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Exposure to perceived male rivals raises men's testosterone on fertile relative to nonfertile days of their partner's ovulatory cycle

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ABSTRACT

The challenge hypothesis posits that male testosterone levels increase in the presence of fertile females to facilitate mating and increase further in the presence of male rivals to facilitate male–male competition. This hypothesis has been supported in a number of nonhuman animal species. We conducted an experiment to test the challenge hypothesis in men. Thirty-four men were randomly assigned to view high-competitive or low-competitive male rivals at high and low fertility within their partner's ovulatory cycle (confirmed by luteinizing hormone tests). Testosterone was measured upon arrival to the lab and before and after the manipulation. Based on the challenge hypothesis, we predicted that a) men's baseline testosterone would be higher at high relative to low fertility within their partner's cycle, and b) men's testosterone would be higher in response to high-competitive rivals, but not in response to low-competitive rivals, at high relative to low fertility within their partner's cycle. Contrary to the first prediction, men's baseline testosterone levels did not differ across high and low fertility. However, consistent with the second prediction, men exposed to high-competitive rivals showed significantly higher post-test testosterone levels at high relative to low fertility, controlling for pre-test testosterone levels. Men exposed to low-competitive rivals showed no such pattern (though the fertility by competition condition interaction fell short of statistical significance). This preliminary support for the challenge hypothesis in men builds on a growing empirical literature suggesting that men possess mating adaptations sensitive to fertility cues emitted by their female partners.

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Introduction

In many species, the fleeting period of peak fertility that precedes and includes the day of ovulation is the only time when a female can conceive. Given the crucial significance of the fertile window for male reproductive success, a straightforward hypothesis is that males will evolve to detect any available cues of impending ovulation in females. In turn, males might respond to these cues with hormone changes that facilitate mating with fertile females and also facilitate competing with male rivals to prevent them from usurping potential reproductive opportunities.

The challenge hypothesis posits that male testosterone increases in the presence of fertile females and increases further in the presence of fertile females *and* male rivals (Wingfield et al., 1990). A substantial literature has supported the challenge hypothesis across a range of non-human animals, including species of fish (Hirschenhauser et al., 2004; Pankhurst and Barnett, 1993), lizards (Moore, 1986), and primates (Cavigelli and Pereira, 2000; Rose et al., 1972). For example, in one landmark study, male chimpanzees' testosterone levels increased in the presence of parous females in the fertile phase of their cycles (parous females are those who have successfully reproduced in the past; Muller and Wrangham, 2003). Further, this increase in testosterone was associated with increased rates of male–male aggression.

Human ovulation cues

Emerging evidence indicates that men can detect cues of ovulation (reviewed by Haselton and Gildersleeve, 2011). For example, men give higher attractiveness ratings to body odor samples (e.g., Doty et al., 1975; Gildersleeve et al., 2012) and vocal clips (Pipitone and Gallup, 2008; Puts et al., 2013) collected on high- as compared with low-fertility days of the cycle. Moreover, in one study, male patrons at gentlemen's clubs gave lap dancers larger tips on high- as compared with low-fertility days of the dancers' cycles (Miller et al., 2007). In the context of romantic relationships, in two studies, women reported that their male partners were more jealous and possessive on high- relative to low-fertility days of their cycles (Gangestad et al., 2002;

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Haselton and Gangestad, 2006; see also Pillsworth and Haselton, 2006b). In sum, a growing body of evidence indicates that there are cues of ovulation that women's male partners might detect and respond to with shifts in both attraction (facilitating mating) and mate guarding (facilitating male–male competition).

Testosterone and human mating

Consistent with the challenge hypothesis, several key pieces of evidence hint that testosterone might underlie shifts in men's responses to women and male rivals across the ovulatory cycle. First, there is support for the notion that men's testosterone increases in the presence of cues to potential reproductive opportunities in order to facilitate courtship behavior. Several studies have shown that men's testosterone levels increase in the presence of physically attractive women (Roney et al., 2003, 2007; van der Meij et al., 2008) and in response to higher ratios of women relative to men (Miller et al., 2012). In addition, in two studies, men who smelled body odor samples collected from women on high-fertility days of the cycle subsequently showed higher levels of testosterone than did men exposed to body odor samples collected from women on low-fertility days of the cycle (Miller and Maner, 2010; but see Roney and Simmons, 2012). Second, consistent with the notion that men's testosterone increases in the presence of male rivals in order to increase competitive motivation, several studies have shown that men's testosterone levels increase prior to competitive interactions with other men (e.g., tennis matches and judo competitions; Booth et al., 1989; Mazur et al., 1997; Salvador et al., 2003; Suay et al., 1999). In sum, consistent with the challenge hypothesis, current findings point to testosterone as a plausible mediator of changes in men's motivations and behaviors in response to fertile women and male rivals.

The current study

We devised a test of the challenge hypothesis involving romantic couples. The present study tested two predictions that follow from the challenge hypothesis: a) men's baseline testosterone will be higher at high relative to low fertility within their partner's cycle, and b) men's testosterone will be higher in response to high-competitive rivals (but not in response to low-competitive rivals) at high relative to low fertility within their partner's cycle.

Methods

Participants

Participants were thirty-five heterosexual romantic couples, the majority of whom were university students. Women reported regular menstrual cycles and had not used any form of hormonal contraception (e.g., birth control pills, Norplant, vaginal ring, birth control patch, Depo-Provera, Mirena IUD) in the three months prior to their participation. Couples were ineligible if the woman reported an average cycle length shorter than 24 or longer than 35 days or rated her confidence in her cycle length as less than seven on a 9-point scale (1 = not at all confident; 9 = very confident) and, in a follow-up question, reported that she was usually "off" by more than four days in her prediction of her next menstrual onset. The mean age of female participants was 20.51 years (*S.D.* = 3.01, range = 18–32). The sample of female participants was ethnically diverse; 37.1% self-identified as Asian, 20.0% as Caucasian, 11.4% as African-American, 5.7% as Hispanic, and 25.8% as "other" or multiple ethnicities. The mean age of male participants was 21.46 years (*S.D.* = 3.06, range = 18–33). The sample of male participants was also ethnically diverse; 48.6% self-identified as Caucasian, 25.7% as Asian, 8.6% as Hispanic, 2.9% as African-American, and 14.2% as "other" or multiple ethnicities. Mean relationship length was 16.25 months (*S.D.* = 11.58; range = 2–53 months).

Scheduling and LH testing

Prior to enrolling in the study, women completed an initial phone interview that included questions about their average cycle length, regularity, and past two dates of menstrual onset. We used this information to schedule each couple to complete two lab sessions—one session on an estimated high-fertility day of the female partner's cycle and one session on an estimated low-fertility day of her cycle. We used the reverse counting method to identify high- and low-fertility target days for scheduling these sessions (e.g., see Gangestad et al., 2002; Haselton and Gangestad, 2006). We assumed that ovulation occurs, on average, approximately 15 days prior to next menstrual onset (Dixon et al., 1980; Wilcox et al., 1995; but see Cole et al., 2009 for evidence suggesting that ovulation occurs slightly later in the cycle). Specifically, we scheduled couples to complete their high-fertility lab session 16 to 18 days prior to the female partner's predicted date of next menstrual onset (one to three days prior to her predicted date of ovulation) and their low-fertility session three to 10 days prior to her predicted date of next menstrual onset. Actuarial data indicate that these target days generally fall within the high- and low-fertility phases of the menstrual cycle, respectively (Wilcox et al., 2001). The order of couples' high- and low-fertility sessions depended on the female partner's position in the ovulatory cycle at the time of her initial phone interview. If a woman's next predicted menstrual onset was between four and 17 days away, we scheduled her and her partner to complete their low-fertility session first ($n = 16$). Otherwise, we scheduled them to complete their high-fertility session first ($n = 19$).

To verify that high-fertility sessions took place just prior to or on the day of ovulation (when fertility is highest), women completed a series of five ovulation tests in their predicted high-fertility window. All but three women completed ovulation tests from two days before to two days after their high-fertility session. Due to scheduling constraints, the remaining three women completed ovulation tests from one day before to three days after their high-fertility session. We removed the ovulation test wrappers so that participants could not easily identify the purpose of the tests. The tests measured luteinizing hormone (LH) in urine, which typically rises 24–48 h prior to ovulation (Testart and Frydman, 1982). In one study, LH tests were 97% accurate in verifying ovulation as detected by ultrasound (Guermandi et al., 2001). An LH surge was observed, on average, 0.60 days before the high-fertility session, ranging from three days before to two days after (*S.D.* = 1.59). Therefore, on average, high-fertility sessions took place approximately one day before ovulation.

Although LH tests are widely regarded as one of the most rigorous methods for determining women's position in the ovulatory cycle, recent evidence indicates that there is variation in the amplitude, duration, and number of LH peaks women experience (Direito et al., 2012). This variation might introduce error into estimates of the timing of ovulation based on LH tests alone. To increase confidence that high- and low-fertility sessions took place within the appropriate phases of the menstrual cycle, we followed up with women via phone or email to obtain a *confirmed* date of their next menstrual onset following completion of the study. Participants who completed their low-fertility session first also reported the date of menstrual onset between their low- and high-fertility sessions. If women could not be reached to confirm their date of menstrual onset following completion of the study, we *estimated* this date using their self-reported date of last menstrual onset and average cycle length ($n = 6$). Based on these dates, high-fertility sessions occurred, on average, 16.9 days before menstrual onset, ranging from 20 to 13 days prior to menstrual onset (*S.D.* = 1.73). Low-fertility sessions occurred, on average, 5.0 days before menstrual onset, ranging from 12 days prior to menstrual onset to two days after menstrual onset (*S.D.* = 1.73).

To be eligible for inclusion in the analyses, participants had to show evidence of an LH surge within two days prior to and three days after their high-fertility session and fall into one of two categories:

1) high-fertility session took place 20 to 13 days before *confirmed* next menstrual onset ($n = 29$), or 2) high-fertility session took place 19 to 15 days before *estimated* next menstrual onset ($n = 6$). We used a narrower, and thus more conservative, inclusion window for *estimated* than for *confirmed* dates of next menstrual onset in order to maintain a low likelihood of scheduling putative high-fertility sessions on true low-fertility days despite potential error in estimates of next menstrual onset (e.g., as could result from error in participants' reports of their last date of menstrual onset and average cycle length; Wegienka and Baird, 2005).

Of a larger sample who participated in the study ($N = 59$), nine women did not show evidence of an LH surge within two days prior to and three days following their high-fertility session. An additional 15 women completed high-fertility sessions outside of the predetermined windows specified above based on confirmed ($n = 13$) or estimated ($n = 2$) next menstrual onset. These 24 women were therefore removed from all analyses, resulting in a retention rate and sample size (59.3% and $N = 35$) comparable to previous studies using similar inclusion criteria (e.g., 58% and $N = 41$ in Gildersleeve et al., 2012).

High- and low-fertility sessions

Lab sessions lasted approximately 2 h. In order to minimize the effects of circadian fluctuations in testosterone levels, we sought to schedule sessions to begin in the afternoon between the hours of 12:00 p.m. and 7:00 p.m. (Axelsson et al., 2005). On average, sessions began at 3:30 p.m. Due to scheduling constraints, three high-fertility sessions and five low-fertility sessions were scheduled prior to 12:00 p.m. (time since waking is controlled for in analyses, as we describe below).

Partners reported to high- and low-fertility lab sessions together. Upon a couple's arrival, we directed male and female partners to different rooms. Once separated from his partner, the male partner immediately provided a saliva sample to be assayed for *baseline* testosterone. Following this saliva sample, male and female partners completed a questionnaire to collect demographic information and an additional questionnaire for a different study. After completing these questionnaires, we directed partners into the same room to engage in a couple interaction task. We designed this task to ensure that the male partner was exposed to a wide array of possible fertility cues in his female partner (i.e., scent, voice, facial attractiveness; Haselton and Gildersleeve, 2011). First, partners gave each other a 10-second hug while the researcher looked away. Following the hug, we informed the couple that they would have 15 min to complete several tasks in privacy. The first task involved choosing a song from an iTunes library of over 50 songs and "slow-dancing" to that song. At the end of their slow-dance, the male partner smelled his partner's neck. Finally, the couple used Photo Booth (a software application for taking photos and video with an iSight camera by Apple, Inc.) to take at least 10 "cute, couply photos" together. We provided the couple with an instruction sheet that guided them through each step of the couple interaction task and, to assure them of their privacy, informed them that they were not being videotaped.

Following the couple interaction task, we again directed male and female partners into separate rooms, where they remained for the rest of the study. While the female partner completed tasks for a different study, the male partner completed several tasks for the current study. Upon separation from his partner, the male partner provided a second saliva sample. This sample was analyzed to determine *pre-test* testosterone levels just prior to the intrasexual competition manipulation task.

For the intrasexual competition manipulation task, each man viewed a series of 10 male profiles adapted from those described by Li et al. (2010). Each profile included a photograph of a male student and a one-paragraph self-description purported to have been written by the student. To encourage participants to perceive the male students featured in the profiles as potential rivals, we told them that the students currently attended their university and that their partner would view photos of the same students and rate them for physical attractiveness. Also, to encourage participants to pay close attention to the profiles and as a manipulation check, we asked participants to rate each male profile on competitiveness, dominance, and physical attractiveness on a 9-point Likert scale (1, extremely [noncompetitive, non-dominant, unattractive]; 9, extremely [competitive, dominant, attractive]).

To manipulate the degree of the threat imposed by the putative male rivals, men were randomly assigned to view and rate either 10 "high-competitive" or 10 "low-competitive" profiles. High-competitive profiles featured photos of men pre-rated as attractive ($M = 5.45$, $S.D. = .79$, as rated on a 9-point Likert scale) whose self-descriptions indicated that they were relatively high in competitiveness (e.g., "I am a natural leader."). In contrast, low-competitive profiles featured photos of men pre-rated as average-looking ($M = 3.99$, $S.D. = .79$; $F(1,12) = 10.52$, $p = .01$) whose self-descriptions indicated that they were relatively low in competitiveness (e.g., ". . . the world has enough leaders . . . we need some people who are good at following. I guess I was made to be one of those people."). Each man rated the same 10 profiles at his high- and low-fertility session.

Fifteen minutes after completing the intrasexual competition manipulation task, men provided a third and final saliva sample. This sample was analyzed to determine *post-test* testosterone levels. Past research has shown that a fifteen-minute waiting period is sufficient for changes in testosterone to register in saliva (Riad-Fahmy et al., 1987).

Hormone assays

Saliva samples were frozen at −80 °C (Granger et al., 2004) until shipped on dry ice for overnight delivery to the Endocrine Core Lab at the California Regional Primate Research Center, Davis, CA. Prior to assay, samples were centrifuged at 3000 rpm for 20 min to separate the aqueous component from mucins and other suspended particles. Salivary concentrations of testosterone were estimated in duplicate using the Expanded Range Salivary Testosterone EIA Kit (Salimetrics LLC, State College, PA). Intra- and inter-assay coefficients of variation were 4.35% and 4.29%, respectively, and assay sensitivity was 1.0 pg/mL.

Participants with testosterone values greater than three standard deviations from the mean were excluded from all analyses (Roney et al., 2007). There were three such outliers.

Data analytic strategy

The study design involved high- and low-fertility observations nested within participants. To account for the nonindependence of these nested observations, we ran multilevel models with observations at Level 1 and participants at Level 2. Analyses were carried out using the HLM2 module in HLM 7.0 (Raudenbush et al., 2011). We estimated fixed effects and variance components using restricted maximum likelihood estimation procedures, which tend to reduce downward bias in variance components as compared with full maximum likelihood estimation procedures (O'Connell and McCoach, 2008). The analyses included several dummy-coded dichotomous variables: *Fertility*, an observation-level predictor coded as 0 for high fertility and 1 for low fertility; *SessionNumber*, an observation-level predictor coded as 0 for first session and 1 for second session; and *Condition*, a participant-level predictor coded as 0 for high competition condition and 1 for low competition condition.

We first present results from the model testing whether men's baseline testosterone was higher in their partner's high-fertility phase than in her low-fertility phase and then present results from the model testing whether men's testosterone was higher in response to high-competitive rivals (but not in response to low-competitive rivals) at high relative to low fertility within their partner's cycle. All analyses controlled for participants' waking time (*WakeUp*; grand-mean

centered) in order to account for testosterone's diurnal rhythm (Axelsson et al., 2005). We also tested and controlled for effects of *SessionNumber*. The second analysis controlled for pre-test levels of testosterone (*PreT*; grand-mean centered). Due to researcher error, one participant was assigned to different experimental conditions at his high- and low-fertility sessions and therefore was removed from the second analysis. Thus, *N*s differ across the two analyses.

We report the proportional reduction in variance (*PRV*) as a measure of effect size (Raudenbush and Bryk, 2002; Singer and Willett, 2003). *PRV* is the proportion of residual variance at a given level of the model that is reduced by adding a predictor to that level of the model (i.e., how much variance is explained).

Results

Baseline testosterone at high and low fertility within the female partner's cycle

The first prediction derived from the challenge hypothesis is that men's baseline testosterone will be higher on high- as compared with low-fertility days of their partner's cycle. Results failed to support this hypothesis. Men's baseline testosterone levels did not differ significantly across high and low fertility, $t(29) = -.12$, $p = .91$. However, as shown in Fig. 1, men generally had higher baseline testosterone levels at their first session than at their second session, regardless of their partner's fertility status, $t(29) = 2.87$, $p = .01$, $PRV = 17.5\%$.

Competition manipulation check

We expected men to rate the high-competitive male rivals as more competitive, dominant, and physically attractive than we expected men to rate the low-competitive male rivals. As predicted, men randomly assigned to view high-competitive rivals rated them as more competitive ($M = 5.98$, *S.D.* $= 1.13$) than did men randomly assigned to view low-competitive rivals ($M = 4.08$, *S.D.* $= 1.06$), $t(32) = -4.55$, $p < .001$. Likewise, men who viewed high-competitive rivals rated them as more dominant ($M = 5.98$, *S.D.* $= 1.23$) than did men who viewed low-competitive rivals ($M = 3.57$, *S.D.* $= 1.02$), $t(32) = -5.44$, $p < .001$. However, men who viewed high-competitive rivals did not rate them as significantly more physically attractive ($M = 5.65$, *S.D.* $= 1.08$) than did men who viewed low-competitive rivals ($M = 5.09$, *S.D.* $= .53$), $t(32) = -1.56$, $p = .13$. Therefore, the manipulation check indicated that we were successful in manipulating participants' perceptions of the competitiveness and dominance, but not necessarily the physical attractiveness, of putative male rivals. An exploratory analysis revealed that men's ratings of the competitiveness, dominance, and physical attractiveness of putative male rivals did not differ across high- and low-fertility sessions (all *p*s > .42).

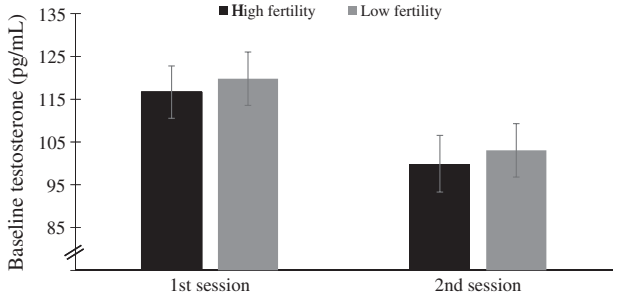
Testosterone response to high-competitive vs. low-competitive male rivals across the cycle

The second prediction derived from the challenge hypothesis is that men will respond to high-competitive rivals (but not to low-competitive rivals) with higher testosterone levels at high relative to low fertility within their partner's cycle. We first included session number and all higher-order interactions with session number (*SessionNumber* × *Condition*, *SessionNumber* × *Fertility*, *SessionNumber* × *WakeUp*, *SessionNumber* × *PreT*) in the model but found no statistically significant effects involving session number. We verified that dropping interactions from the model did not significantly worsen the model's fit. Likelihood ratio tests indicated the models were not significantly different from one another (all *p*s > .10). Therefore, we removed session number and all interactions with session number from the final model reported below. Doing so negligibly changed regression coefficients and did not change the pattern of statistical significance.

Although the predicted fertility (high vs. low fertility) by condition (high vs. low competition) interaction did not reach statistical significance, $t(26) = 1.09$, $p = .29$, analyses revealed the predicted pattern of simple effects. Among men exposed to high-competitive male rivals, the simple effect of fertility on testosterone was statistically significant, $t(26) = -2.12$, $p = .04$, $PRV = 24.3\%$. For these men, post-test testosterone was higher (controlling for pre-test testosterone) at high relative to low fertility within their partner's cycle. In contrast, among men exposed to low-competitive male rivals, the simple effect of fertility on testosterone was not statistically significant, $t(26) = -1.01$, $p = .85$. For these men, post-test testosterone did not substantially differ (controlling for pre-test testosterone) between high and low fertility within their partner's cycle (see Fig. 2).

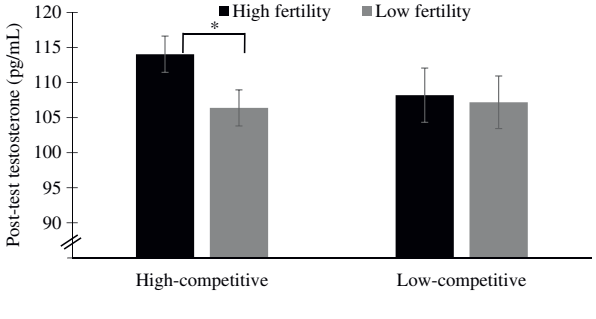
Follow-up analyses

It is possible that men are more responsive to *any* potential male rivals when their partners are at high relative to low fertility. Therefore, we ran follow-up analyses to examine whether there was an effect of fertility on men's testosterone responses to male rivals after removing competition condition from the model. As with previous models, we first included session number and higher-order interactions with session number in the model but found no statistically significant effects involving session number. Therefore, we removed session number and all interactions with session number from the final model reported below. There was a marginally significant main effect of fertility on men's testosterone responses to male rivals. Controlling for pre-test testosterone levels, men exhibited higher post-test testosterone levels at high fertility relative to low fertility, $t(27) = -1.80$, $p = .08$. However, we interpret this marginally significant main effect of fertility with caution because it is in the presence of a potential interaction. The pattern of results shown in Fig. 2 suggests that the marginal main effect of fertility is driven by men in the high-competitive condition, rather



Note. Error bars represent standard errors.

Fig. 1. Baseline testosterone as a function of session number and fertility status (main effect of session number, p = .01).



Note. * $p < .05$. Error bars represent standard errors.

Fig. 2. Marginal means of post-test testosterone levels as a function of fertility status and competition condition, controlling for pre-test testosterone levels.

than men in the low-competitive condition. Lastly, men's testosterone levels did not differ in response to viewing high-competitive versus low-competitive male rivals, $t(32) = -.72$, $p = .48$.

Discussion

Summary

This study provides the first direct test of the challenge hypothesis in the context of a romantic partnership. Contrary to the first prediction derived from the challenge hypothesis, we did not find that men's baseline testosterone levels were higher at high relative to low fertility within their partner's ovulatory cycle. However, consistent with the second prediction derived from the challenge hypothesis, men exposed to high-competitive male rivals exhibited higher testosterone levels in response to these rivals at high relative to low fertility within their partner's cycle (controlling for pre-test testosterone levels), whereas men exposed to low-competitive rivals did not show this pattern. This finding is consistent with a large nonhuman literature showing that male testosterone increases in the presence of fertile females and increases further in the presence of fertile females *and* male rivals (e.g., chimpanzees, Muller and Wrangham, 2003; monogamous avian species, Wingfield et al., 1990). It is also consistent with earlier studies in humans documenting an increase in men's testosterone in response to possible mating opportunities (Roney et al., 2003, 2007; van der Meij et al., 2008) and in competitive contexts (e.g., Booth et al., 1989).

Several studies suggest that men possess psychological adaptations involved in mating that are sensitive to their romantic partner's current fertility status and that lead to increases in men's partner-directed possessiveness and jealousy at high relative to low fertility within their partner's cycle (Gangestad et al., 2002; Haselton and Gangestad, 2006; see also Pillsworth and Haselton, 2006b). However, these studies have relied on *women's reports* of their male partner's behaviors. These reports provide indirect measures of changes in male behavior and are open to alternative explanations. For example, it is possible that the findings reflect changes in women's *perceptions* of their male partner, rather than actual changes in male partner's behavior. The present study importantly advances this literature by directly assessing changes in men across their female partner's ovulatory cycle. The evidence of a hormone shift in men in response to a competitive threat is perhaps the best evidence to date that men are sensitive to and might behaviorally respond to cues of ovulation in their female partners. This finding is consistent with preliminary evidence from an earlier study examining men's perception of other men's dominance as a function of their female romantic partner's position in the cycle. Men tested in their partner's high-fertility phase, according to their reports of their partner's last date of menstrual onset, perceived male faces pre-rated as highly dominant as even more dominant than did men tested in their partner's putative low-fertility phase (Burriss and Little, 2006).

Although there is strong evidence in the nonhuman literature (e.g., Muller and Wrangham, 2003) and suggestive evidence in humans (Miller and Maner, 2010; but see Roney and Simmons, 2012), we did not observe an increase in men's baseline testosterone at high relative to low fertility within their female partner's ovulatory cycle. The lack of this effect (if the effect is truly absent) could reflect differences between the mating patterns of humans as compared with the species on which previous tests of the challenge hypothesis have focused. For example, whereas previous tests of the challenge hypothesis have focused on seasonal breeders (e.g., Wingfield et al., 1990), humans breed year-round and maintain testosterone levels high enough for reproduction throughout the year (see Archer, 2006). In addition, humans have sex throughout the ovulatory cycle (Bullivant et al., 2004). Furthermore, previous tests of the challenge hypothesis have focused on polygynous species with minimal paternal care (Muller and Wrangham, 2003). In contrast to these species, human mating systems involve high rates of pairbonding and paternal care (Flinn and Low, 1986; Geary, 2000; Marlowe, 2003; Pillsworth and Haselton, 2006a), and past research has shown that baseline testosterone levels are lower among men in committed relationships than among single men (Gettler et al., 2011; Gray et al., 2002). One or more of these factors could explain the lack of a clear pattern of change in men's baseline testosterone across their female partner's ovulatory cycle, although further research is certainly warranted.

Unexpectedly, men's testosterone levels were higher at their first lab session relative to their second lab session. A possible explanation of this effect is that men experienced feelings of uncertainty in response to the novel lab situation at their first session and responded to these feelings with threat or challenge appraisals (Lazarus and Folkman, 1984), which have been associated with increases in men's testosterone (e.g., Salvador et al., 2003). We could not locate studies reporting differences in baseline testosterone across testing sessions (but see Wallen and Rupp, 2010 for a related effect involving interactions of session order and women's cycle phase in studies of sexuality). However, our study is different from others examining baseline testosterone across testing sessions because men arrived at the laboratory with their romantic partners. Therefore, men's threat or challenge appraisals could have been present at their first session because these men were accompanied by their female partners, and eased at their second due to the familiarity of the laboratory situation. Whatever explains the difference across sessions, researchers should be mindful that testosterone levels upon men's arrival to novel laboratory situations might not reflect true "baselines" and that declines in testosterone over the course of several lab sessions could reflect men's psychological arousal, uncertainty, or some other effect of the lab situation dissipating over time.

Strengths

A key strength of the present study is that we used rigorous methods to increase confidence that participants included in the analyses completed sessions on true high- and low-fertility days of the ovulatory cycle. Given these methods, the present study should provide relatively precise estimates of ovulatory cycle effects as compared with studies that rely solely on retrospectively recalled dates of menstrual onset to estimate current cycle position (which are frequently "off" by three or more days; Wegienka and Baird, 2005) or otherwise use less rigorous methods.

An additional strength is that couples engaged in an interaction task shortly before the competition manipulation task. This novel task, which we designed for the purpose of this study, ensured that men were exposed to a variety of potential cues of ovulation in their female partner (e.g., appearance, voice, scent, etc.). This task involved positive interactions between partners. Future studies could examine whether negative interactions between partners (e.g., having partners discuss a recent conflict) further increase men's testosterone responses to manipulations of intrasexual competition.

A final strength is that we tested the challenge hypothesis in the context of a romantic partnership. Ancestral men probably encountered the majority of their reproductive opportunities in the context of pairbonded relationships, rather than in casual sexual encounters (Pillsworth and Haselton, 2006a). Furthermore, men might be better able to detect fertility cues in romantic partners, with whom frequent close contact provides a potential opportunity to detect subtle day-to-day variation in scent, voice, and other changes (see Haselton and Gildersleeve, 2011). Taken together, these considerations suggest that the predictions of the challenge hypothesis for humans are especially pertinent in the context of romantic partnerships.

Limitations

Using longitudinal cycle methods is challenging, requiring close monitoring of participants over time (e.g., to ensure compliance with the LH testing protocol), scheduling participants to complete multiple

laboratory sessions on specific days of the ovulatory cycle, and, ultimately, excluding participants who do not meet the inclusion criteria outlined above. In this study, these challenges were compounded by the requirement that both members of a couple complete multiple lab sessions together. These issues constrained the sample size we could achieve. Indeed, a key limitation of this study is its relatively small sample size, which could account for the predicted fertility by competition condition interaction falling short of statistical significance.

In sum, although this study is limited by its sample size, this limitation reflects a conscious decision to trade off a larger sample size in order to achieve greater methodological precision.

Conclusions

This study represents the first direct test of the challenge hypothesis in the context of a romantic partnership. Results provide preliminary evidence that men respond to competitive male rivals with higher testosterone at high fertility as compared with low fertility within their female partner's ovulatory cycle. Men therefore appear to experience higher testosterone in precisely those conditions in which, historically, a failure to successfully compete with male rivals could have been most reproductively costly (i.e., when a potential reproductive opportunity with a female partner could be lost to a male rival). This study contributes to a growing theoretical and empirical literature suggesting that evolutionary forces might have produced male psychological adaptations that are sensitive to cues of their partner's fertility within the cycle (see Haselton and Gildersleeve, 2011). This study also adds to the growing literature on human social endocrinology by providing additional evidence that men's hormone levels are responsive to social context and likely play a role in courtship and competitive behavior (e.g., Miller et al., 2012). In sum, this work supports the idea that ancient adaptations shared by a wide range of nonhuman species—from birds (e.g., Wingfield et al., 1990) to other primates (e.g., Muller and Wrangham, 2003)—are also present, at least in some form, in modern humans. The existence of such adaptations has important implications for understanding ultimate and proximate mechanisms that contribute to systematic day-to-day variation in human social behavior.

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