**Interactive role of endocrine stress systems and reproductive hormones in the effects of stress on declarative memory**

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**Abstract**

The effects of stress on memory performance, and the neuroendocrine mechanisms mediating such effects, are not well understood. Given the interrelationship between **reproductive** hormones and both the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis (HPA-A), we examined their combined effect on stress-induced modulation of declarative memory. Before and after exposure either to the Trier Social Stress Test (TSST) procedure or to a non-stress condition, 112 participants completed the Rey Auditory Verbal Learning Test. We analyzed participants’ HPA-A and SNS reactivity by measuring cortisol and salivary alpha-amylase (sAA, an SNS activation marker) in four saliva samples. In addition, testosterone, estradiol, and progesterone were sampled prior to the stress exposure. Exposure to the TSST attenuated recall following an interference during the declarative memory task. Importantly, controlling for testosterone, estradiol, and progesterone diminished this effect of stress, suggesting the involvement of baseline **reproductive** hormones in stress-induced modulation of memory functions. Furthermore, a moderated regression analysis revealed that stress-induced declines in memory performance were negatively associated with participants’ stress-induced cortisol reactivity, but only among individuals with high testosterone levels, and with the stress-induced increases in sAA, but only in individuals with low progesterone levels. These findings suggest that the effects of stress on memory performance may be modulated by baseline **reproductive** hormones and provide a preliminary indication for specific modulatory interrelationships between **reproductive** hormones and neuroendocrine stress mechanisms in mediating the effects of stress on memory.

Keywords: Trier Social Stress Test; alpha-amylase; cortisol; **reproductive** hormones; memory

1. **Introduction**

Exposure to stress can affect cognition, including declarative memory (Espin et al., 2013). Stress activates two neurobiological stress systems: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis (HPA-A). The hypothalamus induces the SNS to secrete adrenaline and noradrenaline and stimulates the HPA-A, leading to cortisol secretion; it also regulates the secretion of **reproductive** hormones (estradiol, progesterone, and testosterone) through the hypothalamic-pituitary-gonadal axis (HPG-A) (Handa and Weiser, 2014). Given the interrelationships between the neuroendocrine stress systems and **reproductive** hormones (Juster et al., 2016), this study examined their combined effect on stress-induced modulation of declarative memory.

The literature on the effects of acute stress on declarative memory has produced inconsistent findings. Some studies have demonstrated stress-induced impairments in encoding (e.g., Payne et al., 2007), whereas others found enhancements (e.g., Smeets et al., 2007). Additionally, studies investigating the involvement of physiological stress mechanisms on stress-induced changes to declarative memory have yielded inconsistent results. Specifically, the association between cortisol reactivity to stressors and declarative memory was shown to be negative in some studies (Kirschbaum et al., 1996a), but positive in others (Nater et al., 2007). **This discrepancy may be explained by methodological differences, such as the memory testing procedure used or the time of testing (morning vs. afternoon).** The few studies that have examined the impact of SNS activation on memory performance generally have not found an association between stress-induced memory impairments and SNS activation (e.g., Hidalgo et al., 2015).

Given that **receptors of reproductive** hormones are expressed in cognition-related brain structures, including the hippocampus (McEwen and Milner, 2017), there is reason to believe that they may affect declarative memory. For example, women’s tendency to outperform men on verbal memory tasks (Maki, 2015) and patterns that show verbal memory decline during perimenopause (Weber et al., 2013) suggest that estradiol and progesterone may play a role in enhancing declarative memory, and particularly verbal memory. However, although a positive association between verbal memory and women’s basal levels of estradiol (Drake et al., 2000) and progesterone (Henderson et al., 2013) has been demonstrated in some studies, other studies did not indicate such associations (e.g., Halari et al., 2005). Research on the influence of testosterone on verbal memory is also inconsistent; several studies have demonstrated a positive association between basal levels of free testosterone and verbal memory among middle-aged and elderly men (Barrett-Connor et al., 1999; Moffat et al., 2002), whereas others indicated either no association (Aleman et al., 2001) or a negative association (Martin et al., 2007). Thus, the involvement of **reproductive** hormones in memory performance, particularly among healthy young individuals, is not fully understood.

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As reproduction and survival are necessary for life, it follows that their underlying neurobiological mechanisms would be interlinked (Acevedo-Rodriguez et al., 2018). Indeed, androgen and estradiol receptors are expressed across different sections of the HPA-A (Handa et al., 2014). **Reproductive** hormones may also affect stress signaling indirectly. For example, high estradiol levels stimulate the production of cortisol-binding globulin which, in turn, removes free cortisol from circulation (Juster et al., 2016). An HPA-HPG cross-talk may explain why cortisol stress reactivity is typically lower in women as compared to men (Dickerson and Kemeny, 2004). Further, cortisol stress reactivity is higher among women in the luteal phase of their menstrual cycle as compared to women in the late follicular phase (high estradiol levels) (Kajantie and Phillips, 2006). Estradiol is also negatively associated with the SNS stress response (Sita and Miller, 1996). Interestingly, although estradiol delivery has been shown to lower SNS stress response in menopausal women (Del Rio et al., 1998), it was demonstrated to increase SNS and HPA response stress response in men (Kirschbaum et al., 1996b), suggesting that the interaction between estradiol and stress response is sex-dependent.

Similar to estradiol, progesterone also appears to modulate stress reactivity in a sex-dependent manner. Specifically, basal progesterone levels have been shown to be negatively associated with cortisol response to psychosocial stress in men (Juster et al., 2016), but positively associated with cortisol response to physical stress in women at the follicular stage of their menstrual cycle (Herrera et al., 2016). In addition, whereas in menopausal women, progesterone administration suppressed cortisol response and SNS responses to a psychological stressor (Del Rio et al., 1998), in men, progesterone somewhat attenuated the stress-induced increase in plasma cortisol but enhanced the SNS response (Childs et al., 2010). Testosterone also appears to have a modulatory impact over the physiological stress response. Specifically, Juster et al. (2016) demonstrated a negative correlation between post-stress testosterone levels and cortisol response in both men and women. However, in another study, basal pre-stress levels of testosterone were negatively associated with the cortisol stress-response in men but not in women (Stephens et al., 2016), again suggesting a sex-dependent relationship. Importantly, the HPA-HPG interaction is bidirectional; studies have demonstrated that acute stress enhances the secretion of progesterone (Gaffey and Wirth, 2014; Herrera et al., 2016) and testosterone (Bedgood et al., 2014) in men and women, and of estradiol in animals (Shors et al., 1999).

Evidence suggests that the modulation of stress mechanisms by **reproductive** hormones may influence the effects of stress on memory. Specifically, increases in stress-induced cortisol levels and declarative memory performance were found to be negatively associated in men, but not in women in the luteal phase (Wolf et al., 2001). However, in other studies they were positively associated in men, and in women who were using oral contraceptives, but not women in the luteal phase or the follicular phase (Espin et al., 2013). Currently, details of the putative interactions between particular **reproductive** hormones and neuroendocrine stress reactivity in influencing the effects of stress on declarative memory are lacking, which substantially limits the ability to predict the effects of stress on memory functioning and to develop effective strategies for maximizing memory performance under conditions of stress.

The current study examined the interaction between the stress systems (SNS and HPA-A) and **baseline** **reproductive** hormones in mediating the effects of psychosocial stress on declarative memory. HPA activation and SNS activation were evaluated noninvasively via the measurement of salivary cortisol and alpha-amylase levels, respectively. Salivary alpha-amylase (sAA) secretion increases in response to adrenergic stimulation and, consequently, is recognized as a sensitive biomarker for stress-related SNS activation (Nater and Rohleder, 2009). **We hypothesized that stress, induced via the Trier Social Stress Test (TSST), would hinder declarative memory performance, as measured by the Rey Auditory Verbal Learning Test, and that this effect would depend on the interaction between baseline levels of reproductive hormones and the reactivity of stress factors (i.e., cortisol and sAA).**

1. **Method**
   1. **Participants**

The study sample included 112 young men (*n*= 39) and women (*n* = 73). Of the women, 37 were taking oral contraceptives (Oral Contraceptives group; OC). The remaining 36 women were not using oral contraceptives and were in the mid-luteal phase (day 21) of their menstrual cycle at the time of the study (Luteal Phase group; LP). Participants were college students and were recruited through advertisements on campus. After signing an informed consent form, participants completed a questionnaire regarding their health, habits, and demographic details to verify that they met the inclusion criteria. Exclusions included serious medical, gynecological, or hormonal problems; psychopathologies that may affect hormonal regulation (e.g., depression); ADHD or other learning disabilities; and being a smoker. In addition, to be included in the OC group, women had to be taking contraceptive pills containing 25 mg of estradiol (Ethinylestradiol) and 75 mg of progestin (Gestodene). These doses are considered moderate and are commonly prescribed. Inclusion criteria of women in the LP group included not having used oral contraceptives for at least six months prior to the study, having a regular menstrual cycle, and not being pregnant or lactating. Participants in the LP group were monitored for at least 3 months prior to the study to verify the regularity of their cycles and arrived to the research laboratory to participate in the study on the twenty-first day of their cycle, using the day of onset of their last menstruation as the reference point. All participants had to be awake for at least 1 hour before testing to control for circadian fluctuations in cortisol. **The final sample had a mean age of 24.61 years (*SD* = 2.60) and a mean BMI of 23.09 (*SD* = 3.21). All participants** **were native Hebrew speakers**.

The Institutional Ethics Review Board approved the complete study protocol. Participants received $25 in compensation for taking part in the study.

* 1. **Experimental Procedure**

The experimental sessions took place in the laboratory of the YVC Psychology Department between 8:00–10:00 AM, a time period during which testosterone levels are at their peak, before they subsequently decline gradually throughout the day (Dabbs and de La Rue; 1991; Diver et al., 2003). All participants were tested at least one hour after awakening to avoid the possibility that the cortisol increase would be due to the awakening response (which reaches its peak 30 minutes after awakening) (Ghiciuc et al., 2011), rather than the stress induction.

Participants from each group (men, OC, LP) were randomly assigned to one of the two experimental groups: stress or control. The study design allowed for all participants to undergo all of the procedures in a single experimental session, which was comprised of three consecutive stages (see Fig. 1): (1) The Rey Auditory Verbal Learning Test (RAVLT); (2) the Trier Social Stress Test procedure or the control condition (20 minutes); and (3) the second completion of the RAVLT (20 minutes). The stimuli included in the RAVLT, and the presentation of their order, differed in Stages 1 and 3. The participants provided saliva samples at four assessment points: T1 (baseline: 8:00–8:30 AM), T2 (immediately following the TSST/control), T3 (T2 + 10 minutes), and T4 (T3 + 10 minutes). For the T1 sample, participants provided 5 ml of saliva, which was used to evaluate levels of testosterone, estradiol, and progesterone, as well as baseline levels of cortisol and sAA. For the remaining samples, participants provided 2 ml of saliva, which was used to evaluate levels of reactive cortisol and sAA.

* 1. **Saliva sampling procedure and biochemical analysis**

The participants were instructed to refrain from eating, drinking (aside for water), or smoking for at least 1 hour prior to the experimental session. Before each saliva sampling, participants were asked to chew on a piece of parafilm for several seconds to increase saliva secretion. They then deposited a sample of saliva in a SaliCap sampling vial (IBL International GMBH, Hamburg, Germany). **Notably, chewing may affect the relative amount of alpha amylase in the saliva (Rohleder & Nater, 2009). However, this factor was kept constant within the study, as both the control group and the stress group chewed parafilm prior to saliva sampling**.

Saliva samples were stored at -20°C immediately after collection. For each biochemical analyte, tests were performed using commercial CE-IVD-approved ELISA kits: 17 Beta Estradiol Saliva ELISA (mean intra-assay CV% = 4.8, mean inter-assay CV% = 3.4, assay sensitivity = 0.4 pg/mL), Cortisol Saliva ELISA (mean intra-assay CV% = 4.8, mean inter-assay CV% = 8.1, assay sensitivity =0.005 µg/dL), Testosterone Saliva ELISA (mean intra-assay CV% = 9.1, mean inter-assay CV% = 5.7, assay sensitivity = 2.0 pg/mL), Progesterone Saliva ELISA (mean intra-assay CV% = 5.2, mean inter-assay CV% = 7.0, assay sensitivity = 3.1 pg/mL), Alpha Amylase Saliva ELISA (mean intra-assay CV% = 4.6, mean inter-assay CV% = 6.2, assay sensitivity = 3.6 U/mL). All kits were obtained from IBL International GMBH, Hamburg, Germany. All tests were run in an SQII ELISA processor (AESKU Systems, Wendelsheim, Germany). A calibration curve using standard duplicates was performed for each analyte in every run. The performance of all the kits were validated in our laboratory according to good laboratory practice (GLP) guidelines, complying with ISO 9001 certification and JCI accreditation standards.

* 1. **Trier Social Stress Test and the non-stress control condition**

Psychological stress was induced by employing the TSST procedure (Kirschbaum et al., 1993). This procedure consists of a stress task that includes 5 minutes of free speech, in which participants were instructed to speak as if they were at job interview for their “dream job,” and 5 minutes of a mental arithmetic task. Both parts of the tasks were conducted in front of a video camera and a committee comprising of a man and a woman sitting at a distance of 1.5 m from the participant. At the beginning of the procedure, the committee members provided instructions to the participants regarding the task at hand and explained that their performance would be recorded for subsequent behavioral analysis. The participants were then taken to an empty room in which they had 10 minutes to formulate their speech. After this period of time, the participants entered the committee room and performed the free speech and arithmetic tasks. In total, the procedure, including the preparation period, lasted for approximately 20 minutes.

The control condition was devised to be as comparable as possible to the TSST in terms of the mental and physical workload, but without the stress-inducing elements of social-evaluative threat and uncontrollability (Dickerson and Kemeny, 2004). The procedure consisted of a 10-minute phase during which each participant was instructed to read the entry, "England," in Wikipedia silently, followed by 5 minutes of reading the entry, "transport in Israel," out loud and another 5 minutes of counting out loud. During the entirety of the task, the participant was alone in a room (the same room used for the TSST procedure), but with no people or cameras present.

* 1. **Rey Auditory Verbal Learning Test (RAVLT)**

The Hebrew version of the RAVLT (Vakil and Blachstein, 1993) was used to assess declarative memory. Each participant received different versions of the test before and after the stress or control procedure to avoid learning effects, and the order of the two versions were randomized and counterbalanced. The RAVLT was composed of seven trials. On each of the first five consecutive trials, an experimenter read a list of 15 common nouns to the participants, at the rate of one word per second. Each reading was followed by a free recall task, in which participants were asked to repeat as many of those words as possible. The performance on these five trials reflected the rate of learning. In trial 6, an interference list of 15 *new* common nouns was presented, followed by participants’ free recall of these new nouns, which tested retention of the new words. In trial 7, participants were asked to recall the words from the first list. This last trial tested the level of recall after interference.

* 1. **Statistical analyses**

To test the impact of stress exposure on memory performance, we performed a three-way mixed analysis of variance (ANOVA). The independent variables included the sum of participant performance on the first 7 RAVLT trials, group (men, OC, and LP) and stress exposure (stress vs. control), and the dependent variable was participant performance on the RAVLT after stress exposure.

Because cortisol, sAA, and sex hormone levels were not normally distributed, we performed a log10 transformation. **To account for the large variability among participants in their cortisol reactivity to stress, the sample was divided into responders (*n* = 23)and non-responders (*n* = 33), inaccordance with the criteria put forth by Hidalgo et al. (2012). Participants who exhibited an increase in salivary cortisol concentration from baseline levels (-40 min) to the third cortisol measurement (+10 min) after the TSST were considered “responders.” The distribution of responders among hormonal status groups did not differ significantly: *χ2* (2) = 2.70, *p* = .26; see supplementary Table 1 for further analyses.** For sAA, all participants demonstrated elevated levels in T2 as compared to T1 [no significant difference was found in the increase levels between hormonal groups: *F* (2, 43) = .19, *p* = .83]. To examine stress-induced differences in hormonal response, a repeated-measures ANOVA was used, with group (males, OC, LP**), stress exposure (stress, control),** and time (T1, T2, T3, T4) as the independent variables and either cortisol (for responders) or sAA (for the whole sample) as the dependent variables. Significant main effects were further analyzed using Bonferroni post-hoc tests.

To examine the modulating role of stress markers and **baseline reproductive hormones** on the association between stress on memory, we conducted a two-way mixed ANOVA with time and group as the independent variables, performance on the RAVLT as the dependent variable, and r**eproductive** hormones and stress biomarkers reactivity as covariates. For this analysis, cortisol and sAA reactivity were calculated as the change from baseline values to post-stress values: T3 (ΔC) and T2 (ΔsAA), respectively. These two time points were selected because the SNS releases catecholamines immediately after the onset of a stressor, whereas the HPA release of glucocorticoids is slower, with cortisol reaching peak levels only 21–45 minutes after the onset of a stressor (for a meta-analysis, see Dickerson and Kemeny, 2004). For all the ANOVA tests, whenever Mauchly's test indicated a violation of sphericity assumption, Greenhouse-Geisser corrections were used. Post-hoc comparisons were performed using Bonferroni adjustments for multiple comparisons of *p* values.

Finally, to further examine the relation between the HPA-A/SNS and HPG-A cross-talk and stress-induced changes in memory performance, we performed interaction analyses. Moderated regression analyses were conducted using **mean-centered predictors to calculate interaction terms.** The interaction terms were inserted as predictors in the second step of each analysis to predict memory performance (calculated as the difference between memory performance scores before and after the stress exposure or control condition). Significant interactions were probed using the procedures described by Aiken and West (1991).

1. **Results**

**Table 1 presents the mean baseline concentrations of cortisol (separated by responders and non-responders), sAA, testosterone, estradiol, and progesterone for each group—men, OC women, and LP women. One-way ANOVAs, followed by Bonferroni post-hoc tests, verified that testosterone levels were higher among men compared to OC women and LP women, and that estrogen progesterone levels were higher among LP women as compared to OC women and men.**

Table 1

*Means, Standard Deviations, t values, and F tests for group differences in baseline raw scores of the biomarkers* ***for the full******sample***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***Men (N = 21)*** | ***OC (N=20)*** | ***LP (N = 17)*** | ***F*** |
| Cortisol (responders) (µg/dL) | **0.29 (0.15)** | **0.45 (0.19)** | **0.26 (0.13)** | **2.86** |
| Cortisol (non-responders) (µg/dL) | **0.53 (0.27)** | **0.58 (0.37)** | **0.69 (0.40)** | **0.55** |
| Cortisol (full sample) | **8.52 (12.62)** | **9.02 (11.55)** | **8.41 (10.84)** | **0.03** |
| sAA(U/mL) | **70.18 (41.66)** | **59.27 (46.05)** | **35.01 (5.84)** | **0.68** |
| Testosterone (pg/mL) | **135.14 (78.30)** | **31.38 (20.35)** | **43.19 (29.71)** | **47.12\*\*\*** |
| Estradiol (pg/mL) | **2.75 (1.05)** | **2.53 (0.78)** | **3.23 (1.13)** | **4.70\*** |
| Progesterone (pg/mL) | **30.78 (30.19)** | **19.79 (17.19)** | **136.12 (177.14)** | **13.29\*\*\*** |

***Note.* OC = oral contraceptives; LP = luteal phase; sAA = salivary alpha-amylase. Data presented as mean ± SD in absolute values*.***

***\* p<.05; \*\*\* p<.001***

* 1. **Stress response**

The patterns of cortisol reactivity and sAA reactivity for each study group are depicted in Figure 2. **A three-way repeated-measures ANOVA with group (men, OC, and LP), stress exposure (stress, control), and time (T1, T2, T3, T4) as the independent variables**, and with cortisol reactivity as the dependent variable, revealed **no significant stress X group interaction [*F* (2, 66) = 1.36, *p* = .264; *η2p* = .04], and no significant time X group interaction [*F* (6, 158) = 1.69, *p* = .125; *η2p* = .05]. A significant time X stress X group interaction was found [*F* (6, 198) =2.93, *p* < .01; *η2p* = .08], as well as a significant time X stress interaction [*F* (3, 198) =42.64, *p* < .001; *η2p* = .39]. Further analyses revealed a significant effect of time in the stress group.** A **post-hoc analysis revealed that the cortisol level at T3 was higher compared with other cortisol measurements. A significant effect of time was also found in the control group. A post-hoc analysis for** **the control group revealed that the cortisol level at T1 was higher compared with other cortisol measurements, and that there were significant declines in cortisol levels from one timepoint to the next. Further investigation of the three-way interaction (time X stress X group) revealed similar patterns of results as those found in the two-way interaction (time X stress), such that there was a significant effect of time in each hormonal group [*F* (3, 198) = 23.26, *p* <.001; *η2p* = .26] (see Fig. 2).**

**In a three-way repeated-measures ANOVA with group (men, OC, LP), stress exposure (stress, control), and time (T1, T2, T3, T4) as the independent variables and sAA reactivity as the dependent variable, no significant time X group X stress interaction was found [*F* (6, 285) =.54, *p* = .777;** *η****2p* = .01]. Additionally, no significant time x group interaction was found [*F* (6, 285) = 1.03, *p* = .400;** *η****2p* = .02], nor was there a significant stress x group interaction [*F* (2, 95) =.18, *p* = .839;** *η****2p* = .00]. However, a significant time X stress interaction was found [*F* (3, 285) = 19.11, *p* <.001;** *η****2p* = .17]. Further analyses revealed a significant effect of time in the stress group. Post-hoc analyses revealed that the sAA level at T1 was lower than other sAA measurements, that the sAA level at T2 was higher than other sAA measurements, and that the sAA level at T4 was significantly higher than that observed at T3. A significant effect of time was also found in the control group. Post-hoc analyses revealed that the sAA level at T4 was higher than sAA measurements at the other timepoints, but that there were no significant differences in sAA measurements across T1, T2, and T3 (See Fig. 3).**

* 1. **Memory performance**

Table 2 presents the mean scores of the declarative memory test after exposure to either the TSST or the control condition (see Table 2 in the supplementary material for memory scores before exposure to the TSST or the control condition).

**Table 2**

***Means and Standard Deviations for group differences in memory performance following exposure to either the stress procedure or a non-stress control condition***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | ***Men (N = 39)*** | | ***OC (N = 37)*** | | ***LP (N = 36)*** | |
|  | ***Control*** | ***Stress*** | ***Control*** | ***Stress*** | ***Control*** | ***Stress*** |
| **Trial 1** | **8.28 (2.87)** | **6.86 (1.82)** | **6.59 (1.84)** | **7.45 (1.99)** | **8.79 (2.68)** | **7.12 (1.97)** |
| **Trial 2** | **11.28 (2.42)** | **9.19 (2.27)** | **10.29 (1.96)** | **10.5 (2.09)** | **11.89 (2.49)** | **9.59 (2.83)** |
| **Trial 3** | **12.22 (2.42)** | **10.52 (3.12)** | **12.06 (1.82)** | **11.95 (2.26)** | **12.84 (2.29)** | **10.94 (2.49)** |
| **Trial 4** | **13.00 (1.68)** | **11.71 (2.61)** | **13.18 (1.33)** | **12.40 (2.23)** | **13.42 (2.12)** | **12.65 (2.34)** |
| **Trial 5** | **13.44 (1.65)** | **12.24 (2.45)** | **13.71 (1.21)** | **12.10 (2.38)** | **13.95 (1.75)** | **12.47 (2.07)** |
| **Trial 6** | **7.89 (3.36)** | **6.76 (1.84)** | **6.82 (2.10)** | **6.65 (2.28)** | **8.00 (2.21)** | **6.59 (2.60)** |
| **Trial 7** | **12.17 (2.15)** | **8.71 (5.14)** | **11.53 (2.10)** | **7.65 (3.79)** | **12.37 (3.37)** | **8.24 (3.90)** |

***Note:* Abbreviations: OC: oral contraceptives; LP: luteal phase. Data presented as mean ± SD.**

* + 1. **Learning curve (Trials 1-5)**

A three-way mixed ANOVA with hormonal group (men, OC, LP), stress exposure (stress, control) and memory trial (1 to 5) as the **independent variables and declarative memory as the dependent variable revealed that, after the TSST/control procedure, the interaction between hormonal group and stress exposure was not significant [*F* (2, 106) = 1.87, *p* = .159; *η2p* = .03], nor were the interactions between trial and stress [*F* (4, 424) = 1.02, *p* = .397; *η2p* = .07] and between hormonal group and trial [*F* (8, 424) = 1.29, *p* = .247; *η2p* = .02]. Additionally, the three-way interaction of hormonal group, trial, and stress was not significant [*F* (8, 424) = 1.97, *p* = .068; *η2p* = .04].** There was no significant main effect for group [*F* (2, 106) =.44, *p* = .646; *η2p* = .01]. However, a significant main effect was found for memory trial [*F* (4, 424) = 258.42, *p* = .000; *η2p* = .71], with post-hoc analyses revealing a positive learning curve occurring from trial 1 to trial 5 (*p <* .001).

* + 1. **Recall of the interference list (Trial 6)**

A two-way ANOVA with hormonal group (men, OC, LP) and stress exposure (stress, control) as the independent variables and memory of the interference list (trial 6) as the dependent variable revealed that, after the TSST/control procedure, **the interaction between hormonal group and stress exposure was not significant [*F* (2, 106) =.63, *p* = .537; *η2p* = .01].** There was no significant main effect for group [*F* (2, 106) =.66, *p* = .519; *η2p* = .01] and no significant main effect for stress exposure condition [*F* (1, 106) = 3.69, *p* = .057; *η2p* = .03].

* + 1. **Recall after interference (Trial 7)**

A two-way ANOVA with hormonal group (men, OC, LP) and stress exposure (stress, control) as the independent variables and memory after interference (trial 7) as the dependent variable revealed that, after the TSST/control procedure, the interaction between hormonal group and stress exposure was not significant [*F* (2, 106) =.08, *p* = .919; *η2p* = .00]. There was no significant main effect for group [*F* (2, 106) =.59, *p* = .557; *η2p* = .01]. However, a significant main effect for stress exposure was found [*F* (1, 106) = 30.83, *p* = .000; *η2p* = .23], such that the group exposed to the stress condition (TSST) demonstrated poorer recovery after interference as compared to the control group.

* 1. **Cortisol, sAA, reproductive hormones, and memory**

To test the relationship between the HPA-A and HPG-A cross-talk and declarative memory, we performed an analysis only for trial 7, as there was a significant effect found for stress exposure during that trial (see Fig 3). A two-way mixed ANOVA with recall after interference (trial 7) as the dependent variable before and after the TSST among the group exposed to stress and the hormonal group as a between-subject factor revealed a significant main effect for stress [*F* (1, 55) = 11.38, *p* = .001; *η2p* = .17]. Memory performance after interference was higher before stress exposure than after stress exposure. However, the main effect for stress became non-significant after controlling for **baseline reproductive** hormone levels [*F* (1, 47) =.48, *p* = .490; *η2p* = .01].

Hierarchical regressions were conducted to further examine the combined effect that **baseline reproductive** hormones, cortisol, and sAA reactivity have on memory performance after interference. The analysis revealed that there was a significant two-way interaction between testosterone and ΔC in predicting the difference in memory performance before and after stress exposure (*β* = -1.51, *p* = .026, 95% CI: -523.36, -36.74) among responders. Simple slope analyses (Hayes, 2013) revealed a negative association between ΔC and the magnitude of decrease in memory performance following stress exposure for individuals with higher testosterone levels (*b* = -178.45, *t*(22) = 2.54, *p* = .020); however, the slope did not reach significance in individuals with lower testosterone levels (*b* = 27.67, *t*(22) = .70, *p* = .493).

In addition, the analysis revealed a significant two-way progesterone X ΔsAA interaction in predicting the difference in memory performance before and after stress exposure (*β* = .34, *p* = .042, 95% CI: .31, 16.57). Simple slope analyses (Hayes, 2013) revealed a negative association between ΔsAA and the magnitude of decrease in memory performance following stress exposure among individuals with lower progesterone levels (*b* = -4.09, *t*(41) = 2.13, *p* = .040); the slope did not reach significance for individuals with higher progesterone levels (*b* = 4.03, *t*(41) = 1.34, *p* = .187).

1. **Discussion**

To the best of our knowledge, the current study is the first to examine the effects of the interaction between the major neurobiological stress systems (ANS and HPA-A) and **reproductive** hormones (estradiol, progesterone, and testosterone) in mediating the association between psychosocial stress (TSST) and declarative memory performance. We hypothesized that participants’ performance under conditions of stress would be affected by the interaction between their **baseline** levels of specific **reproductive** hormones relative to their individual or joint levels of reactive cortisol or alpha-amylase.

* 1. **Sex differences in baseline levels of** r**eproductive hormones, stress markers and memory**

As expected, women had higher basal levels of progesterone and estradiol compared to men, and men had higher basal levels of testosterone. The comparison between men and women (both LP and OC women) revealed significantly higher levels of estradiol among women (**see Table 3 in the supplementary material for detailed sex differences in baseline hormone levels**).

Consistent with the view that oral contraceptives suppress the secretion of gonadal hormones (D'Arpe et al., 2016), progesterone **and estrogen** levels were significantly lower in OC women than LP women. Consistent with previous reports (e.g., Hidalgo et al., 2014), there were no sex differences in the basal pre-stress levels of cortisol and sAA across the three groups.

* 1. **Effects of psychosocial stress on declarative memory** **and neurobiological stress reactivity**

Psychosocial stress, induced by the TSST, before learning did not affect the learning curve (trials 1–5 on the RAVLT). These results are consistent with several earlier studies on the effects of the TSST on verbal memory, which used similar designs and study samples (Espin et al., 2013; Hidalgo et al., 2012; 2014; Smeets et al., 2006). Yet, in the current study, the TSST did disrupt recall after interference (regardless of hormonal group), which may suggest that **coping** with interference is more sensitive to the effects of psychosocial stress than the initial encoding processes. Notably, a few previous studies with young adults reported no effect of stress on recall after interference (Hidalgo et al., 2014) and one even showed an enhancing effect (Espin et al., 2013). The source for this discrepancy is unclear, but it may due to differences in the characteristics of the samples, **as well as the timing of the testing. Specifically, the two aforementioned studies were conducted in the afternoon, whereas the current study was conducted in the morning (8:00–10:00 AM). Indeed, a meta-analysis by Het et al. (2005) demonstrated that administration of cortisol (somewhat equivalent to the cortisol reactivity induced by stress) caused memory impairments in participants who took part in studies conducted in the morning and memory enhancements in participants who took part in studies conducted in the afternoon. In this regard, it is important to note that most previous studies examining stress reactivity have been conducted during the afternoon, when cortisol levels are relatively low. In contrast, during the morning hours, diurnal cortisol levels are at their highest (Ghiciuc et al., 2011). Thus, the fact that the proportion of participants who demonstrated increased cortisol secretion in response to the TSST (i.e., "responders") in the current study was lower than that typically reported (e.g., Reschke –Hernández et al., 2017; Stephans et al., 2016) may have partially resulted from a ceiling effect.**

Indeed, studies that induced stress in the afternoon demonstrated a significantly larger cortisol increase than studies conducted in the morning (Dickerson and Kemeny, 2004); in the current study, responders had significantly higher basal levels of cortisol (but not of **reproductive** hormones) compared to non-responders. Moreover, in the morning, testosterone levels are at their peak **in both men and women** (Dabbs and de La Rue; 1991; Diver et al., 2003). Consistent with previous evidence suggesting that testosterone may inhibit cortisol stress reactivity (Stephens et al., 2016), in the current study there was a significant negative correlation between basal testosterone levels and the post-stress cortisol levels (**see Table 3 in the supplementary material**). Thus, it is reasonable to deduce that the high levels of testosterone accounted for the relatively modest cortisol response. **Moreover, the difference between men and women in morning testosterone levels may account for the fact that, in the current study, dissimilar to findings of previous studies (Reschke –Hernández et al., 2017), cortisol stress reactivity was lower among men compared to LP women. However, this possibility will need to be verified in future studies.**

**Analyses regarding sAA reactivity revealed an increase in sAA throughout the experiment, similar to findings that have been reported in previous studies (e.g., Sänger, Bechtold, Schoofs, Blaszkewicz, & Wascher, 2014). This increase has been assumed to be caused by the sAA sensitivity to environmental factors (Skoluda et al., 2015). It may be that the continuing cognitive demand had triggered sAA secretion.**

* 1. **Interaction between reproductive hormones and stress systems in the effects of stress on declarative memory**

The literature regarding the role of SNS and HPA activation in stress-induced changes in cognitive functions is not straightforward. Although the exogenous delivery of cortisol or hydrocortisone before learning has often been reported to inhibit declarative memory (e.g., Brunner et al., 2006), some studies have reported no effect (Het et al., 2005). Moreover, cortisol in such studies is often administered in higher doses than the increase that naturally occurs. Supporting the role of cortisol secretion in stress-induced changes to cognitive functions, the TSST altered performance on the RAVLT only among cortisol responders (e.g., Nater et al., 2007). In addition, a negative correlation was found between post-stress cortisol and the delayed retrieval of earlier learned words after exposure to stress (Elzinga and Roelofs, 2005). However, in other studies, the effects of psychosocial stress on participants’ declarative memory performance were only marginally associated with cortisol levels (Hidalgo et al., 2015). Similarly, in the current study, the correlation between stress-induced changes in declarative memory performance and SAA reactivity or cortisol reactivity was not significant. Moreover, there were no differences between cortisol responders and non-responders in performance on the memory task before or after the TSST.

Thus, the effects of cortisol and SNS activation on declarative memory appear to be complex, and to depend on factors such as the valence of the stimuli and the type of memory process (encoding, consolidation, immediate recall, delayed recall, etc.). Moreover, given the interactions between the HPA-A and the HPG-A (Handa and Weiser, 2014), the relationship between stress-induced levels of cortisol and SNS activation may also depend on the basal levels of **reproductive** hormones.Indeed, several prior studies have provided indirect support for this hypothesis by demonstrating sex differences, or differences between women in different hormonal states (follicular phase, LP, OC) in the effects of psychosocial stress on memory (e.g., Wolf et al., 2001). However, the current study is, to the best of our knowledge, the first to directly examine this possibility.

**Reproductive** hormones appeared to modulate the effects of the stressor on declarative memory in the current study: the effects of stress on memory performance were no longer statistically significant when controlling for basal testosterone, estradiol, and progesterone. To further analyze the possible interactive effects of stress factors (cortisol and sAA) on memory, we examined the relationship between the levels of each sex hormone and stress factor in predicting memory performance. To do so, we conducted a moderation analysis through a series of hierarchical regressions, which included the interactions between each sex hormone and cortisol or sAA stress reactivity (i.e., the increase following the TSST). This analysis did not reveal significant associations between stress-induced changes in memory performance and the interactions between levels of estradiol and the stress-induced changes in cortisol or sAA. However, the stress-induced decline in memory performance was negatively associated with the stress-induced cortisol reactivity in individuals with higher testosterone levels; however, in individuals with lower testosterone levels, there was no association between the two. This pattern of results suggests that when basal testosterone levels are high, a greater cortisol reactivity is somewhat "protective" of the negative impact of stress on memory.

In addition**, the TSST induced both an increase in sAA and a decline in memory performance, and there was a negative association between these two effects among individuals with lower progesterone levels. However, there was no association between these effects in individuals with higher progesterone levels**. This pattern of results implies that when basal progesterone levels are low, a greater SNS activation in response to stress is somewhat "protective" of the negative impact of stress over memory. Further investigation aimed at uncovering the source of the interaction by conducting a series of moderated regression analyses, separately by hormonal group. Interestingly, the interaction was significant only among LP women **(see Table 4 in the supplementary material)**, which was likely due to the wide range of progesterone levels in this group. On the other hand, as progesterone levels were particularly low among women in the OC group, it is not surprising that only within this group there was a significant negative correlation between baseline progesterone levels and the level of stress-induced reduction in recall after interference **(see Table 5 in the supplementary material)**. This finding may suggest that, among women taking oral contraceptives, memory performance may be less affected by stress exposure, as long as their SNS activation remains high. However, as the influence of sympathetic activation in general, and its interaction with the HPG in particular, on memory has been little studied to date, a conclusive interpretation of the effect of sAA reactivity and basal progesterone levels on memory performance is difficult to ascertain. Nevertheless, this novel preliminary finding suggests complexity in the SNS-progesterone interaction and points to the need for continued research on the interactive role of **reproductive** hormones and sympathetic activation as it relates to the effects of stress on cognitive functioning.

The current study focused on the relationship between basal sex hormone levels and stress-induced effects on verbal memory performance. However, it is important to emphasize that acute stress may increase the secretion of **reproductive** hormones (Bedgood et al., 2014; Herrera et al., 2016; Shors et al., 1999) and, thus, it is possible that stress-induced elevations in the levels of **reproductive** hormones, at least partially, accounted for the observed reduction in memory performance. This intriguing possibility will be the subject of future studies in our laboratory.

* 1. **Limitations of the Current Study**

Interpretation of the present findings should be viewed in light of a few limitations. First, as the proportion of responders (i.e., participants who exhibited increased cortisol levels after the TSST) was relatively small, the sample size available for many of the analyses was rather small and it precluded comparisons between the different hormonal status groups. Second**, a comparison of the findings of the current study with those of previous studies on the effects of psychosocial stress on the role of stress hormones and reproductive hormones is complicated as the prior studies were primarily conducted in the afternoon and the current study was conducted in the morning. However, this difference can also be viewed as a strength of the current study: the morning is an important time of the day, and it has been understudied in research involving stress reactivity. Third, reproductive** hormones were analyzed from a single saliva sample, which may have led to considerable variability because of the pulsating dynamics of sex hormone secretion **(Keenan & Veldhuis, 2016).** However, the fact that significant results were still obtained despite this variability attests to the importance of **reproductive** hormones in the effects of the stress response on cognition. Nevertheless, taking multiple saliva samples in future studies would yield more accurate assessments of hormonal levels and may increase statistical power**. Post hoc statistical estimates** (Onwuegbuzie & Leech, 2004) **ranged from .12 to .81 for memory performance (learning curve, interference, and following interference trial), which represent low to high effect sizes. Therefore, replications are needed to evaluate the extent of influence that the TSST has on memory performance. Fourth, in the current study the participants chewed on a piece of parafilm in order to stimulate saliva secretion. Such stimulation of saliva secretion may affect the relative amount of alpha amylase in the saliva (Rohleder & Nater, 2009). However, this factor was kept constant within the study as both the control group and the stress group chewed parafilm prior to saliva sampling. Fifth,** a lack of psychological and physiological disorders among the participants was determined solely by self-report. Thus, it is possible that a few of the participants had conditions that could have affected their hormonal regulation**. Moreover, the fact that the study sample was composed only of young, healthy participants limits the generalizability of the results. Sixth**, because psychosocial stressors are the most common stressors in modern life, the current study used the TSST, which is the most validated measure of psychosocial stress (Skoluda et al., 2015). However, various stressors may elicit differentiated responses and physiological mechanisms. Therefore, examining the impact of other stressors in future studies is warranted. Lastly, the memory task used in the current study examined verbal memory. Other forms of declarative memory**, such as long-term visual memory and spatial memory**, may be differentially affected by the interaction between **reproductive** hormones and stress mechanisms.

* 1. **Conclusion**

This study demonstrated that psychosocial stress disrupts aspects of declarative memory. Moreover, these effects of stress appear to depend on the activity of **reproductive** hormones. The evidence suggests that modulatory interrelationships between SNS activation and progesterone may be particularly influential in mediating the effects of stress on declarative memory. These novel findings strengthen the importance of HPA–HPG interactions on behavior and are among the first demonstrations of the role of SNS–HPG interactions on cognitive function. Taken together, these results suggest that the HPG needs to be addressed when studying the effects of neurobiological stress mechanisms on cognitive performance.

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**Figures**

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**Figure 3**

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**Figure captions**

Figure 1. Study design

The experimental session was composed of three consecutive stages: (A) completion of the Rey Auditory Verbal Learning Test (RAVLT); (B) the Trier Social Stress Test procedure or the control procedure; and (C) a second completion of the RAVLT. Participants provided saliva samples at four timepoints, referred to as T1-T4. Participants provided 5 ml of saliva at T1, which was used to evaluate baseline levels of testosterone, estradiol, progesterone, cortisol, and sAA. At the following timepoints (T2-T4), participants provided 2 ml of saliva, which were used to evaluate levels of reactive cortisol and sAA.

Figure 2. Physiological stress response: Cortisol

Salivary concentrations of cortisol before and after either the TSST procedure (Panel A) or the non-stress control procedure (Panel B). For participants in the stress group (data include responders only), cortisol secretion increased significantly from baseline (T1) to T3 (10 minutes after completion of the TSST). Among participants in the control group, cortisol secretion was lower at T2-T4 as compared to baseline (T1) levels.

\**p*<.05. Values depict means of cortisol concentrations. Error bars represent SEM.

Figure 3. Physiological stress response: Alpha amylase

Salivary concentrations of alpha amylase before and after either the TSST procedure (Panel A) or the non-stress control procedure (Panel B). In the stress group, alpha amylase secretion was significantly higher on all 3 post-TSST timepoints as compared to baseline (T1). In the control group, alpha amylase levels were higher than baseline levels only at T4, which occurred 20 minutes after the control procedure. \**p*<.05. Values depict means of alpha amylase concentrations. Error bars represent SEM.

Figure 3. Effects of the TSST over declarative memory

Number of words recalled (Rey Auditory Verbal Learning Test; RAVLT) before and after exposure to the TSST procedure among men, women in the luteal phase of the menstrual cycle (LP women) and women taking oral contraceptives (OC women). The TSST did not affect the learning curve (trials 1-5) or memory of the interference list (trial 6). However, the TSST disrupted recall following interference (trial 7). These effects did not differ across the three groups. \**p*<.05

**Supplementary data**

**Table 1**

***Differences between cortisol responders and non-responders in baseline levels of biomarkers***

|  |  |  |  |
| --- | --- | --- | --- |
|  | *Responders (N = 23)* | *Non-responders (N = 33) (N=20)* | *t* |
| Cortisol (responders) (µg/dL) | 0.56 (0.15) | 0.75 (0.21) | 3.71\* |
| sAA(U/mL) | 77.73 (46.81) | 73.73 (47.58) | 0.31 |
| Testosterone (pg/mL) | 102.15 (105.09) | 68.71 (56.82) | 1.54 |
| Estradiol (pg/mL) | 2.09 (0.54) | 2.34 (0.92) | 1.14 |
| Progesterone (pg/mL) | 35.22 (43.56) | 61.02 (85.96) | 1.44 |

*Note.* sAA = salivary alpha-amylase. Data presented as mean ± SD **in absolute values***.\* p<.05*

**Table 2**

***Means and Standard Deviations for group differences in memory performance before exposure to either the stress procedure or a non-stress control condition***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | ***Men (N = 39)*** | | ***OC Women (N = 37)*** | | ***LP Women (N = 36)*** | |
|  | ***Control*** | ***Stress*** | ***Control*** | ***Stress*** | ***Control*** | ***Stress*** |
| **Trial 1** | **8.56 (2.06)** | **7.19 (1.91)** | **8.29 (1.83)** | **7.25 (1.45)** | **8.95 (1.78)** | **7.35 (1.69)** |
| **Trial 2** | **10.44 (2.66)** | **9.62 (2.74)** | **10.94 (1.82)** | **8.90 (2.10)** | **11.26 (1.97)** | **9.12 (2.78)** |
| **Trial 3** | **12.33 (1.97)** | **10.67 (2.61)** | **12.41 (1.37)** | **11.00 (2.87)** | **12.68 (2.00)** | **10.59 (2.21)** |
| **Trial 4** | **12.56 (2.06)** | **11.29 (2.81)** | **12.65 (1.94)** | **11.95 (2.19)** | **13.26 (1.70)** | **11.18 (1.98)** |
| **Trial 5** | **13.11 (1.94)** | **12.00 (2.07)** | **13.59 (1.00)** | **11.60 (1.79)** | **13.74 (1.45)** | **11.88 (1.65)** |
| **Trial 6** | **7.89 (3.36)** | **5.76 (1.92)** | **6.82 (2.10)** | **6.90 (1.68)** | **8.00 (2.21)** | **5.71 (1.61)** |
| **Trial 7** | **12.17 (2.88)** | **10.52 (2.58)** | **11.94 (1.82)** | **9.10 (3.16)** | **12.68 (2.00)** | **10.41 (2.69)** |

***Note.* OC- oral contraceptives; LP = luteal phase. Data presented as mean ± SD.**

***Differences between cortisol responders and non-responders in declarative memory performance***

There were no differences between cortisol responders and non-responders in declarative memory performance on the Rey Auditory Verbal Learning Test (RAVLT) before the TSST. Moreover, a three-way mixed ANOVA with hormonal group (men, OC, LP), respondent group (responders, non-responders)and trial (1 to 7) as the independent variables and declarative memory as the dependent variable revealed that, after the TSST, the interaction between respondent group and hormonal group was not significant [*F* (2, 50) =2.93, *p* = .063; *η2p* = .11], nor was the interaction between respondent group and trial [*F* (6, 300) =.72, *p* = .632; *η2p* = .01].

**Table 2**

***Sex differences in baseline levels of biomarkers***

|  |  |  |  |
| --- | --- | --- | --- |
|  | *Men (N = 21)* | *Women (N = 37) (N=20)* | *t* |
| Cortisol (responders) (µg/dL) | 0.41 (0.24) | 0.55 (0.35) | 1.63 |
| sAA(U/mL) | 81.94 (49.64) | 73.46 (55.73) | 0.63 |
| Testosterone (pg/mL) | 148.51 (99.81) | 46.44 (30.24) | 4.57\*\*\* |
| Estradiol (pg/mL) | 1.98 (0.48) | 2.35 (0.88) | 2.06\* |
| Progesterone (pg/mL) | 16.73 (3.34) | 68.18 (84.74) | 3.59\*\* |

*Note.* OC = oral contraceptives; LP - luteal phase; sAA - salivary alpha-amylase. Data presented as mean ± SD **in absolute values***.*

*\* p<.05 \*\* p<.01 \*\*\* p<.001*

**Table 3**

***Correlations between basal gonadal hormones and post-stress cortisol and alpha amylase response***

|  |  |  |
| --- | --- | --- |
|  | ***Cortisol – T3*** | ***sAA – T2*** |
| Testosterone | **-.53\*\*** | **.28\*** |
| Estradiol | **-.02** | **.12** |
| Progesterone | **.03** | **.01** |

***Note.* Abbreviations: Cortisol – T3: post stress cortisol levels; sAA - post stress salivary alpha-amylase. Correlations were calculated using log10 transformed biomarker values.**

***\* p<.05 \*\* p<.01***

**Table 4**

***Multiple regression models testing the interactions between (a) basal testosterone × cortisol and (b) progesterone × sAA reactivity to stress interactions in predicting memory performance across each hormonal group***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***β*** | ***B*** | ***SEB*** | ***95% CI*** | ***R2*** | ***ΔR2*** | ***F*** |
| T × ΔC |  |  |  |  |  |  |  |
| Men | **-.11** | **-.02** | **.27** | **(-.67, .62)** | **.40** | **.00** | **1.53** |
| OC | **-.90** | **-.60** | **1.48** | **(-5.31, 4.11)** | **.15** | **.05** | **.17** |
| LP | **2.28** | **.96** | **1.03** | **(-12.13, 14.05)** | **.59** | **.36** | **.47** |
| P × ΔsAA |  |  |  |  |  |  |  |
| Men | **-.11** | **.00** | **.00** | **(-.00, .00)** | **.02** | **.00** | **.06** |
| OC | **-.53** | **-.00** | **.00** | **(-.00, .00)** | **.60** | **.03** | **.95** |
| LP | **.80** | **.00** | **.00** | **(.00, .00)** | **.68** | **.56\*\*** | **6.33\*** |

***Note*. *B* indicates unstandardized regression coefficients. β indicates standardized regression coefficients. CI = confidence interval (95% confidence intervals of unstandardized regression coefficients). Biomarker values were log10 transformed.**

**OC = oral contraceptives; LP = luteal phase**

***\* p<.05 \*\* p<.01***

**Table 5**

***Correlations between basal gonadal hormone levels and the level of stress-induced reduction in recall after interference***

|  |  |  |  |
| --- | --- | --- | --- |
|  | ***Men*** | ***OC women*** | ***LP women*** |
| Testosterone | **.17** | **.03** | **-.45** |
| Estradiol | **.01** | **-.30** | **-.19.17** |
| Progesterone | **-.27** | **-.46\***  **\*** |  |

***Note.* OC = oral contraceptives; LP = luteal phase.**

**Correlations were calculated using log10 transformed biomarker values.**

***\* p=.05***