Facial appearance is a cue to oestrogen levels in women

M. J. Law Smith1,*, D. I. Perrett1, B. C. Jones1,†, R. E. Cornwell1, F. R. Moore1, D. R. Feinberg1, L. G. Boothroyd1,‡, S. J. Durrani1,¶, M. R. Stirrat1, S. Whiten2, R. M. Pitman2 and S. G. Hillier3

1School of Psychology, University of St Andrews, St Andrews, Fife KY16 9JB, UK
2School of Medicine, University of St Andrews, St Andrews, Fife KY16 9TS, UK
3Centre for Reproductive Biology, University of Edinburgh, Edinburgh EH16 4SB, UK

Although many accounts of facial attractiveness propose that femininity in women's faces indicates high levels of oestrogen, there is little empirical evidence in support of this assumption. Here, we used assays for urinary metabolites of oestrogen (oestrone-3-glucuronide, E1G) and progesterone (pregnanediol-3-glucuronide, P3G) to investigate the relationship between circulating gonadal hormones and ratings of the femininity, attractiveness and apparent health of women's faces. Positive correlations were observed between late follicular oestrogen and ratings of femininity, attractiveness and health. Positive correlations of luteal progesterone and health and attractiveness ratings were marginally significant. Ratings of facial attributions did not relate to hormone levels for women wearing make-up when photographed. There was no effect of sex of rater on the relationships between oestrogen and ratings of facial appearance. These findings demonstrate that female facial appearance holds detectable cues to reproductive health that are considered attractive by other people.

Keywords: facial attractiveness; sexual dimorphism; oestrogen; progesterone

1. INTRODUCTION
An evolutionary approach to facial attractiveness proposes that male preferences for feminine female faces (Perrett et al. 1994, 1998; Jones 1995; Rhodes et al. 2000) reflect an adaptation to identifying healthy and fertile mates (Thornhill & Gangestad 1999). Although there have been attempts to demonstrate that facial appearance in females signals some measure of underlying health, results of such studies have been equivocal.

Kalick et al. (1998) conducted the most comprehensive study using lifetime health records and adolescent photographs for a large group of participants (n = 333). They found adolescent facial attractiveness was unrelated to health (as indexed by annual health scores based on detailed medical histories during adolescence) at any stage of life for both males and females. Perceived health rated from the photographs was, however, weakly associated with hormone levels for women wearing make-up when photographed. There was no effect of sex of rater on the relationships between oestrogen and ratings of facial appearance. These findings demonstrate that female facial appearance holds detectable cues to reproductive health that are considered attractive by other people.

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reproductive health as indexed by hormonal profiles may also represent an objective measure of health. The two main hormones that impact on reproductive health are oestrogen and progesterone. Levels of both produced during menstrual cycles have been demonstrated to be good predictors of success of conception (Stewart et al. 1993; Lipson & Ellison 1996; Baird et al. 1997, 1999). There are several mechanisms which may mediate increased fecundity or fertility. Oestrogen levels correlate with follicle size, oocyte quality, thickness of endometrium (Eissa et al. 1986; Dickey et al. 1993) and penetrability of cervical perfusion (Roumen et al. 1982). Progesterone is also essential for endometrial maturation (Santoro et al. 2000) and cell growth (Chaffkin et al. 1993). As oestrogen and progesterone represent an accurate index of fecundity or reproductive health, they might provide useful measures of health to relate to facial appearance.

Although there has been no direct empirical test of relating reproductive hormones to facial appearance in women, there are studies which provide some indirect evidence for a link between hormones and facial appearance. The facial attractiveness literature has consistently demonstrated that facial femininity increases attractiveness of female faces, and that this is a cross-culturally stable preference (Perrett et al. 1994, 1998; Jones 1995). The interpretation for this preference relies on the assumption that facial femininity is oestrogen dependent, and so feminine faces are preferred because they are indicative of youth and high fecundity.

A recent study of body shape and hormone levels by Jasieńska et al. (2004) reported that women with the body shape that is found most attractive by males (large breasts and a low waist-to-hip ratio (WHR), e.g. Singh 1993; Henss 2000) have higher oestrogen levels than other categories of body shape. They also found that WHR related to progesterone levels; women with a more attractive (low) WHR have higher luteal progesterone levels. This result, alongside the finding that rated attractiveness of women’s bodies correlates highly with ratings of facial attractiveness (Thornhill & Grammer 1999), generates the prediction that women with high oestrogen and progesterone will also possess feminine attractive faces. A similar logic can be applied to the findings that women with high-pitched voices have higher oestrogen than those with low-pitched voices (Abitbol et al. 1999), and that men prefer the faces of women with higher pitched voices over those with lower pitched voices (Feinberg et al. in press). A recent study in males has also shown that men with higher testosterone have more masculine faces (Penton-Voak & Chen 2004).

The aim of the current experiment is to test the assumption that oestrogen levels relate positively to women’s facial appearance, by rating faces for perceived femininity, attractiveness and health. The hypothesis that progesterone relates to facial appearance is also tested.

2. MATERIAL AND METHODS

(a) Participants

Participants were 59 white women from the student undergraduate population at the University of St Andrews (age, $M=20.4$, s.d. $=1.5$, range 18–24). No participants were currently using the contraceptive pill or had been in last 90 days. All received monetary payment for participation.

(b) Photographs

Participants were photographed each time they came to the laboratory, weekly for four to six weeks. Participants were photographed in a neutral expression, under standard conditions with diffuse flash lighting from two lateral flashguns. Images were captured on a digital camera at a resolution of $1200 \times 1000$ pixels in uncompressed TIFF format using 24 bit RGB encoding. No restrictions were made for make-up use during photography, however the use of make-up was recorded in self-reports. Consequently, 32 participants were not wearing any make-up when photographed and 27 were wearing make-up. The first photograph taken (week 1) was used for ratings if the participant had either always worn make-up ($n=27$) or always not worn make-up ($n=14$) in all the photographs. If there was a combination of no make-up and make-up photographs ($n=18$), the first photograph with no make-up was used. For presentation to raters, the faces were aligned on interpupillary distance and masked around the face line, so cues to hair and clothing were reduced.

Average faces were created in order to visualize the differences in facial appearance between women with high and low reproductive hormones. Composites were constructed from the faces of the females with the highest 10 and lowest 10 oestrogen using the methods outlined in Benson & Perrett (1993) and Tiddeman et al. (2001). See figure 1 for composite faces. Separate composites were not constructed for progesterone levels, because they were highly intercorrelated with oestrogen levels (see §3b); therefore, the composites would have contained the majority of the same faces. Composites were created from oestrogen rather than progesterone as the latter was not as strongly related to the face ratings (see §3b).

(c) Hormone measurement

Participants were instructed to provide their sample of urine from the midstream of the first urination of the morning of each day of testing. Participants collected samples once a week for four to six weeks, in order to cover all stages of the menstrual cycle. All samples were stored at $-20{\degree}C$ until assays were performed.

The assays involved a direct competitive ELISA 96-well plate system to assess oestrone-3-glucuronide (E1G) and pregnanediol-3-glucuronide (P3G) (major metabolites of oestradiol and progesterone, respectively). Urine samples, diluted in assay buffer, were incubated with labelled antigen (E1G or P3G conjugated to horseradish peroxidase) in the presence of rabbit anti-steroid antibody (anti-P3G antibody (RAB F 27/7/87) or anti-E1G antibody (RAB 1), respectively). Bound and free antigens were separated using solid-phase goat anti-rabbit IgG. The plates were washed and bound antigen was detected by incubation with the substrate o-phenylene-diamine and the developed reaction was detected using a plate reader at 492 nm. For full methods, see Joseph-Horne et al. (2002). The intra-assay variation for both was less than 10%. Hormone level results were expressed as steroid : creatinine ratio. Assays were not available for five subjects’ late follicular oestrogen and two subjects’ luteal progesterone.

Menstrual cycle information was collected via self-report (diary data). To determine day of menstruation and length of menstrual cycle, participants reported the number of days since the onset of their last period of menstrual bleeding and their average menstrual cycle length. Date of onset of period following study completion was also collected via email. Cycle day was calculated by the backwards counting method.
Facial appearance and oestrogen levels  M. J. Law Smith and others

previously used by Jones et al. (2005). The levels of oestrogen in the late follicular stage of the menstrual cycle (14–21 days before next period) were used for comparison as this is the stage at which females are most likely to conceive. This stage may show greatest variation in fertility and thus be most likely to show the greatest associations with physical condition and attractiveness. Previous research reported heightened attractiveness (1 not attractive to 7 very attractive) at the follicular (fertile) stage of the menstrual cycle (Roberts et al. 2004). For progesterone, an average of the luteal (non-fertile) stage was analysed (13–1 day before next period), as progesterone levels are very low until ovulation and then rise until onset of menses. Previous research with progesterone and WHR has used average luteal levels (Jasińska et al. 2004).

(d) Questionnaire
Participants completed the following questionnaire on menstrual status and make-up use at each testing session.

(i) As best you can recollect, when was the date of the beginning of your last menstrual period—i.e. the first day of bleeding?
(ii) When do you expect your next menstrual period to begin?
(iii) Is your menstrual cycle fairly regular? Yes/No
(iv) Normally, how long is your menstrual cycle? (i.e. 28 days, 30 days)
(v) Are you currently wearing any make-up? Yes/No

(e) Face ratings
Participants for the ratings task were 15 female and 14 male students from the University of St Andrews (age, M=22.3, s.d.=1.6, range 19–25). All participants rated the pair of composite faces for which was more attractive along the 8-point preference scale; much more attractive (left image), more attractive (left image), slightly more attractive (left image), guess (left image), guess (right image), slightly more attractive (right image), much more attractive (right image). The composites were rated in the same way for femininity and health.

The composite faces were rated in a forced-choice paradigm by 11 female and 10 male students from the University of St Andrews (age, M=22.3, s.d.=1.6, range 19–25). All participants rated the pair of composite faces for which was more attractive along the 8-point preference scale; much more attractive (left image), more attractive (left image), slightly more attractive (left image), guess (left image), guess (right image), slightly more attractive (right image), more attractive (right image), much more attractive (right image). The composites were rated in the same way for femininity and health.

3. RESULTS
Progesterone and oestrogen metabolite levels and each of the three facial ratings were normally distributed (Kolmogorov–Smirnov: progesterone, p=0.83; oestrogen, p=0.09; femininity, p=0.58; attractiveness, p=0.28; health, p=0.63; all Zs<1.2). Therefore, parametric statistics were used in subsequent analyses. Ratings of faces were highly consistent across raters (all Cronbach’s α >0.9).

(a) Controlling for potential confounds
(i) Make-up use
To determine if women who always choose to wear make-up differ from those that choose not to wear make-up, independent samples t-tests were used to compare hormone levels and age. There was no significant difference between make-up wearers and non-make-up wearers in age (M=20.5, s.d.=1.2; M=20.3, s.d.=1.7; t=−0.64, d.f.=57, p=0.53), oestrogen levels (M=13.3, s.d.=6.5; M=13.1, s.d.=7.9; t=−0.14, d.f.=52, p=0.89) or progesterone levels (M=0.27, s.d.=0.10; M=0.25, s.d.=0.13; t=−0.52, d.f.=55, p=0.60).

Faces wearing make-up were rated as significantly more feminine (M=4.3, s.d.=1.0), attractive (M=3.40, s.d.=0.73) and healthy (M=4.36, s.d.=0.68) than those not wearing make-up (M=3.5, s.d.=1.1; M=3.01, s.d.=0.92, M=3.99, s.d.=1.12; t=−2.84, p=0.006; t=−2.34, p=0.023; t=−2.13, p=0.038; respectively, all d.f.=57).

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Figure 1. Composite faces of the (a) 10 women with highest and (b) 10 with lowest levels of late follicular oestrogen metabolite (oestrone-3-glucuronide, E1G).
There was no significant difference in day of cycle when the rated photographed was taken (make-up, M=18.7, s.d.=5.7; non-make-up, M=14.9, s.d.=8.7) t = -1.30, d.f.=57, p = 0.21. Therefore, rating differences between make-up and no make-up images cannot be due to cyclic change in attractiveness (see Roberts et al. 2004) as a result of make-up use being potentially biased to a particular part of the cycle.

(ii) Effect of age
As there was no difference in age or hormones between the make-up and non-make-up wearers, the following correlations between hormones and age were conducted with the total sample, using Pearson’s product moment correlation. Age did not correlate with either of the hormone levels (oestrogen, r= 0.11, p = 0.44, n = 54; progesterone, r=0.18, p = 0.21, n = 57), or any of the face ratings (femininity, r=0.11, p = 0.40; attractiveness, r = 0.09, p = 0.51; health, r=0.19, p = 0.16; all n = 59). Therefore, age was not controlled for in any of the following analyses.

(b) Hormone levels and facial attributions
As the use of make-up influenced attributions, the following analyses investigating the hormone appearance relationship were conducted for make-up and non-make-up wearers separately.

For those wearing no make-up, late follicular oestrogen levels were significantly positively correlated with femininity (r = 0.48, p= 0.007, n = 30), attractiveness (r = 0.48, p= 0.007, n = 30) and health ratings (r = 0.52, p= 0.003, n = 30). For those wearing make-up, however, oestrogen levels were not related to femininity (r = -0.02, p > 0.05, n = 24), attractiveness (r = -0.08, p = 0.71, n = 24) or health ratings (r = 0.07, p = 0.74, n = 24).

For females wearing no make-up, luteal progesterone levels were not related to femininity (r = 0.28, p = 0.13, n= 30). There was a trend for progesterone to positively correlate with attractiveness (r = 0.33, p = 0.075, n = 30) and health ratings (r = 0.35, p = 0.055, n = 30). For those wearing make-up, however, progesterone levels were not related to femininity (r = 0.09, p = 0.66, n = 27), attractiveness (r = 0.04, p = 0.63, n = 27) or health ratings (r = 0.17, p = 0.61, n = 27).

As the hormone levels did not relate to attributions for those wearing make-up, the following further analysis of hormone appearance relationships was restricted to the non-make-up group.

The three face ratings are highly interrelated (attractiveness and femininity, r = 0.84; attractiveness and health, r = 0.81; femininity and health, r = 0.60; all p<0.001, n=31). The three face ratings were entered into a principal component analysis (PCA). One factor with eigenvalue greater than 1 was extracted (eigenvalue = 2.51, accounting for 83.69% of the variance) on which all face ratings loaded (femininity, r = 0.89; attractiveness, r = 0.97; health, r = 0.88). This factor was interpreted as a general ‘quality’ factor. Oestrogen was significantly positively correlated with the quality factor (r = 0.54, p = 0.002, n = 30) and there was a trend for progesterone to positively correlate with the quality factor (r = 0.35, p = 0.058, n = 30). As progesterone is highly correlated with oestrogen (r = 0.67, p < 0.001), a linear regression was performed to determine the extent to which both hormones were independently related to the quality factor.

A significant regression model was produced for predicting quality rating (adj $r^2$ = 0.28, p = 0.017) with only oestrogen as a significant predictor ($\beta$ = 0.51, p = 0.035). Progesterone was a non-significant predictor ($\beta$ = 0.03, p = 0.89).

(i) Effect of sex of rater
To determine if there was any effect of sex of the rater on the relationship between oestrogen levels and the face ratings, the faces were divided into three equal groups (high, mid and low hormone levels) for a mixed ANOVA, with sex of rater as a between-subjects factor (two levels), oestrogen level as the within-subjects factor (three levels) and face ratings as the dependent variable.

There was a highly significant main effect of oestrogen level with the faces corresponding to high oestrogen levels receiving higher ratings (all $F_{2,50} > 38.98$, p < 0.001). There was no main effect of sex of rater on any of the ratings (all $F_{1,25} < 0.63$, p > 0.44). There were no interactions between rater sex and oestrogen level (all $F_{2,50} < 0.93$, p > 0.40). Thus, rater sex did not affect ratings or qualify the relationship between oestrogen and face ratings.

(ii) Composite faces

The high oestrogen face was rated as much more feminine, attractive and healthy than the low reproductive hormone face (all $t > 6.31$, p < 0.001, d.f. = 20) using a one-sample t-test on strength of preference. These results cannot be due to a mediating effect of age on facial appearance and hormones as there was no significant difference in age of the composites (low, $M=20.8$, s.d. = 1.5; high, $M=20.4$, s.d. = 1.8; t = 0.54, p = 0.60, d.f. = 18). All face ratings were highly consistent (a > 0.9).

4. DISCUSSION

The major finding of this study is that women (not using make-up) with higher levels of late follicular oestrogen have more feminine, attractive and healthy looking faces than those with lower levels.

The initial inclusion of those participants wearing make-up allowed investigation into the effect of make-up on attributions and also its role in mediating the relationships between hormones and appearance. No differences in age, progesterone or oestrogen level were found between those women choosing to wear make-up and those choosing not to wear make-up. Differences were, however, found in attributions ascribed to the faces of the two groups: faces with make-up were seen as more attractive, feminine and healthy. While our study used a between-groups comparison, this result may support the assumption that women use make-up to improve facial appearance.

The main findings of this study are the statistically significant associations between late follicular oestrogen levels and three perceptual ratings of facial appearance; femininity, attractiveness and health, in those women wearing no make-up. There were marginally significant correlations of luteal progesterone and health and attractiveness ratings. Interestingly, these relationships were not seen in those women’s faces with make-up on. The use of make-up may compensate for or mask cues indicating low hormone levels, making perceivers unable to form attributions based on natural hormonal cues.
The relationships found here between oestrogen and appearance in natural images of faces are important because they are the first evidence for a link between facial femininity and oestrogen that has previously been assumed in facial attractiveness research. This provides evidence that the sexually dimorphic appearance of female faces is related to oestrogen levels. The associations of oestrogen with attractiveness and health ratings also provide evidence that markers of oestrogen are consistently seen as attractive and healthy.

Luteal progesterone levels were found to relate marginally to health and attractiveness ratings of faces. Progesterone levels have not been discussed in the facial attractiveness literature, although Jasienska et al. (2004) recently found that gonadal hormone levels were related to femininity in body shape (WHR and breast size): both oestrogen and luteal progesterone levels were related to an increasingly attractive WHR and breast size.

All three face ratings were highly interrelated, and a PCA revealed one factor, interpreted as ‘quality’, which all ratings heavily loaded on. Late follicular oestrogen and luteal progesterone were correlated indicating that, for young adult females levels of the two gonadal hormone levels may both reflect reproductive quality. A linear regression showed oestrogen to be the only predictor of overall ‘quality’ in appearance.

There was no effect of the sex of the rater on the relationships found between oestrogen and the face ratings, and inter-rater reliability was high. Both results indicate that judgments of female faces are consistent.

The construction of the composite faces of the women highest and lowest in oestrogen levels allowed a visualization of our correlational results. There appear to be multiple visual differences between the two face groups evident in the composites. The oestrogen female face was consistently rated as more attractive, feminine and healthy looking. Future studies should address the nature of facial cues related to hormone levels.

The current finding that oestrogen predicts facial appearance has implications for the evolutionary approach to facial attractiveness. The female face does seem to hold detectable cues to underlying health and fertility, as indexed by oestrogen levels. These cues are used in judgements relevant to mate choice decisions. These detectable cues to reproductive hormones may have shaped male preferences, and could therefore provide an adaptive explanation for the cross-cultural tendency for feminine female faces to be found most attractive.

This work was supported by BBSRC, MRC and Unilever Research. The authors thank Lesley Ferrier for recruiting and scheduling of participants; Alexandra Boyden, William Calderhead, Carolyn Cheetham, Catherine Dutton, Fiona Elder, Suzanne Hall, Jennifer Hardingham, Laura Harper, Laura Johnson, Jamie Lawson, Gillian McBride, Jennifer McChesney, Anne-Marie Morgan, Joanna Souter and Katherine Timmins for assistance with data collection. We also thank the Reproductive Medicine Laboratory Staff of the University of Edinburgh and Martha Urquhart for assistance in hormone analysis.

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