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Sexual activity modulates shifts in T_H1/T_H2 cytokine profile across the menstrual cycle: an observational study

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Objective: To investigate if sexual activity moderated menstrual cycle–related shifts in cytokines associated with T-helper type 1 (T_H1) cells (e.g., interferon [IFN] γ) and T-helper type 2 (T_H2) cells (e.g., interleukin [IL] 4). Immune activity shifts across the menstrual cycle, with higher follicular-phase T_H1-cell activity but higher luteal-phase T_H2-cell activity. Little is known about how social behaviors alter T_H1-T_H2 ratios, despite evidence that psychosocial factors can influence immunity. Of particular interest is how sexual activity influences immune responses that may support conception, such as the T_H1-T_H2 balance.

Design: Participants provided saliva samples at four time points (menstrual, follicular, ovulatory, and luteal phases), which were assayed by means of ELISA.

Setting: Academic laboratory.

Participant(s): Thirty healthy premenopausal women (16 sexually abstinent, 14 sexually active) not taking hormonal or immunoactive medications.

Intervention(s): None.

Main Outcome Measure(s): Salivary E_2, P, IFN-γ, and IL-4.

Result(s): Sexually active, but not abstinent, women were significantly more likely to express T_H2-like cytokine ratios (IFN-γ < IL-4) in the luteal phase than in other phases. Similarly, sexually active women had significantly higher P, and higher P-E_2 ratios, in the luteal phase than did abstinent women. The P-E_2 ratio mediated menstrual variations in cytokine ratios in sexually active women.

Conclusion(s): These results support the hypothesis that shifts in immune response across the menstrual cycle may reflect tradeoffs between reproduction and immunity. These findings point to the need for further research on the interaction between sexual behavior, the menstrual cycle, and immune response. (Fertil Steril® 2015;104:1513–21. ©2015 by American Society for Reproductive Medicine.)

Key Words: Immunity, sex differences, menstrual cycle, T_H1-T_H2, cytokine

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There is increasing interest in the mechanisms underlying sex differences in immune health. In women, a coordinated shift in immune parameters occurs during reproductive events, such as pregnancy and parturition, to accommodate the changing needs of mother and offspring (1). Intriguingly, changes in immunity have been documented across the menstrual cycle as well and similarly thought to represent tradeoffs between reproduction and immunity. For example, several studies have documented lower inflammation and other innate immune parameters at midcycle, corresponding to ovulation, compared with other phases of the menstrual cycle (2–4); this is thought to prevent immune interference with conception during fertile windows.

Similarly, researchers have suggested that there may be cycle-related changes in adaptive immunity, particularly in T-helper (T_H) cells, which coordinate activity of other immune actors,

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such as B cells and macrophages. Several studies have tracked shifts in the relative proportions of cytokines, immune signaling molecules, associated with different subtypes of T_H cells. Those studies have shown that cytokines expressed during the follicular phase are predominantly those associated with T_H type 1 (T_H1) cells, but luteal-phase cytokines are more predominantly those associated with T_H type 2 (T_H2) cells (5–8). Typical development of T_H cells requires instruction from dendritic cells that locks in what type of cytokines (and thus what subtype of T_H cell) they will secrete. The T_H1-associated cytokines (e.g., interferon [IFN] γ) drive intracellular defense, whereas T_H2-associated cytokines (e.g., interleukin [IL] 4) are characteristic of extracellular defense as well as self-cell tolerance (9). Higher levels of T_H2-type cytokines are expressed during pregnancy, because these cytokines facilitate embryo implantation and placentation (10). In contrast, higher levels of T_H1-type cytokines stimulate macrophages and cytotoxic T cells, key components of immune defense in the tissues. Thus, the ratio of T_H1/T_H2-associated cytokines can be characterized as reflecting immune priorities: defending against viruses and bacteria (T_H1 dominant) versus preparing for and/or tolerating the semiallogenic fetus (T_H2 dominant) (11).

Progesterone (P) and estradiol (E_2), steroid hormones that regulate much of the endocrine-immune coordination during pregnancy, fluctuate across the menstrual cycle, with the luteal phase characterized by a high P-E_2 ratio (i.e., the concentrations ratio of unbound P and E_2). Similarly, pregnancy is associated with a marked increase in the P-E_2 ratio. Owing to the parallels between luteal phase and pregnancy in endocrine and immune factors, the female body has been described as being in a "pregnancy-like" state (7). These reproductive-immune interactions may also explain sex differences in the T_H1-T_H2 ratio, with women typically expressing more T_H2-like cytokine ratios than men (12). Of note, sexually active women typically express higher luteal-phase P and E_2 across the menstrual cycle than women who are sexually abstinent (13). Therefore, there may also be significant differences in how T_H1-T_H2 ratios change across the menstrual cycle in sexually active and abstinent women.

Understanding the potential role for partnered sexual behaviors in T_H1-T_H2 ratios is important also because of increasing research suggesting that social behaviors moderate immune activity (14). In addition to indirect effects on stress or mood (15), social interactions can provide information about one's environment or life events (e.g., reproduction) that may require immune modulation (16). In particular, partnered sexual behavior may signal that conception is possible, and as such, downshift immune responses that may interfere with reproduction and/or up-regulate immune responses that may promote conception. Among women who are sexually abstinent, however, there would be no such signal and therefore, potentially, no significant shifts in the T_H1-T_H2 ratio. In support of this hypothesis, several studies have shown that sexual activity may modulate immune activity. Increased frequency of partnered sexual activity is associated with lower levels of secretory immunoglobulin A, an antibody important for first-line immune defense (17, 18). Moreover, sexual activity appears to moderate shifts in inflammation, with increased frequency of sexual activity associated with greater midcycle

decrease in C-reactive protein, a marker of inflammation (2). It is unknown whether sexual activity within a relationship affects cytokine ratios or moderates changes in cytokine ratios across the menstrual cycle. If shifts in cytokine ratios are related to coordination of tradeoffs between reproduction and immunity, they may be more critical for reproductively active (i.e., sexually active) than reproductively inactive (i.e., sexually abstinent) women. As such, partnered sexual activity—even that which occurs outside the fertile window and thus can not directly lead to a conception—may promote fertility via priming the immune system to engage in shifts that promote reproduction.

In the present study, we examined changes in IL-4 (a T_H2-associated cytokine) and IFN-γ (a T_H1-associated cytokine) and their ratio across the menstrual cycle. We compared these changes in women who were sexually active with a partner and women who were sexually abstinent. We hypothesized that sexually active women would demonstrate significant shifts in cytokine ratios across the menstrual cycle; however, such shifts in sexually abstinent women would be weaker and potentially nondetectable. Extending the findings of Faas et al. (7), we hypothesized that sexually active women, and to a lesser extent abstinent women, would show a significantly more T_H2-like cytokine ratio in the luteal phase. Also, following the findings of Prasad et al. (13), we predicted that luteal-phase P would be higher in sexually active versus abstinent women. Novel to the present study, we predicted that the P-E_2 ratio would be associated with cytokine ratios, such that higher P-E_2 ratios in the luteal phase would mediate the effect of group (sexually active vs. abstinent) on cytokine ratios.

MATERIALS AND METHODS
Participants

All study procedures were approved by the Indiana University Institutional Review Board, and all participants provided informed consents. Thirty-five healthy premenopausal women were recruited from the community. The inclusion criterion was premenopausal with regular menstrual cycles every 26–34 days. Exclusion criteria were any self-reported use of hormonal medications or medications with a known immune effect (e.g., antibiotics), regular use of any other medication type (prescription or nonprescription), pregnancy or lactation within the past 12 months, or any medical condition known to affect immune response (e.g., cancer). Women reporting vitamin or herbal supplement use or occasional ($<1\times$/wk) use of over-the-counter antihistamines or analgesics (acetaminophen, aspirin, or nonsteroidal antiinflammatory drugs) were included. Sexually abstinent participants included women who reported no partnered genital sexual activity within the past 4 months; however, women with a lifetime history of sexual activity were included. Sexually active participants included women who reported penile-vaginal intercourse at least once a week and with only one current sexual partner. Because women taking oral contraceptives (OCs) were excluded, sexually active participants used either condoms or nonhormonal intrauterine devices as contraception. Study size was determined by prospective power analyses, which

indicated that 12–16 participants per group would adequately (∼85% power) capture small effect sizes ($d = 0.20$). Of the 35 women recruited, three dropped out during the study and two could not produce adequate saliva volumes to be assayed, leaving data from 30 participants.

Sample Collection

Participants completed two laboratory visits: one at menses (within 2 days of onset of menstrual bleeding) and one at ovulation (within 2 days of ovulation). Date of ovulation was estimated by means of backwards counting according to the onset of menstrual bleeding and the participant's typical cycle length [19]. To further confirm the date of ovulation, participants were given a packet of five dipstick urine tests for LHe (Onestep Urine Ovulation Test; Bluecross Biomedical) at their first laboratory visit (i.e., at menses). They were instructed to complete the tests daily at 1–5 p.m. in the days before their estimated ovulation date; if participants noticed a positive test strip before the estimated date of ovulation, they were scheduled for a laboratory visit within 48 hours. If participants had not noticed a positive test strip by the estimated date of ovulation, they were instructed to continue daily testing, and come into the laboratory as soon as they did notice a positive test. All participants had a positive test strip (i.e., evidence of ovulation) within 48 hours of the second laboratory visit (the "ovulation" sample).

During laboratory visits, participants completed surveys and were measured for height, weight, and body fat with a floor-unit body composition scale (Fitscale 585F; Tanita). All participants (sexually active and abstinent) also completed urine tests for hCG at both laboratory visits to confirm that no participant was pregnant during the study. All participants provided information on any illness and significant stressful events during the study period by means of surveys completed alongside sample collection (see below). Sexually active participants additionally reported on each sexual event during the study by means of online diaries; from these, sexual intercourse events were tallied.

Because salivary measures of cytokines have been shown to correlate well with serum measures [20], but are significantly less invasive to collect, we used saliva as our main medium for endocrine and cytokine measures. During lab visits, participants provided saliva samples in polypropylene tubes via a passive drool with no stimulation; samples were frozen immediately after collection. Additionally, participants completed two at-home saliva samples, which were frozen in their home freezers and transported frozen to the lab on deep-freeze ice packs in Styrofoam boxes [19]. At-home samples were collected during the follicular phase (7–10 days after menses onset) and the luteal phase (7–10 days after ovulation). All saliva samples were stored at $-80°C$ until analysis, and no sample was subjected to more than two freeze-thaw cycles.

Cytokine and Hormone Assay

Saliva samples were assayed for P, E_2, IFN-γ, and IL-4 with the use of commercially available ELISA kits and procedures recommended by the kit manufacturers (P and E_2: kits from Salimetrics; IFN-γ and IL-4: Cytoset kits from Invitrogen). Intra-assay and interassay coefficients of variance were low (4.71%–6.35% and 2.24%–10.48%, respectively). Sensitivity limits for the assays were as follows: IFN-γ 3.9 pg/mL; IL-4 2.0 pg/mL; P 5.0 pg/mL; and E_2 0.1 pg/mL. P and E_2 were measured in pmol/L to standardize across different molecular weights.

Analytic Plan

Undetectably low cytokine values were replaced with the lowest detectable value for the assay. A total of 11% of IL-4 and 38% of IFN-γ values were below the limit of detection, which is typical for a sample of young healthy participants [20]. In addition, 8 participants did not provide luteal-phase samples, leading to 27% missing data in the luteal phase (7% of total values). Because the missing of these samples was due to pragmatic reasons (e.g., samples collected at home were returned to the lab unfrozen) not related to the underlying relationships to be tested, for the purposes of our statistical tests these values were considered to be missing at random [21]. We further confirmed that luteal-phase samples were missing at random by means of the Dixon test, in which the mean values of key variables are compared across missing and nonmissing observations [22]. In no case were key variables (IL-4, IFN-γ, P, or E_2) at menses, follicular, or ovulation time points significantly different in women with or without missing luteal-phase samples. Missing values were addressed with the use of bootstrapping and other robust statistical techniques for missing data (see below). We used a natural log transformation to correct for right skew of cytokine distributions.

To examine changes in IL-4, IFN-γ, P, E_2, and the P-E_2 ratio across the menstrual cycle, we used repeated-measures mixed generalized linear models, specifying subject-level intercepts (which accounted for individual differences in baseline cytokine levels) and controlling for important covariates. We included age, race (dummy coded as white/nonwhite), and body mass index (BMI) as covariates, because they have been shown to be important sources of variation in cytokine values in healthy individuals in both cross-sectional studies [23–25] and short-term longitudinal studies (e.g., predicting changes in T_H1/T_H2 profiles across pregnancy [26]). In each model we included the following fixed effects: group (sexually active vs. sexually abstinent), time (menstrual, follicular, ovulatory, luteal) as a repeated measure, and the interaction of group and time.

To examine changes in the cytokine ratio across the menstrual cycle, we used the methods recommended by Lin et al. [27] to characterize within-subject shifts in cytokine ratios. We first calculated the standardized (i.e., z-scored) value for each cytokine within each participant. That is, the value for each time point was compared with the other values collected from the same participant. These scores represented the relative expression of cytokine within the cycle: high values would indicate high expression of that cytokine and vice versa. We were thus able to characterize the relative expression of IFN-γ/IL-4 into two categories: T_H1-like (z-scored IFN-γ > z-scored IL-4), or T_H2-like (z-scored IFN-γ < z-

scored IL-4). The average absolute difference between IFN-γ and IL-4 standardized cytokine values within a time point was $z = 0.61$ (that is, 0.61 standard deviations from each other), and in only 21 cases (18% of total data) were the standardized cytokine values within 0.10 standard deviations from each other. In other words, cytokine ratios were generally either strongly T_H1-like or T_H2-like, with few values close to the classification cutoff.

We conducted a repeated-measures generalized estimating equation (GEE) with cytokine ratio category (T_H1-like vs. T_H2-like) as the outcome variable, again entering group, time, and their interaction as fixed effects and controlling for age, race, and BMI. Finally, to test for mediation of differences between groups in change in T_H1-T_H2 ratio via hormonal factors, we conducted bootstrapped path analyses testing indirect effects. All analyses were performed with the use of IBM SPSS Statistics version 22.0 for Windows; path analyses were conducted with the use of the PROCESS and MEMORE macros for SPSS (28, 29). Contrasts were considered to be significant if $P<.05$ and marginally significant if $P<.10$.

RESULTS
Participant Demographics

A total of 16 sexually abstinent and 14 sexually active women were enrolled; full demographics are presented in Table 1. Participants reported their race as predominantly white (70%), with 16% East Asian and 13% other; one white woman reported Hispanic/Latina ethnicity. The average age was 23.44 years (SD 5.31 y). There was a significant difference in relationship status between sexually active and abstinent participants ($\chi^2 = 27.47$; $P<.001$), with all sexually active participants reporting being in a sexually active relationship and only three sexually abstinent participants reporting being in a nonsexual dating relationship. Because all sexually active participants were in a relationship, these variables were collinear and therefore we were unable to control for relationship status in the analyses below. Average length of the current relationship was 4.14 years (SD 7.38 y). On average, sexually active women reported 6.67 intercourse events during the study period (range 1–18); there was no significant difference across menstrual cycle phases in the number of intercourse events ($F[3,7.55] = 1.34$; $P=.34$; Cohen $f = 0.04$).

Participants had an average BMI of 23.31 (SD 3.60), and average body fat percentage of 27.09% (SD 7.43%). There was no significant change in body fat ($t[31] = 0.62$; $P=.49$) nor BMI ($t[31] = 0.82$; $P=.23$) from menses to ovulation, and therefore in the analyses below we used the average across time points for each participant as a covariate. No participant reported significant traumatic events or colds/infections during the study period. There were no significant differences between sexually active and abstinent participants on any demographic variable other than relationship status (Table 1 and Supplementary Table 1).

Endocrine Measures

The interaction between group and time significantly predicted P ($F[3,85.90] = 5.26$; $P<.01$; Cohen $f = 0.32$). Across groups, P was highest during the luteal phase (main effect of time, $F[3,85.95] = 15.76$; $P<.001$, Cohen $f = 0.48$); however, sexually active women had significantly greater P during the luteal phase than did sexually abstinent women (mean difference 148.70 pg/mL, SE 37.41; P(contrast) $<.001$; Fig. 1). Similarly, the interaction between group and time significantly predicted E_2 ($F[3,85.54] = 3.25$; $P<.05$; Cohen $f = 0.18$). In this case, the effect was driven by differences in the sexually abstinent group, because there was no significant change over time in E_2 among sexually active women (all specific contrast P values = ns). Among sexually

TABLE 1

Demographics and baseline characteristics by sexual activity status (unadjusted).

Characteristic	Sexually active		Sexually abstinent		Total		F	P value
	Mean	SD	Mean	SD	Mean	SD	F	P value
Age, y	24.87	7.02	22.16	2.92	23.44	5.31	2.214	.155
Education, y	15.96	4.12	15.53	2.42	15.73	3.24	0.003	.960
Body mass index	23.54	3.19	23.5	4.67	23.31	3.6	0.001	.978
	n	%	n	%	n	%	χ^2	
Race							4.974	.083
White	12	86	8	50	20	67		
Asian	2	14	5	31	7	23		
Mixed/other	0	0	3	19	3	10		
Ethnicity							1.182	.277
Hispanic/Latina	1	7	0	0	1	3		
Not Hispanic/Latina	13	93	16	100	29	97		
Relationship status							21.233	.000
Married/cohabiting	6	43	0	0	6	20		
Dating/in a relationship	8	57	3	19	11	37		
Single/casually dating	0	0	13	81	13	43		

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.

FIGURE 1

Progesterone concentration across the menstrual cycle, separated by sexual activity status (adjusted for age, body mass index, and race). Luteal-phase P was significantly higher in sexually active versus sexually abstinent women.

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.
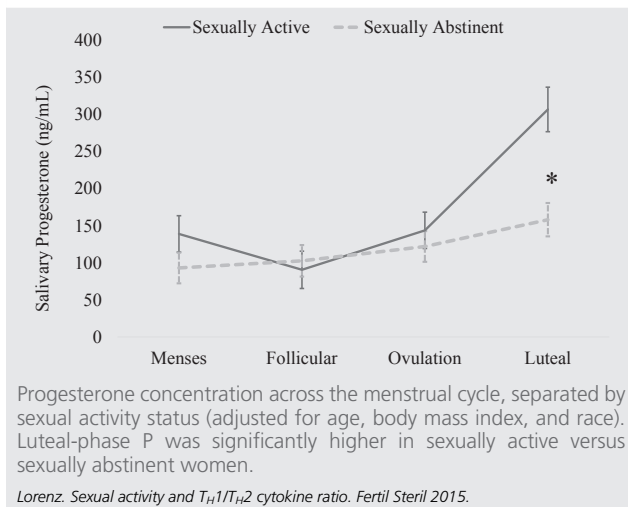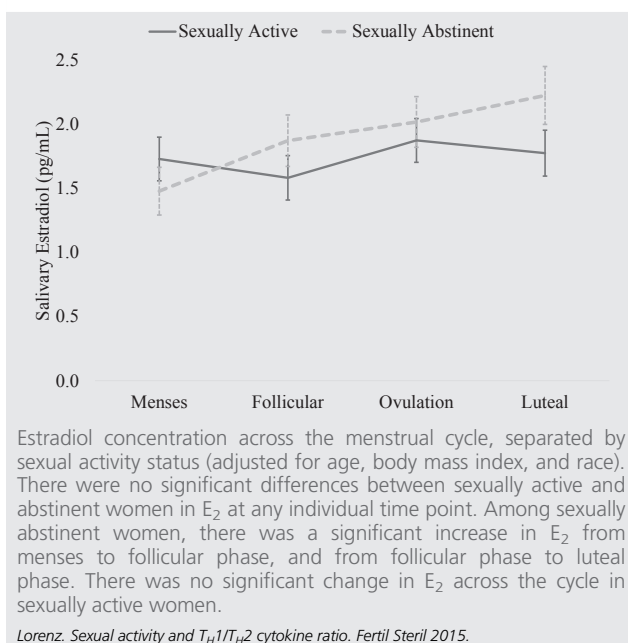
abstinent women, E_2 at menses was significantly lower than at the follicular time point (mean difference $-.39$ pg/mL, SE 0.15, P(contrast) $< .05$), which in turn was significantly lower than at the luteal time point (mean difference $-.353$, SE 0.16, P(contrast) $< .05$; Fig. 2). Finally, the P-E_2 ratio was significantly predicted by the interaction of group and time (F[3,85.55] $= 7.39$; $P < .001$; Cohen $f = 0.41$; Supplemental Fig. 1 [available online at www.fertstert.org]). As with P, sexually active women had significantly higher P-E_2 ratios than sexually abstinent women in the luteal phase (mean difference 104.48, SE 20.74, P(contrast) $< .001$).

FIGURE 2

Estradiol concentration across the menstrual cycle, separated by sexual activity status (adjusted for age, body mass index, and race). There were no significant differences between sexually active and abstinent women in E_2 at any individual time point. Among sexually abstinent women, there was a significant increase in E_2 from menses to follicular phase, and from follicular phase to luteal phase. There was no significant change in E_2 across the cycle in sexually active women.

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.

Cytokine Measures

For IFN-γ and IL-4, neither group nor time, nor their interaction, was a significant predictor (Supplemental Table 1 [available online at www.fertstert.org]). However, the interaction of group and time on IFN-γ was marginally significant (F[3,26.95] $= 2.82$; $P = .064$; Cohen $f = 0.12$). Sexually active women had a marginally significant decrease in IFN-γ between ovulation and luteal phases (mean difference $-.56$ pg/mL, SE 0.34, P(contrast) .108), although sexually abstinent women did not (mean difference 0.08 pg/mL, SE 0.26, P(contrast) .777).

For the cytokine ratio, there was a significant interaction between group and time (Wald $\chi^2 = 7.53$; $P < .05$; Cramer $V = 0.29$). In sexually abstinent women, there was no significant change in cytokine ratio across the menstrual cycle ($\chi^2 = 0.62$; $P = .89$; Cramer $V = 0.10$). However, among sexually active women, the proportion of women showing T_H2-like cytokine ratios significantly shifted from 35% during the follicular phase and ovulation to 78% at luteal phase ($\chi^2 = 10.75$; $P < .05$; Cramer $V = 0.59$; Fig. 3).
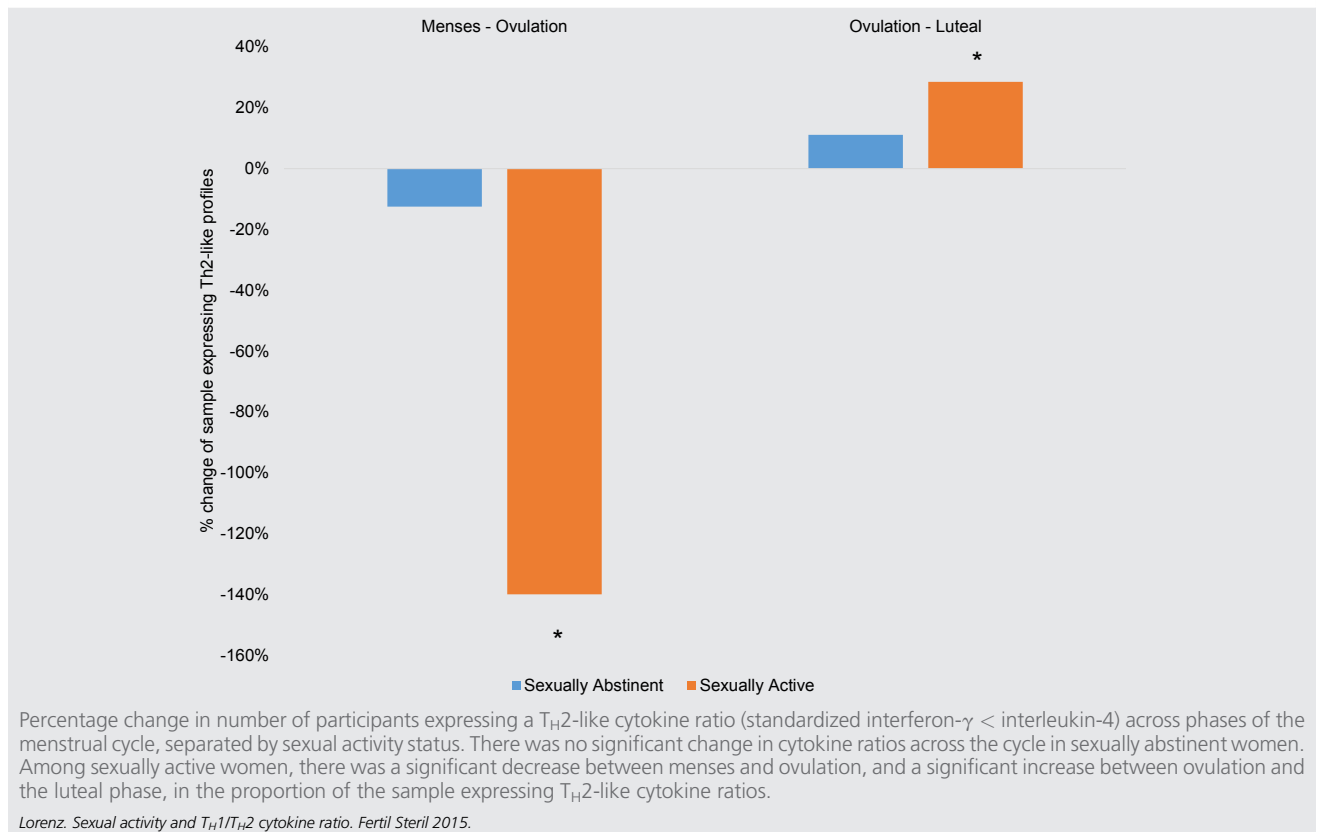
Interactions between Endocrine and Cytokine Measures

We tested the association of P-E_2 ratio and cytokine ratio, controlling for the effects of cycle phase, age, BMI, and race. There was a significant association between the P-E_2 ratio and cytokine ratio (F[1,86.65] $= 3.70$; $P = .05$; Cohen $f = 0.19$), such that samples with T_H2-like cytokine ratios had higher P-E_2 ratios (mean 77.53) than did samples with T_H1-like cytokine ratios (mean 58.96; mean difference 18.56, SE 9.65, P(contrast) .05).

We then examined if endocrine measures could explain the observed group-level differences in cytokine ratio change across the menstrual cycle. We specified a moderated mediation model, with cycle phase as the exposure, T_H1-T_H2 ratios as the outcome, P-E_2 ratios as the mediator, group (sexually active vs. abstinent) as the moderator of the mediator, and age, BMI, and race as covariates (effect estimates are presented in Supplemental Table 2 and a conceptual diagram in Supplemental Fig. 2 [available online at www.fertstert. org]). The confidence intervals of the estimated index of moderated mediation did not include zero (index estimate 0.17, SE 0.17, 90% confidence interval [CI] 0.01–0.54), thus supporting a moderated mediation. Specifically, among sexually active women, the CIs of the conditional indirect effect of cycle phase on T_H1-T_H2 ratio (mediated by P-E_2 ratio) did not include zero (index estimate 0.17, SE 0.16, 90% CI [0.01–0.49]). Among sexually abstinent women, however, the confidence intervals of the conditional indirect effect did include zero (index estimate 0.00, SE 0.06, 90% CI [-0.08 to 0.10]. In other words, there was evidence that P-E_2 ratios mediated cycle-related change in T_H1-T_H2 ratios in sexually active women, but not in abstinent women.

Because the predominant group-level differences in the T_H1-T_H2 ratio were observed in the luteal phase, we examined if the P-E_2 ratio mediated the effect of group (sexually active vs. abstinent) on the T_H1-T_H2 ratio in the luteal phase only. Here, we specified a mediation model, with group (sexually

FIGURE 3

Percentage change in number of participants expressing a T_H2-like cytokine ratio (standardized interferon-γ < interleukin-4) across phases of the menstrual cycle, separated by sexual activity status. There was no significant change in cytokine ratios across the cycle in sexually abstinent women. Among sexually active women, there was a significant decrease between menses and ovulation, and a significant increase between ovulation and the luteal phase, in the proportion of the sample expressing T_H2-like cytokine ratios.

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.

active vs. abstinent) as the exposure, T_H1-T_H2 ratios as the outcome, P-E_2 as the mediator, and age, BMI, and race as covariates. The CIs of the estimated indirect effect included zero (effect estimate 1.62, SE 11.67, 90% CI −24.55 to 25.00]); therefore, this mediation effect was not supported.

DISCUSSION

The present study is the first to examine how partnered sexual behaviors influence cytokine ratios in healthy women, and how these ratios change across the menstrual cycle. There was a marginally significant decrease in IFN-γ during the luteal phase among sexually active women, but no significant change in IL-4 across the menstrual cycle for either sexually active or abstinent women. When considering both cytokines in relation to each other in terms of the T_H1-T_H2 cytokine ratio, sexually active, but not abstinent, women expressed more T_H2-like cytokine ratios (IFN-γ < IL-4) in the luteal phase than in other phases. Finally, there were significant differences in endocrine ratios between groups, with sexually active women showing greater increase of P-E_2 ratios in the luteal phase than sexually abstinent women.

Many studies have shown immune effects of intimate relationships, such as increased inflammatory cytokine expression in individuals in hostile or unsatisfying marriages (30, 31); similarly, there has been considerable work suggesting that poor relationship or sexual satisfaction is associated

with infertility (32, 33). However, beyond the indirect role of stress (34), the mechanisms linking intimate relationships with fertility is unclear. The present study is among the first to examine these effects with respect to a specific, and evolutionarily unique, social behavior, namely partnered sexual behavior. Our findings support the broader hypothesis that sexual activity within a relationship—even that which does not result in conception—serves a variety of functions that affect reproductive health, from improving pair bonds (35) and improving relationship satisfaction across the lifespan (36) to engaging endocrine and immune responses (this study) that may promote fertility.

Specifically, as predicted, there were significant differences between sexually active and abstinent women in T_H1-T_H2 cytokine ratios across the menstrual cycle. Patterns of T_H1 and T_H2 cells are associated with a variety of clinically relevant reproductive outcomes, such as pregnancy and infertility (37). A T_H2-dominant ratio is predictive of normal pregnancy whereas T_H1-type cytokines are predictive of preeclampsia (38). This may be because T_H1 cytokines, such as IFN-γ, can promote production of proteases such as thrombin, which disrupt the placental vasculature (39). Therefore, a T_H2-like cytokine ratio during the luteal phase may help the female body to prepare for possible pregnancy.

In the few days before and immediately after ovulation, however, a T_H1-dominant cytokine ratio may be more beneficial. T_H1-associated cytokines suppress vaginal immune

responses that impair sperm motility (40), and promote production of IL-1, which assists in embryo implantation (41, 42). Thus, shifting from a T_H1-like cytokine ratio around ovulation, to a T_H2-like cytokine ratio during the luteal phase, may promote reproduction. Indeed, several studies have documented a midcycle surge in IL-1, a T_H1-associated cytokine (43, 44). In the present study, shifts in T_H1-T_H2 cytokine ratios occurred in sexually active women but not in abstinent women, suggesting that partnered sexual behavior itself may act as a trigger for these immune events necessary for reproduction. If so, this may help to explain why women attempting to conceive have greater success with increased regularity of sexual activity, even that which occurs well outside the fertile window (45).

Of note, although the greatest groupwise differences in cytokines were seen in the luteal phase, there was no evidence that absolute hormone levels in the luteal phase mediated the difference between sexually active and abstinent women. This implies that effects were driven by patterns of change in hormones and cytokines, rather than absolute levels per se. One study similarly found that although absolute levels of ovarian hormones did not determine women's inflammation levels at any one time point within the cycle, differential patterns of change in ovarian hormones between sexually active and abstinent women predicted differential patterns of change in inflammation (46). Because each cycle phase represents different reproductive priorities, the immune system likely uses relative endocrine signals—such as increases or decreases—to determine the response most appropriate to that phase.

Subtle shifts in the T_H1-T_H2 ratio may be relevant in caring for patients with autoimmune conditions in which cytotoxicity is chronically high (associated with very high T_H1-T_H2 ratio, e.g., type 1 diabetes) or low (associated with very low T_H1-T_H2 ratio, e.g., systemic erythematous lupus). Our findings, along with other studies examining menstrual cycle–related shifts in immunity (4), indicate the need to consider cycle phase, and likely partnered sexual behavior, in the clinical interpretation of immune biomarkers such as T_H1-T_H2 ratios. These findings call for further study of the behavioral health implications of sexual activity within a relationship above and beyond the traditional sexual risk-taking paradigm. Moreover, cytokines such as IFN-γ and IL-4 are neuroactive, and their central action has been implicated in mood disturbances (47), including menstrual cycle–related mood disorders (48). Separately, it has been noted that women reporting regular sexual intercourse (several times per month) are significantly more likely to meet criteria for premenstrual dysphoric disorder than women reporting infrequent sexual intercourse (a few times per year) (49). Our data suggest a link between these separate findings: Specifically, it is possible that, for vulnerable individuals, (normal) cyclical variations in T_H1-associated cytokines are exacerbated by sexual activity which then lead to greater premenstrual mood disturbance.

Shifts in the cytokine ratio were associated with corresponding shifts in the P-E_2 ratio, suggesting coordination between endocrine and immune systems. Many earlier studies have similarly documented an effect of menstrual hormones on T_H1-T_H2 ratios (50, 51). These findings imply that hormonal manipulations may be used clinically to replicate immune effects of sexual activity. However, studies that have attempted to manipulate T_H1-T_H2 ratios ex vivo in blood samples from women at follicular and luteal phases failed to find any significant differences across increasing concentrations of P and/or E_2 (12). It is likely that endocrine factors work in concert with other systems (e.g., the autonomic nervous system, which is immunoregulatory (52) and which facilitates female sexual arousal (53)) to regulate the impact of partnered sexual activity on women's immunity.

These findings also support the need to consider the broader context of interactions across behaviors and endocrine and immune systems. That is, there are likely ongoing inputs from behavioral patterns that modulate endocrine activity which in turn modulates immune activity. For example, it is possible that sexual activity and relationship quality interact to amplify effects on immune response. We were unable to control for relationship status in the present study, because all participants in the sexually active group were in relationships; future work examining women in long-distance or otherwise pragmatically sexually abstinent but committed relationships may help tease apart these effects.

Limitations to the present study include its small sample of predominantly white participants. We were also limited in the number of menstrual cycles we could sample. There is much variation in hormonal measurements between cycles within a woman, let alone differences between ethnic groups (54). This limited sampling may be why we did not find higher E_2 among sexually active women, an effect that has been previously noted to be significant but small (13). It is also possible that the effect of sexual activity on E_2 across the cycle is moderated by parity; because our sample was predominantly nulliparous young women, this hypothesis could not be tested. However, at least one study has shown that parity does not differentially predict the effect of intimate relationship status on E_2 or P across the menstrual cycle (55), suggesting that the effect of sexual activity on ovarian hormones may not depend on parity. Similarly, our sample comprised relatively young women early in their reproductive careers. Life history theory suggests that among older women who have fewer future opportunities for reproduction, tradeoffs such as the ones observed in this study should be shifted in favor of reproduction (56). That is, among older (but still reproductive-age) women, we should expect the immuno-modulatory effect of sexual activity to be larger than that seen in this young sample. In women past reproductive age (that is, post-menopausal), however, the expected effects of sexual activity are unclear. Certainly there are significant changes to both endocrine and immune systems during menopause, and there are also aging-related changes in response to environmental or psychosocial cues (57, 58). However, many aspects of response to sexual stimuli (e.g., vaginal arousal) are similar in pre- and post-menopausal women, including hormonal response (59). Further study is needed to examine the immunomodulatory effects of sexual activity across the lifespan.

We examined women not currently taking OCs and found evidence for mediation of partnered sexual activity's impact

on immune function via hormonal changes across the cycle. Thus it is likely that, to the extent that OCs limit the degree of hormonal fluctuation across the menstrual cycle, the effect of sexual activity on cytokine ratios also would be limited. Although likely not the case in our sample (because all women showed evidence of ovulation), it is also possible that more broadly, immune differences in sexually active versus abstinent women are at least partially driven by differences in rates of ovulation (13). Because women taking OCs are significantly less likely to ovulate (this being one of the main mechanisms by which OCs prevent pregnancy), we would expect fewer immune effects of sexual activity than in women not taking OCs.

Because most of the sexually active women in the present study were in committed long-term monogamous sexual relationships with men, it is also unknown if these results would extend into other types of sexual relationships, e.g., with multiple partners, with women, or with casual sexual partners outside of a steady relationship. It is likely that the immune response to a novel partner would differ from a regular sexual partner, both regarding exposure to the partner's microbiome (60) and in the psychosocial context of the sexual behavior. In short, this work would benefit from replication in a broader population, with longer follow-up, canvassing a broader array of sexual behaviors. Such follow-up studies may be able to tease apart the specific effects of vaginal penetration/stimulation compared with (or in concert with) the effects of sexual activity on relationship satisfaction and other psychosocial factors. Of note, there may be interactions between these effects and exposure to chronic stress, pathogens, and other immune challenges that were not observed in this sample of young healthy women living in a post-industrial environment.

The ratio of T_H1/T_H2 cells was originally proposed as a marker of immune dysregulation, particularly in autoimmune and allergic conditions (61). However, the "T_H1/T_H2 paradigm" does not universally explain reproductive events (37) nor directly account for the more recently discovered T_H17 cells (62). It is also possible that measures of stimulated lymphocyte cytokine production, or other measures of immune activity, would have differing results from our measures of circulating cytokine levels. Finally, this preliminary study investigated immune responses in healthy women who were actively contracepting, and as such it did not directly address the impact of the observed effects on fertility or infertility. As such, much more work is needed to contextualize our findings in the broader scope of the immune system and to examine the proposed mechanisms on clinically relevant outcomes, such as pregnancy.

CONCLUSION

The findings from this study suggest that partnered sexual behavior is related to cycle-related shifts in endocrine and cytokine parameters in healthy women. Recent studies have shown interactions between menstrual cycling and sexual activity on other aspects of immunity, such as mucosal antibodies (18), C-reactive protein (2), and antiviral activity (63, 64). This work underscores the importance of considering the environmental—including social—context of endocrine-immune interactions (65) and points to a novel behavioral paradigm for research and clinical care in reproductive-age women.
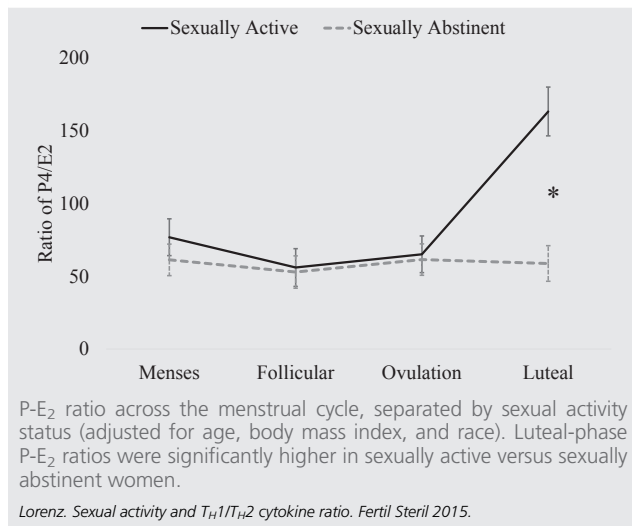
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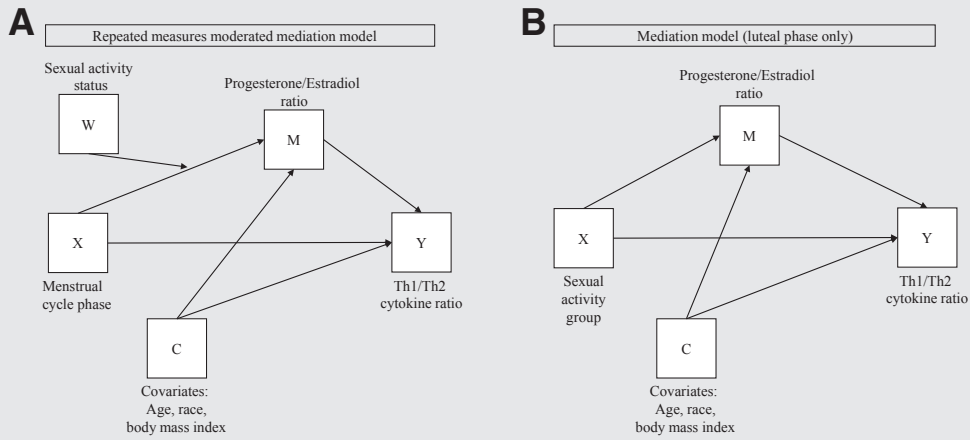
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SUPPLEMENTAL FIGURE 1

P-E$_2$ ratio across the menstrual cycle, separated by sexual activity status (adjusted for age, body mass index, and race). Luteal-phase P-E$_2$ ratios were significantly higher in sexually active versus sexually abstinent women.

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.

SUPPLEMENTAL FIGURE 2

Conceptual diagram for mediation analyses.

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.

SUPPLEMENTAL TABLE 1

Peripheral unstimulated serum cytokine concentrations, by cycle phase and sexual activity status (unadjusted).

| | Interferon-γ | | | | | | | | Interleukin-4 | | | | | | | |
| | Menstrual | | Follicular | | Ovulatory | | Luteal | | Menstrual | | Follicular | | Ovulatory | | Luteal | |
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sexually active	10.76	13.39	13.77	17.59	19.30	27.42	12.30	13.75	7.28	21.45	5.44	14.20	6.53	10.82	7.82	14.37
Sexually abstinent	10.12	11.43	35.67	98.02	9.87	11.06	13.52	21.17	4.54	11.45	14.37	44.50	8.13	17.33	3.94	7.17

Note: All cytokine values are expressed in pg/mL. There were no significant differences in the mean cytokine values between groups at any time point.

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.

SUPPLEMENTAL TABLE 2

Effect estimates for mediation analyses.

Parameter	Effect estimate	Standard error
Repeated-measures moderated mediation (corresponding to Supplemental Fig. 2A)		
Direct effect of X on Y	−0.13	0.19
Effect of X on M	0.10	5.93
Effect of W on M	−20.28	24.51
Effect of XW on M	19.05	9.33
Effect of M on Y	0.01	0.00
Conditional effect of X on Y through M at level 1 of moderator (Sexually active)	0.17	0.16
Conditional effect of X on Y through M at level 2 of moderator (Sexually abstinent)	0.00	0.06
Index of moderated mediation	0.17	0.17
Luteal phase–only mediation model (corresponding to Supplemental Fig. 2B)		
Direct effect of X on Y	−0.06	1.90
Effect of X on M	115.78	38.00
Effect of M on Y	0.01	0.01
Indirect effect of X on Y through M	1.66	11.67

Lorenz. Sexual activity and T_H1/T_H2 cytokine ratio. Fertil Steril 2015.