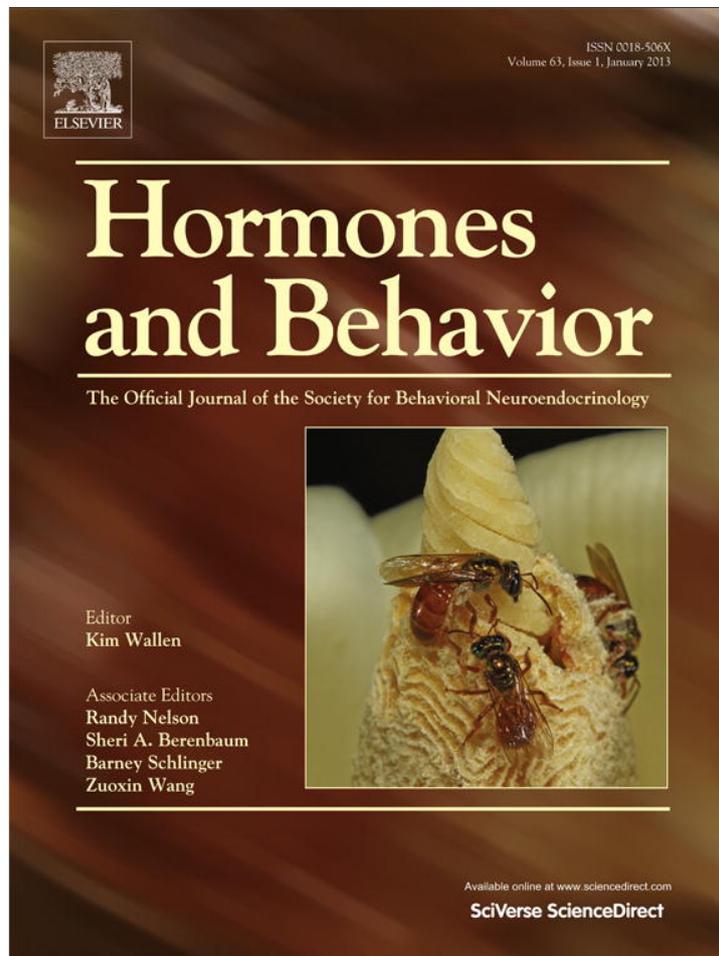


Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at SciVerse ScienceDirect

## Hormones and Behavior

journal homepage: [www.elsevier.com/locate/yhbeh](http://www.elsevier.com/locate/yhbeh)

## Women's attractiveness changes with estradiol and progesterone across the ovulatory cycle

David A. Puts<sup>a,\*</sup>, Drew H. Bailey<sup>b</sup>, Rodrigo A. Cárdenas<sup>c</sup>, Robert P. Burriss<sup>d</sup>, Lisa L.M. Welling<sup>a</sup>, John R. Wheatley<sup>a</sup>, Khytam Dawood<sup>c</sup>

<sup>a</sup> Department of Anthropology, Pennsylvania State University, University Park, PA 16802, USA

<sup>b</sup> Department of Psychological Sciences, University of Missouri, Columbia, MO 65211, USA

<sup>c</sup> Department of Psychology, Pennsylvania State University, University Park, PA 16802, USA

<sup>d</sup> Department of Psychology, University of Sterling, Sterling, FK9 4LA Scotland, UK

### ARTICLE INFO

#### Article history:

Received 10 May 2012

Revised 6 November 2012

Accepted 8 November 2012

Available online 15 November 2012

#### Keywords:

Attractiveness

Estradiol

Face

Menstrual cycle

Progesterone

Voice

### ABSTRACT

In many species, females are more sexually attractive to males near ovulation. Some evidence suggests a similar pattern in humans, but methodological limitations prohibit firm conclusions at present, and information on physiological mechanisms underlying any such pattern is lacking. In 202 normally-cycling women, we explored whether women's attractiveness changed over the cycle as a function of two likely candidates for mediating these changes: estradiol and progesterone. We scheduled women to attend one session during the late follicular phase and another during the mid-luteal phase. At each session, facial photographs, voice recordings and saliva samples were collected. All photographs and voice recordings were subsequently rated by men for attractiveness and by women for flirtatiousness and attractiveness to men. Saliva samples were assayed for estradiol and progesterone. We found that progesterone and its interaction with estradiol negatively predicted vocal attractiveness and overall (facial plus vocal) attractiveness to men. Progesterone also negatively predicted women's facial attractiveness to men and female-rated facial attractiveness, facial flirtatiousness and vocal attractiveness, but not female-rated vocal flirtatiousness. These results strongly suggest a pattern of increased attractiveness during peak fertility in the menstrual cycle and implicate estradiol and progesterone in driving these changes.

© 2012 Elsevier Inc. All rights reserved.

### Introduction

Estrus is the phase of the ovulatory cycle immediately preceding ovulation and is accompanied by changes in appearance, odor and behavior across a variety of female mammals (Gangestad and Thornhill, 2008). Males respond to these cues by intensifying their mating efforts with estrous females. In *Homo sapiens*, estrus is not heralded by obvious signs, leading multiple researchers to conclude that natural selection favored the suppression of such signs in our species (Alexander and Noonan, 1979; Bensch and Thornhill, 1979; Gangestad and Thornhill, 2008; Strassman, 1981; Symons, 1979).

Suppression of ovulatory cues by females would pose an adaptive problem for males. Males who could detect ovulatory cues, however slight, would be at an advantage in more efficiently directing their mating effort toward fertile females. Recent research indicates that some observable characteristics in women change over their cycles, including voice pitch (Bryant and Haselton, 2009) and skin color (Van den Berghe and Frost, 1986). Other research suggests that men may be capable of detecting these cues, generally preferring women's

odors (Doty et al., 1975; Gildersleeve et al., 2012; Havlicek et al., 2006; Kuukasjärvi et al., 2004; Singh and Bronstad, 2001; Thornhill et al., 2003), faces (Roberts et al., 2004), and voices (Fischer et al., 2011; Pipitone and Gallup, 2008, 2011) during the late follicular (fertile) phase (see also Haselton and Gildersleeve, 2011 for a review).

Ancestral women may also have benefited from detecting the ovulatory status of other women. Not only do women appear to be more attractive at mid-cycle, but they are also more sexually attracted to men of putatively high genetic quality (e.g., Gangestad and Thornhill, 1998; Penton-Voak et al., 1999; Puts, 2005) and report more extra-pair sexual interests (Gangestad et al., 2002, 2005, 2010; Garver-Apgar et al., 2006; Haselton and Gangestad, 2006; Pillsworth and Haselton, 2006) and behavior (Bellis and Baker, 1990) at this time. At mid-cycle, women may therefore pose a greater threat to their same-sex rivals' ability to attract and retain mates. Some evidence suggests that women rate the odors (Doty et al., 1975; Kuukasjärvi et al., 2004), faces (Roberts et al., 2004) and voices (Pipitone and Gallup, 2008) of women near ovulation as being more attractive.

However, Gildersleeve et al. (2012) noted several methodological limitations of previous work in this area, including suboptimal data analysis (treating raters rather than stimulus donors as the unit of analysis) and design (between-subjects rather than within-subjects), reliance on

\* Corresponding author. Fax: +1 814 863 1474.

E-mail address: [dap27@psu.edu](mailto:dap27@psu.edu) (D.A. Puts).

self-report data to establish ovulatory cycle position, and small sample sizes. Ultimately, the strongest support for cyclic changes in attractiveness will come from elucidation of the physiological mechanisms underlying them. Estradiol and progesterone are likely candidates for mediating any such changes (Haselton and Gildersleeve, 2011; Kuukasjärvi et al., 2004). For example, estradiol and progesterone receptors are expressed in laryngeal tissues (Ferguson et al., 1987; Marsigliante et al., 1996; Voelter et al., 2008), and puberty, pregnancy, menopause (Caruso et al., 2000), hormone replacement therapy (Firat et al., 2009) and hormonal contraceptive use (Amir et al., 2002) involve changes in both these hormones and vocal acoustics. Moreover, the day of ovulation can be precisely estimated using estrogen and progesterone (Baird et al., 1995), so if women's attractiveness varies with fertility, then it should also vary with these hormones. Yet, these associations remain unexplored.

We therefore investigated relationships between menstrual cycle fluctuations in women's estradiol and progesterone levels and their attractiveness to men and perceived mating threat to women (measured by attractiveness and apparent flirtatiousness). We explored these relationships in two characteristics highly salient to human mating, faces and voices (Puts et al., 2012b), using a within-subjects design and the largest sample yet collected for these purposes.

## Material and methods

### Participants

Two hundred and two normally-cycling women (mean age  $19.6 \pm 1.6$  years) from 159 unique sibling groups (43 sister pairs, plus 116 singletons) participated in this research as part of a larger study involving siblings at a large Midwest U.S. university. Self-reported ethnicities were 91.6% White, 3.5% Asian, 2.0% Black or African American, 0.5% Hispanic or Latino, 0.5% Native Hawaiian or Other Pacific Islander, and 1.5% Other. Participants were scheduled for two laboratory sessions according to self-reported menstrual cycle length and date of the beginning of last menstrual bleeding. One laboratory session was scheduled within one day of expected peak estradiol production during the follicular phase, and the other session was scheduled within two days of expected peak progesterone production (mid-luteal phase), according to the methods of Puts (2006). Session order was counterbalanced across participants, and sessions occurred between 1300 h and 1600 h to minimize the influence of circadian hormonal fluctuations. Because we statistically analyze hormone levels rather than self-reported cycle phase, our use of the term "session" henceforth refers to first or second session rather than presumed follicular or luteal session. Approximately 12% of women attended only the first session.

### Saliva collection and hormonal analysis

Participants collected approximately 9 ml of saliva in sodium azide-treated polystyrene test tubes during both sessions. Contamination of saliva samples was minimized by having participants not eat, drink (except plain water), smoke, chew gum, or brush their teeth for 1 h before each session. Participants rinsed their mouths with water before chewing a piece of sugar-free Trident gum (inert in salivary hormone assays) to stimulate saliva flow. The tube was capped and left upright at room temperature for 18–24 h to allow mucins to settle. Tubes were then frozen at  $-20$  °C until analysis by the Neuroendocrinology Assay Laboratory at the University of Western Ontario, Canada.

Per previous research (e.g., Hampson et al., 2005; Oinonen and Mazmanian, 2007), progesterone was assayed using  $^{125}\text{I}$  Coat-A-Count assay kits (Diagnostic Products Corporation, Los Angeles, CA) modified for use with saliva. Similar to previous research (e.g., Finstad et al., 2009), estradiol was assayed using  $^{125}\text{I}$  Ultra-Sensitive  $\text{E}_2$  RIA DSL-4800 kit (Diagnostic Systems Laboratories, Webster, TX) modified for use

with saliva. Each sample was assayed twice, and average hormone levels for each sample were used in our analyses. Assay sensitivities were 0.65 pg/ml and 5 pg/ml, and intra-assay coefficients of variation were 5.1% and 10.7%, for estradiol and progesterone, respectively.

### Facial photographs

Participants were provided wet wipes and instructed to remove any makeup, jewelry or spectacles and to assume a neutral expression. Facial photographs were taken with a tripod-mounted Canon PowerShot S10 digital camera at a distance of approximately 1 m, a height adjusted to the participant, and using constant lighting across participants. All face images were cropped beneath the chin, normalized on interpupillary distance, and rotated so that both pupils lay on the same horizontal plane.

### Voice recording and analysis

Participants were recorded reading an excerpt from a standard voice passage (Fairbanks, 1960) in an anechoic, soundproof booth using a Shure SM58 vocal cardioid microphone. A curved wire kept the participant's mouth approximately 9.5 cm from the microphone. Voices were recorded using Goldwave software in mono at a sampling rate of 44,100 Hz and 16-bit quantization, and saved as uncompressed .WAV files.

Each recording was analyzed using Praat software (version 4.4.11). Pitch floor and ceiling were 100 Hz and 500 Hz, in accordance with the programmers' recommendations (Boersma and Weenik, 2009); otherwise, default settings were used. Across each recording, we measured mean (mean =  $208.6 \pm 17.6$  Hz) and standard deviation (mean =  $38.1 \pm 9.4$  Hz) of fundamental frequency ( $F_0$ , the acoustic correlate of pitch), duration (mean =  $5.36 \pm .90$  s), number of voice breaks (mean =  $14.6 \pm 2.8$ ), harmonics (mean =  $15.5 \pm 1.4$  Hz), four measures of jitter (cycle-to-cycle variation in fundamental frequency), and five measures of shimmer (cycle-to-cycle variation in amplitude) using the 'voice report' function in Praat. All jitter ( $r > .90$ , mean  $r = .94$ ) and shimmer ( $r > .47$ , mean  $r = .79$ ) variables were correlated, so they were standardized (mean = 0,  $SD = 1$ ) and summed (jitter: mean =  $-.41 \pm 3.40$ ; shimmer: mean =  $-.07 \pm 4.32$ ).

We also measured formant frequencies  $F_1$  through  $F_4$ . Lower, more closely spaced formants correspond with a deeper vocal timbre. Formants were measured at each glottal pulse and averaged across measurements, as in Puts et al. (2012a). Formant measurements obtained by this method correlate highly ( $.93 \leq r \leq .98$ ) with measurements obtained by measuring and averaging across individual vowels (Puts et al., 2012a). We then computed formant position ( $P_f$ , mean =  $0.85 \pm 0.40$ ), defined as the average standardized formant value for the first four formants, using the method described in Puts et al. (2012a). The following between-sexes means and SDs were used to standardize formants:  $F_1 = 482.6 \pm 49.8$  Hz,  $F_2 = 1643.2 \pm 145.7$  Hz, mean  $F_3 = 2544.7 \pm 173.9$  Hz and mean  $F_4 = 3618.8 \pm 266.8$  Hz.

### Face and voice ratings

Face photographs and voice recordings were rated by 568 men (mean age:  $19.4 \pm 1.8$  years) and 558 women (mean age:  $19.1 \pm 2.4$  years) from a large northeast U.S. university. Raters had a comparable ethnic distribution to the women who provided the photographs and recordings. Each rater assessed  $24.9 \pm 2.6$  voice recordings and  $24.1 \pm 2.4$  face photographs (including those of men and hormonally contracepting women not used here). Raters were presented a random sample of face photographs and voice recordings, except that no rater was presented with more than one photograph or recording from each participant. Using 7-point Likert scales, men rated stimuli on attractiveness for short- and long-term relationships, and women rated

stimuli on flirtatiousness and attractiveness to men. The order in which participants completed the rating tasks (e.g., short- or long-term first) was random across participants, as was the order in which stimuli were presented. Each stimulus was rated by  $\geq 15$  raters of each sex (mean female raters = 18.6, mean male raters = 18.9). The first 15 ratings obtained of each voice and face stimulus for each type of rating (e.g., short-term attractiveness) were averaged to produce composite ratings of short- and long-term attractiveness (male-rated), attractiveness to men and flirtatiousness (female-rated) for each photograph and recording.

#### Data treatment

Estradiol (session 1:  $2.4 \pm 4.6$  pg/ml, session 2:  $2.3 \pm 6.0$  pg/ml) and progesterone (session 1:  $81.1 \pm 70.0$  pg/ml, session 2:  $68.1 \pm 63.0$  pg/ml) levels were obtained for both sessions for 171 and 176 participants, respectively. Hormone values were positively skewed and thus natural logarithm-transformed.

Short- and long-term female attractiveness ratings were highly correlated within both sessions (face: both  $r = .95$ , voice:  $r = .94$  and  $.91$ , respectively) and were thus summed to create composite attractiveness measures (face mean:  $5.5 \pm 1.7$ ; voice mean:  $7.4 \pm 1.7$ ).

#### Analyses

Data were analyzed using random intercept multilevel models (using maximum likelihood estimation) with an unstructured covariance structure, using the *nlme* package in R (Pinheiro et al., 2012). Results from multilevel models can be interpreted similarly to the way that one would interpret the results from a regression model, but multilevel modeling is preferred when observations are not completely independent of each other (nested structure). Ignoring such structure leads to underestimation of standard errors in the model. Therefore, to account for the possibility that sessions within participants were correlated with each other, we nested sessions within participants, and to account for the possibility that siblings were correlated with each other, we nested participants within sibling pairs. Hormones were treated as time varying (Level 1) predictors of women's facial and vocal attractiveness and apparent flirtatiousness. Within (Level 1)- and between (Level 2)-participants variation in hormone levels were assessed separately as predictors of facial and vocal perceptions. As siblings were recruited for research purposes unrelated to the present study, we did not assess the influence of any sibling level variables (Level 3) on female attractiveness.

When assessing the interaction between progesterone and estrogen on attractiveness, we centered and standardized ( $M = 0$ ,  $SD = 1$ ) each predictor to reduce collinearity among predictors and facilitate interpretation of the main effects intercepts. Univariate plots were used to screen for outliers, as were inspections of residual plots from the random intercept models. Effect sizes are not straightforward in multilevel modeling, there is currently no consensus as to the effect sizes that are most appropriate (Peugh, 2010), and we therefore opted not to include them.

## Results

#### Hormones and overall attractiveness to men

To explore cyclic changes in overall attractiveness to men, we followed Cohen's (1990) recommendations for producing composite variables by standardizing and summing women's facial and vocal attractiveness ratings. In separate models, progesterone ( $t(156) = -4.21$ ,  $p < .0001$ ;  $n = 194$ ; regression weight =  $-.30$ ;  $SE = .07$ ; Figs. 1 and 2), but not estradiol ( $t(151) = -1.16$ ,  $p = .25$ ;  $n = 193$ ; regression weight =  $-.12$ ;  $SE = .10$ ), predicted attractiveness to men. When estradiol, progesterone and their interaction were included in a

single model, after first standardizing progesterone and estradiol, progesterone ( $t(146) = -4.24$ ,  $p < .00005$ ) and the interaction of estradiol and progesterone ( $t(146) = -2.09$ ,  $p = .04$ ) were statistically significant predictors, but estradiol was not ( $t(146) = -.04$ ,  $p = .97$ ; for this model,  $n = 193$ ) (Fig. 3).

A random intercept model with facial attractiveness regressed on vocal attractiveness indicated that facial and vocal attractiveness were positively associated ( $t(159) = 3.01$ ,  $p = .003$ ;  $n = 194$ ). To test whether this was due to individual differences in attractiveness or hormone-mediated within-subjects variation, we partitioned facial attractiveness into between-participants components (each participant's mean facial attractiveness across sessions) and within-participants components (the difference between a participant's facial attractiveness for each session and that participant's mean facial attractiveness across sessions). Only participants with data from both sessions were used. Facial attractiveness was significantly related to voice attractiveness at the between-participants ( $t(31) = 2.45$ ,  $p = .02$ ), but not within-participants ( $t(159) = 1.37$ ,  $p = .17$ ; for this model,  $n = 162$ ), level. Because cyclic changes in women's attractiveness were not due to coordinated changes in facial and vocal attractiveness, we proceeded to analyze the two traits separately.

#### Hormones and facial attractiveness

Hormones were first entered into separate models to predict facial attractiveness to men. Only progesterone significantly predicted attractiveness ( $t(158) = -2.53$ ,  $p = .01$ ;  $n = 196$ ; regression weight =  $-.19$ ;  $SE = .08$ ; Figs. 1 and 2). When estradiol, progesterone and their interaction were included in a single model, after first standardizing progesterone and estradiol as above, the interaction was not statistically significant ( $t(148) = -.34$ ,  $p = .73$ ;  $n = 195$ ) (Fig. 4).

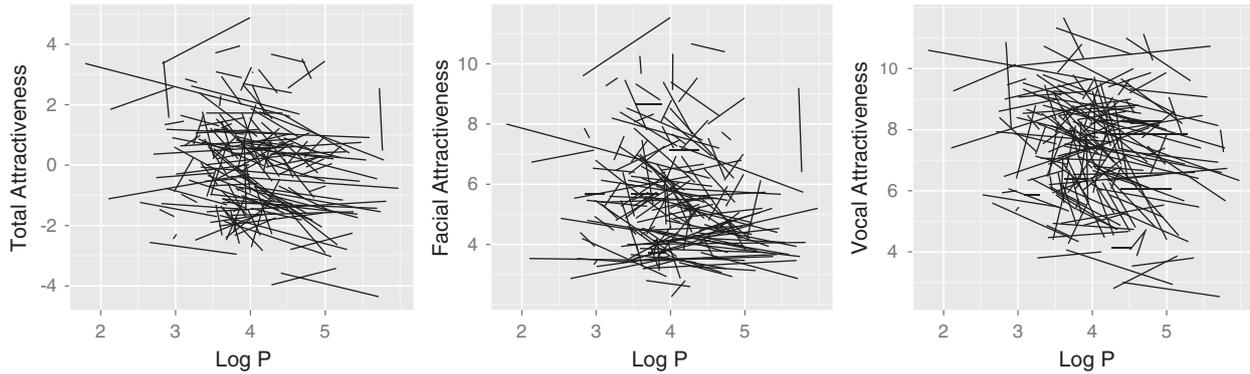
Next, we partitioned progesterone values into between-participants components (each participant's mean progesterone value across sessions) and within-participants components (for each session, the difference between a participant's progesterone for that session and her mean progesterone across sessions) (Puts et al., 2010). Only participants with data from both sessions were used. Progesterone was related to attractiveness only at the within-participants level ( $t(159) = -3.07$ ,  $p = .003$ ;  $n = 174$ ).

#### Hormones and vocal attractiveness

Each hormone was entered into a separate model to predict vocal attractiveness to men. Only progesterone significantly predicted attractiveness ( $t(170) = -4.34$ ,  $p < .00005$ ;  $n = 197$ ; regression weight =  $-.37$ ;  $SE = .08$ ; Figs. 1 and 2). Estradiol, progesterone and their interaction were included in a single model, after first standardizing progesterone and estradiol. The interaction was statistically significant ( $t(160) = -2.02$ ,  $p = .046$ ;  $n = 197$ ) (Fig. 4).

We partitioned hormone values into between- and within-participants components as above. Progesterone negatively predicted attractiveness only at the within-participants level ( $t(162) = -4.85$ ,  $p < .00005$ ;  $n = 166$ ). We estimated a model using within- and between-level predictors of estradiol and progesterone, and four interactions (progesterone within  $\times$  estradiol within, progesterone between  $\times$  estradiol between, progesterone between  $\times$  estradiol within, progesterone within  $\times$  estradiol between). Of the interactions, only within-participants estradiol by between-participants progesterone was statistically significant ( $t(158) = -2.34$ ,  $p = .02$ ;  $n = 166$ ), indicating that for low levels of between-participants progesterone, within-participants estradiol is positively related to voice attractiveness.

Acoustic mediators of the relationships between hormones and vocal attractiveness should be significantly related to progesterone and/or estradiol  $\times$  progesterone. However, progesterone and estradiol  $\times$  progesterone were not significantly related to any acoustic variable that we measured, and thus we did not perform mediation analyses.



**Fig. 1.** Changes in individual participants' attractiveness across sessions as a function of changes in progesterone levels. Multiple observations from the same participants are connected by line segments.

*Women's perceptions*

To explore whether women could detect menstrual cycle changes in the faces and voices of other women, we first standardized and summed women's face and voice ratings to create overall (face plus voice) attractiveness and overall apparent flirtatiousness measures. Progesterone significantly predicted female-rated attractiveness ( $t(156) = -2.69, p = .008; n = 194$ ; regression weight =  $-.21; SE = .08$ ) and marginally significantly predicted flirtatiousness ( $t(156) = -1.79, p = .08; n = 194$ ; regression weight =  $-.14; SE = .08$ ), but estradiol did not (both  $|t| < 1, p > .30$ ). Controlling for estradiol and progesterone, their interaction also did not significantly predict overall attractiveness ( $t(146) = -1.45, p = .15$ ) but marginally significantly predicted flirtatiousness ( $t(146) = -1.82, p = .07; n = 193$ ).

We then entered estradiol and progesterone into separate models to predict facial and vocal attractiveness and flirtatiousness. Progesterone negatively predicted female-rated facial attractiveness ( $t(158) = -2.35, p = .02; n = 196$ ; regression weight =  $-.11; SE = .05$ ), facial flirtatiousness ( $t(158) = -2.09, p = .04; n = 194$ ; regression weight =  $-.10; SE = .05$ ), and vocal attractiveness ( $t(170) = -2.45, p = .02; n = 199$ ; regression weight =  $-.11; SE = .05$ ), but not vocal flirtatiousness ( $t(170) = -1.22, p = .22; n = 199$ ; regression weight =  $-.05; SE = .04$ ). Estradiol and estradiol  $\times$  progesterone did not significantly predict female-rated facial or vocal flirtatiousness or attractiveness (all  $|t| < 1.4, p > .15$ ).

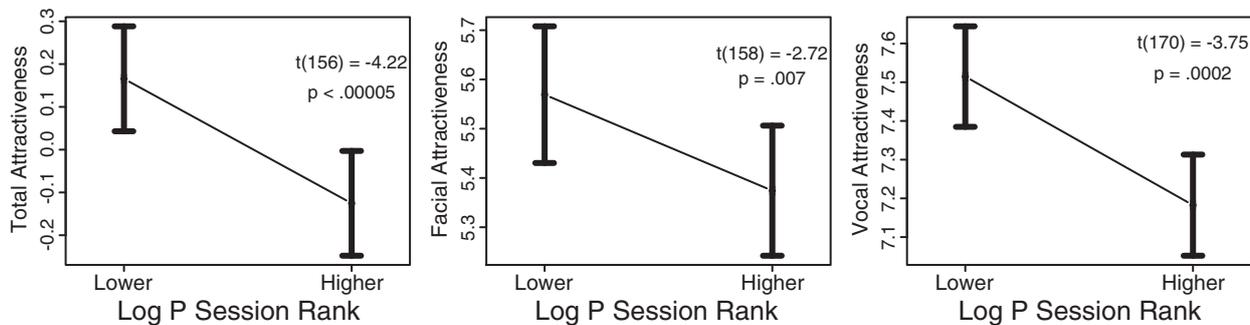
**Discussion**

This is the first study to examine the hormonal mediators of cyclic variation in women's attractiveness. It is also the first to explore cyclic

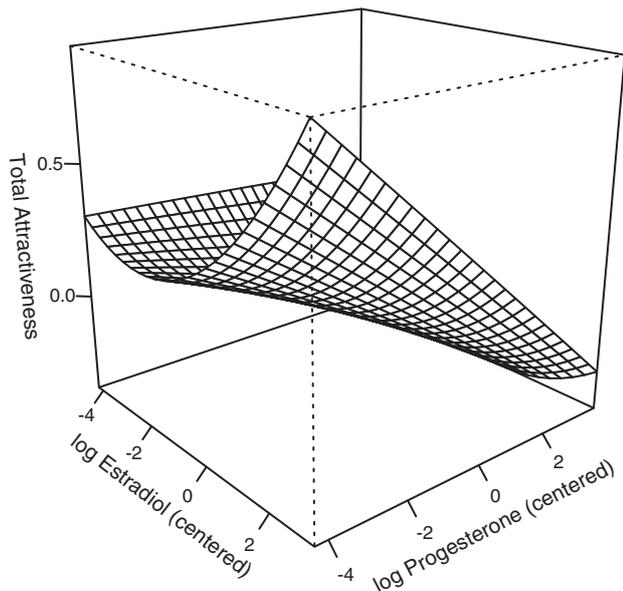
changes in attractiveness involving multiple sensory modalities. Previous studies have employed small to moderate samples (Gildersleeve et al., 2012), but this study has the advantage of utilizing a sample size nearly equal to the combined samples of all previous studies on women's facial, vocal, or olfactory attractiveness across the cycle.

We found that estradiol and progesterone predicted cyclic changes in women's attractiveness to men and measures of perceived mating threat to women. Men found women most attractive when progesterone levels were low, a state corresponding with the follicular (fertile) phase of the ovulatory cycle, and when estradiol levels were high relative to progesterone levels, a state corresponding with peak conception risk (Baird et al., 1995). In separate models, progesterone negatively predicted facial and vocal attractiveness, and high estradiol relative to progesterone predicted vocal attractiveness. Because estradiol peaks during both the late follicular (fertile) and luteal (non-fertile) phases of the cycle, it was unsurprising that estradiol did not predict attractiveness except through its interaction with progesterone.

All hormonal effects were within-subjects with the exception of the interaction of within-subjects estradiol and between-subjects progesterone on women's vocal attractiveness. This interaction with between-subjects progesterone probably owes to large within-subjects effects of this hormone combined with considerable between-subjects variation in the scheduling of sessions according to self-report. It is possible that some between-subjects hormonal effects would have been observed if women were assessed at more precisely identical times in their cycles and/or if measures were collected from each woman at additional intervals across her cycle. However, it is likely that adult hormonal differences account for a greater proportion of the changes in women's attractiveness across their cycles than they account for between-women differences in attractiveness, which are probably multifactorial and may



**Fig. 2.** Changes in mean ( $\pm SE$ ) attractiveness ratings as a function of higher or lower progesterone session. Only participants with attractiveness and progesterone data from both sessions were used. Sessions were rank-ordered on the observed progesterone level. Included  $t$ -values are tests of the effect of high progesterone session from a random intercept model with observations nested in participants and participants nested in sibling pairs.



**Fig. 3.** Overall (facial plus vocal) attractiveness to men by estradiol and progesterone levels, calculated from second-degree polynomial regression model and constructed using the RSM package (Lenth, 2009) in R.

depend more on pubertal levels of sex hormones than adult levels (Puts et al., 2012b). Overall, these results provide strong evidence that men's mate preferences target fertile women.

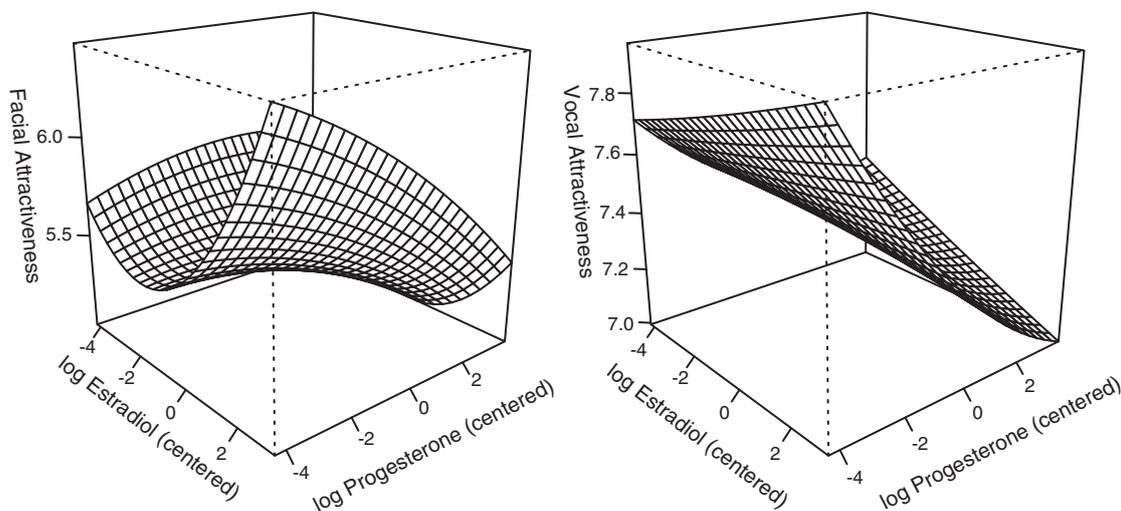
Progesterone levels also predicted women's ratings by other women. Progesterone decreased the appearance of attractiveness and flirtatiousness in women's faces and the appearance of attractiveness in their voices. It should be noted that some differences between male and female raters' ability to detect cyclic changes in women's faces and voices (e.g., the generally stronger results for male raters) could reflect the different dimensions on which these groups assessed women's faces and voices. Nevertheless, given menstrual cycle variation in women's attractiveness and extra-pair sexual interest (Gangestad and Thornhill, 2008), the ability to detect fertility status (Pipitone and Gallup, 2008; Roberts et al., 2004) may have evolved in women to mitigate the risks to romantic relationships imposed by their fertile-phase competitors.

These results demonstrate that ovulation is not entirely cryptic in women. However, our results also appear to support the conclusion that selection has not favored broadcast signals of ovulatory status. Facial and vocal attractiveness were related only at the between-subjects level, suggesting no selection for coordinated changes in attractiveness across the cycle. In addition, we found no systematic change with estradiol, progesterone or their interaction in fundamental and formant frequencies—acoustic features of voice known to affect attractiveness to men (Puts et al., 2011). Although previous research found an increase in fundamental frequency, but not formant frequencies, near ovulation (Bryant and Haselton, 2009), these acoustic variables did not appear to track conception risk in our sample, as they would have been related to changes in estradiol and progesterone (Baird et al., 1995). From these data, it appears that strong selection to detect cyclic changes in fertility attuned men and women to whatever cues were available. As Gangestad and Thornhill (2008) note, such cyclic changes in women's appearance may represent “leakage” of fertility information rather than signaling of ovulatory status. For example, cyclic changes in facial attractiveness could be due to fluctuations in acne (Stoll et al., 2001).

It is worth considering how these results might shed light on the evolution of concealed ovulation. According to Strassman (1981), in an ancestral polygynous mating system, lower-ranking males with few mating opportunities would benefit reproductively from defending and investing in a single mate. However, if females advertized ovulation, then high-ranking males would displace low-ranking males at mid-cycle, decreasing low-ranking males' paternity confidence and thus their investment. To maintain investment, females suppressed cues to ovulation, hiding their fertility status from extra-pair males.

Suppressing ovulatory cues could have two additional benefits to females. First, it could decrease their mates' ability to achieve polygyny and divert their investment toward other females (Alexander and Noonan, 1979). However, as noted above, less dominant males should prefer to defend and invest in a mate (especially, given the lengthy development of hominoid offspring). For low-ranking males, monogamy and high paternity certainty would be preferable to the alternative of having no mate.

Second, suppressing ovulatory cues could allow the female to recruit high-quality genes outside of the pair-bond by reducing her ability to concentrate mate guarding around ovulation (Benshoof and Thornhill, 1979; Symons, 1979). There is evidence that women employ



**Fig. 4.** Facial and vocal attractiveness to men by estradiol and progesterone levels, calculated from second-degree polynomial regression model and constructed using the RSM package (Lenth, 2009) in R.

this strategy, especially if their long-term mate is low in genetic quality (Bellis and Baker, 1990; Gangestad et al., 2002, 2005, 2010; Garver-Apgar et al., 2006; Haselton and Gangestad, 2006; Pillsworth and Haselton, 2006). However, infidelity may lead to lower male investment (Welling et al., 2011), relationship dissolution (Betzig, 1989) and male violence (Wilson and Daly, 1996). Also, if cuckoldry had been too common over human evolution, then men would have evolved not to invest in the offspring of their long-term mates. In fact, extra-pair paternity is modest in modern human populations, probably below 10% (Simmons et al., 2004).

These benefits (promoting investment from a pair-bonded mate, reducing the ability of a mate to practice polygyny, and facilitating female extra-pair mating) are not mutually exclusive. Given that we observed within- but not between-subjects changes in attractiveness with hormone levels, it is reasonable to infer that ovulation is concealed more from extra-pair males than from long-term mates (Provost et al., 2008). Indeed, some evidence indicates that men increase their mate guarding of their partners at mid-cycle (Gangestad et al., 2002; Haselton and Gangestad, 2006). On the one hand, this would seem to support Strassman's hypothesis that concealed ovulation evolved to prevent high-ranking males from supplanting low-ranking, investing males, as ovulation would be more concealed from extra-pair males. On the other hand, even if concealed ovulation evolved for other functions, counter-adaptations for detecting ovulation would be favored in males, and those with repeated exposure to females would have the most information available for detecting ovulation.

## Acknowledgments

We thank Marc Breedlove and Cynthia Jordan for research support, Bradly Alicea, Jill Armington, Emily Barben, Julia Barndt, Michael Burla, Kathryn Cheney, Michael DiRaimo, Melina Durhal, Rebecca Frysinger, Sana Khan, Mallory Leinenger, Heather Malinowski, Samantha Melonas, Ernestine Mitchell, Joe Morehouse, Diana Rosaleyra, Charlene Scheld, Kevin Singh, Sara Sutherland, Lisa Vroman, Tyasha Washington, and Molly Zolianbawi for assistance with study preparation and data collection, Andrew Littlefield for advice on statistical analysis, and Elizabeth Hampson and Bavani Rajakumar for assistance with hormonal assays.

## References

- Alexander, R., Noonan, K.M., 1979. Concealment of ovulation, parental care, and human social evolution. In: Chagnon, N., Irons, W. (Eds.), *Evolutionary Biology and Human Social Behavior: An Anthropological Perspective*. Duxbury Press, North Scituate, MA, pp. 436–453.
- Amir, O., Kishon-Rabin, L., Muchnik, C., 2002. The effect of oral contraceptives on voice: preliminary observations. *J. Voice* 16, 267–273.
- Baird, D.D., McConaughey, D.R., Weinberg, C.R., Musey, P.I., Collins, D.C., Kesner, J.S., et al., 1995. Application of a method for estimating day of ovulation using urinary estrogen and progesterone metabolites. *Epidemiology* 6, 547–550.
- Bellis, M.A., Baker, R.R., 1990. Do females promote sperm competition? *Anim. Behav.* 40, 997–999.
- Benshoof, L., Thornhill, R., 1979. The evolution of monogamy and concealed ovulation in humans. *J. Soc. Biol. Struct.* 2, 95–106.
- Betzig, L.L., 1989. Causes of conjugal dissolution: a cross-cultural study. *Curr. Anthropol* 30, 654–676.
- Boersma, P., Weenik, D., 2009. Praat: doing phonetics by computer (Version 5.1.03). Retrieved March 21, 2009, from <http://www.praat.org/>.
- Bryant, G.A., Haselton, M.G., 2009. Vocal cues of ovulation in human females. *Biol. Lett.* 5, 12–15.
- Caruso, S., Roccasalva, L., Sapienza, G., Zappala, M., Nuciforo, G., Biondi, S., 2000. Laryngeal cytological aspects in women with surgically induced menopause who were treated with transdermal estrogen replacement therapy. *Fertil. Steril.* 74, 1073–1079.
- Cohen, J., 1990. Things I have learned (so far). *Am. Psychol.* 45, 1304–1312.
- Doty, R.L., Ford, M., Preti, G., Huggins, G.R., 1975. Changes in the intensity and pleasantness of human vaginal odors during the menstrual cycle. *Science* 190, 1316–1318.
- Fairbanks, G., 1960. *Voice and Articulation Drillbook*, 2nd ed. Harper & Row, New York.
- Ferguson, B.J., Hudson, W.R., McCarty Jr., K.S., 1987. Sex steroid receptor distribution in the human larynx and laryngeal carcinoma. *Arch. Otolaryngol. Head Neck Surg.* 113, 1311–1315.
- Finstad, S.E., Emaus, A., Tretli, S., Jasienska, G., Ellison, P.T., Furberg, A., et al., 2009. Adult height, insulin, and 17 $\beta$ -estradiol in young women. *Cancer Epidemiol. Biomarkers Prev.* 18, 1477–1483.
- Firat, Y., Engin-Ustun, Y., Kizilay, A., Ustun, Y., Akarçay, M., Selimoglu, E., et al., 2009. Effect of intranasal estrogen on vocal quality. *J. Voice* 23, 716–720.
- Fischer, J., Semple, S., Fickenscher, G., Jurgens, R., Kruse, E., Heistermann, M., et al., 2011. Do women's voices provide cues of the likelihood of ovulation? The importance of sampling regime. *PLoS One* 6, e24490.
- Gangestad, S.W., Thornhill, R., 1998. Menstrual cycle variation in women's preferences for the scent of symmetrical men. *Proc. R. Soc. B Biol. Sci.* 265, 927–933.
- Gangestad, S.W., Thornhill, R., 2008. Human oestrus. *Proc. R. Soc. B Biol. Sci.* 275, 991–1000.
- Gangestad, S.W., Thornhill, R., Garver, C.E., 2002. Changes in women's sexual interests and their partners' mate-retention tactics across the menstrual cycle: evidence for shifting conflicts of interest. *Proc. R. Soc. B Biol. Sci.* 269, 975–982.
- Gangestad, S.W., Thornhill, R., Garver-Apgar, C.E., 2005. Women's sexual interests across the ovulatory cycle depend on primary partner developmental instability. *Proc. R. Soc. B Biol. Sci.* 272, 2023–2027.
- Gangestad, S.W., Thornhill, R., Garver-Apgar, C.E., 2010. Fertility in the cycle predicts women's interest in sexual opportunism. *Evol. Hum. Behav.* 31, 400–411.
- Garver-Apgar, C.E., Gangestad, S.W., Thornhill, R., Miller, R.D., Olp, J.J., 2006. Major histocompatibility complex alleles, sexual responsiveness, and unfaithfulness in romantic couples. *Psychol. Sci.* 17, 830–835.
- Gildersleeve, K.A., Haselton, M.G., Larson, C.M., Pillsworth, E.G., 2012. Body odor attractiveness as a cue of impending ovulation in women: evidence from a study using hormone-confirmed ovulation. *Horm. Behav.* 61, 157–166.
- Hampson, E., Finestone, J.M., Levy, N., 2005. Menstrual cycle effects on perceptual closure mediate changes in performance on a fragmented objects test of implicit memory. *Brain Cogn.* 57, 107–110.
- Haselton, M.G., Gangestad, S.W., 2006. Conditional expression of women's desires and men's mate guarding across the ovulatory cycle. *Horm. Behav.* 49, 509–518.
- Haselton, M.G., Gildersleeve, K.A., 2011. Can men detect ovulation? *Curr. Dir. Psychol. Sci.* 20, 87–92.
- Havlicek, J., Dvorakova, R., Bartos, L., Flegr, J., 2006. Non-advertized does not mean concealed: body odour changes across the human menstrual cycle. *Ethology* 112, 91–90.
- Kuukasjärvi, S., Eriksson, C.J.P., Koskela, E., Mappes, T., Nissinen, K., Rantala, M.J., 2004. Attractiveness of women's body odors over the menstrual cycle: the role of oral contraceptives and receiver sex. *Behav. Ecol.* 15, 579–584.
- Lenth, R.V., 2009. Response-surface methods in R, using rsm. *J. Stat. Softw.* 32, 1–17.
- Marsigliante, S., Muscella, A., Resta, L., Storelli, C., 1996. Human larynx expresses isoforms of the oestrogen receptor. *Cancer Lett.* 99, 191–196.
- Oinonen, K.A., Mazmanian, D., 2007. Facial symmetry detection ability changes across the menstrual cycle. *Biol. Psychol.* 75, 136–145.
- Penton-Voak, I.S., Perrett, D.I., Castles, D.L., Kobayashi, T., Burt, D.M., Murray, L.K., et al., 1999. Menstrual cycle alters face preference. *Nature* 399, 741–742.
- Peugh, J.L., 2010. A practical guide to multilevel modeling. *J. Sch. Psychol.* 48, 85–112.
- Pillsworth, E.G., Haselton, M.G., 2006. Male sexual attractiveness predicts differential ovulatory shifts in female extra-pair attraction and male mate retention. *Evol. Hum. Behav.* 27, 247–258.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., the R Development Core Team, 2012. *nlme: Linear and Nonlinear Mixed Effects Models (Version 3.1-103)*: R package.
- Pipitone, N.R., Gallup Jr., G.G., 2008. Women's voice attractiveness varies across the menstrual cycle. *Evol. Hum. Behav.* 29, 268–274.
- Pipitone, N.R., Gallup, G., 2011. The unique impact of menstruation on the female voice: implications for the evolution of menstrual cycle cues. *Ethology* 118, 281–291.
- Provost, M.P., Quinsey, V.L., Troje, N.F., 2008. Differences in gait across the menstrual cycle and their attractiveness to men. *Arch. Sex. Behav.* 37, 598–604.
- Puts, D.A., 2005. Mating context and menstrual phase affect women's preferences for male voice pitch. *Evol. Hum. Behav.* 26, 388–397.
- Puts, D.A., 2006. Cyclic variation in women's preferences for masculine traits: potential hormonal causes. *Hum. Nat.* 17, 114–127.
- Puts, D.A., Cardenas, R.A., Bailey, D.H., Burriss, R.P., Jordan, C.L., Breedlove, S.M., 2010. Salivary testosterone does not predict mental rotation performance in men or women. *Horm. Behav.* 58, 282–289.
- Puts, D.A., Barndt, J.L., Welling, L.L.M., Dawood, K., Burriss, R.P., 2011. Intrasexual competition among women: vocal femininity affects perceptions of attractiveness and flirtatiousness. *Personal. Individ. Differ.* 50, 111–115.
- Puts, D.A., Apicella, C.L., Cardenas, R.A., 2012a. Masculine voices signal men's threat potential in forager and industrial societies. *Proc. R. Soc. B Biol. Sci.* 279, 601–609.
- Puts, D.A., Jones, B.C., DeBruine, L.M., 2012b. Sexual selection on human faces and voices. *J. Sex Res.* 49, 227–243.
- Roberts, S.C., Havlicek, J., Flegr, J., Hruskova, M., Little, A.C., Jones, B.C., et al., 2004. Female facial attractiveness increases during the fertile phase of the menstrual cycle. *Proc. R. Soc. B Biol. Sci.* 271 (Suppl. 5), S270–S272.
- Simmons, L.W., Firman, R.C., Rhodes, G., Peters, M., 2004. Human sperm competition: testis size, sperm production and rates of extrapair copulations. *Anim. Behav.* 68, 297–302.
- Singh, D., Bronstad, P.M., 2001. Female body odour is a potential cue to ovulation. *Proc. R. Soc. B Biol. Sci.* 268, 797–801.
- Stoll, S., Shalita, A.R., Webster, G.F., Kaplan, R., Danesh, S., Penstein, A., 2001. The effect of the menstrual cycle on acne. *J. Am. Acad. Dermatol.* 45, 957–960.

- Strassman, B.I., 1981. Sexual selection, paternal care, and concealed ovulation in humans. *Ethol. Sociobiol.* 2, 31–40.
- Symons, D., 1979. *The Evolution of Human Sexuality*. Oxford University Press, New York.
- Thornhill, R., Gangestad, S.W., Miller, R., McCollough, J.K., Franklin, M., 2003. Major histocompatibility complex genes, symmetry, and body scent attractiveness in men and women. *Behav. Ecol.* 14, 668–678.
- Van den Berghe, P.L., Frost, P., 1986. Skin color preference, sexual dimorphism and sexual selection: a case of gene culture coevolution? *Ethn. Racial Stud.* 9, 87–113.
- Voelter, C., Kleinsasser, N., Joa, P., Nowack, I., Martinez, R., Hagen, R., et al., 2008. Detection of hormone receptors in the human vocal fold. *Eur. Arch. Otorhinolaryngol.* 265, 1239–1244.
- Welling, L.L.M., Burriss, R.P., Puts, D.A., 2011. Mate retention behavior modulates men's preferences for self-resemblance in infant faces. *Evol. Hum. Behav.* 32, 118–126.
- Wilson, M.I., Daly, M., 1996. Male sexual proprietariness and violence against wives. *Curr. Dir. Psychol. Sci.* 5, 2–7.