility (n=26) as the control group. Women who fulfilled the criteria of infertility and had no known cause were accepted as having unexplained infertility. The patients' oligo-anovulation status, polycystic ovarian morphology (PCOM) on transvaginal ultrasonography, and Ferriman-Gallwey scoring as clinical indications of hirsutism were used to diagnose PCOS.

Name, surname, age, weight, height, BMI, menstrual pattern, number of days of menstruation, duration of unprotected intercourse, acne status, and past pregnancies were all noted in patients who participated in the study. The hirsutism of the patients was assessed using the modified Ferriman Gallwey scoring method. The results of a baseline ultrasound, which is performed routinely throughout the evaluation, were recorded. In addition, routinely requested basal hormones (FSH, LH, E2, Prolactin, and AMH) were recorded. Chronic diseases, drug use, smoking, and alcohol use were questioned.

BMI greater than 30 kg/m^2, FSH levels >25 mIU/ml, a male component in infertility, excessive alcohol consumption in the previous six months, smoking at least 20 cigarettes per day, long-term illness, taking melatonin-containing medications or supporting treatments are exclusion criteria.

Patient Blood Serum and Follicular Fluid Collection

Since our study consisted of patients who applied for infertility treatment and were planned for IVF, patients who used the antagonist protocol were included to standardize the number and quality of oocytes and pregnancy status. Oocyte retrieval was conducted at the 35th hour (between 10:00 and 12:00) after hCG treatment when enough follicular development was visible in serial transvaginal ultrasonography. Five ccs of the remnant ovarian follicular fluid was put into a dry tube and stored at -20 °C after the oocytes were separated from the follicular fluid. Approximately 8 cc of patient blood was put into a 10 cc empty dry tube at the same time as the routine blood tests performed

during the patient's hospitalization. In 15 minutes, the blood sample was centrifuged at 4000 rpm for 10 minutes. The obtained supernatants were kept at -20 °C in an Eppendorf test tube (1,5 ml, FIRADMED, polypropylene). The samples were delivered to the laboratory using the cold chain rule once the number of patients had been completed.

Melatonin levels were determined using the Enzyme-linked Immunosorbent Assay Melatonin Kit (catalog number CEA908Ge). After washing with the Human brand, Combi Wash model washing device and reading with the Next Level brand, Alisei model equipment. The sensitivity of the kit is 4.63 pg/mL.

Pregnancy and Oocyte Quality Follow-up

The embryologist noted the number of oocytes, their morphological parameters (MII, PNII, etc.), and the number of embryos after the oocyte pick-up process. On the 14th day after embryo transfer, patients tested for hCG. Patients whose tests were positive for hCG were monitored until fetal heartbeats were detected. The patient was accepted as clinically pregnant when fetal heartbeat was detected. It took about five months to complete the study.

Statistical Analysis

Data were analyzed using the IBM SPSS Statistics Client Documentation 26.0 program. Unexplained infertility and PCOS groups were evaluated with Kolmogorov-Smirnov and Shapiro-Wilk normal distribution test, Skewness- Kurtosis tests, and visually normal Q-Q Plot distribution curve, histogram, and box plot. Data that did not fit the normal distribution were analyzed with the Mann-Whitney U Test. The relationship between melatonin level and oocyte number and quality was evaluated with Spearman's correlation test. p<0.05 in the analysis results was considered statistically significant.

Results

In terms of age and BMI, there was no statistically significant difference between unexplained infertility and PCOS infertility groups (*p>0.05* for all). When comparing PCOS to the unexplained group, the cycle duration was significantly longer, and the total number of follicles were significantly higher (*p<0.01*). Table I displays descriptive statistics.

Table I. Descriptive Statistics

	Unexplained Infertile		PCOS Infertile		*p*
	Min-Max (Median)	**Mean±SS**	**Min-Max (Median)**	**Mean±SS**	
Age (year)	21-35 (26.7)	27.1±4.09	23-38 (26.5)	27.92±4.22	*0.762*
BMI (kg/m²)	19-33.3 (24.4)	24.35±3.11	16.9-30 (26.5)	25.72±3.52	*0.084*
Cycle Time	21-45 (28)	27.57±4.5	28-54 (41)	40.08±6.07	***<0.010***
Total Number of Antral Follicles	8-20 (14)	12.15±3.04	10-32 (26)	24.1±4.08	***<0.010***

Independent-Samples, Mann-Whitney U Test, *p<0.05* was considered statistically significant.

When the groups' baseline hormone levels were evaluated, there was no statistically significant difference in FSH, estradiol, or prolactin levels (*p>0.05* for all). LH and AMH levels were considerably higher in PCOS infertile women than in the unexplained group (*p=0.022* and *p=0.01*, respectively). The PCOS infertility group had a statistically low amount of serum melatonin (*p=0.018*). Melatonin levels in follicular fluid were equal in both groups (*p=0.701*). Table II shows the basal hormone and melatonin levels of the groups.

Table II. Basal Hormone and Melatonin Levels of the Groups

	Unexplained Infertile		PCOS Infertile		*p*
	Min-Max (Median)	**Mean±SS**	**Min-Max (Median)**	**Mean±SS**	
FSH (mIU/ml)	1.4-11.4 (6.6)	6.75±2.29	4.3-10.9 (6.65)	6.89±1.74	*0.812*
LH (mIU/ml)	1.02-15.3 (5.4)	5.97±3.17	2.2-19 (7.8)	7.98±3.56	***0.022***
Estradiol (pg/ml)	12-116 (40.45)	46.9±19.7	29.6-70.8 (42.35)	44.22±10.58	*0.777*
Prolactin (ng/ml)	0.5- 42 (15.5)	18.09±10.1	7.5-39 (19.65)	20.31±8.91	*0.282*
AMH (ng/ml)	1.2- 5.7 (2.76)	2.97±1.43	2-13 (4.55)	5.51±2.84	***<0.010***
Serum Melatonin Level (pg/mL)	7.18-50.89 (23.21)	26.13±11.26	5.35-44.29 (17.86)	20.66±9.02	***0.018***
Follicle Fluid Melatonin Level (pg/mL)	6.16-18.3 (12.38)	12.16±3.22	5.94-19.97 (12.81)	12.27±3.63	*0.701*

Independent-Samples, Mann-Whitney U Test, *p<0.05* was considered statistically significant.

There was no statistically significant association between serum melatonin and follicular fluid melatonin levels (*p>0.05*). In terms of total oocyte count, M2

oocyte count, PN2 oocyte count, grade A embryo count, second-day embryo count, and embryo transfer number, there was no significant correlation between serum melatonin level and follicular fluid melatonin level (*p>0.05*, for all). Table III shows the relationship between Melatonin levels, Oocyte Quality, and Embryo Count.

Table III. Correlation of Melatonin Level with Oocyte Quality and Embryo Count

		Correlation Coefficient	*p*
Number of Oocytes Collected	Serum Melatonin Level	-0.143	*0.312*
	Follicular Fluid Melatonin Level	0.155	*0.273*
M2 Oocyte Count	Serum Melatonin Level	-0.147	*0.298*
	Follicular Fluid Melatonin Level	0.153	*0.279*
PN2 Oocyte Count	Serum Melatonin Level	-0.007	*0.958*
	Follicular Fluid Melatonin Level	0.038	*0.791*
Number of Grade A Embryos	Serum Melatonin Level	-0.082	*0.562*
	Follicular Fluid Melatonin Level	0.037	*0.797*
Number of Embryos on Day 2	Serum Melatonin Level	0.050	*0.725*
	Follicular Fluid Melatonin Level	0.049	*0.729*
Number of Patients Who Underwent Embryo Transfer	Serum Melatonin Level	0.049	*0.731*
	Follicular Fluid Melatonin Level	-0.200	0.156

Spearman's correlation test, *p<0.05* was considered statistically significant.

Discussion

In our study, the PCOS group had considerably low serum melatonin levels. According to several studies, oxidative stress caused by reactive oxygen radicals (ROS) is higher in PCOS, and infertility is more common than in the general population (5). While cells need a physiological amount of oxygen to live, free oxygen radicals emerge as a result of these reactions; oxygen superoxide (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl (-OH) reagents must be removed from the environment. During ovulation, reactive oxygen radicals in the follicular environment accelerate oocyte aging (6). Antioxidants like superoxide dismutase (SOD) and glutathione, which eliminate reactive oxygen radicals from the environment, are also in good shape. Melatonin is a powerful antioxidant produced from the pineal gland into the bloodstream and then into the follicular fluid. According to the findings, melatonin treatment reduced oxidative stress by lowering nitric oxide levels and inhibited proinflammatory cytokines (7). It has also been shown that adding melatonin to the culture medium enhances oocyte maturation and lowers the generation of reactive oxygen radicals (8). Various studies have shown that more oxidative stress and melatonin usage are higher in PCOS patients (9-11). Melatonin metabolites, in addition to the direct action of melatonin in lowering oxidative damage, also contribute to reducing oxidative stress through physiological and metabolic effects. Furthermore, because of its anti-inflammatory properties, it aids in reducing free radical production and damage that occurs as a result of the inflammatory response (12). Free oxygen radicals and oxidative stress factors are likely to be higher in the PCOS group, it's reasonable to assume that melatonin consumption will be higher to detoxify them, and that serum melatonin levels will be lower in the PCOS group.

The urinary level of 6-sulphatoxymelatonin, an essential marker for melatonin production, was higher in patients with PCOS than in non-PCOS women (13). Although we did not test 6 sulfateoxymelatonin as a melatonin metabolic marker, endogenous serum melatonin levels were lower in the PCOS group than in the unexplained infertility group. These indicators can be used to determine whether the production is low, or the consumption is high.

Oral melatonin administration in PCOS patients has been shown to reduce oxidative stress, increase oocyte quality, and maybe protect the reproductive system (14). In our study, the serum melatonin level of infertile women with PCOS was lower than the unexplained infertility group, suggesting that it may adversely affect fertility and oocyte quality. However, it was not statistically significant. There was no significant relationship between clinical pregnancy and melatonin levels.

Human studies have found a favorable association between day and night melatonin concentrations and total antioxidant capacity (15). In this regard, increasing endogenous melatonin levels through exogenous melatonin supplementation during

fertilization appears to positively influence the fertilization rate.

The median follicular fluid melatonin level in the PCOS group was 12.81 pg/ml, whereas the follicular fluid melatonin level in the unexplained infertility group was 12.38 pg/ml, and the follicular fluid melatonin level was similar in both groups.
In vitro experiments have shown that women with PCOS have lower levels of melatonin in follicular fluid compared to healthy women (16). Melatonin is hypothesized to minimize oxidative stress, inhibit follicle atresia, and increase follicular maturation in this group of people (17). Unlike in vitro studies, there was no significant difference between the PCOS and unexplained infertility groups in our study.

The total number of oocytes retrieved, the total number of oocytes progressing to MII, the total number of oocytes with PN2, the number of grade A embryos, the number of days two embryos, and the number of embryos that can be transferred were all recorded. One of the most common causes of IVF failure is poor oocyte quality and maturation. Oxidative stress factors in the oocyte may play a role in reduced embryo quality (18). Reactive oxygen radicals and melatonin have directly affected oocyte maturation and fertilization. In a study of 56 patients taking 3 mg/day melatonin supplements and 59 controls, fertilization was found to be higher in the melatonin supplement group (19). The melatonin levels in follicular fluid and serum did not affect the quality and maturation of oocytes in our study. Although exogenous melatonin has been demonstrated to have a favorable effect on oocyte maturation, endogenous follicular fluid and serum melatonin levels did not correlate with the number of oocytes or the number of good-quality oocytes in our study (14). There was no significant relationship between clinical pregnancy and melatonin levels. The effect of melatonin on infertility is not fully understood (20).

Because melatonin is produced in various tissues and organs, the mean serum melatonin level is likely to be higher than the mean follicular fluid. Recent studies show that melatonin is synthesized in small amounts in a wide variety of tissues and has paracrine and autocrine effects (21). In our study, the mean serum melatonin level was 23.39±10.12 pg/ml, and the mean follicular fluid melatonin fluid level was 12.21±3.42 pg/ml. In another study, the follicular fluid melatonin level was found to be 20.9 ± 3.6 pg/ml in the PCOS group, and it was higher than we found (22). Brzezinski et al. discovered that the level of melatonin in the follicular fluid before ovulation was higher than the level of melatonin in the plasma (22,23).

In contrast to this study, serum melatonin levels were higher than follicular fluid melatonin levels. This may be because Brzezinski et al. utilized the RIA method while we used the ELISA. Because melatonin is produced by various tissues and melatonin consumption may be higher during ovulation due to increased oxidative stress, the follicular fluid level of melatonin is expected to be lower than the serum level. The PCOS group had a median AMH level of 4.55 ng/ml, while the unexplained infertile group had a median AMH level of 2.76 ng/ml. The PCOS group's AMH level was statistically greater. Due to the lack of an international standard, AMH currently plays no significant role in the diagnosis and treatment of PCOS. Many studies have shown that AMH levels are higher in PCOS patients (24,25). The total number of oocytes collected in both groups was compared, the PCOS group had a total oocyte count of 9.5.

In contrast, the unexplained infertility group had a median total oocyte count of 4. It was statistically higher in the PCOS group ($p<0.01$). The level of AMH can help us forecast ovarian reserve and oocyte count.

The median LH level measured on the second day of the menstrual cycle in women in the PCOS group was 7.83 mIU/ml, and 5.41 mIU/ml in the unexplained infertile group. It was statistically higher in PCOS. In the past, a higher LH level than FSH level was used as an indicator for PCOS. In some studies, it has been shown that the LH level is high in PCOS (26). However, an elevated LH level is not a diagnostic criteria for PCOS. Androgen increase and phenotypic changes due to LH elevation may be symptoms of PCOS.

When the two groups were compared, the median FSH value was 6.65 mIU/ml in the PCOS group and 6.60 mIU/ml in the unexplained infertility group, and it was similar in both groups. FSH level above

10 mIU/mL is considered a poor ovarian response, indicating a decreased ovarian reserve (27). In addition, estradiol levels were also found to be similar in both groups. Therefore, it shows that the groups are similar in ovarian response.

When the menstruation cycles of the two groups were compared, women with PCOS had a longer period. This is because women with PCOS are more likely to have oligo-ovulation. Women with oligomenorrhea whom have fewer than nine menstrual cycles per year is frequently seen in PCOS patients (28). The cycle in the PCOS group was 40.08±6.07 days in our study. Compared to the unexplained infertility group, these women's cycles lasted much longer.

There was no difference between the two groups in terms of age, height, weight, and BMI. However, the unexplained infertility group had a median age of 26.7 years, while the PCOS group had 26.5 years. The unexplained infertility group had a median BMI of 24.4 kg/m^2, while the PCOS group had a median BMI of 26.5 kg/m^2, with no statistical difference. More than half of women with PCOS are obese (29). Therefore, to exclude obesity-related factors in the PCOS group, patients having a BMI of more than 30 kg/m^2 were excluded from the study.

The limitations of this study, the patients' levels of endogenous melatonin were measured and compared to the follicular fluid. Therefore, melatonin's production and elimination processes were not studied. These processes were not included because the study's principal objective was to correlate follicular fluid and serum melatonin and its effect on IVF. To investigate these phases, more comprehensive in-vitro experiments can be planned.

Conclusion

The levels of melatonin in follicular fluid were similar in the PCOS and unexplained infertility groups. The antioxidant impact of melatonin in the follicular fluid is identical to the unexplained infertility group, despite the high oxidative stress reported in PCOS. This shows that oxidative stress markers in PCOS follicular fluid may be lower than previously thought. Additionally, melatonin levels did not affect oocyte and embryo quality. Although exogenous melatonin treatment has been shown to improve the rate of pregnancy in numerous studies, we discovered that endogenous melatonin levels did not affect the pregnancy rate. In our study, the serum melatonin level was lower in the PCOS group than in the unexplained infertility group. However, melatonin levels were high in PCOS patients in various studies. Therefore, it is expected that serum melatonin levels may be low in PCOS due to the consumption of melatonin, which is an antioxidant.

References

1. Luo L, Zeng X, Huang Z, Luo S, Qin L, Li S. Reduced frequency and functional defects of CD4+ CD25 high CD127 low/− regulatory T cells in patients with unexplained recurrent spontaneous abortion. Reprod Biol Endocrinol 2020;18:1-10.

2. Athaullah N, Proctor M, Johnson NP. Oral versus injectable ovulation induction agents for unexplained subfertility. Cochrane Database Syst Rev. 2002;2002(3):CD003052.

3. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. JCEM 2005;1929-1935.

4. Brzezinski A, Seibel MM, Lynch H J, Deng MH, Wurtman R J. Melatonin in human preovulatory follicular fluid. JCEM 1987;64(4):865-867.

5. Giannetto A, Fernandes JM, Nagasawa K, et al. Influence of continuous light treatment on expression of stress biomarkers in Atlantic cod. DCI 2014;44(1):30-34.

6. Goud AP, Goud PT, Diamond MP, Gonik B, Abu-Soud HM. Reactive oxygen species and oocyte aging: role of superoxide, hydrogen peroxide, and hypochlorous acid. Rad Biol Med 2008;44(7):1295-1304.

7. Lemos AJJM, Peixoto CA, Teixeira ÁAC, et al. Effect of the combination of metformin hydrochloride and melatonin on oxidative stress before and during pregnancy, and biochemical and histopathological analysis of the livers of rats after treatment for polycystic ovary syndrome. Toxicology and Applied Pharmacology 2014;280(1):159-168.

8. Kang JT, Koo OJ, Kwon DK, et al. Effects of melatonin on in vitro maturation of porcine oocyte and expression of melatonin receptor RNA in cumulus and granulosa cells. JPR 2009;46(1):22-28.

9. Shreeve N, Cagampang F, Sadek K, et al. Poor sleep in PCOS; is melatonin the culprit. Hum Reprod 2013;28(5):1348-1353.

10. Lemos AJJM, Peixoto CA, Teixeira ÁAC, et al. Effect of the combination of metformin hydrochloride and melatonin on oxidative stress before and during pregnancy, and biochemical and histopathological analysis of the livers of rats after

treatment for polycystic ovary syndrome. Toxicology and Applied Pharmacology 2014;280(1):159-168.
11. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fert Stert 2003;80(1):123-127.
12. Mauriz JL, Collado PS, Veneroso C, Reiter RJ, González-Gallego J. A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives. JPR 2013;54(1):1-14.
13. Luboshitzky R, Qupti G, Ishay A, Shen-Orr Z, Futerman B, Linn S. Increased 6-sulfatoxymelatonin excretion in women with polycystic ovary syndrome. Fert Stert 2001;76(3):506-510.
14. Pacchiarotti A, Carlomagno G, Antonini G, Pacchiarotti A. Effect of myo-inositol and melatonin versus myo-inositol, in a randomized controlled trial, for improving in vitro fertilization of patients with polycystic ovarian syndrome. Gynecological Endocrinology 2016;32(1):69-73.
15. Reiter RJ, Tan DX, Maldonado MD. Melatonin as an antioxidant: physiology versus pharmacology. JPR 2005;39(2):215-216.
16. Tamura H, Nakamura Y, Korkmaz A, et al. Melatonin and the ovary: physiological and pathophysiological implications. Fert Stert 2009;92(1):328-343.
17. Jain P, Jain M, Haldar C, Singh TB, Jain S. Melatonin and its correlation with testosterone in polycystic ovarian syndrome. Journal of Human Reproductive Sciences 2013;6(4):253-258.
18. Tamura H, Takasaki A, Taketani T, et al. The role of melatonin as an antioxidant in the follicle. Journal of Ovarian Research 2012;5:1-9
19. Tamura H, Takasaki A, Miwa I, et al. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. JPR 2008;44(3):280-287.
20. Nakamura Y, Tamura H, Takayama H, Kato H. Increased endogenous level of melatonin in preovulatory human follicles does not directly influence progesterone production. Fert Stert 2003;80(4):1012-1016.
21. Acuña-Castroviejo D, Escames G, Venegas C, et al. Extrapineal melatonin: sources, regulation, and potential functions. CMLS 2014;71:2997-3025.
22. Kim MK, Park EA, Kim HJ, et al. Does supplementation of in-vitro culture medium with melatonin improve IVF outcome in PCOS?. RBMO 2013;26(1):22-29.
23. Brzezinski A, Seibel MM, Lynch HJ, Deng MH, Wurtman RJ. Melatonin in human preovulatory follicular fluid. JCEM 1987;64(4):865-867.
24. Dumont A, Robin G, Catteau-Jonard S, Dewailly D. Role of Anti-Müllerian Hormone in pathophysiology, diagnosis and treatment of Polycystic Ovary Syndrome: a review. Reprod Bio End 2015;13:1-10.
25. Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod bio online 2016; 33(1):15-28.
26. Fleming R, Coutts JR. 11 LHRH analogues for ovulation induction, with particular reference to polycystic ovary syndrome. Baillière's Clin Obs Gyn 1988;677-687.
27. Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. Bio of Reprod 1994;653-663.
28. Hull M, Joyce D, Turner G, Wardle P. Undergraduate Obstetrics & Gynaecology. Acta Obst Gyn Scandinavica 1997;76(8):809.
29. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycystic ovary syndrome. Inter J of Obesity 2002; 26(7):883-896.