Melatonin and its correlation with testosterone in polycystic ovarian syndrome

Priyanka Jain, Madhu Jain, Chandana Haldar[1], Tej Bali Singh[2], Shuchi Jain

Departments of Obstetrics and Gynaecology, [1]Zoology, [2]Community Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Address for correspondence:
Dr. Madhu Jain,
11, Lal Bhadur Shastri Colony, Karaundi Crossing,
Varanasi - 221 005,
Uttar Pradesh, India.
E-mail: drmadhujainbhu@gmail.com

Received: 27-09-2013
Review completed: 27-11-2013
Accepted: 31-12-2013

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/0974-1208.126295

ABSTRACT

CONTEXT: Polycystic ovarian syndrome (PCOS) is considered to be the most common endocrine disorder affecting women. Melatonin, a small lipophilic indoleamine, and reproductive hormones may be interrelated. Melatonin influences sex steroid production at different stages of ovarian follicular maturation as melatonin receptors have been demonstrated at multiple sites in ovary and in intrafollicular fluid. It plays role as an antioxidant and free radical scavanger which protects follicles from oxidative stress, rescuing them from atresia, leading to complete follicular maturation and ovulation. **AIMS:** To study the role of melatonin in PCOS and to investigate its correlation with testosterone in patients suffering from PCOS. **SETTINGS AND DESIGN:** A total of 50 women with PCOS (Rotterdam criteria, 2003) and 50 age and weight matched healthy controls were selected and serum melatonin estimation was done in both the groups and correlated with serum total testosterone levels. **MATERIALS AND METHODS:** In a case-control study, detailed history, clinical examination and hormonal evaluation [basal levels of leutinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, prolactin, insulin, total testosterone, progesterone and melatonin] were carried out in all the participants including both cases and controls. For melatonin estimation, blood samples were collected between 12:00 am and 04:00 am on day 2nd of menstrual cycle and analyzed by using commercially available enzyme-linked immunosorbent assay kit. **STATISTICAL ANALYSIS:** Student's t-test was used to compare the significant difference in mean values between cases and control groups. Chi-square test was used to test the significant association between the qualitative variables. Linear correlation coefficient and regression analysis were done to see the amount and direction of relationship between quantitative variables. **RESULTS:** The mean melatonin level was observed to be significantly increased in patients (63.27 ± 10.97 pg/mL) than in controls (32.51 ± 7.55 pg/mL). Melatonin was found to be raised in all the cases of PCOS (above cut-off value of ≥45 pg/mL, $P < 0.001$). Total testosterone level was also raised in 72% of patients. Melatonin levels were found to be positively associated with increased testosterone ($P < 0.001$). In regression analysis using melatonin as dependent variable and testosterone as an independent variable, the value of R2 × 100 (percent variation) was found to be 72.1%. **CONCLUSIONS:** Women with PCOS have significantly raised serum melatonin levels and hyperandrogenemia along with increased number of atretic follicles. Further studies are required to establish a definite role of melatonin in PCOS cases with disturbed hormonal milieu. This could open up the way for therapeutic role of melatonin in treatment of patients suffering from PCOS.

KEY WORDS: Hyperandrogenemia, infertility, melatonin, Polycystic ovarian syndrome

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is considered to be the most common endocrine disorder affecting women.[1] It is the most common cause of anovulatory infertility and hirsutism.[2,3] About 15% women of reproductive age group are affected.[4] Women with PCOS in long term are known to exhibit adverse Cardiovascular risk profile such as obesity, dyslipidemia, hypertension, insulin resistance and hyperinsulinemia,[5] with an increased risk of premature coronary artery disease.[6]

The role of melatonin (N–acetyl-5-methoxytryptamine), a small lipophilic indoleamine,[7] in human reproduction is

still unknown. A large body of information suggests that melatonin and the reproductive hormones are interrelated. This concept is based on observation of increased melatonin levels in hypogonadal patients with gonadotropin-releasing hormone (GnRH) deficiency,[8] in patients of hypothalamic amenorrhea, and in anorexia nervosa.[9] Increased melatonin has been seen to influence sex steroid production at different stages of ovarian follicular maturation.[10]

Melatonin, as well as its metabolites, are claimed to be broad-spectrum antioxidants and free radical scavengers,[11,12] and their role is to quench reactive oxygen species (ROS) as well as reactive nitrogen species.[13] Elevated melatonin in preovulatory follicles, as seen in normal women, is likely to protect granulosa cells and oocyte from free radicals that are induced during ovulation. In addition, melatonin regulates the antioxidant enzymes and antiapoptotic/proapoptotic protein gene expression.[10]

Melatonin is detectable in virtually every compartment of body and its wide distribution allows melatonin to carry out its pleiotrophic actions. Till now, only few studies [Luboshitzky *et al*., 2001; 2003; Prata Lima *et al*., 2004; Tamura *et al*.; 2009; 2012] have been carried out to show association between melatonin and PCOS in human population. Since no study of such type has been done on Indian population, we, therefore, have designed this study in an effort to investigate the role of melatonin and its correlation with testosterone in PCOS patients.

MATERIALS AND METHODS

A prospective case-control study was designed taking 50 PCOS patients and 50 age- and weight-matched healthy controls, attending outpatient department of Department of Obstetrics and Gynecology, Sir Sunderlal Hospital, Banaras Hindu University, Varanasi from September 2011 to December 2012. Sample size was calculated and it came out to be 50 by taking the level of significance 1%, power of study 80% and combined standard deviation 10.

Inclusion criteria for selection of cases

The Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine-sponsored PCOS consensus criteria was used to diagnose PCOS and women with presence of any two of the following three features were included in the study:

1. Oligomenorrhea and/or amenorrhea (oligoamenorrhoea >45 days or <8 cycles per year and amenorrhea >3 months in a women with pervious periodic menses) for a period of 6 months
2. Clinical and/or biochemical hyperandrogenemia, presence of acne, hirsutism (FG score > 8), and alopecia
3. Polycystic ovaries on sonography (>12 follicles in one or both ovaries, 2-9 mm in diameter and/or increased ovarian volume >10 mL).

Inclusion criteria for selection of controls

1. Regular menstrual cycle
2. Absence of hirsutism, alopecia, and acne
3. Absence of polycystic ovary on sonography
4. Normal hormonal parameters including thyroid-stimulating hormone (TSH), testosterone, prolactin, lieutinizing hormome (LH), follicle-stimulating hormone (FSH), LH: FSH ratio.

Exclusion criteria

All patients with diabetes mellitus, hypertension, hyperprolactinaemia, thyroid disorder, cushing's syndrome, acromegaly, premature ovarian failure, virilising adrenal or ovarian tumors, and history of using oral contraceptive pill within last 6 months were excluded from the study. None of the subjects were alcoholic or smoker.

A written and informed consent was taken from all the participants.

Biochemical and hormonal analysis

Blood samples were drawn on day 2 of menstrual cycle or progesterone-induced bleeding after an overnight fast. Plasma LH, FSH, prolactin, total testosterone, progesterone and insulin were measured by chemiluminescent enzyme immunoassay using commercially available kits (Immulite 1000 systems, Siemens). Plasma Glucose was measured by glucose oxidase peroxidase method (selectra XL analyzer, Vital Scientifics, Holland). Serum cholesterol, triglycerides, low-density lipoprotein, and low-density lipoprotein levels were measured using kits by ERBA diagnostic, Mannheim, Germany. TSH levels were measured using IRMA kit (BARC, Mumbai, India). Cut off points for diagnosis of hyperandrogenemia was set at ≥ 118 ng/dL (according to chemiluminescent enzyme immunoassay), for insulin resistance it was set at a fasting glucose/insulin (G: I) ratio ≤4.5 and for hypersecretion of LH, LH: FSH ratio ≥2 was considered to be a cut-off point.

Sonography

Pelvic sonography (Nermio 30, Toshiba, Japan) was carried out on day 2 of menstrual cycle in both cases and controls.

Melatonin estimation

Blood samples for melatonin estimation were collected between 12:00 am and 04:00 am on day 2nd of menstrual cycle. A total of 5 mL of heparinized blood was taken and samples centrifuged for 15 min at 2500 RPM within 30 min of collection. Plasma was separated and stored samples in aliquot at −20°C or −80°C. Plasma melatonin concentration was measured using a commercially available enzyme-linked immunosorbent assay kit for melatonin

(Manufacturers: USCN Life Science Inc, USA). This assay employs the competitive inhibition enzyme immunoassay technique.

Statistical analysis

SPSS 16.0 version for Windows was used for statistical analysis. All quantitative variables were expressed as mean ± standard deviation, while qualitative data were shown in the form of number and percentage. Student's t-test was used to compare the significant difference in mean values between cases and control groups. Chi-square test was used to test the significant association between the qualitative variables. Linear correlation coefficient and regression analysis were done to see the amount and direction of relationship between quantitative variables. A *P* value less than 5% ($P < 0.05$) was considered statistically significant.

RESULTS

The demographic, anthropometric, biochemical, and hormonal parameters of the study population (cases and controls) were compared [Table 1].

Melatonin concentration in PCOS patients varied from 47.9 pg/mL to 87.6 pg/mL, whereas in controls it ranged from 20.0 pg/mL to 44.04 pg/mL. Mean melatonin level was 63.27 ± 10.97 pg/mL in PCOS cases as compared with 32.51 ± 7.55 pg/mL in controls. Cut off level of melatonin was found to be 45 pg/mL. We found that all PCOS patients had significantly elevated serum melatonin levels (above cut-off value of 45 pg/mL, $P < 0.001$ and $t = 16.33$).

The mean melatonin level was shown in relation to demographic, anthropometric, biochemical, and hormonal profile of PCOS patient [Table 2].

Mean melatonin level in PCOS patients having regular menstrual cycle was 68.31 ± 11.18 pg/mL and in patients with oligomenorrhoea, it was 61.84 ± 10.62 pg/mL. The difference in mean levels of melatonin between the groups was not statistically significant ($P = 0.84$ and $t = 1.76$) [Table 2].

We observed a significant positive correlation of serum melatonin with serum total testosterone levels in PCOS patients. Mean Melatonin level was 74.53 ± 10.99 pg/mL in patients with serum total testosterone level 128-140 ng/dL, whereas it was 53.28 ± 3.78 pg/mL in patients with total testosterone levels of ≤118 ng/dL. Thus, significant difference has been observed in mean melatonin level among different testosterone categories ($P < 0.001$, Chi-square = 20.97 and df = 2). Melatonin level was also correlated with serum LH: FSH ratio and fasting glucose: insulin ratio (G: I). Mean melatonin level in patients with LH: FSH ratio ≥2 was 59.35 ± 10.02 pg/mL, whereas it was 71.24 ± 8.89 pg/mL in patients with LH: FSH ratio <2. These two variables seem to be inversely correlated ($P = 0.013$). We did not find any significant correlation between serum melatonin level and fasting glucose: Insulin ratio ($P = 0.290$) [Table 2].

Figure 1 represents the linear correlation coefficient (R) (cases: 0.85; controls: 0.06), percent variation explained (R2 × 100)) (cases: 72.1%; controls: 3%), regression constant (cases: -101.0; controls: 29.57) and regression coefficient (cases: ×1.337; controls: ×0.034) for melatonin as dependent

Table 1: Demographic, anthropometric, biochemical, and hormonal parameters of the study population (polycystic ovarian syndrome cases and controls)

Parameters	Study population		*P* value
	PCOS (*n*=50)	Control (*n*=50)	
Age (years)	24.87±4.43	22.60±4.033	0.161
BMI (kg/m^2)	28.19±2.31	27.38±2.46	0.352
Waist: hip ratio	1.63±0.49	1.00±0.00	<0.001*
LH (mIU/mL)	16.13±7.95	7.18±1.97	<0.001*
FSH (mIU/mL)	5.43±1.53	6.60±2.86	0.106
LH/FSH ratio	3.10±1.69	1.15±0.25	<0.001*
TSH (IU/mL)	2.45±0.97	2.66±1.09	0.563
Prolactin (ng/mL)	14.7±4.48	13.35±3.26	0.380
Total testosterone (ng/dL)	122.84±6.96	84.71±12.75	<0.001*
Melatonin (pg/mL)	63.27±10.97	32.51±7.55	<0.001*
Progesterone (ng/mL)	2.58±2.83	16.87±4.29	<0.001*
Fasting glucose (mg/dL)	113.01±11.25	93.68±7.53	<0.001*
Fasting insulin (mU/mL)	26.06±4.44	16.48±3.54	<0.001*
Glucose insulin ratio	4.38±0.43	5.91±1.37	<0.001*

*P<0.05 was considered statistically significant. BMI=Body mass index, FSH=Follicle-stimulating hormone, LH=Lieutinizing hormone, PCOS=Polycystic ovarian syndrome, TSH=Thyroid-stimulating hormone

Table 2: Distribution of melatonin in relation to demographic, anthropometric, biochemical, and hormonal profile of polycystic ovarian syndrome patient

Parameters	Melatonin (pg/mL) Mean±SD		*P* value
Regular menstrual cycle	68.31±11.18	1.761	0.84
Oligomenorrhea	61.84±10.62		
Primary infertility	66.33±11.83	1.481	0.162
Secondary infertility	56.74±8.12		
Obese (>30 kg/m^2)	60.89±10.45	0.319	0.752
Overweight (25-<30 kg/m^2)	62.21±11.25		
LH: FSH ratio <2	71.24±8.89	2.649	0.013*
LH: FSH ratio ≥2	59.35±10.02		
Glucose insulin ratio ≤4.5	63.71±12.66	1.078	0.290
Glucose insulin ratio ≥4.5	59.46±8.07		
Testosterone (ng/dL) <118	53.28±3.78	12.68	0.001*
Testosterone (ng/dL) 118-128	62.45±6.74		
Testosterone (ng/dL) 128-140	74.53±10.99		

*P<0.05 was considered statistically significant. FSH=Follicle-stimulating hormone, LH=Lieutinizing hormone, SD=Standard deviation

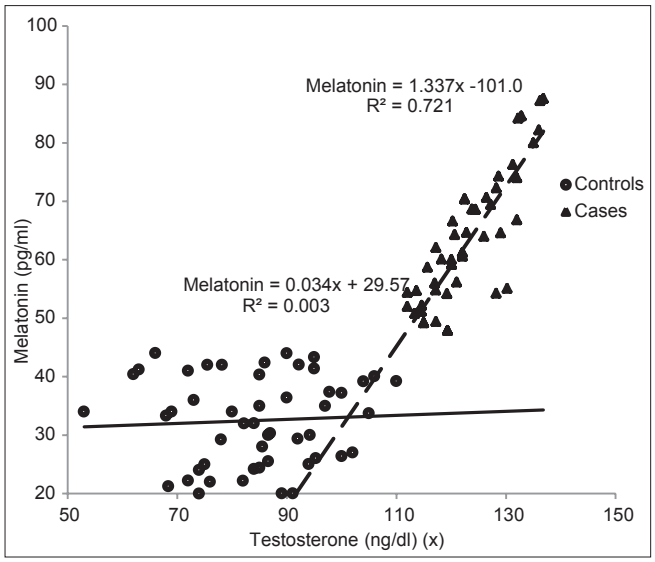

Figure 1: Linear regression of melatonin with testosterone

variable and testosterone as independent variable in both cases and controls. Testosterone explained 72.1% of total variability of melatonin in PCOS cases.

DISCUSSION

In present study, we found that patients with PCOS had significantly raised plasma melatonin levels (> cut off value of 45 pg/mL) as compared with age- and weight-matched controls (*P* < 0.001). Melatonin is normally synthesized in the ovary, as both melatonin synthesizing enzymes AANAT (arylalkylamine N-acetyltransferase) and HIOMT (hydroxyindole-O-methyl transferase) are present in ovarian tissue.[14] Melatonin, synthesized by the ovary, may be released into the follicular fluid. However, the bulk of melatonin detected in the ovary and preovulatory follicular fluid is derived from the circulation.[10] There may be a reduction in the uptake of melatonin from circulation into the ovarian follicles of PCOS cases due to anovulation and increased number of atretic follicles and consequently serum melatonin concentration may increase in PCOS as a feedback response to decreased follicular concentration.[10] Studies done by Luboshitzky *et al*.,[9,15] gave the consistent results of raised plasma melatonin production in PCOS. They found that mean level of urinary 6-sulfatoxymelatonin (αMT6s), a major enzymatic metabolite of melatonin, in their study was 54.0 ± 20.3 μg/24 h (*P* = <0.001). The study carried out by Lima *et al*.,[16] confirmed the development of ovarian cysts in rats similarly to that observed in human PCOS under conditions of melatonin deficiency caused due to pinealectomy or continuous light in female rats.[16]

Melatonin is a documented powerful free radical scavenger and a broad-spectrum antioxidant.[11,12] Atresia is an apoptotic process that is highly regulated by proapoptotic and antiapoptotic factors. In PCOS, ROS generation from mononuclear cells and serum lipid peroxidation products are significantly elevated,[17,18] and levels of antioxidants superoxide dismutase, glutathione peroxidase, catalase get reduced, which ultimately may contribute to oxidative stress mediated apoptosis in atretic follicles.[19] Melatonin has also been shown to regulate the gene expression of antioxidant enzymes superoxide dismutase, glutathione peroxidase, catalase and antiapoptotic/proapoptotic protein Bcl2 and Casp3. Melatonin prevents apoptosis by inducing Bcl2 expression and reducing Casp3 activity.[10] Melatonin also acts as antioxidant by increasing insulin-like growth factor-1[20] and transforming growth factor − beta (TGF−β) production.[21] Normally, insulin-like growth factors act as mitogenic and antiapoptic peptides. Thus, normally the increase in follicular melatonin concentration in the growing follicle could be an important factor in avoiding atresia. The follicle may be rescued by melatonin and this would allow a preovulatory follicle to fully develop and provide an oocyte for fertilization.[10]

Melatonin influences sex steroid production at different stages of ovarian follicular maturation.[10] It (100 mM) has been shown to increase progesterone and androgen production in mouse preantral follicles after incubation for 12 days.[22] and melatonin (100 ng/mL) also stimulates progesterone and androgen production in 30-h cultures of porcine antral follicles without having any effect on estrogen levels.[23] Similarly, the raised serum melatonin level seen our PCOS cases was then found to be positively associated with serum testosterone level (*P* < 0.001). However, it has been seen that melatonin gets decreased in ovarian follicular fluid of PCOS patients and this intrafollicular decrease in melatonin is responsible for follicular atresia because of increased oxidative stress and consequent follicular damage in PCOS.[10] These atretic follicles, escape full maturation and lead to formation of multiple small follicular cysts, surrounded by hyperplastic theca cells. Atretic follicles ultimately contribute to an expanding stroma that increases in volume over time, further increasing the cellular mass producing androgens, sets in another self-propagating cycle that predisposes to chronic anovulation and leads to increased concentration of androgens.[24] Thus, melatonin correlates with hyperandrogenemia and anovulation in PCOS as found in our study (R2 = 0.721). Women with ovarian hyperandrogenism have increased melatonin production only, which remains normal in women with hyperandrogenemia due to idiopathic hirsutism.[9]

Hyperandrogenism is the key feature of PCOS, resulting primarily from excess androgen production in the ovaries and, to a lesser extent, in the adrenals. The primary mechanisms driving increased ovarian androgen production in PCOS include hypersecretion of LH and increased LH

bioactivity, hyperinsulinemia due to insulin resistance and increased volume of theca cells in an expanded ovarian stroma.[24] In our study, serum melatonin was found to be positively associated with serum total testosterone levels, whereas it was inversely correlated with LH: FSH ratio and no correlation has been seen with fasting glucose: insulin ratio. This can be explained by diverse mode of action of melatonin, first by a direct action at receptors MT1 and MT2 leading to alteration in ovarian steroidogenesis and secondly by an action at follicular level as antioxidant.[25]

High level of melatonin in the follicular fluid is essential for follicle growth, ovulation, and oocyte quality, whereas reduced follicular melatonin concentration may be responsible for anovulation and poor oocyte quality in PCOS. Follicles fail to mature fully and become atretic. Small follicles respond poorly to gonadotropins and undergo atresia.[10]

Infertility is a major concern in PCOS patients and important causes of infertility in PCOS are follicular atresia, anovulation, and consequent hyperandrogenemia. Thus, melatonin can be used as a therapeutic agent to treat infertile patients undergoing *in vitro* fertilization in whom infertility occurs due to poor oocyte quality and anovualtion and can create a new ray of hope for infertile patients. Melatonin may become the medicine of choice for improving oocyte quality for women who are unable to become pregnant because of poor oocyte quality.[10,26] Melatonin works as a potent radical scavenger and antioxidant and thus may increase oocyte yield. In future, this may translate into improved oocyte quality as well. However, this needs further research and validation from larger studies.

Lacunae

Being the first study of its kind in India, we did not measure melatonin level in follicular fluid of PCOS patients. Correlation with midnight levels of melatonin was another hindrance in the study.

Scope of study

Future field of research would be to find out correlation between serum melatonin level and the level of antioxidants in PCOS patients. Determination of the quality of oocytes in relation to melatonin would be another area of exploration in patients of PCOS suffering from infertility.

REFERENCES

1. Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853-61.
2. Adams J, Franks S, Polson DW, Mason HD, Abdulwahid N, Tucker M, *et al*. Multifollicular ovaries: Clinical and endocrine features and response to pulsatile gonadotrophin releasing hormone. Lancet 1985;2:1375-9.
3. Hull MG. Epidemiology of infertility and polycystic ovarian disease: Endocrinological and demographic studies. Gynecol Endocrinol 1987;1:235-45.
4. Legro RS. Polycystic ovary syndrome and cardiovascular disease: A premature association? Endocr Rev 2003;24:302-12.
5. Glueck CJ, Papanna R, Wang P, Goldenberg N, Sieve–Smith L. Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. Metabolism 2003;52:908-15.
6. Talbott EO, Zborowskii JV, Boudraux MY. Do women with polycystic ovary Syndrome have an increased risk of cardiovascular disease? Review of the evidence. Minerva Ginecol 2004;56:27-39.
7. Carlomagno G, Nordio M, Chiu TT, Unfer V. Contribution of myo-inositol and melatonin to human reproduction. Eur J Obstet Gynecol Reprod Biol 2011;159:267-72.
8. Kadva A, Djahanbakhch O, Monson J, Di WL, Silman R. Elevated nocturnal melatonin is a consequence of gonadotropin-releasing hormone deficiency in women with hypothalamic amenorrhea. J Clin Endocrinol Metab 1998;83:3653-62.
9. Luboshitzky R, Qupti G, Ishay A, Shen-Orr Z, Futerman B, Linn S. Increased 6-sulfatoxymelatonin excretion in women with polycystic ovary syndrome. Fertil Steril 2001;76:506-10.
10. Tamura H, Nakamura Y, Korkmaz A, Manchester LC, Tan DX, Sugino N, *et al*. Melatonin and the ovary: Physiological and pathophysiological implications. Fertil Steril 2009;92:328-43.
11. Reiter RJ, Tan DX, Maldonado MD. Melatonin as an antioxidant: Physiology versus pharmacology. J Pineal Res 2005;39:215-6.
12. Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, *et al*. Chemical and physical properties and potential mechanisms: Melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem 2002;2:181-97.
13. Tan DX, Manchester LC, Terron MP, Flores LJD, Reiter RJ. One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and reactive nitrogen species? J Pineal Res 2007;42:28-42.
14. Itoh MT, Ishizuka B, Kuribayashi Y, Amemiya A, Sumi Y. Melatonin, its precursors, and synthesizing enzyme activities in the human ovary. Mol Hum Reprod 1999;5:402-8.
15. Luboshitzky R, Shen-Orr Z, Herer P, Nave R. Urinary 6-sulfatoxymelatonin excretion in hyperandrogenic women with polycystic ovary syndrome: The effect of ethinyl estradiol-cyproterone acetate treatment. Gynecol Endocrinol 2003;17:441-7.
16. Prata Lima MF, Baracat EC, Simões MJ. Effects of melatonin on the ovarian response to pinealectomy or continuous light in female rats: Similarity with polycystic ovary syndrome. Braz J Med Biol Res 2004;37:987-95.
17. Gonzalez F, Rote NS, Minium J, Kirwan JP. Reactive oxygen speciesinduced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:336-40.
18. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. Clin Biochem 2001;34:407-13.
19. Gupta RK, Miller KP, Babus JK, Flaws JA. Methoxychlor inhibits growth and induces atresia of antral follicles through an oxidative stress pathway. Toxicol Sci 2006;93:382-9.
20. Schaeffer HJ, Sirotkin AV. Melatonin and serotonin regulate the release of insulin-like growth factor-I, oxytocin and progesterone by cultured human granulosa cells. Exp Clin Endocrinol Diabetes 1997;105:109-12.
21. Liu F, Ng TB, Fung MC. Pineal indoles stimulate the gene expression of immunomodulating cytokines. J Neural Transm 2001;108:397-405.
22. Adriaens I, Jacquet P, Cortvrindt R, Janssen K, Smitz J. Melatonin has

dose-dependent effects on folliculogenesis, oocyte maturation capacity and steroidogenesis. Toxicology 2006;228:333-43.
23. Tanavde VS, Maitra A. *In vitro* modulation of steroidogenesis and gene expression by melatonin: A study with porcine antral follicles. Endocr Res 2003;29:399-410.
24. Fritz MA, Speroff L. Chronic Anovulation and the Polycystic Ovary Syndrome. In, Clinical Gynecologic Endocrinology and Infertility, 8th ed. New Delhi: Wolter Kluwer Pvt Ltd; 2011. p. 497-99
25. Woo MM, Tai CJ, Kang SK, Nathwani PM, Pang SF, Leung PC. Direct action of melatonin in human granulosa-luteal cells. J Clin Endocrinol Metab 2001;86:4789-97.
26. Tamura H, Takasaki A, Taketani T, Tanabe M, Kizuka F, Lee L, *et al*. The role of melatonin as an antioxidant in the follicle. J Ovarian Res 2012;5:5.

How to cite this article: Jain P, Jain M, Haldar C, Singh TB, Jain S. Melatonin and its correlation with testosterone in polycystic ovarian syndrome. J Hum Reprod Sci 2013;6:253-8.
Source of Support: Nil, **Conflict of Interest:** None declared.