



# Investigating effects of sex hormones, cycle phases and age on female fundamental frequency

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## Abstract

Mean fundamental frequency (f0) has been found to be affected by hormones. In females, studies show contradicting results regarding the influence of hormonal changes across the cycle on f0. Some studies have suggested hormonal changes due to menopause are responsible for the decrease in female f0 with age, while others point to an earlier onset of this decrease.

Here, the effect of hormones (estradiol, progesterone, testosterone) on mean f0 within speakers across the cycle and between speakers was investigated in 62 German participants. In addition, the factors age, height and speech material were included.

Results reveal that mean f0 was not affected by cycle phase. Also, variation in mean f0 across speakers could not be explained by variation in investigated hormones. In contrast, a clear effect of speakers' age on mean f0 was found, irrespective of hormone levels, height and speech material, pointing to other factors contributing to a lower mean f0 with increasing age in females.

**Index Terms:** mean f0, hormones, estrogen, progesterone, testosterone, age

## 1. Introduction

Mean fundamental frequency (f0) exhibits strong sexual dimorphism and contributes to a speaker sounding more male or more female [1,2]. Mean f0 affects perceptions of attractiveness, dominance, and masculinity/femininity (e.g., [3–5]). Whether a speaker's mean f0 also reflects their hormonal constitution and indexes underlying characteristics that are relevant to mate quality (in terms of male formidability, mate quality, female fertility) is still a matter of debate [6–11].

Mean f0 is determined by biological parameters such as vocal fold length and thickness, but also sociocultural (learned) factors (cf. [12]) – as speakers can adjust their f0 by e.g., increasing the tension of the vocal folds. The vocal folds are sensitive to sex hormone levels, which fluctuate throughout the life cycle. Sex hormones may act on the neuromotor control of the larynx ([13]) or directly on the vocal fold mucosa that contain specific hormone receptors (which differ across age and gender) for androgens, estrogens and progesterone [14].

Hormones have been found to affect speech, in particular mean f0 [6–7, 15–17]. In males, higher testosterone levels have been found to correlate with lower mean f0 within speakers (diurnal variation) and between speakers ([15–17] but see also [18] for a contrary result). In addition, the relationship was found to be weakened by a high cortisol level [6, 16] and varied

between the analyzed speech materials (stronger effect in sustained vowels than in semi-spontaneous speech, see [16]). In females, estrogens, progesterone, and testosterone have been found to affect voice characteristics in relation to fertility cycle, menopause and pregnancy [7, 10, 13, 19–20]. The *good genes ovulatory shift hypothesis* (GGOSH, [21]) assumes that hormonal changes due to the fertility cycle help to identify the gene quality of potential partners. However, the hypothesis and the assumed relationship is far from clear: an increase in f0 has been detected prior to ovulation in some studies [7,8] while another very comprehensive study including 202 women did not find such a relationship [9]. On the perception side, female voices have been rated as more attractive on midcycle days [8–9, 22]. The present study adds to this line of research by looking at the effect of cycle phase on mean f0 in healthy German females.

After menopause studies found a decrease in mean f0 [22, 23], which has been explained in terms of a decrease in estrogens and an increase in androgens affecting the vocal folds via hormone receptors ([24]). Estrogen injections have been shown to counteract, at least in part, these postmenopausal decreases in f0 [25], while hormone therapies with testosterone have been shown to lower female f0 [26]. Moreover, administering testosterone can lead to a significant and desired lowering of f0 in transmen (e. g. [27–29]).

Pregnancy also affects estrogen and progesterone levels, while the effect of pregnancy on voice is still unclear. [20] investigated British English mothers and found a lowering of mean f0 one year after giving birth compared to during pregnancy or before. However, [30,31] found contradictory results with a lowering of mean f0 after pregnancy in German mothers but not in Swedish mothers, thereby pointing to the role of sociopsychological factors (e.g. level of support from other parent) besides hormonal influences due to pregnancy. Similarly, more recent studies point to social factors playing a role in variation in mean f0 across the life span (e.g. 30, with respect to becoming a parent). In addition, some studies report a lowering of mean f0 in females already in younger age groups, long before hormonal changes related to menopause are supposed to have this effect on voice [32, 33]. The present study adds to this line of research by investigating the interaction of age, hormone levels and mean f0 in women before menopause (mean age of menopause for German women is 49.7 [34]).

### 1.1. This study

The present study investigates the potential impact of hormonal changes in estradiol, progesterone and testosterone on mean f0 in healthy German females. On the one hand, we highlight intra-individual variation in hormone levels across the menstrual cycle and its effect on mean f0. On the other hand, we shed light on inter-individual variation in hormone levels

across age (18-44 years) and its potential relationship with mean f0. Our research questions are the following:

- 1) Is female mean f0 affected by cycle phase (fertile vs. luteal phase)?
- 2) Is female mean f0 affected by inter-individual variation in hormone levels (estradiol, progesterone, testosterone)?
- 3) Does the decrease in mean f0 in females with age start before menopause and can it be explained by changes in hormone levels (estradiol, progesterone, testosterone)?

## 2. Method

### 2.1. Participants

80 female German participants between 18 and 44 years (mean age: 28.9) took part in the investigation. They were divided into three age groups (F1: 18-25 years, F2: 26-34 years, F3: 35-44 years). None of them used hormonal contraception and all of them reported having a normal and regular cycle. The majority were non-smokers, while seven participants reported smoking up to two cigarettes per day. To control potential effects of smoking on voice characteristics they were excluded from the subsequent analysis.

Prior to the recording sessions we asked participants to monitor their cycle and count their average cycle length across three months. We then recorded them during two sessions. One session took place during the fertile phase, on the days prior to ovulation (i.e. reverse cycle days 16-18, with reverse cycle day 16 as the ideal date). Another session took place during the luteal phase (after ovulation and prior to the next menstrual onset), i.e. reverse cycle days 4-11, with reverse cycle days 6 to 8 as the ideal dates (cf. [35]). The order of the recording sessions (fertile or luteal phase first) was reversed for half of the participants. Figure 1 shows the distribution of cycle day reported by the participants at the two sessions separated by estimated phase (f=fertile, l= luteal). Participants who accidentally came on days that did not lie within the desired intervals of the respective phases were not recorded.

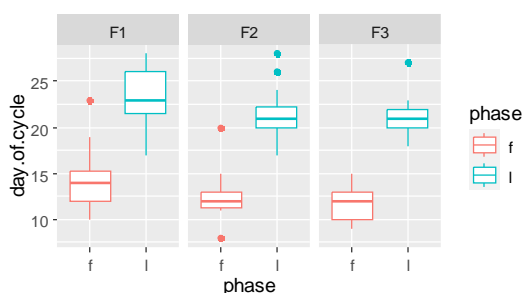


Figure 1: Cycle day separated by phase and age group.

### 2.2. Data collection

Data collection, which is part of a larger project investigating the role of hormones in males and females on speech production and perception [36], took part in a quiet room at the university and was conducted by one of four female

experimenters. During each session, participants filled out a questionnaire on personal background data, were recorded giving various speech samples, gave saliva samples and conducted a perception experiment, which is not part of the present study. The speech material consisted of three different tasks: sustained vowels /a: e: i: o: u:/, 15 read sentences, and a short sequence of spontaneous unscripted speech elicited through the description of a picture.

Saliva samples were gathered at each session (three times within one hour) and frozen on the same day at -18 °C until data collection was finished and then transported on dry ice to the laboratory for hormone analysis (Institute for Special Diagnostics (ISD), Malente, Germany). Estradiol, progesterone and testosterone levels were estimated. Figure 2 shows estradiol and progesterone levels separated by phase and age group.

As expected, progesterone levels were higher in the luteal phase than in the fertile phase, while the difference in estradiol between the phases is more subtle. Looking at individual participants, ten females had to be excluded due to a higher progesterone level in the estimated fertile than in the estimated luteal phase and another speaker was excluded based on extreme values in progesterone (due to a microprolactinoma). A t-test between estradiol/progesterone ratio across phases was conducted on the remaining subjects, confirming the expected higher values in the fertile than in the luteal phase ( $t = 3.6$ ,  $df = 68.9$ ,  $p < .001$ ,  $95\%CI = [0.05; 0.17]$ ).

Altogether, 62 participants were part of the present analysis, distributed into three age groups (F1 (n = 28, 22.5 years, SD = 2.0), F2 (n = 21, 29.2 years, SD = 3.1), F3 (n = 13, 40.3 years, SD = 3.1)).

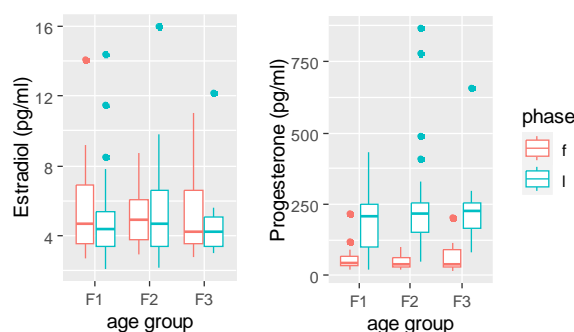


Figure 2: Estradiol and progesterone levels separated by phase and age group.

### 2.3. Analyses

Acoustic analyses were performed using *Praat* [37]. Mean f0 was measured for each speaker separately for the different speech materials and recording sessions (analysis parameters: time step = 0.01 s, minimum pitch = 50 Hz, maximum pitch = 500 Hz). For statistical analysis we used linear mixed models in the *R* environment [38]. Test variables were added successively to the model. We used sum coding for categorical variables. P-values were estimated using model comparisons, i.e., by comparing models with and without the respective variables or interactions in question using likelihood ratio tests provided by the *anova* function (package *lme4*, [39]). For post hoc comparisons, the package *emmeans* was used [40].

### 3. Results

#### 3.1. Graphical exploration

First, a graphical exploration of the potential relationships between changes in hormonal levels and changes in mean  $f_0$  within speakers across the cycle phases was made. Changes in both  $f_0$  and hormone levels were expressed in percent by dividing the values of the fertile phase by the values measured in the luteal phase for each speaker. Values around 100 reflect no variation across the cycle phases, while values above 100 reflect *higher* values in the fertile phase (expected for  $f_0$  and estradiol), and values below 100 reflect *lower* values in the fertile phase (expected for progesterone). Figure 3 (panels A1-A3) shows changes in mean  $f_0$  measured in the reading task of the 62 speakers in relation to each speaker's changes in hormone levels (A1: estradiol, A2: progesterone, A3: testosterone).

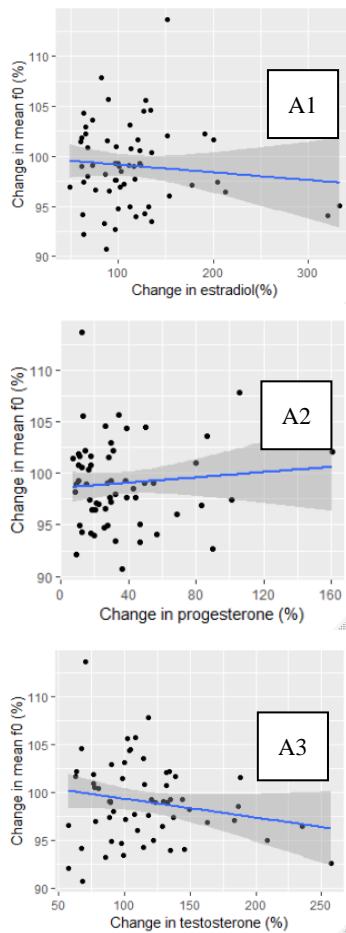


Figure 3: Relationships between changes in mean  $f_0$  and changes in hormone levels across cycle phases (>100 reflect higher values in the fertile than in the luteal phase)

It can be seen that speakers'  $f_0$  values are equally distributed above and below 100, thereby reflecting no clear picture in the direction of change. Also, with respect to potential relationships between changes in mean  $f_0$  and changes in hormone levels, no

clear and systematic patterns are obvious. Only for testosterone, a slight tendency towards a negative relationship can be seen.

In addition, Figure 4 (B1) visualizes the potential effect of absolute hormone levels (estradiol) on mean  $f_0$  across speakers separated by cycle phase (f: fertile, l: luteal). Similarly, no systematic relationship is apparent, which is also true for the other hormones (not shown here). The lower two plots of Figure 4 (B2, B3) highlight the potential effect of age on mean  $f_0$  (B2) and estradiol (B3). While for estradiol, no systematic effect of age is apparent, mean  $f_0$  decreases constantly with increasing age.

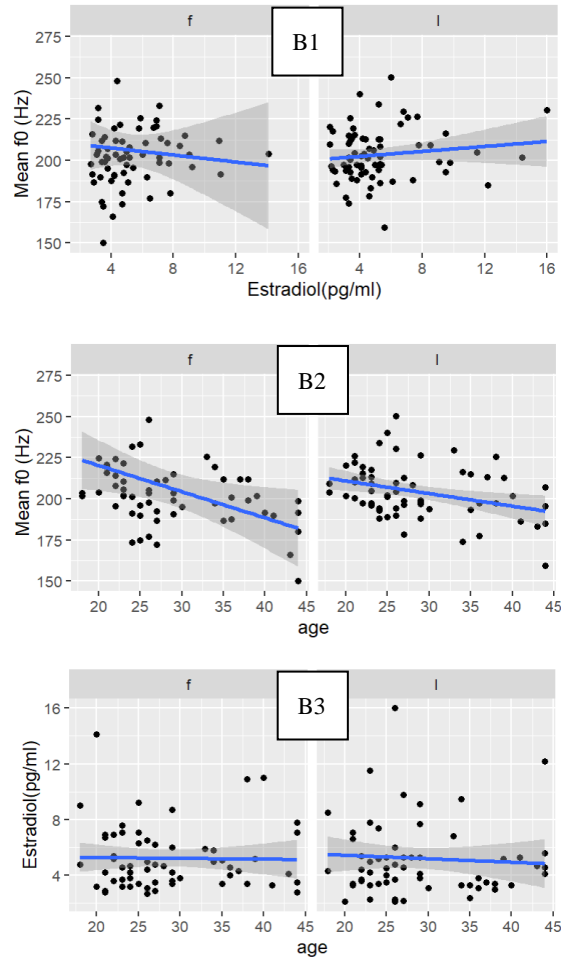


Figure 4: Relationships between absolute estradiol values and mean  $f_0$  (B1) and between age and mean  $f_0$  (B2) and age and estradiol (B3), separated by cycle phase

#### 3.2. Effects of cycle phase, hormone levels and age

Figure 5 shows mean  $f_0$  values across the three age groups (F1, F2, F3) separated by speech material (picture description, read speech, sustained vowels) and phase (f: fertile, l: luteal). A decrease in mean  $f_0$  with increasing age can be seen here, too, independent of speech material and in both cycle phases. However, no systematic pattern with respect to differences between cycle phases in mean  $f_0$  is apparent.

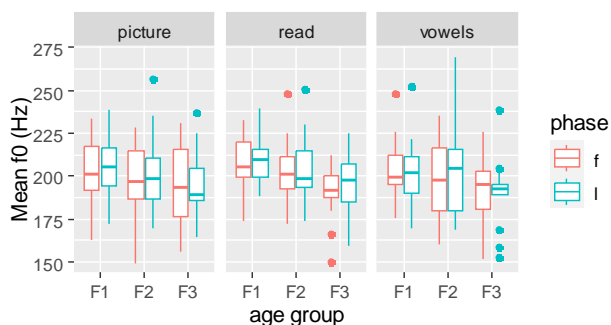


Figure 5: Mean  $f_0$  for the three speech tasks separated by age group and cycle phase.

For the statistical analysis linear mixed models were run, and the fixed factors *speech material* (picture, read, vowels), *age* (numerical), *phase* (f, l), *estradiol* (numerical), *progesterone* (numerical), *testosterone* (numerical), as well as *height* (numerical) as a control variable were entered successively to the model. Speaker was added as a random intercept. In addition to analyzing main effects, we looked at interactions between all factors. The final model which explained the data best included the main effects of age ( $\chi^2(1) = 8.5, p < .05$ ) and task ( $\chi^2(2) = 8.1, p < .05$ ), while none of the hormone related factors reached significance, and neither did any interaction. Table 1 gives the summary statistics of the final model including the post hoc comparisons between all speech tasks. Speakers showed a significantly higher mean  $f_0$  in the reading task than in the other two tasks (sustained vowels and picture description task). More interestingly, speakers mean  $f_0$  decreased with increasing age, independent of hormonal changes, cycle phases or speech material.

Table 1: Summary statistics of the final model with mean  $f_0$  as dependent variable (Number of obs: 366, groups: speaker, 62)

Fixed effects	Estimate	Std. Error	df	Pr(> t )
Intercept	224.9	8.69	62.8	< .001
pic vs. read	-5.6	2.41	302	0.05
pic vs. vowels	0.61	2.41	302	0.96
read vs vowels	6.22	2.41	302	<.05
age	-0.87	0.29	59.5	< .01

#### 4. Discussion

Regarding our research questions, we found that female mean  $f_0$  was not affected by cycle phase (fertile vs. luteal) within our speakers. Also, female mean  $f_0$  was not affected by inter-individual variation in hormone levels regarding estradiol, progesterone or testosterone. Our results are thus in line with [9], but differ from [7,8]. In [8] the authors found a marginally significant variation in  $f_0$  with an increase prior to and a distinct drop during ovulation. However, they also point to a large variation throughout the cycle, which “precluded unequivocal identification of the period with the highest conception risk”. Thus, methodological differences (comparing two cycles instead of a day-to-day analysis) might explain the different results. In line with our results, they found that neither estrogen nor progesterone levels predicted the changes in  $f_0$  (see also

[9]). Our study contributes to these findings pointing to a complex relationship between variation in mean  $f_0$  and hormonal fluctuations across the cycle.

On the other hand, a clear effect of speakers’ age on mean  $f_0$  was found, with  $f_0$  decreasing with age. This is particularly interesting since our speakers were women between 18 and 44 years with regular menstrual cycles, and individual differences in hormonal levels (regarding estradiol, progesterone and testosterone) were not responsible for this decrease. Thus, the start of menopause or hormonal fluctuations in sex hormones across adulthood cannot be the sole reason for the decrease in female mean  $f_0$  with increasing age [24]. Like other studies [32,33], our results point to a lowering of mean  $f_0$  in females already starting in younger age groups, long before hormonal changes related to menopause are supposed to have this effect on voice. We suggest that social factors play a role in the variation in mean  $f_0$  across the life span. We can only speculate about the specific reasons but sociopsychological factors related to different life stages such as becoming a parent or changing gender role self-concepts [30] could lead to changes in voice cues. Also, achieving higher job positions with increasing age might affect mean  $f_0$  given the findings of [41] who found  $f_0$  influenced the perception of leadership capacity in both male and female speakers.

#### 5. Conclusions

Variation in female mean  $f_0$  across the menstrual cycle or across age cannot solely be explained by fluctuations in sex hormones. A speaker’s age affects mean  $f_0$  irrespective of hormone levels pointing to other (social) factors contributing to a lower mean  $f_0$  with increasing age in females.

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