

# Fundamental Frequency of Crying in Two-month-old Boys and Girls: Do Sex Hormones During Mini-puberty Mediate Differences?

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**Summary: Objective.** To evaluate whether the puberty-like sex hormone surge during the first months of life (mini-puberty) affects fundamental frequency (fo) in infant crying as one would derive from hormone influences on voice in adults.

**Study Design.** Populational prospective study.

**Participants.** Twenty healthy normal-hearing infants (nine boys) were recruited for participation.

**Methods.** Spontaneously uttered cries were collected from each infant at 8 weeks of age. The cries were acoustically analyzed for mean fo and fo range. The fo properties were correlated to the average serum levels of bioavailable estradiol (E2) (mean E2/sex hormone-binding globulin [SHBG]) and testosterone (T) (mean T/SHBG) across the second month of life.

**Results.** Whereas no significant hormone effect was found for mean fo, a significant negative correlation ( $r = -0.55$ ) was found between fo range and mean E2/SHBG. No indication for a T influence on fo features was found at this age. Although girls showed a slightly higher mean E2 concentration than boys did, the observed differences in cry fo range were judged to be reflective of an infant's serum concentration of E2 rather than a sex-based difference.

**Conclusion.** In the absence of laryngeal size differences between female and male infants, the result was interpreted as indicative of an E2 influence on viscoelastic properties of the vocal folds. In our opinion, the investigation of young infants' vocalizations during the early postnatal surge of sex steroids (mini-puberty) may advance our understanding of the mechanisms mediating average sex differences in vocal development and early communication.

**Key Words:** fundamental frequency–cry–sex differences–infant–sex hormones.

## INTRODUCTION

The vocal fundamental frequency (fo) characteristics of adults and older children are well documented across speakers of several languages who differ in age and sex.<sup>1–10</sup> Adults can identify the sex of speakers as young as 4 years of age by listening to their voice.<sup>6</sup> However, at this early age, gender ratings are primarily dependent upon vowel formant frequencies rather than fo. Although a sexual dimorphism in fo properties caused by physiological or behavioral factors is somewhat controversial in prepubertal children,<sup>7,8,11</sup> gender is clearly differentiated by fo after 12 years.<sup>6</sup> The onset of puberty triggers a distinctive sexually dimorphic trait, with boys exhibiting a decrement in fo from infancy to adulthood of approximately two octaves and girls showing a drop of one octave.<sup>12</sup> The typical mutational voice change in boys was found to correlate to changes in vocal fold length, and even stronger to structure and mass of the vocal folds at this time period.<sup>13</sup> These changes were assumed to be at least partly affected by hormonally induced changes occurring during

puberty. For example, pubertal exposure to androgens was found to cause a 60% increase in men's vocal fold length relative to women, and a corresponding decrease in its inverse acoustic correlate, mean fo.<sup>13,14</sup> The female voice is also affected by sex hormones such as estrogen, progesterone, and testosterone (T) throughout life.<sup>15,16</sup>

Recently, Nacci and coworkers<sup>17</sup> postulated an alternative mechanism causing gender differences in voice to the classical assumption that sex hormones directly influence laryngeal function. These researchers suggested an estradiol (E2)-dependent expression of certain growth factors in the laryngeal tissue, for example, basic fibroblast growth factor (bFGF) or transforming growth factor beta1 (TGF- $\beta$ 1). This E2 expression of growth factors may be influenced by hormonal variations.<sup>17</sup> This is in agreement with findings of E2 facilitating mitosis, fibroblast migration, and the production of extracellular matrix through the production of growth factors.<sup>18</sup>

The assumption of an E2-dependent expression of growth factors in laryngeal tissue is of particular interest with respect to vocal fold development in infants. Three strong arguments can be raised in favor of this provocative assumption from a developmental perspective:

- (1) At birth, the lamina propria of the vocal folds consists of a hypercellular monolayer changing to the three-layered structure only within the first year of life.<sup>19</sup> However, by 2 months of age (the age period considered in the present study), there are early signs of differentiation into a bilaminar structure of distinct cellular population densities.<sup>19</sup> Synchronously, maximal

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respiratory muscle strength increases significantly during the first 6 weeks of life,<sup>20</sup> contributing to respiratory control, which is also influenced by sex hormones.<sup>21</sup>

- (2) A significant correlation between blood serum concentrations of E2 and the melodic patterns of infant spontaneous crying at this age (8 weeks old) was demonstrated in a previous study by our research team.<sup>22</sup> In contrast, no significant relationship was found between the melodic pattern and the serum T concentrations in the same study, a finding in agreement with Grisa *et al*<sup>23</sup> who studied women treated for adrenocortical tumor in childhood. These researchers found that female laryngeal tissue is less sensitive to androgen exposure between birth and adrenarche than during other age periods.
- (3) The first postnatal months are characterized by an elevated sex hormone surge comparable with puberty, and hence called “mini-puberty.”<sup>24–29</sup> During this period, boys exhibit higher T concentrations than girls, whereas girls show slightly higher E2 concentrations than boys. These differing levels of sex hormones could mediate the sex differences noted in cry melody,<sup>22</sup> as well as the consistently found “female advantage” in early language development.<sup>30–32</sup>

The female advantage in language development is evidenced by young girls scoring higher than young boys on cognitive and language abilities, particularly vocabulary comprehension and production,<sup>32–37</sup> and the observation that males seem to be more susceptible to heritable forms of specific language impairment.<sup>38,39</sup> Despite these common observations, there are still substantial gaps in our understanding of the molecular interactions that mediate language-relevant anatomical maturation, as well as neural circuitries in human infants. The consideration of early sex hormone differences may provide clues as to why girls and boys differ in their early language development.

The rationale of the present study rests on the well-known influence of sex steroid hormones on voice features in both sexes after puberty. It further relies on the finding that a sex hormone surge, comparable in its extent with that of later puberty, occurs during the first months of life. There are reports of sex differences in the *fo* of infant crying; however, the possible contributing factors for these sex differences have not been reported. Because past research examining the effects of body size on acoustic features of infant crying found no effects on vocal *fo*<sup>40</sup> and in the absence of any reports on a sexual dimorphism in infant laryngeal anatomy,<sup>41–44</sup> the observed sex differences in cry *fo*<sup>45–48</sup> could be attributed to hormonal factors.

Consequently, the purpose of the present study was to explore possible associations between serum concentrations of E2 and T and *fo* features (mean *fo*, *fo* range) in spontaneous crying of healthy 2-month-old infants. Accordingly, the research question posed in the present study was: Does mini-puberty, that is, the puberty-like sex-specific postnatal surge of hormones affect *fo* features in spontaneous crying at 8 weeks?

In our opinion, the investigation of young infants’ mean *fo* and *fo* range upon the early postnatal surge of sex steroids (mini-

puberty) provides a suitable and promising approach to advance our understanding of the mechanisms mediating possible sex differences in vocal development and early communication.<sup>5,32,49</sup>

## METHODS

### Participants

Twenty healthy full-term infants (nine boys) with normal hearing and normal thyroid hormone values (TSH < 20 mU/L) served as the sample for the present investigation. All infants were born at the Sana Hospital Lichtenberg in Berlin, Germany. Infants were a subsample of a larger cohort in a broader study examining genetic and external factors influencing language development from birth to about 6 years of age. The second and last authors were two of the project leaders. The hormone subproject associated to this study was dedicated to the investigation of the potential short- and long-term influence of sex hormones on infant vocalization during mini-puberty.<sup>22,30,31</sup> Following ethical approval, the parents of each infant were informed about the purpose of the study and each parent provided written consent for their infant’s participation. All infants selected for the study demonstrated normal development throughout the data collection period and undertook regular medical and developmental checkups. Anthropometric features of body length (cm), body weight (g), and head circumference (cm) were obtained for each infant.

### Data collection

#### *Cry samples*

Digital cry recordings were made in the home environment (quiet room) at 8 weeks of life (mean age 57 days, range 53–60 days) using a TASCAM DA-P1 recorder (TEAC Corporation, USA) and an Earthworks (New Hampshire, USA) directional microphone (TC20). Cry recording began when an infant started to fuss or at a time when the mother would normally feed the child. None of the cries were associated with the administration of a painful stimulus to the infant. Sampling frequency was 48 kHz and the dynamic range was 16 bits. The microphone was positioned approximately 10 cm from the infant’s mouth. The average duration of each infant’s crying episode was approximately 2 minutes.

#### *Hormone data*

Mini-puberty begins in human infants about 1 week after birth, and then rapidly rises over the next weeks, followed by a fading to prepubertal values until the fifth to sixth month.<sup>25–29</sup> The rise is characterized by sex-specific hormone profiles: peak values of serum level T in boys are as high at mini-puberty as they will be in late puberty and adulthood, respectively. In girls, a continuous T decline after delivery is typical.<sup>25</sup> In both sexes, E2 secretions increase (in girls slightly more than in boys) during the postnatal period with highest concentrations found within the first 2 months. This pattern of sex-specific hormones was observed in the present group of infants as displayed in [Figure 1](#). Although the focus of the present study was on the 8-week period of life, the display spans the period of 4–20 weeks to illustrate the typical postnatal pattern of change.

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