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MEDICALLY ASSISTED PROCREATION

The efficacy of melatonin administration on oocyte quality

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The aim of the study was to evaluate the efficacy of melatonin administration on oocyte quality in women underwent *in vitro* fertilization (IVF) cycles. Eighty-five women undergoing IVF cycles were randomized in two groups during IVF–embryo transfer (ET) procedure, 40 women with melatonin treatment (A) and 45 women without melatonin treatment (B). Primary endpoint was the number of morphologically mature oocytes retrieved (MII oocytes). Secondary endpoints were fertilization rate per number of mature oocytes, embryo quality and pregnancy rate. There were no differences between two groups according to age, and peak estradiol levels. The mean number of oocytes (15.33 vs. 14.27) and the mean number of mature oocytes did not differ between the two groups (12.63 vs. 10.94), whereas the percentage of mature oocytes (M2/oocytes retrieved) was significantly different in melatonin-treated group ($p < 0.05$). Fertilization rate (72.75 vs. 71.16) did not differ between the two groups. The mean number of class 1 embryos resulted higher in the group A (3.28 vs. 2.53) ($p < 0.05$). Clinical pregnancy rate was in tendency higher in the group treated with melatonin, although the differences did not reach statistical significance. Melatonin is likely to improve oocyte and embryo quality in women undergoing IVF or intracytoplasmic sperm insemination (ICSI).

Keywords: Assisted reproductive technology, melatonin, ovulation induction

Introduction

It is estimated that one in five couples of reproductive age experience some form of fertility problem, and up to half of those may require treatment. Currently, the most advanced and successful treatment option available to these couples is *in vitro* fertilization (IVF).

The availability of assisted reproductive technology (ART) and delivery rates per oocyte retrieval vary greatly between countries. Per aspiration, pregnancy rates range from 17% to 42% and delivery rates from 8% to 34% [1].

In couples with repeated implantation failures, a comprehensive investigation and many therapeutic strategies were offered. These include hysteroscopy, endometrial injury, change in the stimulation protocol, blastocyst transfer, assisted hatching and preimplantation genetic screening for aneuploidy, vitamin and antioxidants supplementation [2–4].

The quality of oocytes plays a key role in the development of a clinical pregnancy. In humans, in fact oocytes of poor quality may be the cause of women infertility and an important obstacle in successful IVF [5].

Despite great advances in ART, poor oocyte quality remains a profound problem for female infertility. Reactive oxygen species (ROS) are produced within the follicle, especially during the ovulatory process [6]. It is believed that oxidative stress may be a cause of poor oocyte quality. The role of ROS and antioxidants in relation to female reproductive function has been a subject of recent research interest [6,7]. Reduced levels of glutathione peroxidase (GSH-Px) are reported in the follicular fluid of women with unexplained infertility [8]. Yang et al. [9] found higher levels of the oxidant, H_2O_2 in fragmented embryos compared with non-fragmented embryos, whereas Paszkowski and Clarke [10] reported the elevated consumption of antioxidants, which suggests increased ROS levels, during incubation of poor-quality embryos. Several antioxidant enzymes protect oocytes and embryos against oxidative stress; these enzymes include superoxide dismutase (SOD), catalase and GSH-Px [11]. Cu,Zn-SOD and Mn-SOD dismutate the superoxide anion radical to the non-radical species, H_2O_2. Melatonin as well as its metabolites are potent direct free radical scavengers [12–15] and indirect antioxidants by virtue of their ability to modulate gene transcription for antioxidant enzymes [16].

The aim of this study was to evaluate the efficacy of a treatment with melatonin compared with control on oocyte quality in women underwent IVF cycles.

Materials and methods

Starting on the day of GnRH administration, 85 women undergoing IVF cycles were randomized in two groups using a computer-assisted 1:1 randomization. During the next IVF–embryo transfer (ET) procedure, 40 women with melatonin treatment (3 mg/day) and 45 women without melatonin treatment, no blinding procedure could be adopted toward both patients and doctors. Embryologists were "blind" as they did not know to which treatment arm were the patients assigned.

Primary endpoint was number of morphologically mature oocytes retrieved (MII oocytes). Secondary endpoints were fertilization rate per number of mature oocytes, embryo quality and pregnancy rate.

Inclusion criteria

Patients, who were at the primary infertility age, 20–40 years, were included. All patients had regular menstrual cycles (21–35 days), had no hormonal or non-hormonal therapy at least for the last 3 months and had no systemic illness.

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Table I. Demographic characteristics and *in vitro* fertilization outcomes.

Variables	Melatonin (−) group	Melatonin (+) group	*p* Value
Variable numbers	45	40	
Age	29.7 ± 4.9	30.4 ± 5.6	0.495[a]
Peak E_2	2102.5 ± 872.96	2171.0 ± 1041.09	0.739[a]
Oocyte count	14.27 (4–25)	15.33 (4–27)	0.666[b]
Mature oocyte count	10.9 ± 4.0	12.0 ± 6.0	0.139[a]
Percentage of mature oocyte	75.8% (31–100)	81.9% (28–100)	0.034[b]
Fertilized oocyte count	7.8 (2–16)	9 (2–17)	0.213[b]
Percentage of fertilized oocyte	71% (22–100)	72.7% (29–100)	0.870[b]
G1 embryo	2.5 (0–5)	3.2 (0–6)	0.035[b]
Percentage of G1 embryo	30.4% (0–41)	33.7% (0–50)	0.004b
Result			
No pregnancy	20 (44.4%)	14 (35.0%)	0.513c
Chemical pregnancy	7 (15.6%)	6 (15%)	
Clinical pregnancy rates	18 (40.0%)	20 (50%)	

[a]Student's *t*-test.
[b]Mann–Whitney *U* test.
[c]Pearson Ki-Kare test.

Patients with serious endometriosis (American Fertility Society Stage III vs. IV), serious male factor (azoospermia), hypogonatropic hypogonadism, FSH >13 were excluded from the study. All couples had previously been evaluated by Day 3 FSH level, estradiol levels and pre-ovulatory ultrasound evaluation of antral follicles count, hysterosalpingography or ofis hysteroscopy and semen analysis. Motile ejaculate sperms were used for all patients. Patients with the cycles cancelled were excluded from the study.

Follicle stimulation protocol

All the women enrolled were down-regulated with a GnRH agonist (Lucrin daily. Abbott, Turkey) from mid-luteal phase onward and, when optimally down-regulated, were stimulated with rec-FSH (Puregon; MSD, Turkey) with a starting dose of 150 IU/day. The FSH dose was adjusted according to the individual response. Follicular size was monitored regularly by ultrasound and serum estradiol assays. Rec-hCG (Ovitrelle 250, Serono, Turkey) was administered when average diameter of the leading follicles reached at least 18 mm. Cycle was cancelled if E2 level was >4000 pg/mL because of high risk for ovarian hyperstimulation syndrome.

IVF procedure

Oocyte retrieval was performed 36 h after rec-hCG injection with transvaginal guidance. Cumulus and corona radiate cells were immediately removed 2 h after retrieval by a short exposure to Hyase10X (Vitrolife; IVF Science, Sweden) medium and gentle aspiration in and out of a Pasteur pipette and mechanically cleaned from the remaining surrounding cumulus cells by aspiration using a denuding pipette (Swemed Denudation Pipette) with a 130–133 μm diameter.

The denuded oocytes were then assessed for their meiotic maturation status. In preparation for IVF, oocytes with an extruded first polar body presumably at the metaphase II stage (MII) were selected.

Embryo quality classification

The embryos were classified according to the criteria proposed by Veeck's morphological criteria [17] as follows: grade 1, equally sized blastomeres with no fragmentation; grade 2, equally or unequally sized blastomeres with <20% overall fragmentation; grade 3, equally or unequally sized blastomeres with 20–50% fragmentation and grade 4, equally or unequally sized blastomeres with >50% fragmentation. Embryo quality was assessed before the transfer that occurred in all patients at ~48 h (four-cell stage) after insemination.

Statistical analysis

Statistical analysis was carried out using the computer program SPSS for windows 11.5. Shapiro Wilk, Student's *t*-test, Mann–Whitney *U* test, Pearson Ki-Kare test were used as appropriate. A value of $p < 0.05$ was considered significant.

Results

There were no differences between two groups according to age and peak estradiol levels.

The mean number of oocytes retrieved (15.33 vs. 14.27) and the mean number of mature oocytes did not differ between the two groups. Although the group treated with melatonin reported a greater mean number of mature oocytes (12 ± 6 vs. 11 ± 4), the percentage of mature oocytes (M2/oocytes retrieved) was significantly different in melatonin-treated group ($p < 0.05$).

Fertilization rate (72.75 vs. 71.16) did not differ between the two groups. The mean number of embryos of top quality (class 1) resulted higher in the group A (3.28 vs. 2.53) ($p < 0.05$).

A total of 38 pregnancies were obtained (20 in group A and 18 in group B). Clinical pregnancy rate was in tendency higher in the group treated with melatonin, although the differences did not reach statistical significance. Biochemical pregnancy rate was similar in both groups (Table I).

Discussion

A number of the treatment modalities used as adjuncts to ovulation induction claim to increase the pregnancy rate. Medications include aspirin, glucocorticoids, growth hormone (GH), dehydroepiandrosterone (DHEA), sildenafil, heparins and intravenous immunoglobulin (IV Ig) and antibiotics [18].

The use of adjuvant therapy may improve the outcome of IVF, and may be particularly beneficial for women with a history of repeated IVF failure.

In a study, Rizzo et al. [19] evaluated the efficacy of a treatment with myoinositol plus folic acid plus melatonin compared with myoinositol plus folic acid alone on oocyte quality in women underwent IVF cycles. The group co-treated with melatonin reported a significantly greater mean number of mature oocytes. The mean number of embryos of top quality resulted higher in the group A. Fertilization rate did not differ between the two groups. Clinical pregnancy rate and implantation rate were in tendency higher in the group co-treated with melatonin, although the differences did not reach statistical significance. They emphasized that melatonin ameliorated the activity of myoinositol and folic acid by improving oocyte quality and pregnancy outcome in women with low oocyte quality history [19].

Tamura et al. [20] investigated the relationship between oxidative stress and poor oocyte quality and whether the antioxidant melatonin improved oocyte quality. Follicular fluid was sampled at oocyte retrieval during IVF–ET. Patients who failed to become pregnant in the previous IVF–ET cycle were divided into two groups during the next IVF–ET procedure; 56 patients with melatonin treatment (3 mg/day) and 59 patients without melatonin treatment. In conclusion, they emphasized that the fertilization rate was improved by melatonin treatment compared with the previous IVF–ET cycle [20].

Our results are in line with other studies, suggesting the positive effect that melatonin plays on oocyte and embryo quality, and pregnancy outcome.

Declaration of interest

The authors report no declarations of interest.

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