Adolescent Development of Biological Rhythms: Estradiol Dependence and Effects of Combined Contraceptives

Azure D. Grant^a, Linda Wilbrecht^{a,b}, Lance J. Kriegsfeld^{a-d}

^aThe Helen Wills Neuroscience Institute, University of California, Berkeley, CA, 94720, United States;

^bDepartment of Psychology, University of California, Berkeley, CA, 94720, United States;

^cDepartment of Integrative Biology, University of California, Berkeley, CA, 94720, United States;

^dGraduate Group in Endocrinology, University of California, Berkeley, CA, 94720, United States

Short Title: Adolescent Development of Biological Rhythms

Key Words: puberty, estrous, birth control, metabolism, signal processing, wavelet analysis.

Address Correspondence to:

Lance J. Kriegsfeld, PhD
Department of Psychology, Integrative Biology,
Graduate Group in Endocrinology and
The Helen Wills Neuroscience Institute
2121 Berkeley Way West, University of California, Berkeley,
CA, 94720, United States
e-mail: kriegsfeld@berkeley.edu
Phone: 510-642-5148
Fax: 510-642-5293

Abstract

Purpose: Adolescence is a period of continuous development, including the maturation of endogenous rhythms across systems and timescales. Although these dynamic changes are well recognized, their continuous structure and hormonal dependence have not been systematically characterized. Given the well-established link between core body temperature (CBT) and reproductive hormones in adults, we hypothesized that high-resolution CBT can be applied to passively monitor pubertal development and disruption with high fidelity.

Methods: To examine this possibility, we used signal processing to investigate the trajectory of CBT rhythms at the within-day (ultradian), daily (circadian), and ovulatory timescales, their dependence on estradiol, and the effects of hormonal contraceptives.

Results: Puberty onset was marked by a rise in fecal estradiol (fE2), followed by an elevation in CBT and circadian power. This time period marked the commencement of 4-day rhythmicity in fE2, CBT, and ultradian power marking the onset of the estrous cycle. The rise in circadian amplitude was accelerated by E2 treatment, indicating a role for this hormone in rhythmic development. Contraceptive administration in later adolescence reduced CBT and circadian power and resulted in disruption to 4-day cycles that persisted after discontinuation.

Conclusions: Our data reveal with precise temporal resolution how biological rhythms change across adolescence and demonstrate a role for E2 in the emergence and preservation of multiscale rhythmicity. These findings also demonstrate how hormones delivered exogenously in a non-rhythmic pattern can disrupt rhythmic development. These data lay the groundwork for a future in which temperature metrics provide an inexpensive, convenient method for monitoring pubertal maturation and support the development of hormone therapies that better mimic and support human chronobiology.

Introduction

Adolescence is a period of rhythmic reorganization during which physiology transitions from a non-reproductive juvenile state into reproductive early adulthood(1–5). Historically, researchers have relied on self-report, tanner staging, or infrequent salivary or blood hormone samples to track milestones of pubertal development. We reasoned that a time series characterization of continuous core body temperature (CBT)(6–10) could reflect dynamic, rhythmic features of hormonal development across the pubertal trajectory. If feasible, CBT could become a highly convenient and more accurate pubertal staging tool with unprecedented temporal resolution. Its application could also improve our understanding of the range of typical pubertal trajectories and factors that drive deviation from these trajectories.

Rhythmic development occurs at multiple timescales, including within-a-day (ultradian rhythms; URs)(11), daily (circadian rhythms; CRs)(12,13), and multi-day ovulatory cycles in females (ovulatory rhythms; ORs)(14). These rhythms occur across physiological systems, serving to increase the efficiency of signal transduction(15–17), temporally segregate incompatible processes(18), synchronize internal systems to the environment(19), and maximize reproductive success(20). Although structure at one timescale can be modulated by changes at another(21,22), distinct mechanisms underlie each rhythmic frequency. This suggests individual rhythmic frequencies may each follow distinct developmental trajectories.

Coordinated URs are observed in hypothalamic-pituitary-peripheral axes and beyond in adult mammals, with broad manifestation in systems including cardiovascular outputs, thermoregulation, and even cognition (reviewed in(6,23–26)). Many URs, including those in reproductive and growth hormones, are present early in life(27) and increase in amplitude from pre-to-mid puberty(28–30). Some URs increase markedly in frequency and amplitude around puberty onset (e.g., gonadotropin releasing hormone (GnRH) luteinizing hormone (LH) and estradiol (E2)(11,28,31–35)(28,35)). In adults, URs are modulated by time of day(36) and

phase of the ovulatory cycle(21), suggesting that pubertal modifications at the CR and OR timescales likely impact UR structure. Although URs are thought to be centrally controlled, potentially via interaction of dopaminergic and hypothalamic circuits(23,37–42), relatively little is understood about mechanisms and progression of URs across adolescence.

Whereas the mechanisms and phenomenology of ultradian development require much further study, those underlying circadian rhythms and pubertal changes to CRs are well documented. Circadian rhythms are nearly ubiquitous, generated intracellularly via interlocked transcription-translation feedback loops, and are governed by a central pacemaker within the suprachiasmatic nucleus (SCN) of the hypothalamus(43–45). Although changes in SCN output and connectivity during adolescence are not well characterized, integration of new neurons into the central clock(46) and reproductive neurocircuitry(47) alongside adolescent increases in SCN input to the GnRH system(48) may contribute to downstream CR changes. For example, circadian amplitude appears to increase across puberty in many systems (e.g., cortisol(49), activity(45), and potentially temperature(50)), while emerging for the first time in others (e.g., LH(34) and FSH(29)). Finally, circadian activity(45) and sleep-wake(51) rhythms are phase delayed during puberty(52) and are more vulnerable to disruption by mistimed light and food cues than in adults(53).

In contrast to ultradian and circadian rhythms, which are apparent to variable degrees in juveniles, the female ovulatory, or estrous, cycle emerges for the first time in adolescence(14). Briefly, in spontaneously-ovulating rodents, the 4 to 5 day cycle begins with rising E2 levels that maintain LH at low concentrations through negative feedback. When E2 is sufficiently high, and a pool of ovarian follicles has matured, E2 positive feedback integrates with circadian signaling and progesterone of neural origin(47,54,55) to induce a preovulatory LH surge that initiates ovulation(55–60). Subsequent formation of the corpus luteum leads to a brief rise in circulating progesterone prior to beginning the next cycle. As with URs and CRs, ORs manifest as

changes in a number of systems, including metabolic hormones, cardiac output, and thermoregulation(61–63). Although it is clear that ORs emerge at puberty, the continuous patterns of commencement and stabilization are poorly understood. Pre-pubertal ovarian follicles typically undergo development and atresia without substantial release of sex steroids(30). Soon after the emergence of the first cycle, at menarche in girls(20), cycles have a higher likelihood of anovulation or low post-ovulatory progesterone compared to adults(64). Although high temporal resolution patterns are unknown, large increases in plasma E2 and FSH occur from pre to mid puberty(28,34,65). Given these changes in hormones across puberty, one aim of the present investigation was to employ continuous, longitudinal, and high-resolution CBT monitoring alongside daily E2 measures to characterize the emergence of the ovulatory cycle in rats.

By characterizing rhythmic outputs that reflect underlying physiological change across adolescence, a greater understanding of typical progression can be garnered and the impact of exogenous hormone manipulation on temporal trajectories can be observed. Temporal disruption at all three timescales is associated with negative health outcomes in adults(66–71), and adolescence may be a sensitive period where disruptions have rapid(1) and potentially long-term health impacts(2,53,72,73). A growing proportion of teenage girls (estimated between 22-54% across the first two decades of the 21st century(74,75)) receive hormonal contraceptives for a variety of purposes, including pregnancy prevention(76) or treatment of menstrual symptoms(77), and acne(78). These hormonal contraceptives aim to prevent ovulation, sperm penetration, and implantation by maintaining elevated progestin levels akin to the post-ovulatory portion of the cycle (with or without estrogen derivatives)(79–83). As they are delivered at static or once daily bolus concentrations that differ from the endogenous, multiscale rhythmic pattern of release(84–86), hormonal contraceptives can be considered a form of temporal endocrine disruption(87,88). Hormonal contraceptive use is associated with elevated body

temperature(89), decoupling of follicular maturation cycles within the ovary(88,90), weight change(91,92), mental health risks(93–95), lasting luteal phase deficiency(96), and a variety of other off target effects(97–100). Furthermore, women under 21 are more likely to exhibit anovulatory cycles following birth control cessation than are older individuals(101), suggesting that contraceptives taken during late adolescence may be more disruptive than in adulthood. Although currently considered safe, discontinuation rate is high(102) and impact on the temporal progression of development is unclear.

CBT as a continuous surrogate marker for pubertal status may provide convenience and new information to longitudinal research studies on this topic and beyond. Continuous measures of activity have been used for rhythmic monitoring(52), but this measure is not as closely tied to high-frequency changes driven by hormonal systems(9,10) and does not appear to predict individual health events with comparable specificity(9,10,103). In contrast, CBT exhibits clear URs, CRs, and ORs that reflect underlying hormonal changes(6,61,104), including ultradian rhythmic CBT patterns that mirror LH(21,105) and estradiol(21) prior to ovulation. Likewise, CBT is a reliable phase marker for circadian rhythmicity(106,107). Hormonal alterations across the ovulatory cycle directly influence CBT, with E2 decreasing temperatures prior to ovulation, and E2 with progesterone increasing temperature following ovulation(8,106). As a result, we hypothesized that insight into changes in the temporal structure of underlying physiology across adolescence can be garnered via assessment of continuous CBT levels and rhythms.

The present study employed continuous CBT to characterize rhythmic change across adolescent development and examine the role of pubertal onset of E2 production in guiding the typical developmental trajectory. Additionally, because late pubertal contraceptive use might act to disrupt the typical progression of rhythmic developmental changes, we examined the impact of a common contraceptive regimen (i.e., ethinyl estradiol and levonorgestrel) on endogenous estradiol concentrations and CBT rhythms. As the pulse amplitude of multiple hormones

increases across adolescence, we hypothesized that the amplitude of CBT URs would be similarly impacted. We also hypothesized that CR amplitude and overall body temperature would increase during adolescence, and that these increases would be influenced by E2. As changes to rhythmicity have primarily been reported from early to mid adolescence, we hypothesized that rhythmic restructuring would be most pronounced during this period. Finally, we hypothesized that rhythmic patterns of body temperature change identified during adolescent development would be disrupted during and potentially after, the cessation of contraceptive administration.

Materials and Methods

Animals. Female Wistar rats were purchased at 250 g from Charles River (Charles River, Wilmington, MA) and bred in the lab and weaned at p21. Weanlings were housed in standard translucent propylene (96 x 54 x 40 cm) rodent cages, and provided *ad libitum* access to food and water, wood chips for floor cover, bedding material, and chew toys for the duration of the study. To minimize social isolation stress, which is known to affect pubertal development(108,109), animals were housed with a same sex, non-experimental sibling. Animals were maintained on a 12:12 light dark (LD) cycle; light intensity during the photo- and scotophases were ~500 lux white light and <1 lux red light, respectively, with lights on at 1 AM and off at 1 PM. Animals were gently handled before weighing daily to minimize stress. To prevent mixing of feces used for hormone analysis, cage mates were separated by a flexible stainless steel lattice that permitted aural, scent, and touch interaction between siblings. A total of 64 animals were included in the study: 32 as experimental animals (Intact, Intact + Contraceptives, Ovariectomized (OVX) and OVX + E2; n=8/group), and 32 as social, littermate partners. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California, Berkeley.

Core Body Temperature Data Collection. Data were gathered with G2 E-Mitter implants that chronically record CBT (Starr Life Sciences Co., Oakmont, PA). At weaning, G2 E-Mitters were implanted in the intraperitoneal cavity under isoflurane anesthesia with analgesia achieved by subcutaneous injections of 0.03 mg/kg buprenorphine (Hospira, Lake Forest, IL) in saline (administered every 12 h for 2 days after surgery). E-Mitters were sutured to the ventral muscle wall to maintain consistent core temperature measurements. Recordings began immediately, but data collected for the first 4 days post-surgery were not included in analyses. Recordings were continuous and stored in 1-min bins.

Ovariectomy and Silastic Capsule Replacement. Ovariectomies were performed at weaning (p21) at the same time as the implantation of the E-Mitter, as previously described(10,57). The E-Mitter surgery served as a control operation in non-OVX animals. Incisions were closed using dissolvable sutures and wound clips. At p29, OVX animals were anesthetized and implanted with silastic capsules (0.78 mm I.D., 1.25 O.D.; Dow Corning, Midland, M). Capsules were implanted subcutaneously and intrascapular. Capsules were 20mm in length with with 5mm silicone sealant at each end (Sigma Aldrich, St. Louis, MO) and contained either 112 μ g (180 μ g/mL) 17 β estradiol in sesame oil, or sesame oil alone. E2 treatment results in plasma E2 concentrations averaging ~ 5 μ g/day, beginning in animals of 80-100g(110,111). Capsules were primed for 24 h prior to implantation via submersion in 0.9% saline in order to avoid delivering a large initial bolus of E2. Although these doses have been tested previously, large variability in serum E2 levels following silastic implant is typically reported(110–113). Incisions were closed using dissolvable suture and a wound clip, and buprenorphine was delivered as above for post-operative analgesia.

Contraceptive Administration. Ethinyl Estradiol (EE2, 30 μ g/day) and Levonorgestrel (30 μ g/day), a progestin, were dissolved in in 0.01 mL of sesame oil and delivered subcutaneously at the nape of the neck daily for the duration of two estrous cycles during mid to late

adolescence (p50 to p58). Although a wide range of rodent doses of EE2 and Levonorgestrel have been reported(114–116), the doses chosen here aimed to match those used consistently for suppressing ovulation and mimicking effects observed in humans, such as increased blood pressure(117), and for comparability to existing rodent literature on subcutaneous delivery of Levonorgestrel and EE2(118–120). Many doses for orally delivered EE2 and Levonorgestrel in rats also fall in this range(82,115,121,122). These drugs have been available as human contraceptives for decades under several brand names in widely varying doses(100,123).

Fecal Sample Collection. Fecal E2 (fE2) concentrations were assessed across puberty from feces generated over 24 h periods. Fecal samples provide hormone concentrations more representative of average daily hormone concentrations than single timepoint blood samples(124–128) and eliminates associated stress and infeasibility of high-frequency, longitudinal blood collection. Samples were collected in small, airtight bags at the end of dark phase under dim red light (<5 lux) from p25 to p37 (pre puberty and first cycle), p45 to p51 (mid-puberty), and p55 to p65 (late puberty to early adulthood) in all groups, and additionally to p75 in Intact+C and Intact groups (adulthood). Samples soiled with urine were discarded, and all other droppings generated over each 24-h segment were combined. Within 1 h of collection, samples were stored at -20°C until processed. Sample collection was rapid (~ 1 min per animal).

Before assessment of hormone concentration, samples were processed according to manufacturers' instructions(129). Briefly, samples were placed in a tin weigh boat and heated at 65°C for 90 minutes, until completely dry. Dry samples were ground to a fine powder in a coffee grinder, which was wiped down with ethanol and dried between samples to avoid cross contamination. Powder was weighed into 0.2 mg aliquots. For hormone extraction, 1.8mL of 100% ethanol was added to each test tube, and tubes were shaken vigorously for 30 minutes. Tubes were then centrifuged at 5,000 RPM for 15 minutes at 4°C. Supernatant was moved to a

new tube and evaporated under 65°C until dry (~ 90 minutes). Sample residue was reconstituted in 100uL of 100% ethanol. 25μL of this solution was diluted for use in the assay and remaining sample was diluted and stored.

ELISA Assays. A commercially available fE2 enzyme-linked immunosorbent assay (ELISA) kit was used to quantify E2 in fecal samples (Arbor Assays, Ann Arbor, MI). These assays have been previously published in species ranging from rats and mice(130–132), to wolves(133), to humans(134). ELISAs were conducted according to manufacturer's instructions. To ensure each sample contained $\leq 5\%$ alcohol, 25μL of concentrate were vortexed in 475μL assay buffer. All samples were run in duplicate, and an inter-assay control was run with each plate. Sensitivity for the assay was 39.6 pg/mL and the limit of detection was 26.5 pg/mL. fE2 intra-assay coefficient of variation (COV) was 5.94% and inter-assay COV was 5.71%.

Data Availability and analysis. All code and data used in this paper are available at A.G.'s and L.J.K.'s Github Respository (<https://github.com/azuredominique>; <https://github.com/Kriegsfeld-Lab>)(135). Code was written in MATLAB 2020b with Wavelet Transform (WT) code modified from the Jlab toolbox and from Dr. Tanya Leise(136,137). Briefly, data were imported to MATLAB at 1-min resolution. Any data points outside ± 4 standard deviations were set to the median value of the prior hour, and any points showing near instantaneous change, as defined by $\text{local abs(derivative)} > 10^5$ as an arbitrary cutoff, were also set to the median value of the previous hour. Small data gaps resulting from intermittent data collection (<10 minutes) were linearly interpolated. Continuous data from p26 to p74 were divided into three equal-length phases: early to mid puberty (p26 to p41), mid to late puberty (p42 to p58), and late puberty to early adulthood (p59 to p74).

Wavelet Analyses and Statistics of CBT Data. Wavelet Transformation (WT) was used to generate a power estimate, representing amplitude and stability of oscillation at a given periodicity, within a signal at each moment in time. Whereas Fourier transforms allow

transformation of a signal into frequency space without temporal position (i.e., using sine wave components with infinite length), wavelets are constructed with amplitude diminishing to 0 in both directions from center. This property permits frequency strength calculation at a given position. In the present analyses we use a Morse wavelet with a low number of oscillations (defined by $\beta=5$ and $\gamma=3$, the frequencies of the two waves superimposed to create the wavelet(138)), similar to wavelets used in many circadian and ultradian applications(9,10,136–139). Additional values of β (3–8) and γ (2–5) did not alter the findings (data not shown). As WTs exhibit artifacts at the edges of the data being transformed, only the WT from p26 to p74 were analyzed further. Periods of 1 to 39 h were assessed. For quantification of spectral differences, WT spectra were isolated in bands; circadian periodicity power was defined as the max power per minute within the 23 to 25 h band; ultradian periodicity power was defined as the max power per minute in the 1 to 3 h band. The latter band was chosen because this band corresponded with the daily ultradian peak power observed in ultradian rhythms (URs) across physiological systems in rats(6,140–142).

For statistical comparisons of any two groups, Mann Whitney U (MW) rank sum tests were used to avoid assumptions of normality for any distribution. Non-parametric Kruskal-Wallis tests were used instead of ANOVAs for the same reason; for all Kruskal-Wallis (KW) tests, χ^2 and p values are listed. In cases of repeated measures within an individual over adolescence, Friedman's tests were used. Data from each estrous cycle were treated as independent. We chose to treat estrous cycles as independent for 3 reasons: 1) the estrous cycle is the longest periodicity rhythm within the study, 2) the adolescent estrous cycle develops rapidly from one iteration to the next in rats, and 3) because the interventions of ovariectomy and birth control administration exert their effects by removal or modification of estrous cycles. Dunn's test was used for multiple comparisons. Mann Kendall (MK) tests were used to assess trends over time in wavelet power and linear CBT over three equally sized temporal windows

described above. For short term (<3 days of data) statistical comparisons, 1 data point per hour was used; for longer term (>3 days of data) statistical comparisons, 1 data point per day was used. Continuous wavelet power data were smoothed with a 24 h window using the MATLAB function “movmean”. Violin plots, which are similar to box plots with probability density of finding different values represented by width(143), were calculated using the MATLAB function “violin”. Median daily circadian power was regressed against each day’s fE2 for each group using a mixed effects linear regression (MATLAB function “fitlme”, formula: circadian daily medians~1+E2 values + (1+ E2 |Individual ID).

Data alignment and Analysis of E2 Concentrations and Estrous Cyclicity. The day of fE2 rise before cycling began was defined as the first day fE2 level rose > 2 standard deviations above its starting value at p25. The initial rise in fE2 was used as an alignment point for CBT, ultradian power, and Z-Score(CBT) - Z-Score(UR power) group averages. Group differences in fE2 area under the curve by cycle were assessed using the MATLAB function “trapz” and KW tests with Dunn’s post hoc correction. As estrous cycles are not all aligned in time or by age, samples were aligned with the highest value in a collection period (e.g., mid puberty) where a ‘fall’ was observed three days later. Fast Fourier Transforms (FFT) were used to assess the presence or absence of 4-5 day power in CBT in each individual from the period of fE2 rise until p50 (when Intact+C animals started receiving daily contraceptive injections), and from p50 to p74. In order to further assess commencement and stability of estrous cycling after first rise in fE2, as well as any potential perturbation during and after contraceptive administration, metrics were divided into 4 day blocks, with each day labelled 1,2,3, and 4: repeating for subsequent cycle lengths. Groups for statistical comparison were constructed from all data corresponding to 1’s, 2’s, 3’s and 4’s. Friedman’s tests with Dunn’s correction for multiple comparisons were used to determine if values associated with each day of cycle (e.g., all day 1’s) varied significantly from other days of the cycle by group.

Results

Impact of Hormonal Status on Estradiol Concentrations and Weight Gain Across Adolescence.

Frequent fecal estradiol (fE2) measurements were collected to assess if hormonal status affected the level or temporal patterning of fE2 across puberty. FE2 concentrations did not differ between groups from p25-p31, a baseline period prior to puberty onset ($\chi^2=4.48$, $p=0.214$; **Figure 1A**). Vaginal opening occurred between p31 and p33 in Intact rats, and fE2 rose 2 standard deviations above its p25 starting value between p31 and p36.

During this window, Intact and Intact+C animals' fE2 concentrations exceeded that of OVX animals ($\chi^2=15.9$, $p=0.001$; $p=0.0134$ and $p=0.003$, respectively; **Figure 1B**). This difference was maintained at mid puberty (cycles aligned from p40 to p47) ($\chi^2=13.7$, $p=0.003$; $p=0.003$ and 0.032 , respectively) and early adulthood (cycles aligned from p55 to p61) ($\chi^2=17.1$, $p=0.001$; $p=0.001$ and 0.009 , respectively; **Figure 1C-D**). OVX+E2 animals were not different from other groups at any timepoint, with intermediate values between Intact and OVX groups ($p>0.05$ in all cases). Unlike Intact animals, Intact+C animals did not exhibit days of elevated fE2 every 4th day (See **Supplemental Figure 1**). However, fE2 concentrations did not differ between Intact and Intact+C groups approximately 4 to 5 cycles after contraceptive administration ceased, between p69 to p75 ($\chi^2=1.62$ $p=0.203$). See **Methods** for details of within-cycle alignment.

Additionally, daily weights were measured to recapitulate known effects of E2 on pubertal growth trajectory, and to assess if contraceptive administration modulated weight gain. Intact animals gained weight consistently across puberty. Pre-pubertal OVX was associated with increased body weight at mid puberty, with OVX animals weighing more than animals from all other groups, and OVX+E2 animals weighing more than Intact or Intact+C groups ($\chi^2=57.9$, $p=1.65 \times 10^{-12}$; $p<0.05$ for all individual comparisons). By early adulthood, both OVX and OVX+E2 groups weighed significantly more than Intact or Intact+C groups and did not differ from one

another ($\chi^2=76.1$, $p=2.08 \times 10^{-16}$; $p=0.99$ for OVX vs. OVX+E2; $p<0.05$ for all other comparisons). Eight days of contraceptive administration did not significantly impact weight relative to Intact animals ($\chi^2=0.28$, $p=0.594$; See **Supplemental Figure 2**).

Circadian, but not Ultradian, Power of Body Temperature Increased Across Pre-to-Mid Adolescence. As reproductive circadian and ultradian rhythms change markedly across adolescence and may be coupled to CBT(32,49,144), we investigated the impact of estradiol status on the timing and tempo of CBT rhythmicity. All animals exhibited a significant positive trend in CR power from pre-to-mid adolescence (p26 to p41) ($p=0.002$, 0.007 , 0.029 , 0.04 for Intact, Intact+C, OVX, and OVX+E2 animals, respectively). CR power stabilized thereafter ($p>0.05$ in all cases; **Figure 2A-B**). To examine the relative rate of this increase across groups, we set a criterion of 2 standard deviations above the mean. CR power rose 2 standard deviations above the mean significantly faster in OVX+E2 animals compared to Intact or OVX animals ($\chi^2=19.0$, $p=3 \times 10^{-4}$; $p=0.025$ and 0.001 , respectively; **Figure 2L**).

Ultradian power did not show a significant upward or downward trend across the study period in any group ($p>0.05$ for all groups at all time windows) (**Figure 2A-D**). Although directionality of CR power change across adolescence was similar (i.e., an early increase followed by a plateau) in all individuals, magnitude of circadian power differed among groups. Specifically, circadian power for OVX animals was depressed compared to all other groups from pre to mid adolescence ($\chi^2=125$, $p=4.02 \times 10^{-27}$, $p<0.02$ for OVX vs. all other groups; **Figure 2E**). From mid to late adolescence (p42 to p58), CR OVX power remained depressed and OVX+E2 trended toward lower power ($\chi^2=112$, $p=2.82 \times 10^{-24}$; $p<0.01$ Intact and Intact+C vs OVX; **Figure 2F**). In early adulthood (p59 to p74), following contraceptive administration, the CR power for Intact+C was depressed compared to Intact rats ($\chi^2=37.9$, $p=2.93 \times 10^{-08}$, $p=0.04$; **Figure 2G**).

Body Temperature Circadian Power was Most Correlated to Fecal Estradiol Level in Unmanipulated Animals. Temperature level, UR power, and E2 exhibited coupled patterning,

and appeared to change markedly during adolescence. However, it was unclear if CBT circadian rhythmicity was coupled to estradiol, if such a relationship existed during adolescence, and if the relationship could be modified by hormonal state. FE2 and normalized circadian power were strongly correlated in Intact ($R^2=0.226$, $p=2.90 \times 10^{-4}$) and Intact+C rats prior to p50 when contraceptive administration began ($R^2=0.166$, $p=0.025$), and weakly correlated in OVX+E2 animals ($R^2=0.062$, $p=0.001$; **Figure 2 H-K**). This positive correlation was abolished during and after hormonal contraceptive administration in the Intact+C group ($R^2=1.06 \times 10^{-5}$, $p=0.58$; **Figure 2I, inset**). Circadian power and fE2 was not significantly correlated in OVX animals ($R^2=0.013$, $p=0.06$; **Figure 2J**).

Ovulatory Rhythms in CBT and Perturbations During and After Contraceptive Administration.

We next investigated the relationship between ORs and cycles in body temperature. Our goal was to determine if interactions at this timescale a) could be observed in continuous body temperature in adolescents, b) if previously described hormonal UR modulations by phase of cycle(145,146) translates to ovulatory cycles in CBT URs, and c) if exogenous hormone administration disrupts these continuous dynamics. In intact rats, we observed a significant 4-day modulation of combined CBT and UR power corresponding to the estrous cycle (Intact $\chi^2=59.1$, $p=9.26 \times 10^{-13}$, Intact+C group prior to BC administration $\chi^2=13.7$ $p=0.003$; **Figure 3A-B, 4A-B**). This 4-day pattern commenced in Intact and Intact+C rats (prior to treatment) with a significant increase in mean daily CBT following the first rise in fE2 above 2 standard deviations ($p=0.03$ in each case). Intact and Intact+C (prior to treatment) animals also exhibited a 4-day pattern of UR power modulation. This modulation manifested as a significant trough of UR power within 4 days of the first rise of fE2, as previously reported in adult rodents(9,10,147,148) ($p=0.04$, $p=0.03$, respectively). The combination of UR power and linear temperature yielded a more easily separable metric which rose significantly the day after first rise of fE2 in Intact and

Intact+C ($p=0.01$, $p=0.02$, respectively; **Figure 3A-B**). (For individual metric comparisons see **Supplemental Figure 3**).

We noted an absence of significant 4-day differences in combined CBT and UR power in the Intact+C group during hormonal contraceptive administration, even following 4 cycle-lengths of recovery (Intact+C group $\chi^2=7.2$, $p=0.07$; Intact group over same time period $\chi^2=58.9$, $p=1.00 \times 10^{-12}$). A FFT of data in Intact and Intact+C animals prior to contraceptive administration revealed comparable AUCs for 4 to 5 day oscillations (no group difference; $\chi^2=0.54$, $p=0.46$; **Figure 3C, Inset**). However, after hormonal contraceptive administration, AUC for Intact animals was significantly greater for 4 to 5 day oscillations than in Intact+C animals ($\chi^2=3.98$, $p=0.046$; **Supplemental Figure 4**). As expected, a 4-day pattern was also absent in OVX and OVX+E2 animals ($p>0.05$ in both cases). Note that 5-day cycles occurred rarely in Intact rats and using 5-day bins rather than 4-day bins abolished significant differences by day of cycle for all groups (*data not shown*).

CBT Increased in Early to Mid Adolescence. As growth and metabolism speed in adolescence, we hypothesized that CBT would also increase during the period of most rapid growth, pre to mid puberty. Pre to mid puberty (p26-41) was associated with a significant positive trend in CBT in Intact ($p=5.75 \times 10^{-5}$) and Intact+C animals prior to contraceptive administration ($p=1.20 \times 10^{-4}$; **Figure 4A-B**). Notably, implantation of the silastic capsule in OVX and OVX+E2 animals resulted in a transient (one day) increase in CBT (OVX $p=0.01$, OVX+E2 $p=0.03$; **Figure 4C-D, Supplemental Figure 5**). This surgical-recovery-associated rise was highly variable and did not differ between OVX and OVX+E2 animals ($p=0.65$; **Supplemental Figure 5**). Interestingly, the early pubertal CBT increase did not require E2, as OVX animals also exhibited a significant positive trend ($p=0.034$; **Figure 4C,E**). For a summary guide to CBT features that may be useful for Intact pubertal staging, see **Supplemental Figure 6**).

CBT Maintenance in Late Adolescence to Adulthood Required Estradiol. Complex interactions exist between metabolism, growth, and E2 level during adolescence. As estrogen deficiency in puberty is associated with weight gain and reduced metabolism, we investigated if maintenance of elevated temperature would be impacted by hormonal status. The maintenance of increased temperature in late puberty and adulthood was E2-dependent, with OVX animals exhibiting a significant downward trend in CBT from mid to late puberty (p58-p74; $p=0.01$) relative to Intact and OVX+E2 animals ($p>0.05$ in each case; **Figure 4C-D**). E2 treatment in the OVX+E2 group prevented intra-individual CBT decline in late puberty; correspondingly, temperatures in the OVX but not OVX + E2 groups were lower than that of Intact animals (late puberty to early adulthood $\chi^2=62.8$ $p=1.46 \times 10^{-13}$, $p=4 \times 10^{-4}$ for Intact vs. OVX, $p=0.16$ for Intact vs. OVX+ E2; **Figure 4F-G, Supplemental Figure 5**).

Contraceptive Administration Longitudinally Depressed CBT. CBT power did not exhibit a positive or negative trend from mid puberty through early adulthood (p42 to p58) in Intact animals ($p=0.12$), but exhibited a significant downward trend in Intact+C animals during the period of contraceptive administration ($p=0.028$; **Figure 4A-B**), resulting in a trend toward depressed temperatures following administration in mid to late adolescence ($\chi^2=21.84$ $p=7.04 \times 10^{-5}$, $p=0.1$ for Intact vs. Intact+C) that persisted into early adulthood ($\chi^2=62.83$ $p=1.46 \times 10^{-13}$, $p=0.1$ for Intact vs. Intact+C; **Figure 4B, 4F-G**).

Discussion

Adolescent Development and Estradiol Dependence of CBT Rhythmicity. Female adolescence is characterized by stereotyped development of CBT rhythms at the ultradian, circadian, and ovulatory timescales. The present findings reveal that early adolescence in the female rat is marked by rising CBT and CBT circadian power, and commencement of 4-day cycling in CBT and CBT URs. These early circadian and ultradian changes likely reflect maturation of the reproductive axis, as 1) the rate of CR power rise was hastened by estradiol, 2) the

commencement of 4-day temperature cycling was preceded by a rise in fE2, and 3) CBT CR power was correlated with fE2 concentration(65,149). These observations confirm and extend reports of early pubertal development of ultradian-circadian-ovulatory interactions(46) (47), and suggest that reproductive-thermoregulatory coupling may develop prior to, or in tandem with, pubertal onset.

Adolescent increases in UR amplitude for many endocrine outputs are well-documented, but UR structure across all of puberty is not well mapped(28,65). However, from the time of rise in fE2 (a defining marker of pubertal onset), CBT UR power retained the same mean value but commenced a 4-day, ovulatory cycle-associated pattern. Four day cycles in URs are consistent with data collected on URs in adult rodents(10,147,148) and humans (at a longer time scale)(139). Four-day patterning was not present in ovariectomized or E2-replaced animals, consistent with dependence on the ovarian cycle. These results support that URs in CBT achieve adult amplitude and stability early in life, prior to maturity of CRs(150–152), and that interactions among thermoregulatory and reproductive circuits permitting rapid *ultradian* coupling are established well before pubertal onset.

Additionally, the present findings suggest that some dynamics of CBT development require specific patterns of E2 rather than simply concentrations above a particular threshold. Ovariectomy eliminated ORs, increased weight, reduced CR power, rate of CR power rise, and correlation between CR power and fE2, and overall temperature(153,154). E2 replacement partially rescued circadian metrics and reduced weight but did not recapitulate ORs. Short-term exposure to contraceptives in late adolescence longitudinally altered CBT metrics by reducing temperature and CR power, and abolishing ORs in CBT and UR power. Despite the apparently early maturation of substrate for thermoregulatory and reproductive coupling, the impact of E2 replacement and the enduring effects of short-term contraceptives suggest that these systems

are sensitive to both level and patterning of reproductive hormones across the adolescent period.

Together, temperature amplitude and oscillation stability at the UR, CR, and OR timescales are modulated across the adolescent transition and are influenced by endogenous and exogenous E2. CBT URs mature to their adult amplitude and stability prior to CRs, and ORs and are rapidly modulated by ovulatory phase in intact animals (**See Supplemental Figure 6**). Conversely, CRs increase in magnitude and stability in early adolescence, are not significantly impacted by the phase of OR, and are impacted by ovariectomy. These results support the notion that ultradian and ovulatory systems are tightly coupled and reflected in CBT, and that circadian effects on the ovulatory cycle may be unidirectional(22,139). Ovariectomy, E2 replacement, and pubertal contraceptive administration have a number of effects on rhythmic dynamics at each timescale, overall indicating that ‘intact’ dynamics are not easily recapitulated and that exogenous sex steroids can have enduring impact.

Limitations. The ovulatory cycle of the female rat differs from that of humans, in that rats do not exhibit prolonged elevation of progesterone in the absence of pregnancy or pseudopregnancy(155). This lack of a true luteal phase means that post-ovulatory temperature elevation in the rat follows a more compressed trajectory than in humans(156). Therefore, exposure to estrogen and progesterone analogs in rats for prolonged periods may be associated with a different phenotype in rats than in humans(157). Finally, the laboratory environment imposes artificially stable environmental conditions on animals; recapitulation of these patterns under naturalistic conditions in future experiments will strengthen the translational potential of this work.

Considerations of Rhythmicity Perturbation Through Adolescent Contraceptive Use. Although rats cannot fully model human biology, it was notable that contraceptive administration imposed lasting structural changes on CBT rhythmicity and its relationship with E2. Levonorgestrel and

EE2 administration abolished the 4-day modulation of fE2, UR power, and temperature level, eliminated the correlation between CR power and fE2, and significantly depressed CR power. As these changes required continuous monitoring to detect and occurred in the absence of significant group changes to fE2 level, it is not unreasonable to speculate that studies of less frequently timed samples, or samples averaged across individuals, could make similar disruptions in humans difficult to detect.

Perturbation of body temperature rhythms is associated with diverse health insults across species, both reflecting perturbation in underlying systems and potentially acting as a causative agent. Circadian disruption to CBT rhythms occurs in, and is proportional to, severity of jetlag(158), depression(159), sepsis severity(160,161), post-traumatic injury(162), cognitive decline(163), and has even been proposed as a root cause of disease dubbed “Circadian Syndrome”(164) . Disruption to ovulatory temperature rhythms occurs in anovulatory and atypical luteal phase cycles(165–167), including those arising from polycystic ovarian syndrome (PCOS)(168). The impact of ultradian rhythmic disruption of the reproductive axis requires additional study(169–171), but existing work in other hormonal systems suggests that preservation of pulsatility in drug delivery (e.g., of cortisol in Addison’s disease or insulin in diabetes) can lead to better patient outcomes when compared to conventional non-rhythmic treatment(66,67,104,172,173). It is likely that disruption of URs may result in negative impact analogous to disruption at longer timescales. It may appear counter-intuitive that thermoregulation could be both a reporter for such diverse maladies and a potential mediator for disease progression. However, temperature rhythm disruption is associated with a wide range of temporal, inflammatory, and endocrine insults, in part, because thermoregulatory circuits are directly impacted by the master clock, and modulated by inflammatory factors, autonomic status, and a variety of endocrine factors including estradiol(8,174) and progesterone(7). Lastly, CBT itself acts as a synchronizing cue for peripheral circadian oscillators(175). Further

research is needed to disentangle if disruption of CBT rhythmicity itself causes harm, or if CBT rhythms are merely reporting perturbations in underlying systems (e.g., sex hormones or metabolic factors).

Our observations suggest that a non-physiological pattern of contraceptive administration may act as ‘hormonal jetlag’. The degree of lasting impact to other systems that rely on temperature as an entraining stimulus, or disrupted systems reported indirectly by temperature (e.g., SCN, endocrine, autonomic) remain to be assessed. Furthermore, the extent to which such disruptive effects differ among species(157), contraceptive agents, administration methods, or between adolescent populations and adults requires further investigation. Encouragingly, the observation that rodents and humans exhibit similar CBT patterning during the peri-ovulatory period(10,139,140) points to potential translational relevance

Together, despite established societal benefits of widely available hormonal contraception(176), especially in individuals experiencing hormonal irregularities(177), the present findings suggest that administration of exogenous estrogens and progestins during adolescence leads to persistent rhythmic disruption across timescales. Future research is needed to determine if rhythmic patterns of sex steroid administration more closely mimicking endogenous release, analogous to those implemented in cortisol(67) and closed loop insulin therapy(178), can minimize rhythmic disruption. Conversely, future studies that validate an “updated” symptom-thermal method using signal processing of continuous CBT data may provide feasible, non-disruptive alternatives for contraception(106,139,179–181). Contraceptive administration to adolescent girls is on the rise(74), and there are a paucity of data on the impact of chronic hormonal perturbation on endogenous rhythmicity, or if such disruptions during the sensitive window of adolescence have lasting effects(93,94,101,182,183). Further research is needed to characterize the impact of adolescent hormonal contraceptive use so that

further improvements can be made, individuals at-risk for side effects can be identified, and informed decisions about family planning can be made.

Utility of CBT for Monitoring Pubertal Development in Rodents and Potential Translational Relevance. Continuous monitoring of CBT may have great utility for passive detection of pubertal milestones in rodents in preclinical research. Existing methods for pubertal staging in rodents carry considerable downsides: frequent blood sampling and vaginal lavage(184) are repeatedly invasive, and fecal hormone analysis is time consuming and costly(124,185). Moreover, our results indicate that individual rats do not traverse identical pubertal trajectories by day of life, and that this assumption could lead to considerable errors in staging. Conversely, signal processing of passively collected CBT can add temporal resolution, greater quantitative power, and limit repeated invasive procedures for staging puberty(186).

In human subjects, continuous temperature monitoring via wearables may similarly facilitate the study of pubertal development. The signal characteristics of rat CBT exhibit remarkable similarities to human peripheral temperature (See:(61,148,187)). Monitoring human peripheral temperature during adolescence could serve broad purposes – from personalizing health education based on self-collected data(188,189), to adapting teaching style to an individual's developmental phase(190–193), to enabling research into the impact of teen contraceptive use(194,195) or the process of gender transition(196,197). Together, pubertal monitoring via continuous body temperature is worthy of further investigation in both animal models and human subject populations for its potential utility to individuals, researchers, families, and clinicians.

Conclusion. In conclusion, body temperature monitoring provides a window into the development of biological rhythms in puberty over multiple timescales. These rhythms may serve as convenient and high temporal resolution indicators of developmental stage and trajectory for application in research and clinical studies. Our study of body temperature also

reveals unintended side effects of tonic hormonal manipulations. We anticipate that these findings will inform creative improvements to female reproductive research and healthcare.

Acknowledgements: The authors would like to thank Andrew Ahn, Ronald Dahl, Frederic Théunissén, and Albert Qü for their helpful feedback on study design and methods and invaluable comments and suggestions on an earlier version of this manuscript.

Funding: This work was funded by SL-CN grant #1640885, NIH grant HD-050470

Figure Legends

Figure 1. High Frequency Fecal Estradiol Enables Monitoring of Estrous Cycle Emergence, Sex Steroid Manipulation, and Ovarian Status During Adolescence. Group mean ($\pm$ S.E.M.) fecal estradiol concentrations of Intact (light blue), intact + short-term pubertal contraceptives (Intact+C; dark blue), OVX (gray), and OVX+E2 (orange) groups did not significantly differ prior to puberty (p24-30) (A). Fecal estradiol in Intact and Intact+C groups increased over that of OVX animals beginning at the first cycle following vaginal opening or relative to the time of silastic implant in OVX+E2 animals (p30-37) (B) and remained significantly elevated thereafter at mid puberty (p43-p49; C) and during late puberty (p55-61; D). Dark horizontal bar in D indicates Intact+C data were gathered during contraceptive administration. Color of letters at the top of B, C, and D indicate experimental group; letters indicate statistical differences, with groups not sharing the same letter being significantly different ($p < 0.03$).

Figure 2. Level and Rate of Early Adolescent Rise in CBT Circadian Power are Tied to Estradiol. Circadian, but not ultradian power rises across early adolescence (A-D). Linear plots of group mean ($\pm$ S.E.M.) of CBT circadian (solid) and ultradian (dashed) power in Intact (light blue, A), Intact+C (dark blue, B), OVX (gray, C) and OVX+E2 (orange, D) animals. * Indicates significant trend over time for the bracketed time region ($p < 0.05$). Phase of adolescent time periods (pre to mid, mid to late, and late to adult) are indicated by black dividers in the colored x-axis at p42 and p58. Although CBT circadian power rises over early adolescence in all groups, estradiol increases the rate of this rise (L). Violin plots (E-G) of circadian power in each group analyzed by segment of life: pre to mid adolescence from p26-41 (E), mid to late adolescence from p42-57 (F), and late adolescence through early adulthood from p58-73 (G) illustrate that CR power is highest in Intact animals, with reduction following hormonal contraceptive administration and OVX, with a partial rescue in OVX+E2 animals. Black lines indicate mean and red lines indicate median of each plot. Scatters of fE2 level by CR power indicate that

hormonal manipulation reduces or eliminates the correlation between CR power and fE2 concentrations (H-K). Note that each individual within a group is plotted in a unique color. CR power and fE2 are significantly correlated in Intact (light blue, H), Intact+C animals prior to contraceptive administration (dark blue, I), with inset depicting abolished correlation during and after contraceptive administration. CR power and fE2 are weakly correlated in OVX+E2 (orange, K) but not OVX animals (J). * Indicates significant positive correlation between fE2 and circadian power. AIC indicates relative performance of the mixed effects model. Color of letters at the top in E-G and L indicate experimental group; letters indicate statistical differences, with groups not sharing the same letter being significantly different ($p < 0.03$).

Figure 3. Contraceptive Administration in Adolescence Persistently Perturbs 4-Day Temperature Rhythms. Normalized CBT mean ($\pm$ S.E.M.) minus UR power relative to within-individual first day of fE2 rise in Intact (A) and Intact+C (B) rats (see: Methods and Supplemental Figure 3). Dark bars along the x-axis for Intact+C animals indicate average time of contraceptive administration relative to fE2 rise. * Indicates regions of time over which every 4th day's CBT values are significantly elevated compared to other days of cycle ($p < 0.003$). Twenty-four hour smoothed average plots of normalized linear CBT in Intact (C) and Intact+C (D) individuals from the time of contraceptive administration illustrate a reduction in regularity of oscillations. Insets show FFT centered at 4-5 days. * Indicates significantly higher AUCs in the 4-5 day range for Intact (panel C) compared to Intact+C rats (panel D). Individual animals (E-T) comprising Intact (light blue) and Intact+C (dark blue) groups prior to and following hormonal contraceptive administration. Dark bars along horizontal axes indicate time of contraceptive administration; administration days differ based on an individual's day of fE2 rise.

Figure 4. Female Adolescence is Associated with Sex Steroid-Dependent CBT Levels and Trends. CBT linear group means ($\pm$ S.E.M.) in Intact (A, light blue) compared to Intact+C (B, dark blue), OVX + sham (C, gray), and OVX+E2 (D, orange) animals. * Indicates significant

trend during the bracketed time period for the group matching the color of the bracket ($p < 0.03$). Phase of adolescence time periods (pre to mid, mid to late, and late to adult) are indicated by breaks in the colored x-axis at p42 and p58. Time of hormonal contraceptive administration in panel B is indicated by the tall horizontal bar. Violin plots of temperature for all groups at early to mid adolescence (E), mid to late adolescence (F), and early adulthood (G) indicate that hormonal contraceptive administration leads to reductions in CBT relative to controls after administration. Ovariectomy, even with E2 replacement, is also associated with significantly reduced temperatures by early adulthood (G). Color of letters at the top in E-G indicate experimental group. Letters indicate statistical differences, with groups of different letters being significantly different ($p < 0.001$).

Supplemental Figure Legends

Supplemental Figure 1. Estradiol Group Averages from Pre-Puberty to Adulthood Post-Contraception: Contraceptives Longitudinally Alter the Pattern but Not Level of Fecal Estradiol. Pre-puberty (p24-p30), early puberty (p30-p37), mid puberty (p43-p49), late puberty (p55-p61) and early adulthood (p69-p76) in Intact (A), Intact+C (B), OVX (C) and OVX+E2 (D). Early adulthood post-contraceptive administration, captured only in Intact and Intact+C animals, are overlaid in (E), illustrating persistent perturbation of estrous-cycle estradiol patterning. Dark bar indicates samples were collected during contraceptive administration in Intact+C animals. * Indicates significant elevation of day 3 compared to day 1 ($p < 0.05$).

Supplemental Figure 2. OVX and OVX + E2 are Heavier than Intact Animals in Mid Puberty and Early Adulthood. Prepubertal OVX did not significantly impact weight gain before mid-puberty. OVX animals (gray) are significantly heavier than all other groups at mid-puberty. OVX animals remain significantly heavier into adulthood. In early adulthood, OVX+E2 animals (orange) are

not different from OVX animals and are significantly heavier than either Intact (light blue) or Intact+C animals (dark blue). Colors of * Indicate groups that are significantly heavier ($p < 0.05$).

Supplemental Figure 3. Day of First Estradiol Rise Occurs Near Vaginal Opening and Coincides with Markers of Estrus: Subsequent Drop in UR Power and Rise in CBT. Estradiol has a unique day in proximity to, but distinct from, vaginal opening, during which fE2 rises > 2 Standard Deviations within an individual (A-B). Alignment to this day as a proxy for puberty onset provides a convenient point from which to average across animals to permit easy visualization of the estrous cycle. Daily mean UR power decreases significantly following this day (C), and daily mean CBT rises significantly following this day (D), The difference between these two metrics exaggerates the difference, and the differences across subsequent cycles (E). After this point, all three metrics commence the 4-day estrous pattern. * Indicates significant difference from all other time periods.

Supplemental Figure 4. FFT Power of Intact Temperature Across Life Peaks Unique at 4 Days and is Disrupted During and After Contraceptive Administration. Fast Fourier Transform of Each Individual's CBT Log in Intact (light blue) and Intact + BC (dark blue) prior to contraceptive administration (left), and during and after contraceptive administration (right). * Indicates significant difference for AUC between the groups in 4-5 periodicity.

Supplemental Figure 5. Estradiol Administration in Ovariectomized Animals is Associated with Lower Temperatures Across Adolescence. Core CBT linear group means ($\pm$ standard deviation) in OVX (gray) and OVX+E2 animals from p28-p72. Box plots indicate values used in KW comparisons between groups during silastic implant recovery (left) and across adolescence (right) with independent y-axes. * Indicates significant difference between groups during the marked time period ($p < 0.03$).

Supplemental Figure 6: Summary of CBT Feature Use for Pubertal Staging in an Intact Female.

Features of CBT and CBT rhythmicity can be used to complement and extend hormonal and external markers of adolescence. Here, we illustrate derived features on an example Intact female from p26 to p74, with fE2 rise and vaginal opening occurring on p31 and p32, respectively. Dots indicate vaginal opening and “e” indicates first rise of fE2 > 2 standard deviations. Rising CBT, emergence of ORs in UR power, and Rising CR power can be used to characterize pubertal onset. Mid to late adolescence is characterized by a plateau of CR power, as well as the emergence of ORs in raw CBT their persistence in UR power. Post-natal day 60 did not correspond with any overt transitions in CBT or CBT metrics.

References

1. Jain Gupta N, Khare A. Disruption in daily eating-fasting and activity-rest cycles in Indian adolescents attending school. *PloS One*. 2020;15(1):e0227002.
2. Pereira LR, Moreira FP, Reyes AN, Bach S de L, Amaral PL do, Motta JDS, et al. Biological Rhythm Disruption Associated with Obesity in School Children. *Child Obes Print*. 2019 Apr;15(3):200–5.
3. Smarr BL, Schirmer AE. 3.4million real-world learning management system logins reveal the majority of students experience social jet lag correlated with decreased performance. *Sci Rep*. 2018 Mar 29;8(1):4793.
4. Mohr MA, Sisk CL. Pubertally born neurons and glia are functionally integrated into limbic and hypothalamic circuits of the male Syrian hamster. *Proc Natl Acad Sci*. 2013 Mar 19;110(12):4792–7.
5. Sisk CL, Foster DL. The neural basis of puberty and adolescence. *Nat Neurosci*. 2004 Oct;7(10):1040–7.
6. Grant AD, Wilsterman K, Smarr BL, Kriegsfeld LJ. Evidence for a Coupled Oscillator Model of Endocrine Ultradian Rhythms. *J Biol Rhythms*. 2018 Aug 22;33(5):475–96.
7. Forman RG, Chapman MC, Steptoe PC. The effect of endogenous progesterone on basal body temperature in stimulated ovarian cycles. *Hum Reprod Oxf Engl*. 1987 Nov;2(8):631–4.
8. Williams H, Dacks PA, Rance NE. An Improved Method for Recording Tail Skin Temperature in the Rat Reveals Changes During the Estrous Cycle and Effects of Ovarian Steroids. *Endocrinology*. 2010 Nov 1;151(11):5389–94.
9. Smarr BL, Zucker I, Kriegsfeld LJ. Detection of Successful and Unsuccessful Pregnancies in Mice within Hours of Pairing through Frequency Analysis of High Temporal Resolution Core Body Temperature Data. *PloS One*. 2016;11(7):e0160127.
10. Smarr BL, Grant AD, Zucker I, Prendergast BJ, Kriegsfeld LJ. Sex differences in variability across timescales in BALB/c mice. *Biol Sex Differ*. 2017;8:7.
11. Bourguignon JP. Time-related neuroendocrine manifestations of puberty: a combined clinical and experimental approach extracted from the 4th Belgian Endocrine Society lecture. *Horm Res*. 1988;30(6):224–34.
12. MacKinnon PCB, Puig-Duran E, Laynes R. Reflections on the attainment of puberty in the rat: have circadian signals a role to play in its onset? *Reproduction*. 1978 Mar 1;52(2):401–12.
13. Garcia J, Rosen G, Mahowald M. Circadian rhythms and circadian rhythm disorders in children and adolescents. *Semin Pediatr Neurol*. 2001 Dec;8(4):229–40.
14. Vidal JD. The Impact of Age on the Female Reproductive System. *Toxicol Pathol*. 2017 Jan;45(1):206–15.

15. Brodsky VY, Lloyd D. Self-Organized Intracellular Ultradian Rhythms Provide Direct Cell-Cell Communication. In: Lloyd D, Rossi EL, editors. *Ultradian Rhythms from Molecules to Mind: A New Vision of Life* [Internet]. Dordrecht: Springer Netherlands; 2008 [cited 2021 Jan 8]. p. 85–104. Available from: https://doi.org/10.1007/978-1-4020-8352-5_3
16. Lloyd D, Stupfel M. The occurrence and functions of ultradian rhythms. *Biol Rev Camb Philos Soc*. 1991 Aug;66(3):275–99.
17. Walker JJ, Terry JR, Tsaneva-Atanasova K, Armstrong SP, McArdle CA, Lightman SL. Encoding and Decoding Mechanisms of Pulsatile Hormone Secretion. *J Neuroendocrinol*. 2010;22(12):1226–38.
18. Panda S. Circadian physiology of metabolism. *Science*. 2016 Nov 25;354(6315):1008–15.
19. The Mammalian Circadian Timing System: Organization and Coordination of Central and Peripheral Clocks. *Annu Rev Physiol*. 2010;72(1):517–49.
20. Carlson LJ, Shaw ND. Development of Ovulatory Menstrual Cycles in Adolescent Girls. *J Pediatr Adolesc Gynecol*. 2019 Jun;32(3):249–53.
21. Backstrom C, McNeilly AS, Leask R, Baird D. Pulsatile secretion of LH, FSH, Prolactin, oestradiol, and progesterone during the human menstrual cycle. *Clin Endocrinol (Oxf)*. 1982;17:29–42.
22. Shechter A, Boivin DB. Sleep, Hormones, and Circadian Rhythms throughout the Menstrual Cycle in Healthy Women and Women with Premenstrual Dysphoric Disorder. *Int J Endocrinol*. 2010 Jan 18;2010:e259345.
23. Goh GH, Maloney SK, Mark PJ, Blache D. Episodic Ultradian Events—Ultradian Rhythms. *Biology*. 2019 Mar 14;8(1):15.
24. Shannahoff-Khalsa DS, Kennedy B, Yates FE, Ziegler MG. Ultradian rhythms of autonomic, cardiovascular, and neuroendocrine systems are related in humans. *Am J Physiol*. 1996 Apr;270(4 Pt 2):R873-887.
25. Brodsky VY. Circadian (Ultradian) metabolic rhythms. *Biochem Mosc*. 2014 Jun 1;79(6):483–95.
26. Brandenberger G, Simon C, Follenius M. Ultradian endocrine rhythms: A multioscillatory system. *J Interdiscip Cycle Res*. 1987 Dec 1;18(4):307–15.
27. Ojeda SR, Urbanski HF, Ahmed CE. The Onset of Female Puberty: Studies in the Rat. In: Greep RO, editor. *Proceedings of the 1985 Laurentian Hormone Conference* [Internet]. Boston: Academic Press; 1986 [cited 2021 Mar 3]. p. 385–442. (Recent Progress in Hormone Research; vol. 42). Available from: <https://www.sciencedirect.com/science/article/pii/B9780125711425500136>
28. Dunger DB, Matthews DR, Edge JA, Jones J, Preece MA. Evidence for temporal coupling of growth hormone, prolactin, LH and FSH pulsatility overnight during normal puberty. *J Endocrinol*. 1991 Jul 1;130(1):141–9.

29. Albertsson-Wikland K, Rosberg S, Lannering B, Dunkel L, Selstam G, Norjavaara E. Twenty-Four-Hour Profiles of Luteinizing Hormone, Follicle-Stimulating Hormone, Testosterone, and Estradiol Levels: A Semilongitudinal Study throughout Puberty in Healthy Boys. *J Clin Endocrinol Metab.* 1997 Feb 1;82(2):541–9.
30. Apter D. Development of the hypothalamic-pituitary-ovarian axis. *Ann N Y Acad Sci.* 1997 Jun 17;816:9–21.
31. Jenner MR, Kelch RP, Kaplan SL, Grumbach MM. Hormonal Changes in Puberty: IV. Plasma Estradiol, LH, and FSH in Prepubertal Children, Pubertal Females, and in Precocious Puberty, Premature Thelarche, Hypogonadism, and in a Child with a Feminizing Ovarian Tumor. *J Clin Endocrinol Metab.* 1972 Mar;34(3):521–30.
32. Shaw ND, Butler JP, McKinney SM, Nelson SA, Ellenbogen JM, Hall JE. Insights into Puberty: The Relationship between Sleep Stages and Pulsatile LH Secretion. *J Clin Endocrinol Metab.* 2012 Nov;97(11):E2055–62.
33. Apter D, Bützow T, Laughlin GA, Yen SS. Accelerated 24-hour luteinizing hormone pulsatile activity in adolescent girls with ovarian hyperandrogenism: relevance to the developmental phase of polycystic ovarian syndrome. *J Clin Endocrinol Metab.* 1994 Jul;79(1):119–25.
34. Ojeda SR, Skinner MK. Puberty in the rat. *Knobil Neills Physiol Reprod.* 2006;2061–126.
35. Dunger DB, Villa AK, Matthews DR, Edge JA, Jones J, Rothwell C, et al. Pattern of secretion of bioactive and immunoreactive gonadotrophins in normal pubertal children. *Clin Endocrinol (Oxf).* 1991 Sep;35(3):267–75.
36. Refinetti R. Circadian modulation of ultradian oscillation in the body temperature of the golden hamster. *J Therm Biol.* 1994 Aug 1;19(4):269–75.
37. Ootsuka Y, de Menezes RC, Zaretsky DV, Alimoradian A, Hunt J, Stefanidis A, et al. Brown adipose tissue thermogenesis heats brain and body as part of the brain-coordinated ultradian basic rest-activity cycle. *Neuroscience.* 2009 Dec 1;164(2):849–61.
38. Prendergast BJ, Zucker I. Ultradian rhythms in mammalian physiology and behavior. *Curr Opin Neurobiol.* 2016 Oct;40:150–4.
39. Blum ID, Zhu L, Moquin L, Kokoeva MV, Gratton A, Giros B, et al. A highly tunable dopaminergic oscillator generates ultradian rhythms of behavioral arousal. *eLife.* 2014 Dec 29;3:e05105.
40. Lehman MN, He W, Coolen LM, Levine JE, Goodman RL. Does the KNDy Model for the Control of Gonadotropin-Releasing Hormone Pulses Apply to Monkeys and Humans? *Semin Reprod Med.* 2019 Mar;37(2):71–83.
41. Merkley CM, Porter KL, Coolen LM, Hileman SM, Billings HJ, Drews S, et al. KNDy (kisspeptin/neurokinin B/dynorphin) neurons are activated during both pulsatile and surge secretion of LH in the ewe. *Endocrinology.* 2012 Nov;153(11):5406–14.

42. Merkle CM, Coolen LM, Goodman RL, Lehman MN. Evidence for Changes in Numbers of Synaptic Inputs onto KNDy and GnRH Neurons during the Preovulatory LH Surge in the Ewe. *J Neuroendocrinol*. 2015 Jul;27(7):624–35.
43. Takahashi JS. Transcriptional architecture of the mammalian circadian clock. *Nat Rev Genet*. 2017;18(3):164–79.
44. Hastings MH, Maywood ES, Brancaccio M. Generation of circadian rhythms in the suprachiasmatic nucleus. *Nat Rev Neurosci*. 2018;19(8):453–69.
45. Hagenauer MH, King AF, Possidente B, McGinnis MY, Lumia AR, Peckham EM, et al. Changes in circadian rhythms during puberty in *Rattus norvegicus*: developmental time course and gonadal dependency. *Horm Behav*. 2011 Jun;60(1):46–57.
46. Mohr MA, DonCarlos LL, Sisk CL. Inhibiting Production of New Brain Cells during Puberty or Adulthood Blunts the Hormonally Induced Surge of Luteinizing Hormone in Female Rats. *eNeuro*. 2017 Oct;4(5).
47. Mohr MA, Wong AM, Tomm RJ, Soma KK, Micevych PE. Pubertal development of estradiol-induced hypothalamic progesterone synthesis. *Horm Behav*. 2019 May;111:110–3.
48. Kriegsfeld LJ, Silver R, Gore AC, Crews D. Vasoactive intestinal polypeptide contacts on gonadotropin-releasing hormone neurons increase following puberty in female rats. *J Neuroendocrinol*. 2002 Sep;14(9):685–90.
49. Duan XN, Yan SQ, Wang SM, Hu JJ, Fang J, Gong C, et al. [Developmental characteristics of circadian rhythms in hypothalamic-pituitary-adrenal axis during puberty]. *Zhonghua Liu Xing Bing Xue Za Zhi Zhonghua Liuxingbingxue Zazhi*. 2018 Aug 10;39(8):1086–90.
50. Pronina TS, Orlova NI, Rybakov VP. [Circadian rhythm of skin temperature of children during puberty]. *Fiziol Cheloveka*. 2015 Apr;41(2):74–84.
51. Hummer DL, Lee TM. Daily timing of the adolescent sleep phase: Insights from a cross-species comparison. *Neurosci Biobehav Rev*. 2016 Nov;70:171–81.
52. Hagenauer MH, Lee TM. The neuroendocrine control of the circadian system: adolescent chronotype. *Front Neuroendocrinol*. 2012 Aug;33(3):211–29.
53. Crowley SJ, Cain SW, Burns AC, Acebo C, Carskadon MA. Increased Sensitivity of the Circadian System to Light in Early/Mid-Puberty. *J Clin Endocrinol Metab*. 2015 Nov;100(11):4067–73.
54. Kim SH, Lundgren JA, Bhabhra R, Collins JS, Patrie JT, Burt Solorzano CM, et al. Progesterone-Mediated Inhibition of the GnRH Pulse Generator: Differential Sensitivity as a Function of Sleep Status. *J Clin Endocrinol Metab*. 2018 Mar 1;103(3):1112–21.
55. Angelopoulou E, Quignon C, Kriegsfeld LJ, Simonneaux V. Functional Implications of RFRP-3 in the Central Control of Daily and Seasonal Rhythms in Reproduction. *Front Endocrinol [Internet]*. 2019 Apr 10 [cited 2021 Feb 16];10. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6467943/>

56. Ginther OJ, Pinaffi FLV, Khan FA, Duarte LF, Beg MA. Circadian influence on the preovulatory LH surge, ovulation, and prolactin concentrations in heifers. *Theriogenology*. 2013 Feb;79(3):528–33.
57. Russo KA, La JL, Stephens SBZ, Poling MC, Padgaonkar NA, Jennings KJ, et al. Circadian Control of the Female Reproductive Axis Through Gated Responsiveness of the RFRP-3 System to VIP Signaling. *Endocrinology*. 2015 Jul;156(7):2608–18.
58. Piet R, Fraissenon A, Boehm U, Herbison AE. Estrogen Permits Vasopressin Signaling in Preoptic Kisspeptin Neurons in the Female Mouse. *J Neurosci*. 2015 Apr 29;35(17):6881–92.
59. Gibson EM, Humber SA, Jain S, Williams WP, Zhao S, Bentley GE, et al. Alterations in RFamide-related peptide expression are coordinated with the preovulatory luteinizing hormone surge. *Endocrinology*. 2008 Oct;149(10):4958–69.
60. Williams WP, Jarjisian SG, Mikkelsen JD, Kriegsfeld LJ. Circadian control of kisspeptin and a gated GnRH response mediate the preovulatory luteinizing hormone surge. *Endocrinology*. 2011 Feb;152(2):595–606.
61. Grant AD, Newman M, Kriegsfeld LJ. Ultradian Rhythms in Heart Rate Variability and Distal Body Temperature Anticipate the Luteinizing Hormone Surge Onset. *bioRxiv*. 2020 Jul 17;2020.07.15.205450.
62. Brar TK, Singh KD, Kumar A. Effect of Different Phases of Menstrual Cycle on Heart Rate Variability (HRV). *J Clin Diagn Res JCDR*. 2015 Oct;9(10):CC01-04.
63. Buxton CL, Atkinson WB. Hormonal factors involved in the regulation of basal body temperature during the menstrual cycle and pregnancy. *J Clin Endocrinol Metab*. 1948 Jul;8(7):544–9.
64. Peña AS, Doherty DA, Atkinson HC, Hickey M, Norman RJ, Hart R. The majority of irregular menstrual cycles in adolescence are ovulatory: results of a prospective study. *Arch Dis Child*. 2018 Mar;103(3):235–9.
65. Delemarre-Van De Waal HA, Wennink JM, Odink RJ. Gonadotrophin and growth hormone secretion throughout puberty. *Acta Paediatr Scand Suppl*. 1991;372:26–31; discussion 32.
66. Lightman SL, Birnie MT, Conway-Campbell BL. Dynamics of ACTH and Cortisol Secretion and Implications for Disease. *Endocr Rev*. 2020 Jun 1;41(3).
67. Kalafatakis K, Russell GM, Harmer CJ, Munafo MR, Marchant N, Wilson A, et al. Ultradian rhythmicity of plasma cortisol is necessary for normal emotional and cognitive responses in man. *Proc Natl Acad Sci U S A*. 2018 24;115(17):E4091–100.
68. Casper RF, Gladanac B. Introduction: circadian rhythm and its disruption: impact on reproductive function. *Fertil Steril*. 2014 Aug;102(2):319–20.
69. Wang F, Xie N, Wu Y, Zhang Q, Zhu Y, Dai M, et al. Association between circadian rhythm disruption and polycystic ovary syndrome. *Fertil Steril*. 2020 Dec 23;

70. Gibson EM, Wang C, Tjho S, Khattar N, Kriegsfeld LJ. Experimental “jet lag” inhibits adult neurogenesis and produces long-term cognitive deficits in female hamsters. *PloS One*. 2010 Dec 1;5(12):e15267.
71. Gotlieb N, Moeller J, Kriegsfeld LJ. Circadian Control of Neuroendocrine Function: Implications for Health and Disease. *Curr Opin Physiol*. 2018 Oct;5:133–40.
72. Logan RW, Hasler BP, Forbes EE, Franzen PL, Torregrossa MM, Huang YH, et al. Impact of Sleep and Circadian Rhythms on Addiction Vulnerability in Adolescents. *Biol Psychiatry*. 2018 Jun 15;83(12):987–96.
73. Carskadon MA, Harvey K, Duke P, Anders TF, Litt IF, Dement WC. Pubertal changes in daytime sleepiness. 1980. *Sleep*. 2002 Sep 15;25(6):453–60.
74. Birth Control Pill Use - Child Trends [Internet]. [cited 2021 Jan 8]. Available from: <https://www.childtrends.org/indicators/birth-control-pill-use>
75. Products - Data Briefs - Number 327 - December 2018 [Internet]. 2019 [cited 2019 Dec 7]. Available from: <https://www.cdc.gov/nchs/products/databriefs/db327.htm>
76. Apter D. Contraception options: Aspects unique to adolescent and young adult. *Best Pract Res Clin Obstet Gynaecol*. 2018 Apr;48:115–27.
77. Adeyemi-Fowode OA, Santos XM, Dietrich JE, Srivaths L. Levonorgestrel-Releasing Intrauterine Device Use in Female Adolescents with Heavy Menstrual Bleeding and Bleeding Disorders: Single Institution Review. *J Pediatr Adolesc Gynecol*. 2017 Aug;30(4):479–83.
78. Mwanthi M, Zaenglein AL. Update in the management of acne in adolescence. *Curr Opin Pediatr*. 2018 Aug;30(4):492–8.
79. Knobil and Neill’s Physiology of Reproduction - 4th Edition [Internet]. [cited 2021 Jan 7]. Available from: <https://www.elsevier.com/books/knobil-and-neills-physiology-of-reproduction/plant/978-0-12-397175-3>
80. Gemzell-Danielsson K. Mechanism of action of emergency contraception. *Contraception*. 2010 Nov;82(5):404–9.
81. Li C, Zhang H-Y, Liang Y, Xia W, Zhu Q, Zhang D, et al. Effects of Levonorgestrel and progesterone on Oviductal physiology in mammals. *Reprod Biol Endocrinol RBE*. 2018 Jun 20;16(1):59.
82. Nowaczyk-Dura G, Czekaj P. Effects of ethinylestradiol and levonorgestrel on morphology, ultrastructure and histoenzymatic activity of rat kidney. *Physiol Res*. 1998;47(4):241–51.
83. Krishnan S, Kiley J. The lowest-dose, extended-cycle combined oral contraceptive pill with continuous ethinyl estradiol in the United States: a review of the literature on ethinyl estradiol 20 µg/levonorgestrel 100 µg + ethinyl estradiol 10 µg. *Int J Womens Health*. 2010 Aug 10;2:235–9.

84. Strom JO, Theodorsson E, Holm L, Theodorsson A. Different methods for administering 17 β -estradiol to ovariectomized rats result in opposite effects on ischemic brain damage. *BMC Neurosci.* 2010 Mar 17;11:39.
85. Zhou X, Shao Q, Han X, Weng L, Sang G. Pharmacokinetics of medroxyprogesterone acetate after single and multiple injection of cyclofem[®] in Chinese women. *Contraception.* 1998 Jun;57(6):405–11.
86. Naqvi RH, Mitra SB, Saksena IF, Lindberg MC. Pharmacokinetics of levonorgestrel in the rat. *Contraception.* 1984 Jul;30(1):81–8.
87. Lucaccioni L, Trevisani V, Marrozzini L, Bertoncelli N, Predieri B, Lugli L, et al. Endocrine-Disrupting Chemicals and Their Effects during Female Puberty: A Review of Current Evidence. *Int J Mol Sci.* 2020 Mar 18;21(6).
88. Landersoe SK, Forman JL, Birch Petersen K, Larsen EC, Nøhr B, Hvidman HW, et al. Ovarian reserve markers in women using various hormonal contraceptives. *Eur J Contracept Reprod Health Care.* 2020 Jan 2;25(1):65–71.
89. Baker FC, Mitchell D, Driver HS. Oral contraceptives alter sleep and raise body temperature in young women. *Pflugers Arch.* 2001 Aug;442(5):729–37.
90. Obruca A, Korver T, Huber J, Killick SR, Landgren B, Struijs MJ. Ovarian function during and after treatment with the new progestagen Org 30659. *Fertil Steril.* 2001 Jul;76(1):108–15.
91. Lopez LM, Ramesh S, Chen M, Edelman A, Otterness C, Trussell J, et al. Progestin-only contraceptives: effects on weight. *Cochrane Database Syst Rev.* 2016 Aug 28;(8):CD008815.
92. Okunola TO, Bola-Oyebamiji SB, Sowemimo O. Comparison of weight gain between levonorgestrel and etonogestrel implants after 12 months of insertion. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet.* 2019 Oct;147(1):54–8.
93. Skovlund CW, Mørch LS, Kessing LV, Lange T, Lidegaard Ø. Association of Hormonal Contraception With Suicide Attempts and Suicides. *Am J Psychiatry.* 2018 01;175(4):336–42.
94. Skovlund CW, Mørch LS, Kessing LV, Lidegaard Ø. Association of Hormonal Contraception With Depression. *JAMA Psychiatry.* 2016 Nov 1;73(11):1154–62.
95. Fruzzetti F, Fidecicchi T. Hormonal Contraception and Depression: Updated Evidence and Implications in Clinical Practice. *Clin Drug Investig.* 2020 Dec;40(12):1097–106.
96. Gnoth C, Frank-Herrmann P, Schmoll A, Godehardt E, Freundl G. Cycle characteristics after discontinuation of oral contraceptives. *Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol.* 2002 Aug;16(4):307–17.
97. Benagiano G, Benagiano M, Bianchi P, D’Elios MM, Brosens I. Contraception in autoimmune diseases. *Best Pract Res Clin Obstet Gynaecol.* 2019 Oct;60:111–23.

98. Combination birth control pills - Mayo Clinic [Internet]. [cited 2021 Apr 29]. Available from: <https://www.mayoclinic.org/tests-procedures/combo-birth-control-pills/about/pac-20385282>
99. Barr NG. Managing Adverse Effects of Hormonal Contraceptives. *Am Fam Physician*. 2010 Dec 15;82(12):1499–506.
100. Levonorgestrel And Ethinyl Estradiol (Oral Route) Description and Brand Names - Mayo Clinic [Internet]. [cited 2021 Mar 3]. Available from: <https://www.mayoclinic.org/drugs-supplements/levonorgestrel-and-ethinyl-estradiol-oral-route/description/drg-20406441>
101. Pinkerton GD, Carey HM. Post-pill anovulation. *Med J Aust*. 1976 Feb 21;1(8):220–2.
102. Coukell AJ, Balfour JA. Levonorgestrel subdermal implants. A review of contraceptive efficacy and acceptability. *Drugs*. 1998 Jun;55(6):861–87.
103. Smarr BL, Aschbacher K, Fisher SM, Chowdhary A, Dilchert S, Poldon K, et al. Feasibility of continuous fever monitoring using wearable devices. *Sci Rep* [Internet]. 2020 Dec 14 [cited 2021 Feb 25];10. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7736301/>
104. Smarr BL, Burnett DC, Mesri SM, Pister KSJ, Kriegsfeld LJ. A Wearable Sensor System with Circadian Rhythm Stability Estimation for Prototyping Biomedical Studies. *IEEE Trans Affect Comput*. 2016 Jul;7(3):220–30.
105. Moenter SM, Caraty A, Locatelli A, Karsch FJ. Pattern of Gonadotropin-Releasing Hormone (GnRH) Secretion Leading up to Ovulation in the Ewe: Existence of a Preovulatory GnRH Surge. *Endocrinology*. 1991 Sep 1;129(3):1175–82.
106. Webster WW, Smarr B. Using Circadian Rhythm Patterns of Continuous Core Body Temperature to Improve Fertility and Pregnancy Planning. *J Circadian Rhythms*. 2020 Sep 24;18:5.
107. Refinetti R, Menaker M. The circadian rhythm of body temperature. *Physiol Behav*. 1992 Mar;51(3):613–37.
108. Bakshi VP, Geyer MA. Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats. *Physiol Behav*. 1999 Sep;67(3):385–92.
109. Boggiano MM, Cavigelli SA, Dorsey JR, Kelley CEP, Ragan CM, Chandler-Laney PC. Effect of a cage divider permitting social stimuli on stress and food intake in rats. *Physiol Behav*. 2008 Sep 3;95(1–2):222–8.
110. Ojeda, Andrews. The maturation of estradiol-negative feedback in female rats: evidence that the resetting of the hypothalamic “gonadostat” does not precede the fir... - PubMed - NCBI [Internet]. [cited 2019 Nov 22]. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/6796387>
111. Ström JO, Theodorsson A, Ingberg E, Isaksson I-M, Theodorsson E. Ovariectomy and 17 β -estradiol Replacement in Rats and Mice: A Visual Demonstration. *J Vis Exp JoVE* [Internet]. 2012 Jun 7 [cited 2021 Jan 7];(64). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3471296/>

112. Clark RG, Tarttelin MF. Some effects of ovariectomy and estrogen replacement on body composition in the rat. *Physiol Behav.* 1982 Jun;28(6):963–9.
113. day, kinder. Influence of prepubertal ovariectomy and estradiol replacement therapy on secretion of luteinizing hormone before and after pubertal age in heifers. 1986;
114. Prakapenka AV, Hiroi R, Quihuis AM, Carson C, Patel S, Berns-Leone C, et al. Contrasting effects of individual versus combined estrogen and progestogen regimens as working memory load increases in middle-aged ovariectomized rats: one plus one does not equal two. *Neurobiol Aging.* 2018;64:1–14.
115. Olaniyi KS, Olatunji LA. Oral ethinylestradiol-levonorgestrel attenuates cardiac glycogen and triglyceride accumulation in high fructose female rats by suppressing pyruvate dehydrogenase kinase-4. *Naunyn Schmiedeberg's Arch Pharmacol.* 2019;392(1):89–101.
116. Santoru F, Berretti R, Locci A, Porcu P, Concas A. Decreased allopregnanolone induced by hormonal contraceptives is associated with a reduction in social behavior and sexual motivation in female rats. *Psychopharmacology (Berl).* 2014 Sep;231(17):3351–64.
117. Geraghty DP, Byrne KB, McPherson GA, Burcher E. Renal and myocardial adrenoceptors in steroid contraceptive-induced hypertension in rats. *Clin Exp Pharmacol Physiol.* 1990 Aug;17(8):567–78.
118. Simone J, Bogue EA, Bhatti DL, Day LE, Farr NA, Grossman AM, et al. Ethinyl estradiol and levonorgestrel alter cognition and anxiety in rats concurrent with a decrease in tyrosine hydroxylase expression in the locus coeruleus and brain-derived neurotrophic factor expression in the hippocampus. *Psychoneuroendocrinology.* 2015 Dec;62:265–78.
119. Follesa P, Porcu P, Sogliano C, Cinus M, Biggio F, Mancuso L, et al. Changes in GABAA receptor gamma 2 subunit gene expression induced by long-term administration of oral contraceptives in rats. *Neuropharmacology.* 2002 Mar;42(3):325–36.
120. Dehghan M, Mente A, Zhang X, Swaminathan S, Li W, Mohan V, et al. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *The Lancet [Internet].* 2017 Aug 29 [cited 2017 Sep 4];0(0). Available from: [http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(17\)32252-3/abstract](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(17)32252-3/abstract)
121. Olatunji LA, Olaniyi KS, Usman TO, Abolarinwa BA, Achile CJ, Kim I-K. Combined oral contraceptive and nitric oxide synthesis inhibition synergistically causes cardiac hypertrophy and exacerbates insulin resistance in female rats. *Environ Toxicol Pharmacol.* 2017 Jun;52:54–61.
122. Guerra MDO, Souza ERD, Peters VM. Reproductive performance of female wistar rat, descendent of mothers treated with levonorgestrel during the lactation. *Rev Assoc Médica Bras.* 2002 Jun;48(2):135–9.
123. JADELLE (levonorgestrel implants) for subdermal use. :35.

124. Woodruff JA, Lacey EA, Bentley G. Contrasting fecal corticosterone metabolite levels in captive and free-living colonial tuco-tucos (*Ctenomys sociabilis*). *J Exp Zool Part Ecol Genet Physiol*. 2010;313A(8):498–507.
125. Touma C, Palme R, Sachser N. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. *Horm Behav*. 2004 Jan;45(1):10–22.
126. Millspaugh JJ, Washburn BE. Within-sample variation of fecal glucocorticoid measurements. *Gen Comp Endocrinol*. 2003 Jun 1;132(1):21–6.
127. Harper JM, Austad SN. Fecal glucocorticoids: a noninvasive method of measuring adrenal activity in wild and captive rodents. *Physiol Biochem Zool PBZ*. 2000 Feb;73(1):12–22.
128. Auer KE, Kußmaul M, Möstl E, Hohlbaum K, Rüllicke T, Palme R. Measurement of Fecal Testosterone Metabolites in Mice: Replacement of Invasive Techniques. *Anim Open Access J MDPI [Internet]*. 2020 Jan 18 [cited 2021 Apr 7];10(1). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7023058/>
129. Place 1514 Eisenhower, Ann Arbor M 48108-3284. Steroid Solid Extraction [Internet]. Arbor Assays. [cited 2021 Mar 3]. Available from: <https://www.arborassays.com/resource/steroid-solid-extraction/>
130. Neurodevelopmental Consequences of Maternal Omega-3 Fatty Acid Deficiency - ProQuest [Internet]. [cited 2021 Mar 3]. Available from: <https://search.proquest.com/openview/2b9eaffc7b3f492b60daf6e1fababebd/1?pq-origsite=gscholar&cbl=2026366&diss=y>
131. Lv X, He C, Huang C, Hua G, Chen X, Timm BK, et al. Reprogramming of ovarian granulosa cells by YAP1 leads to development of high-grade cancer with mesenchymal lineage and serous features. *Sci Bull*. 2020 Aug 15;65(15):1281–96.
132. Mathew L, Gaikwad A, Gonzalez A, Nugent EK, Smith JA. Evaluation of Active Hexose Correlated Compound (AHCC) in Combination With Anticancer Hormones in Orthotopic Breast Cancer Models. *Integr Cancer Ther*. 2017 Sep 1;16(3):300–7.
133. Franklin AD, Waddell WT, Behrns S, Goodrowe KL. Estrous cyclicity and reproductive success are unaffected by translocation for the formation of new reproductive pairs in captive red wolves (*Canis rufus*). *Zoo Biol*. 2020;39(4):230–8.
134. Righetti F, Tybur J, Van Lange P, Echelmeyer L, van Esveld S, Kroese J, et al. How reproductive hormonal changes affect relationship dynamics for women and men: A 15-day diary study. *Biol Psychol*. 2020 Jan 1;149:107784.
135. azuredominique. azuredominique/Rat-Puberty-Lab-Conditions [Internet]. 2021 [cited 2021 Mar 3]. Available from: <https://github.com/azuredominique/Rat-Puberty-Lab-Conditions>
136. Leise TL. Wavelet analysis of circadian and ultradian behavioral rhythms. *J Circadian Rhythms*. 2013 Jul 1;11:5.

137. Leise TL. Chapter Five - Wavelet-Based Analysis of Circadian Behavioral Rhythms. In: Sehgal A, editor. *Methods in Enzymology* [Internet]. Academic Press; 2015 [cited 2016 May 18]. p. 95–119. (Circadian Rhythms and Biological Clocks, Part A; vol. 551). Available from: <http://www.sciencedirect.com/science/article/pii/S0076687914000123>
138. Lilly JM, Olhede SC. Generalized Morse Wavelets as a Superfamily of Analytic Wavelets. *IEEE Trans Signal Process.* 2012 Nov;60(11):6036–41.
139. Grant AD, Newman M, Kriegsfeld LJ. Ultradian rhythms in heart rate variability and distal body temperature anticipate onset of the luteinizing hormone surge. *Sci Rep.* 2020 Nov 23;10(1):20378.
140. Sanchez-Alavez M, Tabarean IV, Osborn O, Mitsukawa K, Schaefer J, Dubins J, et al. Insulin causes hyperthermia by direct inhibition of warm-sensitive neurons. *Diabetes.* 2010 Jan;59(1):43–50.
141. Kottler ML, Coussieu C, Valensi P, Levi F, Degrelle H. Ultradian, circadian and seasonal variations of plasma progesterone and LH concentrations during the luteal phase. *Chronobiol Int.* 1989;6(3):267–77.
142. de Kloet ER, Sarabdjitsingh RA. Everything has rhythm: focus on glucocorticoid pulsatility. *Endocrinology.* 2008 Jul;149(7):3241–3.
143. Violin Plots 101: Visualizing Distribution and Probability Density [Internet]. [cited 2021 Mar 3]. Available from: <https://mode.com/blog/violin-plot-examples/>
144. Wu FC, Butler GE, Kelnar CJ, Huhtaniemi I, Veldhuis JD. Ontogeny of pulsatile gonadotropin releasing hormone secretion from midchildhood, through puberty, to adulthood in the human male: a study using deconvolution analysis and an ultrasensitive immunofluorometric assay. *J Clin Endocrinol Metab.* 1996 May;81(5):1798–805.
145. Gabriel SM, Roncancio JR, Ruiz NS. Growth hormone pulsatility and the endocrine milieu during sexual maturation in male and female rats. *Neuroendocrinology.* 1992 Nov;56(5):619–25.
146. Hoeger KM, Kolp LA, Strobl FJ, Veldhuis JD. Evaluation of LH secretory dynamics during the rat proestrous LH surge. *Am J Physiol.* 1999 Jan;276(1):R219–225.
147. Prendergast BJ, Beery AK, Paul MJ, Zucker I. Enhancement and Suppression of Ultradian and Circadian Rhythms across the Female Hamster Reproductive Cycle. *J Biol Rhythms.* 2012 Jun 1;27(3):246–56.
148. Sanchez-Alavez M, Alboni S, Conti B. Sex- and age-specific differences in core body temperature of C57Bl/6 mice. *Age Dordr Neth.* 2011 Mar;33(1):89–99.
149. Apter D, Bützow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab.* 1993 Apr;76(4):940–9.

150. Tenreiro S, Dowse HB, D'Souza S, Minors D, Chiswick M, Simms D, et al. The development of ultradian and circadian rhythms in premature babies maintained in constant conditions. *Early Hum Dev.* 1991 Nov;27(1-2):33-52.
151. Menna-Barreto L, Benedito-Silva AA, Marques N, de Andrade MM, Louzada F. Ultradian components of the sleep-wake cycle in babies. *Chronobiol Int.* 1993 Apr;10(2):103-8.
152. Bueno C, Menna-Barreto L. Development of sleep/wake, activity and temperature rhythms in newborns maintained in a neonatal intensive care unit and the impact of feeding schedules. *Infant Behav Dev.* 2016 Aug;44:21-8.
153. Curtis KS, McCracken K, Espinosa E, Ong J, Buck DJ, Davis RL. Temporal and Site-Specific Changes in Central Neuroimmune Factors During Rapid Weight Gain After Ovariectomy in Rats. *Neurochem Res.* 2018 Sep;43(9):1802-13.
154. Wegorzewska IN, Walters K, Weiser MJ, Cruthirds DF, Ewell E, Larco DO, et al. Postovariectomy weight gain in female rats is reversed by estrogen receptor α agonist, propylpyrazoletriol. *Am J Obstet Gynecol.* 2008 Jul 1;199(1):67.e1-67.e5.
155. Paccola CC, Resende CG, Stumpp T, Miraglia SM, Cipriano I. The rat estrous cycle revisited: a quantitative and qualitative analysis. 2013;7.
156. Marrone BL, Gentry RT, Wade GN. Gonadal hormones and body temperature in rats: effects of estrous cycles, castration and steroid replacement. *Physiol Behav.* 1976 Sep;17(3):419-25.
157. Liechty ER, Bergin IL, Bell JD. Animal models of contraception: utility and limitations. *Open Access J Contracept.* 2015 Apr 17;6:27-35.
158. Ariznavarreta C, Cardinali DP, Villanúa MA, Granados B, Martín M, Chiesa JJ, et al. Circadian rhythms in airline pilots submitted to long-haul transmeridian flights. *Aviat Space Environ Med.* 2002 May;73(5):445-55.
159. Polugrudov AS, Panev AS, Smirnov VV, Paderin NM, Borisenkov MF, Popov SV. Wrist temperature and cortisol awakening response in humans with social jetlag in the North. *Chronobiol Int.* 2016;33(7):802-9.
160. Drewry AM, Fuller BM, Bailey TC, Hotchkiss RS. Body temperature patterns as a predictor of hospital-acquired sepsis in afebrile adult intensive care unit patients: a case-control study. *Crit Care Lond Engl.* 2013 Sep 12;17(5):R200.
161. Granger JJ, Ratti P-L, Datta SC, Raymond RM, Opp MR. Sepsis-induced morbidity in mice: effects on body temperature, body weight, cage activity, social behavior and cytokines in brain. *Psychoneuroendocrinology.* 2013 Jul;38(7):1047-57.
162. Culver A, Coiffard B, Antonini F, Duclos G, Hammad E, Vigne C, et al. Circadian disruption of core body temperature in trauma patients: a single-center retrospective observational study. *J Intensive Care.* 2020;8:4.

163. Sharma A, Sethi G, Tambuwala MM, Aljabali AAA, Chellappan DK, Dua K, et al. Circadian Rhythm Disruption and Alzheimer's Disease: The Dynamics of a Vicious Cycle. *Curr Neuroparmacol*. 2021;19(2):248–64.
164. Zimmet P, Alberti KGMM, Stern N, Bilu C, El-Osta A, Einat H, et al. The Circadian Syndrome: is the Metabolic Syndrome and much more! *J Intern Med*. 2019 Aug;286(2):181–91.
165. Akin A, Elstein M. The value of the basal temperature chart in the management of infertility. *Int J Fertil*. 1975;20(2):122–4.
166. He H. [Diagnosis of basal body temperature, serum progesterone and endometrial biopsy for luteal phase defect]. *Zhonghua Fu Chan Ke Za Zhi*. 1993 Feb;28(2):82–4, 122–3.
167. Bopp B, Shoupe D. Luteal phase defects. *J Reprod Med*. 1993 May;38(5):348–56.
168. Mitwally MF, Kescu NK, Yalcinkaya TM. High ovulatory rates with use of troglitazone in clomiphene-resistant women with polycystic ovary syndrome. *Hum Reprod Oxf Engl*. 1999 Nov;14(11):2700–3.
169. Veldhuis JD, Christiansen E, Evans WS, Kolp LA, Rogol AD, Johnson ML. Physiological Profiles of Episodic Progesterone Release During the Midluteal Phase of the Human Menstrual Cycle: Analysis of Circadian and Ultradian Rhythms, Discrete Pulse Properties, and Correlations With Simultaneous Luteinizing Hormone Release. *J Clin Endocrinol Metab*. 1988 Feb 1;66(2):414–21.
170. Sir-Petermann T, Piwonka V, Pérez F, Maliqueo M, Recabarren SE, Wildt L. Are circulating leptin and luteinizing hormone synchronized in patients with polycystic ovary syndrome? *Hum Reprod Oxf Engl*. 1999 Jun;14(6):1435–9.
171. Herbison AE. The Gonadotropin-Releasing Hormone Pulse Generator. *Endocrinology*. 2018 01;159(11):3723–36.
172. Lightman S, Terry JR. The importance of dynamic signalling for endocrine regulation and drug development: relevance for glucocorticoid hormones. *Lancet Diabetes Endocrinol*. 2014 Jul;2(7):593–9.
173. Bong Choi S, Shil Hong E, Hee Noh Y. Open Artificial Pancreas System Reduced Hypoglycemia and Improved Glycemic Control in Patients with Type 1 Diabetes | *Diabetes* [Internet]. 2018 [cited 2020 Sep 10]. Available from: https://diabetes.diabetesjournals.org/content/67/Supplement_1/964-P
174. Marui S, Uchida Y, Nagashima K. Daily Changes of Body Temperature and Heart Rate are Modulated after Estradiol Depletion in Female Rats. In 2016.
175. Brown SA, Zimbrunn G, Fleury-Olela F, Preitner N, Schibler U. Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr Biol CB*. 2002 Sep 17;12(18):1574–83.
176. Peachman RR. Weighing the Risks and Benefits of Hormonal Contraception. *JAMA*. 2018 Mar 20;319(11):1083–4.

177. Bahamondes L, Valeria Bahamondes M, Shulman LP. Non-contraceptive benefits of hormonal and intrauterine reversible contraceptive methods. *Hum Reprod Update*. 2015 Oct;21(5):640–51.
178. Lewis DM. Automated Insulin Delivery [Internet]. 2019 [cited 2020 Sep 10]. Available from: <https://www.artificialpancreasbook.com/>
179. Webster W, Godfrey EM, Costantini L, Katilius J. Passive fertility prediction using a novel vaginal ring and smartphone application. *Fertil Steril*. 2015 Sep 1;104(3):e98.
180. Aptekar D, Costantini L, Katilius J, Webster W. Continuous, Passive Personal Wearable Sensor to Predict Ovulation [21G]. *Obstet Gynecol*. 2016 May;127:64S.
181. Shilaih M, Goodale BM, Falco L, Kübler F, De Clerck V, Leeners B. Modern fertility awareness methods: Wrist wearables capture the changes of temperature associated with the menstrual cycle. *Biosci Rep*. 2017 Nov 14;Epub ahead of print.
182. Mørch LS, Skovlund CW, Hannaford PC, Iversen L, Fielding S, Lidegaard Ø. Contemporary Hormonal Contraception and the Risk of Breast Cancer. *N Engl J Med*. 2017 07;377(23):2228–39.
183. Westhoff CL, Pike MC. Hormonal contraception and breast cancer. *Contraception*. 2018 Sep;98(3):171–3.
184. McLean AC, Valenzuela N, Fai S, Bennett SAL. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. *J Vis Exp JoVE*. 2012 Sep 15;(67):e4389.
185. Chelini MOM, Souza NL, Rocha AM, Felipe ECG, Oliveira CA. Quantification of fecal estradiol and progesterone metabolites in Syrian hamsters (*Mesocricetus auratus*). *Braz J Med Biol Res*. 2005 Nov;38(11):1711–7.
186. van der Vinne V, Potheary CA, Wilcox SL, McKillop LE, Benson LA, Kolpakova J, et al. Continuous and non-invasive thermography of mouse skin accurately describes core body temperature patterns, but not absolute core temperature. *Sci Rep*. 2020 Nov 26;10(1):20680.
187. Shechter A, Boudreau P, Varin F, Boivin DB. Predominance of distal skin temperature changes at sleep onset across menstrual and circadian phases. *J Biol Rhythms*. 2011 Jun;26(3):260–70.
188. Fowler LR, Gillard C, Morain S. Teenage Use of Smartphone Applications for Menstrual Cycle Tracking. *Pediatrics* [Internet]. 2020 May 1 [cited 2021 Feb 25];145(5). Available from: <https://pediatrics.aappublications.org/content/145/5/e20192954>
189. Eschler J, Menking A, Fox S, Backonja U. Defining Menstrual Literacy With the Aim of Evaluating Mobile Menstrual Tracking Applications. *Comput Inform Nurs CIN*. 2019 Dec;37(12):638–46.
190. Master SL, Eckstein MK, Gotlieb N, Dahl R, Wilbrecht L, Collins AGE. Distangling the systems contributing to changes in learning during adolescence. *Dev Cogn Neurosci*. 2020 Feb;41:100732.

191. Piekarski DJ, Johnson CM, Boivin JR, Thomas AW, Lin WC, Delevich K, et al. Does puberty mark a transition in sensitive periods for plasticity in the associative neocortex? *Brain Res.* 2017 Jan 1;1654(Pt B):123–44.
192. Davidow JY, Foerde K, Galván A, Shohamy D. An Upside to Reward Sensitivity: The Hippocampus Supports Enhanced Reinforcement Learning in Adolescence. *Neuron.* 2016 Oct 5;92(1):93–9.
193. DePasque S, Galván A. Frontostriatal development and probabilistic reinforcement learning during adolescence. *Neurobiol Learn Mem.* 2017 Sep;143:1–7.
194. Martinez GM. Sexual Activity, Contraceptive Use, and Childbearing of Teenagers Aged 15–19 in the United States. 2015;(209):8.
195. Patseadou M, Michala L. Usage of the levonorgestrel-releasing intrauterine system (LNG-IUS) in adolescence: what is the evidence so far? *Arch Gynecol Obstet.* 2017 Mar;295(3):529–41.
196. Strittmatter E, Holtmann M. [Gender identities in transition]. *Z Kinder Jugendpsychiatr Psychother.* 2020;48(2):93–102.
197. Castilla-Peón MF. Medical management of transgender children and adolescents. *Bol Med Hosp Infant Mex.* 2018;75(1):7–14.

bioRxiv preprint doi: <https://doi.org/10.1101/2021.07.20.459145>; this version posted July 21, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

NAF Puberty Manuscript Figures

Figure 1. High Frequency Fecal Estradiol Enables Monitoring of Estrous Cycle Emergence, Manipulation, and Ovarian Status During Adolescence

Figure 2. Circadian Power Increases in Early Adolescence with Level and Rate of Increase Tied to Estradiol

[illegible]

Figure 4. Female Adolescence is Associated with Sex Steroid-Dependent CBT Trends and Levels

Supplemental Figures

Supplemental Figure 1: Estradiol Group Averages from Pre-Puberty to Adulthood Post-Contraception:
Contraceptives Longitudinally Alter the Pattern but Not Level of Fecal Estradiol

Supplemental Figure 2: OVX and OVX + E2 are Heavier than Intact Animals in Mid Puberty and Early Adulthood

bioRxiv preprint doi: <https://doi.org/10.1101/2021.07.20.453145>; this version posted July 21, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

bioRxiv preprint doi: <https://doi.org/10.1101/2021.07.20.453145>; this version posted July 21, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

A.

B.

C.

Supplemental Figure 3:
Estradiol Rises Near
Vaginal Opening and is
Followed by Decreased
UR Power and Increased
Temperature

D.

E.

Supplemental Figure 4: FFT Power of Intact Temp Across Life Peaks Uniquely at 4 days and is Disrupted During and After Contraceptive Administration

bioRxiv preprint doi: <https://doi.org/10.1101/2021.07.20.453145>; this version posted July 21, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

Supplemental Figure 5. Estradiol Administration in Ovariectomized Animals is Associated with Lower Temperatures Across Adolescence

Supplemental Figure 6. Continuous Body Temperature Features Enable Remote Assessment of Female Puberty