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Average ovarian hormone levels, rather than daily values and their fluctuations, are related to facial preferences among women

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ABSTRACT

Hormones are of crucial importance for human behavior. Cyclical changes of ovarian hormones throughout women's menstrual cycle are suggested to underlie fluctuation in masculinity preference for both faces and bodies. In this study we tested this hypothesis based on daily measurements of estradiol and progesterone throughout menstrual cycle, and multiple measurements of women's preference towards masculinity of faces and bodies of men. We expected that due to a large variation among daily hormonal levels we would not observe a direct effect of daily hormone levels, but rather that average levels of ovarian hormones throughout the cycle (a reliable marker of a probability of conception) would better predict women's preferences. We found a negative relationship between average progesterone levels and facial masculinity preference, but only among women who were in long-term relationships. There was no relationship between facial masculinity preference and either of the estradiol or progesterone daily levels. Similarly, only average levels of hormones were significantly related to facial symmetry preference. For women who were in relationships estradiol was positively related to symmetry preference, while for single women this relationship was opposite. For body masculinity preference there were no significant relationships with neither averaged nor daily hormonal levels. Taken together, our results further suggest that overall cycle levels of ovarian hormones (averaged for a cycle) are better predictors of facial masculinity and symmetry preference than daily levels assessed during preferences' tests. Importantly, including information about relationship status in the investigations of hormonal bases of preferences is crucial.

1. Introduction

Hormones play an important role in human behavior and mating preferences. Women's levels of ovarian hormones (mainly progesterone and estradiol) are not constant, but change during their lifetime (being highest during peak reproductive years) and fluctuate throughout the menstrual cycle. Levels of hormones are under influence of many factors (Ellison, 2001; Jasienska, 2013), including genetics (Jasienska et al., 2006a; Jasienska et al., 2015) developmental conditions (Jasienska et al., 2006b; Jasienska et al., 2006c; Nunez-de la Mora et al., 2007), body size and shape (Jasienska et al., 2004; Ziomkiewicz et al., 2008) and lifestyle during adulthood (Jasienska & Ellison, 2004; Jasienska et al., 2006d). In fully functioning cycles, during the initial (follicular) phase of the cycle, a mature follicle is produced, which then releases an egg in a process called ovulation, allowing fertilization to occur. By the end of the follicular phase the ovary secretes high doses of estradiol. Mid-cycle drop in estradiol is a reasonable estimate of the day of ovulation (Lipson & Ellison, 1996). Following the ovulation, levels of estradiol still fluctuate, while levels of progesterone rise, preparing the endometrium for possible implantation of the fertilized egg. If implantation does not occur or if the embryo is not able to send hormonal signals to maternal organism, progesterone levels drop and menstrual bleeding starts.

It is hypothesized that women's preferences also fluctuate throughout the menstrual cycle due to hormonal changes, ovulation and, consequently, conception probability. “The ovulatory shift” hypothesis states that women around ovulation (when estradiol levels are high and progesterone levels are low) prefer partners with genes beneficial for their offspring, rather than partners providing high paternal investment. By contrast, during the low conception probability phase (i.e. luteal phase) women should prefer partners providing paternal investment and parenting skills. Therefore, women should express (*i*) increased masculinity preference around ovulation when searching for good quality in partner, and (*ii*) lowered masculinity preference in non-

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fertile phase when body is preparing for pregnancy and preference shifts towards social or economic support (Johnston et al., 2001). A number of articles testing this hypothesis have been published (for meta-analyses see (Gildersleeve et al., 2014; Wood et al., 2014), see also (Gildersleeve et al., 2013)), however a common agreement on the occurrence of such shifts is still missing, as some studies found shifts in masculinity preferences (Penton-Voak et al., 1999; Puts, 2005; Little et al., 2008), while other did not (Harris, 2012; Marcinkowska et al., 2016; Peters et al., 2009).

One of cues to good genes is exaggerated sexual dimorphism. Masculinity was suggested to be related to robust immune system, overall good health (Rhodes et al., 2003), strength (Fink et al., 2007), dominance (Geniole et al., 2015), increased reproductive success (Apicella et al., 2007) and men's anatomy (and related to this masculinity) shows evidence of adaptive design for contest competition (Puts, 2005). It has also been found that muscular men obtain higher attractiveness rating, than lean or heavily-set men (Dixson et al., 2014).

Nevertheless, exaggerated sexual dimorphism in men is also related to negative traits, such as dominant demeanor and aggressive behavior. Moreover, highly masculine men were found to be more sexually open (Boothroyd et al., 2008). Hence, the overall women's preference for masculinity is a trade-off between obtaining genetically “high quality” partner and paternal investment. Based on this trade-off in preferences for exaggerated sexual dimorphism in men it has been suggested that women's preferences should vary accordingly to the conception probability. In this study we test this hypothesis, by monitoring relationship of women's preferences for both facial and body masculinity to their daily ovarian hormones levels during multiple meetings throughout menstrual cycle.

We hypothesize that due to large variation in women's daily hormonal levels (Jasienska & Jasienski, 2008) and high irregularity of the natural, healthy cycles we would not observe a direct effect of hormone levels during a particular day of the cycle on the masculinity preference during the same day. Nonetheless, we propose that average levels of hormones throughout the cycle (a reliable marker of a probability of conception) should be a better predictor of women's preferences. We also hypothesize that in ovulatory cycles effects of hormone levels on preferences should be stronger than in non-ovulatory cycles (Roney & Simmons, 2008).

2. Methods

2.1. Participants

Women from the region of Malopolska in Poland were recruited personally or via mailing lists. Inclusion criteria for participation in the study were as follows: regular menstrual cycles (difference between lengths of consecutive cycles less than ± 5 days), no diabetes, no medically diagnosed problems of reproductive system, and no pregnancy, breast-feeding or use of hormonal contraception for a time period of at least 3 months prior to participation in the study. All participants provided a written consent. Out of 110 recruited participants 105 completed the study and 102 provided daily saliva samples collected throughout the entire menstrual cycle. All women completed a socio-demographic survey providing information on their age, past use of hormonal contraception, menstrual cycles, and reproductive (pregnancies and lactation) history. Participants also filled questionnaires on sexual orientation (on a 7 point Kinsey Scale, (Kinsey et al., 1948/1998)), relationship status and relationship satisfaction, partner's attractiveness and masculinity (on a 7 point Likert scale). Participants were between 21 and 37 years of age (Mean = 30, SD = 4.7), and 71 of them were in a long-term relationship (defined as longer than 6 months). Eight participants scored 4 or higher on Kinsey Sexual Orientation Scale (self-defined themselves as bi- or homo-sexual and, because the sexual orientation influences sexual preferences (Glassenberg et al., 2010), were excluded from further analysis. Women, on average, judged their partners to be attractive (Mean = 5.86, SD = 1.20) and masculine (Mean = 5.99, SD = 0.85), and overall relationship satisfaction was high (Mean = 5.92, SD = 1.20).

2.2. Procedure

All participants were asked to collect daily saliva samples each morning throughout one full menstrual cycle. All women were instructed about proper saliva collection and storing, and received written instructions and a set of 2 ml centrifuge tubes. Participants were also given 10 Lutenizing Hormone (LH) Ovulation Kits together with urine cups and written instruction. LH tests were individually conducted by each participant from 10th until 20th day of the cycle (days of the cycle with highest probability of ovulation occurrence) or until obtaining a positive result.

To account for hormonal variation throughout the cycle and possible caveats mentioned in previous studies, multiple testing sessions in different cycle phases were scheduled for each participant. All women attended 3 meetings throughout the cycle: the first meeting took place in early follicular phase (between 2nd and 8th day), the second meeting around ovulation (not later than 72 h after the positive result of ovulation detected by LH test or on 20th day of the cycle if there was no positive result) and the third meeting about one week after the ovulation (in mid-luteal phase).

During each meeting women chose via 2-alternative forced choice participants from 10 pairs of male faces varying in masculinity and from 3 pairs of male torsos. Visual stimuli constituted of 20 facial pictures of Caucasian men (aged 18–24). All faces were transformed with the PSYCHOMORPH Programme (Tiddeman et al., 2001) on a femininity–masculinity scale (Little et al., 2011). Two versions of each of the 20 original faces were created by adding or subtracting 50% of the linear difference between age matched 40 adult-male composite and a 40 adult-female composite. Thanks to such technique of facial transformation two stimuli pictures showed simultaneously to participants differed only in sexually dimorphic cues of face shape (DeBruine et al., 2010). Same technique was used for creation of torso visual stimuli (Little et al., 2007; Little et al., 2011). Visual stimuli were presented in a randomized order and location (left- or right-hand side of the screen).

2.3. Hormonal samples and measurements

Per each participant, hormonal profiles of 17-β-estradiol (E2) and progesterone (P) were obtained based on 15 daily measurements centered around the ovulation for E2 and the last 14 days of the cycle for P. Saliva samples were collected at least 30 min after eating, drinking or smoking and were frozen immediately after collection. All saliva samples were transported in portable freezers to the laboratory. Hormonal measurements were conducted using commercially available hormonal assays of DRG International Incl.: Elisa plates SLV4188 for 17-β-estradiol (sensitivity: 0.4 pg/ml, standard range: 1–100 pg/ml) and SLV3140 for 17-α-hydroxy-progesterone (sensitivity: 2.5 pg/ml, standard range: 10–5000 pg/ml). To achieve high standard of the measurements, all hormonal assays were conducted in duplicates. Quality of measurements was controlled for each plate separately by including (in duplicates) samples of known concentrations (“pools”) of P and E2 (in total these control measurements consisted of 19 pools per plate). Average levels of P for a cycle ranged from 17.2 to 416.3 pg/ml (mean = 113.53 pg/ml, SD = 70.88) and average levels of E2 ranged from 0.9 to 32.1 pg/ml (mean = 7.96 pg/ml, SD = 5.20). For P, inter-assay CV was 14.1%, and intra-assay was 4.9% and for E2, inter-assay CV was 10.01%, and intra-assay was 7.5%.

2.4. Statistical analysis

Our response variable was the preference for the masculinity (0, 1) or for the symmetry of the face (0, 1) of each participant, for each item and for each meeting. Due to binomial nature of the data, mixed multiple logistic regressions were used for analysis. Explanatory variables for these models included the daily hormonal levels: estradiol (E2), progesterone (P) and E2/P ratio, averaged hormone levels (counted as 15 days centered around ovulation for E2 and as last 14 of the cycle for P) and relationship status. We performed the analyses in two steps: first with all participants, then narrowing the sample to only ovulatory cycles. All predictors were considered fixed-effects variables. Due to repeated measurements for each individual, we included the participant's and item's identities as random effects. As meeting sessions were nested within participants, we also enter it as random effect. To test if the daily hormonal levels differed among the three days of the meeting, we used a linear mixed-effect model with participant's identity as random effects. To perform the analyses, hormone levels were centered on subject-mean and we assessed for multicollinearity among them using the Variation Inflation Factor (VIF). Means and odd-ratios (OR) were computed, with the 95% confidence intervals. Inter- and intra-assay coefficients of variability (CVs) were computed for both hormones (Schultheiss & Stanton, 2009). All data analyses were done using R version 3.4.3.

3. Results

3.1. Menstrual cycle and hormonal levels

Average length of the menstrual cycle was 28.2 days (SD = 2.95). Ovulation (based on the positive result of the LH ovulation kit) was observed in 72% of the cycles ($N = 68$), and occurred between −8 and −22 days before the onset of the next menses (mean = −14.5, SD = 2.34), 81% of participants attended the second (around ovulatory) meeting up to 48 h after the positive results of the ovulation test (64% in the first 24 h after the positive result). The average of daily hormonal levels (P, E2 and ratio) are summarized in Table 1 (for more details see Electronic Supplementary Material, ESM 1 Tables S1–S3, Figs. S1–S5). The daily P levels were significantly different among the three meetings ($F_{2,\ 3752} = 503$, $p < 0.0001$, $R^2 = 0.65$), but did not differ between single and paired participants ($F_{1,\ 89} = 0.23$, $p = 0.63$). The daily E2 levels were significantly different among the three meetings ($F_{2,\ 3752} = 31.7$, $p < 0.0001$, $R^2 = 0.64$, Table 1), but did not differ between single and paired participants ($F_{1,\ 89} = 2.56$, $p = 0.11$). The daily E2/P ratio levels were significantly different among the three meetings ($F_{2,\ 3752} = 29.1$, $p < 0.0001$, $R^2 = 0.37$), but did not differ between single and paired participants ($F_{1,\ 89} = 1.21$, $p = 0.27$). Average levels of ratio (E2/P) for a cycle ranged from 0.005 to 0.48 (mean = 0.12, SD = 0.10).

3.2. Facial masculinity preferences, relationship status and hormonal levels

Women judged masculinized male faces as less attractive than the feminized ones on average (0.36 [95% CI 0.34–0.37], $p < 0.0001$), and for all three meetings throughout the menstrual cycle (0.32 [95% CI 0.29–0.36] for the first meeting, 0.36 [95% CI 0.34–0.37] for the second and 0.34 [95% CI 0.31–0.38] for the last, all $p < 0.0001$).

Table 1
Daily hormone levels (mean, SD) for the three meetings.

	1st meeting	2nd meeting	3rd meeting
P level (pg/ml)	53.4 (50.6)	87.5 (68.6)	100.6 (73.7)
E2 level (pg/ml)	6.7 (5.8)	7.2 (4.8)	6.6 (5.1)
E2/p ratio	0.14 (0.11)	0.11 (0.10)	0.09 (0.09)

Facial masculinity preferences were significantly different among the three meetings ($Chi^2 = 8.70$, df = 2, $p = 0.013$; Fig. S6), however, post-hoc analyses showed that this effect was significant only between the first and the second meeting, Table S4). There was significant difference in face masculinity preference between ovulatory (0.32 [95% CI 0.29–0.34]) and non-ovulatory cycles (0.46 [95% CI 0.42–0.51]; $Chi^2 = 7.73$, df = 1, $p = 0.005$; OR = 0.39 [95% CI 0.20–0.76]; Fig. S7). When the relationship status was included in the model, there was a trend for partnered women to have almost 2 times stronger preferences for feminine faces than for single women (OR = 1.96 [95% CI 0.96–4]; $Chi^2 = 3.50$, df = 1, $p = 0.061$; Fig. S8).

For facial masculinity preferences, our analysis showed a significant interaction between average cycle P level and masculinity preference, when the relationship status was included in the model ($Chi^2 = 5.59$, df = 1, $p = 0.018$; Fig. 2). In partnered women, with the increase in average progesterone levels masculinity preferences decreased (OR = 0.57 [95% CI 0.20–1.62]), whereas for single women, an increase in progesterone was related to increase in masculinity preferences (OR = 2.34 [95% CI 0.87–6.29]). No other effects with averaged hormonal levels on the facial masculinity preference were significant (Table S11). We did not find a general effect of daily P levels ($Chi^2 = 2.58$, df = 1, $p = 0.11$; OR = 1.24 [95% CI 0.95–1.62]), daily E2 levels ($Chi^2 = 2.29$, df = 1, $p = 0.13$; OR = 1.14 [95% CI 0.92–1.41]), nor E2/P levels ($Chi^2 = 0.01$, df = 1, $p = 0.95$, Table S5). The same pattern of results was found when analyzing data only for ovulatory cycles (Table S6). All results of analyses on daily hormonal levels (Fig. 1) remained statistically non-significant when analyses were conducted for each day of the meeting separately (except with the daily P levels at second meeting (OR = 2.72 [95% CI 1.16–6.38], Tables S7–S9). All interactions involving relationship status and daily hormonal levels were non-significant (all $p > 0.18$, Table S10).

3.3. Facial symmetry preferences, relationship status and hormonal levels

Female participants judged more symmetric faces as more attractive than the less symmetric ones on the average (0.55 [95% CI 0.51–0.57], $p = 0.004$), but this difference was significant only for the last of the three meetings of the menstrual cycle (0.57 [95% CI 0.51–0.63], $p = 0.011$; Fig. S9). Nonetheless, symmetry preference was not significantly different among the three meetings ($Chi^2 = 1.98$, df = 2, $p = 0.37$). There was no difference in face symmetry preference between ovulatory (0.55 [95% CI 0.52–0.59]) and non-ovulatory cycles (0.52 [95% CI 0.46–0.58]; $Chi^2 = 1.09$, df = 1, $p = 0.29$). When the relationship status was included in the model, results showed effect of the interaction between the day of the meeting and relationship on symmetry preference ($Chi^2 = 6.64$, df = 2, $p = 0.036$, Fig. S11), however post-hoc analyses revealed no statistically significant differences (Table S12).

Regarding the symmetry preferences, our analysis showed a significant interaction between average cycle E2 level and relationship status on the symmetry preferences ($Chi^2 = 4.29$, df = 1, $p = 0.038$; Fig. 3). In single women, the increase in average cycle E2 level decreased symmetry preferences (OR = 0.70 [95% CI 0.56–1.06]), whereas for partnered women increase of E2 was related to increase in symmetry preferences (OR = 1.18 [95% CI 0.83–1.67]). No other effects with averaged hormonal levels on the symmetry preference were significant (Table S18). We did not find a general effect of daily P levels ($Chi^2 = 0.08$, df = 1, $p = 0.79$), daily E2 levels ($Chi^2 = 0.51$, df = 1, $p = 0.47$), nor of the E2/P levels ($Chi^2 = 1.69$, df = 1, $p = 0.19$, Table S13). All results of analyses on daily hormonal levels remained statistically non-significant when analyses were conducted for each day of the meeting separately and when only data for ovulatory cycles were analysed (Table S14–S16, Table S19). All interactions involving relationship status and daily hormonal levels were non-significant (all $p > 0.07$; Table S17).

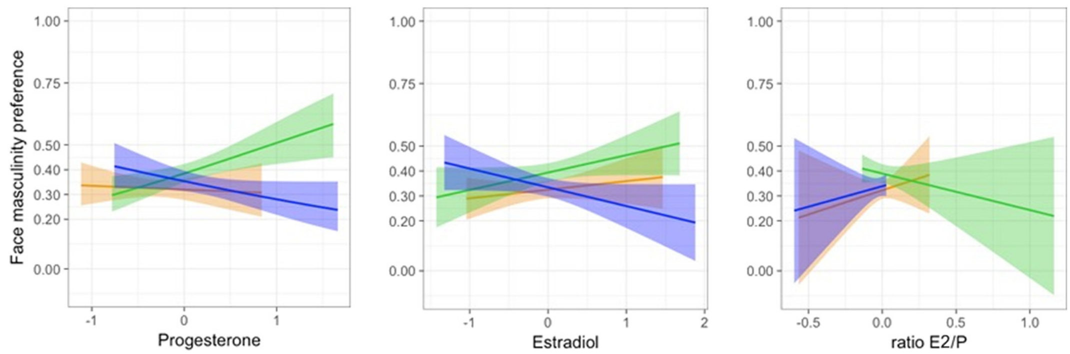

Fig. 1. Effect of hormone levels on the facial masculinity preference during three meetings (1st meeting in orange, 2nd meeting in green and 3rd meeting in blue). Lines and shaded areas represent regression lines and confident intervals for preference. To perform the analyses, hormone levels were centered on subject-mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

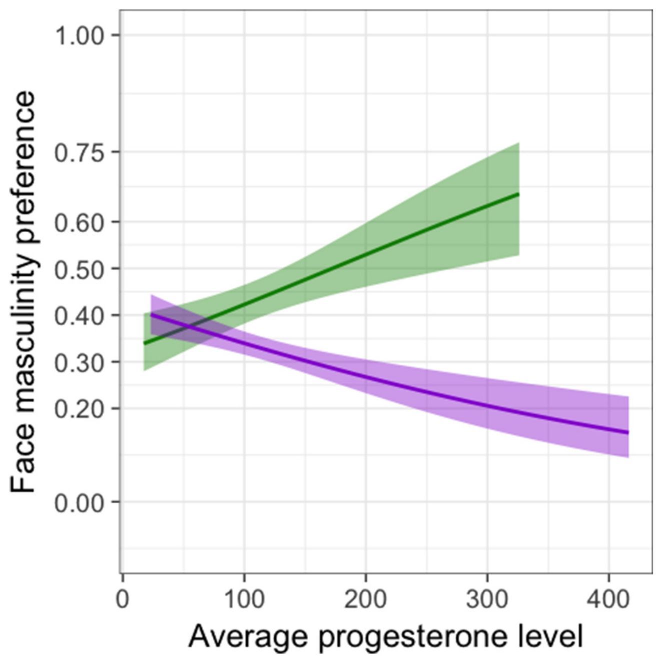

Fig. 2. Interaction between average progesterone level and facial masculinity preference (green: single participants; violet: paired women). Lines and shaded areas represent regression lines and confident interval for preference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

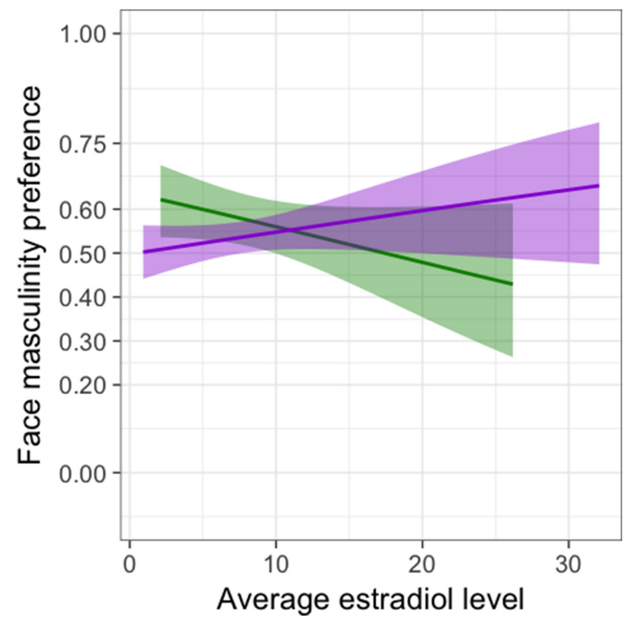

Fig. 3. Interaction between average estradiol and facial symmetry preference (green: single participants; violet: paired women). Lines and shaded areas represent regression lines and confident intervals for preference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Body masculinity preferences, relationship status and hormonal levels

In general, women preferred masculinized torsos (0.56 [95% CI 0.53–0.60], $p < 0.0002$) and this preference tended to be expressed regardless of the menstrual phase during which preference tests were conducted (0.56 [95% CI 0.49–0.62], $p = 0.074$ for the first meeting, 0.56 [95% CI 0.50–0.62], $p = 0.047$ for the second and 0.58 [95% CI 0.52–0.65], $p = 0.009$ for the last meeting, Fig. S12). There was no difference between ovulatory (0.56 [95% CI 0.52–0.59]) and non-ovulatory (0.52 [95% CI 0.46–0.58]) cycles in torso masculinity preference overall ($Chi^2 = 0.29$, df = 1, $p = 0.59$, Fig. S13), or among the three meetings ($Chi^2 = 0.52$, df = 2, $p = 0.77$). When the relationship status was included in the model, the analyses showed no difference between single and paired participants in torso masculinity preference (Fig. S14).

Analyses did not show an effect of daily P levels ($Chi^2 = 2.34$, df = 1, $p = 0.12$; OR = 1.24 [95% CI 0.94–1.64]), daily E2 levels ($Chi^2 = 0.01$, df = 1, $p = 0.93$; OR = 0.99 [95% CI 0.78–1.26]), nor daily E2/P ratio levels ($Chi^2 = 0.55$, df = 1, $p = 0.45$) on the torso masculinity preference (Table S20). All results of relations of daily hormonal levels and body preferences remained statistically non-significant when analyses were conducted for each day of the meeting separately (Tables S21–S23), or when the analysis was restricted to only ovulatory cycles (Table S26). Interaction involving relationship status and daily P levels was significant ($Chi^2 = 4.22$, df = 1, $p = 0.04$, Table S24). In paired participants, analyses did not show an effect of the daily P levels ($Chi^2 = 0.01$, df = 1, $p = 0.96$; OR = 0.99 [95% CI 0.70–1.40]). However, in single participants, the daily P levels were positively related to torso masculinity preference ($Chi^2 = 6.26$, df = 1, $p = 0.012$; OR = 1.87 [95% CI 1.15–3.06]). No simple effects or interaction effects were found when averaged cycle hormonal levels were used for the analyses (Table S25).

3.5. Additional tests for narrowed subsample of fertile window

To ensure that we are indeed testing women only during their most fertile phase of the cycle we conducted additional analyses restricting the sample to a more narrow subgroup. We have first selected only women who attended peri-ovulatory meeting met not > 24 h after LH surge ($N = 43$, 64% of total sample). Secondly, we have expanded the selection by including all women who attended peri-ovulatory meeting not > 48 h after LH surge ($N = 54$, 81% of the total sample). No effects involving hormone levels were significant in these restricted analyses (all $ps > 0.08$, see the Supplemental Material, Tables S26–S31).

4. Discussion

In our study, preferences for facial masculinity differed depending on average levels of progesterone (P) levels, but direction of these differences depended on relationship status. In women who were in long-term relationships, higher levels of average P during the cycle were related to weaker masculinity preference. In single women, the opposite trend was observed – higher levels average P were related to stronger masculinity preference. Similarly to some previous studies we did not observe a relationship between estradiol (E2) levels and facial masculinity preference (Bobst et al., 2014). The negative relationship between overall P levels and masculinity preference among paired women could be explained based on the fact that high P levels may signal pregnancy (Gilbert, 2000). Hence women with high P levels who are in a stable relationship direct their attention more towards parenthood skills (and relationship stability), rather than putative signals of good genes (i.e. masculinity) of their actual or potential partners. An opposite trend observed among single women could be explained by suggesting that single women who are currently not in a stable union may have a different strategy towards men. Their preferences thus are manifested in a different way than in women in a long-term relationship, i.e. lowered preference towards masculinity when P is high would not be as beneficial for them as for paired women.

Contrary to some previously published studies (Peters et al., 2009) we did not find significant relationships between any of the E2 measurements and facial masculinity preference. It is possible that because participants of the study judged their partners as very attractive and very masculine, and their relationship satisfaction was high, no around ovulation increase in masculinity preference (related to increased E2 levels around ovulation) was to be expected in the first place. According to the cyclical shifts hypothesis around ovulation peak in masculinity preference (following E2 peak) is a result of searching for good genes for the progeny (Little & Jones, 2012). We did find, however, a positive correlation between average E2 levels and symmetry preference for women who were in a relationship. We postulate that for women in a stable relationship, there would be no adaptive advantage of peak of E2 (and in general high E2 concentrations) influence on masculinity preference (as suggested by (Gangestad et al., 2005)), and symmetry would be a better cue to good genes. It is possible that masculinity is not as good of a cue to good genes as previously suggested (Scott et al., 2013) and that body muscularity and craniofacial shape are not traits that vary temporally and hence are unlikely to be condition-dependent signals. It is possible that other traits are better biomarkers of good genes. For example, another possible signal of masculinity that is beardedness, which has much stronger effects on judgments of men's masculinity, dominance and attractiveness (Dixson et al., 2016; Dixson et al., 2017) or facial symmetry, which might be a better long-term health predictor (Rhodes, 2006). We did observe the opposite correlation between E2 and symmetry preference among single women, suggesting that overall peak in facial masculinity preference found in previous studies (Little et al., 2008) is most probably not based on the high levels of E2 around ovulation.

Discrepancies between our and previously published studies can also stem from the fact, that our study was based on measurements of E2 and P in daily collected samples throughout the entire cycle (average levels of ovarian hormones were calculated based on 15 for P and 14 for E2 daily measurements, as opposed to average of 3 days of the meetings) and large sample size. In addition, in contrast to previous studies, we were able to control for a potentially confounding influence of relationship status. Moreover, we used LH tests to detect ovulation (which is a reliable way of defining the fertile window, in contrast to counting days method used in previously published hormone based studies (following very closely approach outlined in recent methodological paper (Blake et al., 2016), and measured preferences on three (and not two as previously done) meetings throughout the cycle.

Women's preferences for torso masculinity were not related to the average ovarian hormone levels, regardless of their relationship status. It is possible that we did not observe fluctuations in torso masculinity preferences because this is not as important signal to women as the facial masculinity is. Firstly, face is of crucial importance in human cognition (Perrett, 2010), and exaggerated sexual dimorphism of the face can be more informative for observers than the bodily one. Secondly, it is also plausible that when judging body of putative partner other features, like for example, body fat distribution is of more importance, as it is better correlated with testosterone levels and immune-response in men than body masculinity (Rantala et al., 2012).

We also did not find a significant relationship between fluctuating daily hormonal measurements and masculinity preferences when the analyses were restricted to only ovulatory cycles. This does not follow previously suggested idea that only in ovulatory cycles women should experience increased preferences towards masculinity due to hormonal surge around ovulation (Roney & Simmons, 2008). Our results are more consistent with the idea that changes in facial preferences are based on averaged, more stable levels rather than on daily changes in ovarian hormone levels, which often substantially fluctuate during the cycle (Jasienska & Jasienski, 2008; Jasienska et al., 2017). This finding supports our hypothesis that women should base their preferences on more reliable cues of fertility than hormone levels during a particular day. While in most cycles there is a predictable overall trend of changes in levels of hormones (e.g. levels of estradiol increase during follicular phase, or levels of progesterone increase following the ovulation during the luteal phase) daily levels of hormones are very variable. Even given an overall trend of raising levels of hormones, daily levels on a given day are not necessary higher than levels on a preceding day or a day after (Jasienska et al., 2017). Thus, levels observed during a single day cannot be used to predict a cycle quality. We suggest that important, fitness-influencing decisions (i.e. mating preferences) should not be based on such unreliable cues of fertility status as levels of hormones on a single day. Such decisions should be made based on more integrated information about a given cycle, such as hormone levels produced during at least several days, or the entire cycle phase. Integrated information provides much more reliable cue of cycle quality, signaling a chance of conception, implantation and successful pregnancy. Results of this article further support multiple recent null findings in studies examining per-ovulatory shifts (Dixson et al., 2018; Jones et al., 2018; Marcinkowska et al., 2018; Milkowska et al., 2018).

In the current study we were unable to account for the order effect of the testing sessions – participants followed all three sessions in same order. However, we believe that due to randomization of questionnaires, all questions and stimuli presentation on three consecutive meetings the possible order effect was minimised as much as possible. Also, time of the meeting has been included in the statistical model as a random factor. Another putative pitfall of the data collection is that not all participants who obtained a positive result of the ovulation test attended the meeting up to 48 h after the result – time ensuring highest probability of actually measuring fertile window. To account for this possible pitfall (and taking advantage again of the big sample size and detailed hormonal measurements) we have conducted two additional analyses based only on women who attended second meeting not later than i) 24 and ii) 48 h after the marked ovulation (ESM 1, Tables 26–31). The results of the additional analyses did not yield any significant relation between daily hormonal measurements and women's preferences, providing further, more robust confirmation that women's preferences for facial and body masculinity, and facial symmetric are not modulated by the ovulatory shift model.

In our study we did not find a support for the hormone-mediated increase in masculinity preference in most fertile phase of the cycle. We did, however, find a partial support for lowered masculinity preference when conception probability is lower, i.e. when levels of the overall progesterone are high (i.e., in luteal phase or during onset of pregnancy), and heightened symmetry preference when fertility is high (i.e. when cycle is characterized by a high overall estradiol levels) but only among paired women and only based on averaged levels of ovarian hormone. We suggest that relationship status is far more important than previously thought and should be a compulsory variable included in cyclical shifts preferences studies.

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Appendix A. Supplementary data

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