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

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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 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 imply estradiol and progesterone in driving these changes.

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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 suppressing such signs in our species (Alexander and Noonan, 1979; Benshoof 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 maintain 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...
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; Marsiglante et al., 1996; Voelter et al., 2008), and puberty, pregnancy, menstruation (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 ± 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 mucus to settle. Tubes were then frozen at −20 °C until analysis by the Neuroendocrinology Assay Laboratory at the University of Western Ontario, Canada.

#### Face and voice ratings

Face photographs and voice recordings were rated by 568 men (mean age: 19.4 ± 1.8 years) and 558 women (mean age: 19.1 ± 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 ± 2.6 voice recordings and 24.1 ± 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 attractiveness changes with estradiol and progesterone across the ovulatory cycle, Horm. Behav. (2012), http://dx.doi.org/10.1016/j.hbeh.2012.11.007
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 ≥ 15 raters of each sex
(mean female raters = 18.6, mean male raters = 18.9). The first 15 rat-
ings 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), attractive-
tness to men and flirtatiousness (female-rated) for each photograph
and recording.

Data treatment

 Estradiol (session 1: 2.4 ± 4.6 pg/ml, session 2: 2.3 ± 6.0 pg/ml)
and progesterone (session 1: 81.1 ± 70.0 pg/ml, session 2: 68.1 ±
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 at-
tractiveness measures (face mean: 5.5 ± 1.7; voice mean: 7.4 ± 1.7).

Analyses

 Data were analyzed using random intercept multilevel models (using
maximum likelihood estimation) with an unstructured covariance struc-
ture, 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 indepen-
dent 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 attrac-
tiveness 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 inter-
pretation of the main effects intercepts. Univariate plots were used to
screen for outliers, as well as inspections of residual plots from the ran-
dom 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 var-
iables by standardizing and summing women’s facial and vocal attract-
tiveness ratings. In separate models, progesterone (τ(156) = −4.21,
\( p < .0001; n = 194\); regression weight = − .30; SE = .07; Figs. 1 and 2),
but not estradiol (τ(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 (τ(146) = −4.24, \( p < .00005\)) and the interaction of es-
tradiol and progesterone (τ(146) = −2.09, \( p = .04\)) were statistically
significant predictors, but estradiol was not (τ(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 (τ(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 ses-
sion and that participant’s mean facial attractiveness across sessions).

Only participants with data from both sessions were used. Facial attract-
tiveness was significantly related to voice attractiveness at the
between-participants level (τ(31) = 2.45, \( p = .02\), but not within-participants
(τ(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 attract-
tiveness (τ(158) = −2.53, \( p = .01; n = 196\); regression weight = − .19;
SE = .08; Figs. 1 and 2). When the estradiol, progesterone and their in-
teraction were included in a single model, after first standardizing pro-
gesterone and estradiol as above, the interaction was not statistically
significant (τ(148) = −3.4, \( p = .73; n = 195\) (Fig. 4).

Next, we partitioned progesterone values into between-participants
components (each participant’s mean progesterone value across ses-
sions) and within-participants components (for each session, the differ-
ence between a participant’s progesterone for that session and her
mean progesterone across sessions) (Puts et al., 2010). Only partici-
pants with data from both sessions were used. Progesterone was related
to attractiveness only at the within-participants level (τ(159) = −3.07,
\( p = .003; n = 174\).

Hormones and vocal attractiveness

Each hormone was entered into a separate model to predict vocal at-
tractiveness to men. Only progesterone significantly predicted attractive-
ness (τ(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 progester-
one and estradiol. The interaction was statistically significant
(τ(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 (τ(162) = −4.85,
\( p < .00005; n = 166\). We estimated a model using within- and
between-level predictors of estradiol and progesterone, and four interac-
tions (progesterone within × estradiol within, progesterone between ×
estriadiol between, progesterone between × estradiol within, progester-
one within × estradiol between). Of the interactions, only within-
participants estradiol by between–participants progesterone was statisti-
cally significant (τ(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
attractiveness (female-rated) for each photograph

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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 × 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 evolve...
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 advertised 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 ovulatory 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 mate’s ability to concentrate mate guarding around ovulation (Benshoof and Thornhill, 1979; Symons, 1979). There is evidence that women employ 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

![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.](image1)

![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.](image2)

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(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, men would have evolved not to invest in the offspring of their long-term mates. In fact, extra-pair maternity is modest in modern human populations, probably below 10% (Simmons et al., 2004).

These benefits (promoting investment from a partner-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 mates than from long-term mates (Provoet 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 Trivers'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 mates.

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.

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