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The relationship between the menstrual cycle and cortisol secretion: Daily and stress-invoked cortisol patterns

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PII: S0167-8760(17)30269-6
DOI: doi:10.1016/j.ijpsycho.2018.03.021
Reference: INTPSY 11427

To appear in: *International Journal of Psychophysiology*

Received date: 26 April 2017
Revised date: 19 March 2018
Accepted date: 22 March 2018

Please cite this article as: Eva Montero-López, Ana Santos-Ruiz, M. Carmen García-Ríos, Manuel Rodríguez-Blázquez, Heather L. Rogers, María Isabel Peralta-Ramírez , The relationship between the menstrual cycle and cortisol secretion: Daily and stress-invoked cortisol patterns. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Intpsy(2017), doi:10.1016/j.ijpsycho.2018.03.021

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Title: The relationship between the menstrual cycle and cortisol secretion: Daily and stress-invoked cortisol patterns

Running head: Cortisol levels during the menstrual cycle phases

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Abstract

The menstrual cycle involves significant changes in hormone levels, causing physical and psychological changes in women that are further influenced by stress. The aim of this study was to understand the relationship between menstrual cycle phase and salivary cortisol patterns during the day as well as the salivary cortisol response to the Virtual Reality Version of the Trier Social Stress Test (TSST-VR).

Forty two women not taking oral contraceptives (24 in follicular phase and 18 in luteal phase) participated in the study. Five samples of salivary cortisol collected during the day and another five samples of cortisol during the TSST-VR were analyzed. Psychological stress measures and psychopathological symptomatology were also evaluated. A 2x4 mixed ANCOVA showed an interaction between the two groups on the TSST-RV invoked cortisol response to the [$F(3,42) = 3.681$; $p = 0.023$) where women in luteal phase showed higher cortisol post exposure levels (5.96 ± 3.76 nmol/L) than women in follicular phase (4.31 ± 2.23 nmol/L). No other significant differences were found.

Our findings provide evidence that menstrual cycle phase tended to influence cortisol response to laboratory-induced mental stress, with more reactivity observed in the luteal phase.

KEYWORDS: Cortisol during the day, HPA axis, menstrual cycle, psychological stress, TSST, virtual reality

1. Introduction

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Menstrual cycle phases involve significant changes in hormone levels. Physical and psychological changes in women are caused by such hormonal changes, in combination with changes in patterns of eating, exercise, sleep, and health (Brown, Morrison, Calibuso, & Christiansen, 2008), and influenced by other factors such as tobacco, oral contraceptive use, and stress (al'Absi, Hatsukami, & Davis, 2005; Boisseau et al.,2013; Huttlin, Allena, Tosuna, Allena, & al'Absi, 2015; Kajantie & Philips, 2006; Kudielka & Kirschbaum, 2005; Kudielka, Hellhammer, & Wüst, 2009).

However, these physical and psychological changes vary among women, and the mechanisms involved are not fully understood (Manikandan, Nillni, Zvolensky, Rohan, Carkeek, & Leyro, 2016). Manikandan and colleagues carried out a study about emotional control, specifically related to anxiety, in different menstrual cycle phases. They found that women in the late luteal phase who with better emotional regulation had better anxiety control compared to women with less emotional regulation. Therefore, they concluded that emotional regulation might explain the differences that exist among women with respect impairments associated with menstrual phase with interventions specific for the vulnerability every woman.

Nillni, Rohan, Mahon, Pineles, & Zvolensky (2013) examined the association between anxiety sensitivity and menstrual cycle related symptoms over the entire menstrual cycle. They found that women with more anxiety sensitivity (vs. those with less) reported more typical menstrual cycle symptoms, regardless of the phase.

With respect to psychological changes, cortisol is the main hormone produced in response to stress. It is considered one of the main markers of the activated hypothalamic-pituitary-adrenal (HPA) axis in response to a stressful event (Hellhammer, Wüst, & Kudielka, 2009). Given the hormonal changes that occur during the menstrual cycle, various studies have examined the ways in which cortisol levels are related to the distinct menstrual cycle phases (e.g., Duchesne & Pruessner, 2013; Gordon & Girdler, 2014; Kudielka et al., 2009; Lustyk,

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Olson, Gerrish, Holder, & Widman, 2010). Overall, although changes are apparent, in general the differences between phases do not reach statistical significance in these studies. Walder and colleagues (2012) report non-significant phase-related differences in salivary cortisol levels, with non-significant differences in the impact of an acute stressor across phases, yet differing in time course and severity (Walder, Statucka, Daly, Axen, & Haber, 2012). Gordon and Girdler (2014) report no significant effects of cycle phase on plasma cortisol reactivity using a modified Trier Social Stress Test (TSST), while Lustyk and colleagues (2010) describe more variations in salivary cortisol levels during luteal as compared to follicular phase testing of women performing the Paced Auditory Serial Addition Task (PASAT) and cold pressor test. Maki and colleagues (2015) also used the TSST to study changes in salivary cortisol. They concluded that menstrual phase was not related to cortisol responses to this laboratory stressor, but estradiol and progesterone levels were. Awakening cortisol levels have been found to be similar across the menstrual cycle phases, however a significant increase was detected during ovulation (Wolfram, Bellingrath, & Kudielka, 2010).

Villada et al. (2017) examined differences in post-menopausal women vs. those in luteal phase and those in follicular phase in the TSST. They did not find significant differences between groups with respect to heart rate reactivity, but did find that, compared to post-menopausal women, those in luteal and follicular phases had higher cortisol stress reactivity and less active coping patterns, therefore worse autonomic regulation.

There are other influences on the stress response with respect to the menstrual cycle, and oral contraceptives (OC) in particular play an important role. With respect to OCs, Kudielka et al. (2009) reviewed their possible influence, and the findings of Kirschbaum et al. (1999) showed lower salivary cortisol levels in response to TSST in women taking OCs. However, these results were not confirmed in a more recent study conducted by Boisseau et al. (2013) involving a physical (not mental) stress test. Use of OCs was associated with high diurnal

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cortisol levels, but with a low activation of the HPA axis in response to a physical stressor, regardless of the menstrual phase.

In general, among the studies that used the TSST as a form of mental stress to activate the HPA axis, there seems to be some agreement that when under stress, cortisol increases are greater in the luteal phase relative to the other phases in women who are not taking OC (e.g., Kudielka et al., 2009; Rohleder et al., 2001; Rohleder et al., 2003; Schoof and Wolf, 2009). However, no studies have investigated whether menstrual cycle phases are related to both cortisol levels during the day and salivary cortisol reactivity evoked by a mental stress task such as the TSST in the same sample of women.

Liu et al. (2017) conducted a meta-analysis in order to determine sex differences in levels of cortisol in saliva after the TSST. They were able to take into account difference procedures and protocols used to carried out the TSST and their conclusions suggested that future studies should include additional analytic methods, such as Area Under the Curve (AUC), to help better understand the effects of methodological differences.

This recommendation, along with the fact that existing studies have been unable to elucidate the hormonal mechanisms implicated in the stress response/reactivity associated with menstrual cycle phases and the fact that prior studies have examined either stress reactivity in the laboratory or daily life stress, the aim the present study was to test whether daily cortisol levels of women in luteal vs. follicular at different menstrual cycle phases were associated with subsequent reactivity to stress in the classic tasks of public speaking and arithmetic in a modified version of the TSST. Cortisol levels during the day (salivary cortisol levels collected every four hours over a full day) and salivary cortisol levels in the laboratory were measured in response to a virtual reality TSST evoking mental stress in women participants not taking OC. This design was crafted to attempt to clarify the changes in cortisol

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levels that occur in the menstrual cycle phases, and their possible relationship with an increased HPA stress response to a mental stressor.

2. Methods

2.1. Participants

Forty-two women with a mean age of 33.62 years (*SD* = 7.75) participated in this study. Group 1 was composed of 24 women who self-reported to be in the follicular phase (cycle days 2-8) with a mean age of 33.96 years (*SD* = 8.22). Group 2 was composed of 18 women who self-reported to be in the luteal phase (cycle days 18-26) with a mean age of 33.17 years (*SD* = 7.29).

Participants were recruited from the University of Granada through classroom visits, campus fliers, and posters in public institutions, newspapers, and local radio. The inclusion criteria were women, aged 18–50, literate, body mass index (BMI) between 18.5 and 24.9, regular menstrual cycles ranging from 25 to 35 days, not taking OC, and not presenting a physical or mental disease at the time of the study. The following additional exclusion criteria were adopted because of their potentially negative effect on cortisol levels (Williams et al., 2004): use of alcohol, nicotine, amphetamines, barbiturates, methadone, muscle relaxants, and/or lithium.

2.2. Procedure

A brief interview was conducted by telephone with each woman interested in the study to ensure they met the inclusion criteria. Potential participants attended an information session and they then read and signed informed consent form, which had been approved by The Ethics Committee of the University of Granada and followed the recommendations of the Helsinki Declaration. Afterwards, the participant was interviewed and completed different

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psychometric scales. Subsequently, each participant performed the TSST-virtual reality (VR) public speaking and then the arithmetic task.

All the participants were tested in the lab on a weekdays Monday to Thursday, and they collected saliva samples the next day. In this way, the cortisol samples during the day were collected only on weekdays, not on weekends.

At the end of the laboratory portion of the study, participants were given instructions to measure cortisol levels during the day during the following day and were provided with a kit containing 5 Salivette® tubes, a detailed instruction-sheet explaining sample collection and a record sheet for indicating the day and the time the sample was collected. We instructed them to avoid eating (neither chewing gum nor candy) or drinking except water and were not allowed to smoke during the half hour preceding each sample collection. For sample collection, they were told to introduce and soak the cotton swab during one minute. For example, they self-collected the salivary sample in the lab during the TSST-VR, to assure they could collect the sample correctly and that saliva did not have red blood cells or other contaminating elements. To ensure the participation we paid them 20 € when they delivered the samples.

Seven participants were excluded because they did not have regular cycles or were in menopause, eight because of their overweight problems or obesity, and seven due to illness or allergy problems. The menstrual phase of the participants was estimated by gathering self-report information about the date and duration of the last menses during semi-structured interviews in the experimental laboratory session. Finally, participants were assessed for psychopathological symptoms with the SCL-90-R Symptoms Inventory (Derogatis, 1994; Spanish adaptation by Gonzalez de Rivera & De las Cuevas, 1988) to rule out potential psychopathology. All participants who completed the scale were included in the study, as none scored more than one and a half standard deviations above the mean.

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2.3. Instruments

2.2.1. Semi-structured interview

The participants provided information on socio-demographic factors, daily life and sleep habits, medication, and history of psychiatric or psychological treatment.

2.2.2. Stress Vulnerability Inventory (SVI)

The SVI (Beech, Burns, & Scheffield, 1986; Spanish adaptation validated by Robles-Ortega, Peralta-Ramirez, & Navarrete-Navarrete, 2006) consists of 22 items and evaluates an individual's predisposition to be affected by perceived stress with higher scores indicating higher levels of stress vulnerability. The Spanish adaptation has a Cronbach's alpha of 0.87 (internal consistency). As for convergent validity, the results show a significant positive correlation ($p<0.01$) with the following assessment scales: Trait Anxiety measured by the State Trait Anxiety Inventory, the Beck Depression Inventory, the Somatic Symptom Scale, and the Survey of Recent Life Experiences.

2.2.3. Perceived Stress Scale (PSS)

The PSS (Cohen, Kamarak, & Mermeistein, 1983; Spanish adaptation by Remor, 2006) is a self-report scale used to evaluate perceived stress levels and the degree to which people find their lives unpredictable, uncontrollable, or overwhelming (aspects that contribute to stress). The PSS consists of 14 items with five response alternatives. Higher scores on the scale correspond to higher levels of perceived stress. The Spanish version of the PSS (14 items) has adequate reliability (internal consistency = 0.81 and test-retest = 0.73), concurrent validity, and sensitivity (Remor, 2006). In the present study, scores over 22 (the mean score for the Spanish population; Remor, 2006) were indicative of high levels of perceived stress.

2.2.4. SCL-90-R Symptoms Inventory

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The SCL-90-R (Derogatis, 1994; Spanish adaptation by González de Rivera et al., 1988) was used to rule out potential psychopathology in the participants. This self-report questionnaire was developed to assess symptoms of psychopathology and includes 90 items with five response alternatives (0-4) on a Likert scale. The Spanish version has an internal consistency = 0.79 - 0.90 and reliability after a week of 0.78 – 0.90. Women respond according to how they have felt within the past seven days, including the day the inventory is administered. The inventory is scored and interpreted according to nine main dimensions (somatization, obsessive-compulsive symptoms, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism) and three global indices of psychological distress (Global Severity Index [GSI], Positive Symptom Total [PS], and Positive Symptom Distress Index [PSDI]). The Spanish language version of the instrument has satisfactory reliability (internal consistency = 0.81) and validity (De las Cuevas et al., 1991).

2.4. Virtual reality version of the Trier Social Stress Test (TSST-VR)

This task is based on the traditional *Trier Social Stress Test* (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) and was adapted into a virtual environment by Montero-López et al. (2016). It consists of the presentation of a virtual audience in a 3D display, with the sounds of the virtual environment conveyed to the participants through headphones and a microphone that is used to simulate the recording of the participant's speech (at the end of the study, it is revealed to the participants that their speech was not recorded). The investigator controls the virtual reality program throughout the task. The virtual reality task contains the same phases as the traditional TSST.

The first phase (*adaptation to the virtual environment*) participants were seated and instructed to relax and remain still during 3 minutes. They faced a screen showing a 3D image of a stage curtain with sounds that one would expect to hear from an actual audience.

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The second phase (*anticipatory stress period*) consisted of the initiation of the virtual environment. This phase lasted 5 minutes and participants were required to prepare a speech about their positive and negative traits, to be delivered in front of the virtual audience..

The third phase (*exposure period*) began as the curtain rose and the virtual audience appeared (Figure 1). The participant had to being deliver their speech. They were instructed to speak for the whole 5 minutes without interruption and attend to the form and content of the speech, as that would greatly determine the audience's response to their speech. Special emphasis was placed on the requirement to speak continuously for the entire five minutes, and the audience does react accordingly. At two and a half minutes into the speech, the investigator changed the audience reaction from normal to restless for all participants, regardless of participant performance of the speech task.

INSERT FIGURE 1

Once the speech ended, the last stage (*the arithmetic task*) began. This task consisted of serially subtracting the number 13 from the number 1022 as quickly as possible for five minutes. Participants were asked to re-start from 1022 whenever they made an error.

Although cortisol levels are reportedly stable between 14:00 and 16:00 hours (Kudielka & Kirschbaum, 2005), Spanish metabolic and circadian rhythms have been found to be different from those of other European citizens due to the greater number of light hours and the fact that most Spanish eat lunch, their main meal of the day at 14:00 or 15:00. Therefore, following a pilot study, we determined that cortisol levels were stable between 15:30 and 18:00 hours, and all participants were subsequently tested in the afternoon within this window of time (Santos-Ruiz et al., 2010).

A diagram of the protocol of the TSST-VR is shown in Figure 2.

INSERT FIGURE 2

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2.5. Cortisol measures

Salivary cortisol sample collection was performed using a Salivette® Cortisol kit (Sarstedt, Numbrecht, Germany, Ref.51.1534), which consists of two small tubes, one containing a small piece of cotton. Participants chewed the cotton for approximately 60 seconds, after which it was placed into the salivette for analysis. Samples were analyzed at the San Cecilio University Hospital using the electrochemiluminescence immunoassay "ECLIA" method. This method is designed for use in Roche Elecsys 1010/2010 automated analyzers with the Elecsys MODULAR ANALYTICS E170 module.

2.5.1. Measurement of cortisol response under stress (TSST-VR)

The first sample of cortisol (baseline cortisol) was collected when the participants arrived at the laboratory after completing the semi-structured interview, the SVI, and the PSS. The first sample always, occurred within the first 20 minutes of arrival at the laboratory. Immediately afterwards, the TSST-VR was explained to the participant. The second salivary cortisol sample (pre-exposure cortisol) was collected 30 minutes after the baseline cortisol collection when they get the instructions to prepare the speech, and immediately before the stressful task started (anticipatory stress to a situation of speech delivery and arithmetic task). Right after the end of arithmetic task, the third cortisol sample (post-exposure cortisol) was taken. This occurred 18 minutes after the pre-exposure cortisol sample. Then, 10 minutes after the post-exposure cortisol sample , the fourth (post-exposure cortisol +10') cortisol sample was collected. And then 10 minutes later, the fifth (post-exposure cortisol +20') cortisol sample was obtained.. Participants were given interior design magazines to leaf through in the interval between the fourth and fifth drawing of salivary cortisol.

2.5.2. Measurement of cortisol levels during the day

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To assess cortisol levels during the day, each participant collected five samples of their salivary cortisol. The first sample (Cortisol 1) was collected 30 minutes after waking up (while still fasting), the second (Cortisol 2) at +4 hours, the third (Cortisol 3) at +8 hours, the fourth (Cortisol 4) at +12 hours, and the fifth (Cortisol 5) at +16 hours. During the half hour preceding each sample collection, the participants we asked not to have anything to eat or drink except water and not to smoke. We asked them to write down on the record sheet the times each sample was collected, and we set an alarm on the participants' phones to ensure they collected the saliva samples at the appropriate times.

2.6. Statistical analyses

To determine whether there were statistically significant differences between sociodemographic and psychological variables in the two groups, Student's *t-tests* were performed using menstrual cycle phase as the independent variable with two levels (follicular and luteal) and the following dependent variables: age, schooling, and scores on the SVI, the PSS, and the SCL-90-R. Chi-square analyses were used to check for differences between menstrual cycle phase and use of tobacco.

Several statistical analyses were performed to evaluate the influence of the potential confounders of cortisol levels. Student's t-tests were performed with the independent variable tobacco consumption (smoker/non-smoker), psychological factors, and the dependent variables cortisol during the day levels and cortisol levels in response to the stressor.

To determine whether there were differences between menstrual cycle phases in the TSST-VR, a 2x5 mixed ANOVA was performed. The first factor was the independent between-groups variable (follicular phase group and luteal phase group) and the second factor was the within-subjects variable (repeated measures of cortisol at baseline, pre-exposure, post-exposure, post-exposure +10', and post-exposure +20'). Afterwards, a 2x4 mixed ANCOVA (with the baseline cortisol level as covariate) was performed.

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Finally, to determine whether there were statistically significant differences between the menstrual cycle phase of the participants and their cortisol levels during the day, a 2x5 mixed ANOVA was performed. The first factor included two between group levels (follicular phase group and luteal phase group), and the second factors was a within-subject variable of repeated measures across five time-points: 30 minutes after waking (cortisol 1), +4 hours (cortisol 2), +8 hours (cortisol 3), and +12 hours (cortisol 4), and +16 hours (cortisol 5). In all repeated measures analyses, the Greenhouse-Geisser correction was applied and tests of simple effects were conducted if an interaction was found. Data were analyzed using SPSS version 20.

For cortisol levels under stress and stress levels during the day, we measured the area under the total response curve with respect to the ground (AUCG), providing information about total cortisol production. Second, we measured the area under the curve with respect to increase (AUCI) for cortisol levels under stress, which allowed for an assessment the overall intensity and sensitivity of the cortisol levels obtained during the TSST-VR. Both areas were calculated using the trapezoid formula following (Pruessner, Kirschbaum, Gunther, Meinlschmid, & Hellhammer, 2003).

Afterwards, we carried out Student's *t-test* to determine whether there were any differences between the AUCG and the AUCI in the two groups (follicular phase and luteal phase). Finally, correlation Pearson's analyses were used to test the relationship between the AUCG of the TSST-VR and the AUCG of cortisol levels during the day.

3. Results

3.1. Sample description

The follicular phase and luteal phase groups were similar in their socio-demographic characteristics and psychological and psychopathological scale scores (Table 1).

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INSERT TABLE 1

In the sample overall, neither socio-demographic characteristics nor smoking was significantly correlated with cortisol at any daily collection time point or any experimental stress time point. The lack of significant findings suggests that these factors are not confounders that needed to be controlled in later analyses.

3.2. Cortisol levels under stress (TSST-VR) at different phases of the menstrual cycle

There were no significant differences between the luteal vs. follicular phase groups and the 5 cortisol measurements over time collected before, during, and after the TSST-VR.

There were significant differences in the level of baseline cortisol between the two groups [$F(1,42) = 7.860$; $p = 0.008$], and therefore this variable was included as a covariate in the mixed ANCOVA conducted afterwards.

The 2x4 ANCOVA showed a significant interaction effect between the two menstrual phase groups and the salivary cortisol response to the TSST-VR over time [$F(3,42) = 3.681$; $p = 0.023$]. The between-groups analysis performed using cortisol levels at different time-points relative to the TSST-VR revealed differences in the post-exposure cortisol levels [$F(3,42) = 5,617$; $p = 0.029$). Women in the luteal phase had higher levels of post-exposure cortisol (Mean ± Standard Deviation, 5.96 ± 3.76) than the women in the follicular phase (4.31 ± 2.23). See Figure 3.

INSERT FIGURE 3

There were no significant differences between groups on AUCG nor AUCI.

3.3. Cortisol levels during the day and menstrual cycle phases

The 2x5 ANOVA did not show any main effects or interaction between the follicular and luteal groups with regard to cortisol levels during the day over time. Nor were there any significant

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differences between the two phases of the menstrual cycle and the AUCG for cortisol levels during the day .

4. Discussion

The aim of this study was to examine the relationship between cortisol levels during the day and stress-invoked cortisol levels in different menstrual cycle phases. To accomplish this objective, after ensuring that the two menstrual phase groups were similar with respect to socio-demographics and most major psychological variables, daily stress was assessed using the cortisol levels during the day measured over a full day; as a baseline indicator of HPA axis activity. Furthermore, the response to psychosocial stress was captured by measuring cortisol levels during exposure to the TSST-VR HPA axis as measure of reactivity to stress. The results revealed no differences between the follicular and luteal phase group on cortisol levels during the day. These findings are consistent with those of Kudielka & Kirschbaum (2003) and Wolfram et al. (2010) who likewise did not find any differences in cortisol levels during the day for women in the follicular vs. luteal phases.

Despite there being no major menstrual phase-related differences in cortisol levels during the day levels, women in the luteal phase had significantly higher cortisol levels than women in the follicular phase immediately following the laboratory stressors. These results are similar to those found by Walder et al. (2012), where women in the luteal phase had higher cortisol levels in the stressful task than did women in the follicular phase. That study differed from the present study in several ways. For instance, Walder and colleagues used a different type of stressful task in the laboratory under a different schedule (they used a morning schedule and we used an afternoon schedule). Another difference was the way in which salivary cortisol samples were taken. In the study conducted by Walder et al. (2012), three samples were taken each hour (9:00 am, 10:00 am, and 11:00 am), while in the present study; five samples were collected (taking into account the baseline cortisol level). Moreover, they

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collected the samples at varied time intervals, following the protocol previously described. Despite these differences, both studies arrive at similar conclusions.

Maki et al. (2015) also found that menstrual cycle phases influenced cortisol response to laboratory psychosocial stress, however, in their study, compared to women in the follicular phase, women in the luteal phase had higher levels of progesterone and estradiol and lower cortisol reactivity to the stressor. Similar to our study, subjective assessment of anxiety and stress was not influenced by menstrual cycle phase. In the present study, psychological and stress-related factors were controlled, and thus are not likely to have confounded the results; however, unlike Maki and colleagues, estradiol and progesterone levels were not taken into account and may have influenced subsequent cortisol levels measured during stress.

The results of the present study coincide with those of the meta-analysis conducted by Villada et al. (2017). Specifically, cortisol differences in response to an acute social stressor were found between women in luteal and follicular phases compared those post-menopausal women but not between menstrual cycle phases. These findings highlight the importance of including age and hormonal status with respect to the study of the physiological stress coping response, including stress reactivity and recovery to stressful situations. The results of the present study showed that, in a stressful, women in the luteal phase of the menstrual cycle tended to experience a greater increase in salivary cortisol levels, although this difference was found in reflected cortisol levels during the day. Thus, the menstrual cycle does not appear to influence the amount of perceived daily stress nor subjective evaluations of general stress or anxiety, although some differences tend to be revealed upon exposure to a stressful situation. Similarly, Manikandan et al. (2016) concluded that it is each woman's emotional regulation capacity, and not her menstrual cycle phase, that determines her ability to control stress/anxiety. Nillni et al. (2013) also described how anxiety sensitivity levels determined typical menstrual cycle symptoms independent of phase of the cycle. The higher stress-

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induced cortisol levels experience by women in the luteal phase suggests increased activation of the HPA axis, along with all of the consequences that this activation entails. It is possible that hormonal levels associated with menstrual cycle phases influence the magnitude of HPA axis response to an acute stressor. This could, in turn, increase their stress levels and the risk of worsening physical and mental health. Our findings may help to better understand higher level mechanisms and better elucidate how hormone levels associated with the menstrual cycle affect women's health, adding evidence to the importance of adequate hormonal regulation as related to health and wellbeing.

4.1. Limitations

Our study has limitations that need to be taken into account when considering the findings. In future research, it would be valuable to collect additional information with regards to ovarian hormone levels such as progesterone and estradiol, and examine their relationship with cortisol directly. The cortisol awakening response (CAR) could also be measured, with cortisol samples during the day collected over a period of various days, or even during an entire month so that both phases of the menstrual cycle are fully captured in the same person. Participants could keep a simple diary using their smartphones to identify stressful events throughout the day that could be correlated to their cortisol samples. This ecological momentary assessment methodology and other other changes would allow better clarification of the mechanisms underlying the potential relationship between menstrual phase and the stress response.

4.2. Conclusions

This study provides preliminary evidence to suggest that menstrual cycle phase tends to influence cortisol response immediately after a laboratory mental stress task, with more stress induced observed in those women in the luteal phase of their cycle. However, cortisol levels during the day were not found to be influenced by menstrual cycle phase. These findings suggest that differential hormonal levels associated with menstrual cycle phases may be

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implicated in cortisol reactivity to an acute mental stressor and future research to clarify these interactions is warranted.

Acknowledgement

This study is a part of a Thesis Doctoral and was supported by the I+D Project "PSI2010-15780", funded by the Spanish Ministry of Economy and Competitiveness. The Ministry was not involved in the study design, in the collection, analysis, or interpretation of data. The played no role, in the writing of the report or in the decision to submit the paper for publication.

Disclosure statement

The authors report no conflicts of interest.

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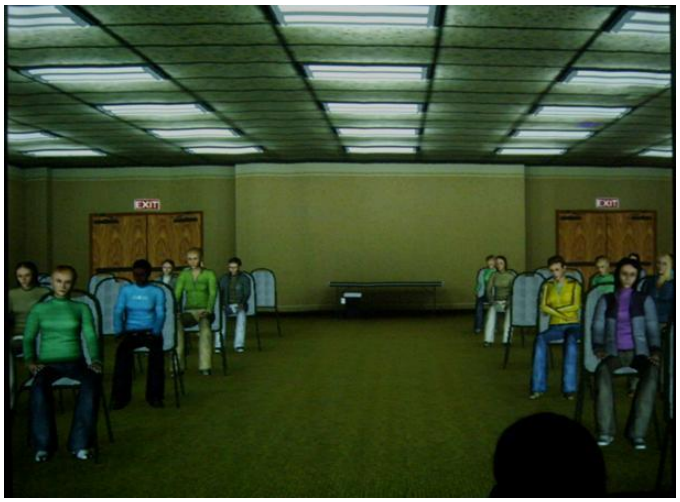

*Figure 1.*Virtual audience displayed during the speech

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Initial assessment	**Stress period**					**Final assessment**		
Baseline Cortisol Interview SVI PSS SCL 90-R	**Pre Cortisol (20')**	TSST -VR			Arithmetic task (5')	**Post Cortisol (18')**	**Post+10 Cortisol**	**Post+20 Cortisol**
		Adaptation to VR (3')	Anticipatory Stress (5')	Speech delivery(5')				

Figure 2. Diagram of the TSST-VR protocol. *Note*: Post Cortisol: post-exposure cortisol; Post +10 Cortisol: cortisol at 10 minutes after exposure; Post +20 Cortisol: cortisol at 20 minutes after exposure; Pre Cortisol: pre-exposure cortisol; PSS: Perceived Stress Scale; SCL-90-R: Symptom Checklist SCL-90-R; SVI: Stress Vulnerability Inventory; TSST-VR: Trier Social Stress Test adapted to Virtual Reality

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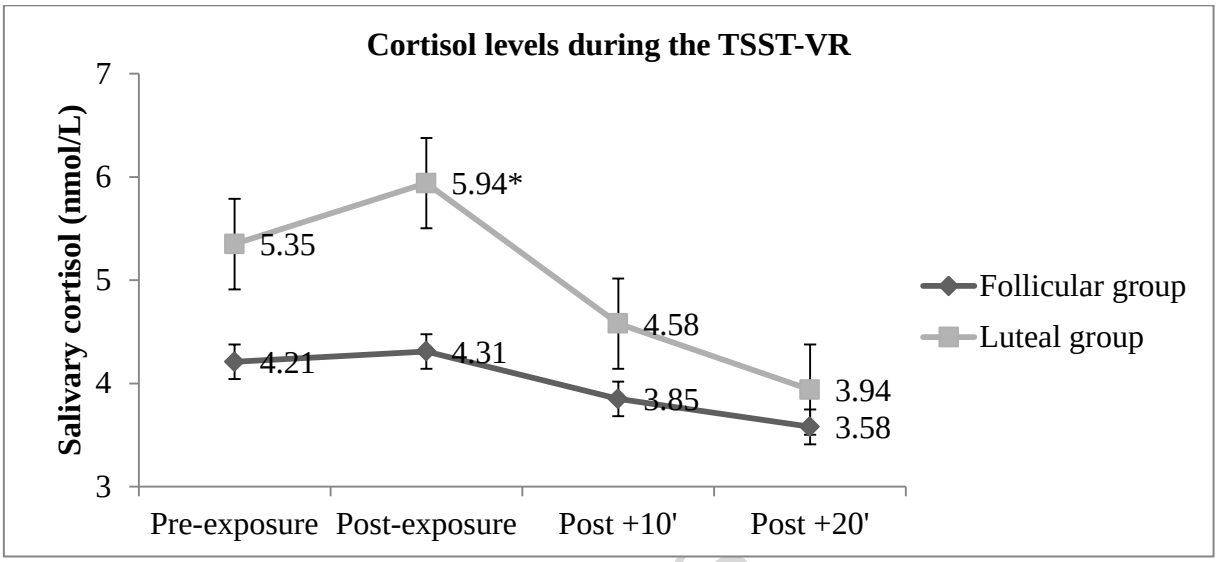

Figure 3. Levels of salivary cortisol during the TSST-VR according to phase in the menstrual cycle.

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Table 1. Means (*M*) and standard deviations (*SD*) of socio-demographic and psychological variables for the participants in both groups.

	Follicular (n=24) Mean ± SD	Luteal (n=18) Mean ± SD	t/x^2	*p*
Age (Years)	33.96 ± 8.23	33.17 ± 7.29	0.115	0.748
Education (Years)	16.50 ± 3.06	16.67 ± 2.93	0.032	0.860
Tobacco (%, x^2)	18.8%	30%	1.558	0.219
Stress Vulnerability Inventory	7.04 ± 4.921	5 ± 4.704	1.776	0.190
Perceived Stress Scale	23.13 ± 8.81	19.88 ± 9.05	1.317	0.258
SCL-90-R				
Somatization	53.48 ± 10.45	48.72 ± 9.19	2.318	0.136
Obsessions and Compulsions	55.26 ± 11.98	50.72 ± 9.74	1.701	0.200
Interpersonal Sensitivity	53.22 ± 11.77	50.06 ± 11.97	0.709	0.402
Depression	49.13 ± 9.60	45.44 ± 10.65	1.352	0.252
Anxiety	52.78 ± 10.81	47.61 ± 9.67	1.701	0.120

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Hostility	50.13 ± 9.32	46.28 ± 8.96	0.594	0.446
Phobic Anxiety	42.57 ± 11.68	40 ± 8.96	1.694	0.213
Paranoia	52.48 ± 11.60	48.11 ± 12.76	1.311	0.259
Psychoticism	50.22 ± 13.35	46 ± 13.04	1.027	0.317

Note: x^2: Chi-square analysis; *t:* t-student value

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Highlights:

- There was an interaction in the cortisol levels invoked in response to the stressor
- There were no differences between the two groups in cortisol levels during the day
- The menstrual cycle phase tends to influence cortisol response to laboratory-induced mental stress

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