ABSTRACT

Background: It has not yet been established whether men in heterosexual relationships adapt their hormone levels to their female partner’s menstrual cycle to allocate reproductive resources to the period when the female is actually fertile.

Aim: This prospective observational study tested the hypothesis that some males have peaks in testosterone or acne (a possible biomarker for androgen activity) near their partners’ ovulation, whereas other males display the opposite pattern.

Methods: 48 couples supplied menstrual cycle data, male salivary samples, and a protocol of daily activities for 120 days. Daily saliva samples were analyzed for testosterone concentrations by enzyme-linked immunosorbent assay. The main hypothesis was tested by analyzing whether each individual male’s testosterone/acne response to ovulation (either an increase or a decrease in comparison to the individual’s average levels) was stable over time. To do this, we analyzed the Spearman correlation between individually normalized periovulatory testosterone and acne during the first half of the study versus the second half of the study.

Outcomes: Correlation between each male individual’s periovulatory testosterone and acne patterns during the first half of the study versus the second half of the study.

Results: No predictability in the male individuals’ testosterone (Spearman’s rho = −0.018, P = .905) or acne (Spearman’s rho = −0.036, P = .862) levels during ovulation was found.

Clinical translation: The study being “negative,” there is no obvious translational potential in the results.

Strengths and limitations: The main strength of this study lies in the excellent compliance of the study participants and the large number of sampling timepoints over several menstrual cycles, thereby allowing each male individual to be his own control subject. A limitation is that samples were only obtained in the morning; however, including later timepoints would have introduced a number of confounders and would also have hampered the study’s feasibility.

Conclusions: The current results strongly indicate that male morning testosterone levels neither increase nor decrease in response to the partner’s ovulation. This discordance to previous laboratory studies could indicate either that (i) the phenomenon of hormonal adaptation of men to women does not exist and earlier experimental studies should be questioned, (ii) that the phenomenon is short-lived/acute and wanes if the exposure is sustained, or (iii) that the male testosterone response may be directed toward other women than the partner.


Key Words: Acne; Menstrual cycle; Ovulation; Reproduction; Saliva; Testosterone
INTRODUCTION

In animals, it has repeatedly been demonstrated that male testosterone levels adapt to the female’s reproductive status.\(^1\)\(^–\)\(^4\) Such an adaptation is thought to mobilize the male’s resources in the form of courtship, aggressiveness toward other males, and an increased sexual drive to the period when the female is fertile. However, it has not been established whether men in heterosexual relationships adapt their hormone levels to their female partner and join in the rhythmicity of the menstrual cycle. Even if it has been shown in laboratory settings that female fertility cues acutely affect male testosterone levels,\(^5\)\(^–\)\(^6\) real-life observational studies in couples have failed to provide evidence for an induced hormone cycle in males.\(^7\)\(^–\)\(^8\)

One of the studies searching for hormonal synchronization in heterosexual couples came from our laboratory.\(^7\) Even if testosterone surges near ovulation were seen in some of the 29 males, the summarized results from all individuals indicated that no ovulatory testosterone peak existed. Because the study only included 1 month per couple, it could not be assessed whether the subgroup with testosterone increases near ovulation continued to display this pattern in coming months, which could indicate that at least some couples were hormonally synchronized. The results could be explained by one of the following: (i) either all male testosterone variations were random in regard of the female cycle, or (ii) there were subgroups of males with diametrically different testosterone responses to ovulation that in summary ruled each other out. The second explanation would not merely suggest that some men had testosterone peaks near ovulation, whereas the other men’s testosterone levels varied randomly, but that there were males who actually reacted on ovulation with a decrease in testosterone. Otherwise it could not be explained that a tendency of an ovulation peak was not seen in the summarized results.\(^7\) It could be speculated that such an ovulation-related decrease in testosterone, if it existed, could serve as a form of endogenous birth control for males in relationships.

This study was therefore initiated to test the hypothesis that some males have peaks in testosterone or acne, the latter a possible biomarker for androgen activity, near their partners’ ovulation, whereas other males have an opposite pattern. If the 2 testosterone patterns could be found, then our secondary aim was to explain the dichotomy by comparing these groups in terms of relationship quality, perceived stress, demographics, intent to reproduce with the current partner, and other variables. Information on possible confounders affecting the hypothesized hormonal synchronization was also collected, such as sexual intercourse and masturbation on a day-to-day basis, which are factors known to acutely affect testosterone concentrations.

METHODS

Participants

Before participant recruitment, the study protocol was approved by the Regional Ethical Review Board of Uppsala (2014/360). The study was performed in accordance with relevant guidelines and regulations. Informed consent was obtained from all participants.

Heterosexual couples were recruited by posters and flyers on the university campuses in Linköping, (Sweden) and Örebro (Sweden) and by an advertisement in the local newspaper in Örebro. The posters, flyers and advertisement specified that the participants must live together in a heterosexual relationship, be 18 to 50 years of age and must not use hormonal contraceptives. After the participants contacted the research team, they were provided with more detailed information about the study and asked to answer an inclusion/exclusion questionnaire. It was described that the study aimed to investigate the effect of social conditions on testosterone concentrations, but the exact hypothesis was not disclosed to the participants during the entire study. The final selection of participants was made on the basis of the inclusion/exclusion questionnaires, aiming to recruit a healthy and fertile group in which the hypothesized phenomenon was thought to be most likely to occur.

Inclusion criteria:

- Couple living together in a heterosexual relationship
- Both persons at least 18 years old

Exclusion criteria:

- Previous gonadectomy
- Transsexuality
- Postmenopausal female
- Oligomenorrhea
- Disease that could affect the sex hormone system, social interaction, or olfaction (the only 3 diseases that were reported from potential participants were anxiety, celiac disease, and well-regulated hypothyreosis, and none of these were considered to be a reason for exclusion)
- Consumption of prescription drugs that could alter the sex hormone system
- Current or recent use of hormone-containing contraceptives
- Pregnancy or planned pregnancy during the time of the study
- Long period of absence from the partner planned during the study
- Consumption of narcotics
- Consumption of anabolic steroids
- Tobacco-smoking

From the 163 couples initially signing up as volunteers, 50 couples were finally included, and 48 completed the study (Figure 1). Before commencing the study, at least 1 person from
each included couple attended a start-up meeting with the research team for detailed practical instructions. For participating in the study, each male and female participant received the Swedish krona equivalent of €300 and €60, respectively.

Data Collection
The study started at a random time point in the menstrual cycle and proceeded for 120 days. Saliva samples were taken at home at the same time every morning by the males (Salivette test tubes with synthetic oral swabs, product no. 51.1534.500; Sarstedt AG & Co, Nümbrecht, Germany). Any temporal deviations were registered in a protocol, but, to avoid such errors, all male participants were offered the option to receive a text message reminder on their cellular phones at the same time every morning. Participants were told not to eat or brush their teeth before sampling. The saliva samples were immediately put in a plastic bag and stored in the home freezer until the end of the sampling period. After the sampling period, all samples were collected by the research team, and stored at −80°C until analysis. The saliva samples were not allowed to thaw during transport. The other outcome variable, acne, was registered daily by each male participant. Only acne lesions on the face, from forehead to the lower edge of the mandible and in front of the ears, were counted.

In earlier studies, intense physical exercise,9 sexual activity,10,11 and illness12 have been reported to acutely influence testosterone levels. To control for these factors, all males received protocols in which they were instructed to register time of masturbation, intercourse (defined as sexual activity leading to male orgasm, with involvement of the partner), training (defined as physical activity with the intent of exercising), illness (defined as fever or other illness preventing the participant to go to work, had it been a workday), absence from partner overnight, and sampling mishaps (such as forgetting to put the sample in the freezer or eating before sampling).

The females received protocols in which they were instructed to record first day of the menstrual cycle and day of ovulation. For detection of ovulation, luteinizing hormone-tests for home use were provided (Wondfo LH Urine Test, Cat. No. W1—MII; Guangzhou Wondfo Biotech Co, Guangzhou, China).

In addition to the inclusion/exclusion questionnaire, participants were requested to answer an additional set of questionnaires. These included the 2 relationship scales Quality of Dyadic Relationships—36 (QDR36)13 and the Swedish Relationship Assessment Scale (RAS),14 as well as the Perceived Stress Scale (PSS),15 as well as questions regarding general health, economic situation, female hygiene habits, body weight, body length, use of tobacco products (other than smoking, which was already asked for before inclusion in the study), length of relationship, family situation, frequency of night shifts at work, how often each partner made sexual approaches, and how much the well-being of the female differed between the premenstrual period and the rest of the menstrual cycle.

Testosterone Analysis
Saliva samples were thawed, spun in a centrifuge for 15 minutes at 1500g, and analyzed by testosterone enzyme-linked immunosorbert assay (ELISA; DES6622; Demeditec
Statistical Analyses

First, the 3-day-long periovulatory periods were defined as the day before until the day after a positive LH-test result. If no positive LH-test result had been obtained, the periovulatory periods were defined as 15 to 13 days before initiation of the next menstrual period. Second, mean concentrations of salivary testosterone and mean prevalence of acne spots were calculated for each male participant. Each daily salivary testosterone concentration and acne number was then normalized by dividing it by the individual’s mean value. In effect, if the normalized salivary testosterone concentration on a specific day was calculated to be 2, it meant that the concentration was twice as high as the mean salivary testosterone of that individual. The normalization was done to make sure that each individual, irrespective of average testosterone concentrations, contributed equally in the analysis. If a testosterone concentration value was missing, the specific day was omitted from the analysis.

Before the main analysis we aimed to test the conclusion from our previous article” that there was no overall peak in testosterone during ovulation, taking all participants into account. This was done by calculating the mean value and confidence interval of all periovulatory, normalized testosterone values, and likewise for acne values. If the confidence interval had included 1, it would mean that the normalized testosterone or acne values were not significantly higher or lower during the periovulatory period than during other parts of the cycle.

The main analysis aimed to investigate whether the individual male periovulatory testosterone (or acne) pattern during the first half of the study would be predictive of the individual’s periovulatory pattern during the second half of the study. Such a correlation would indicate that some males have a tendency of periovulatory hormone peaks, whereas other males tended to display periovulatory hormone valleys. In contrary, if all testosterone and acne variations were random with regard of the menstrual cycle, a peak in the first half of the study would not be predictive of a peak in the second half of the study. To perform the main analysis, the periovulatory periods for each couple were first split in a “first half” and a “second half” of the study. Second, the individual average of normalized salivary testosterone concentrations and normalized acne values was calculated for the periovulatory periods in the first and second half of the study, respectively. An average above 1 would indicate a peak, whereas a number below 1 would indicate a valley. The relation between each individual’s first and second half result (in terms of average normalized salivary testosterone concentrations and average normalized acne) was tested by Spearman correlation.

If the null hypothesis was rejected in the main analysis, men displaying consistent ovulatory peaks were to be compared with men displaying consistent valleys in terms of relationship quality (according to RAS and QDR36), average testosterone concentration, average acne number, age, intercourse frequency, sexual approach frequency, stress level, family situation and intent to reproduce with the current partner. For these analyses, t-test was to be used for ratio scale variables and Mann-Whitney test for ordinal scale variables.

Furthermore, if the null hypothesis was rejected in the main analysis, mediator/confounder analyses were planned to be carried out regarding intercourse the day before sampling, masturbation the day before sampling, training the day before sampling, disease and sleeping away from the partner.

QDR36 index was calculated according to the QDR36 manual (https://toneahlborg.files.wordpress.com/2013/09/qdr36-manual-eng.pdf, accessed 2016-12-14). Average scores for the 5 items were calculated, ranging from 1–6. Then the item scores were summarized, giving a range from 5–30, with higher points indicating better quality of the relationship. RAS median was calculated by inverting item 4 and 7. The range was 7–35 points, with higher points indicating better quality of the relationship. PSS was summarized as described in the original article, giving a range of 0–56 points, where the highest score indicates the highest level of perceived stress.

All statistical analyses were performed using SPSS (Version 23; IBM corporation, Armonk, NY, USA), and P < .05 were considered significant. Data are expressed as mean ± standard deviation (SD) for ratio scale variables and median (interquartile range) for ordinal scale variables throughout.
iovulatory normalized acne was 0.852, with a 95% confidence interval of 0.66. The main analysis showed that there was no correlation between the male individuals’ average normalized salivary testosterone concentrations near ovulation in the first and second half of the study (n = 48, Spearman’s ρ = −0.018, P = .905). In other words, a certain periovulatory testosterone pattern (peak or valley) during the first part of the study did not predict similar pattern during the second part of the study (Figure 2 and 3). Therefore no further mediator/confounder analysis was performed.

Forty of the males reported at least 1 acne lesion during the study. Because some of the males reported only 1 or a few acne lesions during the entire study, many of the average normalized acne values at periovulatory periods equaled zero. This heavily biased the Spearman correlation analysis between the first and second part of the study, making the association falsely significant (n = 40, Spearman’s ρ = 0.51, P = .001). However, when repeating the analysis after omitting males with very few acne lesions, the correlation was not nearly significant (n = 26, Spearman’s ρ = −0.036, P = .862). In other words, that a certain male had an acne peak near ovulation in the first part of the study did not predict an acne peak near ovulation in the second part of the study (and likewise for valleys; Figure 3).

### RESULTS

During the periovulatory periods, normalized testosterone was on average 0.99, with a 95% confidence interval of 0.96—1.03, meaning that the levels during the periovulatory period did not significantly differ from the remaining cycle. Similarly, the periovulatory normalized acne was 0.852, with a 95% confidence interval of 0.66—1.05.

The main analysis showed that there was no correlation between the male individuals’ average normalized salivary testosterone concentrations near ovulation in the first and second half of the study (n = 48, Spearman’s ρ = −0.018, P = .905). In

![Figure 3](image-url)

**Figure 3.** Average, normalized testosterone and acne values are plotted against standardized cycle day. Also, the defined periovulatory periods, on which the main analyses were based, is pointed out.

### Table 1. Daily collected variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary testosterone concentration (pg/mL ± SD)</td>
<td>149.3 ± 103.8</td>
</tr>
<tr>
<td>Time-point for saliva sample (hh:mm ± SD)</td>
<td>07:24 ± 00:50</td>
</tr>
<tr>
<td>Sample miss-happenings</td>
<td></td>
</tr>
<tr>
<td>Forgotten or lost sample [% of samples]</td>
<td>5.02</td>
</tr>
<tr>
<td>Intake of food or drink prior to sampling [% of samples]</td>
<td>0.64</td>
</tr>
<tr>
<td>Delay before sample put in freezer, ≤12 h [% of samples]</td>
<td>1.4</td>
</tr>
<tr>
<td>Delay before sample put in freezer, &gt;12 h [% of samples]</td>
<td>0.8</td>
</tr>
<tr>
<td>Acne lesions (n/d)</td>
<td>0.45</td>
</tr>
<tr>
<td>Masturbation frequency (n/d)</td>
<td>0.21</td>
</tr>
<tr>
<td>Intercourse frequency (n/d)</td>
<td>0.20</td>
</tr>
<tr>
<td>Training frequency (n/d)</td>
<td>0.22</td>
</tr>
<tr>
<td>Sleeping away from partner frequency (n/d)</td>
<td>0.082</td>
</tr>
<tr>
<td>Illness frequency (n/d)</td>
<td>0.020</td>
</tr>
<tr>
<td>Detected ovulations per female participant (n ± SD)</td>
<td>3.50 ± 0.94</td>
</tr>
<tr>
<td>Menstruations per female participant (n ± SD)</td>
<td>4.35 ± 1.20</td>
</tr>
</tbody>
</table>

Before commencing the study, a conservative power calculation was performed based on data from the previous study from our group and a small pilot study. This power calculation was performed with the intent to focus only on males displaying ovulatory testosterone peaks, so with the final design focusing both on peaks and valleys, the power was in effect much higher. In the acne pilot study, acne increased 100% from just after menses to ovulation, with a standard deviation of the increase of 140%. This demanded that 18 couples be included if all males displayed peaks during ovulation to get statistical power of 80%. Because 11/29 males in the earlier study from our group had testosterone peaks near ovulation, we assumed that approximately 40% of males would display this pattern, resulting in 50 couples to be included with a calculated dropout of 10%.

Population Data

An overview of the studied variables is found in Table 1. Participant data from questionnaires are summarized in Table 2.
no participant was below the prespecified reference interval, and the median was higher than expected.

**Protocol Violations**

As mentioned, 2 of the initially recruited couples did not finish the study. One of them contacted the researchers after half the study time had passed, admitted not having taken any saliva samples, and wanted to opt out. The other couple did not come to the final meeting where samples and data would be handed in and remained unreachable despite numerous attempts to make contact. Also, one of the 48 included couples did not submit the additional set of questionnaires. Furthermore, 5.02% of saliva samples were either missed by the participants or impossible to analyze for technical reasons (such as too small sample volume).

**DISCUSSION**

In this study, no effect of the female ovulation on male testosterone could be demonstrated. This was in spite of the study being powered to detect subgroup patterns of male testosterone either rising or falling near ovulation. The substantial number of participants and samples, excellent compliance of the participants and lack of even a slight trend to support the hypothesis strongly indicates that this negative finding is valid. Furthermore, no synchronization of male acne to the female menstrual cycle was detected, although this analysis was based on fewer participants.

There are several possible explanations for the negative findings. The most obvious one is that the hypothesized phenomenon—male testosterone synchronization to the partner’s menstrual cycle—does not exist or is too subtle to be detected. The present results are in line with a previous real-life partner study from our research group and also corroborate the findings regarding baseline testosterone in a real-life partner study by Fales et al. This interpretation would also be in line with the notion that primates in general, and Old World species in particular, rely more on visual than olfactory communication in comparison to other animals. This has been a matter of intense debate and was thoroughly reviewed by Drea in 2015. The current results do, however, seem to contradict results from a couple of experimental studies, in which males displayed testosterone peaks in response to olfactory ovulation cues. In the strongest of these, Cerda-Molina et al demonstrated testosterone peaks after exposure to near-ovulation vaginal and axillary pads, whereas a testosterone decrease was seen after exposure to non-ovulation olfactory cues. A negative experimental study by Roney and Simmons also deserves to be mentioned, however; in that study the controls to the olfactory ovulation cues were not nonovulatory cues, but only water, which makes the results more difficult to interpret.

If we assume that both the positive experimental study results and the negative results from current study (and the other real-life partner studies) are valid, there are at least 2 possible explanations for this discordance. First, in contrast to the current study, the testosterone response in the experimental studies was acute. This design difference could suggest that the male hormonal adaptation to ovulatory cues is restricted to an acute, short-lived reaction but wanes if the ovulatory stimulus persists, as it does when sleeping next to each other overnight. A second important difference between the experimental and the real-life studies that also offers an explanation to the result differences is that the males in the experimental studies were exposed to females that were not their partners. A possible interpretation of this could be that male testosterone surges mainly occur in response to women with whom they are not in
a relationship. The evolutionary benefits of both mating with your partner and cheating are obvious and well recognized, but the mobilization of reproductive resources could be so costly that the males need to prioritize. It may be more relevant for males to mobilize reproductive efforts toward a sporadic low-cost, no-commitment reproduction opportunity than to invest extra efforts in the female with whom they are already in a relationship.

In the study by Fales et al.,8 it was not only investigated whether male baseline testosterone was higher during the partner’s ovulation (which it was not) but also how male testosterone response to competition was affected by the partner’s cycle phase. They did find that the competition-triggered testosterone increase actually was higher during the partner’s ovulation, which however does not necessarily contradict the line of thought regarding males’ economic priorities when it comes to testosterone peaks in response to partners versus non-partners. It could be argued that the cost of a testosterone surge is more reasonable when another male threatens to take advantage of the partner’s ovulation.

The validity of the testosterone ELISA analyses is an important issue in the assessment of the current negative results. To assure reliable results, we chose the only commercial salivary testosterone ELISA kit that, with excellent results, has been tested against the gold standard mass spectrometry.18 Furthermore, this kit from the German company Demeditec has proven to correlate well with ELISA kits from DRG and Salimetrics.19 During the analysis, the controls supplied from the vendor consistently showed excellent results, and the intrasay variability was low. Furthermore, because the participants timed the initiation of the study randomly in regard of the menstrual cycle, the placement of the salivary samples corresponding to the partner’s ovulation on the assay plate was random. Also, the effect of the samples being stored in the participants home refrigerators were tested by checking for correlation between sample tube number (lowest number indicating longest time in home fridge) and normalized testosterone values. The correlation was very low (Spearman’s rho = 0.035) and therefore not deemed to be a relevant source of bias. All taken together, inaccuracy or invalidity of the testosterone assay is a highly unlikely explanation for the negative results.

The reliability of the ovulation tests is another matter to consider. The manufacturer declares that the reliability of the tests is 99.6%. We have not, however, performed any type of validation study, nor are we aware of the existence of any such publication. On the other hand, if the ovulation tests were negative or missed despite other signs of ovulation, the females were prompted to note this as a “possible ovulation.” The entire analysis has been performed including and excluding these “possible ovulations” with the same results. In summary, we deem it very unlikely that malfunction of the ovulation tests would explain the negative study results.

Another issue is the chosen time point for obtaining saliva samples. In the current study, we chose to take morning samples for a number of reasons. First, in the morning the male is relatively unbiased with regard to exposure to other women, training activities, stress, and eating/drinking-related alterations of the saliva’s chemical properties. Second, a morning routine is probably the most easy to adhere to if samples are to be taken at a specific timepoint every day. Third, testosterone concentrations are highest in the morning,9 which could be beneficial for detecting fluctuations. And last, it seemed advantageous that the males had spent the night sleeping beside their partner, guaranteeing an intense olfactory exposure before saliva sampling. However, the fact that we have only taken morning samples limits our conclusions to the morning setting. It is possible that hormonal synchronization could be found in a study measuring day, evening, or night testosterone concentrations or perhaps by continuous sampling during the entire 24 hours. Such studies would, however, struggle with the challenges mentioned above, not least the great variability in how the daily activities could affect testosterone. It should also be noted that the couples in the current study were not trying to get pregnant. It could be speculated that the results would have been different if “trying to get pregnant” had been an inclusion criterion instead of an exclusion criterion.

CONCLUSIONS

In conclusion, the current results strongly indicate that male morning testosterone levels neither increase nor decrease in response to the partner’s ovulations. This discordance to previous laboratory studies could either indicate that (i) the phenomenon of hormonal adaptation of men to women does not exist and earlier experimental studies should be questioned, (ii) the phenomenon is short-lived/acute and wanes off if the exposure is too long, or (iii) the male testosterone response is directed toward other women than the partner. In our eyes, the most important step forward now is attempting to replicate the findings by Cerda-Molina et al.,5 because these results to date constitute the strongest evidence for effects of the female menstrual cycle on male hormones. Also, it would be interesting to see whether men in relationships are as responsive to other women’s ovulatory cues as single men are, and whether this responsiveness is affected by the partner’s current hormonal status. Also, is a man in a relationship responsive toward his own partner’s olfactory ovulatory cues if he has not met his partner for a few hours? If so, does a positive response predict fertility of the couple? Answering questions such as these will be crucial in the investigation of whether, when, and how human reproductive hormonal interactions take place.

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(b) **Acquisition of Data**
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(c) **Analysis and Interpretation of Data**
Jakob O. Ström; Edvin Ingberg; Julia K. Slezak

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(b) **Revising It for Intellectual Content**
Jakob O. Ström; Edvin Ingberg; Julia K. Slezak; Annette Theodorsson; Elvar Theodorsson

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(a) **Final Approval of the Completed Article**
Jakob O. Ström; Edvin Ingberg; Julia K. Slezak; Annette Theodorsson; Elvar Theodorsson

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**SUPPLEMENTARY DATA**

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jsxm.2018.06.003.