Published in final edited form as:

Fertil Steril. 2014 June ; 101(6): 1773–1780.e1. doi:10.1016/j.fertnstert.2014.02.047.

Differences in ovarian hormones in relation to parity and time since last birth

Emily S. Barrett, Ph.D.¹, Lauren E. Parlett, Ph.D.², Gayle C. Windham, Ph.D.³, and Shanna H. Swan, Ph.D.⁴

¹Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, Rochester, NY, United States

²Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

³Division of Environmental and Occupational Disease Control, California Department of Public Health, Richmond, CA

⁴Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai; New York, NY

Abstract

Objective—To examine ovarian function in relation to parity and time since last birth.

Design—Women collected daily urine samples for up to 8 menstrual cycles. Urinary estradiol and progesterone metabolite concentrations (E1C and PdG) were measured using enzyme-linked immunoassay. Cycle phase lengths were calculated from hormone profiles and daily diaries.

Setting—California, U.S.A.

Patients—Three hundred forty-six naturally cycling women, age 18–39.

Intervention(s)—None.

Main Outcome Measure(s)—Mean follicular E1C (cycle days –8 to –1), mean luteal PdG (days 0 to +10), and cycle phase lengths in ovulatory cycles.

Results—Women who had given birth within the previous three years had lower E1C than nulliparas ($\beta = -0.22$, 95% CI: $-0.33, -0.11$) and women who last gave birth >3 years earlier ($\beta = -0.13$, 95% CI: $-0.23, -0.03$). Among parous women, E1C was positively associated with time since last birth ($\beta = 0.02$, 95% CI: $0.01, 0.04$). Women who last gave birth >3 years earlier had

© 2014 American Society for Reproductive Medicine. Published by Elsevier Inc. All rights reserved.

Corresponding author: Emily S. Barrett, PhD, Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Box 668, Rochester, NY 14642 UNITED STATES, Phone: (585)275-9187; Fax: (585)276-2171, Emily_barrett@urmc.rochester.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Authors' contributions to the manuscript: ESB developed the analysis plan and composed the manuscript; LEP conducted statistical analyses; GCW and SHS developed and conducted the WRHS and helped to edit the current manuscript.

Financial disclosures: ESB has nothing to disclose. LEP has nothing to disclose. GCW has nothing to disclose. SHS has nothing to disclose.

longer follicular phases than nulliparas ($\beta=1.18$, 95% CI: 0.02, 2.34). There were no associations between parity and PdG or luteal phase length.

Conclusions—Our cross-sectional data suggest that ovarian function differs in nulliparous and parous women and is positively associated with time since last birth. Longitudinal research is needed to explore within-woman changes in ovarian function pre- and post-partum.

Keywords

ovarian function; fecundity; motherhood; menstrual cycle; estradiol

Introduction

Concentrations of the ovarian steroid hormones, estradiol and progesterone, can vary considerably from cycle-to-cycle and woman-to-woman, which is not surprising given that the ovary is highly responsive to ecological cues (1). This is most clearly illustrated by extreme examples, such as the prevalence of amenorrhea and oligomenorrhea among highly trained athletes including dancers, gymnasts, and distance runners (2-6). Within ovulatory cycles, ovarian hormone production varies in relation to more subtle cues, including minor weight gain (7, 8) and loss (9, 10), recreational physical activity (11, 12), dietary intake (13-17), sleep variation (18), and possibly even psychosocial stress (19-21). Ovarian function also varies in relation to demographic factors, such as age and race (22-29).

Somewhat surprisingly, there has been relatively little research on whether reproductive history, particularly parity, predicts ovarian steroid hormone levels in reproductive-age women. Recently, in a Norwegian cohort, we found that testosterone, a hormone partly ovarian in origin, is lower in parous women as compared to nulliparous women (30), results that echoed earlier findings in Filipina women (31). In both cases, the studies' authors proposed that there might be down-regulation of testosterone production in relation to a transition from mating to parenting (30, 31). However it is also possible that lower testosterone levels among parous women are indicative of a more general suppression of ovarian function, which could include modulation of estradiol and progesterone as well. Only a handful of studies have examined these hormones in relation to parity, and most, but not all, reported that estrogen is lower in parous than nulliparous women, adjusting for age and other covariates (26, 28, 32-34).

Understanding predictors of variation in these hormones is important because of their relevance to fecundity as well as other health outcomes. Within and across women, naturally-occurring conception cycles are characterized by higher average follicular estradiol levels and luteal progesterone levels than non-conception cycles (35-37) and assisted reproductive technologies are more successful in cycles with higher follicular estradiol prior to ovarian stimulation (38, 39). Beyond fertility, ovarian hormones may play an important role in osteoporosis (40, 41), reproductive cancers (42-44), and cardiovascular disease (45, 46).

To that end, in this analysis, our primary objective was to examine ovarian function in relation to parity in a large population-based sample of cycling, reproductive-age women in

California, USA. We first asked whether levels of urinary ovarian hormone metabolites differ in relation to parity, comparing nulliparous women to women who gave birth within the last three years, and women who last gave birth more than three years ago, a time cutoff selected based on our previous work (30). To examine the possibility that parity-related differences in the rate of follicular development might contribute to any relationships found, we looked at whether cycle phase lengths (most notably follicular, but also total and luteal phase lengths) differed in these three groups. Finally, we examined whether, within parous women, estradiol and progesterone metabolite levels were related to time since last birth.

Methods

Study Population

Women were recruited into the Women's Reproductive Health Study (WRHS) from 1990-1991. To be eligible, women had to be currently enrolled in the Kaiser Permanente Medical Care Program in Northern California, age 18-39, married, at risk of becoming pregnant (e.g. not using hormonal contraception, no history of hysterectomy, neither woman or her partner sterilized), have had a menstrual period within the past six weeks, and be willing to collect and freeze morning urine samples for up to six months (or their second missed menstrual period). Nearly 6,500 women were screened by phone interview, of which 1,092 were eligible. Of those, 561 agreed to participate, however 150 dropped out or became ineligible leaving 411 subjects who completed urine collection and all study activities. Women who either collected 60 or more days of daily urine samples through 2+ menstrual cycles or became clinically pregnant during their participation were considered to have successfully completed the study. More detailed summaries of study recruitment and methods have been previously published (26, 47). The human subjects review boards at all participating institutions approved the study prior to implementation (including University of Rochester, RSRB#00039941) and all subjects signed informed consent prior to participation.

Questionnaire data

At intake, all women were interviewed over the phone by trained examiners on topics including demographics, reproductive history, and lifestyle. Subjects reported on age, race, weight, height, alcohol use, smoking, and employment. Height and weight were used to calculate body mass index (BMI; weight in kg/(height in m)²). Subjects reported how much time they spent doing various sports and physical activities and from that, a metabolic units (MET)/week composite was created using the 2011 Compendium of Physical Activities (48). In a series of questions about reproductive characteristics, subjects reported their age at menarche and history of use of oral contraceptives. They also reported the month and year of all previous pregnancies and their outcomes. This information was used to calculate the time since last birth at baseline.

Urine sample collection and laboratory methods

Participants collected and froze first morning urine samples daily. These samples were regularly collected by study staff and sent to the University of California at Davis, where enzyme-linked immunoassay was used to assay samples for creatinine, a progesterone

metabolite (pregnanediol-3-glucuronide [PdG]), and estradiol metabolites (estrone sulfate and estrone glucuronide, collectively referred to as E1C). Hormone concentrations under the limit of detection were assigned the minimum value of detection for analysis (PdG<0.15 µg/ml; E1C<7.8 ng/ml). To adjust for differences in urine concentration, PdG and E1C levels were divided by creatinine levels; only creatinine-adjusted values are used in the current analyses. Samples within a single menstrual cycle were assayed on the same microtiter plate (along with internal controls and standards). The intraplate coefficient of variation (CV) for E1C was 1.6% and the interplate CVs for the high, medium, and low pools were 4.9%, 6.6%, and 11.2% respectively. The intraplate CV for PdG was 1.8% and the interplate CVs for the high, medium, and low pools were 5.2%, 6.9%, and 11.0% respectively.

Ovarian hormone concentrations and cycle phase determination

Urine samples were split into cycles based on prospectively recorded menstrual diary data combined with urinary ovarian steroid concentrations, using methods published elsewhere (47). Subjects contributed data and urine samples from 1 to 8 cycles, depending on their cycle length and the duration of their participation. Ovulatory status and day of ovulation were assigned using a validated algorithm (47, 49) and quality control procedures have been described elsewhere (26). Follicular phase length was the number of days from the first day of bleeding in a cycle up to and including the day of ovulation (47). Luteal phase length was the number of days after ovulation, up until the start of menstrual bleeding in the next cycle. Total cycle length was follicular phase length plus luteal phase length. Anovulatory cycles, by definition, do not have a day of ovulation, nor can they be “aligned” to calculate follicular and luteal hormone concentrations and cycle phase lengths, thus they were excluded from the current analyses (n=5 cycles). The current analyses looked at mean follicular E1C (cycle days -8 to -1) and mean luteal PdG (cycle days 0 to +10), where day 0 indicates the day of ovulation.

Statistical analysis

We first looked at descriptive statistics for all relevant variables and conducted crude analyses, including Pearson’s correlations to examine the relationships among the continuous variables. In the primary set of general linear models, we examined whether parity predicted creatinine-adjusted urinary concentrations of E1C and PdG. E1C and PdG data were natural log-transformed to better fit assumptions of linearity between covariates and outcomes. E1C and PdG concentrations were then averaged across cycles within each woman to create the outcome measures. Throughout our analyses, we used weights to account for the variable number of cycles contributed by each woman, whereby women who contributed more cycles would be more influential than women who contributed fewer cycles.

Because previous research has indicated that the effects of parity on gonadal hormone levels may differ based on time since last birth (30, 31), we categorized women into three groups: nulliparous women (reference group), women who had given birth within the previous three years, and women who had last given birth more than three years prior. The three-year cutoff was chosen based on our previous work on marriage, motherhood, and reproductive

hormones (30). Based on the existing literature, we selected several additional covariates *a priori* for inclusion in all models: age, BMI, age at menarche, physical activity level (in “mets”), smoking (current/former/never), alcohol use (any/none), and race (non-Hispanic Caucasian, Hispanic or Latina, other).

We then fit linear models limited to the parous women in our cohort, in which we examined whether the time since last birth (as a continuous variable) predicted E1C and PdG (keeping all other covariates the same). We explored whether parity confounded the relationship between time since last birth and ovarian steroid metabolites by including it as a covariate, and, in a separate model, as an effect modifier (one child versus two or more).

In secondary analyses, we examined whether parity predicted cycle phase lengths, keeping all other covariates the same. We fit three linear models, one for each of the cycle length outcomes: follicular phase length, total cycle length, and luteal phase length. Finally, we conducted two sets of sensitivity analyses. In the first, we shifted the cut off used to categorize time since last birth (< 3 yrs vs. >3 years) to other time points (1, 2, 4, 5, 6, 7, 8 and 9 years), to examine the robustness of our results. In the second, we excluded women who had given birth within the previous year to because of the hormonal effects of breast-feeding. In all of our models, we checked assumptions of linearity between covariates and outcome and normal distribution of errors with constant variance. Finally, for all analyses, we identified outliers and influential points and ran sensitivity analyses excluding those subjects. Exclusion of those subjects did not affect results, so all subjects were retained in the final models. All analyses were performed with SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA) and all p-values reported are two-tailed with an alpha-level of 0.05.

Results

Four hundred and eleven women contributed one or more ovulatory cycles to the Women’s Reproductive Health Study; of these, subjects were missing data on physical activity (n=38), age at menarche (n=26), and BMI (n=1), resulting in a sample size of 346 women for the current analyses (84%). The subjects were predominantly white (73%), and educated (41% with at least a college degree), with a mean age of 32 years (Table 1). Eighty percent were parous and of those, 60% had two or more children. Woman’s age was strongly correlated with time since last birth ($r=0.42$, $p<0.0001$). Nulliparous women were more likely to be employed than women with children, and were more likely to have completed at least some college as compared to women whose last birth was over three years prior. In crude analyses, mean follicular E1C and luteal PdG were not correlated with one another ($r=0.02$, $p=0.66$). E1C concentrations were associated with time since last birth ($r=0.15$, $p=0.008$) and PdG concentrations were associated with BMI (-0.14 , $p=0.007$).

In multivariable models, women who had given birth within the previous three years had significantly lower follicular E1C concentrations than nulliparous women ($\beta=-0.22$, $p<0.0001$) and women who had last given birth over three years earlier ($\beta=-0.13$, $p<0.01$) (Figure 1). Women who had last given birth over three years earlier had lower follicular E1C concentrations than nulliparas as well, but the difference was not statistically significant ($\beta=-0.09$, $p<0.15$). Both groups of parous women had lower luteal PdG

concentrations than nulliparas, however these differences were not statistically significant either (Table 2). The results of the hormone analyses were unchanged after excluding women who had given birth within the last year ($n=78$; not shown). In sensitivity analyses in which the cutoff for time since last birth categorization was shifted from 3 years to other durations (1, 2, 4, 5, 6, 7, 8, and 9 years), women with a shorter time since last birth consistently had lower log E1C levels than the other groups and for most (but not all) cutoff points, these differences were significant at $p < 0.05$ (Supplemental Table 1). In analyses limited to parous women, time since last birth (continuous) was significantly and positively associated with E1C concentrations ($\beta=0.02$, $p=0.005$; Table 2, Figure 2), but not PdG concentrations ($\beta=-0.008$, $p=0.37$; Table 2). In analyses stratified by number of children, we saw no differences in the relationship between time since last birth and hormone levels between women who had one child versus those who had two or more.

In our secondary analyses examining the association between parity and cycle phase lengths, there were no significant differences in follicular phase length ($\beta=0.79$, $p=0.15$) or total cycle length ($\beta=0.86$, $p=0.11$) in women who had given birth within the last three years as compared to nulliparous women. Women who last gave birth more than three years ago had longer follicular phases ($\beta=1.18$, $p=0.05$) than nulliparas and showed a trend towards longer total cycle lengths ($\beta=1.09$, $p=0.06$) as compared to nulliparas (Table 2). There were no differences in luteal phase length among the groups.

Discussion

In this cross-sectional study, we examined ovarian hormone concentrations in relation to reproductive history in healthy, pre-menopausal women with ovulatory cycles. We found that women who had given birth in the previous three years had, on average, 22% lower follicular E1C concentrations than nulliparous women and 13% lower follicular E1C concentrations than women who had last given birth more than three years earlier. Within parous women, furthermore, time since last birth was positively associated with E1C, a pattern that was evident until 9 years post-partum (at which point there were too few subjects to draw conclusions). We found no significant associations between time since last birth and PdG concentrations. In this population of cycling women, superficially, ovarian function appeared to be fully restored post-partum, and yet estradiol levels, which may be indicative of fecundability (35-37), were lower for years after the last birth. Surprisingly, in sensitivity analyses shifting the cutoff for time since last birth, we found that even when we used 9 years post-partum as a cut-off, women who had given birth within the previous 9 years had lower E1C concentrations than women who had last given birth more than 9 years ago.

In addition, we showed that the follicular phase in parous women was roughly one day longer than in nulliparous women, on average. That parous women have both lower E1C levels and longer follicular phases is not entirely surprising given previous work showing that cycles with longer follicular phases often have lower estradiol concentrations as well (50, 51). Variation in follicular phase length is believed to be the result of differences in the time needed for follicular recruitment, followed by emergence of the antral follicle (51, 52). Based on the cycle-long hormone profiles we observed (Figure 1), in parous women

compared to nulliparas, E1C is lower throughout the cycle, even at ovulation. Thus the hormonal differences observed among groups appear to reflect differences across the cycle, not simply slower initial growth of the cohort of developing follicles.

Our results on ovarian hormone concentrations and parity confirm and extend previous work. The limited research on this topic to date has typically considered parity in a strictly binary way—that is, as parous versus nulliparous (32, 33). Indeed, in a previous publication on predictors of ovarian function in this cohort, parity was dichotomized in that way (26). That publication, and others, found that parous women have lower estradiol and/or progesterone concentrations than nulliparous women while cycling (26, 32) and during pregnancy (28, 34, 53). The only relevant longitudinal study, to our knowledge, found no difference in early follicular estradiol before and 7-19 months after a pregnancy. However it relied on a single estradiol measurement per cycle and had a very small sample size (54).

It is widely accepted that ovarian function (and by extension, fecundity) is typically suppressed in the early post-partum period (55). In non-lactating women, menstruation may begin approximately 8-11 weeks postpartum, with ovulation resuming shortly thereafter (56-58). Among women who breast-feed, the longer duration of amenorrhea is so widely recognized that breast-feeding has been presented as a method of family planning (59-61). In the current study, we do not have data on breast-feeding, thus it is possible that women with a very short time since last birth could still be lactating, resulting lower steroid hormone profiles. However this would not explain why the positive association between E1C metabolite levels and time since last birth extends so far beyond the typical period of breast-feeding and why our main results persisted even after excluding women with children younger than one year of age (who might reasonably still be breast-feeding).

Across the menstrual cycle, parous women's E1C profiles were lower than those of nulliparous women (Figure 1). Although these differences are subtle, because they appear to extend over many years they may be relevant to numerous clinical outcomes linked to estradiol concentrations. These include fertility-related concerns, such as subfecundity and family planning. Approximately 3.6 million U.S. women are affected by secondary impaired fecundity (62), and our results suggest that the time lapsed since last birth may be an important factor to consider. Our results may also be relevant to understanding the protective effects of parity on risk of reproductive cancers, particularly some breast cancers (63, 64). If giving birth culminates in extended down-regulation of ovarian function (lasting several years or more), lifetime exposure to free estradiol is likely to be reduced, which may, in turn, lower risk of estrogen-dependent cancers (65-67). Additional research is needed to examine the extent to which parity and time since last birth are related to fecundity and other hormone-related health outcomes.

Our assessment of ovarian steroid levels is both a strength and limitation. Because we used convenient, non-invasive urine sampling as our medium for steroid assay, we were able to collect samples daily, allowing us to capture steroid production across the cycle. We assayed daily hormone levels in up to 8 cycles per woman, moreover, in contrast to the many clinical studies which commonly rely on a single (or at best, several) opportunistic serum sample(s) for estradiol and progesterone measurement (28, 32, 33, 54, 68). However, although urinary

E1C metabolites are widely studied in reproductive epidemiology, they are not a direct measure of circulating hormone levels, and are influenced by inter-individual metabolic differences (26, 36, 51, 69). It is unclear how this might affect our results. However, we would predict that if anything, it would obscure our ability to detect differences among groups, leading to an underestimate of effect size. Future research in this vein should consider salivary hormone measurement, which is convenient and also reflects only the free fraction, making it potentially more biologically relevant.

The cross-sectional nature of our study is an additional limitation, in that it is impossible to determine whether within individual women, ovarian steroid levels rise with increasing time since last birth. Although our findings may suggest a causal relationship, we cannot conclude that parity directly causes a reduction in hormone levels. Ideally, a longitudinal study, following women through pregnancies and the subsequent post-partum years would help to clarify change over time and possible causal relationships. Based on our results, we hypothesize that ovarian function may be down-regulated for several years after childbirth, gradually returning to higher levels after several years. Longitudinal studies of this sort are costly and time-intensive, but analogous work in men showing changes in testosterone levels in relation to parenting proves that such studies are feasible (70). Such a study is arguably more important in females, moreover, given the clinical relevance of possible extended suppression of ovarian steroid production.

Finally, our results may not be generalizable to all women given that our population was predominantly Caucasian and highly educated. Subjects had to adhere to a strict urine collection protocol over several months, moreover, and had to speak English. Future research is needed to see if these findings can be replicated in more diverse populations. Most importantly, to participate in WRHS all subjects had to have had a recent menstrual cycle and to be included in the current analyses, they had to have an ovulatory cycle during participation (in order to calculate hormone indices). Thus our sample may have less variation in ovarian parameters than the general population, and in particular, women with low levels of ovarian function may be underrepresented. We do not have data on the use of assisted reproductive technologies (ART) in our population, thus we cannot rule out the possibility that associations between time since last birth and ovarian hormones may differ in women who used ART versus those who conceived naturally.

The current results suggest that time since last birth is related to ovarian function in healthy, cycling women. They raise several directions for future research, including taking a longitudinal approach and investigating possible underlying mechanisms. They may have important clinical ramifications for estrogen-related health outcomes. Given the paucity of data on this topic, confirmation in other cohorts is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to thank the entire WRHS study team and the women who participated in the study.

Financial support: The original WRHS study was supported in part by NIH grants ESO 6198, ESO 4699, and ESO 5707. The current analyses were completed under NIH grant K12 ES019852. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Works cited

1. Barrett ES, Thune I, Lipson SF, Furberg AS, Ellison PT. A factor analysis approach to examining relationships among ovarian steroid concentrations, gonadotrophin concentrations and menstrual cycle length characteristics in healthy, cycling women. *Hum Reprod.* 2013; 28:801–11. [PubMed: 23250924]
2. Hetland ML, Haarbo J, Christiansen C, Larsen T. Running induces menstrual disturbances but bone mass is unaffected, except in amenorrheic women. *Am J Med.* 1993; 95:53–60. [PubMed: 8328497]
3. Bacchi E, Spiazzi G, Zendrini G, Bonin C, Moghetti P. Low body weight and menstrual dysfunction are common findings in both elite and amateur ballet dancers. *J Endocrinol Invest.* 2013; 36:343–6. [PubMed: 23027765]
4. Bale P, Doust J, Dawson D. Gymnasts, distance runners, anorexics body composition and menstrual status. *The Journal of sports medicine and physical fitness.* 1996; 36:49–53. [PubMed: 8699838]
5. Castelo-Branco C, Reina F, Montivero AD, Colodron M, Vanrell JA. Influence of high-intensity training and of dietetic and anthropometric factors on menstrual cycle disorders in ballet dancers. *Gynecol Endocrinol.* 2006; 22:31–5. [PubMed: 16522531]
6. Zanker CL, Swaine IL. The relationship between serum oestradiol concentration and energy balance in young women distance runners. *Int J Sports Med.* 1998; 19:104–8. [PubMed: 9562218]
7. Bates GW, Bates SR, Whitworth NS. Reproductive failure in women who practice weight control. *Fertil Steril.* 1982; 37:373–8. [PubMed: 6800847]
8. Chapman JF, McIntyre MH, Lipson SF, Ellison PT. Weight change and ovarian steroid profiles in young women. *Fertil Steril.* 2009; 91:858–61. [PubMed: 18314121]
9. Panter-Brick C, Lotstein DS, Ellison PT. Seasonality of reproductive function and weight loss in rural Nepali women. *Hum Reprod.* 1993; 8:684–90. [PubMed: 8314959]
10. Bailey RC, Jenike MR, Ellison PT, Bentley GR, Harrigan AM, Peacock NR. The ecology of birth seasonality among agriculturalists in central Africa. *J Biosoc Sci.* 1992; 24:393–412. [PubMed: 1634568]
11. De Souza MJ, Toombs RJ, Scheid JL, O'Donnell E, West SL, Williams NI. High prevalence of subtle and severe menstrual disturbances in exercising women: confirmation using daily hormone measures. *Hum Reprod.* 2010; 25:491–503. [PubMed: 19945961]
12. De Souza MJ, Miller BE, Loucks AB, Luciano AA, Pescatello LS, Campbell CG, et al. High frequency of luteal phase deficiency and anovulation in recreational women runners: blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. *J Clin Endocrinol Metab.* 1998; 83:4220–32. [PubMed: 9851755]
13. Gaskins AJ, Mumford SL, Chavarro JE, Zhang C, Pollack AZ, Wactawski-Wende J, et al. The impact of dietary folate intake on reproductive function in premenopausal women: a prospective cohort study. *PloS one.* 2012; 7:e46276. [PubMed: 23050004]
14. Gaskins AJ, Mumford SL, Zhang C, Wactawski-Wende J, Hovey KM, Whitcomb BW, et al. Effect of daily fiber intake on reproductive function: the BioCycle Study. *Am J Clin Nutr.* 2009; 90:1061–9. [PubMed: 19692496]
15. Schliep KC, Schisterman EF, Mumford SL, Pollack AZ, Perkins NJ, Ye A, et al. Energy-containing beverages: reproductive hormones and ovarian function in the BioCycle Study. *Am J Clin Nutr.* 2013; 97:621–30. [PubMed: 23364018]
16. Schliep KC, Schisterman EF, Mumford SL, Pollack AZ, Zhang C, Ye A, et al. Caffeinated beverage intake and reproductive hormones among premenopausal women in the BioCycle Study. *Am J Clin Nutr.* 2012; 95:488–97. [PubMed: 22237060]
17. Kapiszewska M, Miskiewicz M, Ellison PT, Thune I, Jasienska G. High tea consumption diminishes salivary 17beta-estradiol concentration in Polish women. *Br J Nutr.* 2006; 95:989–95. [PubMed: 16611391]

18. Merklinger-Gruchala A, Ellison PT, Lipson SF, Thune I, Jasienska G. Low estradiol levels in women of reproductive age having low sleep variation. *Eur J Cancer Prev.* 2008; 17:467–72. [PubMed: 18714190]
19. Sanders KA, Bruce NW. A prospective study of psychosocial stress and fertility in women. *Hum Reprod.* 1997; 12:2324–9. [PubMed: 9402304]
20. Sanders KA, Bruce NW. Psychosocial stress and the menstrual cycle. *J Biosoc Sci.* 1999; 31:393–402. [PubMed: 10453249]
21. Barsom SH, Mansfield PK, Koch PB, Gierach G, West SG. Association between psychological stress and menstrual cycle characteristics in perimenopausal women. *Womens Health Issues.* 2004; 14:235–41. [PubMed: 15589774]
22. Fitzgerald CT, Seif MW, Killick SR, Elstein M. Age related changes in the female reproductive cycle. *Br J Obstet Gynaecol.* 1994; 101:229–33. [PubMed: 8193098]
23. Schwartz D, Mayaux MJ. Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. *Federation CECOS. N Engl J Med.* 1982; 306:404–6. [PubMed: 7057832]
24. van Noord-Zaadstra BM, Looman CW, Alsbach H, Habbema JD, te Velde ER, Karbaat J. Delaying childbearing: effect of age on fecundity and outcome of pregnancy. *BMJ.* 1991; 302:1361–5. [PubMed: 2059713]
25. Lipson SF, Ellison PT. Normative study of age variation in salivary progesterone profiles. *J Biosoc Sci.* 1992; 24:233–44. [PubMed: 1583036]
26. Windham GC, Elkin E, Fenster L, Waller K, Anderson M, Mitchell PR, et al. Ovarian hormones in premenopausal women: variation by demographic, reproductive and menstrual cycle characteristics. *Epidemiology.* 2002; 13:675–84. [PubMed: 12410009]
27. Pinheiro SP, Holmes MD, Pollak MN, Barbieri RL, Hankinson SE. Racial differences in premenopausal endogenous hormones. *Cancer Epidemiol Biomarkers Prev.* 2005; 14:2147–53. [PubMed: 16172224]
28. Arslan AA, Zeleniuch-Jacquotte A, Lukanova A, Afanasyeva Y, Katz J, Levitz M, et al. Effects of parity on pregnancy hormonal profiles across ethnic groups with a diverse incidence of breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2006; 15:2123–30. [PubMed: 17119037]
29. Marsh EE, Shaw ND, Klingman KM, Tiamfook-Morgan TO, Yialamas MA, Sluss PM, et al. Estrogen levels are higher across the menstrual cycle in African-American women compared with Caucasian women. *J Clin Endocrinol Metab.* 2011; 96:3199–206. [PubMed: 21849524]
30. Barrett ES, Tran V, Thurston S, Jasienska G, Furberg AS, Ellison PT, et al. Marriage and motherhood are associated with lower testosterone concentrations in women. *Horm Behav.* 2013; 63:72–9. [PubMed: 23123222]
31. Kuzawa CW, Gettler LT, Huang Y-y, McDade TW. Mothers have lower testosterone than non-mothers: evidence from the Philippines. *Horm Behav.* 2010; 57:441–7. [PubMed: 20122934]
32. Bernstein L, Pike MC, Ross RK, Judd HL, Brown JB, Henderson BE. Estrogen and sex hormone-binding globulin levels in nulliparous and parous women. *J Natl Cancer Inst.* 1985; 74:741–5. [PubMed: 3857369]
33. Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, et al. Relationships of age and reproductive characteristics with plasma estrogens and androgens in premenopausal women. *Cancer Epidemiol Biomarkers Prev.* 1995; 4:381–6. [PubMed: 7655334]
34. Toriola AT, Vaarasmaki M, Lehtinen M, Zeleniuch-Jacquotte A, Lundin E, Rodgers KG, et al. Determinants of maternal sex steroids during the first half of pregnancy. *Obstet Gynecol.* 2011; 118:1029–36. [PubMed: 22015870]
35. Lipson SF, Ellison PT. Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. *Hum Reprod.* 1996; 11:2090–6. [PubMed: 8943508]
36. Venners SA, Liu X, Perry MJ, Korrick SA, Li Z, Yang F, et al. Urinary estrogen and progesterone metabolite concentrations in menstrual cycles of fertile women with non-conception, early pregnancy loss or clinical pregnancy. *Hum Reprod.* 2006; 21:2272–80. [PubMed: 16798842]
37. Baird DD, Wilcox AJ, Weinberg CR, Kamel F, McConnaughey DR, Musey PI, et al. Preimplantation hormonal differences between the conception and non-conception menstrual cycles of 32 normal women. *Hum Reprod.* 1997; 12:2607–13. [PubMed: 9455822]

38. Kummer NE, Feinn RS, Griffin DW, Nulsen JC, Benadiva CA, Engmann LL. Predicting successful induction of oocyte maturation after gonadotropin-releasing hormone agonist (GnRHa) trigger. *Hum Reprod.* 2013; 28:152–9. [PubMed: 23077235]
39. Liu HC, Jones GS, Jones HW Jr. Rosenwaks Z. Mechanisms and factors of early pregnancy wastage in in vitro fertilization-embryo transfer patients. *Fertil Steril.* 1988; 50:95–101. [PubMed: 3384123]
40. Baczyk G, Opala T, Kleka P, Chuchracki M. Multifactorial analysis of risk factors for reduced bone mineral density among postmenopausal women. *Arch Med Sci.* 2012; 8:332–41. [PubMed: 22662008]
41. Finigan J, Gossiel F, Gluer CC, Felsenberg D, Reid DM, Roux C, et al. Endogenous Estradiol and The Risk of Incident Fracture in Postmenopausal Women: The OPUS Study. *Calcif Tissue Int.* 2012
42. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, Banerjee S, Koenig KL, Shore RE, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. *J Natl Cancer Inst.* 1995; 87:190–7. [PubMed: 7707406]
43. Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, et al. Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocr Relat Cancer.* 2008; 15:485–97. [PubMed: 18509001]
44. Potischman N, Hoover RN, Brinton LA, Siiteri P, Dorgan JF, Swanson CA, et al. Case-control study of endogenous steroid hormones and endometrial cancer. *J Natl Cancer Inst.* 1996; 88:1127–35. [PubMed: 8757192]
45. Gaskins AJ, Wilchesky M, Mumford SL, Whitcomb BW, Browne RW, Wactawski-Wende J, et al. Endogenous Reproductive Hormones and C-reactive Protein Across the Menstrual Cycle: The BioCycle Study. *Am J Epidemiol.* 2012; 175:423–31. [PubMed: 22306563]
46. Matthews KA, Santoro N, Lasley B, Chang Y, Crawford S, Pasternak RC, et al. Relation of cardiovascular risk factors in women approaching menopause to menstrual cycle characteristics and reproductive hormones in the follicular and luteal phases. *J Clin Endocrinol Metab.* 2006; 91:1789–95. [PubMed: 16492698]
47. Waller K, Swan SH, Windham GC, Fenster L, Elkin EP, Lasley BL. Use of urine biomarkers to evaluate menstrual function in healthy premenopausal women. *Am J Epidemiol.* 1998; 147:1071–80. [PubMed: 9620051]
48. Ainsworth, B.; Haskell, W.; Hermann, S.; Meckes, N.; Bassett, D., Jr; Tudor-Locke, C., et al. The Compendium of Physical Activities Tracking Guide. Center HLR. , editor. Vol. 2013. College of Nursing and Health Innovation, Arizona State University; 2011.
49. Baird DD, Weinberg CR, Wilcox AJ, McConaughy DR, Musey PI. Using the ratio of urinary estrogen and progesterone metabolites to estimate day of ovulation. *Stat Med.* 1991; 10:255–66. [PubMed: 2052803]
50. Landgren BM, Uden AL, Diczfalusy E. Hormonal profile of the cycle in 68 normally menstruating women. *Acta Endocrinol (Copenh).* 1980; 94:89–98. [PubMed: 6770571]
51. Harlow SD, Baird DD, Weinberg CR, Wilcox AJ. Urinary oestrogen patterns in long follicular phases. *Hum Reprod.* 2000; 15:11–6. [PubMed: 10611180]
52. Faundes A, Brache V, Alvarez F. Functional life-span of the dominant follicle in pharmacologically induced anovulatory cycles. *Hum Reprod.* 1996; 11:114–6. [PubMed: 8671171]
53. Bernstein L, Depue RH, Ross RK, Judd HL, Pike MC, Henderson BE. Higher maternal levels of free estradiol in first compared to second pregnancy: early gestational differences. *J Natl Cancer Inst.* 1986; 76:1035–9. [PubMed: 3458941]
54. Musey VC, Collins DC, Brogan DR, Santos VR, Musey PI, Martino-Saltzman D, et al. Long term effects of a first pregnancy on the hormonal environment: estrogens and androgens. *J Clin Endocrinol Metab.* 1987; 64:111–8. [PubMed: 2946715]
55. Jackson E, Glasier A. Return of ovulation and menses in postpartum nonlactating women: a systematic review. *Obstet Gynecol.* 2011; 117:657–62. [PubMed: 21343770]
56. Campbell OM, Gray RH. Characteristics and determinants of postpartum ovarian function in women in the United States. *Am J Obstet Gynecol.* 1993; 169:55–60. [PubMed: 8333476]

57. Cronin TJ. Influence of lactation upon ovulation. *Lancet*. 1968; 2:422–4. [PubMed: 4174155]
58. Howie PW, McNeilly AS, Houston MJ, Cook A, Boyle H. Fertility after childbirth: post-partum ovulation and menstruation in bottle and breast feeding mothers. *Clin Endocrinol (Oxf)*. 1982; 17:323–32. [PubMed: 7139965]
59. Kennedy KI, Rivera R, McNeilly AS. Consensus statement on the use of breastfeeding as a family planning method. *Contraception*. 1989; 39:477–96. [PubMed: 2656086]
60. Howie PW, McNeilly AS. Contraceptive effect of breast feeding. *J Trop Pediatr*. 1982; 28:ii–v. [PubMed: 7097829]
61. Howie PW, McNeilly AS. Breast-feeding and postpartum ovulation. *IPPF Med Bull*. 1982; 16:1–3. [PubMed: 12311528]
62. Chandra, A.; Copen, CE.; Stephen, EH. National Health Statistics Reports. Vol. 67. National Center for Health Statistics: U.S. Department of Health and Human Services; 2013. Infertility and Impaired Fecundity in the United States, 1982-2010: Data from the National Survey of Family Growth.
63. Gleicher N. Why are reproductive cancers more common in nulliparous women? *Reproductive biomedicine online*. 2013; 26:416–9. [PubMed: 23518034]
64. Russo J, Moral R, Balogh GA, Mailo D, Russo IH. The protective role of pregnancy in breast cancer. *Breast cancer research: BCR*. 2005; 7:131–42. [PubMed: 15987443]
65. Henderson BE, Ross RK, Judd HL, Krailo MD, Pike MC. Do regular ovulatory cycles increase breast cancer risk? *Cancer*. 1985; 56:1206–8. [PubMed: 4016708]
66. Garland M, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Spiegelman D, et al. Menstrual cycle characteristics and history of ovulatory infertility in relation to breast cancer risk in a large cohort of US women. *Am J Epidemiol*. 1998; 147:636–43. [PubMed: 9554602]
67. Bernstein L. Epidemiology of endocrine-related risk factors for breast cancer. *J Mammary Gland Biol Neoplasia*. 2002; 7:3–15. [PubMed: 12160084]
68. Nagata C, Wada K, Nakamura K, Hayashi M, Takeda N, Yasuda K. Associations of body size and reproductive factors with circulating levels of sex hormones and prolactin in premenopausal Japanese women. *Cancer Causes Control*. 2011; 22:581–8. [PubMed: 21287259]
69. Gann PH, Giovanazzi S, Van Horn L, Branning A, Chatterton RT Jr. Saliva as a medium for investigating intra- and interindividual differences in sex hormone levels in premenopausal women. *Cancer Epidemiol Biomarkers Prev*. 2001; 10:59–64. [PubMed: 11205490]
70. Mazur A, Michalek J. Marriage, divorce, male testosterone. *Soc Forces*. 1998; 77:315–30.

Figure 1. Mean E1C concentrations by cycle day (where '0' indicates day of ovulation) in nulliparous women (n=68), women who last gave birth over three years ago (n=100), and women who gave birth within the past three years (n=178).

Figure 2.
Mean follicular E1C (days -8 to -1) in parous women in relation to time since last birth
(n=278).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

	Parous women by time since last birth									
	Total cohort (n=346)	Nulliparous (n=68)	3 years (n=178)	>3 years (n=100)						
Drinks alcohol (ever)	250	72.3	52	76.5	122	68.5	0.22	76	76.0	0.94
Used oral contraceptives (ever)	275	79.5	58	85.3	138	77.5	0.18	79	79.0	0.30
Number of children							--			--
0	68	19.7	68	100	0	0		0	0	
1	231	66.8	0	0	145	81.5		86	86.0	
2 or more	47	13.6	0	0	33	18.5		14	14.0	
Number of cycles contributed to current analyses							0.34 ^c			0.15 ^c
1-2 cycles	96	27.8	23	33.8	49	27.5		24	24.0	
3-5 cycles	170	49.1	32	47.1	88	49.4		50	50.0	
6-7 cycles	80	23.1	13	19.1	41	23.0		16	26.0	

^aTotal n may not equal 346 in some cases (and total % may not equal 100) due to missing data.

^bCompared to reference group, nulliparas; p-values estimated using unadjusted t-test

^cP-values estimated using Mantel-Haenszel Chi-Square due to ordinal nature of the cycles

Table 2
Associations between urinary ovarian steroid metabolite concentrations (E1C and PdG),
cycle phase lengths, and time since last birth in the WRHS (n=346)^a

	Time since last birth				Parous women only (time since last birth, continuous; n=278)	
	^b 3 years (n=178)		^b >3 years (n=100)		β	p-value
	β	p-value	β	p-value		
Log E1C (ng/mg creatinine)	-0.22	<0.0001	-0.09	0.15	0.02	0.005
Log PdG (ng/mg creatinine)	-0.04	0.50	-0.10	0.11	-0.008	0.37
Total cycle length (days)	0.86	0.11	1.09	0.06		
Follicular phase length (days)	0.79	0.15	1.18	0.05		
Luteal phase length (days)	0.07	0.71	-0.08	0.70		

^aWeighted general linear models adjusted for smoking status (current/former/never), use of alcohol (ever/never), BMI, age, race (non-Hispanic White, Hispanic or Latina, other), former use of oral contraception (ever/never), age at menarche (years), physical activity (mets).

^bReference group is nulliparous women.