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Stress in couples undergoing assisted reproductive technology

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Abstract

Purpose To determine the associations between pre-treatment self-reported stress level, salivary cortisol levels, and clinical pregnancy outcome in couples undergoing assisted reproductive technology treatment (ART).

Study design Seventy-five couples (150 patients) undergoing ART treatment were enrolled in this study. Psychological variables were assessed using the Perceived Stress Scale, Beck Anxiety Inventory, State–Trait Anxiety Inventory, and Beck Depression Inventory. Salivary cortisol levels were obtained from each couple prior to commencing gonadotropin treatment at several times (upon waking and at 15, 30, and 60 min after waking).

Results There was no statistically significant association between the self-reported stress levels and the ART treatment outcome in couples. Women with a successful outcome after ART treatment had higher median salivary cortisol levels than women who had an unsuccessful result [24.7 (19.9–63.1) vs. 20.7 (10.4–30.4), respectively]. There was no statistically significant difference in the median salivary cortisol levels of men in relation to ART treatment outcome. Salivary cortisol levels of the couples were not correlated.

Conclusions Women who had higher median salivary cortisol levels in the pre-treatment period had a higher clinical pregnancy rate. This result suggests that moderately increased activity of the hypothalamic–pituitary–adrenal axis during ART treatment might be associated with successful conception.

Keywords Assisted reproductive technology · Salivary cortisol · Psychology · Stress

Introduction

Infertility as an important determinant of the course of one's life and often gives rise to unpredicted stressors and depressors in couples [1]. Infertile couples may experience negative changes in their family relationships and social interactions. Assisted reproduction technology (ART), such as in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI), is an additional stress, being physically rigorous, emotionally difficult, and financially costly [2].

The glucocorticoid cortisol, a biological concomitant of stress, plays a major role in stress regulation via two main pathways: the hypothalamic–pituitary–adrenal (HPA)/hypothalamic–pituitary–gonadal (HPG) axis and HPA–immune interactions [3, 4]. Cortisol has an effect at the level of both the hypothalamus and the gonads. It inhibits the release of gonadotrophin-releasing hormone (GnRH) and the secretion of GnRH-induced luteinizing hormone [3]. It also acts directly at the level of the gonads via the suppression of steroid hormone production or glucocorticoid-induced apoptosis [3]. As regards the HPA–immune pathway, cortisol may alter the activity of the immune system, which plays an important role in reproductive physiology [4]. Cortisol awakening response (CAR) describes increased cortisol release in the first hour following awakening in the morning [5]. It is measured non-invasively in the saliva [6] and represents the

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activity of the HPA axis. The specific role of CAR is still unknown [7], but it has been associated with stress and anxiety [8] and depressive symptoms [9]. Therefore, its measurement during ART might represent an objective assessment of stress, anxiety, and depression.

There is growing evidence that psychological factors (such as anxiety and depression) are also associated with ART outcomes. Some studies have reported that stress has an adverse effect on fertility and that a reduction in stress levels increases pregnancy rates [10, 11]. By contrast, some studies found no association between anxiety and infertility [12, 13]. Although some studies have investigated the association between psychological factors and ART outcomes in women, few studies have considered the psychological factors of couples (females and males) in relation to ART outcomes [2].

The present study aimed to determine the prevalence and severity of anxiety/depression in relation to gender differences and to the underlying etiologies of infertility in women who underwent ART treatment. It also aimed to determine the impact of anxiety/depression on the ART treatment outcome.

Materials and methods

Study subject

Seventy-five couples with diagnosed infertility who underwent IVF/ICSI treatment at the Inonu University School of Medicine's Division of Reproductive Endocrinology and Infertility between January 2016 and June 2017 enrolled in this study. Two reproductive endocrinologists (A.K., G.T.) evaluated the couples, who were also visited by one psychologist (I.R.). After a signed informed consent form was obtained, couples were requested to complete surveys on psychological variables and provide salivary samples for cortisol analyses before the start of gonadotropins in GnRH antagonist cycle (first day of their ART cycle). The local Ethical Committees of the Institutions approved this study (Approval no. 2017/127).

All the couples were primary infertile and prepared for the first ART treatment cycle. Only couples (women and men) with a body mass index of 20.0–29.9 kg/m^2 were enrolled in this study. The exclusion criteria eliminated subjects with untreated endocrine disorders (such as diabetes mellitus, hypo- or hyper-thyroidism, hyperprolactinemia, Cushing's disease or Addison's disease), those concurrently taking a corticosteroid, those with psychiatric diseases according the ICD-10 classification of mental and behavioral disorders [history of (or current) schizophrenia, schizotypal, delusional disorder], and patients receiving psychotherapy.

Diagnosis of the female factor was made by the assessment of ovulation tests, a hysterosalpingogram, and an ovarian reserve test [including day 3 serum follicle stimulating hormone (FSH) level and antral follicle count (AFC)]. Female factor infertility was defined as women with ovulatory disorders, endometriosis, tubal factor, and poor ovarian reserve. The definition of the male factor was based on WHO criteria 2010 [14]. At least two semen analyses within minimum 1 month apart were evaluated. The average of the separate semen analysis parameters was used. A diagnosis of unexplained infertility was made after the above-recommended testing failed to reveal any abnormalities. To illuminate the effect of the underlying cause of infertility on stress, couples were asked whether the causes of infertility were caused by them individually. Only couples defined as male factor were included in the study if they knew that the cause of infertility was due to the male partner and only couples defined as female factor were included in this study if they knew that the cause of infertility was due to the female partner. If the two partners do not know that the cause of infertility is due to both of them, we defined these couples as unexplained. Couples carrying male and female factors together as the cause of infertility were excluded from the study.

Clinical pregnancy was determined by the presence of a fetal heartbeat 6–7 weeks after embryo transfer.

Measures

Two psychological measurements of stress were evaluated in this study: anxiety and depression. Anxiety was assessed using the Turkish version of the Perceived Stress Scale, the Beck Anxiety Inventory, and the State–Trait Anxiety Inventory (STAI-I and STAI-II). Depression was estimated using the Turkish version of the Beck Depression Inventory. All scales have shown acceptable reliability and validity.

Cortisol analysis

Four salivary samples (upon waking and at 15, 30, and 60 min after waking) were collected from the study participants for cortisol assessment. The protocol and procedures of this study were explained to the participants before the commencement of sampling. Saliva samples were collected according to the method of Ozgocer et al. [5] following expert-consensus guidelines [15]. Polypropylene tubes (1.5 mL, ISOLAB, Germany) were labeled with both participants' IDs and saliva sampling time (e.g., 0, 15, 30, or 60 min). Saliva samples were collected by the passive drool method and were used to determine the cortisol awakening response (CAR), area under curve with respect to ground (AUCg), and area under curve with respect to increase (AUCi) according to the protocol described in previous

studies [6, 16]. At the time of saliva collection, the couples were warned to avoid drinking, eating, and tooth brushing in the first 30 min, and avoid drinking milk or coffee in the first 60 min after waking up. Saliva samples were collected on the same day on which they arrived at the facility and were kept at − 20 °C until analysis.

Saliva samples were analyzed for cortisol levels by an enzyme-linked immunosorbent assay (ELISA) developed in our laboratory.

Statistical analysis

All statistical analyses were conducted using the SPSS program (version 11.0 for Windows, SPSS, Chicago, IL, USA). Data are reported as mean ± standard deviation (SD) for normal distributed parameters and median (min–max) for abnormal distributed parameters. Student's *t* test was used for parametric data sets and Mann–Whitney *U* test for non-parametric data sets. The Pearson χ^2 and Fisher exact tests were used for categorical data. The salivary cortisol levels were not normally distributed, and log transformations were conducted prior to analysis. Multivariable modeling was employed to determine which characteristics were associated with clinical pregnancy. In this model, we included age, BMI, cigarette smoke, serum FSH, and salivary cortisol level. A p value ≤ 0.05 was considered statistically significant.

Results

The sociodemographic characteristics of the study participants are shown in Table 1. The mean age of the women was 32.9 ± 4.7, and that of the men was 33.9 ± 3.9. All study participants had no pregnancy history prior to the treatment, and all of them were undergoing ART treatment for the first time. The average duration of infertility was 5.25 years (min: 2.5 years–max: 19 years). There was no statistically significant difference between women with positive ART results and negative ART results regarding age, BMI, duration

Table 1 Clinical data and treatment cycle summaries of study participants

	Pregnancy (+) $n=23$	Pregnancy (–) $n=52$	p value
Female age (years)	30.9 ± 4.9	33.9 ± 3.9	0.19
Male age (years)	32.2 ± 4.1	35.3 ± 3.5	0.29
Female BMI (kg/m^2)	25.4 ± 4.0	25.9 ± 4.0	0.69
Duration of infertility (years)	5.5 ± 3.5	5.5 ± 2.4	0.67
FSH (IU/mL)	9.7 ± 6.9	7.1 ± 2.2	0.13
LH (mIU/mL)	6.6 ± 3	5.5 ± 2.4	0.26
E_2 (pg/mL)	58 ± 75	42 ± 18	0.38
PRL (ng/mL)	16.4 ± 6.5	20.1 ± 15.5	0.37
TSH (ng/mL)	1.8 ± 1.1	1.9 ± 1.2	0.70
Cigarette smoke	3/23 (13%)	5 /52 (9.6%)	0.69
Regular physical exercise	1/23 (4.3%)	1/52 (1.9%)	0.52
Total gonadotropin dose (IU)	1837 ± 889	2067 ± 963	0.61
Duration of induction (day)	9 (7–12)	9 (7–11)	0.52
Endometrial thickness on hCG (mm)	10.9 (9–14)	10.5 (7–15)	0.35
Number of oocytes retrieved	12.4 ± 7.8	9.6 ± 6.3	0.14
Number of mature oocytes	8.3 ± 4.5	6.7 ± 3.7	0.28
Number of fertilized oocytes	5.7 ± 4.0	4.8 ± 3.0	0.32
Fertilization rate (%)	71.2 ± 22	68.4 ± 23.3	0.54
Number of cleaved embryos	4.6 ± 2.9	3.9 ± 2.3	0.48
Cleavage rate (%)	83.3±12.5	82.1±17.9	0.72
Number of embryos obtained	3.9±1.9	3.7±2.1	0.73
Good quality embryo rate (%) (grade I and II embryos)	72	55	0.15
Number of transferred embryos	1.29 ± 0.47	1.24 ± 0.43	0.69

Data are reported as mean ± standard deviation, median (min–max) or frequency (percentage)

BMI body mass index, *FSH* follicle stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *PRL* prolactin, *TSH* thyroid-stimulating hormone, *Mature oocytes* oocytes with the first polar body visible, *Fertilization rate* number of fertilized oocytes/number of injected oocytes, *Cleavage rate* number of cleaved embryo/number of fertilized oocytes

of infertility, day 3 serum follicular stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) levels, cigarette smoke and regular exercise history. There was no statistically significant difference between the two groups in terms of treatment characteristics (Table 1).

In the present study, 54% of the women had a normal/minimal anxiety (score 0–9), 33% had minimal/moderate anxiety (score 10–18), 11% had moderate/severe anxiety (score 19–29), and 2% had severe anxiety as measured by the Beck Anxiety Inventory. As to the men, 68% of them had normal/minimal anxiety, 23% had minimal/moderate anxiety, 6% had moderate/severe anxiety, and 3% had severe anxiety.

In the present study, 56% of the women scored in the normal range (score 0–10), 23% had a mild mood disturbance (score 11–16), 10% had borderline clinical depression (score 17–20), 5% had moderate depression (score 21–30), and 6% had severe depression (score 31–40) as measured by the Beck Depression Inventory. Among the men, 67% had a normal score, 16% had a mild mood disturbance, 6% had borderline clinical depression, 8% had moderate depression, and 3% had severe depression.

Twenty-five couples were diagnosed with male-factor infertility, 25 couples were diagnosed with female-factor infertility, and 25 couples were diagnosed with unexplained infertility. Interestingly, women in couples with male-factor infertility had a greater median STA-I scores than women with other factors (Table 2). By contrast, there was no statistically significant difference between the two groups regarding the women's score on the Perceived Stress Scale, Beck Anxiety Inventory, Beck Depression Inventory, or STAI-II (Table 2). There was no statistically significant difference between the males 'anxiety, stress, or depression scores in regard to the underlying cause of infertility (Table 2).

There was no statistically significant difference in the women's and men's scores on the Perceived Stress Scale, Beck Anxiety Inventory, Beck Depression Inventory, STAI-I, or STAI-II between women who became pregnant and women who did not become pregnant after ART treatment (Table 3).

There was no statistically significant difference between three groups regarding women's and men's salivary (upon waking, at 15, 30, and 60 min after waking, and mean) cortisol levels (Table 4).

The median measures of salivary cortisol level were higher in women who subsequently became pregnant than in women who did not become pregnant [24.7 (19.9–63.1) vs. 20.7 (10.4–30.4), respectively] (Table 5, Fig 1a). There was no statistically significant difference regarding men's cortisol levels between two groups (Fig. 1b). In the final logistic model, there was a significant association between serum cortisol levels and clinical pregnancy (adjusted OR 3.2, 95% CI 1.01–10.3, $p=0.04$). By contrast, there was no statistically significant difference in salivary cortisol levels at 0, 15, 30, or 60 min after waking between two groups. In addition to, there was no difference in AUCg and AUCi salivary cortisol levels between the subsequently pregnant and non-pregnant women.

Table 2 Anxiety, stress and depression scores in relation to underlying cause of infertility

	Female factor ($n=25$)	Male factor ($n=25$)	Unexplained infertility ($n=25$)	p
Perceived Stress Scale				
♀	19 (13–21)	22 (16–31)	21 (14–28)	0.18
♂	20 (13–23)	23(18–30)	18 (11–24)	0.21
Beck Anxiety Inventory				
♀	5 (2–16)	9 (3–12)	10 (5.5–12.5)	0.64
♂	9 (5–16)	5 (2–15)	5 (2–10)	0.26
Beck Depression Inventory				
♀	4 (1–12)	8 (4–12)	12 (7.5–17)	0.06
♂	12(2–23)	7 (3–17)	4 (2–8)	0.27
STA-I				
♀	44 (40–49)	47 (41–49)	41 (38–45)	0.02*
♂	33 (30–42)	36 (27–47)	30.5 (26.2–38)	0.17
STA-II				
♀	51 (46–52)	47 (44–52)	48 (42–53)	0.66
♂	39 (35–49)	41(34–49)	40 (35–43)	0.94

The data represents median (min–max) values

*$p<0.05$

Table 3 Anxiety, stress and depression scores in relation to ART outcome

	Pregnancy (+) $n=23$	Pregnancy (−) $n=52$	p
Perceived Stress Scale			
♀	19 (15–26)	21 (14–27)	0.77
♂	22 (15–26)	20 (13–24)	0.25
Beck Anxiety Inventory			
♀	7.5 (5–11)	9 (3–14)	0.63
♂	6.5 (3–10.5)	6 (2–15)	0.51
Beck Depression Inventory			
♀	7.5 (2.2–16.2)	10 (4–15)	0.51
♂	5.5 (2.2–15.2)	4 (2–12)	0.84
STA-I			
♀	43.5 (39.5–45.7)	43.5 (38.7–47.2)	0.76
♂	34.5 (28–47.2)	33 (27.7–39.2)	0.27
STA-II			
♀	48 (46–52)	49 (43–53)	0.79
♂	40.5 (36–49)	39 (34–45)	0.58

The data represent median (min–max) values

Table 4 Salivary cortisol levels (upon waking, at 15, 30, and 60 min after waking, and mean) in relation to underlying cause of infertility

	Female factor ($n=25$)	Male factor ($n=25$)	Unexplained infertility ($n=25$)	p
Cortisol (0 min)				
♀	12 (10–22)	15.7 (7.2–26.1)	18 (13–27)	0.21
♂	18.8 (11.6–70)	18.7 (9.2–36.8)	15 (7–26)	0.37
Cortisol (15 min)				
♀	13 (11–23)	17 (9.5–26.6)	22.6 (11.4–42.4)	0.18
♂	17.1 (8.6–39)	15.5 (8.2–26.2)	13.2 (8.9–26.9)	0.77
Cortisol (30 min)				
♀	21 (13–31)	17.4 (10.1–32.3)	26.6 (11.8–43.9)	0.36
♂	24.7 (16.7–61.5)	19.8 (12.8–28.5)	16.4 (10.9–58.8)	0.49
Cortisol (60 min)				
♀	20 (11–74)	25.1 (10.4–35.4)	30 (13–77)	0.53
♂	20 (12–183)	23.2 (9.9–40.4)	19.5 (10.5–48.8)	0.55
Cortisol (median)				
♀	20.2 (12.4–24.8)	21.2 (11.6–30.9)	25.6 (10.2–44.8)	0.42
♂	29 (13.9–175)	24.2 (14.2–47.2)	19.6 (11.7–43.7)	0.39

The data represent median (min–max) values

Table 5 Salivary cortisol levels (upon waking, at 15, 30, and 60 min after waking, and mean) in relation to ART outcome

	Pregnancy (+) $n=23$	Pregnancy (−) $n=52$	p
Cortisol (0 min)			
♀	21.1 (12–28.8)	13.7 (10–23)	0.12
♂	22.5 (8.7–49.8)	17.8 (8.6–32.4)	0.43
Cortisol (15 min)			
♀	19.2 (12.6–29.2)	15.4 (9.7–30.2)	0.36
♂	20.6 (6.2–46.5)	13.2 (8.6–26.6)	0.27
Cortisol (30 min)			
♀	24.6 (15.4–39.7)	21.8 (10.3–34.7)	0.21
♂	20.7 (11.2–53.5)	19.6 (12.3–42.4)	0.83
Cortisol (60 min)			
♀	28 (19–92)	22.8 (10.2–43.9)	0.07
♂	21.3 (14.3–64.8)	19.9 (10.5–38.9)	0.42
Cortisol (median)			
♀	24.7 (19.9–63.1)	20.7 (10.4–30.4)	0.04*
♂	36.8 (16.1–93.9)	22.1 (11.9–33.8)	0.13

The data represent median (min–max) values

*$p<0.05$

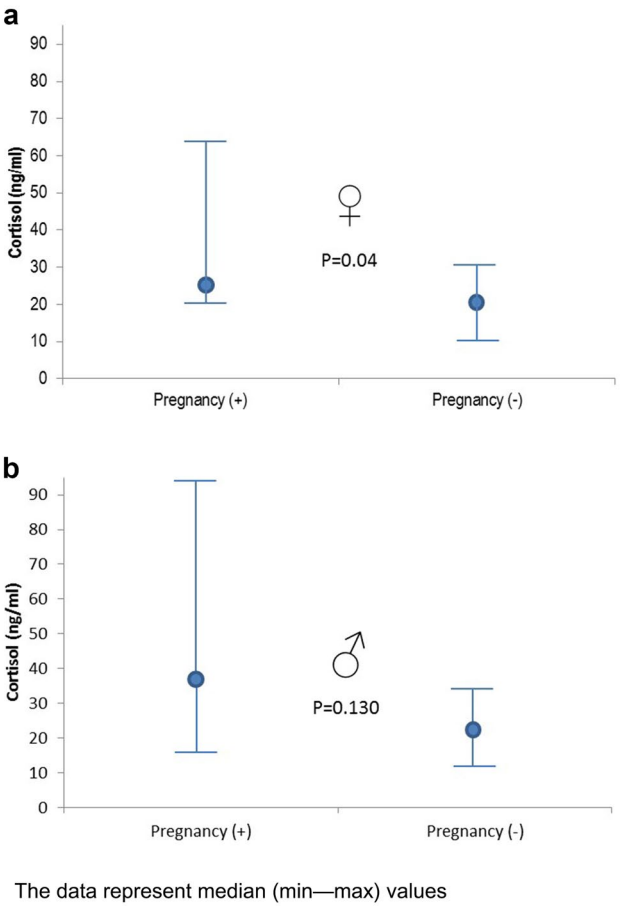

Fig. 1 **a** Saliva cortisol levels in women included in the study. **b** Salivary cortisol levels in men included in the study

Discussion

In the present study, we found that women with a successful outcome after ART treatment had higher median salivary cortisol levels than those found in women who had an unsuccessful result. There was no statistically significant difference in median salivary cortisol levels in men in relation to ART treatment outcome.

The current study included couples suffering from female-factor, male-factor, and unexplained infertility. We found that women in couples with male-factor infertility have a greater STA-I level than women with unexplained infertility. Increased anxiety levels in women in couples with male-factor infertility suggest that the psychological stress is higher in women with male-factor infertility than in women with unexplained infertility. We did not find any difference in either self-reported anxiety or depression score and salivary cortisol levels between the study groups in relation to the cause of infertility. The comparison between couples with unexplained infertility and couples with male or female infertility was inconclusive. Some studies have found no differences between the groups [17, 18], while several studies indicate that women with unexplained infertility were more anxious and dissatisfied with themselves and their lifestyle than women in the other groups [19–21].

In the present study, there was no statistically significant relationship between the self-reported stress level of couples and ART treatment outcome. In agreement with this study, a meta-analysis by Boivin et al. [22] supported the theory that the stress and anxiety caused by infertility and/or infertility treatment do not have any effect on the potential pregnancy outcome. In contrast, in another meta-analysis by Matthiesen et al. [1] revealed that statistically significant but small negative association between stress and clinical pregnancy, and between state and trait anxiety and clinical pregnancy rate. Interestingly, Smeenk et al. [10] reported that the pretreatment baseline scores of anxiety status and depression were significantly positively related to IVF/ICSI outcome.

The current study also searched whether male's awakening cortisol levels and perceived stress were associated with ART success. There was only one study in the literature regarding the effect of male's cortisol levels on ART results. In agreement with this study, Butts et al. [23] found no significant association between urine cortisol levels of couples (both females and males) and ART results.

In the present study, we found that a successful outcome after ART treatment was associated with higher median salivary cortisol levels than in women who had an unsuccessful treatment result. In agreement with this study, several studies have shown that elevated cortisol levels (all of them in follicular fluid) were observed in patients who became pregnant after fertility treatment [24–26]. By contrast, a study by An et al. [27] reported that higher follicular-fluid cortisol levels are associated with a negative IVF treatment result. These conflicting results were evident in a recent systematic review, which found that both low and high cortisol levels were associated with an increased probability of pregnancy in women undergoing IVF treatment [28].

Nouri et al. [29] investigated awakening cortisol levels in women undergoing IVF treatment and found no correlation between cortisol release and IVF outcome. In their study, salivary cortisol was measured at 30 min post-awakening and at the time before going bed in the night for three consecutive days of IVF procedure. Cesta et al. [30] reported that perceived stress, infertility-related stress, and cortisol levels were not associated with IVF cycle outcomes. Massey et al. [11], on the other hand, measured both salivary cortisol (taken at awakening and 30 min post-awakening) and hair cortisol and found that hair cortisol but not salivary cortisol predicted clinical pregnancy. Hair cortisol represents the long-term accumulation of cortisol and, therefore, might results in a better mean cortisol levels. However, in the current study, we took salivary samples for four times per participants and this appears to strengthen the mean cortisol levels. There are several possible reasons for these inconsistencies. One is that individuals may not accurately report their level of distress when completing psychological questionnaires [31].

Cortisol, known as stress hormone, is necessary for normal functioning of the body under unfavorable conditions. Blunted or lower cortisol levels have been associated with chronic stress, anxiety, depression, and anger, with low memory function, and with ill health or burnout [32]. Hence, higher awakening median cortisol levels in the current study and its positive association with pregnancy outcome suggest that cortisol might be necessary for normal conception process. Current study showed that higher awakening salivary cortisol levels were associated with higher pregnancy rates but in the hair cortisol study by Massey et al. [11], the opposite was true. Cortisol in each centimeters of hair represents the cortisol levels of the preceding month as hair is accepted to grow 1 cm per month. Thus, cortisol levels in the current study covers the immediate days of ART treatments while that of the study of Massey et al. [11] covers the last month before ART. Although hair cortisol might represent individual's chronic cortisol level, we think that cortisol levels just before the ART treatment might be more appropriate as hormones have relatively short half-lives. Nevertheless, taking into account the dynamic process of cortisol secretion and the complexity of establishment of pregnancy, new studies are warranted as there are sufficient data suggesting the effects of cortisol on pregnancy.

In conclusion, the current study showed that (i) the infertile couples had moderate-to high psychological stress, (ii) median salivary cortisol levels were higher in women who became pregnant after ART procedure than those of women who did not become pregnant, (iii) males' cortisol levels did not differ between the groups and (iv) there was no correlation between the males' and women's cortisol levels. Further studies with larger populations are required to confirm the effects of anxiety/depression on the ART treatment outcome.

Author contributions GT: protocol/project development, data collection, and manuscript writing/editing. SY: protocol/project development, data analysis, and manuscript writing/editing. AK: protocol/project development, data collection, data analysis, and manuscript writing/editing. İR: data analysis and manuscript writing/editing. TÖ: data analysis and manuscript writing/editing. CU: data analysis and manuscript writing/editing. UK: data collection and manuscript writing/editing. SÜ: data analysis and manuscript writing/editing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards Institutional review boards approved the study (Approval no. 2017/127). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964

Helsinki Declaration and informed consent forms had been obtained from all study participants.

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