World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Melatonin levels in the follicular fluids and its correlation with ICSI Outcomes

Salim Abd Mohammed Ghanim [1,*] and Neamat Abdulkadhim Obaida [2]

[1] *Department of Biochemistry, Alkindi Teaching Hospital, Baghdad, Iraq,*
[2] *Department of Infertility and Assisted Reproductive Techniques, Kamal Alsamarai Teaching Hospital, Iraq, Baghdad.*

World Journal of Biology Pharmacy and Health Sciences, 2024, 20(01), 409–413

Publication history: Received on 04 September 2024; revised on 15 October 2024; accepted on 17 October 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.20.1.0746

Abstract

Background: Melatonin is a hormone mainly synthesized and secreted from the pineal gland. The daily rhythm of circulatory melatonin is released in very low concentrations during the day and high levels at night. Melatonin has many functions beside regulation of circadian rhythm including immune system and autonomic cardiovascular regulation but the main melatonin function is antioxidant action and detoxification of free radicals.

Patients and method: This cross sectional study was conducted during the period starting from November 2018 to May 2019. Eighty infertile female aged range 18–46 years were enrolled in the present study, the patients were undergoing controlled ovarian induction for IVF /ICSI treatment. All patents were divided into 3 groups according to age, FSH, E2, AMH and ovarian respond to controlled ovarian stimulation (COS) protocols. The follicular fluid melatonin measured at oocyte retrieval day using enzyme linked immunosorbent assay (ELISA).

Results: There was significant difference in follicular fluid melatonin level between the three studied groups (p=0.031). There was also a positive significant correlation between follicular fluid melatonin with MII oocytes (r=0.343; p= 0.024), grade I embryo (r=0.375; p= 0.003) and AMH (r = 0.279; p= 0.027), on the other hand there was a negative significant correlation between follicular fluid melatonin and serum FSH (r = - 0.331; p= 0.016). There was also no significant difference of follicular fluid melatonin levels between pregnant and non-pregnant females (p=0.083).

Conclusion: Melatonin levels in follicular fluid improve the oocytes and embryo qualities but have no effect in positive pregnancy rate.

Keywords; Melatonin; Infertility; ICSI (Intracytoplasmic sperms injection); IVF; Metaphase oocytes; Embryos grading

1. Introduction

Infertility is a complex disorder with serious medical, psychosocial, and economic problems and defined as failure to achieve a successful pregnancy within 12 months or more of regular unprotected sexual intercourse. Earlier evaluation should be achieved after six months without conception for women who are older than 35 years because of the age-related decline in fertility (1).

Follicular fluids contain proteins, sugars, hormones, growth factors, reactive oxygen species and other factors (2). Melatonin in the follicular fluid is responsible for a very important microenvironment in which the oocyte matures (3). Therefore; follicular fluids content may have an important role in the prediction of the successful rate of Assisted Reproductive Technology (ART) (4).

* Corresponding author: Salim Abd Mohammed Ghanim

Formerly, melatonin was known as a hormone, produced by the pineal gland, with the ability to modulate the circadian and reproductive physiology in photoperiod-dependent, seasonally breeding mammals but in 1993 it's discovered to be a potent endogenous scavenger of free radicals (5). In present, it is known that melatonin through its receptors in the suprachiasmatic nuclei of the brain and in pars tuberalis in the pituitary gland acts to modulate the reproductive function, furthermore the distribution of melatonin receptors in many other tissues of the reproductive system e.g. epididymis, vas deferens, prostate, ovary, mammary gland and in other tissues including the gastrointestinal tract, skin, liver, spleen kidney and immune system cells. These findings may relate to a hypothesis of multiple melatonin functions (antioxidant, anti-inflammatory properties, genomic stabilizing effects, and modulator of mitochondrial homeostasis) (6).

During IVF induction protocols and oocyte manipulation, oocyte and embryos may be exposed to high levels of superoxide free radicals with alteration of the level of endogenous oxygen scavengers (7). The culture media has no antioxidants unlike the follicular fluids, including melatonin which protects oocyte from oxidative stress (8).

2. Materials and Methods

All the patients undergo a controlled ovarian hyperstimulation for IVF/ICSI treatment, the exclusion criteria are smoking, major medical illness, ovarian tumors and chronic use of any medication.eg beta blocker and glucocorticoid

The patients were divided into three groups:

- Group I normal responders: Normal ovarian response was defined as follows: Age < 35 years, and normal ovarian reserve test (FSH level < 10 IU/ml and AMH level 1.5–4.0 ng/ml).
- Group II poor responders: Poor ovarian response is defined by the presence of two of the following features; either advanced maternal age (≥40 years) or an abnormal ovarian reserve test (i.e. antral follicle count < 5–7 follicles, or AMH level 0.5–1.1 ng/mL).
- Group III high responders: High ovarian response was defined as follows; either the number of retrieved oocytes is more than 15 or the number of follicles (>12–14 mm) is more than 20 or the serum E2 level is higher than 5000μU/mL in controlled ovarian hyperstimulation.

According to Ovarian Induction Protocols (OIP) seventy eight patients were treated with GnRH antagonist and another eleven infertile females were treated with GnRH long agonist.

In the 2 protocols transvaginal ultrasound guided oocyte retrieval was done 34 -36 hours following the HCG injection.

At the day of ova picked up, the follicular fluid were aspirated, then all follicular fluids samples were centrifuged for 10 min at 3000 rpm and aliquots of the supernatants were stored at –20°C until melatonin assay by ELISA. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 22.0. The study groups were compared by independent sample t test, chi square and one way ANOVA. The degree of association between variables was calculated by Pearson's correlation coefficient and *p* value equal to or less than 0.05 was considered to be statistically significant.

3. Results

The patients in the present study were divided into 3 groups; normal responder, poor responder and high responders (35, 22 and 23 females respectively).

The comparisons of clinical data between the studied groups were presented in table 1, the results were revealed a significant differences concerning female's age (p=0.002), FSH levels (p=0.001) and AMH levels (p=0.001). There was also a significant differences between normal, poor and high responders regarding follicular fluids melatonin (61.29 ± 20.19 vs. 51.54 ± 11.97 vs.52.14 ± 13.4; p=0.031); however there were no significant differences between the three studied groups regarding to the BMI (p=0.321), LH (p=0.411), E2 (p=0.385) and prolactin levels (p=0.202).

The correlations between follicular fluids melatonin with patient's age, FSH levels, AMH levels and ICSI outcomes were demonstrated in table 2; according to the results there were a significant positive correlation between melatonin with total oocytes count (r=0.311 ; p=0.012) MII oocytes (r=0.343 ; p=0.024), grade I embryo (r=0.375 ; p=0.003) and AMH levels (r=0.279 ; p=0.027); on the contrary, melatonin showed a significant negative correlation with serum FSH (r= -0.331 ; p=0.016); however there were insignificant correlations between follicular fluids melatonin with patient's age, MI oocytes, fertilization rate, grade II embryos and grade III embryos.

Table 1 Comparison of clinical data between the studied groups

Parameters	Normal responder N.=35	Poor responder N.=22	High responder N.=23	*p* value
Age (years)	29.55 ± 5.31	35.89 ± 7.45	27.75 ± 5.53	0.002
BMI (Kg/m^2)	26.16 ± 4.13	28.64 ± 4.32	25.29 ± 3.61	0.321
F.F.Melatonin ng/ml	61.29 ± 20.19	51.54 ± 11.97	52.14 ± 13.4	0.031
FSH mU/l	6.52 ± 3.22	9.42 ± 4.42	6.09 ± 2.31	0.001
LH mU/l	4.60 ± 2.36	5.83 ± 3.41	5.12 ± 2.09	0.411
E2 mU/l	36.99 ± 23.09	28.99 ± 25.32	32.08 ± 11.28	0.385
AMH mU/l	2.23 ± 1.19	1.63 ± 1.94	3.66 ± 1.17	0.001
Prolactin mU/l	22.01 ± 10.87	20.86 ± 10.56	18.75 ± 13.89	0.202

BMI: Body mass index; F.F: Follicular fluids; FSH: Follicular stimulating hormone; LH: Luteinizing hormone; AMH: Antimullerian hormone

Table 2 Correlation between follicular fluids melatonin with patient's age, FSH, AMH and ICSI outcome

Parameters	Pearson's correlation coefficient	*p* value
Age	- 0.187	0.089
FSH	- 0.331	0.016
AMH	0.279	0.027
Retrieved oocytes count	0.311	0.012
MI	-0.009	0.899
MII Oocyte	0.343	0.024
Fertilization rate	0.163	0.174
grade I embryo	0.375	0.003
grade II embryo	0.168	0.298
grade III embryo	-0.157	0.364

FSH: Follicular stimulating hormone; AMH: Antimullerian hormone; MI: Metaphase I; MII: Metaphase II

Seventeen females out of 80 females were became pregnant (Pregnancy rate =21.25%). The follicular fluids melatonin levels were insignificantly higher among pregnant females; however there was no significant differences of melatonin levels between pregnant and non-pregnant females (58.36 ± 16.14 vs. 53.68 ± 18.56; *p*=0.083) as illustrated in table 3.

Table 3 Comparison of melatonin levels between pregnant and non-pregnant females

Parameters	Pregnant females N.=17	Non-Pregnant females N.=63	*p* value
Follicular fluids melatonin ng/ml	58.36 ± 16.14	53.68 ± 18.56	0.083

4. Discussion

Melatonin levels in the follicular fluid displayed good correlations with MII oocytes (r =0.343, *p*=0.024) and this agree with Jing Tong *et al*. (10) which also showed a significant positive correlation between MII oocyte and follicular fluid melatonin. Other studies (12,11) also demonstrate an improvement of oocytes numbers and quality after oral melatonin

supplements such as in batioglu *et al*. (12) which demonstrated higher percentage of MII in oocytes in melatonin group versus control group, Erlimaz *et al* (11) also demonstrated even higher MII oocytes in melatonin groups versus control groups (11.5 vs. 6.9). Bahia *et al.* (14) also demonstrate a significantly higher in the number of the patients who had mature MII oocytes among the women who received melatonin supplements (14).

Some studies showed that melatonin supplementation reduces the intra-follicular concentrations of eight-hydoxy-20-deoxyguanosine (8-OHdG), which is a sensitive indicator of DNA damage as the result of oxidative stress (11). In the present study there was also a significant correlation between follicular fluid melatonin and embryo quality. Jing tong *et al.* (10) revealed a very significant correlation between follicular fluid melatonin and top quality or grade I embryo (10). The use of in vitro melatonin on embryo culture has been shown to cause a reduction of oxidative stress and apoptosis. Indeed, scientific studies demonstrated the ability of melatonin to reduce oxidative stress and apoptosis, increasing Glutathione levels and produce a protective effect on embryos (14).

Few studies (12,11) also showed a good embryo quality in patients taking oral melatonin supplements, Batioglu *et al* (12) and Nishihara *et al* (11) demonstrated a higher number of grade I embryos in patients received 3mg melatonin supplement.

Regarding the fertilization rate and pregnancy rate there was no significant correlation between follicular fluid melatonin and fertilization and pregnancy rate. Tamura *et al.* (13) investigated the role of melatonin supplementation in patients with failure of pregnancy to in a previous cycle of IVF and embryo transfer, with a fertilization rate of less than 50% and in the next IVF cycle they used a dose of 3 mg/day from day 5 of the menstrual cycle until oocyte retrieval (13). The fertilization rate was significantly higher in the melatonin group when compared with their first cycle, while in other study design with controls there was no differences in fertilization and pregnancy rate. Erlymaz *et at.* (11) and Batioglue *et al* (12) showed no differences in fertilization rate and clinical pregnancy rate after melatonin oral supplements. Also Nishihara *et al* (11) demonstrate a significant number of fertilized oocytes without increasing pregnancy rate. It's clear that there is no correlation between follicular fluid melatonin and fertilization and pregnancy rate, but oral melatonin supplements may improve fertilization rate only without affecting pregnancy rate. The failure of increasing pregnancy rate in our study may be explained due to male factor effects e.g. abnormal spermatogenesis i.e. azozoospermia, oligozoospermia, asthenozoospermia and teratozoospermia.

The ovarian reserve associated with both quantity and quality of oocytes and reflects the outcomes of IVF as a result (12). Many methods are used in the assessment of ovarian reserve in order to predict the outcome in IVF including age, FSH level and AMH level but there is not useful predictive test assess the ovarian reserve accurately (12).

In the present study there was a good positive correlation between the melatonin levels in follicular fluid and AMH and a negative correlation with FSH. The goals in IVF good outcome is the optimization of AMH with minimization of FSH. Walecka-Kapica *et al.* (14) and Jing Tong *et al* (10) demonstrate a significant correlation between follicular fluid melatonin and plasma AMH and FSH levels; there was also a significant correlation with MII oocytes and top quality embryo (10). AMH and melatonin are produced exclusively by granulosa cells of secondary preantral and small antral follicles in the ovary the (10) and this may be explaining the relationship between melatonin and AMH level.

Bahia *et al.* (14) assessed the effect of oral melatonin supplements on a diminished ovarian reserve between case and control groups and the results showed higher number of MII oocytes in case group without reaching statistical significance but mature MII and top quality embryo significantly different(14). As the age is reflecting the ovarian reserve, so melatonin levels in the follicular fluid may be used as a markers of ovarian reserve and its level diminished with increasing the patients age, possibly because of the dysfunction of ovarian granulosa cells and pineal gland (12).

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Funding

The authors declare that there is no funding.

References

[1] Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertility and Sterility 2013; 99(1):63 https://www.ncbi.nlm.nih.gov/pubmed/32115183 .

[2] Fahiminiya S, Gerard N. Follicular fluid in mammals. Gynecologie, Obstetrique & Fertilite 2010;38(6):pp.402-404. https://www.sciencedirect.com/science/article/abs/pii/S1297958910001487

[3] Revelli A, Piane L, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reproductive Biology and Endocrinology 2009; 7: 40. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2685803/

[4] Bedaiwy MA, Elnashar SA, Goldberg JM, Sharma R, Mascha EJ, Arrigain S, Agarwal A, Falcone T. Effect of follicular fluid oxidative stress parameters on intracytoplasmic sperm injection outcome. Gynecological Endocrinology 2012; 28(1): pp.51–55. https://www.researchgate.net/publication/307915753_Antioxidants_reduce_oxidative_stress_in_follicular_fluid_of_aged_women_undergoing_IVF

[5] Salvatore Giovanni Vitale, Paola Rossetti, Francesco Corrado, Agnese Maria Chiara Rapisarda, Sandro La Vignera, Rosita Angela Condorelli, Gaetano Valenti, Fabrizio Sapia, Antonio Simone Laganà and Massimo Buscema , International Journal of Endocrinology;Volume 2016, ArticleID4987436.https://www.hindawi.com/journals/ije/2016/4987436/

[6] D. Acu˜na-Castroviejo, G. Escames, C. Venegas et al., "Extrapineal melatonin: sources, regulation, and potential functions,"Cellular and Molecular Life Sciences, vol. 71, no. 16, pp. 2997– 3025, 2014.https://www.researchgate.net/publication/260271540_Extrapineal_melatonin_Sources_regulation_and_potential_functions

[7] S. Palini, S. Benedetti, M. C. Tagliamonte et al., "Influence of ovarian stimulation for IVF/ICSI on the antioxidant defence system and relationship to outcome," Reproductive BioMedicine Online, vol. 29, no. 1, pp. 65–71, 2014. https://www.sciencedirect.com/science/article/abs/pii/S1472648314001771

[8] S. Fernando and L. Rombauts, "Melatonin: shedding light on infertility? A review of the recent literature," Journal of Ovarian Research, vol. 7, article 98, 2014. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4209073/

[9] Shavi Fernando and Luk Rombauts, Melatonin: shedding light on infertility? - a review of the recent literature 2014 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4209073/

[10] Jing Tong, Shile Sheng, Yun Sun, Huihui Li, Wei-Ping Li, Cong Zhang and Zi-Jiang Chen, Melatonin levels in follicular fluid as markers for IVF outcomes and predicting ovarian reserve2017 https://rep.bioscientifica.com/view/journals/rep/153/4/443.xml

[11] Takuji nishihara and hashimoto chu Article in Gynecological Endocrinology · March 2014 Vol.30,No.5, pp. 359-362. https://www.tandfonline.com/doi/abs/10.3109/09513590.2013.879856

[12] Hossam I Abdalla, FRCOG,London, U.K., Assessment of ovarian reserve prior to IVF,Middle East Fertility Society Journal Vol. 12, No. 1, 2007. https://tspace.library.utoronto.ca/bitstream/1807/39527/1/mf07003

[13] Tamura, H.; Takayama, H.; Nakamura, Y.; Reiter, R.J.; Sugino, N. Fetal/ placental regulation of maternal melatonin in rats. J. Pineal Res. **2008**, 44, 335–340. https://scholars.uthscsa.edu/en/publications/fetalplacental-regulation-of-maternal-melatonin-in-rats

[14] Bahare Salehi , Farukh Sharopov , Patrick Valere Tsouh Fokou , Agnieszka Kobylinska ,Lilian de Jonge , Kathryn Tadio , Javad Sharifi-Rad , , Malgorzata M. Posmyk ,, Miquel Martorell , Natália Martins , and Marcello Iriti : Melatonin in Medicinal and Food Plants: Occurrence, Bioavailability, and Health Potential for Humans,2019 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6678868/