**Comparison of affect changes during the ovulatory phase**

**in women with and without hormonal contraceptives**

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Abstract: Studies about affect changes during the menstrual cycle and the role of hormones in these changes have yielded contradictory results. Often research has focused on the pre-menstrual phase, with few studies paying specific attention to the affect changes around ovulation. In this research thirty women completed a daily questionnaire measuring the positive and negative affect during their menstrual cycle. These women were divided in two groups: hormonally-contracepting and naturally-cycling. The Positive and Negative Affect Schedule was used to measure the affect. A Digital Ovulation Test was used to determine the day of ovulation in the participants not taking hormonal contraceptives by measuring the Luteinizing Hormone (LH) peak. The differences in positive affect (PA) and negative affect (NA) between groups were examined. The results indicate that during the ovulatory phase, PA scores are significantly higher in naturally-cycling women’s than hormonally-contracepting women’s.

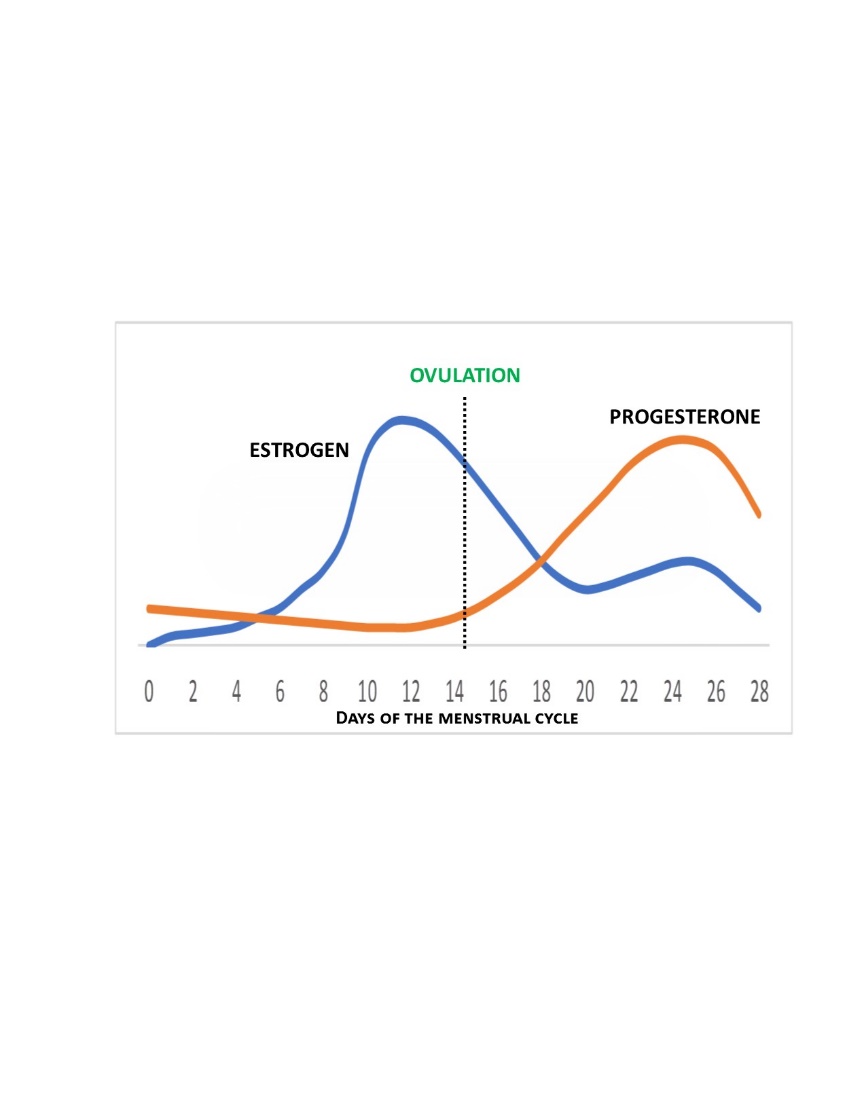
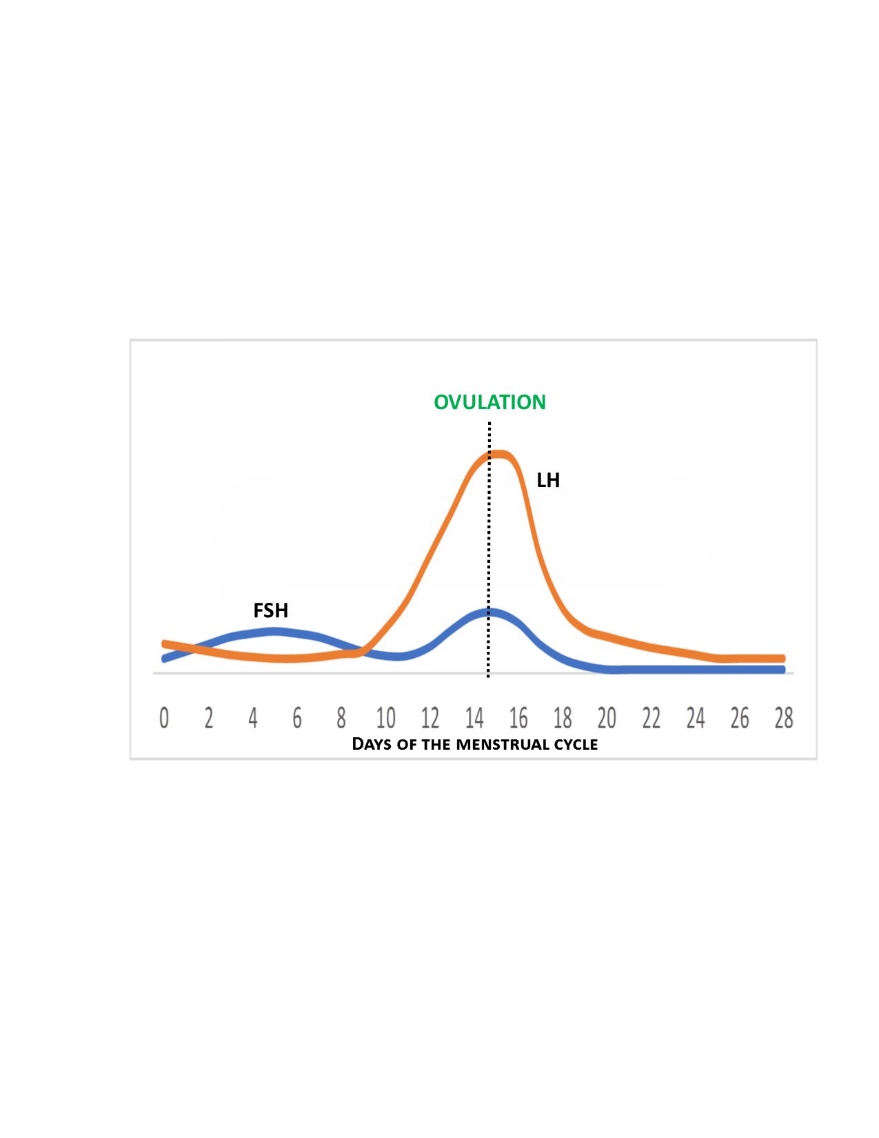
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**1. Introduction**

Around 75-80% of reproductive age women present with some psychophysiological symptoms during the menstrual cycle (Pearlstein and Steiner, 2008; Storck, 2012). According to the Mayo Clinic, these include mood changes (anxiety, depression, lability, irritability, anger, etc.) as well as physical changes (headaches, fatigue, water retention, breast sensitivity, acne, bloating, etc.). Numerous studies (Almagor et al., 1991; Backstrom et al., 1983; Schwartz et al., 2012; Woods, 1986) have observed a positive correlation between these symptoms and changes in hormone levels during the menstrual cycle but the results of these studies have been inconsistent and, at times, contradictory.

There are four main hormones involved in the menstrual cycle (Fig. 1): two of them, Progesterone and Estrogen, are released by the ovaries while the other two, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH), are released by the pituitary gland. This gland is a brain structure that also plays a role in emotional processing (Daban et al., 2005). Could the high levels of LH and FSH needed for ovulation to occur, cause mood changes?

Fig. 1. Hormone levels during the menstrual cycle

 **OVARIAN HORMONES PITUITARY HORMONES**

The rhythm of the menstrual cycle is set by the hypothalamus through the intermittent release of the LH and FSH-releasing hormone which stimulates the synthesis and secretion of the pituitary hormones (Mock, 2002; Silberstein and Merriam, 2000). Depressed women showed a decrease in frequency and a lack of rhythm in the LH pulses which points to a relationship between LH and mood (Meller et al., 2001).

In the 70s and 80s, Rossi & Rossi, Woods, Sanders et al. and Backstrom et al. carried out a series of studies around psychological changes during the menstrual cycle, and found that women experience cyclic changes in mood and that affect is more positive in the middle of the cycle (around ovulation) and more negative during the premenstrual phase.

The HC suppress the LH peak and therefore, the ovulation. Could lower levels of LH influence affect during ovulation? This is the question the present research is trying to answer. This research explores the change in affect during the ovulatory phase, and the direction of this change.

There are many studies about the effects of hormonal contraceptives (HC) in mood (Coffee et al., 2006; Graham et al., 2007; Grant and Pryse-Davies, 1968; Joffe et al., 2003; Shakerinejad et al., 2013; Wyatt et al., 2004). Many of them use women taking HC to better understand the role of hormones in mood changes. These women are often chosen in research because HC alter predictively the hormone levels block the LH peak and prevent ovulation. The most popular HC include a combination of estrogen and progesterone.

In 2002, Oinonen and Mazmanianpublished a study where they reviewed research on HC-related affect changes. These authors found that “HC users experience less variability in affect across the entire menstrual cycle, and less negative affect during menstruation” than non-users. Theyalso reported that only negative affect was measured in most of the studies.

However, other studies (Almagor and Ben-Porath, 1991; Graham et al., 2007) obtained opposite results finding no side effects due to HC use. Almagor and Ben-Porath (1991) found that women taking HC scored higher in PA in the follicular and luteal phases, and during menstruation, but did not observe any significant differences in NA. In the research by Graham et al. (2007), no relationship was found between HC and negative mood (measured with Beck’s depression questionnaire).

Because of the inconsistencies in these results, it is important to continue the research about hormone-related affect changes, taking into account, and attempting to resolve, methodological issues encountered by other researchers. Even though there are many studies about the effects of ovarian hormones in mood and affect, and the effects of HC on mood and affect, none were found which focused on the ovulatory phase while measuring the LH.

**2. Method**

**2.1. Participants**

Thirty women with regular menstrual cycles, between 18-33 years of age, participated in this research. Following Sanders’ method, the following women were excluded from the study: women with medical, psychological and gynecological problems, or important problems in the daily life, as well as women who were breastfeeding or had given birth in the last 6 months, and women taking any medication (with the exception of HC).

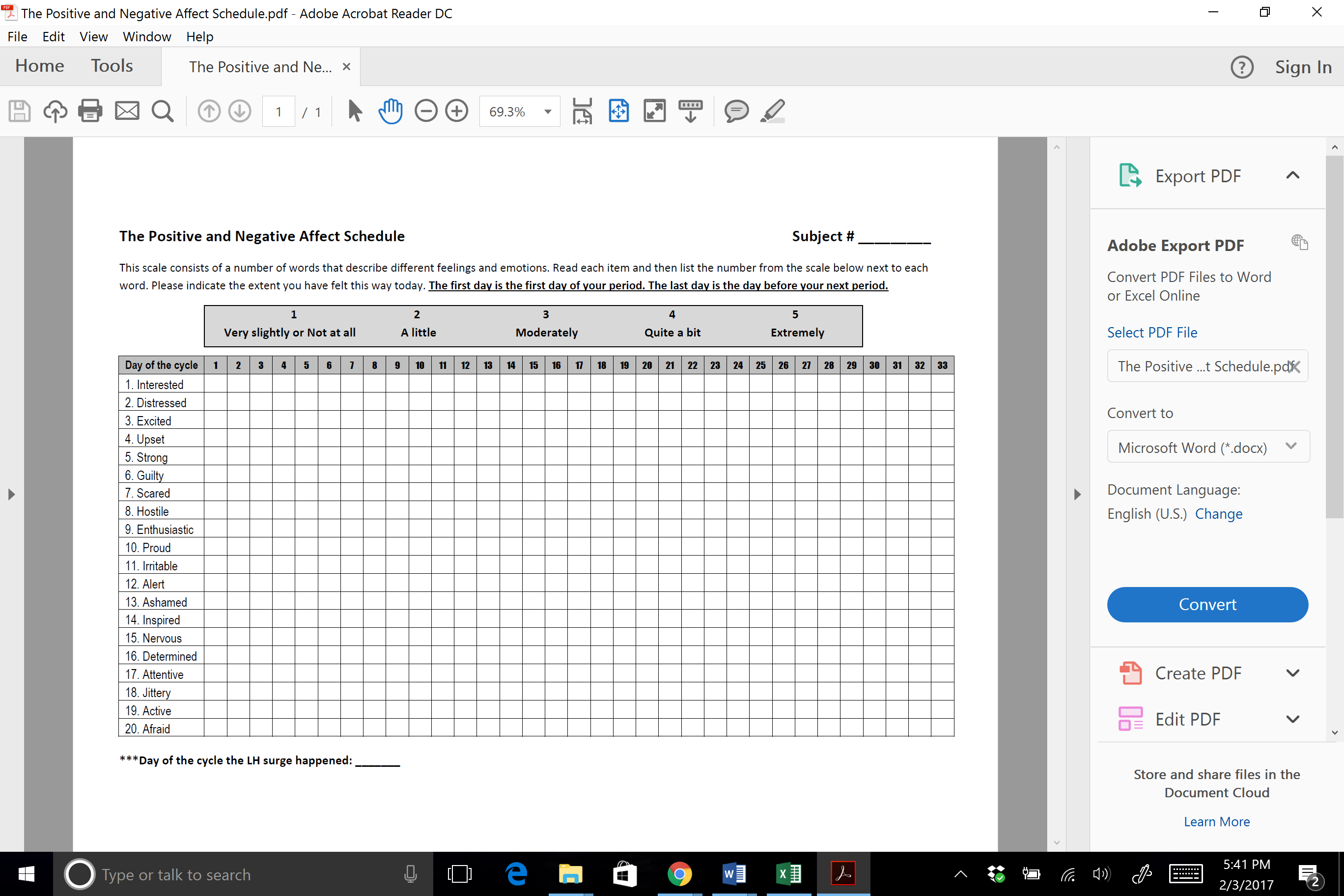
The target sample size for this study was 50 participants. Twenty-five hormonally-contracepting women and twenty-nine naturally-cycling women agreed to participate in this study but only 17 hormonally-contracepting women and 15 naturally-cycling women returned the completed PANAS. One of the naturally-cycling participants reported a cycle of 23 days with the LH peak on day 5. If she had followed the instructions given, she would not have started checking for the LH peak around day 8, therefore the LH peak could never have been day 5. Her data was excluded. Another naturally-cycling participant reported a cycle length of 33 days. The LH was expected to happen around days 16 or 17 but she reported happening on day 26. She would have run of test strips before day 26 had she followed the instructions provided. Her results were also excluded.

All hormonally-contracepting participants reported that they had been compliant with HC use while they were participating in this study.

The study was reviewed and approved (Certificate of Approval # 20131183) by Western Institutional Review Board (www.wirb.com) and the participants were compensated for their time ($20) by the main investigator (personal funds). No funding from any other sources was used. Participants were given a ‘Consent Information Sheet’ and consented to participate without coercion or undue influence. Because only de-identified data was used, a signed Informed consent was not required and a waiver was obtained.

**2.2. Measures**

A quasi-experiment research with two groups was used in this study. A standardized measure frequently selected to be used to measure affect, the Positive and Negative Affect Schedule (PANAS) developed by Watson et al. (1988), was used in the present research because it is a short questionnaire, extensively validated, culturally appropriate and with high internal consistency. The PANAS measures the two main dimensions of affect, PA and NA, with two scales of only 10 items each. This subjective self-reports are adequate to determine the affect at a specific point in time (Sanders et al., 1983). The PANAS (Fig. 2) was completed daily at the same time in the afternoon during a menstrual cycle. Participants were asked to keep the questionnaire in their purses at all times and to set a daily alarm in their cell phones for 6 pm to remind them to fill out the PANAS at that time.

Fig. 2. PANAS with instructions

Many researchers have used the PANAS to measure the PA and NA such as Fernández-Megias (2012), Fredrickson and Joiner (2002), Chico (2006), Chico (2011), Shiota et al. (2006), Wright and Cropanzano (1998), Crede et al. (2005), and Kashdan and Roberts (2004), among others.

The LH levels were measured with the Digital Ovulation Test Clearblue. This test has shown in clinical studies to have a 99% accuracy detecting an increase in the LH. An advantage of this type of test is that the results are visualized (in 3 min.) in a digital screen which eliminates the need for interpreting the results. This test was used every morning at the same time (9 a.m.) on the days the LH increase was expected. Some researchers (Rossi and Rossi, 1977; Woods, 1986) determined the menstrual cycle phases by counting the days since the end of the last menstrual cycle. Since the exact length of the menstrual cycle frequently varies from one month to the next and the ovulation phase lasts only one day, counting the days is not a reliable method to determine when ovulation happens. To accurately pinpoint the day of ovulation, an objective test needs to be used such as a digital ovulation test. This is an important methodological improvement the present research offers over past studies.

**2.3. Statistical Analysis**

SPSS (version 23) was used to calculate a one-way ANOVA between the two groups using the PA and NA scores. Microsoft Excel was used to calculate the means and standard deviation.

**2.4. Procedure**

The menstrual cycle starts the first day of menses and ends the day before the next period. The length of the cycle varied among the participants: the shortest was 19 days and the longest was 33 days. As a cautionary measure, the 19-day cycle was excluded from the analysis due to being significantly outside of the norm which could indicate incorrect use of the tester. Women were asked to participate for only one menstrual cycle to limit participant attrition. The women completed the PANAS every day at the same time (6 p.m.). The time and consistency of the time are both important because Watson et al. (1988) realized that the PA is sensitive to the time of day, tending to increase in the morning and decrease after 9 p.m., but remains stable between noon and 9 p.m. The NA did not show that sensitivity. This is another methodological improvement over other studies since this was not considered in any other research.

The participants were assigned to the hormonally-contracepting (n=17) or naturally-cycling (n=13) group depending on whether they were taking HC or not. The women taking the contraceptives were assigned to the hormonally-contracepting group and the ones that were not taking HC were assigned to the naturally-cycling group.

The women in both groups completed the PANAS daily but only the women in the naturally-cycling group used the digital ovulation test. This tester measures the LH level in urine with a test stick to identify the day of the LH peak. To determine the first day for testing, the participants were instructed to use the Clearblue manufacturer’s recommended online calculator (www.clearblueeasy.com/start-testing-with-clearblue-easy-ovulation-tests.php). Once the result was positive (detection of an increase in the LH), the participant stopped using the test and recorded the day of the peak in the PANAS. Each participant was given a new, unopened ovulation tester.

Participants were assigned a random number to be able to link the results of the PANAS with the demographic data collected: age, race, socio-economic level, relationship status, whether they were attending college/university and the brand of contraceptive taken (for the hormonally-contracepting group only).

**3. Results**

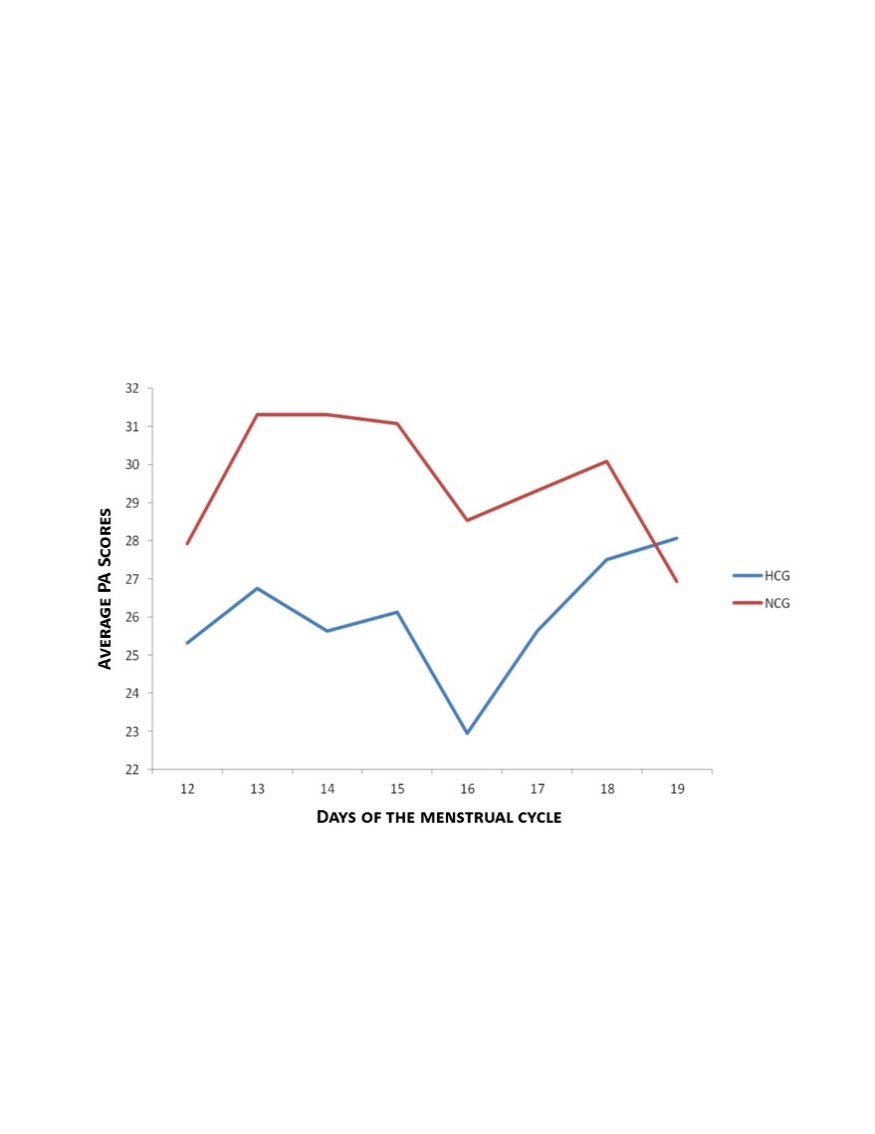
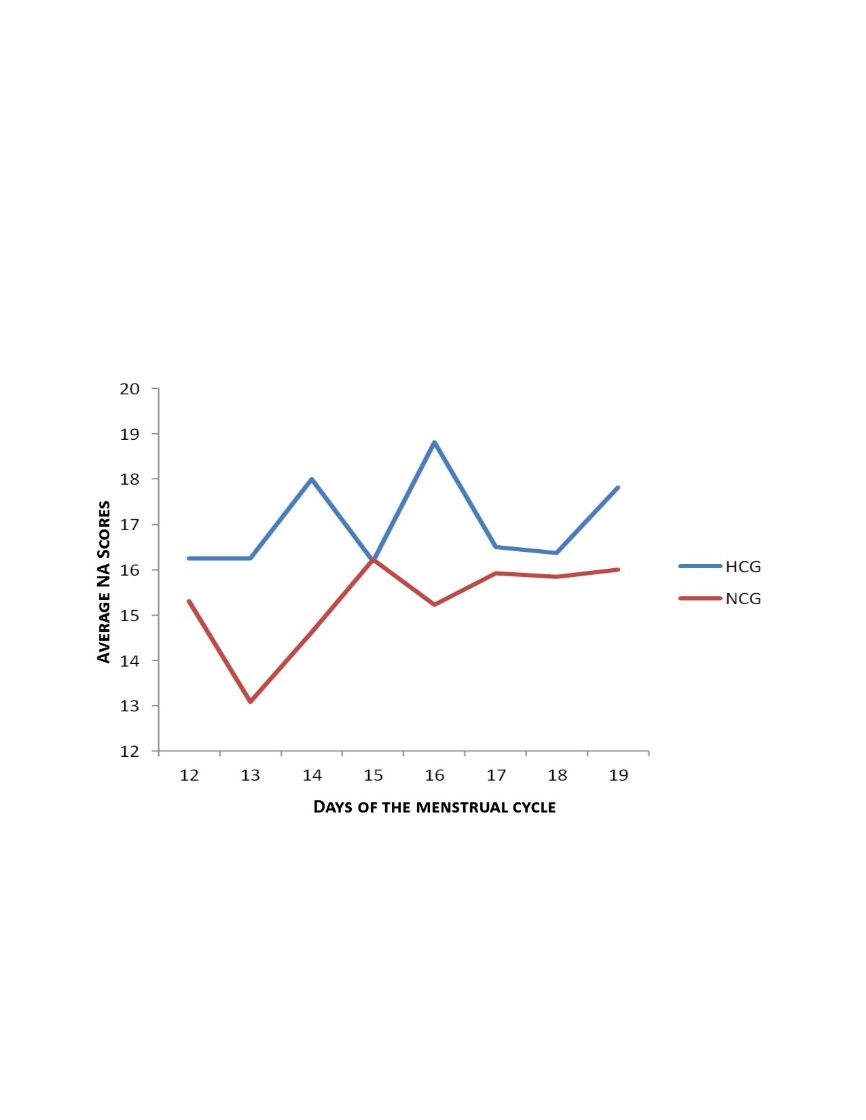
Demographic data breakdown shows that the sample was heterogeneous. The race was diverse: 37% Caucasian, 27% Hispanic, 20% Asian-American, 10% African-American, and 6% other races (Indian and Persian). The socioeconomic data was also varied: 10% lower class, 13% lower-middle class, 50% middle class, and 27% upper-middle class. Around 63% reported being in a relationship. The most homogeneous data was the education level with 77% of participants attending university. Moreover, the hormonally-contracepting women group participants reported using a variety of HC brands: Ortho-cycle, Gildess, Chateal, Yaz and Dian 35 among others.

**3.1. Measures**

To measure the LH, a tester was used. This tester returns a positive result above a certain threshold (40 mlU/ml). The LH peak in the naturally-cycling group was reported between the 12th and 26th days of the menstrual cycle. Ovulation usually occurs within 10-24 hours after LH reaches its peak. Using a one-way ANOVA (in SPSS), the difference in NA and PA the day of the LH peak was compared in both the naturally-cycling group (NCG) and hormonally-contracepting group (HCG) to determine if the groups were significantly different.

**3.1.1. Days of the LH peak: days 12-19**

The analysis of the averages per day of the data for the days 12-19, when 92.3% of the participants reported the LH peak, was performed without aligning the LH peaks. For this analysis, the data from the participant who reported the peak on the 26th day was excluded for being significantly outside of the range of days where it would have been expected. This analysis showed a statistically significant difference in both PA (F(1,14) = 25.032, p =.000), Fig. 3, and NA (F(1,14) = 10.180, p =.007), Fig. 4.

**** Fig. 3. PA data for the days 12-19 Fig. 4. NA data for the days 12-19

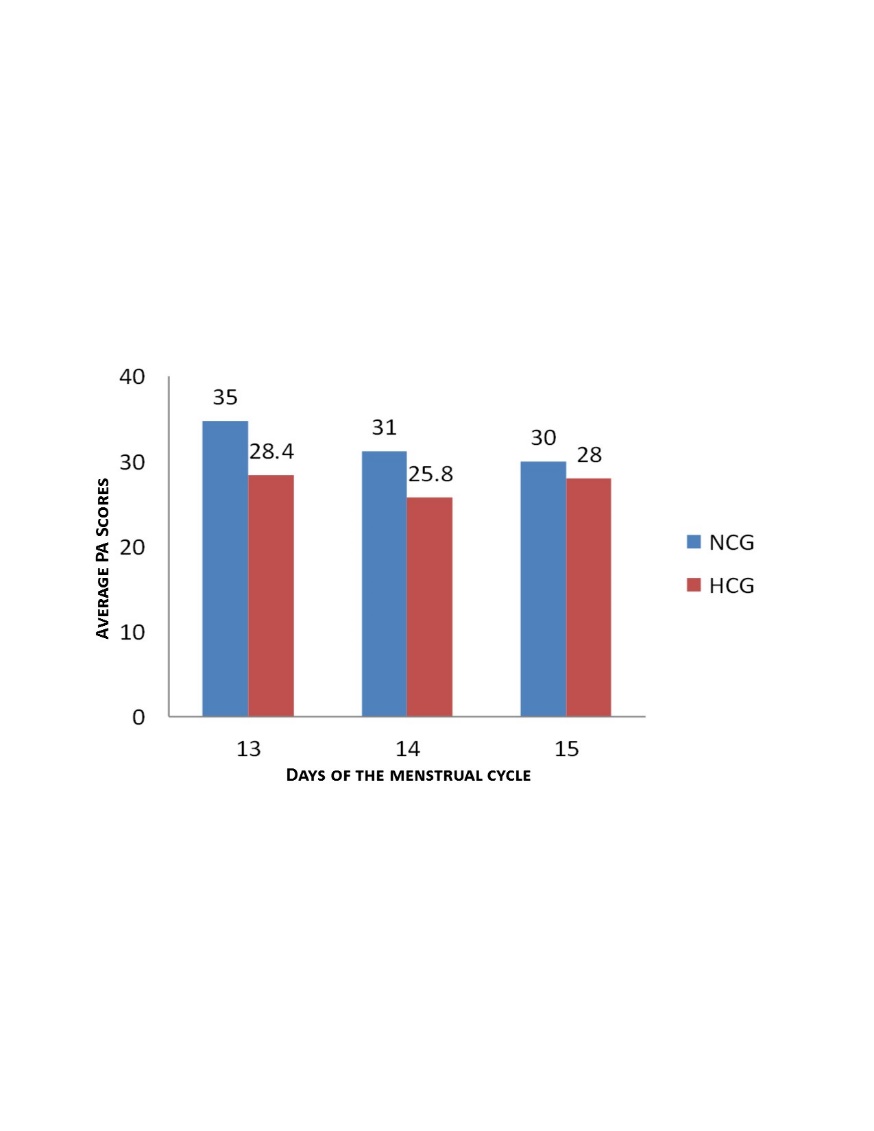
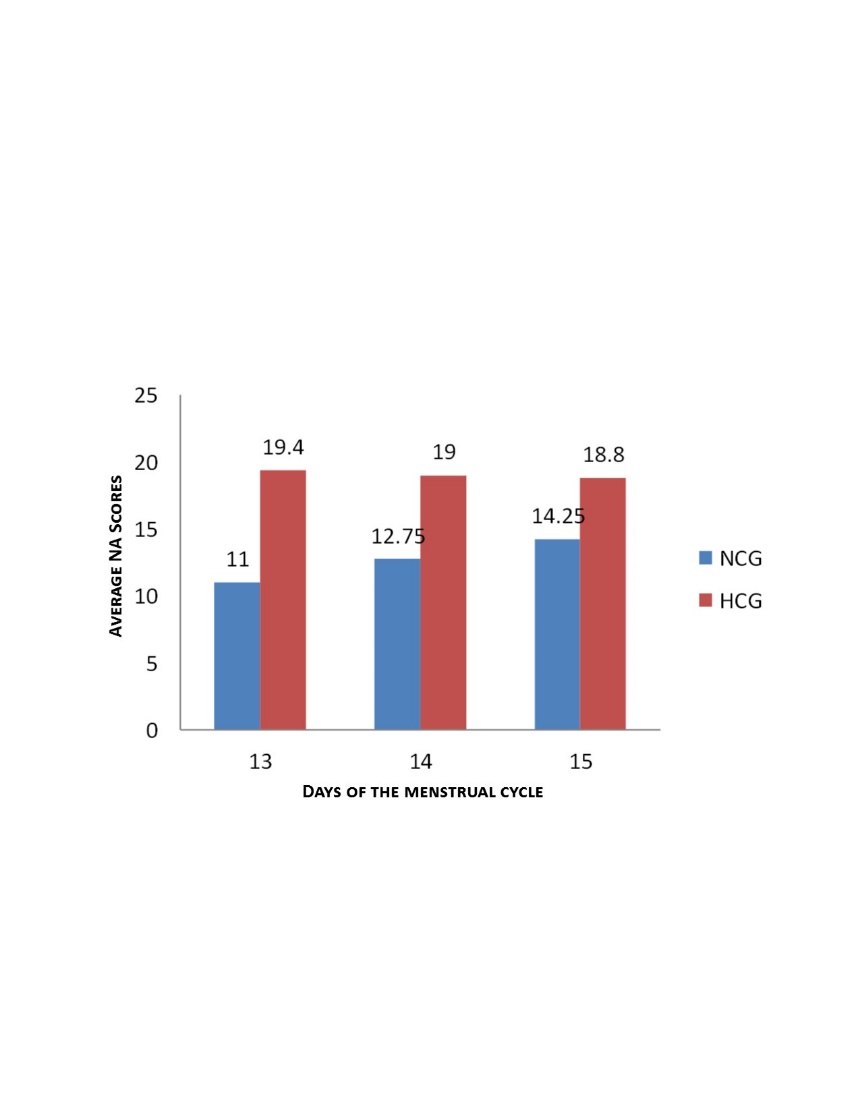
**3.1.2. The day of the LH peak**

Since the hormonally-contracepting group does not experience the LH peak, for the statistical analysis the day that the LH peak would have occurred if these women were not taking HC was used. Typically, this peak happens in the middle of the cycle (day 14 in a 28-day cycle) and since the cycles were of different lengths, this middle day had to be calculated for each participant separately. For the naturally-cycling group, the day that the LH peak was detected was used.

When comparing the data between both groups using all of the participants individual data, no statistically significant differences in NA (F(1,28) = .598, p =.446) were observed during ovulation, but there were statistically significant differences in PA (F(1,28) = 7.161, p =.012).

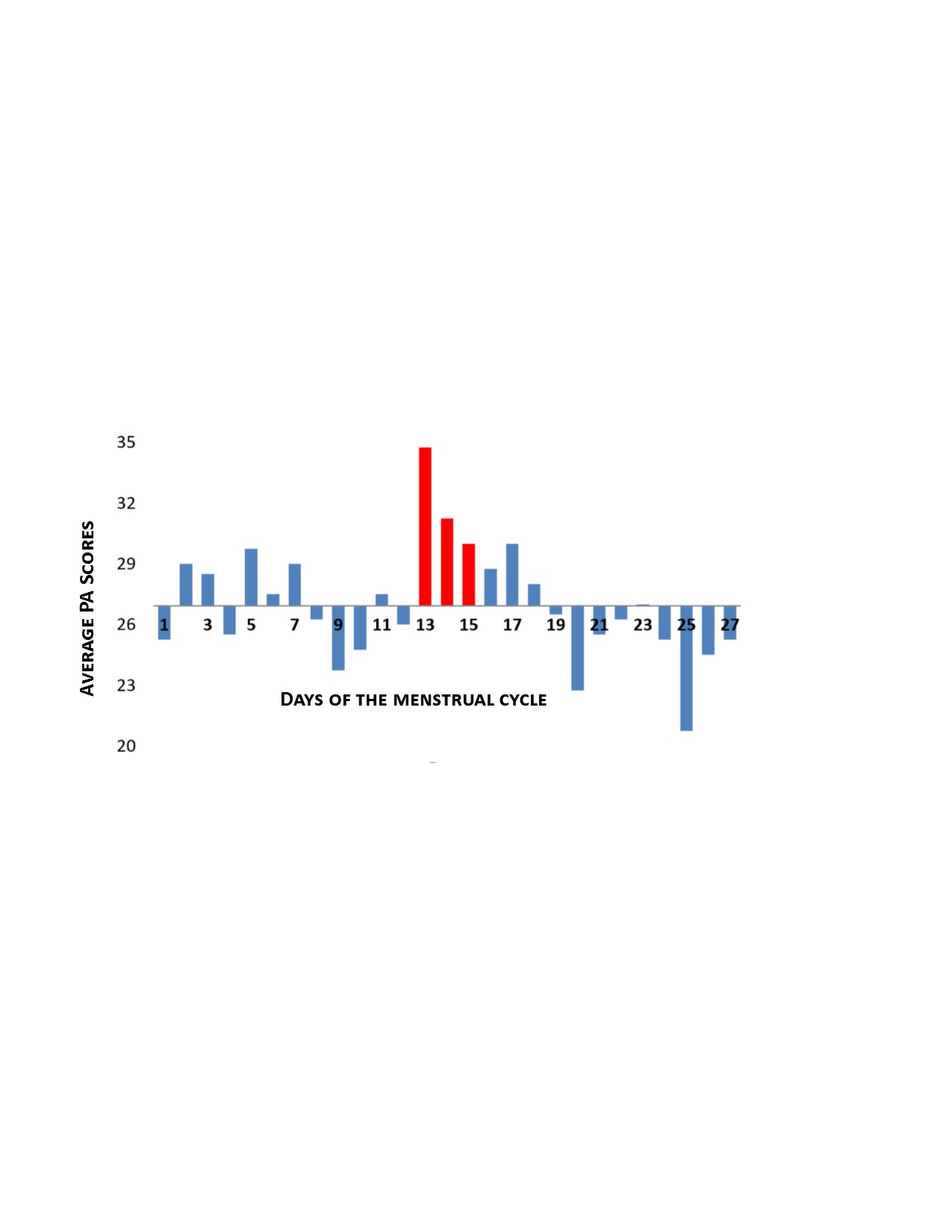
**3.1.3. LH peak in the same-length cycles: 28-day**

Nine of the participants had 28-day cycles: four in the naturally-cycling group and five in the hormonally-contracepting group. The LH peaks of the naturally-cycling participants all occurred in the middle of the cycle (days 13-15). These are the means of PA and NA for those days.

Fig. 5. Means of PA in days 13-15 Fig. 6. Means of NA in days 13-15

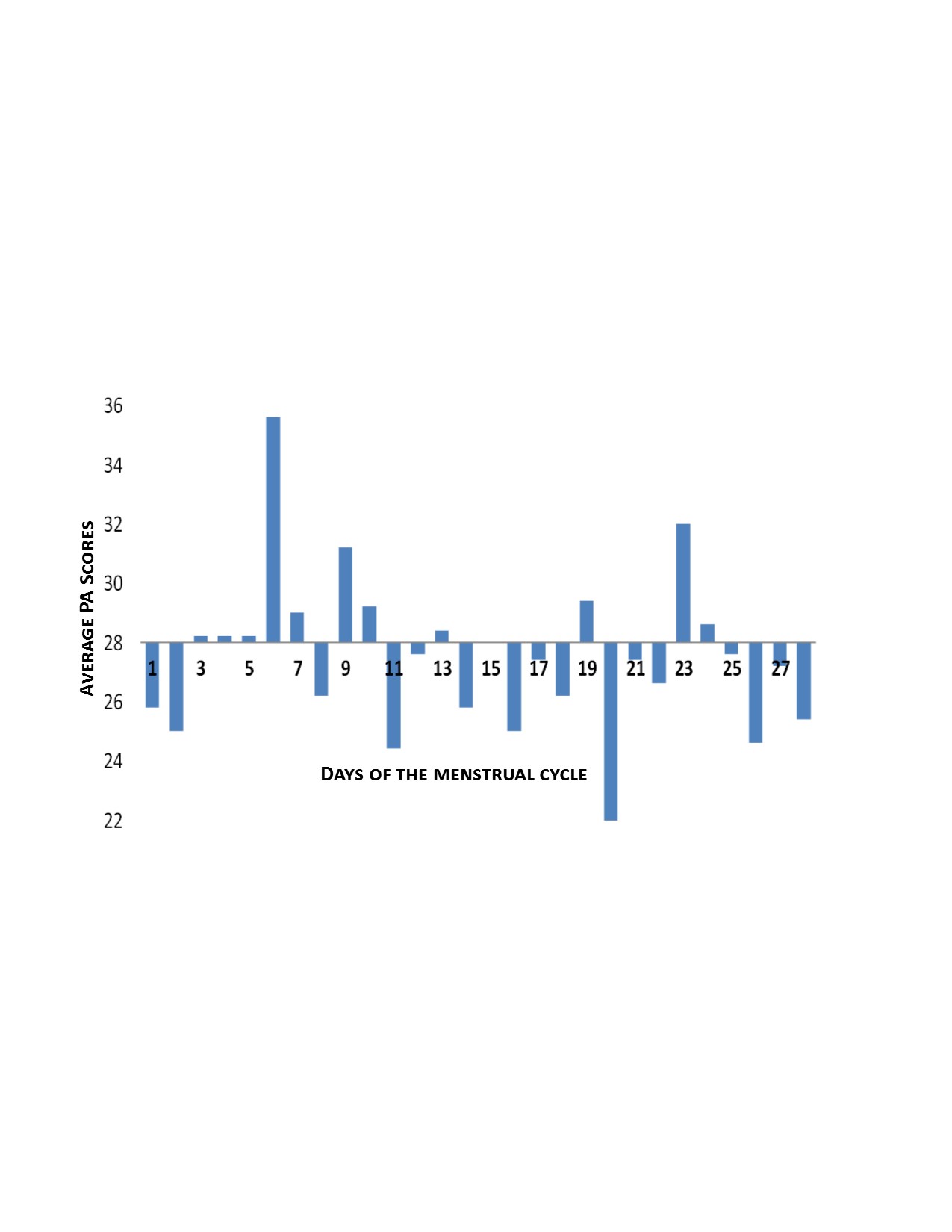
The mean PA scores per day (Fig. 5) were higher in the naturally-cycling group and the mean NA scores per day (Fig. 6) were higher for the hormonally-contracepting group during ovulation. When looking at all the data for all the days of the cycle, the naturally-cycling group’s PA mean scores (Fig. 7) were higher during ovulation (days 13-15 in red) while the hormonally-contracepting group scores (Fig. 8) were lower.

**Fig. 7. Naturally-cycling daily PA mean scores in the 28-day cycle participants**



*The Standard Deviation is 2.85*

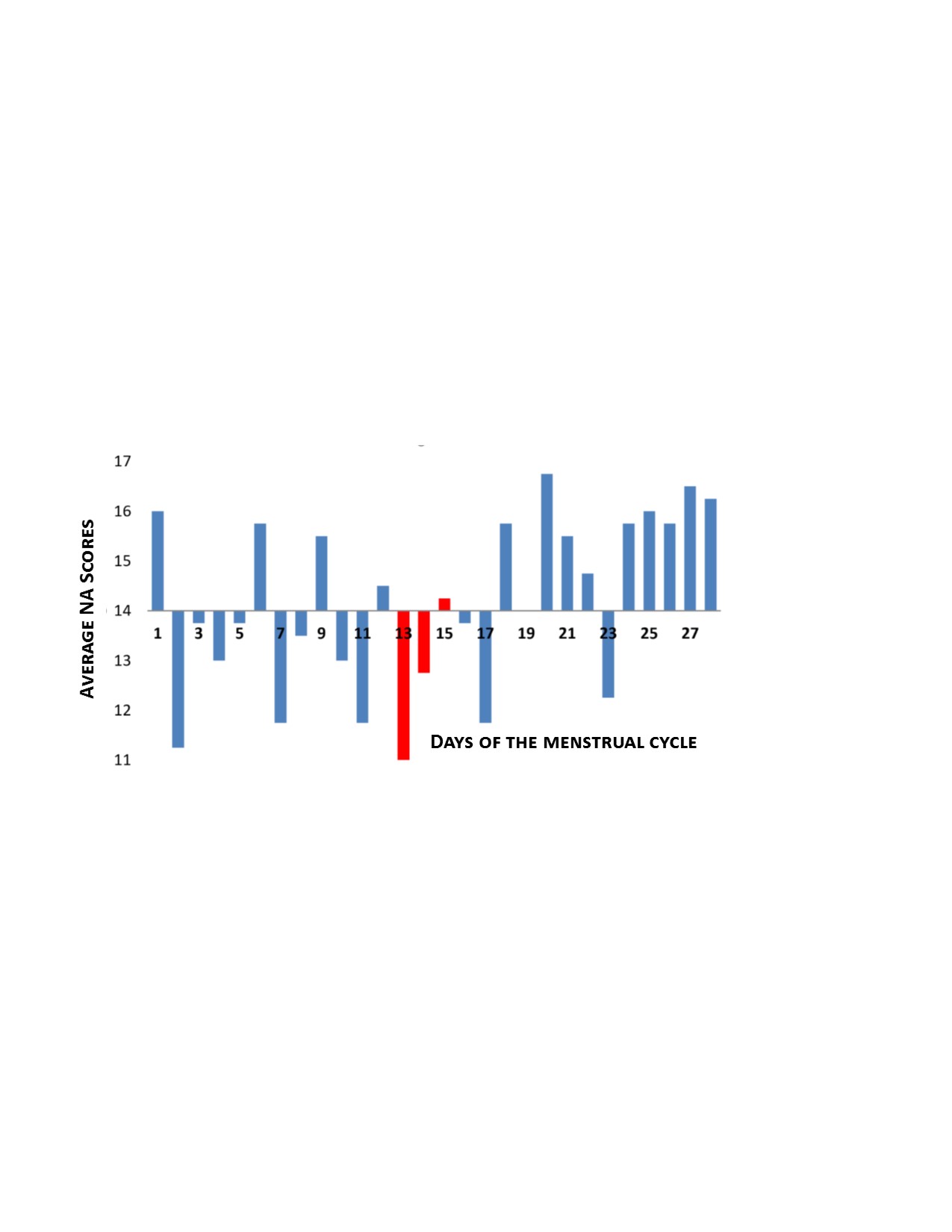
**Fig. 8. Hormonally-contracepting daily PA mean scores in the 28-day cycle participants**



*The Standard Deviation is 2.6*

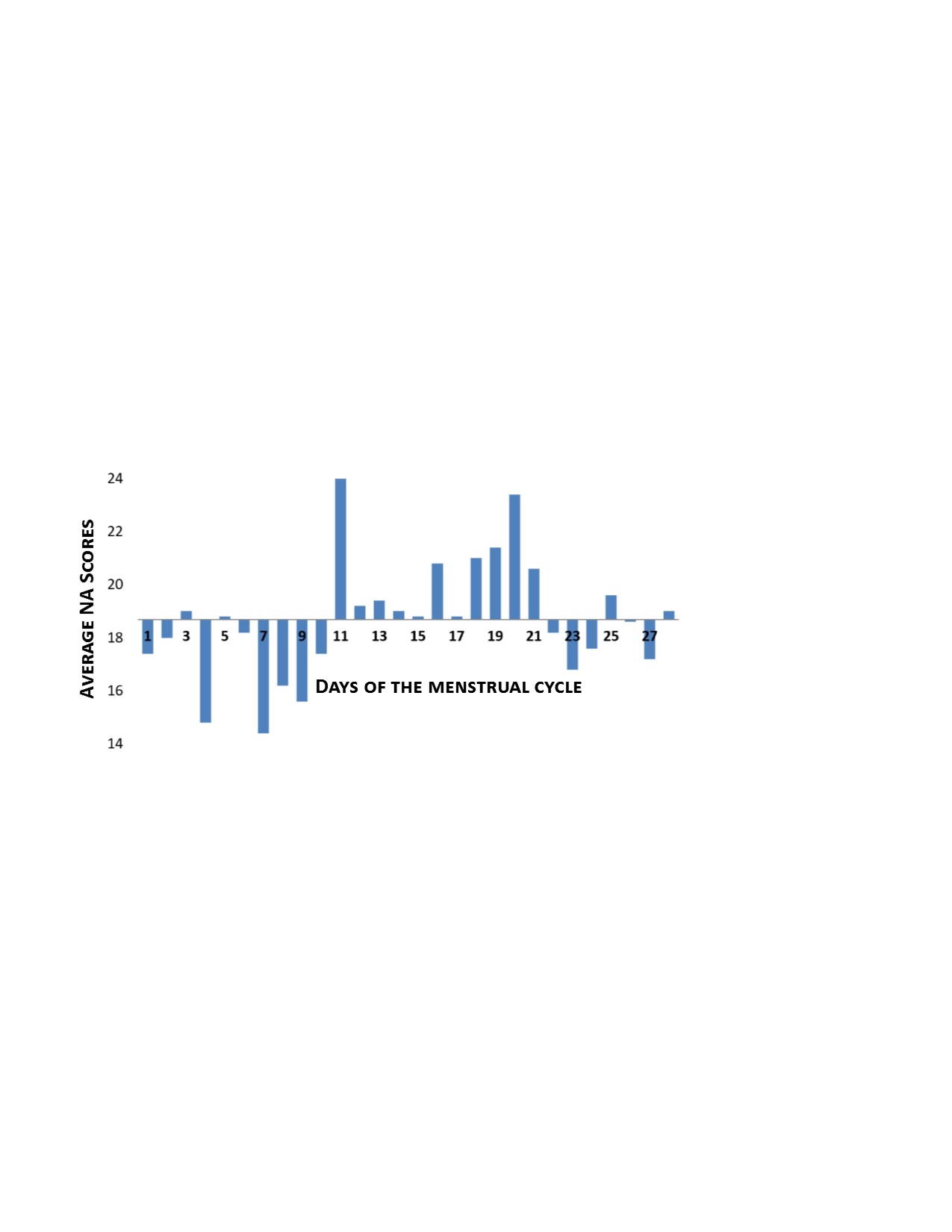
The naturally-cycling group’s NA mean scores (Fig. 9) per day showed a decrease during ovulation (days 13-15 in red) while the hormonally-contracepting group (Fig. 10) showed very little variation from the mean.

**Fig. 9. Naturally-cycling daily NA mean scores in the 28-day cycle participants**

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*The Standard Deviation is 1.75*

**Fig. 10. Hormonally-contracepting daily NA mean scores in the 28-day cycle participants**

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*The Standard Deviation is 2.22*

**4. Discussion**

The most relevant result of this data analysis is that PA is higher in the naturally-cycling group than in the hormonally-contracepting group during ovulation in all data analysis conditions. In this research, the affect was measured daily in a group of women without mental or physical problems. The LH was also measured to determine the ovulation phase with accuracy in naturally-cycling participants. The increase in PA in the middle of the menstrual cycle observed in Fig. 6 is in line with the results obtained by Sanders and Backstrom in 1983.

These same results were not observed in the same-length (28-day) cycle participants. This could be due to the small number of participants (n=9) in this condition. Also, NA is lower in the naturally-cycling group than in the hormonally-contracepting group during ovulation in the same-length (28 days) cycles data analysis. Women not taking HC have less NA than women taking HC.

Approximately 40-50% of women taking HC stop taking them after a few months due to the negative side effects, which included mood changes (Sanders et al. 2001). For example, one study found an increase in the symptoms of depression in women using HC (Carrier, 2012). In another study, women taking HC scored lower in positive affect (PA) and showed less change in PA due to environmental influences (Jarva and Oinonen, 2007). Research has shown that a balance between PA and NA positively correlates with the subjective feeling of wellbeing or satisfaction with life (Chico, 2006), while a reduced PA is characteristic of depression (Clark and Watson, 1989).

The effect of HCs’ use in daily affect was studied by Oinonen and Mazmanian in 2001. These researches observed that the variations in affect were associated with personal and family psychiatric histories, and the type HC (monophasics and triphasics) and duration of use. The results showed that in long time HC users, withdrawal from triphasics (which cause changing hormonal levels) produced greater variability in positive affect than withdrawal from monophasics (which cause constant hormonal levels). This seems to point to a connection between hormone levels and affect.

Also, there are still many questions about how hormones have an effect on affect. For example, suppression of the ovarian function, which reduces estrogen, LH and FSH levels, improves PMS symptoms only in some women (Pincus et al., 2011). Moreover, not fully understood are the physical changes in the brain as a result of menstrual cycle, specifically in the morphology of the amygdala. Before the onset of menses, Ossewaarde et al. (2013) observed an increase in gray matter in the amygdala that positively correlated with an increase in NA. The amygdala and the hypothalamus are both part of the limbic system and are involved in processing emotional stimuli (Karlssona et al., 2010; Swenson, 2006).

From an evolutionary perspective, it makes sense that naturally-cycling women score higher in PA in the days they are fertile, the periovulatory time. Specifically, women who ovulate self-report more enthusiasm, determination, attentiveness and activity levels during those days, which could be conductive to attracting and engaging a potential mate. These changes were not observed in the PA scores of hormonally-contracepting women which also makes sense since they do not ovulate. The limbic system could be implicated directly or indirectly in these findings since it is responsible for both triggering ovulation and the processing of emotions and motivation.

The negative affect (NA) does not seem to be caused by the menses since the Premenstrual Syndrome (PMS) symptoms can happen even when the menstruation does not. A study with hysterectomized women (uterus was removed but ovaries were not) indicated that hormones are responsible for the psychological distress (Backstrom et al., 1981). In 1983, Backstrom et al. found that cyclic changes in mood are present even when there are no changes in ovarian hormone levels. More recent studies (Bannbers et al., 2012; Carrier, 2012; Childs et al., 2010; Schwartz, 2012) also measured the ovarian hormones to determine if they were responsible for mood changes and obtained mixed results.

Some inconsistencies, and possible methodological issues, were found in studies about hormones and mood which were addressed in this study to increase the reliability of the results.

Previous researchers (Almagor and Ben-Porath, 1991; Ossewaarde et al., 2013) used well-validated and reliable standardized questionnaires, such as the PANAS while others (Grant and Pryse-Davies, 1968; Rossi and Rossi, 1977; Sanders et al., 2001; Woods, 1986) developed questionnaires specifically designed for their studies. However; even in the studies where the PANAS was used, there was no mention of instructing the participants to complete it during the hours of the day when results are considered stable. The present research used a trustworthy measure of affect, the PANAS, and also gave instructions to the participants on when to complete this questionnaire to maximize reliability.

Another important aspect of the present study is that an objective test was used to measure the LH peak. Sit et al. (2011) and Schwartz et al. (2012) warned that the use of an objective test to identify the day of ovulation is essential in research involving the menstrual cycle. Two very similar studies about mood and the menstrual cycle, one by Sit et al. (2011) and the other by Rasgon et al. (2003), obtained contradictory results. To determine the phase of the cycle, the LH was measured in the study by Sit et al. (2011), but it was not in the study by Rasgon et al. (2003). Given the importance of identifying the phases correctly in studies about the menstrual cycle, it is likely that this could this have contributed to the disparity in the results.

Backstrom et al. (1983) and Sanders et al. (1983) measured the hormone levels in blood in their studies. But in the research conducted by Sanders et al., these levels were only analyzed two or three times per week which presents a problem if the purpose is to determine the day of the LH peak since this peak can only be detected for a day during the menstrual cycle.

For this reason, to identify the timing of the LH surge, it is recommended to test daily to avoid missing the peak. Moreover, Backstrom and Sanders only measured the LH in the experimental group and not in their control group. This poses a problem because in their research, the women in the control group also were naturally-cycling. Other studies didn’t objectively measure the hormone levels: Rossi and Rossi (1977), Jarva and Oinonen (2007), Woods (1986), and Almagor and Ben-Porath (1991) among others.

Research involving the menstrual cycle presents with the added difficulty that the length of the menstrual cycle varies significantly from woman to woman. For example, in the present study, there was a reported difference of 14 days between the shortest and longest cycles. For the data analysis, Almagor and Ben-Porath (1991) tried to address this problem by artificially manipulating the length of the cycles so they would all measure 28 days. To accomplish this, they had to add days for some of the women and remove days for others.

The majority of these researches (Almagor and Ben-Porath, 1991; Jarva and Oinonen, 2007; Patkai et al., 1974; Rossi and Rossi, 1977; Woods, 1986) asked the participants to report the first day of their menstruation and then, using the average length of each phase, counted days to try to determine the phases of the cycle. The actual length of each phase, which varied among women, was disregarded. In the case of Sanders et al. (1983), these researchers also did an average of the results after they grouped them by phase. Schwartz et al. (2012) cautions against averaging hormone levels for each phase because it can lead to the loss of subtle changes. The present study did not estimate the length of the phases, did not artificially change the length of the menstrual cycle and did not group the results by phases.

This research has tried to rectify some of the methodological errors made by similar studies and provides three improvements over other research about mood and/or affect, and the menstrual cycle: (a) the LH level was measured in urine to determine the ovulation period with accuracy, (b) the length of the cycle was not manipulated and (c) a standardized and valid measure to assess affect, the PANAS, was used while controlling for the time of day the questionnaire was completed. By objectively measuring the LH peak, it was possible to more precisely correlate the measures of affect. By not artificially manipulating the lengths of the cycles, the internal validity was increased. By giving the participants specific instructions to complete the PANAS at a specific time each day, the reliability of the questionnaire’s results was increased.

To be able to generalize the results of the present study to the general population and avoid bias, participants from diverse socio-economic status were selected. However, one of the variables didn’t show a high variability, the educational level (77.4% of the participants were enrolled in a college or university). For future research, it would be interesting to make an effort to include more women without a college education to determine if this variable affects the results.

**6. Conclusions**

These results represent empirical evidence in favor of higher PA when there is ovulation since this scores are higher during the same phase of the cycle in the ovulating women when compared to the OC-using, non-ovulating participants. In the same-length (28 days) cycle participants, lower NA was observed around the ovulatory phase in ovulating women (NCG) when compared to non-ovulating women (HCG).

Why didn’t the PA in the same-length analysis mimic the results obtained in the all-data analysis and by other authors? Why were the NA changes observed in the same-length analysis not observed in the all-data analysis? It would be interesting to replicate this study with a larger sample size.

The socioeconomic status could be a confounding factor for the PA/NA so it would be interesting to separate by this variable and see if there are differences in affect. Another confounding factor is the HC therefore it could also be interesting to look at the different HC to see if different types have different effects in mood. Another possible improvement to consider for a future study is introducing a placebo pill for the NCG to avoid the potential confounding situation of taking a pill.

There are many questions left unanswered: are there biological processes triggered by the release of the LH that contribute to these results? Is there a confluence of biological events during ovulation that contribute to changes in affect? Future studies could address these questions and focus on the specific mechanisms that lead to the discrepancies in affect observed in this study.

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