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ORIGINAL ARTICLE *Reproductive epidemiology*

Cannabis use while trying to conceive: a prospective cohort study evaluating associations with fecundability, live birth and pregnancy loss

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STUDY QUESTION: Is cannabis use assessed via urinary metabolites and self-report during preconception associated with fecundability, live birth and pregnancy loss?

SUMMARY ANSWER: Preconception cannabis use was associated with reduced fecundability among women with a history of pregnancy loss attempting pregnancy despite an increased frequency of intercourse.

WHAT IS KNOWN ALREADY: Cannabis use continues to rise despite limited evidence of safety during critical windows of pregnancy establishment. While existing studies suggest that self-reported cannabis use is not associated with fecundability, self-report may not be reliable.

STUDY DESIGN, SIZE, DURATION: A prospective cohort study was carried out including 1228 women followed for up to six cycles while attempting pregnancy (2006 to 2012), and throughout pregnancy if they conceived.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women aged 18–40 years with a history of pregnancy loss ($n = 1228$) were recruited from four clinical centers. Women self-reported preconception cannabis use at baseline and urinary tetrahydrocannabinol metabolites were measured throughout preconception and early pregnancy (up to four times during the study: at baseline, after 6 months of follow-up or at the beginning of the conception cycle, and weeks 4 and 8 of pregnancy). Time to hCG-detected pregnancy, and incidence of live birth and pregnancy loss were prospectively assessed. Fecundability odds ratios (FOR) and 95% CI were estimated using discrete time Cox proportional hazards models, and risk ratios (RRs) and 95% CI using log-binomial regression adjusting for age, race, BMI, education level, baseline urine cotinine, alcohol use and antidepressant use.

MAIN RESULTS AND THE ROLE OF CHANCE: Preconception cannabis use was 5% (62/1228), based on combined urinary metabolite measurements and self-report, and 1.3% (11/789) used cannabis during the first 8 weeks of gestation based on urinary metabolites only. Women with preconception cannabis use had reduced fecundability (FOR 0.59; 95% CI 0.38, 0.92). Preconception cannabis use was also associated with increased frequency of intercourse per cycle (9.4 ± 7 versus 7.5 ± 7 days; $P = 0.02$) and higher LH (percentage change 64%, 95% CI 3, 161) and higher LH:FSH ratio (percentage change 39%, 95% CI 7, 81). There were also suggestive, though imprecise, associations with anovulation (RR 1.92, 95% CI 0.88, 4.18), and live birth (42% (19/45) cannabis users versus 55% (578/1043) nonusers; RR 0.80, 95% CI 0.57, 1.12). No associations were observed between preconception cannabis use and pregnancy loss (RR 0.81, 95% CI 0.46, 1.42). Similar results were observed after additional adjustment for parity, income, employment status and stress. We were unable to estimate associations between cannabis use during early pregnancy and pregnancy loss due to limited sample size.

LIMITATIONS, REASONS FOR CAUTION: Owing to the relatively few cannabis users in our study, we had limited ability to make conclusions regarding live birth and pregnancy loss, and were unable to account for male partner use. While results were similar after

excluding smokers, alcohol use and any drug use in the past year, some residual confounding may persist due to these potential co-exposures.

WIDER IMPLICATIONS OF THE FINDINGS: These findings highlight potential risks on fecundability among women attempting pregnancy with a history of pregnancy loss and the need for expanded evidence regarding the reproductive health effects of cannabis use in the current climate of increasing legalization.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland (Contract numbers: HHSN267200603423, HHSN267200603424, HHSN267200603426, HHSN275201300023I). Jeannie G. Radoc has been funded by the National Institutes of Health Medical Research Scholars Program, a public–private partnership supported jointly by the National Institutes of Health and generous contributions to the Foundation for the National Institutes of Health from the Doris Duke Charitable Foundation (DDCF Grant # 2014194), Genentech, Elsevier, and other private donors. The authors report no conflict of interest in this work and have nothing to disclose.

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Introduction

The multibillion dollar cannabis industry continues to expand as medical and recreational use skyrockets (Carliner *et al.*, 2017). As popularity grows, so does normalization, with cannabis currently legalized in 34 US states and counting (NCSL, 2019). Parallel with the movement toward decriminalization, the prevalence of cannabis use has continued to rise, with self-reported use nearly doubling among women of reproductive age over the past 20 years (Brown *et al.*, 2017; Kerr *et al.*, 2018). While the American College of Obstetricians and Gynecologists discourages cannabis use in pregnant women and those trying to conceive (Committee on Obstetric Practice, 2017), recent studies show that use during pregnancy and in the year prior to pregnancy has increased substantially (Ko *et al.*, 2015; Corsi *et al.*, 2019a; Young-Wolff *et al.*, 2019), likely due to increasing perceptions of overall safety and acceptability (Ko *et al.*, 2015; Jarlenski *et al.*, 2017; Chang *et al.*, 2019).

Despite growing perceptions of harmlessness, data to support or refute the safety of cannabis use before and during pregnancy are limited. Some evidence suggests that exogenous cannabinoids may inhibit release of GnRH, a key factor involved in ovulatory function, uterine receptivity and implantation (Paria *et al.*, 1995; Paria *et al.*, 2001; Brown and Dobs, 2002; Brents, 2016; Walker *et al.*, 2019), which, in turn, could have implications for fecundability and pregnancy loss. To date, epidemiologic research on cannabis and reproductive outcomes has mostly focused on neonatal outcomes (Hingson *et al.*, 1982; Hatch and Bracken, 1986; Jutras-Aswad *et al.*, 2009; Varner *et al.*, 2014; Ryan *et al.*, 2018; Sharapova *et al.*, 2018; Corsi *et al.*, 2019b; El Marroun *et al.*, 2019; Metz *et al.*, 2019; Rodriguez *et al.*, 2019). The few prior studies on fecundability and pregnancy loss suggest that self-reported cannabis use is not detrimental to fecundability (Joesoef *et al.*, 1993; Kasman *et al.*, 2018; Wise *et al.*, 2018) or pregnancy viability in noninfertility treatment settings (Wilcox *et al.*, 1990; Kline *et al.*, 1991; Ness *et al.*, 1999; Nassan *et al.*, 2019a). Importantly, these studies assessed cannabis use using self-report that may underestimate use particularly during preconception and early pregnancy due to stigma (Fendrich *et al.*, 2004), even in the setting of legalization (Metz *et al.*, 2019). Longitudinal assessment of urinary metabolites is needed to objectively capture cannabis use over preconception and early pregnancy as exposures during these sensitive windows may have important consequences for the establishment and maintenance of a healthy pregnancy.

Therefore, our objective was to examine associations of cannabis use during critical windows of pregnancy establishment, including preconception and early pregnancy, with fecundability, live birth and pregnancy loss. Cannabis use was assessed using urinary metabolites measured at multiple time points in conjunction with self-report. We also evaluated associations with reproductive hormones and ovulation to provide insight into potential mechanisms.

Materials and methods

Study design and population

This study was a secondary analysis of the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, a multicenter, block-randomized, double-blind, placebo-controlled clinical trial investigating preconception low-dose aspirin on pregnancy outcomes in four US medical centers (Pennsylvania, New York, Utah and Colorado; 2006–2012) (Schisterman *et al.*, 2014). Participants were 18–40 years old, actively trying to conceive, and had 1–2 prior pregnancy losses ($n = 1228$). Exclusion criteria included known current or recent alcohol or illicit drug abuse, any self-reported major psychiatric diagnosis (including bipolar illness, schizophrenia, uncontrolled depression and uncontrolled anxiety disorder), and any prior diagnosis of infertility. More study details are available elsewhere (Schisterman *et al.*, 2013).

Women were followed for up to six menstrual cycles while attempting pregnancy, and throughout pregnancy if conception occurred. Women completed questionnaires at baseline regarding sociodemographics, lifestyle, health and reproductive history. Height and weight were measured using standardized protocols and were used to calculate BMI. During the first two menstrual cycles of follow-up, women completed daily diaries, which included information on intercourse, and they provided daily first-morning urine samples, which were used to measure reproductive hormones and identify early pregnancy losses (described in detail below).

Ethical approval

Institutional Review Board approval was obtained at each study site and the data coordinating center. All participants provided written informed consent. The parent trial is registered with ClinicalTrials.gov (#NCT 00467363).

Assessment of cannabis use

Cannabis use was assessed using biomarker measurement and self-report. Self-reported cannabis use was obtained from a self-administered baseline questionnaire which asked, 'How often have you used marijuana, pot, or hashish in the past 12 months'? with responses ranging from never, rarely, occasionally, sometimes, often, to daily; results were categorized as any versus no use in the past 12 months for analysis given small numbers in the frequent use categories (70% of self-reported users reported 'rarely', corresponding to 1/month or less). Longitudinal measurement of urinary metabolites occurred after study completion using stored urine samples, with each woman contributing up to four urinary metabolite measurements (two during preconception and two in early pregnancy). Preconception samples were measured at baseline, after 6 months of follow-up for women who did not conceive (median 20.4 weeks after baseline, 25th percentile 18.9 weeks, 75th percentile 22.1 weeks), and at the beginning of the cycle of conception for women who conceived (median 8.4 weeks after baseline, 25th percentile 4.6 weeks, 75th percentile 13.4 weeks). For women who became pregnant, two samples in early pregnancy were measured at weeks 4 and 8 of pregnancy. Metabolites were measured via a biochip array (Drugs of Abuse-Ultra) chemiluminescent immunoassay measured on the Evidence Investigator (Randox Toxicology, County Antrim, UK). The assay measured tetrahydrocannabinol (THC) concentrations with the following metabolites: (-)-11-nor-9-Carboxy-Δ9-THC, (±)-11-Hydroxy-Δ9-THC, Δ8-THC and Δ9-THC. A woman was identified as positive based on the standard manufacturer-recommended cut-off (100 ng/ml), and the interassay coefficients of variation (CVs) were 16.3% at mean concentrations of 21.4 ng/ml and 19.0% at 102.9 ng/ml. Confirmatory testing was not performed.

Outcome measures

The primary outcomes of interest included time to hCG-detected pregnancy, and incidence of live birth and pregnancy loss. Secondary outcomes included urinary reproductive hormone concentrations and anovulation.

Time to pregnancy was defined as the number of menstrual cycles until hCG-detected pregnancy. As previously described (Schisterman *et al.*, 2014; Mumford *et al.*, 2016), pregnancies were identified by the following: a positive urine pregnancy test (Quidel Corporation, San Diego, CA, USA) sensitive to 25 mIU/ml hCG, which was conducted on spot urine samples at end-of-cycle clinic visits when a participant reported missing menses; or free beta-hCG testing performed on daily first-morning urine samples from the last 10 days of the first two study cycles and on spot urine samples from all end-of-cycle visits (Diagnostic Automation, Inc., Calabasas, CA, USA; BioVendor, Asheville, NC, USA). This testing allows for more sensitive detection of very early pregnancies compared to conventional pregnancy tests (Mumford *et al.*, 2016). Live birth was ascertained through medical record abstraction. Pregnancy loss included hCG-detected losses and clinically recognized losses. hCG-detected losses were defined in two ways: a positive hCG pregnancy test at home or the clinic followed by a lack of clinical signs of pregnancy at the study ultrasound, or a positive free beta-hCG test followed by a lack of positive pregnancy test at home or the clinic (Mumford *et al.*, 2016). Clinically recognized pregnancy losses were all losses detected after ultrasound confirmation of pregnancy, including pre-embryonic, embryonic, ectopic and fetal losses, and stillbirths.

Reproductive hormones, including estrone-1-glucuronide (E1G), pregnanediol-3-glucuronide (PdG), FSH and LH, were assayed at four time points during each of the first two preconception cycles, timed to specific menstrual cycle phases. Urinary E1G and PdG were measured by competitive chemiluminescence duplex assay (Quansys Biosciences, Logan, UT, USA), and LH and FSH via reagent/sandwich immunoassay (Roche Diagnostics, Indianapolis, IN, USA). The interassay laboratory CVs were 20% for E1G, 23% for PdG, 1.6% for LH and 1.8% for FSH.

Androgens and sex hormone-binding globulin (SHBG) were measured in serum from the baseline visit. Total testosterone concentration (TT; nanograms per deciliter) was determined by liquid chromatography and tandem mass spectrometry using a Shimadzu Prominence liquid chromatogram (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) with an ABSceix 5500 tandem mass spectrometer (AB SCIEX, Framingham, MA, USA). Interassay CVs for TT were 2.0% at 189.81 and 1.4% at 809.54 ng/ml. Free testosterone (fT) was calculated as $24.00314 \times TT/\log_{10}S—0.0499 \times TT^2$ and free androgen index (FAI) as $100 \times (TT/SHBG)$, where TT was measured in nanomoles per liter and SHBG in nanomoles per liter (Sartorius *et al.*, 2009). SHBG concentration was determined by SHBG reagent/sandwich immunoassay method/electrochemiluminescence (Roche Diagnostics, Indianapolis, IN, USA) utilizing a Roche COBAS 6000 chemistry analyzer (Roche Diagnostics). Interassay CVs were 3.0% at 55.64 nmol/L and 3.8% at 19.74 nmol/L. Dehydroepiandrosterone sulfate (DHEAS) was determined by DHEA sulfate reagent/competitive immunoassay method/electrochemiluminescence using a Roche COBAS 6000 chemistry analyzer (Roche Diagnostics). Interassay CVs were 4.6% at 5.43 μmol/L and 4.9% at 13.01 μmol/L. Anovulation was defined as the absence of ovulation detected using fertility monitors (ClearBlue Easy Fertility Monitor; Inverness Medical, Waltham, MA, USA) across up to six cycles of preconception follow-up, with urinary luteal PdG measurements to improve sensitivity of ovulation detection in the first two cycles of study participation, as previously described (Behre *et al.*, 2000; Park *et al.*, 2007; Johnson *et al.*, 2015).

Statistical analysis

All 1228 women had information on cannabis use, either via preconception urine assessment ($N = 1218$) or self-report ($N = 1220$). Baseline sociodemographic and lifestyle characteristics were compared among cannabis users and nonusers.

To assess time to pregnancy, we estimated fecundability odds ratios (FOR) and 95% CI comparing users and nonusers using discrete time Cox proportional hazard models accounting for right censoring and left truncation (cycles attempted to conceive prior to enrollment; median two cycles, 25th percentile one cycle, 75th percentile four cycles). For live birth and pregnancy loss, we estimated risk ratios (RRs) and 95% CI using log-binomial regression. The primary exposure for the

fecundability and live birth models was any exposure to cannabis (indicated from either the baseline self-report or urinary preconception assessments). We also evaluated baseline self-report, baseline urinary metabolites, and any urinary preconception assessment separately. Pregnancy loss models were restricted to women who became pregnant, with inverse probability weights to account for possible selection bias due to restricting based on factors associated with pregnancy (Hernan et al., 2004). Given the low numbers of women exposed to cannabis in early pregnancy, we were unable to estimate associations between early pregnancy use and pregnancy loss. As cannabis use was associated with withdrawal from the study, analyses for live birth and pregnancy loss were also weighted to account for participant withdrawal (of the 797 women who became pregnant, 12 were missing information on pregnancy outcome) using inverse probability weights based on factors associated with withdrawal including cannabis use, opioid use, antidepressant use, treatment arm, age, marital status, number of prior losses, parity, race, education and cotinine level (Supplementary Table SI).

We evaluated associations between recent cannabis use and reproductive hormones and anovulation to provide insight into potential mechanisms of action. Associations between urinary assessment of baseline cannabis use and log-transformed hormone concentrations in the first cycle of participation were estimated using linear mixed models with polynomial terms to account for hormone periodicity. Percentage differences were calculated as ((exp(coefficient)-1)*100). Log-binomial regression was used to estimate associations between urinary assessment of baseline cannabis use and risk of anovulation in the first cycle, as well as with a pattern of anovulatory cycles over follow-up (≥50% of cycles anovulatory).

All models were adjusted for age, race, BMI, education, urinary cotinine levels (Jeemon et al., 2010), alcohol use and antidepressant use based on factors known to be associated with both reproductive outcomes and cannabis use. Results were compared with models that additionally adjusted for parity, income, employment status and stress. We further compared results after excluding women reporting smoking, alcohol or other drug use. Missingness in covariates ranged from 0% for age, race and antidepressant use to 1.4% for cotinine, 3.6% for employment, and 15.9% for stress, with missingness for the exposures ranging from 0.7% for self-reported use at baseline to 1.4% for urine assessment at baseline. Multiple imputation with fully conditional specification was used to account for missing exposure and covariate data.

As information on male partner cannabis use was not available in this study, there may be the potential for residual confounding, as behaviors such as drug use are likely to have concordance within couples (Leonard and Homish, 2005; Meyler et al., 2007), and cannabis has been associated with male reproductive health and semen quality (Kolodny et al., 1974; Gammon et al., 2005; Nassan et al., 2019b; Rajanahally et al., 2019; Carroll et al., 2020). We utilized two sensitivity analysis approaches for unmeasured confounding, including the e-value method (VanderWeele and Ding, 2017) and a simulation study to address this concern. We performed a simulation study where we varied the strength of a confounder needed to explain the associations observed across a range of potential scenarios, and compared results after adjusting for this simulated confounder. Specifically, we varied the association between male partner and female partner cannabis use from OR 1.5 to 5.0, and the association between male cannabis use and fecundability from FOR 0.60 to 2.00.

Analyses were completed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Overall, 5% (62/1228) of women used cannabis during the preconception period as identified either by a positive urine test or by self-report. Of these, 71% self-reported use ($n = 44$) and 53% had a positive urine test ($n = 33$). Forty-five percent of women with a positive urine test also self-reported use (15/33). Of the women who became pregnant, only 1.3% (11/789) used cannabis during the first 8 weeks of pregnancy as identified by urine testing. Cannabis use identified by either urine test or self-report was more common among women who self-identified as a non-white race and had lower education (Table I). Cannabis use was also associated with higher urine cotinine levels, more frequent alcohol use, antidepressant use, early withdrawal from the study and a higher frequency of intercourse. Women from the Colorado site were more likely to self-report cannabis use than those from other study sites (11% (8/73) versus 3% (29/993) in Utah, 3% (2/74) in Pennsylvania and 6% (5/77) in New York).

Forty-two percent (26/62) of women who used cannabis any time before conception became pregnant (based on combined urine testing and self-report), whereas 66% (771/1166) of women who did not use cannabis became pregnant (Table II). Women who used cannabis during the preconception period (assessed either by self-report or urinary metabolites) had 41% reduced fecundability in both unadjusted and adjusted models (aFOR 0.59, 95% CI 0.38, 0.92). Point estimates were consistent when evaluating self-report and urinary assessments individually as well. Specifically, self-reported cannabis use was associated with a 42% reduction in fecundability (FOR 0.58, 95% CI 0.35, 0.98) and urine metabolite measurement with 47% reduction, though results were more imprecise after adjustment (FOR 0.53, 95% CI 0.29, 0.96; aFOR 0.60, 95% CI 0.32, 1.12). Similar results were observed after additional adjustment for parity, income, employment status and stress. Exclusion of smokers (FOR 0.54, 95% CI 0.31, 0.97), alcohol users (FOR 0.58, 95% CI 0.24, 1.39) or women who reported any drug use in the past year (FOR 0.54, 95% CI 0.34, 0.86) also yielded similar results (Supplementary Table SII). Using a simulation study to address potential unmeasured confounding by male partner cannabis use, we found that male partner cannabis use would need to be associated with fecundability in the range of 0.60 to 0.75 to attenuate our findings such that the upper confidence bound includes 1.0, associations not observed in prior literature (Kasman et al., 2018; Wise et al., 2018) (Supplementary Fig. S1). Similar results were found using the e-value method, with even stronger confounding needed to bring the point estimate of the observed association to the null.

Preconception cannabis use based on urine testing or self-report was suggestive of a potential association with a lower risk of live birth before adjustment (42% (19/45) versus 55% (578/1043): RR 0.66, 95% CI 0.47, 0.92; Supplementary Table SIII), though the number of cannabis users with live births was small and results were attenuated after adjustment (RR 0.80, 95% CI 0.57, 1.12). Similar results were also observed for self-reported use (unadjusted RR 0.60, 95% CI 0.40,

Table I Prevalence of urine metabolites and self-reported cannabis use according to sociodemographic characteristics among participants in the Effects of Aspirin in Gestation and Reproduction trial.

Characteristics[a, b]	Urine test any time before conception[c]			Self-reported use[d]		
	Positive (N = 33)	Negative (N = 1185)	P	Any (N = 44)	None (N = 1176)	P
Age, years	27.2 ± 5.1	28.8 ± 4.8	0.10	29.1 ± 6	28.7 ± 4.7	0.61
BMI, kg/m^2	27.6 ± 6.2	26.3 ± 6.5	0.10	27.2 ± 7.1	26.3 ± 6.5	0.43
Race, % white	24 (73)	1128 (95)	<0.0001	36 (82)	1121 (95)	<0.0001
Education, % with greater than high school	25 (76)	1022 (86)	0.08	32 (73)	1020 (87)	0.008
Income, % ≥$75 000	14 (42)	619 (52)	0.26	22 (50)	615 (52)	0.76
Employed, %	18 (64)	870 (76)	0.16	31 (72)	861 (76)	0.58
Physical activity level, %			0.28			0.30
Low	6 (18)	313 (26)		16 (36)	305 (26)	
Moderate	12 (36)	484 (41)		15 (34)	481 (41)	
High	15 (46)	387 (33)		13 (30)	390 (33)	
Average stress in cycle 1[e]	0.74 (0.56)	0.84 (0.52)	0.26	0.96 (0.56)	0.83 (0.52)	0.18
Urine cotinine level ≥10.95 ng/ml, %	21 (64)	121 (10)	<0.0001	18 (41)	123 (11)	<0.0001
Any alcohol intake in past 12 months, %	22 (69)	379 (32)	<0.0001	36 (82)	370 (32)	<0.0001
Antidepressant use, % any	11 (33)	196 (17)	0.01	11 (25)	196 (17)	0.15
Opioid use, % any	8 (24)	202 (17)	0.28	10 (23)	196 (17)	0.29
Number of prior live births, %			0.61			0.25
0	18 (55)	555 (47)		25 (57)	548 (47)	
1	11 (33)	422 (36)		15 (34)	420 (36)	
2	4 (12)	208 (18)		4 (9)	208 (18)	
Number of prior pregnancy losses, %			0.12			0.61
1	18 (55)	800 (68)		28 (64)	792 (67)	
2	15 (46)	385 (33)		16 (36)	384 (33)	
Aspirin treatment group, %	16 (49)	595 (50)	0.84	20 (46)	589 (50)	0.55
Withdrew from study, %	12 (36)	125 (11)	<0.0001	11 (25)	124 (11)	0.003
Study site, %			<0.0001			0.002
Pennsylvania	2 (6)	73 (6)		2 (5)	72 (6)	
New York	9 (27)	67 (6)		5 (11)	72 (6)	
Utah	19 (58)	976 (82)		29 (66)	964 (82)	
Colorado	3 (9)	69 (6)		8 (18)	65 (6)	
Intercourse frequency per cycle, daily diary; mean (SD)	9.4 (7)	7.5 (7)	0.02	8.4 (9)	7.5 (6)	0.19
Intercourse frequency/month at baseline, %			0.05			0.20
A lot	15 (56)	359 (33)		16 (42)	357 (33)	
Average	12 (44)	668 (61)		22 (58)	663 (61)	
A few	0 (0)	68 (6)		0 (0)	68 (6)	
Time since last miscarriage, %			0.01			0.23
≤4 months	8 (26)	637 (55)		17 (40)	629 (54)	
5–8 months	8 (26)	212 (18)		10 (23)	210 (18)	
9–12 months	3 (10)	96 (8)		5 (12)	94 (8)	
>12 months	12 (39)	223 (19)		11 (26)	225 (19)	

[a]Values are N (%) or mean ± SD with *P*-values from χ^2 tests or Fisher's exact test as appropriate.
[b]Missingness in covariates is as follows: BMI, n = 16; education, n = 1; cotinine, n = 17; alcohol, n = 16; income, n = 1; employment, n = 44; and stress, n = 195.
[c]Urine tested for tetrahydrocannabinol at baseline or the last preconception cycle.
[d]Any self-reported cannabis use during the 12 months before baseline.
[e]Likert scale of 0 (no stress) to 3 (a lot of stress).

Table II Time to pregnancy by urine tested[a] and self-reported[b] cannabis use among women in the EAGeR cohort.

Cannabis use exposure[c]	N[d]	N (%), pregnancies		Unadjusted FOR (95% CI)[e]	Adjusted FOR (95% CI) Model 1[f]	Adjusted FOR (95% CI) Model 2[g]
Overall	1228	797	(64.9)			
Combined urine sample and self-reported use						
At or before baseline visit						
Negative and none	1168	772	(66.1)	Reference	Reference	Reference
Positive or any	60	25	(41.7)	**0.56 (0.37, 0.87)**	**0.62 (0.39, 0.97)**	**0.62 (0.40, 0.98)**
Any time before conception						
Negative and none	1166	771	(66.1)	Reference	Reference	Reference
Positive or any	62	26	(41.9)	**0.55 (0.36, 0.84)**	**0.59 (0.38, 0.92)**	**0.59 (0.38, 0.92)**
Urine tested						
Baseline visit						
Negative	1181	773	(65.5)	Reference	Reference	Reference
Positive	30	12	(40.0)	0.58 (0.31, 1.08)	0.67 (0.35, 1.30)	0.64 (0.33, 1.23)
Any time before conception						
Negative	1185	777	(65.6)	Reference	Reference	Reference
Positive	33	13	(39.4)	**0.53 (0.29, 0.96)**	0.60 (0.32, 1.12)	0.56 (0.30, 1.05)
Self-reported use						
During 12 months before baseline						
None	1176	777	(66.1)	Reference	Reference	Reference
Any	44	18	(40.9)	**0.55 (0.33, 0.91)**	**0.58 (0.35, 0.98)**	0.60 (0.36, 1.02)

EAGeR: Effects of Aspirin in Gestation and Reproduction, FOR: fecundability odds ratios. Bold indicates $P<-0.05$.
[a]Urine tested for tetrahydrocannabinol.
[b]Includes any amount of self-reported cannabis use.
[c]Missingness for cannabis use: urine testing at baseline, n = 17; urine testing at baseline + last preconception follow-up cycle, n = 10; self-reported use, n = 8.
[d]Numbers include participants with measured data. Multiple imputation was used to account for missing exposure and covariate information, thus all 1228 participants were included in analyses of FOR.
[e]From discrete time Cox proportional hazard models accounting for right censoring and left truncation.
[f]Adjusted for age, race, BMI, education level, baseline urine cotinine, alcohol use, and antidepressant use.
[g]Includes covariates included in Model 1 as well as parity, income, employment status, and stress.

0.90; adjusted RR 0.78, 95% CI 0.52, 1.17). No associations were observed with urine metabolite measurement alone and live birth.

No associations were observed between preconception cannabis use any time before conception (based on combined urine testing and self-report) and pregnancy loss (27% (7/26) versus 24% (181/759); RR 0.91, 95% CI 0.54, 1.52; Supplementary Table SIV). The number of cannabis users who experienced a pregnancy loss was too few to estimate loss risk with use during the first 8 weeks of pregnancy.

Cannabis use was associated with higher LH concentrations across the cycle (percentage change 64%, 95% CI 3, 162), and higher LH:FSH ratio (percentage change 39%, 95% CI 7, 81) though not with E1G, PdG, FSH, SHBG or DHEAS (Table III). There was a suggestion of increased TT and fT associated with cannabis use, though this association was attenuated after adjustment. Cannabis use was suggestive of an increased risk of anovulation in the first cycle (23% versus 13%; RR 1.92, 95% CI 0.88, 4.18), and with a pattern of anovulatory cycles over follow-up (≥50% of cycles, anovulatory: 19% versus 9%; RR 2.09, 95% CI 0.84, 5.21), though associations were not statistically significant.

Discussion

Despite the growing perception that recreational use of cannabis is harmless, we found that among women with a history of loss who were trying to become pregnant again, preconception cannabis use was associated with impaired fecundability despite an increased frequency of intercourse. Similar results were observed using both urine metabolite measurements during real-time study observation and self-report reflecting the preceding 12 months. Thus, past use may influence fecundability, which may not be immediately reversible and highlights the need for future studies to utilize multiple exposure assessment methodologies. Cannabis use was also associated with higher LH concentrations and LH:FSH ratio, suggesting mechanisms central to menstrual and ovulatory function, though associations were not observed with other reproductive hormones. There were also suggestive, though imprecise, associations with anovulation and live birth, though numbers were limited, and we were unable to account for male partner cannabis use in this study. These findings highlight potentially harmful associations between cannabis use and reproductive health outcomes among women with a history of pregnancy loss trying to conceive (Ko *et al.*, 2015; Corsi *et al.*, 2019a; Young-Wolff *et al.*, 2019).

Table III Reproductive hormone concentrations during the first cycle of follow-up and risk of anovulation by baseline urinary cannabis metabolites[a] among women in the EAGeR cohort.

	Urine test at baseline					
	Positive	Negative				
Anovulation	**N (%)**	**N (%)**	**Fisher's exact *P*-value**	**Unadjusted RR (95% CI)**	**Adjusted RR (95% CI) Model 1[b]**	**Adjusted RR (95% CI) Model 2[c]**
Cycle 1[d]	6 (23%)	148 (13%)	0.15	1.92 (0.88, 4.19)	1.87 (0.86, 4.07)	1.74 (0.85, 3.58)
≥50% of cycles anovulatory[e]	5 (19%)	106 (9%)	0.09	2.12 (0.85, 5.27)	2.00 (0.79, 5.05)	1.75 (0.85, 3.60)
Reproductive hormones	**Median (IQR)**	**Median (IQR)**	***T*-test *P*-value**	**Percentage change (95% CI)[f]**	**Percentage change (95% CI)[g]**	**Percentage change (95% CI)[h]**
E1G, ng/ml	41.1 (24.8, 75.5)	32.9 (15.7, 62.6)	0.12	31% (−7, 85)	12% (−22, 60)	10% (−23, 57)
PdG, μg/ml	7399 (2853, 17528)	5493 (2165, 12521)	0.20	39% (−6, 106)	19% (−20, 78)	21% (−19, 81)
FSH, mIU/ml	2.9 (0.8, 7.6)	1.7 (0.8, 4.2)	0.12	30% (−9, 83)	16% (−18, 65)	14% (−20, 62)
LH, mIU/ml	1.7 (0.2, 3.7)	0.5 (0.2, 1.7)	0.02	**80% (14, 185)**	**63% (2, 159)**	**64% (3, 162)**
LH: FSH ratio	0.4 (0.2, 0.8)	0.3 (0.2, 0.6)	0.03	**33% (3, 73)**	**38% (6, 79)**	**39% (7, 81)**
Total testosterone	2 3.5 (17.5, 29.0)	20.2 (15.2, 26.7)	0.06	**19% (2, 38)**	9% (−6, 27)	8% (−7, 26)
SHBG	48.6 (37.3, 74.6)	61.4 (42.5, 85.3)	0.06	−13% (−4, 28)	2% (−14, 21)	2% (−14, 22)
Free testosterone	0.34 (0.23, 0.43)	0.27 (0.21, 0.36)	0.06	**23% (6, 43)**	8% (−7, 25)	7% (−8, 24)
DHEAS	4.7 (3.5, 5.8)	4.6 (3.3, 6.2)	0.71	7% (−10, 28)	−8% (−10, 23)	−9% (−9, 24)

IQR, interquartile range; RR, risk ratios; E1G, estrone-1-glucuronide; PdG, pregnanediol-3-glucuronide; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate. Bold indicates $P<0.05$.

[a]Urine tested for tetrahydrocannabinol.

[b]Adjusted for age, race, BMI, education level, baseline urine cotinine, alcohol use, and antidepressant use.

[c]Includes covariates included in Model 1 as well as parity, income, employment status, and stress.

[d]Anovulation assessed in the first cycle of participation. Models estimate RRs using log-binomial regression.

[e]Percentage of anovulation calculated based on up to six cycles of follow-up.

[f]Unadjusted model: hormone∼cannabis + t + t^2 + t^3 + t^4, where t=day/cycle_length. The polynomial terms of t is used to mimic the curve of the hormone.

[g]Adjusted model: hormone∼cannabis + age + race + BMI + education level + cotinine + alcohol use + antidepressant use + t + t^2 + t^3 + t^4, where t=day/cycle_length. The polynomial terms of t are used to mimic the curve of the hormone.

[h]Adjusted model: hormone∼cannabis + age + race + BMI + education level + cotinine + alcohol use + antidepressant use + parity + income + employment status + stress + t + t^2 + t^3 + t^4, where t=day/cycle_length. The polynomial terms of t are used to mimic the curve of the hormone.

Previous studies on cannabis use and fecundability have yielded mixed results and have all relied on self-report (Mueller *et al.*, 1990; Joesoef *et al.*, 1993; Kasman *et al.*, 2018; Wise *et al.*, 2018). The present study extends prior work by incorporating longitudinal urine assessments in addition to self-report, which is especially important in a population of women attempting pregnancy, for whom stigma may result in underreporting (Fendrich *et al.*, 2004). Indeed, more than 50% of participants with measurable urinary metabolites did not self-report use, highlighting the need for urinary assessments. Other studies have also shown a similar level of under-reporting with no change after legalization (Claudius *et al.*, 2019), and reporting remains to be a concern especially among pregnant women (Garg *et al.*, 2016; Metz *et al.*, 2019). Moreover, the prevalence of self-reported use varied widely by site, with the Colorado site reporting the highest usage rates, likely due to legalization during the study period. Since results were largely consistent for fecundability using both measures, this may reflect a nontransient effect of cannabis on fecundability. However, this does not support the hypothesis that exposure misclassification alone may explain differences across studies. It is unknown why harmful associations were observed with fecundability in this study in contrast to prior work, though this finding could potentially be due to a lower rate of withdrawal in EAGeR compared to other studies (11% in EAGeR versus 20% in PRESTO (Wise *et al.*, 2018)), a lower prevalence of cannabis use compared to previous work (Hasin *et al.*, 2015; Kasman *et al.*, 2018; Wise *et al.*, 2018), or that women had to have a history of pregnancy loss to enroll in the study and may have tried to reduce their exposures to prevent future losses (Coleman *et al.*, 2005). Importantly, the time trying to conceive prior to study entry was handled differently in prior studies, and there is the possibility that the couples were trying to conceive for a longer period of time in prior studies and prior results may be influenced by subfertility. In addition, one prior study was cross-sectional and relied on self-report of both cannabis use and time to pregnancy (Kasman *et al.*, 2018). We did find that the association was robust to adjustment for numerous factors, including relevant lifestyle, demographic and socioeconomic factors, though we were unable to account for partner cannabis use. Interestingly, the cannabis users in our study reported more frequent intercourse, as has been shown in other studies (Sun and

Eisenberg, 2017). That we observe a potential reduction in fecundability associated with cannabis use, despite increased sexual frequency, may imply that a biological rather than behavioral mechanism is at play (Stanford and Dunson, 2007).

Several potential biologic mechanisms could explain our observed associations. Animal studies, particularly in rhesus monkeys, suggest cannabis disrupts the hypothalamic pituitary gonadal (HPG) axis (Brown and Dobs, 2002), altering GnRH pulsatility, subsequently affecting ovulation (Brents, 2016). Our findings are consistent with altered HPG function as we observed hormonal perturbations, and suggestive associations with an increased risk of anovulation in the cycle of cannabis use. It is also possible that cannabinoids may decrease uterine receptivity, as bound/activated CB1 cannabinoid receptors in the uterus have been shown to have embryotoxic effects (Schmid et al., 1997; Paria et al., 2001), which may in turn influence implantation. Finally, it is also possible that decreased fecundability may be a result of impaired endocannabinoid signaling, which is proposed to play a role in sperm transport in the female reproductive tract, capacitation and fertilization (Schuel et al., 2002).

We extended prior work on fecundability to evaluate possible associations with live birth and pregnancy loss in a preconception cohort, including early pregnancy losses not often captured in studies recruiting women in early pregnancy. However, given the small number of cannabis users in this cohort, we were unable to make conclusions regarding associations with these outcomes. The expression of cannabinoid receptors within the reproductive tract and developing embryo indicates a potential role in the transport of the embryo and synchronous development of the embryo and uterus (Karasu et al., 2011), and exposure to cannabinoids has resulted in failure of embryo development in mouse studies (Paria et al., 1995). Though most previous studies in women have not found associations with pregnancy loss (Wilcox et al., 1990; Kline et al., 1991; Ness et al., 1999), one recent study observed an increased risk among a cohort of women seeking infertility treatment (Nassan et al., 2019a). Indeed, the role of cannabis use on pregnancy loss is an area of important future research.

Our study has several strengths and limitations. First, we used urine testing for cannabis exposures and augmented with self-report to more completely capture cannabis use in this cohort. Though some studies suggest that cannabis may be more accurately reported than other drugs, such as cocaine and heroin, in some settings (Buchan et al., 2002; Fendrich et al., 2004; Zaldivar Basurto et al., 2009), others note that self-report during pregnancy may be especially inaccurate (Markovic et al., 2000; Garg et al., 2016; Metz et al., 2019), even in the setting of legalization (Metz et al., 2019). Importantly, urinary assessment also captures combined exposures via smoking, vaporization and ingestion, and has longer detection time of metabolites than measures in saliva or blood (Sharma et al., 2012). The half-life of cannabis also tends to be longer compared to other illicit drugs, and more frequent use increases the detectable half-life (half-life for infrequent users is about 1.3 days and for frequent users ranges from 5 to 13 days) (Huestis et al., 1996; Huestis and Cone, 1998; Smith-Kielland et al., 1999; Vandevenne et al., 2000; Sharma et al., 2012), which likely improved the sensitivity of our assessment of cannabis use. However, some users may have been missed due to infrequent urine testing. Though secondhand exposure may have inappropriately classified some nonusers as users, this is unlikely given a recent study that reported that 1 hour of passive exposure in ventilated and unventilated settings produced no positive tests when using a similar immunoassay (Cone et al., 2015). Although only 45% of women with a positive urine test also self-reported use, results regarding the agreement between self-report and urinary metabolite measurement are complicated by the different time periods assessed. Since self-report referred to the previous 12 months of use, we cannot disentangle self-report of past versus current use; this could explain lack of a positive urine test (i.e. current) among those that self-reported prior use. Overall, though there may be some degree of misclassification, we do not expect that this would explain our findings. Further, we were able to account for potential confounders, including cotinine, alcohol and antidepressant use, as well as minimize confounding by drug abuse, and related factors by design as women with a history of illicit drug abuse were excluded (though no details were asked regarding cannabis use disorder specifically). Given that many substances are used together, it can be challenging to tease apart associations for individual exposures even with covariate adjustment, though reassuringly results were also similar after excluding smokers, alcohol users and those reporting any drug use during the past year. However, information on male partner cannabis use was not collected, and there may be the potential for residual confounding by cannabis use in the male partners, though detailed sensitivity analyses suggest that associations between male cannabis use and fecundability would need to be quite strong (0.60–0.75) to make the associations we observed be nonstatistically significant. Prior studies suggest this association is approximately 0.87–1.24 (Kasman et al., 2018; Wise et al., 2018). Importantly, causality cannot be inferred based on the results of this study. We were limited in having small numbers of cannabis users, which limited our ability to make conclusions regarding associations with live birth and pregnancy loss. We observed that cannabis users were more likely to withdraw from the study, though only 11.4% of women withdrew from the study, which is lower than observed in other cohorts (Wise et al., 2018). There is the potential for bias for the findings with fecundability if withdrawal is also associated with fecundability, and this may have influenced the findings for live birth and loss as well, though inverse probability weights were used to address loss to follow up. Further, the lack of racial/ethnic diversity in our cohort reduces the generalizability of our findings. However, our study is strong in its prospective design and comprehensive evaluation of outcomes (such as very early pregnancy loss and reproductive hormones) which provide some insight into specific periods of susceptibility and plausible biological mechanisms.

Overall, among women attempting pregnancy with a history of pregnancy loss, cannabis use while trying to conceive was associated with reduced fecundability, potentially through effects on menstrual cycle function. These results highlight potentially harmful associations between cannabis use and reproductive health outcomes, and the need for expanded evidence regarding the effects of cannabis use on reproductive health in the current climate of increasing legalization.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

S.L.M., L.A.S., N.J.P., R.M.S. and E.F.S.: study design and data acquisition. S.L.M., K.S.F. and N.J.P.: data analysis. S.L.M., V.C.A. and Z.A.: wrote the first draft of the manuscript. All authors contributed to critical discussion, revisions to the manuscript and approval of the final submission.

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Conflict of interest

The authors have no conflicts to declare.

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