Title: Exogenous progesterone for smoking cessation in men and women: a pilot double-blind, placebo-controlled randomized clinical trial

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DECLARATION OF INTEREST

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ABSTRACT

Background & Aims: In some clinical studies men and women have been found to differ in their ability to quit smoking, perhaps as a result of progesterone. The primary aim of this study was to provide a preliminary test of whether progesterone (PRO), as compared with placebo (PBO), was more effective for smoking cessation in men and women.

Design: Pilot double-blind, placebo-controlled randomized clinical trial.

Setting: Minneapolis/St. Paul metro area, Minnesota, USA.

Participants: A total of 216 participants were enrolled including 113 men (18-60 years; PRO=56, PBO=57) and 103 women (18-50 years, premenopausal with self-reported regular menstrual cycles; PRO=51, PBO=52).

Intervention: Participants were randomized (1:1 within sex group) to either PRO (200mg twice daily) or PBO. Participants were assigned a quit date approximately 7 days after starting medication (luteal phase for women) and were followed for 12 weeks to assess relapse.

Measurements: The primary outcome was self-reported 7-day point prevalence abstinence (PPA) at week 4. Secondary outcomes included 7-day PPA at weeks 8 and 12, prolonged abstinence, continuous abstinence, urine cotinine <50ng/mL, expired carbon monoxide ≤5ppm and days to relapse.

Findings: There was a significant difference in 7-day PPA at week 4 among women (PRO: 18 [35.3%] vs. PBO: 9 [17.3%], Odds Ratio [95% confidence interval]=2.61 [1.04, 6.54], $p=0.041$) but not among men (PRO: 13 [23.2%] vs. PBO:12 [21.1%], 1.13 [0.47, 2.76], $p=0.782$). There was some evidence that PRO delayed relapse in women (Days to Relapse; PRO: 20.5 ± 29.6 vs. PBO: 14.3 ± 26.8 , $p=0.03$) but not in men (PRO: 13.4 ± 25.9 vs. PBO: 13.3 ± 23.8 , $p=0.69$).

Conclusions: Oral micronized progesterone may aid smoking cessation in women.

INTRODUCTION

Despite the declining prevalence, cigarette smoking persists as the leading cause of preventable morbidity and mortality worldwide (1). Extensive research has been conducted to identify effective smoking cessation interventions [i.e., computer/electronic aids (2), behavioral interventions (3) and pharmacotherapy (4)] however, nearly 70–85% still relapse within one year of a quit attempt (5).

Some research has shown that men and women differ in their ability to quit smoking (6–8) while others have disputed this gender disparity (9). One study found that women smoke fewer cigarettes per day, they smoke cigarettes with lower nicotine content and do not inhale as deeply as men (10). Other studies have found that women have greater nicotine dependency (11), have higher relapse rates (12,13), are less likely to achieve long-term abstinence (6) and have greater difficulty quitting than men (14,15). Given the health consequences (16) and economic burden of smoking (17,18), it is important to continue elucidating factors that help explain these sex/gender differences. One factor that has gained considerable attention is sex hormones, specifically progesterone.

The literature on the role of sex hormones in substance use disorders (including tobacco use disorder) continues to grow (19). Pre-clinical literature provides strong evidence indicating that progesterone may be protective against drug abuse behaviors, whereas estrogen may facilitate drug abuse behaviors (20–27). The clinical literature, however, is less clear (28). Our prior work in a randomized controlled trial observed improved smoking cessation outcomes among women who quit smoking during the luteal phase (high progesterone) compared to the follicular phase (low progesterone) of the menstrual cycle (29). In a retrospective cohort study using bupropion as a smoking cessation aid, Mazure and colleagues (2011) also found that women experienced improved smoking cessation outcomes when they quit smoking in the luteal phase compared to the follicular phase (30). However, two studies of nicotine replacement therapy reported improved smoking cessation outcomes when quitting occurred in the follicular rather than the luteal phase (31,32). A fifth study by Saladin and colleagues (2015) found that increases in progesterone levels (rather than menstrual phase described above) may be associated with increased abstinence in women treated with nicotine patch, but not varenicline (33). There were several methodological differences between these studies, however, one of the primary differences is that the first two did not use nicotine replacement therapy whereas the other three did. This suggests that there may be an interaction between nicotine and sex hormones (34).

Given this current evidence, several studies have investigated the acute effect of exogenous progesterone (i.e., progesterone treatment) versus placebo on cigarette smoking. Sofuoglu and colleagues (2001) found that, following a single dose of progesterone (200 mg), female smokers (who quit in the early follicular phase; low progesterone) demonstrated attenuated craving and subjective effects of smoking compared with placebo during a self-administration smoking paradigm (35). Another study by the same group, which included men, demonstrated that administration of progesterone (200 mg) was associated with lower ratings of “drug-liking”

than placebo and 200- and 400-mg doses were associated with lower ratings of “drug strength” in both men and women (36).

Among clinical trials examining the effects of longer-term administration of progesterone versus placebo (8-12 weeks), research is limited and has focused on the prevention of postpartum smoking relapse. In a recent 12-week double-blind, placebo-controlled randomized pilot trial (N=46), we observed that at four weeks postpartum, 7-day point prevalence abstinence rates were higher in the progesterone-treated group vs. the placebo group (75.0% vs. 68.2%; $p=.75$) (37). A similar study by Forray and colleagues (2017; N=41) found that postpartum women taking progesterone were 1.8 times more likely to be abstinent during week 8 of treatment (38).

No published studies to date have examined the effect of exogenous progesterone on smoking cessation in a large group of men and women. The overall goals of this pilot double-blind, placebo-controlled randomized clinical trial were to (1) preliminarily test whether oral micronized progesterone (200mg twice daily for 12 weeks) was more effective than placebo on smoking cessation at weeks 4, 8 and 12 and (2) test the association of progesterone levels at week 2 with each of the abstinence outcomes, as well as with smoking-related symptomatology (e.g., withdrawal, craving, urges to smoke, mood and perceived stress) at week 4. We expected that for both men and women, the group randomized to active progesterone would see a higher proportion of participants with 7-day point-prevalence, prolonged and continuous abstinence compared to those randomized to placebo. We also expected that serum levels of progesterone would be associated with smoking abstinence and smoking-related symptomatology.

METHODS

This pilot double-blind, placebo-controlled randomized clinical trial was conducted at the University of Minnesota. Specifically, we enrolled men and women who were daily smokers, motivated to quit smoking and otherwise healthy. Participants were stratified by sex then randomly assign (1:1 ratio) to either active (200mg twice daily) or placebo progesterone. All participants were followed for 12 weeks after an assigned quit date with in-person clinic visits to monitor for smoking relapse via self-report and biochemical verification. Compliance and safety were also monitored throughout the follow-up period. All procedures were approved by the Institutional Review Board and all participants provided informed consent. A Data and Safety Monitoring Board (DSMB) provided ongoing oversight in patient safety and treatment efficacy throughout the trial.

Participants

Recruitment occurred throughout the Minneapolis/St. Paul metro area, Minnesota, USA. Recruitment efforts included flyers, pamphlets, and advertisements on social and mass media. Eligibility criteria included: women and men who were daily smokers (≥ 5 cigarettes per day); motivated to quit (≥ 7 on a Likert-type scale when asked “On a scale of 1-10 [with 10 being the highest], how motivated are you to quit smoking?” used in prior studies) (29,39); women 18-50 years and premenopausal with self-reported regular menstrual cycles for at least the past six

months; men 18-60 years. Exclusionary criteria included: current use of other types of tobacco, nicotine replacement therapy or smoking cessation medications; use of illicit drugs with the exception of non-daily marijuana; diagnosis of psychotic, bipolar, ADHD, or major depressive disorder using the Structured Clinical Interview for DSM-IV by trained research staff (40); substance dependence with the exception of nicotine; unstable psychotropic medications; current use of exogenous hormones; current/recent (past 3 months) pregnancy or breastfeeding; or conditions contraindicated to progesterone treatment. All participants completed the Fagerstrom Test for Nicotine Dependence (FTND) at the screening visit however, this measure was not a factor in determining eligibility (41).

Randomization

Participants were stratified by sex and randomized (1:1) to either 12 weeks of oral micronized progesterone (200mg twice daily) or placebo. The study statistician generated a randomization list that included identification numbers and treatment assignment (A/B) using mixed permuted blocks of size four and six; the pharmacy randomly assigned placebo to one letter and progesterone to the other. A pharmacy technician prepared sequentially numbered pill containers that masked any information about the study medication and had no other participation in the study. This ensured that all study staff remained blind to participant allocation. Randomization occurred at the baseline visit.

Study Medication

Oral micronized progesterone (Teva Pharmaceuticals) were over-encapsulated and identical appearing placebo capsules were prepared by Investigational Drug Services at the University of Minnesota, Fairview Medical Center. Both types of capsules also contained riboflavin which caused the urine to change color, allowing for a real-time indication of adherence. Typically, 50–60% of micronized progesterone is absorbed after oral administration; it reaches its peak plasma levels in two to three hours and has an elimination half-life of three to four hours (42). As found in our previous study with postpartum women, a dose of 200 mg of micronized progesterone results in serum progesterone levels comparable to those found in the mid-luteal phase of the menstrual cycle (3-25ng/ml) (37). For men, a dose of 200 mg of micronized progesterone results in similar serum progesterone levels (36). Due to the relatively short half-life, we utilized 200 mg administered twice daily to maintain stable plasma levels. Common side effects include fatigue, nausea, breast tenderness, irregular menstrual/vaginal bleeding (women only) and weight gain (43). Study medication was started one week prior to the study-assigned quit day.

Procedures

All female participants attended a screening visit during the follicular phase of their menstrual cycle (cycle days 1-7, with onset of menses defined as day 1), a baseline visit during the early luteal phase (1-3 days after ovulation as determined by First Response Ovulation Tests detailed elsewhere) (44,45), and were assigned a quit date during the mid-luteal phase (7 days after the baseline visit; approximate menstrual cycle day 21). Men attended a screening visit at any time, were randomly assigned a baseline date based on typical wait times for female participants, and were assigned a quit date seven days after their baseline visit. Following their assigned quit

dates, participants attended weekly clinic visits, continued on medication for an additional 11 weeks, and were followed for 12 weeks. Participants were compensated at all clinic visits.

At each clinic visit, expired carbon monoxide was measured, smoking Timeline Followback (46,47) interview was completed, and blood samples for progesterone and urine samples for cotinine were collected. Medication adherence and adverse events were assessed and participants completed smoking-related symptomatology measures. Brief smoking cessation counseling was offered by a certified Tobacco Treatment Specialist. A study-specific facilitator's manual was developed based on principles of the Mayo Clinic Nicotine Dependence Center. Blood and urine samples were frozen and sent for batch analyses to University of Minnesota labs. Progesterone was analyzed in serum using a solid phase enzyme-linked immunosorbent assay (ELISA) kit per the manufacturer's instruction (IBL International, RE52231). The assay sensitivity is 0.045 ng/mL with a standard range of 0–40 ng/mL. Cotinine concentrations were determined in 20 µL of a 1:10 diluted sample by liquid chromatography tandem mass spectroscopy as previously described (48). The limit of quantitation for these analyses was 1.5 ng/mL and the coefficient of variation was 6%.

Outcomes and Measures

The primary outcome was self-reported 7-day point prevalence abstinence (PPA) from smoking defined as having no slips (i.e., a puff or more from a lit cigarette) in the seven days prior to week 4. Secondary outcomes included: (A) self-reported 7-day point prevalence abstinence at weeks 8 and 12, (B) self-reported prolonged abstinence defined as having fewer than seven consecutive slips without a 24-hour period between any two slips, prior to each of weeks 4, 8 and 12, (C) self-reported continuous abstinence defined as having no slips at all prior to week 12, (D) urine cotinine, with <50 ng/mL indicative of abstinence (49,50), at each of weeks 4, 8 and 12, (E) expired carbon monoxide, with ≤5 ppm indicative of abstinence (51), at each of weeks 4, 8 and 12, and (F) self-reported days to relapse defined as the number of days from assigned quit date to the first day with a slip (for those who quit and then relapsed) or to last TLFB date (for those who quit and did not relapse) (52).

Other secondary analyses for this study examined the associations of progesterone levels at week 2 with each of the abstinence outcomes, as well as with smoking-related symptomatology at week 4. Smoking-related symptomatology was assessed by questionnaires of withdrawal, craving, urges to smoke, mood and perceived stress. The Minnesota Withdrawal Scale (MNWS) (53,54) is an 8-item scale measuring nicotine withdrawal symptoms and craving. The Brief Questionnaire of Smoking Urges (QSU-Brief) (55) is a 10-item scale measuring two sub-scales: desire to smoke (Factor 1) and anticipated relief from negative affect (Factor 2). The Profile of Mood States (POMS) (56,57) is a 65-item scale measuring six subscales; tension-anxiety, anger-hostility, fatigue-inertia, depression-dejection, vigor-activity and confusion-bewilderment. It also yields a total mood disturbance score. The Perceived Stress Scale (PSS) (58,59) is a 10-item scale measuring the degree to which situations in one's life are appraised as stressful. All data were collected and managed using REDCap electronic data capture tools hosted at the University of Minnesota (60).

Statistical Power

The trial was originally designed to have 80% power, with type I error rate 0.025 for *each* sex group, to detect an odds ratio of 3.5 for a two-tailed test of progesterone vs. placebo for the primary outcome of 7-day PPA at week 4 (assuming a PPA of 0.34 in the placebo group), requiring 100 completed week 4 visits per sex. However, the type I error rate correction for the testing of the two sex groups was deemed too conservative and unnecessary by the DSMB in light of recruitment difficulties and was replaced with a type I error rate of 0.05 per sex group upon their recommendation in October 2014. The trial was thus re-designed to have 80% power, with type I error rate of 0.05 per sex group, to detect an odds ratio of 3.5 (assuming a PPA of 0.34 in the placebo group), requiring 84 completed week 4 visits per sex.

Statistical Analysis

In accordance with the trial's design, all treatment group comparisons were performed separately by sex. All statistical analyses were performed using SAS 9.4. Per sex, a *p*-value less than 0.05 was considered statistically significant.

Descriptive statistics were used to examine the characteristics of randomized patients by sex and treatment group. Means were reported with their standard deviation (SD) and medians were reported with interquartile ranges (IQR). *P*-values for the comparison of participant characteristics between treatment groups per sex were performed using the Chi-Square test or Fishers Exact Test for categorical variables, and ANOVA or Kruskal-Wallis test for continuous variables.

For all analyses, participants were included in the group to which they were randomized (intent to treat, ITT). For the primary outcome (7-day point prevalence abstinence at week 4) and secondary outcomes (7-day point prevalence abstinence at weeks 8 and 12, prolonged abstinence at weeks 4, 8 and 12, continuous abstinence at week 12, urine cotinine <50 ng/mL at weeks 4, 8 and 12, expired carbon monoxide ≤5 ppm at weeks 4, 8 and 12, and days to relapse), any randomized participant who was missing the outcome variable (e.g., drop-outs, missed visits, or missing data) was included in the analysis by assuming they were a smoker in that week. Primary and secondary abstinence outcomes were compared between treatment groups per sex using logistic regression (binary variables) or the Kruskal-Wallis Test (days to relapse). A similar analysis approach was followed for the secondary analyses comparing smoking-related symptomatology (continuous variables) between treatment groups per sex using the Kruskal-Wallis Test.

Secondary analyses of the association between week 2 progesterone (natural log) and binary abstinence outcomes were performed using logistic regression. Generalized linear models with link=log option (GLIMMIX) were used for the secondary analysis of the association between week 2 progesterone (natural log) and week 4 smoking-related symptomatology due to the skewedness of the outcome variables. The models were adjusted for the screening variables of age, cigarettes per day, FTND score and POMS Total Mood Disturbance (TMD) score, except that the model with POMS TMD at week 4 as the outcome was not adjusted for POMS TMD at screening due to their high correlation.

RESULTS

A total of 6,440 people expressed interest in the study either by phone or email. Of these, 1,580 (25%) completed a brief phone screen (see Figure 1). Eight hundred and fifty (54%) were found to be ineligible after completing the brief phone screen. The three most common reasons for exclusion were insufficient motivation to quit smoking (<7 on a Likert-type scale), use of other types of tobacco or nicotine products, and self-reported irregular menstrual cycles for women. Of the 312 people who completed an in-person screening visit, 76 (24%) were found to be ineligible and 20 (6%) were lost to follow-up between screen and baseline (randomization). The three most common reasons for exclusion at this point were unstable or unsafe health conditions, current major depressive disorder, and irregular menstrual cycles for women. Participants not lost to follow-up were significantly older than those lost to follow-up (37 ± 9.6 years vs. 31 ± 9.1 years respectively, $p=0.008$); there were no other statistically significant differences in participant characteristics (see Table 1; Supplementary Table 1).

Adherence to Study Treatment, Losses to Follow-up and Adverse Events

Figure 2 shows the difference in progesterone levels at week 2 between the progesterone and placebo groups for both men and women. The bimodal distributions indicate that the progesterone treatment increased serum progesterone as expected for both men and women (Kruskal-Wallis test of treatment group difference, $p<0.0001$ for both men and women).

Medication adherence was assessed by visual inspection of participant urine for riboflavin (contained in the blinded capsules) as well as by pill count. Adherence based on detectable riboflavin at week 4 was 85.7%. No statistically significant difference in adherence was found by treatment (see Table 2). Losses to follow-up at week 4 were similar for both treatments for both sexes; at week 12, losses to follow-up for men were similar across treatments; however, losses to follow-up were nearly double for women in the placebo group compared to the progesterone group).

The most commonly reported adverse events that were likely related to the study medication were fatigue, irregular menstrual bleeding and nausea. One male participant in the progesterone group experienced a non-fatal stroke which was reported as a “possibly related” serious adverse event (see Table 3).

Primary Outcome: 7-Day Point Prevalence Abstinence at Week 4

Among women, the odds of achieving 7-day PPA at week 4 were 2.6 times higher in the PRO group versus the PBO group (Odds Ratio [95% confidence interval]=2.61 [1.04, 6.54], $p=0.041$); whereas for men, the odds of 7-day PPA at week 4 were similar in the PRO and PBO groups (1.13 [0.47, 2.76], $p=0.782$; see Table 4).

Secondary Outcomes

Mean days to relapse was significantly different for women by randomization group. Participants in the PRO group had an average of 20.5 ± 29.6 days to relapse while the PBO group had an average of 14.3 ± 26.8 days to relapse ($p=0.03$). No other statistically significant

differences between groups in secondary outcomes were observed in women. In men, the days to relapse did not differ by randomization (PRO: 13.4 ± 25.9 vs. PBO: 13.3 ± 23.8 , $p=0.69$), and there were no statistically significant differences by randomization group in secondary outcomes were observed in men (see Table 4).

Other Secondary Analyses

Week 2 progesterone levels were not significantly associated with 7-day point prevalence abstinence, prolonged abstinence, urine cotinine <50 ng/mL or expired carbon monoxide ≤ 5 ppm at week 4 in men or women (see Table 5). Week 2 progesterone levels among women were not significantly associated with week 4 MNWS withdrawal or craving, QSU factor 1 or 2, PSS or POMS scores. In contrast, among men, a higher week 2 progesterone level was significantly associated with a higher POMS fatigue and total mood disturbance scores at week 4 (see Table 6).

DISCUSSION

This double-blind placebo-control pilot randomized trial is among the first to examine the effect of exogenous progesterone (200mg twice daily for 12 weeks) compared to placebo on smoking cessation and smoking-related symptomatology (SRS; e.g., withdrawal, craving, urges to smoke, mood and perceived stress) in both men and women. As expected, exogenous progesterone increased serum progesterone levels and was well tolerated in both men and women. In line with our hypotheses, women randomized to active progesterone, as compared to women on placebo, had nearly three times higher odds of abstinence at Week 4 and averaged six more days to relapse. In contrast, there was no evidence of progesterone improving cessation outcomes in men at any time point. Furthermore, in both men and women, levels of progesterone at week 2 were not associated with SRS nor with biochemically verified markers of abstinence at week 4.

Although this pilot study is strengthened by its double-blind, placebo-controlled randomized clinical trial design, as well as the inclusion of both men and women, there are limitations worth noting. First, our sample size was too small for a formal statistical hypothesis test between sexes. Second, our primary outcome was not biochemically-verified abstinence and participants were only followed for 12 weeks. Third, generalizability is limited, especially in women, as our sample included only premenopausal women with varying levels of endogenous progesterone. Studying postmenopausal women (with less varying levels) may provide further insight to the effects of exogenous progesterone as a potential smoking cessation aid for women. Lastly, we did not adjust for multiple testing across the many secondary outcomes and a Type I error in our observations is possible. Despite these limitations, this pilot study offers some observations that advance the field and offer directions for future research.

Our observations suggest that progesterone may improve cessation outcomes in women. These novel findings help to untangle the complex factors that contribute to sex differences in smoking cessation. Pre-clinical literature provides strong evidence that progesterone is protective against drug abuse behaviors (11–18), however, clinical research has been less clear. Results of this study support our and others prior findings that women who attempt to quit

smoking in the luteal phase of the menstrual cycle (high progesterone) may have better smoking cessation outcomes (29,30). Results of this study also complement previous findings that exogenous progesterone may be helpful in preventing postpartum smoking relapse (37,38). However, the lack of an association between endogenous progesterone levels and study outcomes suggests that the progesterone itself may not be the direct cause of improved cessation outcomes in women. Future research should explore the causal mechanisms involved. One possible explanation may be allopregnanolone, which is a metabolite of progesterone that has neurobiological stress-reducing effects (61,62) and has been implicated in preclinical literature as a risk factor for drug-taking behaviors (63). Our prior research indicate that more allopregnanolone is produced during acute smoking abstinence in the luteal (when more progesterone is naturally available) as opposed to the follicular phase (when less progesterone is naturally available) (64). Further, the physiological, subjective and cognitive effects of nicotine vary by allopregnanolone levels. This suggests that the reinforcing effects of nicotine, and perhaps cigarette smoking, may vary by allopregnanolone levels (65). Thus, future research could examine metabolism levels of progesterone to allopregnanolone, as well as absolute levels of allopregnanolone, as possible effect modifiers in the relationship between exogenous progesterone administration and cessation outcomes in women.

The null results in men are similar to those observed by Sofuoglu and colleagues (2007) who found that in their study of male cocaine users, the placebo group, rather than progesterone group, had better cessation outcomes at the end of treatment (66). Additional research is needed to elucidate why progesterone may have an effect in women but not men. Interestingly, allopregnanolone may also be responsible for this sex difference as preclinical research has indicated that greater allopregnanolone levels is related to lower levels of self-administration in female cocaine-week rats, but not their male counterparts (63). Future research may also explore sex and hormonal differences in the neurobiological reward response to smoking cues as a possible explanation for the lack of an effect in men as previous research has observed varying response by sex (67) and menstrual phase (68).

Interestingly, dropout rates by week 12 were nearly double for women in the placebo group compared to the progesterone group while dropout rates for men were comparable between groups. One possible explanation for this is that women in the progesterone group, although blinded to their treatment, may have felt like the intervention was helpful and therefore were more apt to adhere to the protocol and remain in the study. They may have also had clues to their randomization assignment given the higher reported vaginal bleeding in this group. These results further validate the potential for exogenous progesterone as an effective smoking cessation aid for women.

In conclusion, this pilot double-blind, placebo-controlled randomized clinical trial has shown that 200mg twice daily of oral micronized progesterone may be effective for smoking cessation in women, but not in men. Further research is needed to confirm this observation with a larger and more diverse study sample, longer follow-up period, and with biochemically-verified outcome measures, as well as examine the effect of pairing progesterone with smoking cessation pharmacotherapies (such as nicotine replacement therapy and varenicline).

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Figure 1. CONSORT Diagram of Participant Flow

Figure 2. Log Progesterone Levels at Week 2 by Sex and Treatment Group (log ng/mL)

Table 1. Baseline Characteristics by Sex and Treatment Group				
	Males		Females	
	Progesterone	Placebo	Progesterone	Placebo
	Mean $\pm$ SD or N (%)	Mean $\pm$ SD or N (%)	Mean $\pm$ SD or N (%)	Mean $\pm$ SD or N (%)
Number of Participants	56	57	51	52
Demographics				
Age (years)	37.9 $\pm$ 10.6	36.8 $\pm$ 11.8	37.6 $\pm$ 7.4	36.0 $\pm$ 7.8
Race (% non-white)	22 (39.3)	25 (43.9)	20 (39.2)	21 (40.4)
Education (% $\geq$ college grad)	10 (18.2)	6 (10.7)	12 (23.5)	16 (30.8)
Smoking Verification				
CO Level (ppm)	17.1 $\pm$ 9.3	14.5 $\pm$ 8.6	17.4 $\pm$ 11.1	16.6 $\pm$ 9.4
Cotinine (ng/mL)	4609 $\pm$ 3874	3731 $\pm$ 3086	3976 $\pm$ 4583	3145 $\pm$ 2393
Smoking Behavior				
Cigarettes Smoked (cigs/day)	15.6 $\pm$ 6.7	15.4 $\pm$ 7.2	12.8 $\pm$ 5.0	12.6 $\pm$ 6.6
Age Started Smoking (years)	17.4 $\pm$ 4.2	18.5 $\pm$ 6.9	17.5 $\pm$ 4.0	17.2 $\pm$ 3.3
Quit Attempts (number)	4.9 $\pm$ 4.0	7.0 $\pm$ 7.9	6.8 $\pm$ 8.6	6.3 $\pm$ 13.8
FTND Total Score	4.9 $\pm$ 2.1	5.1 $\pm$ 2.3	4.5 $\pm$ 2.3	4.2 $\pm$ 2.1
Smoking-related Symptomatology				
MNWS Withdrawal	12.5 $\pm$ 4.9	12.6 $\pm$ 4.5	14.2 $\pm$ 4.9	13.5 $\pm$ 5.2
MNWS Craving	3.3 $\pm$ 1.0	3.6 $\pm$ 0.8	3.8 $\pm$ 0.7	3.3 $\pm$ 0.9
QSU Factor 1	18.1 $\pm$ 8.0	20.3 $\pm$ 8.9	17.0 $\pm$ 8.9	16.8 $\pm$ 7.9
QSU Factor 2	9.4 $\pm$ 4.9	11.7 $\pm$ 7.1	10.0 $\pm$ 6.6	9.6 $\pm$ 5.6
POMS Tension-anxiety	6.9 $\pm$ 5.2	6.8 $\pm$ 4.4	7.3 $\pm$ 4.8	7.5 $\pm$ 4.9
POMS anger-hostility	5.0 $\pm$ 5.5	6.0 $\pm$ 5.4	5.1 $\pm$ 6.8	5.5 $\pm$ 5.4
POMS fatigue-inertia	5.5 $\pm$ 5.0	5.6 $\pm$ 4.5	6.7 $\pm$ 5.9	6.7 $\pm$ 5.2
POMS depression-dejection	5.3 $\pm$ 7.7	5.4 $\pm$ 5.5	4.9 $\pm$ 5.6	6.5 $\pm$ 7.5
POMS vigor-activity	17.1 $\pm$ 6.2	16.3 $\pm$ 5.0	14.9 $\pm$ 5.8	14.8 $\pm$ 6.2
POMS confusion-bewilderment	5.0 $\pm$ 3.7	5.3 $\pm$ 3.1	5.3 $\pm$ 3.5	6.0 $\pm$ 3.6
POMS Total Mood Disturbances	10.7 $\pm$ 28.2	12.8 $\pm$ 21.8	14.4 $\pm$ 24.5	17.3 $\pm$ 25.7
PSS Total	17.3 $\pm$ 8.0	17.8 $\pm$ 7.9	18.1 $\pm$ 8.6	19.8 $\pm$ 6.7
The P-value column is for the comparison between treatment groups. Chi-Square or Fishers Exact Test for categorical variables, ANOVA or Kruskal-Wallis for continuous variables.				
Abbreviations -> FTND = Fagerstrom Test for Nicotine Dependence, CO = Carbon Monoxide, SD = Standard Deviation, MNWS = Minnesota Nicotine Withdrawal Scale, QSU-Brief = Questionnaire of Smoking Urges-Brief POMS = Profile of Mood States, PSS = Perceived Stress Scale				

Table 2. Adherence - Riboflavin & Pill Counts by Sex and Treatment Group						
	Males			Females		
	Progesterone	Placebo	P-value	Progesterone	Placebo	P-value
	N (%)	N (%)		N (%)	N (%)	
Number of Participants	56	57		51	52	
Week 4						
Riboflavin Tested	42	40		45	40	
Detectable Riboflavin	38 (90.5)	36 (90.0)	>0.990	37 (82.2)	32 (80.0)	>0.990
Pill Counts Tallied	42	44		47	46	
Percent of Pills Taken Median (IQR)	82.4 (54.7 - 95.7)	63.8 (34.8 - 93.8)	0.101	83.3 (53.0 - 97.1)	80.9 (51.3 - 92.4)	0.580
Week 8						
Riboflavin Tested	31	32		40	30	
Detectable Riboflavin	26 (83.9%)	25 (78.1%)	0.750	32 (80.0%)	27 (90.0%)	0.331
Pill Counts Tallied	42	44		47	46	
Percent of Pills Taken Median (IQR)	50.4 (34.6 - 56.3)	37.6 (20.1 - 55.2)	0.103	44.8 (28.7 - 54.8)	50.4 (31.7 - 57.0)	0.418
Week 11						
Riboflavin Tested	21	30		35	28	
Detectable Riboflavin	19 (90.5%)	19 (63.3%)	0.048	27 (77.1%)	26 (92.9%)	0.164
Pill Counts Tallied	42	44		47	46	
Percent of Pills Taken Median (IQR)	39.0 (28.2 - 43.8)	31.6 (16.1 - 43.9)	0.144	35.2 (21.3 - 42.8)	38.4 (23.8 - 47.1)	0.343
The P-value column is for the comparison between treatment groups. The P-values are from Chi-Square or Fishers Exact Test (binary variables), ANOVA or Kruskal-Wallis (continuous variables).						
Abbreviations -> IQR = Interquartile range						

Table 3. Summary of Adverse Events by Sex and Treatment Group				
	Males		Females	
	Progesterone	Placebo	Progesterone	Placebo
	N (%)	N (%)	N (%)	N (%)
Number of Participants	56	57	51	52
Gastrointestinal	8 (9.8)	6 (10.3)	11 (10.3)	9 (10.8)
Nausea	3 (3.7)	1 (1.7)	5 (4.7)	2 (2.4)
Constipation	2 (2.4)	1 (1.7)	3 (2.8)	0
Stomach Flu Symptoms	1 (1.2)	1 (1.7)	0	4 (4.8)
Diarrhea	0	1 (1.7)	2 (1.9)	2 (2.4)
Acid Reflux	2 (2.4)	0	1(0.9)	1 (1.2)
Hemorrhoid	0	2 (3.4)	0	0
General	38 (46.3)	27 (46.6)	35 (32.7)	29 (34.9)
Cold/Flu Symptoms	24 (29.3)	13 (22.4)	20 (18.7)	16 (19.3)
Fatigue	7 (8.5)	8 (13.8)	9 (8.4)	7 (8.4)
Body Aches/Pain	7 (8.5)	6 (10.3)	6 (5.6)	6 (7.2)
Genital/Urinary	3 (3.7)	0	26 (24.3)	14 (16.9)
Irregular Menstrual Bleeding	0	0	18 (16.8)	5 (6.0)
Breast Tenderness/Pain	0	0	3 (2.8)	2 (2.4)
Cramping	1 (1.2)	0	0	3 (3.6)
Sexual Dysfunction	2 (2.4)	0	1 (0.9)	1 (1.2)
Change in vaginal discharge	0	0	0	2 (2.4)
Herpes Outbreak	0	0	2 (1.9)	0
Urinary Tract Infection	0	0	1 (0.9)	1 (1.2)
Hot Flashes	0	0	1 (0.9)	0
Heart & Lungs	3 (3.7)	3 (5.2)	2 (1.9)	4 (4.8)2
High Blood Pressure (> 160/100)	3 (3.7)	2 (3.4)	1 (0.9)	3 (3.6)
Shortness of Breath	0	0	1 (0.9)	1 (1.2)
Heart Palpitations	0	1 (1.7)	0	0
Mental Health	1 (1.2)	0	5 (4.7)	6 (7.2)
Increased Emotions/Anxiety	0	0	3 (2.8)	4 (4.8)
Depression/Suicidal Thoughts	1 (1.2)	0	2 (1.9)	2 (2.4)
Neurological	9 (11.0)	7 (12.1)	11 (10.3)	7 (8.4)
Dizziness	6 (7.3)	3(5.2)	6 (5.6)	2(2.4)
Headache	1 (1.2)	3 (5.2)	5 (4.7)	5 (6.0)
Numbness and Tingling	1 (1.2)	1 (1.7)	0	0
Stroke	1 (1.2)*	0	0	0
Skin & Hair	1 (1.2)	1 (1.7)	2 (1.9)	3 (3.6)
Acne/Pimples	1 (1.2)	0	0	1 (1.2)
Itching	0	0	0	2 (2.4)
Thinning Hair/Hair Loss	0	1 (1.7)	1 (0.9)	0
Skin Irritation	0	0	1 (0.9)	0
Other	19 (23.2)	14 (24.1)	15 (14.0)	11 (13.3)
Car/bike accident, fall, sprain	2 (2.4)	4 (6.9)	4 (3.7)	4 (4.8)

Infection	2 (2.4)	1 (1.7)	1 (0.9)	1 (1.2)
Allergies	3 (3.7)	0	1 (0.9)	0
Insomnia/Trouble Sleeping	1 (1.2)	0	3 (2.8)	0
Nightmares	0	1 (1.7)	0	2 (2.4)
Appetite Increase/Decrease	1 (1.2)	1 (1.7)	0	0
Aversion to cigarettes	1 (1.2)	1 (1.7)	0	0
Dry Mouth	0	1 (1.7)	0	1 (1.2)
Blurred Vision	0	0	1 (0.9)	0
Other	9 (11.0)	5 (8.6)	5 (4.7)	3 (3.6)
Total Number of Events	82	58	107	83

* Documented as a serious adverse event

Table 4. Primary & Secondary Abstinence Outcomes by Sex and Treatment Group Odds Ratios Added								
	Males				Females			
	Progesterone	Placebo	Odds Ratio (95% CI)	P- value	Progesterone	Placebo	Odds Ratio (95% CI)	P- value
	N (%) or Mean ± SD	N (%) or Mean ± SD			N (%) or Mean ± SD	N (%) or Mean ± SD		
Number of Participants	56	57			51	52		
Week 4								
7-day Point Prevalence Abstinence↑	13 (23.2)	12 (21.1)	1.13 (0.47, 2.76)	0.782	18 (35.3)	9 (17.3)	2.61 (1.04, 6.54)	0.041
Prolonged Abstinence↑	11 (19.6)	15 (26.3)	0.68 (0.28, 1.66)	0.400	18 (35.3)	10 (19.2)	2.29 (0.93, 5.62)	0.070
Cotinine <50ng/mL	11 (19.6)	9 (15.8)	1.30 (0.49, 3.44)	0.592	10 (19.6)	8 (15.4)	1.34 (0.48, 3.73)	0.573
CO Level ≤5 ppm	20 (35.7)	17 (29.8)	1.31 (0.60, 2.87)	0.505	22 (43.1)	18 (34.6)	1.43 (0.65, 3.18)	0.375
Week 8								
7-day Point Prevalence Abstinence↑	10 (17.9)	10 (17.5)	1.02 (0.39, 2.69)	0.965	16 (31.4)	10 (19.2)	1.92 (0.77, 4.76)	0.159
Prolonged Abstinence↑	7 (12.5)	9 (15.8)	0.76 (0.26, 2.21)	0.616	15 (29.4)	8 (15.4)	2.29 (0.87, 6.01)	0.091
Cotinine <50ng/mL	8 (14.3)	7 (12.3)	1.19 (0.40, 3.54)	0.753	10 (19.6)	8 (15.4)	1.34 (0.48, 3.73)	0.573
CO Level ≤5 ppm	13 (23.2)	14 (24.6)	0.93 (0.39, 2.21)	0.866	17 (33.3)	16 (30.8)	1.13 (0.49, 2.58)	0.780
Week 12								
7-day Point Prevalence Abstinence↑	7 (12.5)	12 (21.1)	0.54 (0.19, 1.48)	0.228	20 (39.2)	12 (23.1)	2.15 (0.91, 5.06)	0.079
Prolonged Abstinence↑	6 (10.7)	7 (12.3)	0.86 (0.27, 2.73)	0.794	13 (25.5)	7 (13.5)	2.20 (0.80, 6.07)	0.128
Continuous Abstinence↑	4 (7.1)	4 (7.0)	1.02 (0.24, 4.29)	0.979	8 (15.7)	2 (3.8)	4.65 (0.94, 23.09)	0.060
Cotinine <50ng/mL	9 (16.1)	6 (10.5)	1.63 (0.54, 4.92)	0.388	10 (19.6)	9 (17.3)	1.17 (0.43, 3.16)	0.763
CO Level ≤5 ppm	14 (25.0)	9 (15.8)	1.78 (0.70, 4.53)	0.227	19 (37.3)	20 (38.5)	0.95 (0.43, 2.11)	0.899
Days to Relapse↑								
Mean (SD)	13.4 ±25.9	13.3 ±23.8		0.690	20.5 ±29.6	14.3 ±26.8		0.030
Median (IQR)	2.0 (0.0-8.0)	3.0 (1.0-11.0)			6.0 (2.0-21.0)	2.5 (0.0-8.5)		
Range (Min, Max)	0.0, 84.0	0.0, 84.0			0.0, 84.0	0.0, 84.0		
The P-value column is for the comparison between treatment groups. The P-values are from logistic regression (binary variables) or the Kruskal-Wallis Test (continuous variables). Odds ratios are interpreted as progesterone group compared to placebo group.								

7-day point prevalence abstinence is defined as having no slips (i.e., a puff or more from a lit cigarette) in the seven days prior to a given time point (week 4, week 8, week 12). Prolonged abstinence is defined as having less than seven consecutive slips without a 24-hour period between any two slips prior to a given time point (week 4, week 8, week 12). Continuous abstinence is defined as having no slips at all prior to a given time point (week 12).
Abbreviations -> CO = Carbon Monoxide, SD = Standard Deviation, IQR = Interquartile range, ppm = parts per million, ng/mL = nanograms per milliliter
↑: Self-reported

Table 5. Other Secondary Analyses by Sex: Effect of Week 2 Progesterone (log) on Week 4 Smoking Outcomes				
	Males		Females	
	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value
Number of Participants with Week 2 Progesterone Level	69		69	
Smoking Outcomes				
7-day Point Prevalence Abstinence [†]	1.04 (0.71, 1.53)	0.832	1.17 (0.86, 1.61)	0.317
Prolonged Abstinence [†]	0.77 (0.52, 1.15)	0.202	1.38 (0.99, 1.92)	0.061
Cotinine <50 ng/mL	1.00 (0.67, 1.51)	0.983	1.05 (0.73, 1.51)	0.808
CO Level ≤5 ppm	1.05 (0.72, 1.52)	0.815	1.10 (0.81, 1.50)	0.529
Odds Ratios, 95% Confidence Intervals (CI) and p-values are from logistic regression adjusted for screening variables of age, cigarettes per day, FTND total score, and POMS total mood disturbance score. Odds ratios are interpreted per one unit higher natural log progesterone (measured in ng/mL).				
Abbreviations -> CI = Confidence Interval, CO = Carbon Monoxide, ppm = parts per million, ng/mL = nanograms per milliliter				
[†] : Self-reported				

Table 6. Other Secondary Analyses by Sex: Effect of Week 2 Progesterone (log) on Week 4 Continuous Smoking Symptoms (log)				
	Males		Females	
	Estimate (Standard Error)	P-value	Estimate (Standard Error)	P-value
Number of Participants	65		68	
<i>MNWS</i>				
MNWS Craving	-0.022 (0.03)	0.511	0.020 (0.03)	0.495
MNWS Withdrawal	0.009 (0.03)	0.759	0.039 (0.02)	0.119
<i>QSU-Brief</i>				
QSU Factor 1	0.011 (0.04)	0.773	-0.023 (0.03)	0.514
QSU Factor 2	0.037 (0.04)	0.335	-0.002 (0.04)	0.964
<i>POMS</i>				
Tension-Anxiety	0.061 (0.05)	0.220	0.034 (0.05)	0.492
Anger-Hostility	0.057 (0.07)	0.418	0.114 (0.07)	0.086
Fatigue-Inertia	0.114 (0.06)	0.046	0.055 (0.05)	0.263
Depression-Dejection	-0.047 (0.10)	0.634	0.083 (0.08)	0.331
Vigor-Activity	-0.001 (0.03)	0.965	-0.039 (0.03)	0.183
Confusion-Bewilderment	0.015 (0.05)	0.744	0.082 (0.05)	0.082
Total Mood Disturbance	0.441 (0.21)	0.039	0.119 (0.11)	0.278
<i>PSS</i>				
PSS Total	-0.006 (0.03)	0.854	0.006 (0.03)	0.840
Estimates, standard errors and p-values are from a Generalized Linear Model with link=log (GLIMMIX) Adjusted for screening variables of age, cigarettes per day, FTND score and POMS total mood disturbance score EXCEPT POMS Total Mood Disturbance score model was not adjusted for POMS TMD at screening - too closely related				
Abbreviations -> MNWS = Minnesota Nicotine Withdrawal Scale, QSU-Brief = Questionnaire of Smoking Urges-Brief POMS = Profile of Mood States, PSS = Perceived Stress Scale				

Supplemental Table 1. Baseline Characteristics by Sex			
	Males	Females	P-value
	Mean $\pm$ SD or N (%)	Mean $\pm$ SD or N (%)	
Number of Participants	113	103	
Demographics			
Age (years)	37.3 $\pm$ 11.2	36.8 $\pm$ 7.6	0.693
Race (% non-white)	47 (41.6)	41 (39.8)	0.789
Education (% $\geq$ college grad)	16 (14.4)	28 (27.2)	0.021
Smoking Verification			
CO Level (ppm)	15.8 $\pm$ 9.0	17.0 $\pm$ 10.3	0.372
Cotinine (ng/mL)	4170 $\pm$ 3514	3561 $\pm$ 3662	0.104
Smoking Behavior			
Cigarettes Smoked (cigs/day)	15.5 $\pm$ 6.9	12.7 $\pm$ 5.8	0.002
Age Started Smoking (years)	17.9 $\pm$ 5.7	17.3 $\pm$ 3.7	0.377
Quit Attempts (number)	6.0 $\pm$ 6.4	6.6 $\pm$ 11.5	0.204
FTND Total Score [†]	5.0 $\pm$ 2.2	4.3 $\pm$ 2.2	0.037
The P-value column is for the comparison between sexes. Chi-Square or Fishers Exact Test for categorical variables, ANOVA or Kruskal-Wallis for continuous variables.			
Abbreviations -> FTND = Fagerstrom Test for Nicotine Dependence, CO = Carbon Monoxide, SD = Standard Deviation, ppm = parts per million, ng/mL = nanograms per milliliter			